A STUDY OF PYRIDINIUM BETAINES

A Thesis

by

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GENERAL INTRODUCTION

The biological significance of certain pyridinium betaines, such as diphosphopyridine nucleotide and triphosphopyridine nucleotide, has been fully recognized. The vital importance of these substances in the life of organisms provided an impetus for extensive research in this field, with the result that our knowledge concerning the oxidation-reduction processes in living organisms has been greatly enriched.

There were, however, additional factors which stimulated this research. Since pyridinium betaines, such as trigonelline and homarine were isolated from natural sources, it was expected that investigation of these substances would afford information about the biological methylation process. Other pyridinium betaines were found to be highly reactive intermediates of the alkaline cleavage of certain pyridinium salts. Investigations which began in the early thirties revealed that pyridinium salts were invaluable intermediates in the synthesis of a great variety of organic compounds, and thus a new branch of unusual versatility and importance in organic chemistry was opened.

The object of the present research was to study the synthesis of a series of pyridinium betaines which possessed a keto or a carboxylic group as their main anionic function. Certain pyridinium betaines containing the 1,3-indandione group were synthesized successfully. The behaviour of the isomeric pyridinemonocarboxylic acids in the formation of 1,3-indandione pyridinium betaines was noted with interest. In this case it was discovered that the carbonyl group became the negative centre of the dipolar ion in preference to the carboxylic group when both these functional groups were present in the same molecule. This type of betainization involving the

carbonyl group as the main negative centre, also was found to occur when 2-bromo-acetophenone reacted with pyridinecarboxylic acids.

The uncertainty which existed about the nature of addition compounds obtained from the reaction of certain α-halo ketosteroids with pyridine bases was elucidated. That these addition products were typical pyridinium salts was established. Elimination of hydrogen halide from these substances resulted in the formation of the corresponding coloured betaines. The study of pyridinium salts in ketosteroids, in conjunction with the attempted synthesis of pyridinium betaines containing a carboxylic group as the anionic function of the dipole, was an integral part of the investigation concerning the synthesis of nitrogen-containing steroidal compounds, which is in progress in this laboratory.

HISTORICAL INTRODUCTION

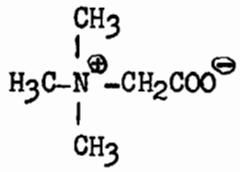
Structure and character of the betaines

A great number and variety of organic compounds belong to the class of betaines. For the most part, these betaines are obtained by synthesis in vitro, although some of them are distributed in nature.

In spite of the variation among these compounds their chemical, and more particularly their physical, properties are quite similar. This similarity is due apparently to the dipolar character of the molecule, especially to the cationic function which is always located at a tetra-substituted quaternary nitrogen atom.

Consequently betaines may be defined as dipolar ions, the positive and negative charges of which are located at different centres in the molecule, and in which the positive charge is situated at a quaternary nitrogen atom.

Originally the term betaine was used (1) to designate the simplest member of this class of compounds, the anhydride of carboxymethyltrimethyl-ammonium hydroxide (I). This compound, which is widely distributed in



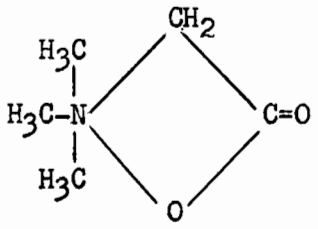
I

plants, was given the name betaine by Scheibler (2) when he first isolated it from the plant "Beta vulgaris". Scheibler isolated the compound in its hydrate form, and by heating this hydrate at 100° he obtained the free betaine (I).

The name betaine was extended later by Brühl (3) to include the

higher homologues of the original betaine. This investigator prepared the trimethyl-α-propiobetaine using ethyl α-chloropropionate and trimethylamine. In addition, Brühl (4) arbitrarily proposed a cyclic structure for the betaines based on a pentavalent nitrogen atom. This proposal appeared to contradict the fact, which he had observed and reported, that the betaines had a basic and acidic character (4).

In early investigations of this class of compounds, only the cyclic structure was used for betaines. However more and more experimental results were accumulated which could not be explained on the basis of such a cyclic structure (II), for example the solubility of betaines in polar solvents, the quite high melting points of these compounds, and their basic



II

and acidic nature. In view of these experimental facts, a revision of the established structure for betaines seemed necessary.

The disagreement between the proposed structure and physical properties of betaines reflected the confusion which existed at that time about the character of amino acids and proteins. It was obvious that the first members of the betaines were closely related to the corresponding amino acids, since these betaines could be considered as exhaustive N-methylation products of the amino acids. However, the theoretical knowledge at the end of the nineteenth century was not sufficient to clarify this problem. Bredig (5,6) suggested that betaines, which had an amphoteric character,

should be considered as inner salts. He assumed that the positive and negative charges were located at separate centres of the molecule, and that the molecule as a whole was electrically neutral. A few years later, Küster (7,8) attributed the difference in colour of the dye methyl orange in acidic and alkaline media to the existence of the molecule in the form of a "zwitterion". These ideas concerning amphoteric electrolytes were met by other authors with skepticism (9) and criticism (10).

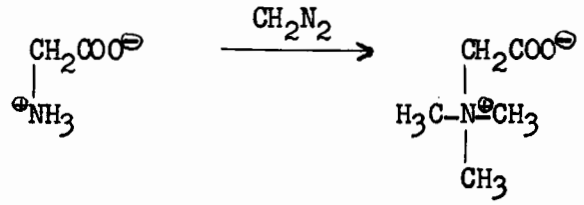
Once the importance of proteins and amino acids in the life of organisms was fully recognized, it became imperative that precise knowledge concerning these compounds should be obtained. In the research which followed, important contributions were made by Waterman (11) who claimed that betaines and amino acids had an open structure in acidic medium, and by Adams (12) who pointed out clearly that the dissociation constants of amino acids could be explained only on the basis of their dipolar character. However, no serious consideration was given to this idea at that time.

A number of years later, Pfeiffer in a series of papers (13,14,15) proved conclusively that betaines possessed an open dipolar structure. The arguments used by this investigator were based on the melting points of these compounds and on sound steric factors. Pfeiffer (13) observed that the N-trialkyl derivatives of the isomeric o-, m- and p-amino-benzoic acids formed betaines with the same ease, by a typical elimination of a hydrogen halide molecule and betainization. If these betaines had cyclic structures, then their formation in the case of the m- and p-amino-benzoic acids should be sterically inhibited. For the same reason, it could be predicted that trans-amino-cinnamic acids would not form betaines (14). The ease with which such betaines were obtained, constituted strong additional evidence in support of the open dipolar structure for these compounds. Pfeiffer (13)

used the concept, advanced by Debye and Sherrer (16), that crystals of salts possessed an ionic lattice structure, as further justification of the open dipolar structure for betaines.

The foregoing evidence presented by Pfeiffer was considered as conclusive proof of the dipolar character of betaines. In addition, the nature of dipolar ions was eluciated by Bjerrum (17) who discussed the constitution of ampholytes and their dissociation constants in his famous paper of 1923. In the same year, Debye and Hückel (18) proposed their well-known theory which dealt with the interaction of ions in dilute solution. These developments clarified the situation and a new and important epoch opened in the physical and chemical studies of dipolar ions.

Further evidence of the ampholytic character of betaines was obtained from measurements of the dielectric constants (19), and of the observed dielectric increments of solvents such as water in the presence of betaines (20). Kuhn and Brudowna (21) treated solid amino acids with ethereal diazomethane, and obtained the corresponding betaines instead of the methyl esters which might have been expected. This result could be explained only in terms of the dipolar nature of amino acids and betaines:



Recently, infrared spectroscopy (22) confirmed the established structure for betaines. No absorption maximum due to the stretching frequency of a carbonyl group was observed in the infrared spectra of betaines, but instead an absorption characteristic of a carboxyl group was detected. Since a cyclic structure for these betaines had to involve a maximum indicating the presence of a carbonyl group, infrared spectroscopy eliminated the possib-

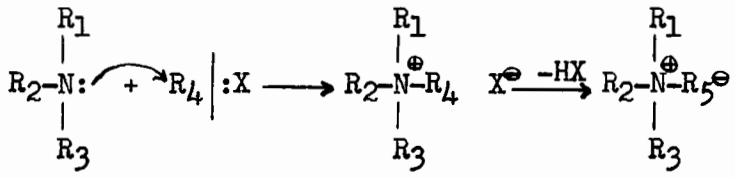
ility of such a structure.

The name "zwitterion" for these ampholytic compounds was used first by Küster (7, 8). Later Ingold (23) employed the term "dipolar ion" which was probably the most satisfactory equivalent of the German word "zwitterion". Several other names such as "inner salt", "double ion", "hybrid ion", "amphoteric ion" and "ion dipole" were used to denote this type of molecule.

According to the electronic conception (24), the nitrogen atom possesses five electrons in its outer or valence shell. Three of these electrons occupy the orbitals $2p_x$, $2p_y$ and $2p_z$, one electron being in each orbital. The remaining two electrons form an unshared electron pair and occupy a 2s atomic orbital. The representation of these five electrons, based on quantum mechanics, is $2s^2$, $2p^3$ or $2s^2$, $2p_x$, $2p_y$, $2p_z$. The unpaired electrons can unite with electrons of some other element to form three typical covalent bonds. This constitutes the electronic interpretation of the trivalency of nitrogen. The unshared electron pair is responsible for the formation of a coordinate covalent bond.

While this coordinate covalent bond is being formed, the nitrogen atom becomes quaternary, and simultaneously positively charged. As has been previously noted, the quaternary nitrogen atom is the common characteristic of all betaines. It should be emphasized that quaternization and betainization are different, and quite independent processes. In the formation of betaines, the nitrogen is quaternized first, forming a substituted ammonium or cyclammonium salt. The betainization which follows involves an elimination process, such as the displacement of a hydrogen halide, and is possible only when there is a potential or actual electronegative centre in the cationic function of the ammonium salt. A proton is

split off from the cation of the ammonium salt, together with the whole anionic function, leaving behind the betaine. The above reactions may be represented by the following scheme:



where $R_5^{\ominus}H^{\oplus} = R_4$

Pyridinium betaines

a. Classification

The unshared electron pair of the annular nitrogen in pyridine and its homologues can give rise to quaternization and in some instances to pyridine betaine formation.

Pyridine reacts readily with alkyl halides (25), usually to form pyridinium salts. It is evident that elimination of a proton from the alkyl radical attached to the annular nitrogen of an alkylpyridinium halide is not probable because the conditions (26) for the splitting off a proton with simultaneous formation of a carbanion are not present in this particular case. Thus the betainization process theoretically is possible only in two cases, namely when a real or potential negative pole is attached to the pyridine nucleus or when such a centre is present in the side chain attached directly to the annular nitrogen. Both of these possibilities have been investigated thoroughly and a great number of pyridinium betaines have been synthesized.

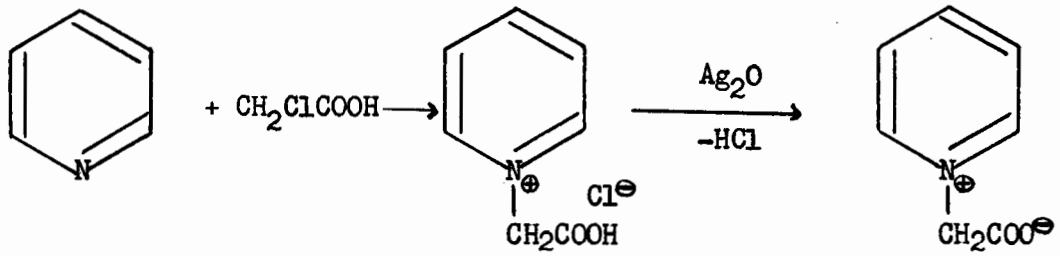
Since a great deal of information about these betaines had accumulated it was felt that the material should be organized for this presentation. The best method of classification appeared to be that based on the

difference in the anionic functions of the pyridinium betaines, since the cationic centre of these compounds was always the cyclammonium ion, and consequently did not provide a basis for classification.

Pyridinium betaines with carboxylate anionic function

The carboxylic group is able to release one proton, and the carboxylate anion thus obtained is quite a strong electronegative functional group. The presence of a free carboxylic group in a pyridinium salt provides the possibility for formation of a betaine, since it is likely that the proton of the carboxylic group will be eliminated with the anionic function of the salt.

The first pyridinium betaine, which was prepared by von Gerichten (27), was of this type. He allowed a mixture of monochloroacetic acid and pyridine to react on the steam bath and treated the pyridinium salt which formed with silver oxide. Hydrogen chloride was split off and the product obtained was 1-(carboxymethyl)pyridinium betaine (III).



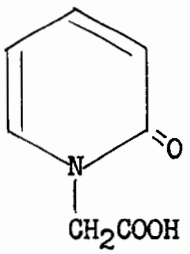
III

Many derivatives of this compound (III) were synthesized by Krüger (28,29) who also made a quite thorough investigation of its chemical properties. The physicochemical properties of 1-(carboxymethyl)pyridinium betaine (III), such as Raman spectra (30), dipole moments (31), viscosities (32) and dielectric constants (31,33) of solutions containing this betaine (III), and infrared (34) spectra were studied intensively.

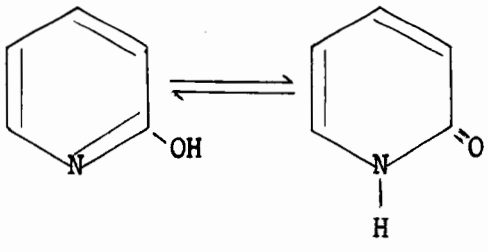
Von Gerichten (27) found that a similar betainization process

occurred when quinoline was allowed to react with monochloroacetic acid. Roussopoulos (35) prepared the same quinolinium betaine following the procedure of Hoffmann (36). This method involved the reaction of tertiary amines with the esters of α-halogenated aliphatic acids instead of with the free acids. As the first step of the reaction, the ammonium salt was formed. Then silver oxide was added, and betainization proceeded with the simultaneous elimination of hydrogen halide and hydrolysis of the ester.

The reaction of monohalogenated acetic acid with pyridine was used as the basis for investigating the possibility of preparing the corresponding salts and betaines from pyridine derivatives. Alkylpyridines (29), aminopyridines (37) and 3-hydroxy-pyridine (38) gave the expected substituted pyridinium betaines. The reaction of 2-hydroxy-pyridine with monochloroacetic acid gave (IH)-2-pyridone-1-yl-acetic acid (IV)(38). This "abnormal" reaction was explained on the basis of the tautomerism of 2-hydroxy-pyridine (V). The formation of (IH)-2-pyridone-1-yl acetic acid (IV) was considered (38) to be the result of the reaction of 2-hydroxy-pyridine (V) in its 2-(IH)-pyridone tautomeric form with monochloroacetic



IV



V

acid. In contrast, although similar tautomeric forms existed in the case of 4-hydroxy-pyridine, the latter reacted with monochloroacetic acid to give the corresponding betaine after the elimination of hydrogen chloride (39).

Other investigators (40,41,42) used different procedures for preparing pyridinium betaines with the carboxylate anion as the negative centre of the dipolar ion. In none of these cases were yields reported, and it seemed possible that they might be low or not reproducible.

The difficulty in obtaining a pure product in high yield was observed by Edsall (19) who claimed that the yield and purity of the betaine were improved by allowing the reaction mixture of pyridine and monochloroacetic acid to react at a lower temperature for a longer time. Von Gerichten (27) made a further observation concerning the effect of temperature when he heated the 1-(carboxymethyl)pyridinium chloride above 200°. He found that no betainization occurred but instead the pyridinium salt decomposed.

Attempts were made to carry out the reaction of pyridine with di- and trichloroacetic acid but neither salt formation nor betainization took place (40).

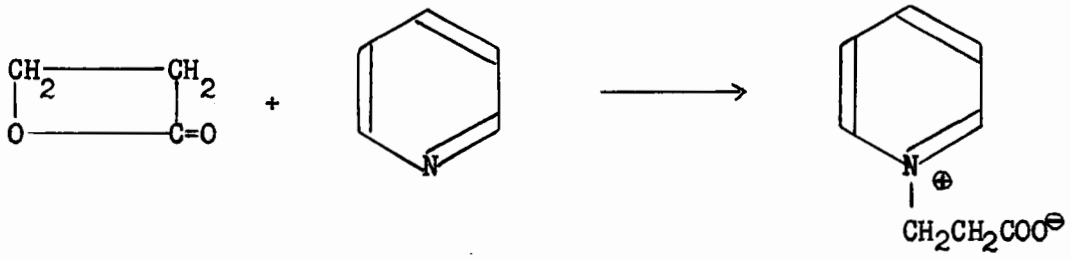
A further study of the reaction of α-halo carboxylic acids with pyridine was carried out by Bezzi (32). He found that, in all cases except the one reported by von Gerichten (27), it was impossible to isolate pyridinium betaines with the carboxylate anion as the negative centre. This was explained by the decarboxylation of the pyridinium salt which occurred during the course of the reaction. In addition to confirming earlier experimental work by Weinhagen (43), Bezzi (32) was able to detect the formation of pyridinium betaines as unstable intermediate products of the reaction of pyridine with α-halo carboxylic acids, by viscosity measurements of the reaction mixture in toluene.

Kirpal (44) tried to synthesize pyridinium betaines by reacting pyridine with β-halo carboxylic acids. He observed that the pyridinium salt

was formed readily, but attempts to obtain the corresponding betaine by elimination of hydrogen halide were unsuccessful. The proton involved in hydrogen halide displacement, instead of being provided by the carboxylic group, was afforded by the methylene group in α-position to the carboxylic function. Thus the elimination of hydrogen halide which occurred simultaneously with the cleavage of the bond between the annular nitrogen and the carbon atom in β-position to the carboxylic group, resulted in the formation of acrylic acid, and liberation of the pyridine base.

The betaines which Kirpal (44) tried to obtain by reacting β-halo propionic acids with pyridine bases were detected and in some cases isolated by a new and quite elegant procedure. This procedure involved the reaction of pyridine bases with β-propiolactone (V), 45,46).

β-Lactones are quite stable compounds, and exhibit all the reactions of typical lactones, which are internal esters. In addition, these compounds enter into the characteristic reactions of bimolecular displacements at the β-carbon atom (47). The unshared electron pair of the nitrogen atom of pyridine bases makes possible a nucleophilic attack at the β-carbon atom of a β-lactone. As a result of this attack, the lactone ring is opened and simultaneously 1-(2-carboxyethyl)pyridinium betaine (VI) is formed.

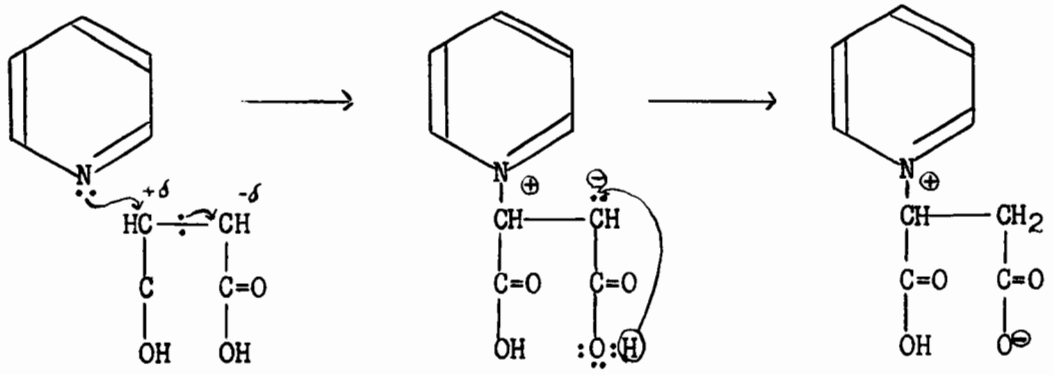


V VI

It was found that halogenated dicarboxylic acids reacted with pyridine bases to give the corresponding pyridinium salts and betaines (48).

A modification of this procedure was introduced by Ortoleva (42) who allowed a mixture of the dicarboxylic acid, pyridine and iodine to react. When he used malonic acid, he obtained, after partial decarboxylation, the 1-(carboxymethyl)pyridinium betaine (III) reported by von Gerichten.

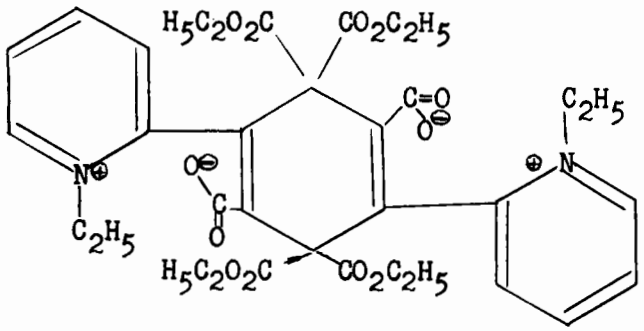
Lutz in a series of papers (44,50,51,52,53) reported his experimental work with pyridine bases and unsaturated dicarboxylic acids. In the case of maleic and fumaric acids, 1-(1-carboxy-2-carboxyethyl)pyridinium betaine was obtained. This compound had been isolated also from the reaction of pyridine bases with monohalogenated succinic acid (48) or sym dibromosuccinic acid (54). At the time of this investigation by Lutz, no electronic theory had been proposed, and consequently no adequate basis for an explanation of this reaction was available. However, this type of betainization appeared to be somewhat similar to that which occurred during β-lactone ring opening. In the latter case the reaction was inititated by polarization of the $-CH_2-O-$bond while in the former case, polarization of the π bond of the olefinic link could be condidered responsible for the bimolecular nucleophilic displacement. The following scheme outlines a possible mechanism for this reaction.



In the course of his research during which fission of the pyridine ring was accomplished, Zincke (55) prepared pyridinium betaines which had the carboxylate anion attached to an aromatic ring. It should be pointed

that, in this instance, pyridinium betaine formation could occur only when a strongly electron-attracting substituent was attached to the aromatic nucleus. Several other interesting reactions of this type were reported by Gabriel (56) and Boese (57). They obtained pyridinium betaines from the salts formed by the reaction of pyridine bases with phthalimido-N-alkyl halides. When these salts were treated with aqueous alkali at an elevated temperature their anhydride ring opened and the corresponding betaine was formed.

As a side product of the condensation of 2-methylpyridine with oxomalonic ester, McElvain and Johnson (58) isolated the only known coloured pyridinium betaine with the carboxylate group as the negative centre of the dipolar salt. They postulated the following structure (VII) for that compound.



VII

The reaction of pyridinecarboxylic acids with alkyl halides or halo carboxylic acids usually yielded the corresponding pyridinium salts. Betainization frequently occurred upon the elimination of a hydrogen halide molecule. Many pyridinium betaines of this type were synthesized (50,60,61, 62,63,64), and some, such as trigonelline (65) and homarine (66,67), were isolated from natural sources. In addition, it was discovered that the amides of 3- and 4-pyridinecarboxylic acids respectively possessed anti-pellagra and tuberculostatic properties. The amide of 3-pyridinecarboxylic

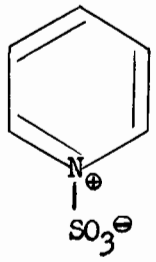
(nicotinamide) was present in diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). These two nucleoproteides, which possessed betaine structures, were indispensable in the oxidation-reduction processes in living organisms (68). The factor involved in these biological processes, the mechanism of which was elucidated recently (69) was found to be the nicotinamide function of the pyridine nucleotides. The biological importance of these compounds led to intensive research, most of which was biological in nature and beyond the scope of this thesis.

In the early investigations of the reactions of alkyl halides with di- and poly-pyridinecarboxylic acids considerable confusion existed. The betaines formed were considered to possess a cyclic structure and it was assumed (60) that the cyclization occurred preferentially through the carboxylic group located in the position which would permit the formation of a strainless ring. Kirpal (60) assumed that the negative pole of the betaine derived from 3,4 pyridinedicarboxylic acid was the carboxylic group in 3 position of the pyridine nuclei. When a carboxylic group was present in the chain attached directly to the annular nitrogen atom he considered (70,71) that the elimination process rendered this carboxylic group the anionic function of the dipolar ion. These ideas of course were incorrect on the basis of the open ring structure of betaines. In addition, their erroneous nature was shown by experimental evidence (72).

Pyridinium betaines with sulphonate anionic function

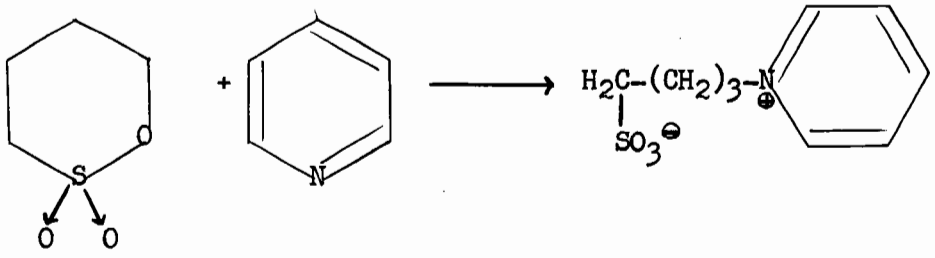
It was possible also for the sulphonic group to be the negative centre of the dipolar molecule of pyridine betaines. In some of these inner salts the sulphonic group was attached directly to the pyridine in 3 position (73,74,75). Compounds of this type were used to facilitate dyeing and printing in the textile industry (74,75).

In other cases the sulphonic group was attached to the annular nitrogen, or to the side chain which quaternized this nitrogen atom. Baumgarten (76) prepared the 1-(sulpho)pyridinium betaine (VIII) by treating pyridine in chloroform with sulphur trioxide.



VIII

This compound (VIII) was better known as pyridine-sulphur trioxide complex and had been synthesized previously by Wagner (77), who allowed pyridine to react with free or esterified chlorosulphonic acid. Helberger (78) obtained several pyridinium betaines possessing the sulphonate anionic function by a procedure involving the reaction of pyridine with sultones. The reaction of butane sultone (IX) and pyridine yielded the 1-(sulphobutyl)pyridinium betaine (X).

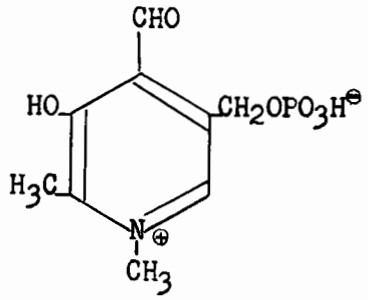


IX X

Pyridinium betaines with phosphate anionic function

In connection with his studies of the vitamin B6, Folkers and his collaborators (79) prepared pyridinium betaines having the phosphate group as the anionic function of the inner salt. An example of the kind of

betaine was 4-formyl-3-hydroxy-5-(hydroxymethylphospho)-1,2-dimethylpyridinium betaine (XI).



XI

Enol and Phenol pyridinium betaines

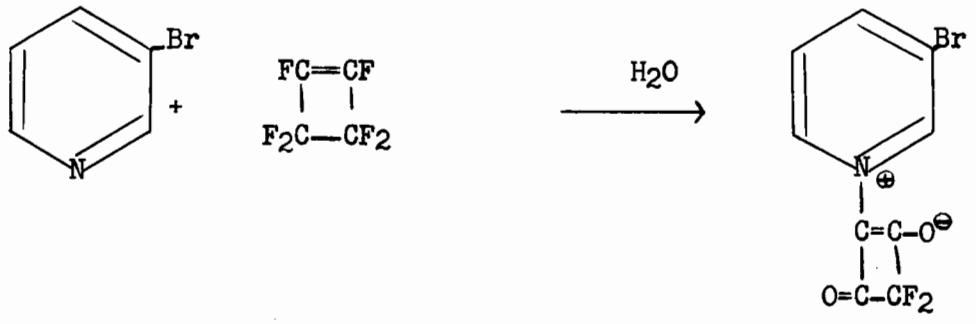
This large and interesting group of pyridinium betaines was characterized by a negatively charged oxygen atom being one of the poles of the inner salt. The negatively charged oxygen atom could arise from a phenolic hydroxyl group or an enolized ketonic group. This difference in the origin of the negative oxygen atom which remained after a proton had split off from the hydroxyl group was the basis for dividing these compounds into two classes, the enol and phenol pyridinium betaines.

A. Enol pyridinium betaines

The existence of enol betaines depended on the presence of a carbonyl group which could be enolized, and a quaternary nitrogen atom within the same molecule. This situation occurred in cyclammonium salts which were obtained easily by the action of α-halo ketones on tertiary bases. In the latter reaction pyridine bases behaved as typical tertiary amines, and reacted to form pyridinium salts. These salts were isolated without difficulty and were found, in most cases, to be stable.

Pyridinium enol betaines could be divided into three categories: I. This group of pyridinium enol betaines consists of compounds which are colourless, neutral and which melt at high temperatures. The first repres-

entative of this type was synthesized by Benary (80). Later, similar compounds were prepared by reacting hexafluorocyclobutene (XI) with tertiary amines (81). When aliphatic amines were used in this reaction, the desired products were obtained readily. Although pyridine failed to give the corresponding betaine, 3-bromopyridine (XII) yielded the 1-(3,3-difluoro-2,4-dioxocyclobutyl)-3-bromopyridinium betaine (XIII).



XII XI XIII

II. The methine enol pyridinium betaines are slightly or fairly basic and melt, generally with decomposition, at low temperatures. All methine enol betaines are characterized by their deep colour.

An extensive study of these compounds was carried out by Krohnke over a number of years. This author has written (82) an excellent and comprehensive review which could be consulted for information concerning the work done in this particular field. Krohnke, called these substances "methine enol betaines" because each molecule possessed one methine group between the quaternary annular nitrogen and the enolized keto group $-\overset{O^{\ominus}}{\overset{|}{C}}=CH-\overset{\oplus}{N}$.

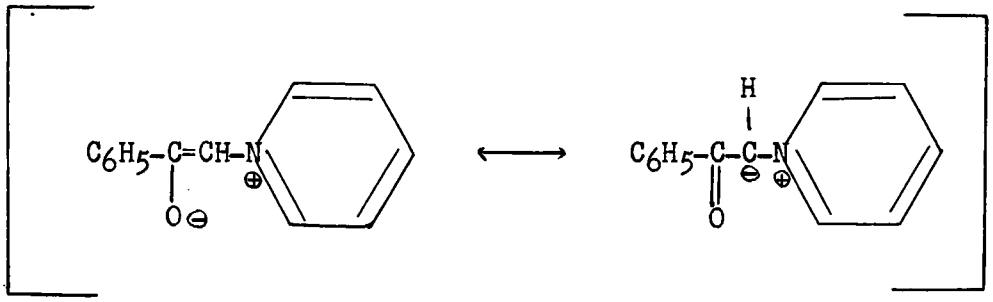
The existence of this methine group gave rise to colour reactions with chloranil and picryl chloride (83,84). The methine enol betaines also reacted with the Zerevitinov reagent (85).

III. The third category of pyridinium enol betaines contain the dibenzoyl-

methyl enol betaines (86). These inner salts resemble the methine enol betaines because both have the enolate anion as the negative centre, and are deeply coloured. The main difference between the two types of compounds is that the dibenzoylmethyl enol betaines have no methine group between the quaternary nitrogen atom and the keto-enol anionic function of the inner salt. The hydrogen atom originally present in the methine group has been replaced by one benzoyl group. The lack of a mobile hydrogen atom in these compounds is responsible for their failure to exhibit the characteristic colour reaction of the pyridinium methine enol betaines (83,84).

Krollpfeiffer and Müller (87) contributed the first evidence for the existence of a general enol betaine structure. These investigators isolated reactive, crystalline, orange-coloured intermediates in the alkaline cleavage of pyridinium salts derived from alkyl-o-halogenoacyl phenyl sulphides. Subsequently the structure of enol betaines was established, and later confirmed by Kröhnke (83). The latter author in his study of the alkaline cleavage of "phenacyl pyridinium bromide" and its derivatives obtained independently, and by a different approach, the same results as Fuson and his associates (88,89). They found that the "acidic" cleavage of these pyridinium salts brought about by alkalies was very similar to the alkaline fission of the acetoacetic ester or generally of α,γ-diketo compounds.

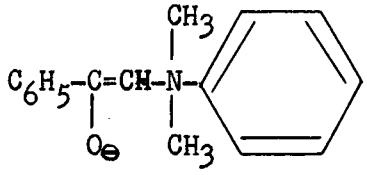
Kröhnke (83,90) attributed the colour of pyridinium enol betaines to the combination of an extended conjugated system, and the polar nature of the molecule. He also explained the colour by resonance, and considered the possibility of resonating structures which had the carbon atom of the methine group negatively charged instead of the oxygen atom of the carbonyl group (XIV). Kröhnke recognized that these additional resonating structures



XIV

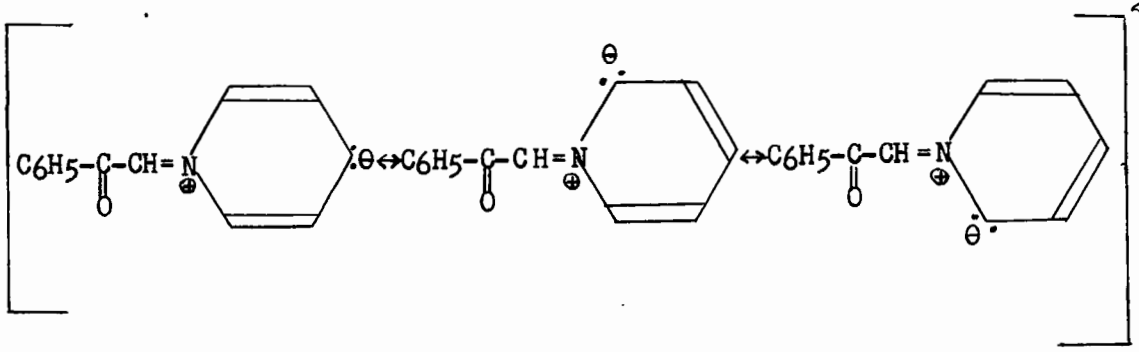
contributed to the resonance hybrid, to some extent, causing a positive bathochromic and hyperchromic effect on the electronic absorption spectrum of the molecule.

These assumptions by Kröhnke were not completely satisfactory because they did not explain the great difference in colour between the pyridinium and ammonium or anilinium enol betaines. For example the 1-(phenacyl)pyridinium betaine (XIV) was deeply coloured but the phenacyl dimethylanilinium betaine (XV) was colourless.



XV

Stafford (91) explained this phenomenon by proposing that the presence of the pyridine ring in the molecule gave rise to additional resonating structures which contributed to the resonance hybrid of the 1-(phenacyl)pyridinium betaine (XIV). These resonating structures were considered to arise from the separation of electrical charges within the aromatic cyclammonium cation as shown below (XVI).



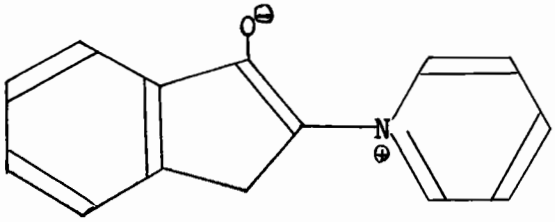
XVI

The same investigator (91) pointed out that the observed difference (83) in the colour of the methylpyridinium, quinolinium and isoquinolinium enol betaines was due to a variation in the contribution of the resonating structures to the resonance hybrids of these compounds. The anilinium enol betaines on the other hand appeared colourless because the presence of resonating structures with separated electrical charges in the portion of the molecule containing the quaternary nitrogen atom was restricted.

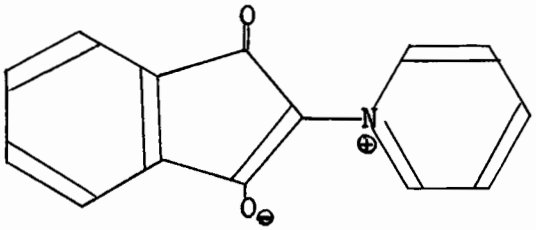
Stafford (91) also reported the synthesis and electronic absorption spectra of two enol betaines of the indane-pyridinium series. These betaines were derivatives of 1-indanone and 1,3-indandione. In addition the author discussed the relation existing among these inner salts, "N-methyl pyrophthalones", and sydnones. In later papers, Stafford (92,93) correlated the dipole character of betaines with the fine structure of azulenes. The latter study provided evidence that the colour of betaines was due to the association of the cationic and anionic functions with two aromatic rings and to the anionic function being at least partially associated with one or more carbon atoms.

The pyridinium enol betaines derived from pyridine and either 1-indanone or 1,3-indandione, were the 1-(1-hydroxy-2-indenyl)pyridinium hydroxide, betaine (XVII) and 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) respectively. It should be mentioned here that Vitkovsky and Shemyakin (94) also obtained the 1-(3-hydroxy-1-oxo-2-indenyl)

pyridinium hydroxide betaine (XVIII) during their extensive reinvestigation of the Hooker reaction (95). In these two betaines (XVII) and (XVIII), only one carbon atom was situated between the nitrogen atom and the keto-enol



XVII



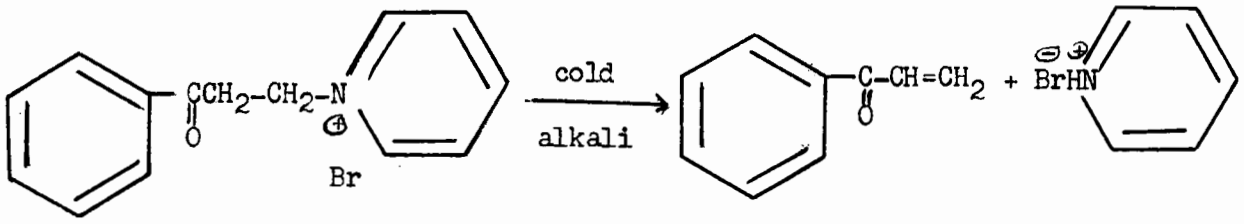
XVIII

group. For this reason these compounds were very similar to those betaines which possessed the group $-C=C-N\langle$ (with $O^{\ominus}$ and $O^{\ominus}R$ substituents and $N^{\oplus}$), in which R was either a hydrogen atom or some other group and consequently could be considered as enol but not methine pyridinium enol betaines.

A certain similarity was observed (91) between 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide betaine (XVIII) and the "N-alkyl pyrophthalones". The bright yellow colour of the latter was attributed by Kuhn and Bär (96) to the possibility of a type of tautomerism in these compounds. Actually the tautomeric formulae proposed by these authors were resonating structures because only a shift of electrons was involved, and not a tautomeric migration of a proton. Krönke (83) on the other hand, differentiated between phthalones and enol betaines on the basis that, in the former, two carbon atoms were located between the quaternary nitrogen and the keto-enol functional group while in the latter only one carbon atom was present in that position.

As a result of his studies, Pfeiffer (13) stated that betaine formation was, to a great extent, independent of the relative position and the distance between the actual or potential polar groups of the inner salt.

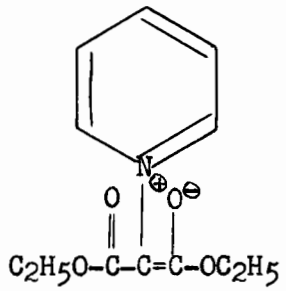
According to this, pyridinium salts possessing the group $-\overset{O}{\overset{\|}{C}}-(CH_2)_n-\overset{\oplus}{N}\overset{X^{\ominus}}{<}$ should be able to form enol betaines, if the methylene group adjacent to the carbonyl group possessed sufficiently labile hydrogen atoms. However it was found (97) that the 1-(benzoylethyl)pyridinium bromide (XIX) when treated with cold alkalies did not give even traces of an enol betaine. Instead, a cleavage of the pyridinium salt (XIX) occurred immediately and acrylphenone (XX), pyridine and hydrogen bromide were formed. Apparently, in place of an alkaline cleavage, a type of fission similar to that previously reported in the substituted ammonium series (98) took place and a substituted ethylene was obtained. The olefinic double bond formation



XIX XX

proceeded in accordance with Hoffmann's rule. A detailed discussion of the Hoffmann and Saytzeff rules for olefinic formation is contained in an excellent review by Ingold (99).

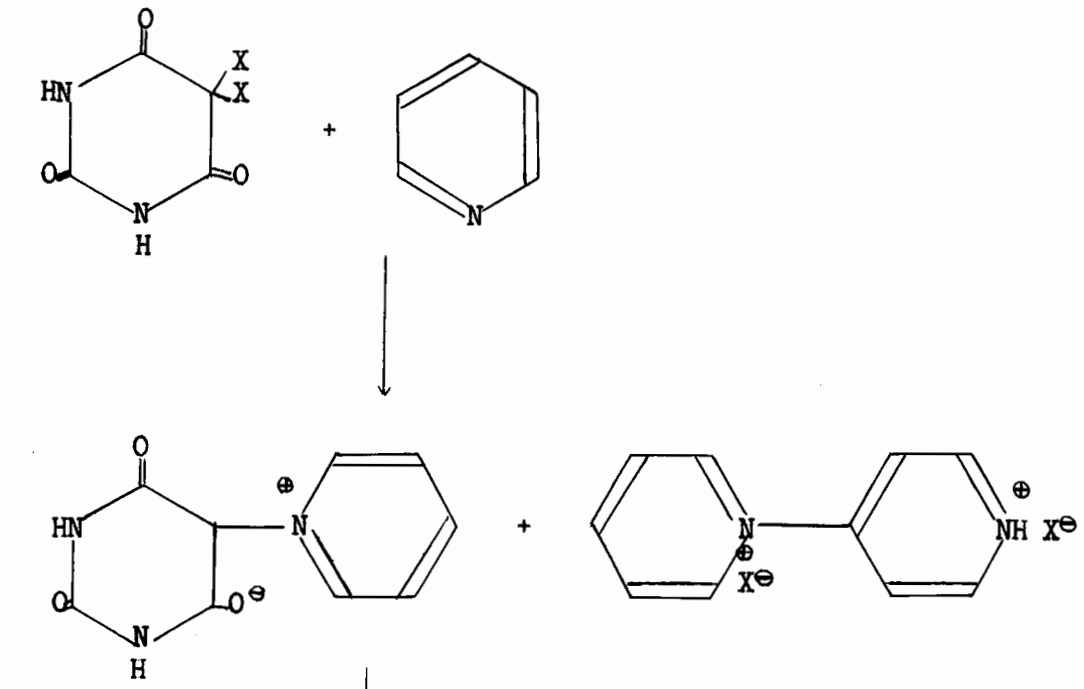
An interesting type of enol betaine was obtained (100) when the carbonyl group of the keto-enol function was situated in the carbethoxy group of the molecule of an acalkylcyclammonium salt such as the 1-(dicarbethoxymethyl)pyridinium betaine (XXI). These compounds were of theoretical interest because they were the first ones in which the enol group of the ester enolates of malonic acid was not attached to a metal as a cation.



XXI

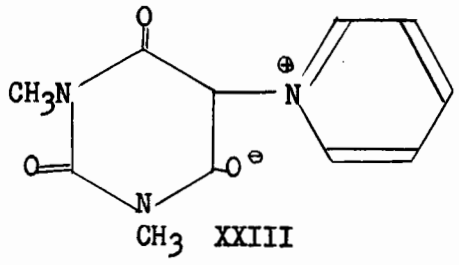
Taylor, Jr. and his collaborators (101), obtained pyridinium betaines from the reaction of 5,5-dihalobarbituric acids with pyridine and 4-methylpyridine. However, they did not obtain a product with 2-methylpyridine, and attributed this failure to steric hindrance by the methyl group in 2 position of the pyridine nucleus. These investigators did not designate their products as enol betaines, but the result of methylation indicated that the enol betaine form predominated or else was the only form present. The following reaction scheme was given for the synthesis of 1- 5(2,4,6-trioxohexahydropyrimidyl) pyridinium betaine (XXII). The methylation product of (XXII) was the 1- 5(1,3-dimethyl-2,4,6-trioxohexahydropyrimidyl) pyridinium betaine (XXIII) which was also obtained from the reaction of 1,3-dimethyl-5,5-dihalobarbituric acid (XXIV) pyridine.

The previous discussion, dealing with pyridinium enol betaines, could be summarized by stating that enol betaines were formed when a carbonyl group and at least one mobile hydrogen atom in the position to the keto group were present in the molecule. This assumption was substantiated by Krollpfeiffer and Müller (97) who found that pyridinium enol betaines were not formed from ω,ω-dialkyl substituted phenacyl pyridinium halides because there was no mobile hydrogen atom in the vicinity of the keto group.

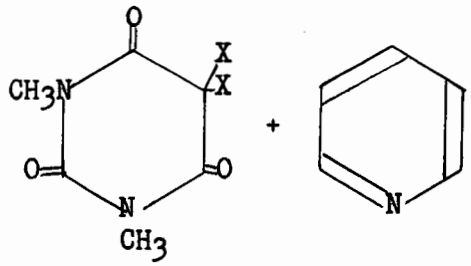


XXII

CH_2N_2
$(CH_3)_2SO_4$



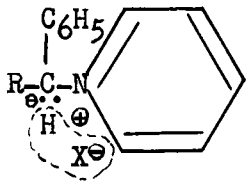
XXIII



XXIV

Pyridinium betaines with a carbanion as the anionic function

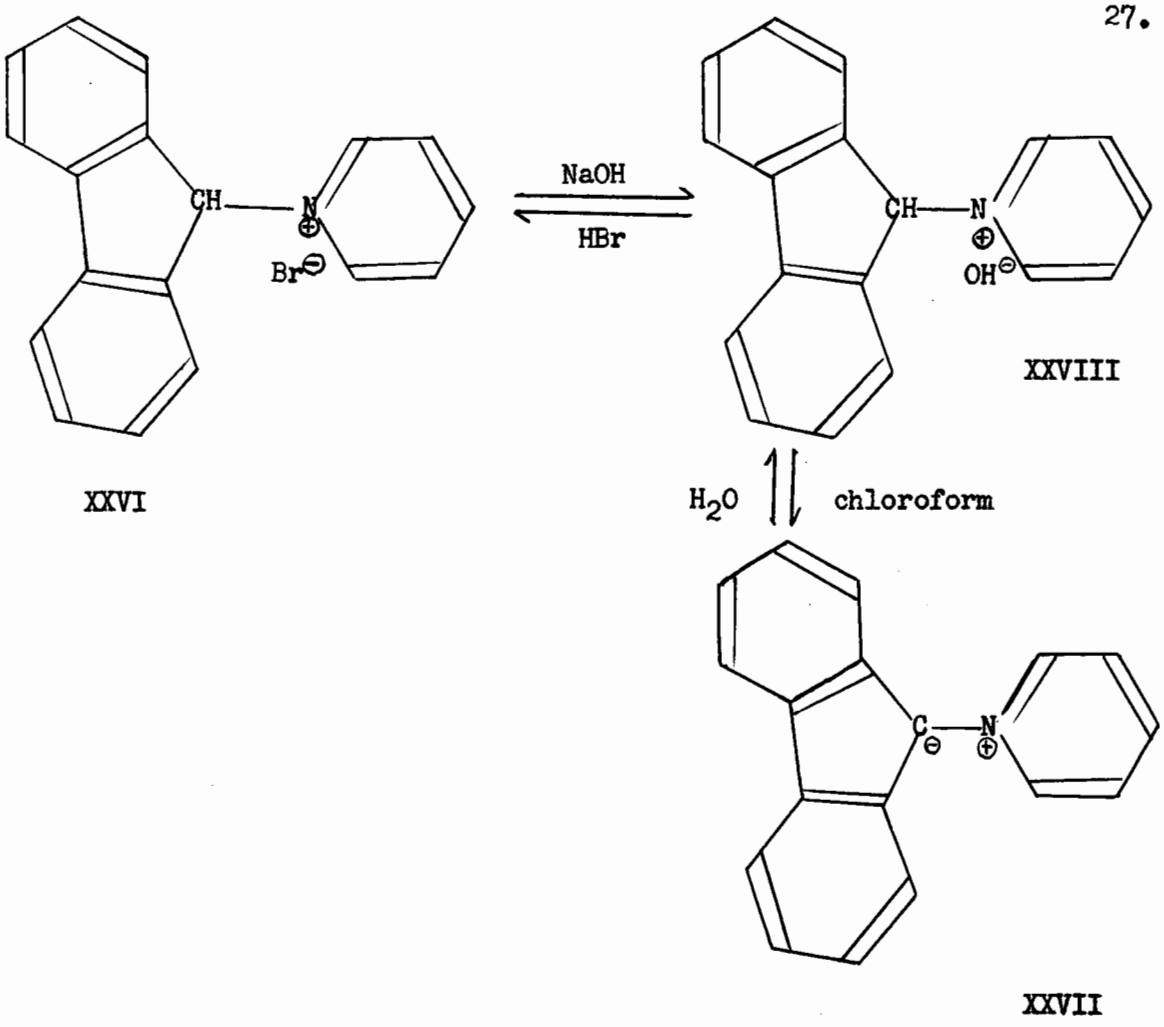
The presence of carbanions in some of the resonating structures which contributed to the resonance hybrids of enol betaines was emphasized by Krönhke (83) and later by Stafford (91). The assumption of these canonical structures, which facilitated the interpretation of the deep colour of pyridinium enol betaines, was verified by experimental evidence. Krönhke (102) showed that pyridinium salts of the general type (XXV) when



XXV

treated with alkalies, gave a pyridinium base which was coloured, particularly in the presence of chloroform. These betaines (XXV) could react also with aromatic nitroso compounds to form nitrones. However both of the above reactions occurred only when the group R was able to activate the hydrogen atom of the phenylmethylene group in α position to the annular nitrogen. When this hydrogen atom was eliminated as a proton, a carbanion remained, and became the negative centre of the pyridinium betaine which formed.

The 1-(9-fluorenyl)pyridinium betaine (XXVII) was a typical example of a pyridinium inner salt which could possess only a carbanion as the negative centre of the dipole. The following sequence of reactions outlined the formation of the 1-(9-fluorenyl)pyridinium betaine (XXVII) from 1-(9-fluorenyl)pyridinium bromide (XXVI). The fact that the 1-(9-fluorenyl)pyridinium bromide (XXVI) did not give the betaine (XXVII) immediately in aqueous alkaline solution was due probably to the formation



of the free base 1-(9-fluorenyl)pyridinium hydroxide (XXVIII) (103).

B. Pyridinium phenol betaines

In these types of pyridinium inner salts the anionic function was a phenolic oxygen atom. Many of these betaines were known, and they could be considered as products of the reaction between pyridine bases and halo quinones or hydroquinones. A survey of the literature in this field was included in the review article by Krönke (82).

Hünig and Rosenthal (104) from a study of spectroscopic data have presented a discussion of the relation between the colour and constitution of the phenol betaines. Although a thorough review of the voluminous material available about phenol betaines is beyond the scope of this survey, the practical importance of some members of this series as vat dyes, should

be mentioned (105,106).

Harris and his collaborators (107) obtained pyridinium betaines from the alkaline hydrogen peroxide oxidation of the codecarboxylase and pyridoxal. Since the anionic centre of these betaines was the oxygen atom of the hydroxyl group attached to 4 position of the pyridine nucleus, these compounds could be considered as pyridinium phenol betaines.

"Isonitroso" and "aci-nitro" pyridinium betaines

These two groups of pyridinium betaines possess as their main anionic function an oxygen atom of either the isonitroso- or aci-nitro group present in their molecule. Many of these inner salts have been described and further information about them may be found in the review article by Krönhke (82).

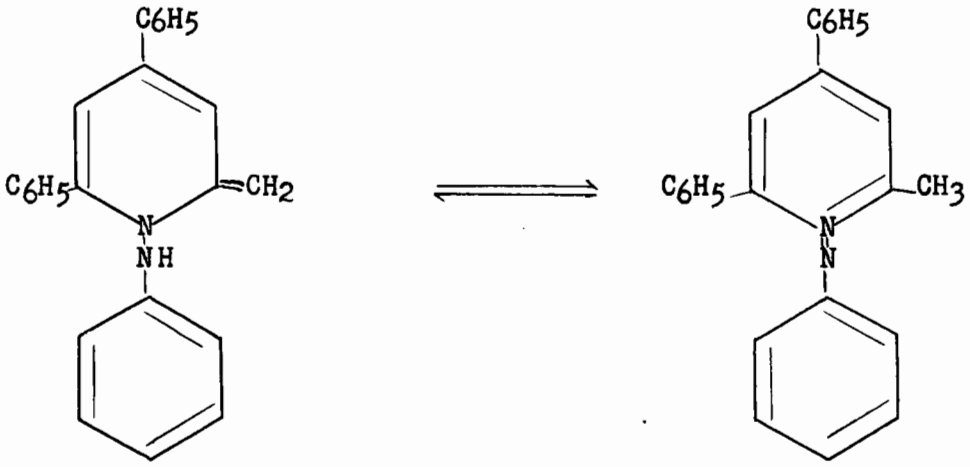
Pyridinium betaines with a nitrogen atom as the anionic function ("Azeniate" Pyridinium betaines)

Recently, dipolar ions, which possessed two nitrogen atoms in their molecule were reported. In such molecules one of the nitrogen atoms was quaternary and positively charged, while the other was the negative centre of the dipole. In a series of papers, Simonov (108, 109,110, 111) described the synthesis of these inner salts. The compounds prepared by this investigator had a negatively charged nitrogen which was either primary or secondary. In contrast, the "enimine" pyridinium betaines possessed a tertiary nitrogen atom which, for example, could be located in the nitrile group of a pyridinium salt, and which became secondary and negatively charged by a mesomeric electron shift (112). The earlier work in this field was reviewed by Krönhke (82).

During the past fifteen years, an extensive study has been carried out at Marburg University concerning the effect of solvents

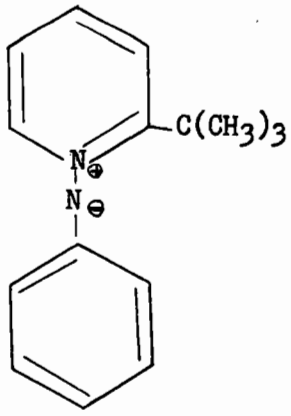
on the colour of a great variety of compounds. These studies have revealed that the N-aryl pyridine imine compounds, originally prepared and characterized by Schneider and his collaborators (113,114,115,116,117), actually possess an azeniate pyridinium betaine structure (118).

Schneider attempted to explain the colour of the compound (XXIX) in terms of tautomerism.



XXIX

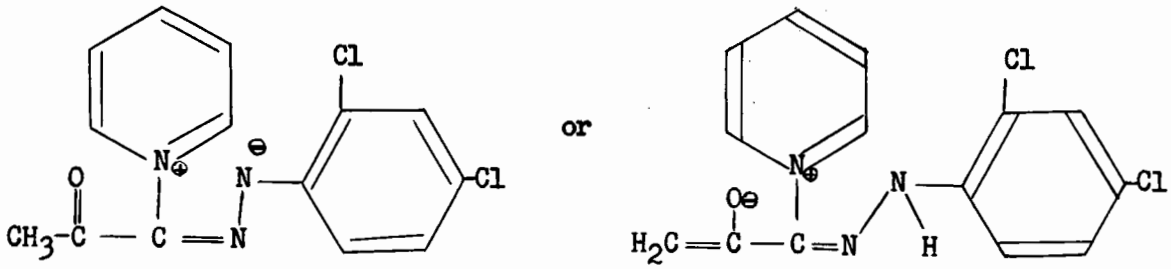
However this explanation proved to be erroneous, because the 2-tert-butyl pyridinium compound (XXX), in which no tautomeric shift of a proton could occur, was also coloured.



XXX

Eicken (119) and Arnoldy (120) demonstrated that resonance was responsible for the colour of these compounds and they assigned the "azeniate" structure (XXX) to them. In addition, ultraviolet absorption spectra showed that these compounds should be dipolar, because their spectra were similar to those of pyridinium phenol betaines (119).

A group of pyridinium inner salts similar to the "azeniate" pyridinium betaines was described. The structures originally assigned to these compounds (121) were revised by Neber and Wörner (122) who suggested that due to tautomerism the inner salts could be considered either as enol or "azeniate" betaines. The two structures (XXXI) and (XXXII) presenting respectively the 1-[α-[(2,4-dichlorophenyl)hydrazono]acetonyl]pyridinium hydroxide, betaine and 1-[1-(2,4-dichlorophenylhydrazono)2-hydroxyallyl] pyridinium hydroxide betaine illustrated this tautomerism.



XXXI or XXXII

b. Pyridinium salts as intermediates in the introduction of olefinic double bonds in steroids

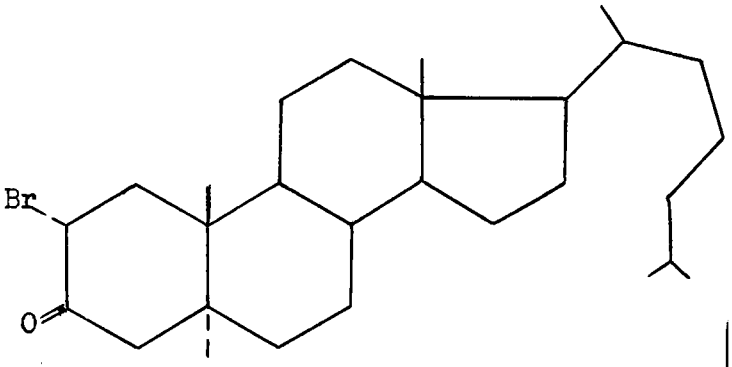
The biological significance of steroidal compounds and the pharmacological importance of certain members of this class were widely recognized. One of the problems encountered in the partial or total synthesis of these compounds was the introduction of an olefinic double bond, particularly in the α position to a keto group.

As one approach to this problem, 3-cholestanone was submitted to

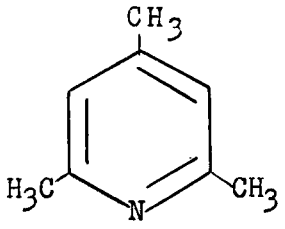
a halogenation reaction, in an effort to introduce a halogen atom in α position to the keto group. In all cases 2-halo-3-cholestanone was the sole product. The latter was treated with pyridine and gave an addition product in high yield (123,124,125,126). Pyrolysis of this addition product afforded 4-cholesten-3-one, but in low yield (125).

Butenandt and his collaborators (127) improved the procedure for eliminating hydrogen halide by using 2,4,6-trimethylpyridine (XXXIII) instead of pyridine. Treatment of 2-bromo-3-cholestanone (XXXIV) with 2,4,6-trimethylpyridine (XXXIII) resulted in the immediate formation of 1-cholesten-3-one (XXXVI). In no instance was an addition product formed as an intermediate.

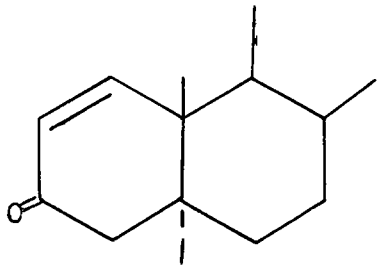
Later it was found (128) that the reaction of 2-bromo-3-cholestanone (XXXIV) with 2,4,6-trimethylpyridine (XXXIII) did not follow the straightforward path described by Butenandt and his colleagues (127). Jacobsen (128) observed that this reaction yielded a small amount of 3-cholestanone (XXXV) in addition to the main product, 1-cholesten-3-one (XXXVI). This unexpected formation of the corresponding saturated compound as a side product of the elimination of hydrogen halide was shown later by Djerassi and his collaborators (129) to be characteristic of 2-halo-3-ketosteroids. In addition the latter author demonstrated that the corresponding saturated allo ketosteroids were obtained when 2-iodo-3-ketosteroids of the allo series were reacted with 2,4,6-trimethylpyridine (XXXIII). It was difficult to propose a mechanism which would explain this result because it was impossible to determine the composition of the black semi-solid 2,4,6-trimethylpyridine portion of the reaction. However, a plausible scheme was postulated by Stork in a personal communication to Djerassi (129).



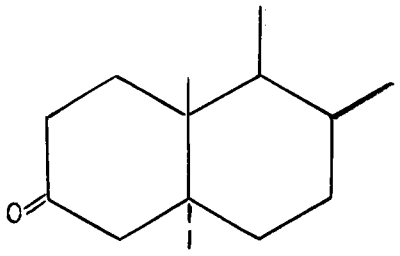
XXXIV



XXXIII

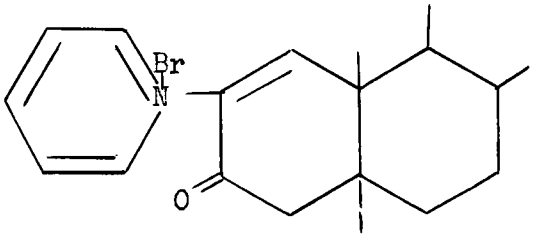


XXXVI

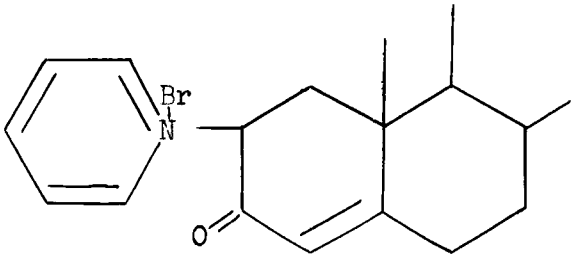


XXXV

Schwenk and Whitman (124), during the course of their investigation of the debromination of brominated sterols, obtained the addition compound first isolated by Butenandt (113). In addition, they isolated two unsaturated pyridinium salts one from the reaction mixture of 2,2-dibromo-3-cholestanone and pyridine and the other from 2,4-dibromo-3-cholestanone and pyridine. To these respective salts they assigned, arbitrarily, the structural formulae (XXXVII) and (XXXVIII).



XXXVII

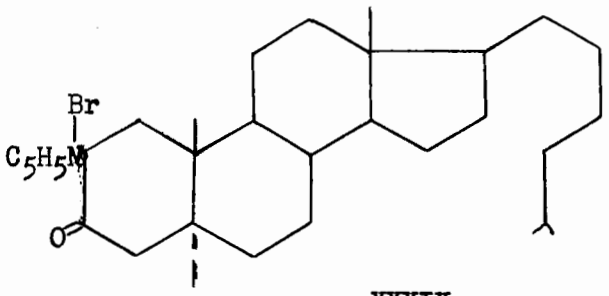


XXXVIII

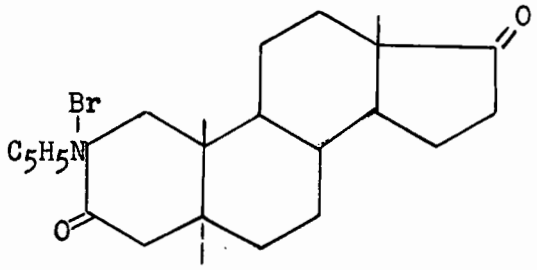
Ruzicka and his co-workers (125) in their study of sex hormones, prepared the addition products which resulted from the reactions of 2-bromo-3-cholestanone (XXXIV) and 2-bromo-3,17-androstanedione with pyridine. Ruzicka (125) was the first to assign the structure of a typical pyridinium salt to these compounds, even though he considered the nitrogen atom pentavalent. The structures were proposed on the basis mainly of experimental and theoretical data made available by Krönke (130,131) who was carrying out research in this field at almost the same time. The high melting points of these compounds, and the negative ionic character of the bromine atom present in the molecule, were considered by Ruzicka to confirm his assumption. Furthermore the analytical data were in agreement with the empirical formula for a salt which could be considered as an addition product.

Both the compounds prepared by Ruzicka, 1-(3-oxo-2-cholestanyl) pyridinium bromide (XXXIX) and 1-(3,17-dioxo-2-androstanyl)pyridinium bromide (XL), when tested with silver nitrate solution gave quantitative, voluminous precipitates of silver bromide immediately. The addition of an aqueous alkaline solution to crystals of 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX) caused them to turn yellow. In the case of 1-(3, 17-dioxo-2-androstanyl)pyridinium bromide (XL) the addition of alkali produced a yellow substance which turned red on heating. When this coloured substance was treated with hydrogen bromide, the original addition product (XL) was recovered. Ruzicka (125) considered that the yellow material was the free base, or in other words, that in the presence of alkali the bromine anion was replaced by the hydroxyl anion.

Inhoffen and his associates (132) followed a procedure similar to that of Butenandt (123,127) for introducing olefinic double bonds in

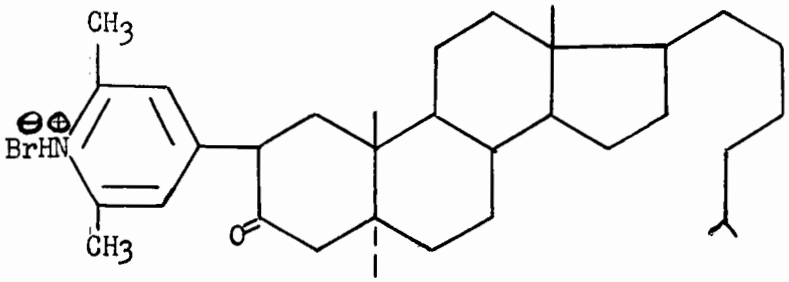


XXXIX



XL

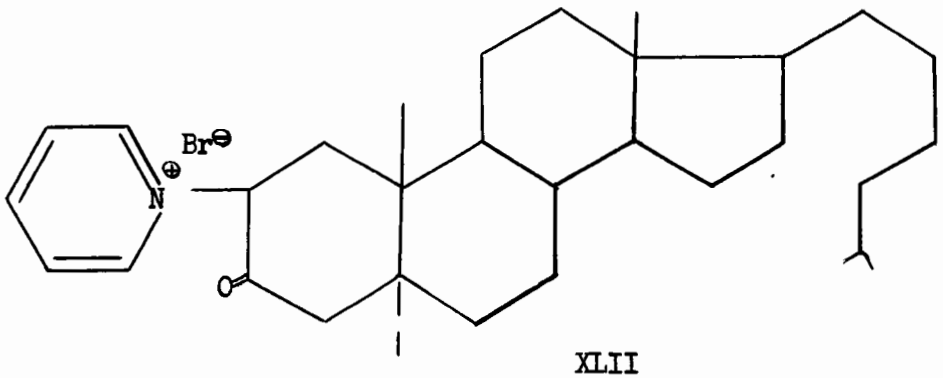
the A ring of certain 3-ketosteroids. In the same manner, he attempted the elimination of hydrogen bromide using various methyl derivatives of pyridine. He observed that the reaction of 2-bromo-3-cholestanone (XXXIV) with 2,4-dimethylpyridine proceeded smoothly to yield 1-cholesten-3-one (XXXVI) without any formation of an intermediate nitrogen containing steroidal adduct. However, when 2,6-dimethyl pyridine was used, an intermediate addition product was obtained readily. Inhoffen (132) attributed the difference in behaviour of these two dimethylpyridines towards 2-bromo-3-cholestanone (XXXIV), to the presence of a methyl group in 4 position of the pyridine nucleus. He assumed that a methyl group in this position inhibited the formation of the steroid-pyridine addition compound although the methyl groups in positions 2 and 6 did not. To explain these facts, Inhoffen postulated that the bond between the ketosteroid (XXXIV) and the pyridine nucleus was in position 4 of the latter ring as shown in structure (XLI). He argued that if pyridine and the steroid were connected through



XLI

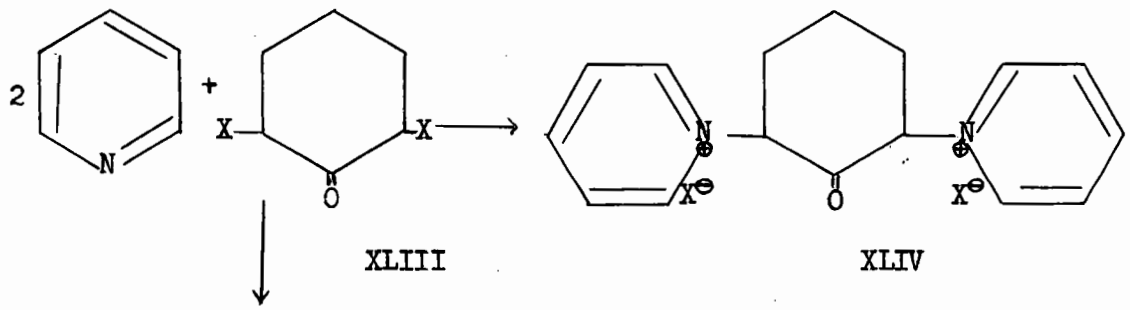
the annular nitrogen atom then the formation of a typical pyridinium salt would be more sterically hindered in the case of 2,6-dimethylpyridine than in the case of 2,4-dimethylpyridine. However, experimental work, as previously indicated, showed that 2,6-dimethylpyridine gave an intermediate addition product while 2,4-dimethylpyridine did not. In addition, both the anionic character of the bromine atom, and the high melting point of the compound could be explained in terms of the structure (XLI) postulated by Inhoffen.

Since close similarity existed between pyridine, and its methyl derivatives, it was felt that similar structures should be assigned to their steroidal adducts. However, application of the previously discussed assumptions made by Ruzicka (125) would lead to the formulation of structure (XLII) for the addition product experimentally obtained by Inhoffen. Since the assignment of both structures (XLI) and (XLII) to this particular compound was based on sound arguments, the actual structure remained problematic (133). However, the introduction of a keto group in the 2-bromo-3-ketosteroids of the allo series by means of the pyridinium salt and nitrone formation (126,134) favoured the structure (XLII) to some extent.

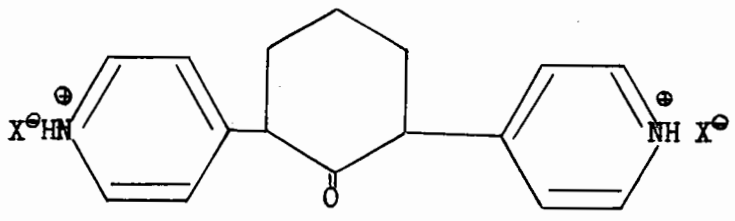


XLII

Galinovsky and his co-workers (135) attempted to establish which of the structures (XLI) or (XLII) was correct. In this connection he investigated in great detail the reaction of pyridine bases with 2,6-dibromo-1-cyclohexanone (XLIII). The treatment of 2,6-dibromo-1-cyclohexanone (XLIII) with pyridine itself, gave an addition product which consisted of the starting materials in the ratio of 1:2 respectively. Although this compound was the main product of the reaction, small amounts of phenol and pyridine hydrobromide were formed simultaneously. The addition product had a high melting point and low solubility in all solvents. When suspended in alkalies, it turned orange-yellow, and the addition of excess hydrochloric acid to the coloured mixture gave a product with the empirical formula $C_{16}H_{18}ON_2Cl_2$. Two structures were possible for the latter compound depending on whether the assumption by Ruzicka (XLIV) or the postulation by Inhoffen (XLV) was accepted.



XLIII XLIV



XLV

Pyrolysis of the addition product produced phenol and pyridine hydrogen halide. When the pyridine rings present in the molecule were completely hydrogenated, a Zerevitinov determination indicated that only one active hydrogen atom was present. These experimental facts, and the result obtained when the compound was treated with methyl iodide favoured structure (XLIV).

The reaction of 2,6-dibromo-1-cyclohexanone (XLIII) with either 2,4,6-trimethylpyridine (XXXIII) or 2-methyl-5-ethylpyridine readily gave phenol and the corresponding hydrobromide of the pyridine base. To explain this Galinovsky (135) proposed that two reactions proceeded simultaneously, namely the formation of a pyridinium salt, and the elimination of hydrogen halide. The proportion of each product obtained was considered to depend on the relative rate of the reaction which, in turn, was a function of the constitution and the basic character of the pyridine base.

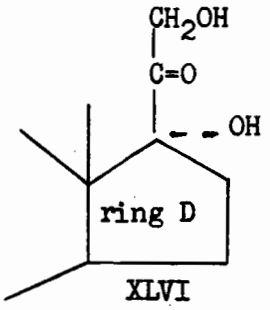
c. Methods of preparation of pyridinium salts

The close relation between pyridinium salts and their betaines was obvious, because the betaines were derived from the salts by an elimination of hydrogen halide or a similar entity. For this reason the preparation of the pyridinium salts was in most cases the first step in a pyridinium betaine synthesis.

Bamberger (136) introduced a simple method for the preparation of certain pyridinium salts which involved the reaction of α-halo ketones with pyridine bases in non-polar solvents. The salts which formed separated immediately because of their very low solubility in non-polar solvents. This procedure was, in principle, the same as that used in the Menschutkin reaction (137). The latter involved the reaction of alkyl halides with amines and afforded the substituted ammonium or cyclammonium

salts.

The procedure introduced by Bamberger was used exclusively for many years, and generally with satisfactory results. In fact, the synthesis of pyridinium salts was only a scientific curiosity until the early thirties, when interest in this field developed for a number of reasons. One of these reasons was the reinvestigation of the kinetics of the Menshutkin reaction which led to the concept of hyperconjugation (138). Later, Krönhke (139) introduced his elegant nitrone synthesis, the importance and versatility of which was recognized almost immediately. This method proved to be extremely valuable in the preparation of α-keto aldehydes, α-keto carboxylic acids, α-keto alcohols etc., and found extensive application (82) in the synthesis of cortical hormones which possessed an α-keto alcoholic group in the side chain (XLVI).



XLVI

Preparation of pyridinium salts according to the method of Bamberger could be achieved only when the α-halo ketone, necessary for the reaction, could be obtained readily and in good yields. Unfortunately some of the ketones were expensive, and in some cases the α-halo derivatives were available in low yields. The latter was a consequence of the many side reactions which occurred during halogenation. The interference by side reactions was greater when the ketone involved in the reaction was a complicated molecule with more than one centre which could be attacked by bromine.

Finally the difficulties were overcome by King (140) who was

able to prepare the pyridinium salts in high yields. The corresponding ketones were allowed to react, usually on a steam bath, with an excess of iodine and pyridine base at or near the reflux temperature. After a certain length of time, the excess of pyridine base and iodine was removed by extraction with a non-polar solvent, such as ether. The pyridinium salts remained and were purified.

During his investigations, King examined the possibility of synthesizing pyridinium salts of steroidal compounds. He observed that no reaction occurred when cholesteryl chloride was treated with pyridine bases (141). Later Tsuda and Hayatsu (142) confirmed the findings of King in so far as the reaction of cholesteryl chloride with pyridine was concerned. However they reported that a rearrangement with the formation of 1-(5-cholesten-3-yl)pyridinium bromide took place when cholesteryl bromide was used in the reaction. The latter observation was only of theoretical significance because of the low yield of pyridinium salt.

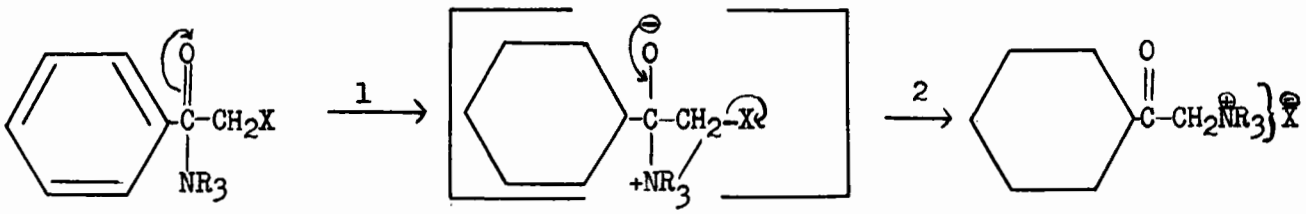
As an alternate procedure, King (141,143) prepared steryl p-toluenesulphonate esters and allowed them to react with anhydrous pyridine bases. Pyridinium salts were formed in all cases, but the yields varied depending on the character of the pyridine base used (141). King (144) established, in some cases, the stereochemical configuration of the salts obtained.

d. Mechanism of the formation and alkaline cleavage of the β-keto alkylpyridinium salts

Numerous kinetic studies concerning the formation of β-keto alkylpyridinium salts were made in attempts to determine the mechanism of aromatic chain substitution. Finally it was proved that this particular reaction exhibited second order kinetics in solvents of high polarity (145).

In non-polar solvents the kinetic picture was more complicated (146). Although a complete literature survey of this field was beyond the scope of this presentation, the work done in connection with the proposal of a mechanism for this type of reaction should be mentioned.

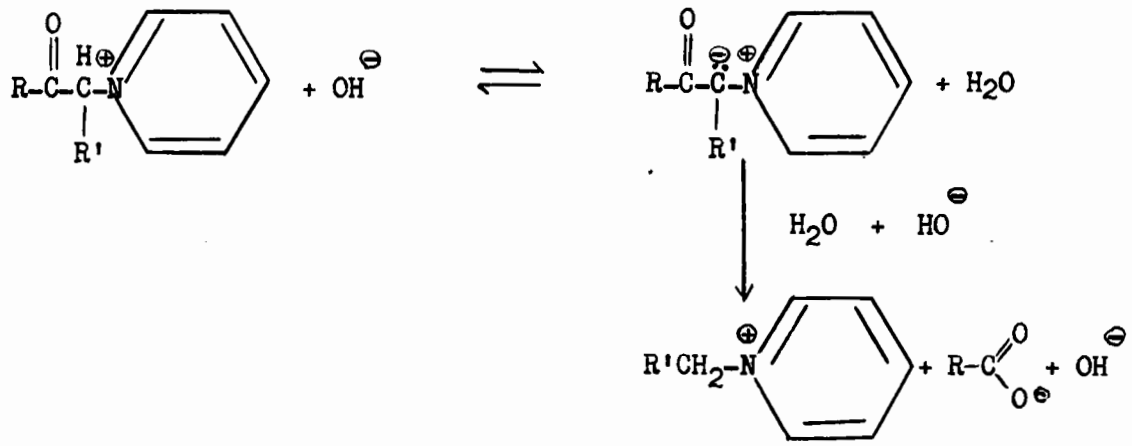
Baker (145) visualized the formation of β-keto alkylpyridinium salts as a typical nucleophilic attack by the pyridine entity. However in the case of the phenacyl group, a possibility existed for resonance similar to the resonance in the ground state of benzoyl halides. This latter fact made it possible to assume that the carbonyl group was the centre of the nucleophilic attack. Theoretically the side methylene group also could be attacked by the pyridine base, but this was highly improbable because of the resonance. Finally, the following mechanism was postulated. Step 1 determined the rate of the reaction. The inter-



mediate product could not be isolated because it underwent a very fast intramolecular rearrangement, similar to the "pinacolic electron displacement" postulated by Ingold and Shoppee (147).

The kinetics of the alkaline cleavage of β-keto alkylpyridinium salts was studied by Krönke (131). Previously, it had been found that the products of this alkaline hydrolysis were a carboxylic acid and a 1-methyl pyridinium salt (148). By kinetic methods, Krönke (131) showed that the cleavage was of pseudo first order in the presence of excess of alkali and second order in excess of salt. He assumed that the formation

of the enol betaine was the first step of the reaction, and that this betaine then reacted with a molecule of water and a hydroxyl anion to form the final products of the cleavage. The reaction was illustrated by the following scheme:



The kinetic studies of Krȍhnke were confirmed by Pearson (149, 150) but he postulated a different mechanism for the alkaline hydrolysis of these salts. He assumed that the reaction proceeded by the quaternary nitrogen atom being attacked by two hydroxyl ions.

e. Absorption spectra of pyridinium betaines

The pyridinium enol betaines were generally coloured, very reactive, and decomposed in air and light (91). The pronounced tendency of these compounds to form hydrates of varying constitution was an additional factor which frequently made the analytical results unreliable, (97). In spite of this, no attempts were made in the early investigations to obtain additional information about these pyridinium betaines by spectroscopic methods. Later it was found (91) that their photosensitivity, tendency to form hydrates, and strong light absorption in the visible region of the spectrum made spectroscopic measurements difficult.

The absorption spectra of several phenol pyridinium betaines

were measured during the lengthy investigation at the University of Marburg which dealt with the effect of solvents on the colour of organic compounds. The earlier work in this field was reviewed by Dimroth (118) and more recent studies were reported by Hünig and Rosenthal (104).

Krönke and Bohlmann (90) studied the light absorption of coloured pyridinium betaines. They found that dibenzoylmethyl pyridinium enol betaines exhibited an absorption maximum at 320 mμ. This was in agreement with theoretical calculations which predicted that the absorption maximum of these betaines should occur in the region 300-330 mμ. The same investigators showed that enol betaines derived from phenacyl pyridinium salts absorbed in the region 440-460 mμ. In addition they (90) reported that when the possibilities for resonance in a molecule were increased, the absorption maxima were shifted to longer wavelengths.

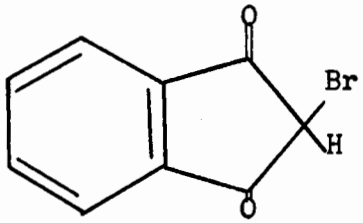
Stafford (91) measured the absorption maxima of pyridinium betaines of the indane series. He found that solutions of 1-(1-hydroxy-2-indenyl) pyridinium hydroxide, betaine (XVII) did not obey the Lambert-Beer law and that the maxima for this compound were in the region of 252-257 mμ and 425-440 mμ. The measurements were made in several solvents and a solvent effect on the maxima was detected. The electronic absorption spectrum of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) showed three maxima in the region of 238.5 mμ, 310 mμ and 393 mμ respectively.

Taylor, Jr. and his collaborators (101) found that the absorption maximum of the 1- 5-(2,4,6-trioxohexahydropyrimidyl)pyridinium betaine (XXII) occurred at 246 mμ in a 0.1N sodium hydroxide solution.

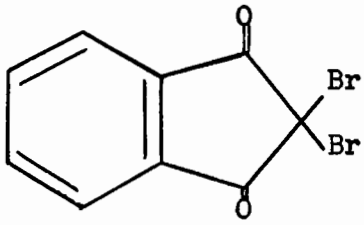
DISCUSSION

A. Pyridinium betaines of the 1,3-indandione series

The object of this phase of the present research was to study the reaction of pyridine with 2-bromo-1,3-indandione (XLVII) and with 2,2-dibromo-1,3-indandione (XLVIII). By a comparison of the products obtained from these two reactions, it was hoped that information concerning the mechanism by which they proceeded would be obtained. Such information, it was expected, would help to solve the major problem of synthesizing



XLVII

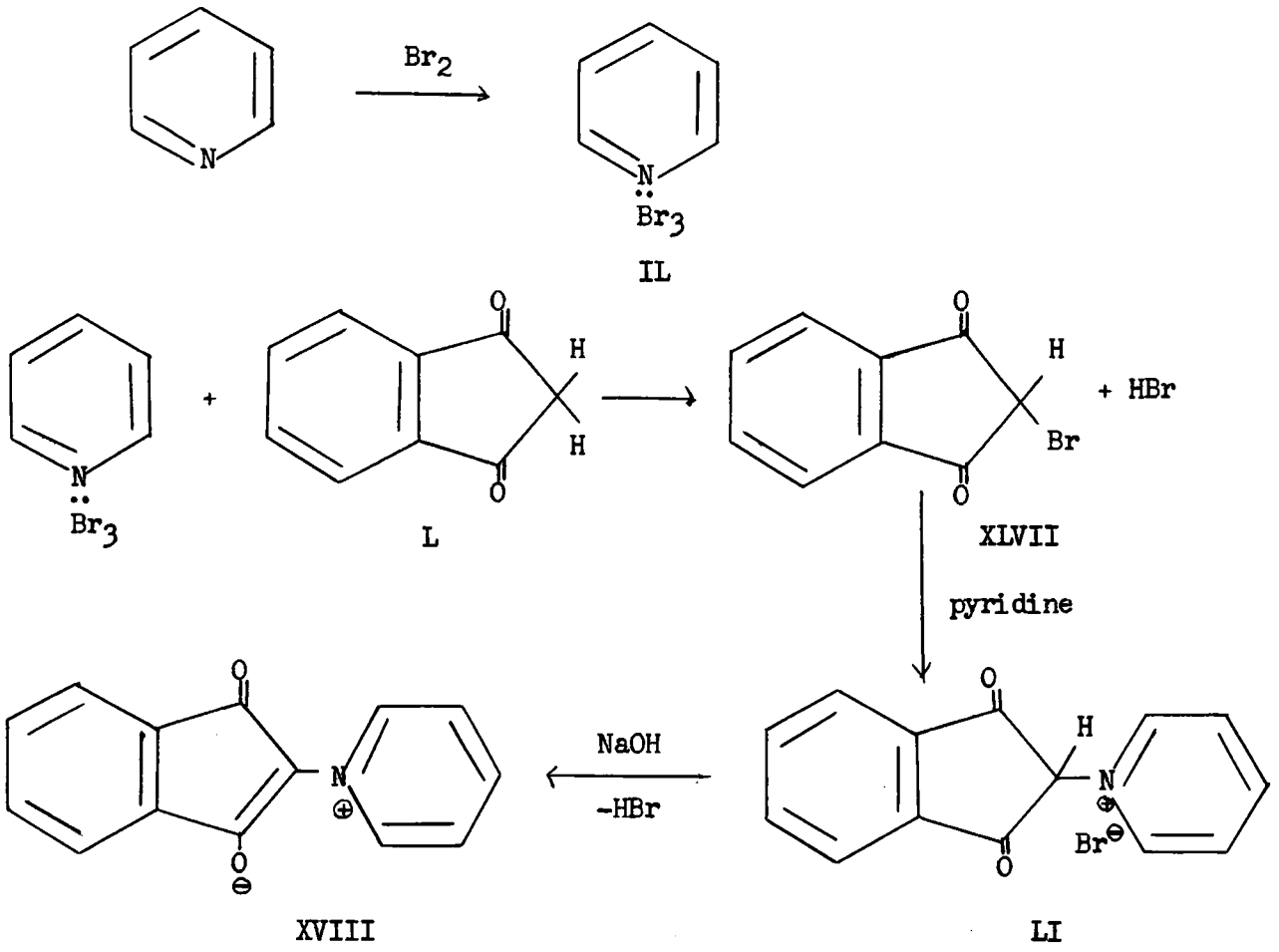


XLVIII

betaines of the 1,3-indandione series when a methylpyridine or pyridinecarboxylic acid was used as the entity providing the positive centre of the inner salt.

In his paper dealing with the synthesis of the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII), Stafford (91) gave a sound explanation for the colour of this inner salt in terms of resonance. However, his proposed sequence of reactions, outlined below, left some doubt about the mechanism and the intermediate steps which led to the formation of the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII).

The addition of bromine to pyridine resulted in the formation of pyridine bromide perbromide (IL), a mild brominating reagent. The perbromide (IL) with 1,3-indandione (L) gave 2-bromo-1,3-indandione (XLVII) which reacted further with pyridine to form the corresponding pyridinium salt (LI). On treatment with alkali, this salt yielded the 1-(3-hydroxy-1-oxo-2-indenyl)

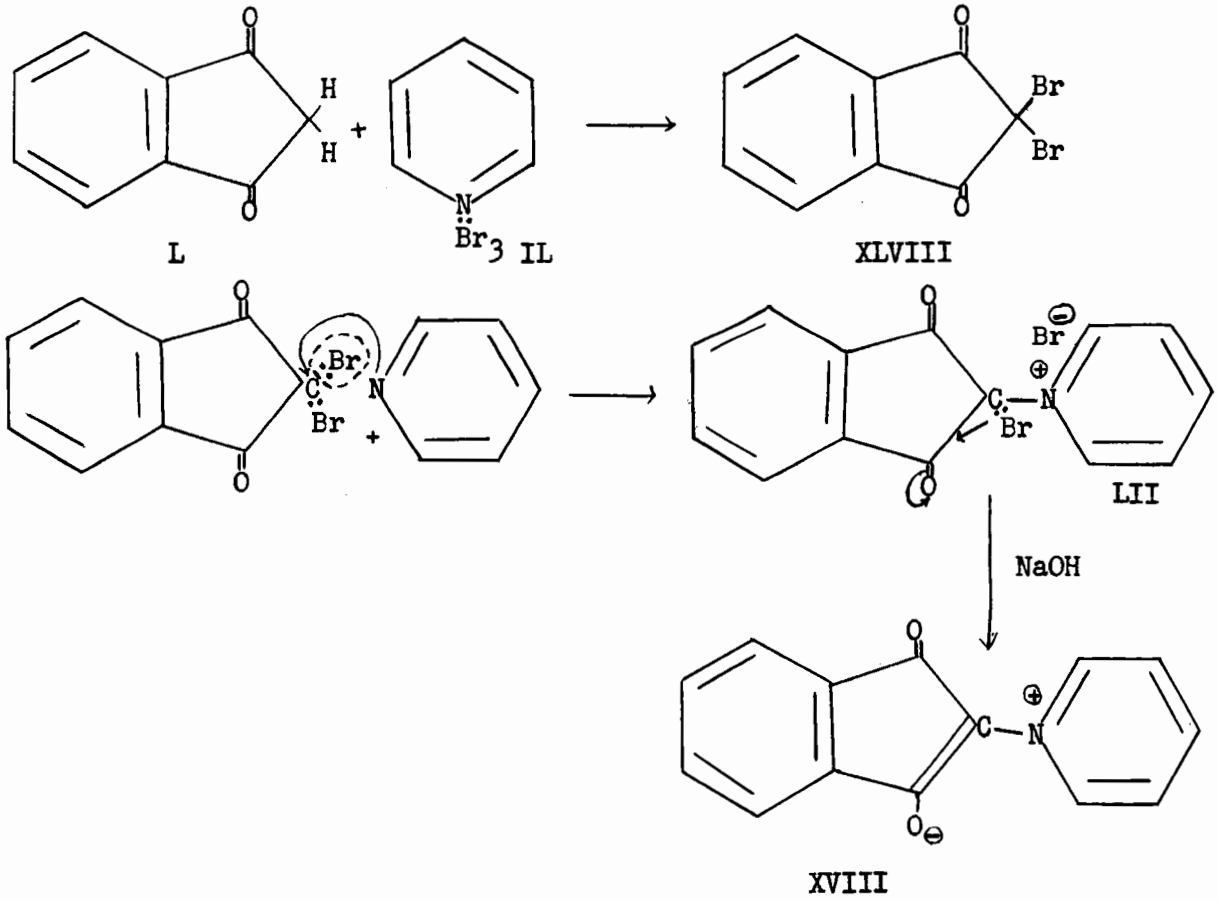


pyridinium hydroxide, betaine (XVIII). Apparently the alkali caused elimination of hydrogen bromide from 1-(1,3-dioxo-2-indanyl)pyridinium bromide (LI), and betainization, during which the 1-(3-hydroxy-1-oxo-2-indenyl) pyridinium hydroxide, betaine (XVIII) was formed. These intermediate steps appeared plausible, but actually no experimental proof was offered to support these assumptions.

Pyridine bromide perbromide (IL) always behaves as a mild brominating agent but without specificity (151). In other words, the products obtained from the reaction of this reagent (IL) with substances whose molecules are susceptible to bromination, are identical with those isolated from the reaction of free bromine on the same substances. Only quantitative, and not qualitative, differences have been detected between pyridine bromide

perbromide (IL) and free bromine in their use as brominating agents.

When free bromine is allowed to react with 1,3-indandione (L), only 2,2-dibromo-1,3-indandione (XLVIII) is obtained, and 2-bromo-1,3-indandione (XLVII) has never been isolated as an intermediate from this bromination. On the basis of the foregoing experimental evidence, it can be argued that the reaction between pyridine bromide perbromide (IL) and 1,3-indandione (L) should yield 2,2-dibromo-1,3-indandione (XLVIII). Furthermore, the formation of the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) can be explained readily by assuming that 2,2-dibromo-1,3-indandione (XLVIII) is an intermediate product of the reaction between pyridine bromide perbromide (IL) and 1,3-indandione (L). Consequently the following scheme is proposed to represent the steps of the reaction.



According to this scheme, the reaction of pyridine bromide perbromide (IL) with 1,3-indandione (L) results in the formation of 2,2-dibromo-1,3-indandione (XLVIII). The latter compound then reacts with pyridine to give 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII) which when treated with alkalies, yields 1-(3-hydroxy-1-oxo-2-indenyl) pyridinium hydroxide, betaine (XVIII).

Reaction of 2-bromo-1,3-indandione with pyridine

2-Bromo-1,3-indandione (XLVII) (152) is quite reactive, rather unstable, and undergoes self-polymerization at elevated temperatures. The bromine atom present in the molecule possesses a "positive" character which can be detected by the liberation of iodine upon treatment of 2-bromo-1,3-indandione (XLVII) with an aqueous solution of potassium iodide in the presence of starch. The "positive" nature of this particular bromine atom is not surprising because cases have been reported in the literature (153) in which β-halo α,γ-diketones possess a "positive" halogen atom.

When 2-bromo-1,3-indandione (XLVII) was added to pyridine, a yellowish solution was obtained, the colour of which deepened gradually as the reaction proceeded. Upon standing at room temperature for several hours, or short heating on the steam bath, the solution turned very dark. Then the reaction mixture was diluted with water, the excess pyridine extracted with ether, and the aqueous layer made alkaline. The 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) deposited slowly and was identified with the betaine (XVIII) obtained by Stafford (91), by a comparison of their melting points and electronic absorption spectra. (All spectroscopic data will be discussed in a later chapter). The yield obtained in this case was much higher than that reported by Stafford (91). Apparently, in the latter procedure the side reactions which occurred were

responsible for the low yields.

It is emphasized that the reaction of 2-bromo-1,3-indandione (XLVII) with pyridine described above gives the same product 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII), as Stafford obtained when he allowed 1,3-indandione (L) to react with pyridine in the presence of bromine. This seems to confirm his proposal that 2-bromo-1,3-indandione (XLVII) is an intermediate in the latter case.

It was felt that 1-(1,3-dioxo-2-indanyl)pyridinium bromide (LI) should be formed from 2-bromo-1,3-indandione (XLVII) in the first step of the reaction as the result of a simple nucleophilic attack by pyridine, because the final product of the reaction (XVIII), a betaine, contained both the pyridine and indane nuclei. This assumption followed from a consideration of the normal course of betainization.

Consequently attempts were made to isolate the intermediate 1-(1,3-dioxo-2-indanyl)pyridinium bromide (LI). For this reason the reaction mixture of 2-bromo-1,3-indandione (XLVII) and pyridine was diluted with water and evaporated to dryness over phosphorus pentoxide at room temperature. The black residue gave a strong positive test for anionic bromine and nitrogen. After having been thoroughly dried in vacuo, the residue was a brittle resinous mass which appeared partially crystalline, and partially amorphous. When sublimation of this material was attempted, a white substance sublimed first. It was purified by recrystallization from a mixture of chloroform and styrene and found to be pyridine hydrobromide. At a higher temperature, a lustrous yellow substance sublimed which was identified with the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) prepared by Stafford (91).

The isolation and identification of the pyridine hydrobromide and

the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII), led to the conclusion that the intermediate 1-(1,3-dioxo-2-indanyl)pyridinium bromide (LI) is very reactive and undergoes spontaneous dehydrobromination. The latter process apparently is due to the presence of pyridine. Furthermore the isolation of these two products indicates that the "positive" bromine present in 2-bromo-1,3-indandione (XLVII) can be released also in an anionic form.

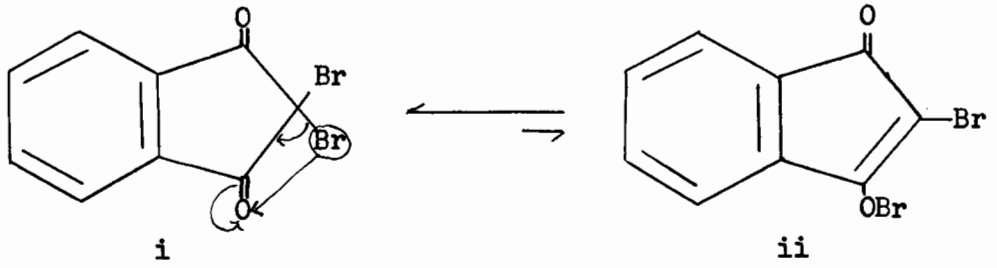
Reaction of 2,2-dibromo-1,3-indandione with pyridine

2,2-Dibromo-1,3-indandione (XLVIII) (154) was obtained directly from the bromination of 1,3-indandione (L) in glacial acetic acid. Upon treatment of a suspension of 2,2-dibromo-1,3-indandione (XLVIII) in water with an aqueous solution of potassium iodide, iodine was liberated revealing that the compound contained "positive" bromine.

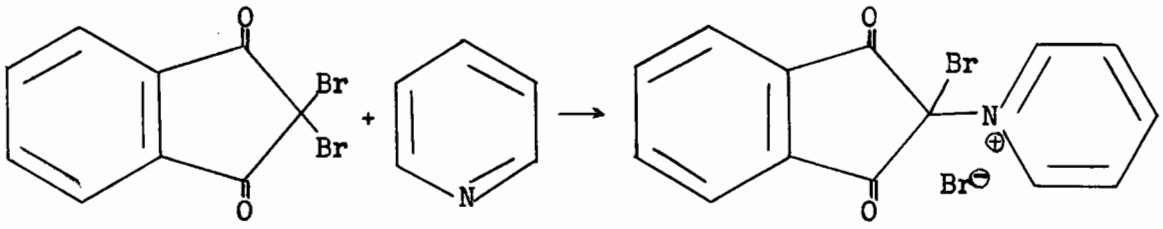
When the colourless 2,2-dibromo-1,3-indandione (XLVIII) was added to pyridine, an orange solution was obtained the colour of which deepened gradually with time. This served as qualitative evidence that some kind of reaction was occurring.

It is difficult to explain the progressive change in colour of the reaction mixture in terms either of the shift of a bromonium ion or the formation of a typical pyridinium salt. In the former case, the migration of one positively charged bromine atom in the molecule of 2,2-dibromo-1,3-indandione (XLVIII) is not likely to cause such a pronounced change in colour because only one double bond is shifted. In addition, it is well known that the group -OBr is not stable, because both the oxygen and bromine are strongly electronegative, and consequently the (ii) form of 2,2-dibromo-1,3-indandione (XLVIII) can not predominate in an equilibrium of forms (i) and (ii) of the compound (XLVIII). The latter explanation for

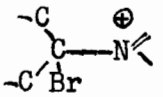
the change in colour based on the assumption that a pyridinium salt, 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII), can form by the splitting off of a bromine anion and quaternization of the annular nitrogen, is not satisfactory either.

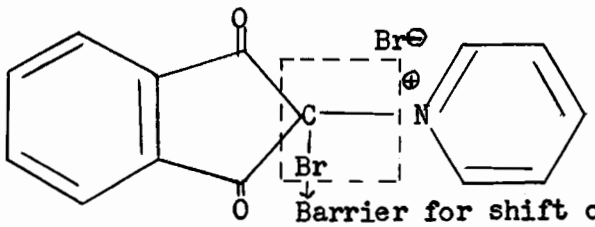


i XLVIII ii



XLVIII LII

When the salt (LII) is produced, a molecule of pyridine containing three aromatic double bonds is added to the molecule of 2,2-dibromo-1,3-indandione (XLVIII). The resonating structures which contribute to the resonance hybrid of 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide, molecule (LII), arise from shifts of π electrons within the indane, and within the pyridine part of the molecule. The group  acts as a barrier and prevents the free shift of π electrons throughout the cation of 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII). The existence of this barrier limits considerably the number of resonating structures, and consequently the great change in colour during the reaction between pyridine and 2,2-dibromo-1,3-indandione (XLVIII) can not be explained adequately by resonance.



LII

The observed solvatochromy of the reaction mixture under discussion emphasized the doubts existing about the formation of 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII) as the main product of the reaction between 2,2-dibromo-1,3-indandione (XLVIII) and pyridine. It was found that when water was added, the colour of the reaction mixture became lighter, but when the same quantity of ethanol was added no significant effect on the colour was observed other than that expected due to dilution. The decrease in intensity of colour upon the addition of water definitely was not a linear function of the concentration of reaction product, but appeared to be a complicated function of a varying rate and degree of hydration of this product. If the decrease in intensity of colour were simply a function of the concentration of reaction product, then this decrease should be almost independent of the nature of the solvent and should depend on the amount of solvent added.

Additional experimental work verified these assumptions and left no doubt that the product of the reaction of pyridine with 2,2-dibromo-1,3-indandione was not 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII), but 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII). The latter compound was isolated by removing the excess pyridine in vacuo and recrystallizing the semisolid residue from alcohol. The residue also could be purified by fractional sublimation, and this method was preferable because two different crystalline compounds, pyridine hydrobromide and 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII), could be separated easily. It is pointed out that the sublimation of the reaction mixture was carried out in an all-glass

sublimation apparatus, because the pyridine hydrobromide present attacked rubber.

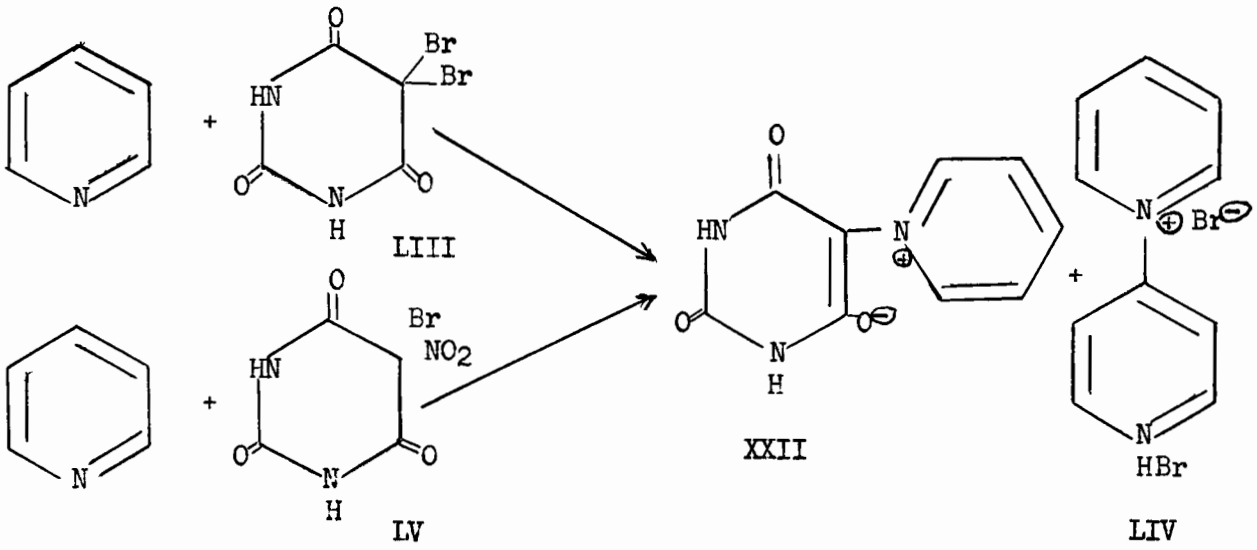
In addition to the pyridine hydrobromide and the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII), there remained in the sublimation apparatus, a black residue which did not sublime but began to soften at an elevated temperature. Attempts to crystallize this amorphous material or to isolate a homogeneous substance from it were unsuccessful. Examination of the residue under the microscope showed that it was completely amorphous, and a hard, brittle, resinous polymeric substance, which did not possess any tendency to form fibres. The latter characteristic was a qualitative indication that the material was not a linear polymer. Elementary analysis of the substance indicated the presence of nitrogen and anionic bromine.

The reaction between pyridine and 2,2-dibromo-1,3-indandione (XLVIII) may be considered as an elimination of two bromine atoms from each molecule of 2,2-dibromo-1,3-indandione (XLVIII), followed by the formation of the corresponding pyridinium betaine (XVIII). This reaction seems to be similar to that of pyridine with 5,5-dibromobarbituric acid (LIII) reported recently by Taylor, Jr., and his collaborators (101), in which the products were 1-[5-(2,4,6-trioxohexahydropyrimidyl)]pyridinium betaine (XXII) and 4-pyridyl-pyridinium bromide hydrobromide (LIV). According to the mechanism proposed (101) for the latter reaction, the first step was a nucleophilic attack by pyridine and displacement of a bromonium ion.

The similarity between the reaction of pyridine with 2,2-dibromo-1,3-indandione (XLVIII) and 5,5-dibromobarbituric acid (LIII) is considered to arise from the fact that both compounds possess a β,β-dibromo-α,γ-diketo group in their molecule which can be attacked by pyridine. In both cases, also, the corresponding pyridinium betaine is formed.

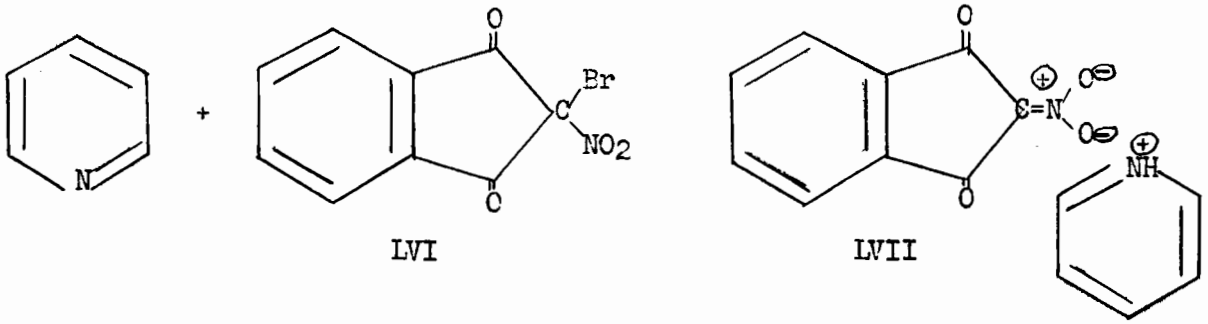
However, differences are apparent between these two reactions. Taylor, Jr., and his co-workers (101) found that the second product of the reaction between pyridine and 5,5-dibromobarbituric acid (LIII) was 4-pyridyl-pyridinium bromide hydrobromide (LIV). In the reaction mixture of pyridine and 2,2-dibromo-1,3-indandione (XLVIII), pyridine hydrobromide was isolated.

Subsequently, Taylor, Jr., and his colleagues (101) found that the reaction of pyridine with 5-bromo-5-nitrobarbituric acid (LV) gave the same pyridinium betaine (XXII) as that obtained from the reaction of pyridine and 5,5-dibromobarbituric acid (LIII). However, Metro and Taurins (155) studied



LIII LV XXII LIV

the reaction of 2-bromo-2-nitro-1,3-indandione (LVI) with pyridine and found that the pyridinium salt of the aci-nitro form of 2-nitro-1,3-indandione (LVII) was formed, as shown by the following scheme.



LVI LVII

A consideration of the experimental evidence presented above leads to the assumption that the mechanism for the reaction of 2,2-dibromo-1,3-

indandione (XLVIII) with pyridine is not identical with that proposed by Taylor, Jr., and his co-workers (101) for the reaction of pyridine with 5,5-dibromobarbituric acid (LIII). Consequently, the following scheme is postulated to represent the mechanism by which pyridine reacts with 2,2-dibromo-1,3-indandione (XLVIII),(Fig. 1).

The first step of the reaction of pyridine with 2,2-dibromo-1,3-indandione (XLVIII) is considered to be a nucleophilic attack by pyridine. This nucleophilic attack causes an elimination of one bromine anion from each molecule of 2,2-dibromo-1,3-indandione (XLVIII) and the subsequent formation of 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII). From the point of view of the products obtained, it does not matter whether the pyridine attacks the carbon atom of one of the carbonyl groups first, or the carbon atom in 2 position of 2,2-dibromo-1,3-indandione (XLVIII). However, a careful analysis of the electronic distribution and polarization of the keto groups of this compound (XLVIII), indicates that the nucleophilic attack occurs at the carbon atom of one of the keto groups of 2,2-dibromo-1,3-indandione (XLVIII). An unstable intermediate (LVIII) is formed which rapidly undergoes a 1,2-shift of the type discussed by Ingold and Shoppee (147). This rapid rearrangement results in the formation of 1-(2-bromo-1,3-dioxo-2-indanyl)pyridinium bromide (LII), which is attacked by another molecule of pyridine. The latter nucleophilic attack brings about the removal of the remaining bromine atom, in 2 position of the indane nucleus, as a bromonium ion. This ion forms an unstable complex (LIX) with pyridine while 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) is being produced simultaneously. The pyridine-bromine complex (LIX) reacts further with pyridine giving pyridine hydrobromide and 4-pyridyl-pyridinium bromide (LX). The inability to isolate the latter

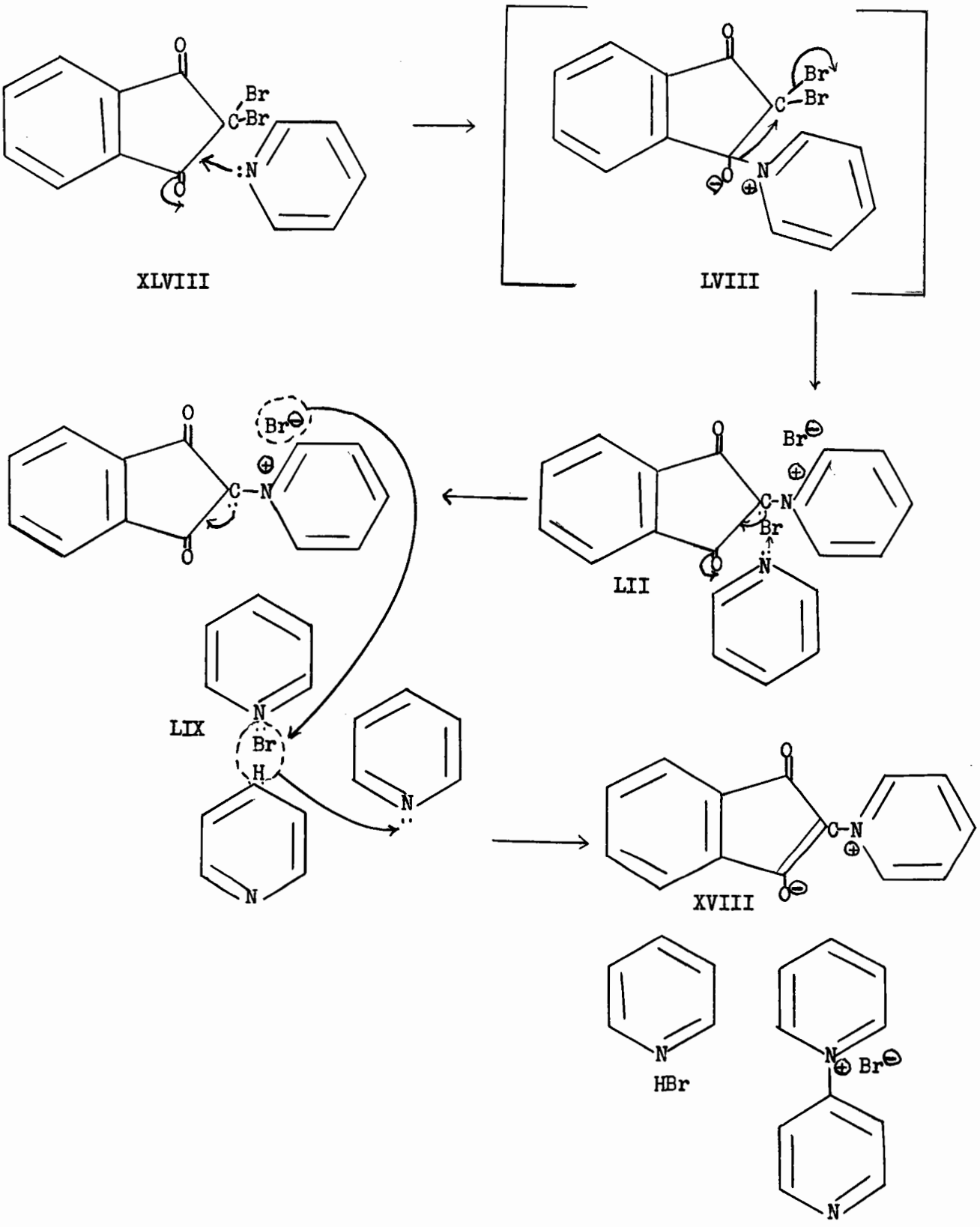
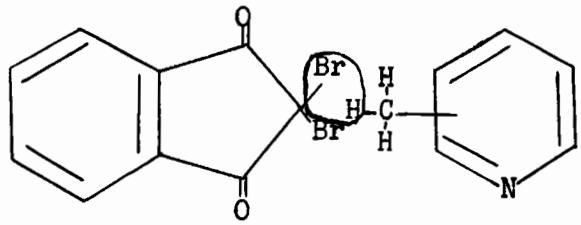


FIG. 1. Schematic representation of the reaction of pyridine and 2,2-dibromo-1,3-indandione

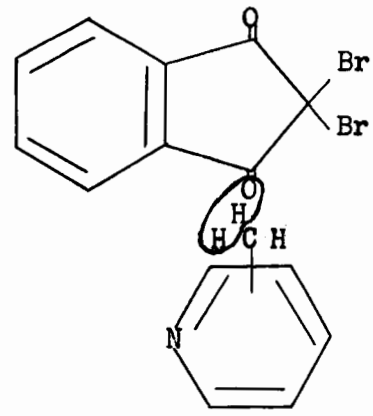
salt (LX) from the reaction mixture of 2,2-dibromo-1,3-indandione (XLVIII) is attributed to the probable polymerization of 4-pyridyl-pyridinium bromide (LX) in the presence of pyridine and anionic or cationic bromine, the latter being provided by 2,2-dibromo-1,3-indandione (XLVIII) as previously described. The high reactivity of the latter compound (XLVIII) makes possible the participation of this substance in the initial or final stages of the polymerization, the probable mechanism for which is outlined schematically in Fig. 2.

Reaction of 2-, 3-, and 4-methylpyridines with 1,3-indandione in the presence of bromine

The 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) may be considered as the simplest pyridinium betaine of the 1,3-indandione series. The possibility of synthesizing similar inner salts using the three isomeric methylpyridines (picolines) instead of pyridine was examined, in an effort to determine how the methyl group attached to the pyridine molecule would affect the synthesis. It appeared possible that instead of the formation of a bond between the carbon in 2 position of 2,2-dibromo-1,3-indandione (XLVIII) and the annular nitrogen of the methyl-pyridines, an elimination of hydrogen bromide or water might occur as shown below.



Elimination of hydrogen bromide?



Elimination of water?

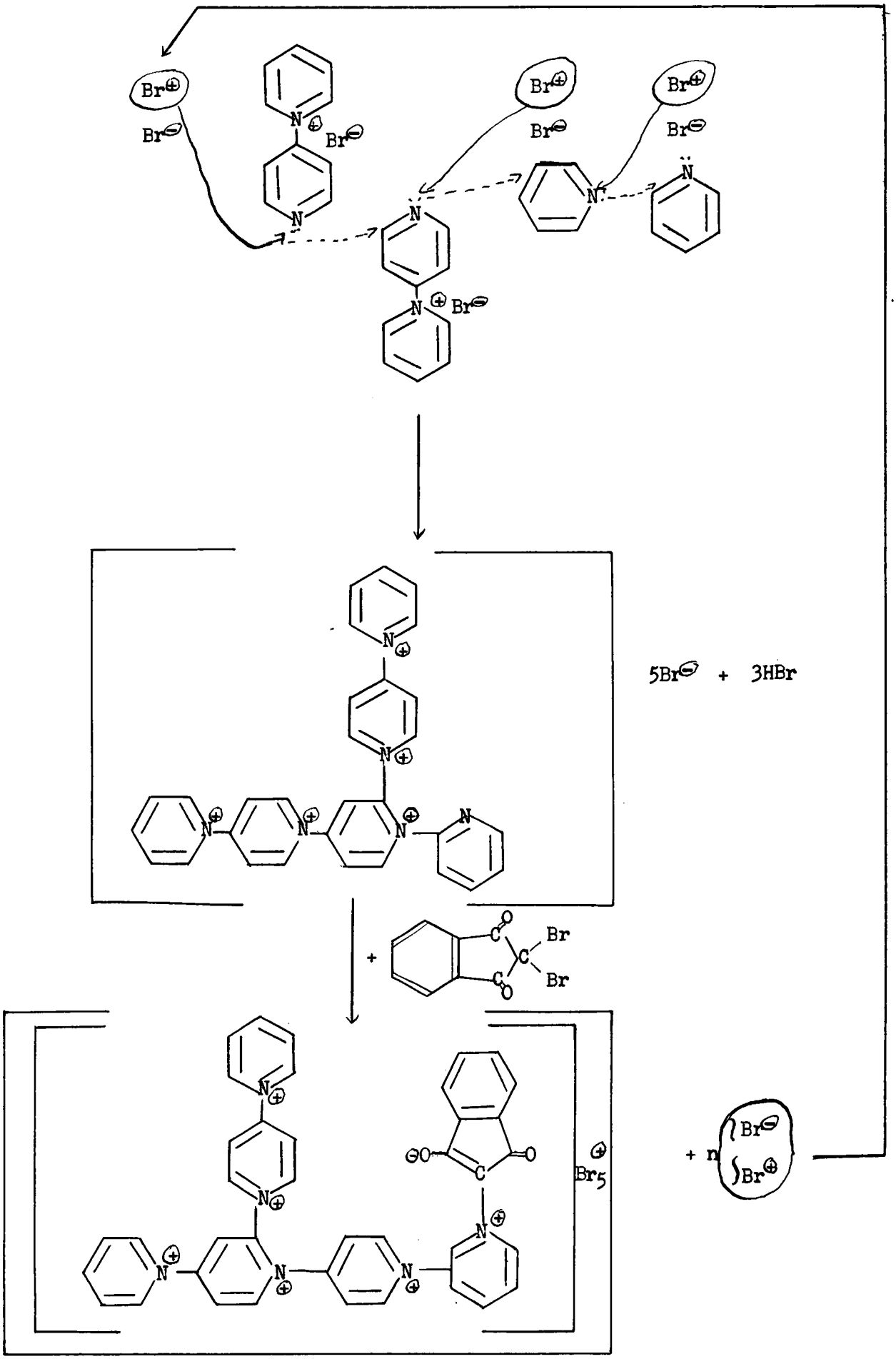

Br⊕
Br⊖
N⊕
Br⊖
N
Br⊕
Br⊖
Br⊕
Br⊖
N
N
N⊕
Br⊖
N⊕
N⊕
N⊕
N
5Br⊖ + 3HBr
+
C
O
Br
C
Br
O
N⊕
N⊕
N⊕
N⊕
N⊕
N⊕
N⊕
N⊕
N⊕
O⊖
O
C
Br5⊕
+ n
Br⊖
Br⊕

FIG. 2

As the first approach to this problem it seemed logical to follow the previously mentioned procedure of Stafford (91). If the isomeric 2-, 3-, and 4-methylpyridines reacted similarly to pyridine, then the reaction of the methylpyridines with 1,3-indandione (L) in the presence of bromine would be expected to give the desired betaines.

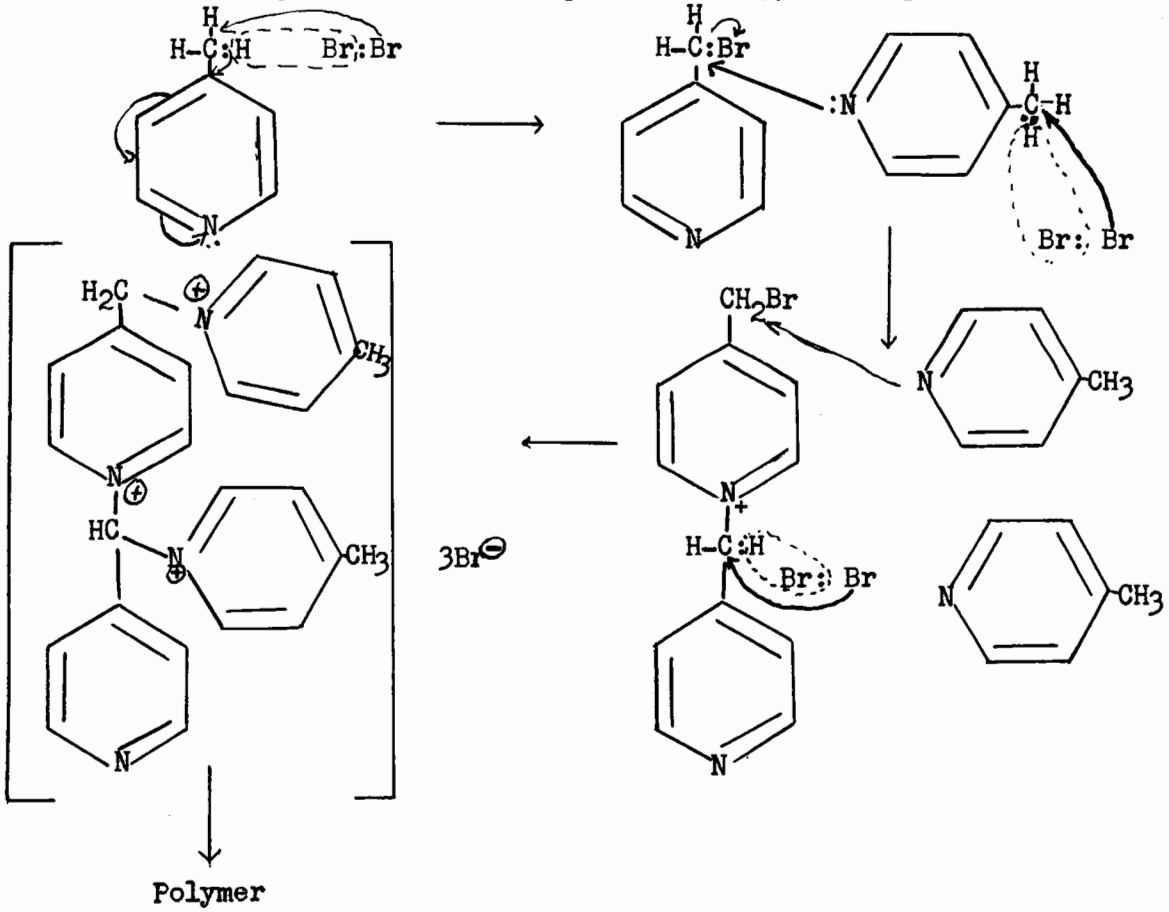
However, this method of approach was viewed with a certain amount of skepticism because of the experimental data available about the reaction of bromine with methylpyridines. It has been reported (156) that when bromine was added to any of the three isomeric methylpyridines, the reaction mixture turned black and rapid polymerization ensued. Only in the case of 3-methylpyridine was it possible, under carefully controlled conditions, to obtain "β-picolyl bromide" (156). The latter tended to undergo self-polymerization rapidly.

Attempts were made to determine whether the reaction of methylpyridines with bromine would pursue the same path in the presence of 1,3-indandione (L). In these attempts essentially the same procedure as Stafford had used for the reaction with pyridine was followed. It was thought that the molecule of 1,3-indandione (L), being highly reactive, might alter the direction of the reaction of bromine with methylpyridines. In other words that the intermediate bromination of 1,3-indandione (L) might be the predominant reaction and inhibit polymerization of the methylpyridines.

However this did not prove to be the case. When bromine was added dropwise into a solution of 1,3-indandione (L) in any one of the methylpyridines, even at 0°, a rapid polymerization was the only reaction which occurred. In all cases, a black semisolid product was obtained which was extracted with ether to remove any free methylpyridine present in the reaction mixture. After this extraction, the material dried and a hard resinous

residue was obtained. Sublimation of this material yielded a white substance which was identified as the corresponding methylpyridine hydrobromide. The hydrobromides were rapidly decomposed by alkalies to the corresponding free methylpyridine bases. The latter were identified by their boiling points and their derivatives with picric acid.

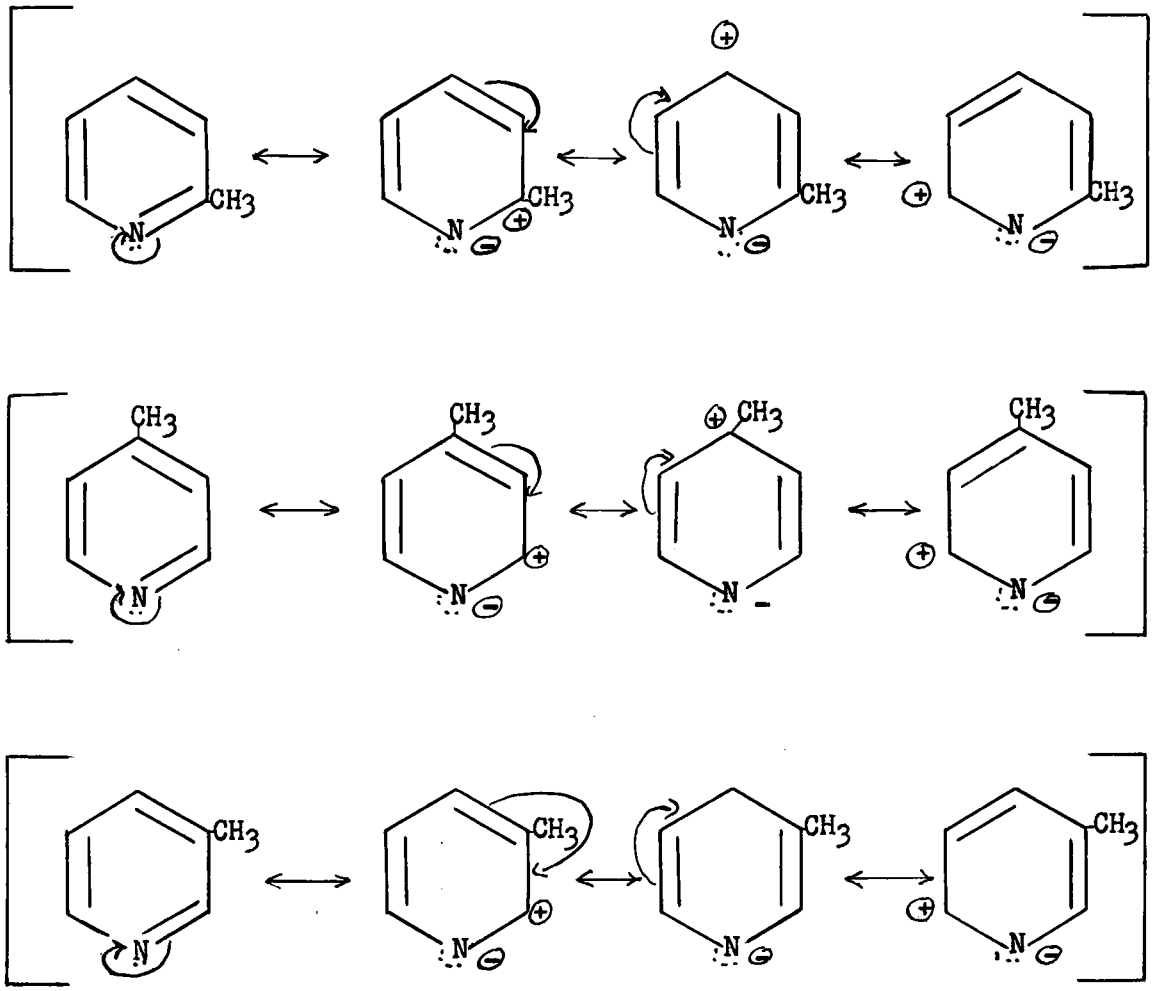
The mechanism by which this polymerization of the methylpyridines proceeds is somewhat uncertain, but the following outline is proposed to represent a possible sequence of reactions. In this outline 4-methylpyridine is used as the reacting entity. The first step of the reaction is the replacement of one, or perhaps more, hydrogen atoms of the methyl group of 4-methylpyridine by bromine. The electron-attracting effect exerted by the annular nitrogen brings about the release of a proton from the methyl group attached to the pyridine ring, leaving the carbon atom of



the methyl group negatively charged. In other words, a carbanion is formed. Simultaneously with the release of the proton and formation of a carbanion, a bromine molecule present in the reaction mixture cleaves heterolytically. The anionic bromine atom thus formed combines with the displaced proton giving hydrogen bromide, which then reacts with the organic base present producing 4-methylpyridine hydrobromide. The bromonium ion combined with the carbanion forming 4-methylpyridyl bromide. The latter product, by reacting with another molecule of 4-methylpyridine or 4-methylpyridyl bromide, initiates a polymerization which proceeds very rapidly.

The assignment of the same mechanism to the polymerization reaction of 2-methylpyridine in the presence of bromine is based on a consideration of the resonating structures contributing to the resonance hybrid of 2-, and 4-methylpyridine. These canonical structures show clearly that there is quite a low electron density in the 2 and 4 position of pyridine and that this is a result of the electron attraction by the annular nitrogen atom. The resonating structures which contribute to the resonance hybrid of the 3-methylpyridine molecule however, reveal that the electron density in 3 position is relatively higher than in 2 or 4 position. Apparently the inductive effect of the annular nitrogen atom does not affect considerably the electron density in 3 position. Thus, the hydrogen atoms of the methyl group attached to the pyridine nucleus in 3 position do not exhibit a strong tendency to be released as protons. However, the replacement of a hydrogen atom of the methyl group in 3 position by a bromine atom can occur but does so with less vigour than in the case of 2-, and 4-methylpyridine.

The polymerization of 2-, 3-, and 4-methylpyridine in the presence



of bromine which occurred even at 0°, inhibited the subsequent reaction with 1,3-indandione (L). Consequently attempts were made to prevent this polymerization by lowering the temperature of the reaction. In a series of experiments, the flask containing the methylpyridine was immersed in a dry ice-acetone bath. The organic base rapidly solidified and bromine was added to it dropwise. The bromine also solidified immediately, so that a two phase solid system was formed. A slow reaction proceeded at the interface between the two phases, yielding an orange-yellow solid product. This product was not identified but presumably it was a methylpyridine-bromine complex similar to the pyridine-bromine complexes (151). When

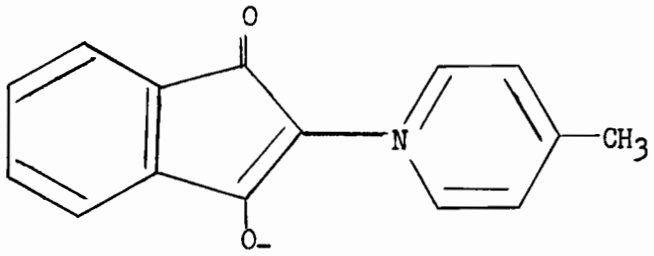
the reaction mixture was allowed to reach the temperature at which both reactants became liquid, polymerization started immediately and a semisolid tar was produced. The same result was obtained when 1,3-indandione (L) was present in the reaction mixture.

These experiments demonstrated that the three methylpyridines did not react with 1,3-indandione (L) in the presence of bromine to give methylpyridinium betaines of the 1,3-indandione series. Consequently further experimental work in this direction was abandoned.

Reaction of 4-methylpyridine with 2,2-dibromo-1,3-indandione

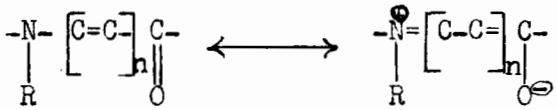
Since the application of the Stafford procedure (91) failed to yield, in the case of methylpyridines, the desired betaines of the 1,3-indandione series, the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with 4-methylpyridine was attempted. The addition of 4-methylpyridine to a boiling solution of 2,2-dibromo-1,3-indandione (XLVIII) in ethanol caused an immediate change in the colour of the solution. As the reaction proceeded the colour became a deep red. Upon cooling the reaction mixture, a dark red crystalline material separated which was identified as the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI). Assignment of the following structural formula to the latter compound (LXI) was based on the molecular weight determination and elementary analysis. A comparison of the electronic absorption spectra of this compound and the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (XVIII) revealed a similarity in pattern which served to confirm the postulated formula (LXI) for the reaction product of 4-methylpyridine and 2,2-dibromo-1,3-indandione (XLVII).

An explanation in terms of resonance can be offered for the deep colour of the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI), analogous to that given by Stafford (91) for the corresponding



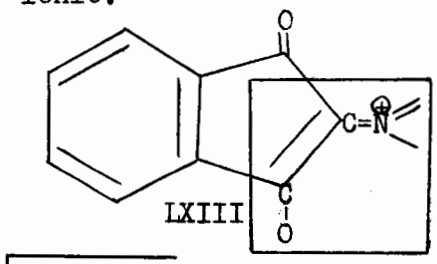
LXI

betaine of pyridine (XVIII). In this connection, the difference between the pyridinium betaines of the 1,3-indandione series and the merocyanine dyes (157) should be pointed out. This class of dyes possesses the function (LXII).

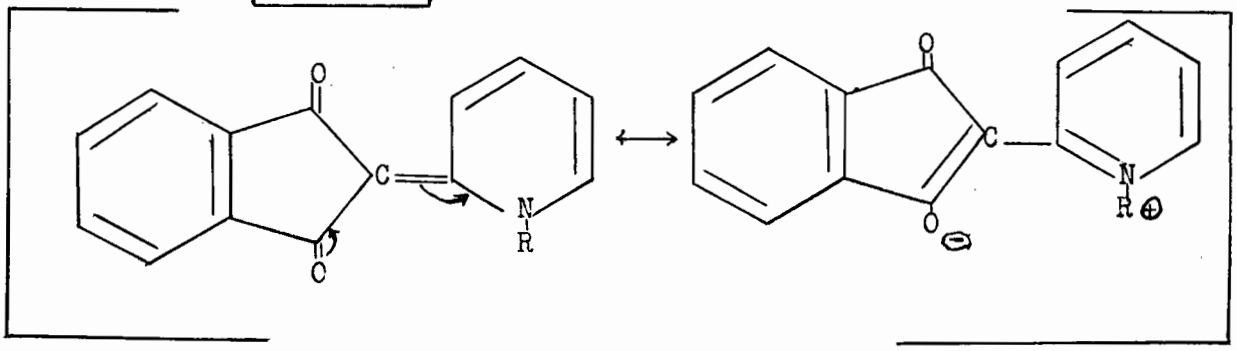


LXII

When n=1, the function exists also in the 1,3-indandione series of pyridinium betaines (LXIII). In contrast with the betaines (LXIII) which are always dipolar ions, the merocyanine molecule (LXIV) has resonating structures contributing to its resonance hybrid which may or may not be ionic.



LXIII



LXIV

Concurrent research (158) in this laboratory, dealing with the synthesis of certain phthalones, afforded the opportunity to compare typical merocyanines with the pyridinium betaines of the 1,3-indandione series. It was observed that the merocyanines had a lighter colour than the corresponding pyridinium betaines and the dissimilarity in colour was attributed to the difference in the degree of polarity within the molecules of the two types of compounds.

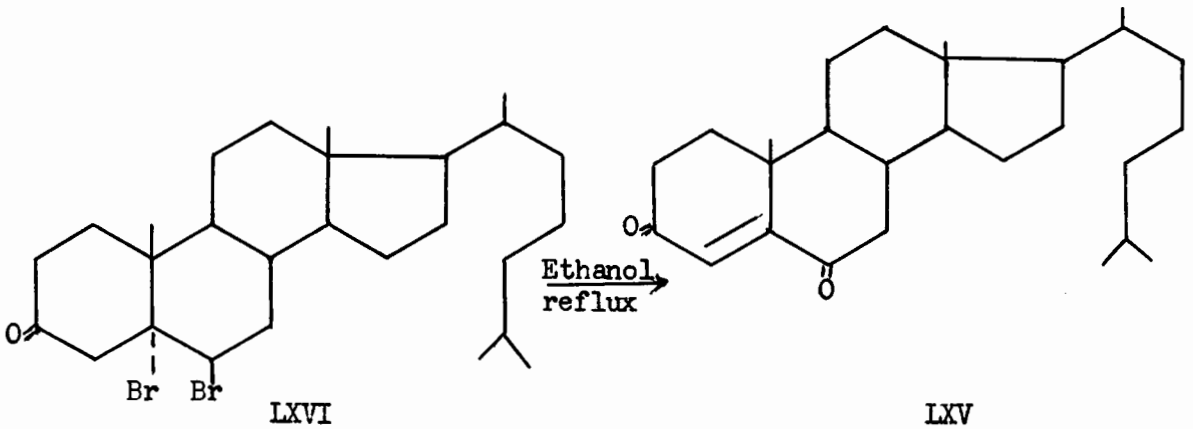
The elimination of one molecule of bromine from each molecule of 2,2-dibromo-1,3-indandione (XLVIII) and the formation of the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI) can be considered as the result of a typical nucleophilic attack by a molecule of 4-methylpyridine. The mechanism for the reaction appears to be the same as that proposed for the reaction of pyridine with 2,2-dibromo-1,3-indandione (XLVIII) since the same type of product is isolated from the reaction mixture in each case.

In the reaction of 4-methylpyridine with 2,2-dibromo-1,3-indandione the corresponding betaine (LXI), 4-methylpyridine hydrobromide, and a dark polymer were isolated as products. In this case, polymerization followed a complicated path, since the methyl group attached to the pyridine ring could participate in the formation of intermolecular links and in this way propagate polymerization. The participation of the methyl group was possible because one of its hydrogen atoms could be replaced by a bromine atom in a typical electrophilic attack by a bromonium ion.

Elimination of a molecule of bromine from each molecule of 2,2-dibromo-1,3-indandione (XLVIII) raised the question whether this elimination was due exclusively to nucleophilic attack of the compound (XLVIII) by 4-methylpyridine or whether the solvent exerted some effect on this partic-

ular elimination process.

The reaction of 2,2-dibromo-1,3-indandione and 4-methylpyridine was carried out in alcoholic solution, although cases had been reported in which elimination of a bromine molecule from a dibromo compound was catalyzed by ethanol. Urushihara and Ando (159) had obtained 4-cholestene-3,6-dione (LXV) by refluxing an alcoholic solution of 5,6-dibromo-3-cholestanone (LXVI).



LXVI LXV

The latter type of bromine elimination which was followed by oxidation was different from the elimination of bromine from 2,2-dibromo-1,3-indandione (XLVIII) because in 5,6-dibromo-3-cholestanone (LXVI), the bromine atoms are attached to vicinal carbon atoms, while in 2,2-dibromo-1,3-indandione (XLVIII) both bromine atoms are attached to the same carbon atom. In an effort to determine the effect of solvent, the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with 4-methylpyridine was carried out in dry toluene. The products in this case were the same as those obtained previously in an alcoholic medium but the yield of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI) was lower. In toluene, polymerization occurred to a greater extent. The explanation for this phenomenon is based on the following two factors.

1. The effect of temperature on the reaction

The reaction of 2,2-dibromo-1,3-indandione (XLVIII) and 4-methyl-

pyridine was carried out either in boiling ethanol or boiling toluene. Since ethanol has a considerably lower boiling point than toluene, the rate of the reaction should be greater in toluene according to the Arrhenius law of chemical kinetics. As already mentioned, this reaction yielded two main products, 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI), and 4-methylpyridine hydrobromide, and in addition a polymer. The process is considered to consist of two reactions, polymerization and betaine formation which proceed simultaneously. A temperature increase seems in this particular case to favour the rate of polymerization rather than that of betaine formation. Consequently, the yield of polymer is higher at higher temperatures.

2. The effect of the chemical nature of the solvent

It was observed that in toluene polymerization occurred predominantly even when the reaction was carried out at the temperature of boiling ethanol. Since a lower yield of polymer was obtained when ethanol was used as solvent at the same temperature, it was concluded that the ethanol inhibited the polymerization process to some extent. This inhibition is attributed to the ethanol being an efficient acceptor of the "positive" bromine liberated during the reaction. As previously shown, this "positive" bromine acts as a propagator of polymerization. Consequently, if some of the positive bromine is consumed by the ethanol during the reaction, then polymerization will occur to a lesser extent than when the reaction is carried out in toluene.

Reaction of 2,2-dibromo-1,3-indandione with 4-methylpyridine in the presence of styrene or phenol

The next question which arose was whether free bromine was released in any of the steps of the reaction between 2,2-dibromo-1,3-ind-

andione (XLVIII) and 4-methylpyridine. As previously mentioned, 2,2-dibromo-1,3-indandione liberated iodine upon treatment with an aqueous solution of potassium iodide. Obviously then, this reaction would interfere with the iodometric determination of any free bromine released during the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with pyridine bases. Consequently, the detection and determination of the liberated bromine in the reaction mixture was difficult. Ordinarily, the elegant procedure of Bartlett and Altschul (153) for determining "positive" halogen could be used to detect free halogen. However, because 2,2-dibromo-1,3-indandione (XLVIII) is able to react with cyclohexene (160), the method of Bartlett and Altschul could not be applied in this case.

As one approach to the problem, the reaction of 2,2-dibromo-1,3-indandione (XLVIII) and 4-methylpyridine was attempted in the presence of phenol, or styrene which had been stabilized against polymerization by tertiary butyl catechol. Then if bromine was liberated during the reaction, either styrene dibromide or 2,4,6-tribromophenol should be formed. However, neither of these bromination products were obtained from the reaction mixture and in each case either styrene or phenol was recovered. In the case of styrene the few milligrams of styrene dibromide isolated was considered to arise from a minor side reaction of 2,2-dibromo-1,3-indandione (XLVIII) and styrene.

The failure to isolate any free bromine from the reaction mixture of 2,2-dibromo-1,3-indandione (XLVIII) and 4-methylpyridine served to confirm the mechanism previously postulated for the reaction, according to which the elimination of both bromine atoms from the molecule of 2,2-dibromo-1,3-indandione (XLVIII) does not proceed simultaneously but in two different steps.

Reaction of 2-bromo-1,3-indandione with 4-methylpyridine

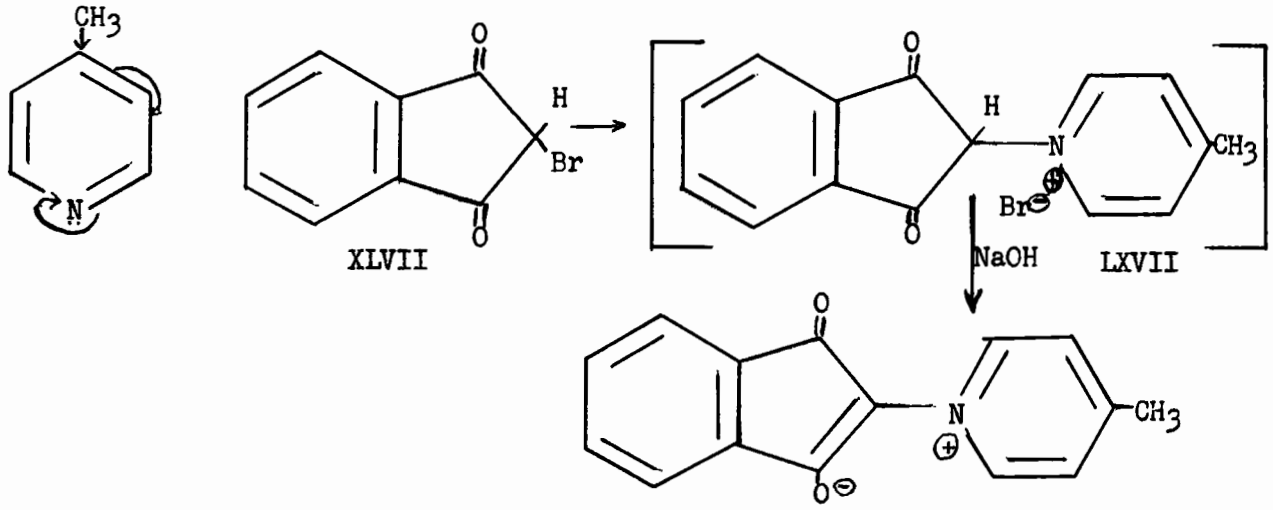
4-Methylpyridine reacted with 2-bromo-1,3-indandione (XLVII) in the same way as pyridine, and the ultimate product of the reaction was the same betaine (LXI) which had been obtained from the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with 4-methylpyridine. It was observed that 4-methylpyridine reacted with greater ease than pyridine with 2-bromo-1,3-indandione (XLVII) and for this reason, the reaction of 4-methylpyridine with 2-bromo-1,3-indandione (XLVII) was carried out at room temperature over a period of 24 hours.

When the reaction was attempted at an elevated temperature, the main product was a black semisolid polymer. The intermediate product, which should be a cyclammonium salt (LXVII), formed from the addition of 4-methylpyridine and 2-bromo-1,3-indandione (XLVII) could not be isolated for the same reasons that the intermediate pyridinium salt (LI) could not be obtained.

The formation of the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (LXI) was thought to be the result of a typical nucleophilic attack of 2-bromo-1,3-indandione (XLVII) by 4-methylpyridine. This attack was similar to that previously described in the reaction of 2-bromo-1,3-indandione (XLVII) with pyridine, but proceeded with greater ease.

Theoretical considerations confirm the experimentally observed fact that 4-methylpyridine has a higher reactivity than pyridine towards 2-bromo-1,3-indandione (XLVII). In 4-methylpyridine, the methyl group has an electron repelling effect which is in the same vectorial direction as the inductive effect of the annular nitrogen atom. These combined effects cause a high electron density around the nitrogen atom. Because of this higher electron density, 4-methylpyridine has a stronger nucleophilic

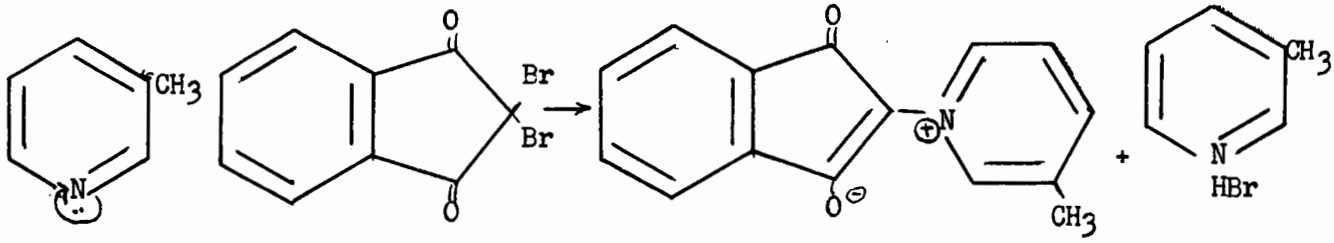
character than unsubstituted pyridine, and consequently a higher reactivity towards 2-bromo-1,3-indandione (XLVII).



XLVII LXVII LXI

Reaction of 3-methylpyridine with 2,2-dibromo-1,3-indandione and 2-bromo-1,3-indandione

The products of the reaction of 3-methylpyridine and 2,2-dibromo-1,3-indandione (XLVIII) were 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine (LXVIII), 3-methylpyridine hydrobromide and a polymeric material. The similarity of these products with those obtained from the reaction of pyridine or 4-methylpyridine with 2,2-dibromo-1,3-indandione (XLVIII) led to the conclusion that all of these reactions proceeded by a similar mechanism.



XLVIII LXVIII + polymer

However it was observed that 3-methylpyridine exhibited less reactivity than 4-methylpyridine toward 2,2-dibromo-1,3-indandione (XLVIII)

and more drastic conditions of time and temperature were necessary to accomplish the reaction of these compounds. Lengthy refluxing of 3-methylpyridine and 2,2-dibromo-1,3-indandione (XLVIII) in methanol or ethanol, resulted in almost quantitative recovery of the starting materials. Only the slight increase in the intensity of the yellow colour of the reaction mixture indicated that an extremely slow reaction was occurring. The desired product, 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine (LXVIII), was obtained, when the mixture of reactants was dissolved in hot isoamyl alcohol and refluxed for a long time. It was noted that if the reaction time was not sufficiently long, and unreacted 2,2-dibromo-1,3-indandione (XLVIII) was still present in the reaction mixture, a molecular compound of 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine (LXVIII) and 2,2-dibromo-1,3-indandione (XLVIII) was obtained. Analysis revealed that this molecular complex had the empirical formula $(C_{15}H_{11}NO_2)_5C_9H_4O_2Br_2$, that is, five molecules of the inner salt (LXVIII) were combined with one molecule of 2,2-dibromo-1,3-indandione. The formation of such a molecular compound in the field of pyridinium salts and betaines is very common and well known (161).

The betaine (LXVIII) obtained from the reaction of 3-methylpyridine and 2,2-dibromo-1,3-indandione (XLVIII) was identified by elementary analysis, by its deep yellow colour, and by comparison of its electronic absorption spectrum with the absorption spectra of the inner salts obtained from the reaction of pyridine or 4-methylpyridine with 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII). A close similarity in the patterns of these spectra was observed.

As has been mentioned previously, 3-methylpyridine exhibits a lower reactivity toward 2,2-dibromo-1,3-indandione than does 4-methyl-

pyridine. This is due to the fact that the methyl group in 3 position has a negligible electron repelling effect toward the annular nitrogen atom. Consequently this methyl group is unable to increase the electron density of the nitrogen and the nucleophilic character of 3-methylpyridine.

The reaction of 3-methylpyridine with 2-bromo-1,3-indandione (XLVII) proceeded smoothly, without complications, to yield the same inner salt (LXVIII) as was obtained from the reaction of 3-methylpyridine with 2,2-dibromo-1,3-indandione (XLVIII).

Reaction of 2-methylpyridine with 2,2-dibromo-1,3-indandione and 2-bromo-1,3-indandione

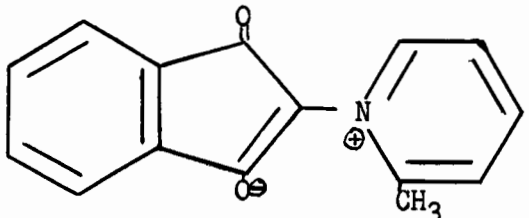
The reaction of 2-methylpyridine with either 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII) did not produce the corresponding betaine with the 2-methylpyridinium group as the cationic function, although both reactions were attempted under varying conditions of temperature, solvent and time. In all cases, the reaction mixture turned yellow-red immediately and this colour became darker as the reaction proceeded. Tests for the amount of unreacted 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII) indicated that it decreased rapidly and in this way revealed that some kind of reaction occurred.

The reaction product isolated was a tar, and no traces of the 1-(3-hydroxy-1-oxo-2-indenyl)2-methylpyridinium hydroxide, betaine could be detected, although the latter was expected as one of the products of the reaction by analogy with the betaines obtained from the reaction of 2,2-dibromo-1,3-indandione (XLVIII) and 2-bromo-1,3-indandione (XLVII) with pyridine, 4-methylpyridine and 3-methylpyridine.

In this connection, it was noted that Taylor, Jr., and his collaborators (101), failed to isolate a definite product from the reaction

of 5,5-dibromobarbituric acid with 2-methylpyridine. They assumed that this failure was due to steric factors. However, in the present case, the explanation could not be based on steric hindrance factors for the following reasons.

1. The construction of a scale model of 1-(3-hydroxy-1-oxo-2-indenyl)2-methylpyridinium hydroxide, betaine (LXIX) shows clearly that no considerable steric factor is involved. It is difficult to imagine the methyl group which is neither bulky nor polar exerting such a strong steric effect that the reaction would be prevented completely from taking place.



LXIX Not isolated

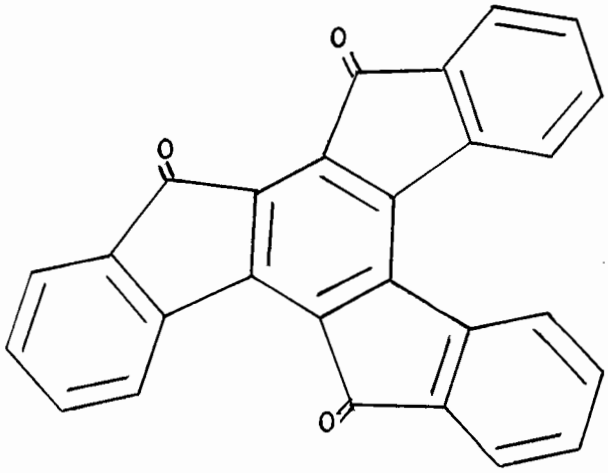
2. As will be discussed later, when 2-pyridinecarboxylic acid (picolinic acid) is used in the reaction in place of 2-methylpyridine, the corresponding betaine is formed. This fact demonstrates clearly that the failure of the reaction of 2-methylpyridine with 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII) to produce the 1-(3-hydroxy-1-oxo-2-indenyl)2-methylpyridinium hydroxide, betaine (LXIX) cannot be attributed to steric hindrance by the methyl group present in the 2 position of pyridine ring. If the failure was due to steric hindrance, then the polar and more bulky carboxylic group of 2-pyridinecarboxylic acid should inhibit the formation of the inner salt to an even greater extent than the methyl group of 2-methylpyridine.

It is postulated that the failure of the reaction of 2-methylpyridine with 2,2-dibromo-1,3-indandione (XLVIII) and 2-bromo-1,3-indandione (XLVII) to produce the betaine (LXIX), is due to the high susceptibility of

the methyl group in 2 position of pyridine nucleus to an electrophilic attack in which a hydrogen atom is replaced by the attacking electrophilic entity. Since it is known that bromonium ions provided by 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII), are present in the reaction mixture, it is assumed that these ions attack the methyl group. This electrophilic attack initiates a polymerization similar to that which occurs during the reaction of free bromine with methylpyridines. In the present case, the polymerization process follows a more complicated pattern because 2,2-dibromo-1,3-indandione (XLVIII) or 2-bromo-1,3-indandione (XLVII) may participate.

Additional evidence which favours this explanation is that 2-aminopyridine also reacts with 2,2-dibromo-1,3-indandione (XLVIII) and 2-bromo-1,3-indandione (XLVII), giving a polymeric material. The chemical character of the hydrogen atoms of the amino and methyl groups in 2 position of pyridine nucleus, are qualitatively similar because protons can be displaced from both groups by an electrophilic attack. It is pointed out that polymerization proceeds more rapidly in the case of 2-aminopyridine, probably because the hydrogen atoms of the amino group are more mobile than those of the corresponding methyl group.

In another experiment, the amino group of 2-aminopyridine was protected by benzoylation, and the benzoyl derivative allowed to react with 2,2-dibromo-1,3-indandione (XLVIII). No reaction between the reactants occurred because of the steric hindrance of the bulky dibenzoylamino group. However, 2,2-dibromo-1,3-indandione (XLVIII) underwent self-condensation giving tri-o-benzoylene-benzene (LXX).

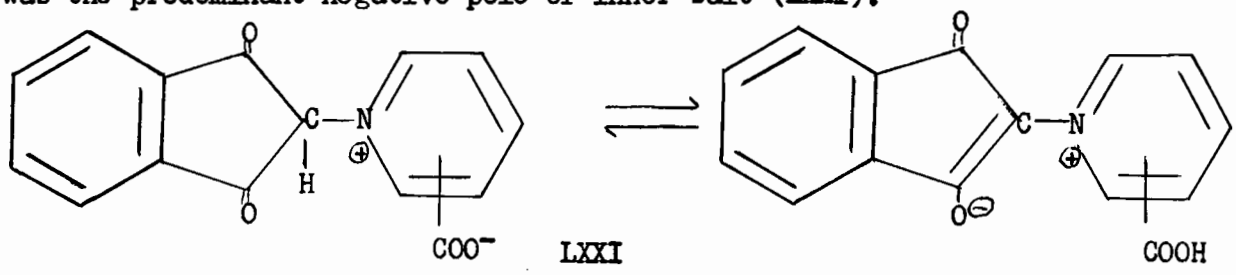


LXX

Reaction of 2,2-dibromo-1,3-indandione with pyridinemonocarboxylic acids

The fact that 2-, and 4-methylpyridine exhibited a higher reactivity towards 2,2-dibromo-1,3-indandione (XLVIII) than unsubstituted pyridine, was explained previously in terms of a higher electron density around the annular nitrogen atom. This increased electron density strengthened the nucleophilic character of the organic base.

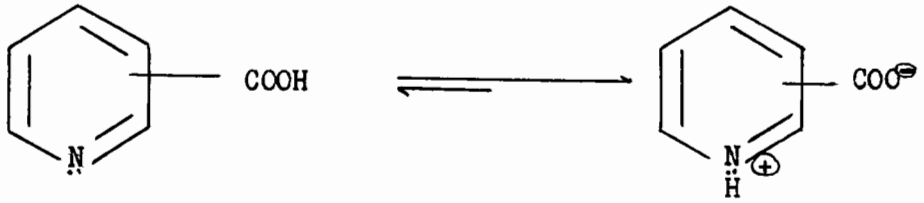
Although this explanation seemed plausible, it was felt that it should be supported by confirmatory experimental evidence. Consequently the reaction of 2-, 3-, and 4-pyridinecarboxylic acids with 2,2-dibromo-1, 3-indandione (XLVIII) was studied. These reactions were of interest because the electron attracting carboxylic group was attached to the pyridine nucleus, and this functional group could be considered as a potential negative centre in the betaine which might be formed during the reaction. It was expected that this study would reveal whether the carboxylic or enolized keto group was the predominant negative pole of inner salt (LXXI).



LXXI

The measurement of the dissociation constants of pyridinemonocar-

boxylic acids by ultraviolet spectrophotometry (162,163,164,165,166) provided the information that these acids exist in a tautomeric equilibrium, in which the zwitterion form is predominant.



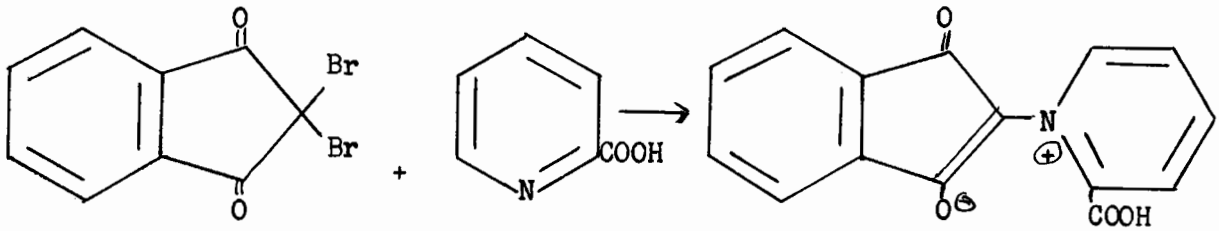
LXXII LXXIII

The tautomeric form (LXXII) may be considered as a nucleophilic reagent, because the annular nitrogen atom possesses an unshared electron pair, while the nitrogen in the zwitterion tautomeric form (LXXIII) shares an electron pair with the proton which has been released from the carboxylic group.

The electron attracting property of the carboxylic group and the fact that the tautomeric form (LXXII) existed to a much less extent than (LXXIII), led to the conclusion that the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with pyridinemonocarboxylic acids would proceed with difficulty. This assumption, based on theoretical considerations, was confirmed by experimental evidence. In order for the reaction to take place, drastic conditions of time and temperature had to be applied. Furthermore reaction occurred only in the presence of a large excess of the pyridinecarboxylic acid, because such a small proportion of this substance existed in the reacting tautomeric form (LXXII). On cooling the reaction mixture, the golden coloured product precipitated together with the unreacted excess of pyridinecarboxylic acid. It is pointed out that isoamyl alcohol was used as the solvent for the reaction when 3-, and 4-pyridinecarboxylic acids were the reactants. The unreacted excess of acid was removed by extraction with boiling water or more effectively by treatment with strong

mineral acids. In the strongly acidic medium the reaction product remained insoluble, but the amphoteric pyridinecarboxylic acid passed into solution and was removed. Further extraction with chloroform removed any remaining unreacted 2,2-dibromo-1,3-indandione (XLVIII).

2-Pyridinecarboxylic acid (LXXIV) reacted with 2,2-dibromo-1,3-indandione (XLVIII) in boiling n-butyl alcohol. In this case, a poor yield of product was obtained and the reaction was accompanied by fairly extensive polymerization. The low yield of 2-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (LXXV), was explained to some extent by steric hindrance caused by the carboxylic group in 2 position, but mainly by the very weak basic character of 2-pyridinecarboxylic acid (LXXIV). The extremely weak basic properties of this particular acid decreased, to a



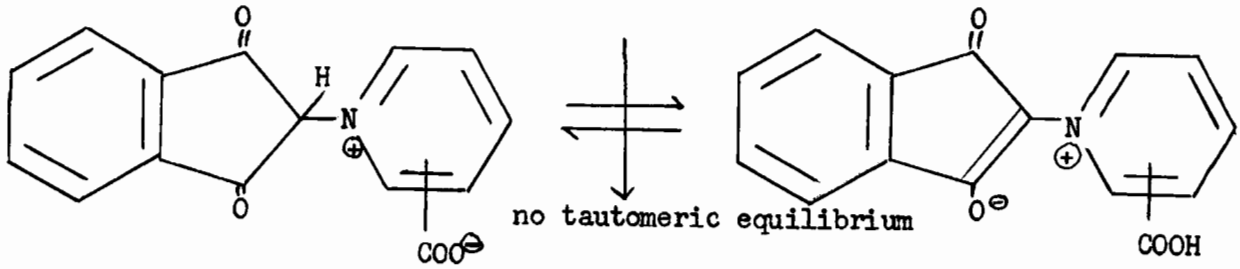
XLVIII LXXIV LXXV

great extent its nucleophilic nature and made the elimination of the bromine atoms from 2,2-dibromo-1,3-indandione (XLVIII) difficult.

The reaction products of 2-, 3- and 4-pyridinecarboxylic acids with 2,2-dibromo-1,3-indandione (XLVIII) were bright yellow crystalline compounds, high melting and bromine-free. The mechanism by which these reactions proceeded was considered to be similar to that postulated for the reactions of pyridine and methylpyridines with 2,2-dibromo-1,3-indandione (XLVIII) since the products obtained in all cases were similar.

Nevertheless there appeared to be some uncertainty about the betaines obtained from the reaction of 2,2-dibromo-1,3-indandione (XLVIII)

with pyridinecarboxylic acids, particularly concerning the negative centre of these zwitterions. As previously mentioned it was possible to assign one, or both tautomeric structures (LXXI) in an equilibrium, to these particular betaines. The similarity in the patterns of the electronic spectra of these compounds, and the inner salts obtained from the reaction of 2,2-dibromo-1,3-indandione (XLVIII) with pyridine, or the methylpyridines, revealed that the betaines (LXXI) existed in the form in which the oxygen atom of one of the keto groups is mainly the negative centre of the dipole (ii) and not in the form in which the carboxylic group is the anionic function (i).

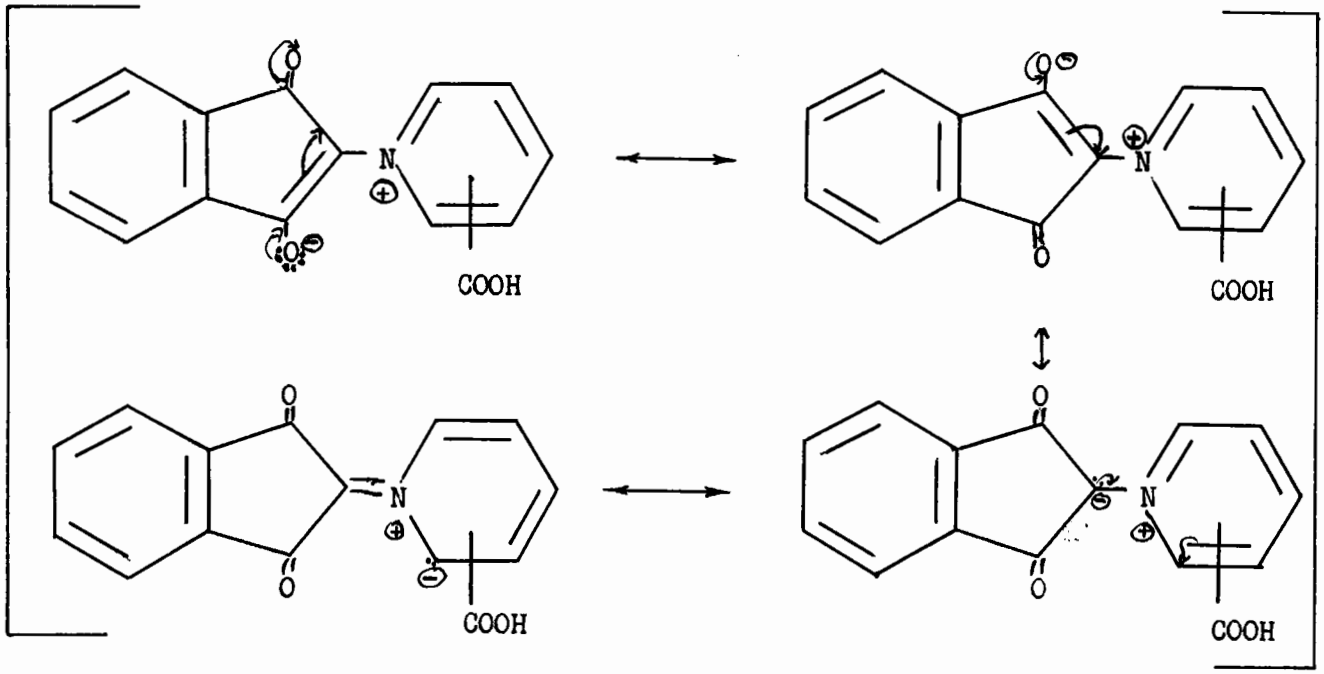


i LXXI ii

This is confirmed by the deep colour of the betaines (LXXI) which is due to the existence of resonance. The intensity of the colour can hardly be explained by the tautomeric form (i) because the group $>CH-N^{\oplus}<$ acts as barrier preventing the free shift of electrons throughout the whole molecule. Thus, since the limited resonance in (i) is not in agreement with the deep colour of the betaines (LXXI), the tautomeric form (ii) must be accepted as correct. It is emphasized, however, that the structural formula (ii) is only one of the numerous resonating structures which can be assigned to the resonance hybrid of (LXXI).

Degradation of the pyridinium betaines of the 1,3-indandione series

The inner salts obtained from the reaction of either methylpyridines or pyridinemonocarboxylic acids with 2,2-dibromo-1,3-indandione (XLVIII) were



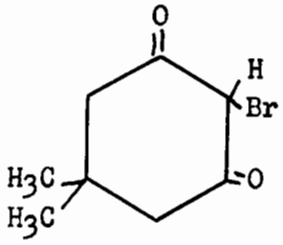
sensitive to light, and darkened when exposed to sunlight. Therefore all of these compounds were kept in the dark and samples for analysis could be obtained only after many recrystallizations. This difficulty in obtaining reliable analysis of the pyridinium enol betaines had been reported in several cases (91,97).

This series of compounds was very stable toward alkalies, and alkaline hydrogen peroxide. Only potassium permanganate at elevated temperatures caused degradation, and the main product of this degradation was phthalic acid which appeared to come from the indane part of the molecule.

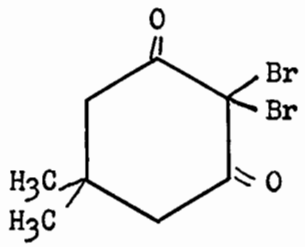
Attempted reaction of pyridine bases with 2-bromo-5,5-dimethyl-1,3-cyclohexanedione and 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione

An effort was made to determine whether the reaction between pyridine bases and 2-bromo-1,3-indandione (XLVII) and 2,2-dibromo-1,3-indandione (XLVIII) was typical of a general reaction between pyridine bases and all β-bromo- and β,β,-dibromo- α,γ-diketo compounds.

In this connection the reaction of 2-bromo-5,5-dimethyl-1,3-cyclohexanedione (LXXVI) (167) with pyridine was attempted. In all attempts both reactants were recovered quantitatively. This is explained by the strong electropositive character of the bromine which liberated iodine from an aqueous solution of potassium iodide with ease. In these compounds in which it was strongly positive, the halogen apparently could not split off as a negatively charged entity which subsequently would be the anion of the pyridinium salt formed (155).



LXXVI



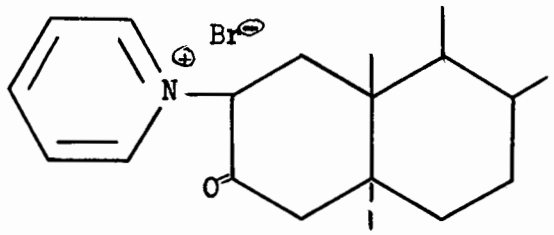
LXXVII

However, it should be pointed out that in the case of 2-bromo-1,3-indandione (XLVII) such a reaction did take place. In this instance, the electropositive character of the bromine atom of 2-bromo-1,3-indandione (XLVII) was not very strong and consequently the possibility existed that it could be released as an anion by a different process. 2,2-Dibromo-5,5-dimethyl-1,3-cyclohexanedione (LXXVII) (168) was allowed to react with pyridine, methylpyridines and pyridinecarboxylic acids. It was evident that reactions occurred because the solutions became darker and darker in colour. When the solvent had been evaporated the substances obtained exhibited the typical characteristics of pyridinium enol betaines. They were insoluble in water and ether, but soluble in alcohols. In all cases, they tended to separate as oils, and all attempts to crystallize and purify these oils were unsuccessful.

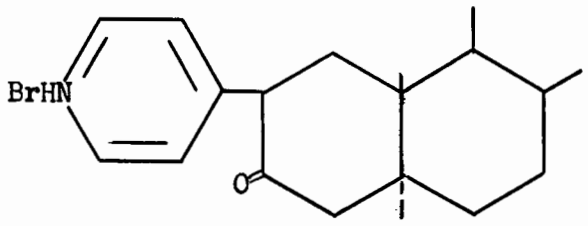
B. Reaction of α-bromo-ketosteroids with pyridine bases

Reaction of 2α -bromo-3-cholestanone with pyridine

As mentioned in the historical section of this presentation two entirely different structural formulae (XXXIX) and (XLI) were proposed by Ruzicka (125) and Inhoffen (132) respectively, for the product of the reaction between 2α -bromo-3-cholestanone (XXXIV) and pyridine. Consequently the present work was undertaken in an effort to establish which



XXXIX



XLI

formula was correct, and in agreement with the chemical character of the addition compound obtained from this particular reaction.

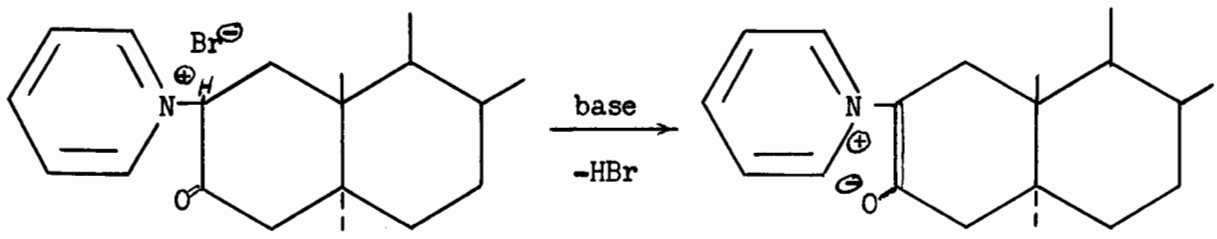
From a consideration of the proposed structural formulae (XXXIX) and (XLI), it appears that if hydrogen bromide is eliminated from this molecule, the product obtained will depend on which one of these structures represents the true character of the molecule. If the formula proposed by Ruzicka (XXXIX), is correct, then the elimination of one molecule of hydrogen bromide should result in the formation of a pyridinium inner salt of the enol betaine type. On the other hand, elimination of hydrogen bromide from a molecule with the structural formula proposed by Inhoffen (XLI), should produce a 4-substituted pyridine.

With this in mind, it was decided to attempt to isolate and identify the substance remaining after the elimination of a hydrogen bromide molecule from the reaction product of pyridine with 2α -bromo-3-cholestanone.

In order to achieve this, the reaction product was dissolved in chloroform, and treated with solid anhydrous potassium carbonate in a nitrogen atmosphere. The chloroform solution became orange-yellow in colour and later wine-red. The inorganic salts were separated by filtration, and the chloroform removed by blowing nitrogen into the filtrate. The deep red oil which remained could not be crystallized by any method and exhibited a pronounced tendency to form thin films. In addition, it was sensitive to heat and photodecomposed gradually. Qualitative elementary analysis of the oil indicated the presence of nitrogen but not of bromine. Upon treatment with hydrogen bromide the product afforded the starting material which was the addition product of the reaction of pyridine with 2α -bromo-3-cholestanone (XXXIV). The corresponding colourless perchlorate salt was formed readily when the oil was treated with an aqueous solution of perchloric acid.

These experimental facts supported the assumption that the reaction product of pyridine and 2α -bromo-3-cholestanone (XXXIV) was a typical pyridinium salt (XXXIX) which formed the 1-(3-hydroxy-2-cholesten-2-yl) pyridinium hydroxide, betaine (LXXVIII) upon elimination of a molecule of hydrogen bromide. The betaine (LXXVIII) in this case was unstable and did not crystallize.

In other attempts to obtain the betaine (LXXVIII), 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX) was suspended in water or ethanol and



XXXIX LXXVIII

an aqueous solution of base added to the suspension in excess. Various bases were used, such as potassium carbonate, potassium hydroxide, sodium carbonate, sodium hydroxide, guanidine carbonate, ammonia, to bring about the displacement of a hydrogen bromide molecule from 1-(3-oxo-2-cholestanyl) pyridinium bromide (XXXIX). In all cases, tests for the presence of bromine anion indicated that the elimination of hydrogen bromide occurred readily. The product (LXXVIII) formed, separated as oil and possessed a very pronounced tendency to form films. The latter could be understood since the molecule of steroids of the allo series, being almost planar and elongated (169,170) favours the formation of thin films.

The organic liquid bases, piperidine and morpholine also were used to bring about the elimination of hydrogen bromide from 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX). In this case, the pyridinium salt (XXXIX) was dissolved in the organic base, and the colour changed immediately. Even at 0° the elimination of hydrogen bromide from 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX) proceeded rapidly, and was followed by the precipitation of piperidine or morpholine hydrobromide. The latter precipitated almost quantitatively, because of their insolubility in the organic bases. By collecting, drying and weighing these salts, it was shown that the elimination of hydrogen bromide from 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX) was complete, because the calculated amount of hydrogen bromide which could be eliminated from the pyridinium salt (XXXIX), was recovered quantitatively in the form of piperidine or morpholine hydrobromide. After evaporation of the piperidine or morpholine from the filtrate, a dark red oil remained, which did not crystallize on standing or by triturating with drops of methanol.

Finally, the 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide,

betaine (LXXVIII) was obtained in crystalline form on dehydrobromination of 1-(3-oxo-2-cholestanyl)pyridinium bromide (XXXIX) in a three component system consisting of ethanol, water and dimethylamine. 1-(3-Oxo-2-cholestanyl)pyridinium bromide (XXXIX) was suspended in a very small amount of ethanol, and the mixture heated to boiling. An aqueous solution of dimethylamine was added dropwise into the reaction mixture, until the solution became turbid. From this solution, fine yellow needles of the 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine (LXXVIII)deposited on cooling. It was obvious that dehydrobromination was brought about, in this case, by the dimethylamine, which had a basic character and appeared to alter the solvent properties of the water and alcohol so that crystallization occurred in the three component system. Subsequent purification of the 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine (LXXVIII) was achieved by recrystallization from the same three component system. Although the betaine was yellow when it precipitated, it became red when thoroughly dried. It was very sensitive to light and decomposed slowly in air. However by placing the pyridinium betaine (LXXVIII) in a vacuum desiccator over phosphorus pentoxide in the dark, it was possible to keep the material practically unchanged for several weeks. Difficulty was encountered in the preparation of an analytical sample due to the sensitivity of the compound. The sample could be dried in an Abderhalden pistol at the temperature of boiling ether, although it decomposed completely if dried at the temperature of boiling ethanol.

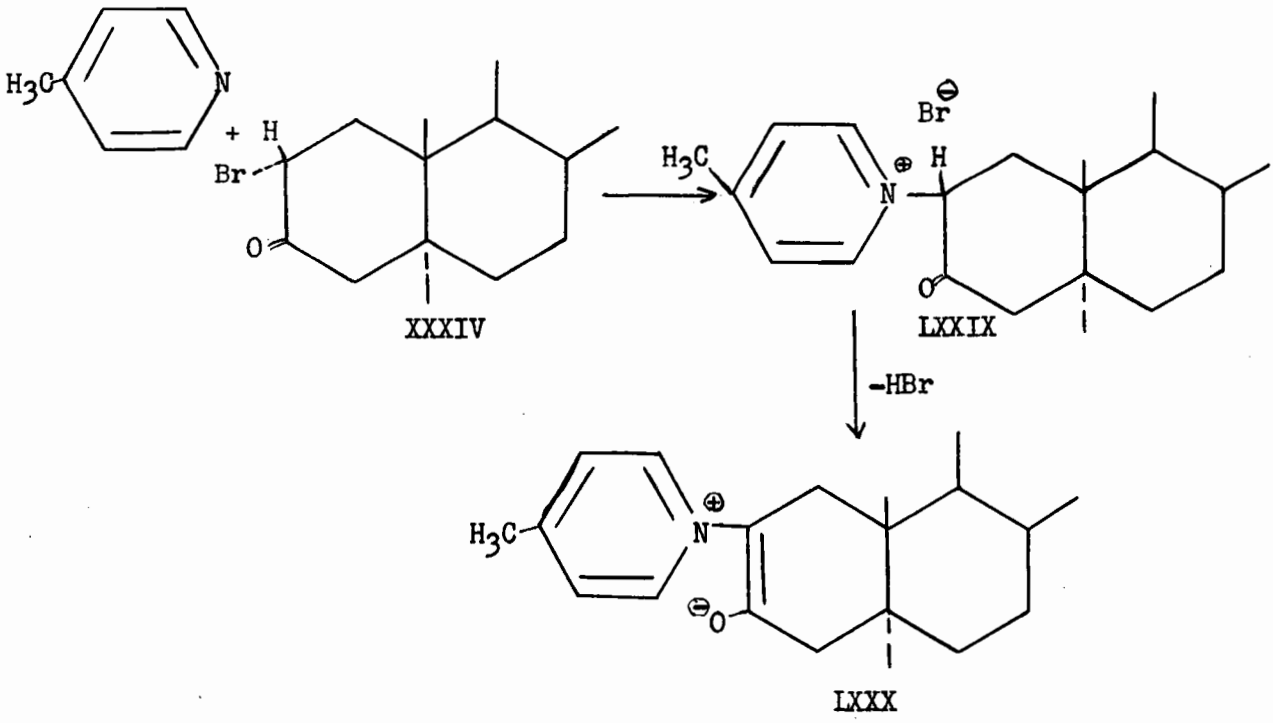
Determination of the electronic absorption spectrum of the 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine (LXXVIII) provided further proof that this compound was a typical pyridinium betaine. Consequently the addition product of the reaction of pyridine with 2α -bromo-3-

cholestanone (XXXIV) was a typical pyridinium salt, 1-(3-oxo-2-cholestanyl) pyridinium bromide (XXXIX).

Reaction of 2 α -bromo-3-cholestanone with 4-methylpyridine

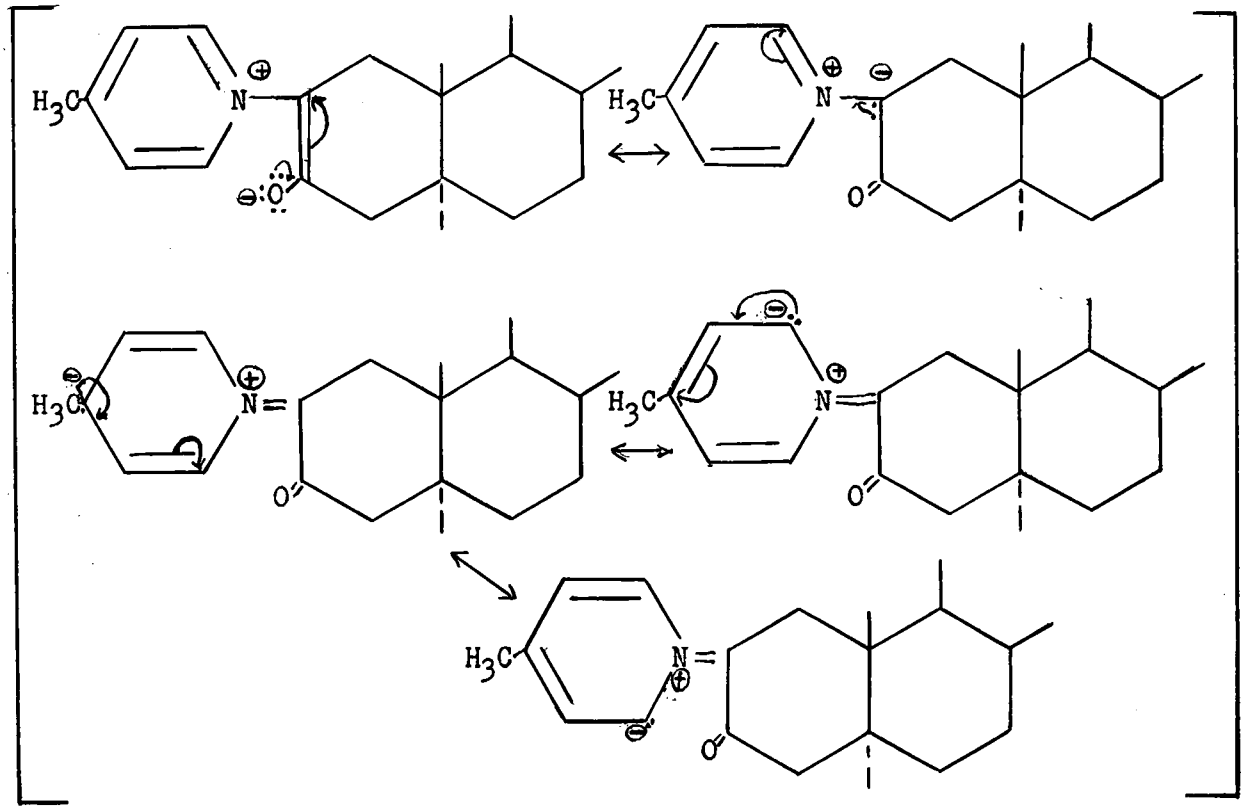
On the basis of the assumptions made by Inhoffen (132), the addition product of the reaction between pyridine bases and 2α -bromo-3-cholestanone (XXXIV) should be a 4-substituted pyridine hydrobromide. If this is correct, then it can be argued that 4-methylpyridine should not react with 2α -bromo-3-cholestanone (XXXIV), because the presence of a substituent in 4 position of the pyridine molecule should prevent the reaction.

However, it was found that the reaction of 4-methylpyridine with 2α -bromo-3-cholestanone (XXXIV) proceeded smoothly and gave the salt 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide (LXXIX). The latter was added to a suspension of anhydrous potassium carbonate in chloroform with stirring. A rapid change in the colour of the reaction mixture, from



colourless to dark red occurred. This change in colour was attributed to a dehydrobromination of 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide followed by the formation of the 1-(3-hydroxy-2-cholesten-2-yl) 4-methylpyridinium hydroxide, betaine (LXXX) which was coloured.

It is pointed out that the structural formula (LXXX) given for the 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine, represents only one of the many resonating structures which contribute to the resonance hybrid of the molecule. The colour of this betaine is an indication of the electronic excitement within the molecule. All of the resonating structures which can be postulated are typical zwitterions because resonating structures without a separation of electrical charges within the molecule (LXXX) are impossible. Similar resonating structures can contribute to the resonance hybrid of a molecule of any inner salt of this particular series. Inner salts of this type can be considered as



LXXX

products of the dehydrobromination of pyridinium salts obtained from the reaction of various pyridine bases with certain α-halo ketosteroids.

The solubility of the 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) in chloroform facilitated its separation from both the unreacted potassium carbonate, and the potassium bromide which was formed during the dehydrobromination process. The latter salts were practically insoluble in cold chloroform and were removed easily by filtration. The filtrate containing the inner salt (LXXX) was evaporated to dryness by blowing dry nitrogen into solution. It was necessary to avoid contact between the 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) and the oxygen of the air, since the latter could readily cause an oxidative degradation or polymerization. The substance remaining after all the chloroform had been removed was an amorphous dark red solid. All attempts to crystallize this substance (LXXX) yielded only oils or films.

The 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) is very reactive and sensitive to light and acids. In sunlight it gradually decomposes, and on treatment with acids, colourless solids are obtained which are obviously the corresponding pyridinium salts. Elementary analysis and the electronic absorption spectrum of 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) are in complete agreement with the assignment of an inner salt structure to this compound.

In contrast with many other compounds of a similar enol betaine type, 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) fails to react with chloranil in chloroform. This failure can be explained by the lack of a mobile hydrogen atom attached to the carbon atom

in 2 position of the steroidal skeleton. This carbon atom is the one directly attached to the annular nitrogen of the 4-methylpyridine molecule. The lack of reactivity shown by 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX) and all inner salts of the steroidal pyridinium betaines series confirms the mechanism proposed by Krönhke and Smeiss (84) for this type of reaction.

Reaction of 2 α -bromo-3-cholestanone and 3-methylpyridine

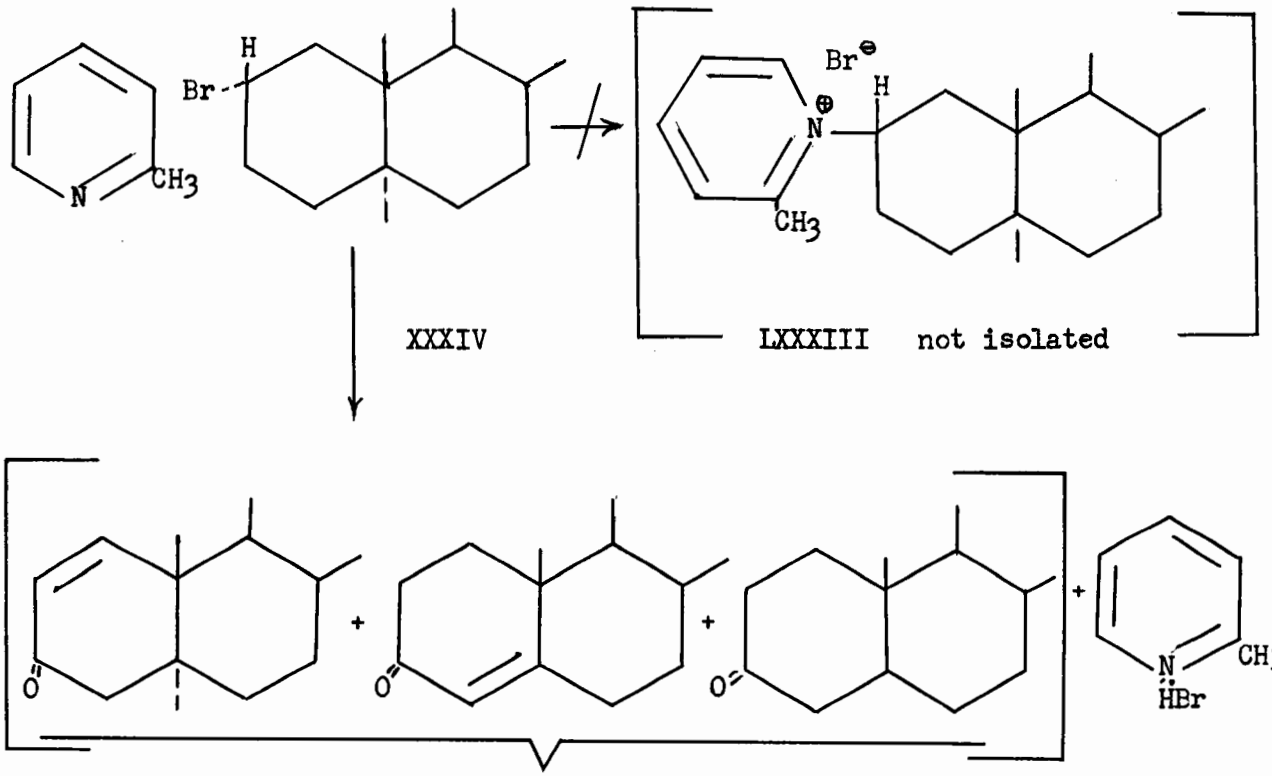
The product 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide (LXXXI) was obtained from the reaction of 2 α -bromo-3-cholestanone (XXXIV) with 3-methylpyridine. The 2 α -bromo-3-cholestanone (XXXIV) was dissolved in a large excess of 3-methylpyridine which apparently served both as a reactant and solvent. The solution, on refluxing, deposited the 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide (LXXXI). It was observed that traces of water did not prevent the reaction between 2 α -bromo-3-cholestanone (XXXIV) and 3-methylpyridine or other pyridine bases, but decreased the yield of the pyridinium salt formed. For this reason, both reactants were dried thoroughly, and the reaction was carried out in a nitrogen atmosphere.

When 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide (LXXXI) was treated with anhydrous potassium carbonate in chloroform, an immediate dehydrobromination of (LXXXI) and formation of the corresponding inner salt 1-(3-hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine (LXXXII) occurred. A rapid change in the colour of the chloroform solution accompanied these processes. The separation of 1-(3-hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine (LXXXII) was accomplished in a way similar to that previously discussed in the case of 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (LXXX). The inner

salt (LXXXII) was obtained as a dark red powder which could not be crystallized, but was purified by dissolving in chloroform, filtering, and evaporating to dryness in a nitrogen atmosphere several times.

Reaction of 2α -bromo-3-cholestanone with 2-methylpyridine

The reaction of 2α -bromo-3-cholestanone (XXXIV) with 2-methylpyridine did not afford the corresponding salt 1-(3-oxo-2-cholestanyl)2-methylpyridinium bromide (LXXXIII). Instead 2-methylpyridine hydrobromide was isolated from the reaction mixture. The formation of the latter compound indicated that dehydrobromination of 2α -bromo-3-cholestanone (XXXIV) had taken place.



LXXXIV

It appeared that the elimination of one molecule of hydrogen bromide from 2α -bromo-3-cholestanone (XXXIV) could be explained by two mechanisms. Either the dehydrobromination proceeded directly, or through

the formation of the intermediate salt, 1-(3-oxo-2-cholestanyl)2-methylpyridinium bromide (LXXXIII). By analogy with the previously discussed cases, in which 2α -bromo-3-cholestanone (XXXIV) was allowed to react with pyridine, 3-, and 4-methylpyridines, the formation of the salt (LXXXIII) in this case seemed improbable since it should interfere with the elimination of hydrogen bromide.

This assumption was confirmed experimentally. Samples were taken from the reaction mixture at different times and treated with alkali. No change in colour was observed. If 1-(3-oxo-2-cholestanyl)2-methylpyridinium bromide (LXXXIII) was present in the reaction mixture, it should undergo a dehydrobromination and form the corresponding inner salt. This process would be accompanied by a change in colour.

It was presumed that dehydrobromination of 2α -bromo-3-cholestanone (XXXIV) yielded a mixture of compounds (LXXXIV) similar to those isolated by Jacobsen (128) from the reaction of 2α -bromo-3-cholestanone (XXXIV) with 2,4,6-trimethylpyridine. The separation and identification of the components of the mixture (LXXXIV) was felt to be beyond the scope of the present work.

The formation of 2-methylpyridine hydrobromide during the reaction reveals that 2-methylpyridine reacts in a different way from 3-, and 4-methylpyridines with 2α -bromo-3-cholestanone (XXXIV). 2,6 Dimethylpyridine reacts with 2α -bromo-3-cholestanone (XXXIV) to form the corresponding addition compound (132) while 2,4-dimethylpyridine reacts in a manner similar to 2-methylpyridine, yielding a mixture of dehydrobromination products (LXXXIV) of 2α -bromo-3-cholestanone (XXXIV). No analogous cases could be found in the literature on which to base an explanation for these experimental results. Therefore the following interpretation is offered.

An electron repelling methyl group (- Is effect) attached to the pyridine nucleus increases the electron density around the annular nitrogen and strengthens the nucleophilic nature of the molecule. The latter is reflected by the relative basicity of the substance and consequently may be determined experimentally. Measurements of the K_A values for 2-, 3- and 4-methylpyridine (171,172) show clearly that the increase in nucleophilic character brought about by the presence of a methyl group attached to the pyridine ring, is less in the case of 2-methylpyridine than in 3- or 4-methylpyridine. This fact may be explained in the following way. The electron repelling methyl group, in 2 position of the pyridine nucleus, is in the immediate vicinity of the annular nitrogen atom which already has a high electron density. Consequently the electron repulsion is decreased, with the result that the nucleophilic character of 2-methylpyridine is lower than that of 3-, or 4-methylpyridine. The proximity of the methyl group to the annular nitrogen atom in 2-methylpyridine is considered to be responsible for the fact that elimination of hydrogen bromide occurs during the reaction with 2α -bromo-3-cholestanone (XXXIV) instead of formation of an addition compound.

It is assumed that the methyl group in 2 position probably increases the steric hindrance in the activation complex of the reaction which leads to the formation of the addition salt and consequently this reaction is inhibited. In contrast, this methyl group decreases the steric hindrance in the activation complex of the reaction in which hydrogen halide is eliminated. In other words, when 2-methylpyridine reacts with 2α -bromo-3-cholestanone (XXXIV) there is steric retardation of the formation of an addition product and steric acceleration of the elimination of hydrogen bromide. These assumptions are in agreement with those reported (173,174)

in certain elimination reactions in the aliphatic series.

The stronger basic character of 3-, and 4-methylpyridines in comparison with 2-methylpyridine, indicates that the former are more nucleophilic in nature than the latter. In addition, since the methyl groups are situated further away from the annular nitrogen in these molecules, no steric effect is present. The absence of a steric factor allows the reaction with 2α-bromo-3-cholestanone (XXXIV) to proceed in such a way that an addition salt is formed.

In the case of 2,6-dimethylpyridine, both the steric factor and the nucleophilic character of the molecule affect its reaction with 2α-bromo-3-cholestanone (XXXIV). As a result, an addition salt is formed. Measurements of K_A (171,172) show that the -Is effects of the two methyl groups are additive. Because these two methyl groups are situated close to the annular nitrogen, the steric effect is also greatly increased. It seems probable that this effect is increased sufficiently to cause a steric retardation of the elimination of hydrogen bromide and a decrease of the steric hindrance in the activated complex of the reaction in which the addition product is formed, when 2,6-dimethylpyridine is allowed to react with 2α-bromo-3-cholestanone. This assumption is in accord with the fact that although 2,6-dimethylpyridine is more basic than other pyridine bases it does not react with the Lewis acid trimethylboron (175).

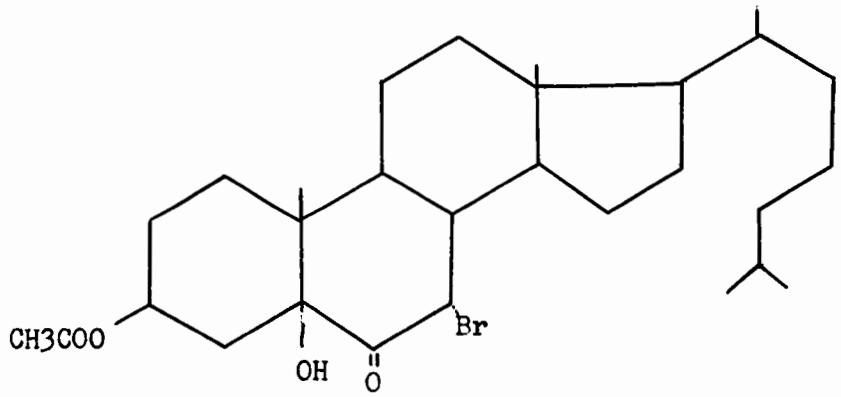
The same arguments which were used to explain the reaction between 2-methylpyridine and 2α-bromo-3-cholestanone (XXXIV) can be applied in the case of 2,4-dimethylpyridine and the latter compound (XXXIV). It is obvious that although the nucleophilic character of 2,4-dimethylpyridine is considerably stronger than that of 2-methylpyridine, the steric effect in both these molecules is similar. Consequently there is steric retardation

of the reaction leading to the formation of the addition product and steric acceleration of the elimination reaction.

Reaction of 7α -bromocholestane-3β ,5α -diol-6-one 3-acetate with pyridine

Fieser and Rajagopalan (176) reported the synthesis of 7α -bromocholestane-3β ,5α -diol-6-one 3-acetate (LXXXV), and assigned the β orientation to this particular bromine atom. The β orientation was chosen mainly because of the difficulty encountered in dehydrobrominating this compound (LXXXV) with basic reagents.

More recent work by Corey (177) has shown unequivocally that the bromine atom in 7 position of any 7-bromo-6-keto-steroid of the allo series is always α-oriented. The conformation of this α oriented bromine atom is axial. On the other hand, the conformation of the bromine atom of 2α -bromo-3-cholestanone (XXXIV) is known to be equatorial.

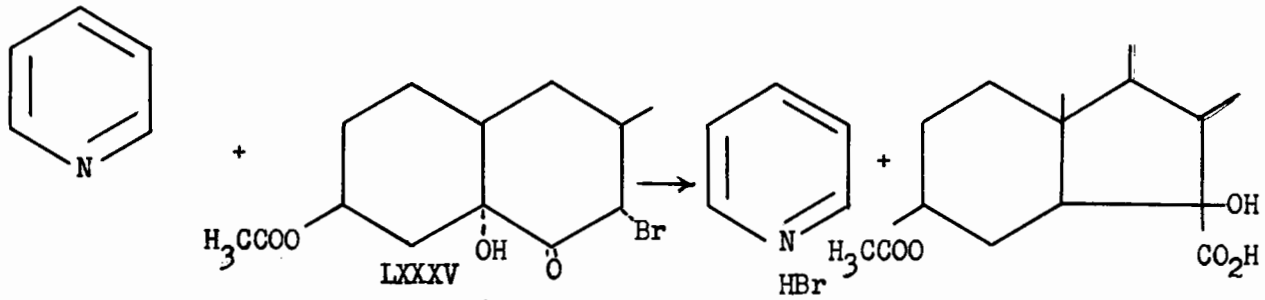


LXXXV

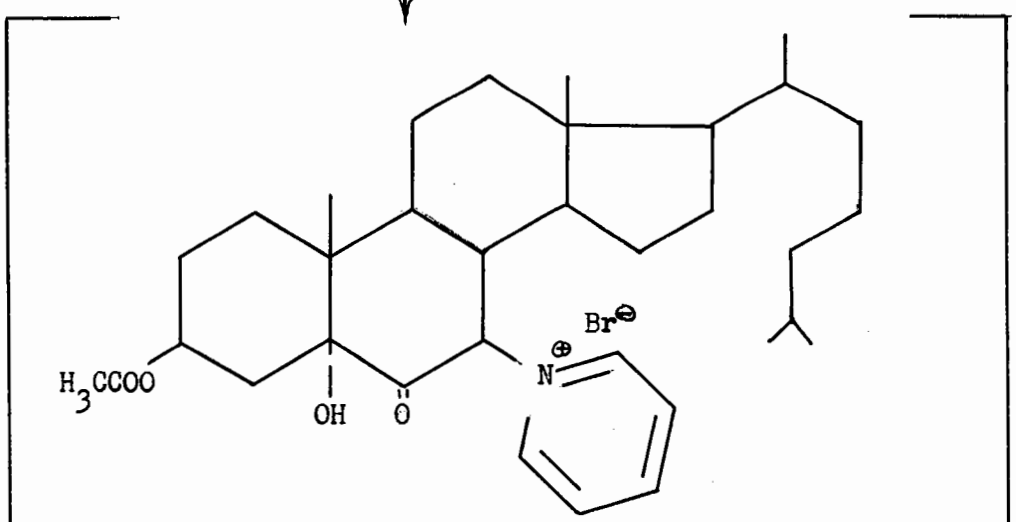
The difference in the conformation of the bromine atoms in 7α -bromocholestane-3β ,5α -diol-6-one 3-acetate (LXXXV) and 2α -bromo-3-cholestanone (XXXIV), provided the stimulus for an effort to determine whether the conformation of the bromine atom had any effect on the way in which the reaction of these two components with pyridine proceeded.

7α -Bromocholestane-3β ,5α -diol-6-one 3-acetate (LXXXV) resisted dehydrobromination. It was only after a very lengthy refluxing of (LXXXV) in pyridine, that a small amount of the bromine-free compound X was isolated. This compound had been obtained from the reaction mixture of 7α -bromocholestane-3β ,5α-diol-6-one 3-acetate (LXXXV) and triethylamine by Fieser and Rajagopalan previously (176).

During the lengthy reaction of pyridine with 7α -bromocholestane-3β ,5α -diol-6-one 3-acetate (LXXXV), small samples were taken out of the reaction mixture at different intervals, and treated with alkali. The absence of any change in colour indicated that no addition salt (LXXXVI) was being formed.



Fieser's compound X



LXXXVI not detected

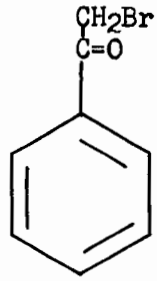
The assumption that (LXXXVI) was formed, but during treatment with alkali suffered an alkaline fission of the bond between the quaternary nitrogen atom and the carbon atom at the 7 position of the steroidal skeleton, was incorrect for the following reasons.

1. There was no change in colour even when the sample of the reaction mixture of 7α -bromocholestane-3β ,5α -diol-6-one 3-acetate (LXXXV) and pyridine was treated at low temperatures with mild reagents such as potassium carbonate, an aqueous solution of dimethylamine, or ammonia. These mild reagents could not cause a rapid alkaline fission of the salt (LXXXVI).

2. When the sample was treated with water, the excess pyridine and pyridine hydrobromide passed into solution. The steroidal compound, being insoluble in water, was collected and dried. It was found to contain no nitrogen.

C. Reaction of pyridinecarboyxlic acids with 2-bromo-acetophenone

Since no information could be found in the literature concerning the reaction of 2-bromo-acetophenone (LXXXVII) or its derivatives with pyridinecarboxylic acids it was felt that investigation of this reaction



LXXXVII

might prove interesting. Because the presence of a free carboxylic group in the molecule decreased the nucleophilic character of the pyridine nucleus, it was uncertain whether pyridinecarboxylic acids would react with 2-bromo-acetophenone (LXXXVII).

If the reaction between a pyridinecarboxylic acid and 2-bromo-

acetophenone (LXXXVII) proceeded in the ordinary way and a typical pyridinium salt was formed, then two possibilities existed for the formation of an inner salt from the pyridinium salt by elimination of a molecule of hydrogen bromide. This was so, because the proton involved in the elimination of hydrogen bromide could originate from either the carboxylic group or the enolized carbonyl group of the pyridinium salt. Thus, it was decided that an attempt should be made to determine which group released the proton. In doing this it was hoped that some information about the relative tendencies of the carboxylic and carbonyl groups to participate in the formation of pyridinium betaines would be obtained.

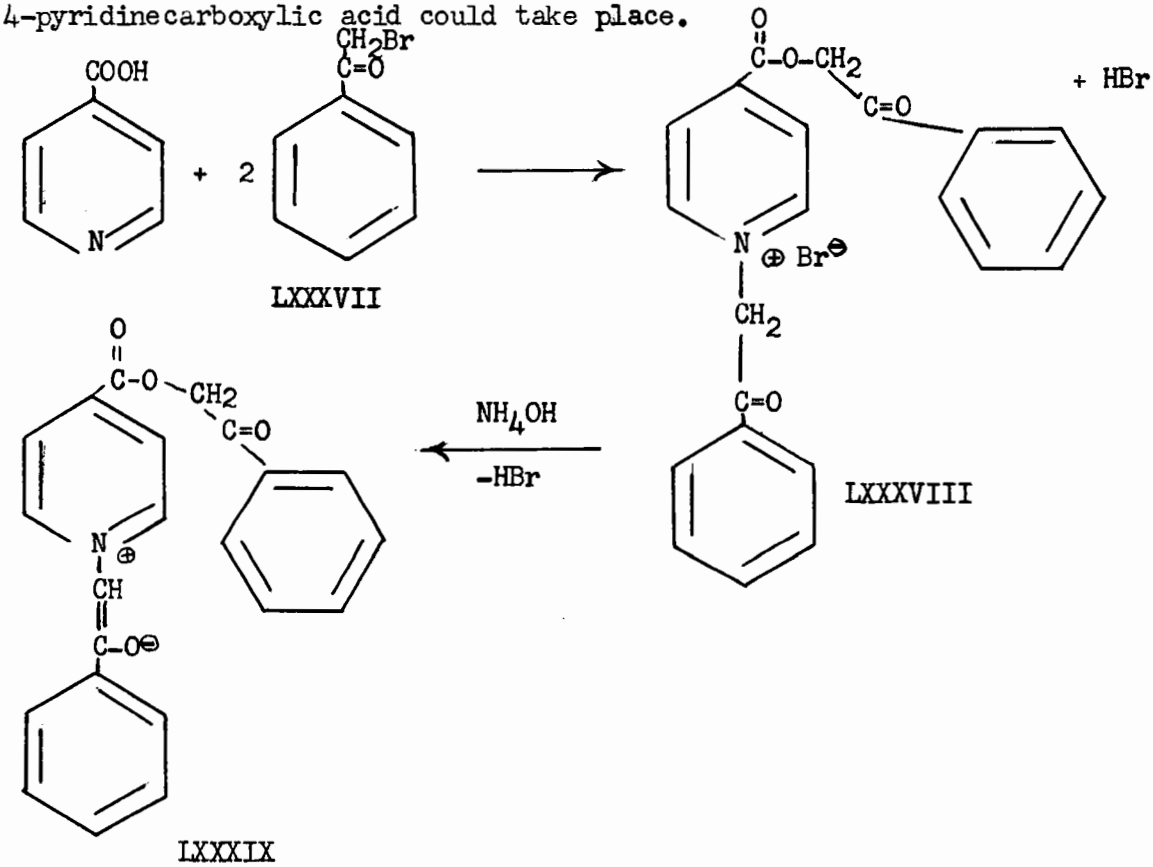
Reaction of 2-bromo-acetophenone with 4-pyridinecarboxylic acid

The reaction of 2-bromo-acetophenone (LXXXVII) with 4-pyridinecarboxylic acid was carried out in boiling ethanol over a long period of time. The product obtained, 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII), gave a strong positive test for bromine in contrast to the starting material, 2-bromo-acetophenone (LXXXVII), which did not. The reaction product (LXXXVIII) decomposed slowly in air with a change in colour from white to yellow. During this decomposition the characteristic odour of benzaldehyde was detected. On treatment with alkalies, hydrogen bromide was eliminated from 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) and a dark red intermediate product was formed.

The 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine (LXXXIX) was very unstable, and decomposed rapidly in the presence of strong alkalies. For this reason, aqueous ammonia at 0° was used as the reagent for the enolization, elimination of hydrogen bromide, and betainization of the salt (LXXXVIII). The pyridinium betaine (LXXXIX) was also very sensitive to light and heat. It was recrystallized from hot absolute ethanol as

quickly as possible because complete decomposition occurred upon prolonged heating.

Analysis of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) and the corresponding inner salt (LXXXIX) showed that 4-pyridinecarboxylic acid reacted with 2-bromo-acetophenone (LXXXVII) in a manner similar to that in which pyridinecarboxylic acids reacted with alkyl halides (178). In all these cases, esterification of the free carboxylic group proceeded simultaneously with quaternization of the annular nitrogen of the pyridine nucleus. Apparently, elimination of one molecule of hydrogen bromide was necessary before esterification of the carboxylic group of 4-pyridinecarboxylic acid could take place.



An examination of the structural formulae of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) and the corresponding betaine (LXXXIX) reveals that their sensitivity to alkalies and susceptibility to

alkaline cleavage arises from the presence of two highly reactive methylene or methine groups. The latter are located between the carbonyl group and the carboxylic function and between the carbonyl group and the quaternary nitrogen atom.

The products of the alkaline cleavage of the pyridinium betaine (LXXXIX), after acidification, were identified as benzoic acid and 4-pyridinecarboxylic acid. Both the phenacyl groups, originally present in the molecule of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII), yielded benzoic acid and carbon dioxide. One of these phenacyl groups was attached to the quaternary nitrogen atom of (LXXXVIII), while the other was connected to the carboxylic group in 4 position of the pyridine nucleus, through an ester grouping. In the latter instance, it is considered that the phenacyl group provides 2-hydroxy-acetophenone (XC), the alcoholic function of the ester.

The latter compound (XC) is produced by a saponification of the ester group at the same time as the alkaline fission of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) occurs. 2-Hydroxy-acetophenone (XC) is very sensitive toward alkali especially at elevated temperatures, and undergoes a spontaneous autoxidation to benzaldehyde and then to benzoic acid. This sequence of reactions explains the presence of benzaldehyde which was detected during the alkaline cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII).

The presence of 4-pyridinecarboxylic acid as a product of the alkaline fission of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) was detected by paper chromatography according to the procedure of Huebner (179).

The formation of 4-pyridinecarboxylic acid during the alkaline

cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) is unusual, because in most alkaline fission reactions involving 1-(phenacyl) pyridinium halides and their substituted derivatives, a product is formed in which a methyl group is still attached to the annular nitrogen atom (148). This unusual alkaline fission is attributed to the presence of the strong electron-attracting carbobenzoylmethoxy group, which obviously decreases the electron density around the annular nitrogen atom, and weakens the bond between the quaternary nitrogen and the carbon atom of the methylene group. The latter group is provided by the phenacyl group attached directly to the annular nitrogen atom. The alkaline cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII) is shown in Fig. 3.

Reaction of 3-pyridinecarboxylic acid with 2-bromo-acetophenone

The reaction of 3-pyridinecarboxylic acid with 2-bromo-acetophenone (LXXXVII) proceeded similarly to the reaction of 4-pyridinecarboxylic acid with (LXXXVII) and yielded the corresponding salt, 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (XCI).

This pyridinium salt (XCI) was more sensitive to alkalies than the salt (LXXXVIII) obtained from the reaction of 4-pyridinecarboxylic acid and 2-bromo-acetophenone (LXXXVII), and underwent alkaline cleavage rapidly even at low temperatures. The unstable yellow intermediate 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine which was produced had a tendency to form hydrates. The betaine decomposed very easily giving the cleavage products 3-pyridinecarboxylic acid and benzoic acid which were similar to those obtained from the alkaline fission of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (LXXXVIII). It is pointed out that the formation of hydrates is common with enol pyridinium betaines and has been reported on numerous occasions, (83,91).

FIGURE 2

Schematic representation of the polymerization occurring during the reaction of 2,2-dibromo-1,3-indandione and pyridine

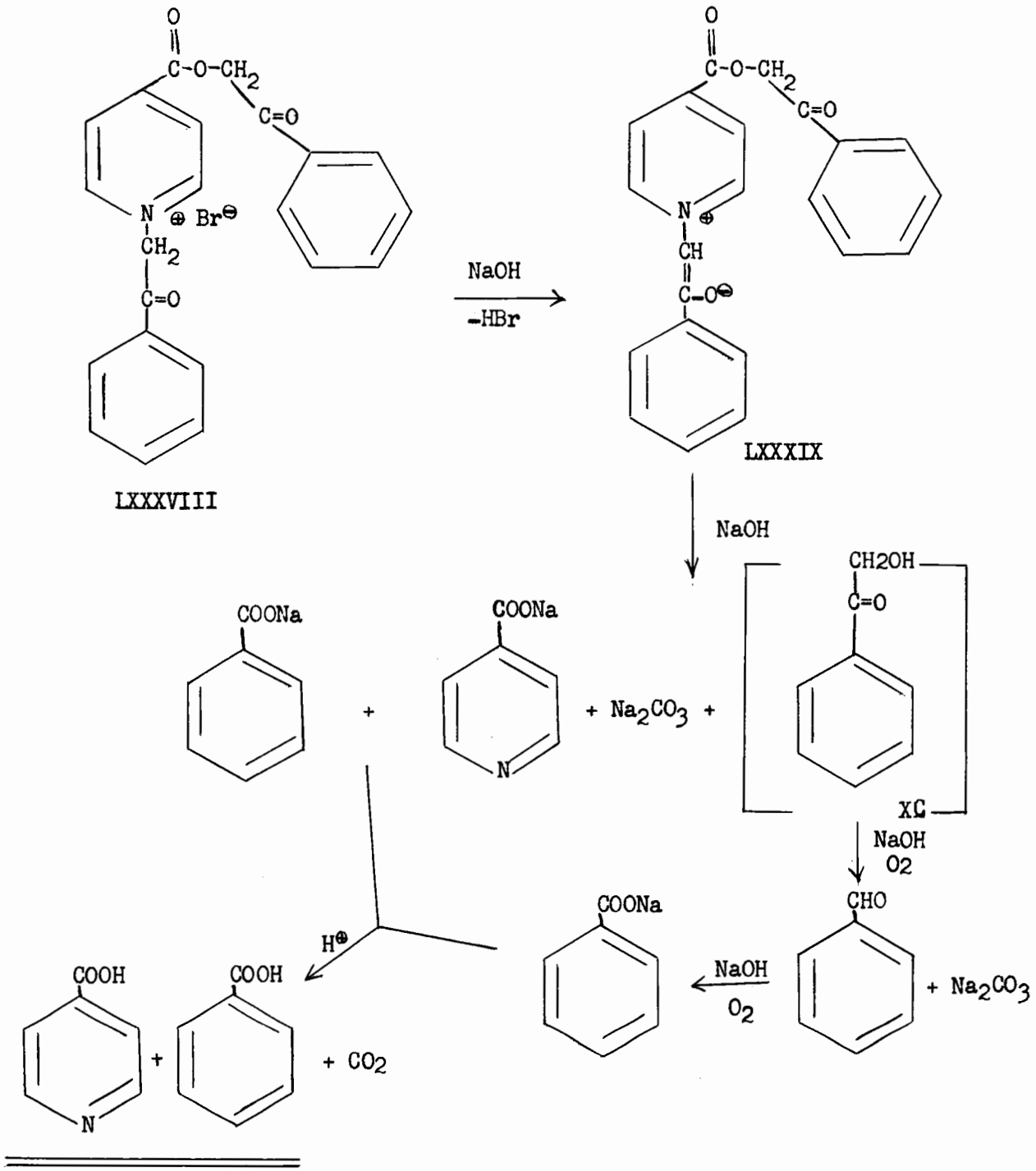


FIG. 2.

Schematic representation of the alkaline cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide

Many basic substances were used to eliminate hydrogen bromide from, and bring about the betainization of, 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (XCI). The best results were obtained when guanidine carbonate was used in this capacity.

The reactions of 3-, and 4-pyridinecarboxylic acids with 2-bromoacetophenone (LXXXVII), and subsequent betainizations, showed that there was no preferential elimination of a proton from the carboxylic groups of the pyridinium salts formed. This was due to the fact that during the quaternization of the annular nitrogens, esterification of the carboxylic groups occurred. Since the carboxylic groups were blocked in this way, the products of the reactions were typical pyridinium enol betaines.

Reaction of 2,4'-dibromo-acetophenone with methyl 4-pyridinecarboxylate

This particular reaction was studied in an effort to establish whether the reaction of α-halo ketones with esters of pyridinecarboxylic acids proceeded by a mechanism similar to that followed by the reaction of α-halo-ketones with pyridine bases.

It could be predicted from theoretical considerations that the carbomethoxy group in 4 position of methyl 4-pyridinecarboxylate (XCIII), being electron attracting in nature, would decrease, to a certain extent, the electron density around the annular nitrogen and the nucleophilic character of the pyridine nucleus.

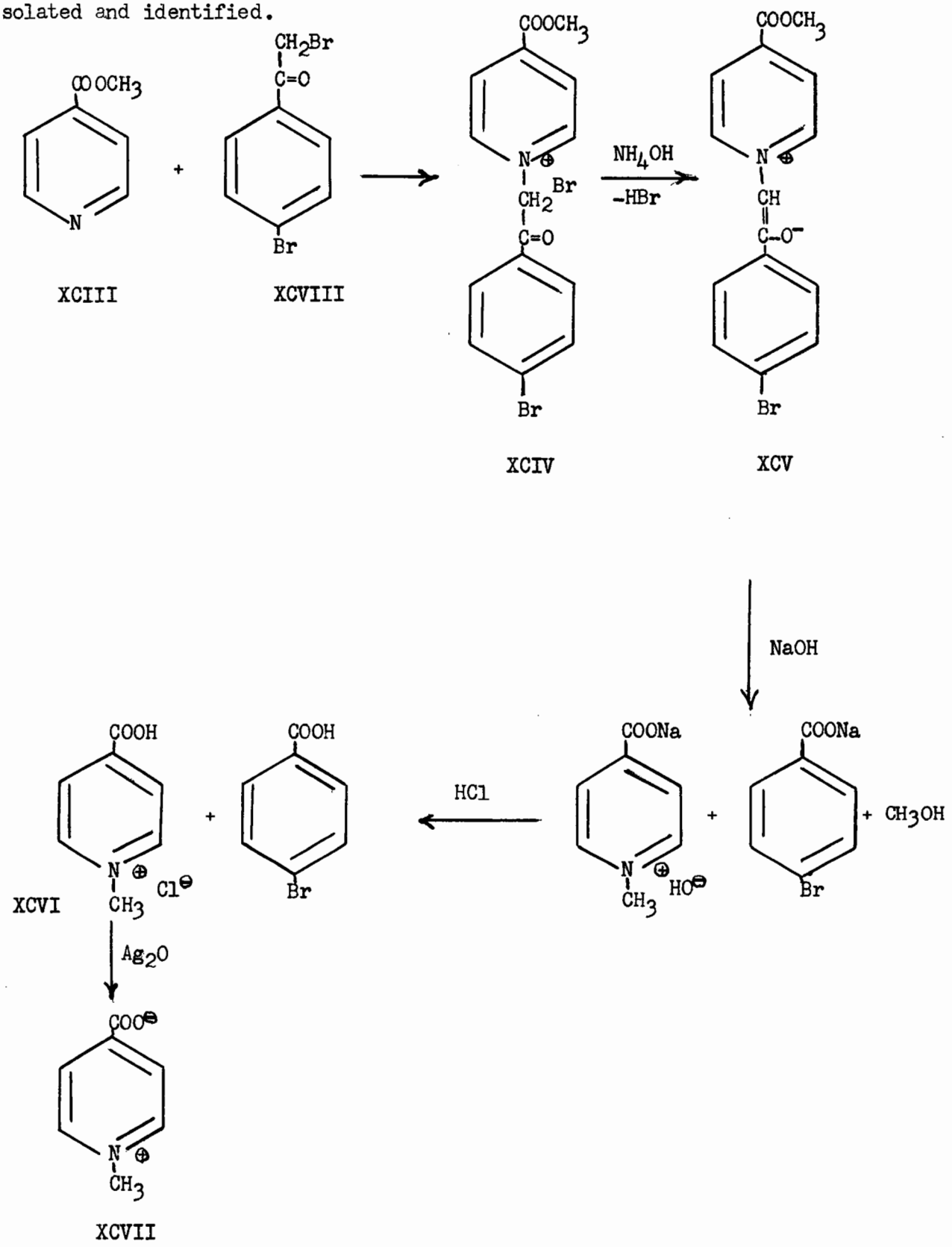
However, this decrease in the nucleophilic character of the pyridine nucleus was not sufficient to prevent the formation of the corresponding pyridinium salt, 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide (XCIV) and the quite stable 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine (XCV).

The latter compound (XCV) was obtained by treating 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide (XCIV) with ammonia. The alkaline cleavage of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide (XCIV) was accomplished in an aqueous solution of sodium hydroxide. The progress of this alkaline fission could be followed easily by the decrease in intensity of the red colour of the solution. The refluxing of the intermediate pyridinium betaine (XCV) with sodium hydroxide was continued until the colour remained slightly yellow, or became colourless.

During the alkaline cleavage of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine (XCV), hydrolysis of the ester group also occurred. The products of the cleavage of the inner salt (XCV) were isolated only after acidification of the reaction mixture. Upon acidification with hydrochloric acid, p-bromo-benzoic acid separated immediately and was removed by filtration. The filtrate was treated with freshly precipitated silver oxide which apparently caused hydrogen chloride to be eliminated from 4-carboxy-1-methyl pyridinium chloride (XCVI), and 4-carboxy-1-methyl pyridinium hydroxide, betaine (XCVII) to be formed. The excess silver oxide, and the silver chloride produced, were insoluble in water, and were filtered off, while the 4-carboxy-1-methyl pyridinium hydroxide, betaine (XCVII) was soluble and remained in the filtrate. It was obtained by evaporating off the water.

2,4'-Dibromo-acetophenone (XCVIII) was used instead of 2-bromo-acetophenone (LXXXVII) in the reaction with methyl 4-pyridinecarboxylate (XCIII) because in the former case, one of the products of the alkaline cleavage of the pyridinium salt (XCIV) was p-bromo-benzoic acid which, being practically insoluble in cold water, could be removed from the reaction mixture. Then the other product (XCVII) of the alkaline fission

of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide (XCIV) could be isolated and identified.



D. Pyridinium betaines with a carboxylic group as the anionic function

Reaction of pyridine with monochloroacetic acid

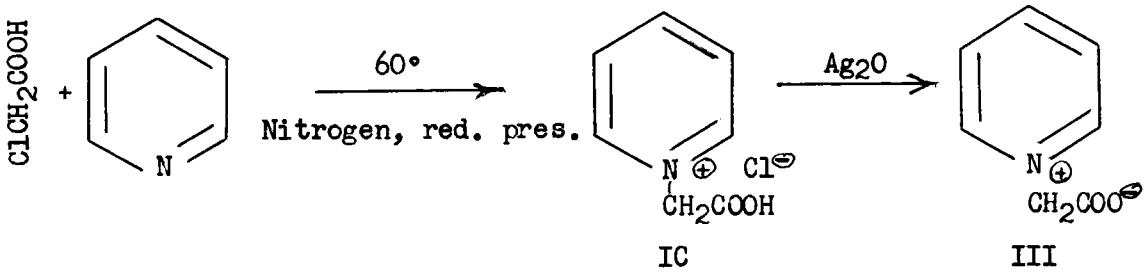
As has been previously mentioned, no information concerning the yield which could be expected in the synthesis of 1-(carboxymethyl)pyridinium betaine (III), was found in the literature. For this reason, a reinvestigation of the reaction between pyridine and monochloroacetic acid was undertaken with the object of establishing the optimum conditions for the reaction.

In all cases, in which the von Gerichten procedure was followed, a brown syrup was obtained which later crystallized, but was very impure and required many recrystallizations. It became apparent that side reactions were proceeding simultaneously with the main reaction. These side reactions were due presumably to the nucleophilic character of pyridine, and gave decarboxylation and polymerization products which seemed similar to those reported by Kirpal (44) and Bezzi (32).

The effect of the relative concentrations of the reactants was examined. The proportion of both pyridine and monochloroacetic acid in the reaction mixture was varied over a wide range, but no improvement in the yield or purity of 1-(carboxymethyl)pyridinium chloride (IC) was achieved. The effect of solvent was also studied. Both reactants were soluble in most nonpolar solvents while the reaction product (IC) was practically insoluble in these solvents. Although in a 1,4-dioxane solution, 1-(carboxymethyl)pyridinium chloride (IC) separated as soon as it was formed, no improvement in the purity of the product was achieved by using this solvent. The reaction of pyridine and monochloroacetic acid was also carried out at various temperatures, and the observation that the purity and yield of

1-(carboxymethyl)pyridinium chloride (IC) were improved at low temperatures (19) was confirmed. This improvement however was not pronounced.

Finally, excellent results were obtained when the reaction was carried out at 60°, in a dry nitrogen atmosphere under reduced pressure. Under these conditions 1-(carboxymethyl)pyridinium chloride (IC) was obtained quantitatively in a very pure state. This modified procedure for preparing the salt (IC) made it possible, after a typical elimination of hydrogen chloride, to obtain the 1-(carboxymethyl)pyridinium betaine (III) in a high yield.



IC III

Reaction of quinoline and isoquinoline with β-chloropropionic acid

When quinoline and isoquinoline reacted with β-chloropropionic acid on the steam bath, a sticky dark polymeric material was produced. However, the same reaction, under reduced pressure and at moderate temperatures yielded 1-(2-carboxyethyl)quinolinium chloride and 2-(2-carboxyethyl)isoquinolinium chloride respectively. These compounds were identical with those observed when the products of the reactions of quinoline and isoquinoline with β-propiolactone were treated with hydrogen chloride (45).

The reaction of quinoline and isoquinoline with β-chloropropionic acid produced the corresponding salts in very poor yields because the polymerization reaction which accompanied the desired reaction could not be prevented. The polymerization is considered to arise as the result of

acrylic acid being formed during the dehydrochlorination of β-chloropropionic acid. The acrylic acid initiates subsequently the polymerization. The elimination of a molecule of hydrogen chloride from β-chloropropionic acid is considered to be an ordinary displacement caused by the nucleophilic attack of quinoline or isoquinoline.

All attempts to obtain the corresponding betaines from 1-(2-carboxyethyl)quinolinium chloride and 2-(2-carboxyethyl)isoquinolinium chloride were unsuccessful. When these salts were treated with freshly precipitated silver oxide, the corresponding free bases 1-(2-carboxyethyl)quinolinium hydroxide and 2-(2-carboxyethyl)isoquinolinium hydroxide were formed. Upon treatment of the bases with hydrogen chloride, the salts 1-(2-carboxyethyl) quinolinium chloride and 2-(2-carboxyethyl)isoquinolinium chloride were regenerated. These salts were highly hygroscopic. Attempted dehydration of 1-(2-carboxyethyl)quinolinium hydroxide and 2-(2-carboxyethyl)isoquinolinium hydroxide did not result in betainization but led instead to complete decomposition.

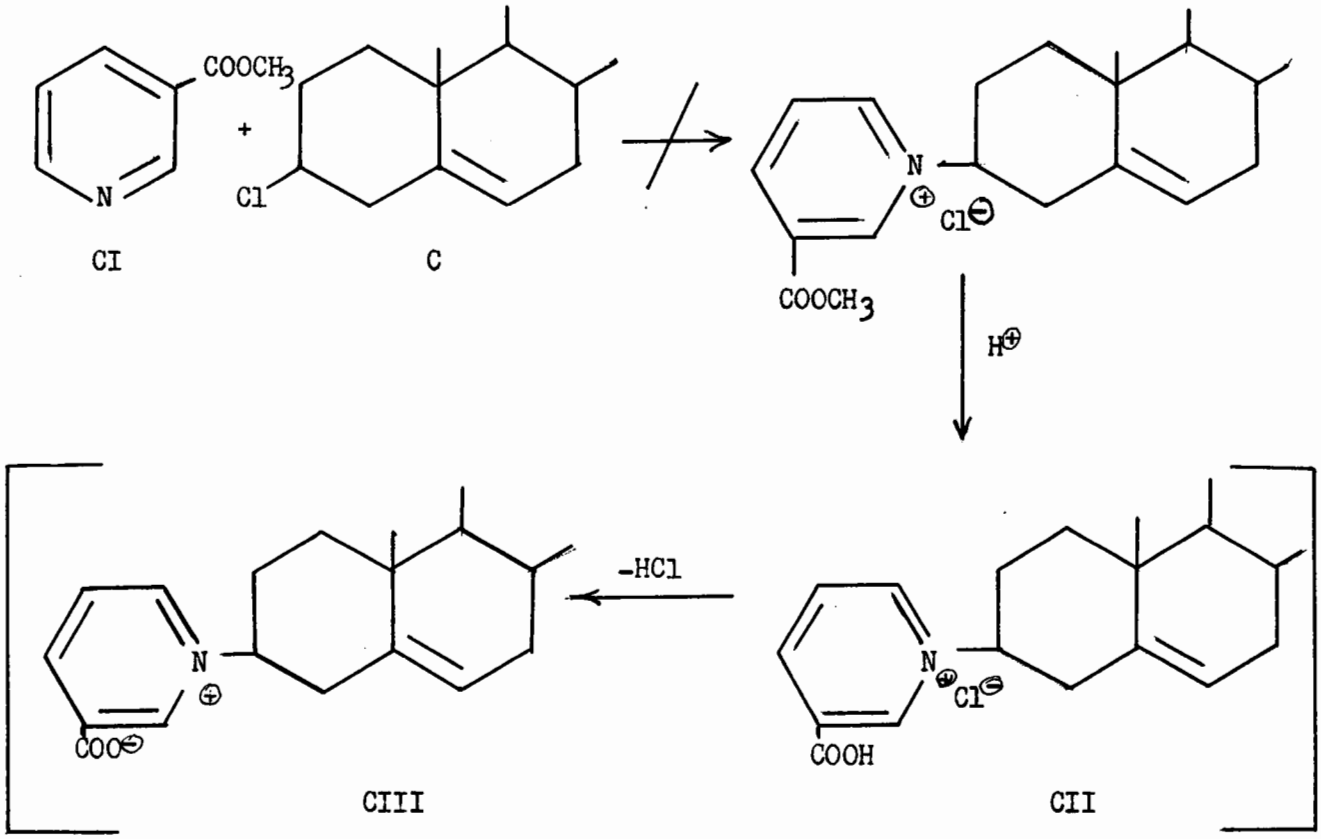
Attempted reaction of pyridinecarboxylic acids with cholesteryl chloride

A number of attempts were made to introduce the pyridinecarboxylic function into a steroid molecule. The purpose of these attempts was to synthesize pyridinium betaines which had a carboxylic group as the anionic centre of the dipole and a steroidal group as function quaternizing the annular nitrogen atom. All attempts in this connection were unsuccessful and in each case the starting material was recovered.

As the first approach to this problem, the reaction of 3-pyridinecarboxylic acid with cholesteryl chloride was undertaken. However, after a lengthy refluxing in alcohol, both reactants were recovered. The failure

of this reaction appeared to be due partly to the weak nucleophilic character of 3-pyridinecarboxylic acid, but mainly to the established (141) low reactivity of the chlorine atom present in the molecule of cholesteryl chloride.

When attempts to carry out the reaction of cholesteryl chloride (C) with 4-pyridinecarboxylic acid also were unsuccessful, then another approach to the problem was considered. If a reaction occurred between methyl 3-pyridinecarboxylate (methyl nicotinate) (CI) and cholesteryl chloride (C), then the ester group could be hydrolyzed, a molecule of hydrogen chloride eliminated from the resulting salt, (CII) and the pyridinium betaine (CIII) obtained. This sequence of reactions is outlined below. However, this endeavour also failed, and the starting materials



methyl 3-pyridinecarboxylate (CI) and cholesteryl chloride were recovered.

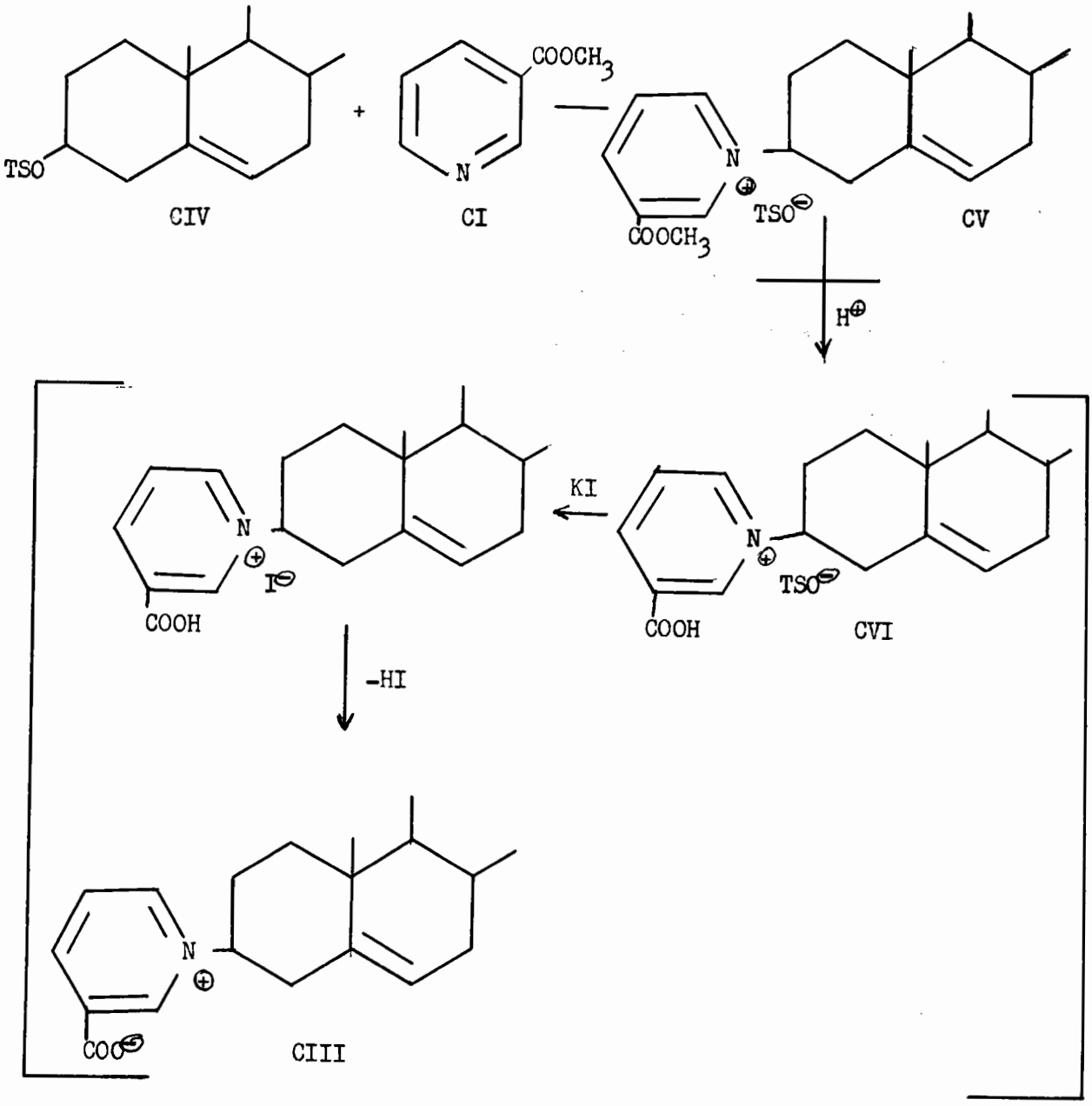
Attempted reaction of pyridinecarboxylic acids with cholesteryl p-toluenesulphonate

The failure of pyridinecarboxylic acids or their esters to react with cholesteryl chloride (C) led to another attempt, and a different approach to the problem. In this investigation it was found that no reaction occurred between cholesteryl p-toluenesulphonate (CIV) and 3- and 4-pyridinecarboxylic acids. In all cases the starting materials were recovered.

However, when methyl 3-pyridinecarboxylate (CI) was allowed to react with cholesteryl p-toluenesulphonate (CIV), 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (CV) was isolated. A large excess of methyl 3-pyridinecarboxylate (CI) was added to cholesteryl p-toluenesulphonate (CIV), because the reaction was carried out at a temperature at which methyl 3-pyridinecarboxylate (CI) was liquid, and it acted both as a reactant and a solvent.

The hydrolysis of the carbomethoxy ester group present in 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (CV) was attempted, but failed. It was thought that the hydrolysis of the carbomethoxy group in 3 position of the pyridine nucleus should be an acidic one, because 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (CV) like a typical pyridinium salt was sensitive to alkalies. The treatment of the latter compound (CV) with alkali appeared to cause a cleavage of the bond between the annular nitrogen atom and the carbon atom in 3 position of the steroid skeleton. Attempted acidic hydrolysis did not yield the desired product 3-carboxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate CVI), but instead, a dark resinous polymer.

The purpose of hydrolysing the ester, was to synthesize the corresponding betaine after the p-toluenesulphonic acid molecule had been eliminated from 3-carboxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (CVI). It had been planned to accomplish this by replacing the p-toluenesulphonate anion by an iodine anion and then eliminating the hydrogen iodide.



It was felt that the main reason for the failure of this hydrolysis was the well-known resistance of ester groups, attached directly to the pyridine nucleus, to acidic hydrolysis. Consequently mild acidic conditions were unable to bring about the ester hydrolysis, while more drastic conditions caused decomposition of 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (CV) and polymerization.

Electronic Absorption Spectra

The electronic absorption spectra of the betaines obtained by reacting 2,2-dibromo-1,3-indandione with mono-methylpyridines or pyridine-carboxylic acids were determined and a great similarity in the patterns of these spectra was revealed. Three absorption maxima occurred in each spectrum in the regions of 237-243 mμ, 311-316 mμ, and 386-418 mμ, and the molecular extinction coefficients were of the same order of magnitude. In the case of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine a fourth absorption maximum appeared in the region of 300 mμ. The other pyridinium betaines of the 1,3-indandione series did not exhibit this particular peak in their absorption curves but instead showed a smooth curve in the same region (300 mμ).

The absorption maxima which were observed for these betaines are listed in Table II of the experimental part of this presentation. Comparison of these data also reveals a great similarity between the two types of betaines of the 1,3-indandione series previously mentioned. This similarity serves to confirm the structure assigned to the betaines obtained from the reaction of 2,2-dibromo-1,3-indandione and pyridinecarboxylic acids, and leaves no doubt that these dipolar ions are typical enol betaines. Thus, it is clear that the carboxylic group does not participate in the formation of the inner salt by becoming the negative centre of the dipole, because if such were the case, the pattern of the absorption spectrum should not be similar to that of a typical enol betaine.

The electronic absorption spectra of the pyridinium betaines of the 1,3-indandione series were determined in absolute alcohol. When attempts were made to measure the absorption spectra of these compounds in chloroform, it was found that the wavelengths, at which the absorption

maxima appeared, were reproducible but the molecular extinction coefficients were not. This phenomenon had been observed in a similar case by Stafford (91). As shown in Table II, a pronounced shift of the absorption maxima towards longer wavelengths occurred when chloroform was used as solvent instead of alcohol. The maxima in the region 237-243 mμ observed in alcoholic solution did not appear in chloroform solution, apparently because the chloroform absorbed the light completely below 245 mμ.

The extremely low solubility of pyridinium betaines of the 1,3-indandione series in chloroform was an additional disadvantage, because it was impossible to prepare a more concentrated solution. This low solubility presumably was responsible for the fact that the third absorption maximum in the region 300-316 mμ could not be detected except in the case of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine.

The electronic absorption spectra of the betaines obtained by elimination of hydrogen bromide from the addition products of the reaction between 2α-bromo-3-cholestanone and pyridine bases also showed three absorption maxima, in the regions 245-255 mμ, 304-314 mμ, and 430-432 mμ. Such absorption maxima justified the assignment of typical enol betaine structures to these compounds. The spectra were determined in alcoholic solution and the results are listed in Table II. Because the compound was not stable to radiation the molecular extinction coefficients for 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine were not reproducible.

The structures proposed for the 3- and 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaines were verified by determining the electronic spectra of these compounds. It was found that they exhibited two absorption maxima at 248 mμ and 482 mμ. A comparison of these spectra

with that of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine which showed two maxima at 243 mμ and 480 mμ, revealed the shift of absorption maxima in the spectra of the former compounds toward longer wavelengths. This was due apparently to the resonance of the carbobenzoyl-methoxy group. However, the shift was not very pronounced because the methylene group between the carboxylic and the benzoyl groups acted as a barrier to resonance through the whole molecule of 3- and 4-carbobenzoyl-methoxy-1-(phenacyl)pyridinium hydroxide, betaines. Since the latter compounds decomposed rapidly, their molecular extinction coefficients were not reproducible.

Infrared Absorption Spectra

The infrared absorption spectra of

(i) 1,3-indandione

(ii) 2-nitro-1,3-indandione

(iii) 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine

(iv) 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine

(v) 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

were determined by Dr. A. Taurins with the help of Dr. R.N. Jones and Mr. R. Lauzon at the Laboratories of the National Research Council of Canada in Ottawa.

The infrared spectra of 1,3-indandione and 2-nitro-1,3-indandione were determined in an effort to establish in what way the nitro group affected the spectrum of 1,3-indandione. Since the nitro group possesses

a similar electron withdrawing force (-Is effect) to that of the positively charged annular nitrogen atom of the pyridinium betaines of the 1,3-indandione series, it was expected that the spectra of the latter compounds would exhibit some similarity to that of 2-nitro-1,3-indandione.

Although 1,3-indandione showed two characteristic absorption maxima at 1745 and 1705 cm^{-1} due to the presence of two carbonyl groups, no such maxima occurred in the spectra of either 2-nitro-1,3-indandione or 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine. However in the case of 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine and 3-carboxy -1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine, it was observed that each of these compounds had only one absorption maximum at 1730 cm^{-1} and 1728 cm^{-1} respectively due to the presence of the carbonyl groups.

The two absorption maxima which occurred in the spectrum of 1,3-indandione at 1745 cm^{-1} and 1705 cm^{-1} were noted with interest. They were considered to reflect a dissimilarity between the two carbonyl groups present in the molecule and were due possibly to a difference in the degree of enolization of these two carbonyl groups. Other absorption maxima which occurred in the spectrum of 1,3-indandione could be explained as follows: The band at 1593 cm^{-1} was due to the aromatic ring present in the molecule, those at 920 cm^{-1} and 775 cm^{-1} to the substituted aromatic ring, that at 1260 cm^{-1} to the presence of an aryl ketone system. The rest of the absorption maxima could be attributed to the Nujol used in the determinations.

In the absorption spectrum of 2-nitro-1,3-indandione no absorption maximum due to the carbonyl group was observed because of the strong electron withdrawing force (-Is effect) of the nitro group. Bands at 1650, 1595 and 1400 cm^{-1} were characteristic of the nitro group present in the molecule,

and maxima at 1177 and 1145 cm^{-1} were attributed to the aryl ketone system.

It was observed that the patterns of the spectra of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine, 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine and 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine were similar, with the difference that the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine showed no absorption maximum due to the carbonyl groups. Bands due to the presence of the aromatic rings in these three compounds were observed at 1585, 1590 and 1565 cm^{-1} respectively. In the spectrum of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine maxima characteristic of 4-methylpyridine appeared at 1520 and 1475 cm^{-1} and in the spectrum of 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine. Similar bands occurred at 1490, 1457 and 1442 cm^{-1} due to the 3-methylpyridine present in the molecule. In the spectrum of 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine maxima due to the pyridine nucleus were present at 1490, 1465 and 1450 cm^{-1}. The bands at 1395 and 1385 cm^{-1} were attributed to the methyl groups present in the pyridine part of the betaine molecule though the Nujol also could be considered to exhibit the same absorption. Maxima due to the presence of an aryl ketone were located at 1217 and 1193 cm^{-1} in the spectrum of 1-(3-hydroxy-1-oxo-2-indenyl) 4-methylpyridinium hydroxide, betaine, while 1-(3-hydroxy-1-oxo-2-indenyl) 3-methylpyridinium hydroxide, betaine exhibited maxima at 1210 and 1190 cm^{-1} due to the aryl ketone system. The spectrum of 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine showed two peaks at 1210 and 1190 cm^{-1} due to the aryl ketone system and two absorption maxima at 1410 and 1240 cm^{-1} characteristic of the carboxylic group present in the molecule.

EXPERIMENTAL

The melting points below 225° were determined in a Thiele-Dennis melting point tube containing concentrated sulphuric acid. Melting points above 225° were determined in a melting point block constructed according to the specifications given by Fieser (180). All melting points were uncorrected.

Carbon-hydrogen, nitrogen microanalyses were carried out in the Schwarzkopf microanalytical laboratory.

Pyridinium betaines of the 1,3-indandione series

2,2-Dibromo-1,3-indandione

For the preparation of 2,2-dibromo-1,3-indandione, the procedure of Wislicenus (154) was followed. The sample of the 1,3-indandione was obtained from Pieree Chemical Co.

To a solution of 14.6 g. (0.1 mole) of 1,3-indandione in 150 ml. of glacial acetic acid, 11.5 ml. of bromine was added dropwise at room temperature. The dark red colour of the solution caused by the free bromine, gradually disappeared as the reaction proceeded. After a few minutes a precipitate of 2,2-dibromo-1,3-indandione appeared and hydrogen bromide gas was evolved. After standing 4 hours at room temperature, the mixture was filtered under reduced pressure (water aspirator). The solid precipitate remaining on the filter paper was washed with two 10-ml. portions of ethanol in order to remove the traces of the free bromine present, and recrystallized from ethanol. The filtrate was evaporated to dryness under reduced pressure leaving a solid residue which gave a second crop of 2,2-dibromo-1,3-indandione. This was recrystallized twice from

ethanol. The total yield of 2,2-dibromo-1,3-indandione, m.p. 178°, was 25.5 g. (83% based on the amount of 1,3-indandione used). The m.ps. reported in the literature for 2,2-dibromo-1,3-indandione were 176-177° (154), 177° (181), 177-179° (182).

2-Bromo-1,3-indandione

The synthesis of 2-bromo-1,3-indandione involved the following steps:

1. Preparation of ethyl cinnamate
2. Preparation of ethyl α,β-dibromo-β-phenylpropionate
3. Preparation of phenylpropiolic acid
4. Preparation of racemic dibromo-cinnamic acid
5. Preparation of 2,3-dibromoindone
6. Preparation of 2-bromo-1,3-indandione.

Ethyl cinnamate

Ethyl cinnamate was prepared as described in Organic Syntheses (183). Dry toluene (800 ml.) was placed in a 3 l. three-necked, round-bottomed flask with ground glass joints. To the toluene, 58 g. of sodium, cut in very small pieces, was added. The flask was fitted with a Hershberg stirrer, and reflux condenser. A thermometer was suspended through the condenser so that it reached almost to the bottom of the flask. The third neck was provided with a stopper which could be replaced by a dropping funnel. The flask was immersed in an oil bath and the mixture heated to the reflux temperature of toluene. At that temperature the sodium melted. The mixture was stirred vigorously and the oil bath was removed. The stirring was continued until the mixture had reached room temperature.

During the gradual cooling the sodium solidified in very fine particles. The toluene was decanted off carefully, and dry ethyl acetate (920 ml.) and 5 ml. of absolute ethanol were added to the sodium which remained in the flask. This mixture was cooled to -5° by means of an ice-salt bath. The stopper of the flask was removed and replaced by a dropping funnel containing 212 g. (2 moles) of benzaldehyde. The benzaldehyde was added dropwise, with vigorous stirring, at such a rate that the temperature of the reaction mixture was maintained at -5° throughout the addition. The addition required two and a half hours at the end of which the reaction mixture was treated with 160 ml. of glacial acetic acid. Then water (600 ml.) was added. The ethyl cinnamate with the excess of ethyl acetate was separated at once from the aqueous layer. The latter was extracted with 50 ml. of ethyl acetate and this extract added to the mixture of the esters. The ester mixture was washed with 6N hydrochloric acid (600 ml.) and dried over anhydrous sodium sulphate. When the ethyl acetate had been distilled off, ethyl cinnamate was distilled at 141° under reduced pressure (15 mm.). The yield was 289 g. (82%).

Ethyl α,β-dibromo-β-phenylpropionate

Ethyl α,β-dibromo-β-phenylpropionate was prepared as described in Organic Syntheses (184). In a 500 ml. three-necked flask fitted with a mechanical stirrer, a dropping funnel and a thermometer was placed a solution of 88.1 g. (0.5 mole) of ethyl cinnamate in 50 ml. of carbon tetrachloride. The flask was surrounded by a freezing mixture of ice and salt, and the contents of the flask cooled to 0°. Then 80 g. (0.5 mole) of bromine was added gradually through the dropping funnel, with constant stirring over a period of 25 minutes. The temperature of the reaction

mixture was maintained at 0-5°. The cooling bath was removed and the stirring continued for an additional hour during which time the reaction mixture warmed to room temperature. The mixture was poured into an evaporating dish and the carbon tetrachloride and traces of free bromine were evaporated on the steam bath. The evaporation was accelerated by a funnel which covered the evaporating dish and was connected to a water aspirator. The cake of ethyl α,β-dibromo-β-phenylpropionate obtained was kept between large filter papers for 24 hours. The product was pulverized with ease, and after being dried in a vacuum desiccator it weighed 143 g. After two recrystallizations from petroleum ether (b.p. 70-90°) 112 g. of pure ethyl α,β-dibromo-β-phenylpropionate (m.p. 75°) was obtained. The m.p. reported for ethyl α,β-dibromo-β-phenylpropionate was 74-75° (184).

Phenylpropiolic acid

Phenylpropiolic acid was prepared as described in Organic Syntheses (185). One hundred and twelve grams of ethyl α,β-dibromo-β-phenylpropionate was added to a solution of 84.2 g. (1.5 mole) of potassium hydroxide in 400 ml. of ethanol at 40°. Initially there was a vigorous reaction. Later this reaction ceased and then the mixture was refluxed for 5 hours. The contents of the reaction flask were cooled to 0° and the salts which deposited were filtered. The filtrate was neutralized with concentrated hydrochloric acid and an additional quantity of salts separated. These were filtered and combined with those previously obtained. The filtrate was evaporated on the steam bath until the temperature of the vapour coming off reached 95°. The residue remaining in the evaporating dish was combined with the salts previously separated and dissolved in 250

ml. of water. Crushed ice was added to this solution until the total volume was 600 ml. In order to avoid decarboxylation, the temperature was maintained at 0°, by immersing the flask which contained the solution in an ice-salt bath. Sulphuric acid (2N) was added slowly with mechanical stirring until the solution reached a pH of 3. The phenylpropiolic acid was separated, filtered and washed with four 10-ml. portions of 2N sulphuric acid.

The crude product was dissolved in 350 ml. of 5% sodium carbonate solution. To the brown solution of sodium phenylpropiolate 7 g. of Norit was added and the mixture heated on the steam bath for twenty minutes. The Norit was removed by filtration and the decolourized filtrate cooled to 0°. Then sulphuric acid (2N) was added dropwise to the filtrate with vigorous mechanical stirring. The temperature of the mixture was kept constant at 0° during the addition of the sulphuric acid which was continued until the mixture reached a pH of 3.

The phenylpropiolic acid was separated by suction filtration, washed with 15 ml. of 2N sulphuric acid and 15 ml. of water, and air-dried. After two recrystallizations from carbon tetrachloride 26.5 g. (54%) of pure phenylpropiolic acid, melting at 135-136°, was obtained. The m.p. reported for phenylpropiolic acid was 135-136° (185).

Cis- and trans-dibromo-cinnamic acids

Cis- and trans- dibromo-cinnamic acids were prepared according to the procedure of Roser and Haselhoff (185). In a 300 ml. three-necked, round-bottomed flask, fitted with a separatory funnel, mechanical stirrer, and thermometer, was placed a solution of 21.9 g. (0.15 mole) of phenylpropiolic acid in 150 ml. of dry chloroform. The flask was placed in an

ice-salt bath and, while it cooled, the solution was stirred continuously. When the temperature reached -5°, bromine (7.7 ml., 0.15 mole) was added from the separatory funnel at such a rate that the temperature of the reaction mixture did not exceed -1°. The addition required 40 minutes. When the bromine had been added, the contents of the flask were allowed to stand for a short time and then evaporated. The evaporation was facilitated by blowing nitrogen gas into the reaction mixture. The solid residue which remained was dissolved in a small amount of chloroform and petroleum ether added. A mixture of cis- and trans-dibromo-cinnamic acids separated, was filtered and then washed with 20 ml. of petroleum ether. The yield was 44 g., 96%.

2,3-Dibromoindone

2,3-Dibromoindone was prepared according to the procedure of Roser and Haselhoff (187). In a 125 ml. Erlenmeyer flask, 30.6 g. (0.1 mole) of the mixture of cis- and trans-dibromo-cinnamic acids was dissolved in 50 ml. of concentrated sulphuric acid at room temperature. A deep brown solution resulted and was poured onto 500 g. of crushed ice. A mixture of 2,3-dibromoindone and trans-dibromo-cinnamic acid separated at once in bright yellow flakes. This mixture was filtered with suction and treated with an aqueous 5% solution of sodium bicarbonate. The trans-dibromo-cinnamic acid passed into the alkaline solution as the corresponding sodium salt but the 2,3-dibromoindone remained practically insoluble. The insoluble material was filtered with suction, washed with two 10-ml. portions of 5% sodium bicarbonate solution and 20 ml. of water, and dried in a vacuum desiccator. The crystalline crude product thus obtained, was recrystallized from ethanol giving 125 g. (43.4%) of bright orange needles of 2,3-dibromo-

indone, m.p. 123°. The m.p. reported for 2,3-dibromoindone was 123° (187).

2-Bromo-1,3-indandione

The procedure of Roser and Haselhoff (188) was followed for the preparation of 2-bromo-1,3-indandione. A sample of 5.76 g. (0.02 mole) of 2,3-dibromoindone was suspended in 25 ml. of ethanol, and placed in a 100 ml. three-necked, round-bottomed flask which was provided with a mechanical stirrer, dropping funnel and thermometer. The flask was immersed in an ice-salt bath. When the temperature had reached 0°, the reaction mixture was stirred and 10 ml. of 10% aqueous solution of sodium hydroxide was added dropwise. The yellow-red solution obtained was allowed to stand for 24 hours at room temperature, and then made acid to litmus with 2N hydrochloric acid. Upon acidification, the 2-bromo-1,3-indandione deposited at once. The yellow crude product was filtered, washed with 20 ml. of water, and dried. After one recrystallization from a mixture of benzene and ligroin (b.p. 100-120°) 1.65 g. (36.6%) of pure 2-bromo-1,3-indandione (m.p. 118-119°) was obtained. The m.p. reported for 2-bromo-1,3-indandione was 119° (188).

Purification of pyridine bases

Pyridine

Eastman-Kodak white label pyridine was refluxed over potassium hydroxide pellets for 5 hours, left overnight, and distilled under reduced pressure with moisture excluded.

4-Methylpyridine

A white label sample of 4-methylpyridine was obtained from Eastman-Kodak Co. The purification procedure was the same as that used in

the case of pyridine.

2-Methylpyridine

A "practical" grade, yellow label sample of 2-methylpyridine from Eastman-Kodak Co. was refluxed over potassium hydroxide pellets for 5 hours. Then it was fractionally distilled through a one foot Vigreux column. The fraction distilling at 127.5-128° under 757 mm. pressure was used.

3-Methylpyridine

Only a "practical" grade containing 90-95% of 3-methylpyridine was available from Eastman-Kodak Co. This "practical" 3-methylpyridine was fractionally distilled under reduced pressure using an all glass Dufton fractionating column 20 cm. long. The fraction which distilled at 28.5° under 9 mm. pressure was collected and purified according to the procedure of Taurins and De Souza (189). This method gave 100% pure 3-methylpyridine by oxidizing and removing the 2- and 4-alkylpyridines which contaminated the 3-methylpyridine. Selenium dioxide was used as the oxidizing reagent because it was a mild oxidant and did not attack the 3-methylpyridine.

In a 1 litre three-necked, round bottomed flask provided with a mechanical mercury-sealed stirrer, reflux condenser and thermometer was placed a solution of 93.1 g. (1 mole) of "practical" 3-methylpyridine in 300 ml. of thiophene-free toluene. The solution was heated to the reflux temperature by means of an electric mantle which surrounded the flask. The first portion of selenium dioxide (27.75 g., 0.25 mole) was added to the solution through the condenser by means of a long glass tube reaching

below the surface of the refluxing solution. The reaction mixture was stirred continuously and after 4 hours refluxing, a second portion of selenium dioxide (27.75 g., 0.25 mole) was added. Selenium dioxide which adhered to the walls of the glass tube was rinsed into the solution by pouring 15 ml. of toluene down the tube. The reaction mixture was allowed to reflux for a total of 17 hours. During this time the solution became progressively more yellow in colour and metallic selenium separated as a grey-black amorphous powder mixed with the unreacted excess of selenium dioxide. The liquid layer while still hot was decanted into a 1 litre separatory funnel. The solid residue in the flask was washed with three 35-ml. portions of toluene and these washings were combined with the liquid layer in the separatory funnel. The liquid material was extracted with two 150-ml. portions of 10% hydrochloric acid and the aqueous layer made alkaline with 20% sodium hydroxide solution. The mixture obtained was extracted with three portions of ether; 300 ml., 150 ml., and 150 ml. respectively. When the ether was distilled from the combined ether extracts on the steam bath an oily residue remained. It was refluxed with 45 g. of sodium hydroxide pellets for 5 hours and fractionally distilled under reduced pressure using a one foot Vigreux column. The fraction which came over at 38.5-39° under 13 mm. pressure was pure 3-methylpyridine (53.4 g., 57.35%).

Purification of pyridinecarboxylic acids

The "practical" grade of 2-pyridinecarboxylic acid (picolinic acid) obtained from Eastman-Kodak Co. was purified by recrystallization from benzene. 3-Pyridinecarboxylic acid (nicotinic acid) from Brickman Co., and 4-pyridinecarboxylic acid (isonicotinic acid) from Reilly Co. were

recrystallized twice from water before being used.

1-(3-Hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

a. 1-(3-Hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine was prepared according to the procedure of Stafford (91). Bromine (2.25 ml.) was dissolved in 15 ml. of dry pyridine at 0°, and slowly added to a stirred solution of 4.38 g. (0.03 mole) of 1,3-indandione in 6 ml. of pyridine. The reaction mixture was heated gently on the steam bath for 5 minutes, and then poured into 300 ml. of water. The dark-red solution obtained was extracted with three 10-ml. portions of ether to remove the excess pyridine. The aqueous layer was separated and made alkaline with 2N sodium hydroxide solution. When the pH of the solution reached 10.5 at 19°, the addition of alkali was stopped. The reaction mixture was kept overnight at 0° and 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine deposited in elongated yellow plates which were recrystallized from ethanol. The yield was 1.2 g. (18% based on the amount of the 1,3-indandione used) and the pure product melted at 257° with decomposition. The m.ps. reported for the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine were 255-256° (91) and 256-257° (94).

b. Reaction of 2-bromo-1,3-indandione with pyridine

2-Bromo-1,3-indandione (1.13 g., 0.005 mole) was dissolved in 3 ml. of dry pyridine and the yellowish solution obtained was heated gently on the steam bath for 6 minutes. During this heating the solution became dark red in colour. The reaction mixture was poured into 50 ml. of water and refluxed for 10 minutes. After being allowed to cool to room temperature, the solution was extracted with three 10-ml. portions of

ether. The aqueous layer was separated, and made alkaline (pH 11 at 22°) with 1N aqueous sodium hydroxide. Upon cooling in the refrigerator overnight, the solution deposited 0.75 g. of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (67% yield based on the amount of 2-bromo-1,3-indandione used). A sample obtained by mixing this product with that prepared according to the procedure of Stafford did not show any depression of the melting point 257° (decomp.).

The same procedure as above was followed except that instead of being heated on the steam bath, the reaction mixture of 2-bromo-1,3-indandione and pyridine was allowed to stand for 24 hours at room temperature. The product obtained was identified with that prepared according to the above procedure, by a mixed melting point determination.

Attempted isolation of 1-(1,3-dioxo-2-indanyl)pyridinium bromide

The reaction mixture of 2-bromo-1,3-indandione (1.13 g., 0.005 mole) and 3 ml. of pyridine after standing at room temperature for 24 hours was poured into 50 ml. of water and refluxed for 10 minutes. The mixture was allowed to cool, and then was evaporated to dryness over phosphorus pentoxide in vacuo at room temperature. A black residue was obtained which, when tested with silver nitrate solution, showed that anionic bromine was present. The test for the presence of nitrogen was positive. Numerous attempts were made to recrystallize the material from various solvents, but all failed. Under the microscope the brittle resinous mass appeared partially crystalline, and partially amorphous. Finally an effort was made to purify the black residue by sublimation in an all glass sublimation apparatus. The dry material partially sublimed at 125° under 1 mm. pressure giving a reddish solid m.p. 158-164°. This solid after

recrystallization from a mixture of hot chloroform and styrene melted at 217°. The compound was identified as pyridine hydrobromide for the following reasons:

i. It was shown by a silver nitrate test that the compound contained anionic bromine.

ii. The substance liberated pyridine quantitatively upon treatment with 10% aqueous sodium hydroxide.

iii. The sublimate was very soluble in water and alcohol but insoluble in ether and most of the nonpolar solvents.

iv. The product readily formed a picrate m.p. 164° which was identified by a mixed melting point determination with the picrate obtained from either pyridine or pyridine hydrobromide. The m.p. reported for this picrate was 165-166° (190).

v. No depression of the melting point was observed when this compound was mixed with an authentic sample of pyridine hydrobromide, m.p. 217°. Reported m.p. 215-218° (101). The total amount of pyridine hydrobromide which sublimed was 0.37g. (46.3%).

The sublimation of the crude product from the reaction of 2-bromo-1,3-indandione and pyridine was continued but at higher temperature. At 185-190° under 1 mm. pressure a yellow substance sublimed slowly. This compound melted at 257° with decomposition. A mixed melting point determination with 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine prepared according to the procedure of Stafford (91), showed no depression of the melting point, 257° (decomp.). The total amount of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine sublimed was 0.73 g. (65.5% based on the amount of 2-bromo-1,3-indandione used).

c. Reaction of 2,2-dibromo-1,3-indandione and pyridine

A sample of 2,2-dibromo-1,3-indandione (1 g., 3.3 millimoles) was added to an excess (3 ml.) of pyridine and the mixture shaken until an orange solution was obtained. The colour of this solution deepened gradually on standing. The solution was heated on the steam bath for 3 hours then the excess pyridine was removed under reduced pressure. The solid dark residue which remained was dried in a vacuum desiccator over concentrated sulphuric acid. The dried material was recrystallized from 12 ml. of boiling ethanol to which a small amount of Norit had been added. The alcoholic solution slowly deposited yellow crystals of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine which melted at 257° with decomposition. The mixed melting point determination of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine prepared according to the procedures a, b and c, did not show any depression. The yield based on 2,2-dibromo-1,3-indandione was 0.34 g., 46.5%.

Fractional sublimation of the crude product of the reaction of pyridine with 2,2-dibromo-1,3-indandione

The procedure described previously for the fractional sublimation of the reaction mixture of 2-bromo-1,3-indandione and pyridine was followed in the present case. The sublimation was carried out in an all glass apparatus because pyridine hydrobromide attacked rubber.

The first fraction which sublimed at 120-125° under 1 mm. pressure was identified after purification as pyridine hydrobromide. The purification of this compound was achieved by several sublimations or recrystallization from a mixture of hot chloroform and styrene. The pyridine hydrobromide which sublimed initially was white but as sublimation continued the

substance gradually became orange-yellow. Treatment of this product with aqueous potassium iodide showed that neither free bromine nor any pyridine-bromine complex was present, because no iodine was released. The orange-yellow colouration was due to the presence of a small amount of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine which sublimed simultaneously. The latter compound was isolated by treating the crude pyridine hydrobromide with a very small quantity of water. The pyridine hydrobromide passed into solution while the 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine present remained insoluble and was identified with an authentic sample of the compound. The second fraction sublimed at 185-190° and was identified with 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine by its melting point, and a mixed melting point determination with an authentic sample of the compound (257° dec.). A black material remained in the sublimation apparatus. Examination of this substance under the microscope showed that it was completely amorphous. It appeared polymeric and did not show any tendency to form fibres. Attempts to crystallize the material using various solvents failed. Elementary analysis showed the presence of nitrogen and bromine. The latter was shown to be anionic when tested with silver nitrate solution. On several occasions reaction mixtures of pyridine and 2,2-dibromo-1,3-indandione were sublimed in the manner described above and the quantitative data obtained on these occasions were averaged and are presented below.

Starting with 1 g. of 2,2-dibromo-1,3-indandione and 3 ml. of pyridine, 1.628 g. of solid residue was obtained which upon fractional sublimation gave 0.475 g. of pyridine hydrobromide, 0.587 g. of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (74% based on the amount of 2,2-dibromo-1,3-indandione used), and 0.5 g. of polymeric material. The

observed loss of 1.628 - (0.475 + 0.587 + 0.5) = 0.066 g. was due apparently to the fact that some of the vapours of pyridine hydrobromide and the pyridinium betaine were not solidified on the cold finger of the sublimation apparatus and distilled off instead.

Solvatochromy of the reaction mixture of 2,2-dibromo-1,3-indandione and pyridine

In a 25 ml. two-necked, round-bottomed flask with ground glass joints was placed a solution of 2,2-dibromo-1,3-indandione (2 g., 6.6 millimoles) in 10 ml. of pyridine. The flask was equipped with a reflux condenser and a glass stopper which could be removed for taking samples of the reaction mixture. The solution was heated on the steam bath for 3 hours and at several intervals during this time samples of the reaction mixture were taken. Two drops of each sample were added to each of 10 test tubes. To 5 of these tubes 2, 4, 6, 8 and 10 drops of ethanol were added, so that a series of increasing dilutions was obtained. The intensity of the colour of the solution decreased proportionally to the amount of ethanol added. Therefore the decrease in colour could be attributed to a dilution effect. To the other 5 test tubes 2, 4, 6, 8 and 10 drops of water were added. In this case, since a pronounced decrease in the colour of the solution was observed, irrespective of the quantity of water, the decrease could not be ascribed to a dilution effect.

1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine

a. Attempted synthesis

(i) A solution of 1,3-indandione (1.46 g., 0.01 mole) in 10 ml. of 4-methylpyridine was placed in a 25 ml. Erlenmeyer flask which was

immersed in an ice-salt bath. When the temperature of the solution reached 0°, bromine (0.75 ml.) was added to it dropwise. The reaction mixture became almost black in colour and a semisolid material separated from the solution. After standing for 30 minutes, the reaction mixture was extracted with three 20-ml. portions of ether to remove the unreacted 4-methylpyridine. The dark residue which remained was dried in a vacuum desiccator at room temperature for 24 hours and then transferred to an all glass sublimation apparatus. This apparatus was immersed in an oil bath and connected to a mechanical pump. The pressure in the apparatus, measured by means of a manometer, was 1 mm. When the oil bath had been heated to 125-130°, a few milligrams of material sublimed. This sublimate was identified as 4-methylpyridine hydrobromide for the following reasons:

1. The substance, when tested with silver nitrate solution, was found to contain negative bromine.

2. When treated with a 5% aqueous solution of sodium hydroxide the substance liberated 4-methylpyridine. The latter was extracted with ether and the ethereal extract evaporated carefully on the water bath. The boiling point of the liquid residue was determined by Siwoloboff's micro-method (191) and found to be 143°.

3. The compound readily formed a picrate, which after recrystallization from ethanol melted at 167°. A mixed melting point determination with an authentic sample of the picrate of 4-methylpyridine showed no depression of the melting point (167°).

The residue remaining in the sublimation apparatus was a black polymer. No crystalline product could be obtained from it although many attempts were made to crystallize it from various solvents.

Exactly the same procedure as above was followed in the case of

2- and 3-methylpyridines with very similar results. Upon treatment of these pyridine bases with bromine in the presence of 1,3-indandione, polymerization occurred accompanied by the formation of small quantities of the corresponding methylpyridine hydrobromide.

(ii) In a 25 ml. Erlenmeyer flask immersed in a dry ice-acetone bath was placed 10 ml. of 4-methylpyridine. When the liquid 4-methylpyridine had solidified, bromine (1 ml.) was added dropwise. It solidified immediately. A very slow reaction occurred at the interface of the two phase solid system with the formation of an orange-yellow crystalline product. The reaction mixture was allowed to reach a temperature at which both reactants melted and then was poured into a cold, violet-coloured suspension of 1,3-indandione (2.19 g., 0.015 mole) in 3 ml. of 4-methylpyridine. The mixture darkened in colour and upon heating on the steam bath for 5 minutes deposited a dark-brown semisolid material. The excess of 4-methylpyridine was removed by extraction with two 20-ml. portions of ether and the semisolid residue which remained after the extraction was dried in vacuo at room temperature and then sublimed at 130°/1 mm. Only a small amount (0.16 g.) of a yellow substance sublimed during a period of 2 hours. This crude sublimate was purified by another sublimation. It was identified as 4-methylpyridine hydrobromide by the same tests as were used previously.

The residue remaining in the sublimation apparatus was a black polymer which gave positive results when tested for the presence of nitrogen and anionic bromine.

Exactly the same procedure was followed when 2-, or 3-methylpyridine was used instead of 4-methylpyridine and similar results were obtained.

b. Reaction of 2,2-dibromo-1,3-indandione and 4-methylpyridine

(i) Using ethanol as solvent

In a 50 ml. two-necked, round-bottomed flask equipped with a reflux condenser and dropping funnel was placed 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione and 20 ml. of ethanol. The mixture was refluxed for 2 minutes, then as the solid phase of 2,2-dibromo-1,3-indandione passed completely into solution, 4-methylpyridine (4 ml.) was added dropwise over a period of 10 minutes. The refluxing was continued for an additional 2 hours and during this time the reaction mixture gradually turned red. Most of the ethanol (14 ml.) was removed under reduced pressure (water aspirator) leaving a dark red viscous liquid, which after standing overnight at room temperature, slowly deposited lustrous wine-red flakes. These crystals were collected on the filter and recrystallized from ethanol. Analysis and molecular weight determination indicated that the substance should be the 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine. The electronic absorption spectrum, because it showed a similar pattern to that of 1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine, confirmed the structure assigned to the compound. The yield of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine was 0.82 g. (67% based on the amount of 2,2-dibromo-1,3-indandione used). It melted at 258° with decomposition.

Molec. weight Calc. for $C_{15}H_{11}NO_2$: 237

Found by the method of Rast: 234

Anal. Calc. for $C_{15}H_{11}NO_2$: C, 75.95; H, 4.67; N, 5.9%

Found: C, 76.22; H, 4.74; N, 6.19%

The filtrate obtained from the reaction mixture of 2,2-dibromo-1,3-indandione and 4-methylpyridine after the separation of 1-(3-hydroxy-1-

oxo-2-indenyl)4-methylpyridinium hydroxide, betaine, was evaporated to dryness under reduced pressure, and then transferred to an all glass sublimation apparatus. The apparatus was immersed in an oil bath at 130°. A white powder (0.37 g.) sublimed during a period of 2 hours and a polymeric residue was left in the sublimation apparatus. The white sublimate was identified as 4-methylpyridine hydrobromide by the methods which were described in case (a). The dark polymeric residue did not crystallize. It softened at 280-285°, and elementary analysis showed that it contained nitrogen and bromine.

Reaction of 2,2-dibromo-1,3-indandione and 4-methylpyridine

(ii) Using toluene as solvent

Twenty millilitres of c.p. toluene which had been dried over sodium, and 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione were placed in a 50 ml. two-necked flask fitted with a dropping funnel and a reflux condenser to which was attached a calcium chloride drying tube. The flask was immersed in an oil bath maintained at 120-125°. To the boiling solution of 2,2-dibromo-1,3-indandione an excess of 4-methylpyridine (4 ml.) was added dropwise over a period of 5 minutes. This reaction mixture was refluxed for 40 minutes during which time a solid material began to separate from the dark-coloured solution. After being cooled in an ice bath the reaction mixture was filtered with suction (water aspirator). The solid material which remained on the filter was washed with 10 ml. of toluene and then dried in a vacuum desiccator for 24 hours at room temperature. A mixture weighing 2.4 g. and consisting of 1-(3-hydroxy-1-oxo-2-indenyl) 4-methylpyridinium hydroxide, betaine, 4-methylpyridine hydrobromide and amorphous polymeric material was obtained. This mixture after several

recrystallizations from boiling ethanol gave 0.46 g. of pure 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine in fine dark red leaflets, melting at 258° with decomposition. The yield based on the amount of 2,2-dibromo-1,3-indandione used was 39% (0.46 g.). The filtrates and washings were evaporated to dryness under reduced pressure. The residue obtained was sublimed and yielded 0.11 g. of 4-methylpyridine hydrobromide and 1.83 g. of a polymeric material.

The above procedure was followed when 2,2-dibromo-1,3-indandione and 4-methylpyridine were reacted in toluene at 78-80° for 2 hours. The yield of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine in this case was 44.7% (0.53 g.). After the betaine had been separated, the filtrate and washings were evaporated to dryness, and then sublimed. The white sublimate was identified as 4-methylpyridine hydrobromide, by the procedure used in the case of the reaction of 4-methylpyridine and 1,3-indandione in the presence of bromine. In the sublimation apparatus a black polymeric material remained.

The effect of temperature and solvent on the reaction of 4-methylpyridine and 2,2-dibromo-1,3-indandione is shown in Table I.

TABLE I

Solvent	Temperature	1-(3-hydroxy-1-oxo-2-indenyl) 4-methylpyridinium hydroxide, betaine Yield %*	4-methylpyridine hydrobromide grams	Polymer grams
Ethanol	78.5°	67	0.37	0.74
Toluene	110.6°	39	0.11	1.83
Toluene	78-80°	44.7	0.24	1.4

* Reaction of 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione with 4 ml. of 4-methylpyridine.

Reaction of 4-methylpyridine and 2,2-dibromo-1,3-indandione in the presence of phenol

In a 25 ml. Grignard flask equipped with a nitrogen inlet tube, thermometer, and reflux condenser protected by a calcium chloride drying tube, was placed a mixture of 8 ml. of 4-methylpyridine, 3.06 g. (0.01 mole) of 2,2-dibromo-1,3-indandione and 0.3 g. of phenol. The flask was immersed in an oil bath which was maintained at 105°. The reaction mixture turned orange immediately and this colour deepened as the reaction proceeded. A slow stream of dry nitrogen was passed through the flask during the reaction time of one hour. The hot solution was transferred to a small Claisen flask and distilled under 2 mm. pressure. The temperature of the bath in which the Claisen flask was immersed was not allowed to rise higher than 80° (i.e. below the m.p. of 2,4,6-tribromo-phenol). Phenol solidified and remained in the condenser. It was identified by its m.p. (41°) and solubility in water. The dry residue left in the Claisen flask after the distillation of the liquid phase was transferred carefully to an all-glass sublimation apparatus. This apparatus was placed in an oil bath maintained at 125-130°, and connected with a mechanical pump. A white material sublimed over a period of two hours under 1 mm. pressure. This substance was identified as 4-methylpyridine hydrobromide by the methods previously described. No 2,4,6-tribromo-phenol was present, because the sublimate was completely soluble in water.

Reaction of 4-methylpyridine and 2,2-dibromo-1,3-indandione in the presence of styrene

A mixture of 3.06 g. (0.01 mole) of 2,2-dibromo-1,3-indandione, 4 ml. of 4-methylpyridine and 2.5 ml. of styrene (stabilized against

polymerization by tertiary butyl catechol) in 25 ml. of ethanol was heated to the reflux temperature in a nitrogen atmosphere. Refluxing was continued for 45 minutes, then the reaction mixture was distilled. The fraction distilling at 145-146° (1.8 ml.) was unreacted styrene. The residue which remained was transferred to a Claisen flask and distilled under reduced pressure (15 mm.). When the temperature reached 139-141° styrene dibromide distilled and solidified in the condenser. The amount of this substance collected was 35 mg. and it was identified by its melting point (70-71°) and a mixed melting point determination with an authentic sample of styrene dibromide.

c. Reaction of 2-bromo-1,3-indandione and 4-methylpyridine

(i) A solution of 1.13 g. (0.005 mole) of 2-bromo-1,3-indandione in 4 ml. of 4-methylpyridine was placed in a 10 ml. Erlenmeyer flask and kept at room temperature for 24 hours. The wine-red solution was poured into 20 ml. of water and heated for 15 minutes on the steam bath. Then, the excess of 4-methylpyridine was removed by two extractions of the solution with 15 and 10 ml. of ether. The aqueous layer was separated and part of the water (8 ml.) removed under reduced pressure. The remainder of the solution was made alkaline (pH 11 at 19°) by adding 1N solution of sodium hydroxide dropwise. From the alkaline mixture a dark red solid substance slowly deposited and crystallized in elongated prisms. After recrystallization from ethanol, 0.85 g. (71.7%) of 1-(3-hydroxy-1-oxo-2-indenyl) 4-methylpyridinium hydroxide, betaine melting at 258° (decomp.) was obtained. A sample prepared by mixing the betaines obtained from the reaction of 4-methylpyridine with either 2,2-dibromo-1,3-indandione or 2-bromo-1,3-indandione did not show any depression of the melting point,

258° (decomp.).

(ii) A reaction mixture of 2-bromo-1,3-indandione (1.13 g., 0.005 mole) and 4-methylpyridine (4 ml.) was refluxed for 6 minutes, during which time it turned almost black and underwent a rapid polymerization. The black semisolid material produced did not crystallize. It was soluble in methanol and ethanol but only slightly soluble in ether and water. Sublimation of this product gave a small amount of 4-methylpyridine hydrobromide.

Attempted isolation of 1-(1,3-dioxo-2-indanyl)4-methylpyridinium bromide

In a 10 ml. Erlenmeyer flask was placed a solution of 2-bromo-1, 3-indandione (1.13 g., 0.005 mole) in 4 ml. of 4-methylpyridine. The reaction mixture which immediately turned orange in colour became wine-red while standing for 24 hours at room temperature. The solution was poured into water and heated for 15 minutes, then cooled and extracted with 25 ml. of ether. The aqueous layer was placed in a desiccator over phosphorus pentoxide, and dried. The dark residue which gave 4-methylpyridine hydrobromide and 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine on sublimation. These products were similar to those obtained in the reaction of pyridine with 2-bromo-1,3-indandione. However, all attempts to isolate 1-(1,3-dioxo-2-indanyl)4-methylpyridinium bromide from the reaction mixture failed.

1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine

a. Reaction of 2,2-dibromo-1,3-indandione and 3-methylpyridine

(i) Using ethanol as solvent

To a 50 ml. one-necked, round-bottomed flask equipped with a reflux condenser was added a mixture of 1.53 g. (0.005 mole) of 2,2-dibromo-1, 3-indandione, 5 ml. of 3-methylpyridine and 40 ml. of ethanol. The reaction mixture initially was yellowish but turned orange-yellow while refluxing on the steam bath for 6 hours. After evaporating most of the ethanol (25 ml.) under reduced pressure, the solution was placed in the refrigerator overnight. The solid which separated was recrystallized from alcohol and identified as 2,2-dibromo-1,3-indandione by its melting point and a mixed melting point determination with an authentic sample. The 2,2-dibromo-1,3-indandione was recovered from the reaction mixture in a yield of 94.7% (1.45 g.).

Unreacted 2,2-dibromo-1,3-indandione also was recovered almost quantitatively when methanol was used as solvent instead of ethanol in the above experimental procedure.

(ii) Using isoamyl alcohol as solvent

To a solution of 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione in 40 ml. of warm isoamyl alcohol, 5 ml. of 3-methylpyridine was added. The orange reaction mixture was refluxed for one hour then allowed to cool to room temperature. After standing for several hours, 1.2 g. of a deep yellow product separated from the solution as fine needles. This material was filtered and recrystallized three times from isoamyl alcohol. The substance melted at 238° with decomposition, when the melting point apparatus was preheated to 175°, but decomposed at 207° when the sulphuric acid bath was not preheated. Elementary qualitative analysis showed the

presence of nitrogen and bromine. Microanalysis for C, H and N showed that the product was probably a molecular compound of 1-(3-hydroxy-1-oxo-2-indenyl) 3-methylpyridinium hydroxide, betaine and unreacted 2,2-dibromo-1,3-indandione in the molar ratio 5:1.

Anal. Calc. for $(C_{15}H_{11}NO_2)_5\ C_9H_4Br_2O_2$: C, 67.69; H, 3.99; N, 4.7%

Found: C, 67.22; H, 4.15; N, 4.83%

The above reaction was repeated under exactly the same conditions except that the refluxing was continued for 7 hours. Then most of the iso-amyl alcohol was distilled off under reduced pressure and the viscous orange solution remaining was cooled in an ice bath. The solid material which separated was filtered with suction and washed with two 10-ml. portions of ether. On recrystallization from ethanol it melted at 218° with decomposition. The yield was 0.91 g. (77% based on the amount of 2,2-dibromo-1,3-indandione used).

Anal. Calc. for $C_{15}H_{11}NO_2$: C, 75.95; H, 4.67; N, 5.9%

Found: C, 76.20; H, 4.62; N, 6.15%

(iii) 2,2-Dibromo-1,3-indandione (1.53 g.,0.005 mole) was dissolved in 5 ml. of 3-methylpyridine. The orange solution obtained was heated for 4 hours on the steam bath and then the unreacted 3-methylpyridine was distilled off under reduced pressure. The solid material which remained was transferred to an all glass sublimation apparatus and fractionally sublimed, according to the procedure previously outlined in the description of the reaction between pyridine and 2,2-dibromo-1,3-indandione. Similar products were obtained from the reactions of pyridine and 3-methylpyridine with 2,2-dibromo-1,3-indandione. The first fraction was 0.23 g. of 3-methylpyridine hydrobromide. It was identified by the method used to identify

4-methylpyridine hydrobromide. The second fraction which sublimed at 170-180°/1 mm. was found to be 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine. It melted at 218° with decomposition and no depression of the melting point was observed when it was mixed with a sample obtained from the reaction of 2,2-dibromo-1,3-indandione and 3-methylpyridine in isoamyl alcohol solution. The yield was 0.78 g. (65.8%). In the sublimation apparatus a black polymer (0.64 g.) remained which did not crystallize.

b. Reaction of 2-bromo-1,3-indandione and 3-methylpyridine

A solution of 1.13 g. (0.005 mole) of 2-bromo-1,3-indandione in 3 ml. of 3-methylpyridine was placed in a 10 ml. round-bottomed flask which had a ground glass joint and was provided with a reflux condenser. The reaction mixture was heated gently on the steam bath for 4 minutes, then left overnight at room temperature. The dark red solution was poured into 20 ml. of water, and refluxed for 5 minutes. This aqueous solution was cooled and then extracted with 15 ml. of ether in three portions to remove the excess 3-methylpyridine. The water layer was separated and made alkaline (pH 11 at 21°) by the dropwise addition of a 1N solution of sodium hydroxide. A solid material separated slowly from the solution. It was filtered with suction, air-dried and recrystallized from ethanol in bright yellow needles. The pure dry product weighed 0.97 g. (82% yield based on the amount of 2-bromo-1,3-indandione used) and melted at 218° with decomposition. A mixed melting point determination and comparison of the electronic absorption spectra showed that this compound was identical with 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine.

Attempted synthesis of 1-(3-hydroxy-1-oxo-2-indenyl)2-methylpyridinium hydroxide, betaine

a. Reaction of 2-methylpyridine and 2,2-dibromo-1,3-indandione

A mixture of 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione, 4 ml. of 2-methylpyridine and 20 ml. of ethanol was placed in a 50 ml. two-necked, round-bottomed flask with ground glass joints. One joint was fitted with a reflux condenser, and the other with a glass stopper which could be removed for sampling. The solution was refluxed for one hour and during this time its colour darkened gradually from orange-yellow to a very deep red. While the solution was being refluxed, samples were taken by means of a micropipette at 10 minute intervals. Each sample amounted to three to four drops of the solution and was placed in a small test tube. Water (1 ml.) was added and a yellowish solid material precipitated. This solid material after recrystallization from ethanol was identified as 2,2-dibromo-1,3-indandione by determination of its melting point (178°) and mixed melting point with an authentic sample of 2,2-dibromo-1,3-indandione. In addition the presence of "positive" bromine was detected by the standard potassium iodide-starch test.

As the refluxing was continued the amount of free 2,2-dibromo-1,3-indandione isolated from each sample gradually decreased until the compound no longer was detected. The alcohol and the excess of 2-methylpyridine were distilled off in vacuo, and the semisolid residue was dried in an Abderhalden drying pistol for 24 hours at 35° (boiling ether). A polymeric substance was obtained from which no crystalline material could be isolated. The polymer appeared to be a highly viscous tar and thin films of it under the polarizing microscope did not show any centre of crystal growth.

The reaction of 2,2-dibromo-1,3-indandione and 2-methylpyridine was repeated under the same conditions except that it was carried out in a dry nitrogen atmosphere and absolute ethanol was used as the solvent. Subsequently the reaction was carried out without solvent, and with various other solvents such as methanol, propanol, isoamyl alcohol, 1,4-dioxane and chloroform. In all these cases, the same results as above were obtained.

b. Reaction of 2-methylpyridine and 2-bromo-1,3-indandione

A sample of 1.13 g. (0.005 mole) of 2-bromo-1,3-indandione was dissolved in 4 ml. of 2-methylpyridine. After being heated on the steam bath for 4 minutes, the reaction mixture was diluted with 20 ml. of water and the excess of 2-methylpyridine was extracted with 20 ml. of ether in 4 portions. The aqueous layer was separated and the greater part of the water removed under reduced pressure. The residue which remained was dried in a vacuum desiccator for 72 hours over phosphorus pentoxide. This resulted in the formation of a dark tar from which no crystalline substance was obtained.

Attempted reaction of 2,2-dibromo-1,3-indandione with 2-aminopyridine

A mixture of 3 g. of 2-aminopyridine and 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione was placed in a 10 ml. two-necked flask equipped with a nitrogen inlet tube and reflux condenser protected by a calcium chloride drying tube. The mixture was warmed on the steam bath and as the 2-aminopyridine melted, 2,2-dibromo-1,3-indandione passed immediately into solution. During the gentle heating on the steam bath a slow stream of dry nitrogen was passed into the flask. After 20 minutes the reaction mixture

which had become dark brown in colour was cooled and extracted with 10-ml. portions of ether. The ether was decanted and the tarry residue dried. All attempts to sublime or recrystallize this tar failed. Apparently it was a polymeric material.

Attempted reaction of 2,2-dibromo-1,3-indandione with 2-dibenzoylaminopyridine

To a suspension of 4 g. of 2-dibenzoylaminopyrdine (m.p. 167°, prepared by benzoylation of 2-aminopyridine according to the directions of Tchitchibabin (192)) in 25 ml. of isoamyl alcohol (b.p. 130-132°) was added 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione. The colour of the reaction mixture which became yellow immediately, deepened as the reaction proceeded. After refluxing for 3 hours, the mixture was cooled in an ice bath and filtered. The material remaining on the filter, which consisted of a light brown crystalline substance and a tar, was washed with two 5-ml. portions of isoamyl alcohol. Upon recrystallization from hot pyridine, the tar remained in solution but the crystalline material separated on cooling. A yield of 0.4 g. of tri-o-benzoylene-benzene melting at 427° was obtained.

Attempted reaction of 2-bromo-1,3-indandione with 2-aminopyridine or 2-dibenzoylaminopyridine

The experimental procedure described above was followed when 2-aminopyridine and 2-dibenzoylaminopyridine were allowed to react with 2-bromo-1,3-indandione instead of 2,2-dibromo-1,3-indandione. In each of these cases the reaction failed to give the corresponding betaine and a tar was obtained.

4-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

In a 100-ml. one-necked, round-bottomed flask equipped with a reflux condenser and situated in an electric heating mantle, was placed 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione. A hot solution of 3.69 g. (0.03 mole) of 4-pyridinecarboxylic acid in 40 ml. of isoamyl alcohol was added and the mixture refluxed for a period of 6 hours. The colour of the solution gradually turned deep orange as the 4-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine was formed. Removal of the isoamyl alcohol under reduced pressure left a yellow residue which was extracted with three 10-ml. portions of boiling water and then dried in a vacuum desiccator for 24 hours. The dry yellow substance was extracted with 15 ml. of chloroform and recrystallized twice from absolute ethanol. Finally, 0.78 g. of 4-carboxy-1-(3-hydroxy-1-oxo-2-indenyl) pyridinium hydroxide, betaine melting at 351° with decomposition was obtained. The yield based on the amount of 2,2-dibromo-1,3-indandione used was 58%.

Anal. Calc. for $C_{15}H_9NO_4$: C, 67.41; H, 3.4; N, 5.24%

Found: C, 67.18; H, 3.78; N, 4.72%

3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

To a suspension of 1.53 g. (0.005 mole) of 2,2-dibromo-1,3-indandione in 25 ml. of isoamyl alcohol an excess (4 g.) of 3-pyridine-carboxylic acid was added. The mixture immediately became yellow and this colour deepened as the reaction proceeded. Both reactants passed completely into solution. After refluxing for 8 hours, the hot mixture

was transferred to a Claisen flask and the isoamyl alcohol removed by distillation at 25-28° under 1 mm. pressure. The bright yellow residue was treated with 15 ml. of 2N hydrochloric acid, filtered and washed on the filter with 10 ml. of water and 15 ml. of chloroform. When dry, 0.96 g. (72% based on the amount of 2,2-dibromo-1,3-indandione used) of 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine was obtained. It was purified by three recrystallizations from isoamyl alcohol and melted at 317° with decomposition.

Anal. Calc. for $C_{15}H_9NO_4$: C, 67.41; H, 3.4; N, 5.24%

Found: C, 67.46; H, 3.81; N, 4.72%

2-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

A sample of 2,2-dibromo-1,3-indandione (0.76 g., 2.5 millimoles) was allowed to react with 2 g. of 2-pyridinecarboxylic acid in 30 ml. of boiling n-butanol (b.p. 117-118°) for 3 hours. Then the butanol was removed by distillation under reduced pressure. A solid residue was left which, after extraction with 8 ml. of boiling water to remove the excess of 2-pyridinecarboxylic acid, was dried in vacuo and recrystallized several times from n-butanol. A yield of 0.18 g. (27% based on the amount of 2,2-dibromo-1,3-indandione used) of 2-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine, m.p. 312° with decomposition, was obtained.

Anal. Calc. for $C_{15}H_9NO_4$: C, 67.41; H, 3.4; N, 5.24%

Found: C, 67.05; H, 3.67; N, 4.92%

Oxidation of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine with potassium permanganate

A suspension of 0.1 g. of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine in 2 ml. of water was placed in a 10 ml. round-bottomed flask with a ground glass joint, and treated with 2 ml. of 1N sodium hydroxide solution. Three millilitres of a saturated aqueous solution of potassium permanganate was added to this mixture. Then a reflux condenser was attached to the flask and the latter immersed in an oil bath maintained at 105°. The contents of the flask were refluxed gently for 20 minutes, after which the reaction mixture was cooled, the excess permanganate destroyed with sodium hydrosulphite solution, and the manganese oxide filtered off. The basic filtrate, upon acidification with 2N sulphuric acid, deposited 0.3 g. (43%) of crude phthalic acid. After recrystallization from water the substance melted at 206° and showed no depression in a mixed melting point determination with an authentic sample of phthalic acid.

Attempted oxidation of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine with alkaline hydrogen peroxide solution

In a 10 ml. Erlenmeyer flask was placed a suspension of 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine in 4 ml. of a 0.4N sodium hydroxide solution containing 10% hydrogen peroxide. The reaction mixture was kept at room temperature and shaken by means of a mechanical shaker for 24 hours. Then after the suspension had been made acid to litmus with dilute (1N) sulphuric acid, 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine was recovered quantitatively.

Subsequently, the reaction was carried out using an alkaline solution containing 30% hydrogen peroxide instead of 10%, and the same result was obtained. Attempts to oxidize by the above procedure the betaines of the 1,3-indandione series obtained from pyridinecarboxylic acids and 3-methylpyridine also ended in failure.

2-Bromo-5,5-dimethyl-1,3-cyclohexanedione

This compound was prepared according to the directions of Voitila (167), and Blout and his collaborators (168). To a suspension of 1.4 g. (0.01 mole) of Eastman Kodak 5,5-dimethyl-1,3-cyclohexanedione and 1.96 g. (0.022 mole) of potassium acetate in 20ml. of glacial acetic acid a solution of 3.2 g. (0.002 mole) of bromine in 100 ml. of glacial acetic acid was added dropwise at room temperature with vigorous stirring. The reaction mixture was allowed to stand at room temperature for one hour and then 200 ml. of ether was added. As a result of this addition the potassium salts precipitated at once and were separated by filtration. The filtrate was evaporated to dryness under reduced pressure. A residue remained which after recrystallization from 40% aqueous alcohol gave 1.49 (68%) of 2-bromo-5,5-dimethyl-1,3-cyclohexanedione melting at 175°. The m.p. reported in the literature was 175° (168).

2,2-Dibromo-5,5-dimethyl-1,3-cyclohexanedione

2,2-Dibromo-5,5-dimethyl-1,3-cyclohexanedione was prepared according to the procedure of Blout and his collaborators (168). Bromine (6.4 g., 0.04 mole) was dissolved in 200 ml. of glacial acetic acid and added dropwise to a suspension of 1.4 g. (0.01 mole) of 5,5-dimethyl-1,3-cyclohexanedione in 15 ml. of glacial acetic acid which contained 3.9 g.

(0.04 mole) of potassium acetate. When one third of the bromine solution had been added, the reaction mixture became yellow in colour. Upon completion of the bromine addition the reaction mixture was allowed to stand for one hour with occasional shaking. Ether (300 ml.) was added and the potassium salts (bromide and acetate) which precipitated at once, were collected on a filter. The filtrate was evaporated to dryness under reduced pressure, leaving a white solid residue which was recrystallized from 40% aqueous ethanol and melted at 150-151°. The yield was 1.61 g. (54%). The reported m.p. for 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione was 150.5-151° (168).

Attempted reaction of 2-bromo-5,5-dimethyl-1,3-cyclohexanedione with pyridine

To a solution of 0.55 g. (0.0025 mole) of 2-bromo-5,5-dimethyl-1,3-cyclohexanedione in 4 ml. of ethanol was added 2 ml. of pyridine. The solution was refluxed for 45 minutes during which time the reaction mixture became yellow. This colour did not deepen on continued heating. The solution was allowed to cool to room temperature and then was diluted with 6 ml. of water. The unreacted 2-bromo-5,5-dimethyl-1,3-cyclohexanedione precipitated quantitatively and was collected on the filter. It was identified by its m.p. and the fact that it liberated iodine from potassium iodide. The filtrate was extracted with ether (25 ml.) and when the ether was distilled off from this extract pyridine was recovered.

Reaction of 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione with pyridine

In a 10-ml. two-necked, round-bottomed flask equipped with a

reflux condenser and a nitrogen inlet tube was placed a sample of 0.6 g. (0.002 mole) of 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione. A solution of 1 ml. of pyridine in 5 ml. of ethanol was added and a slow stream of nitrogen was passed through the contents of the flask for 15 minutes. Then the reaction mixture was refluxed on a steam bath in a nitrogen atmosphere for 25 minutes. The initial yellow colour of the reaction mixture deepened during the refluxing. The hot mixture was transferred quickly to a Claisen flask, into which nitrogen was passed through a capillary tube attached to the flask. The ethanol and unreacted pyridine were removed under reduced pressure leaving a dark red oil which could not be crystallized. This oil was very soluble in the lower members of the alcohol series and in chloroform, but was practically insoluble in ether and water. Addition of ether or other nonpolar solvents to an alcoholic solution of the reaction product brought about its immediate precipitation, but in the form of an oil. A similar result was obtained on the addition of a strongly polar solvent such as water, formamide, dimethylformamide. Oily, deep-coloured materials also were obtained when 2-, 3-, and 4-methylpyridines were allowed to react with 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione.

Reaction of 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione with 3-pyridinecarboxylic acid

2,2-Dibromo-5,5-dimethyl-1,3-cyclohexanedione (0.6 g., 0.002 mole) was added in one portion to a suspension of 1.23 g. (0.01 mole) of 3-pyridinecarboxylic acid in 5 ml. of isoamyl alcohol. The yellow reaction mixture thus obtained was refluxed for 4 hours. Both reactants passed completely into solution and the colour deepened as the refluxing

was continued. The solution was treated with 5 ml. of 2N hydrochloric acid and the isoamyl alcohol layer separated. The latter was washed with 10 ml. of water in two portions, dried over anhydrous potassium carbonate and distilled under reduced pressure in a nitrogen atmosphere. The oily residue could not be crystallized.

Reactions of α-bromo-ketosteroids with pyridine bases

1-(3-oxo-2-cholestanyl)pyridinium bromide

The synthesis of 1-(3-oxo-2-cholestanyl)pyridinium bromide involved the following steps:

1. Preparation of cholestanol by hydrogenation of the olefinic double bond of cholesterol.
2. Oxidation of cholestanol to 3-cholestanone.
3. Bromination of 3-cholestanone to 2α-bromo-3-cholestanone.
4. Reaction of 2α-bromo-3-cholestanone with pyridine to give 1-(3-oxo-2-cholestanyl)pyridinium bromide.

Cholestanol

The hydrogenation of cholesterol to cholestanol was carried out according to directions of Hershberg (193). Commercial cholesterol obtained from Brickman Co. was used after purification by the classical bromination-debromination procedure of Fieser (194). The overall yield of pure cholestanol was 78%. The hydrogenation of cholesterol was accomplished as follows:

In the reduction flask of a PARR low pressure hydrogenation apparatus was placed 19.33 g. (0.05 mole) of cholesterol, 0.4 g. of platinic oxide prepared according to the procedure described in Organic Syntheses (195) and 250 ml. of c.p. ethyl acetate. Four drops of 72% perchloric acid were added and the reduction flask was evacuated and connected to the hydrogen tank of the apparatus. The flask was flushed out several times with hydrogen and heated up to 50° by means of an electric resistance heater. Initially the pressure in the flask connected to the tank was 18 lb./sq.in. The heater was turned off and the flask was shaken mechan-

ically for 20 minutes. During this time the temperature of the reaction mixture was maintained between 45° and 50° by the heat evolved from the exothermic reaction of hydrogenation. Absorption of the theoretical amount of hydrogen usually required 15 to 20 minutes. After disconnecting the flask from the tank of the hydrogenation apparatus, the hydrogen was removed from the flask by suction (water aspirator) and replaced by nitrogen. Then the flask was shaken for 5 minutes, so that the catalyst coagulated. The latter was separated by filtration. The filtrate after standing in the refrigerator overnight yielded a first crop of cholestanol which was collected by filtration. The filtrate from the latter filtration was evaporated to dryness under reduced pressure and the residue recrystallized from 250 ml. of boiling methanol in the presence of a small amount of Norit (0.25 g.). The total yield of cholestanol m.p. 139-140° was 16.9 g. (87%). The m.p. reported for cholestanol was 139-141° (193).

3-Cholestanone

3-Cholestanone was prepared according to the procedure of Vavon and Jakubowicz (196) as modified by Fieser and Dominguez (197). To a stirred suspension of 13.05 g. of cholestanol in 78 ml. of glacial acetic acid was added a hot solution of 13.5 g. of sodium dichromate dihydrate. The reaction mixture was heated on the steam bath for 15 minutes and allowed to stand at room temperature overnight. Then water (10 ml.) was added and the mixture filtered with suction. The 3-cholestanone which collected on the filter was washed thoroughly with water (150 ml.), then dried in vacuo at 56° for 24 hours. It was recrystallized from a mixture of ethanol-acetone in the proportion 4:1. The yield was 9.6 g. (74%), m.p. 127-128°. The m.p. reported for 3-cholestanone was 127-128° (197).

2α-Bromo-3-cholestanone

2α-Bromo-3-cholestanone was prepared according to the procedure described by Butenandt and Wolff (123). A solution of 7.73 g. (0.02 mole) of 3-cholestanone in 310 ml. of glacial acetic acid was placed in a 500 ml. Erlenmeyer flask. To this solution was added 2 ml. of a saturated solution of dry hydrogen bromide (prepared by bromination of tetralin (198)) in glacial acetic acid. The flask was immersed in an ice bath until its contents had cooled to a temperature of 16°. Then, 18.5 ml. of a bromine solution (1.05 molarity) in acetic acid was added to the flask with occasional shaking. The reaction mixture was kept at a temperature between 15° and 20° for 10 minutes and then allowed to stand at room temperature for 12 hours. The solid material which separated was collected on a filter. This crude product, after recrystallization from chloroform-acetic acid, gave 8.5 g. (91%) of 2α-bromo-3-cholestanone, m.p. 169°. The m.p. reported for 2α-bromo-3-cholestanone was 169-170° (123).

1-(3-Oxo-2-cholestanyl)pyridinium bromide

The procedure outlined by Ruzicka and his collaborators (125) was followed with some modifications. To a 50 ml. two-necked, round-bottomed flask equipped with a nitrogen inlet tube, and a reflux condenser protected with a calcium chloride drying tube, was added a solution of 7 g. (0.015 mole) of 2α-bromo-3-cholestanone in 34 ml. of dry pyridine. The flask was placed in an oil bath and a slow stream of dry nitrogen was passed through the flask during the reaction. The clear solution was heated to the reflux temperature, and after 10 minutes a solid material separated rapidly. After the reaction mixture had been refluxed for 2 hours, it was allowed to cool to room temperature, and filtered with suction. The solid

material which remained on the filter was washed thoroughly with three 25-ml. portions of petroluem ether (b.p. 60-90°), and dried. The crude product after recrystallization from ethanol gave 6.2 g. (76%) of 1-(3-oxo-2-cholestanyl)pyridinium bromide, m.p. 310° (decomp.). The m.p. reported for the latter compound was 310° with decomposition (125).

Attempted synthesis of 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine

(i) Reaction of 1-(3-oxo-2-cholestanyl)pyridinium bromide with potassium carbonate in chloroform

1-(3-Oxo-2-cholestanyl)pyridinium bromide (1 g., 0.018 mole) was dissolved in 10 ml. of dry chloroform and placed in a 25 ml. Grignard flask fitted with a mercury sealed stirrer, reflux condenser and nitrogen inlet tube. While dry nitrogen was being passed through the flask 1 g. of anhydrous potassium carbonate was added to the solution with stirring. The mixture turned yellow immediately and as the reaction proceeded the colour deepened. After being stirred for one hour at room temperature the reaction mixture was refluxed for 5 minutes. Then it was cooled and filtered. The salts which remained on the filter were washed thoroughly with 30 ml. of chloroform. The filtrate and washings were combined and evaporated to dryness by blowing dry nitrogen into the coloured solution. An orange-yellow oil remained which did not crystallize. Elementary analysis of this oil showed that nitrogen was present but bromine was not. Apparently elimination of hydrogen bromide from 1-(3-oxo-2-cholestanyl)pyridinium bromide had occurred since anionic bromine was detected in the potassium salts which remained on the filter. The oil photodecomposed gradually and was sensitive to heat. On exposing it to light or heat pyridine which could

be detected by its odour, was liberated. This decomposition resulted in a gradual disappearance of the colour of the oil. When the latter was treated with a few drops of ethanol, and 2N hydrobromic acid was added dropwise, 1-(3-oxo-2-cholestanyl)pyridinium bromide, m.p. 310°, was recovered. A mixed melting point determination with an authentic sample of 1-(3-oxo-2-cholestanyl)pyridinium bromide did not show any depression of the m.p. On treatment with perchloric acid the oil gave a salt which melted at 302-303° with decomposition.

(ii) Procedure (i) was followed except that 1-(3-oxo-2-cholestanyl) pyridinium bromide was suspended in alcohol or water instead of in dry chloroform. Upon treatment of this suspension with various basic compounds such as potassium carbonate, potassium hydroxide, sodium hydroxide, sodium carbonate, quanidine carbonate and ammonia, a dark red oil always was obtained. This oil which did not crystallize possessed a tendency to form films, and showed the same characteristics as the oil obtained from the reaction of potassium carbonate and 1-(3-oxo-2-cholestanyl)pyridinium bromide in chloroform solution.

(iii) Reaction of 1-(3-oxo-2-cholestanyl)pyridinium bromide with piperidine

In a 10 ml. Erlenmeyer flask 6 ml. of piperidine and 1.09 g. of 1-(3-oxo-2-cholestanyl)pyridinium bromide (0.002 mole) were placed. The pyridinium salt dissolved immediately in the piperidine to form a red solution. As the colour of this solution deepened a precipitate separated. After standing for half an hour the reaction mixture was cooled to 0° and filtered. The solid material remaining on the filter was washed with 2 ml. of piperidine and dried in a vacuum desiccator. It was a very hygroscopic

substance and liberated piperidine upon treatment with 5% sodium hydroxide. When silver nitrate was added to an aqueous solution of the compound, silver bromide precipitated. These experimental facts showed that the substance was piperidine hydrobromide. The amount of dry piperidine hydrobromide obtained was 0.31 g. (93.5%). Similar results were obtained when morpholine was used instead of piperidine in the above experimental procedure.

1-(3-Hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine

In a 25 ml. three-necked flask equipped with a dropping funnel, mechanical stirrer and reflux condenser, a sample of 0.54 g. (0.001 mole) of 1-(3-oxo-2-cholestanyl)pyridinium bromide was suspended in 3 ml. of alcohol. A stream of nitrogen was passed over the surface of the solution during the reaction by means of a glass tube which extended through the condenser. The heterogeneous reaction mixture was stirred and maintained at the reflux temperature while a 25% aqueous solution of dimethylamine was added dropwise. Gradually the unreacted 1-(3-oxo-2-cholestanyl)pyridinium bromide and the 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine which was being formed passed into solution. Addition of the dimethylamine solution at the reflux temperature was continued until the yellow solution had become turbid. Then the reaction flask was surrounded with cotton so that its contents would cool to room temperature very slowly. The pyridinium betaine separated in fine yellow needles which turned orange-red when dry. Recrystallization from alcohol and aqueous (25%) dimethylamine in a nitrogen atmosphere gave 0.29 g. (63%) of 1-(3-hydroxy-2-cholesten-2-yl) pyridinium hydroxide, betaine which melted at 169° with decomposition.

An analytical sample was dried in vacuo (Abderhalden drying pistol) over phosphorus pentoxide at 34° for 72 hours in the dark. If the sample

was heated at 78°, it decomposed.

Anal. Calc. for $C_{32}H_{49}NO$: N, 3.02%

Found: N, 3.19%

1-(3-Oxo-2-cholestanyl)4-methylpyridinium bromide

2 α-Bromo-3-cholestanone (4.65 g., 0.01 mole) and 15 ml. of dry 4-methylpyridine were added to a 25 ml. round-bottomed flask which was provided with a reflux condenser protected with a calcium chloride drying tube. The flask was placed in an oil bath. After a few minutes refluxing a solid material deposited from the clear solution. The refluxing was continued for 4 hours and then the reaction mixture was allowed to cool to room temperature. The solid material which had separated was collected on a filter, washed with petroleum ether and dried. After recrystallization from hot acetic acid, it gave 3.7 g. (66%) of shiny colourless prisms of 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide melting at 316° with decomposition.

Anal. Calc. for $C_{33}H_{52}BrNO$: C, 70.95; H, 9.36; N, 2.5%

Found: C, 71.35; H, 9.57; N, 2.75%

1-(3-Hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine

To a stirred suspension of 1 g. of anhydrous c.p. potassium carbonate in 10 ml. of dry chloroform was added 0.28 g. (0.005 mole) of 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide. The colour of the mixture turned orange-red immediately and deepened as the elimination of hydrogen bromide and the betainization of 1-(3-oxo-2-cholestanyl)4-methyl-pyridinium bromide proceeded. The reaction mixture was refluxed gently for 5 minutes in a dry nitrogen atmosphere. Then it was cooled to 0° and

filtered. The inorganic salts which collected on the filter were washed with two 5-ml. portions of chloroform. These washings were combined with the filtrate and evaporated to dryness by blowing dry nitrogen through the solution. After drying the residue in vacuo, at 34°, in the dark for 72 hours, 0.19 g. (79.5%) of 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine was obtained. Although the substance softened above 100° it had an indefinite melting point.

Anal. Calc. for $C_{33}H_{51}NO$: N, 2.93%

Found: N, 2.92%

A sample of 50 mg. of 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine was suspended in 3 ml. of aqueous ethanol (40%). When this suspension of the coloured compound was treated with 1 ml. of 2N hydrobromic acid, 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide, m.p. 316° with decomposition was obtained. The latter compound did not show any depression of the m.p. when mixed with an authentic sample of 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide. When 3% perchloric acid was added to a suspension of the betaine in water, a white crystalline substance separated at once. It melted at 312-314° with decomposition and was 1-(3-oxo-2-cholestanyl)4-methylpyridinium perchlorate. 1-(3-Hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine was dissolved in chloroform, and the red solution obtained was treated with a 1% solution of chloranil in chloroform. No colour reaction was observed.

1-(3-Oxo-2-cholestanyl)3-methylpyridinium bromide

The reaction mixture of 2.33 g. (0.005 mole) of 2α-bromo-3-cholestanone and 6 ml. of dry 3-methylpyridine was refluxed for 5 hours, with atmospheric moisture being excluded by means of a calcium chloride

drying tube attached to the reflux condenser. After being cooled, the mixture was filtered with suction (water aspirator). The solid material remaining on the filter was washed with 30 ml. of petroleum ether (b.p. 60-90°), then dried and recrystallized from a mixture of acetic acid and water in the proportion 2:3. A yield of 1.8 g. (64%) of 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide, m.p. 314° (decomp.), was obtained.

1-(3-Hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine

To a 25 ml. Grignard flask which contained 0.28 g. (0.5 millimole) of 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide, and was fitted with a mercury sealed stirrer, reflux condenser and nitrogen inlet tube, were added 1 g. of c.p. anhydrous potassium carbonate and 10 ml. of chloroform. The suspension turned yellow immediately and the colour deepened as the reaction mixture was refluxed gently for 5 minutes in a stream of dry nitrogen. Then the mixture was filtered and the salts remaining on the filter washed thoroughly with 30 ml. of chloroform. The coloured filtrate and washings were combined and evaporated to dryness by blowing dry nitrogen into the solution. An orange-yellow residue was left and this was dissolved in 10 ml. of chloroform and filtered. After evaporation of the chloroform from the filtrate in a stream of nitrogen, 0.17 g. (71%) of 1-(3-hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine was obtained. Although this substance softened at 128°, it did not melt at a definite temperature but gradually decomposed. The electronic absorption spectrum of this betaine was similar to those of the corresponding betaines of pyridine and 4-methylpyridine.

Anal. Calc. for $C_{33}H_{51}NO$: N, 2.93%

Found: N, 2.74%

Reaction of 2 α-bromo-3-cholestanone with 2-methylpyridine

A mixture of 10 ml. of dry 2-methylpyridine and 2.33 g. (0.005 mole) of 2 α-bromo-3-cholestanone was placed in a 25 ml. three-necked flask fitted with a nitrogen inlet tube and reflux condenser. A removable glass stopper was inserted in the third neck of the flask. The reaction mixture was refluxed for 5 hours but no voluminous precipitate was obtained either at the beginning or during the reflux period. Several samples were taken by means of a micropipette at different time intervals. No pronounced change in colour was observed when these samples were added to dilute alkaline solutions. The excess of 2-methylpyridine was removed from the reaction mixture in a stream of nitrogen under reduced pressure. Then the residue was transferred to an all glass sublimation apparatus immersed in an oil bath. A white substance sublimed at 125-130° under 1-2 mm. pressure which was extremely soluble in water and formed a picrate easily, when the sublimate was added to 20 ml. of a saturated alcoholic solution of picric acid. This solution was heated to boiling and allowed to cool slowly. The picrate which deposited was collected on a filter. After one recrystallization from boiling ethanol, 1.3 g. (81%) of long bright yellow needles of the picrate of 2-methylpyridine, m.p. 165°, was obtained. A mixed m.p. determination with an authentic picrate of 2-methylpyridine did not show any depression of the melting point. In addition the presence of anionic bromine in the sublimate showed that it was 2-methylpyridine hydrobromide.

7 α -Bromocholestane-3 β ,5α -diol-6-one 3-acetate

7α -Bromocholestane-3β ,5α -diol-6-one 3-acetate was prepared in a 47% overall yield according to the procedure of Fieser and Rajagopalan (176). This synthesis involved the following steps:

1. Cholesterol, after short heating with 88% formic acid, gave cholesteryl

formate. This was transformed into cholestane-3β,5α,6β-triol by oxidation with 30% hydrogen peroxide. Subsequently the ester group at 3 position of the steroid molecule was hydrolyzed.

2. Cholestane-3β,5α,6β-triol then was oxidized with an excess of N-bromosuccinimide in aqueous 1,4-dioxane to cholestane-3β,5α-diol-6-one.

3. This diolone was partially acetylated to cholestane-3β,5α-diol-6-one 3-acetate by a short refluxing with acetic anhydride and a few drops of pyridine.

4. Bromination of cholestane-3β,5α-diol-6-one 3-acetate in acetic acid solution with a solution of boron fluoride in ether as catalyst, yielded 7α-bromocholestane-3β,5α-diol-6-one 3-acetate.

Reaction of 7α-bromocholestane-3β,5α-diol-6-one 3-acetate with pyridine

A sample of 400 mg. of 7α-bromocholestane-3β,5α-diol-6-one 3-acetate was dissolved in 3 ml. of dry pyridine and heated to the reflux temperature in a dry nitrogen atmosphere. At one hour intervals during the refluxing period one drop of solution was taken out of the reaction mixture by means of a micropipette and added to one ml. of saturated alcoholic solution of potassium hydroxide. Subsequently other basic solutions, such as aqueous 1% potassium carbonate, 25% aqueous dimethylamine and ammonia, were substituted for the alcoholic potassium hydroxide. In none of these cases was a pronounced change in the colour of the basic solution observed. The refluxing was continued, but the voluminous precipitate of pyridinium salt which had been expected did not separate from the reaction mixture. When samples of the latter were treated with water, a white steroidal compound separated at once which, after being dried, was found to contain no nitrogen. After 48 hours refluxing, 60% aqueous ethanol was

added dropwise to the hot reaction mixture, until the solution became turbid. This mixture, upon cooling, deposited a white solid material which melted over a wide range of temperature (152-161°). The crude product after recrystallization from methanol, and then from acetone-petroleum ether gave 35 mg. of a bromine free compound m.p. 169-170°, which was slightly soluble in alkalies as the compound X of Fieser (176). The filtrates were evaporated to dryness and upon sublimation gave 84 mg. (71%) of pyridine hydrobromide melting at 217°.

4-Carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide

A mixture of 3.98 g. (0.02 mole) of 2-bromo-acetophenone, 1.23 g. (0.01 mole) of 4-pyridinecarboxylic acid and 40 ml. of ethanol was refluxed for 5 hours. After standing overnight at room temperature, the reaction mixture was filtered with suction and the solid material which remained on the filter was dried. This crude product gave a positive test for anionic bromine in contrast with 2-bromo-acetophenone which did not. The reaction product was white initially but upon standing in air it turned yellow and gave off a sweet odour similar to that of benzaldehyde. After recrystallization from ethanol 3.7 g. (84.3%) of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide, which darkened at about 180° and melted at 242° with decomposition, was obtained.

Anal. Calc. for $C_{22}H_{18}BrNO_4$: Br, 18.18%

Found: Br, 17.94%.

4-Carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine

To a stirred suspension of 2.2 g. (0.005 mole) of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide in 25 ml. of ethanol at 0°, 15 ml. of 5% aqueous ammonia was added dropwise over a period of ten minutes. This deep red heterogeneous mixture was stirred for twenty minutes and filtered. The red solid material separated by the filtration was dried in vacuo and then recrystallized as quickly as possible from absolute alcohol. After drying in vacuo over phosphorus pentoxide, at 34°, in the dark, for 48 hours 1.3 g. (73%) of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine melting at 152° with decomposition was obtained.

Anal. Calc. for $C_{22}H_{17}NO_4$: N, 3.90%

Found: N, 3.96%

Alkaline cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine

To 10 ml. of a 10% aqueous potassium carbonate was added 0.5 g. of 4-carbobenzoylmethoxh-1-(phenacyl)pyridinium hydroxide, betaine. This deep red mixture was refluxed for 2 hours and as the alkaline cleavage of the betaine proceeded the intense colour gradually decreased and the odour of benzaldehyde was detected. Finally the solution remained slightly yellow. After cooling it was acidified with 2N hydrochloric acid and benzoic acid separated immediately. The latter was removed by filtration with suction. When paper chromatography of the filtrate was carried out according to the procedure of Huebner (179) there was a strong spot on the paper strip (Whatman paper No. 1) due to the presence of 4-pyridinecarboxylic acid.

3-Carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide

3-Carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide was prepared by refluxing together 3.98 g. (0.02 mole) of 2-bromo-acetophenone, 1.23 g. of 3-pyridinecarboxylic acid and 30ml. of ethanol for 6 hours. The reaction mixture was cooled and then filtered with suction. The solid material which collected on the filter was recrystallized from ethanol and yielded 2.9 g. (66%) of 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide melting at 218° with decomposition.

Anal. Calc. for $C_{22}H_{18}BrNO_4$: N, 3.18%

Found: N, 3.07%

3-Carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine

To a suspension of 4.4 g. (0.01 mole) of 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide and 15 ml. of water, 40 ml. of 10% aqueous guanidine carbonate was added dropwise with stirring at 0°. As soon as the first drops of base had been added the mixture turned yellow then upon continued addition, a voluminous amorphous yellow precipitate separated and was accompanied by an evolution of carbon dioxide. After being stirred for 10 minutes at 0°, the reaction mixture was filtered with suction. The yellow solid material thus obtained was dried in a vacuum desiccator in the dark. The concentrated effort to crystallize this substance was unsuccessful. The compound separated as an amorphous aggregate from numerous solvents. Upon being heated in an oven at 100-105° for one hour, it darkened and lost 9% of its weight. This loss of weight was due to the elimination of 2 molecules of water from each hydrated molecule of the betaine. The inner salt underwent alkaline cleavage when the procedure described for the alkaline cleavage of 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine was carried out. The products of this fission upon acidification were benzoic acid and 3-pyridinecarboxylic acid. The yield of 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine, m.p. 132° with decomposition, was 1.3 g. (33%). An analytical sample was dried at 34°, in the dark, for 48 hours in an Abderhalden pistol.

Anal. Calc. for $C_{22}H_{17}NO_4$, $2H_2O$: N, 3.54%, H_2O, 9.11%

Found: N, 3.60%, H_2O, 9.0%

4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide

4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide was obtained from the reaction of methyl 4-pyridinecarboxylate with 2,4'-dibromo-aceto-

phenone. To prepare methyl 4-pyridinecarboxylate, 4-pyridinecarboxylic acid was esterified by absolute methanol in concentrated sulphuric acid according to the procedure of Levine and Sneed (199). The yield of methyl 4-pyridinecarboxylate in this case was 82%. The 2,4'-dibromo-acetophenone used was obtained from Eastman Kodak Co.

A mixture of 1.37 g. (0.01 mole) of methyl 4-pyridinecarboxylate, 2.78 g. (0.01 mole) of 2,4'-dibromo-acetophenone, and 20 ml. of absolute ethanol was refluxed for 3 hours in a 50 ml. round-bottomed flask equipped with a reflux condenser which was protected by a calcium chloride drying tube. Upon cooling, yellowish needles of 4-carbomethoxy-1(p-bromo-phenacyl) pyridinium bromide crystallized from the reaction mixture. This crude product was separated by filtration and washed with 10 ml. of ethanol. The filtrate and washings were evaporated to dryness giving an additional quantity of the compound. This was combined with that previously collected, and recrystallized from ethanol. A yield of 4 g. (96.4%) of 4-carbomethoxy-1(p-bromo-phenacyl)pyridinium bromide melting at 216° with decomposition was obtained.

Anal. Calc. for $C_{15}H_{13}Br_2NO_3$: Anionic Br, 19.30%

Found: Anionic Br, 19.14%.

4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine

In a flask equipped with a dropping funnel and reflux condenser were placed 2.1 g. (0.005 mole) of 4-carbomethoxy-1-(p-bromo-phenacyl) pyridinium bromide and 25 ml. of ethanol. After a short period of refluxing the pyridinium salt passed into solution and then 5 ml. of a 10% aqueous ammonia was added dropwise. The 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine separated at once. It was recrystallized from a mixture of chloroform and ether and melted at 171° with decomposition.

Yield: 1.23 g., 73.6%.

Anal. Calc. for $C_{15}H_{12}BrNO_3$: C, 53.91; H, 3.62; N, 4.19%

Found: C, 54.34; H, 3.59; N, 3.89%

Alkaline cleavage of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine

In a 25 ml. round-bottomed flask equipped with a reflux condenser was placed 0.5 g. of 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide betaine and 10 ml. of 10% aqueous sodium hydroxide. The initial dark red colour of the suspension disappeared gradually during the 2 hours refluxing and finally remained slightly yellow. The reaction mixture was cooled and then treated with 6N hydrochloric acid until strongly acidic. p-Bromo-benzoic acid which separated immediately, was filtered and dried. It melted at 251°. No depression of the m.p. was observed when a mixed m.p. determination with an authentic sample of p-bromo-benzoic acid was made. The yield of p-bromo-benzoic acid was 93.3% (0.28 g.).

To the filtrate, an excess of freshly precipitated silver oxide was added. The silver oxide was obtained by treating 2 g. of silver nitrate with excess alkali and washing the product with water several times. The filtrate and the silver oxide were refluxed for 20 minutes, then the silver salts were filtered off. The filtrate was dried over phosphorus pentoxide and the solid residue recrystallized from absolute alcohol. A yield of 0.14 g. (68.2%) of 4-carboxy-1-methyl pyridinium betaine melting at 264° was obtained. It was identified by its m.p., and a mixed m.p. determination with an authentic sample of the compound.

1-(Carboxymethyl)pyridinium betaine

A mixture of 17 g. (0.215 mole) of pyridine and 18.9 g. (0.2 mole) of monochloroacetic acid was placed in a 250 ml. Grignard flask. This flask was fitted with a thermometer, the bulb of which extended below the surface of the solution, a reflux condenser, and a nitrogen inlet tube which was attached to a capillary reaching the bottom of the flask. The upper end of the 3 foot reflux condenser was fitted with a gas tube which was connected to a water pump through a U-calcium chloride tube. While dry nitrogen was being bubbled into it, the reaction mixture was heated for three hours at a constant temperature of 60° under reduced pressure. Gradually the 1-(carboxymethyl)pyridinium chloride separated as a white microcrystalline mass which melted at 202-205° with decomposition. The yield was quantitative. The 1-(carboxymethyl)pyridinium chloride was refluxed for half an hour in water solution with an excess of freshly precipitated silver oxide. This resulted in the formation of 1-(carboxymethyl)pyridinium betaine. Since the latter remained in solution, the excess silver oxide, and the silver chloride formed during the reaction were separated by filtration, and the filtrate was evaporated to a small volume under reduced pressure. Then it was dried in a desiccator over concentrated sulphuric acid. Recrystallization of the solid residue from absolute ethanol gave 19.4 g. (54%) of 1-(carboxymethyl)pyridinium betaine melting at 150° with partial decomposition. The m.p. reported for 1-(carboxymethyl)pyridinium betaine was 150° (27,29).

1-(2-Carboxyethyl)quinolinium chloride

A sample of 12.9 g. (0.1 mole) of freshly redistilled quinoline (b.p. 237-238°) was heated with an excess of β-chloropropionic acid (20 g.)

for 6 hours at 60° under reduced pressure (water pump). The crude product of the reaction was recrystallized several times from boiling water and 1,4-dioxane. Finally a yield of 6.2 g. (27.7%) of the compound, m.p. 242° with decomposition, was obtained. The m.p. reported for 1-(2-carboxyethyl) quinolinium chloride was 241-243° (45).

Anal. Calc. for $C_{12}H_{12}ClNO_2$: Cl, 14.94%

Found: Cl, 14.80%

Attempted betainization of 1-(2-carboxyethyl)quinolinium chloride

To a solution of 1 g. of 1-(2-carboxyethyl)quinolinium chloride in 10 ml. of water, an excess of freshly precipitated silver oxide was added in one portion with vigorous stirring. The silver oxide was obtained by treating 3 g. of silver nitrate with 10% sodium hydroxide and washing the product thoroughly with water. The reaction mixture was stirred at room temperature for one half hour after which the insoluble silver salts were separated by filtration. The filtrate was boiled for a few minutes with 0.2 g. of Norit. Then the charcoal was removed by filtration, and the solution evaporated to dryness over phosphorus pentoxide in vacuo, at 34°. The residue crystallized in long needles. Because it was highly hygroscopic, further purification was not achieved. This crude product gave 1-(2-carboxyethyl)quinolinium chloride upon treatment with an excess of hydrochloric acid and evaporation to dryness. It also decomposed on treatment with 10% sodium hydroxide and liberated quinoline. On heating at 100° the compound did not undergo dehydration and subsequent betainization, but decomposed rapidly with liberation of quinoline.

2-(2-Carboxyethyl)isoquinolinium chloride

2-(2-Carboxyethyl)isoquinolinium chloride was obtained from the reaction of isoquinoline and β-chloropropionic acid under reduced pressure at 60°. The procedure followed, was essentially the same as that used for the synthesis of 1-(2-carboxyethyl)quinolinium chloride. The crude product of the reaction after several recrystallizations from absolute ethanol gave 2-(2-carboxyethyl)isoquinolinium chloride. This compound melted at 208° and was obtained in yields varying from 11 to 18%. The m.p. reported for 2-(2-carboxyethyl)isoquinolinium chloride was 211-212° (45).

Treatment of 2-(2-carboxyethyl)isoquinolinium chloride with freshly precipitated silver oxide did not result in betainization but instead a complete decomposition of this salt with the liberation of isoquincline occurred.

Attempted reaction of cholesteryl chloride with 3-pyridinecarboxylic acid

Cholesteryl chloride was obtained from the reaction of cholesterol and an excess of thionyl chloride according to the procedure of Diels and his collaborators (200,201) as modified recently by Baker and Squire (202). Pure cholesteryl chloride melted at 96°. Alumina obtained from Brickman Co. (80 mesh) was treated with warm 10% aqueous sodium hydroxide and warm acetic acid, then washed with distilled water until neutral to litmus. It was reactivated by heating at 200° for 30 hours (203).

Three grams of 3-pyridinecarboxylic acid was added in one portion to a mixture of 8.1 g. (0.02 mole) of cholesteryl chloride and 100 ml. of ethanol. After being refluxed for a short time and shaken occasionally the reaction mixture became a homogeneous solution. The mixture was refluxed

gently for 12 hours and then the alcohol was evaporated under reduced pressure. A solid residue remained which was extracted with 20 ml. of boiling water and filtered. On cooling, the filtrate deposited 2.6 g. (86.6%) of 3-pyridinecarboxylic acid. The solid brown material which had collected on the filter was dried thoroughly in vacuo at 56°. Then it was dissolved in 100 ml. of pentane and filtered through a column of alumina. The dimensions of the column were 1.8 x 10 cm. Subsequently the solution was passed through a column (1.8 x 5 cm.) of Norit. The columns were washed with 50 ml. of pentane, the filtrates combined, and the pentane distilled under reduced pressure. The solid residue which remained, was recrystallized from acetone and then melted at 95-96°. A mixed melting point determination with an authentic sample of cholesteryl chloride showed no depression of the melting point and indicated that the residue was unreacted cholesteryl chloride. It was recovered in 90.3% yield (7.4 g.).

Attempted reaction of methyl 3-pyridinecarboxylate and cholesteryl chloride

Methyl 3-pyridinecarboxylate was prepared in 68% yield according to the procedure of Levine and Sneed (199).

A mixture of 13.7 g. (0.1 mole) of methyl 3-pyridinecarboxylate and 2.03 g. (0.005 mole) of cholesteryl chloride was placed in a 50 ml. round-bottomed flask, with a ground glass joint and was fitted with a reflux condenser protected by a calcium chloride drying tube. The reaction mixture was heated for 6 hours by means of an oil bath maintained at 115°. At this temperature methyl 3-pyridinecarboxylate was liquid and cholesteryl chloride passed into solution. The reflux condenser was replaced by a Claisen distilling head to which was attached a short (10 cm.) Vigreux

column. The greater part (13 g.) of the methyl 3-pyridinecarboxylate was distilled under reduced pressure (105-106°/20 mm.) leaving a residue in the flask which when recrystallized from acetone yielded unreacted cholesteryl chloride, m.p. 96°.

Attempted reactions of 4-pyridinecarboxylic acid and methyl 4-pyridinecarboxylate with cholesteryl chloride

The procedures used when 3-pyridinecarboxylic acid and methyl 3-pyridinecarboxylate were reacted with cholesteryl chloride were followed. No reaction took place in either case and the reactants were recovered unchanged.

Attempted reaction of 3-pyridinecarboxylic acid and cholesteryl p-toluenesulphonate

Cholesteryl p-toluenesulphonate was obtained in 61% yield by reacting cholesterol with p-toluenesulphonyl chloride, following essentially the procedure of Freudenberg and Hess (204). After recrystallization from acetone the m.p. of this compound was 131°. The m.p. reported for cholesteryl p-toluenesulphonate was 131° (204).

(i) Using absolute alcohol as solvent

A 300 ml., two-necked, round-bottomed flask having ground glass joints was fitted with a mercury sealed stirrer and a reflux condenser protected by a calcium chloride drying tube. In the flask were placed 5.4 g. (0.01 mole) of cholesteryl p-toluenesulphonate, 1.23 g. (0.01 mole) of 3-pyridinecarboxylic acid, and 100 ml. of absolute ethanol. The reaction mixture was stirred and refluxed for a short time on the steam bath until

the reactants had passed into solution. Then the stirrer was stopped and the refluxing continued for three hours. When the reaction mixture was allowed to cool to room temperature, a white solid material separated, and was collected by filtration of the mixture with suction. This solid material was extracted with boiling water (15 ml.) and filtered immediately. The filtrate deposited, on cooling in an ice bath, 1.2 g. (97.5%) of 3-pyridinecarboxylic acid. The solid residue gave, after recrystallization from a mixture of ethanol-ether, cholesteryl ethyl ether (3.4 g., 82.5%) melting at 88.5°. The m.p. reported for cholesteryl ethyl ether was 88.5° (205).

(ii) Using dry toluene as solvent

The reaction mixture of 5.4 g. (0.01 mole) of cholesteryl p-toluenesulphonate and 1.23 g. (0.01 mole) of 3-pyridinecarboxylic acid was placed in a 300 ml. round-bottomed flask provided with a reflux condenser protected with a calcium chloride drying tube. Toluene (150 ml.) dried over sodium was added to the flask through the condenser. When it had been refluxed for 14 hours, the reaction mixture was cooled and unreacted 3-pyridinecarboxylic acid was recovered from it.

(iii) In a sealed tube

A mixture of 2.7 g. (0.005 mole) of cholesteryl p-toluenesulphonate and 0.62 g. (0.005 mole) of 3-pyridinecarboxylic acid was placed and sealed in a glass tube. The tube was put in an oven and heated for 10 hours at 150°. At that temperature, the cholesteryl p-toluenesulphonate melted and the 3-pyridinecarboxylic acid dissolved in it. The reaction mixture gradually turned dark brown. When the tube had cooled it was opened and its contents extracted with boiling water (10 ml.) and filtered. On cooling,

the filtrate deposited quantitatively unreacted 3-pyridinecarboxylic acid. The solid material which remained on the filter was a sticky brown polymer. Apparently, it was formed from the decomposition and polymerization of cholesteryl p-toluenesulphonate.

3-Carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate

In a 25 ml. round-bottomed flask equipped with a reflux condenser protected by a calcium chloride drying tube were placed 13.7 g. (0.1 mole) of methyl 3-pyridinecarboxylate and 2.7 g. (0.005 mole) of cholesteryl p-toluenesulphonate. This mixture was heated on the steam bath for 8 hours. Methyl 3-pyridinecarboxylate became liquid at 37° and cholesteryl p-toluenesulphonate passed into solution immediately. The hot reaction mixture was diluted with 50ml. of 2-butanone and then cooled. A white material separated in long needles. After one more recrystallization from 2-butanone 2 g. (59%) of 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate melting at 236° with decomposition was obtained.

Anal. Calc. for $C_{41}H_{59}NO_5S$: N, 2.08%

Found: N, 2.38%

Attempted hydrolysis of the ester group of 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate

A mixture of 0.68 g. (0.001 mole) of 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate and 8 ml. of 75% sulphuric acid was placed in a 15 ml. flask equipped with a reflux condenser. Upon being shaken, the pyridinium salt passed into solution. The reaction mixture was heated on the steam bath for 4 hours, and became very dark in colour as the heating continued. Then the dark solution was poured into 50 ml.

of crushed ice and the steroidal compound separated as an oil. The acid layer was decanted and the dark oil washed with water until neutral. After the oil had been dried, it had the appearance of a brown resin. Attempts to crystallize, or purify this material were unsuccessful. Evidently the 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate had decomposed and polymerized. The same results were obtained when 85% phosphoric acid was used for the acidic hydrolysis of the carbomethoxy ester group of the pyridinium salt.

Electronic Absorption Spectra

The electronic absorption spectra of pyridinium betaines and several pyridinium salts were measured in absolute alcohol. Some of the electronic spectra were determined in dry chloroform. For this purpose, reagent grade chloroform first was washed ten times with distilled water to remove the alcohol which had been added as a stabilizer, then was dried over calcium chloride, distilled, and kept in the dark to avoid the formation of phosgene.

The spectra were determined by means of a Beckman recording spectrophotometer, model D.K.2, using the scale A 0-1 and a scanning period of 2 minutes. The concentrations of the solutions containing the substances whose spectra were to be determined, were between 1×10^{-4} and 2.5×10^{-4} molar. Since the spectrophotometer recorded the per cent absorption, and the wavelength at which the absorption occurred, the molecular extinction coefficient , was calculated using the expression

$$\epsilon = \frac{\log_{10} \frac{I_o}{I}}{CL} = \frac{\log_{10} \frac{100}{100 - \text{per cent Absorption}}}{CL}$$

where I_o was the intensity of the incident light and I was the radiation transmitted by a solution of molar concentration C and of length L. The length of the path of light passing through the solution was 1 cm.

Tables II, III, IV, V, VI, VII, and VIII show the light absorption of pyridinium betaines and salts at various wavelengths. In addition plots of $\log_{10}\epsilon$ against wavelength are given in Figures 4, 5, 6, 7, 8, 9 for some of these compounds.

Infrared Absorption Spectra

The infrared absorption spectra of

i) 1,3-indandione

ii) 2-nitro-1,3-indandione

iii) 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine

iv) 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine

v) 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

were determined (see Figures 10, 11, 12, 13 and 14 respectively) in Nujol mull with a sodium chloride prism of 927 resolution, a response of 1:1, a gain of 5.9, a speed of 4 and a supression of 4.

The scale of recordings was 100 cm^{-1}/cm. for the range 3800-2000 cm^{-1} and 100 cm^{-1}/4 cm. for the range 2000-600 cm^{-1}.

The maxima observed are listed in the tables IX, X, XI, XII, XIII and XIV which follow.

TABLE II

Electronic Absorption Spectra of

Pyridinium Salts and Betaines

Absorption maxima

TABLE II

Compound	In Absolute Alcohol Solution		In Chloroform Soln.
	λ max mμ	$\log_{10}\varepsilon$ max	λ max mμ
1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine	237, 300, 316, 386	4.53; 3.34; 3.45; 4.13	313, 403
1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine	241, 313, 400	4.45; 3.36; 3.95	426
4-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine	243, 315, 418	4.49; 3.28; 4.09	463
3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine	241, 312, 398	4.81; 3.44; 4.05	428
2-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine	240, 311, 396	4.79; 3.33; 4.04	426
1-(3-Hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine	245, 304 430	3.84; 3.03; 3.68	
1-(3-Hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine	248, 308 431	Not reproducible	
1-(3-Hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine	255, 314 432	3.24; 3.24; 3.02	
4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine	243, 480	4.48; 4.23	
4-Carbobenzoylmethoxy-1(phenacyl)pyridinium hydroxide, betaine	248, 482	Not reproducible	247, 475
1-(3-Oxo-2-cholestanyl)pyridinium bromide	261	3.49;	
1-(3-Oxo-2-cholestanyl)4-methylpyridinium bromide	225, 257.5	4.00; 3.55	
3-Carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate	266.5	3.49	

TABLE III

Electronic Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine

in Absolute Alcohol. Concentration 2.5×10^{-5} mole/litre

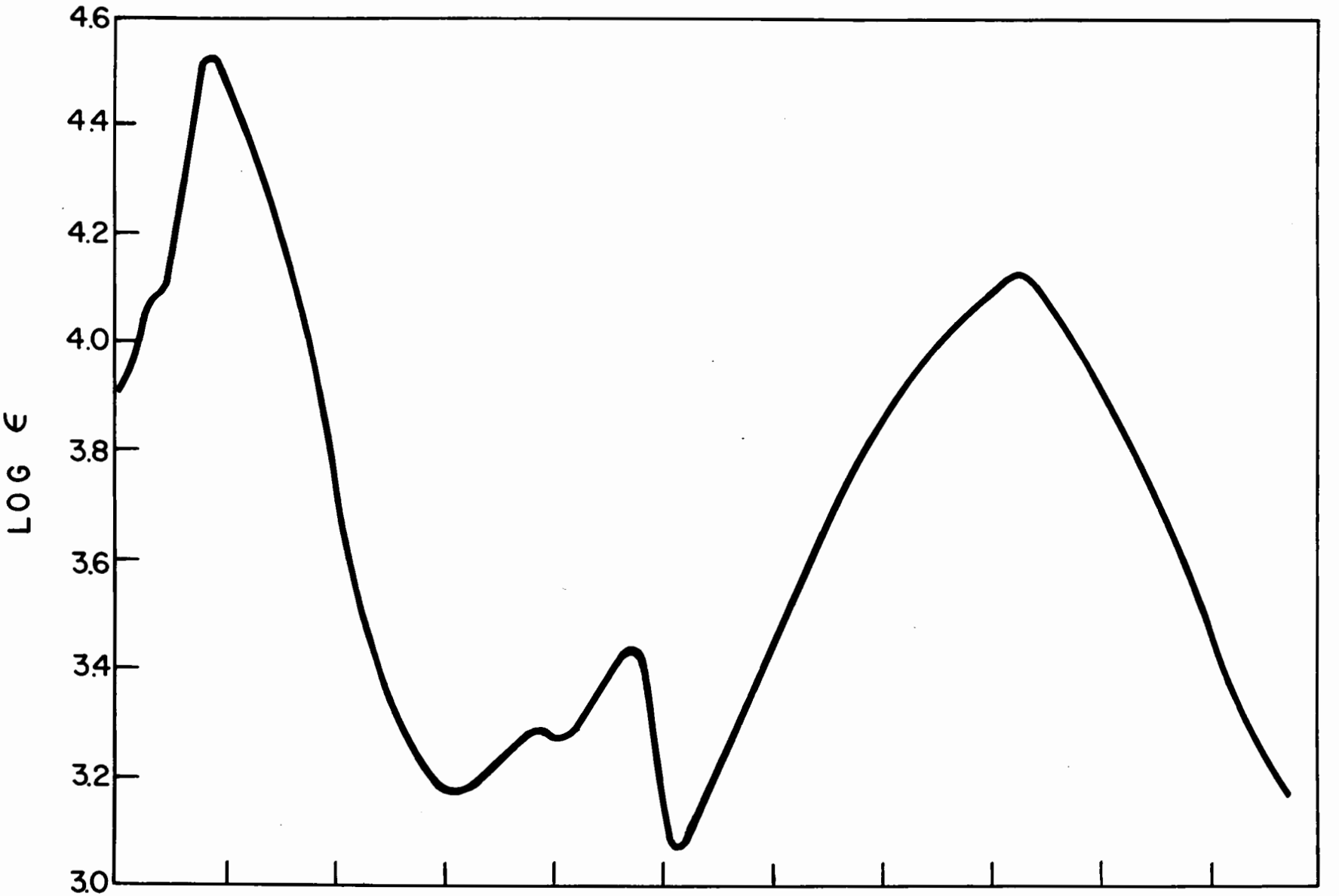
TABLE III

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$	Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$
220	1.615	3.9	300	1.135	3.34
223	1.67	3.96	304	1.13	3.33
225	1.78	4.02	306	1.13	3.33
227	2.00	4.08	308	1.14	3.36
230	2.09	4.11	310	1.16	3.41
232	3.33	4.32	312	1.17	3.43
234	4.54	4.42	316	1.175	3.45
235	5.25	4.46	322	1.07	3.06
236	6.25	4.5	332	1.11	3.26
237	7.17	4.53	340	1.17	3.43
238	6.65	4.52	346	1.25	3.59
239	6.25	4.5	350	1.32	3.68
241	4.77	4.43	354	1.38	3.75
244	3.44	4.33	356	1.43	3.79
247	2.78	4.25	360	1.52	3.86
250	2.25	4.15	366	1.67	3.94
252	2.00	4.08	372	1.82	4.04
254	1.85	4.05	378	1.96	4.06
258	1.54	3.87	380	2.00	4.08
260	1.39	3.76	382	2.1	4.11
265	1.18	3.46	386	2.22	4.13
270	1.09	3.17	390	2.00	4.08
280	1.09	3.17	396	1.82	4.01
282	1.09	3.17	400	1.67	3.94
285	1.1	3.22	405	1.50	3.83
290	1.11	3.26	410	1.37	3.75
292	1.112	3.265	420	1.19	3.47
295	1.12	3.29	435	1.09	3.17
297	1.33	3.33			

FIGURE 4

Electronic Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine



ELECTRONIC ABSORPTION SPECTRUM OF
1-(3-HYDROXY-1-OXO-2-INDENYL) 4-METHYLPYRIDINIUM HYDROXIDE, BETAINE.

TABLE IV

Electronic Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine

in Absolute Alcohol. Concentration 2.5×10^{-5} mole/litre

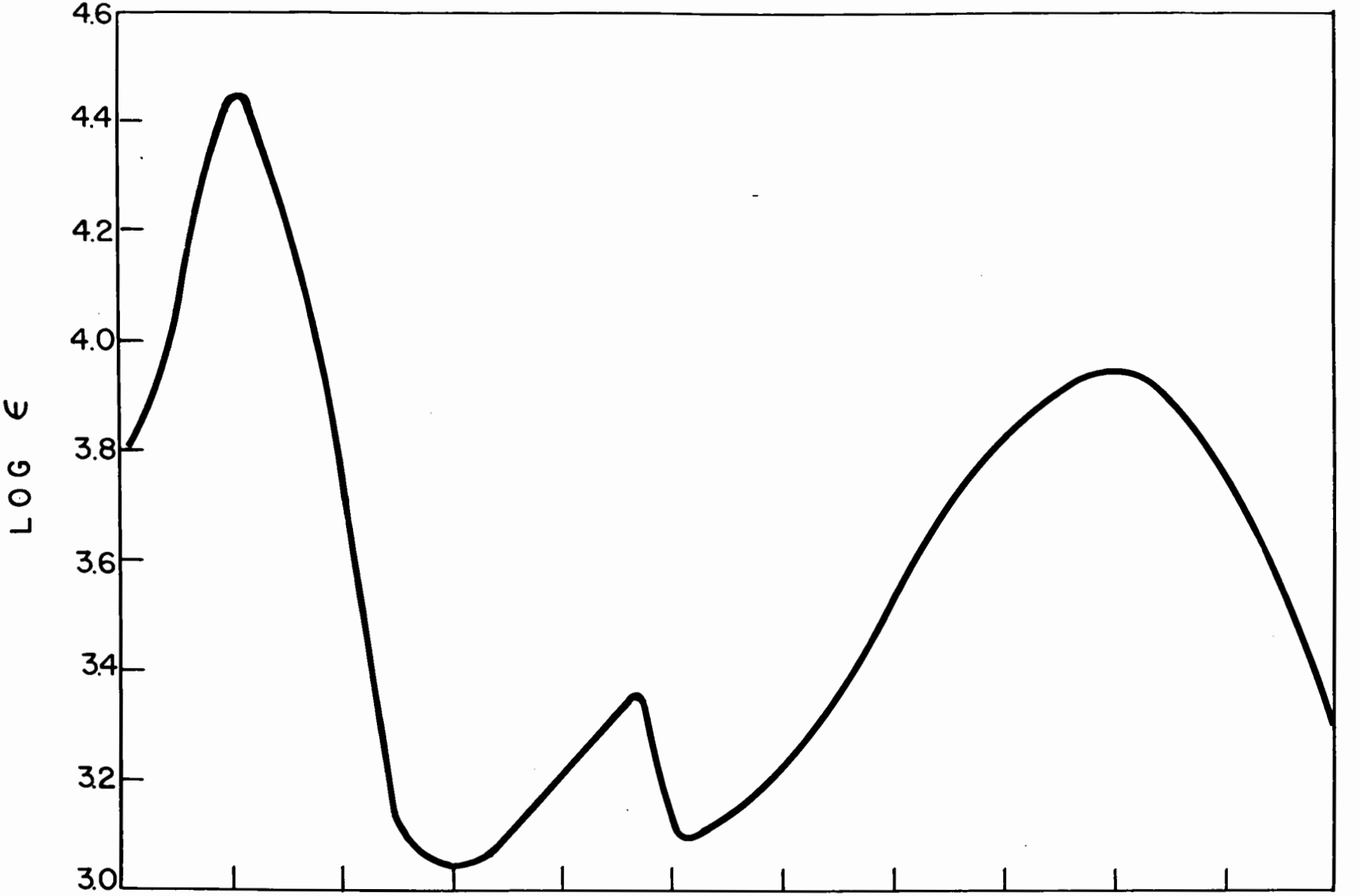
TABLE IV

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$	Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$
222	1.47	3.82	310	1.13	3.33
226	1.59	3.90	312	1.135	3.34
228	1.72	3.97	313	1.14	3.36
232	2.22	4.14	318	1.09	3.17
234	2.63	4.22	322	1.075	3.09
236	3.33	4.32	340	1.1	3.22
238	4.34	4.41	346	1.12	3.29
239	4.54	4.42	350	1.14	3.36
241	5.00	4.45	356	1.19	3.47
242	4.76	4.43	362	1.235	3.55
245	4.00	4.38	370	1.335	3.68
248	2.94	4.27	376	1.41	3.77
250	2.5	4.2	380	1.48	3.83
253	2.0	4.08	386	1.56	3.89
256	1.67	3.95	392	1.64	3.93
259	1.43	3.79	400	1.67	3.95
262	1.25	3.59	405	1.64	3.93
267	1.11	3.26	415	1.47	3.82
270	1.08	3.12	425	1.3	3.66
273	1.07	3.08	440	1.12	3.29
280	1.065	3.03			
290	1.075	3.09			
296	1.08	3.12			
302	1.11	3.25			
304	1.105	3.24			

FIGURE 5

Electronic Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine



ELECTRONIC ABSORPTION SPECTRUM OF
1-(3-HYDROXY-1-OXO-2-INDENYL) 3-METHYLPYRIDINIUM HYDROXIDE, BETAINE.

TABLE V

Electronic Absorption Spectrum of

3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

in Absolute Alcohol. Concentration 1 x 10^{-5} mole/litre

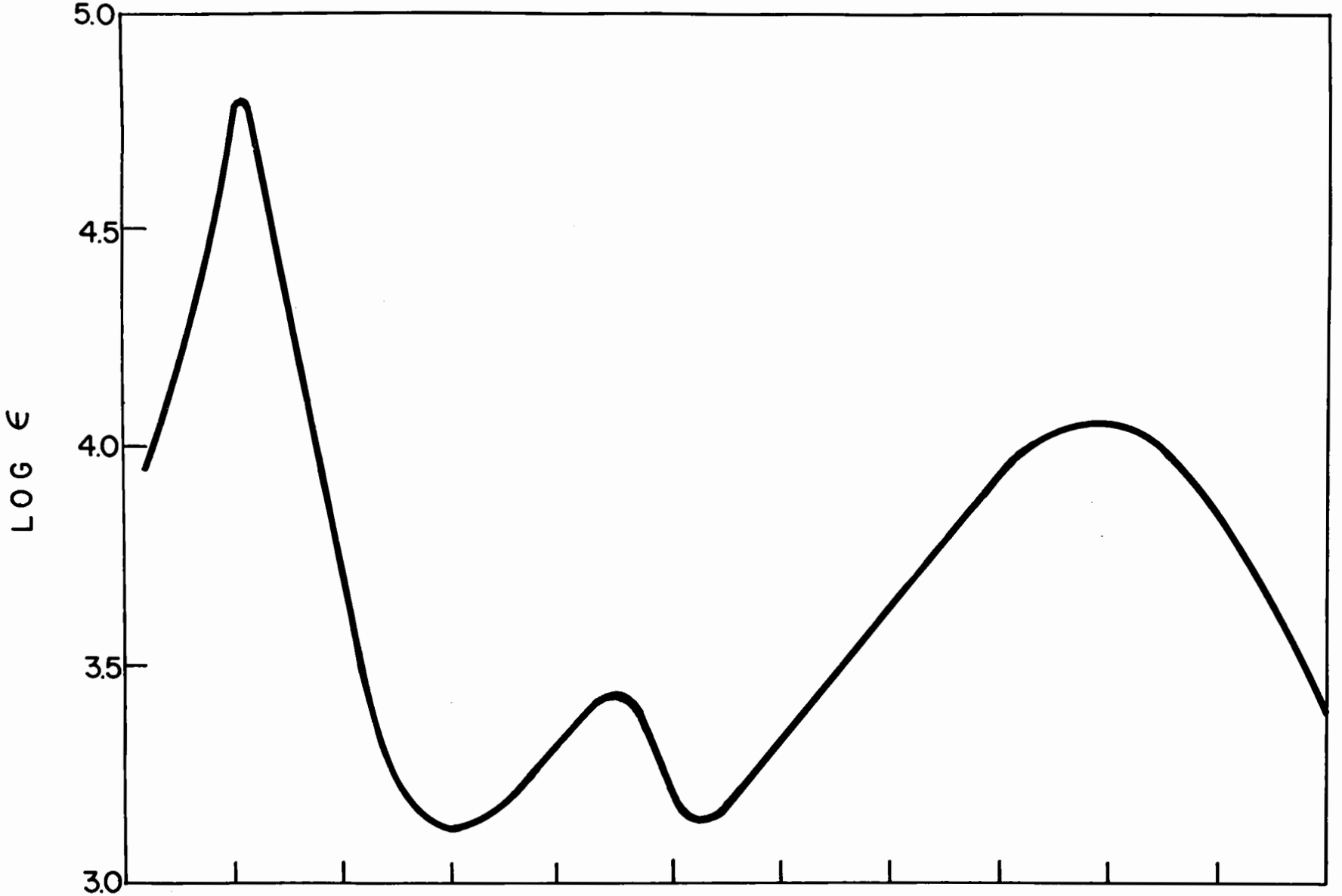
TABLE V

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$	Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$
224	1.66	3.94	322	1.09	3.17
227	1.85	4.05	330	1.09	3.17
230	2.27	4.25	338	1.125	3.3
234	3.7	4.36	340	1.115	3.27
236	5.9	4.49	350	1.18	3.46
239	20.0	4.72	358	1.25	3.59
241	40.0	4.81	364	1.335	3.68
245	10.0	4.60	370	1.43	3.79
248	5.0	4.45	378	1.59	3.90
251	3.23	4.34	386	1.75	3.99
256	1.92	4.05	398	1.94	4.05
260	1.45	3.84	405	1.89	4.04
269	1.11	3.26	415	1.67	3.95
274	1.09	3.17	425	1.41	3.77
280	1.08	3.12	430	1.3	3.66
287	1.09	3.17	445	1.11	3.25
297	1.11	3.26			
302	1.14	3.36			
312	1.172	3.44			

FIGURE 6

Electronic Absorption Spectrum of

3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine



ELECTRONIC ABSORPTION SPECTRUM OF
3-CARBOXY-1-(3-HYDROXY-1-OXO-2-INDENYL) PYRIDINIUM HYDROXIDE, BETAINE.

TABLE VI

Ultraviolet Absorption Spectrum of

1-(3-Oxo-2-cholestanyl)pyridinium bromide

in Absolute Alcohol. Concentration 1 x 10^{-4} mole/litre

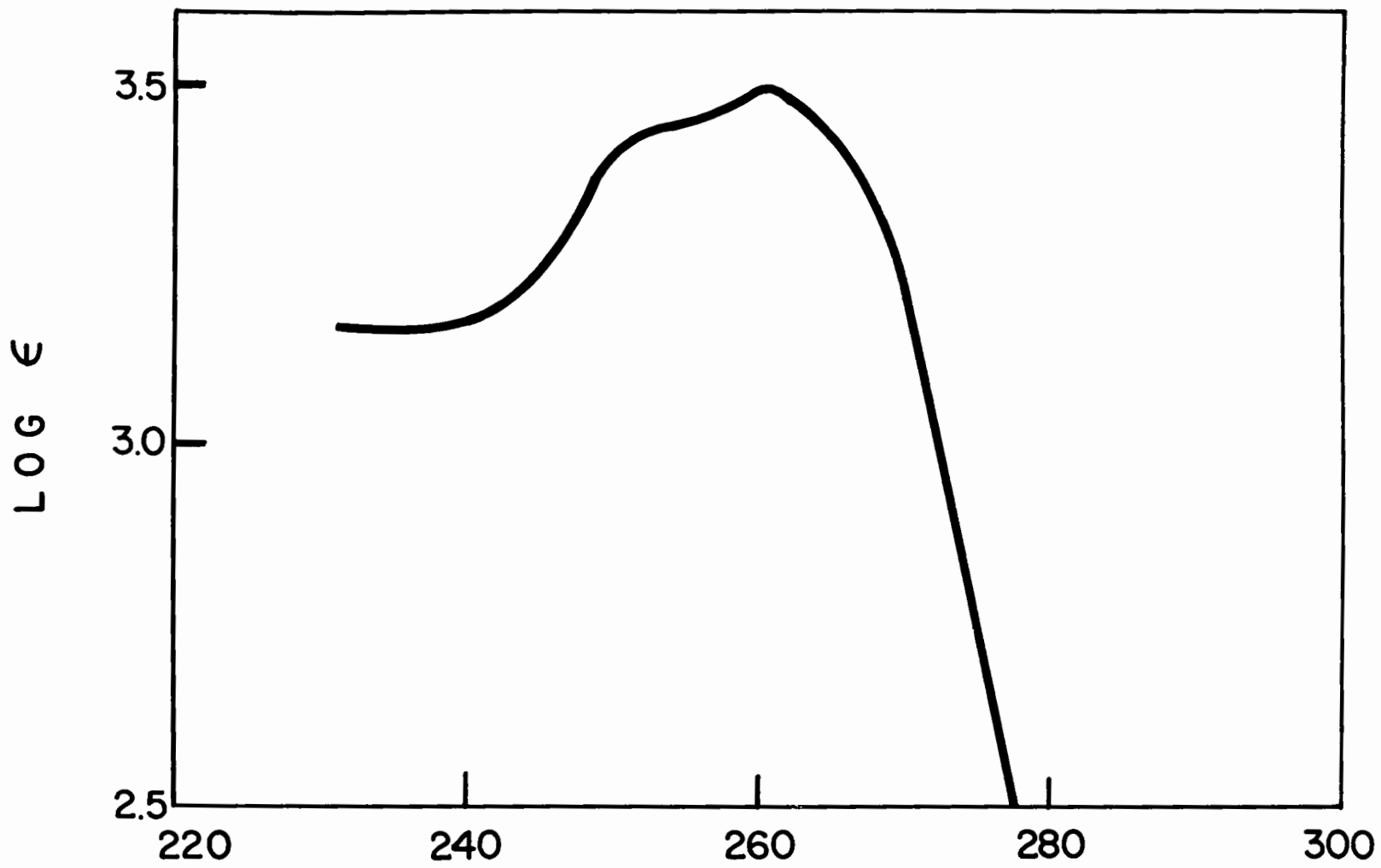
TABLE VI

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$
240	1.41	3.17
244	1.47	3.22
246	1.51	3.25
249	1.79	3.40
253	1.89	3.44
256	1.92	3.45
258	1.96	3.47
259	2.00	3.48
261	2.04	3.49
263	1.96	3.47
266	1.76	3.39
269	1.59	3.30
272	1.3	3.06
274	1.18	2.86
277	1.09	2.57
280	1.05	2.33

FIGURE 7

Ultraviolet Absorption Spectrum of

1-(3-Oxo-2-cholestanyl)pyridinium bromide



ULTRAVIOLET ABSORPTION SPECTRUM OF
1-(3-OXO-2-CHOLESTANYL) PYRIDINIUM BROMIDE.

TABLE VII

Electronic Absorption Spectrum of

1-(3-Hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine

in Absolute Alcohol. Concentration 1 x 10^{-4} mole/litre

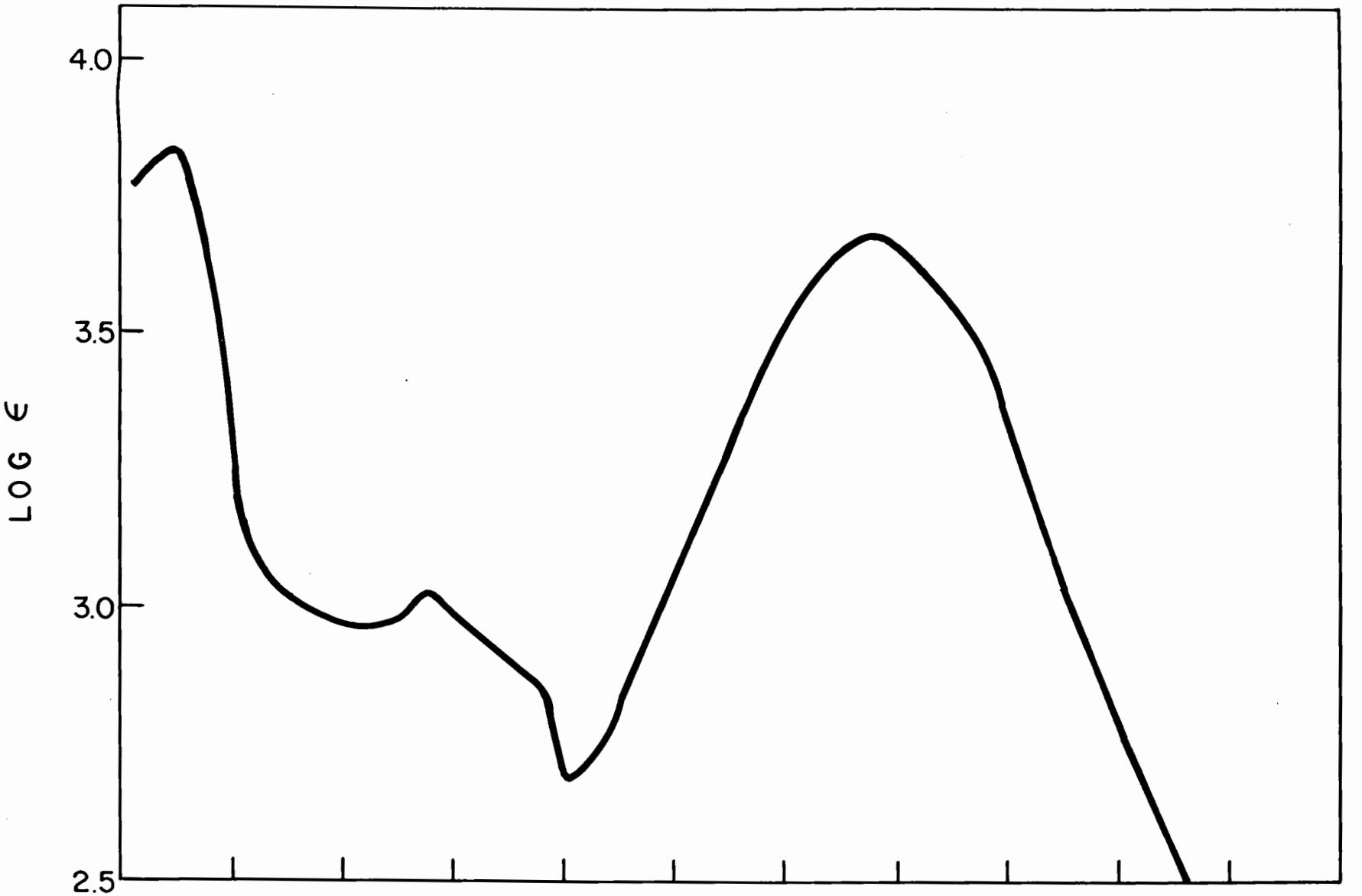
TABLE VII

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$	Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\varepsilon$
239	4.44	3.81	360	1.14	2.75
241	4.55	3.82	370	1.19	2.88
245	4.83	3.84	380	1.29	3.04
248	4.5	3.81	390	1.43	3.19
250	3.85	3.77	400	1.7	3.36
254	2.5	3.60	410	2.04	3.49
257	1.92	3.45	420	2.56	3.61
260	1.59	3.30	430	3.00	3.68
266	1.33	3.09	445	2.63	3.62
274	1.25	2.98	450	2.38	3.57
285	1.25	2.98	460	1.95	3.46
304	1.28	3.03	475	1.49	3.24
310	1.27	3.01	490	1.25	2.98
340	1.2	2.89	500	1.16	2.78
350	1.12	2.69	520	1.06	2.40

FIGURE 8

Electronic Absorption Spectrum of

1-(3-Hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine



ELECTRONIC ABSORPTION SPECTRUM OF
1-(3-HYDROXY-2-CHOLESTEN-2-YL) PYRIDINIUM HYDROXIDE, BETAINE.

TABLE VIII

Electronic Absorption Spectrum of

4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine

in Absolute Alcohol. Concentration 4×10^{-5} mole/litre

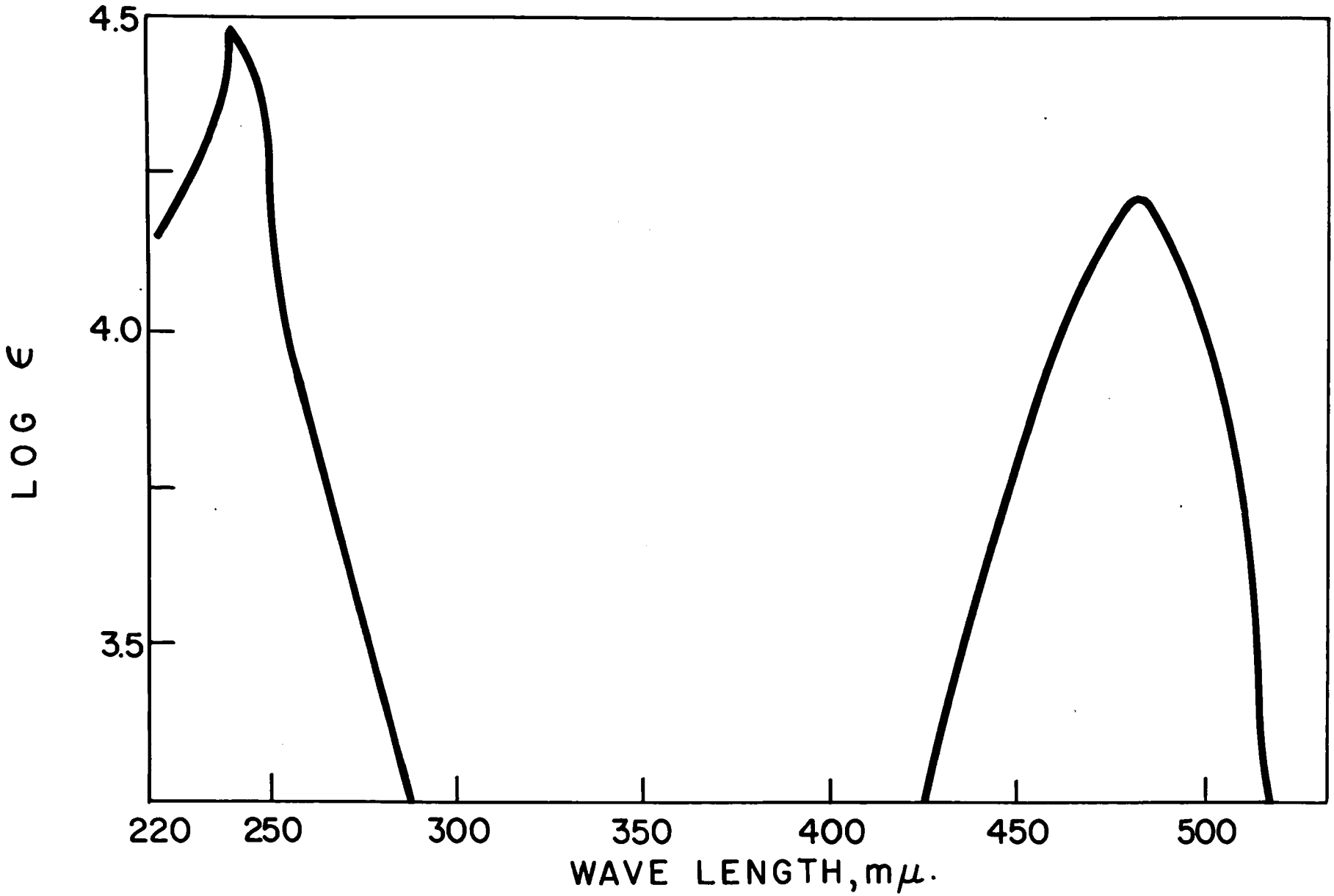
TABLE VIII

Wavelength mμ	$\frac{I_o}{I}$	$\log_{10}\epsilon$
220	3.85	4.16
223	4.34	4.20
230	4.76	4.23
236	6.67	4.31
239	10.0	4.40
243	16.0	4.48
247	10.0	4.4
249	6.67	4.31
253	3.33	4.11
260	1.72	3.78
270	1.47	3.60
276	1.47	3.60
283	1.39	3.54
298	1.11	3.04
425	1.18	3.23
435	1.32	3.48
450	1.76	3.78
465	2.94	4.08
480	4.76	4.23
495	2.94	4.08
510	1.43	3.50

FIGURE 9

Electronic Absorption Spectrum of

4-Carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine



ELECTRONIC ABSORPTION SPECTRUM OF
4-CARBOMETHOXY-1-(p-BROMO-PHENACYL)PYRIDINIUM HYDROXIDE, BETAINE.

TABLE IX

Infrared Absorption Maxima of

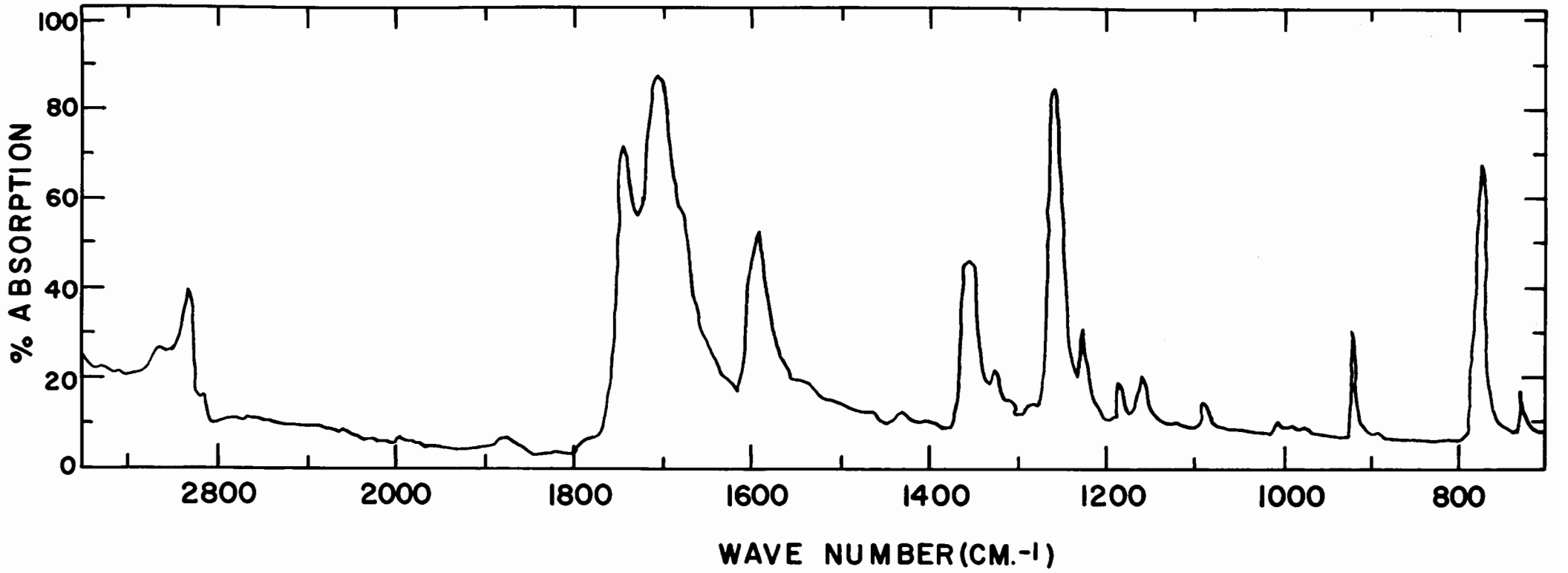
1,3-Indandione

Stretching region (1575 cm^{-1} - 3650 cm^{-1})	Bending region 1350 cm^{-1} - 1500 cm^{-1})	Fingerprint region (below 1350 cm^{-1})
cm^{-1}	cm^{-1}	cm^{-1}
1745 (s)	1355 (s)	1260 (v.s)
1705 (v.s)	1350 (w)	1230 (w)
		1185 (v.w)
		1160 (v.w)
		1090 (v.w)
		920 (w)
		775 (s)
		726 (v.w)

FIGURE 10

Infrared Absorption Spectrum of

1,3-Indandione



INFRARED SPECTRUM OF 1,3 - INDANDIONE

TABLE X

Infrared Absorption Maxima of

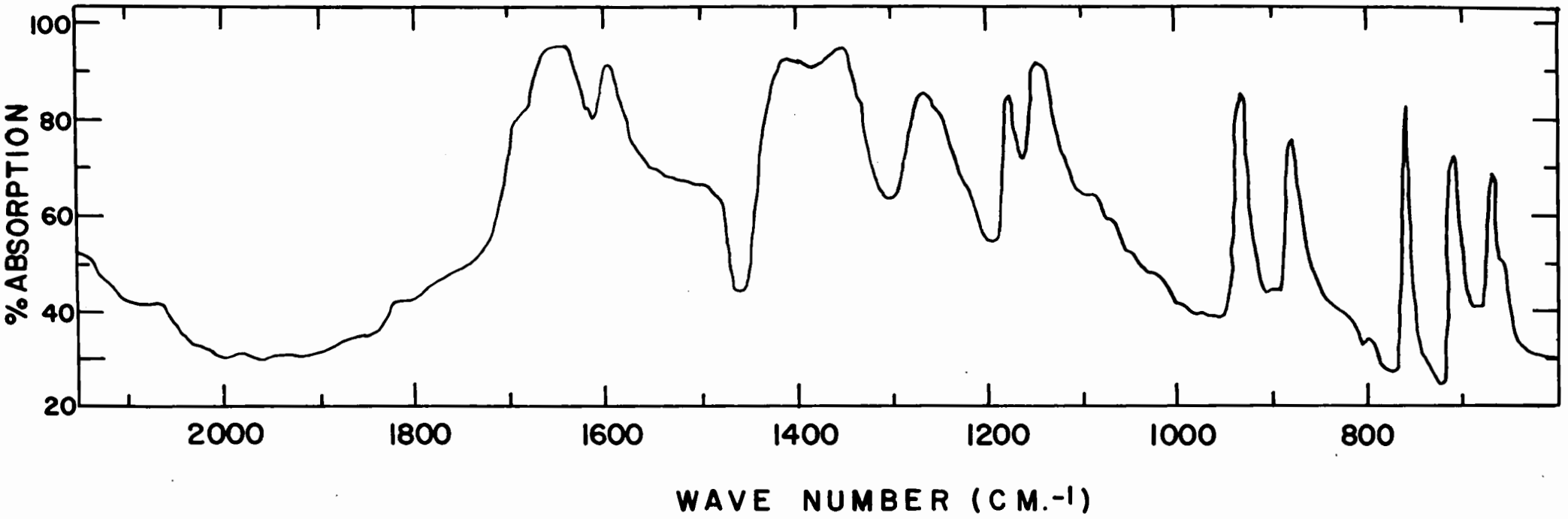
2-Nitro-1,3-indandione

Stretching region (1575 cm^{-1} - 3650 cm^{-1})	Bending region (1350 cm^{-1} - 1500 cm^{-1})	Fingerprint region (below 1350 cm^{-1})
cm^{-1}	cm^{-1}	cm^{-1}
1650 (v.s)	1400 (v.s)	1285 (w)
1595 (v.s)	1350 (v.s)	1265 (v.s)
		1177 (v.s)
		1145 (v.s)
		935 (v.s)
		880 (v.s)
		760
		710
		665

FIGURE 11

Infrared Absorption Spectrum of

2-Nitro-1,3-indandione



INFRARED SPECTRUM OF 2-NITRO-1,3-INDANDIONE

TABLE XI

Infrared Absorption Maxima of

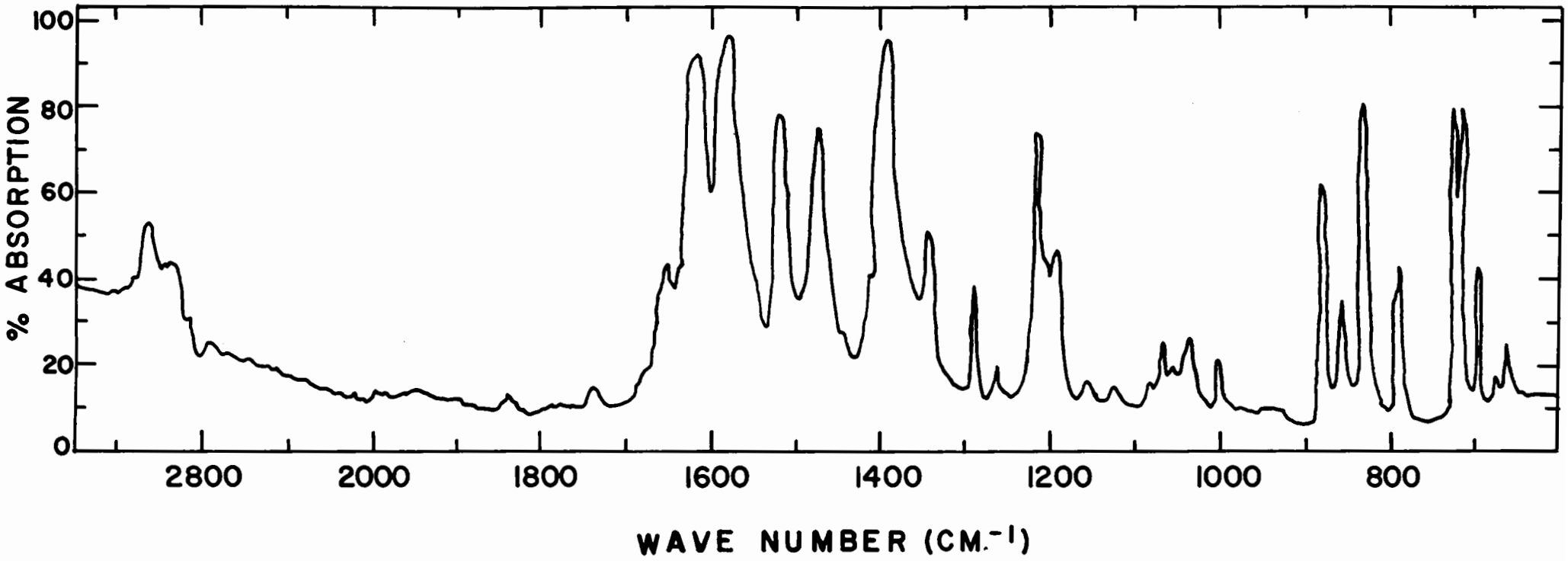
1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine

Stretching region (1575 cm^{-1} - 3650 cm^{-1})	Bending region (1350 cm^{-1} - 1500 cm^{-1})	Fingerprint region (below 1350 cm^{-1})
cm^{-1}	cm^{-1}	cm^{-1}
1655 (w)	1520 (v.s)	1345 (s)
1620 (v.s)	1475 (v.s)	1290 (w)
1585 (v.s)	1395 (v.s)	1262 (v.w)
		1217 (s)
		1193 (w)
		1070 (w)
		1037 (w)
		1005 (w)
		880 (s)
		856 (w)
		830 (v.s)
		790 (s)
		725 (v.s)
		717 (v.s)
		693 (w)
		663 (v.w)

FIGURE 12

Infrared Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine



INFRARED SPECTRUM OF

1-(3-HYDROXY-1-OXO-2-INDENYL)4-METHYLPYRIDINIUM HYDROXIDE, BETAINE.

TABLE XII

Infrared Absorption Maxima of

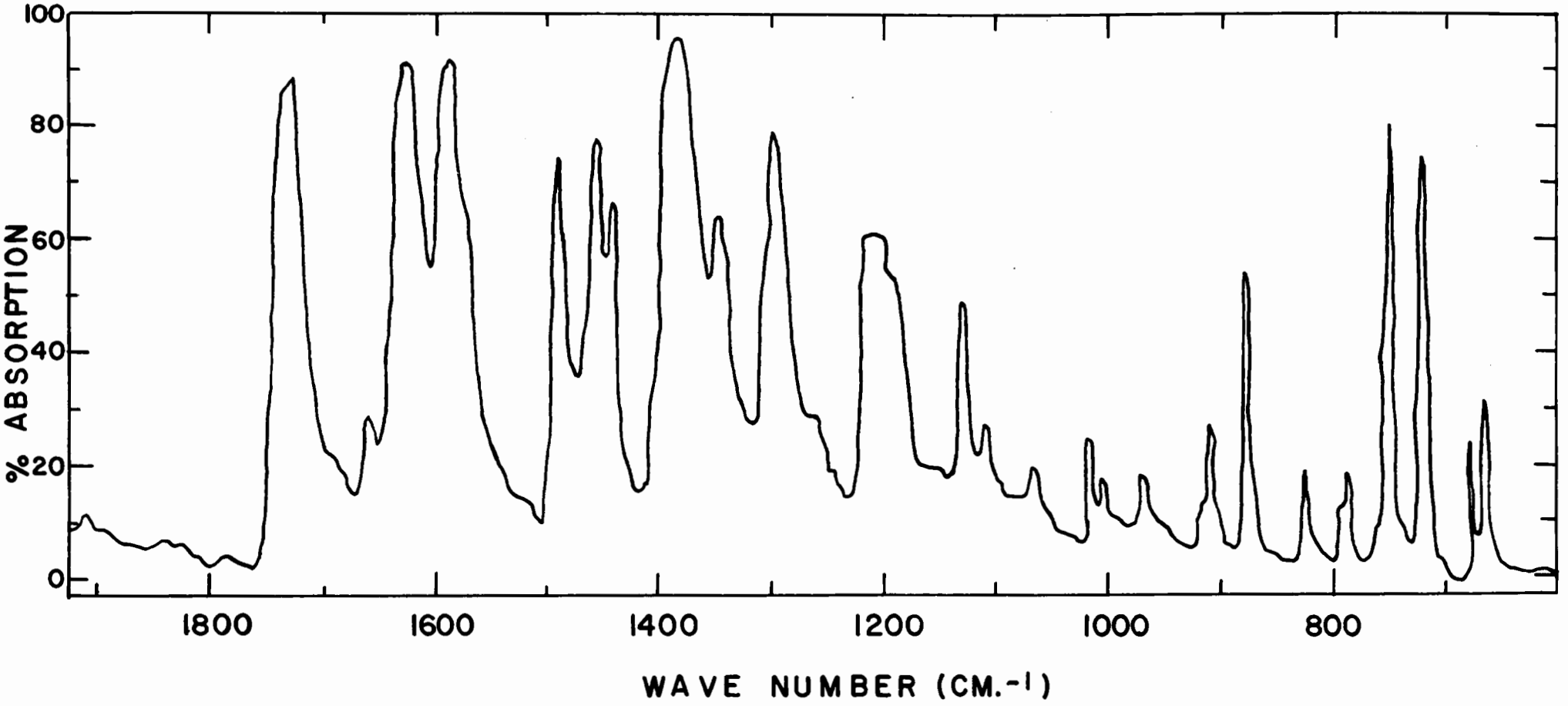
1-(3-Hydroxy -1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine

Stretching region (1575 cm-1 - 3650 cm-1)	Bending region (1350 cm-1 - 1500 cm-1)	Fingerprint region (below 1350 cm-1)
cm^{-1}	cm^{-1}	cm^{-1}
1730 (s)	1490 (v.s)	1300 (v.s)
1660 (v.w)	1457 (v.s)	1205 (s)
1630 (v.s)	1442 (s)	1110 (w)
1590 (v.s)	1385 (v.s)	1065 (w)
	1350 (s)	1017 (w)
		970 (w)
		910 (w)
		877 (s)
		825 (w)
		790 (w)
		750 (s)
		720 (v.s)
		663 (w)

FIGURE 13

Infrared Absorption Spectrum of

1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine



INFRARED SPECTRUM OF

1-(3-HYDROXY-1-OXO-2-INDENYL)3-METHYLPYRIDINIUM HYDROXIDE, BETAINE.

TABLE XIII

Infrared Absorption Maxima of

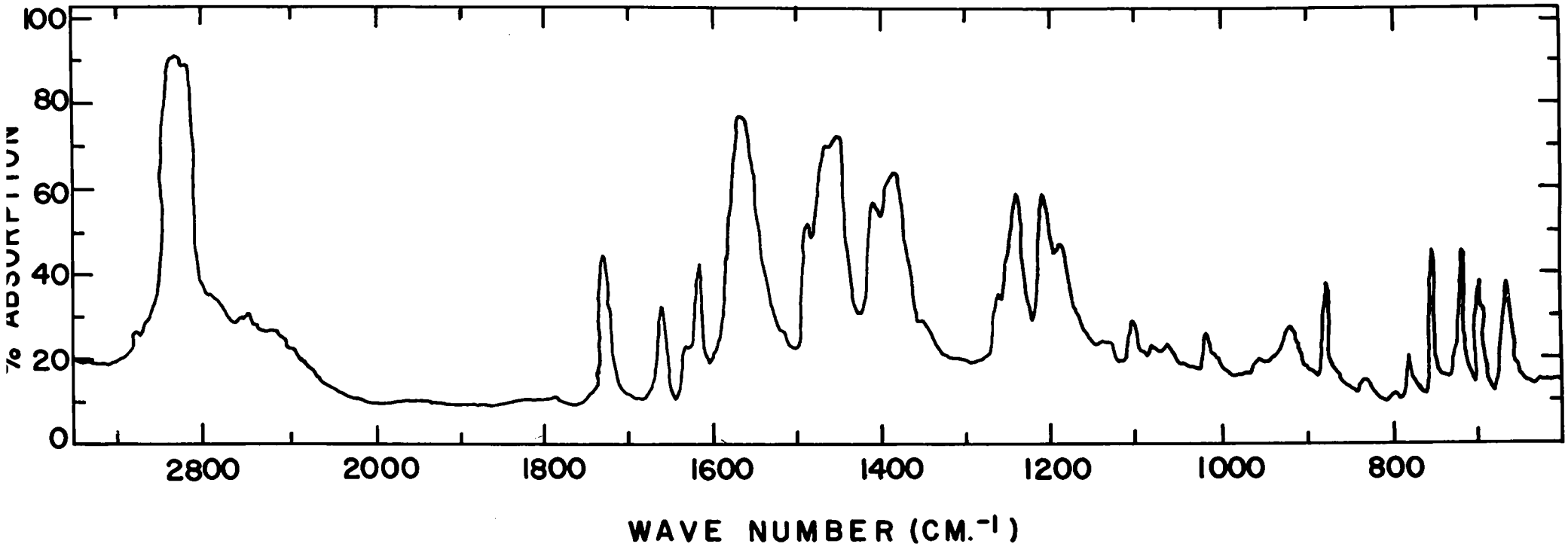
3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine

Stretching region (1575 cm-1 - 3650 cm-1)	Bending region (1350 cm-1 - 1500 cm-1)	Fingerprint region (below 1350 cm-1)
cm-1	cm-1	cm-1
1728 (s)	1490 (s)	1240 (s)
1660 (w)	1465 (s)	1210 (s)
1615 (s)	1450 (v.s)	1190 (s)
1565 (v.s)	1410 (s)	1105 (w)
	1385 (s)	1020 (w)
		920 (w)
		880 (s)
		780 (w)
		758 (s)
		718 (s)
		695 (s)
		665 (s)

FIGURE 14

Infrared Absorption Spectrum of

3-Carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine



INFRARED SPECTRUM OF

3-CARBOXY-1-(3-HYDROXY-1-OXO-2-INDENYL) PYRIDINIUM HYDROXIDE, BETAINE.

TABLE XIV

Infrared Absorption Maxima of

1,3-Indandione	2-Nitro 1,3-indandione	1-(3-Hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine	1-(3-Hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine	3-Carboxy-1-(3-hydroxy-1-oxo-2-indanyl)pyridinium hydroxide, betaine
1745 (s)			1730 (s)	1728 (s)
1705 (v.s)				
	1650 (v.s)	1655 (w)	1660 (v.w)	1660 (w)
		1620 (v.s)	1630 (v.s)	1615 (s)
1593 (s)	1595 (v.s)	1585 (v.s)	1590 (v.s)	1565 (v.s)
		1520 (v.s)		
		1475 (v.s)	1490 (v.s)	1490 (s)
			1457 (v.s)	1465 (s)
			1442 (s)	1450 (v.s)
	1400 (v.s)	1395 (v.s)		1410 (s)
			1385 (v.s)	1385 (s)
1355 (s)				
1350 (w)	1350 (v.s)	1345 (s)	1350 (s)	
	1285 (w)	1290 (w)	1300 (v.s)	
1260 (v.s)	1265 (v.s)	1262 (v.w)		
1230 (w)				1240 (s)
		1217 (s)	1205 (s)	1210 (s)
1185 (v.w)	1177 (v.s)	1193 (w)		1190 (s)
1160 (v.w)	1145 (v.s)			
			1110 (w)	1105 (w)
1090 (v.w)	1070 (w)	1065 (w)		
		1037 (w)	1017 (w)	1020(w)
		1005 (w)		
			970 (w)	
920 (w)	935 (v.s)		910 (w)	920 (w)
	880 (v.s)	880 (s)	877 (s)	880 (s)
		856 (w)		
		830 (v.s)	825 (w)	
		790 (s)	790 (w)	780 (w)
775 (s)	760		750 (v.s)	758 (s)
		725 (v.s)		
726 (v.w)		717 (v.s)	720 (v.s)	718 (s)
	710			
		693 (w)		695 (s)
	665	663 (v.w)	663 (w)	665 (s)

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. An investigation of the reactions of pyridine, 3-methylpyridine and 4-methylpyridine with 2-bromo-1,3-indandione was carried out under a variety of experimental conditions. The corresponding pyridinium betaines of the 1,3-indandione series were obtained, but all efforts to isolate the intermediate addition product were unsuccessful.

2. A reaction mechanism was proposed to explain how the reaction of pyridine bases with 2-bromo-1,3-indandione proceeded, and why the intermediate pyridinium salt in this reaction could not be isolated.

3. When 2-methylpyridine was reacted with 2-bromo-1,3-indandione a polymer was formed instead of the desired betaine. The failure of this reaction to yield an inner salt was attributed to the proximity of the methyl group to the annular nitrogen rather than to steric hindrance. This proximity made the methyl group susceptible to an electrophilic attack by a bromonium ion which initiated polymerization.

4. The reaction of the mono-methylpyridines with bromine was reinvestigated with the hope that addition compounds analogous to those obtained from the reaction of pyridine and bromine could be isolated. However negative results were obtained irrespective of whether 1,3-indandione was present or not.

5. Pyridine, 3- and 4-methylpyridines, and pyridinecarboxylic acids were allowed to react with 2,2-dibromo-1,3-indandione. From these reactions the corresponding pyridinium betaines, pyridine hydrobromides, and polymeric materials were obtained. In no case could the intermediate pyridinium salts be isolated. 2-Methylpyridine failed to produce the corresponding betaine when reacted with 2,2-dibromo-1,3-indandione.

6. A mechanism was proposed for the reaction of 2,2-dibromo-1,3-indandione with pyridine bases. This mechanism was based on a nucleophilic attack by the pyridine base and was supported by the fact that no free bromine could be detected during these reactions.

7. The electronic and infrared spectra of the pyridinium betaines of the 1,3-indandione series were determined and constituted additional evidence that these compounds are dipolar ions. In addition, the spectra of the betaines prepared using pyridinecarboxylic acids as the pyridine base showed that the carboxylic group is not the anionic function of the inner salt. The latter is a typical enol betaine. In the spectra of the pyridinium betaines of the 1,3-indandione series three absorption maxima occurred in the ultraviolet and visible regions, at 237-243 mμ, 311-316 mμ and 386-418 mμ .

8. Attempts to react 2-bromo-5,5-dimethyl-1,3-cyclohexanedione with pyridine were unsuccessful, and their failure was attributed to the strong positive character of the bromine atom present in 2-bromo-5,5-dimethyl-1,3-cyclohexanedione.

9. When 2,2-dibromo-5,5-dimethyl-1,3-cyclohexanedione reacted with pyridine and pyridinecarboxylic acids, there were indications that the corresponding betaines were formed. However the products obtained were not crystalline and all efforts to purify them failed.

10. The product of the reaction between 2α-bromo-3-cholestanone and pyridine, whose structure previously had been the subject of much dispute, was shown to be a pyridinium salt. Similar salts were prepared by reacting 2α-bromo-3-cholestanone with 3- and 4-methylpyridines. A mixture of steroidal compounds, which contained no bromine or nitrogen were obtained when 2-methylpyridine was allowed to react with 2α-bromo-3-cholestanone.

11. An explanation was offered for the difference observed in the way in which pyridine bases reacted with 2α-bromo-3-cholestanone.

12. The reaction of 7α-bromocholestane-3β,5α-diol-6-one 3-acetate with pyridine was undertaken so that a comparison could be made between this reaction and that of pyridine with 2α-bromo-3-cholestanone. In this way, it was hoped that the effect of the conformation of the bromine atom in α-bromo-ketosteroids could be determined. However, 7α-bromocholestane-3β, 5α-diol-6-one 3-acetate resisted all efforts to eliminate hydrogen bromide and attempts to form the corresponding pyridinium salt.

13. The electronic absorption spectra of the salts and betaines obtained from the reaction of 2α-bromo-3-cholestanone and pyridine bases were determined. These spectra justified the structures assigned to these substances. Three absorption maxima were observed in the spectra of these betaines, in the regions 245-255 mμ, 304-314 mμ and 430-432 mμ.

14. The reactions of 3- and 4-pyridinecarboxylic acids with 2-bromo-acetophenone were investigated. It was found that the formation of the corresponding pyridinium salt was accompanied by esterification of the carboxylic group. The alcoholic part of the ester was supplied by 2-bromo-acetophenone after elimination of a molecule of hydrogen bromide.

15. During the course of the alkaline cleavage of the salts obtained from the reaction between pyridinecarboxylic acids and 2-bromo-acetophenone, the corresponding betaines were isolated. The electronic absorption spectra of these unstable betaines were similar in pattern to those prepared by treating with alkali, the salts obtained from the reaction of 2-bromo-acetophenone with esters of pyridinecarboxylic acids.

16. The alkaline cleavage of salts prepared by reacting pyridinecarboxylic acids with 2-bromo-acetophenone was found to proceed in an unusual way,

and resulted in the liberation of the corresponding pyridinecarboxylic acid.

17. The reaction of pyridine with monochloroacetic acid was reinvestigated. Both the yield and purity of the pyridinium salt and the betaine, which were products of this reaction, were greatly improved.

18. The reaction of β-chloropropionic acid with quinoline and isoquinoline yielded the corresponding salts in low yields. However, attempts to synthesize the betaines by eliminating hydrogen chloride from the salts failed.

19. Efforts to introduce a pyridinecarboxylic acid function into a steroid molecule were made under a variety of experimental conditions. However only in the case of the reaction of methyl 3-pyridinecarboxylate with cholesteryl p-toluenesulphonate was the corresponding salt obtained.

20. Attempts to hydrolyze the carbomethoxy ester group of 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate ended in failure and consequently the formation of pyridinium betaines in steroidal compounds possessing a carboxylic group as the anionic function was not achieved.

21. The following compounds, previously unreported have been prepared and characterized:

(a) 1-(3-hydroxy-1-oxo-2-indenyl)4-methylpyridinium hydroxide, betaine (p. 131)

(b) 1-(3-hydroxy-1-oxo-2-indenyl)3-methylpyridinium hydroxide, betaine (p. 137)

(c) 4-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (p. 143)

(d) 3-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (p. 143)

(e) 2-carboxy-1-(3-hydroxy-1-oxo-2-indenyl)pyridinium hydroxide, betaine (p. 144)

(f) 1-(3-hydroxy-2-cholesten-2-yl)pyridinium hydroxide, betaine (p. 155)

(g) 1-(3-oxo-2-cholestanyl)4-methylpyridinium bromide (p. 156)

(h) 1-(3-hydroxy-2-cholesten-2-yl)4-methylpyridinium hydroxide, betaine (p. 156)

(i) 1-(3-oxo-2-cholestanyl)3-methylpyridinium bromide (p. 157)

(j) 1-(3-hydroxy-2-cholesten-2-yl)3-methylpyridinium hydroxide, betaine (p. 158)

(k) 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (p. 162)

(l) 4-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine (p. 162)

(m) 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium bromide (p. 163)

(n) 3-carbobenzoylmethoxy-1-(phenacyl)pyridinium hydroxide, betaine (p. 164)

(o) 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium bromide (p. 164)

(p) 4-carbomethoxy-1-(p-bromo-phenacyl)pyridinium hydroxide, betaine (p. 165)

(q) 3-carbomethoxy-1-(5-cholesten-3-yl)pyridinium p-toluenesulphonate (p. 173).

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