MING P. LI PH.D.

BIOLOGICAL ACTIVITY OF 18-HYDROXYDEOXYCORTICOSTERONE

CHEMICAL SYNTHESIS OF 18-HYDROXYDEOXYCORTICOSTERONE AND SOME ASPECTS OF ITS BIOLOGICAL ACTIVITY

by

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Ph.D. Experimental Medicine

ABSTRACT

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Chemical Synthesis of 18-Hydroxydeoxycorticosterone and Some Aspects of its Biological Activities

A simple synthesis of 18-OH-DOC (20,21-dihydroxy-18,20-epoxy-Δ^4-pregnen-3-one) via 18-hydroxyprogesterone was accomplished. An improved procedure for the preparation of 18-hydroxyprogesterone starting from deoxycorticosterone acetate was developed. The conversion of 18-OH progesterone to 18-OH-DOC was achieved by a new, two-step synthesis. Several steroids with 18,20-cyclohemiketal and 18,20-cyclobutanol structures were isolated and identified, and their mass spectra analyzed.

18-OH-DOC was found to be a hypertensive agent equipotent with deoxycorticosterone in the unilaterally nephrectomized rat maintained on saline. In the adrenalectomized rat, 18-OH-DOC caused sodium retention, affected potassium excretion only at a high dose, and the ratio of the retention of water to sodium was greater than observed with deoxycorticosterone. 18-OH-DOC did not exert a negative feedback effect upon the pituitary adrenal axis of rat but, rather, caused an increased corticosteroid output. It reduced carrageenan-induced edema in the rat paw.

A derivative of metopirone, 2-methyl-1,2-bis(3'-pyridyl)-1-propanol was synthesized from metopirone and was found to cause a three fold enhancement of the biotransformation of deoxycorticosterone to 18-OH-DOC by the rat adrenal in vitro.

Ph.D. Experimental Medicine

ABSTRACT

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Synthèse Chimique de la 18-Hydroxydéoxycorticostérone et Quelques Aspects de son Activité Biologique

Une synthèse simple du 18-OH-DOC (20,21-dihydroxy-18,20-époxy-Δ^4-prégnèn-3-one) via la 18 hydroxyprogestérone fut accomplie. Un procédé amelioré pour la préparation de la 18-hydroxyprogestérone à partir de l'acétate de déoxycorticostérone a été développé. La conversion de la 18-OH progestérone en 18-OH-DOC fut effectuee par une nouvelle synthèse comprenant deux étapes. Plusieurs stéroïdes ayant la structure 18,20-cyclohémicétal et 18,20-cyclobutanol furent isolés et identifiés, et leurs spectres de masse analysés.

Le 18-OH-DOC s'est avéré un agent provoquant l'hypertension au même titre que la déoxycorticostérone chez le rat ayant subi une néphrectomie unilatérale et maintenu avec du sérum physiologique. Dans le cas des rats dont les glandes adrénales avaient été enlevées, 18-OH-DOC a causé une rétention du sodium, affectant l'excrétion du potassium seulement à forte dose; le rapport de la rétention de l'eau à celle du sodium fut supérieur au rapport observé avec la déoxycorticostérone. 18-OH-DOC n'a pas exercé de "feedback" négatif sur l'axe pituitaire-adrénale du rat mais plutôt un taux de production accru de corticosteroïdes.

Il a réduit l'oedème provoqué par la carrageen dans la patte de rat.

Un dérivé de la métopirone, le 2-méthyl-1,2-bis(3'-pyridyl)-1-propanol, fut synthétisé à partir de la métopirone et a causé, dans la glande adrénale de rat, in vitro, une biotransformation de la déoxycorticostérone au 18-OH-DOC trois fois supérieure.

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PURPOSE OF THE INVESTIGATION

18-OH-DOC (20,21-dihydroxy-18,20-epoxy-Δ^4-pregnen-3-one) was identified in 1961 as a major naturally occurring mineralocorticoid in the rat by Birmingham and Ward and independently by Péron. It has now been established that 18-OH-DOC is a natural secretory product of the human adrenal cortex as well. In the adrenalectomized rat, 18-OH-DOC has antidiuretic action and sodium-retaining properties. The nature and extent of the physiological activity of 18-OH-DOC have not been well studied. In rats and in man, 18-OH-DOC has been implicated in the etiology of hypertension. However, direct assessment of the hypertensive effect of 18-OH-DOC by injection into the rat had not been performed, because of the unavailability of the compound in quantities large enough for this type of assay. It was the purpose of the work described here to find a simple method for the synthesis of 18-OH-DOC for adequate exploration of its possible biological activity.

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PART I: ADRENAL 18-OH-DOC

Introduction and Literature Review

1. General consideration of the adrenal gland:

In higher vertebrates the adrenal gland is a composite of two endocrine structures. The inner region or medulla consists of cells of neural origin, whereas the outer region, or cortex, is derived from mesodermal glandular tissue. The nature of the secretory products and their physiological effects are distinct for each of these two sections of the adrenal. The hormones of the cortex are steroids, whereas those of the medulla are catecholamines. The adrenal cortex, as opposed to the adrenal medulla, is essential to life. The steroid hormones of the adrenal cortex exert effects on the metabolism of electrolytes and thus on water metabolism; and on the metabolism of proteins, carbohydrates, and fats. The steroids produced by the adrenals are secreted into the blood stream and carried to the tissues where they perform their functions. In various tissues, but mainly in the liver, the steroids are metabolized or conjugated to inactive forms which are then excreted by the kidney.

Histological studies have revealed that the cells of the adrenal cortex are arranged into three layers (1). The outer layer, which is the thinnest, is called the zona glomerulosa. The middle layer is called the zona fasciculata; this region is the widest of

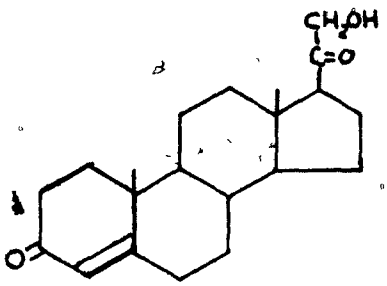
the layers. The innermost layer is called the zona reticularis. Histochemical examination of the adrenal cortex has provided some information about the physiology of the adrenal gland. Administration of deoxycorticosterone to hypophysectomized rats, was shown by Greep and Deane in 1949 (2) to depress the activity of the cells in the zona glomerulosa, as indicated by decreasing numbers of lipid droplets in this zone. The cells in the zona fasciculata, on the other hand, remained unaltered. Deane and Seligman in 1953 (3), demonstrated that the administration of adrenocorticotropic hormone resulted in stimulation of cellular activity in the zona fasciculata of the rat, as evidenced by the accumulation of lipid droplets and the broadening of this zone. The cells in the zona glomerulosa remained normal. These authors suggested that the zona glomerulosa might be the source of hormones responsible for sodium retention and that the zona fasciculata might be the source of the hormones responsible for changes in carbohydrate and protein metabolism. Giroud, Stachenko, and Venning compared the steroid production by rat (4) and by beef (5) adrenal capsules with that of the corresponding decapsulated glands incubated in vitro and suggested that specific pathways of corticoid biosynthesis occur within different zones of the adrenal cortex. This would explain how it is possible for the cortical cells from different zones to synthesize different kinds of steroids

from an identical precursor.

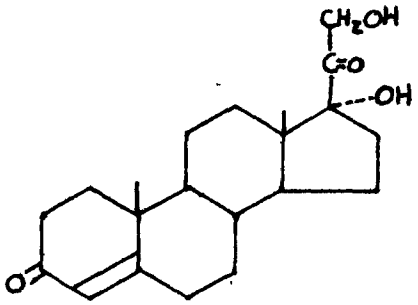
2. Characterization of adrenal corticosteroids:—

(a) Isolation and identification:

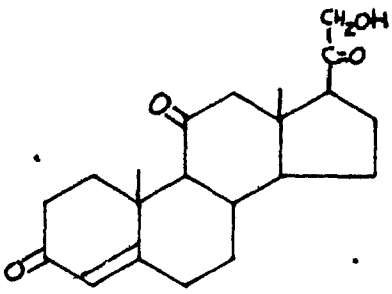
In 1927, Rogoff and Stewart (6) demonstrated that the survival period of adrenalectomized dogs was prolonged by injection of saline extract of whole adrenal glands. An adrenal extract, made with organic solvents, which prolonged the life of adrenalectomized cats was described by Hartman and Brownell (7) in 1939. In 1936, Mason, Myers, and Kendall (8), Wintersteiner and Pfiffner (9), and Reichstein (10) isolated crystalline corticosteroids with marked adrenocortical activity from adrenal extracts. In 1937, Steiger and Reichstein (11) synthesized deoxycorticosterone acetate from stigmasterol, and deoxycorticosterone was later shown by Reichstein and von Euw (12) to be present in minute amounts in adrenal gland extracts. In 1952, Tait, Simpson, and Grundy (13, 14) isolated from beef adrenal extract an amorphous residue possessing a far more potent sodium-retaining activity than deoxycorticosterone. Simpson and Tait, in collaboration with Wettstein, Neher, von Euw, Schindler and Reichstein in 1954 (15) were able to elucidate the chemical structure of this amorphous residue as the 18-aldehyde of corticosterone, later renamed aldosterone. By 1960, about fifty steroids had been obtained from the cortical component of the adrenal gland (16). The compounds that were shown to exhibit glucocorticoid or mineralocorticoid activity are depicted in Fig. A.



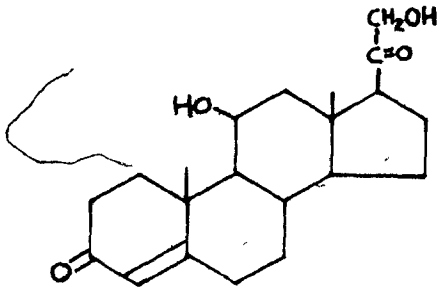
11-Deoxycorticosterone
(DOC)



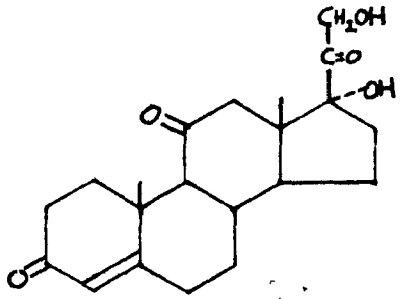
11-Deoxy-17α-hydroxycorticosterone
(11-deoxycortisol)



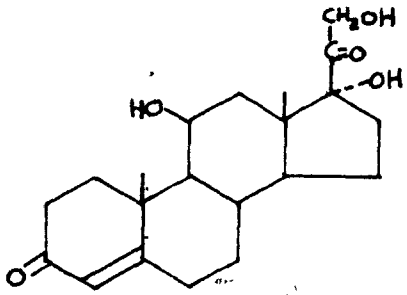
11-Dehydrocorticosterone



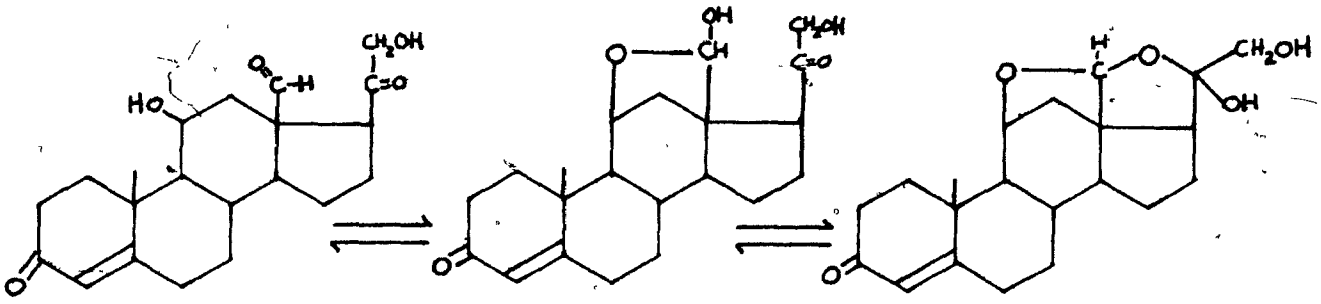
Corticosterone



Cortisone



Cortisol



Aldosterone

Fig. A Some biologically active C_{21} adrenal cortical steroids (16)

The biosynthetic derivation of these steroids from cholesterol is outlined in a later section, as illustrated in Fig. C.

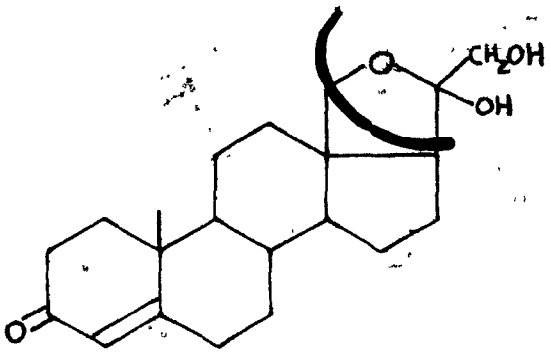
The mineralocorticoid 18-OH-DOC was isolated from rat adrenals in 1961 (17, 18) and from human adrenals in 1970 (19, 20) and will be dealt with in a separate section.

(b) Relationship between structure and physiological action of adrenal steroids:

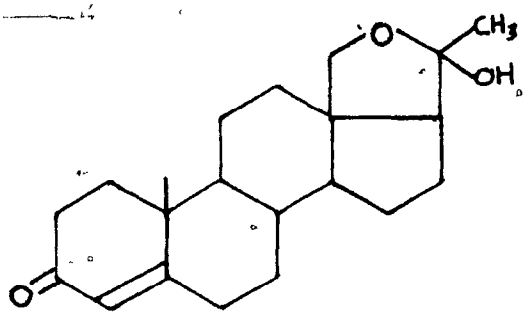
The relationship between the structure and properties of adrenal corticoids was reviewed by Heard in 1948 (21). Three structural features appear to be essential for adrenocortical activity with respect to life maintenance and salt and water metabolism: a Δ^4-3-ketone function, the α-ketol group in the sidechain, and the beta configuration of the sidechain at C-17. Adrenocortical steroids bearing an oxygen atom at the C-11 position, either as an hydroxyl or a ketone group, exert their principal activity on carbohydrate metabolism and have only minor effects on electrolyte and water metabolism. The former effect is classified as glucocorticoid activity (23) and is intensified by the presence of an hydroxyl group at the C-17 position (21, 22). The most important glucocorticoids are cortisol, cortisone, corticosterone, and 11-dehydrocorticosterone (16, 21, 22). Adrenal steroids bearing no oxygen atom at the C-11 position exert potent effects on electrolyte and water metabolism

while the actions on carbohydrate metabolism are markedly diminished (21, 22). Deoxycorticosterone and 11-deoxycortisol belong to this class (16, 21, 22) and are termed mineralocorticoids (23). Aldosterone, the most potent electrolyte-regulating steroid of the adrenal, is an exception; it contains an aldehyde group at the C-18 position in addition to a hydroxyl function at the C-11 position.

Kagawa and Pappo in 1962 (87) studied the structure-activity relationships of some synthetic 18-hydroxy and 18-deoxy steroids and found that 18-OH-DOC-21-acetate was less effective as a mineralocorticoid than DOC acetate. 18-OH Progesterone, lacking an hydroxyl group at the C-21 position (present in 18-OH-DOC), was shown to be less effective as a mineralocorticoid than 18-OH-DOC.

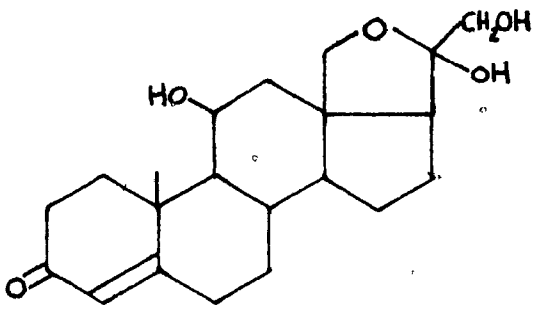


18-OH-DOC

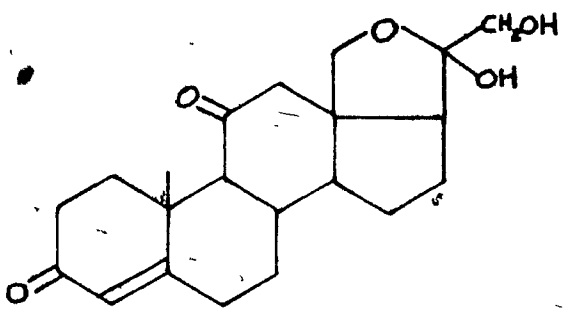


18-OH Progesterone

The unusual chemical feature of the mineralocorticoid 18-OH-DOC among the adrenal steroids is the masking of the 20-ketone by the formation of an 18,20-cyclohemiketal. This cyclic hemiketal structure has been shown so far to occur in only two other Δ^4-3-ketonic adrenal steroids, 18-hydroxycorticosterone and 18-hydroxy-11-dehydrocorticosterone, both of unknown biological function (85, 86, 55).



18-Hydroxycorticosterone



18-Hydroxy-11-dehydrocorticosterone

The biological activity of 18-OH-DOC will be discussed in a separate section.

3. The influence of corticotropin (ACTH) on adrenal steroid production:

Haynes and Berthet in 1957 (24) proposed a theory for the mechanism of action of ACTH in stimulating adrenal steroid biosynthesis. In short, to quote from Hilf (25), that ACTH stimulates the formation

of cyclic adenosine-3',5'-monophosphate (3',5'-AMP), which in turn activates phosphorylase, by the conversion of the inactive form of this enzyme to the active form. Active phosphorylase can then mediate the conversion of glycogen to glucose-1-phosphate (G-1-P), the G-1-P can be converted to glucose-6-phosphate (G-6-P) via phosphoglucomutase, and G-6-P can then be metabolized via the hexose monophosphate shunt. The metabolism of G-6-P by this pathway results in the production of reduced NADP (NADPH), as G-6-P is converted to ribulose-5-phosphate by G-6-P dehydrogenase and 6-phosphogluconate dehydrogenase. Ultimately, the production of NADPH furnishes the necessary co-factor for several steps of steroidogenesis, thus enabling the adrenal gland to synthesize steroids at an increased rate. A summary of the metabolic pathways associated with the action of ACTH on the adrenal cortex, as reported in a review by Hilf in 1965 (25), is depicted in Fig. B. The studies by Grahame-Smith, Butcher, Ney and Sutherland in 1967 (26) with rat adrenals, showed that ACTH increases the concentration of 3',5'-AMP in adrenal quarters in vitro, in intact adrenals in vivo, and in adrenal homogenates. These authors suggested that ACTH acts by stimulating adenyl cyclase activity. Recently, Grower and Bransome (27) proposed that 3',5'-AMP activates the formation of a protein controlling steroid biosynthesis.

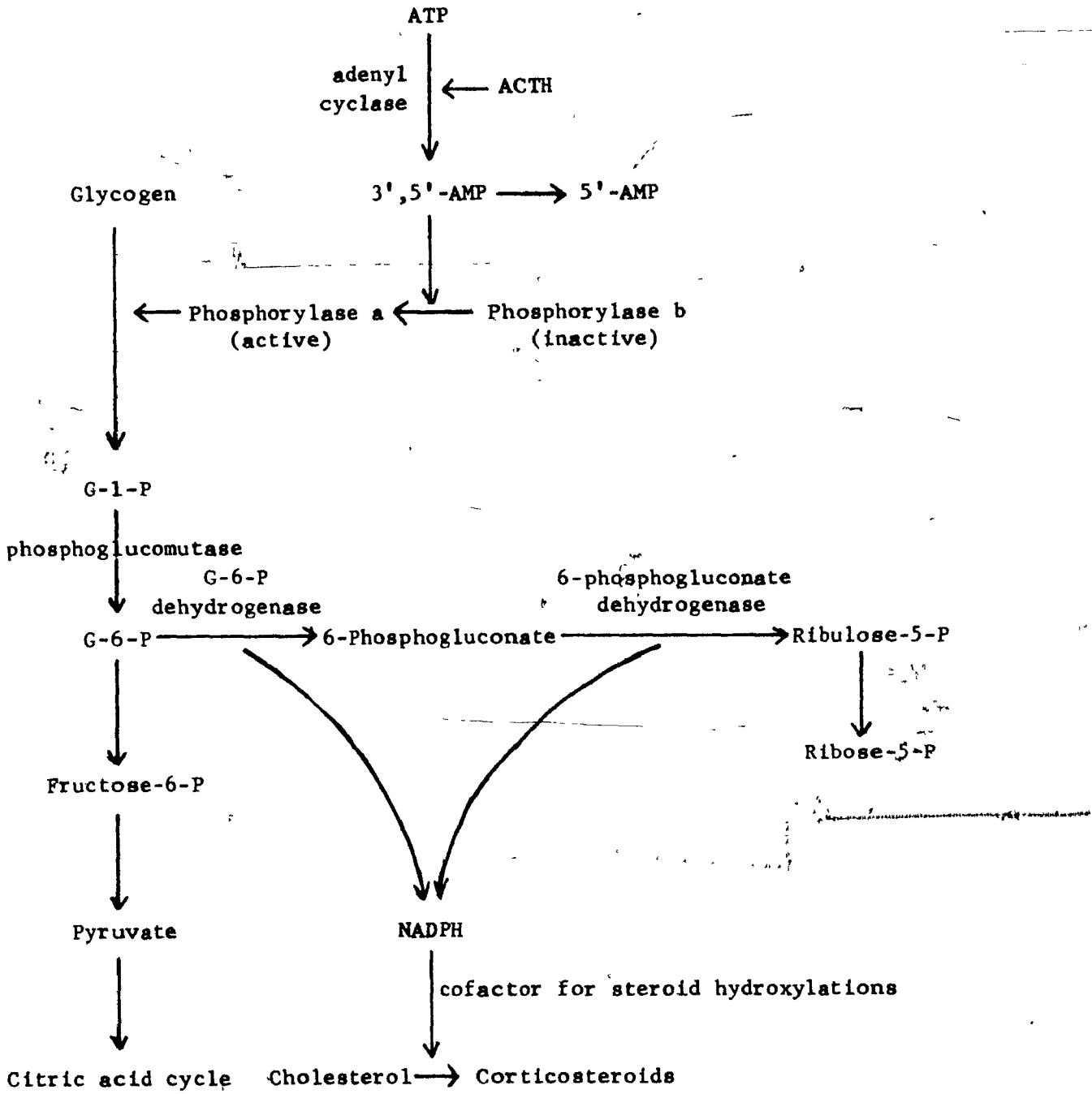


Fig. B Metabolic pathways associated with the action of ACTH upon the adrenal cortex, as adopted from Hilf(25)

4. A brief outline of the biosynthesis of adrenal corticosteroids from cholesterol:

The biosynthetic pathways leading to the formation of adrenal steroids from cholesterol, as outlined by Dorfman and Sharma in 1965 (28), are represented in Fig. C.

Much remains to be elucidated with regard to alternative routes and specific enzyme systems that affect the biosynthesis of adrenal corticosteroids (29). A key step in the pathway of adrenal steroid biosynthesis is the cleavage of the cholesterol sidechain leading to the formation of pregnenolone, known to be one, if not the only, principal precursor of adrenal steroid formation (29). In 1956, Solomon et al. (30, 31) obtained 20β-hydroxycholesterol-4-^{14}C from cow adrenal homogenates incubated with cholesterol-4-^{14}C and proposed that 20β-hydroxycholesterol might be a possible biosynthetic intermediate between cholesterol and pregnenolone. Shimizu et al. in 1961 (32) reported the formation of isocaproic acid, pregnenolone-7-^{3}H, and progesterone-7-^{3}H when synthetic 20α-hydroxycholesterol-7-^{3}H was incubated with a supernatant fraction of bovine adrenal homogenates. Similar findings were reported by Constantopoulous and Tchen in 1961 (33), who showed that, by using a soluble enzyme fraction prepared from beef adrenal homogenates in the presence of added NADPH, 20α-hydroxycholesterol-7α-^{3}H was converted to pregnenolone-7α-^{3}H. Tchen

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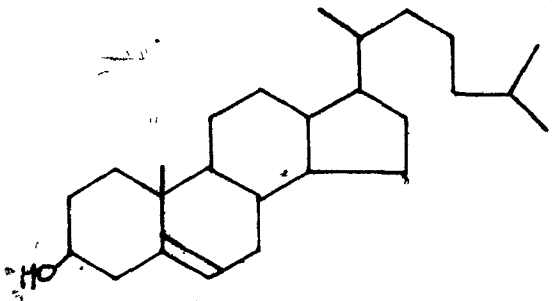
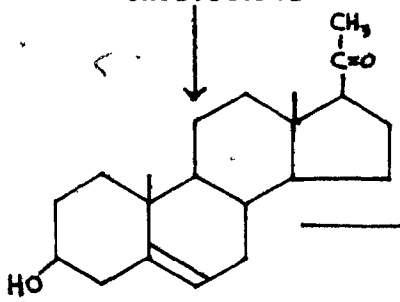
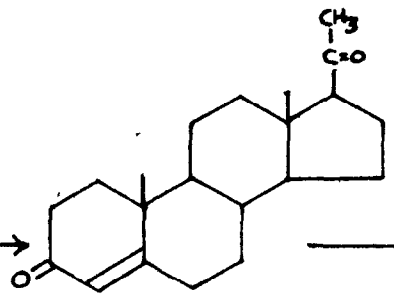


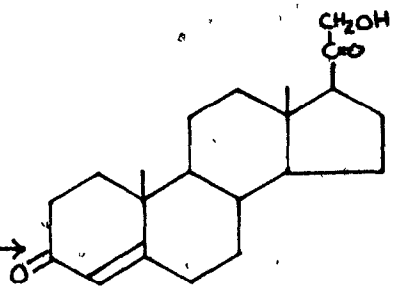
Cholesterol



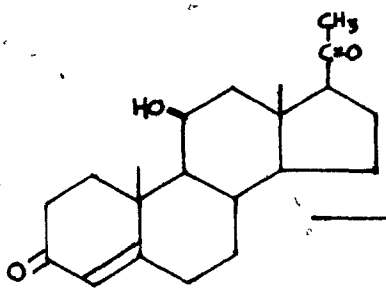
Pregnenolone



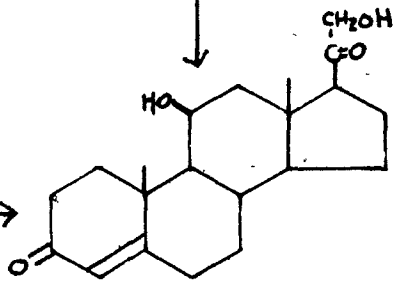
Progesterone



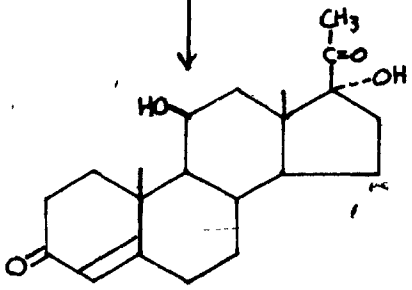
Deoxycorticosterone



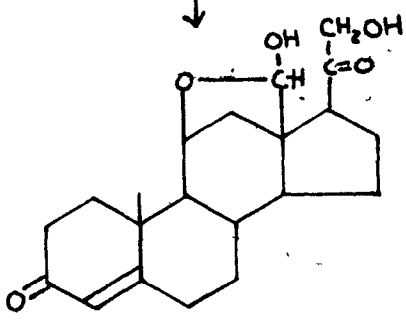
11β-OH Progesterone



Corticosterone



11β,17α-dihydroxy-Progesterone



Aldosterone

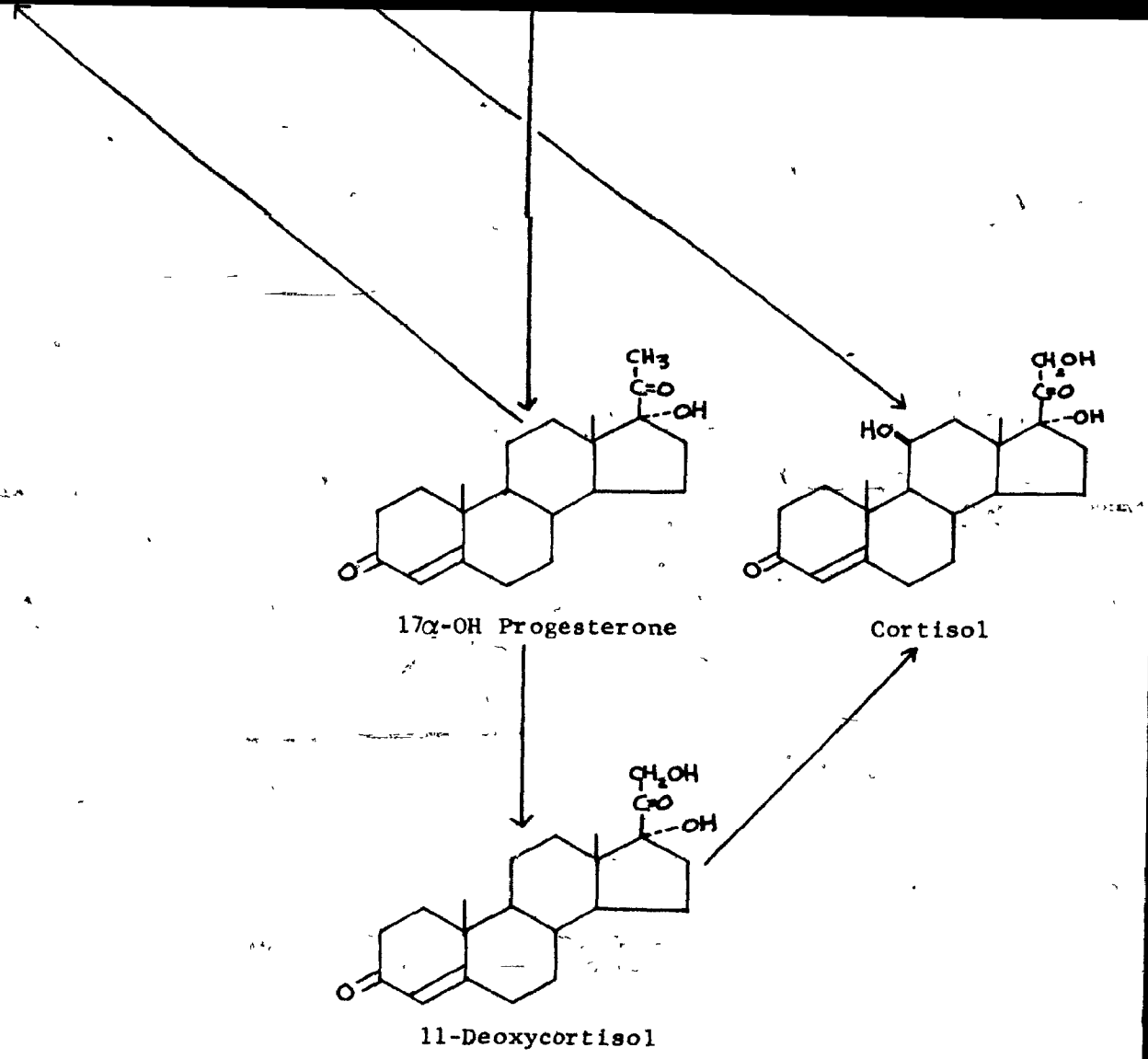


Fig. C An outline of the biosynthesis of adrenal corticoids from cholesterol, as adopted from Dorfman and Sharma (28).

in 1968 (34) proposed that 20α, 22ξ-dihydroxycholesterol is a possible intermediate between 20α-hydroxycholesterol and pregnenolone. The author and his colleagues (35) obtained a labelled polar compound from the mitochondrial fraction of beef adrenal homogenates incubated in the presence of NADPH with cholesterol-4-^{14}C or cholesterol-26-^{14}C. This compound was identified as one of the isomers of 20α, 22ξ-dihydroxycholesterol by lead tetraacetate oxidation to pregnenolone and isocaproaldehyde.

The formation of 18-OH-DOC from steroid precursors, cholesterol, pregnenolone, progesterone, and deoxycorticosterone has been demonstrated in in vitro studies with the implication that the most immediate precursor to 18-OH-DOC might be deoxycorticosterone. This will be described further under a separate section.

5. Adrenal 18-OH-DOC:

(a) Endogenous production of 18-OH-DOC:

18-OH-DOC was first isolated and identified as a naturally occurring steroid from incubated sectioned rat adrenals by Birmingham and Ward in 1961 (17) and by Péron, in the same year (18). It was characterized by Birmingham and Ward (17) to be the 18,20-cyclohemiketal of 18-hydroxy-11-deoxycorticosterone. Part of the criteria for its identification were the oxidation with periodate to the 20→18-lactone of 18-hydroxy-3-keto-4-etien-20-oic acid, a negative test with

tetrazolium chloride for the presence of the α-ketol group, and the absence of an infrared band characteristic for a saturated ketone group. These authors showed that 18-OH-DOC, a steroid containing no 17,21-dihydroxy-20-ketone function nevertheless gave a typical Porter-Silber reaction (36), i.e., it reacted slowly with dinitrophenyl-hydrazine in aqueous sulfuric acid and ethanol, to form a compound absorbing light maximally at 400-410 nm. The in vivo secretion of 18-OH-DOC in rats was demonstrated by Cortés et al. in 1963 (37), who isolated the steroid from adrenal vein blood. The endogenous formation of 18-OH-DOC has also been demonstrated in the camel by Race and Wu in 1964 (38), and in man by Melby and collaborators, (19, 20), who isolated and identified 18-OH-DOC from human adrenal vein blood, by the adrenal vein catherization technique. They obtained levels comparable to those of aldosterone and deoxycorticosterone in normal subjects, and elevated levels in patients suffering from various forms of hypertension, including Cushing's syndrome and essential hypertension.

(b) Biotransformation to 18-OH-DOC:

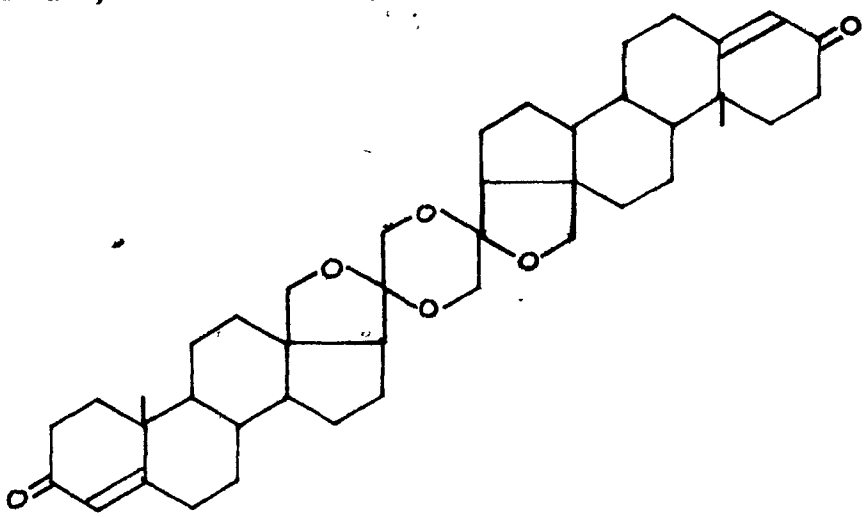
Although the conversion of DOC to 18-OH-DOC is most readily obtained with the rat adrenal, the biosynthesis of 18-OH-DOC from exogenous DOC and other precursors has been demonstrated in various species. Arai and Tamaoki in 1967 (39) reported the conversion of

DOC-4-^{14}C to 18-OH-DOC *in vitro* by the intrarenal tissue of the rainbow trout (Salmon gairdneri). Kahnt, Neher, and Wettstein in 1955 (40) isolated and identified 18-OH-DOC from homogenates of beef adrenals incubated with DOC. Conversion of DOC to 18-OH-DOC was shown to occur in quartered frog adrenal by Kraulis and Birmingham in 1964 (41) and in rabbit adrenal tissue slices by Fazekas and Kokoi in 1967 (42). Relatively large amounts of both 18-OH corticosterone and aldosterone and only a trace of 18-OH-DOC were obtained from quartered mouse adrenals incubated with progesterone-4-^{14}C, in the studies of Raman et al. in 1964 (43). De Nicola and Birmingham in 1970 (44) studied adrenal biosynthetic patterns in subhuman primates. Incubation of sliced adrenals of the squirrel monkey (Saimiri sciureus) with DOC-4-^{14}C in the presence of nicotinamide resulted in the formation of 18-OH-DOC in 0.5% yield, whereas no conversion to 18-OH-DOC was obtained with stumptail monkey (Maccaca speciosa) adrenals.

With quartered rat adrenals, Ward and Birmingham in 1962 (45) showed that DOC, progesterone, and 18-OH progesterone were converted to 18-OH-DOC in 10, 4, and 6% yields, respectively. Vecsei et al. in 1968 (46) compared the conversion of DOC-1,2-^{3}H and progesterone-4-^{14}C to 18-OH-DOC by quartered rat adrenals and concluded that DOC is a more effective precursor than progesterone. Conversion of

progesterone-17α-^{3}H to 18-OH-DOC in good yield, when incubated with sectioned rat adrenals in the presence of NADP and glucose-6-phosphate, was demonstrated by Péron in 1962 (47). Nakano et al. in 1968 (48) isolated and identified 18-OH-DOC from rat adrenal homogenates incubated with progesterone in an atmosphere enriched with $^{18}O_2$. 18-OH-DOC was oxidized by periodic acid and the resulting 20→18-lactone of 18-OH-DOC was subjected to mass spectrometry. The incorporated ^{18}O atom was located at the 18,20-epoxy group of the steroid, by mass spectral fragmentation analysis, implying that 18-hydroxylation had occurred in the biotransformation. These authors emphasized that the principle of locating the ^{18}O atom incorporated into a steroid molecule by mass spectrometry is a valuable analytical technique for structural identification and elucidation of metabolites formed in oxygen-requiring enzyme reactions such as steroid hydroxylation. Levy et al. in 1965 (49) studied the substances formed from DOC-4-^{14}C in beef adrenal perfusion in vitro and isolated from the perfusate, by column chromatography on silica gel, a high-melting substance (not melted at 300°). These authors referred to it as the "dimer" of 18-OH-DOC and remarked that it was probably an artifact, arising by condensation of two molecules of 18-OH-DOC during the work-up process. According to these investigators, this material has an identical infrared spectrum with that of a substance obtained

by Pappo (97) from 18-OH-DOC by treating it with p-toluenesulfonic acid in dioxane for a few minutes at room temperature. Pappo assigned to this substance the structure shown below and gave it the trivial name, 18-OH-DOC dimer.



18-OH-DOC dimer

The biotransformation of DOC-21-^{14}C to 18-OH-DOC by rat adrenal homogenates was studied by Dominguez (50), who obtained evidence, by paper chromatography, for the presence of two interconvertible forms of 18-OH-DOC with differing polarity. The two forms of 18-OH-DOC, upon elution, gave identical spectra in the near-infrared region. According to Dominguez, synthetic 18-OH-DOC supplied by Pappo, exhibited the same interconversion and behavior on paper chromatography. Dominguez suggests that the 18,20-cyclohemiketal of 18-OH-DOC may exist in two tautomeric forms. Lantos, Birmingham and Traikov (51) showed that DOC-1,2-^{3}H and progesterone-7-^{3}H were

converted by incubated quartered rat adrenals to corticosterone, 18-OH-DOC, and a metabolite of corticosterone with properties of allotetrahydrocorticosterone. In the presence of exogenous ACTH in the incubation medium they observed that the yields of corticosterone and 18-OH-DOC were doubled but the fraction corresponding to allotetrahydrocorticosterone was halved. The authors obtained evidence that the increased yield of corticosterone was due to the inhibition by ACTH of the formation of the corticosterone metabolite, but did not test the possibility that an analogous mechanism might account for the increased yield of 18-OH-DOC.

Evidence suggesting the conversion of progesterone-4-^{14}C to 18-OH-DOC by sliced human adrenal tissues was obtained by Carballeira and Venning in 1964 (52). Raman et al. in 1965 (53) showed that adrenal tumor homogenates from a patient with primary aldosteronism readily converted progesterone-4-^{14}C and DOC-1,2-^{3}H to 18-OH corticosterone and aldosterone but not to 18-OH-DOC. The failure of the tumor tissue to synthesize 18-OH-DOC from the labelled precursors was interpreted by the authors to indicate substrate specificity of the steroid 18-hydroxylase.

Conversion of DOC-4-^{14}C to 18-OH-DOC by the human adrenal was first demonstrated unequivocally by de Nicola and Birmingham in 1968 (54) using sectioned adrenals from a patient with a prostatic

carcinoma. Subsequent experiments by these authors with sectioned adrenals from four patients with breast carcinoma and a patient with primary aldosteronism (55) showed that, in all cases, DOC-4-^{14}C was converted to three 18-oxygenated compounds, namely, 18-OH corticosterone, 18-OH-DOC, and aldosterone with yields of 3.8 - 4.6%, 1.0 - 1.4%, and 0.17 - 0.41%, respectively. These metabolites were also formed from progesterone-4-^{14}C, but much lower yields were obtained. These authors contemplated the possible significance of 18-OH-DOC in the case of patients with primary aldosteronism in which DOC-4-^{14}C yielded 6 times as much 18-OH-DOC as aldosterone. Recently, Lucis and Lucis in 1971 (56) reported that both the incubated minced adrenal and adenoma tissues from a patient with primary aldosteronism were capable of transforming progesterone-4-^{14}C to 18-OH-DOC, suggesting the presence of an 18-hydroxylase capable of forming 18-OH-DOC in both types of tissues.

(c) Factors influencing the biosynthesis of 18-OH-DOC:

(i) ACTH Previous investigations by Birmingham and Kurlents in 1958 (57) demonstrated that a brief contact (5 minutes) of quartered rat adrenal tissue with an ACTH-containing solution resulted in an increase of corticosteroid production and a concomitant decrease of ACTH activity in the solution which had been in contact with the tissue for a short period. These observations were explained by the

authors as indicative of the binding of ACTH by the adrenal tissue. Subsequent studies showed that ACTH incubated with rat adrenals causes the formation of two major steroid end products, namely corticosterone, present in greatest quantity, and 18-OH-DOC, the second most abundant steroid (58, 59, 17). Similar results were obtained in rat adrenals incubated by Péron (60). In corroboration of the in vitro work, Cortés, Péron and Dorfman (37) demonstrated that the secretion of corticosterone and 18-OH-DOC in the adrenal vein blood of hypophysectomized rats is increased 5- to 20-fold by administration of ACTH. Recently, Melby et al. (20) found that the basal secretion rate of 18-OH-DOC (determined by the excretion rate of urinary 18-OH-TH-DOC) in five healthy adults was increased 10- to 20-fold by ACTH gel given intramuscularly.

Lucis, Dyrenfurth, and Venning in 1961 (61) studied the in vitro secretion of corticosteroids by rat adrenal capsules and decapsulated adrenal glands. The authors showed that the capsule, consisting mainly of the zona glomerulosa, secreted primarily aldosterone with small amounts of corticosterone. The decapsulated tissue, consisting mainly of the zona fasciculata, secreted corticosterone and 18-OH-DOC and the secretion rate of these two steroids was significantly increased when ACTH was added. However, no aldosterone could be detected in the decapsulated tissues. Sheppard et al. in 1963 (62)

demonstrated that 18-OH-DOC was largely derived from the zona fasciculata whereas only very small amounts were derived from the zona glomerulosa of the rat adrenal cortex. They showed that progesterone-4-^{14}C was converted to 18-OH-DOC to a greater extent by the decapsulated (fasciculata predominantly), as opposed to the capsular (glomerulosa predominantly) portion of the adrenal cortex *in vitro* and that this conversion was stimulated by the addition of ACTH. Stachenko, Laplante, and Giroud in 1964 (63) demonstrated that 18-OH-DOC and corticosterone was produced *in vitro* by the decapsulated portion of the rat adrenal which contains the cells of the zona fasciculata-reticularis, whereas aldosterone, corticosterone and 18-OH corticosterone were found in the capsular portion which contains the cells of the zona glomerulosa.

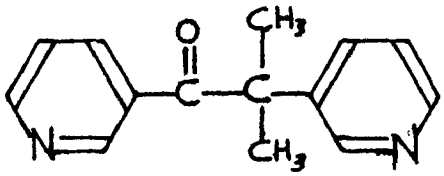
(ii) *3',5'-AMP and NADPH* Haynes, Koritz, and Péron in 1959 (64) showed that 3',5'-AMP is more effective than ACTH in stimulating the steroid production by isolated rat adrenal glands. Birmingham et al. in 1960 (65) showed that 3',5'-AMP stimulates corticosteroid production 3-fold in quartered rat adrenals incubated in the absence of both glucose and calcium. This response is further increased by the presence of calcium, whereas glucose has only a small effect. Increased endogenous production of 18-OH-DOC upon incubation of sectioned rat adrenals in the presence of maximal amounts of 3',5'-AMP or NADPH

was demonstrated by Péron in 1961 (60). The author further showed that cholesterol-4-^{14}C and pregnenolone-7α-^{3}H were converted to 18-OH-DOC by rat adrenal homogenates maximally stimulated with NADPH.

(iii) Calcium and glucose Previous studies by Birmingham, Elliott, and Valère in 1953 (66) and by Schonbaum, Birmingham, and Saffran in 1956 (67) demonstrated that ACTH maximally stimulates the biosynthesis of corticosteroids by rat adrenals in vitro only if calcium and glucose are present in the medium. These authors suggested that calcium might play a role as a cofactor in one of the reactions along the pathways leading to the synthesis of steroids from endogenous precursors, or that calcium might enhance the access of ACTH to cellular sites involved in steroid synthesis. Péron and Koritz in 1960 (68) showed, with particulate preparations from rat adrenal homogenates, that the conversion of endogenous precursors to corticosteroids is stimulated in the presence of calcium. The authors suggested that one locus of calcium action might be the reactions between cholesterol and pregnenolone. According to Kraulis and Birmingham in 1968 (69), the conversion of DOC to 18-OH-DOC by sectioned rat adrenals proceeds equally well in the absence as in the presence of calcium in contrast to the conversion of DOC to corticosterone. The rate of the conversion to either end product is better

maintained with time if glucose is present in the medium.

(iv) SU-4885 (metopirone or 2-methyl-1,2-bis(3-pyridyl)-1-propanone) SU-4885 as a specific inhibitor of 11β-hydroxylation of steroids (70) has become a useful agent for the evaluation of pituitary function as well as for the studies of steroid biosynthesis.



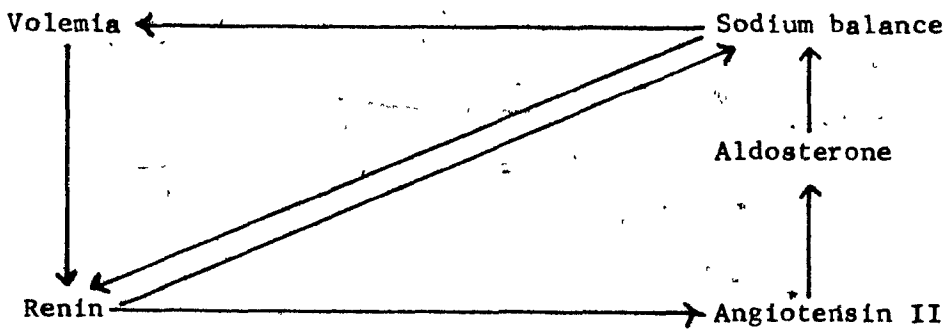
SU-4885

The inhibitory properties of this drug are, however, not restricted to 11β-hydroxylation; it affects hydroxylations at various other sites of the steroid molecule as well, including 18-hydroxylation. In vivo studies in rats by Kahnt and Neher (71) showed that the intravenous injection of a low concentration of SU-4885 greatly decreases aldosterone and 18-OH-DOC levels. Kraulis and Birmingham in 1965 (72) showed that at low doses SU-4885 inhibits the formation of 18-OH-DOC from exogenous DOC or progesterone, as well as from endogenous precursors in incubated rat adrenal sections. The authors suggested that SU-4885 probably exerts a direct inhibitory effect on the 18-hydroxylation of DOC to 18-OH-DOC in the rat since this transformation was shown to be almost completely suppressed by the drug.

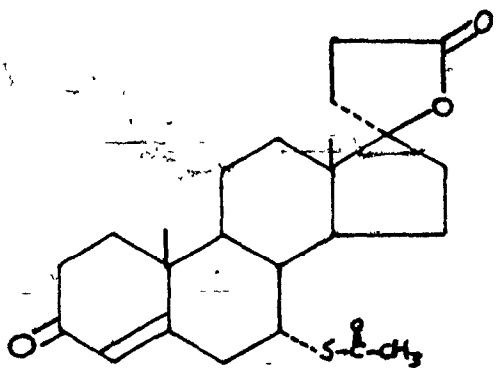
The exact mechanism of action by which SU-4885 acts is not completely understood. Interference with the availability of NADPH required for steroid hydroxylation has been suggested by Ertel and Ungar (73), who found that the conversion of progesterone-4-^{14}C to corticosterone by quartered mouse adrenals was blocked by low concentrations of SU-4885. There is evidence that SU-4885 interferes with steroid hydroxylation by binding to mitochondrial 11β-hydroxylase. Williamson and O'Donnell (74) observed that the transformation of DOC-4-^{14}C to corticosterone by the mitochondrial fraction of beef adrenal homogenates was inhibited by low concentrations of SU-4885. Wilson et al. (75) identified cytochrome P-450 in mitochondria isolated from human adrenals and showed that this hemoprotein was involved in the oxygen-activation required for 11β-hydroxylation of 11-deoxycortisol-4-^{14}C to cortisol. The authors obtained evidence by the ultraviolet spectral method that this transformation is blocked by the addition of SU-4885 in low concentrations and concluded that SU-4885 probably interferes with 11β-hydroxylation by competitive inhibition of the binding of the steroid substrate to cytochrome P-450.

(v) Angiotensin 18-OH-DOC is a mineralocorticoid (69, 87) and its secretion in man, as in the rat, is under the influence of ACTH. There is evidence that 18-OH-DOC is formed, at least in the rat, largely in the zona fasciculata of the adrenal cortex. On the other

hand, aldosterone, the most potent mineralocorticoid produced by the adrenal in man, is believed to be formed exclusively in the zona glomerulosa of the adrenal cortex and its secretion is relatively independent of ACTH (76). Angiotensin, a peptide formed in blood by the action of renin, has been shown by the studies of Genest (77), Laragh (78), and Davis (79) and co-workers to stimulate aldosterone secretion in man. A possible mechanism by which the kidney modulates aldosterone secretion has been proposed by these authors. Their hypothesis suggests that a decrease in renal blood flow activates the juxtaglomerular cells to cause the secretion of renin resulting in the formation of angiotensin II, which then stimulates aldosterone secretion. Aldosterone, in turn, causes the retention of sodium by the renal tubular cells with a resultant increase in blood volume, blood pressure, and renal blood flow; the increased sodium and blood flow act as a negative feedback upon the juxtaglomerular cells to reduce the secretion of renin. A diagrammatic summary of the mechanism for the interaction between aldosterone secretion and angiotensin activity proposed by the authors is illustrated below.



Melby et al. (19, 20) found that 18-OH-DOC concentration in human adrenal vein blood remained unchanged after angiotensin infusions. However, the authors noted that in some hypertensive patients with elevated secretion of 18-OH-DOC, suppressed plasma renin activity, and low or normal aldosterone secretion rates, blood pressure was greatly reduced after administration of spironolactone, a known antagonist of aldosterone.



Spironolactone

(d) Formation of 18-OH-DOC by regenerating rat adrenal glands:

In 1955 Skelton (80) observed a syndrome of hypertension associated with cardiovascular-renal lesions in uni-nephrectomized, salt-treated young, female rats during regeneration of the enucleated adrenal cortex. Enucleation was performed by making an opening in the capsule of the adrenal gland through which the mass of glandular tissue was discharged by compressing the gland with forceps. The cortical tissue of the adrenal gland was restored by cellular prolifer-

ation and hypertension developed as the gland regenerated. The medulla does not regenerate. Skelton observed that vascular disease developed during adrenal regeneration, and noted enlargement of the heart and kidney with damage in the blood vessels of these organs. No significant change in the serum levels of sodium, chloride or potassium was observed by this author. The regenerating rat adrenal gland appeared to be under the regulation of ACTH (81). Adrenal regeneration was prevented by removal of the pituitary or by suppression of ACTH production by steroid hormones. Skelton in his review of 1959 (81) suggested four mechanisms that might be responsible for the hypertensive state in rats bearing a regenerating adrenal cortex: (1) increased secretion of glucocorticoids, (2) inbalance between secretion of corticosterone and aldosterone, (3) secretion of an unknown steroid, and (4) hypersensitivity to adrenal corticoids.

Birmingham, Rochefort, and Traikov in 1965 (82) reported that there was an increased production in vitro of 18-OH-DOC and corticosterone in response to added ACTH, and a decreased formation of material with the mobility, on paper chromatography, of 18-OH corticosterone and aldosterone by bilaterally regenerated adrenal glands of female rats, three weeks after adrenal enucleation. The authors suggested that 18-OH-DOC and corticosterone may be derived mainly from the zona fasciculata-reticularis of the regenerated glands since the production of these two steroids is increased greatly

in response to ACTH. With homogenates prepared from regenerated rat adrenal glands, Brownie and Skelton in 1965 (83) obtained a decreased conversion of progesterone-4-^{14}C to 18-OH-DOC and corticosterone and an accumulation of DOC. They proposed that the impaired utilization of DOC, a known hypertensive agent, by the regenerating adrenals might be the cause for hypertension associated with adrenal regeneration. Vecsei et al. in 1966 (84) studied the biotransformation of DOC-1,2-^{3}H and progesterone-4-^{14}C by regenerating quartered rat adrenal glands and found, by contrast, that the formation of 18-OH-DOC and corticosterone from both labelled precursors was increased, whereas the formation of 18-OH corticosterone and aldosterone was considerably reduced. Increased conversion in vitro of both DOC-1,2-^{3}H and progesterone-4-^{14}C to 18-OH-DOC and corticosterone, and impaired conversion to 18-OH corticosterone and aldosterone by regenerating adrenals of rats before and after onset of the syndrome of adrenal regeneration hypertension was also reported by de Nicola, Oliver, and Birmingham in 1968 (85) and in 1969 (86). The authors suggested that excess secretion of 18-OH-DOC by regenerating adrenals might play a role in the etiology of adrenal regeneration hypertension, in view of the sodium-retaining and antidiuretic properties exhibited by this steroid (87, 69). De Nicola, Oliver, and Birmingham in 1969 (88) found no significant changes in the conversion of progesterone-4-^{14}C to 18-OH-DOC by quartered adrenals of rats with spontaneous

hypertension and suggested that the adrenal cortex is probably not involved in the etiology of this form of hypertension. Rapp in 1970 (89) found no elevation of 18-OH-DOC in the peripheral plasma of rats with adrenal regeneration hypertension. Rapp and Dahl in 1971 (90) compared the adrenal steroid production in female rats bred for susceptibility and resistance to the hypertensive effects of salts. The animals were on a low salt diet (0.4% NaCl) for thirteen weeks. The authors measured 18-OH-DOC levels in adrenal vein blood and in peripheral blood and concluded that the susceptible rats produced twice as much 18-OH-DOC as the resistant rats.

(e) Metabolism of 18-OH-DOC:

Little is known about the metabolism of adrenal 18-OH-DOC. Nicolis and Ulick in 1965 (91) studied the metabolism of DOC-1,2-^{3}H and 18-OH-DOC (randomly labelled with tritium) by bull frog adrenal slices and found that there was only a small conversion of 18-OH-DOC to aldosterone as compared with the conversion of DOC. The authors attributed the poor conversion of 18-OH-DOC to a resistance of 18,20-cyclohemiketal structure to enzymatic oxidation. De Nicola and Birmingham in 1970 (44) reported that in sliced adrenals of the stumptail monkey, 18-OH-DOC-4-^{14}C was converted to 18-OH corticosterone in 0.6% and to aldosterone in 5% yield. Very recently, Melby et al. (19, 20) isolated and identified radioactive 18-OH-TH-DOC (18-hydroxy-tetrahydro-

deoxycorticosterone), a ring A reduced metabolite of 18-OH-DOC, after intravenous injection of radioactive 18-OH-DOC, from the urine of healthy subjects as well as from patients with hypertensive disorders.

The biosynthetic and metabolic transformation reactions for 18-OH-DOC discussed in the preceding sections are summarized diagrammatically in Figure D, along with the appropriate references.

6. Mineralocorticoid activity of synthetic 18-OH-DOC-21-acetate:

The nature and extent of the physiological activity of 18-OH-DOC have not been well studied. In 1960, Ward and Birmingham (59) obtained a purified lipid fraction with properties of the 18,20-cyclohemiketal form of 18-OH-DOC from incubated quartered rat adrenals. This material was shown to have sodium-retaining activity. In three separate assays on adrenalectomized rats, using 2.5, 5, and 5 μg doses per rat, this compound gave effects equivalent to 1.9, 2.2, and 0.5 μg, respectively, of DOC acetate, on urinary Na/K ratios. This lipid fraction was subsequently identified chemically as 18-OH-DOC by the same authors in 1961 (17). Kagawa and Pappo in 1962 (87) found that 18-OH-DOC-21-acetate possessed about 10% of the mineralocorticoid potency of DOC acetate by the criteria of sodium retention, potassium loss, and reduction of the Na/K ratio in the urine of adrenalectomized rats. Birmingham, MacDonald, and Rochefort

in 1968 (69) reported that, using adrenalectomized rats, 18-OH-DOC-21-acetate had a sodium-retaining effect equalling that of DOC acetate at a dose of 10 μg per rat, but was less effective than DOC acetate at higher doses. Uniquely, 18-OH-DOC-21-acetate did not increase the urinary potassium excretion at any of the doses tested (5, 10, 20, and 40 μg per rat), whereas DOC acetate stimulated the excretion of potassium at 10 μg but not at 20 μg. The authors also found that 18-OH-DOC-21-acetate had a dose-dependent antidiuretic action in the adrenalectomized rat that was significant at a dose as low as 5 μg per rat, whereas with DOC acetate no statistically significant reduction in urine volume was obtained with doses of 10 and 20 μg. Porter and Kimsey in 1971 (92) reported that 18-OH-DOC has about one seventh the potency of aldosterone and one fifth the potency of DOC on sodium transport in the isolated urinary bladder of the toad.

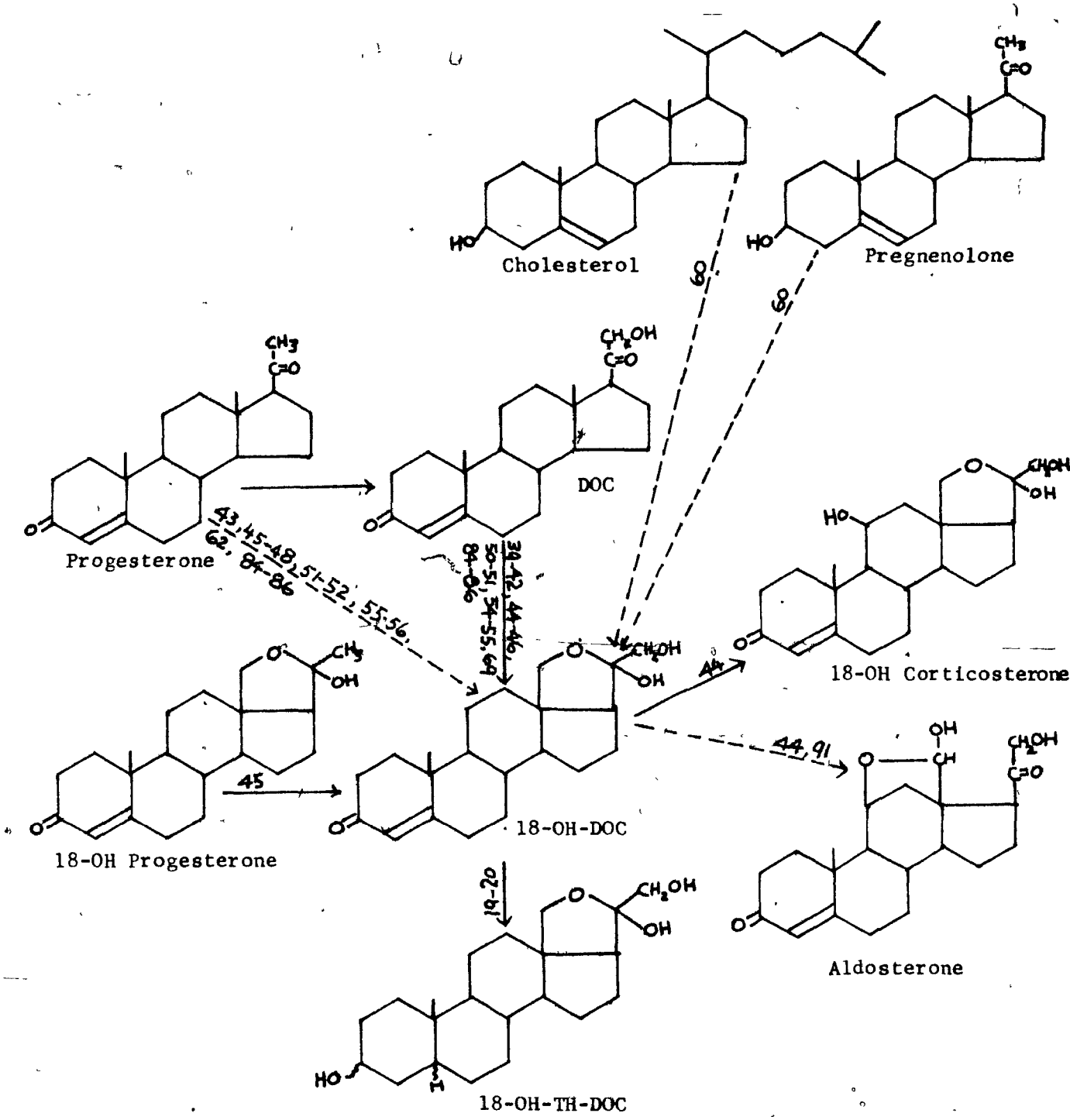


Fig D Summary of biosynthetic and metabolic transformation reactions for 18-OH-DOC. Dotted arrows indicate intermediate steps.

PART II: SYNTHESIS OF 18-OH-DOC

Introduction and Literature Review

The isolation and identification in 1961 of 18-OH-DOC (20,21-dihydroxy-18,20-epoxy-Δ^4-pregnen-3-one) (17, 18) presented the first proof of the existence of a naturally occurring 21-hydroxy, 11-deoxy-corticosteroid with an 18,20-cyclohemiketal linkage. The biosynthesis of 18-OH-DOC from endogenous precursor(s) as well as from exogenous steroid substrate(s) by adrenals has been demonstrated in animal species and in man, as has been described in the preceding section. At present, the physiological significance of 18-OH-DOC is not well understood. It is justifiable to investigate the chemical reactivity, physical properties, and possible biological activity of 18-OH-DOC in view of its structural similarity to aldosterone, the most potent mineralocorticoid in man. The isolation of 18-OH-DOC from adrenal preparations is tedious and the yield is so limited that the chemical synthesis of this compound was attempted.

1. Introduction of an oxygen function at position C-18 of steroids:

The isolation and elucidation of the structure of aldosterone in 1952-54 (13, 14, 15) provided the stimulus to study the chemical synthesis of this steroid. This resulted in active research

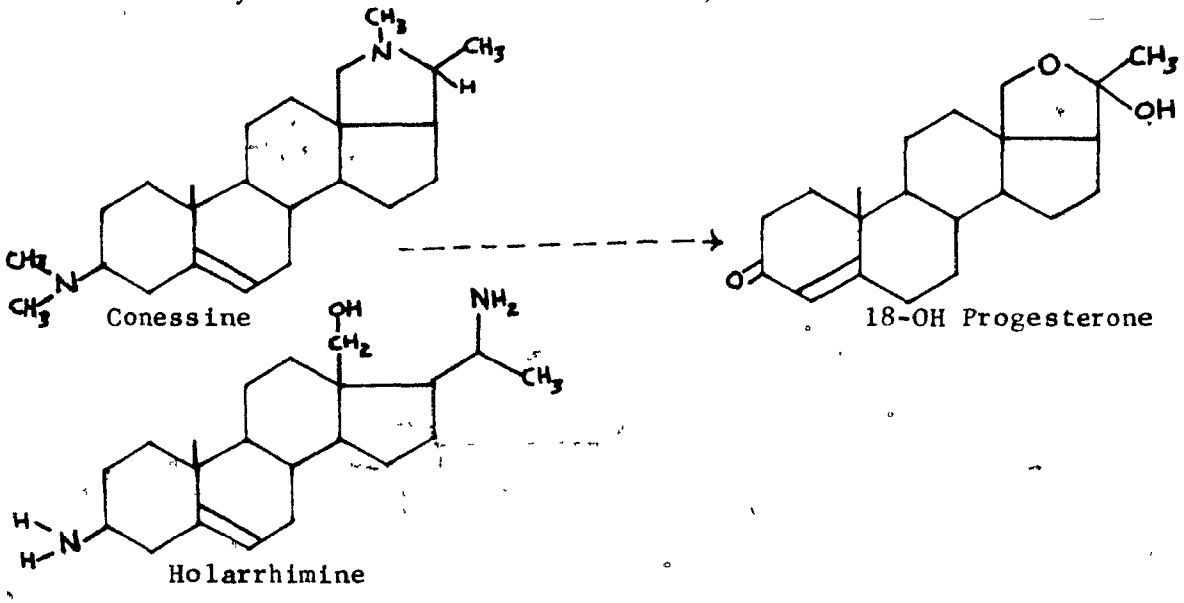
into the methods for the introduction of an oxygen function in place of a hydrogen at the C-18 methyl group.

Two useful methods are known: first, by the degradation of naturally occurring steroidal alkaloids with C-18 appropriately functionalized; second, by the direct introduction of an oxygen at C-18 in steroids lacking a substituent at this position. The literature pertinent to the synthesis of 18-OH progesterone and 18-OH-DOC by the application of these methods is reviewed below.

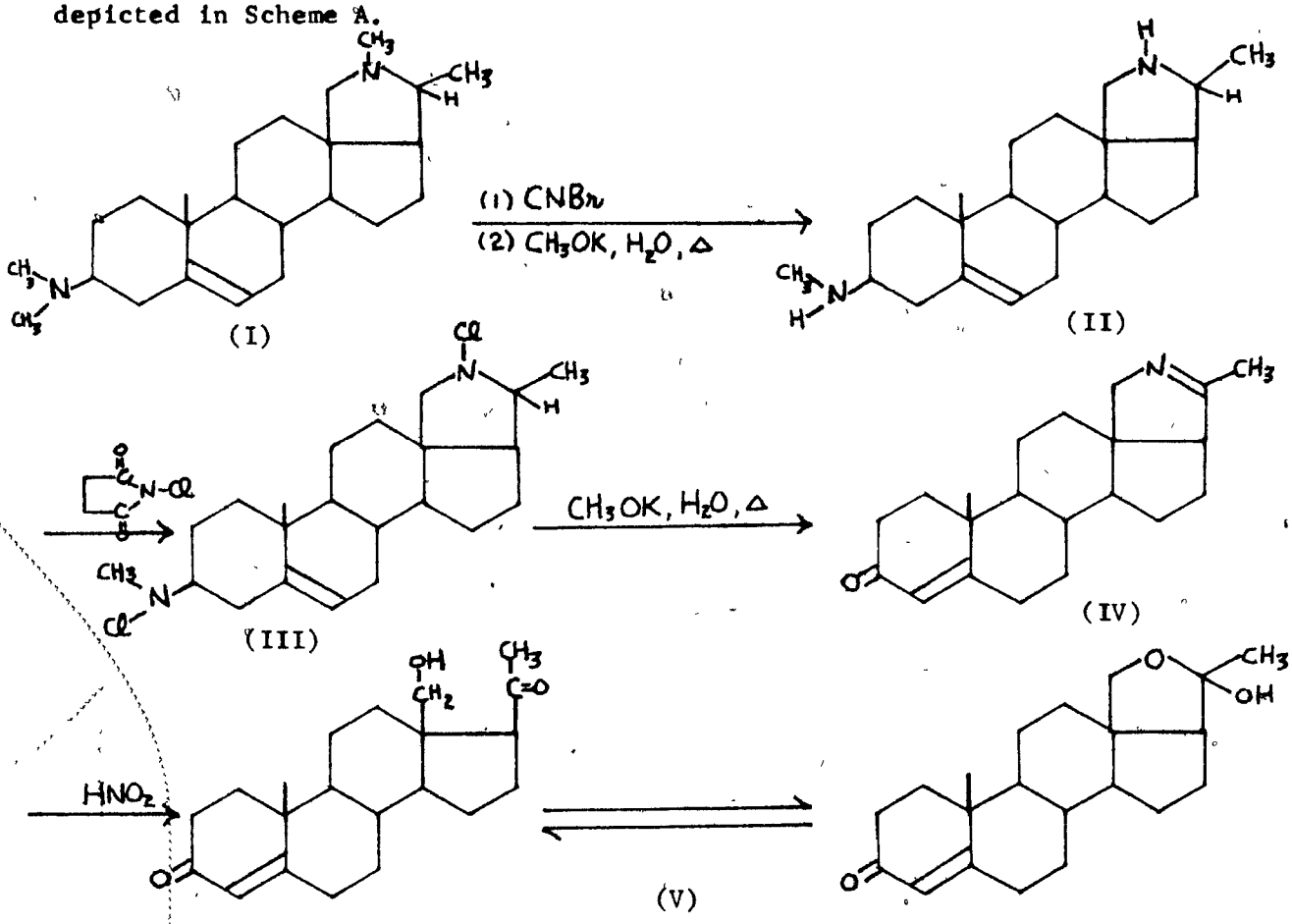
(a) Method one:

The degradation of a steroidal alkaloid, which already is substituted at C-18, to an 18-oxygenated, nitrogen-free steroid.

18-OH Progesterone (20-hydroxy-18,20-epoxy-Δ^4-pregnen-3-one) was synthesized from the alkaloid conessine by Buzzetti et al. in 1959 (93), by Hora and Černý in 1961 (94) and by Goutarel et al. in 1969 (95). It was also synthesized from the alkaloid holarrhimine by Lábler and Šorm in 1960 (96).



These synthetic pathways leading to 18-OH progesterone have one feature in common: the replacement of each amino group in the molecule by an oxygen-containing function via a sequence which is based upon the von Braun's demethylation $\left(>\overset{H}{C}-N<^{CH_3}_{CH_3} \longrightarrow >\overset{H}{C}-N<^{H}_{H}\right)$ followed by Ruschig's deamination $\left(>\overset{H}{C}-N<^{H}_{H} \longrightarrow >\overset{H}{C}-N<^{Cl}_{H} \longrightarrow >C=NH \longrightarrow >C=O\right)$. One representation of this method may be illustrated by Buzzetti's (93) synthesis of 18-OH progesterone from conessine. The sequence of reactions is depicted in Scheme A.



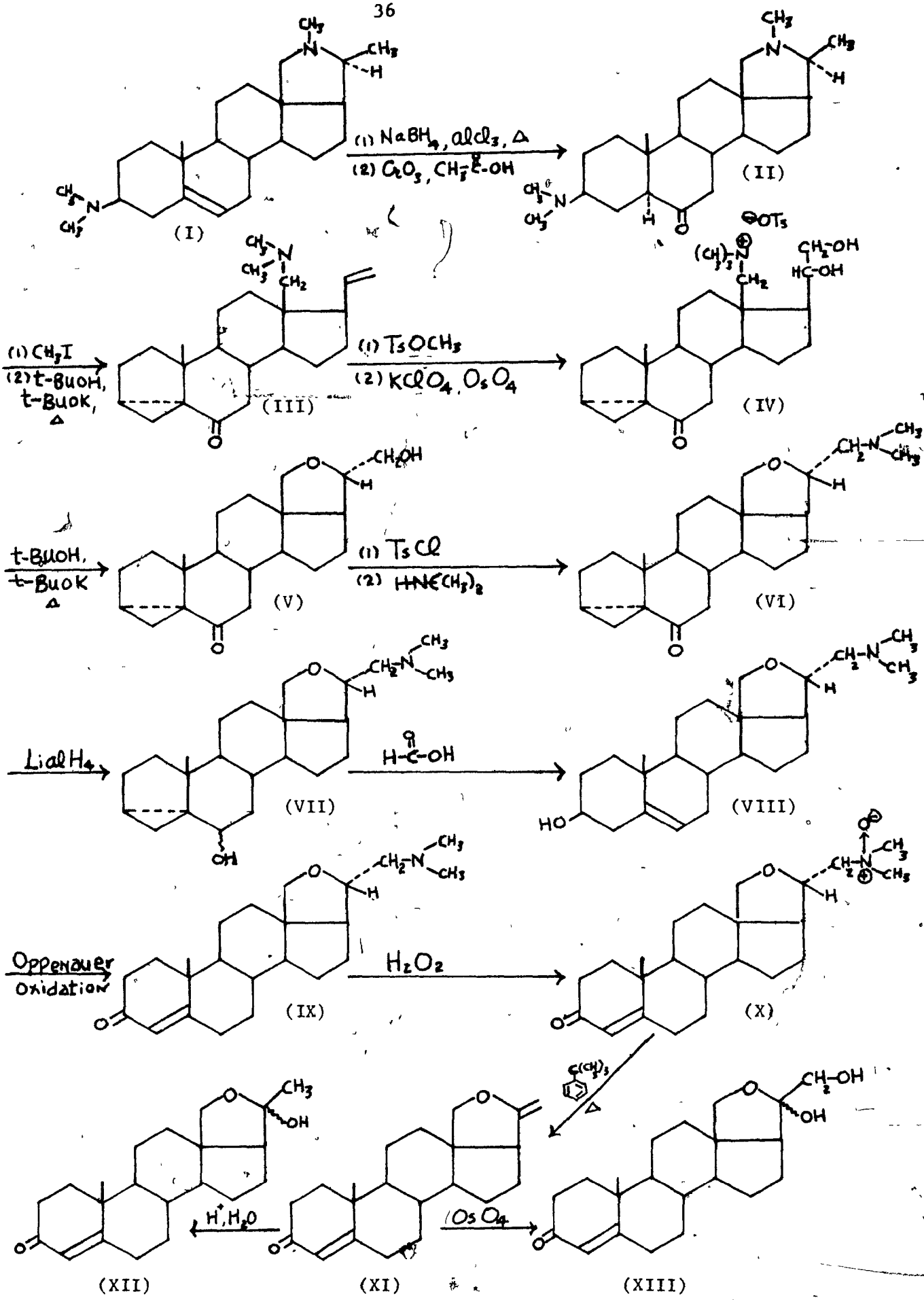
Scheme A Synthesis of 18-OH progesterone (V) from conessine (I) by Buzzetti et al. (93)

von Braun's demethylation of conessine (I) with cyanogen bromide removed two N-methyl groups and gave II, which was converted by Ruschig's deamination with N-chlorosuccinimide into the N,N'-dichloro derivative (III). Treatment of III with aqueous alcoholic potassium hydroxide eliminated two equivalents of hydrogen chloride and effected hydrolysis at C-3 to yield the Δ^4-3-ketone derivative (IV). Hydrolytic fission of ring F and degradation of the amino group with nitrous acid gave the nitrogen-free product 18-OH progesterone (V). The overall yield from I was about 16%.

Alternate routes for the synthesis of some 18-oxygenated steroids from conessine have been developed by Pappo. These are described in greater detail below.

The synthesis of 18-OH-DOC (XIII; Scheme B) has so far been described by Pappo in 1959 only in a preliminary communication (97) and in U.S. Patents (98). It involves a fifteen-step synthesis starting from the alkaloid conessine (I). This long but ingenious synthesis leads not only to 18-OH progesterone (XII) but also to 18-OH-DOC (XIII). The synthetic sequence is depicted in Scheme B.

Conessine (I) was first converted to the 6-ketone (II) by hydroboration with sodium borohydride in the presence of aluminum chloride in diglyme, followed by chromic acid oxidation of the resulting 6-boron intermediate. The 6-ketone (II) was converted, by



Scheme B Synthesis of 18-OH-DOC (XIII) from conessine (I) by Pappo (97, 98)

reaction with methyl iodide, into the bisquarternary methiodide, which on reaction with potassium t-butoxide in boiling t-butanol underwent Hofmann degradation at C-20 and simultaneous 3,5-trans-elimination to give the 3,5-cyclosteroid (III). This intermediate (III) was quaternized with methyl p-toluenesulfonate and the resulting methotosylate was then hydroxylated with aqueous potassium chlorate catalyzed by osmium tetroxide to give a mixture of 20-epimeric diol (IV). Treatment of IV with potassium t-butoxide gave the nitrogen-free product 18,20 β-oxide (V). The 21-tosylate of V was converted to the 21-dimethylamino derivative (VI) with dimethylamine. The 6-keto group in VI was reduced by lithium aluminum hydride to a mixture of 6-alcohols (VII). The 3,5-cyclosteroid (VII) was isomerized to the corresponding Δ^5-3β-ol derivative (VIII) using formic acid. Oppenauer oxidation of VIII yielded the Δ^4-3-ketone (IX). Dimethylhydroxylamine was eliminated from the corresponding N-oxide (X) by refluxing in t-butylbenzene, giving the enol ether (XI). Hydration of XI with dilute acid yielded the 18,20-cyclo-hemiketal form of 18-OH progesterone (XII), whereas hydroxylation of XI with osmium tetroxide in pyridine gave the 18,20-cyclohemiketal form of 18-OH-DOC (XIII).

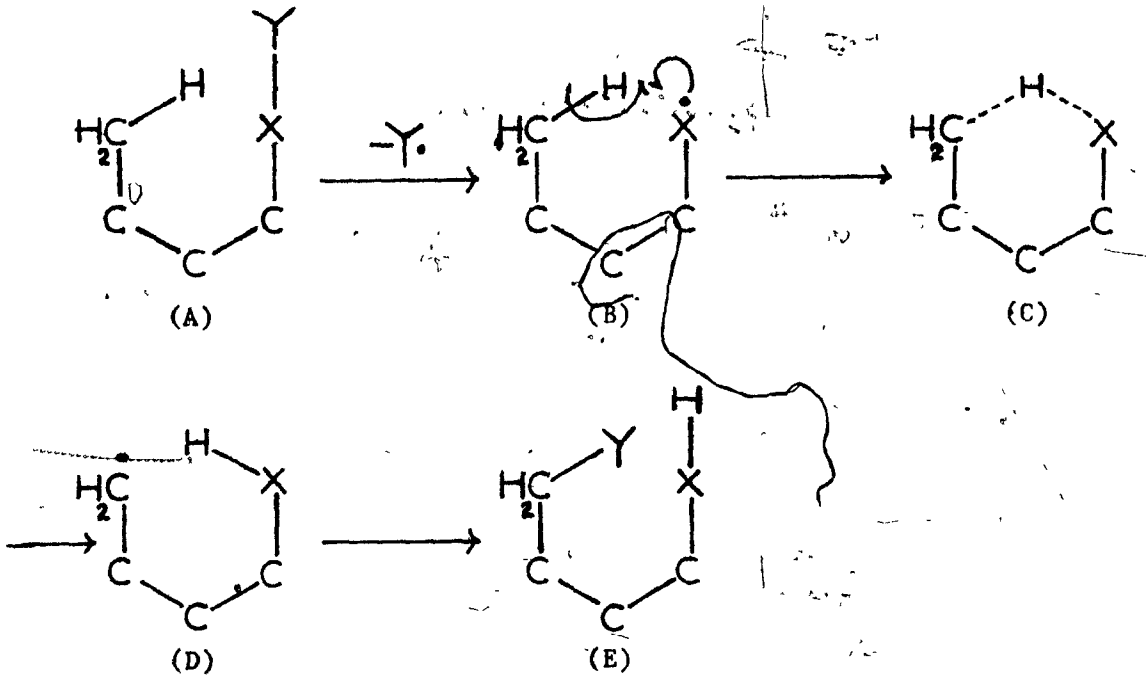
By following Pappo's procedure, all attempts to achieve a cyclization reaction from the 20-epimeric diol (IV) to the 18,20β-oxide (V) were unsuccessful in this laboratory. Alternative routes

to 18-OH progesterone and 18-OH-DOC were therefore sought by the more convenient photochemical method and are described later in a separate section.

(b) Method two:

The direct introduction of an oxygen function at the 18-unsubstituted methyl group by intramolecular radical processes.

Intramolecular free-radical reactions are of practical importance in steroid chemistry in connection with functionalization at non-activated carbon atoms. The most important feature of this reaction is the need for favorable proximity of the groups involved. The intramolecular radical reactions can generally be represented by Scheme C, according to the hypothetical principle of Barton et al. (99).



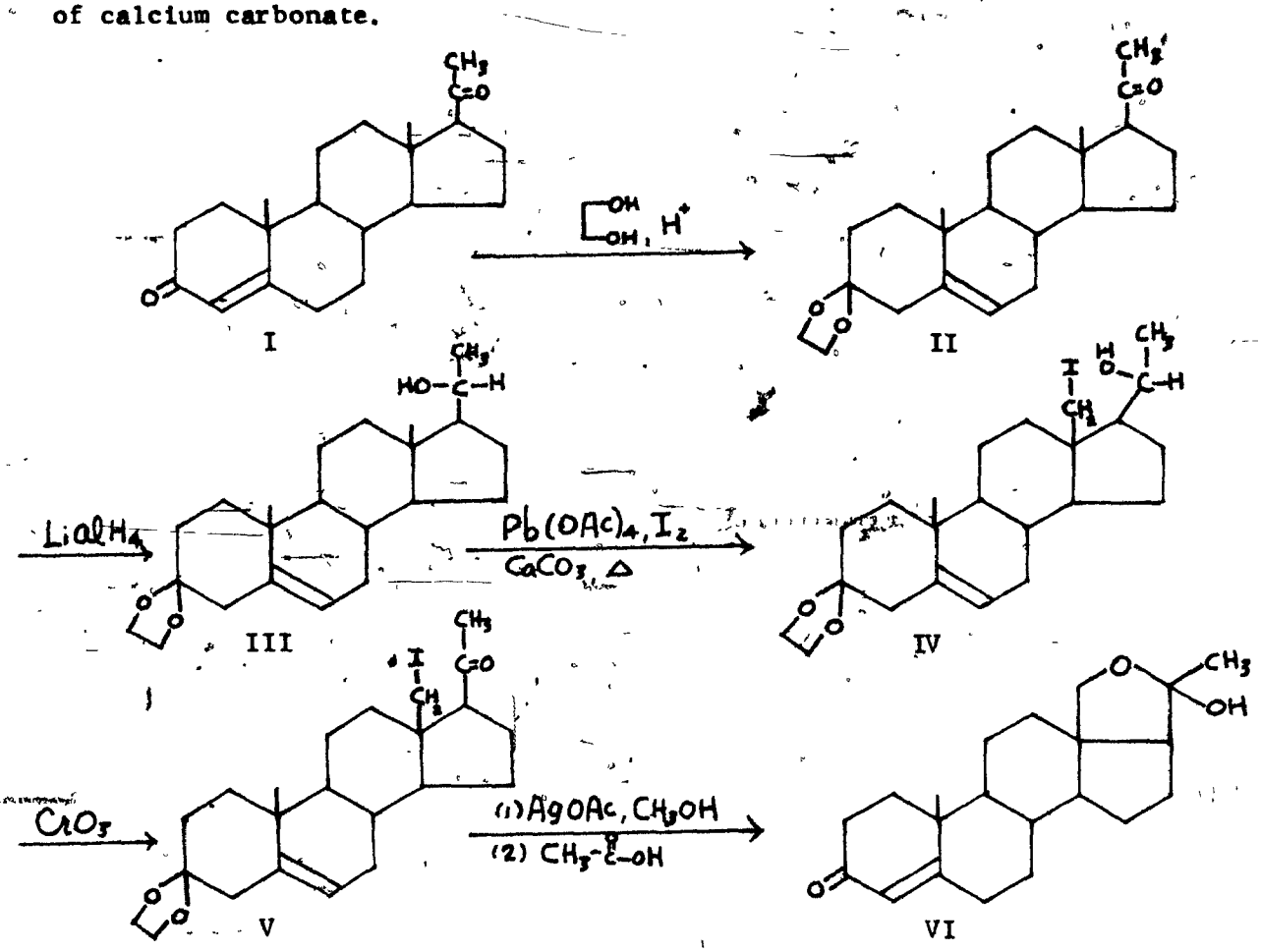
Scheme C Free-radical induced intramolecular hydrogen shift (99)

The important reactions described here are those in which (X) = O and (Y) = I or N = O. The reactive radical (X) may be generated by thermal or photolytic homolysis of the X-Y bond in (A). The resulting free radical or similar reactive species (B) is in a favorable position for the transfer of the hydrogen atom via a six-membered, cyclic transition state (C). The hydrogen transfer leads to a $\cdot CH_2$ radical (D), which may react with a suitable species from the solution, with another free radical, or with a reactive intramolecular site. By suitable choice of steroid substrate, this type of reaction has been used to introduce an oxygen atom into C-18.

For the oxygenation of the angular methyl group at C-18 in steroids three methods involving intramolecular radical reactions are known: (a) oxidation of 20-alcohols with lead tetraacetate in the presence of iodine (the "hypoiodite reaction") (100), (b) photolysis of 20-nitrite esters (99, 101), and (c) photolysis of 21-acetate-20-ketones (102, 103). Of these, the last method appears to be the simplest and most efficient. Methods (a) and (c) share a common advantage: the 20-alcohol and 20-ketone can be used directly as starting material. Method (b), on the other hand, requires an additional step for the formation of a reactive nitrite derivative before homolysis.

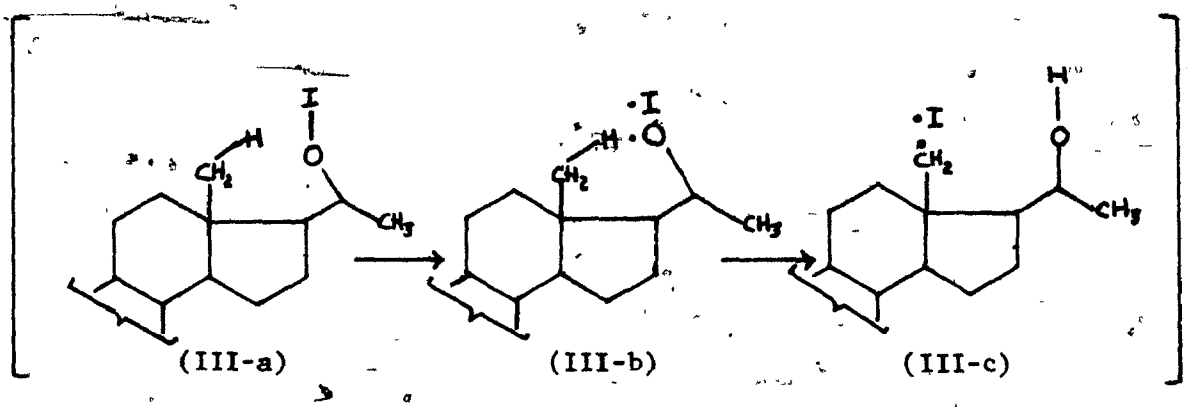
Wettstein et al. in 1962 (100) first reported the synthesis of 18-OH progesterone (VI; Scheme D) from progesterone (I)

by treatment of the 20-β-hydroxyprogesterone derivative (III) with lead tetraacetate and iodine in boiling cyclohexane in the presence of calcium carbonate.



Scheme D. Synthesis of 18-OH progesterone (VI) from progesterone (I) by Wettstein et al. (100)

The initial reaction has been rationalized to be the homolytic cleavage of the presumed intermediate 20-hypoiodite (III-a) leading to the formation of the 20-alkoxy-radical (III-b). This oxygen

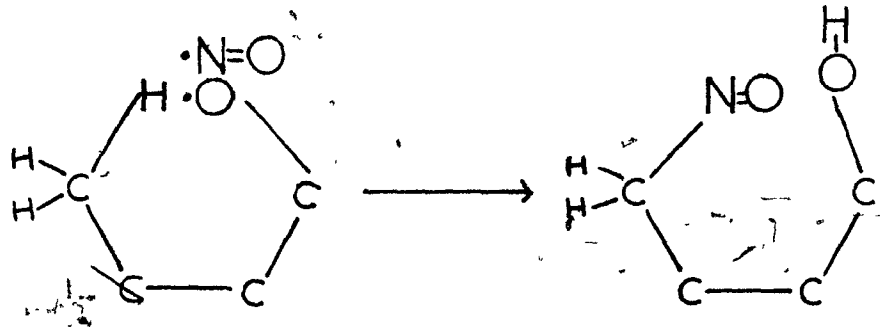


(III-a) (III-b) (III-c)

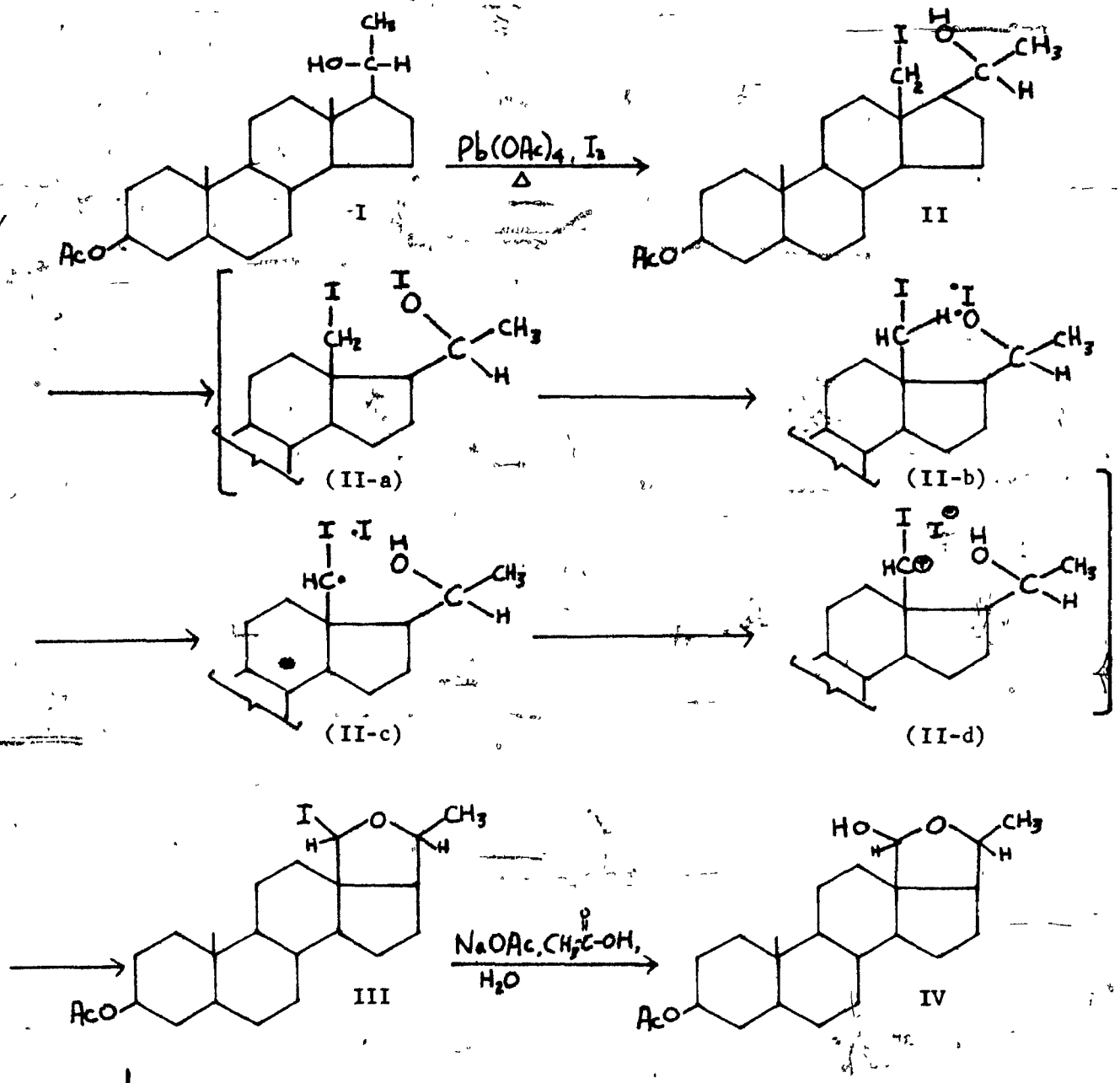
radical (III-b) may abstract a hydrogen from the C-18 methyl group four carbons away to form the 18-iodo-20-hydroxy derivative (IV). Chromic acid oxidation of IV gave 18-iodo-20-ketone (V), which was hydrolyzed with silver acetate in methanol followed by acetic acid to afford 18-OH progesterone (VI) in 20% overall yield from II.

Reactions with lead tetraacetate and iodine are frequently complicated by further attack at C-18 leading to IV (see Scheme E) (100).

Barton et al. in 1960 (99) reported the synthesis of IV (see Scheme F) via irradiation of the 20β-nitrite ester (II) of the 20β-hydroxyl steroid (I). This reaction is now known as Barton's nitrite photolysis.

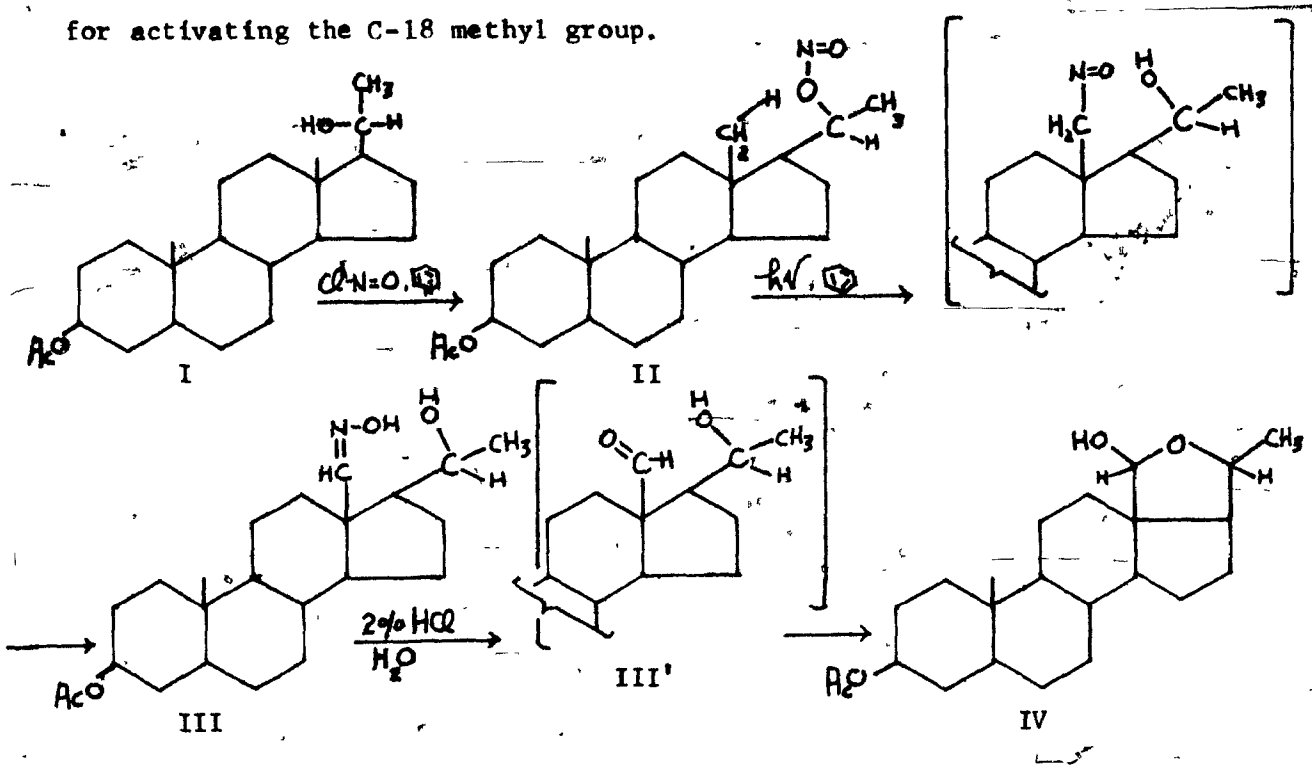


(a) (b)



Scheme E Synthesis of 3β-acetoxy-18-hydroxy-18,20β-epoxy-5α-pregnane (IV) from 3β-acetoxy-20β-hydroxy-5α-pregnane (I) by Wettstein et al. (100)

The reaction involves photolysis of a nitrite ester (a), leading to the intramolecular exchange of the N = O group with a hydrogen atom four carbons away. The nitroso intermediate (b) is formed in the reaction mixture. The eventual product is an oxime, which yields the aldehyde group upon hydrolysis. Barton's discovery has provided another versatile method for activating the C-18 methyl group.

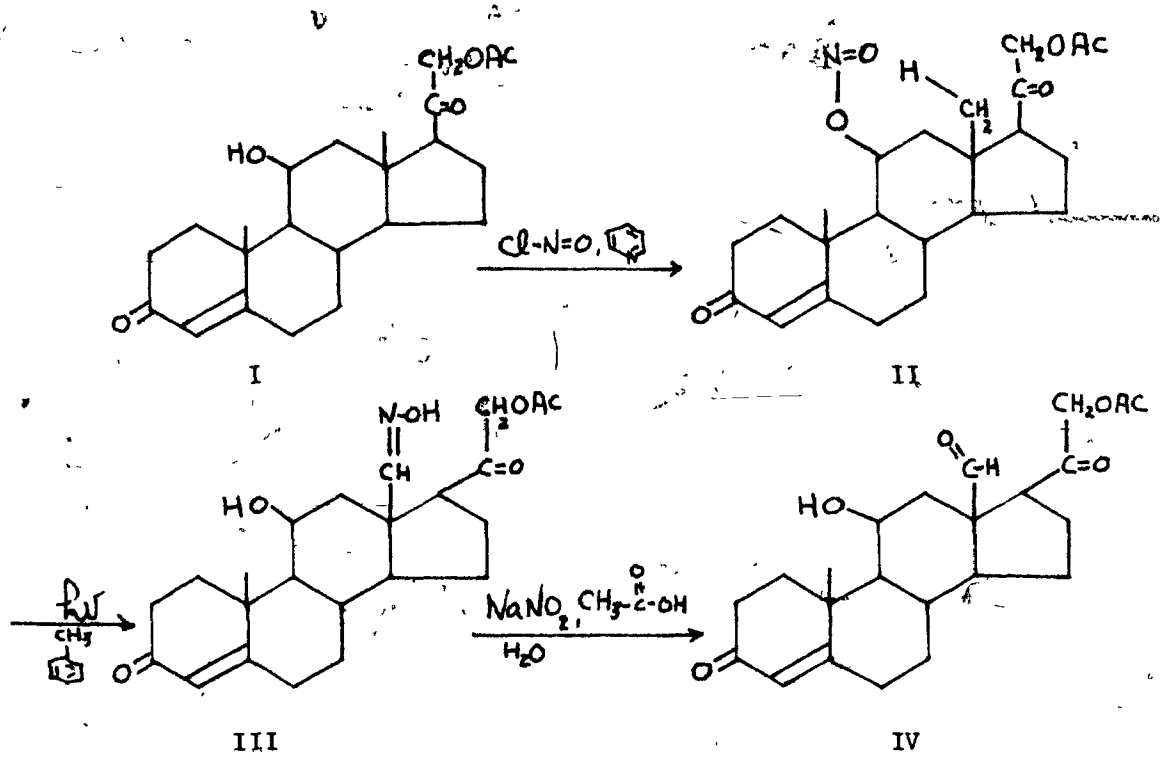


Scheme F Synthesis of 3β-acetoxy-18-hydroxy-18,20β-epoxy-5α-pregnane (IV) from 3β-acetoxy-20β-hydroxy-5α-pregnane (I) by Barton et al. (99)

3β-acetoxy-20β-hydroxy-5α-pregnane (I) was converted to the corresponding 20β-nitrite ester (II) with nitrosyl chloride in pyridine. Photolysis of II in benzene solution allowed the selective introduction of

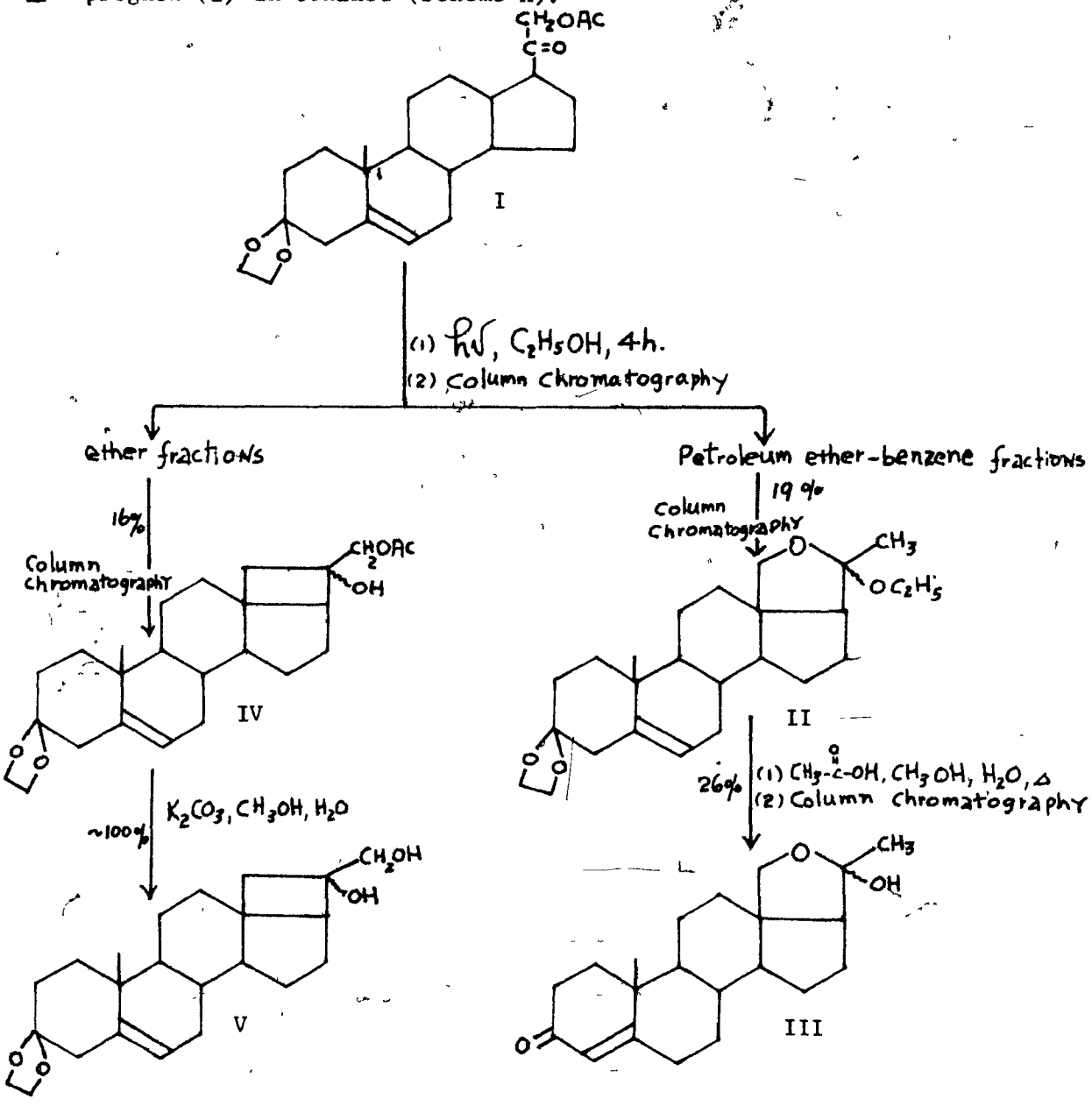
an oxygen function at C-18 affording the masked 18-aldehyde (IV) after acid hydrolysis of the 18-oxime (III). The overall yield was about 33% starting from I.

The three-step synthesis of aldosterone (IV) in 15% yield from corticosterone (I) (Scheme G) via the photolysis of the nitrite ester (II) by Barton and Beaton in 1960 (101) is another application of the free-radical induced intramolecular hydrogen abstraction reactions.



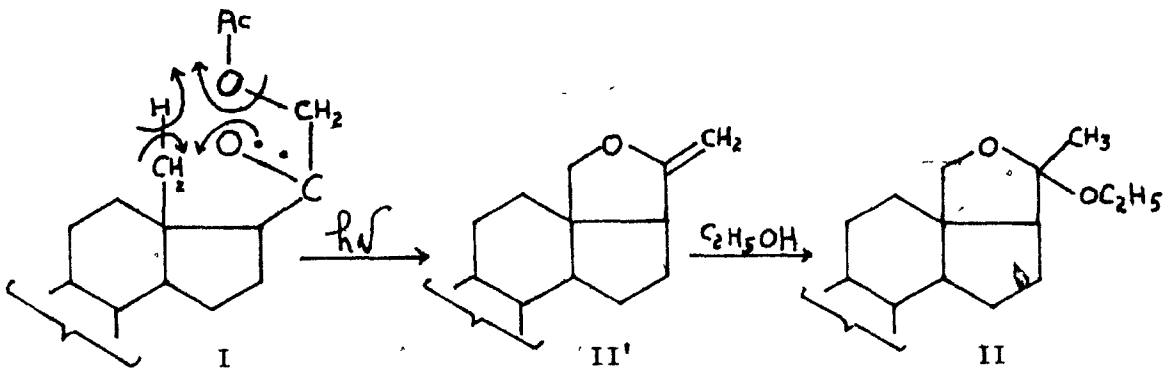
Scheme G Synthesis of aldosterone-21-acetate (IV) from corticosterone-21-acetate (I) by Barton and Beaton (101).

An unusual reaction which permits direct introduction of a C-18 oxygen function has been observed by Jeger and co-workers in 1960 (102, 103) in the photolysis of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (I) in ethanol (Scheme H).



Scheme H Jeger's (102, 103) photochemical reaction of deoxycorticosterone acetate derivative (I)

In addition to the expected 18,20-cyclobutanol derivative (IV), an ethyl ketal (II) having the 18,20-cyclohemiketal structure was obtained. The formation of II, which differs substantially from the usual intramolecular hydrogen transfer process (see Scheme C), has been postulated by Jeger to be proceeding through the following mechanism:



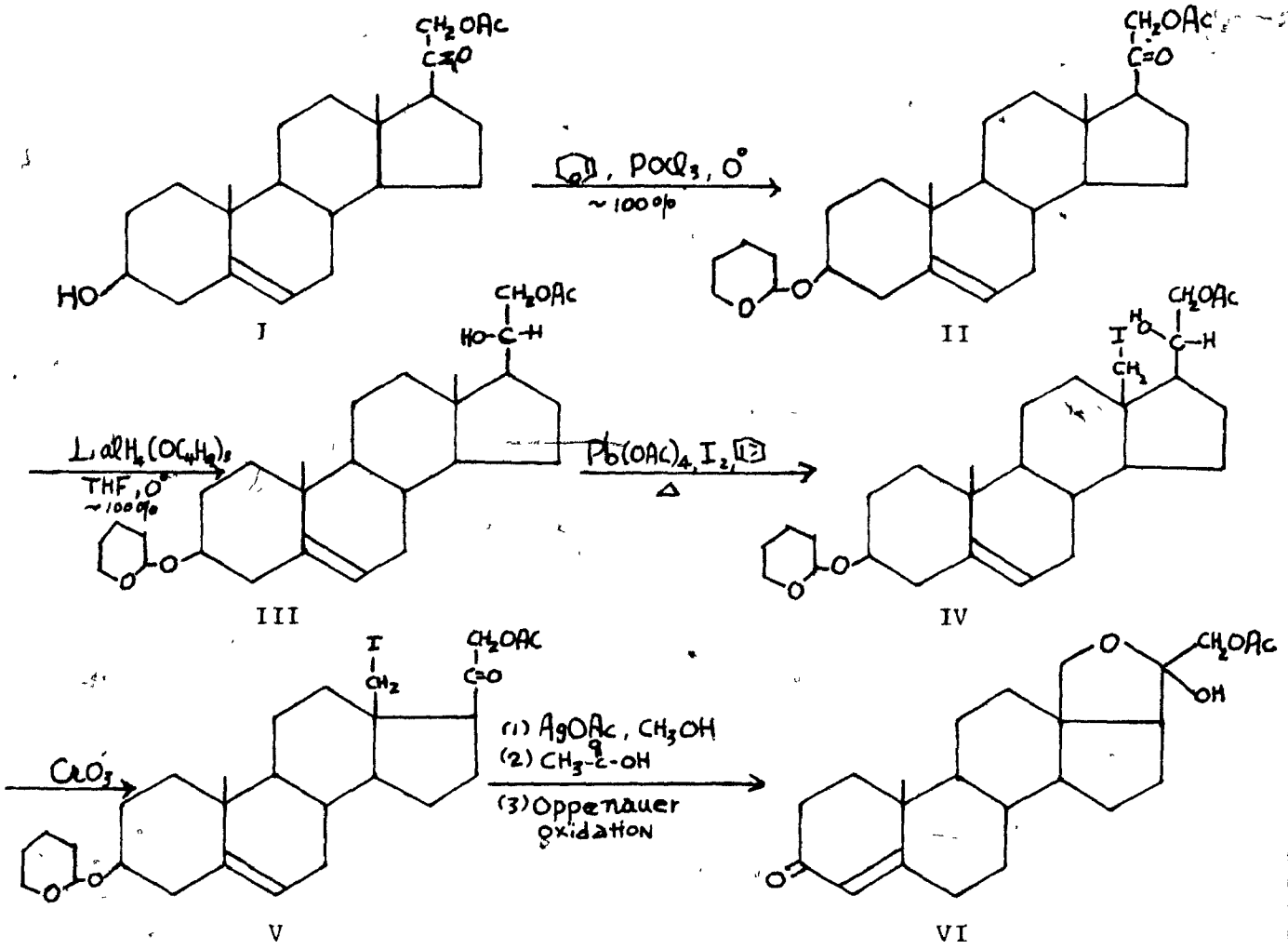
The intermediate of the reaction is the enol ether (II') which was converted by the solvent to II. Acid hydrolysis of the ethyl ketal (II) gave the parent compound 18-OH progesterone (III).

We adopted Jeger's method for the preparation of 18-OH progesterone because of its simplicity and convenience.

PART III: SYNTHESIS OF 18-OH-DOC, RESULTS AND DISCUSSION

1. Attempted conversion of 21-acetoxy pregnenolone (I) to 18-OH-DOC acetate (VI) by reaction with lead tetraacetate and iodine:

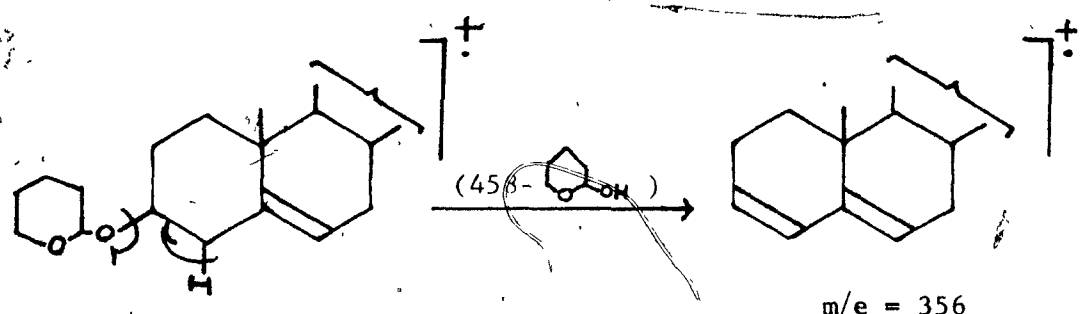
In exploratory experiments, we examined a possible method for the synthesis of 18-OH-DOC acetate (VI) from 21-acetoxy pregnenolone (I) by application of the "hypoiodite reaction" published by Wettstein and co-workers (100). The approach to this synthesis is illustrated by the following sequence of reactions (Scheme 1).



Scheme 1 Proposed route to the synthesis of 18-OH-DOC acetate (VI) from 21-acetoxy pregnenolone (I)

21-Acetoxy pregnenolone (I, m.p. 165-170°) in chloroform was converted to the corresponding tetrahydropyran ether (II, m.p. 115-120°) in quantitative yield by treatment with 2,3-dihydropyran and a catalytic amount of phosphorous oxychloride at 0° for 1.5 h. The yield was 80% when p-toluenesulfonic acid monohydrate was used as a catalyst.

The spectral data were in accord with the structure II. The infrared spectrum of II showed the absence of hydroxyl absorption near 3560 cm^{-1}, but had strong bands at 1750 cm^{-1} (C=O) and 1228 cm^{-1} (C-O) attributable to the 21-acetate group, and at 1723 (C=O) ascrible to the 20-ketone moiety. The N.M.R. spectrum of II indicated the C-6 vinyl proton at τ 4.59 (1H, broad singlet), the 21-methylene protons at τ 5.32 (2H, AB quartet, J_{AB} = 19 c.p.s.), the 21-acetate protons at τ 7.80 (3H, singlet), the C-19 methyl protons at τ 8.95 (3H, singlet), and the C-18 methyl protons at τ 9.27 (3H, singlet). The mass spectrum of II was devoid of the molecular ion (m/e = 458), but showed a base ion at m/e = 356 which probably arose by the following fragmentation:

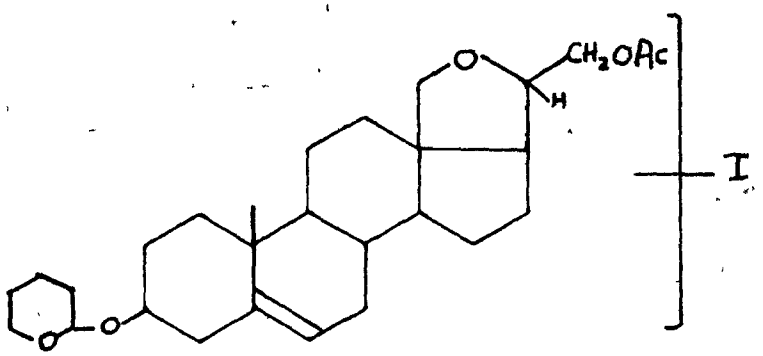


m/e = 458 m/e = 356

Reduction of the 20-ketone (II) to the corresponding 20-alcohol (III, m.p. 125-128°) in quantitative yield was effected using lithium aluminum tri-t-butoxyhydride in tetrahydrofuran at 0° for 1.5 h. The infrared spectrum of III showed the reappearance of hydroxyl bands at 3460, 3598, and 3680 cm^{-1} and the disappearance of the band at 1723 cm^{-1} for the 20-ketone. The continued presence of the 21-acetate carbonyl group was denoted by the band at 1735 cm^{-1}. The presence of an hydroxyl group in III was further supported by the N.M.R. peak at τ 7.63 (1H, singlet) and this signal discharged upon addition of D_2O to the sample. The mass spectrum of III was devoid of the molecular ion (m/e = 460), but showed a base ion at m/e = 358 (460 - ). In preliminary experiments, we found that the reduction by sodium borohydride of the 20-keto-21-acetate (II) in methanol at 0° for 2 - 3 h. (104) gave the 20,21-diol derivative as the principal product due to the concomitant base-catalyzed hydrolysis of the 21-acetate moiety. Reduction of 20-keto-21-acetate (II) using sodium borohydride in dry dimethylformamide or in 80% aqueous dimethylformamide at 0° for 3 h (105) gave approximately equal amounts of the reduction product 20-ol-21-acetate (III) and the starting material (II).

Reaction of 20-ol-21-acetate (III) with lead tetraacetate and iodine (one equivalent) (100) for 2.5 h in boiling benzene under reflux gave a complex mixture of products which were separated by pre-

parative TLC. Three non-crystalline fractions were isolated in low yields and the individual fractions were analyzed by infrared and mass spectrometry. The infrared spectra of these fractions showed similar absorption patterns and the disappearance of the hydroxyl bands near 3400 - 3500 cm^{-1}. The continued presence of the 21-acetate carbonyl moiety was indicated by the band at 1738 cm^{-1}. Bands in the region (1270 - 1000 cm^{-1}) corresponding to C-O stretching vibrations were also observed. The mass spectra of these fractions showed strong peaks in the high mass region (m/e = 500-600) suggesting the presence of iodo substituted compounds; however, no definite structural information could be deduced from these fragment ions. It is possible that an 18,20-epoxy derivative, as depicted in the postulated structural formula below, might have been formed, according to the view of Wettstein et al. (100).

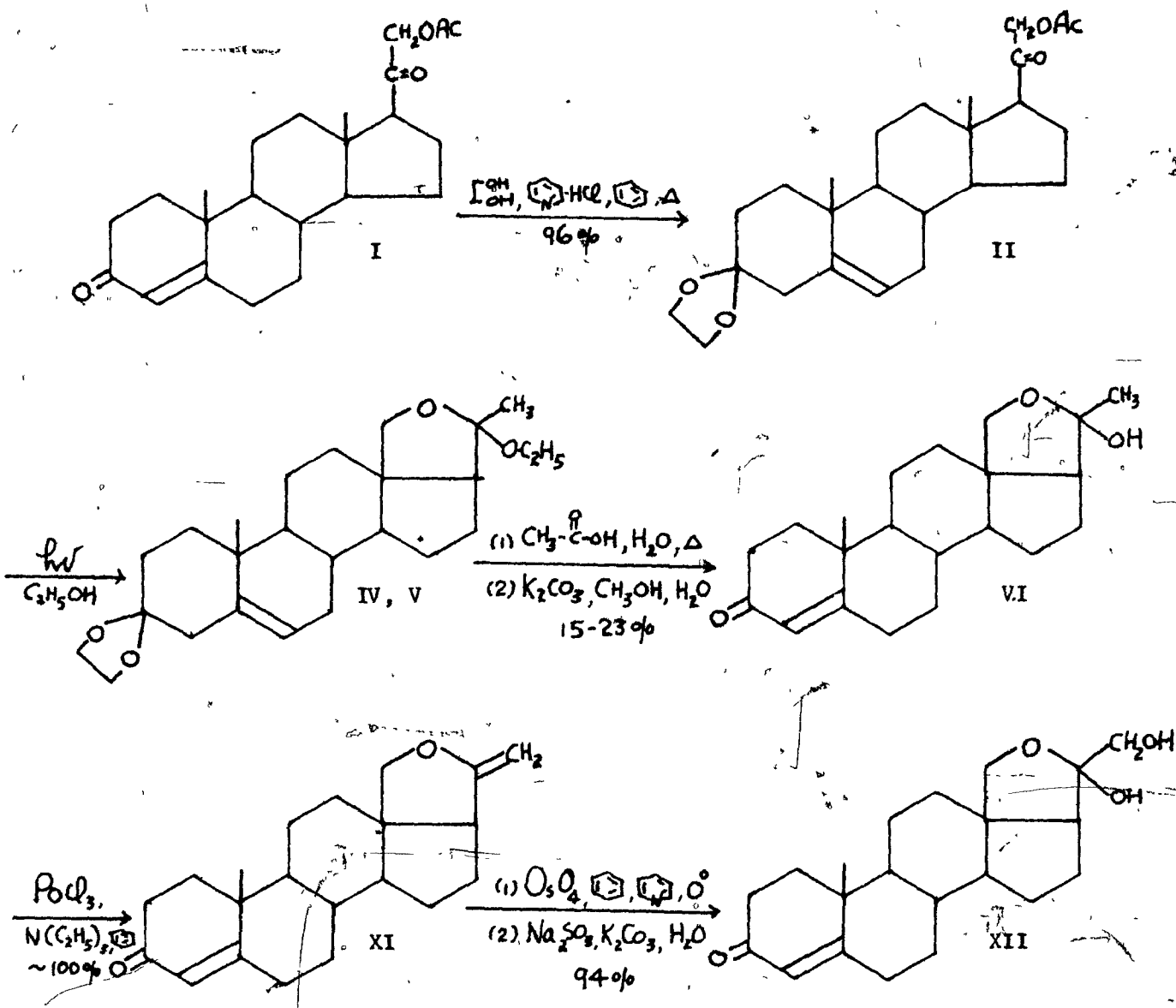


However, no further identification was attempted on these compounds because of the low yield and the fact that the reaction products could be resolved

by chromatographic means only with great difficulty.

2. An outline of the synthesis of 18-OH-DOC from deoxycorticosterone acetate:

The synthetic pathway for 18-OH-DOC developed in this laboratory is represented by the following scheme.



Scheme 2 - Synthesis of 18-OH-DOC (XII) from deoxycorticosterone acetate (I)

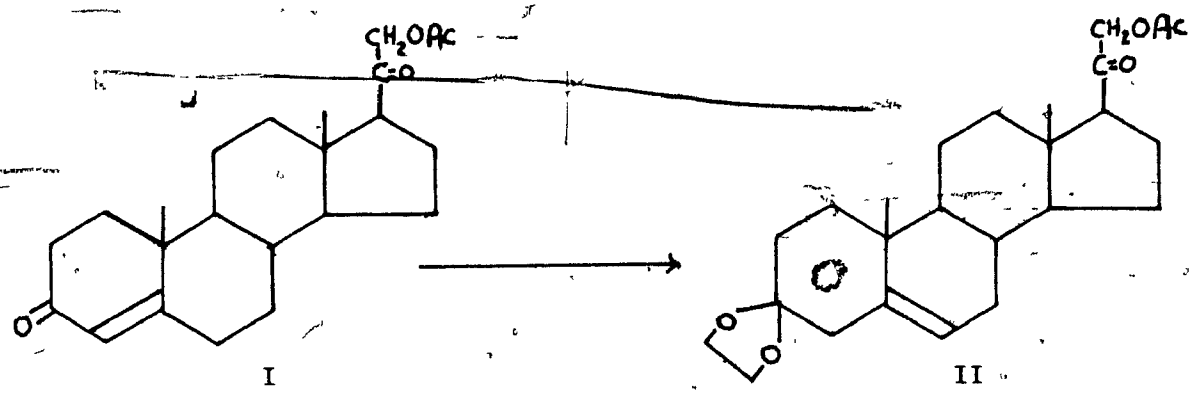
As the immediate precursor for the synthesis of 18-OH-DOC (XII), we chose the structurally similar steroid 18-OH-progesterone (VI). It possesses the Δ^4-3-ketone as well as the 18,20-cyclohemiketal which can be modified to 18-OH-DOC by functionalizing the 21-methyl group.

18-OH Progesterone (VI) was prepared from the readily available deoxycorticosterone acetate (I) by the photochemical method developed by Jeger et al. (102, 103). The advantage of this method is its simplicity and convenience. Thus, 18-OH progesterone can be obtained in a three-step process. Having obtained the intermediate 18-OH progesterone (VI), the next task was the elaboration of the 21-hydroxyl group. Briefly, this was accomplished by converting 18-OH progesterone (VI) into the enol ether (XI) followed by hydroxylation of XI into the desired 18-OH-DOC (XII). The overall yield for the sequence of reactions based on deoxycorticosterone acetate (I) is 15%.

3. Preparation of 18-OH progesterone and identification of photoproducts:

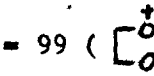
(a) Preparation of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II), the starting material:

Ketalization of deoxycorticosterone acetate (I) in benzene with ethylene glycol and pyridine hydrochloride (0.5 mol equivalent) as the acid catalyst gave pure crystalline 3-ethylene ketal (II; m.p. 194-196°, $[\alpha]_D^{25}$ = + 76.4°) in 96% yield.



I II

The mass spectrum showed an intense molecular ion at m/e=416 corresponding to the molecular weight of II. The presence of the 20-ketone function was corroborated by the infrared band at 1730 cm^{-1}. The absence of the α,β-unsaturated ketone group was verified by the disappearance of infrared absorption at 1668 cm^{-1} and also the lack of ultraviolet absorption at 240 nm. The strong infrared band near 1097 cm^{-1} may be ascribed to the C-O stretching vibrations of ethylene

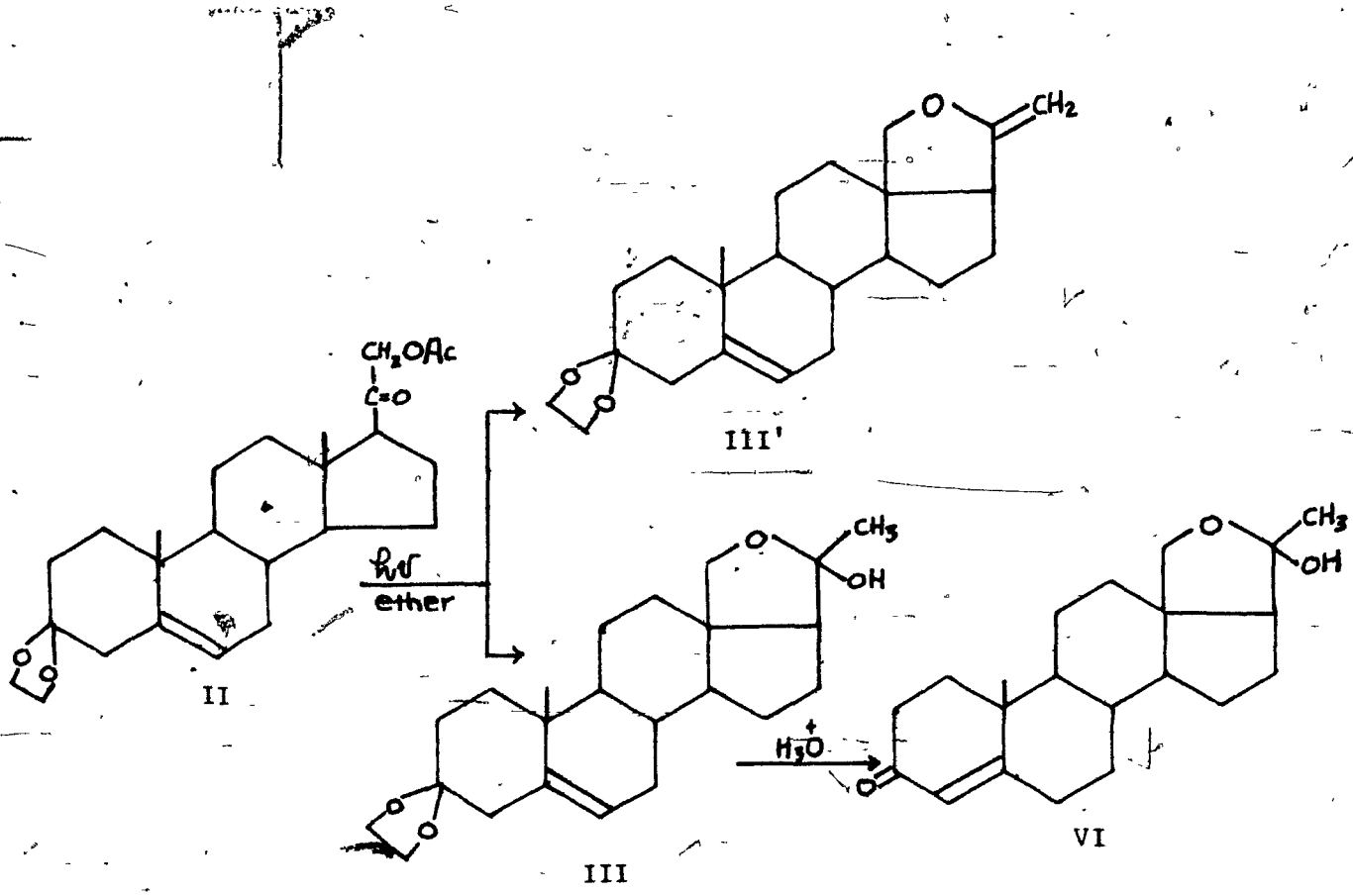
ketals (106). The presence of ethylene ketal was also supported by the N.M.R. signal at τ 5.92 (4H, singlet) and by the base peak at m/e = 99 () in the mass spectrum (132). The presence of the 21-acetoxy group was indicated by infrared absorption at 1748cm^{-1} (C = O) and by the N.M.R. peaks at τ 5.25 (2H, AB quartet, J_{AB} = 20 c.p.s.) for the 21-methylene protons and at τ 7.72 (3H, singlet) for the acetyl methyl protons. The N.M.R. spectrum also showed C-19 methyl protons at τ 8.84 (3H, singlet) and C-18 methyl protons at τ 9.19 (3H, singlet). The vinyl proton exhibited a broad singlet near τ 4.61. This is in agreement with structure II where migration of the double bond from the Δ^4- to the Δ^5-position occurred.

The non-reactivity of the 20-ketone group in this reaction may be ascribed to the steric effects of the neighboring 21-acetoxy group; such effects have been shown with other steroids (106). Pyridine hydrochloride appears to be a useful acid catalyst for the ketalization of I. Bernstein et al. (107) prepared the 3-ethylene ketal (II) from I in 36% yield by the usual procedure employing p-toluene-sulfonic acid as the catalyst.

(b) Photolysis of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II) in absolute ether:

(i) 3,3-Ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnene (III) Jeger et al. reported in a patent literature (103) that the enol ether (III') was isolated in 21% yield from the photolysis of II in absolute ether. The exact

nature of this substance was not disclosed.



It was hoped that III' would be converted to 18-OH-DOC by hydroxylation of the enol ether function followed by acid regeneration of the Δ^4-3-ketone moiety. To our disappointment, attempted repetition of the experiment of Jeger, did not lead to the isolation of III' (see below). In a different approach, the crude reaction mixture (photolyzate) was, without deliberate isolation of III',

directly hydroxylated with osmium tetroxide in benzene containing pyridine followed by treatment of the reaction mixture with warm aqueous methanolic acetic acid. The crude residue was resolved by preparative TLC (Silica Gel G) into five non-crystalline fractions. Direct comparison of their infrared spectra, mass spectral fragmentation patterns, and mobilities on TLC and paper chromatograms with those of an authentic specimen failed to reveal any 18-OH-DOC.

Column chromatography of the photolyzate on neutral alumina (activity II) resulted in the isolation of seven compounds as amorphous residue. Infrared and mass spectral analyses indicated that none of these products were Jeger's enol ether (III'). One of the products (Fractions 44-54) was identified as III. The remaining compounds were not further investigated but their spectral properties are reported in the Experimental section.

Compound III (m.p. 137-140°) was eluted with a mixture of petroleum ether and benzene in 23% yield. The structure was assigned as III on the basis of the following evidence:

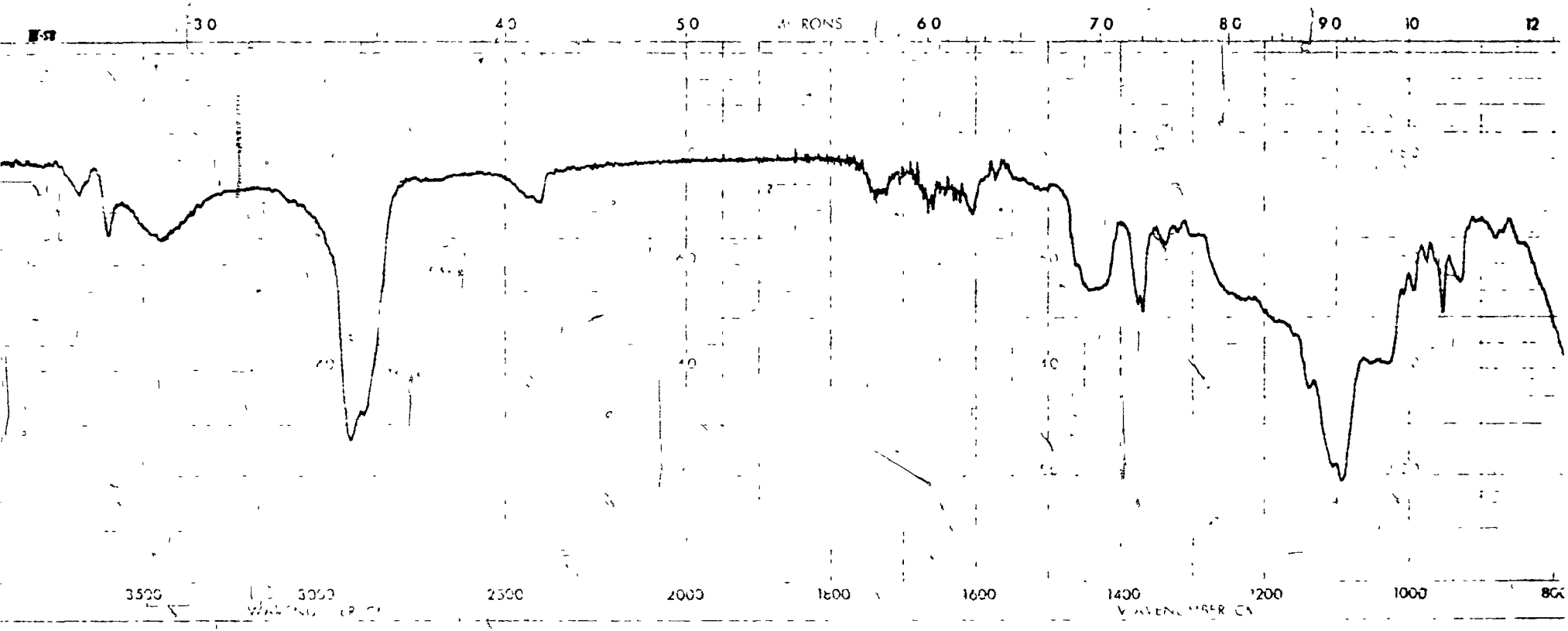
The infrared spectrum showed hydroxyl absorption at 3680, 3590, and 3450 cm^{-1} but no band due to a ketone group. The absence of the absorption maximum near 240 nm in the ultraviolet spectrum and of a band near 1660 cm^{-1} in the infrared spectrum indicated the absence of an α,β-unsaturated ketone moiety. The strong infrared band near 1095 cm^{-1}

is ascribed, at least in part, to the C-O stretching vibrations of the ethylene ketal group. This was corroborated by the signal at τ 5.93 (4H, sharp singlet) in the N.M.R. spectrum and by the base ion at m/e = 99 () in the mass spectrum. The N.M.R. spectrum showed a peak indicating a C-19 methyl group at τ 8.87 (3H, singlet) and a broad signal at τ 4.58 (1H) due to the vinyl proton of the Δ^5 double bond. The absence of the methyl peak at τ 9.19 (present in the starting material II) is indicative of the participation of the C-18 methyl group in the photochemical reaction. The molecular formula, $C_{23}H_{34}O_4$, was assigned for III on the basis of elemental analysis and was substantiated by the molecular weight 374.2457 (calcd. 374.2457) determination by high resolution mass spectrometry. The mass spectral fragmentation pattern can be rationalized on the basis of structure III (see Part IV, p. 127).

The identity of III was confirmed by hydrolysis of III to 18-OH progesterone (VI, m.p. 140°) with warm aqueous methanolic acetic acid. The yield, following purification by preparative TLC (Silica Gel G), was about 10%. The complete characterization of 18-OH progesterone (VI) will be discussed in a later section (see p. 72).

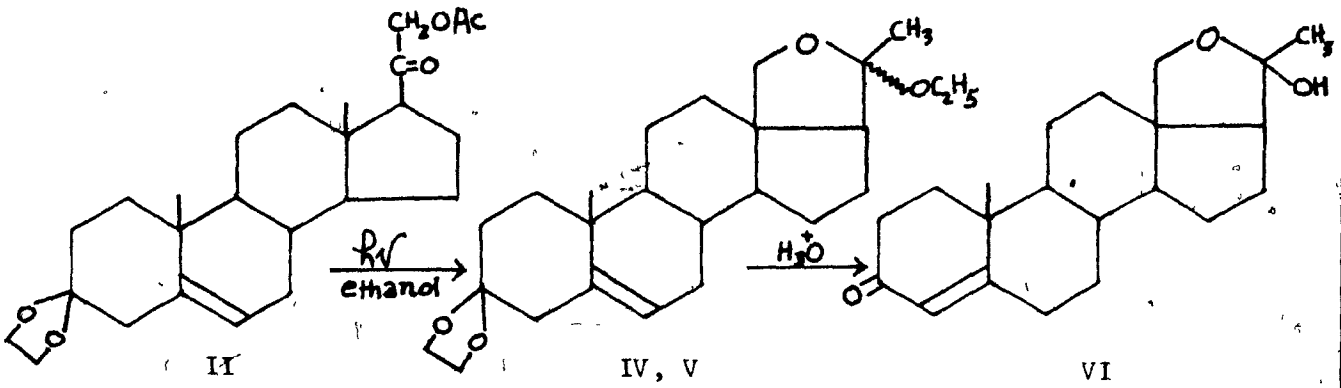
There have apparently been no prior reports on the isolation of III from the photolysis of II. It is possible that III may be formed by hydration of Jeger's enol ether (III') during the isolation procedure. As indicated by osmium tetroxide experiment, efforts to hydroxylate III' in situ were not successful.

I.R. ($CHCl_3$): 3,3-Ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnene (III)



(c) Photolysis of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II) in absolute ethanol:

Photolysis of II was then carried out in absolute ethanol. Chromatography of the photolyzate on a neutral alumina (activity II) column led to the isolation of two crystalline compounds, IV (Fractions 2-6) and V (Fractions 30-81), and a straw-colored residue (Fractions 83-86) which was not identified. IV appears to be an isomer of V and both compounds can be hydrolyzed with acid to 18-OH progesterone (VI).

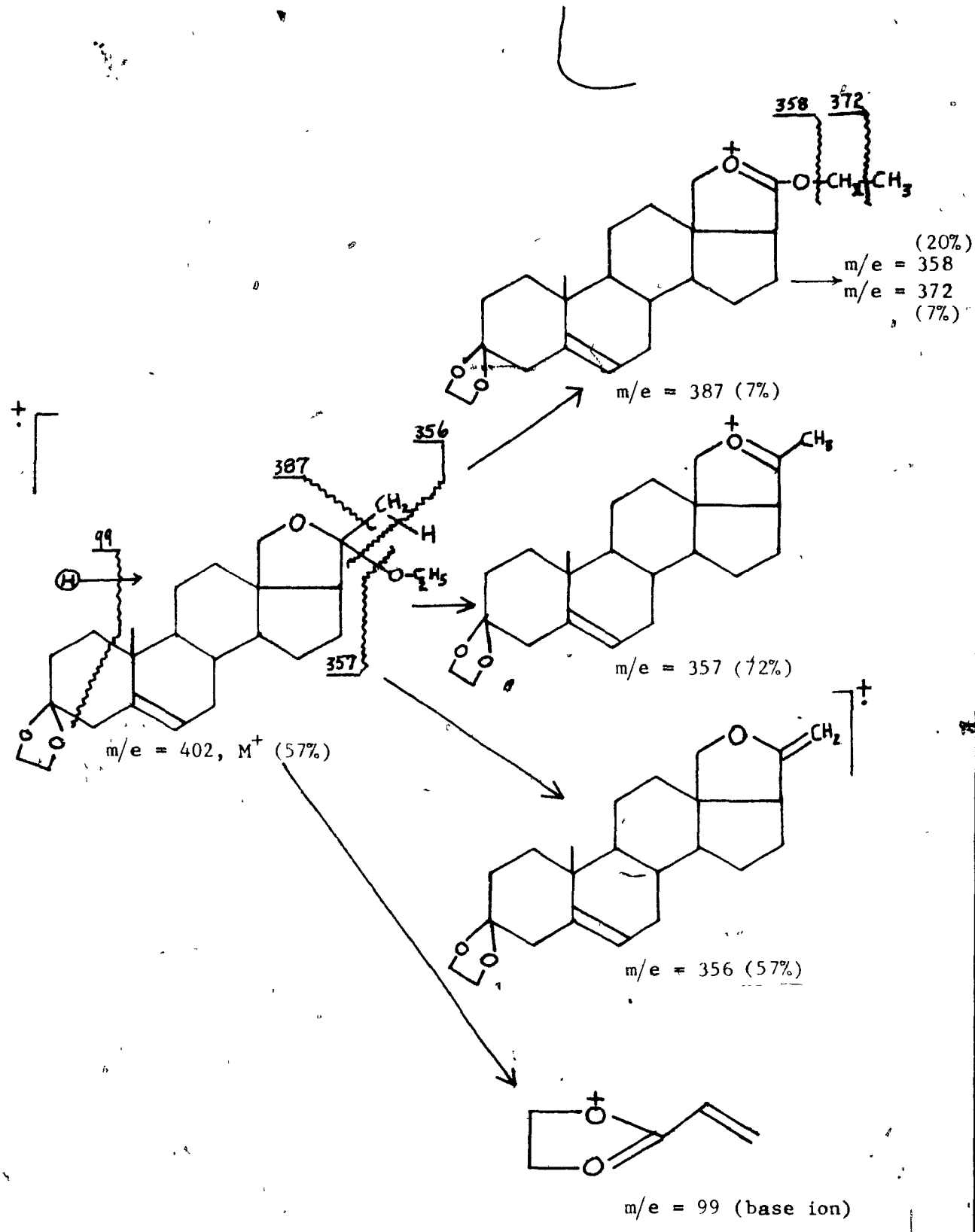


(i) Compound IV (fractions 2-6): Epimer A of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy -Δ^5-pregnene Elution with a mixture of petroleum ether and benzene afforded IV (m.p. 155-158°, $[\alpha]_D^{25}$ = +24.4°) in 18% yield.

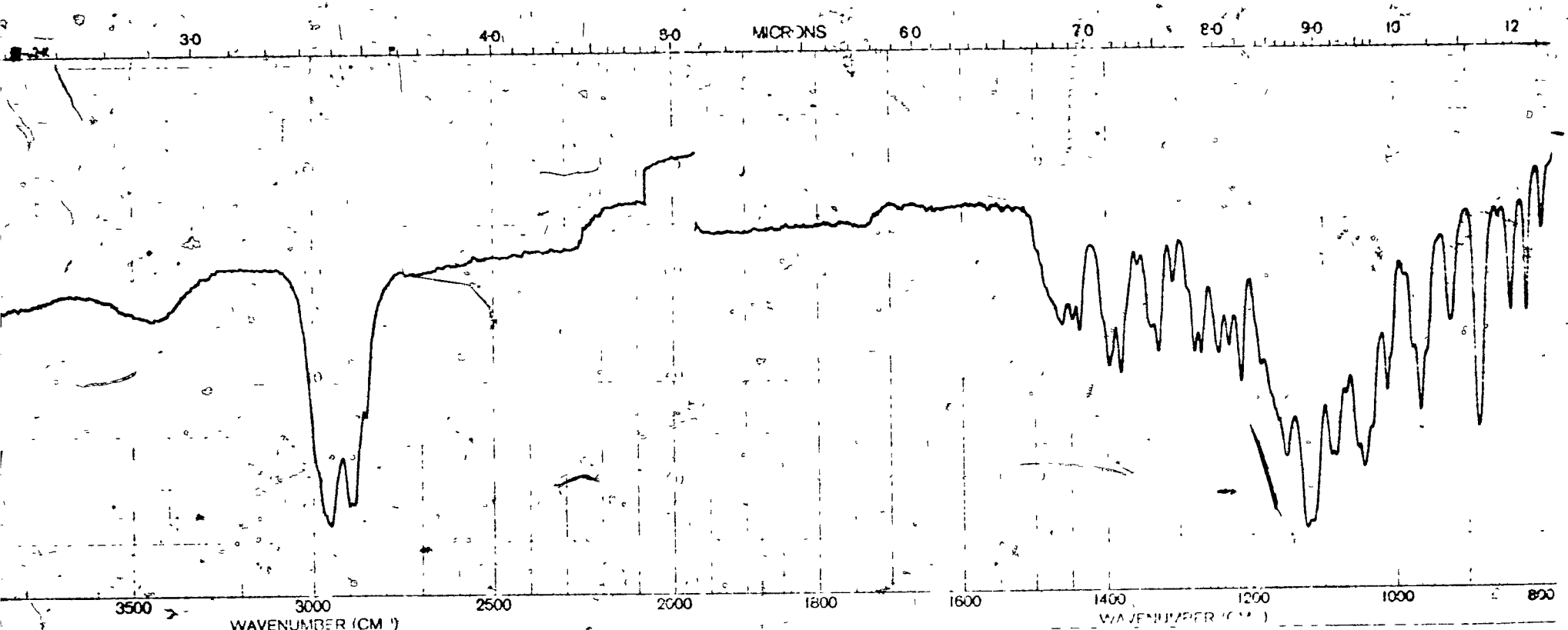
On the basis of elemental analysis, IV was assigned the empirical

formula $C_{25}H_{38}O_4$ which requires a tetracyclic structure in IV. This was supported by the molecular ion at m/e = 402 in themass spectrum. The infrared spectrum showed no evidence of hydroxyl (ca. 3000-4000 cm^{-1}) and carbonyl (ca. 1680-1740 cm^{-1}) absorption. The absence of Δ^4-3-ketone group was also verified by the complete lack of absorption in the ultraviolet (λ_{max} = 240 nm) spectrum. The strong infrared bands near 1105, 1065, and 1024 cm^{-1} might be ascribed, at least in part, to the C-O stretching vibrations of the ethylene ketal moiety. This interpretation was supported by the base ion at m/e = 99 in the mass spectrum. The mass spectral fragmentation pattern was consistent with structure IV.

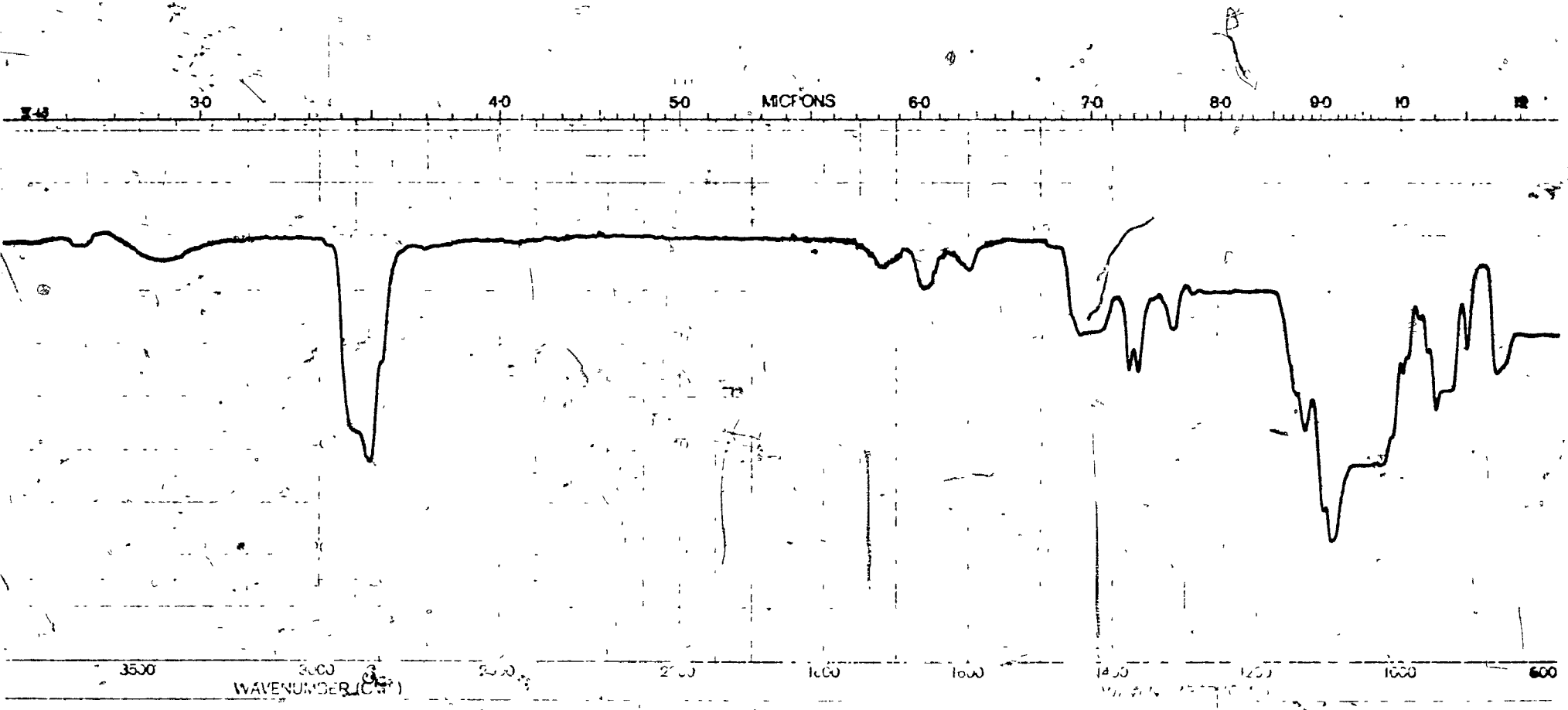
Additional evidence for structure IV was obtained by its hydrolysis with aqueous 30% acetic acid (15 min at 60°) to 18-OH progesterone (VI) as colorless crystals in 96% yield. The identity of VI was confirmed by mixed TLC, and comparison of the infrared and mass spectra with those of authentic 18-OH progesterone.


358
372
(20%)
m/e = 358
m/e = 372
(7%)
m/e = 387 (7%)
356
387
99
357
m/e = 402, M+ (57%)
m/e = 357 (72%)
m/e = 356 (57%)
m/e = 99 (base ion)

I.R. (KBr): Compound IV: Epimer A of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene

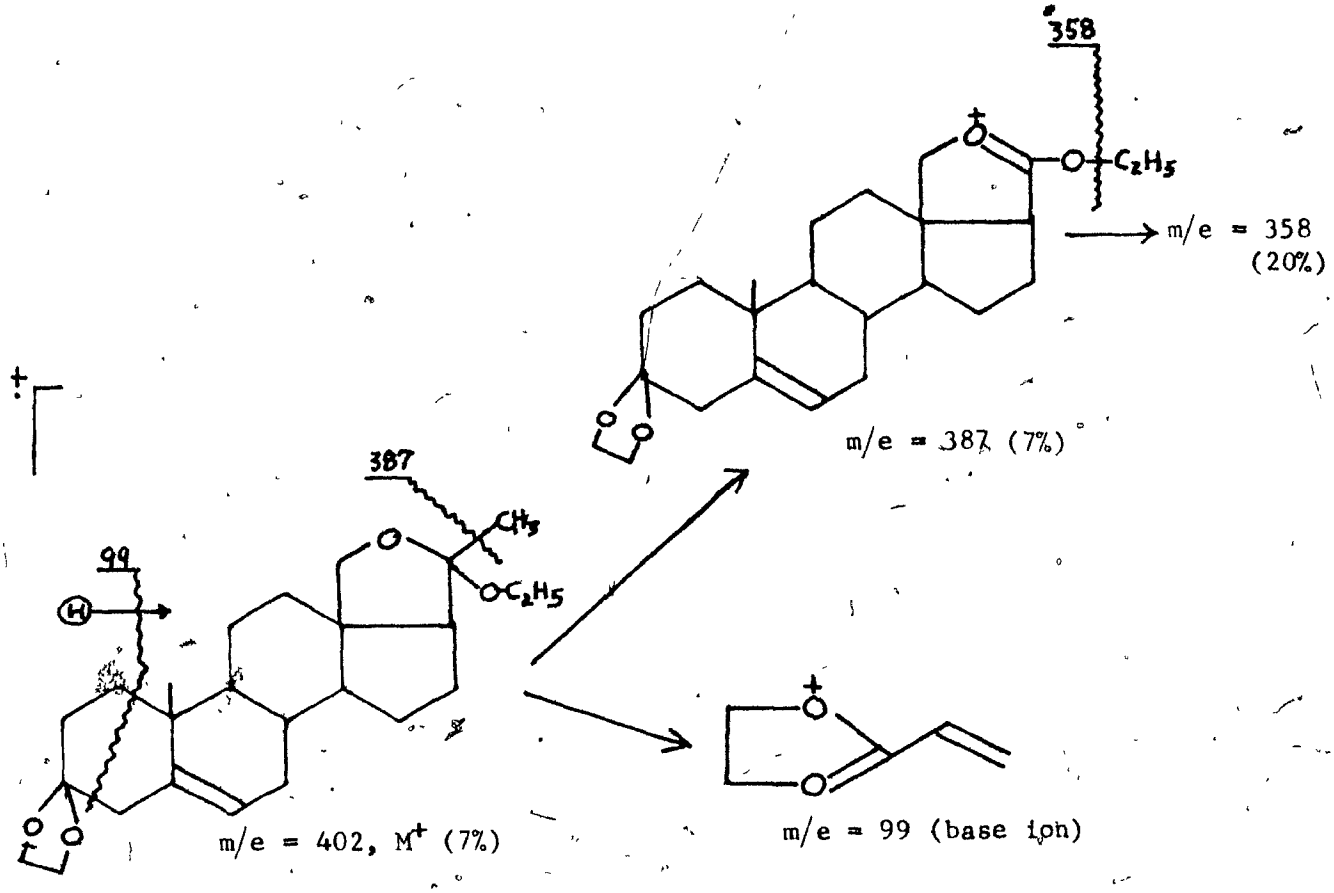


I.R.($CHCl_3$): Compound IV: Epimer A of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene



(ii) Compound V (fractions 30-81): Epimer B of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene Further elution with the same solvent, a mixture of petroleum ether and benzene, gave V (m.p. 187-193°, $[\alpha]_D^{25} = -22.3°$) in 17% yield.

The lack of infrared absorption near 1660-1680cm^{-1} and of an ultraviolet absorption maximum at 240 nm confirmed the absence of an α,β-unsaturated ketone moiety. The strong infrared band near 1090 cm^{-1} might be attributed, at least in part, to the C-O stretching vibrations of the ethylene ketal group. This was supported by the base ion at m/e = 99 in the mass spectrum. The infrared spectrum showed no absorption due to a ketone (ca. 1680-1730 cm^{-1}) function but had an unexpected band near 3500 cm^{-1} for hydroxyl group. Good elemental analysis could not be obtained however, possibly due to the instability of V. Partial hydrolysis of V to 18-OH progesterone occurred upon recrystallization from solvent (acetone-hexane) or on standing at room temperature. This probably explains the anomalous hydroxyl absorption shown in the infrared spectrum. The stability of V on recrystallization can be improved by incorporation of a trace of pyridine into the solvent and by keeping the compound in a desiccator under an atmosphere of pyridine. The mass spectrum showed a molecular ion at m/e = 402 corresponding to the molecular weight of V. The mass spectral fragmentation pattern could be interpreted on the basis of structure V:

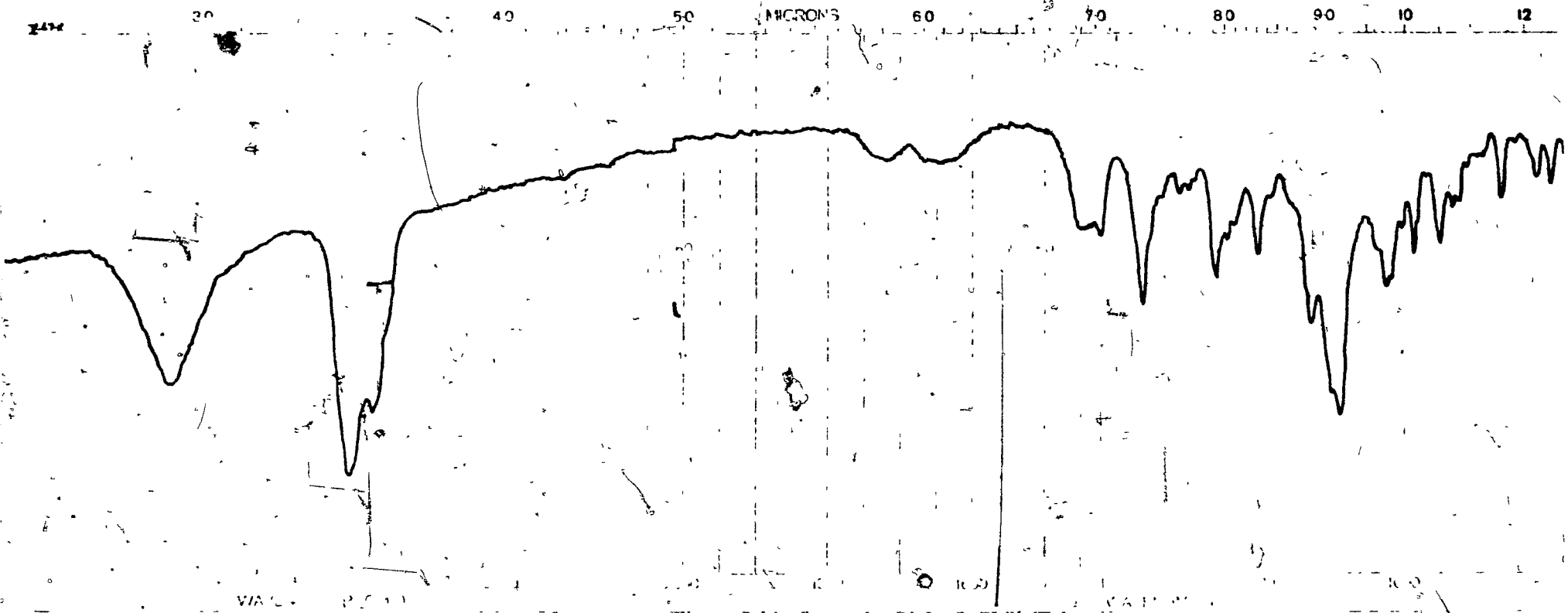


The identity of V was confirmed by hydrolysis (0.5 N hydrochloric acid in dioxane as solvent, 2 h at 60°) to 18-OH progesterone (VI) in 20% yield after separation by column chromatography on neutral alumina. VI (m.p. 155-160°) was identified by comparison of its physical constants (infrared spectrum, mass spectral fragmentation pattern, and

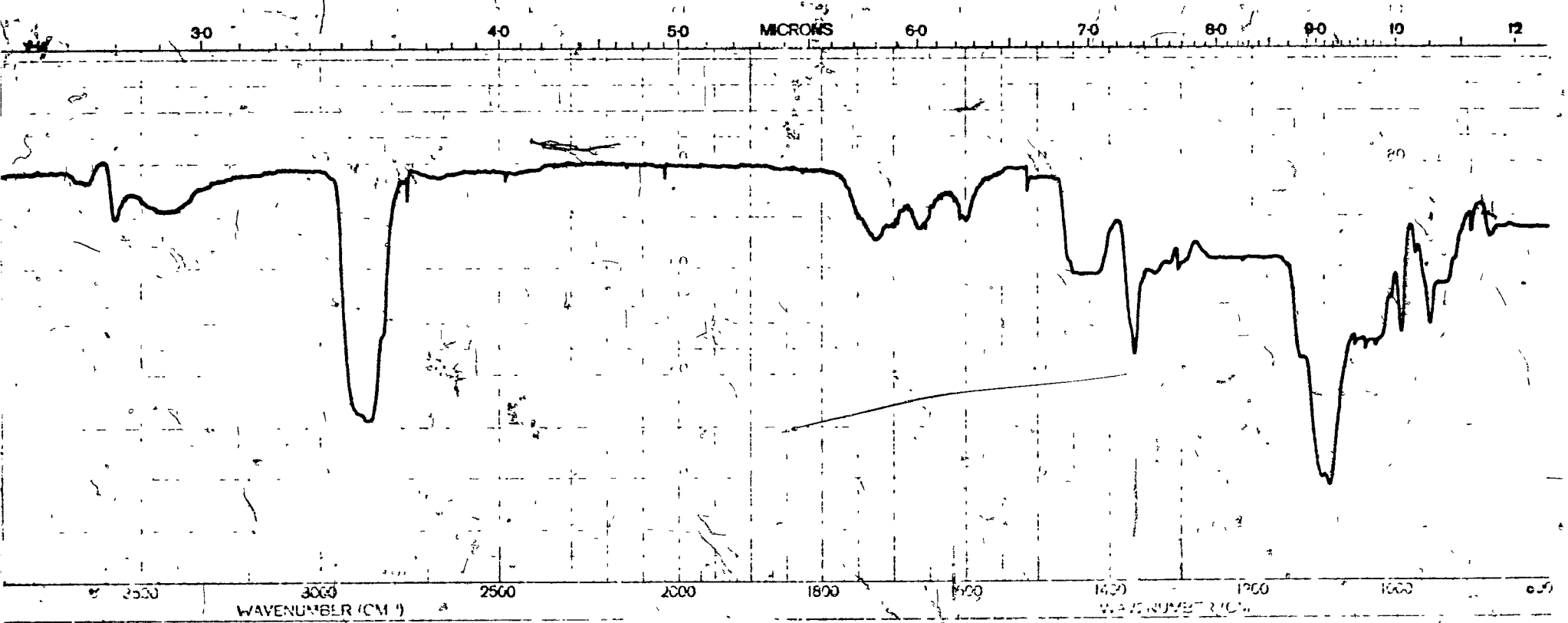
mobility on TLC) with those of authentic 18-OH progesterone. By a similar procedure, Jeger et al. (103) isolated from the photolyzate a compound of m.p. 187-188° but with an optical rotation of $[\alpha]_D = +1°$, which was converted to 18-OH progesterone (VI).

The physical characterization of IV indicated a close relationship to V; the infrared ($CHCl_3$) and mass spectral characteristics were similar, and both formed 18-OH progesterone upon acid hydrolysis. The possibility that IV may be the C-20 epimer of V is suggested. The possibility of crystalline modifications can be ruled out in view of the different optical rotations (IV, $[\alpha]_D^{25} = +24.4°$; V, $[\alpha]_D^{25} = -22.3°$). It is possible therefore that Jeger's compound may be a mixture of IV and V. A vigorous assignment of the stereochemistry at C-20 can not be established at this time.

I.R.(KBr): Compound V: Epimer B of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene

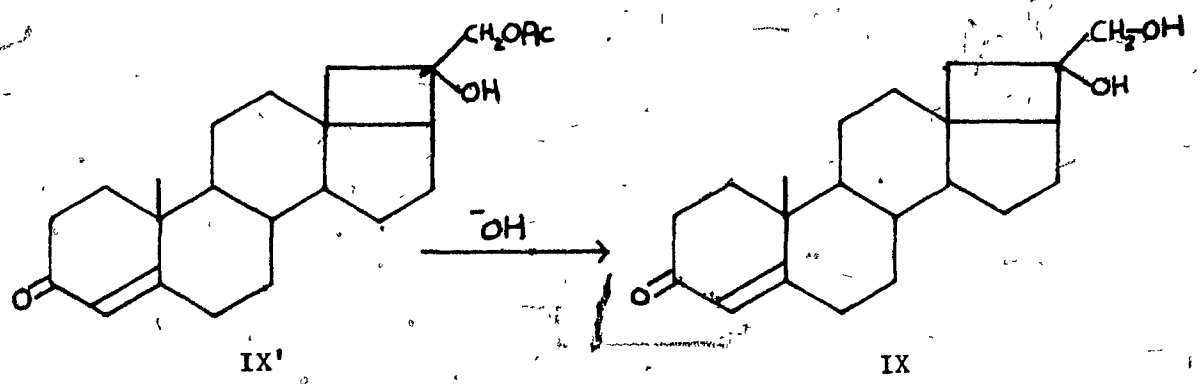


I.R.($CHCl_3$): Compound V: Epimer B of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene



(d) An improved method for the preparation of 18-OH progesterone:

The last procedure allowed us to obtain IV and V in reasonable yield. However, very high losses were encountered in the subsequent conversion to 18-OH progesterone which entailed acid hydrolysis and column chromatography. Jeger reported a 5% overall yield which was substantiated by us. We attempted to improve the yield of 18-OH progesterone by hydrolysing the photolyzate directly. However, it was found that under these conditions, another product IX' was obtained



IX' IX

which had nearly the same polarity as 18-OH progesterone and could only be separated with difficulties. We therefore incorporated another hydrolytic step under alkaline conditions. This resulted in the conversion of IX' to the diol (IX), which can be separated easily from 18-OH progesterone.

In the modified procedure (see Scheme 2 for summary, p. 51) IV and V were not isolated and the photolyzate obtained from the photo-

lysis of II in absolute ethanol was directly hydrolyzed with warm aqueous acetic acid (without addition of methanol, as distinct from Jeger's method) followed by treatment of the acid hydrolyzate with aqueous methanolic potassium carbonate. The products including 18-OH progesterone were isolated from the hydrolyzate by column chromatography on neutral alumina (activity I) (see Table on next page). 18-OH Progesterone was obtained in 15-24% overall yield from II.

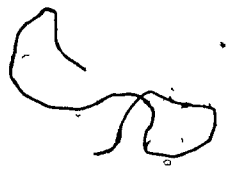
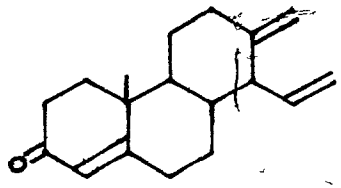
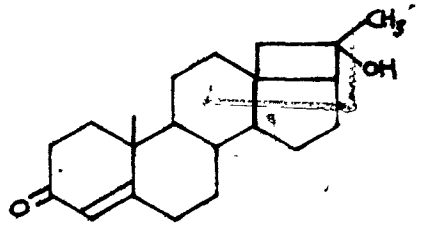
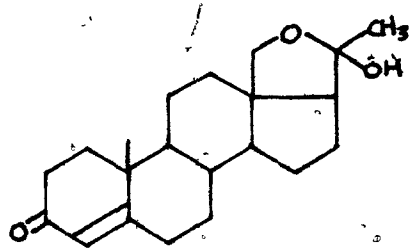
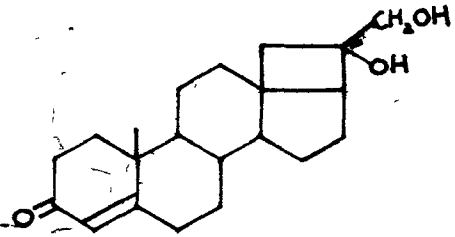
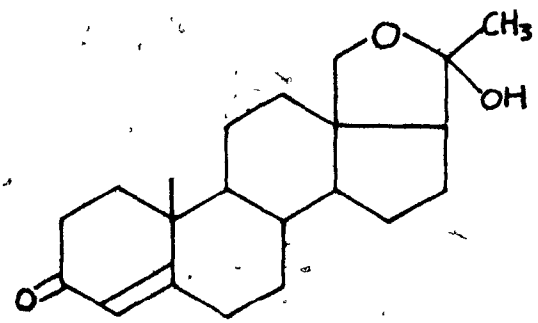


Table 4 - Products Isolated by Alumina Column Chromatography of the Hydrolyzed Ethanolic Photolyzate of II

Fraction	Solvent of elution	Average % yield	m.p. (c°)	$[\alpha]_D^{25}(CHCl_3)$	Structure of compound
1-11	Benzene	4	103-105°	+102.9°	 X
18-57	Benzene Benzene-ether (90:10), (85:15)	2-4	195-197°	+138.1°	 VIII
67-115	Benzene-ether (85:15), (80:20), (75:25)	15-24	150-154°	+152.8°	 VI
122-145	Ether	17-22	183-187°	+128.8°	 IX

We report here the structural characterization of these products. The photolysis and isolation procedure are reported in the Experimental section.

(1) 18-OH Progesterone (18,20-epoxy-20-hydroxy-Δ^4-pregnen-3-one) (VI) ~~18-OH Progesterone~~ (VI, m.p. 150-155°, $[\alpha]_D^{25}$ = +152.8°) was eluted with a mixture of benzene and ether in 15-24% yield.



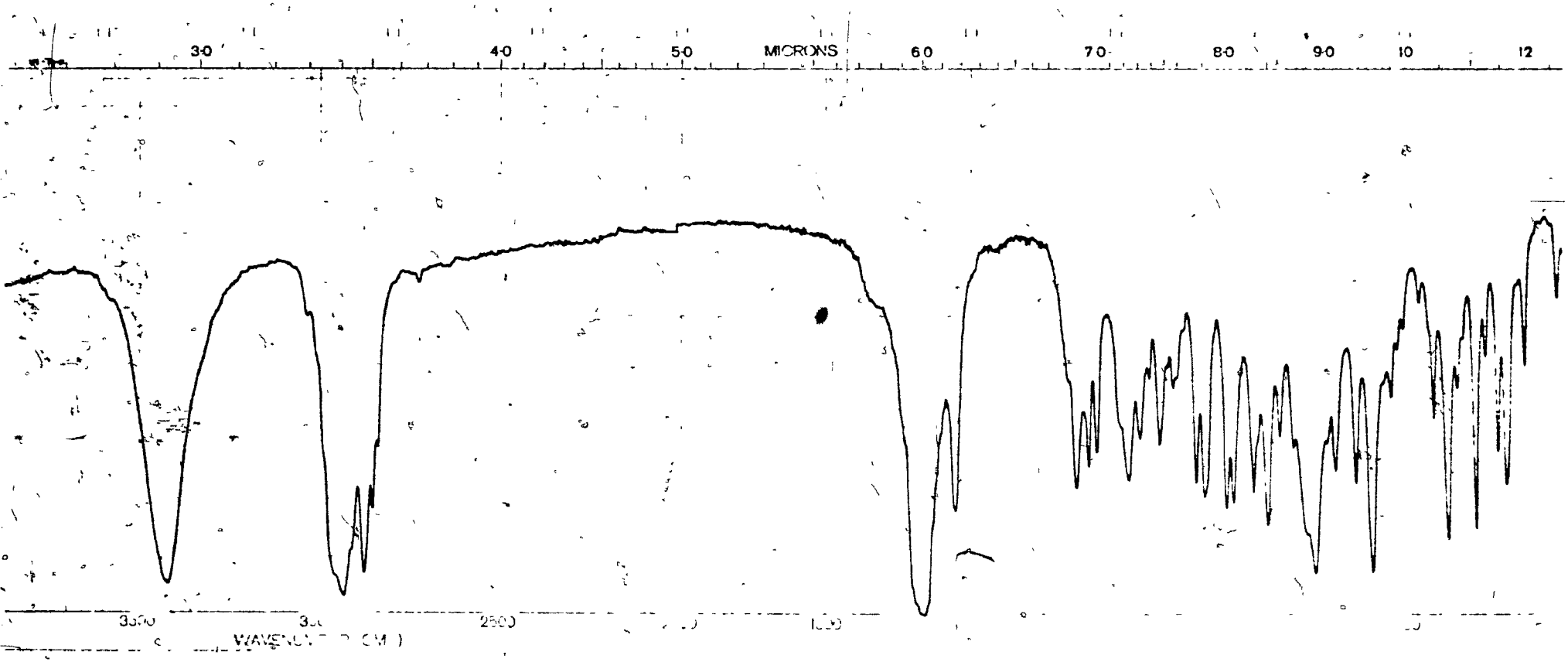
VI

The molecular formula, $C_{21}H_{30}O_3$, was assigned on the basis of elemental analysis. The infrared spectrum showed hydroxyl absorption near 3420 cm^{-1}. The presence of the Δ^4-3-ketone moiety was indicated by the infrared bands at 1665 cm^{-1} (C=O) and 1620 cm^{-1} (C=C) and was supported by the absorption maximum at 241nm (ϵ = 15,000) in the ultraviolet spectrum. The infrared bands at 1121, 1067, 1043, 1285, and 1240 cm^{-1} are attributed to a combination of the C-O stretching vibrations of the ether and alcohol groups and the bands at 860 and

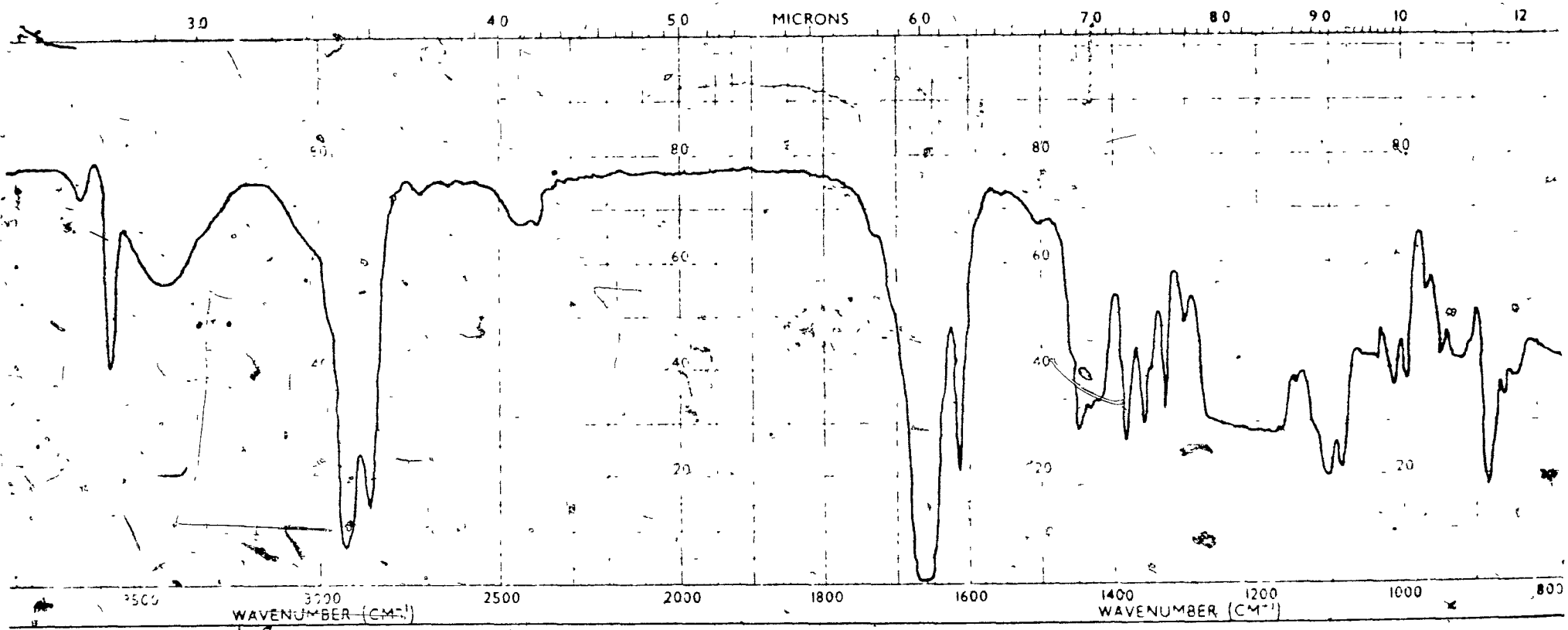
835 cm^{-1}, in conjunction with the one at 1620 cm^{-1}, are ascribed to the olefinic (C=C-H) group. The mass spectral fragmentation pattern can be rationalized on the basis of strucutre VI (see Part IV, p. 110). Direct comparison of VI with an authentic sample of 18-OH progesterone established that infrared and mass spectra as well as mobility on TLC of the two compounds were identical.

The fact that the infrared spectrum (KBr, $CHCl_3$) does not show absorption in the region 1700-1730 cm^{-1} for a 20-ketone group indicates that 18-OH progesterone (VI) exists entirely in the 18,20-cyclohemiketal form. This interpretation is supported by the mass spectral fragmentation pattern of VI (see Part IV, p. 116).

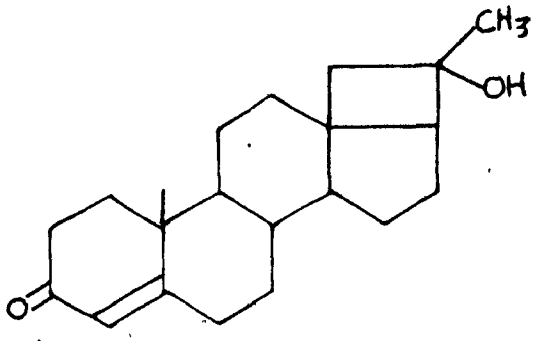
I.R.(KBr): 18-OH Progesterone (VI)



I.R.($CHCl_3$): 18-OH Progesterone (VI)



(ii) 18,20-Cyclo-20-hydroxy-Δ^4-pregnen-3-one (VIII) 18,20-Cyclobutanol (VIII, m.p. 195-197°, $[\alpha]_D^{25}$ = + 138.1°) was eluted with benzene and benzene in ether in 2-4% yield.

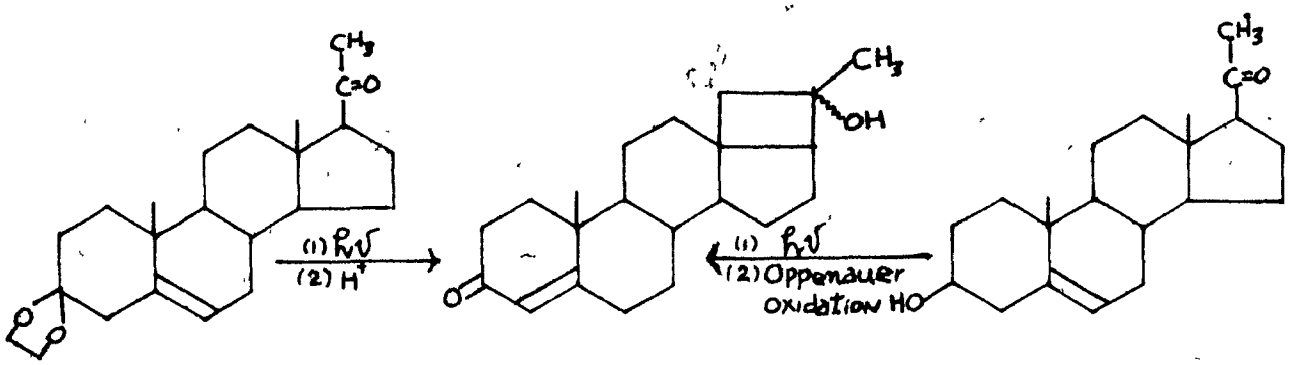


VIII

The infrared spectrum showed strong bands at 3425 cm^{-1} (O-H) and near 1120-1230 cm^{-1} (C-O) due to an alcohol group. The presence of the α,β-unsaturated ketone function was verified by the absorption maximum at 242 nm (ϵ =18,000) in the ultraviolet spectrum and by the bands at 1642 cm^{-1} (C=O) and 1602 cm^{-1} (C=C) in the infrared spectrum. The infrared absorption at 870 cm^{-1}, together with the one at 1602 cm^{-1}, is indicative of the presence of the olefinic (C=C-H) group. On the basis of elemental analysis, VIII was assigned the molecular formula $C_{21}H_{30}O_2$. In agreement, the molecular weight, determined by mass spectrometry, was 314.2162 (calcd. 314.2246). The mass spectral fragmentation pattern was consistent with structure VIII (see Part IV, p. 131).

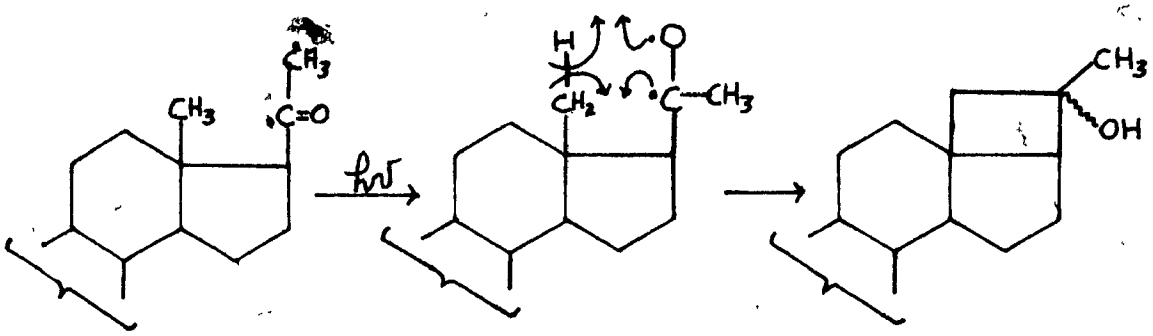
The formation of VIII in the photolysis of II is surprising since 21-unsubstituted 18,20-cyclobutanol derivatives are generally formed by photolysis of the corresponding 20-ketone-pregnane derivatives (see below). It is not clear how VIII was formed in our photolytic reaction because the C-21 was acetylated in the starting material II. However, VIII was formed only in small amounts and it may be an artifact. We have not explored further on the origin of VIII.

Jeger et al. in 1959 (108) reported the formation of single, isomeric 18,20-cyclo-20-hydroxyl steroids (C) with m.p. 191-192°, $[\alpha]_D$ = +130° from the photolysis of 20-ketone steroids (A):



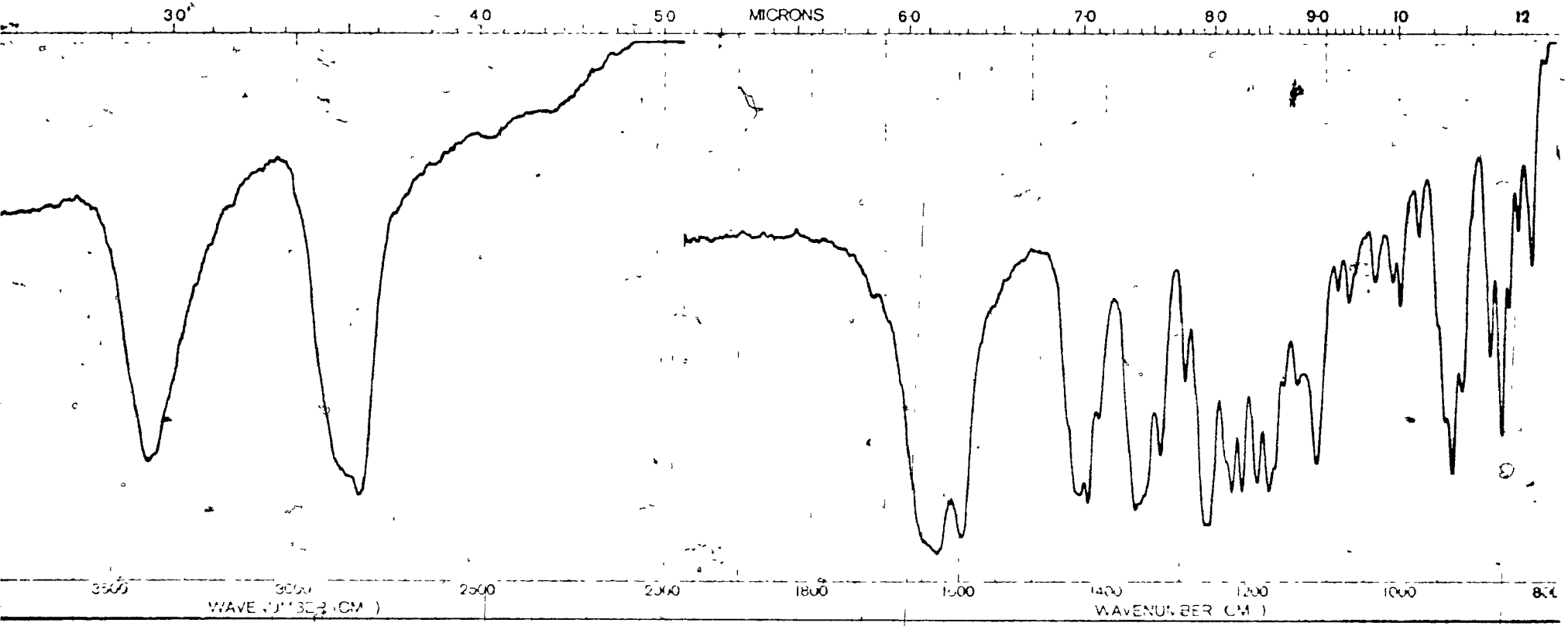
(A) (C) (B)

These authors proposed that the reaction probably proceeds by the following mechanism:

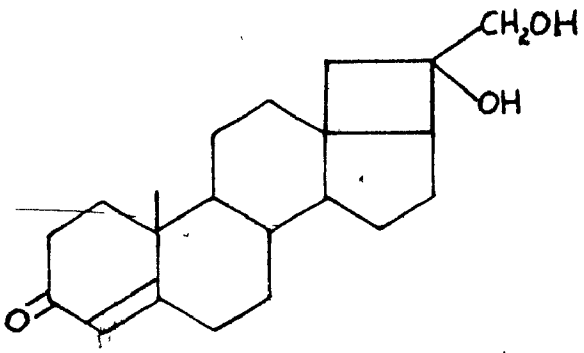


Yang and Yang in 1960 (109) reported that two isomeric 18,20-cyclo-20-hydroxyl steroids (C) with m.p. 204-205° and 163-165° were obtained from the photolysis of pregnenolone (B).

I.R.(KBr): 18,20-Cyclo-20-hydroxy-Δ^4-pregnen-3-one (VIII)



(iii) 18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (IX) 20,21-Dihydroxy-cyclobutanol (IX, m.p. 183-187°, $[\alpha]_D^{25}$ = + 128.8°) was eluted with ether in 17-22% yield.



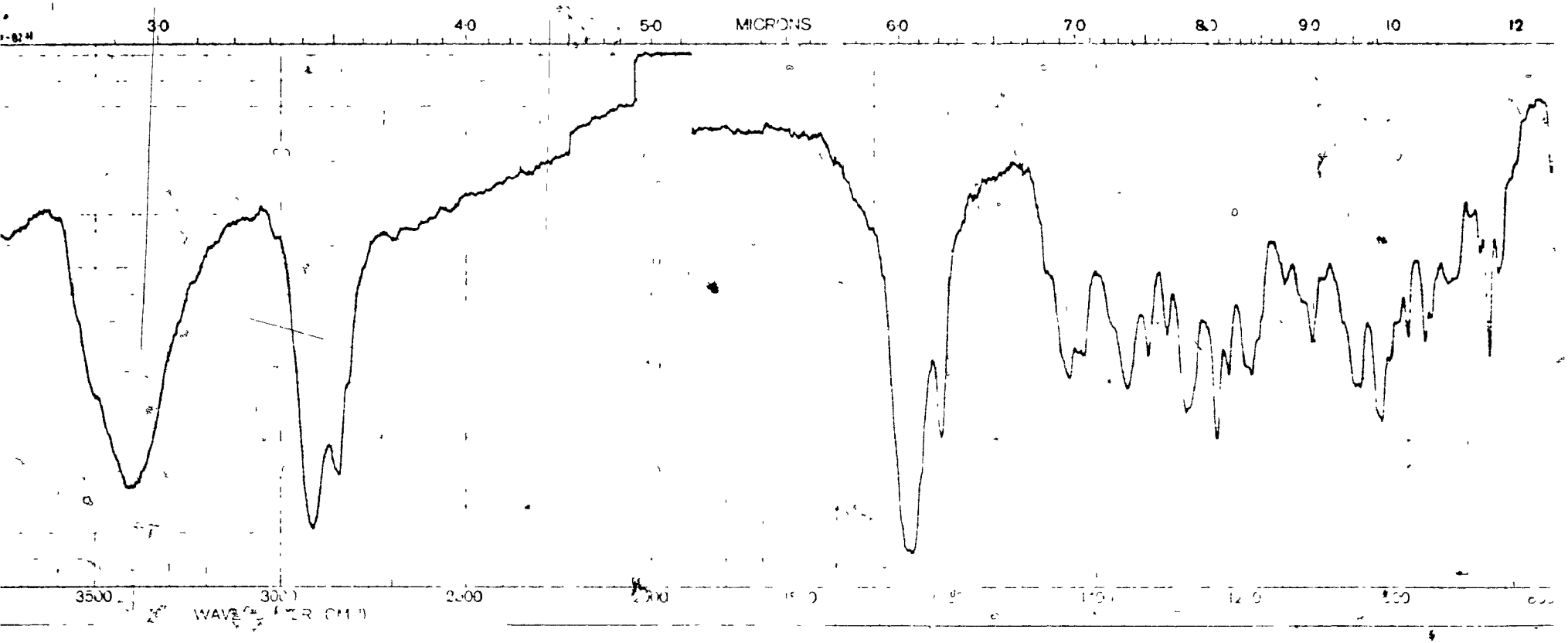
IX

The infrared spectrum showed bands due to alcohol absorption at 3410 cm^{-1} (O-H) and near 1016-1278 cm^{-1} (C-O). The band at 870 cm^{-1}, together with the one at 1610 cm^{-1}, is ascribed to an olefinic (C = C-H) group. The ultraviolet absorption maximum at 242 nm (ϵ = 16,940) and infrared bands at 1650 cm^{-1} (C=O) and 1610 cm^{-1} (C=C) indicated the presence of the Δ^4-3-ketone group. The molecular formula, $C_{21}H_{30}O_3$, was assigned on the basis of elemental analysis. This was supported by the molecular weight, 330.2154 (calcd. 330.2194), determination by mass spectrometry. The mass spectral fragmentation pattern can be rationalized on the basis of structure IX (see Part IV, p. 139).

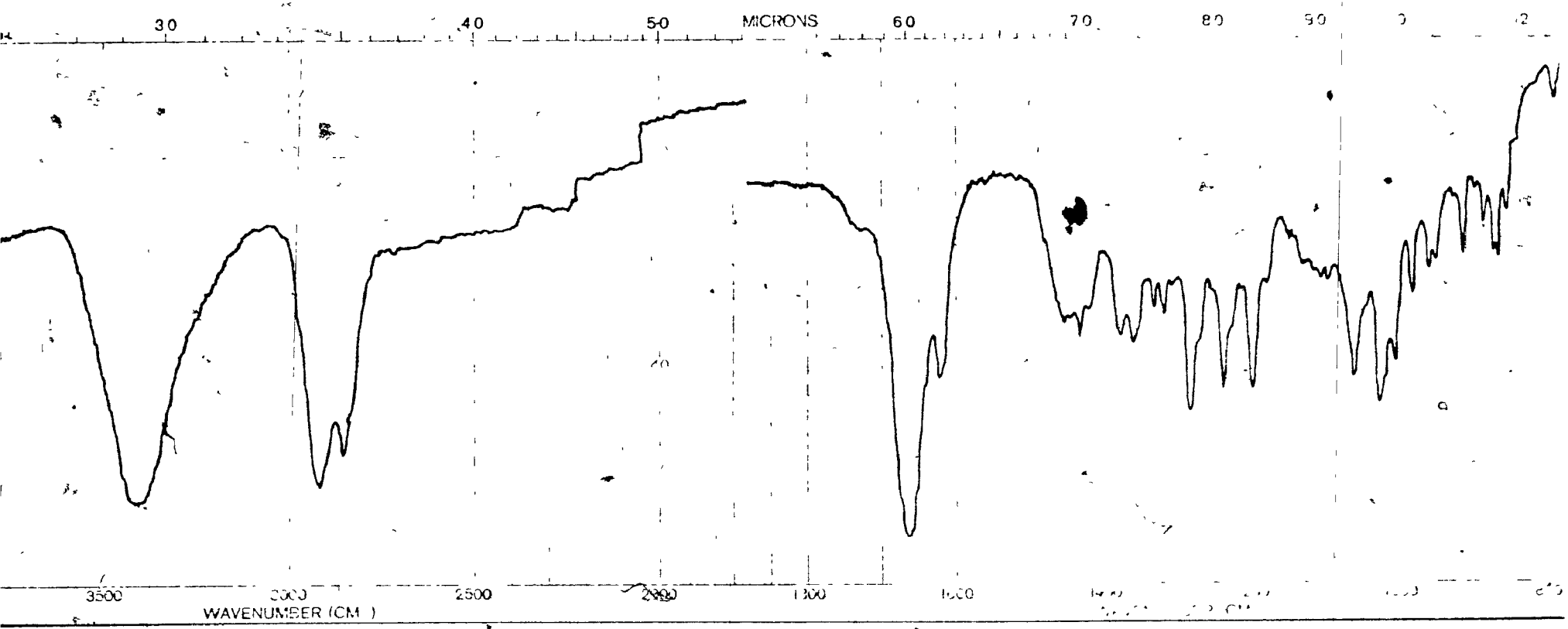
Evidence of structure IX is further supported by the report of Jeger et al. (103) who isolated IX as its 3-ethylene ketal-21-acetate from the photolysis of II (see Scheme H, p. 45).

Upon repeated recrystallization from acetone-hexane the melting point of IX gradually changed to 160-165°, $[\alpha]_D^{25}$ = +117.8°. The behavior is most likely due to crystalline modifications. The two forms of crystal had a similar mass spectral fragmentation pattern, infrared spectrum in chloroform, mobility on TLC, and optical rotation. The only apparent change was the absence of an infrared (KBr) band near 1109 cm^{-1} in the low-melting form.

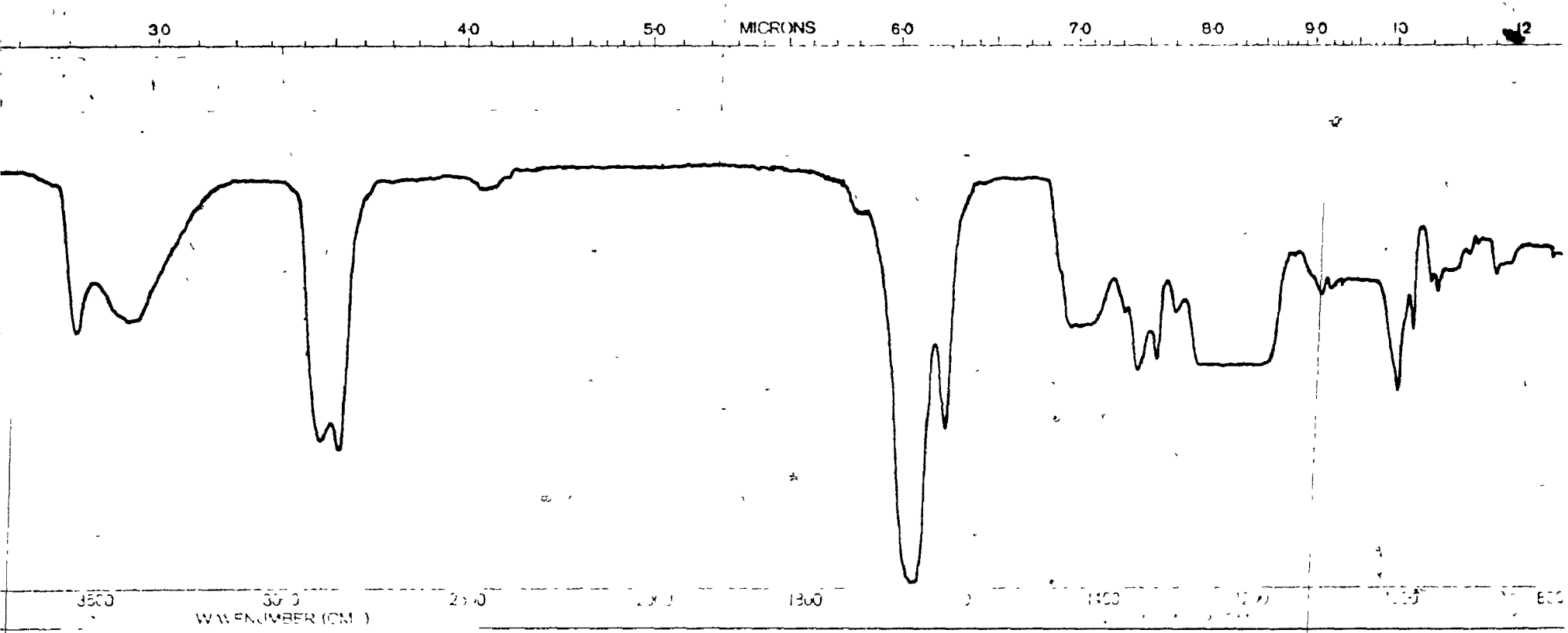
I.R.(KBr): 18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (IX), m.p. 183-187°



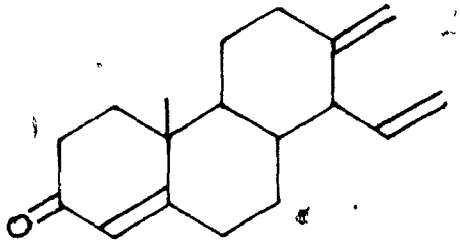
I.R.(KBr): 18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (IX), m.p. 160-165°



I.R.($CHCl_3$): 18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (IX), m.p. 183-187°



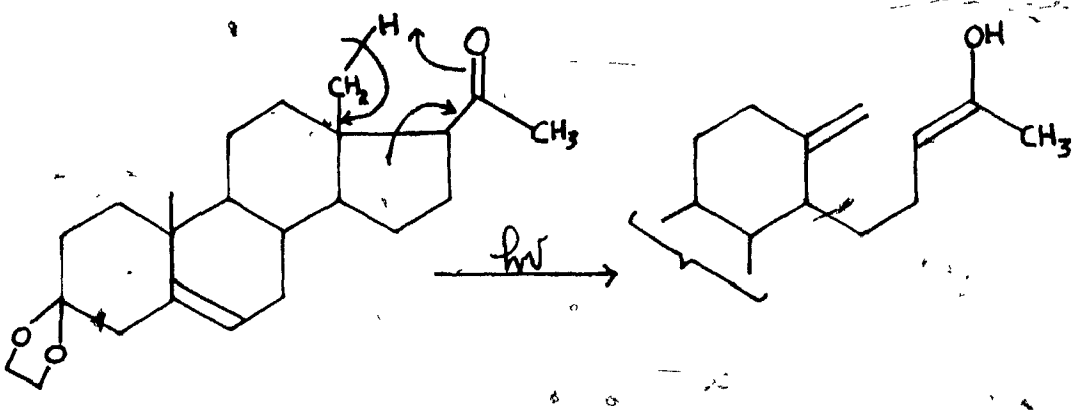
(iv) 17-Nor-13,17-seco-$\Delta^{4,13(18),15}$-androstrien-3-one (X) Compound X (m.p. 103-105°, $[\alpha]_D^{25}$ = +102.9°) was eluted with benzene in 4% yield.

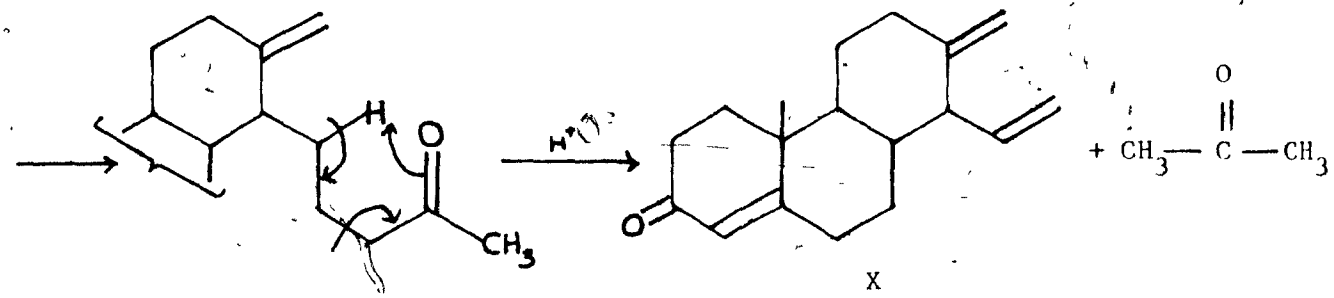


X

The elemental analysis and mass spectrum (M^+ m/e = 256) of X established the molecular formula $C_{18}H_{24}O$. The presence of the α,β-unsaturated ketone group was indicated by infrared absorption at 1665 cm^{-1} (C=O) and 1610 cm^{-1} (C=C), and was supported by the absorption maximum at 241 nm (ε =16,500) in the ultraviolet spectrum. The infrared spectrum showed bands at 1637 cm^{-1} and 860 cm^{-1} assignable to a vinylidine type of unsaturations (110).

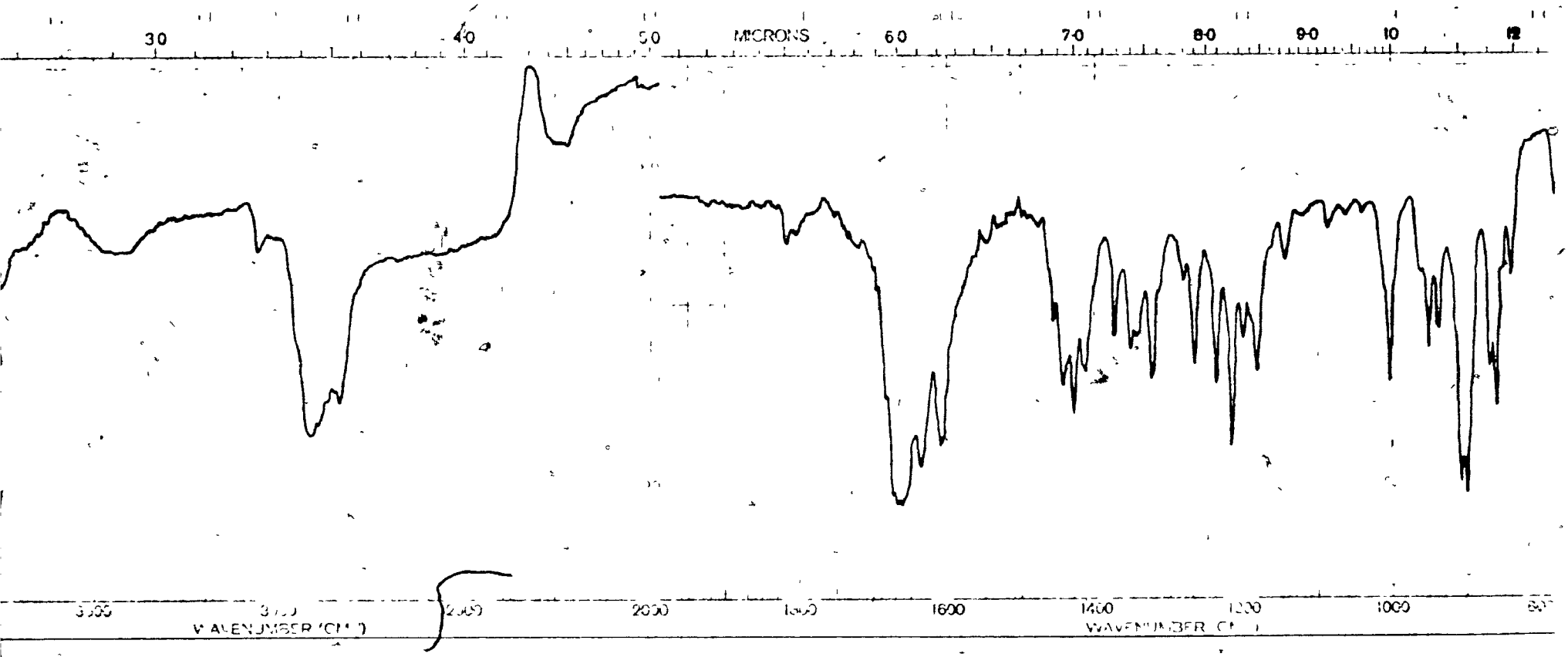
The mode of photochemical formation of X from II in the present study may be similar to that described by Jeger et al. (108) who isolated X (m.p. 109°) from the photolysis of 20-ketone-pregnane derivatives and proposed the following sequence of reactions to account for the formation of X:





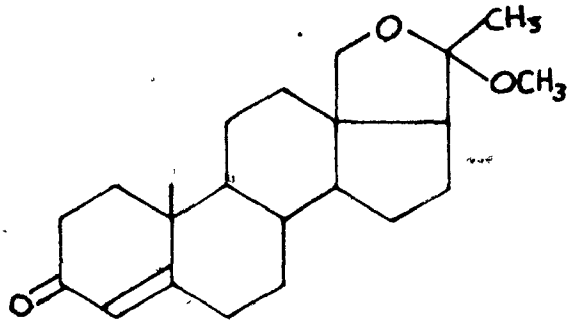
X

I.R.(KBr) 17-Nor-13,17-seco-$\Delta^{4,13(18),15}$-androstrien-3-one (X)



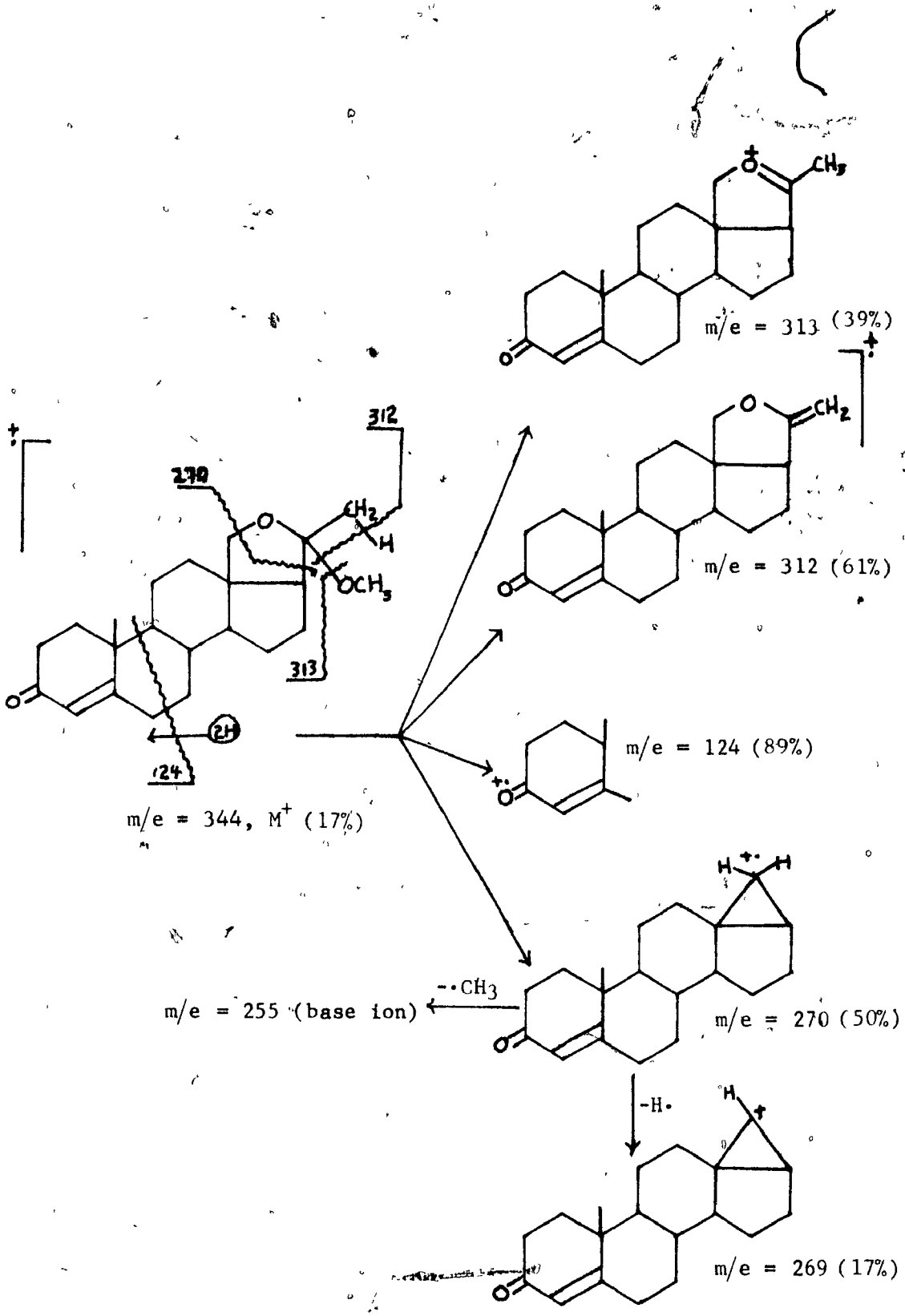
(e) 18,20-Epoxy-20-methoxy-Δ^4-pregnen-3-one (VII):

Hydrolysis of the ethanolic photolyzate of II with warm aqueous acetic acid in the presence of methanol, followed by treatment with aqueous methanolic potassium carbonate and separation of the products by neutral alumina (activity I) column chromatography yielded, in addition to the products already described, the 20-methoxy derivative VII (m.p. 130-134°) upon elution with benzene, in 6 - 8% yield.

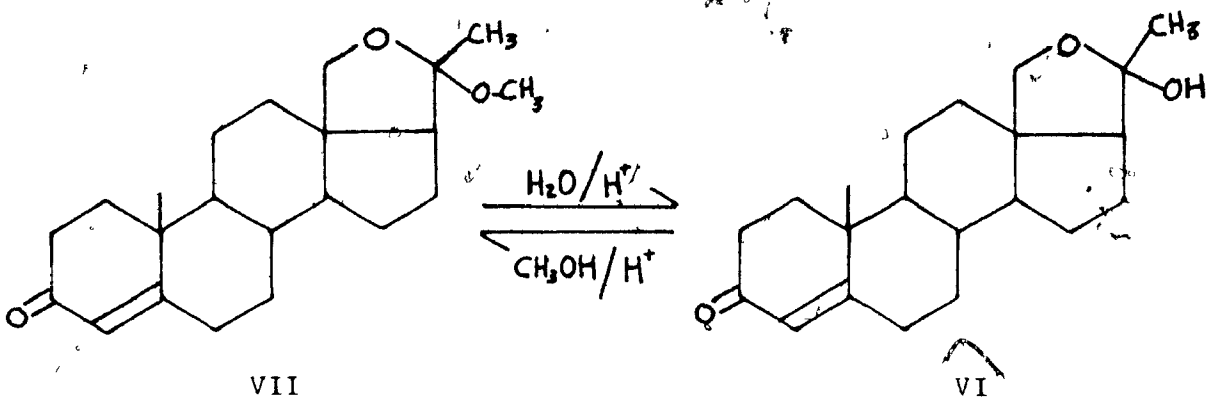


VII

The absorption maximum at 240 nm (ϵ =15,000) in the ultraviolet spectrum and bands at 1668 cm^{-1} (C=O) and 1602 cm^{-1} (C=C) in the infrared spectrum indicated the presence of an α,β-unsaturated carbonyl group. The infrared spectrum showed no absorption in the region 1700-1730 cm^{-1} due to an unconjugated ketone function. The mass spectrum had a molecular ion at m/e=344 corresponding to the molecular weight of VII. The mass spectral fragmentation pattern may be interpreted on the basis of structure VII:


m/e = 313 (39%)
m/e = 312 (61%)
m/e = 124 (89%)
m/e = 344, M+ (17%)
m/e = 255 (base ion)
-·CH3
m/e = 270 (50%)
-H·
m/e = 269 (17%)

Compound VII was characterized by conversion into 18-OH progesterone (VI, m.p. 140-145°) in quantitative yield with aqueous 70% acetic acid (1 h at 60°). On the other hand, 18-OH progesterone (VI) could be quantitatively converted into VII, as detected by TLC, on treatment with aqueous 70% acetic acid in the presence of methanol (1 h at 40°). The results indicated that the 18,20-cyclohemiketal grouping undergoes acid catalyzed reaction with methanol, a reaction characteristic of hemiketal (111).

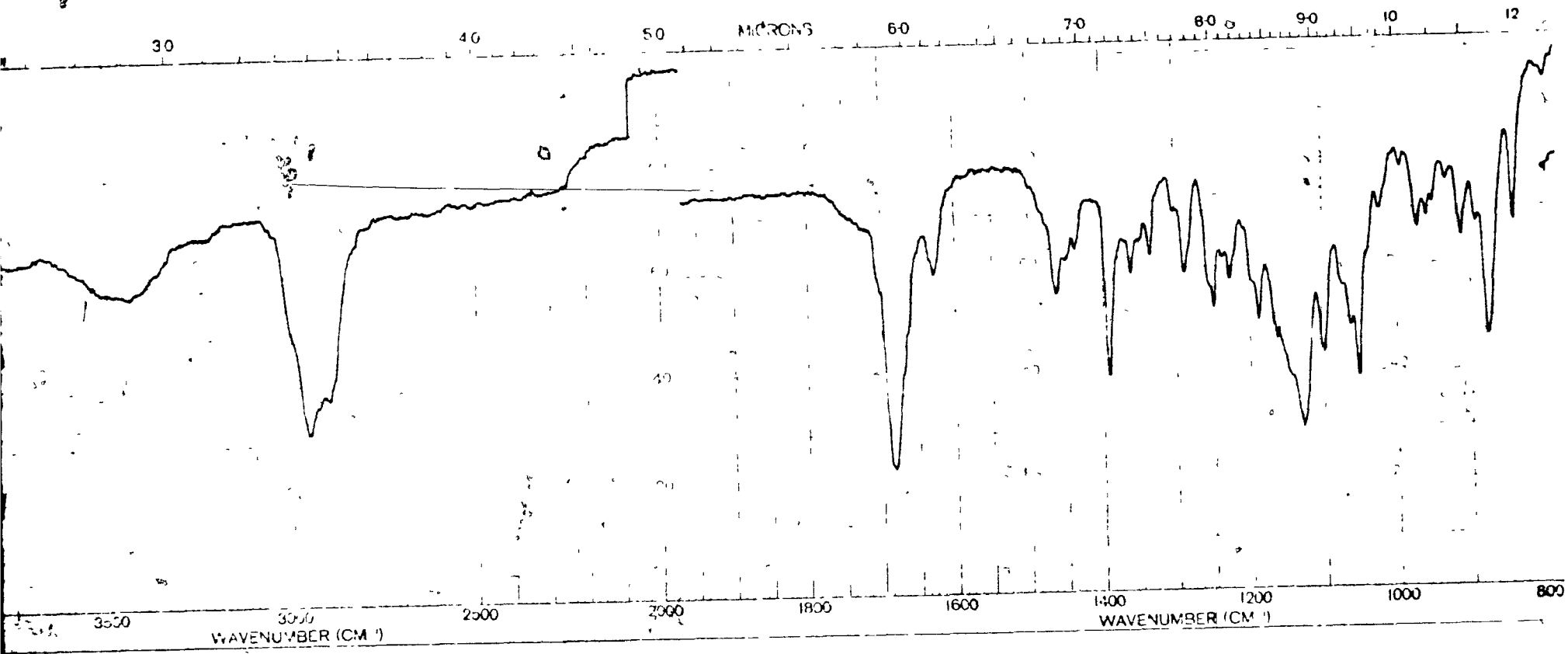


VII VI

Compound VII is probably an artifact formed by methoxylation of 18-OH progesterone during the hydrolysis of the photolyzate with aqueous methanolic acetic acid since VII was not isolated when methanol was omitted. Formation of VII in the saponification step could by discounted since a sample of 18-OH progesterone was recovered unchanged on treatment with aqueous methanolic potassium carbonate under the same condition that was used for the work-up of the photolyzate.

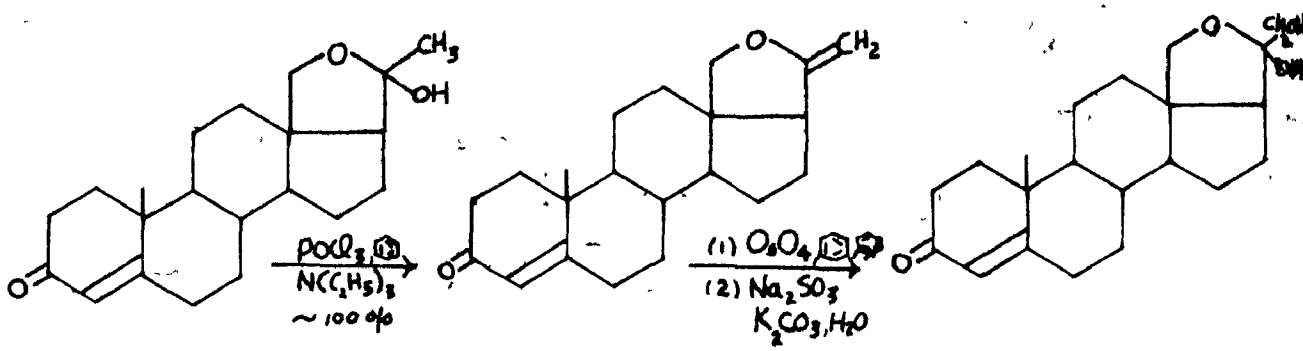
Compound VII was shown to be very unstable. It was converted completely into 18-OH progesterone upon recrystallization from acetone-hexane, as indicated by TLC. The stability of VII could be improved by addition of a trace of pyridine in the solvent or by keeping the compound under an atmosphere of pyridine in a desiccator.

I.R.(KBr): 18,20-Epoxy-20-methoxy-Δ^4-pregnen-3-one (VII)



4. Conversion of 18-OH progesterone to 18-OH-DOC:

Having prepared 18-OH progesterone (VI), the remaining task was to convert this compound to enol ether (XI) by dehydration, thence to the title compound 18-OH-DOC (XII) by hydroxylation.



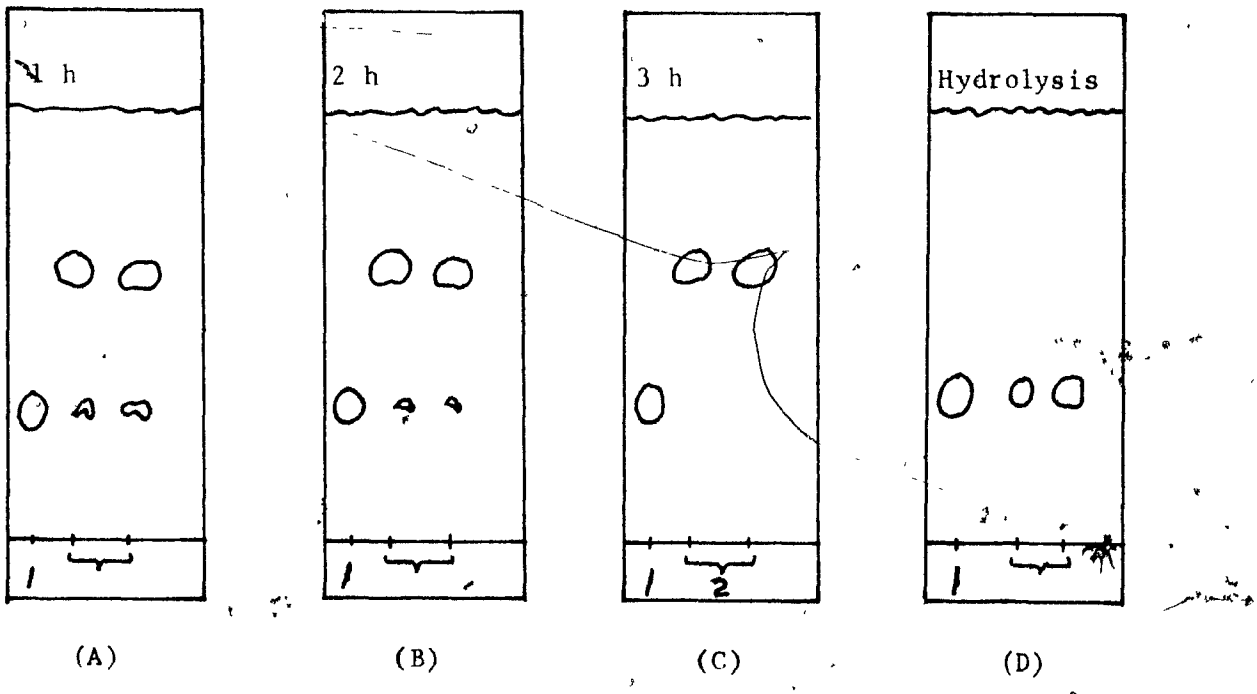
VI XI XII

(a) Preparation of 18,20-epoxy-$\Delta^{4,20}$-pregnadien-3-one (XI):

Although the dehydration of an alcohol in steroids is normally a commonplace reaction, the dehydration of the cyclohemiketal alcohol VI is a more problematical case owing to the instability of the product XI. A suitable dehydration agent was sought. Agents which were examined and found to be unsuitable included p-toluenesulfonyl chloride, DBN (1,5-diazabicyclo [3.4.0]-nonene-5) with methanesulfonyl chloride, p-toluene-sulfonic acid, acetic anhydride, methanesulfonyl chloride, oxalyl chloride, sulfur trioxide-pyridine, and thionyl chloride. The reactions were per-

formed in dry benzene solution under a variety of conditions from room temperature to reflux with or without pyridine as a base.

It turned out that phosphorous oxychloride with triethylamine were the reagents of choice for achieving the dehydration reaction in quantitative yield. This was accomplished by treatment of a solution of 18-OH progesterone (VI) in dry benzene and triethylamine under anhydrous conditions at room temperature with a calculated amount of phosphorous oxychloride (2 mol equivalent) added at hourly intervals until the dehydration reaction was completed (2 to 4 h). The reaction was followed by TLC from the disappearance of VI and the formation of a less polar compound XI. The latter could be hydrolyzed back to VI with aqueous acid.



1 = 18-OH progesterone (VI); R_f = 0.52

2 = 18,20-epoxy-$\Delta^{4,20}$-pregnadien-3-one (XI); R_f = 0.70

(A)→(B) = progress of dehydration of VI

(C) = end of reaction

(D) = acid hydrolysis of XI to VI

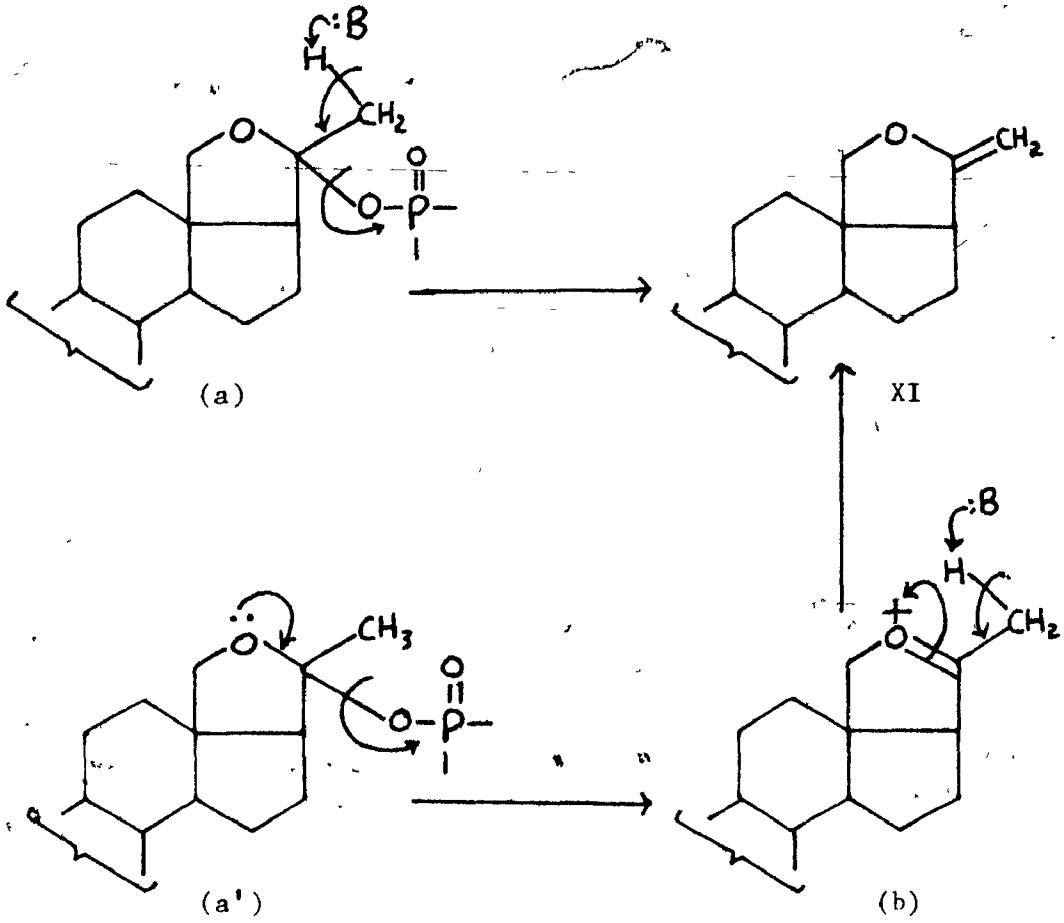
Solvent system: Benzene-ethyl acetate (2:8)

TLC = neutral alumina (Woelm Co.)

The enol ether (XI) could be obtained as a homogeneous (TLC) colorless gum from the reaction mixture.

The reaction conditions are extremely critical for the dehydration to be successful (see Experimental section). It is essential that reagents used should be dried just prior to use and moisture be excluded from the reaction. Incomplete dehydration was observed when the reaction was performed at elevated temperature (60-80°). Benzene, a non-polar solvent, was found to be a more suitable medium than polar solvent (ether) for carrying out the reaction and for extraction of the product.

The present reaction probably proceeds via the phosphate ester intermediate (a→XI); a mechanism analogous to the dehydration of an ordinary alcohol. The elimination reaction may be promoted by participation of the ethereal oxygen (a'→b→XI).



The dehydration reaction of the 18,20-cyclohemiketal of 18-OH progesterone (VI) is without precedent, although another method of preparation of the enol ether (XI) from conessine has been reported by Pappo (97). Jeger et al.(103) claimed to have isolated a 3,3-ethylenedioxy derivative of XI from a mixture obtained by photolysis of deoxycorticosterone acetate 3-ethylene ketal (II) in ether solution

see p. 55.

The enol ether (XI) was too unstable to permit extensive chemical characterization. It may be hydrolyzed to 18-OH progesterone (VI), as detected by TLC, with aqueous 10% acetic acid in a few minutes at room temperature. The enol ether (XI) was therefore osmylated as soon as possible in the next step of synthesis.

(b) Preparation of 18-OH-DOC (XII):

A previous communication (112) from this laboratory has described the preparation of 18-OH-DOC (XII) in situ from the enol ether (XI), a compound in turn prepared from 18-OH progesterone (VI) in 20% overall yield. Since then we were able to isolate pure enol ether (XI) in high yield which was smoothly converted to 18-OH-DOC (XII) in almost quantitative yield. We report herein this improved method of synthesis.

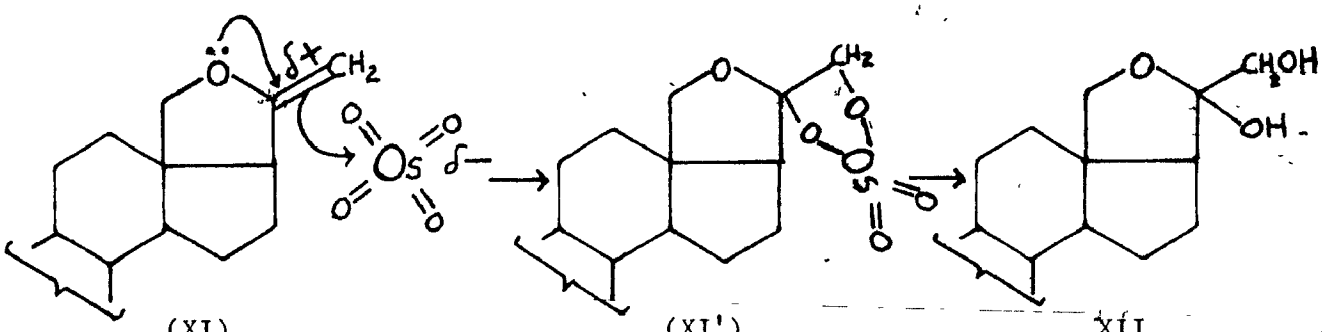
When a benzene solution of XI was treated with a solution of osmium tetroxide (1.1 mol equivalent) in the presence of pyridine at 0° under nitrogen, a rapid reaction (complete in 1 h) occurred. Hydrolysis of the reaction mixture containing the osmate esters under mild conditions at room temperature for 1 h with a mixture of aqueous sodium sulfite and potassium carbonate gave pure, colorless crystalline 18-OH-DOC (XII, m.p. 154-156°). An analytical sample had m.p. 165-168°, $[\alpha]_D^{25} = +110.3°$.

The infrared spectrum (KBr) showed hydroxyl absorption near

3420 cm^{-1}. The presence of bands at 1660 cm^{-1} (C=O) and 1618 cm^{-1} (C=C) in the infrared spectrum and of an absorption maximum at 241 nm (ϵ =15,200) in the ultraviolet spectrum verified the presence of the α,β-unsaturated ketone moiety. The infrared bands in the region 1000-1100 cm^{-1} centered at 1060 cm^{-1} are attributed to a combination of the C-O stretching vibrations of alcohols and ethers. The band at 872 cm^{-1}, in conjunction with the one at 1618 cm^{-1}, indicated the presence of ethylenic (C=C-H) group. The molecular formula, $C_{21}H_{30}O_4$, was assigned on the basis of elemental analysis. This was supported by the peak at m/e = 328 (346-H_2O) in the mass spectrum. The mass spectral fragmentation pattern can be interpreted on the basis of structure XII (see Part IV, p. 119).

The absence of an infrared band near 1700-1730 cm^{-1} characteristic for an unconjugated carbonyl group and a negative reaction with the blue tetrazolium test for an α-ketol side chain implied that 18-OH-DOC (XII) existed in its 18,20-cyclohemiketal form. Support for this view was forthcoming from mass spectrometry (see Part IV, p. 124).

The ease of reaction of the enol ether (XI) with osmium tetroxide is not unexpected. Because of the presence of ethereal oxygen, the π bond is activated and thereby increases the overall reaction rate relative to a simple olefin (113).

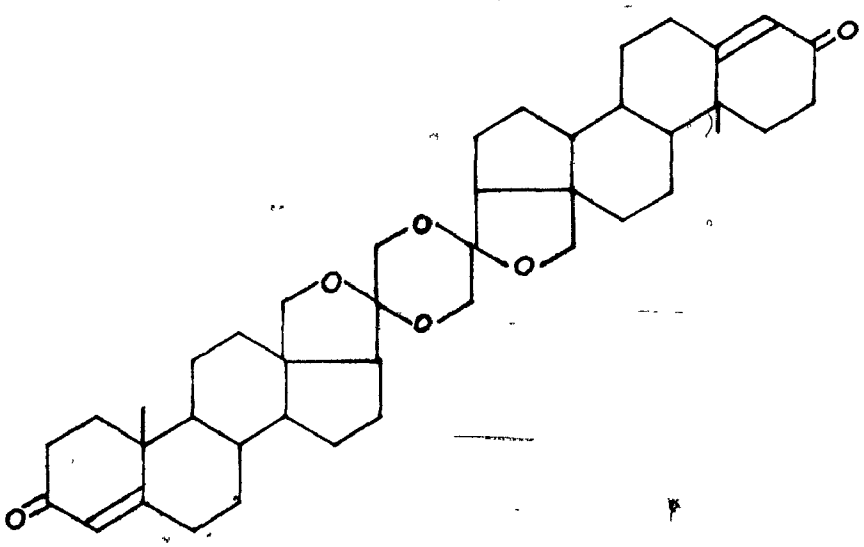


(XI) (XI') XII

Osmium tetroxide would be expected to attack the enol ether (XI) on the α-face (less hindrance side) (114) and the reaction is known to be a stereospecific cis-addition (115). As the hydrolysis results in the cleavage of the osmium-oxygen and not the oxygen-carbon bonds in XI', no inversion of configuration can take place at the carbon atoms (115) and the 20,21-diol XII produced must, like the cyclic osmate ester itself, be cis, e.g., the 20-hydroxyl group would have the α-configuration. However, because of the ready epimerization at C-20 of XII, it is plausible that 18-OH-DOC (XII) exists in two possible diastereoisomeric forms as a mixture. In the present study, only one form of XII was isolated. Nevertheless, 18-OH-DOC (XII) was shown to be, by paper chromatography but not by TLC, a mixture of two closely situated spots. A sample of XII and of

18-OH-DOC obtained from Pappo (97) were chromatographed on paper as single substance or admixture. Each sample resolved into two ultraviolet absorbing compounds. The R_f values of the two fractions contained in XII were the same as those contained in the authentic sample.

We have noted during the isolation procedure that 18-OH-DOC (XII) is rapidly converted to a high-melting material (ca. 285°, decomposed). Its infrared spectrum (KBr) relative to 18-OH-DOC (XII) showed significant reduction of hydroxyl absorption near 3420 cm^{-1} and changes in intensity (reduced) and position of bands near 1000-1300 cm^{-1} region. TLC analysis indicated two spots, one with R_f corresponding to XII and one with a greater R_f. Specific rotation as well as ultraviolet absorption and mass spectra of the high-melting material were similar to those obtained with XII. The molecular weight, as determined by the Rast method, was in the range of 520-645 approaching to a calculated value for a dimer of 18-OH-DOC.



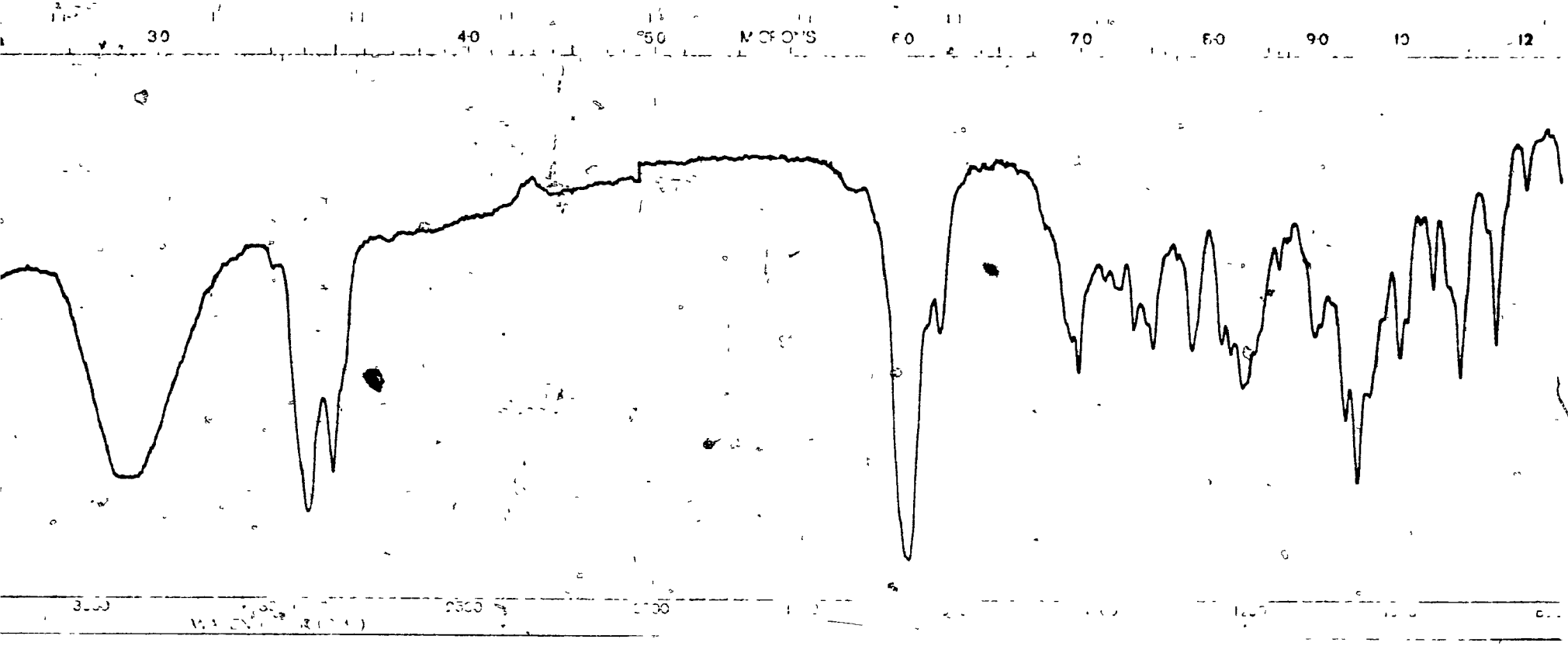
It is possible that this high-melting material might be the 18-OH-DOC "dimer", arising from the acid-catalyzed condensation of two molecules of 18-OH-DOC through the 20,21-diol linkage, as described by Levy et al. (49). It can be said that 18-OH-DOC is an unstable compound especially in the presence of acid and extreme care must be exercised in handling this material.

The reaction of osmium tetroxide with the enol ether (XI) in benzene as the solvent gave a much cleaner reaction than that in dioxane or ether. Hydrolysis of the osmate esters with hydrogen sulfide (116) or with mannitol in aqueous potassium hydroxide (117) led to unidentifiable products. Isolation of product from the reaction mixture by extraction with solvent, including chloroform, methylene chloride, ethyl acetate, dioxane and ether, gave amorphous 18-OH-DOC contaminated with dark brown extraneous matter, presumably due to osmium salts. Solvent recrystallization or treatment with Celite or carbon black (Norite) failed to remove the impurities. Purification of product was more successful by column chromatography on Sephadex LH-20, Florisil or by preparative TLC (SiO_2-7, Baker Co.). Filtration on neutral or basic alumina (Woelm Co.) column led either to low recovery or product destruction. A crucial point in obtaining quality 18-OH-DOC in high yield is to evaporate the reaction mixture under reduced pressure at room temperature to total dryness followed by ether extraction of the crude residue.

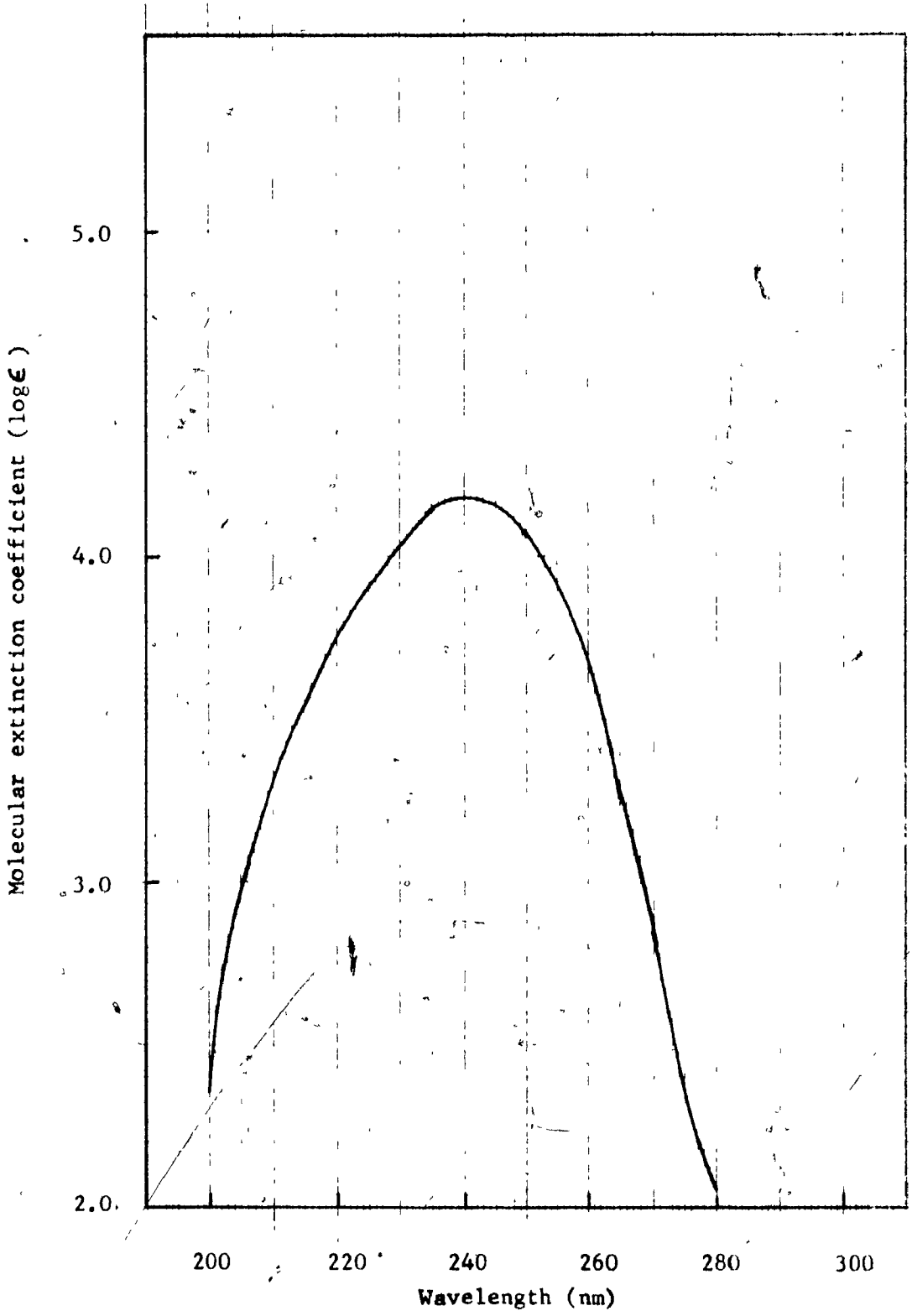
18-OH-DOC (XII) so obtained was identical with an authentic

sample from Pappo (97) by the following criteria: identity of the infrared spectra and the fragmentation patterns of the mass spectra; identical R_f values on TLC and paper chromatograms. 18-OH-DOC (XII) was further characterized by the mobility on paper chromatogram, positive reaction with the Porter-Silber test, negative reaction with the blue tetrazolium test, and mass spectral comparisons with the 18-OH-DOC obtained by incubation with rat adrenals.

I.R.(KBr): 18-OH-DOC (XII)



U.V.(CH_3OH): 18-OH-DOC (XII)



PART IV: THE MASS SPECTRA OF SOME 18,20-CYCLOHEMIKETAL AND 18,20-CYCLOBUTANOL STEROIDS

1. Introduction:

The comprehensive, critical review of Budzikiewicz, Djerassi and Williams in 1964 (118, 119) established mass spectrometry as an important technique for the identification and structural elucidation of steroid molecules. The most valuable aspects of this analytical technique are: (1) the molecular weight of the sample can be deduced from a mass spectrum, (2) useful information with respect to the arrangement of the functional groups in the steroid skeleton may be gained by analysis of the mass spectral fragmentation patterns, and (3) the amount of sample required for a mass spectral determination is less than a fraction of a milligram. In practice, mass spectrometry is best used in conjunction with other analytical techniques available to provide complementary structural information.

2. Nature of steroids studied:

In the course of the synthesis of 18-OH-DOC, two types of compounds, namely 18,20-cyclohemiketal and 18,20-cyclobutanol derivatives of the pregnane series, were obtained. The relative paucity of publications dealing with the mass spectral behaviour of 18,20-cyclohemiketal (120, 126) and 18,20-cyclobutanol steroid derivatives has led us to examine a few of these compounds. Our main interest at

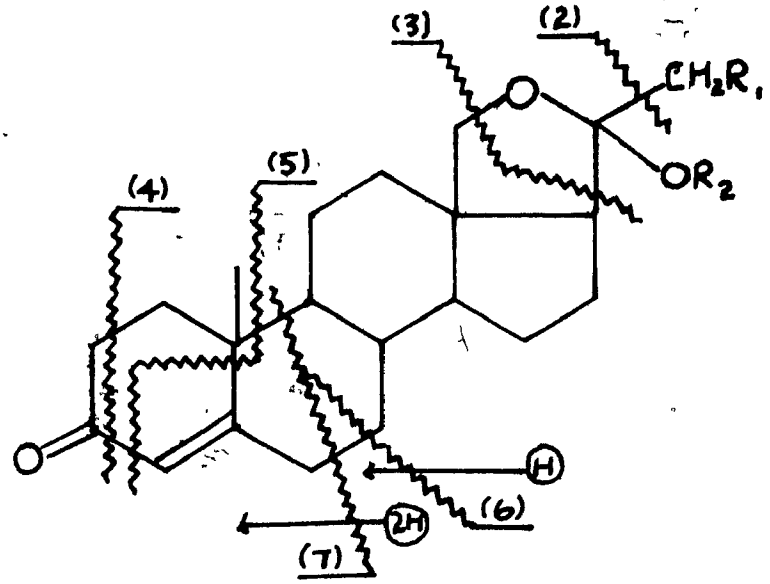
this stage is to correlate the mass spectral fragmentation patterns with structure of the steroid. We recognize that, without labelling experiments our evidence is insufficient to fully substantiate these interpretations. Nevertheless, high resolution mass measurements and metastable peaks were used wherever possible to lend support to the proposed fragmentations. The mass spectra of progesterone described by Peterson (124), of Δ^4-androsten-3-one described by Shapiro and Djerassi (125), and of cortisone acetate 3-ethylene ketal described by Pelah, Williams, Budzikiewicz, and Djerassi (132) were also taken as references.

A brief outline of the fragmentation pathways proposed to rationalize the possible origin of some major fragment ions present in the mass spectra of 18,20-cyclohemiketal (designated as Type I steroids) and 18,20-cyclobutanol (designated as Type II steroids) steroid derivatives is depicted schematically in the following structural formulae. It may serve as a guide for the understanding of the significance of some of the results discussed in the subsequent text.

Type I-A steroids

18-OH Progesterone (I; R_1=R_2=H)

18-OH-DOC (II; R_1=OH, R_2=H)



Major fragmentation possibilities:

(1) Loss of water; (I) R_1=R_2=H, (II) R_1=OH, R_2=H

(2) Cleavage of the hydroxylated C-C bond; (II) R_1=OH, R_2=H

(3) Cleavage of the 18,20-cyclohemiketal bridge; (I) R_1=R_2=H,

(II) R_1=OH, R_2=H

(4) Loss of ketene from ring A; (I) R_1=R_2=H

(5) Extensive cleavage of ring A; (I) R_1=R_2=H

(6) Cleavage of ring B with transfer of one hydrogen atom to ring A;

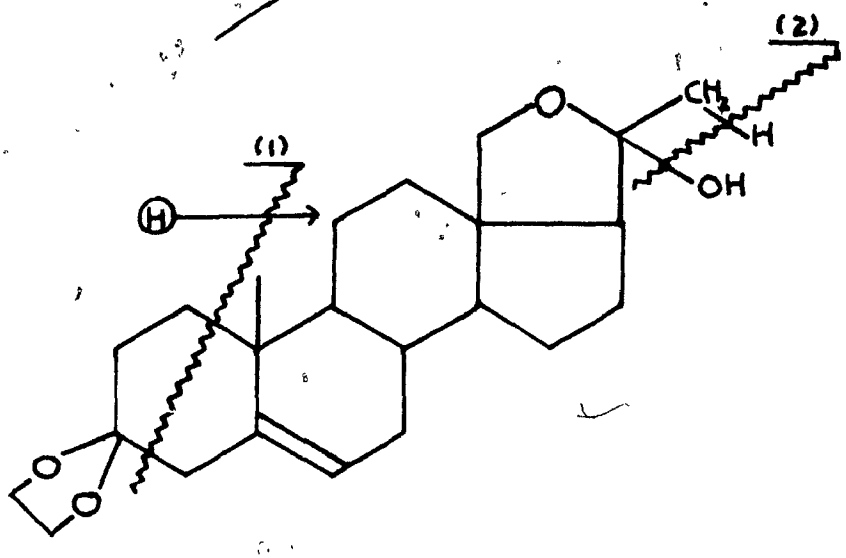
(I) R_1=R_2=H

(7) Cleavage of ring B with transfer of two hydrogen atoms to ring A;

(I) R_1=R_2=H

Type I-B steroid

3,3-Ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnen (III)



Major fragmentation possibilities:

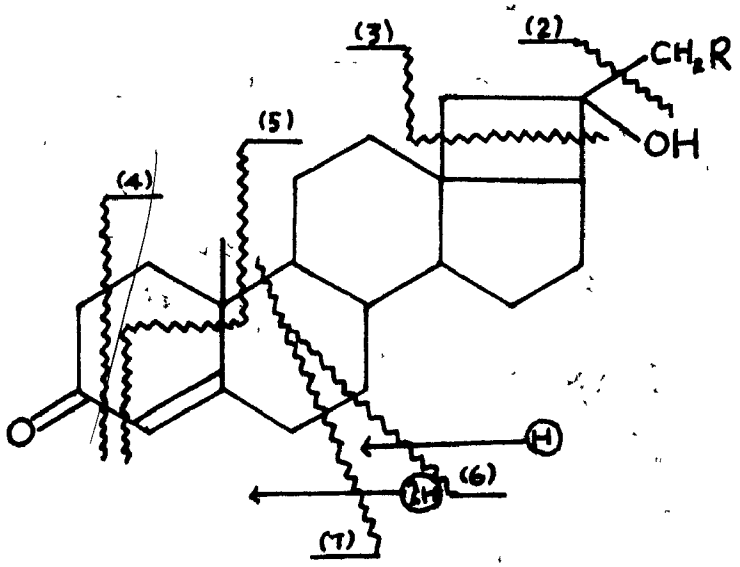
(1) Loss of ethylene ketal

(2) Loss of water

Type II steroids

18,20-Cyclo-20-hydroxy-Δ^4-pregnen-3-one(IV; R=H)

18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one(V; R=OH)



Major fragmentation possibilities:

(1) Loss of water; (IV) R=H, (V) R=OH

(2) Cleavage of the hydroxylated C-C bond; (V) R=OH

(3) Cleavage of the 18,20-cyclobutanol moiety; (IV) R=H, (V) R=OH

(4) Loss of ketene from ring A; (IV) R=H, (V) R=OH

(5) Extensive cleavage of ring A; (IV) R=H, (V) R=OH

(6) Cleavage of ring B with transfer of one hydrogen atom to ring A; (IV) R=H, (V) R=OH

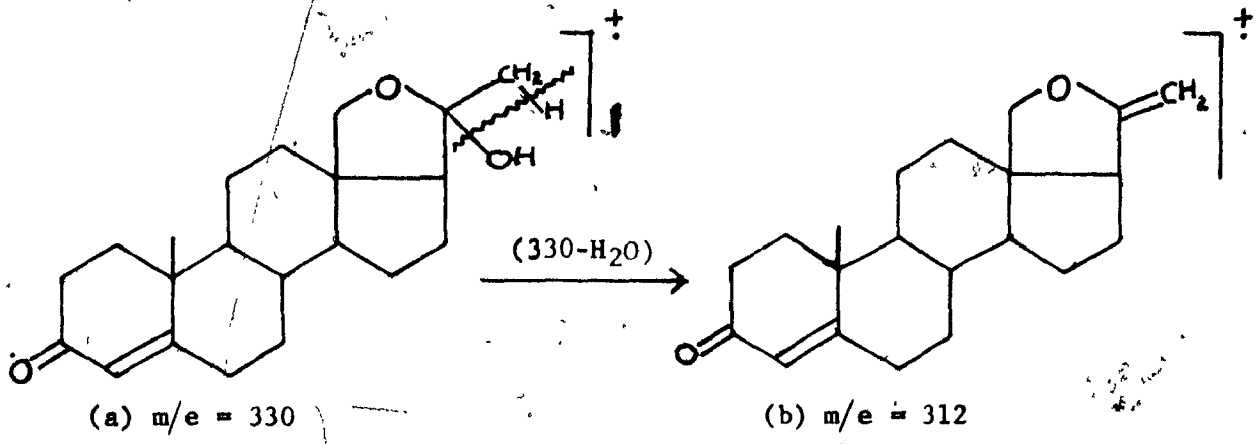
(7) Cleavage of ring B with transfer of two hydrogen atoms to ring A; (IV) R=H, (V) R=OH

3. Mass spectrum of 18-OH progesterone (I; Fig. 1, Scheme 1):

The mass spectrum of Buzzetti's (93) synthetic 18-OH progesterone has appeared in the report of Tschesche et al. (120). The mass spectra of Šorm's (96) and of Goutarel's (95) synthetic 18-OH progesterone were recorded in this laboratory as a reference standard. 18-OH Progesterone was also prepared by us and its mass spectrum shows ion peaks of relative intensities comparable with those of the reference spectra.

Fragment ion m/e = 312.2073 (b, 100%), (a→b)

The mass spectrum was devoid of the molecular ion (m/e = 330) (a), but has a base peak at m/e = 312 (b) corresponding to the loss of a molecule of water from the molecular ion (M^+-18). High resolution mass measurements show the composition of the m/e = 312 (b) ion to be $C_{21}H_{28}O_2$ (calcd. 312.2089).

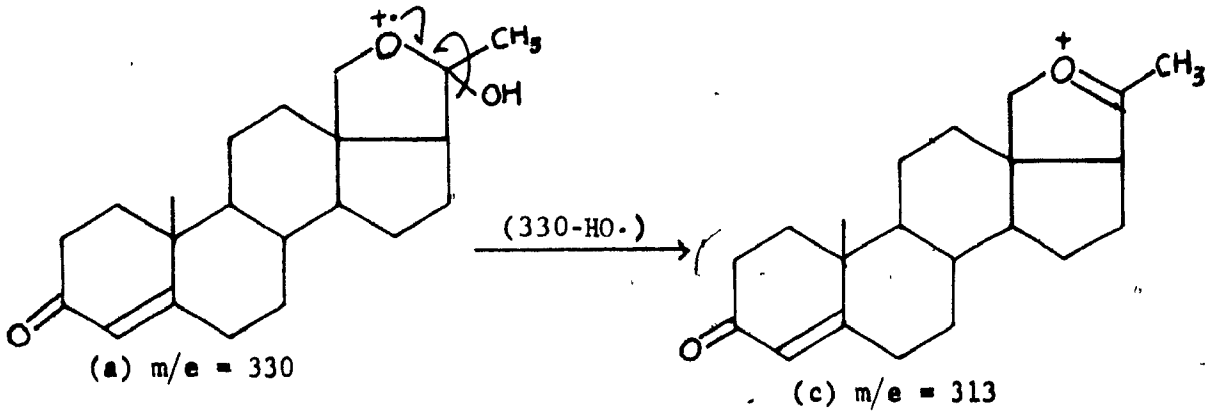


(a) m/e = 330 (b) m/e = 312

It is possible that the dehydration process (a→b) is a thermal one (1,2-elimination of water), since the sample was introduced at a relatively high ion source temperature (165-200°). However, the process is similar to the loss of water for alcohols under electron impact (119).

Fragment ion m/e = 313 (c, 33%), (a→c)

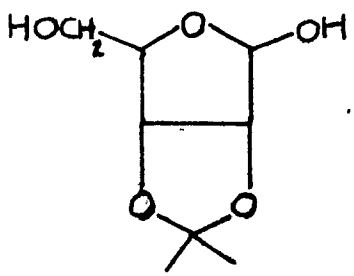
This peak probably arises from the loss of an hydroxyl radical from (a), (330-HO·), giving an oxonium ion (c).



(a) m/e = 330

(c) m/e = 313

Loss of hydroxyl and hydroxylmethyl groups at the alpha carbons of the tetrahydrofuran ring in the mass spectrum of a sugar derivative,

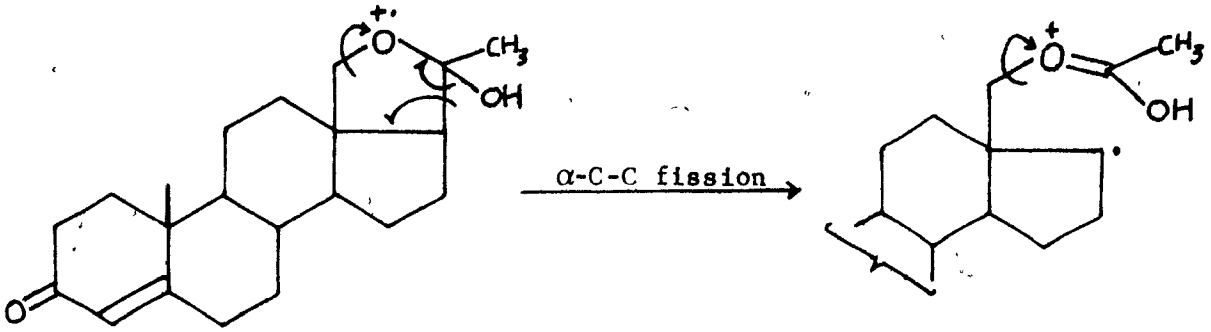


have been observed by De Jong and Biemann (121).

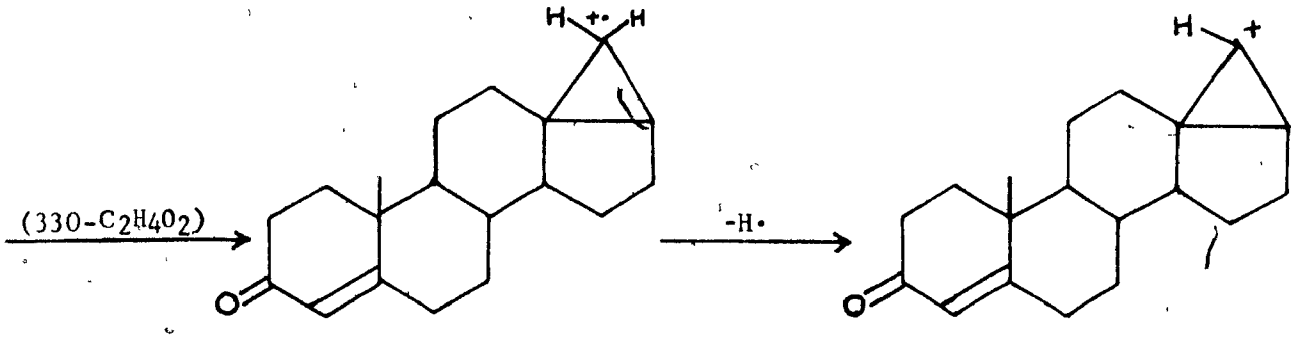
Fragment ions m/e = 270.1926 (d, 22%), (a-d)

m/e = 269 (e, 4%), (a-d-e)

High resolution mass measurements of the m/e = 270 (d) ion gave composition $C_{19}H_{26}O$ (calcd. 270.1983). The origin of the peaks at m/e=270 (d) and m/e = 269 (e) can best be explained as being the result of extensive cleavage of the 18,20-cyclohemiketal moiety in (a), as indicated below.



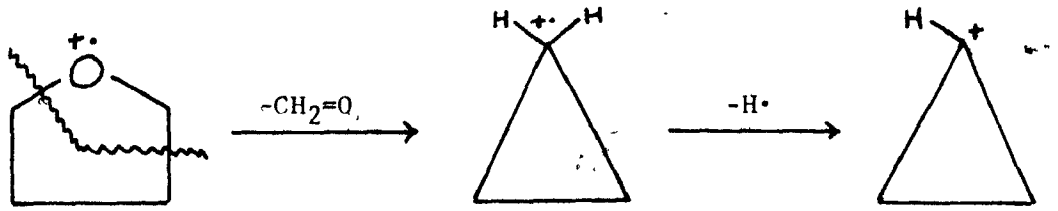
(a) m/e = 330



(d) m/e = 270

(e) m/e = 269

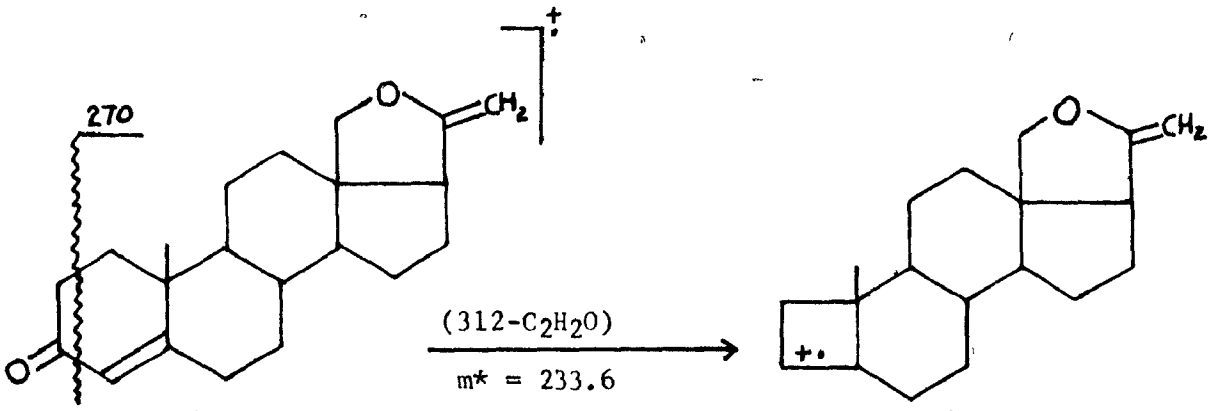
This fragmentation process may be viewed in an analogous manner to that of the mass spectrum of tetrahydrofuran described by Djerassi and co-workers (122) and later by Smakman and de Boer (123).



(A) (B) (C)

They concluded by deuterium labelling experiments that the loss of formaldehyde containing an α-methylene group from the molecular ion (A) leads to the formation of a cyclopropane ion radical (B) as the base ion, which in turn loses a hydrogen atom to form a cyclopropyl ion (C).

Alternatively, the ion at m/e = 270 (f) may be considered to arise as a result of further fragmentation of the base ion (b) in the following way:



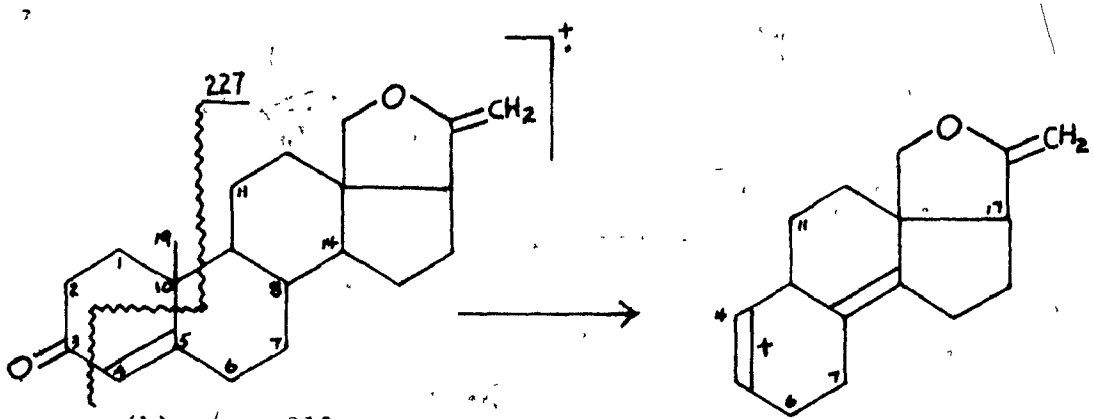
(b) m/e = 312 (f) m/e = 270

This fragmentation is supported by the occurrence of a metastable transition at m* = 233.6 (233.65 = $\frac{(270)^2}{312}$).

Similar fragmentation for progesterone and Δ^4-androsten-3-one has been reported by Peterson (124) and by Shapiro and Djerassi (125), respectively.

Fragment ion m/e = 227.1498 (g, 27%), (b→g)

The ion at m/e = 227 (g) appears to be formed from the base ion (b) by the following cleavage process.



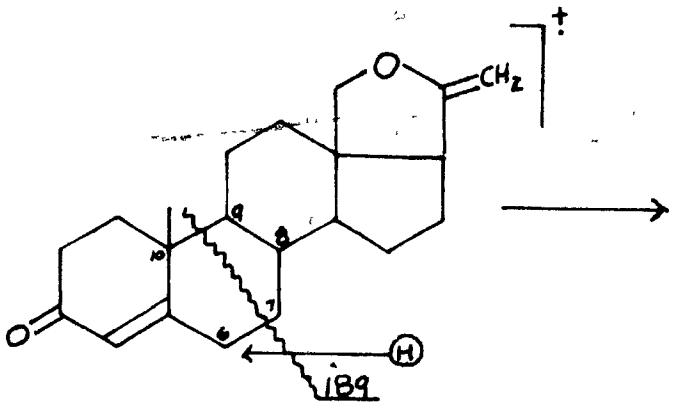
(b) m/e = 312 (g) m/e = 227

High resolution mass measurements show the composition of this ion at m/e = 227 (g) to be $C_{16}H_{19}O$ (calcd. 227.1436), corresponding to the loss of 85 mass units from the base ion at m/e = 312 (b) of composition $C_{21}H_{28}O_2$. This fragmentation process is analogous to that of Δ^4-androsten-3-one, in which the appearance of a "M^+-85" ion has been reported by Shapiro and Djerassi (125). The authors

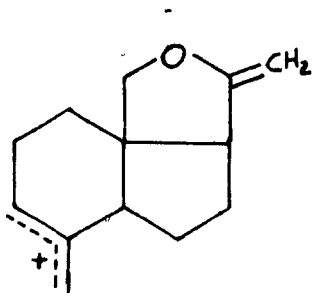
have shown, by deuterium labelling, that C-1, C-2, C-3, C-10, and C-19 as well as the hydrogen at C-8 and at C-14 are lost, whereas C-4, C-6, C-7, C-11, and C-17 are retained in the charged fragment.

Fragment ion m/e = 189 (h, 10%), (b→h)

The ion at m/e = 189 (h) is probably due to the further fragmentation of the base ion (b) by fission of the C-6, 7 and C-9, 10 bonds accompanied by the transfer of one hydrogen atom from C-8. Shapiro and Djerassi (125) has substantiated this mode of fragmentation for Δ^4-androsten-3-one.



(b) m/e = 312

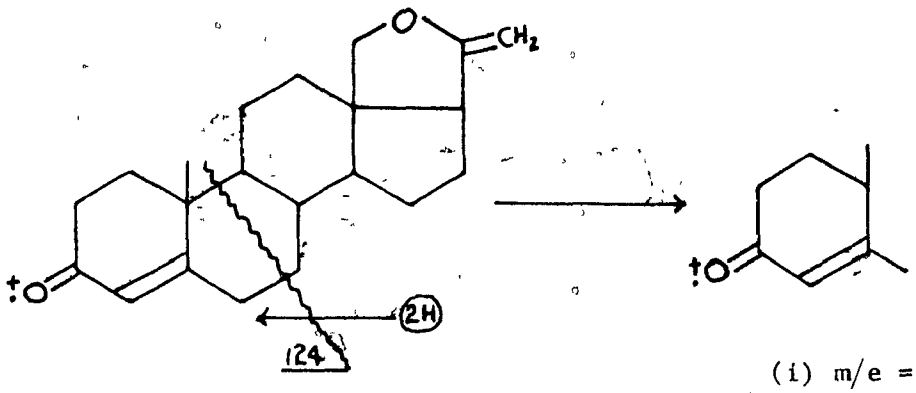


(h) m/e = 189

Fragment ion m/e = 124 (i, 9%), (b→i)

The formation of the m/e = 124 (i) ion by the cleavage (b) → (i) with the transfer of two hydrogens appears to be a general

pathway for Δ^4-3-ketone steroids (124, 125).

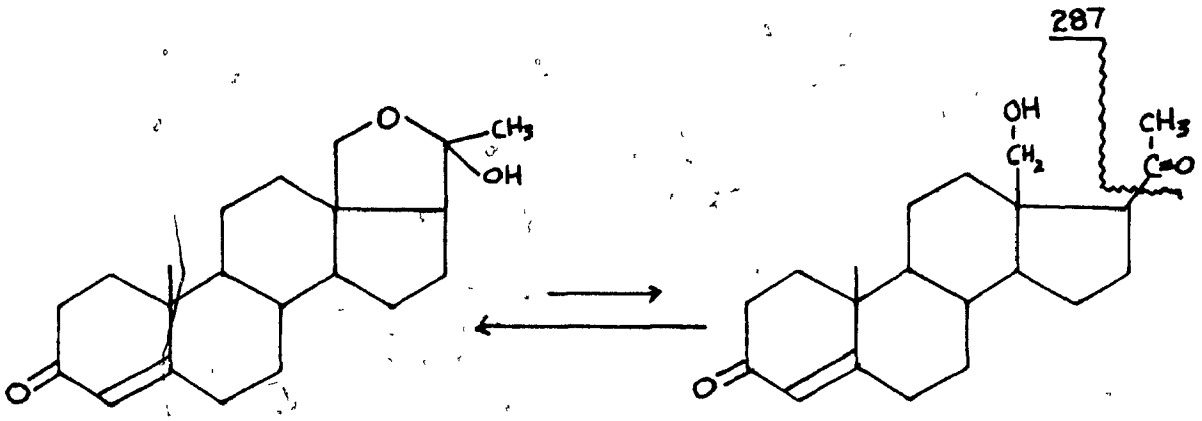


(b) m/e = 312

(i) m/e = 124

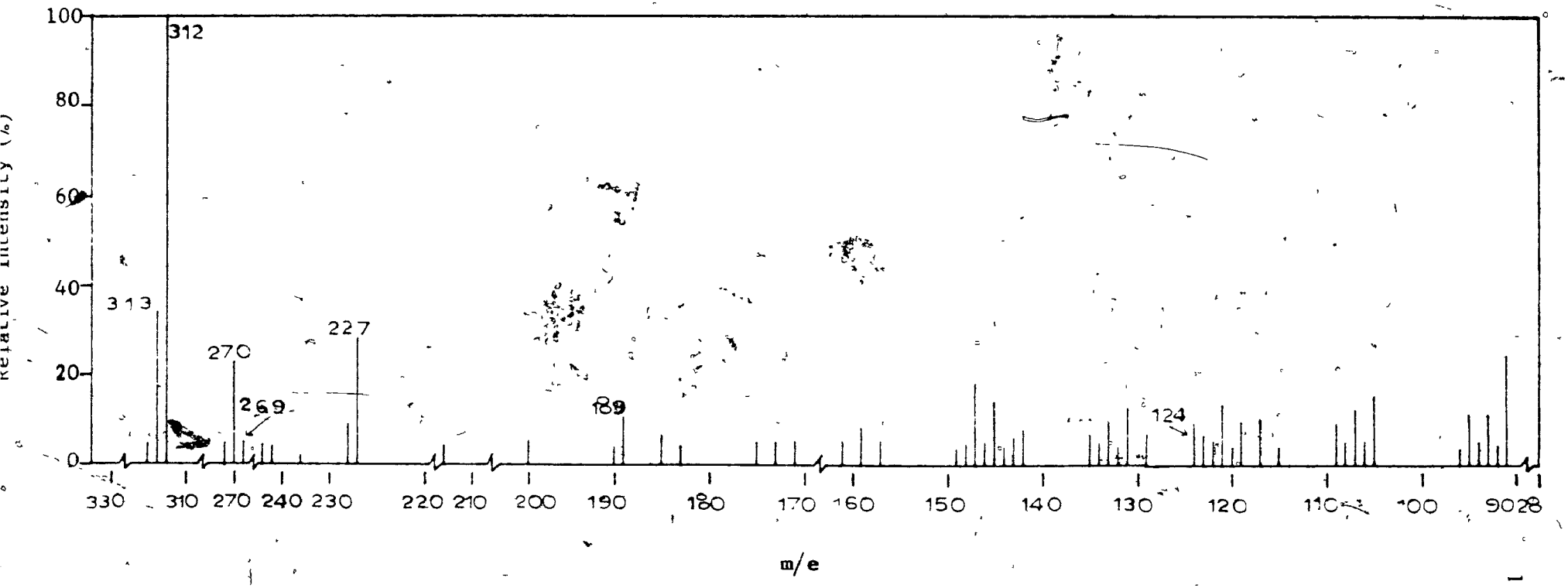
Additional comments

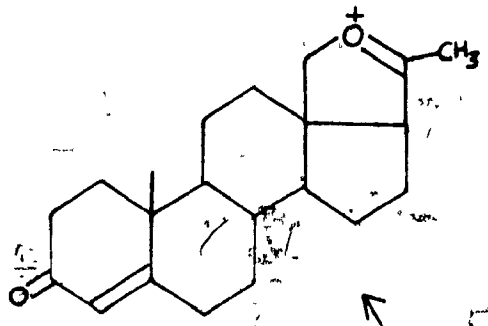
The presence of peaks at m/e = 312 (b) and m/e = 313 (c), and the absence of an ion at m/e = 287 (corresponding to the loss of the whole 17β-side chain from the molecular ion) support an 18,20-cyclohemiketal structure for 18-OH progesterone (I).



A summary of the major fragmentation pathways of 18-OH progesterone (I) is shown in Scheme 1.

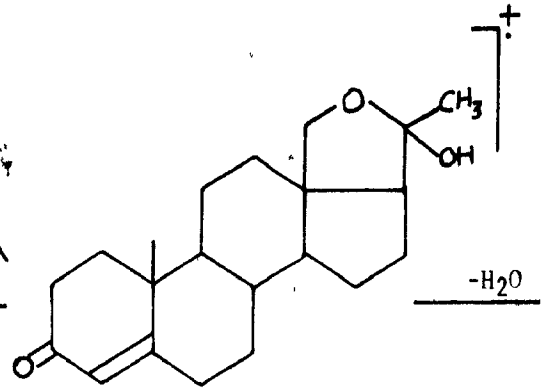
Fig. 1: Mass spectrum of 18-OH progesterone (I)

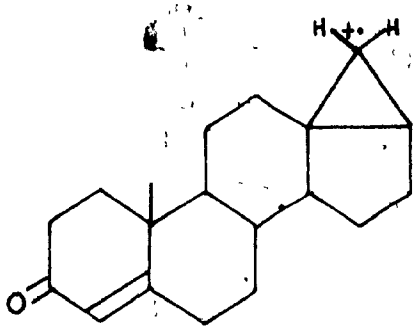




(c) m/e = 313 (33%)

-HO·





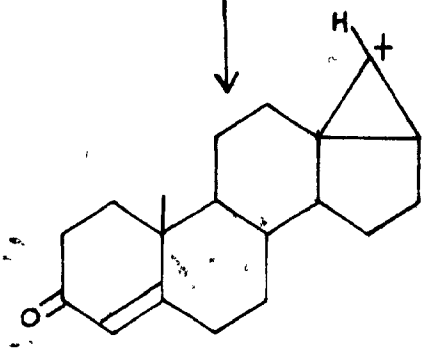
$-C_2H_4O_2$

$-H_2O$

(d) m/e = 270 (22%)

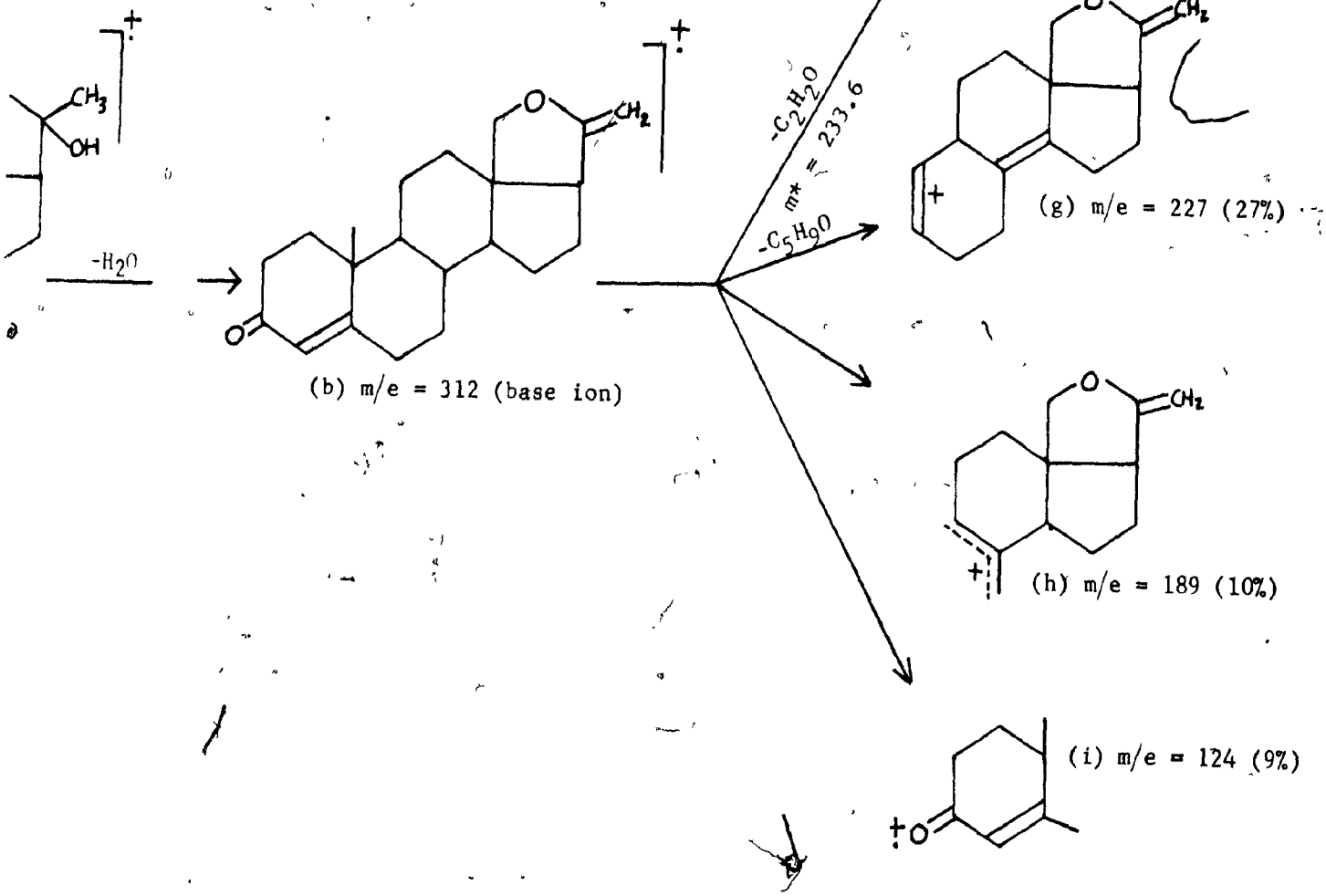
(a) m/e = 330

-H·



(e) m/e = 269 (4%)

Scheme 1 Major Fragmentations in the Mass Spectrum of 18-OH Progesterone (I).


-H2O
(b) m/e = 312 (base ion)
-C2H2O
m* = 233.6
-C5H8O
(f) m/e = 270 (22%)
(g) m/e = 227 (27%)
(h) m/e = 189 (10%)
(i) m/e = 124 (9%)

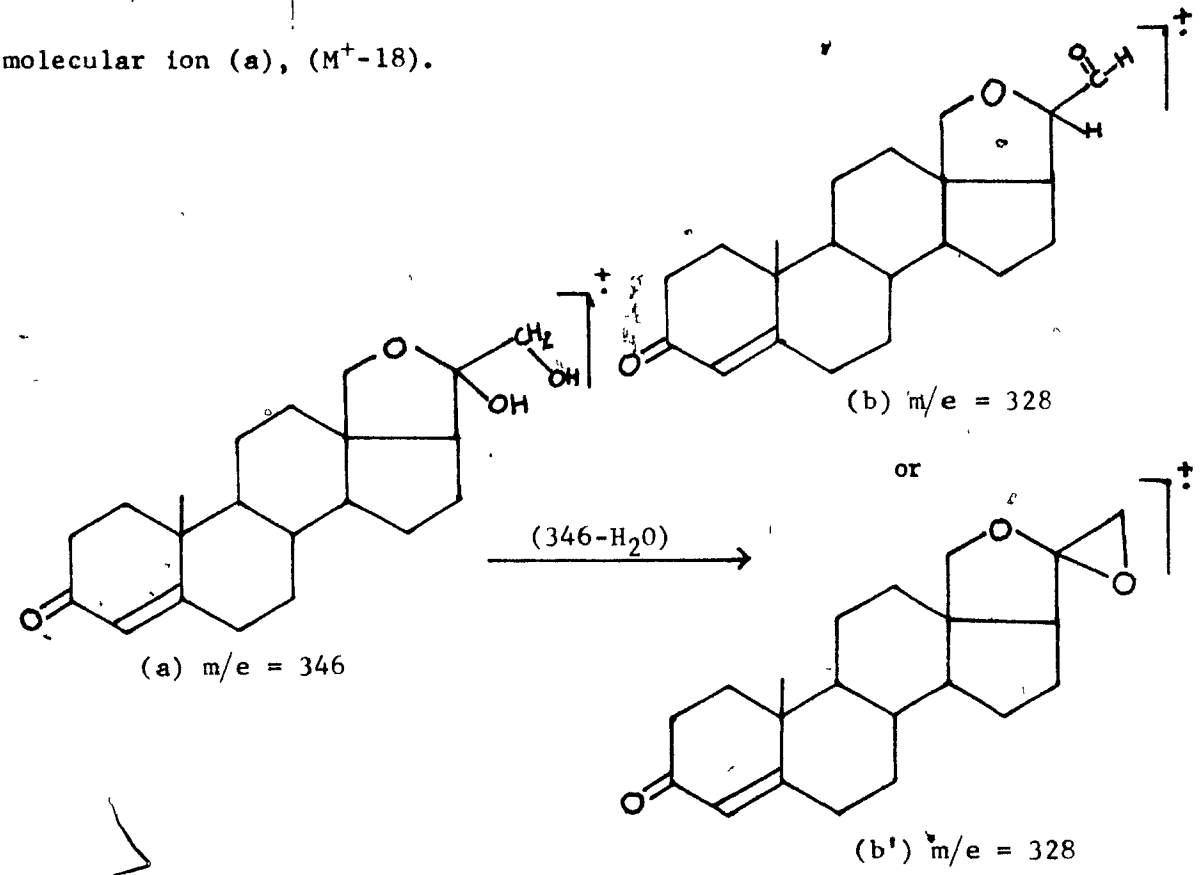
trum

4. Mass spectrum of 18-OH-DOC (II; Fig. 2, Scheme 2):

Recently the mass spectrum of Pappo's (97) synthetic 18-OH-DOC has been reported by Genard et al (126). The mass spectrum of our synthetic 18-OH-DOC and that of Pappo's are in good agreement. The prinicple features of these spectra are the absence of the molecular ion (m/e = 346) and the presence of three prominent peaks in the order of increasing intensity at m/e = 328, 315, and 299, with the last mentioned fragment ion being the base peak. No metastable peak was found in these spectra.

Fragment ion m/e = 328.2044 (b or b', 11%), (a→b or b')

The molecular ion (m/e = 346) (a) was not observed. The ion at m/e = 328 (b or b') corresponds to the loss of water from the molecular ion (a), (M^+-18).



(a) m/e = 346

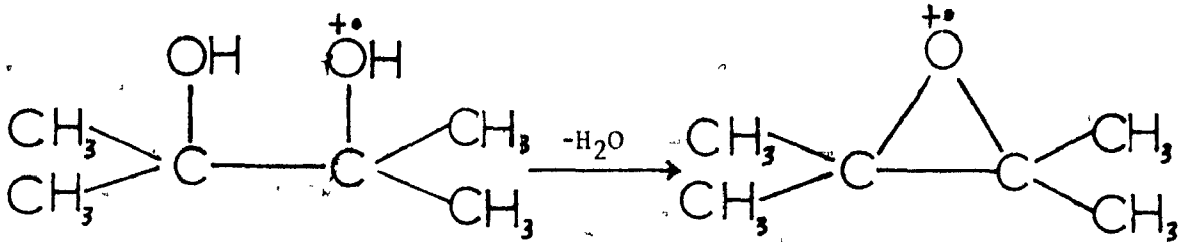
(b) m/e = 328

(b') m/e = 328

High resolution mass measurements established the composition of the m/e = 328 ion to be $C_{21}H_{28}O_3$ (calcd. 328.2038).

The transformation of ethylene glycol to the corresponding carbonyl compound may be considered as a dehydration for which there are ample photochemical precedents (127, 128).

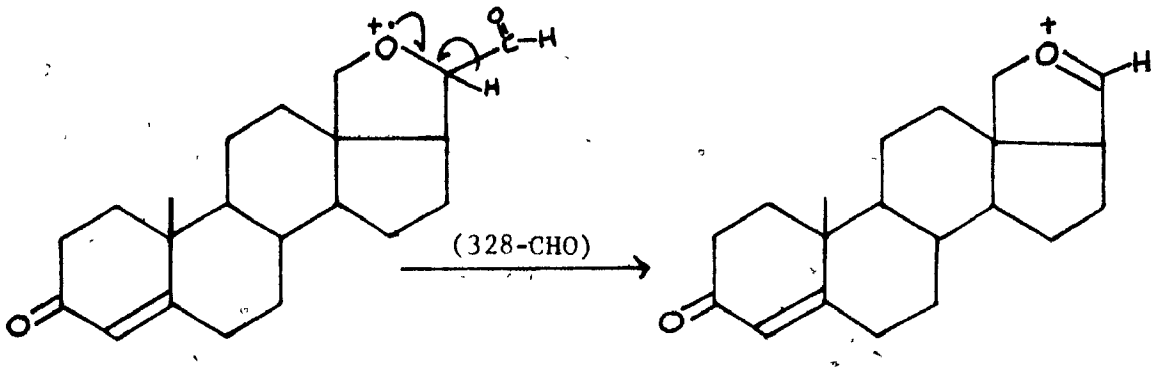
On the other hand, Djerassi et al. (129) reported that the loss of water from the molecular ion occurred in the mass spectra of substituted aliphatic 1,2-glycols (pinacol (A), for example) and the resultant species was formulated as an epoxide ion (B) on the basis of deuterium experiments.



(A) (B)

Fragment ion m/e = 299.2021 (c, 100%), (b→c)

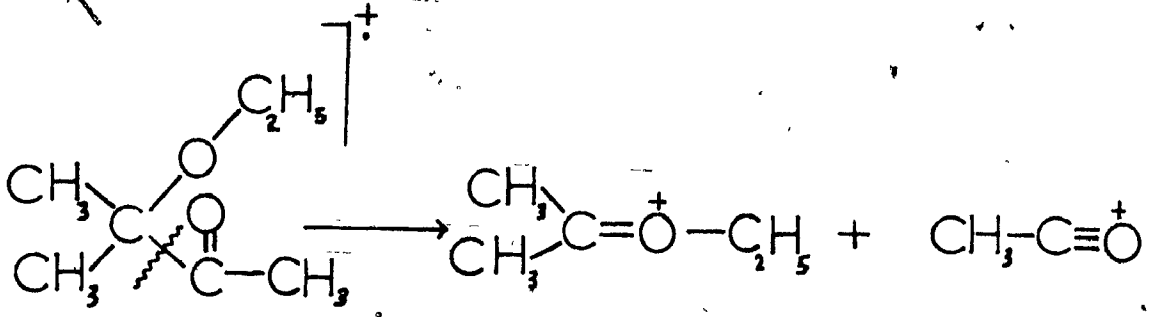
The formation of the base peak at m/e = 299 (c) may be due to further fragmentation of the (M^+-H_2O) ion at m/e = 328 (b) by loss of a CHO fragment (an α-cleavage process).



(b) m/e = 328 (c) m/e = 299

High resolution mass measurements established the composition $C_{20}H_{27}O_2$ (calcd. 299.2011) for the m/e = 299 (c) ion.

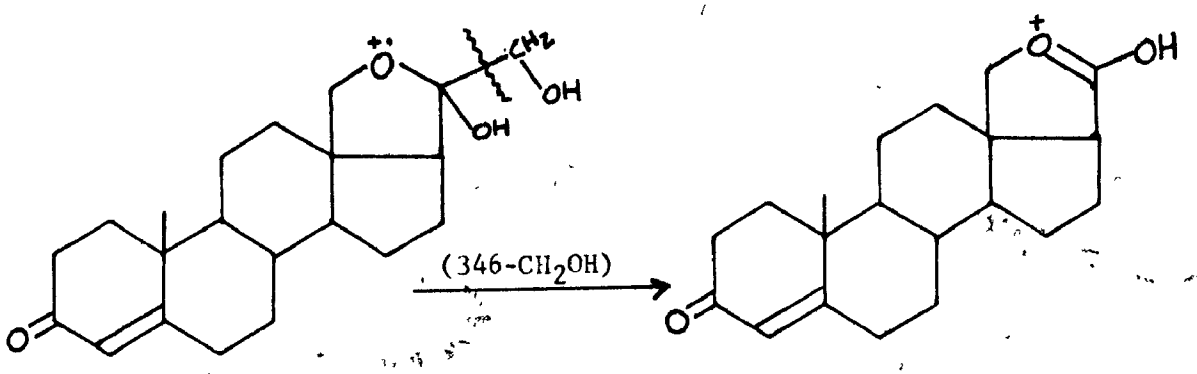
The mode of cleavage (b→c) may be similar to that of the mass spectra of aliphatic α-keto ethers described by Butler (130), (e.g. A → B).



(A) (B) (C)

Fragment ion m/e = 315.1974 (d, 26%), (a→d)

The prominant ion at m/e = 315 (d) is regarded as being derived from the molecular ion (m/e = 346) (a) by α-cleavage of the C_{20}-C_{21} carbon-carbon bond.



(a) m/e = 346 (d) m/e = 315

High resolution mass measurements showed the composition of the m/e = 315 (d) ion to be $C_{20}H_{27}O_3$ (calcd. 315.1960).

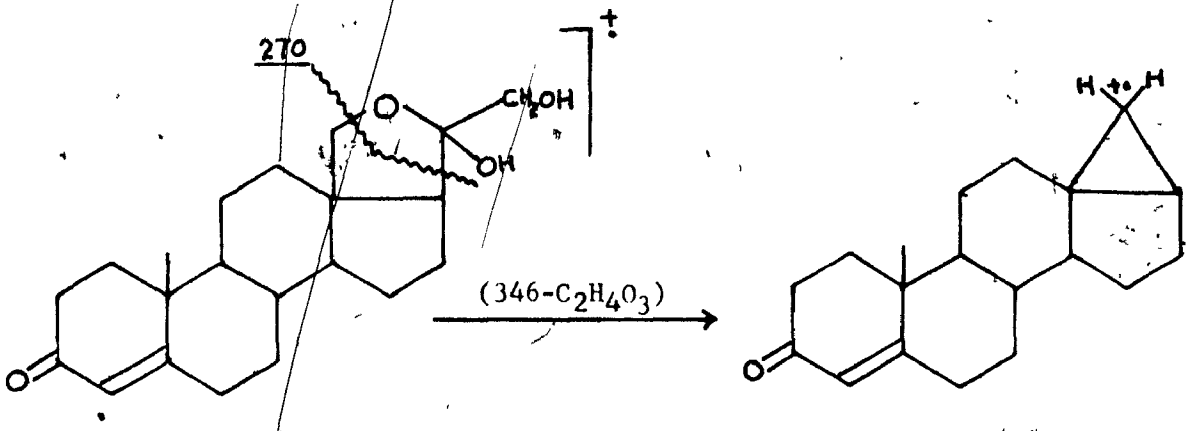
In the mass spectra of aliphatic 1,2-glycols, the cleavage of the carbon-carbon bond joining the two hydroxyl groups has been reported by Djerassi et al. (129) and by Long and Pritchard (131).

Fragment ions m/e = 270 (e, 5%), (a→e)

m/e = 269 (f, 11%), (a→e→f)

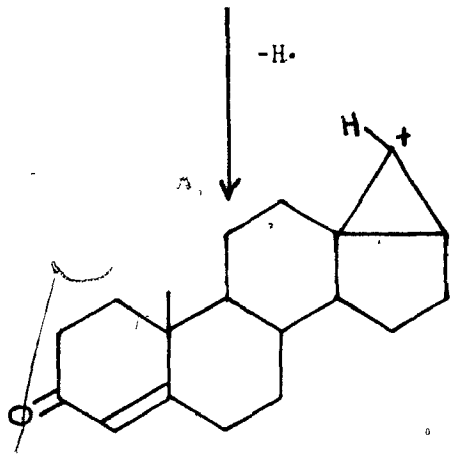
The peaks at m/e = 270 (e) and m/e = 269 (f), albeit of low intensity, may be formed from the molecular ion (a) as a result

of the cleavage of the 18,20-cyclohemiketal moiety in the same way as described in 18-OH progesterone (I) (see p. 112).



(a) m/e = 346

(e) m/e = 270



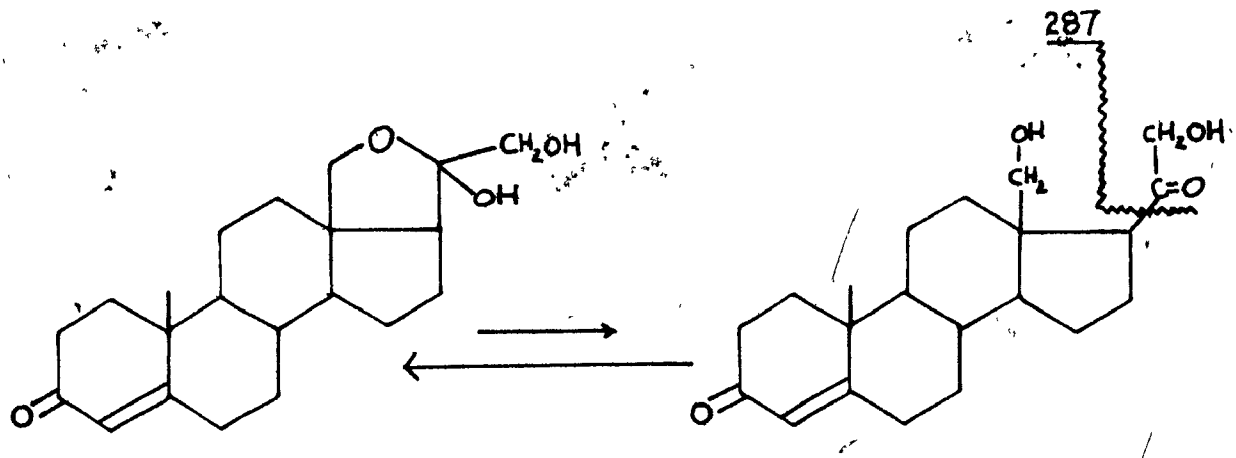
(f) m/e = 269

Fragment ion m/e = 124 (13%)

The fragmentation triggered by the α,β-unsaturated ketone moiety in the A ring (125) of either the molecular ion (m/e = 346) (a) or the base ion (m/e = 299) (c) appears not to be an important feature in the mass spectrum of 18-OH-DOC (I). The characteristic "M^+-42" (loss of ketene from ring A) and "M^+-85" (extensive cleavage of ring A) peaks are virtually absent. However, the spectrum shows a peak at m/e = 124 of 13% relative intensity indicating cleavage of the B ring (see Scheme 2, p. 126).

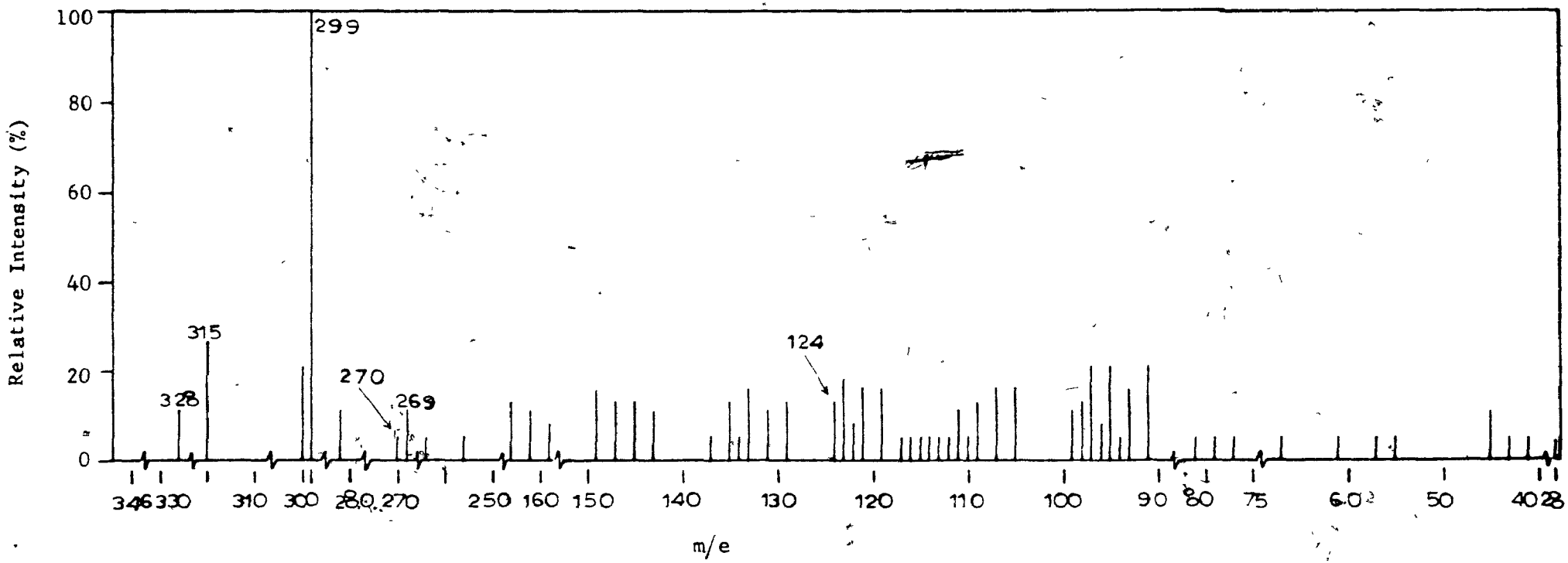
Additional comments

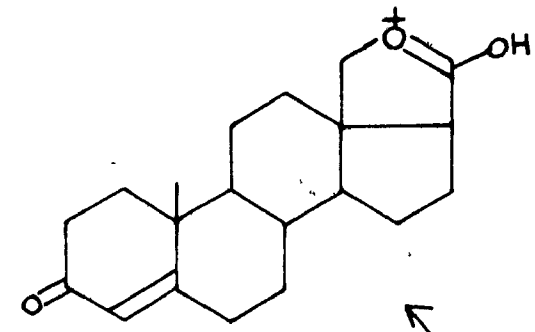
The presence of peaks at m/e = 328 (b or b'), 315 (d), and 299 (c) and the absence of an ion at m/e = 287 (corresponding to the loss of the whole 17β-side chain) strongly favors an 18,20-cyclohemiketal structure for 18-OH-DOC (II).



A summary of the major fragmentation pathways of 18-OH-DOC (II) is shown in Scheme 2.

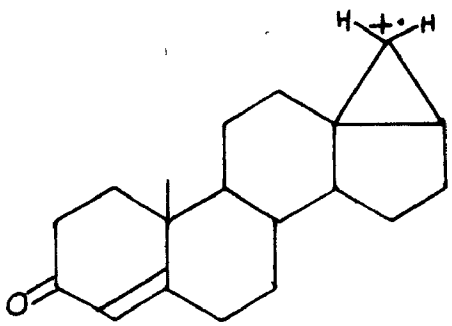
Fig. 2: Mass spectrum of 18-OH-DOC (II)





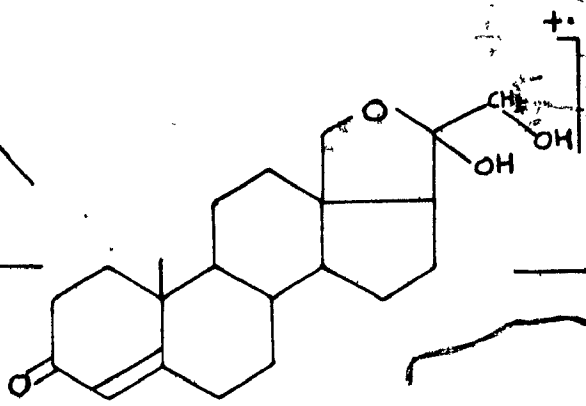
(d) m/e = 315 (26%)

$-CH_2OH$



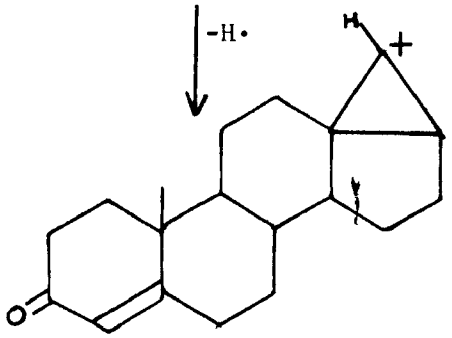
(e) m/e = 270 (5%)

$-C_2H_4O_3$



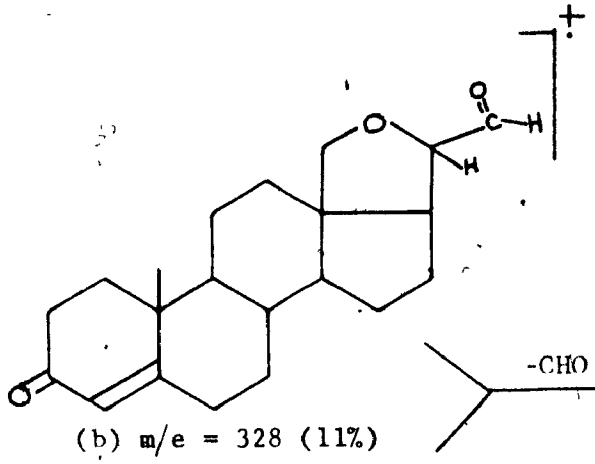
(a) m/e = 346

$-H\cdot$



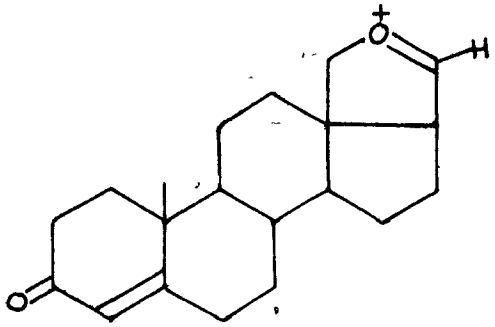
(f) m/e = 269 (11%)

Scheme 2 Major Fragmentations in the Mass Spectrum of 18-OH-DOC (II)



(b) m/e = 328 (11%)

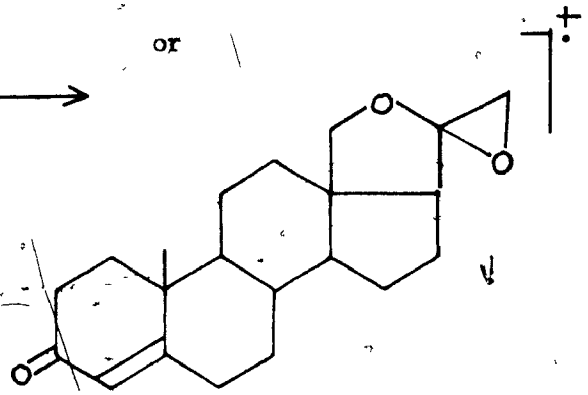
-CHO



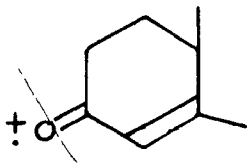
(c) m/e = 299 (base ion)

or

$-H_2O$



(b') m/e = 328 (11%)



m/e = 124 (13%)

5. Mass spectrum of 3,3-ethylenedioxy-18,20-epoxy-20-hydroxy- Δ^5-pregnene(III; Fig. 3, Scheme 3):

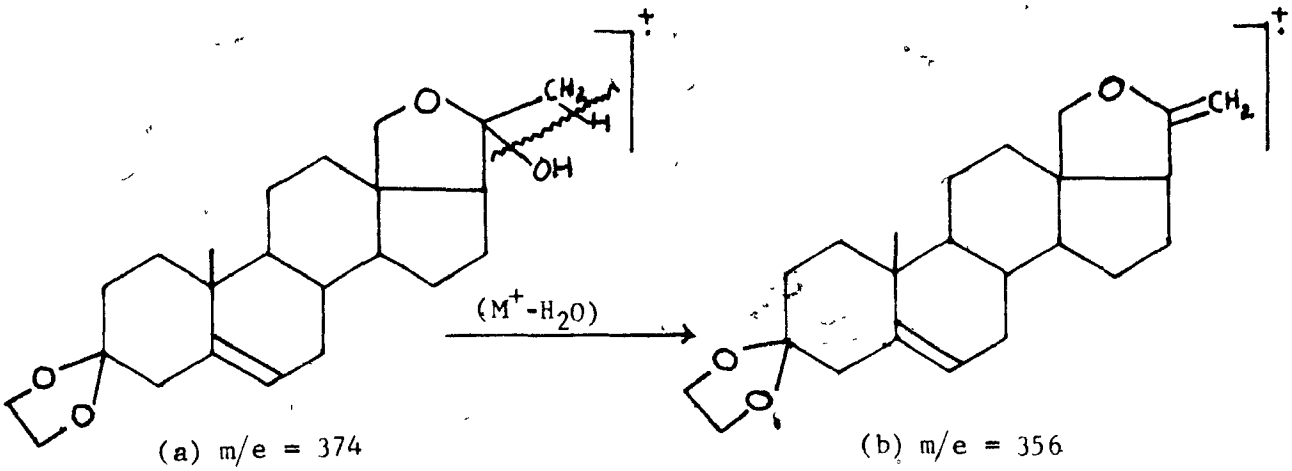
Compound III, the ethylene ketal derivative of 18-OH progesterone (I), shows a fragmentation pattern less complex than that of its parent compound.

Fragment ions m/e = 374.2457 (a, 10%), (M^+)

m/e = 356.2356 (b, 24%), (a→b)

The mass spectrum shows a molecular ion at m/e = 374 (a) with a relative intensity of 10%. High resolution mass measurements established the composition of this ion to be $C_{23}H_{34}O_4$ (calcd. 374.2457).

The abundant ion at m/e = 356 (b) appears to be formed by loss of a molecule of water (M^+-H_2O).

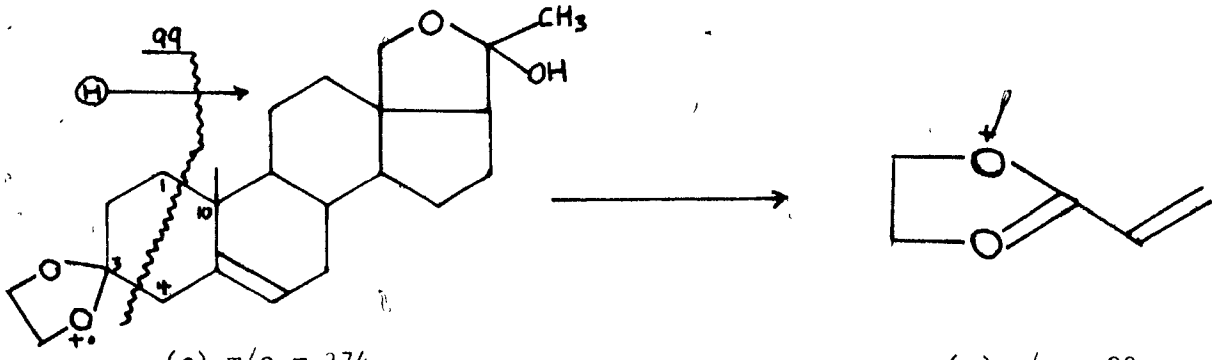


(a) m/e = 374 (b) m/e = 356

High resolution mass measurements confirmed the composition $C_{23}H_{32}O_3^{+\cdot}$ (calcd. 356.2351).

Fragment ion m/e = 99 (c, 100%), (a→c)

In keeping with the results reported by Djerassi et al. (132) on 3,3-ethylenedioxy steroids, the base ion at m/e = 99 (c) is regarded as being derived from the molecular ion (a) by loss of the ethylene ketal moiety.

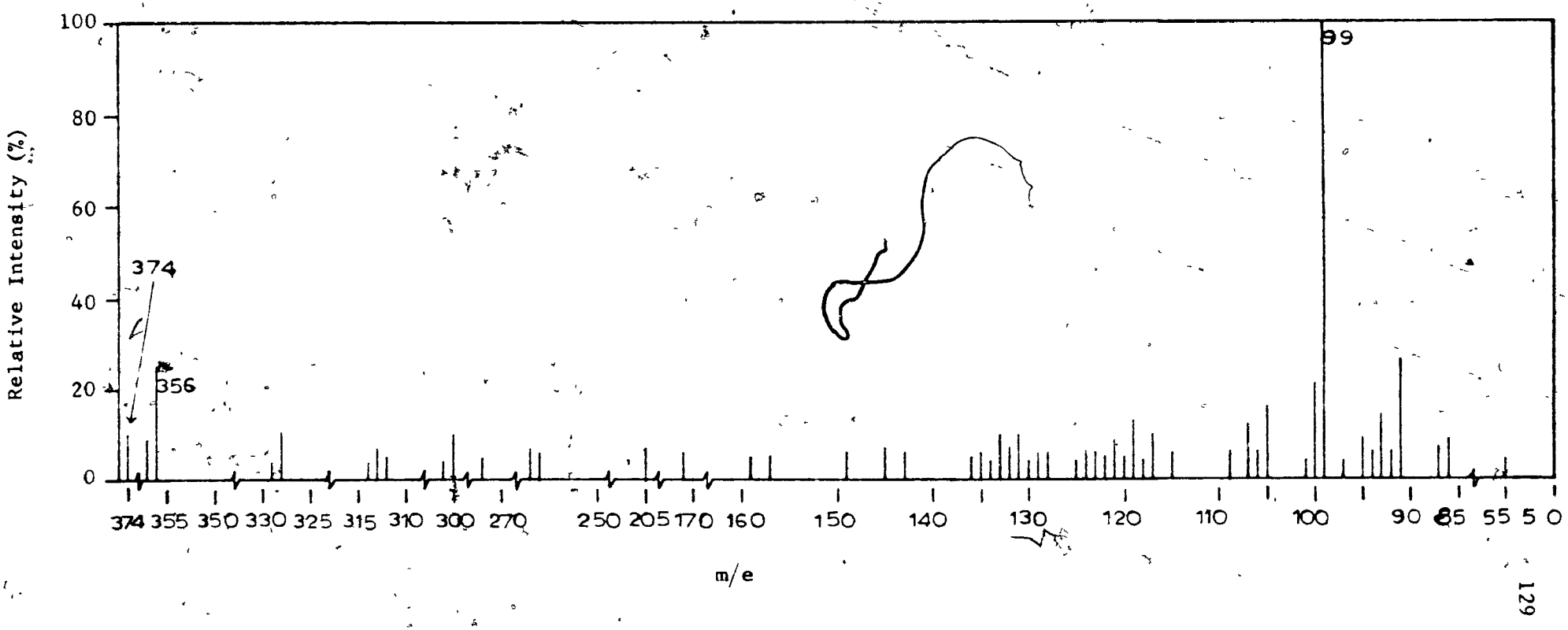


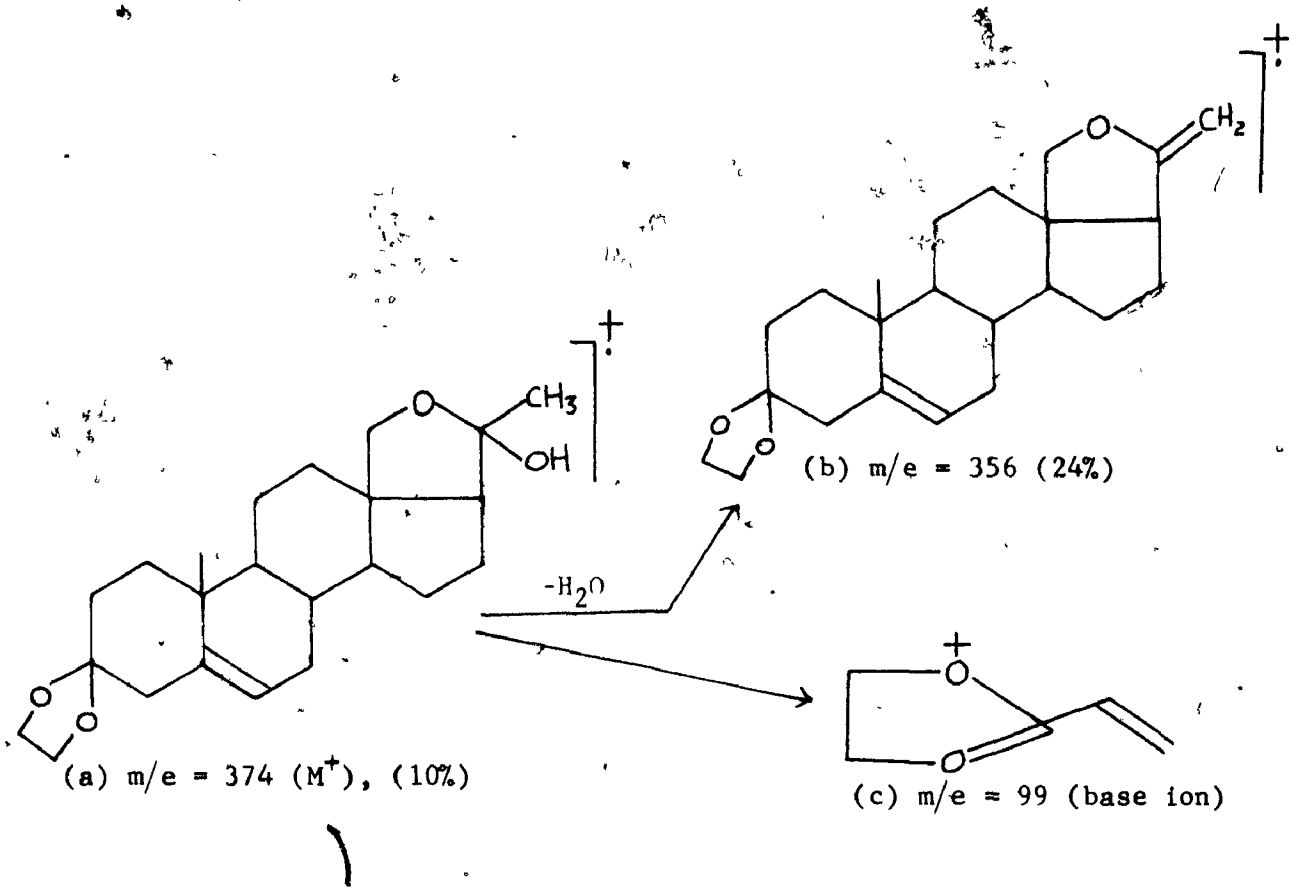
(a) m/e = 374 (c) m/e = 99

Djerassi et al. (132) reported the appearance of a base peak at m/e = 99 in the mass spectrum of cortisone acetate 3-ethylene ketal and suggested that the formation of this ion requires the cleavage of both the allylic activated C-3,4 and C-1,10 bonds with migration of a hydrogen atom. The proposed hydrogen transfer was supported by appropriate deuterium labelling.

A summary of the major fragmentation pathways of III is shown in Scheme 3.

Fig. 3: Mass spectrum of 3,3-ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnene (III)





Scheme 3 Major Fragmentations in the Mass Spectrum of 3,3-Ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnene (III)

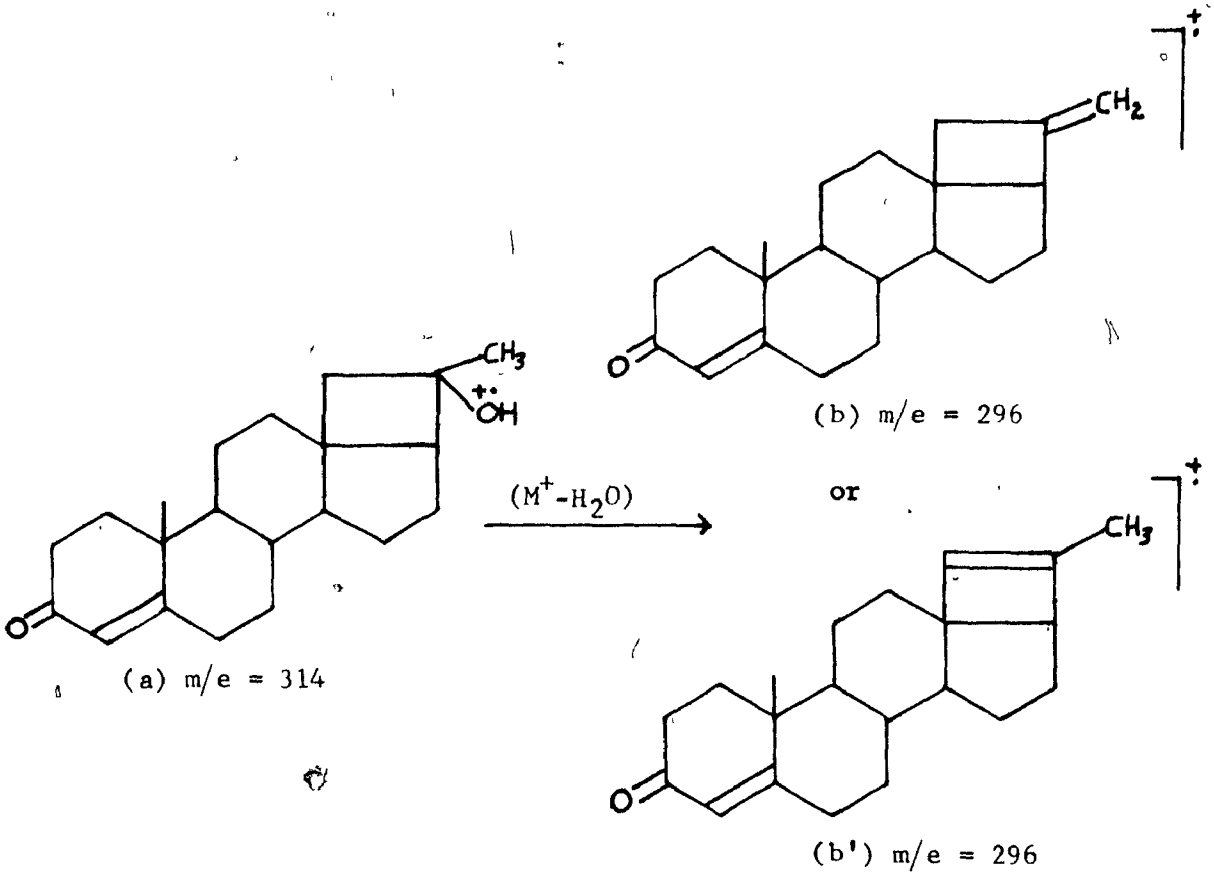
6. Mass spectrum of 18,20-cyclo-20-hydroxy-Δ^4-pregnen-3-one (IV; Fig. 4, Scheme 4):

Fragment ions m/e = 314.2162 (a, 36%), (M^+)

m/e = 296 (b or b', 11%), (a→b or a→b')

The spectrum gives a molecular ion at m/e = 314 (a) with a relative intensity of 36%. The elemental composition of this ion was verified by high resolution mass measurements to be $C_{21}H_{30}O_2$ (calc. 314.2246).

The peak at m/e = 296 (b or b') corresponds to the loss of water from molecular ion (M^+-H_2O).



(a) m/e = 314

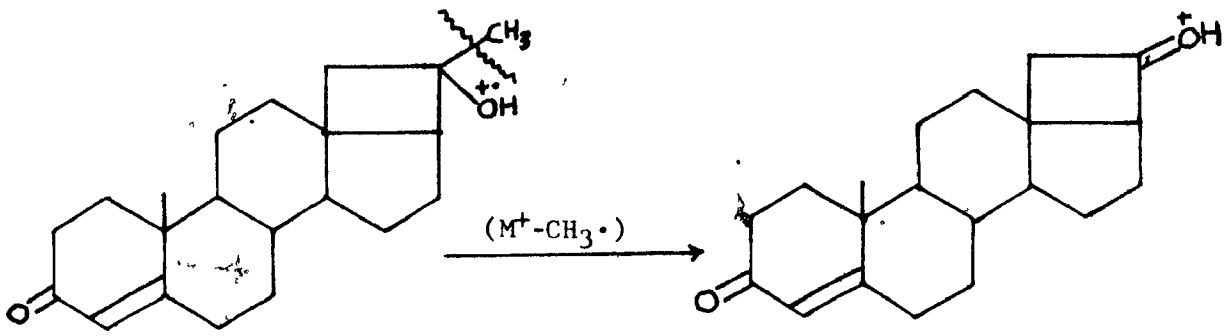
(b) m/e = 296

(b') m/e = 296

Similar fragmentation also occurs in the mass spectrum of 1-methylcyclobutanol under electron impact (133).

Fragment ion m/e = 299 (c, 7%), (a→c)

The appearance of the ion at m/e = 299 (c), albeit of low intensity, suggests its formation from the molecular ion (a) by an α-cleavage of the C-21 methyl group.



(a) m/e = 314 (c) m/e = 299

This process might be viewed as analogous to the mass spectrum of 1-methylcyclobutanol, in which loss of the alpha methyl radical from the molecular ion has been reported (133).

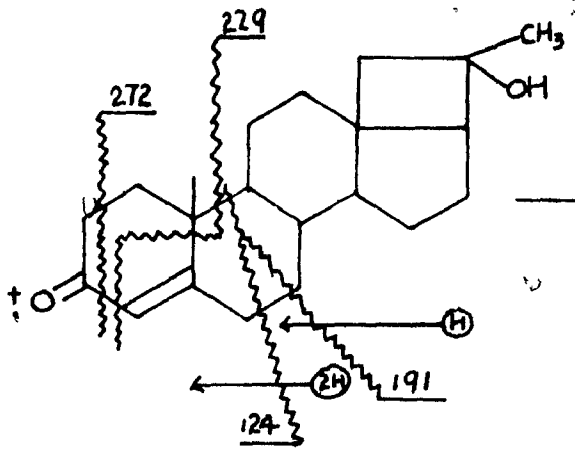
Fragment ions m/e = 272.2085 (d, 37%), (a→d)

m/e = 229 (e, 19%), (a→f)

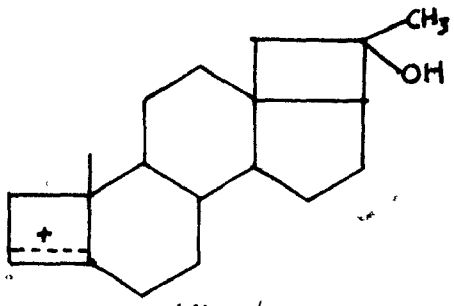
m/e = 191 (f, 28%), (a→g)

m/e = 124 (g, 92%), (a→h)

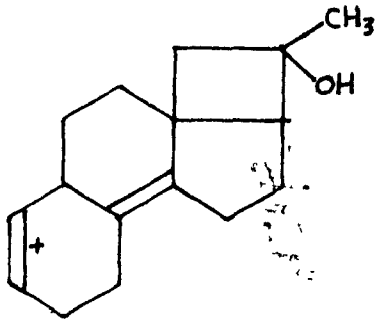
These intense peaks correspond to characteristic cleavages initiated by the α,β-unsaturated ketone moiety in the A ring of the molecular ion (a), (124 , 125). The decomposition pathways leading to the observed ions are illustrated below.



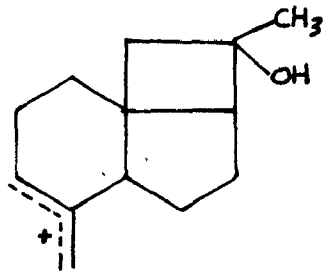
(a) m/e = 314



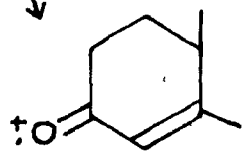
(d) m/e = 272



(e) m/e = 229



(f) m/e = 191

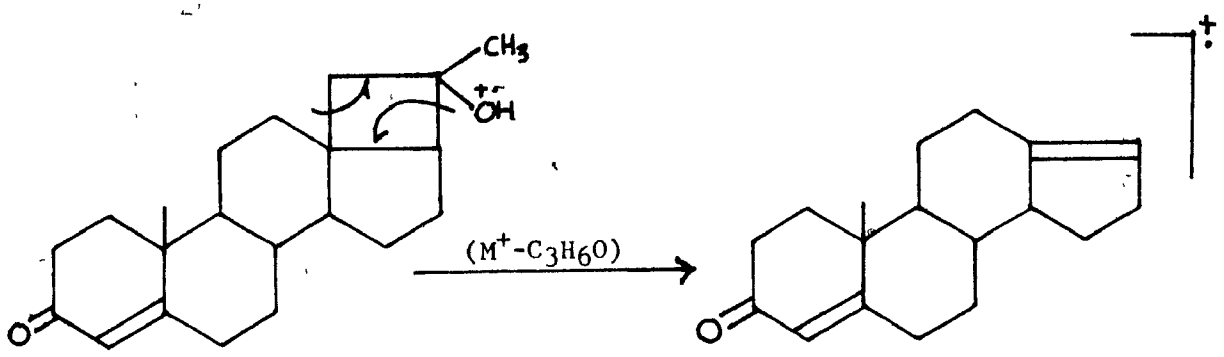


(g) m/e = 124

High resolution mass measurements confirmed the composition $C_{19}H_{28}O$ (calcd. 272.2140) for the peak at m/e = 272 (d). A similar loss of ketene in the mass spectrum of 18-OH progesterone (I) has been described previously (see p. 113).

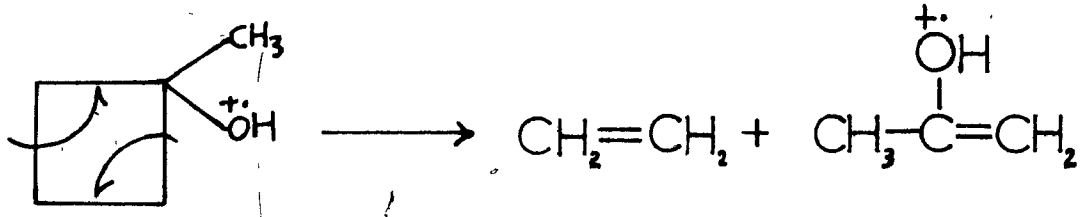
Fragment ion m/e = 256.1797 (h, 100%), (a→h)

The mass spectrum shows a base peak at m/e = 256 (h) ($M^+-C_3H_6O$). High resolution mass measurements proved the composition of the m/e = 256 (h) ion to be $C_{18}H_{24}O$ (calcd. 256.1827).



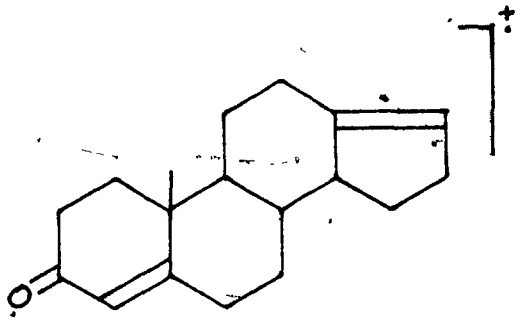
(a) m/e = 314 (h) m/e = 256

Ausloos and Rebbert (133) suggested and substantiated by high resolution mass measurements and deuterium labelling experiments that 1-methylcyclobutanol suffers a similar fragmentation under electron impact.



Fragment m/e = 238.1740 (i, 40%), (h→i)

One major fragmentation reaction of the base ion at m/e = 256 (h) leads to an intense ion at m/e = 238 (i) by loss of 18 mass units, presumably corresponding to the loss of a molecule of water (256 - H_2O). This fragmentation is supported by the appearance of a metastable peak at m* = 221.4 ($\frac{(238)^2}{256}$). High resolution mass measurements verified the composition of the m/e % 238 (i) ion to be $C_{18}H_{22}$ (calcd. 238.1722). However, the mode of its formation and the possible ionic structure of the resulting hydrocarbon fragment ion are uncertain.



(h) m/e = 256

$\xrightarrow[m^*=221.4]{(256-H_2O)}$ (i) m/e = 238

Fragment ions m/e = 214 (j, 17%), (h→j)

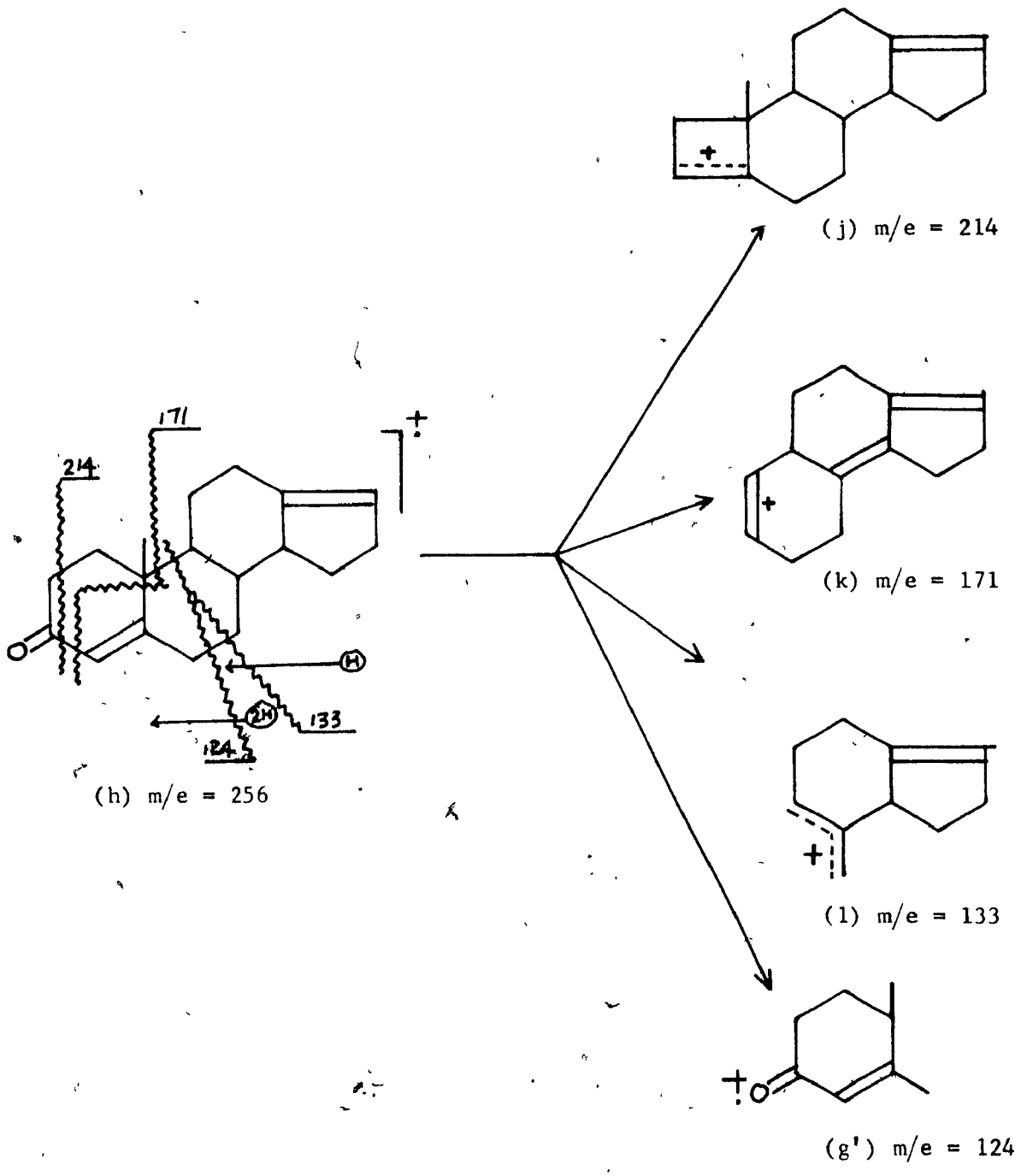
m/e = 171 (k, 26%), (h→k)

m/e = 133 (l, 50%), (h→l)

m/e = 124 (g', 92%), (h→g')

The base ion at m/e = 256 (h) probably undergoes further decompositions to give ions at m/e = 214 (j), 171 (k), 133 (l), and 124 (g'),

which correspond to the fragmentations triggered by the α,β-unsaturated ketone function in the A ring (124, 125).



(h) m/e = 256

(j) m/e = 214

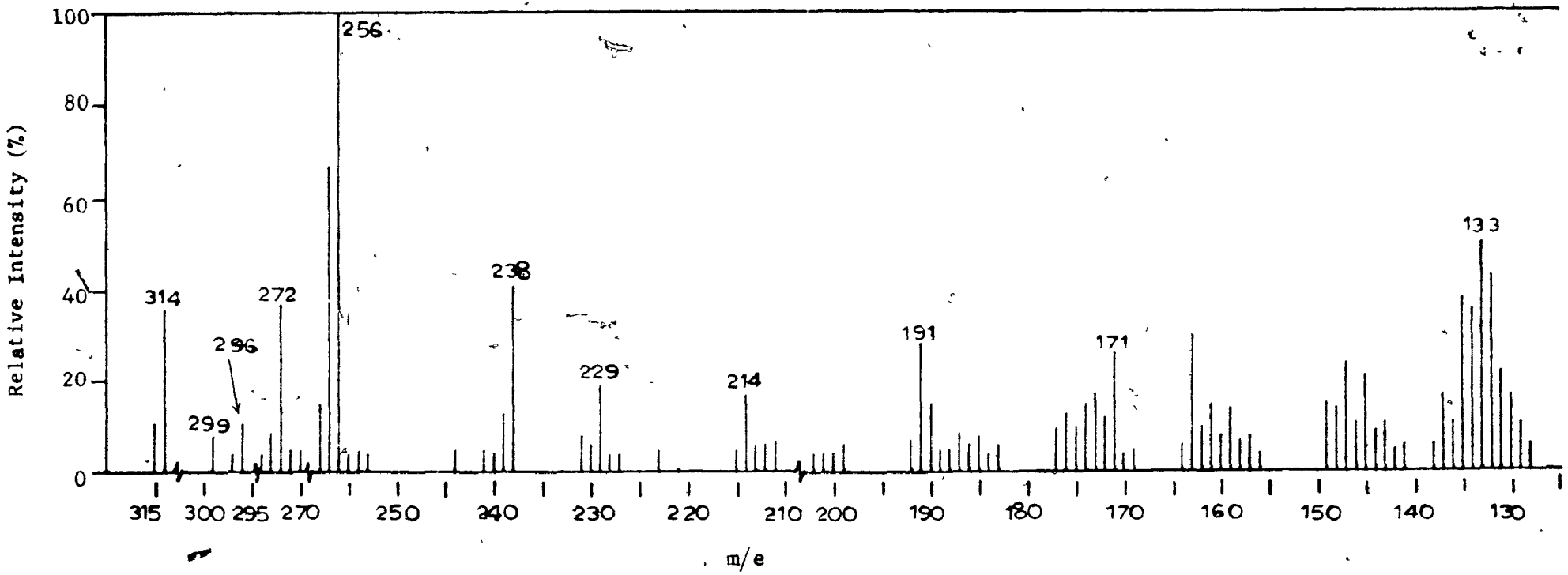
(k) m/e = 171

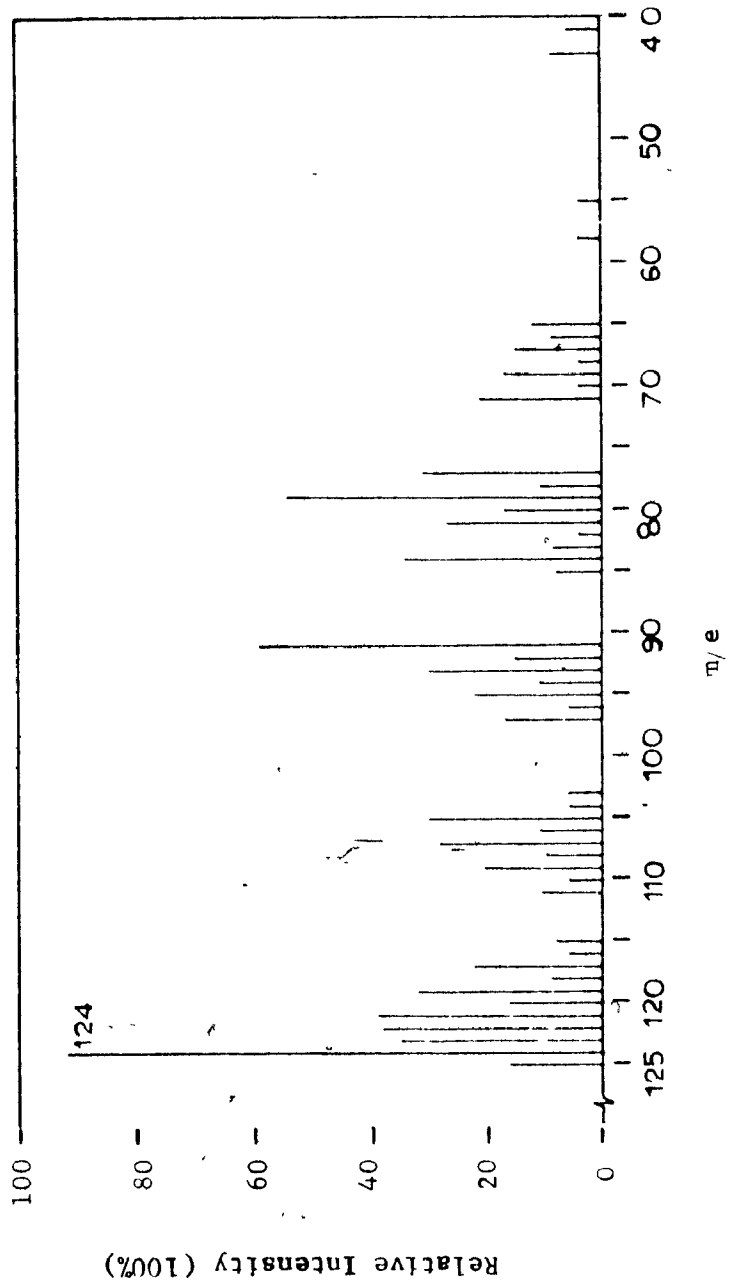
(l) m/e = 133

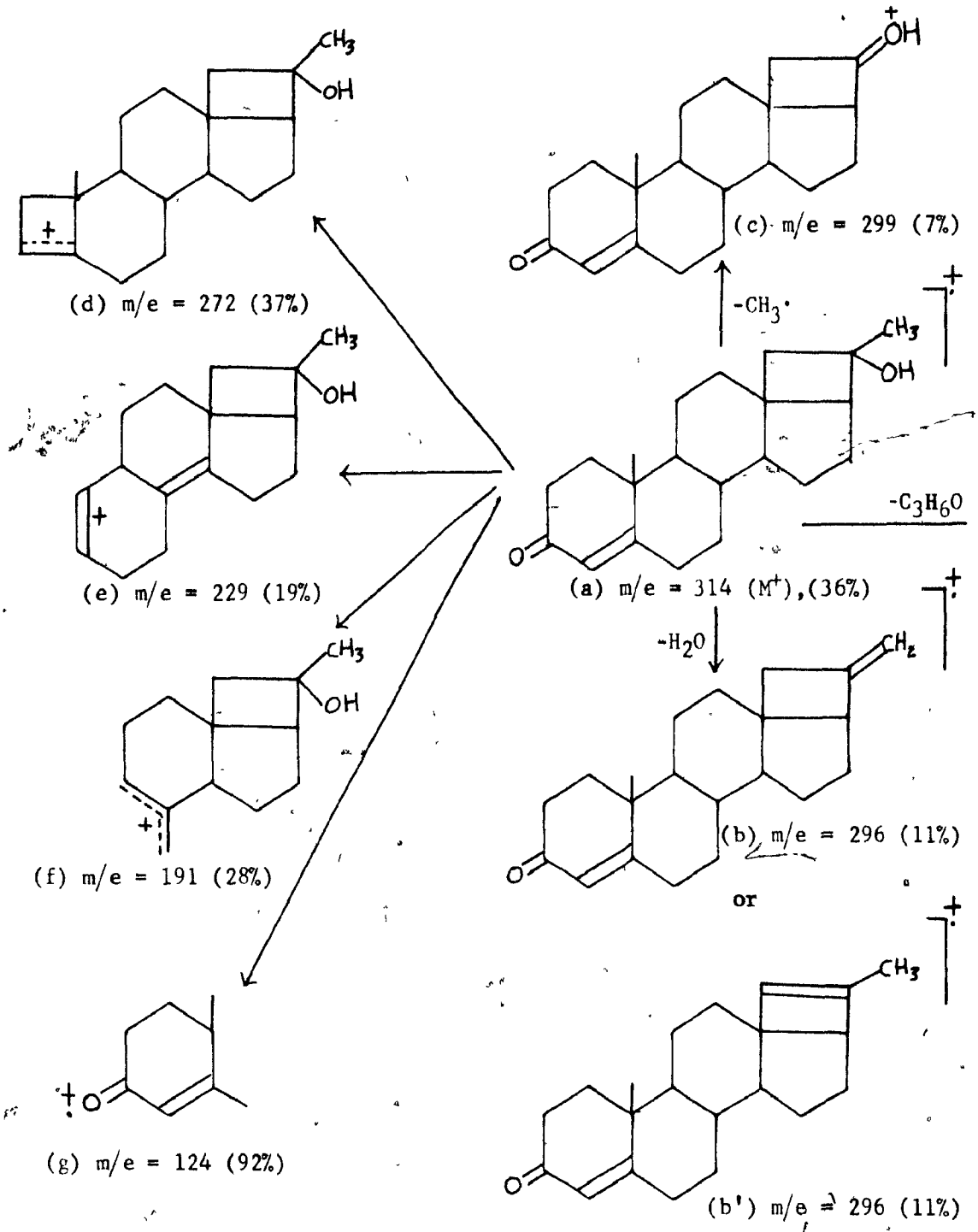
(g') m/e = 124

A summar of the major fragmentation pathways of IV is shown in Scheme 4.

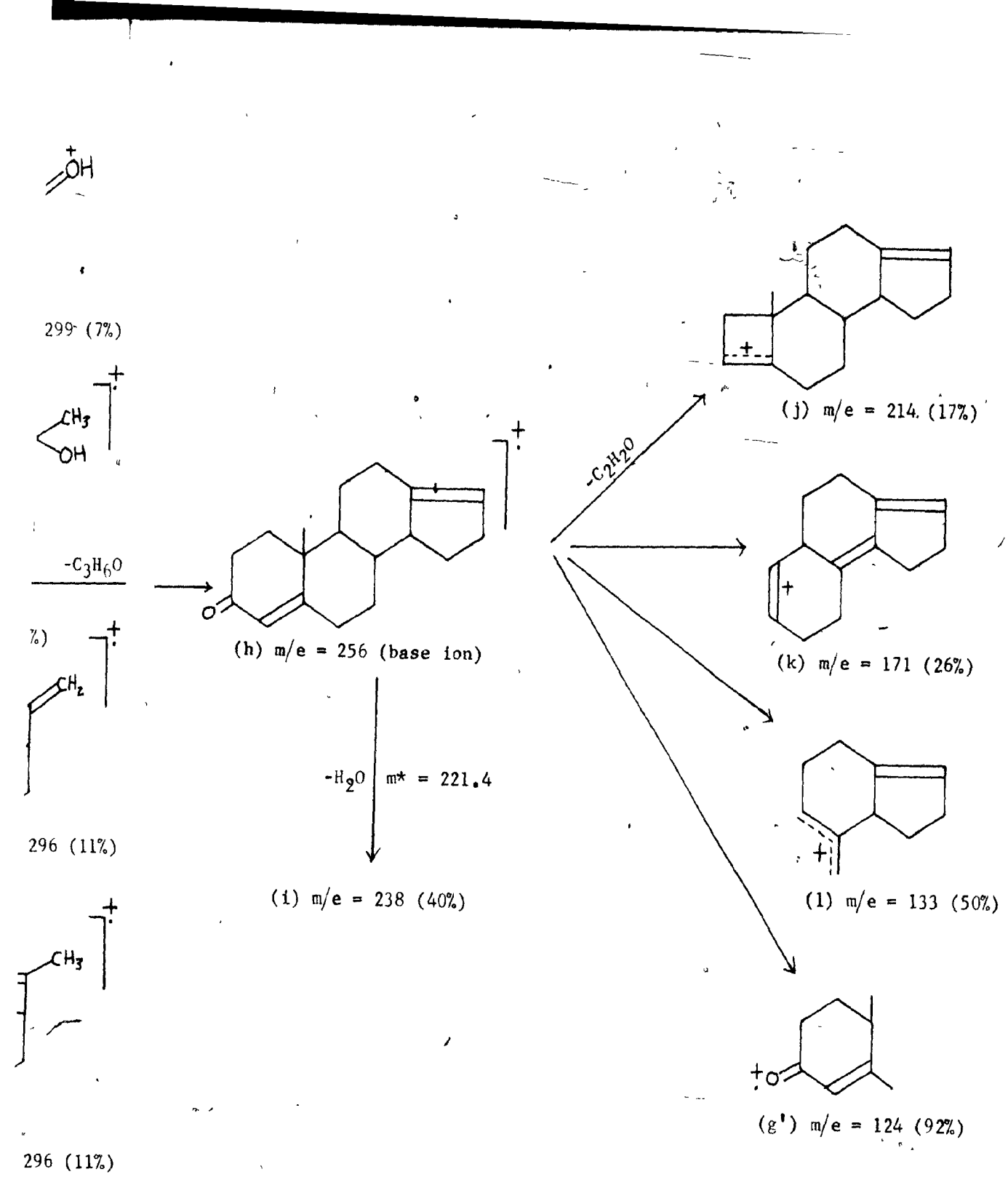
Fig. 4: Mass spectrum of 18,20-cyclo-20-hydroxy-Δ^4-pregnen-3-one (IV)




Relative Intensity (100%)
100
80
60
40
20
0
124
40
50
60
70
80
90
100
110
120
125
m/e



Scheme 4 Major Fragmentations in the Mass Spectrum of 18,20-Cyclo-20-hydroxy-Δ^4-pregnen-3-one (IV)


299 (7%)
$-C_3H_6O$
%)
296 (11%)
296 (11%)
(h) m/e = 256 (base ion)
$-H_2O$ m* = 221.4
(i) m/e = 238 (40%)
$-C_2H_2O$
(j) m/e = 214. (17%)
(k) m/e = 171 (26%)
(l) m/e = 133 (50%)
(g') m/e = 124 (92%)

7. Mass spectrum of 18,20-cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (V; Fig. 5, Scheme 5):

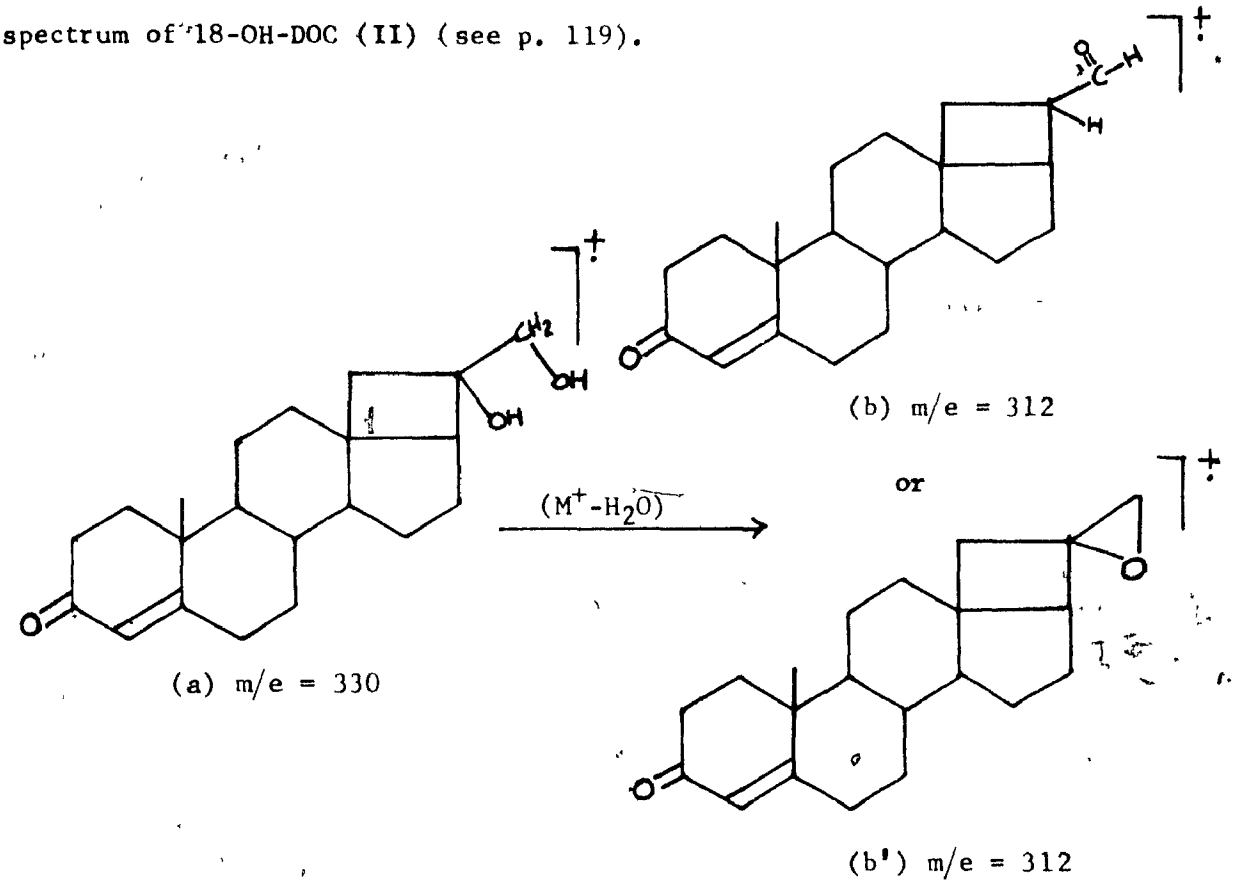
The mass spectrum of V indicates a fragmentation pattern very similar to that of IV (Fig. 4, Scheme 4).

Fragment ions m/e = 330.2154 (a, 36%), (M^+)

m/e = 312 (b or b', 9%), (a→b or a→b')

The spectrum has a peak for the molecular ion at m/e = 330 (a) with a relative intensity of 36%. High resolution mass measurements established the composition of this ion to be $C_{21}H_{30}O_3$ (calcd. 330.2194).

The ion at m/e = 312 (b or b') with a relative intensity of 9% may be considered to arise as a result of the loss of water from the molecular ion (M^+-H_2O) in a fashion analogous to that described for the mass spectrum of 18-OH-DOC (II) (see p. 119).



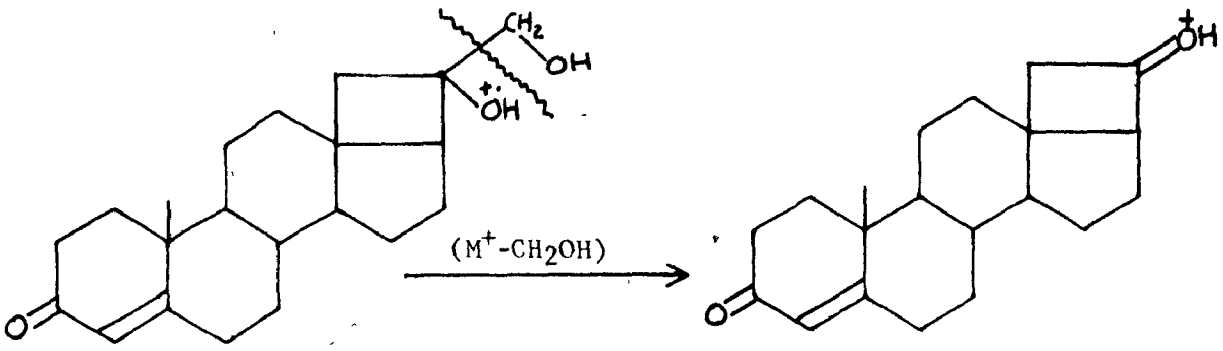
(a) m/e = 330

(b) m/e = 312

(b') m/e = 312

Fragment ion m/e = 299 (c, 9%), (a→c)

The ion at m/e = 299 (c) appears to be formed from the molecular ion at m/e = 330 (a) by loss of 31 mass units, which corresponds to the cleavage of the C_{20}-C_{21} carbon-carbon bond (129, 131) giving the oxonium ion (c).



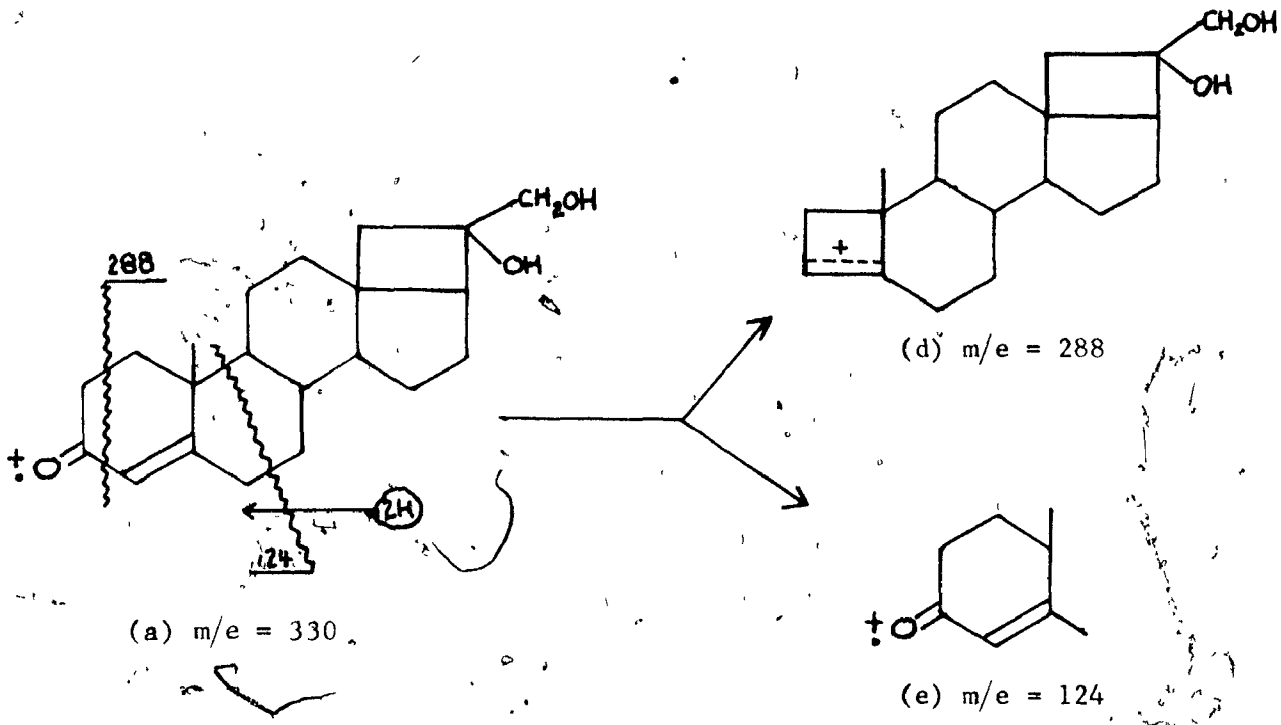
(a) m/e = 330 (c) m/e = 299

A similar fragment was observed in 18-OH-DOC (II) and confirmed by high resolution mass measurements.

Fragment ions m/e = 288 (d, 7%), (a→d)

m/e = 124 (e, 48%), (a→e)

The appearance of peaks at m/e = 288 (d) and m/e = 124 (e) may be indicative of decompositions initiated by the α,β-unsaturated ketone moiety in the A ring of the molecular ion (a), (124, 125).



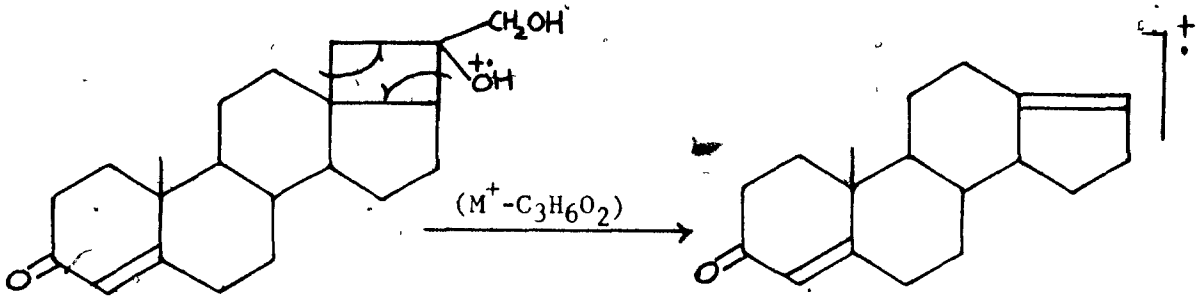
(a) m/e = 330

(d) m/e = 288

(e) m/e = 124

Fragment ion m/e = 256.1834 (f, 100%), (a→f)

The base peak at m/e = 256 (f) may be formed as a result of the loss of a $C_3H_6O_2$ fragment of 74 mass units from the molecular ion (a). High resolution mass measurements established the composition $C_{18}H_{24}O$ (calcd. 256.1827) for the m/e = 256 (f) ion.

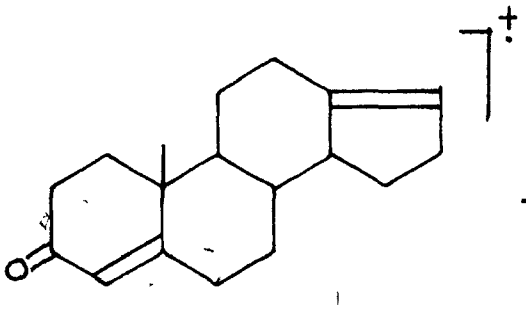


(a) m/e = 330 (f) m/e = 256

The similar mode of fragmentation apparently occurs in the molecular ion of IV (see p. 134).

Fragment ion m/e = 238.1740 (g, 42%), (f→g)

The intense ion at m/e = 238 (g) appears to be formed from the base peak at m/e = 256 (f) by loss of 18 mass units, presumably corresponding to the loss of water (256-H_2O). This transition was confirmed by the appearance of the metastable peak at m* = 221.3 ($221.27 = \frac{(238)^2}{256}$). High resolution mass measurements showed the composition of the m/e = 238 (g) ion to be $C_{18}H_{22}$ (calc. 238.1722). Nevertheless, the mode of its formation is uncertain (see also p. 135).



(f) m/e = 256

$\xrightarrow[m^* = 221.3]{(256-H_2O)}$ (g) m/e = 238

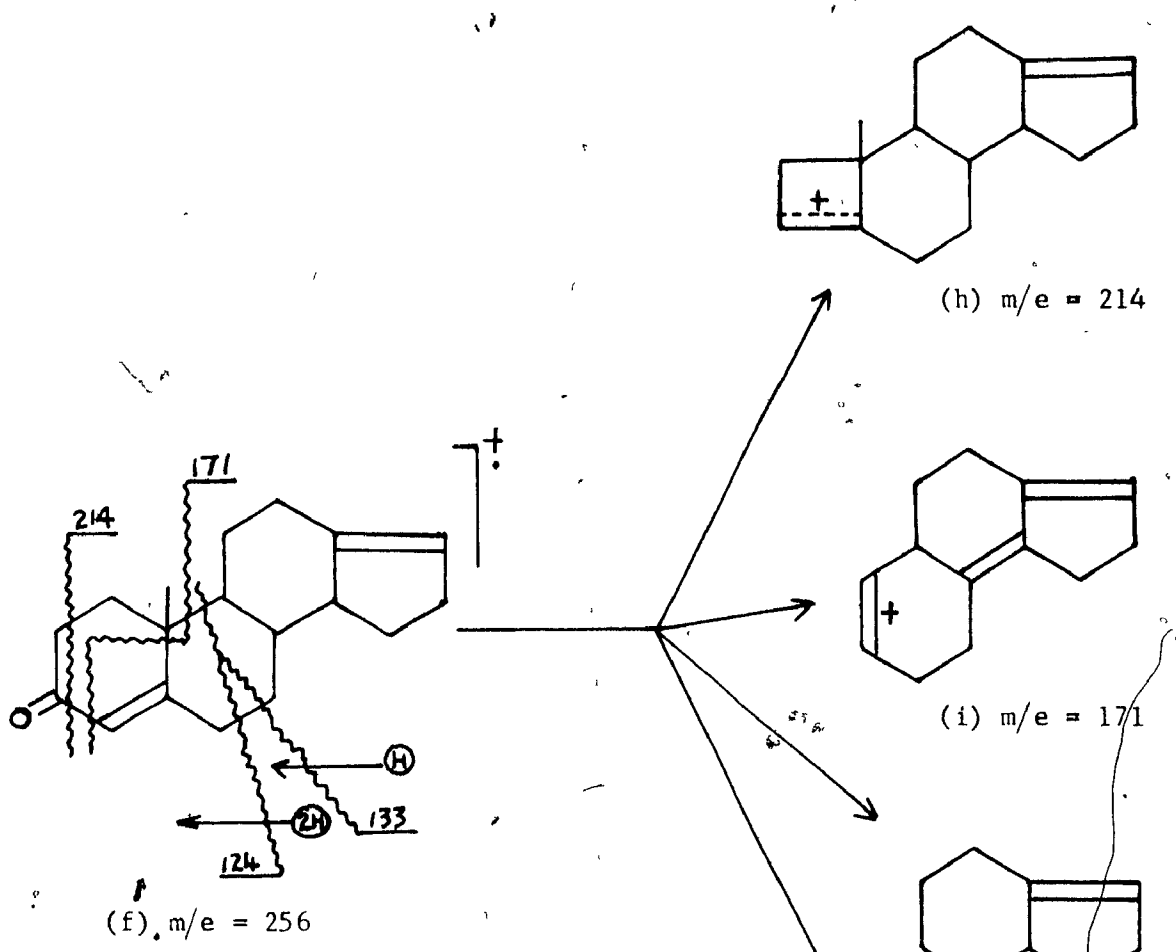
Fragment ions m/e = 214 (h, 17%), (f→h)

m/e = 171 (i, 25%), (f→i)

m/e = 133 (j, 48%), (f→j)

m/e = 124 (e', 48%), (f→e')

The base ion at m/e = 256 (f) appears to undergo further fragmentation giving ions at m/e = 214 (h), 171 (i), 133 (j), and 124 (e'), which correspond to the decompositions initiated by the α, β-unsaturated ketone moiety in the A ring (124, 125). The mode of cleavages is illustrated on the next page.



(f) m/e = 256

(h) m/e = 214

(i) m/e = 171

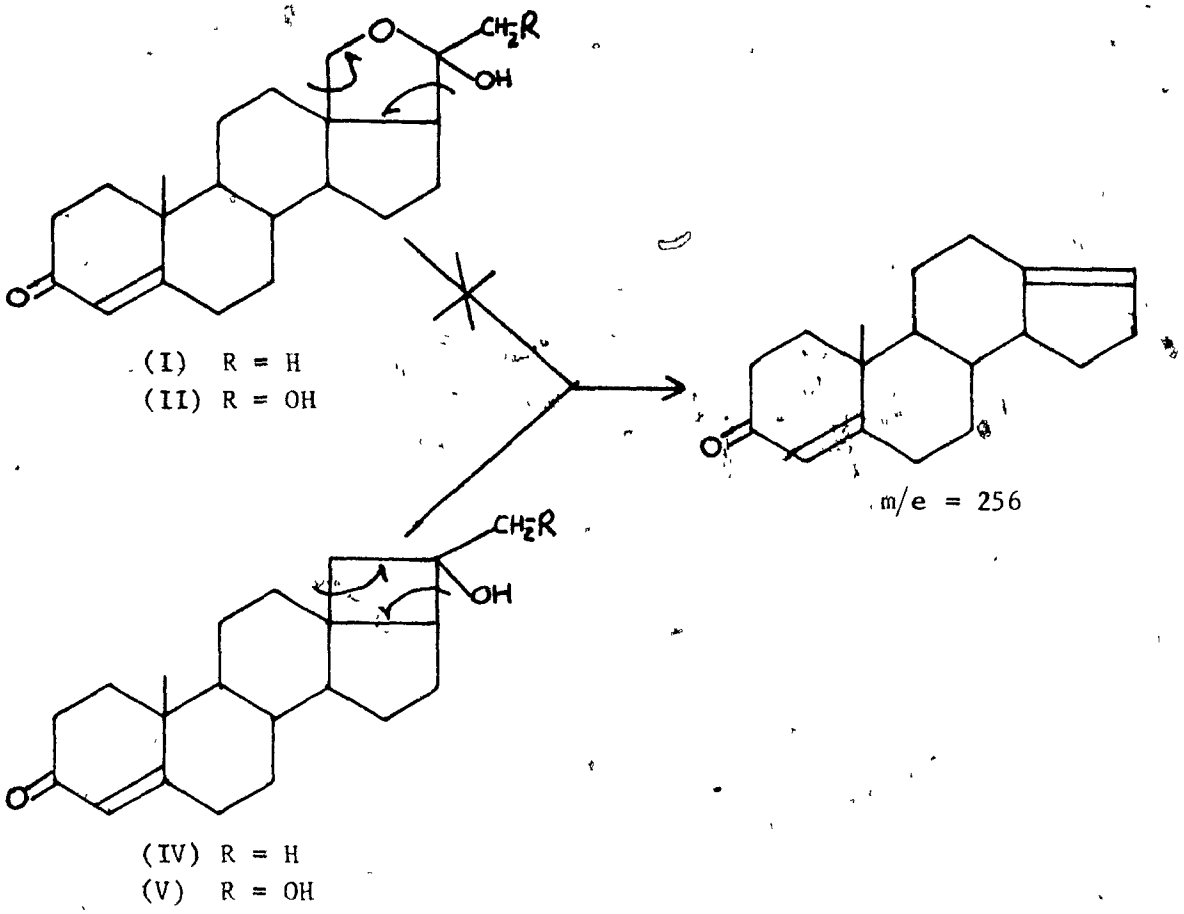
(j) m/e = 133

(e') m/e = 124

Additional comments

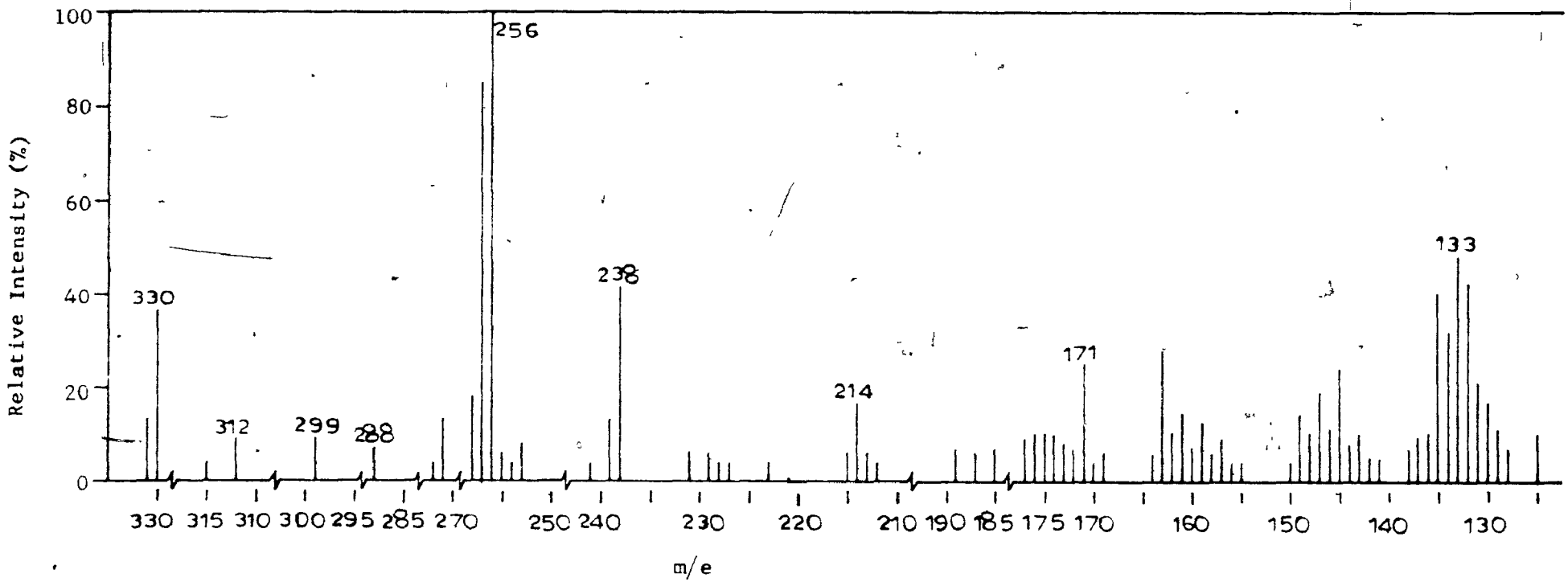
It is noteworthy that the characteristic peak at m/e = 256 of both V and IV is not found in the spectra of 18-OH progesterone (I; Fig. 1) and 18-OH-DOC (II, Fig. 2) despite the fact that the cleavage of the 18,20-

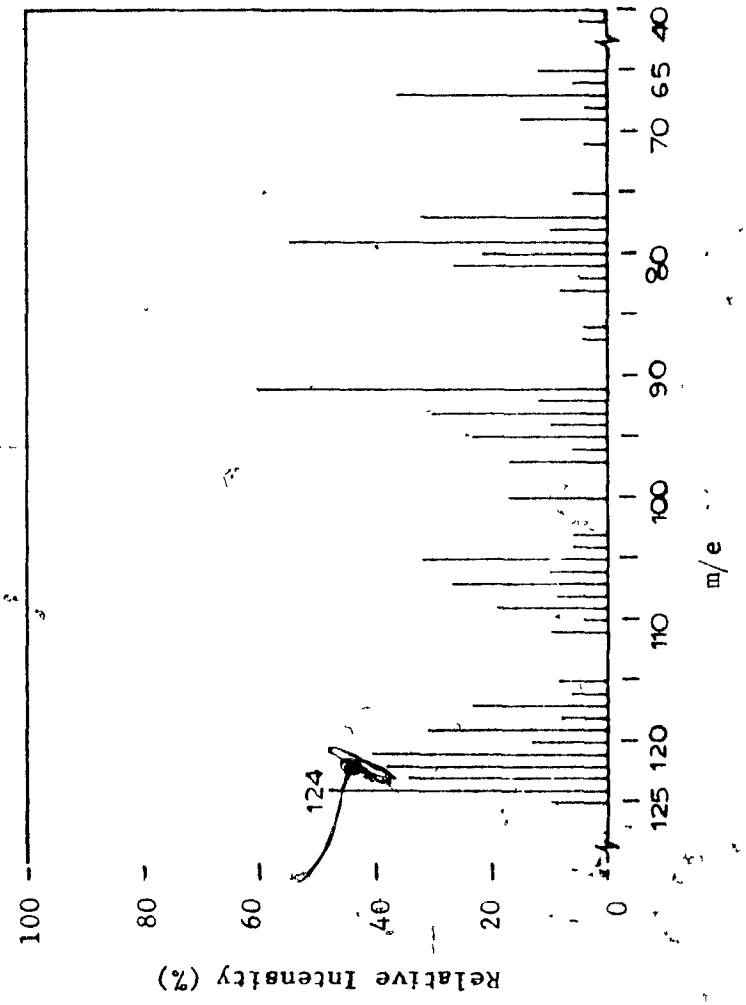
cyclohemiketal moiety of I and II might possibly follow a similar course.

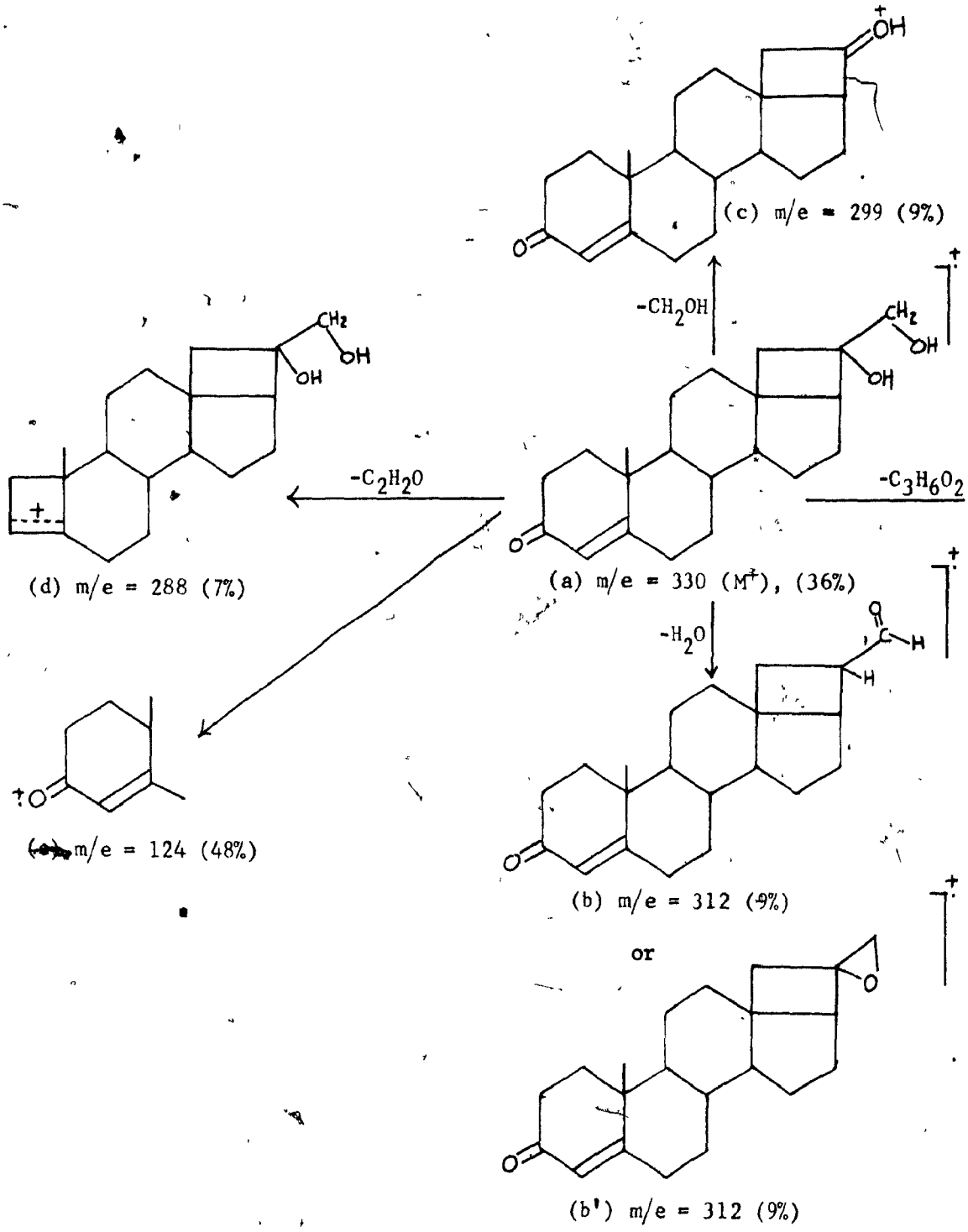


A summary of the major fragmentation pathways of V is shown in Scheme 5.

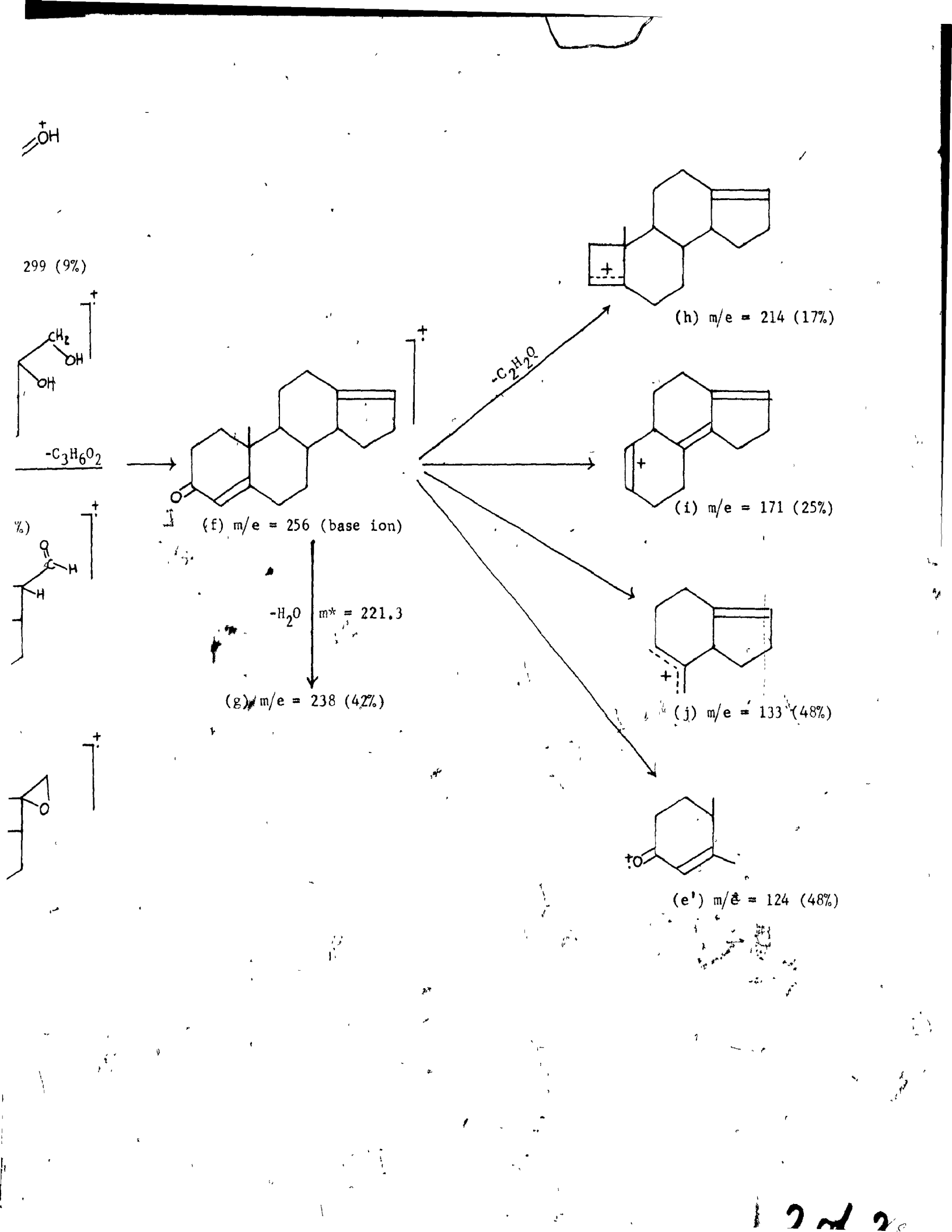
Fig. 5: Mass spectrum of 18,20-cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (V)




Relative Intensity (%)
100
80
60
40
20
0
124
40
65
70
80
90
100
110
120
125
m/e



Scheme 5 Major Fragmentations in the Mass Spectrum of 18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (V)

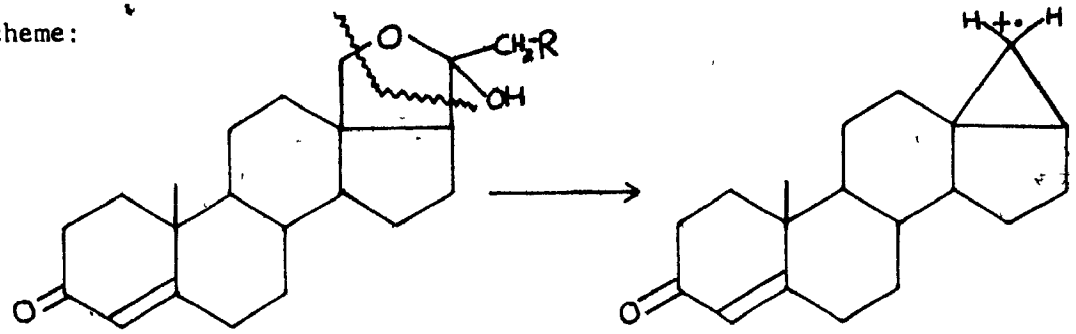

299 (9%)
$-C_3H_6O_2$
%)
(f) m/e = 256 (base ion)
$-H_2O$ m* = 221.3
(g) m/e = 238 (42%)
$-C_2H_2O$
(h) m/e = 214 (17%)
(i) m/e = 171 (25%)
(j) m/e = 133 (48%)
(e') m/e = 124 (48%)

8. Conclusion:

The mass spectra of three 18,20-cyclohemiketal and two 18,20-cyclobutanol steroid derivatives have been recorded and discussed.

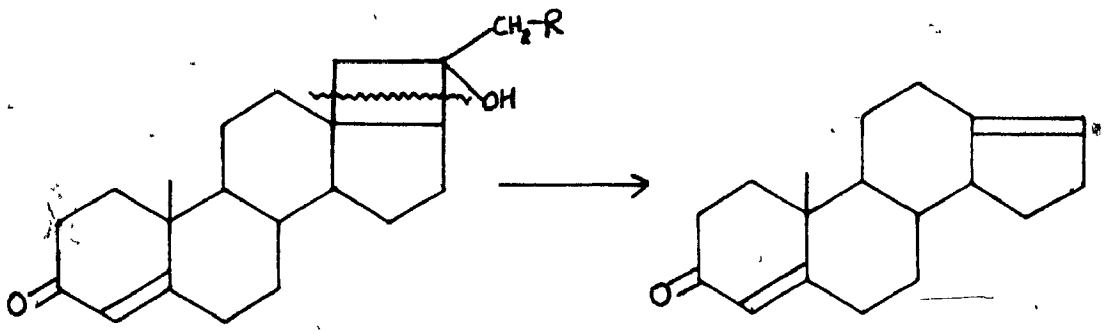
Three main types of fragmentation mode are observed for the compounds studied. 18-OH Progesterone (I) and 18-OH-DOC (II) predominantly give fragmentations initiated by the 18,20-cyclohemiketal grouping. The compounds IV and V show fragmentation patterns initiated by both the 18,20-cyclobutanol and the Δ^4-3-ketone moieties. Compound III, a 3,3-ethylenedioxy derivative of 18-OH progesterone (II), shows prominent cleavages triggered by the ethylene ketal function.

One fragmentation pathway which distinguishes the Type I steroids from the Type II steroids is summed up in the following scheme:



(I) R = H
(II) R = OH.

m/e = 270



(IV) R = H
(V) R = OH

m/e = 256

PART V: EXPERIMENTAL

Preface to Experimental

Melting points - Melting points were determined on a Kofler hot-stage microscope and are uncorrected.

Infrared (I.R.), ultraviolet (U.V.) and nuclear magnetic resonance (N.M.R.) spectra - I.R. spectra were recorded on a Perkin-Elmer Model-257 and/or Model-337 grating infrared spectrophotometer. In reporting these spectra the following abbreviations are used:

vs = very strong

s = strong

m = medium

w = weak

Absorption bands reported in cm^{-1}

U.V. spectra were measured with a Unicam SP-800 spectrophotometer using methanol as the solvent, unless otherwise stated. The N.M.R. spectra in deuterochloroform using tetramethylsilane (TMS) as the external standard were determined on Varian T-60 or A-60 spectrometers. Chemical shifts are reported in τ units relative to TMS. The spectral peaks reported are those of special significance only.

Optical rotations - Unless stated otherwise, samples were determined in chloroform with a Perkin-Elmer Model-141 polarimeter using a 1 dm microcell at the sodium D line.

Mass spectra (M.S.) - Mass spectra were obtained on an AEI MS-902 mass spectrometer by a direct probe method at minimum temperatures (165-200°) necessary to vaporize the sample. The ionizing energy was kept at 70 eV and the ionizing current at 500 μA. High resolution mass measurements were made by the peak-matching method using a perfluorokerosene reference. A molecular ion is indicated as M^+. The metastable ion for a particular process is represented by m*. Percentage relative intensity (% relative abundance) is given in brackets after each m/e value and refers to the intensity of the ion relative to the base peak as 100%. Peaks equal to or greater than 4% of the most intense peak are reported. The fragment ions interpreted are those of special significance only.

Chromatography - If not otherwise stated, thin layer chromatography (TLC) was carried out routinely using neutral aluminum oxide (Woelm Co.). Silica gel refers to SiO_2-G according to Stahl (E. Merck Co.), to SiO_2-7 (Baker Chemical Co.), to SiO_2-film (Eastman Kodak Co.) and to Florisil-TLC (Floridin Co.) and was used for TLC and for preparative layer chromatography (20 x 20 cm glass plate coated with 75-μ thick layer of adsorbent). The TLC plates were viewed under U.V. light and/or sprayed with concentrated sulfuric acid and then heated. Column chromatography was carried out using neutral aluminum oxide (Woelm Co.).

Whenever possible dry ether, benzene and petroleum ether were used for elutions. Paper chromatography was kindly performed by Mrs. H. Traikov at the Allan Memorial Institute of Psychiatry, McGill University. Proportions indicated for solvent mixtures were by volume.

Elemental analysis - The microanalyses were made by Scandanavian Microanalytical Laboratories, Herlev, Denmark, and by Dr. C. Daessle of Organic Microanalyses, Montreal.

Chemicals and reagents - 21-acetoxy pregnenolone and deoxycorticosterone acetate were purchased from Searle Chemicals Inc., Chicago. Lead tetraacetate was dried in a vacuum desicator over potassium hydroxide. Petroleum ether refers to the fraction of boiling point 30-60° and was dried over sodium wire. Phosphorous oxychloride and 2,3-dihydropyrane were redistilled just prior to use. Dry tetrahydrofuran, ether and benzene were obtained by distillation over lithium aluminum hydride or sodium wire. Pyridine and triethylamine were freshly distilled from potassium hydroxide pellets. Solvents were usually removed by rotary evaporation under vacuum at a water-bath temperature of approximately 40°. All compounds reported were purified until no impurities could be detected by TLC analysis.

1. Attempted conversion of 21-acetoxy pregnenolone (I) to 18-OH-DOC acetate (IV) by reaction with lead tetraacetate and iodine (Scheme 1):

(a) Conversion of 21-acetoxy pregnenolone (I) to 3,2'-tetrahydropyranyl-21-acetoxy pregnenolone (II):

To a solution of 21-acetoxy pregnenolone (I, 1.88 g, m.p. 165-170°) in chloroform (15 ml), 2,3-dihydropyran (3 ml) and phosphorous oxychloride (2 drops) were added. The solution was magnetically stirred under anhydrous conditions at ice-water bath temperature for 1.5 h. The mixture was diluted with chloroform (10 ml) and quickly washed with 5% aqueous sodium carbonate (4 ml x 3) followed by water (5 ml x 3). The organic layer was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to give a colorless crystalline product II of m.p. 105°. TLC (SiO_2-G) in benzene-ethyl acetate (5:5) showed that the crude product II was homogeneous (R_f = 0.90) and free of the starting material I (R_f = 0.70). The yield obtained was 2.51 g (100%). Recrystallization twice from petroleum ether raised the melting point to 115-120°.

I.R. ($CHCl_3$) spectrum:

3000 (m, shoulder), 2940 (s),

1750 (s; acetate C=O), 1723 (s; ketone C=O),

1435 (m), 1375 (m), 1228 (s; acetate C-O),

1138 (m), 1116 (m), 1070 (m), 1060 (m),

1030 (m; C-O), 979 (w), 930 (w), 914 (w),

820 (w; C=C-H), 810 cm^{-1} (w).

N.M.R. spectrum:

τ = 4.59 (1H, broad singlet; 6-vinyl proton)

5.32 (2H, AB quartet, J_{AB}=19 c.p.s.; 21-methylene protons)

7.80 (3H, singlet; acetate methyl protons)

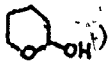
8.32 (multiplet, broad; ring protons)

8.95 (3H, singlet; C-19 methyl protons)

9.27 (3H, singlet; C-18 methyl protons)

Mass spectrum:

The molecular ion (m/e = 458) was not observed.

m/e = 356 (base ion; 458 - ).

(b) Reduction of II to 3,2'-tetrahydropyranyl-21-acetoxy-20-hydroxy-Δ^5-pregnene (III):

To a solution of II (0.783 g, 1.7 mmole) in tetrahydrofuran (8 ml) was added a solution of lithium aluminum tri-t-butoxyhydride (0.894 g, 3.5 mmole) in tetrahydrofuran (5 ml). The mixture was magnetically stirred under nitrogen at ice-water bath temperature. The reaction was completed in 1.5 h, as monitored by TLC. The excess hydride was decomposed by dropwise addition of 5% aqueous acetic acid (5 ml) over a period of 10 min. After addition of water (10 ml), the resulting mixture was repeatedly extracted with chloroform (10 ml x 8). The

combined extracts were washed with water (5 ml x 4). The organic layer was dried over anhydrous sodium sulfate and evaporated to yield a colorless crystalline solid III. TLC (SiO_2-G) in benzene-ethyl acetate (8:2) showed that the product was homogeneous (R_f = 0.40) and free of the starting material II (R_f = 0.70). The yield obtained was 0.77 g (100%). Recrystallization twice from ether gave III with m.p. 125-128°.

I.R. ($CHCl_3$) spectrum:

3680, 3598, 3460 (mw; O-H), 2948 (s), 1735 (s; acetate C=O), 1601 (w; C=C), 1435 (m, broad), 1375 (m), 1220 (m; acetate C=O), 1130 (m), 1113 (m), 1074 (m; C-O), 1020 (m; C-O), 975 (m), 910 (m), 870 cm^{-1} (w; C=C-H).

N.M.R. spectrum:

τ = 4.59 (1H, broad singlet; 6-vinyl proton)

7.63 (1H, singlet; O-H, signal disappeared upon addition of D_2O to the sample)

7.87 (3H, singlet; acetate methyl protons)

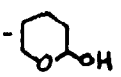
8.32 (multiplet, broad; ring protons)

8.94 (3H, singlet; C-19 methyl protons)

9.17 (3H, singlet; C-18 methyl protons)

Mass spectrum:

The molecular ion (m/e = 460) was not observed.

m/e = 358 (base ion; 460 - ).

(c) Reaction of 3,2'-tetrahydropyranyl-21-acetoxy-20-hydroxy-Δ^5-pregnene (III) with lead tetraacetate and iodine:

Lead tetraacetate (198 mg) and iodine (28 mg, one mole equivalent) were added to a solution of III (51 mg) in benzene (10 ml). The mixture was magnetically stirred and heated to boiling under reflux for 2.5 h. The reaction mixture was cooled to room temperature. The precipitate was removed by filtration and thoroughly washed with benzene (25 ml). The benzene filtrate was washed with 5% aqueous sodium thiosulfate (5 ml x 2) and then with water (5 ml x 4). The organic layer was dried over anhydrous sodium sulfate and evaporated to give a yellow crude residue (54 mg). TLC revealed that the crude residue consisted of a complex mixture of products. Separation by preparative TLC (SiO_2-G) with benzene-ethyl acetate (8:2) gave three major bands, at R_f 0.49, 0.24 and 0.14. Ether extraction gave 32.2, 3.3 and 3 mg of yellowish gum from the respective band.

I.R. ($CHCl_3$) spectra:

The infrared spectra of these three fractions showed similar absorption patterns in the following aspects: the absence of hydroxyl absorption near 3400 - 3600 cm^{-1}, the presence of a strong acetate carbonyl band

at 1738 cm^{-1}, and the occurrence of bands corresponding to C-O stretching vitrations at 1275 (broad), 1125 (m), 1077 (m), and 1020 cm^{-1} (m).

Mass spectra:

Only strong fragment peaks observed in the high mass region are reported.

Fraction of R_f = 0.49 : m/e = 594, 482, 356

Fraction of R_f = 0.24 : m/e = 628, 596, 487, 408

Fraction of R_f = 0.14 : m/e = 596, 486, 408, 356

2. Preparation of 18-OH progesterone and identification of photoproducts:

(a) Preparation of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II), the starting material:

A solution of deoxycorticosterone acetate (I, 15.0 g, 40.3 mmol), ethylene glycol (126.3 g) and pyridine hydrochloride (2.25 g, 0.5 mol equivalent) in dry benzene (650 ml) was refluxed and magnetically stirred for 31 h. The water formed during the reaction was removed by a Dean-Stark moisture trap packed with 10-15 g of anhydrous sodium sulfate. Dry benzene was added at intervals to maintain the starting volume. The process of ketalization was followed by I.R. (complete disappearance of the band at 1668 cm^{-1}), by U.V. (complete disappearance of the absorption maximum at 241 nm), and by TLC (the disappearance of

the starting material I with R_f 0.35 and the formation of the product with R_f 0.55) in benzene-ethyl acetate (8:2). The reaction mixture was cooled to room temperature. The excess ethylene glycol was separated (separatory funnel) and was back-extracted with benzene (20 ml x 3). The combined benzene solutions were washed with 10% sodium carbonate solution (10 ml x 2), water (20 ml x 6), and dried over anhydrous sodium sulfate. The solvent was evaporated to give pure crystalline II (m.p. 185-190°) in 96% yield (15.9 g). Recrystallization twice from hot acetone containing a trace of pyridine gave II with m.p. 194-196° (lit. (107), 206-208° (acetone)), $[\alpha]_D^{25}$ = +76.4° (c = 0.25, $CHCl_3$), (lit. (107), $[\alpha]_D^{20}$ = +42.5 ($CHCl_3$)).

I.R. (KBr) spectrum:

2941 (ms), 1748 (vs, sharp; acetate C=O)

1730 (vs, sharp; ketone C=O), 1427 (mw, doublet),

1385 (s), 1280 (w, shoulder), 1242 (vs, sharp; acetate C-O),

1208 (w, shoulder), 1118 (w), 1097 (s, sharp; ethylene ketal C-O),

1090 (w), 1068 (w), 1054 (w), 1029 (w), 1004 (w), 962 (mw, doublet),

915 (w), 878 (w), 858 (w), 832 (w), 808 (w), 777 (w), 741 (w), 700 cm^{-1} (w).

I.R. ($CHCl_3$) spectrum:

2895 (ms), 1757 (s; acetate C=O), 1733 (s; ketone C=O),

1378 (ms), 1095 (s; ethylene ketal C-O), 948 cm^{-1} (mw).

U.V. spectrum:

transparent above 210 nm

N.M.R. spectrum:

τ = 4.61 (1H, broad; vinyl proton)

5.25 (2H, AB quartet, J_{AB}=20 c.p.s.; 21-methylene protons)

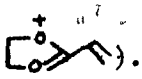
5.92 (4H, singlet; ethylene ketal protons)

7.72 (3H, singlet; acetate methyl protons)

8.84 (3H, singlet; C-19 methyl protons)

9.19 (3H, singlet; C-18 methyl protons)

Mass spectrum:

m/e = 416 (M^+), m/e = 99 (base ion; ).

(b) General procedure for photolysis:

An apparatus similar to that previously described by Jeger et al. (103) was used. The light source was a Hanovia 450-Watt high-pressure mercury lamp (Model 679-A-36) and was fitted with a Corex filter cylinder (1 mm thick; Hanova Model 513-27-114). The lamp was placed in a water-cooled quartz immersion apparatus which was submerged in the irradiation solution. The sample in the appropriate solvent solution was placed in a three-necked flask. The solution was flushed with dry nitrogen for 30 min prior to irradiation and during the irradiation the solution was agitated with a magnetic stirrer and a slow nitrogen flush. The irradiation was performed at room temperature for 4 h. The solvent of the irradiated material was removed under reduced pressure. The residue was dried under vacuum at room temperature for 2-3 h.

Absolute ether was prepared by distillation of anhydrous grade ether over lithium aluminum hydride just prior to use. Absolute ethanol was prepared by distillation of anhydrous grade ethanol over calcium oxide lumps (furnace dried) one day prior to use (L.J. Fieser, Experiments in Organic Chemistry, D.C. Heath Co., Boston, p. 285).

(c) Photolysis of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II) in absolute ether:

A solution of II (1.084 g) in absolute ether (450 ml) was photolyzed. The crude residue (photolyzate) was obtained as a colorless thick syrup (1.144 g). The photolyzate showed a complex mixture of products by TLC (Silica Gel G) in benzene-ethyl acetate (8:2), but no starting material II (R_f = 0.38). It also exhibied no absorption for an α,β-unsaturated ketone chromophore in the I.R. (1668 cm^{-1}) and U.V. (λ max = 240 nm) spectra.

(i) Treatment of the photolyzate with osmium tetroxide To an aliquot of 95 mg of photolyzate (freshly irradiated) in dry benzene (4 ml) containing dry pyridine (1 ml), osmium tetroxide (1.1 mol equivalent, 76 mg) was added. The solution was stirred under anhydrous conditions (calcium chloride drying tube) at room temperature for 12 h. The reaction mixture was treated with a solution of sodium bisulfite (190 mg) in water (3 ml) and pyridine (2 ml) and the resulting mixture stirred

at room temperature for 5 h. The brownish mixture was extracted with chloroform (15 ml x 7), washed with water (8 ml x 3), dried over anhydrous sodium sulfate, and evaporated. The brown gummy residue (111 mg) was treated with a mixture of acetic acid (5 ml), methanol (3 ml), and water (0.5 ml). The solution was heated with stirring at 60° for 5 h. The cooled reaction mixture was neutralized with saturated sodium carbonate solution, extracted with chloroform (15 ml x 4), washed with water (10 ml x 5), dried over anhydrous sodium sulfate, and the chloroform was evaporated. The brown, glassy residue (61 mg) was separated by preparative TLC (Silica Gel G) developed in ethyl acetate-benzene (8 : 2). The bands with R_f ca. 0.68, 0.58, 0.41, 0.26, and 0.10 were repeatedly extracted with ether to give 2.2, 6.3, 6.7, 4.4, and 1.7 mg, respectively, of brown gum with the following common physical properties:

Ultraviolet spectra showed an absorption maximum near 240 nm for the α,β-unsaturated ketone moiety.

Infrared spectra in chloroform showed strong absorption near 3500-3400 cm^{-1} (O-H), 1730 cm^{-1} (unconjugated carbonyl), 1660-1680 cm^{-1} (Δ^4-3-ketone), and 1100 cm^{-1} (C-O).

Paper chromatography:

Solvent system: toluene-propylene glycol (4:1)

Filter paper: Whatman No. 42

Running time: 24 h

All compounds migrated at a faster rate than authentic 18-OH-DOC.

Mass spectra:

The fragmentation patterns were not comparable to that of authentic 18-OH-DOC. On the basis of the physical data, these products were not 18-OH-DOC.

(ii) Isolation of photoproducts Photolyzate (1.048 g) was dissolved in a minimum volume of benzene and chromatographed on a column (3/4 inch O.D.) of neutral alumina (36 g, Woelm activity II) made up of petroleum ether-benzene (9:1). Fractions of 50 ml each were collected and the progress of chromatography was followed by TLC (Silica Gel G) in benzene-ethyl acetate (8:2) and by crystallization of each fraction. Seven compounds were isolated from the photolyzate. Fractions 44-54 was identified as III. The remaining compounds were not further investigated but their spectral data are recorded in this section.

Fractions 5-20 (R_f = 0.57)

Eultion with petroleum ether-benzene (9:1) gave 119 mg (11% by weight) of colorless gum.

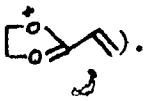
I.R. ($CHCl_3$) spectrum:

3680 (w; O-H), 2900 (s), 1645 (w), 1604 (w), 1440 (m), 1368 (m, sharp), 1135 (w), 1092 (s; C-O), 1030 (m), 952 (w, sharp), 920 (w), 902 cm^{-1} (w, sharp).

U.V. spectrum:

Inflexion at 240 nm

Mass spectrum:

Major fragment ions were observed at m/e = 328, 316, 314, 300, 272, 257, 99 (base ion; ).

Fractions 21-24 (R_f = 0.52)

Elution with petroleum ether-benzene (9:1) gave 8 mg (0.8% by weight) of colorless gum.

I.R. ($CHCl_3$) spectrum:

3680 (w; O-H), 2930 (s), 1602 (w), 1430 (m, broad), 1370 (m), 1095 (s; C-O), 1030 (w, broad), 950 (w), 928 cm^{-1} (w).

Fractions 25-36 (R_f = 0.46)

Elution with petroleum ether-benzene (9:1), (7:3) gave 65 mg (6% by weight) of colorless gum.

I.R. ($CHCl_3$) spectrum:

3678 (w; O-H), 2930 (s), 1605 (w), 1430 (m, broad), 1375 (m), 1260 (w, broad), 1100 (s, broad; C-O), 1018 (s, broad), 951 (w), 925 (w), 869 cm^{-1} (w).

U.V. spectrum:

Inflexions at 224 and 240 nm.

Fractions 40-41 (R_f = 0.33)

Elution with petroleum ether-benzene (7:3) gave 84 mg (8% by weight) of colorless residue (m.p. 160-165°).

I.R. ($CHCl_3$) spectrum:

3678 (w; O-H), 2930 (s), 1604 (w), 1430 (m, broad), 1375 (m), 1095 (s; C-O), 1040 (w, broad), 954 (w), 928 (w), 880 cm^{-1} (w).

U.V. spectrum:

Inflexions at 240 nm

Fractions 44-54 (R_f = 0.28)

3,3-Ethylenedioxy-18,20-epoxy-20-hydroxy-Δ^5-pregnene (III)

Elution with petroleum ether-benzene (2:8) and benzene gave amorphous solid III (m.p. 120-126°) in 23% yield (260 mg). Recrystallization of III from acetone raised the melting point to 137-140° with R_f=0.74 on TLC (Silica Gel G) in benzene-ethyl acetate (5:5).

I.R. ($CHCl_3$) spectrum:

3680, 3590, 3450 (mw; O-H), 2932 (s), 1605 (w; C=C), 1430 (m, broad), 1375 (m, doublet), 1095 (s; C-O), 1026 (w, broad), 951 (w), 925 (w), 892 cm^{-1} (w; C=C-H).

U.V. spectrum:

Transparent above 210 nm

N.M.R. spectrum:

τ = 4.58 (1H, broad; vinyl proton)

5.93 (4H, sharp singlet; ethylene ketal protons)

6.12 (1H, broad; might be an hydroxyl proton)

8.87 (3H, singlet; C-19 methyl protons)

Mass spectrum:

See Part IV, p. 127.

Elemental analysis for $C_{23}H_{34}O_4$:

Found: C, 73.86; H, 9.02

Calcd.: C, 73.76; H, 9.15

Molecular weight for $C_{23}H_{34}O_4$:

Found (mass spectrometry): 374.2457

Calcd.: 374.2457

Fractions 55-58 (R_f = 0.19)

Elution with benzene-ether (5:5) and ether gave a colorless residue (51 mg, 5% by weight) of m.p. 55-65°.

I.R. ($CHCl_3$) spectrum:

3680, 3597, 3440 (mw; O-H), 2930 (s), 1735 (s; unconjugated carbonyl), 1666 (inflexion), 1604 (w), 1430 (m, broad), 1368 (m), 1187 (w, broad), 1090 (s; C-O), 1030 (w), 950 (mw), 928 (mw), 878 cm^{-1} (w).

U.V. spectrum:

Inflexions at 227 and 240 nm

Fractions 59 (R_f = 0.11)

Elution with methanol gave a residue (60 mg, 6% by weight) of m.p. 70-75°.

I.R. ($CHCl_3$) spectrum:

3680, 3593, 3435 (mw; O-H), 2930 (s), 1735 (inflexion), 1668 (inflexion), 1605 (w), 1430 (w, broad), 1370 (w), 1185 (w), 1096 (ms), 1030 (w), 950 (w), 928 (w), 880 cm^{-1} (w).

U.V. spectrum:

High-intensity absorption at 241 nm.

(iii) Hydrolysis of III to 18-OH progesterone (VI) A solution of III (27 mg) in a mixture of methanol (2 ml), acetic acid (2 ml), and water (0.3 ml) was heated under reflux for 10 h. The cooled reaction mixture was neutralized with 2N sodium hydroxide (15 ml), extracted with ether (10 ml x 8), washed with water (5 ml x 4), dried over anhydrous sodium sulfate, and evaporated. The product as colorless gum (22 mg) was purified by preparative TLC (Silica Gel G) in ethyl acetate-benzene (5:5). The band with R_f 0.26 was extracted with ether to give 4 mg (10%) of 18-OH progesterone (VI, m.p. 140°) which had properties (I.R., mass spectrum, and mobility on TLC) identical with those of authentic sample.

(d) Photolysis of 3,3-ethylenedioxy-20-one-21-acetoxy-Δ^5-pregnene (II) in absolute ethanol:

A solution of II (2.01 g) in absolute ethanol (1.85 liter) was photolyzed. The crude residue was obtained as a colorless thick syrup (2.04 g). The photolyzate showed a complex mixture of products by TLC (Woelm neutral alumina) in benzene-ethyl acetate (8:2), but no starting material II (R_f ~ 0.59). It also exhibited no absorption due to an α,β-unsaturated ketone moiety by I.R. (1668 cm^{-1}) and U.V. (λ_{max} = 240 nm) spectroscopy.

(i) Isolation of photoproducts Photolyzate (2.04 g) was dissolved in a minimum volume of benzene and chromatographed on a column (21 mm I.D.) of neutral alumina (50 g, Woelm activity II) made up of petroleum ether-benzene (9:1). Fractions of 100-150 ml each were collected and the progress of chromatography was followed by TLC and by crystallization of each fraction.

Compound IV (Fractions 2-6) :

Epimer A of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy- Δ^5-pregnene

Elution with petroleum ether-benzene (9:1) gave a colorless crystalline solid (368 mg, 18%) with m.p. 140-145°. Repeated recrystallization from benzene-ethanol gave IV m.p. 155-158°, $[\alpha]_D^{25}$ = +24.4° (c=0.39, $CHCl_3$).

I.R. (KBr) spectrum:

2930 (s), 2870 (s), 1428 (m), 1370 (m, doublet), 1308 (m), 1290 (w), 1253 (m, doublet), 1226 (w, sharp), 1210 (w, sharp), 1195 (w, sharp), 1132 (w), 1105 (s; C-O), 1065 (m), 1024 (m), 992 (m), 946 (s), 905 (m), 865 (s, sharp; C=C-H), 820 (m, sharp), 798 (m, sharp), 777 (w, sharp), 732 (w, sharp), 692 cm^{-1} (w, sharp).

I.R. ($CHCl_3$) spectrum:

2858 (s), 1660 (vw), 1430 (m, broad), 1370 (m, doublet), 1315 (w), 1097 (s, broad; C-O), 950 (m), 910 (w), 868 cm^{-1} (w).

U.V. spectrum:

Transparent above 210 nm

Mass spectrum: Major fragment ions were observed at

m/e = 402 (M^+), 387, 372, 358, 357, 356, 99 (base ion)

Elemental analysis for $C_{25}H_{38}O_4$:

Found: C, 74.73; H, 9.52

Calcd.: C, 74.59; H, 9.52

TLC:

R_f = 0.69 in benzene-ethyl acetate (8:2)

0.71 (5:5)

Compound V (Fractions 30-81) :

Epimer B of 3,3-ethylenedioxy-18,20-epoxy-20-ethoxy-Δ^5-pregnene

Further elution with petroleum ether-benzene (75:25), (50:50), (25:75), gave a colorless residue (307 mg, 17%). Repeated recrystallization from acetone-hexane containing a trace of pyridine afforded a crystalline solid V with m.p. 187-193° (lit. (103), m.p. 187-188° (ether-methanol)), $[\alpha]_D^{25}$ = -22.3° (c=0.3, $CHCl_3$) (lit. (103) $[\alpha]_D$ = +1°, $CHCl_3$).

I.R. (KBr) spectrum:

3430 (m; O-H), 2940 (s), 2880 (w, shoulder), 1420 (m, broad), 1361 (m), 1305 (w, doublet), 1263 (m), 1201 (m), 1130 (w, shoulder), 1090 (s; C-O), 1028 (w), 1002 (w), 1030 (w), 950 (w), 930 (w, doublet), 860 (w), 818 (w), 797 (w), 780 (w), 742 (w), 720 (w), 695 cm^{-1} (w).

I.R. ($CHCl_3$) spectrum:

3420 (vw), 2860 (s), 1430 (mw, broad), 1365 (mw), 1090 (s; C-O), 992 (w), 952 cm^{-1} (w).

U.V. spectrum:

Transparent above 210 nm

Mass spectrum: Major fragment ions were observed at

m/e = 402 (M^+), 387, 358, 99 (base ion)

Elemental analysis for $C_{25}H_{38}O_4$:

Found: C, 76.60; H, 9.72

Calcd.: C, 74.59; H, 9.52

TLC:

R_f = 0.31 in benzene-ethyl acetate (8:2)

0.53 (5:5)

Fractions 83-86

Further elution with ether gave a straw colored residue (95 mg, 5% by weight) which was not identified.

(ii) Hydrolysis of IV to 18-OH progesterone (VI) A mixture of IV (8 mg), acetic acid (1 ml) and water (3 ml) was heated at 60° for 15 min. The cooled reaction mixture was taken up in ether (15 ml), neutralized with 0.1 N sodium carbonate solution (10 ml), washed with water (5 ml x 4), dried over anhydrous sodium sulfate, and evaporated. The colorless residue (7 mg, 96%) was homogeneous by TLC. Recrystallization twice from acetone-hexane gave crystalline 18-OH progesterone (VI, m.p. 140-145°). The identity of VI was confirmed by mixed TLC, and comparisons of

the infrared and mass spectra with those of authentic 18-OH progesterone.

(iii) Hydrolysis of V to 18-OH progesterone (VI) A solution of V (415 mg) in dioxane (20 ml) was heated with stirring at 60° for 2 h with 0.5 N hydrochloric acid (8.5 ml). The process of hydrolysis was followed by TLC (the disappearance of the starting material V with R_f 0.53 and the formation of a new spot with R_f 0.21 for 18-OH progesterone) in benzene-ethyl acetate (5:5). The cooled reaction mixture was taken up in ether (60 ml), neutralized with 0.1 N sodium carbonate solution (25 ml), washed with water (10 ml x 4), dried over anhydrous sodium sulfate, and evaporated. The yellowish gummy product (308 mg) was dissolved in a minimum volume of benzene and chromatographed on a column (1.5 cm O.D.) of neutral alumina (12 g, Woelm activity II). Fractions of 25-50 ml each were collected and the progress of chromatography was followed by TLC and by crystallization of each fraction. 18-OH Progesterone (VI) was eluted with benzene-ether (98:2), (90:10), as colorless residue (65 mg, 20%). Recrystallization three times from acetone-hexane gave a crystalline solid VI with m.p. 155-160°. The mobility on TLC, and the infrared and mass spectra of VI were identical with those of authentic 18-OH progesterone.

(e) An improved method for the preparation of 18-OH progesterone:

The original work-up procedure of Jeger et al. (103) was modified with the aim of improving the yield of 18-OH progesterone. The photolyzate, which was obtained from the photolysis of II in abso-

ethanol, was directly subjected to acid hydrolysis followed by treatment of the acid hydrolyzate with alkali. The crude hydrolyzate was separated by column chromatography.

Typical experiment:

A solution of II (2.08-2.09 g), in absolute ethanol (1.85 liter) was photolyzed. The photolyzate (2.10-2.20 g) in aqueous 70% acetic acid (25 ml) was heated with stirring at 80-90° for 4 h under an atmosphere of nitrogen. The hydrolysis was followed by infrared (the appearance of a strong band near 1680 cm^{-1} for the α,β-unsaturated ketone group) and by ultraviolet (the appearance of an absorption maximum near 240 nm) spectroscopy. The solvent was evaporated under reduced pressure by rotary evaporation. For the removal of small amounts of acetic acid, the evaporation was repeated after addition of benzene (15 ml x 15). The acid hydrolyzate (clear reddish syrup) was further dried under vacuum for 2-3 h at room temperature. The product (2.50-2.53 g) was then treated with a mixture of methanol (13 ml), potassium carbonate (1.41 g) and water (13 ml). The resulting solution was stirred under nitrogen at room temperature for 12 h. The process of hydrolysis was followed by infrared spectroscopy (the disappearance of a band near 1740 cm^{-1} for the acetate group). The solvent was removed by azeotropic distillation with benzene (15 ml x 15), as described above. The brown gummy product was taken up in methylene chloride (250 ml), washed with

water (5 ml x 4), dried over anhydrous sodium sulfate, and the solvent evaporated. The residue was further dried under vacuum for 2-3 h at room temperature. The brown glassy product (1.50-1.52 g) was dissolved in a minimum volume of benzene and chromatographed over a column (2.5 cm I.D.) of neutral alumina (60 g, Woelm activity I) made up of petroleum ether. Fractions of 100-150 ml each were collected and the progress of chromatography was followed by TLC and by crystallization of each fraction.

(i) Isolation of photoproducts (also see Table 4, p. 71).

18-OH Progesterone (18,20-epoxy-20-hydroxy-Δ^4-pregnen-3-one) (VI)

Elution with benzene-ether (85:15) afforded a colorless crystalline solid VI of m.p. 145°. The product weighed 0.793 g (24% on the basis of 4.20 g II). Recrystallization twice from acetone-hexane gave VI, m.p. 150-154°, $[\alpha]_D^{25}$ = +152.8° (c=0.53, $CHCl_3$) (lit. (103), $[\alpha]_D$ = +159°).

I.R.(KBr) spectrum:

3420 (s; O-H), 2932 (s), 2878 (s, shoulder), 1665 (s; C=O for Δ^4-3-ketone), 1620 (m; C=C), 1437 (m, triplet), 1381 (m), 1366 (w), 1339 (mw), 1285 (m, doublet), 1240 (m, doublet), 1208 (m), 1189 (m), 1172 (w), 1121 (s; C-O), 1095 (w), 1067 (m), 1043 (s; C-O), 960 (w), 938 (m, sharp), 900 (m, sharp), 888 (w, shoulder), 870 (m; C=C-H), 860 (ms; C=C-H), 835 (w), 790 (w), 778 (w), 750 (w), 694 cm^{-1} (w).

I.R. ($CHCl_3$) spectrum:

3658 (w; O-H), 3578 (m, sharp; O-H), 3420 (w, broad; O-H), 2930 (s), 2860 (w, shoulder), 1660 (s, C=O for Δ^4-3-ketone), 1612 (w, shoulder; C=C), 1425 (m, broad), 1382 (m), 1358 (m), 1328 (m), 1300 (w), 1220 (broad), 1095 (m, doublet; C-O), 1012 (w), 995 (w), 920 (w), 884 cm^{-1} (m; C=C-H).

U.V. spectrum:

λ_{max} = 241 nm (ϵ = 14,980)

Mass spectrum:

See Part IV, p. 110.

Elemental analysis for $C_{21}H_{30}O_3$:

Found: C, 75.85; H, 9.07

Calcd.: C, 76.32; H, 9.15

TLC:

R_f = 0.50 in benzene-ethyl acetate (5·5), (Silica Gel G).

0.40 (Silica Gel Eastman)

0.29 (Alumina Eastman)

The identity of VI was confirmed by mixed TLC, and comparisons of the infrared and mass spectra with those of authentic 18-OH progesterone. The following is a summary of the melting point and specific rotation of 18-OH progesterone as reported in the literature:

Author	Precursor	18-OH Progesterone m.p.	$[\alpha]_D(CHCl_3)$
Pappo(98)	Conessine	173-182° (butanone)	
Buzzetti(93)	Conessine	159-160° (acetone-petroleum ether)	+159°
Lábler(96)	Holarrhimine	164-165° (acetone-hexane)	+157°
Hora(94)	Conessine	169-176° (acetone-hexane)	+157°
Wettstein(100)	Progesterone	154-157° (CH_2Cl_2-ether)	
Goutarel(95)	Conessine	173-177° (ether)	+112°
Jeger(103)	Deoxycorticosterone acetate	178-180° (acetone-hexane)	+159°
present work	Deoxycorticosterone acetate	150-154° (acetone-hexane)	+152.8°

18,20-Cyclo-20-hydroxy-Δ^4-pregnen-3-one (VIII)

Elution with benzene and benzene-ether (90:10), (85:15), gave a colorless crystalline product (26 mg, 2% on the basis of 2.08 g II). Recrystallization twice from acetone-hexane gave VIII with m.p. 195-197° (lit. (108), m.p. 191-192°), $[\alpha]_D^{25}$ = +138.1° (c=0.51, $CHCl_3$) (lit. (108), $[\alpha]_D$ = +130°).

I.R. (KBr) spectrum:

3425 (s; O-H), 2910 (s), 2840 (w, shoulder), 1642 (s; C=O for Δ^4-3-ketone),

1602 (w; C=C), 1440 (s), 1368 (s), 1333 (w, shoulder), 1300 (w), 1273 (s; C-O), 1230 (m, doublet; C-O), 1195 (m, doublet; C-O), 1150 (w), 1121 (m; C-O), 1092 (w), 1077 (w), 1041 (w), 1007 (w), 983 (w), 973 (s), 922 (w), 885 (w), 870 (s; C=C-H), 860 (w, shoulder), 848 (w), 829 (w), 783 (w), 757 (w), 690 cm^{-1} (w).

U.V. spectrum:

λ_{max} = 242 nm (ϵ = 18,000)

Mass spectrum:

See Part IV, p. 131.

Elemental analysis for $C_{21}H_{30}O_2$:

Found: C, 80.24; H, 9.45

Calcd.: C, 80.21; H, 9.62

Molecular weight for $C_{21}H_{30}O_2$:

Found (mass spectrometry): 314.2162

Calcd.: 314.2246

TLC:

R_f = 0.29 in benzene-ethyl acetate (5:5).

18,20-Cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one(IX)

Elution with ether gave a colorless residue (0.573 g, 17% on the basis of 4.20 g II). Recrystallization twice from acetone-hexane gave IX as crystalline solid with m.p. 183-187°, $[\alpha]_D^{25}$ = +128.8° (c=0.51, $CHCl_3$).

I.R. (KBr) spectrum:

3410 (s; O-H), 2918 (s), 2841 (w, shoulder), 1650 (s; C=O for Δ^4-3-ketone), 1610 (w; C=C), 1436 (m), 1420 (w, shoulder), 1359 (m), 1330 (w), 1305 (w), 1278 (m; C-O), 1238 (m; C-O), 1221 (w), 1190 (m; C-O), 1146 (w), 1109 (m), 1045 (m; C-O), 1016 (m; C-O), 980 (w), 955 (mw), 920 (w), 870 (m, sharp; C=C-H), 858 (w), 785 (w), 695 cm^{-1} (w).

I.R. ($CHCl_3$) spectrum:

3560 (ms; O-H), 3400 (ms; O-H), 2880 (s), 1660 (s; C=O for Δ^4-3-ketone), 1610 (mw; C=C), 1430 (mw, broad), 1355 (w), 1325(w), 1230 (mw, broad; C-O), 1205 (w), 1005 (mw), 950 (w), 870 cm^{-1} (w).

U.V. spectrum:

λ_{max} = 242 nm (ϵ = 16,940)

Mass spectrum:

See Part IV, p. 139.

Elemental analysis for $C_{21}H_{30}O_3$:

Found: C, 76.20; H, 9.28

Calcd.: C, 76.32; H, 9.15

Molecular weight for $C_{21}H_{30}O_3$:

Found (mass spectrometry): 330.2154

Calcd.: 330.2194

TLC:

R_f = 0.30 in benzene-ethyl acetate (2/8).

17-Nor-13,17-seco-$\Delta^{4,13(18),15}$-androstrien-3-one (X)

Elution with benzene gave a colorless residue (57 mg, 4% on the basis of 2.08 g). Crystallization from acetone-hexane, X had m.p. 103-105° (lit. (108), m.p. 109°), $[\alpha]_D^{25}$ =+102.9(c=0.51, $CHCl_3$) (lit.(108), $[\alpha]_D$ = +110°).

I.R. (KBr) spectrum:

3420 (vw), 3058 (vw), 2920 (s), 1665 (s; C=O for Δ^4-3-ketone), 1637 (w; $\geq C=C\langle^{H}_{H}$), 1610 (w; C=C), 1430 (m, multiplet), 1375 (w), 1353 (w), 1326 (w), 1268 (w), 1238 (w), 1218 (m), 1200 (w), 1184 (w), 1003 (w), 950 (w), 938 (w), 905 (s, doublet; $^{H}\geq C=C\langle^{H}_{H}$), 860 (ms, doublet; $\geq C=C\langle^{H}_{H}$), 840 (w), 780 (w), 750 (w), 679 cm^{-1} (w).

U.V. spectrum:

λ_{max} = 241 nm (ϵ = 16,500)

Mass spectrum: Major fragment ions were observed at m/e = 256 (M^+, base ion), 241 (8%), 238 (16%), 214 (13%), 199 (10%), 171 (10%), 163 (16%), 149 (23%), 148 (21%), 147 (13%), 145 (13%), 134 (18%), 133 (53%), 132 (33%), 131 (19%), 124 (22%), 119 (19%), 117 (23%), 107 (22%), 105 (29%), 93 (24%), 91 (57%), 79 (52%), 77 (34%).

Elemental analysis for $C_{18}H_{24}O$:

Found: C, 84.29; H, 9.53

Calcd.: C, 84.32; H, 9.44

TLC:

R_f = 0.61 in benzene-ethyl acetate (8:2).

(f) 18,20-Epoxy-20-methoxy-Δ^4-pregnen-3-one (VII):

Hydrolysis of the ethanolic photolyzate of II with warm

aqueous acetic acid in the presence of methanol, followed by treatment with aqueous methanolic potassium carbonate and separation of the products by neutral alumina (activity I) column chromatography yielded, in addition to the products already described in the preceding section, the 20-methoxy derivative (VII) upon elution with benzene in 8% yield (127 mg, on the basis of 2.08 g II). Recrystallization twice from acetone-hexane containing a trace of pyridine gave VII with m.p. 130-134°.

I.R. (KBr) spectrum:

3430 (vw), 2918 (s), 1668 (s; C=O for Δ^4-3-ketone), 1602 (m; C=C), 1440 (m), 1370 (m), 1322 (mw), 1260 (m), 1230 (mw), 1167 (mw), 1100 (s; C-O), 1075 (mw), 1030 (m), 1000 (w), 944 (m), 912 (mw), 849 (s; C=C-H), 818 (m), 775 (w), 743 cm^{-1} (w).

U.V. spectrum:

λ_{max} = 240 nm (ϵ = 15,000)

Mass spectrum: Major fragment ions were observed at

m/e = 344 (M^+), 313, 312, 270, 269, 255 (base ion), 214.

TLC

R_f = 0.59 in benzene-ethyl acetate (4:6)

(i) Hydrolysis of VII to 18-OH progesterone (VI) A solution of VII (10 mg) in aqueous 70% acetic acid (3 ml) was heated with stirring at 60° for 1 h. The solvent was removed by azeotropic distillation with benzene (3 ml x 10). The residue was taken up in ether (30 ml), washed

with water (3 ml x 5), dried over anhydrous sodium sulfate, and the solvent was evaporated. A colorless crystalline compound was obtained in yield of 8 mg. TLC in benzene-ethyl acetate (4:6) indicated that the material was homogeneous (R_f = 0.21) and free of the 20-methoxy derivative (VII) which had an R_f value 0.59. Recrystallization twice from acetone-hexane gave VI, m.p. 145-150°. The identity of VI was confirmed by mixed TLC, and comparisons of the infrared and mass spectra with those of authentic 18-OH progesterone.

3. Conversion of 18-OH progesterone to 18-OH-DOC:

(a) Preparation of 18,20-epoxy-$\Delta^{4,20}$-pregnadien-3-one (XI):

All glass-ware was thoroughly cleaned, oven-dried, and stored in a desiccator prior to use. Phosphorous oxychloride, triethylamine, pyridine, and benzene were freshly redistilled.

In a 25 ml three-necked flask fitted with a calcium chloride tube and nitrogen-inlet tube was placed 51 mg of 18-OH progesterone (VI, 0.51 mmol), 14 ml of benzene, and 0.9 ml triethylamine. The flask was capped with a rubber-septon. The solution was stirred at room temperature and 46 mg of phosphorous oxychloride (2 mol equivalent) was added with a micro-syringe through the rubber-septon. The addition was repeated at intervals of one hour until the dehydration reaction was completed. The progress of the reaction was followed by TLC from the disappearance of VI to the formation of a less polar compound XI which could be hydrolyzed back to VI with aqueous 10% acetic acid in

a few minutes at room temperature.

The reaction was interrupted after 3.5 h (a total of 138 mg of phosphorous oxychloride had been added). The reaction mixture was immediately taken up in benzene (250 ml) containing pyridine (1.5 ml), rapidly washed with cold 10% sodium carbonate solution (5 ml x 1), cold water (6 ml x 7), and dried over anhydrous sodium sulfate. The dried benzene solution was then carefully concentrated to a small volume (5 to 10 ml), but never to dryness, with a rotary evaporator under reduced pressure at room temperature. The excess of triethylamine was removed at this stage. The dehydration product XI was homogeneous and free of the starting material VI by TLC analysis:

18-OH progesterone (VI),	enol ether (XI);	benzene-ethyl acetate
R_f = 0.13	R_f = 0.57	(8:2)
R_f = 0.52	R_f = 0.70	(2:8)

It is essential that the enol ether (XI) obtained be osmylated without delay in the next step of synthesis.

(b) Preparation of 18-OH-DOC (XII):

A solution of the enol ether (XI, ca. 50 mg) in benzene (8 ml) was added dropwise with stirring at 0° under nitrogen a solution of osmium tetroxide (1.1 mol equivalent, 45 mg) in benzene (1 ml) containing pyridine (5 drops). The oxidizing reagent was delivered with a syringe through the rubber-septon. The reaction was completed in 1 h as indi-

cated by TLC (the disappearance of XI and the formation of a prominent dark spot on the base line). The mixture was stirred for an additional hour. The brown solution was treated with a solution of sodium sulfite (195 mg) and potassium carbonate (310 mg) in water (2 ml), and the resulting mixture stirred at room temperature for 2 h. The hydrolysis was essentially completed in first hour as indicated by TLC (the appearance of a single spot with the R_f of XII). The dark brown reaction mixture was evaporated at 30-40° under reduced pressure to total dryness with a rotary evaporator. The dark brown residue was taken up in a small volume of water (10 ml) and repeatedly extracted with ether (20 ml x 10). The ether extract was washed with cold water (5 ml x 3) and dried over anhydrous sodium sulfate. A trace of pyridine (1 drop) was added and the ether solution evaporated at 30-40° under reduced pressure to afford colorless crystalline 18-OH-DOC (XII, m.p. 154-158°) in 94% yield (50 mg). The crude product was homogeneous as indicated by TLC: R_f = 0.23 (SiO_2-G), 0.31 (SiO_2-Eastman) in benzene-ethyl acetate (2:8). Recrystallization twice from acetone-hexane containing a trace of pyridine, gave XII, m.p. 165-168°, $[\alpha]_D^{25}$ = +110.3° (c=0.21, $CHCl_3$) (lit. (50), $[\alpha]_D^{28}$ = +121° (aqueous 70% methanol)).

I.R. (KBr) spectrum:

3420 (s; O-H), 2925 (s), 2860 (m), 1660 (s; C=O for Δ^4-3-ketone), 1618 (w; C=C), 1430 (m), 1358 (w), 1330 (w), 1280 (m), 1212 (ms; C-O), 1114 (w), 1060 (s; C-O), 1000 (mw), 955 (w, sharp), 920 (m, sharp), 872 (m, sharp; C=C-H), 830 (w), 703 cm^{-1} (w).

U.V. spectrum:

λ_{max} = 241 nm (ϵ = 15,200)

Mass spectrum:

See part IV, p. 119.

Elemental analysis for $C_{21}H_{30}O_4$:

Found: C, 72.72; H, 8.68

Calcd.: C, 72.80; H, 8.73

Paper chromatography:

R_B = 0.5 and 0.45

solvent system: toluene-propylene glycol (4:1)

filter paper: Whatman No. 42

running time: 24 h

The infrared and mass spectra of XII were identical with those of an authentic sample of 18-OH-DOC obtained from Pappo (97). The two samples showed identical mobility on TLC and paper chromatogram.

Pappo (98) pointed out that the melting point of 18-OH-DOC can vary depending on the solvent of crystallization:

mp, 18-OH-DOC*	solvent of crystallization*
175-180°	acetone-ethanol with a trace of pyridine
191-195°	acetone
186-190°	ethanol with a trace of pyridine
175-176°	ether
168-170°	butanone with a trace of pyridine

* R. Pappo U.S. Patent 2, 911, 404 (1959), (98)

(c) 18-OH-DOC "dimer":

Origin of sample: a high-melting material (ca. 285°, decomposed) was formed upon recrystallization of 18-OH-DOC (XII, m.p. 154-158°) from acetone-hexane. This material has the following physical properties:

Mass spectrum:

The fragmentation pattern resembled that of 18-OH-DOC.

U.V. spectrum:

λ_{max} = 240 nm (ϵ = 18,860)

Molecular weight:

Found (Rast method): 520-645

Calcd.: 656

$[\alpha]_D^{25}$ = +130° (c=0.21, $CHCl_3$)

TLC (SiO_2-Eastman):

Two spots with R_f 0.31 and 0.47 in benzene-ethyl acetate (2:8).

PART VI: SOME BIOLOGICAL ACTIVITIES OF SYNTHETIC 18-OH-DOC

1. A comparison of the effects of 18-OH-DOC, corticosterone, and deoxycorticosterone on sodium, potassium and water excretion in the adrenalectomized rat:

The effect of synthetic 18-OH-DOC, after subcutaneous administration, on sodium, potassium and water excretion, was compared with that of deoxycorticosterone. It was found that 18-OH-DOC caused a dose-dependent retention of sodium, which was significant at all doses tested, namely 5, 20 and 80 µg. By contrast, an effect on potassium excretion occurred only at the highest dose. A comparison of the effects of 18-OH-DOC and deoxycorticosterone on sodium and potassium excretion in the adrenalectomized rat is given in Table 1.

An antidiuretic action of synthetic 18-OH-DOC occurred only at the higher two dose levels namely 20 and 80 µg. At these levels, the ratio of urine retained to sodium retained was higher than that found at all concentrations of deoxycorticosterone (Table 2).

Corticosterone was tested over the same dose range and caused no sodium or water retention, but it increased potassium excretion at 20 and 80 µg. At the lowest dose, 5µg, it significantly increased sodium and urine excretion (Table 3).

These results suggest that the sodium-retaining and anti-

Table 1 - A Comparison of the Effects of 18-OH-DOC and Deoxycorticosterone on Sodium and Potassium Excretion in the Adrenalectomized Rat

Treatment	No. Animals	Dose	Na Excretion		K Excretion		Na/K
			meq.	meq./liter	meq.	meq./liter	
Control	8	-	0.80 ± 0.06	87.5 ± 4.10	0.14 ± 0.004	15.7 ± 0.99	5.71 ± 0.40
18-OH-DOC	8	5µg	0.65 ± 0.03 p<0.05	66.0 ± 4.36 p<0.01	0.15 ± 0.01 n.s.	14.5 ± 0.61 n.s.	4.60 ± 0.37 p<0.1>0.05
	7	20µg	0.59 ± 0.05 p<0.02	74.8 ± 3.72 p<0.05	0.13 ± 0.009 n.s.	17.3 ± 1.21 n.s.	4.47 ± 0.39 p<0.05
	8	80µg	0.55 ± 0.07 p<0.02	68.2 ± 4.85 p<0.01	0.17 ± 0.008 p<0.01	22.4 ± 2.13 p<0.02	3.34 ± 0.53 p<0.01
DOC	8	5µg	0.34 ± 0.04 p<0.01	40.5 ± 4.97 p<0.01	0.16 ± 0.008 p<0.1>0.05	18.9 ± 0.68 p<0.02	2.12 ± 0.21 p<0.01
	8	20µg	0.44 ± 0.04 p<0.01	52.5 ± 5.22 p<0.01	0.18 ± 0.01 p<0.02	21.0 ± 1.29 p<0.01	2.63 ± 0.40 p<0.01
	8	80µg	0.12 ± 0.02 p<0.01	19.6 ± 3.71 p<0.01	0.22 ± 0.02 p<0.01	33.5 ± 2.66 p<0.01	0.61 ± 0.13 p<0.01

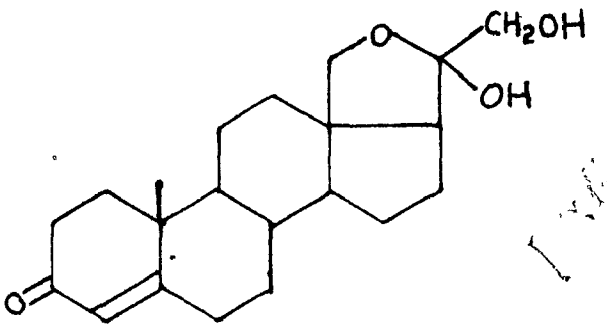
Table 2 - A Comparison of the Effects of 18-OH-DOC and Deoxycorticosterone on Retention of Water and Sodium in the Adrenalectomized Rat

Treatment	Dose	Average Urine Retention(ml)	Average Na Retention meq.	ml Urine Retention / meq. Na Retention
18-OH-DOC	5µg	none	0.15	-
	20µg	1.44	0.21	6.9
	80µg	1.43	0.25	5.7
DOC	5µg	0.85	0.46	1.8
	20µg	0.79	0.36	2.2
	80µg	2.54	0.68	3.7

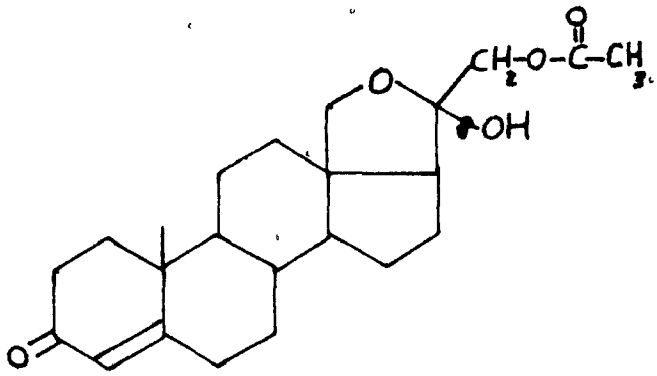
Table 3 - A Comparison of the Effects of Corticosterone and Deoxycorticosterone on Sodium and Potassium Excretion in the Adrenalectomized Rat

Treatment	No. Animals	Dose	Na Excretion		K Excretion		Na/K
			meq.	meq./liter	meq.	meq./liter	
Control	8	-	0.35 ± 0.03	39.2 ± 1.9	0.090 ± 0.008	10.20 ± 0.86	4.06 ± 0.42
Corticosterone	7	5µg	0.48 ± 0.03 p<0.02	47.5 ± 3.8 n.s.	0.090 ± 0.009 n.s.	8.77 ± 0.89 n.s.	5.59 ± 0.67 n.s.
	8	20µg	0.36 ± 0.02 n.s.	36.4 ± 2.4 n.s.	0.122 ± 0.011 0.02<p<0.05	12.51 ± 1.57 n.s.	3.06 ± 0.22 n.s.
	8	80µg	0.40 ± 0.04 n.s.	42.6 ± 3.3 n.s.	0.156 ± 0.009 p<0.01	15.94 ± 1.07 p<0.01	2.76 ± 0.27 p<0.01
DOC	8	5µg	0.300 ± 0.050 n.s.	34.53 ± 5.43 n.s.	0.125 ± 0.011 p<0.02	14.57 ± 1.24 p<0.02	2.35 ± 0.35 p<0.01
	8	20µg	0.103 ± 0.016 p<0.01	12.97 ± 1.94 p<0.01	0.142 ± 0.008 p<0.01	19.04 ± 2.78 p<0.01	0.74 ± 0.12 p<0.01
	8	80µg	0.131 ± 0.025 p<0.01	17.07 ± 2.63 p<0.01	0.150 ± 0.014 p<0.01	20.08 ± 0.82 p<0.01	0.87 ± 0.15 p<0.01
Control	10	-	0.42 ± 0.04	44.61 ± 2.92	0.10 ± 0.004	11.69 ± 1.28	4.07 ± 0.30
Corticosterone	10	80µg	0.44 ± 0.04 n.s.	39.53 ± 2.78 n.s.	0.15 ± 0.009 p<0.01	13.66 ± 1.22 n.s.	3.13 ± 0.38 n.s.
DOC	10	80µg	0.19 ± 0.03 p<0.01	20.39 ± 1.96 p<0.01	0.19 ± 0.013 p<0.01	21.08 ± 1.15 p<0.01	0.99 ± 0.12 p<0.01

diuretic activity of 18-OH-DOC is lower than that of 18-OH-DOC-21-acetate reported by Birmingham, MacDonald, and Rochefort (69). This difference could denote an increased lability of the parent alcohol, 18-OH-DOC, compared to the monoacetate.



18-OH-DOC



18-OH-DOC-21-acetate

The author wishes to thank Dr. M. Givener of Ayerst, McKenna and Harrison Co. Ltd., Montreal, and Dr. M.K. Birmingham of the Allan Memorial Institute of Psychiatry, McGill University for assaying this compound.

2. In vivo effect of 18-OH-DOC on rat adrenal function:

In the rat, ACTH has been shown to greatly stimulate the secretion of both corticosterone and 18-OH-DOC. Corticosterone is known to suppress ACTH secretion but a similar role for 18-OH-DOC has not been established. The effect of 18-OH-DOC on adrenal function in the stressed rat was therefore studied, and compared with that of

corticosterone and deoxycorticosterone. Male rats, weighing 150 gm, were injected with steroid (1 mg/100 gm) for 3 days and decapitated 4 h after the last injection. Plasma corticosterone was measured by acid fluorescence and adrenocortical activity by the in vitro production of ultraviolet absorbing, fluorescent and Porter-Silber positive material. Corticosterone reduced circulating corticosterone levels by 25 to 70%, and reduced corticosterone levels by 40 to 85%. An increase in plasma corticosterone from 12 to 140% was observed following treatment with 18-OH-DOC. The in vitro secretion of ultraviolet-absorbing, Porter-Silber positive and fluorescent steroids was decreased to 50% of control values by both deoxycorticosterone and corticosterone, whereas an increase in all fractions was observed with 18-OH-DOC. The in vitro response of adrenal tissue to administered ACTH was not impaired following treatment with any of the steroids. These findings indicate that 18-OH-DOC is completely inactive in suppressing ACTH secretion, and may, on the contrary, exert a positive feedback effect.

The author wishes to thank Drs. I. Kraulis, M.K. Birmingham, and Mrs. H. Traikov of the Allan Memorial Institute of Psychiatry, McGill University for carrying out this work, which has appeared in abstract form (134).

3. Antiphlogistic effect of 18-OH-DOC:

18-OH-DOC was found to have the ability to prevent carrageenan-induced edema in the rat paw. Male Sprague Dawley rats, weighing 115-160 gm were fasted for 16 hours, the volume of the hind paw was measured by mercury displacement and the animals were subcutaneously injected with adrenal steroids. One hour later carrageenan was administered by subplantar injection and the volume of the hind paw was determined at hourly intervals for 5 hours. 18-OH-DOC at a dose of 15 mg/kg significantly inhibited paw edema one hour after administration of carrageenan. Corticosterone, 30 mg/kg, was active only at the second hour and the effect persisted until the fifth hour. Deoxycorticosterone, 45 mg/kg, was inactive at all periods tested. These findings suggested that 18-OH-DOC also possesses properties associated with glucocorticoids.

These experiments were conducted in collaboration with Dr. G.J. Possanza, J.T. Oliver, and Y. Langlois of Pharma-Research Canada Ltd., Montreal and Dr. M.K. Birmingham of the Allan Memorial Institute of Psychiatry, McGill University (135).

4. In vitro effect of 18-OH-DOC on aerobic glycolysis by intact mouse adrenal glands:

Both aerobic glycolysis (measured by lactic acid production)

and steroid production by intact mouse adrenal glands in vitro are stimulated by ACTH and cyclic 3',5'-AMP, as shown by the studies of Bartová, and Birmingham (136). These authors suggested that the two phenomena might be causally related: one such relation might be a steroid-mediated stimulation of the lactic acid output.

Lactic acid production by intact mouse adrenals in vitro in the presence of ACTH was greatly increased by corticosterone, 11β-hydroxyprogesterone, deoxycorticosterone and progesterone but not by 18-OH progesterone or 18-OH-DOC (137). Steroids not converted to corticosterone by the gland were inactive. The lactic acid production was significantly correlated with the corticosterone content of the gland in the adrenals incubated with steroids. Bartová, Tibagong, and Birmingham (137) suggested that ACTH-induced glycolysis of intact mouse adrenal glands in vitro is mediated to a significant extent, but not exclusively, by the glycolytic action of increased levels of tissue corticosterone.

I with to thank Drs. A. Bartová, M.K. Birmingham and Miss M. Tibagong of the Allan Memorial Institute of Psychiatry, McGill University for testing the action of 18-OH-DOC on adrenal glycolysis.

5. Hypertensive action of 18-OH-DOC:

18-OH-DOC was injected subcutaneously in a daily dose of 200 μg into a group of 10 unilaterally nephrectomized rats maintained

on saline. Other groups received 200 µg deoxycorticosterone acetate (DOCA), 200 µg corticosterone (B) and vehicle only (cotton seed oil). Blood pressure was measured by the tail-cuff method under light ether anesthesia at Pharma-Research Canada Ltd. 18-OH-DOC was effective in raising blood pressure after 2 weeks and was equipotent with DOCA. Corticosterone was without effect:

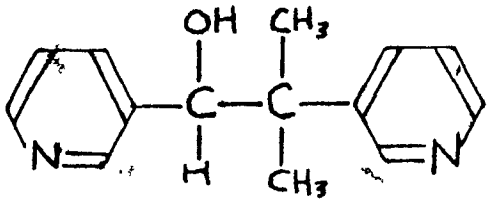
	Day 3	Day 10	Day 16	Day 17
Vehicle	141 + 3	142 + 4	137 + 5	143 + 4
18-OH-DOC	143 + 4	151 + 3	155 + 3**	160 ± 2**
DOCA	134 ± 3	153 + 4	159 + 4*	162 ± 3**
B	145 + 4	140 + 5	140 + 4	148 ± 4

The response was of the same order as that reported by Gross et al. (138) for 250 µg of aldosterone. These findings strengthen the hypothesis that 18-OH-DOC contributes to the etiology of hypertension (139).

The author is indebted to Drs. M.K. Birmingham and A. Bartová of the Allan Memorial Institute of Psychiatry, McGill University, and Drs. J.T. Oliver and P.B. Stewart of Pharma-Research Canada Ltd., Montreal, for conducting the bioassays and for the interpretation of the results.

PART VII: EFFECTS OF REDUCED SU-4885 (Metopirol or 2-methyl-1,2-bis (3'-pyridyl)-1-propanol) ON THE FORMATION OF 18-OH-DOC BY QUARTERED RAT ADRENAL GLANDS

A metabolite of SU-4885 was isolated from the incubation medium of rat adrenal, liver and kidney tissues and identified by the author and collaborators (140) as reduced SU-4885, identical with the Ciba product SU-5236.



Reduced SU-4885

The experiments indicated that the carbonyl function of SU-4885 is reduced to the corresponding hydroxyl group by adrenal and, more actively, by liver and kidney tissue in vitro. ACTH in vitro caused a significant inhibition in the reduction of SU-4885 by both adrenal and liver tissue. The mechanisms involved in the biological reduction of SU-4885 and the inhibition by ACTH of this process remain to be elucidated.

Reduced SU-4885 (prepared in this laboratory by reduction of SU-4885 with sodium borohydride in methanol), was subsequently

shown by Traikov, de Nicola, and Birmingham in 1969 (141) to be an even more selective inhibitor of aldosterone biosynthesis than SU-4885 *in vitro*. When added at a concentration of 2.2×10^{-4}M to medium containing approximately 100 mg of quartered rat adrenal glands, it greatly inhibited the conversion of exogenous DOC-4-^{14}C to aldosterone and 18-OH corticosterone, did not affect the conversion to corticosterone and it caused a three-fold increase in the conversion to 18-OH-DOC.

The author wishes to thank Drs. M.K. Birmingham, I. Kraulis, C.P. Lantos, A.F. de Nicola, and Mrs. H. Traikov of the Allan Memorial Institute of Psychiatry, McGill University, for testing the effects of reduced SU-4885 on the formation of 18-OH-DOC by rat adrenals.

COMMENT

A five-step synthesis of 18-OH-DOC via 18-OH progesterone was accomplished, starting from readily available deoxycorticosterone acetate in 15% overall yield. The synthetic pathway is short. The preparation of 18-OH progesterone from deoxycorticosterone acetate by the photochemical reaction developed by Jeger and improved by us is simple and convenient. The dehydration reaction of 18-OH progesterone and the subsequent hydroxylation of the dehydration product to 18-OH-DOC were performed under mild conditions with quantitative yield. A definite advantage of this method is that 18-OH-DOC is obtained in pure colorless crystalline form upon isolation, obviating the need for extensive purification.

The mass spectra of several steroids with 18,20-cyclohemiketal and 18,20-cyclobutanol structures were analyzed and discussed. Some characteristic features of the fragmentation patterns are recognised and this may prove useful in the identification of naturally occurring 18-hydroxy-18,20-epoxy steroid derivatives.

With respect to the biological actions of 18-OH-DOC, it is of interest that this compound has not only mineralocorticoid action but is also active in tests that are usually associated with glucocorticoid activity. Thus carrageenan-induced edema was reduced, indicating an antiinflammatory action. Furthermore, a significant thymus involution was obtained upon the injection of 18-OH-DOC over a two

Correction - top of page 195 (extra sheet inserted).

week period. Even the short exposure of three days to 18-OH-DOC, in the experiments designed to establish a feedback effect, resulted in some thymus involution, although this was not as marked as that found with corticosterone.

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week period although this was not as marked as that found with corticosterone. Even the short exposure of three days to 18-OH-DOC, in the experiments designed to establish a feedback effect, resulted in some thymus involution.

18-OH-DOC was a potent hypertensive agent suggesting that sodium retention and especially the sodium to potassium ratio is not as reliable a guide with which to gauge hypertensive potency as one might expect. Thus, the hypertensive potencies of 18-OH-DOC, deoxycorticosterone, and aldosterone appeared to be of the same order, at a low dose, whereas vast differences were displayed in the ability of these steroids to alter the urinary sodium to potassium ratio. Perhaps the fact that 18-OH-DOC appeared to exert an antidiuretic action out of proportion to its sodium retaining action and had an anomalous effect on potassium excretion is pertinent to the etiology of hypertension.

The assays which established that 18-OH-DOC is a hypertensive steroid, and has antiinflammatory activity were conducted on rats bearing adrenal glands. It will be of interest to establish whether this compound exerts hypertensive and antiinflammatory potency also in the absence of the adrenal glands. The experiments which indicated that ACTH secretion is not inhibited by 18-OH-DOC but may be enhanced suggest the possibility that some of the biological actions of 18-OH-DOC require an intact pituitary-adrenal axis.

SUMMARY

(1) The literature relevant to the biological activity and the organic synthesis of 18-OH-DOC has been reviewed.

(2) 18-OH Progesterone was prepared according to the method of Jeger. Modification of Jeger's procedure resulted in an improved yield of 18-OH progesterone in 15 to 23% yield. In addition to 18-OH progesterone four other 18,20-cyclohemiketal, two 18,20-cyclobutanol derivatives, and one as photodegradation product were isolated and characterized.

(3) A simple method for the organic synthesis of 18-OH-DOC was established. Deoxycorticosterone acetate was converted into 18-OH progesterone by photochemical reaction. 18-OH Progesterone was dehydrated to the corresponding enol ether, 18,20-epoxy-$\Delta^{4,20}$-pregnadien-3-one in nearly quantitative yield with phosphorous oxychloride in triethylamine. Hydroxylation of the enol ether with osmium tetroxide in the presence of pyridine gave, after hydrolysis of the osmate ester with sodium sulfite in aqueous potassium carbonate, the desired 18-OH-DOC as the 18, 20-cyclohemiketal form of excellent purity in almost quantitative yield. The overall yield based on deoxycorticosterone acetate was about 15%.

(4) The mass spectra of several 18,20-cyclohemiketal and 18,20-cyclobutanol steroid derivatives were determined and discussed.

(5) 18-OH-DOC prepared by organic synthesis was tested for biological action in various in vitro and in vivo systems with the following results:

(a) In the adrenalectomized rat it caused sodium retention

in a dose range from 5 to 80 μg/rat, which was well within the physiological range for this animal. It affected potassium excretion only at the highest dose, 80 μg/rat. The ratio of the retention of water to the retention of sodium was greater than that obtained with deoxycorticosterone. The free compound was not as active as the 21-monoacetate previously assayed, indicating that the free compound may be more liable to destruction when administered parenterally than the acetate.

(b) 18-OH-DOC did not cause an increase in aerobic glycolysis but if anything it had a slight inhibitory effect, as was also the case with aldosterone; it thus differed from the action of corticosterone, deoxycorticosterone and 11β-hydroxyprogesterone in the mouse adrenal in vitro.

(c) 18-OH-DOC had an antiphlogistic action that was anomalous however, in that the peak antiinflammatory effect occurred one hour after injection rather than three hours which is typical for glucocorticoids.

(d) 18-OH-DOC was not able to exert a negative feedback upon the pituitary-adrenal axis of the rat. Rather plasma levels were usually further elevated by the administration of 18-OH-DOC to stressed animals.

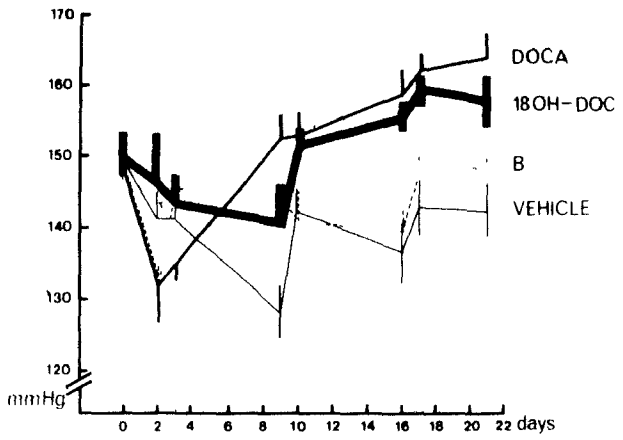
(6) 18-OH-DOC was a potent hypertensive agent equipotent with deoxycorticosterone and, if comparison with other authors' work using the same assay system is justified, equipotent with aldosterone per unit mass.

Corrections:

- the 5th line of pg. 198, should read - per rat for 21 days,
  not per rat for 22 days.

- the 6th line of pg. 198, should read - 140 µg on the 21st day,
  not 140 µg on the 22nd day.

May P.J.C
May 4, 1973



Effects of deoxycorticosterone acetate (DOCA), 18-hydroxy-deoxycorticosterone (18-OH-DOC), and corticosterone (B) on the blood pressure of unilaterally nephrectomized rats, salt-treated rats. DOCA and B were administered daily subcutaneously at a dose of 200 μg per rat for 22 days. The 18-OH-DOC treated animals received daily injections of 200 μg for 21 days and 140 μg on the 22nd day. Control rats received injections of vehicle (cotton seed oil) only. Each group consisted of 10 rats. Blood pressure was determined by the tail cuff method under light ether anesthesia. Vertical bars indicate standard errors.

(7) 2-Methyl-1,2-bis(3'-pyridyl)-1-propanol was prepared from metopirone by organic synthesis and tested for its effect on corticosteroidogenesis. Added to quartered rat adrenals at a concentration of 2.2×10^{-4} M it inhibited the synthesis of aldosterone as effectively as metopirone, and caused a three fold increase in the conversion of deoxycorticosterone to 18-OH-DOC.

CONTRIBUTIONS TO KNOWLEDGE

A simple synthesis of 18-OH-DOC starting from deoxycorticosterone acetate via 18-OH progesterone was accomplished, with an overall yield of about 15%. The synthesis of 18-OH progesterone was performed by modification of a procedure described by Jeger with improved yield. The conversion of 18-OH progesterone to 18-OH-DOC was achieved by a novel, two-step, synthesis, in quantitative yield.

Several steroids with 18,20-cyclohemiketal and 18,20-cyclobutanol structures were isolated and identified, and the fragmentation patterns of their mass spectra were analyzed.

18-OH-DOC prepared by organic synthesis was tested for biological action in various in vitro and in vivo systems with the following results: 18-OH-DOC was found to be a hypertensive agent, equipotent with deoxycorticosterone in the unilaterally nephrectomized rat maintained on saline. In the adrenalectomized rat, 18-OH-DOC caused sodium retention, affected potassium excretion only at a high dose, and caused the retention of water out of proportion to the retention of sodium when compared with the action of deoxycorticosterone. 18-OH-DOC did not exert a negative feedback effect upon the pituitary-adrenal axis of the rat, but instead usually enhanced pituitary-adrenal function. In contrast to corticosterone and precursors of corticosterone, 18-OH-DOC did not increase aerobic glycolysis in mouse adrenal glands but was slightly

inhibitory in this respect. It reduced carrageenan-induced edema of the rat paw.

A derivative of metopirone was synthesized and characterized as 2-methyl-1,2-bis(3'-pyridyl)-1-propanol, and tested for its effect on adrenal steroidogenesis. It caused a three fold enhancement of the biotransformation of deoxycorticosterone to 18-OH-DOC by rat adrenal glands in vitro.

BIBLIOGRAPHY

1. L.J. Soffer, R.I. Dorfman, and J.L. Gabrilove, "The Human Adrenal Gland", Lea and Febiger, Philadelphia (1961), Chapter 2, p. 13.
2. R.O. Greep, and H.W. Deane, Ann. N.Y. Acad. Sci. 50, 596, 1949.
3. H.W. Deane, and A.M. Seligman, Vitamins and Hormones 11, 173, 1953.
4. C.J.P. Giroud, J. Stachenko, and E.H. Venning, Proc. Soc. Exptl. Biol. Med. 92, 154, 1956.
5. J. Stachenko, and C.J.P. Giroud, Endocrinology 64, 730, 1959.
6. J.M. Rogoff, and C.N. Stewart, Science 66, 327, 1927.
7. A.F. Hartman, and K.A. Brownell, Science 72, 76, 1930.
8. H.L. Mason, C.S. Myers, and E.C. Kendall, J. Biol. Chem. 116, 267, 1936.
9. O. Wintersteiner, and J.J. Pfiffner, J. Biol. Chem. 116, 291, 1936.
10. T. Reichstein, Helv. Chim. Acta 19, 1107, 1936.
11. M. Steiger, and T. Reichstein, Helv. Chim. Acta 20, 1164, 1937.
12. T. Reichstein, and J. von Euw, Helv. Chim. Acta 21, 1197, 1938.
13. J.F. Tait, S.A. Simpson, and H.M. Grundy, Lancet 1, 122, 1952.
14. H.M. Grundy, and S.A. Simpson, Nature 169, 795, 1952.
15. S.A. Simpson, J.F. Tait, A. Wettstein, R. Neher, J. von Euw, O. Schindler, and T. Reichstein, Experientia 10, 132, 1954.

16. E. Hefmann, and E. Mosettig, "Biochemistry of Steroids", Reinhold Publishing Corp., New York (1960), Chapter 8, p. 111.

17. M.K. Birmingham, and P.J. Ward, J. Biol. Chem. 236, 1661, 1961.

18. F.G. Péron, Endocrinology 69, 39, 1961.

19. J.C. Melby, T.E. Wilson, and S.L. Dale, J. Clin. Invest. 49, 64-a, 1970.

20. J.C. Melby, S.L. Dale, and T.E. Wilson, Circ. Res. 28 and 29, Suppl. 11, 143, 1971.

21. R.D.H. Heard, "The Hormones" (G. Pincus and K.V. Thimann, eds.), Vol. I, Academic Press Inc. Publishers, New York (1948), Chapter 13.

22. H.C. Harrison, and C.N.H. Long, Endocrinology 26, 309, 1940.

23. H. Selye, "Textbook of Endocrinology", Acta Inc., Montreal (1947).

24. R.C. Haynes, and L.B. Berthet, J. Biol. Chem. 225, 115, 1957.

25. R. Hilf, New Eng. J. Med. 273, 798, 1965.

26. D.G. Grahame-Smith, R.W. Butcher, R.L. Ney, and E.W. Sutherland, J. Biol. Chem. 242, 5535, 1967.

27. M.F. Grower, and E.D. Bransome, Science 168, 483, 1970.

28. R.I. Dorfman, and D.C. Sharma, Steroids 6, 229, 1965.

29. R.I. Dorfman, and F. Ungar, "Metabolism of Steroid Hormones", Academic Press, New York (1965), Chapter 3.

30. S. Solomon, P. Levitan, and S. Lieberman, Rev. Canad. Biol. 15, 282, 1956.

31. S. Solomon, P. Levitan, and S. Lieberman, Proc. Can. Physiol. Soc., Montreal (1956), p. 54.

32. K. Shimizu, M. Hayano, M. Gut, and R.I. Dorfman, J. Biol. Chem. 236, 695, 1961.

33. G. Constantopoulos, and T.T. Tchen, Biochem. Biophys. Res. Comm. 4, 460, 1961.

34. T.T. Tchen, "Functions of the Adrenal Cortex" (K.W. McKerns, ed.), Appleton-Century-Crofts, New York (1968), Vol. I, p. 3.

35. G. Constantopoulos, R.S. Satoh, and T.T. Tchen, Biochem. Biophys. Res. Comm. 8, 50, 1962.

36. C.C. Porter, R.H. Silber, J. Biol. Chem. 185, 201, 1950.

37. J.M. Cortés, F.G. Péron, and R.I. Dorfman, Endocrinology 73, 713, 1963.

38. G.J. Race, and H.M. Wu, Gen. Comp. Endocrinol. 4, 199, 1964.

39. R. Arai, and B. Tamaoki, J. Endocrinol. 39, 453, 1967.

40. F. W. Kahnt, R. Neher, and A. Wettstein, Helv. Chim. Acta 38, 1237, 1955.

41. I. Kraulis, and M.K. Birmingham, Acta Endocrinol. (Kobenhavn) 47, 76, 1964.

42. A.G. Fazekas, and K. Kokai, Europ. J. Steroids 2, 105, 1967.

43. P.B. Raman, R.J. Ertel, and F. Ungar, Endocrinology 74, 865, 1964.

44. A.F. de Nicola, and M.K. Birmingham, Canad. J. Biochem. 48, 85, 1970.

45. P.J. Ward, and M.K. Birmingham, Acta Endocrinol. (Kobenhavn) 39, 110, 1962.

46. P. Vecsei, D. Lommer, and H.P. Wolff, Experientia 24, 1199, 1968.

47. F.G. Péron, Endocrinology 70, 386, 1962.

48. H. Nakano, C. Takemoto, H. Sato, and B. Tamaoki, "Biogenesis and Action of Steroid Hormones" (R.I. Dorfman, K. Yamasaki, and M. Dorfman, eds.), Geron-X, Inc., Los Altos, California (1968), p. 252.

49. H. Levy, D.I. Cargill, C.H. Cha, B. Hood, and J. Carlo, Steroids 5, 131, 1965.

50. O.V. Dominguez, Steroids, Suppl. II, 29, 1965.

51. C.P. Lantos, M.K. Birmingham, and H. Traikov, Acta Physiologica Latino Americana 17, 42, 1967.

52. A. Carballeira, and E.H. Venning, Steroids 4, 329, 1964.

53. P.B. Raman, D.C. Sharma, R.I. Dorfman, and J.L. Gabrilove, Biochemistry 4, 1376, 1965.

54. A.F. de Nicola, and M.K. Birmingham, J. Clin. Endocrinol. Metab. 28, 1380, 1968.

55. A.F. de Nicola, H. Traikov, M.K. Birmingham, J.D. Palmer, and D.W. Ruddick, J. Clin. Endocrinol. Metab. 30, 402, 1970.

56. O.J. Lucis, and R. Lucis, J. Steroid Biochem. 2, 19, 1971.

57. M.K. Birmingham, and E. Kurlents, Endocrinology 62, 47, 1958.

58. M.K. Birmingham, and E. Kurlents, Canad. J. Biochem. Physiol. 37, 510, 1959.

59. P.J. Ward, and M.K. Birmingham, Biochem. J. 76, 269, 1960.

60. F.G. Péron, J. Biol. Chem. 236, 1764, 1961.

61. O.J. Lucis, I. Dyrenfurth, and E.H. Venning, Canad. J. Biochem. Physiol. 39, 901, 1961.

62. H. Sheppard, R. Swenson, and T.F. Mowles, Endocrinology 73, 825, 1963.

63. J. Stachenko, C. Laplante, and C.J.P. Giroud, Canad. J. Biochem. Physiol. 42, 1275, 1964.

64. R.C. Haynes, S.B. Koritz, and F.G. Péron, J. Biol. Chem. 234, 1421, 1959.

65. M.K. Birmingham, E. Kurlents, R. Lane, B. Muhlstock, and H. Traikov, Canad. J. Biochem. Physiol. 38, 1077, 1960.

66. M.K. Birmingham, F.H. Elliott, and P.H.L. Valère, Endocrinology 53, 687, 1953.

67. E. Schonbaum, M.K. Birmingham, and M. Saffran, Canad. J. Biochem. 34, 527, 1956.

68. F.G. Péron, and S.B. Koritz, J. Biol. Chem. 235, 1625, 1960.

69. M.K. Birmingham, M.L. MacDonald, and J.G. Rochefort, "Function of the Adrenal Cortex" (K.W. McKerns, ed.), Vol. 2, Appleton-Century-Crofts, New York (1968), p. 647.

70. J.J. Chart, H. Sheppard, M.J. Allen, W.L. Bencze, and R. Gaunt, Experientia 14, 151, 1958.

71. F.W. Kahnt, and R. Neher, Experientia 18, 499, 1962.

72. I. Kraulis, and M.K. Birmingham, Canad. J. Biochem. 43, 1471, 1965.

73. R.J. Ertel, and F. Ungar, Endocrinology 75, 949, 1964.

74. D.C. Williamson, and V.J. O'Donnell, Canad. J. Biochem. 45, 153, 1967.

75. L.D. Wilson, S.B. Oldham, and B.W. Harding, J. Clin. Endocrinol. 28, 1143, 1968.

76. J. Müller "Regulation of Aldosterone Biosynthesis", Springer-Verlag, New York (1971), pp. 3 and 26.

77. J. Genest, Canad. Med. Assoc. J. 84, 403, 1961.

78. J.H. Laragh, P.J. Cannon, and R.P. Ames, Canad. Med. Assoc. J. 90, 248, 1964.

79. C.C.J. Carpenter, J.O. Davis, and C.R. Ayers, J. Clin. Invest. 40, 2026, 1961.

80. F.R. Skelton, Proc. Soc. Exptl. Biol. Med. 90, 342, 1955.

81. F.R. Skelton, Physiol. Rev. 39, 162, 1959.

82. M.K. Birmingham, G. Rochefort, and H. Traikov, Endocrinology 76, 819, 1965.

83. A.C. Brownie, and F.R. Skelton, Steroids 6, 47, 1965.

84. P. Vecsei, D. Lommer, H.G. Steinacker, A. Vecsei-Görgenyi, and H.P. Wolff, Acta Endocrinol. (Kobenhavn) 53, 24, 1966.

85. A.F. de Nicola, J.T. Oliver, and M.K. Birmingham, Endocrinology 83, 141, 1968.

86. A.F. de Nicola, J.T. Oliver, and M.K. Birmingham, Endocrinology 85, 1205, 1969.

87. C.M. Kagawa, and R. Pappo, Proc. Soc. Exptl. Biol. Med. 109, 982, 1962.

88. A.F. de Nicola, J.T. Oliver, and M.K. Birmingham, Experientia 25, 880, 1969.

89. J.P. Rapp, Endocrinology 86, 668, 1970.

90. J.P. Rapp, and L.K. Dahl, Endocrinology 88, 52, 1971.

91. G.L. Nicolis, and S. Ulick, Endocrinology 76, 514, 1965.

92. E.A. Porter, and J. Kimsey, Endocrinology 89, 353, 1971.

93. F. Buzzetti, W. Wicki, J. Kalvoda, and O. Jeger, Helv. Chim. Acta 42, 388, 1959.

94. J. Hora, and V. Černý, Coll. Czech. Chem. Comm. 26, 2217, 1961.

95. M. Leboeuf, A. Cavé, C. Conreur, K. Lusingchi, and R. Goutarel, Ann. Pharm. Fr. 27, 289, 1969.

96. L. Lábler, and F. Sorm, Coll. Czech. Chem. Comm. 25, 265, 1960.

97. R. Pappo, J. Am. Chem. Soc. 81, 1010, 1959.

98. R. Pappo, U.S. Patents 2, 891, 948, 1959, and 2, 911, 404, 1959.

99. D.H.R. Barton, J.M. Beaton, L.E. Geller, and M.M. Pechet, J. Am. Chem. Soc. 82, 2640, 1960.

100. Ch. Meystre, K. Heusler, J. Kalvoda, P. Wieland, G. Anner, and A. Wettstein, Helv. Chim. Acta, 45, 1317, 1962.

101. D.H.R. Barton, and J.M. Beaton, J. Am. Chem. Soc. 82, 2641, 1960.

102. H. Wehrli, M. Cereghetti, K. Schaffner, and O. Jeger, Helv. Chim. Acta, 43, 367, 1960.

103. O. Jeger, and K. Schaffner, U.S. Patent 3, 131, 180, 1964.

104. J.K. Norymberski, and G.F. Woods, J. Chem. Soc. 3426, 1955.

105. D. Taub, D. Hoffsommer, and N.L. Wender, J. Am. Chem. Soc. 81, 3291, 1959.

106. W.S. Allen, and S. Bernstein, J. Am. Chem. Soc. 77, 1028, 1955.

107. R. Antonucci, S. Bernstein, R. Lenhard, K.J. Sax, and J.H. Williams, J. Org. Chem. 17, 1369, 1952.

108. P. Buchschacher, M. Cereghetti, H. Wehrli, K. Schaffner, and O. Jeger, Helv. Chim. Acta, 42, 2122, 1959.

109. N.C. Yang, and D.H. Yang, Tetrahedron Lett. 10, 1960.

110. L.J. Bellamy, "Advances in Infrared Group Frequencies", Methuen Co., New Fetter Lane, E.C. 4 (1968), p. 24.

111. L. Lábler, and F. Šorm, Coll. Czech. Chem. Comm. 24, 2975, 1959.

112. M.P. Li, C.P. Lantos, H. Traikov, M.K. Birmingham, and T.H. Chan, J. Steroid Biochem. 1, 259, 1970.

113. E.E. van Tamelen, M. Shamma, A.W. Burgstahler, J. Wolinsky, R. Tamm, and P.E. Aldrich, J. Am. Chem. Soc. 91, 7315, 1969.

114. L.F. Fieser, and M. Fieser, "Steroids", Reinhold Publishing Corp., New York, (1959), p. 14.

115. P. Sykes, "A Guidebook to Mechanism in Organic Chemistry", Longmans, Green and Co., London W1, (1961), p. 132.

116. D.H.R. Barton, and D. Elad, J. Chem. Soc., 2085, 1956.

117. R. Criegee, B. Marchand, and H. Wannowius, Ann. Chem. 550, 99, 1942.

118. H. Budzikiewicz, C. Djerassi, and D.H. Williams in "Structure Elucidation of Natural Products by Mass Spectrometry" Vol. II, Holden-Day, Inc., San Francisco (1964), and references cited therein.

119. H. Budzikiewicz, C. Djerassi, and D.H. Williams in "Mass Spectrometry of Organic Compounds", Holden-Day, Inc., San Francisco (1967), and references cited therein.

120. R. Tschesche, I. Morner, and G. Snatzke, Ann. Der Chemie. 670, 103, 1963.

121. D.C. De Jongh, and K. Biemann, J. Am. Chem. Soc. 86, 67, 1964.

122. A.M. Duffield, H. Budzikiewicz, and C. Djerassi, J. Am. Chem. Soc. 87, 2920, 1965.

123. R. Smakman, and Th.J. de Boer, Org. Mass Spectrom. 1, 403, 1968.

124. L. Peterson, Anal. Chem. 34, 1781, 1962.

125. R.H. Shapiro, and C. Djerassi, J. Am. Chem. Soc. 86, 2825, 1964.

126. P. Genard, M. Palem-Vliers, P. Coninx, and M. Margoulies, Steroids 12, 763, 1968.

127. C.E. Burchill, and K.M. Perron, Can. J. Chem. 49, 2382, 1971.

128. A.L. Buley, R.O.C. Norman, and R.J. Pritchett, J. Chem. Soc. B, 849, 1966.

129. J. Kossanyi, J.P. Morizur, B. Furth, J. Wiemann, A.M. Duffield, and C. Djerassi, Org. Mass Spectrom. 1, 777, 1968.

130. P.E. Butler, J. Org. Chem. 29, 3024, 1964.

131. F.A. Long, and J.G. Pritchard, J. Am. Chem. Soc. 78, 2663, 1956.

132. Z. Pelah, D.H. Williams, H. Budzikiewicz, and C. Djerassi, J. Am. Chem. Soc. 86, 3722, 1964.

133. P. Ausloos, and R.E. Rebbert, J. Am. Chem. Soc. 83, 4897, 1961.

134. I. Kraulis, H. Traikov, M. Li, and M.K. Birmingham, Physiology Canada 3, 21, 1972 (abstract); full paper in press with J. Steroid Biochem., March, 1973.

135. G.J. Possanza, M.P. Li, J.T. Oliver, Y. Langlois, and M.K. Birmingham, Fed. Proc., 1973, abstract in press.

136. A. Bartová, and M.K. Birmingham, Endocrinology 88, 845, 1971.

137. A. Bartová, M. Tibagong, and M.K. Birmingham, Endocrinology 89, 1142, 1971.

138. F. Gross, P. Loustalot, and R. Meier, Acta Endocrinol. 26, 417, 1957.

139. M.K. Birmingham, J.T. Oliver, M. Li, T.H. Chan, A. Bartová, and P.B. Stewart, Fed. Proc., 1973, abstract in press.

140. I. Kraulis, H. Traikov, M.P. Li, C.P. Lantos, and M.K. Birmingham, Canad. J. Biochem. 46, 463, 1968.

141. H. Traikov, A.F. de Nicola, and M.K. Birmingham, Steroids 13, 457, 1969.