PHOTOCHEMICAL TRANSFORMATIONS OF 6-FORMYL TESTOSTERONE METHYL ENOLETHER ACETATE, AND ITS SEMICARBAZONE AND OXIME DERIVATIVES

SOME CHEMICAL CONSTITUENTS OF C. PARAPSILOSIS AND C. ALBICANS

by

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Department of Chemistry
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To my parents

Acknowledgments

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Abstract

The Photolysis of 6-formyltestosterone methyl enol ether acetate, and its semicarbazone and oxime derivatives.

Irradiation of 17β -acetoxy-6-formyl-3-methoxy-androsta-3,5-diene in methanol leads to 17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene which is further converted to its acetal. The relative rate constants for these reactions have been determined, thus permitting exclusion of alternate reaction pathways.

Irradiation of syn-semicarbazone enol ether (XXVI) in methanol with 2537 Å light leads to a mixture of syn-ketal (XXVIII) and anti-ketal (XXIX); anti-semicarbazone enol ether (XXVII) is also formed. Irradiation of (XXVI) with 3500 Å light leads very rapidly to an almost quantitative conversion to anti-semicarbazone enol ether (XXVII). It therefore seems that excitation of the long wavelength band (313 mμ) of (XXVI) gives rise to syn – anti isomerization, whereas the short wavelength band (228 mμ) is responsible for ketalization.

Irradiation of syn-oxime enol ether (XXX) leads to syn-oxime ketal (XXXIII), anti-oxime ketal (XXXIV), nitrile enol ether (XXXII) and nitrile ketal (XXXV). The formation of the latter two compounds constitutes, to our knowledge, the first example of a photo-dehydration of

oximes to nitriles. Anti-oxime enol ether (XXXI) could only be detected upon irradiation of XXX with 3500 Å light.

Some chemical constituents of C. parapsilosis and C. albicans.

Thiomethyladenosine was isolated from C. albicans and identified. An amide fraction from C. albicans and a lactone fraction from C. parapsilosis were isolated and studied.

PART I

PHOTOCHEMICAL TRANSFORMATIONS OF 6-FORMYL TESTOSTERONE METHYL ENOL ETHER ACETATE AND ITS SEMICARBAZONE AND OXIME DERIVATIVES.

Introduction

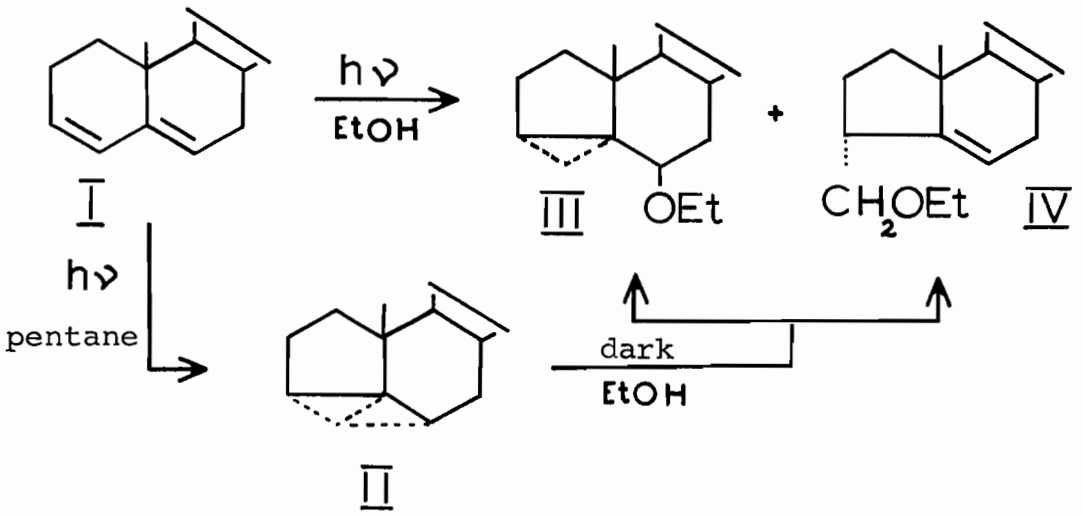
Photochemical transformations have now been known to occur for over half a century since the time when sunlight was first used as a source of irradiation. Many reviews have been published since, covering many aspects of the subject[1-7].

The best known and possibly the most used of the photochemical transformations is that of isomerization about a carbon-carbon double bond[2]. It appears to be the general rule that irradiation favours the less stable isomer in the resulting photoequilibrium[8]. In the transformation of fumaric (trans) acid to maleic (cis) acid, an equilibrium exists in favour of the cis compound[9].

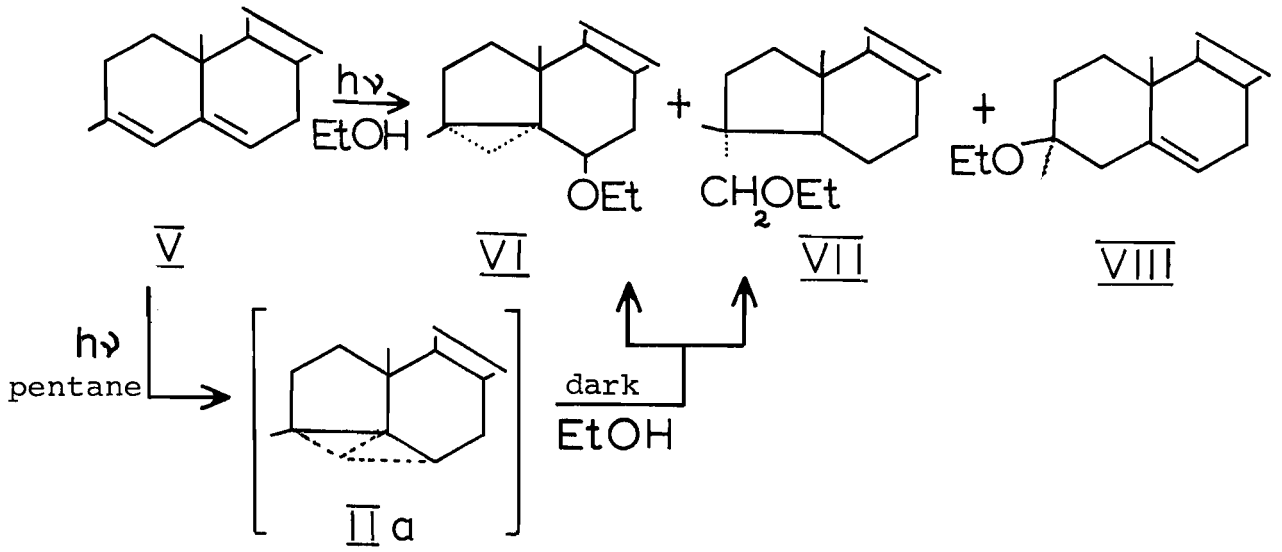
One of the classical examples of the irradiation of a homoannular diene is the photolytic transformation of ergosterol and 7-dehydrocholesterol into the D vitamins[2,3].

The first report on the irradiation of a hetero-annular diene was published in 1959 by Dauben and Ross[10]. The mechanism of the reaction was discussed by Dauben and

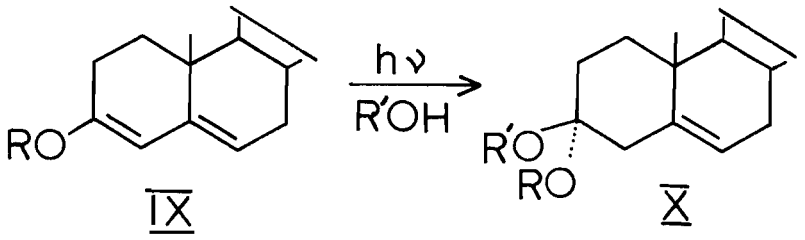
Willey[11]. These authors showed that cholesta-3,5-diene underwent a photochemical transformation in ethanol to ethers III and IV via the bicyclobutane intermediate (II). This intermediate could be isolated when the photolysis was carried out in pentane. Solvolysis of II in ethanol gave III and IV.



When 3-methylcholesta-3,5-diene (V) was irradiated in ethanol[12], VI, VII and VIII were obtained. However, when V was irradiated in pentane followed by a solvolysis reaction in alcohol, compound VIII was not obtained.



When 3-alkoxycholesta-3,5-dienes (IX) were irradiated in alcohol[13], 3,3-dialkoxycholest-5-enes (X) were obtained in good yield. These compounds were



structurally similar to VIII obtained by DiTullio[12]. It was also observed that the alkoxy group entered mainly in the 3β position, whereas protonation at C_4 was non-stereospecific.

At present there seem to be two types of mechanisms for reactions of heteroannular dienes as demonstrated by Bauslaugh[14]. Substituents markedly affect the type of

reaction of a diene. The simplest heteroannular diene, cholesta-3,5-diene, has been found to give products explained solely via a bicyclobutane intermediate derived from a triplet photoexcited state. Substitution of an alkoxy group in the 3-position changes the reaction completely so that the reaction product can probably be explained best by assuming a singlet photoexcited state from which a polar intermediate is formed. In between these two types of reactions lies that of the 3-methylcholesta-3,5-diene; the products obtained can be explained by both mechanisms. When substitution in the 6-position was made with groups such as CH_3 and F, Leznoff[16] found no difference in reaction from the unsubstituted 3-alkoxycholesta-3,5-dienes. When the 6-substituent was a nitro group, photolysis led to an intractable mixture. Thus it seems that substituents which conjugate with the diene have a profound influence on the reaction course.

At this point it should be mentioned that although mechanistic organic photochemistry is developing very rapidly, it is still not possible to state with very much accuracy, the exact nature of the excited species involved.

One of the main objectives of this thesis is to provide some further understanding into the photochemical behaviour of dienes through the irradiation of an enol

ether chromophore conjugated by C=O[17] and C=N[18] functionalities. However before proceeding, mention must be first made of the photochemical behaviour of α,β - unsaturated carbonyl[2,3] compounds.

Saturated carbonyl compounds have been found, in general, to undergo free radical type reactions such as bond cleavage[19], hydrogen abstraction[20], and hydrogen transfer[21]. Conjugated unsaturated carbonyl groups however are quite different, showing geometrical isomerism about the carbon-carbon double bond and polar reactions such as alkyl group migration and dimerization[22]. The products from the latter type of reaction can normally be rationalized through the following Zwitterionic representation in which the β -carbon atom

$$-C{=}C-C{=}O \xrightarrow{h\nu} -\overset{+}{C}-C{=}C-\overset{-}{O}$$

bears a positive charge and the oxygen atom a negative charge[23].

"Photochemical transformations are hypothesized as proceeding by a continuous electron redistribution process of the excited states involved, i.e. chemical changes occur with a minimum electron localization; in other words, the reacting species follow energy valleys and mountain passes but avoid energy maxima[25]."

In carbonyl compounds, n-π^* excitation of the carbonyl group is responsible for the photochemical reaction. Starting with this and the continuous electron redistribution hypothesis, Zimmerman[24] enumerated four steps in the reaction of an α,β-unsaturated ketone:

(1) n-π^* excitation

(2) a continuous electron redistribution process of the n-π^* excited state (bond alteration or rebonding)

(3) π^*-n electron demotion

(4) a continuous electron redistribution process of the species thus formed.

Zimmerman used these 4 steps to explain the photochemical transformation of 4,4-diphenylcyclohexadienone (I) (see scheme I). Step 1 involves the excitation of a non-bonding p_y electron from the oxygen atom, to an antibonding π orbital.+ Step 2 involves a bond alteration between carbons 3 and 5. Step three is an electron demotion (electron transition) from the singly occupied antibonding molecular orbital of XIII, to the low energy, singly occupied oxygen p_y orbital which originally lost the electron in the n-π^* excitation process (in step 1). Step 4 involves a further

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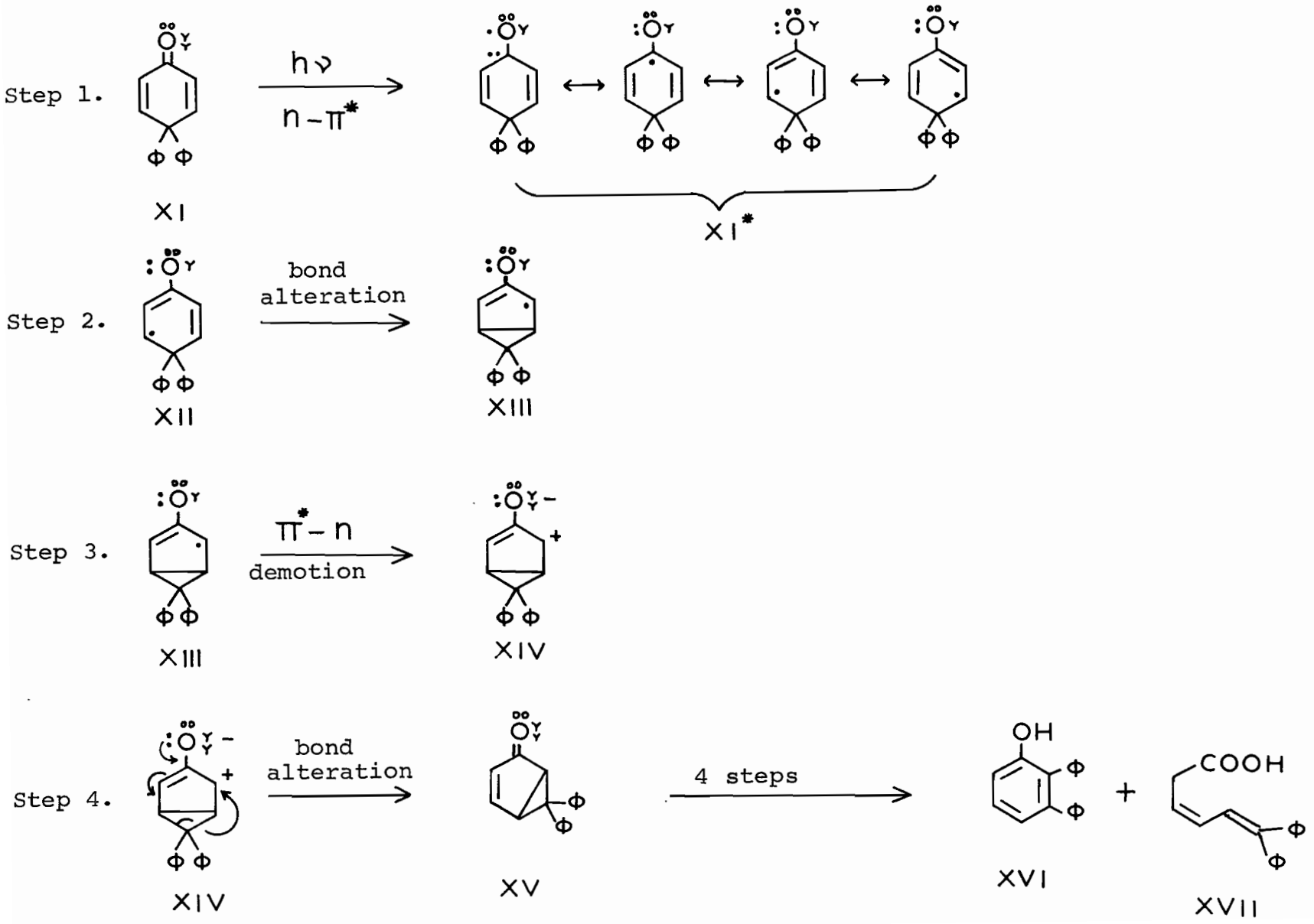
+ A convention has been applied whereby the π electrons are indicated as solid dots • , the heavily s-weighted electrons as circular dots and the p_y electrons as small y's. (See ref. 31).

Scheme I


Step 1.
hν
n-π*
XI
XI*
Step 2.
bond alteration
XII
XIII
Step 3.
π*-n demotion
XIII
XIV
Step 4.
bond alteration
XIV
XV
4 steps
OH
COOH
XVI
XVII

bond alteration of the mesoionic species XIV, leading to the bicyclic ketone XV. The reaction of XV to a 2,3-diphenylphenol can similarly be rationalized through the 4-step sequence.

It was thought an interesting experiment to conjugate an α,β -unsaturated aldehyde with an enol ether chromophore, and to study the effect, if any, of one chromophore on the other. The results are described in this thesis.

Isomerizations about a carbon-nitrogen double bond have been known since 1903, but have not been investigated very extensively[2]. Ciamician and Silber[26,28] used sunlight to convert benzaldoximes to their geometrical isomers. Stoermer[27] found aromatic ketoximes to behave in a similar way. In 1924, when a better source of u.v. light was available, the p-methoxybenzaldoximes and their O-ethers were irradiated[29]. The O-ethers gave nitriles. No geometric isomerism was detected for benzalaniline[30].

As has previously been shown, investigations have been carried out on dienes, substituted dienes, saturated and α,β -unsaturated carbonyl compounds and oximes individually, and their photochemical behaviour described. Part of this thesis involves a study of the photochemical behaviour of a combined chromophore such as an enol ether aldehyde and its semicarbazone and oxime derivatives.

Fig. 1

The nuclear magnetic resonance spectra of

A. 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX).

B. 17β -acetoxy-6-dimethoxymethyl-3,3-dimethoxyandrost-5-ene (XXI) in carbon tetrachloride.

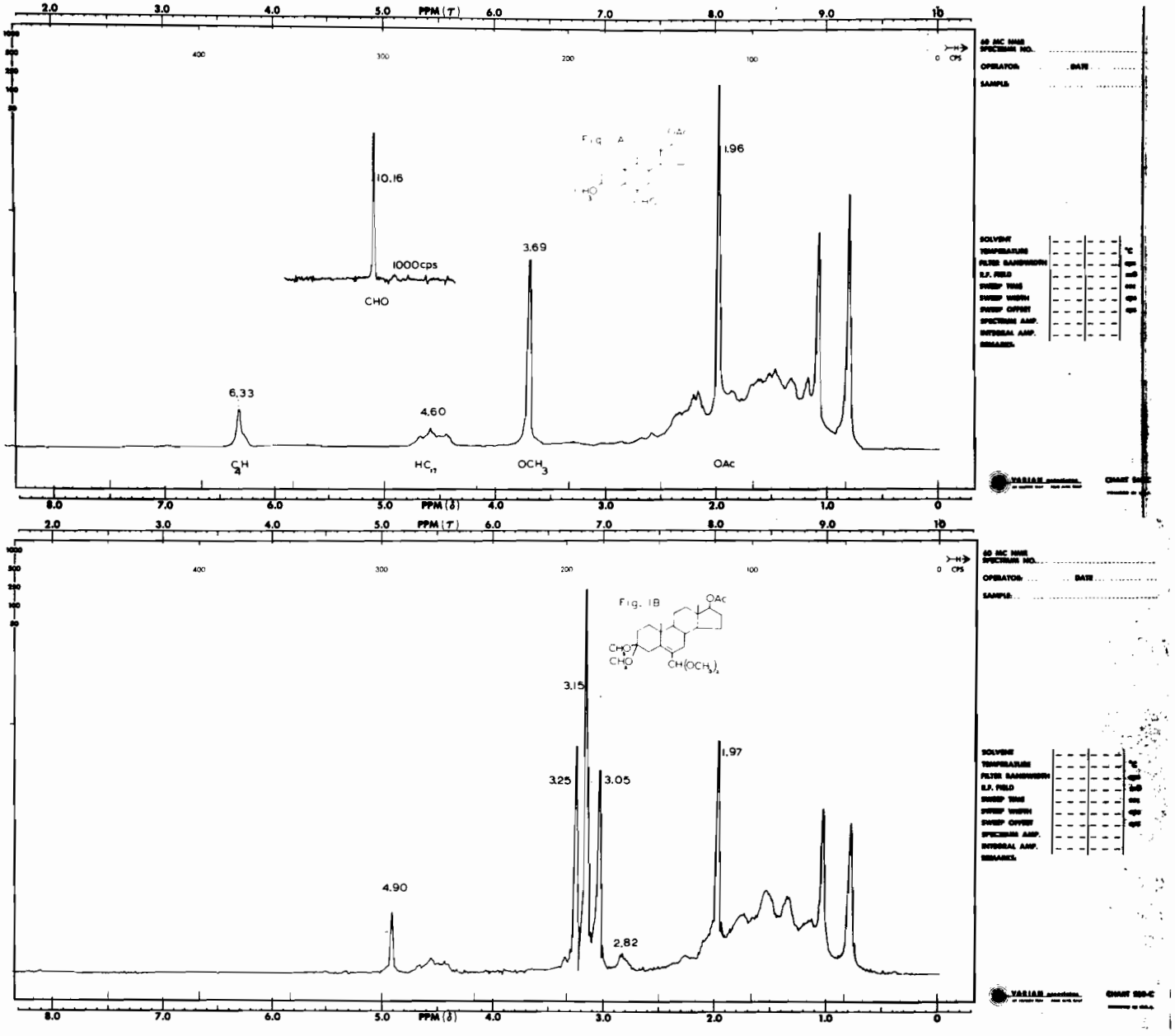

PPM (τ)
PPM (δ)
10.16
1000cps
CHO
6.33
4.60
3.69
1.96
C_4H
HC_{11}
OCH_3
OAc
Fig. A
Fig. 1B
3.15
3.25
3.05
1.97
4.90
2.82
$CH(OCH_3)_2$

Fig. 2

The infrared spectra of

A. 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX).

B. 17β -acetoxy-6-dimethoxymethyl-3,3-dimethoxyandrost-5-ene (XXI).

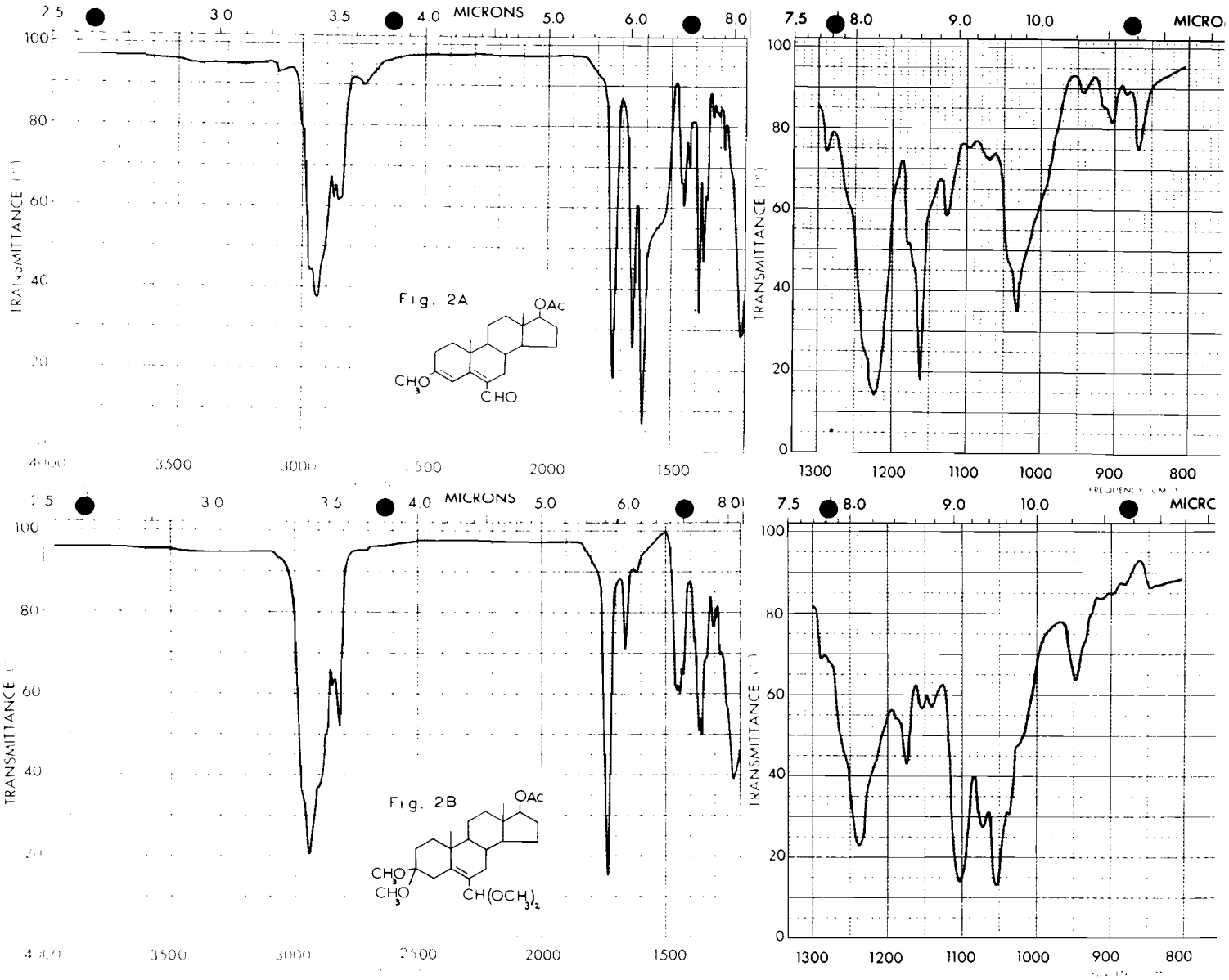

MICRONS
TRANSMITTANCE (%)
Fig. 2A
OAc
CHO
CHO
Fig. 2B
OAc
CHO
CHO
CH(OCH3)2

Fig. 3

The nuclear magnetic resonance spectrum of 17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII) in deuteriochloroform.

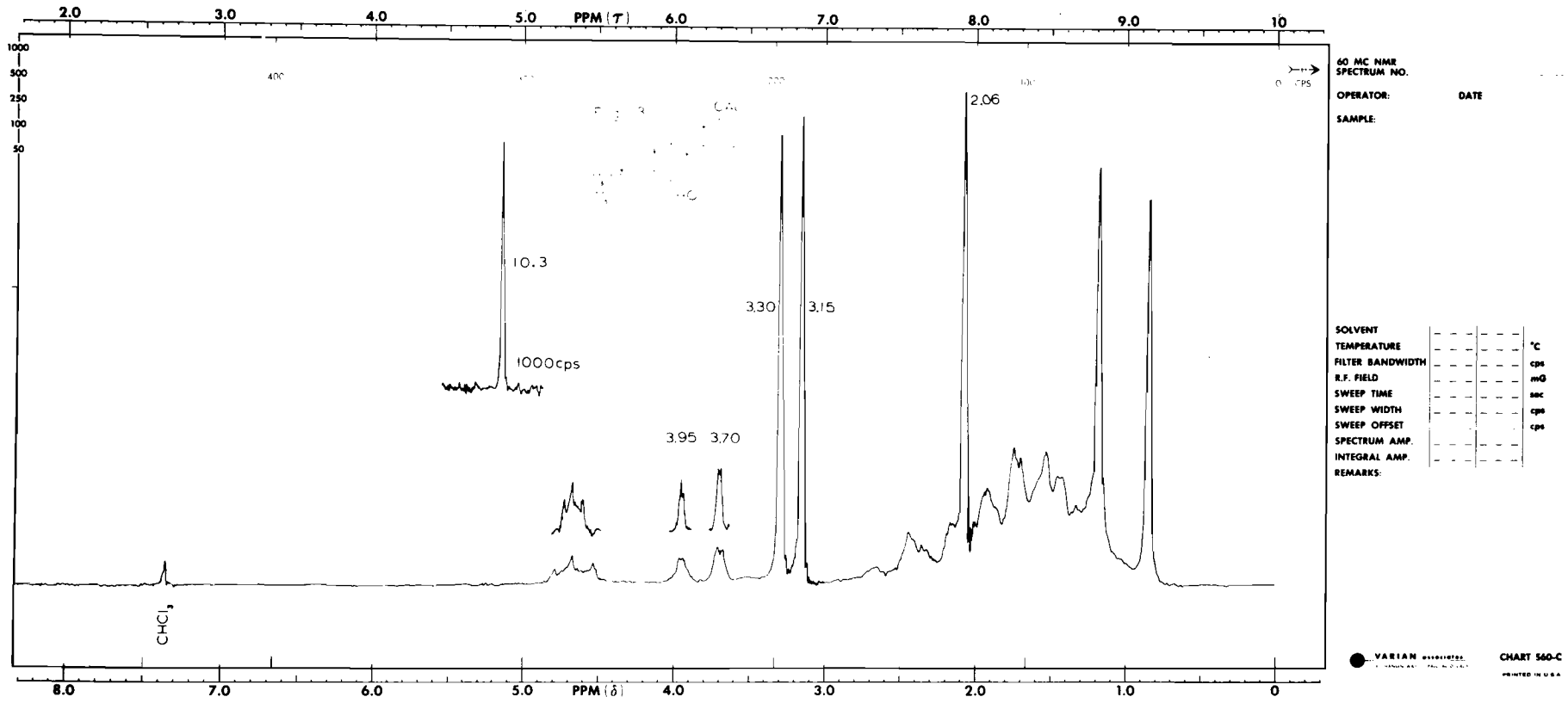

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
1000
500
250
100
50
CPS
10.3
1000cps
3.95
3.70
3.30
3.15
2.06
CHCl3
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0
60 MC NMR
SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT
TEMPERATURE
°C
FILTER BANDWIDTH
cps
R.F. FIELD
mG
SWEEP TIME
sec
SWEEP WIDTH
cps
SWEEP OFFSET
cps
SPECTRUM AMP.
INTEGRAL AMP.
REMARKS:
VARIAN associates
CHART 560-C
PRINTED IN U.S.A.

Fig. 4

The infrared spectrum of

17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII).

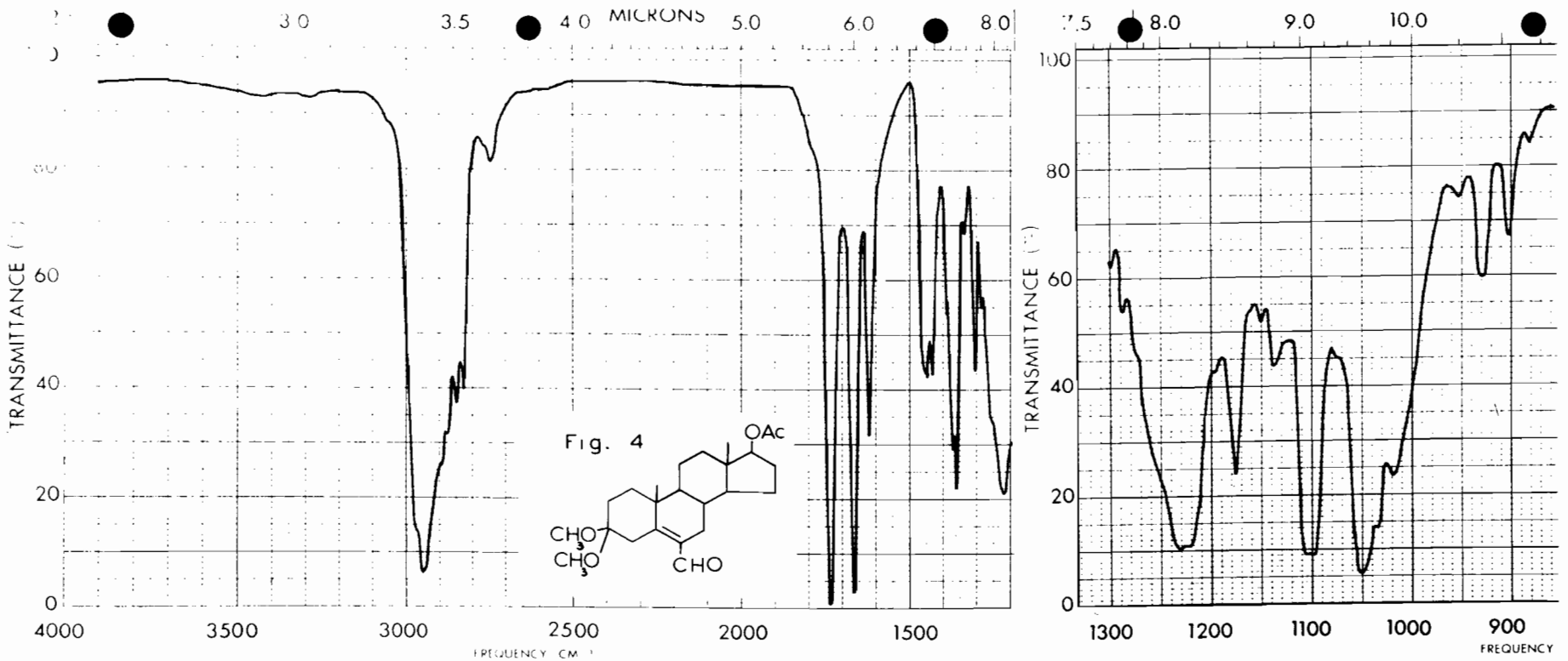

3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
7.5
8.0
9.0
10.0
TRANSMITTANCE (%)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1300
1200
1100
1000
900
FREQUENCY
OAc
CHO
CH3O
CH3O
CHO

Fig. 4

Fig. 5

The nuclear magnetic resonance spectra of

A. 17β -acetoxy-6-hydroxymethyl-3-methoxyandrosta-3,5-diene (XXIII).

B. 17β -acetoxy-6-hydroxymethyl-3,3-dimethoxyandrosta-3,5-diene (XXIV) in deuteriochloroform.

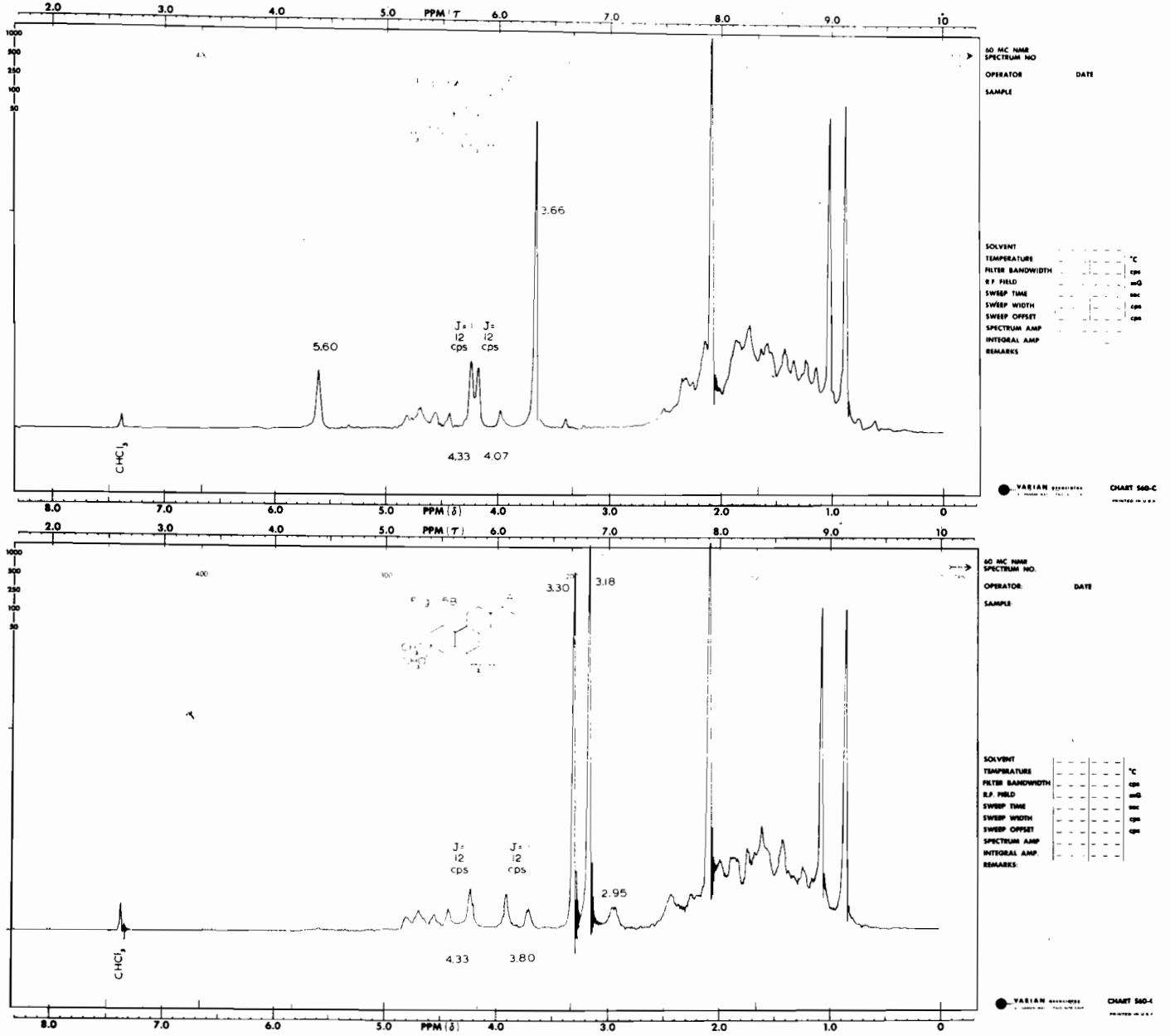

PPM (τ)
PPM (δ)
60 MC NMR SPECTRUM NO.
OPERATOR
DATE
SAMPLE
SOLVENT
TEMPERATURE
FILTER BANDWIDTH
R.F. FIELD
SWEEP TIME
SWEEP WIDTH
SWEEP OFFSET
SPECTRUM AMP
INTEGRAL AMP
REMARKS
5.60
3.66
J= 12 cps
J= 12 cps
4.33
4.07
CHCl3
VARIAN
CHART 560-C
3.30
3.18
2.95
4.33
3.80

Fig. 6

The infrared spectra of

A. 17β -acetoxy-6-hydroxymethyl-3-methoxyandrosta-3,5-diene (XXIII).

B. 17β -acetoxy-6-hydroxymethyl-3,3-dimethoxyandrosta-3,5-diene (XXIV)

in deuteriochloroform.

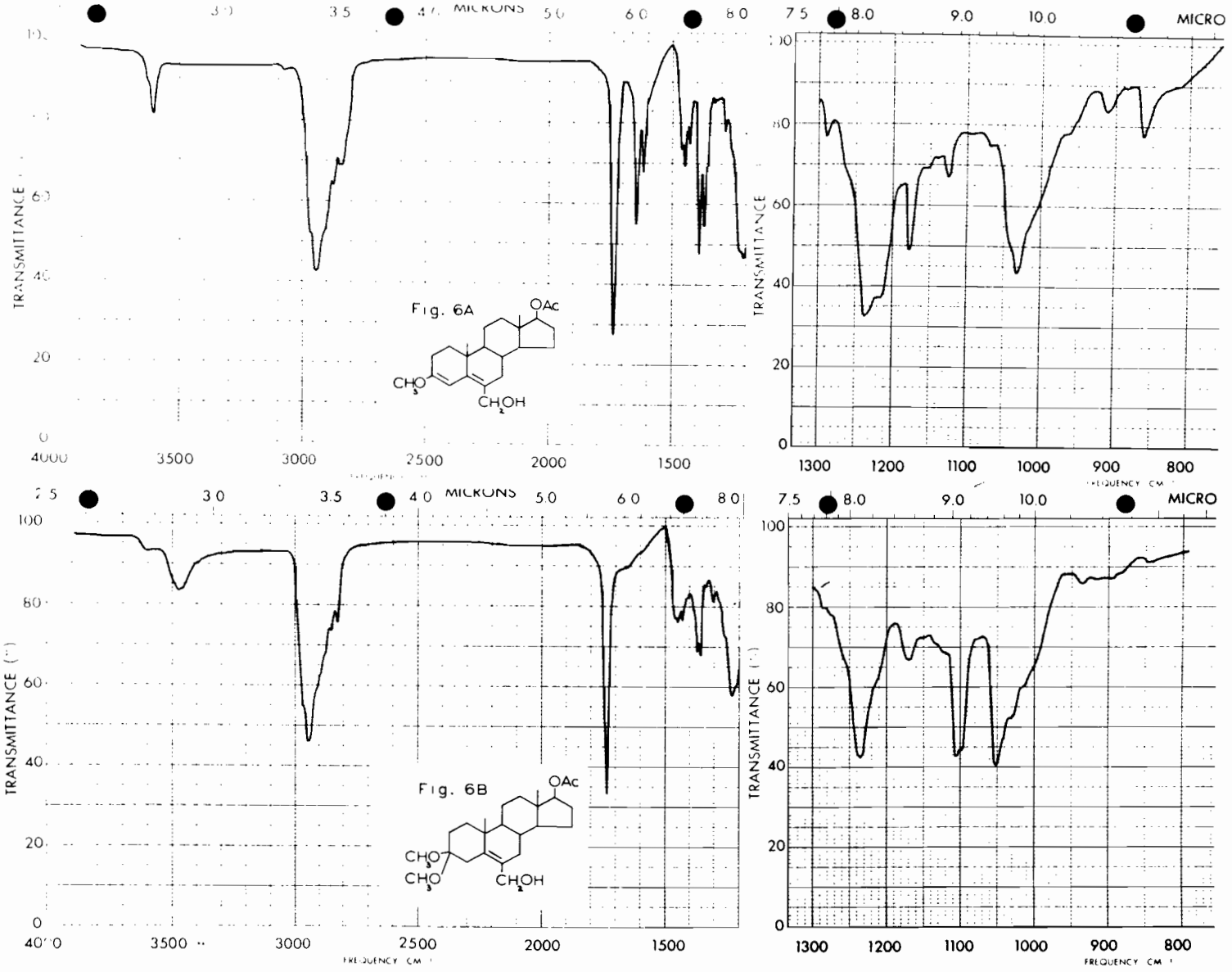

MICRONS
MICRO
TRANSMITTANCE (%)
FREQUENCY CM⁻¹
Fig. 6A
OAc
CH3O
CH2OH
Fig. 6B
OAc
CH3O
CH3O
CH2OH

Fig. 7

The nuclear magnetic resonance spectrum of the hydrolysis product of 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX), in deuteriochloroform.

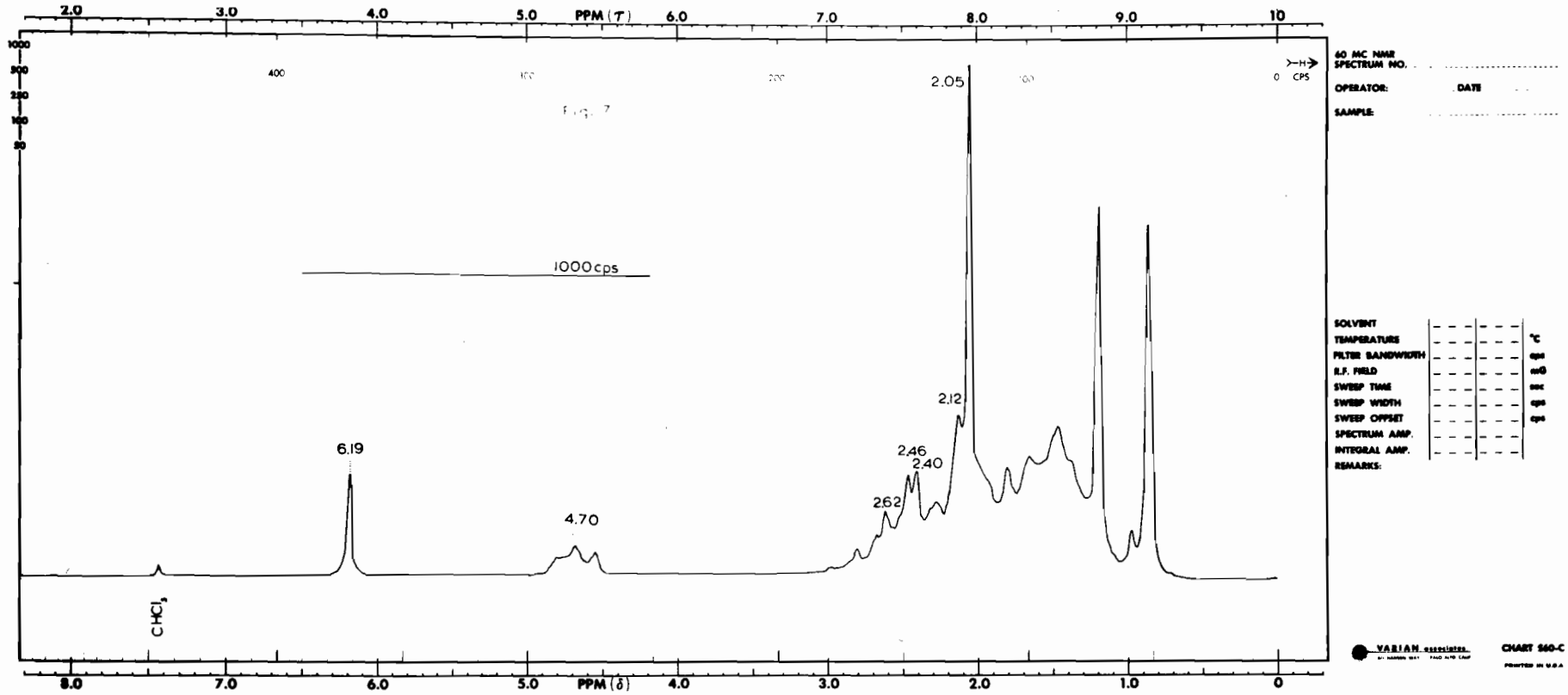

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
1000
500
250
100
50
400
200
0 CPS
2.05
1000 cps
2.12
6.19
2.46
2.40
2.62
4.70
CHCl3
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0
60 MC NMR
SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT
TEMPERATURE
FILTER BANDWIDTH
R.F. FIELD
SWEEP TIME
SWEEP WIDTH
SWEEP OFFSET
SPECTRUM AMP.
INTEGRAL AMP.
REMARKS:
°C
cps
mG
sec
cps
cps
VARIAN associates
CHART 160-C

Fig. 8

The infrared spectrum of the hydrolysis product of 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX).

A. in $CHCl_3$

B. KBr pellet

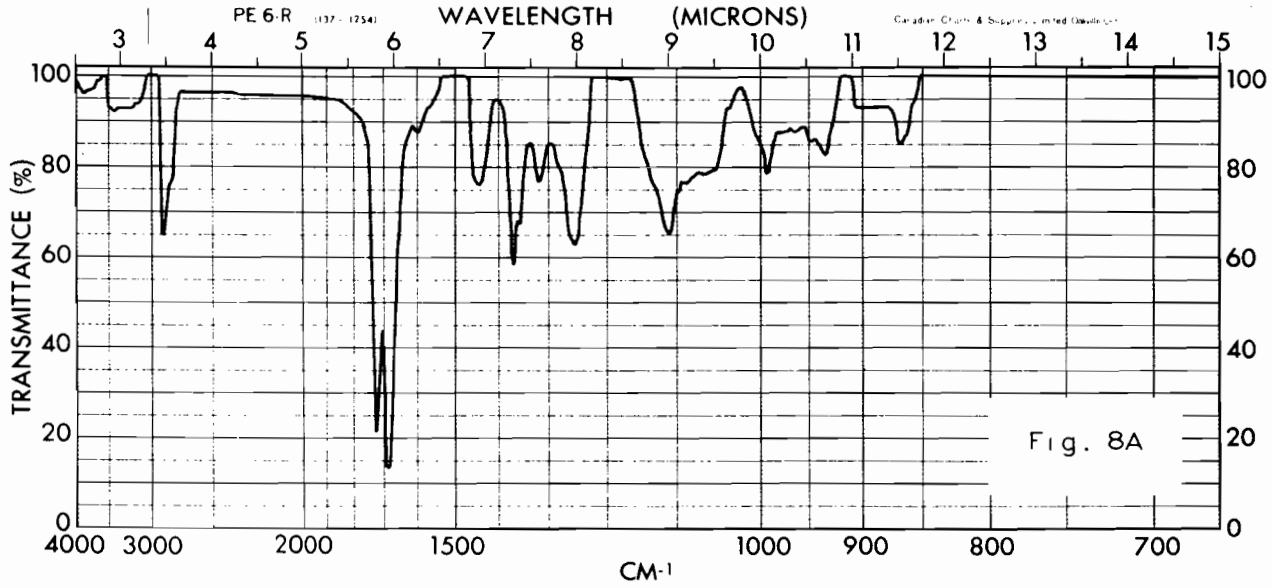

PE 6-R
WAVELENGTH (MICRONS)
3 4 5 6 7 8 9 10 11 12 13 14 15
100 80 60 40 20 0
TRANSMITTANCE (%)
4000 3000 2000 1500 1000 900 800 700
CM-1

Fig. 8A

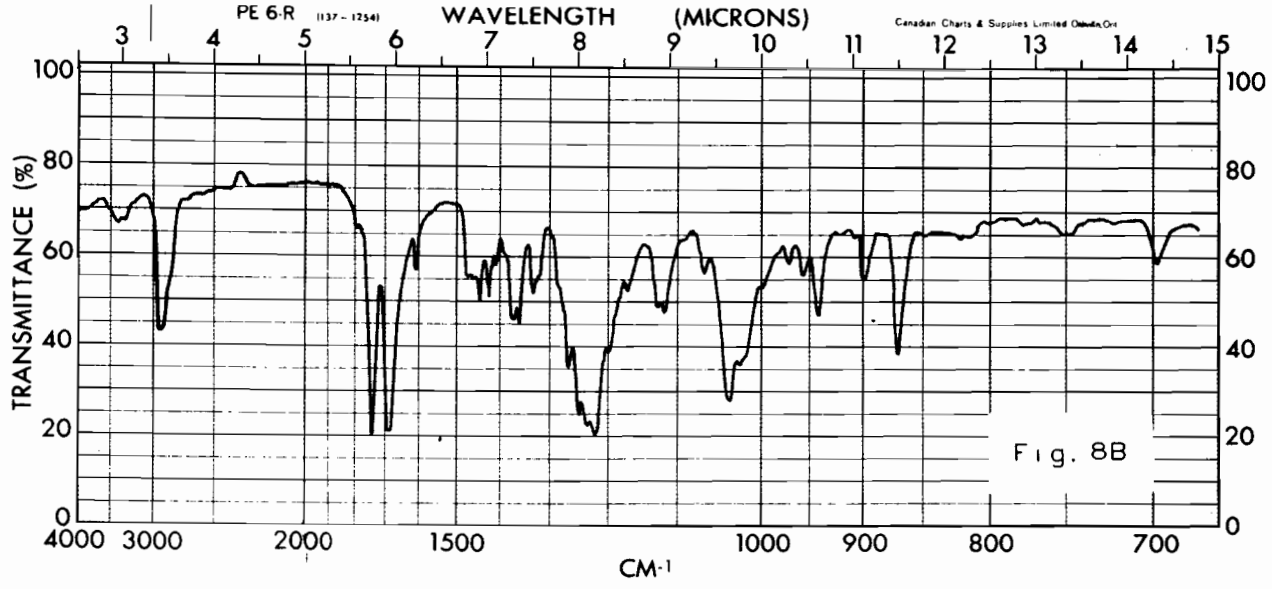

PE 6-R
WAVELENGTH (MICRONS)
3 4 5 6 7 8 9 10 11 12 13 14 15
100 80 60 40 20 0
TRANSMITTANCE (%)
4000 3000 2000 1500 1000 900 800 700
CM-1

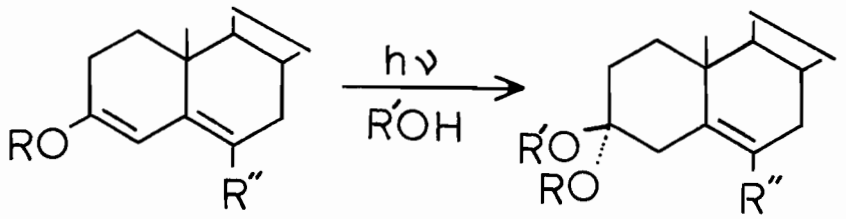
Fig. 8B

Chapter 1

The Photolysis of 6-Formyl Testosterone Methyl Enol Ether Acetate and Related Compounds.

A. Results

It has been stated in the introduction that the photolysis of an alcoholic solution of steroidal 3-alkoxy-3,5-dienes[15,16,32] (XVIII, R" = H, CH_3, F) gave mixed ketals of type XIX in good yield. When the 6-substituent



XVIII XIX

R" = H, CH_3, F

(R") was a nitrogroup[16], the reaction proceeded in a different manner and no product could be isolated in a useful yield. Substituents which conjugate with the enol ether chromophore hence exert a profound influence on the reaction course. By having R" = CHO it was hoped that an insight into the behaviour of a conjugated enol ether chromophore (XX) could be obtained[17].

A methanolic solution of enol ether XX[33] was irradiated until less than 5% of the original ultraviolet chromophore could be detected. Thin-layer chromatography (t.l.c.) indicated the presence of one spot only. The nuclear magnetic resonance (n.m.r.) spectrum (Fig. 1B) of the product (XXI) indicated the presence of an acetate and four methoxy groups, a single proton at 4.90p.p.m. ($C\underline{H}(OCH_3)_2$) and the absence of olefinic protons. The presence of a peak at 2.82p.p.m. (approximately 0.5 H) cast some doubt on the structure assigned, and the possibility that the photoproduct was a 3,4-dimethoxyandrostane derivative could not be excluded at this point. Mild hydrolysis with aqueous methanol converted XXI to the ketal aldehyde XXII. Sodium borohydride reduction of aldehyde XXII gave the corresponding 6-hydroxymethyl ketal XXIV. XXIV was also obtained when enol ether aldehyde XX was reduced with sodium borohydride, and the resulting 6-hydroxymethyl enol ether XXIII photolyzed in methanol. *

Since some doubt persisted concerning the position of two of the methoxy groups in XXI (3,3 or 3,4) and hence XXII and XXIV, XXI was submitted to acid hydrolysis. Under well defined conditions, a crystalline hydrolysis product, identical with that obtained from hydrolysis of XX, could be obtained in 26% yield. Its structure was not obvious and the reaction could not be used to exclude rigorously either

* None of the photochemical reactions occurred in the absence of u.v. light at a comparable rate.

Fig. 9

The nuclear magnetic resonance spectrum of 17β -acetoxy-6-methoxymethyl-3,3-dimethoxyandrost-5-ene (XXIVa) in carbon tetrachloride.

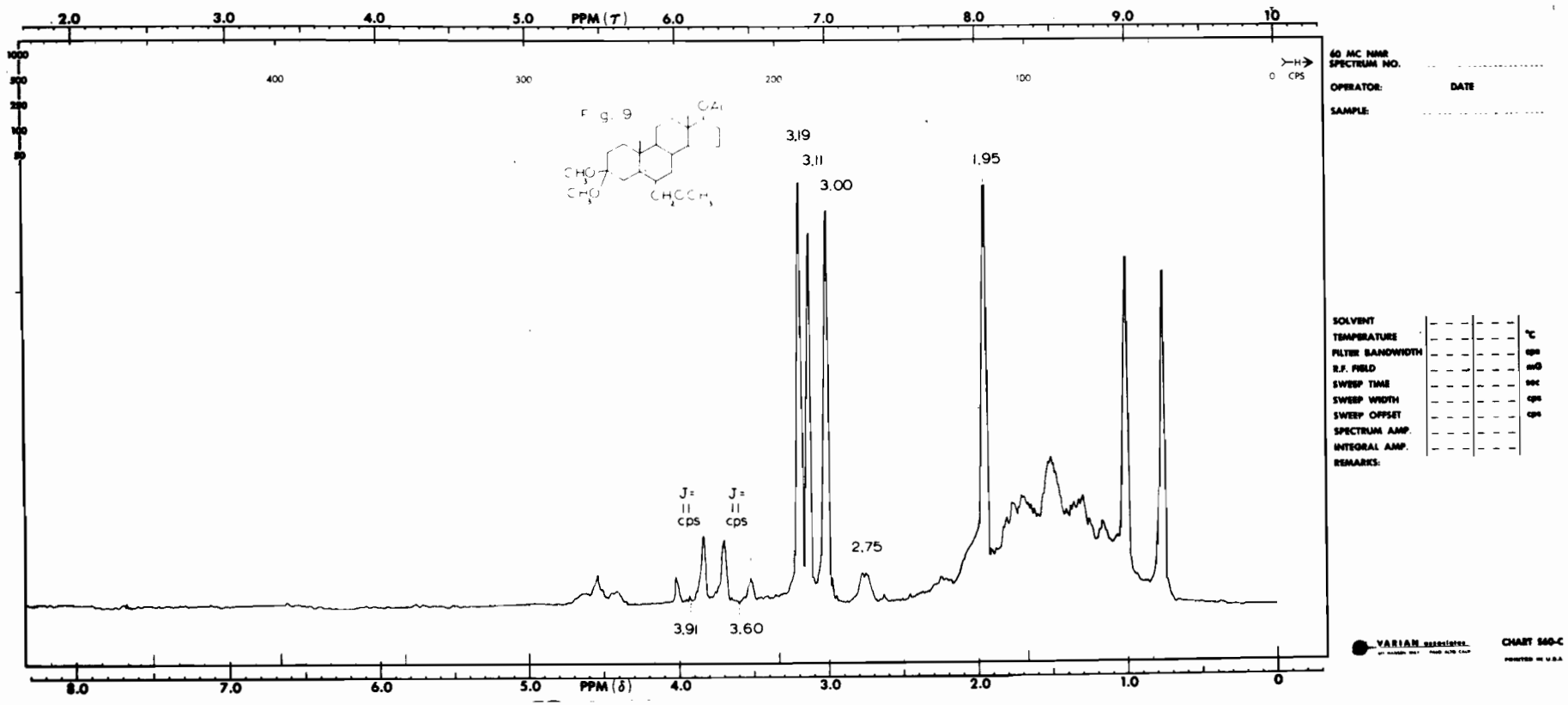

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
1000
500
400
300
200
100
0 CPS
Fig. 9
3.19
3.11
3.00
1.95
2.75
J = 11 cps
J = 11 cps
3.91
3.60
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0
60 MC NMR SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT
TEMPERATURE °C
FILTER BANDWIDTH cps
R.F. FIELD mG
SWEEP TIME sec
SWEEP WIDTH cps
SWEEP OFFSET cps
SPECTRUM AMP.
INTEGRAL AMP.
REMARKS:
VARIAN associates
CHART 560-C

Fig. 10

The infrared spectrum of 17β -acetoxy-6-methoxymethyl-3,3-dimethoxyandrost-5-ene (XXIVa).

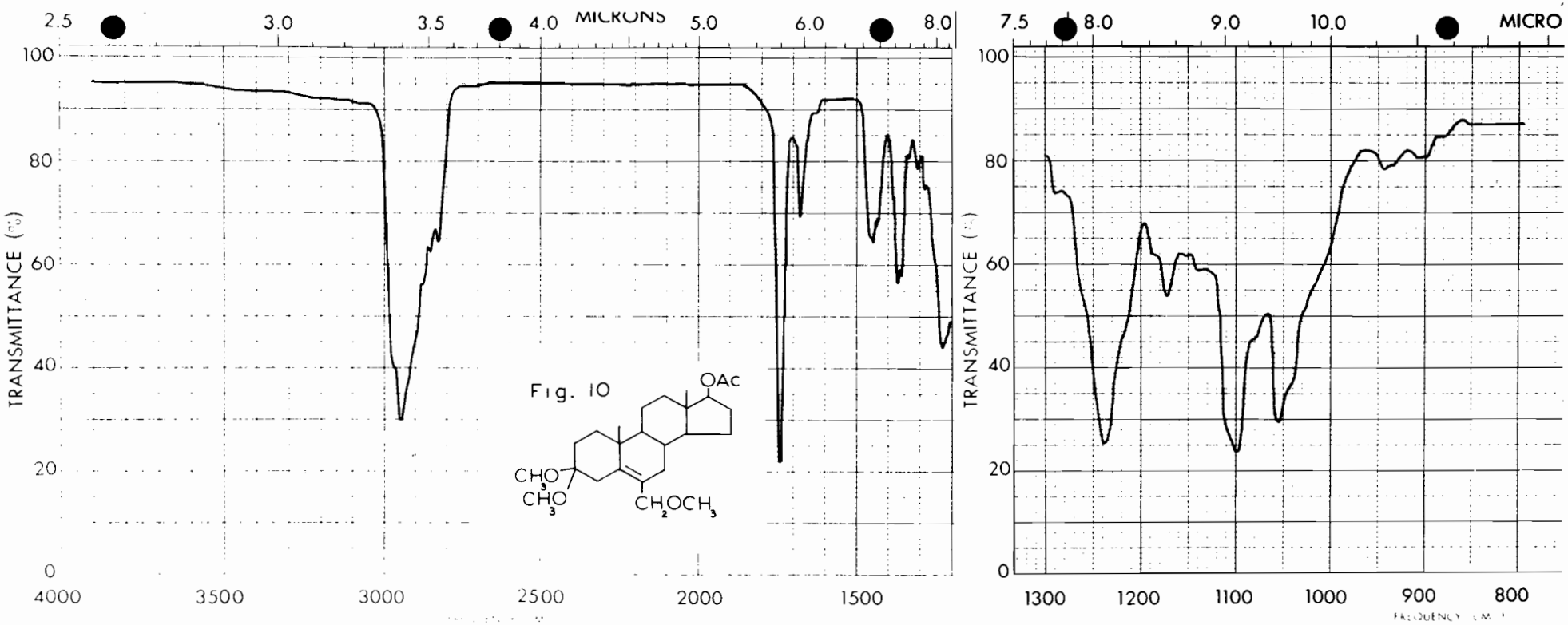

2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
100
80
60
40
20
0
TRANSMITTANCE (%)
4000
3500
3000
2500
2000
1500
OAc
CH3O
CH3O
CH2OCH3
7.5
8.0
9.0
10.0
MICRO
1300
1200
1100
1000
900
800

Fig. 10

Fig. 11

The nuclear magnetic resonance spectrum of 3,3-dimethoxy-6-methyleneandrostan-17β-ol (XXV) in carbon tetrachloride.

Fig. II

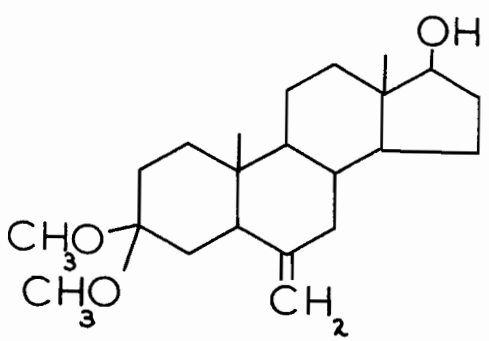


Fig. 12

The infrared spectra of

A. 3,3-dimethoxy-6-methyleneandrostan-17β -ol (XXV).

B. 6-methyleneandrostan-17β -ol-3-one (XXVa).

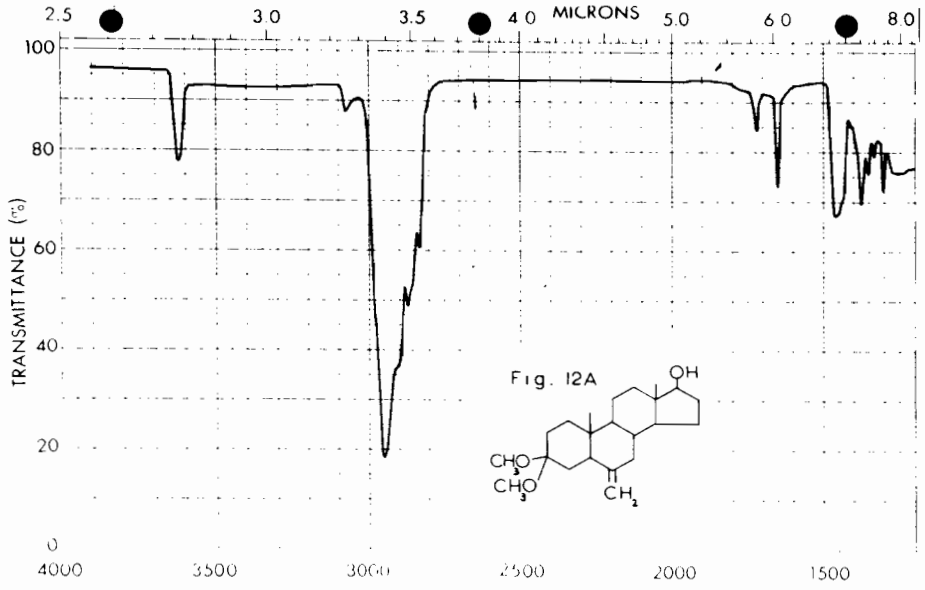
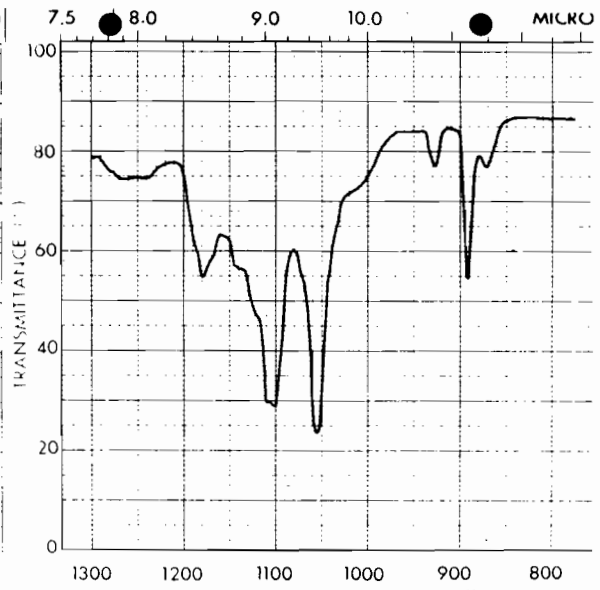


Fig. 12A



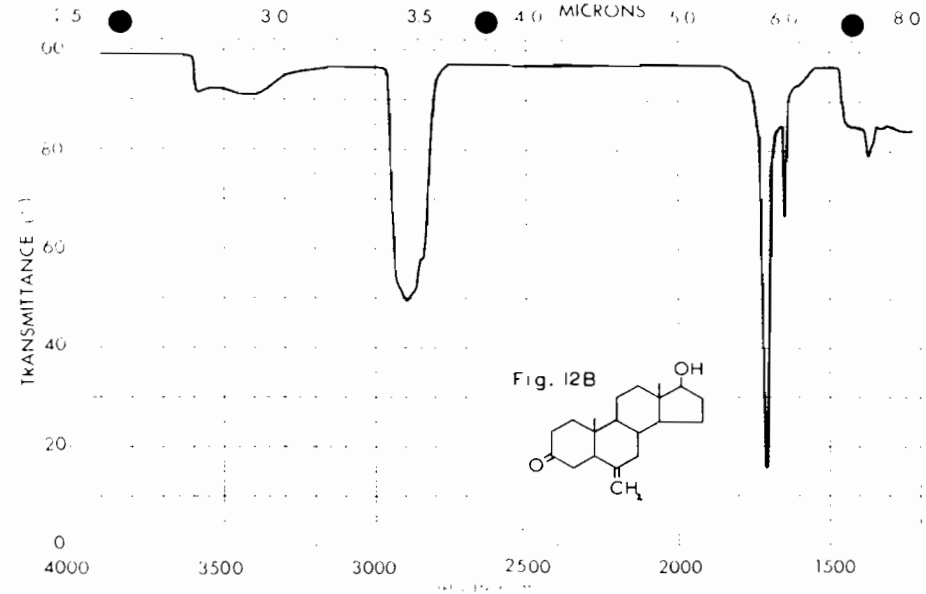
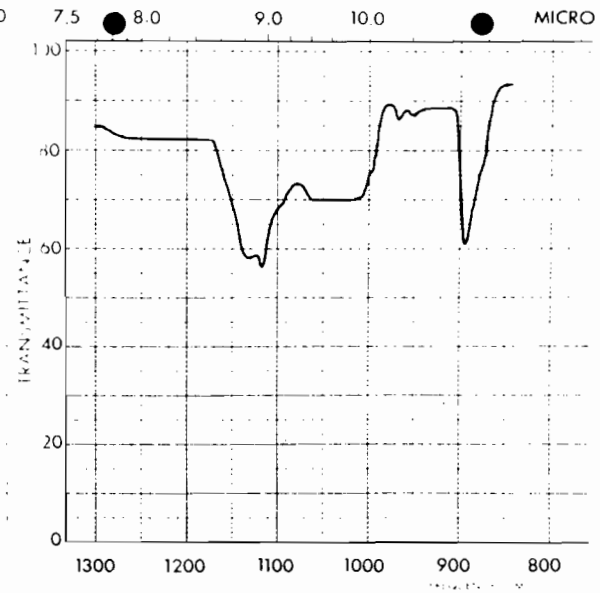


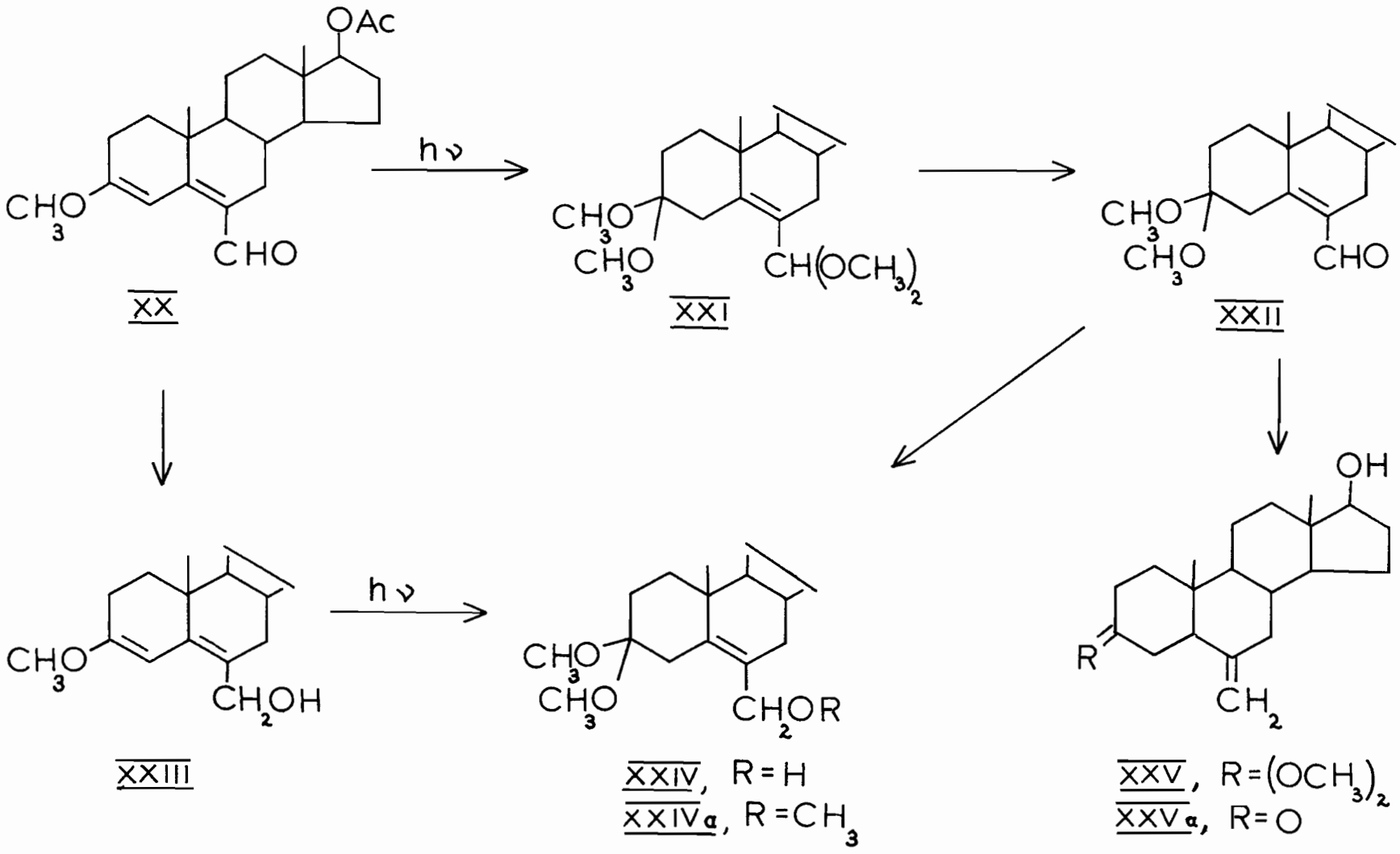
Fig. 12B



possibility. Next, we turned to catalytic hydrogenation. T.l.c. indicated that the product was a mixture. When an attempt was made to convert aldehyde XXII to the corresponding 6-methyl compound by sodium borohydride reduction of the p-toluenesulphonyl hydrazone of XXII[34], the main product isolated had spectral features consonant with structure XXIVa. The Huang-Minlon modification of the Wolff-Kishner reduction[35] of aldehyde XXII gave an amorphous product XXV, which appeared homogeneous (t.l.c.). Its n.m.r. spectrum (Fig. 11) showed the presence of two methoxy groups (3.04 and 3.13 p.p.m.) and two olefinic protons (4.40 and 4.55 p.p.m.). The two olefinic protons must have been part of an exomethylene group, since the infrared spectrum of XXV showed bands at 3075, 1650 and 890 cm^{-1}. The formation of olefin XXV rather than its Δ^5-6 methyl isomer is easily rationalized, since the carbanion[36] preceding the product forming step is allylic. Mild acid hydrolysis of olefinic ketal XXV gave a crystalline olefinic ketone XXVa. Its i.r. spectrum (Fig. 12B) had a peak in the carbonyl region at 1720 cm^{-1} (3-ketone). This reaction sequence rigorously excludes the possibility that XXII, and therefore XXI and XXIV, may be 3,4-dimethyl ethers. The product of photolysis (XXI) was therefore a ketal acetal. (See scheme 1).

The addition of several molecules of methanol to enol ether aldehyde XX proceeds either by formation of enol

Scheme I



XX

XXI

XXII

XXIII

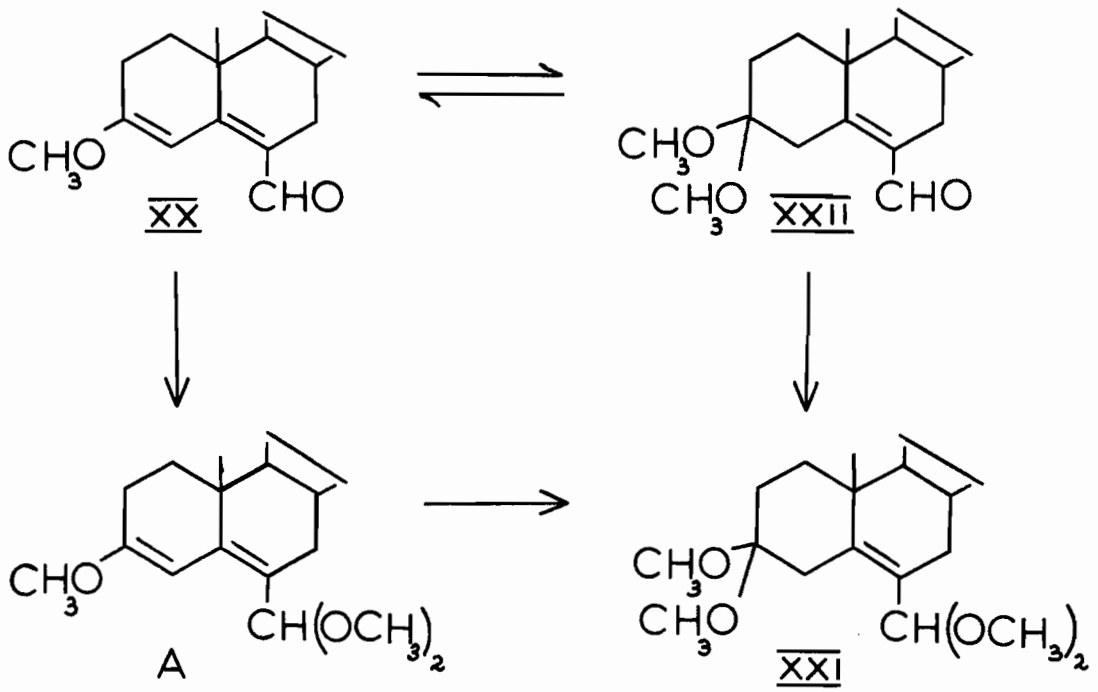
XXIV, R = H

XXIVa, R = CH3

XXV, R = (OCH3)2

XXVa, R = O

ether acetal A, followed by addition of methanol to the enol ether function (XX → A → XXI), or by addition of



methanol to the enol ether, followed by acetal formation (XX → XXII → XXI). In order to distinguish between these two possibilities, a methanolic solution of enol ether aldehyde XX was irradiated. The transitory appearance of XXII could be detected spectrophotometrically (see below). Next, a solution of the ketal aldehyde XXII was irradiated. The product was quantitatively transformed to ketal XXI.* When the reaction was monitored by t.l.c. and u.v., the transitory appearance of 1-2% enol ether aldehyde XX could be detected, and, using optimal conditions, isolated and identified. Since this evidence

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* XXI and XXII had the same R_F-value in all t.l.c. systems used.

Fig. 13

The rate of disappearance of 17β-acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX) and 17β-acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII), log abs. vs. time.

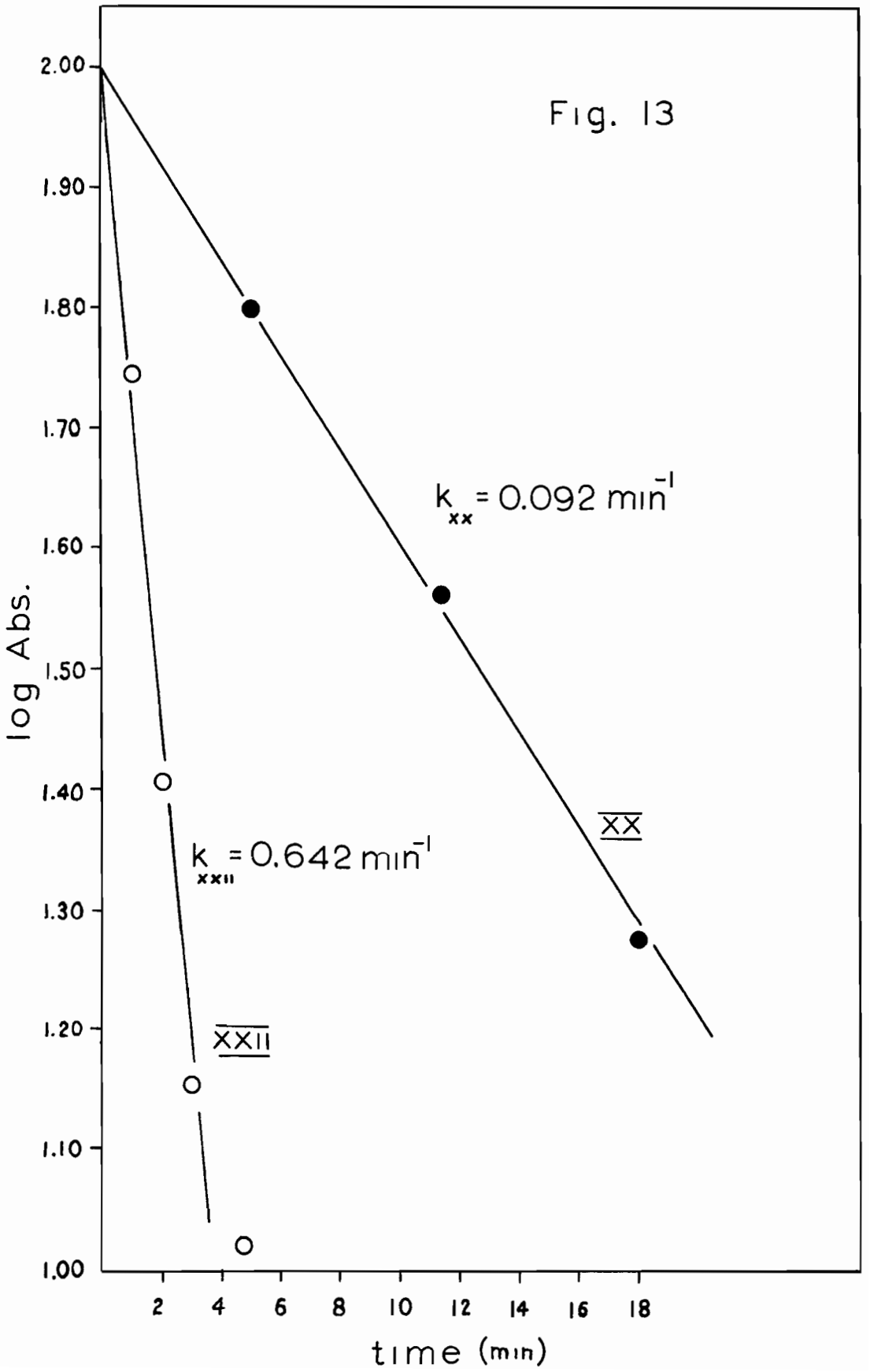


Fig. 13
2.00
1.90
1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
log Abs.
k_{xx} = 0.092 min^{-1}
k_{xxII} = 0.642 min^{-1}
XX
XXII
2
4
6
8
10
12
14
16
18
time (min)

did not exclude either pathway, we next turned to a kinetic approach to determine the reaction sequence.

Kinetics and Discussion

Using a constant light source and a constant initial concentration (0.5×10^{-4} molar) of XX and XXII, the concentration of XX (λ_{max} 320 mμ and 220 mμ), XXII (λ_{max} 250 mμ) and XXI, were determined spectrophotometrically at various time intervals. The rate constants k_{xx} and k_{xx} were obtained from the overall rate of disappearance of XX and XXII (see Fig. 13), according to equations (1) and (2), or more appropriately from the respective integrated forms (1a) and (2a):*

$$- \frac{d[XX]}{dt} = k_{xx} [XX] \qquad (1)$$

after integration,

$$k_{xx} = \frac{2.303}{t_2 - t_1} \log \frac{[XX]_1}{[XX]_2} \qquad (1a)$$

$$- \frac{d[XXII]}{dt} = k_{xx} [XXII] \qquad (2)$$

after integration

$$k_{xx} = \frac{2.303}{t_2 - t_1} \log \frac{[XXII]_1}{[XXII]_2} \qquad (2a)$$

*The photoreaction of XX could be suppressed by addition of a few drops of pyridine or by making the solution 0.006 molar in sodium acetate. However no significant change in reaction rate occurred when XXII was photolysed in sodium acetate.

Fig. 14

The rate of disappearance of 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX) and 17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII), O.D. vs. time.

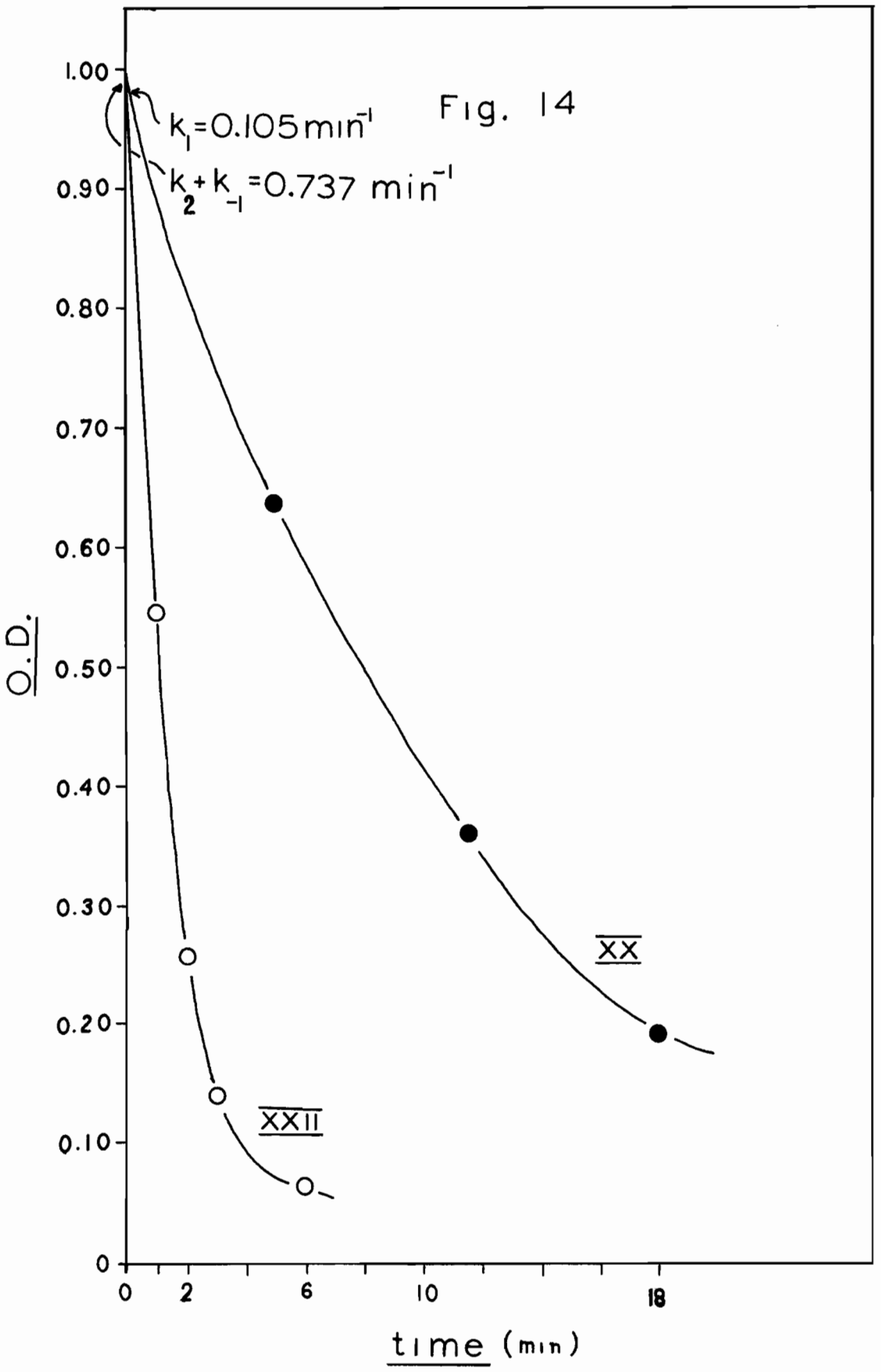


Fig. 14
$k_1 = 0.105 \text{ min}^{-1}$
$k_2 + k_{-1} = 0.737 \text{ min}^{-1}$
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
O.D.
0
2
6
10
18
time (min)
XX
XXII

From these equations

$$k_{xx} = 0.092 \text{ min}^{-1}$$

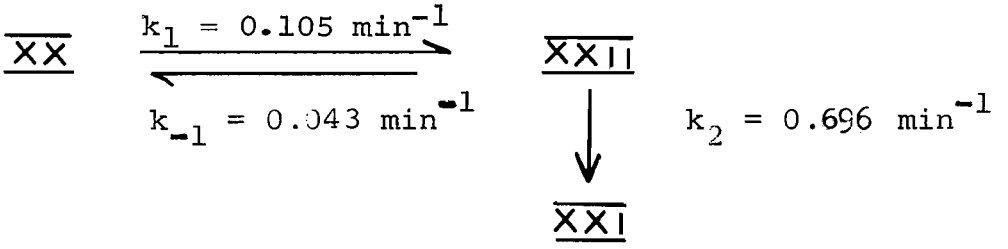
and, $$k_{xx} = 0.642 \text{ min}^{-1}$$

Assuming that only reaction paths denoted by k_1, k_{-1} and k_2 are significant, the following expressions (3) and (4) can be written:

$$-\frac{d[XX]}{dt} = k_1[XX] - k_{-1}[XXII] \qquad (3)$$

$$-\frac{d[XXII]}{dt} = k_2[XXII] + k_{-1}[XXII] - k_1[XX] \qquad (4)$$

The value for k_1 (0.105 min^{-1}) can be obtained directly from a plot (Fig. 14) of XX versus time. Since at near $t = 0$, the term k_{-1} XXII in (3) is very small, and can be neglected, the slope of the curve at $t = 0$ will give a reasonably good value for k_1. Similarly, the value for $k_2 + k_{-1}$ (0.737 min^{-1}) can be obtained from equation (4) and figure 14. Knowing the above values it becomes a simple matter to calculate k_{-1} (0.043 min^{-1}) from (3) and k_2 (0.696 min^{-1})*



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* These values were determined several times. The experimental error is ± 10% for k_1 and k_2. It is considerably larger for k_{-1} (estimated error: ± 80%).

These values provide a self-consistent picture of the reaction and permit to exclude pathway XX → A → XXI.

The values of the rate constants have no further significance. In order to make them meaningful, the relative magnitudes for k_1, k_{-1} and k_2 would have to be corrected for the energy output of the lamp at the wavelength of the absorbing species. This has not been attempted and is beyond the scope of this study.

B. Experimental

All melting points were determined in a concentrated sulphuric acid bath. Optical rotations were performed with a Carl Zeiss automatic polarimeter using a 0.5 dm tube. The infrared spectra were taken on a Perkin-Elmer 337 grating spectrophotometer using 1 millimeter sodium chloride cells. Nuclear magnetic resonance spectra were recorded on a Varian A.60 instrument at 60 megacycles using carbon tetrachloride as solvent unless otherwise stated and tetramethylsilane (0 p.p.m.) as internal standard. The ultraviolet absorption spectra were measured on a Beckman DK-1 recording spectrophotometer. Preparative thin layer chromatographic separation was effected on 0.75 mm thick (20 x 20 cm) unactivated plates coated with Merck A.G. silica gel. All irradiations were performed in a Rayonet photo-

chemical reactor (The Southern New England Co., Middletown, Conn., U.S.A.), using 2537 Å light. Analyses were carried out by Dr. C. Daesslé, Montreal, and Schwarzkopf Microanalytical Laboratory, Woodside 77, N.Y.

Photolysis of 17β-Acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX)

A solution of 0.600 g. of XX in 200 ml. of methanol was irradiated in a helium atmosphere for 16 hours. T.l.c. (benzene-ether 4:1) revealed one spot only. Crystallization from methanol containing a trace of pyridine gave 0.404 g. of XXI, m.p. 119-120°. Recrystallization did not raise the m.p. $[\alpha]_D$ - 68.95° (c, 1.23 in chloroform); ν_{CCl_4} (Fig. 2B) 1740, 1240 cm^{-1} (-OAc), 1660 cm^{-1} (C=C), 1100, 1075, 1055 cm^{-1} (OCH_3); δ (Fig. 1B) 4.90 (singlet, 1H), 3.25 (singlet, 3H), 3.15 (singlet, 6H), 3.05 (singlet, 3H), 2.82 (poorly resolved signal, ~ 0.5H), 1.97 p.p.m. (singlet, 3H).

Calc. for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40.

Found: C, 69.48; H, 9.17.

17β-Acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII)

A methanolic solution of XXI containing a few drops of water was allowed to stand for 18 hours at room temperature, after which time crystals appeared. When necessary, the crystallization could be completed by further addition

of water or cooling. The product, m.p. 159-160.5°, was recrystallized twice from moist methanol and gave pure XXII, m.p. 164-165°; $\alpha]_D$ - 126.9° (c, 0.98 in chloroform); ν_{CCl_4} (Fig. 4) 2750 cm^{-1} (-CHO), 1660 cm^{-1} (α,β-unsaturated aldehyde), 1740, 1230 cm^{-1} (-OAc), 1100, 1050 cm^{-1} ($-OCH_3$); δ (Fig. 3) 10.3 (singlet, 1H), 3.95 (poorly resolved signal, ~ 0.5H), 3.70 (poorly resolved signal, ~ 0.5 H), 3.30 (singlet, 3H), 3.15 (singlet, 3H), 2.06 p.p.m. (singlet, 3H); λ^{MeOH}_{max} 250 mμ (ϵ 12,600).

Calc. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97.

Found: C, 71.44; H, 8.90.

17β-Acetoxy-6-hydroxymethyl-3,3-dimethoxyandrost-5-ene (XXIV)

1. From 17β-Acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII)

To a stirred solution of 60 mg. of XXII in 2 ml. of methanol was slowly added 64 mg. of sodium borohydride and the mixture was stirred for 15 minutes. Ether extraction gave 60 mg. of a crystalline residue. Recrystallization from ether gave 21 mg. of needles, m.p. 180.5-181°; ν_{CCl_4} (Fig. 6B) 3470 cm^{-1} (-OH), 1740, 1240 cm^{-1} (-OAc), 1100, 1050 cm^{-1} ($-OCH_3$).

2. From the Photolysis of 17β-Acetoxy-6-hydroxymethyl-3-methoxy-androsta-3,5-diene (XXIII)

A solution of 99 mg. of XXIII in 70 ml. of methanol was irradiated in a helium atmosphere for 0.5 hours, after

which time t.l.c. (benzene-ether, 3:2) revealed only one spot. Evaporation of the solution and crystallization from methanol gave 73 mg. of needles, m.p. 174.5-176.5°. Recrystallization raised the melting point to 180-181°; $\alpha]_D$ - 50.65° (c, 0.93 in chloroform). This product was identical by i.r., t.l.c., and mixed melting point with that obtained from the sodium borohydride reduction of XXII; δ (Fig. 5B) 4.41, 4.22, 3.91, 3.71 (AB quartet, J = 12 c.p.s., $-\underline{CH_2}OH$), 3.3 (singlet, 3H), 3.18 (singlet, 3H), 2.95 (poorly resolved signal, ~ 0.5H), 2.09 (singlet, 3H) p.p.m.

Calc. for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42.

Found: C, 70.74; H, 9.67.

Hydrolysis of XX

A solution of 530 mg. of XX in 20 ml. dioxane, 0.3 ml. of 50% aqueous perchloric acid and 6 ml. of water was stirred at room temperature for four hours. It was then diluted with water and the solution extracted with ether. The organic layer was washed with 10% aqueous sodium bicarbonate and water, and dried. Evaporation gave a light yellow foam. T.l.c. separated the mixture into two main components. Elution of the slower band with chloroform gave 140 mg. of crystals. Recrystallization from hexane-chloroform gave 102 mg. of plates, m.p. 207.5-209°; $\alpha]_D$

-49.9° (c, 1.10 in chloroform); ν_{CHCl_3} (Fig. 8) 1725 cm^{-1} (-OAc), 1680 cm^{-1} (α,β-unsaturated ketone); ν_{KBr} 1740, 1220, 1020 cm^{-1} (-OAc), 1675 cm^{-1} (broad peak, unsaturated carbonyl(s)), 1600 cm^{-1} (C=C), 1040 cm^{-1} (strong), 940 cm^{-1} (medium), 900 cm^{-1} (weak), 870 cm^{-1} (medium); δ_{CHCl_3} (Fig. 7) 6.19 (singlet, 1H), peaks on the methylene envelope at 2.62, 2.46, 2.40, 2.12 and 2.05 (singlet, 3H) p.p.m.; λ_{max}^{MeOH} 249 mμ (ϵ 8600).

Calc. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44.

Found: C, 73.80; H, 8.44.

Hydrolysis of XXI

A solution of 102 mg. of XXI in 8.0 ml. of acetone containing 2.0 ml. of water and 0.05 ml. of concentrated sulphuric acid was stirred at room temperature for 16 hours. The mixture was extracted with ether and the organic layer washed neutral with H_2O. Evaporation of the dried ethereal solution gave a light yellow foam which was purified by preparative t.l.c. (benzene-ether 4:1): the main component consisted of 20 mg. of a crystalline material identical by i.r., t.l.c. and mixed melting point with that obtained previously from the hydrolysis of XX.

Catalytic Hydrogenation of XXI

A solution of 217 mg. of XXI in 10 ml. of methanol was hydrogenated at 2000 p.s.i. in the presence of 100 mg.

of platinum oxide for 18 hours. T.l.c. of the product revealed at least six components. The n.m.r. spectra of the major components showed that they lacked the required spectral features (namely, four methoxyl groups and two tertiary protons).

17β-Acetoxy-6-methoxymethyl-3,3-dimethoxyandrost-5-ene (XXIVa)

Aldehyde XXII (252 mg.) was slowly added to a stirred solution of p-tosylhydrazine (125 mg.) in methanol (10 ml.) at room temperature. After 30 minutes, t.l.c. revealed one spot and the disappearance of starting material. The solvent was evaporated in vacuo at room temperature to give an orange foam, which could not be crystallized; ν_{CCl_4} 3200, 1740, 1620, 1240, 1170, 1100, 1050, 950, 660, 570 cm^{-1}. The product was dissolved in 25 ml. of methanol and 500 mg. of sodium borohydride was slowly added. The stirred solution was refluxed for 1.5 hours, after which time no tosylhydrazone could be detected by t.l.c. Separation of the resulting mixture by t.l.c. gave one component in 25% yield. Two recrystallizations from methanol containing a trace of pyridine gave crystals, m.p. 126-127°; $[\alpha]_D$ -20.08° (c, 1.24 in chloroform); ν_{CCl_4} (Fig. 10) 1740, 1240 cm^{-1} (-OAc), 1660 cm^{-1} (C=C), 1110, 1100, 1050 cm^{-1} ($-OCH_3$); δ (Fig. 9) pair of doublets centred at 3.91 and 3.60, (AB quartet, J = 11 c.p.s.,

-$\underline{CH_2}$-O), 3.19 (singlet, 3H), 3.11 (singlet, 3H), 3.00 (singlet, 3H), 2.75 (broad signal ~ 0.5H), 1.95 (singlet, 3H) p.p.m.

Calc. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59

Found: C, 72.00; H, 9.41.

3,3-Dimethoxy-6-methyleneandrostan-17β-ol (XXV)

A solution of 256 mg. of aldehyde XXII, 5.0 ml. of diethylene glycol, 0.25 ml. of hydrazine hydrate and 250 mg. of potassium hydroxide was heated under reflux for one hour. The condenser was then removed and the temperature allowed to rise to 210°. After an additional three hours of heating, the cooled colorless solution was extracted with ether. The usual workup gave a foamy residue which was purified by preparative t.l.c.: 132 mg. of amorphous ketal XXV was obtained; ν_{CCl_4} (Fig. 12A) 3620 cm^{-1} (-OH), 3075, 1650, 890 cm^{-1} (-C=CH_2), 1100, 1055 cm^{-1} (-OCH_3). For n.m.r. spectrum see Figure 11.

6-Methyleneandrostan-17β-ol-3-one (XXVa)

Ketal XXV (113 mg.) was heated under reflux for 15 hours in 20 ml. of acetone and 5 ml. of water containing 113 mg. of p-toluenesulphonic acid. Ether extraction gave a crystalline residue. Two recrystallizations from ether gave XXVa, m.p. 194.5-196°; $[\alpha]_D$ -21.03° (c, 1.25 in chloroform); ν_{CHCl_3} (Fig. 12B) 3600, 1120 cm^{-1} (-OH), 1720 cm^{-1}

(saturated ketone), 1650, 895 cm^{-1} ($C{=}CH_2$).

Calc. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00

Found: C, 79.48; H, 10.07

Kinetic Experiments

A magnetically stirred solution (200 ml.) of XX (0.5 x 10^{-4} molar) in absolute methanol was irradiated in a helium atmosphere in a quartz cell fitted with a cold water jacket and a cold water condenser. At various time intervals, 3 ml. aliquots were pipetted out and immediately transferred into a stoppered u.v. cell, and the spectrum recorded on a Beckmann DK1 recording spectrophotometer. Aldehyde XXII was similarly irradiated. Several runs were made in each case. The concentrations of XX, XXII and XXI at various time intervals of a particular run in the controlled photolysis of XX, and the rate constants k_{-1} and k_2 are tabulated below.

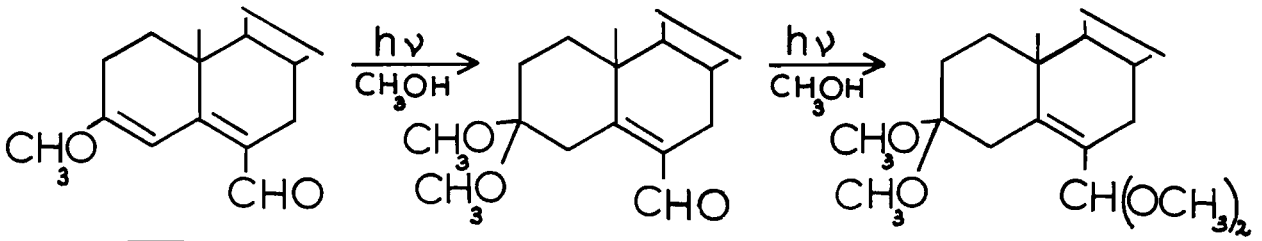
Time min^{-1}	Conc. XX	Conc. XXII	Conc. XXI	k_{-1} min^{-1}	k_2 min^{-1}
0	1.00	0	0	0	0
5	0.635 ± 0.020	0.160	0.205	0.064 ± 61%	0.677 ± 5%
11.5	0.335 ± 0.035	0.170	0.465	0.033 ± 91%	0.704 ± 4%
18	0.189 ± 0.050	0.177	0.634	0.030 ± 98%	0.707 ± 4%

Chapter II

The Photolysis of the Semicarbazone and Oxime Derivatives of 6-Formyl Testosterone Methyl Enol Ether Acetate.

A. Results

In Chapter I it was shown that irradiation of a chromophore constituted of an enol ether conjugated with an aldehyde group XX gave a ketal acetal XXI. It was also shown that ketal aldehyde XXII was an intermediate in the reaction



XX XXII XXI

By irradiation of the 6'semicarbazone (XXVI) and oxime (XXX) derivatives[18], useful information on the behaviour of enol ethers conjugated with nitrogen containing substituents would be obtained.

Semicarbazones

The semicarbazone XXVI of 17β-acetoxy-6-formyl-3-methoxyandrosta-3,5-diene was irradiated* in absolute methanol

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* 2537 Å light was used.

Fig. 15

The nuclear magnetic resonance spectra of

A. syn-semicarbazone enol ether (XXVI)

B. anti-semicarbazone enol ether (XXVII)

in deuteriochloroform.

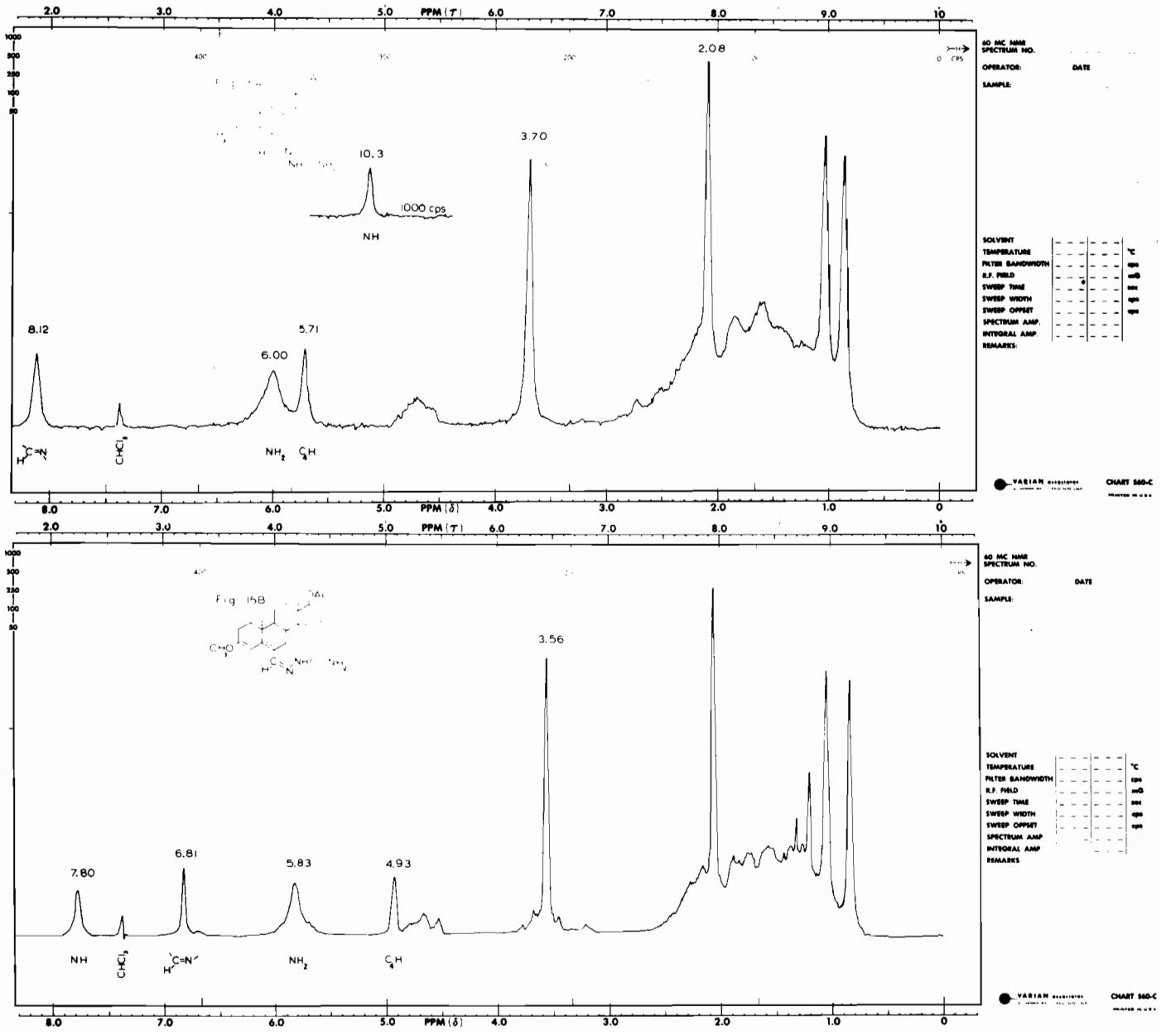

PPM (τ)
PPM (δ)
60 MC NMR SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT
TEMPERATURE
FILTER BANDWIDTH
R.F. FIELD
SWEEP TIME
SWEEP WIDTH
SWEEP OFFSET
SPECTRUM AMP.
INTEGRAL AMP.
REMARKS:
8.12
6.00
5.71
10.3
1000 cps
NH
3.70
2.08
CHCl3
NH2
C4H
VARIAN
CHART 560-C
Fig 15B
7.80
6.81
5.83
4.93
3.56

Fig. 16

The nuclear magnetic resonance spectra of

A. syn-semicarbazone ketal (XXVIII)

B. anti-semicarbazone ketal (XXIX)

in deuteriochloroform.

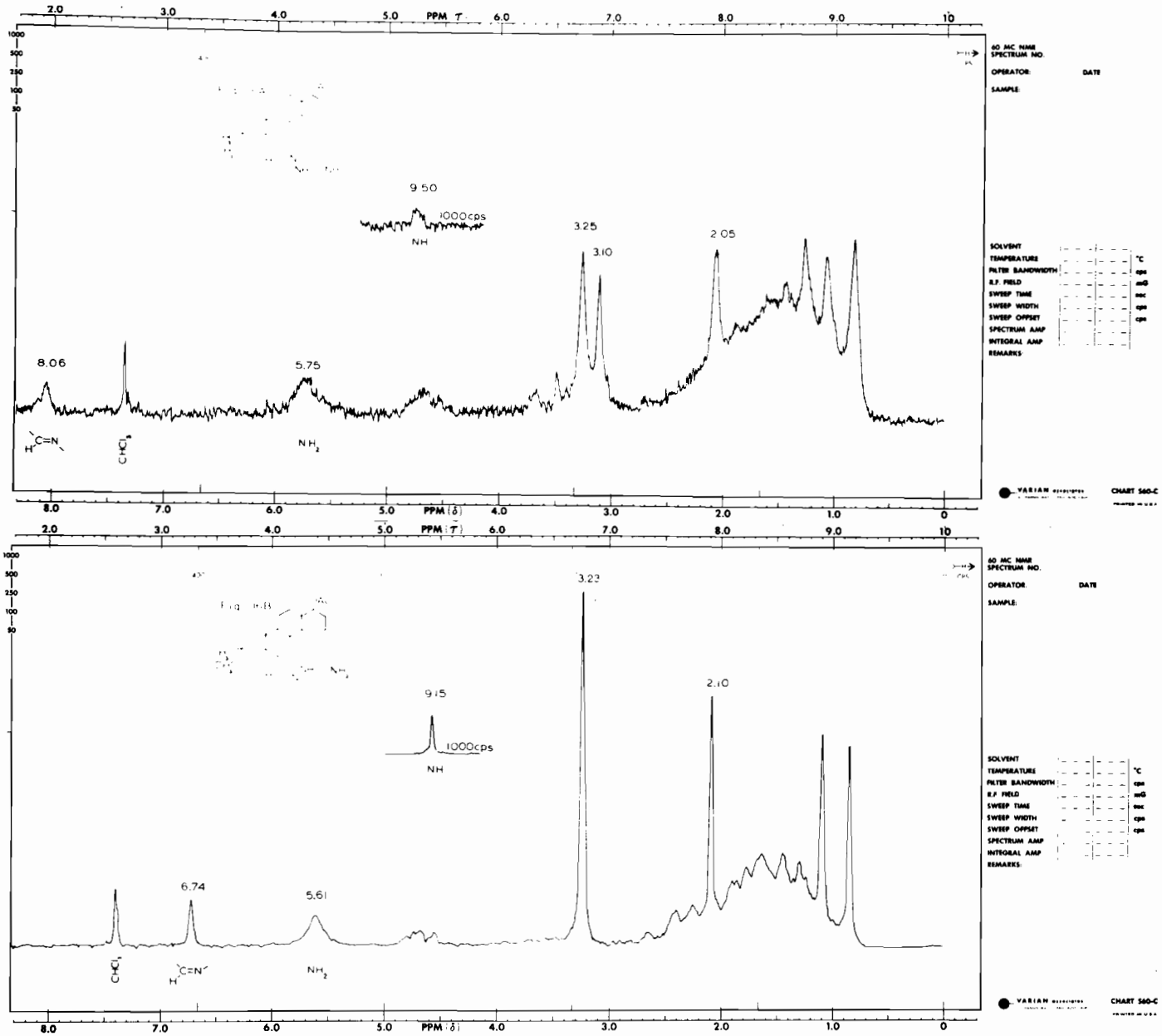

2.0 3.0 4.0 5.0 PPM (τ) 6.0 7.0 8.0 9.0 10
1000 500 250 100 50
9.50
1000cps
NH
3.25
3.10
2.05
8.06
5.75
C=N
H
CHCl3
NH2
8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0
60 MC NMR SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
SOLVENT
TEMPERATURE °C
FILTER BANDWIDTH cps
R.F. FIELD mG
SWEEP TIME sec
SWEEP WIDTH cps
SWEEP OFFSET cps
SPECTRUM AMP
INTEGRAL AMP
REMARKS:
VARIAN
CHART S60-C
2.0 3.0 4.0 5.0 PPM (τ) 6.0 7.0 8.0 9.0 10
1000 500 250 100 50
3.23
2.10
9.15
1000cps
NH
6.74
5.61
CHCl3
C=N
H
NH2
8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0
60 MC NMR SPECTRUM NO.
OPERATOR: DATE
SAMPLE:
SOLVENT
TEMPERATURE °C
FILTER BANDWIDTH cps
R.F. FIELD mG
SWEEP TIME sec
SWEEP WIDTH cps
SWEEP OFFSET cps
SPECTRUM AMP
INTEGRAL AMP
REMARKS:
VARIAN
CHART S60-C

Fig. 17

The infrared spectra of

A. syn-semicarbazone enol ether (XXVI)

B. anti-semicarbazone enol ether (XXVII).

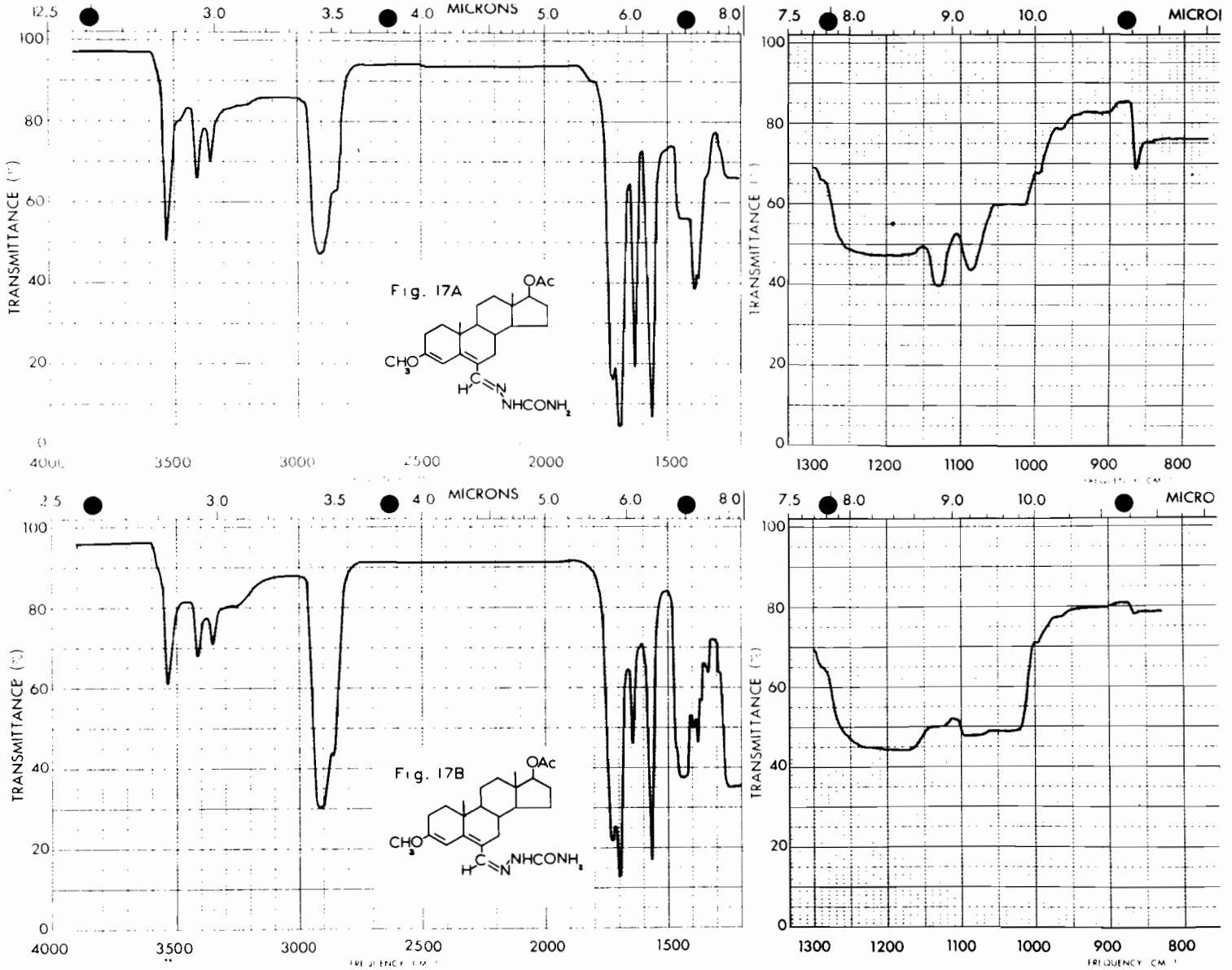
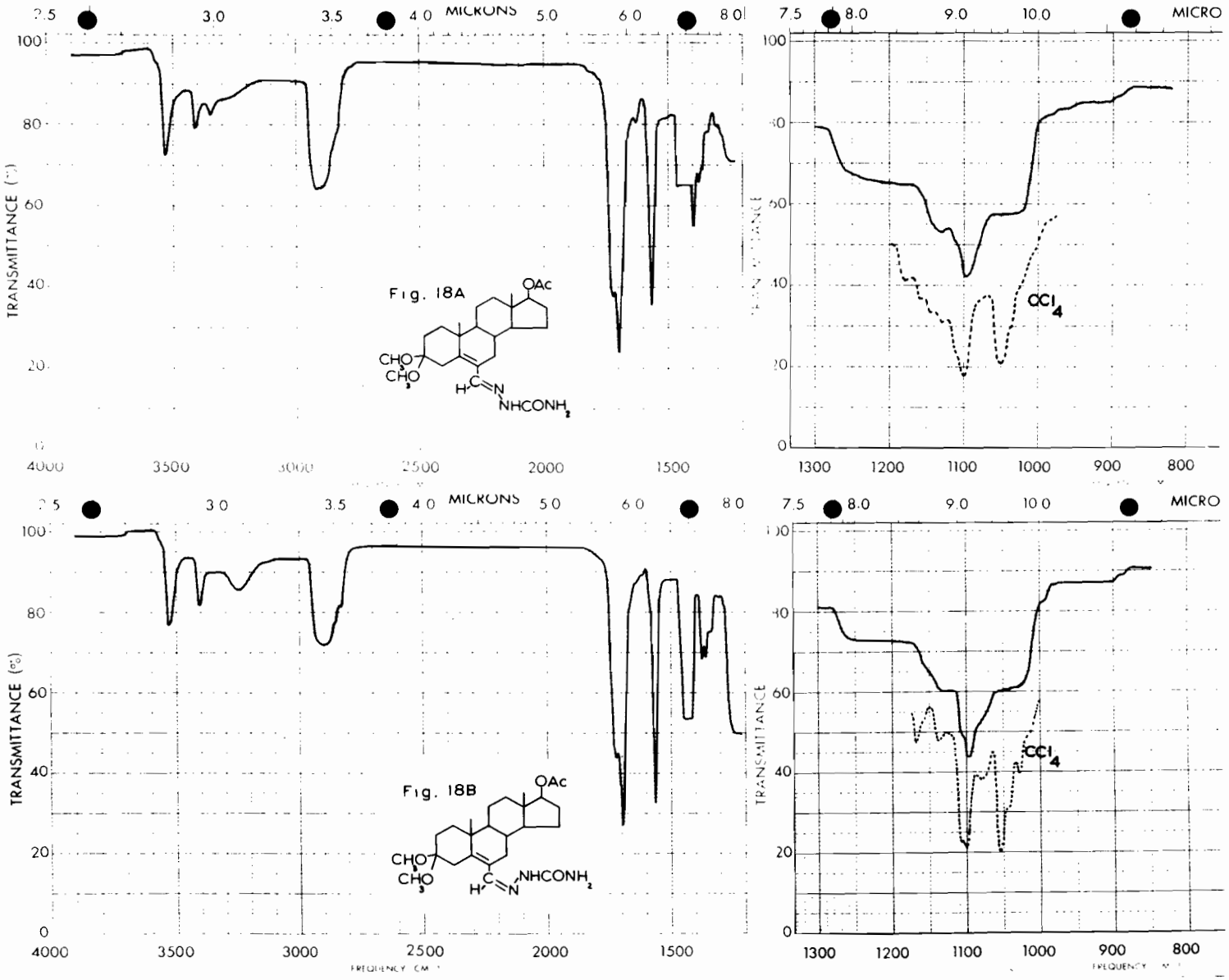

MICRONS
TRANSMITTANCE (%)
Fig. 17A
OAc
CH3O
H
C
N
NHCONH2
4000
3500
3000
2500
2000
1500
1300
1200
1100
1000
900
800
Fig. 17B
FREQUENCY CM-1

Fig. 18

The infrared spectra of

A. syn-semicarbazone ketal (XXVIII)

B. anti-semicarbazone ketal (XXIX).


MICRONS
TRANSMITTANCE (%)
CCl4
OAc
CHO
CH3
NHCONH2
Fig. 18A
Fig. 18B
FREQUENCY CM-1

until less than 5% of the original chromophore could be detected in the ultraviolet (u.v.) spectrum. The resulting mixture was separated by thin layer chromatography (t.l.c.) giving three products XXVII, XXVIII and XXIX in good yield. The structure of these compounds was elucidated through an examination of their nuclear magnetic resonance (n.m.r.) and u.v. spectra, which are summarized in Table I.

The syn-compounds* XXVI and XXVIII (Fig. 15A and 16A) show a signal at 8.12 and 8.06 p.p.m. respectively (H-C=N -), whereas the anti-compounds XXVII and XXIX (Fig. 15B and 16B) absorb at 6.81 and 6.74 p.p.m.** These signals have a similar chemical shift as those observed in the corresponding oximes tabulated in Table II. (See also ref. 37, 38, 39, 41). Syn-semicarbazone ketal XXVIII was also identified by comparison with a sample prepared from ketal aldehyde (XXII) with semi-carbazide hydrochloride. The u.v. spectra are consistent with the assignment. In particular, models show that only the syn-isomers XXVI and XXVIII can exist in a planar arrangement, and hence absorb at a longer wavelength than the corresponding anti-isomers XXVII and XXIX, in which the ureido group is sterically hindered.

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* E.L. Eliel, Stereochemistry of Carbon Compounds, p. 320, McGraw-Hill. "In an aldoxime, the syn-isomer is the one in which the hydroxyl group of the oxime is on the same side as the hydrogen of the aldehyde carbon."

**The HC= and =NNHCO n.m.r. signals of the aldehyde semi-carbazones were distinguished from each other by means of deuterium oxide exchange experiments.

Fig. 19

The ultraviolet absorption spectra of the four semicarbazones; O.D. vs. λ .

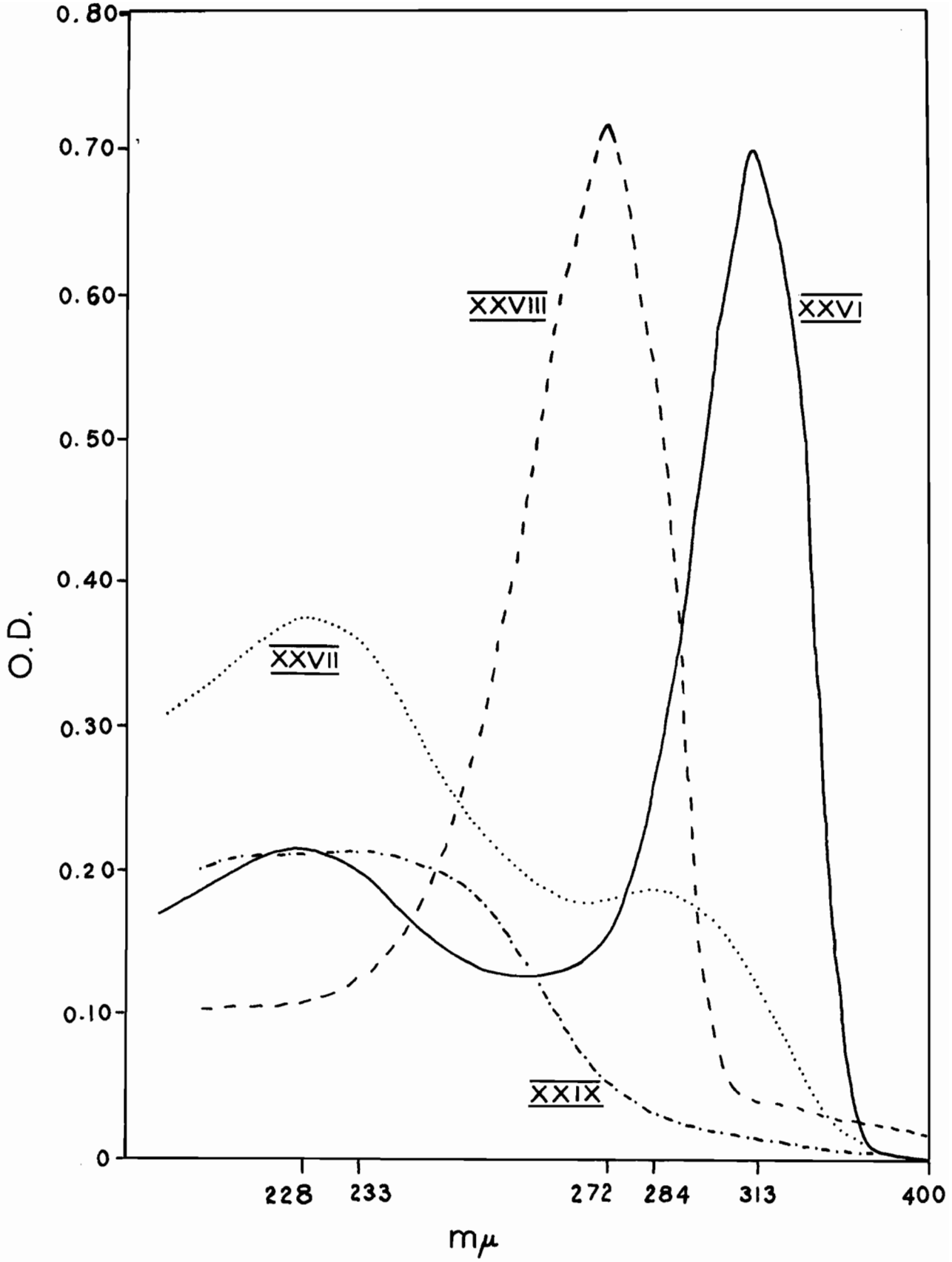


Fig. 19 Ultraviolet spectra of 2.5×10^{-5} molar methanolic solutions of syn-semicarbazone enol ether XXVI ——, anti-semicarbazone enol ether XXVII ····, syn-semicarbazone ketal XXVIII – – – –, and anti-semicarbazone ketal XXIX ·–·–·–·–.

Table I

Some spectral data of syn- and anti-semicarbazones.

Compound	H-C=N-R+ p.p.m.*	$-OCH_3$ p.p.m.	λ^{MeOH}_{max} mμ	(ε)
syn-semicarbazone enol ether (XXVI)	8.12	3.7 (3H)	313 218	(28,000) (8,600)
syn-semicarbazone ketal (XXVIII)	8.06	3.25 (3H) 3.10 (3H)	272	(28,800)
anti-semicarbazone enol ether (XXVII)	6.81	3.56 (3H)	284 230	(7,600) (15,000)
anti-semicarbazone ketal (XXIX)	6.74	3.23 (6H)	233	(8,400)

* Tetramethylsilane was used as internal reference = 0 p.p.m.

\+ R = $-NHCONH_2$.

The infrared spectrum is not very informative in distinguishing between the syn- and the anti- isomers except in that the anti-isomers displayed a peak at 1410 cm^{-1}, the intensity of which is far greater than that of the syn-compounds (Fig. 17 and Fig. 18).

Owing to the considerable overlap of the u.v. bands of the four compounds (Fig. 19), the photolysis could not be followed in a quantitative manner. Nevertheless, the

Fig. 20

The rate of disappearance of syn-semicarbazone enol ether (XXVI) and the rate of appearance of the syn- and anti- ketals (XXVIII and XXIX) and anti-enol ether (XXVII); O.D. vs. time.

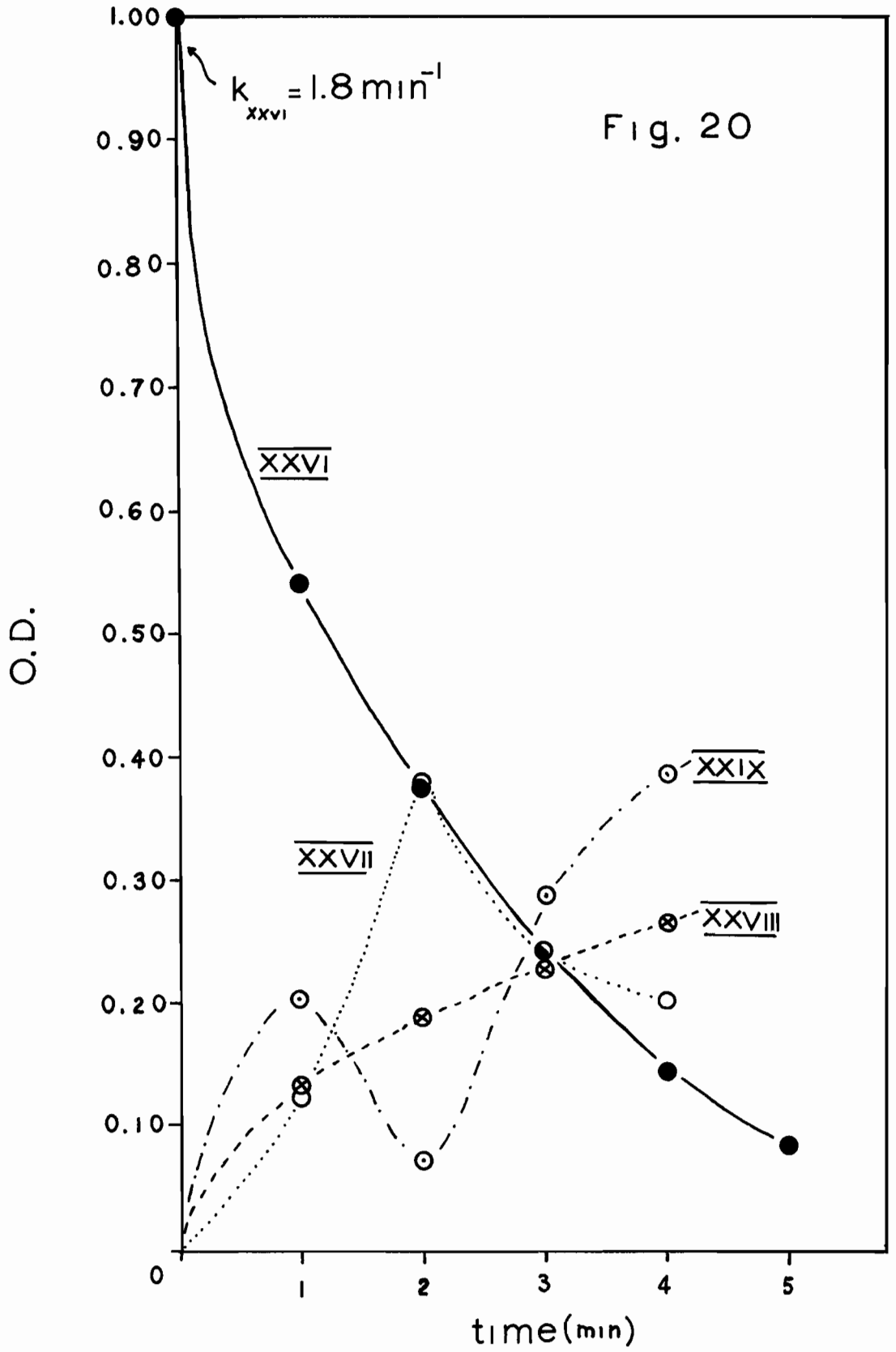

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
O.D.
$k_{XXVI} = 1.8\ min^{-1}$
XXVI
XXVII
XXIX
XXVIII
1
2
3
4
5
time(min)

Fig. 20

following semi-quantitative results were obtained. Irradiation* of a 2.5×10^{-5} molar methanolic solution of syn-semicarbazone enol ether (XXVI) resulted in a rapid disappearance of XXVI. If the concentration of XXVI at any time in the photolysis, were plotted against time (see Fig. 20) the rate of disappearance of XXVI ($k_{XXVI} = 1.8\ min^{-1}$) would be obtained from the slope of the curve at t = 0 according to the expression:

$$- \frac{d[XXVI]}{dt} = k_{XXVI}\ [XXVI]$$

Analysis of the u.v. spectrum of the reaction mixture indicated the fairly rapid appearance of ketals XXVIII and XXIX, together with a somewhat slower appearance of anti-semicarbazone enol ether (XXVII).

The inaccuracy involved in calculating the actual concentration of the four components due to the considerable overlap of the u.v. bands is exemplified in Figure 20. It can easily be seen from the appearance curve of XXVII that a large error is involved.

When XXVII was irradiated*, a rapid conversion to syn-semicarbazone enol ether (XXVI) was observed with a sub-

---

* 2537 Å light was used.

Fig. 21

The rate of disappearance of anti-semicarbazone enol ether (XXVII), and the rate of appearance of syn- and anti- ketals (XXVIII and XXIX) and syn-enol ether (XXVI); O.D. vs. time.

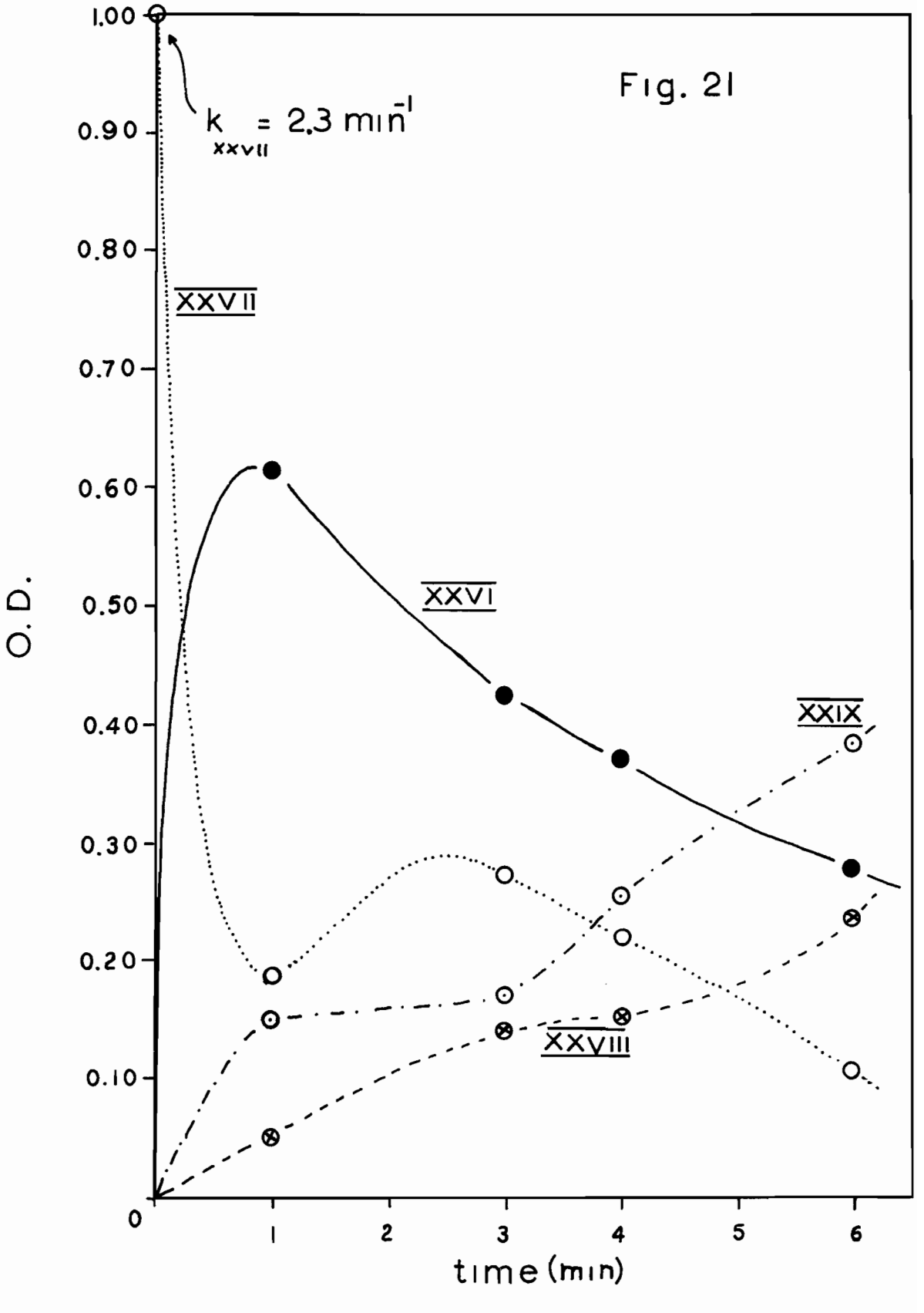


Fig. 21
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
O.D.
k XXVII = 2.3 min⁻¹
XXVII
XXVI
XXIX
XXVIII
1
2
3
4
5
6
time (min)

sequent reaction of XXVI to give ketals as discussed. From a graph (Fig. 21) containing the concentration of XXVII versus time, one can obtain the rate of disappearance of XXVII ($k_{XXVII} = 2.3\ min^{-1}$) from the slope of the curve at t = 0 according to the expression:

$$- \frac{d[XXVII]}{dt} = k_{XXVII}[XXVII]$$

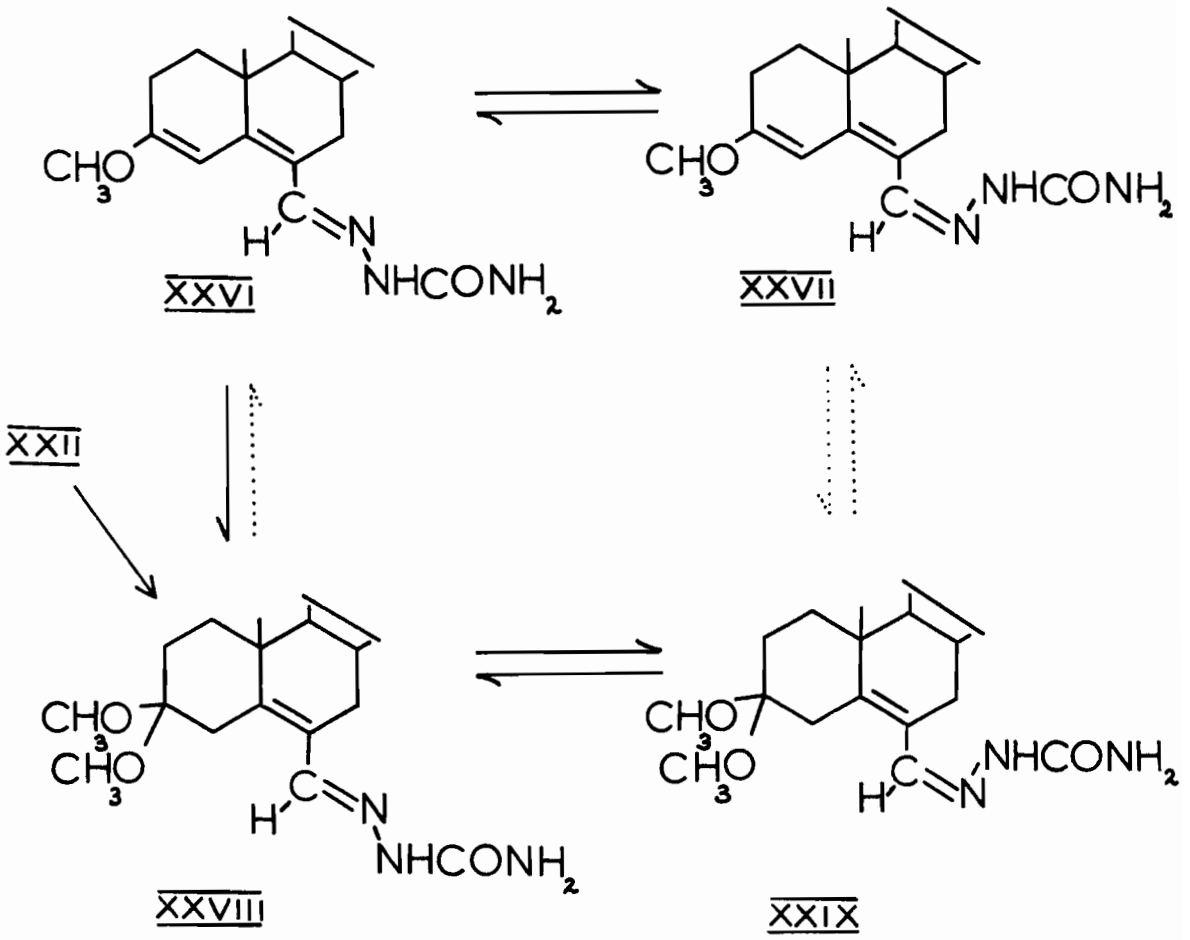
Irradiation* of either ketal (syn- or anti-) resulted in the establishment of a photoequilibrium in which the anti- to syn- ratio was approximately 2.3; since the amount of enol ether formed was below 5% in each case, it could not be determined accurately. Based on this data, the following scheme is proposed in which dotted arrows indicate slow and therefore uncertain paths. (Scheme I).

In an attempt to find out which of the two bands (313 or 228 mμ) in the u.v. spectrum of XXVI gave rise to isomerization or ketalization after excitation with u.v. light, XXVI was irradiated with 3500 Å light in which the energy output of the lamps at 228 mμ is negligible. Anti-semicarbazone enol ether (XXVII) was obtained in almost quantitative yield in 30 seconds. It therefore seems

---

* 2537 Å light was used.

Scheme I



reasonable to assume that the excitation of the 313 mμ band in XXVI leads to isomerization of the syn ⇌ anti type. The excitation of the 228 mμ band is therefore most likely responsible for ketal formation.

Fig. 22

The nuclear magnetic resonance spectra of

A. syn-oxime enol ether (XXX)

B. 1:4.6 photomixture of syn-oxime ketal (XXXIII) and anti-oxime ketal (XXXIV)

in deuteriochloroform.

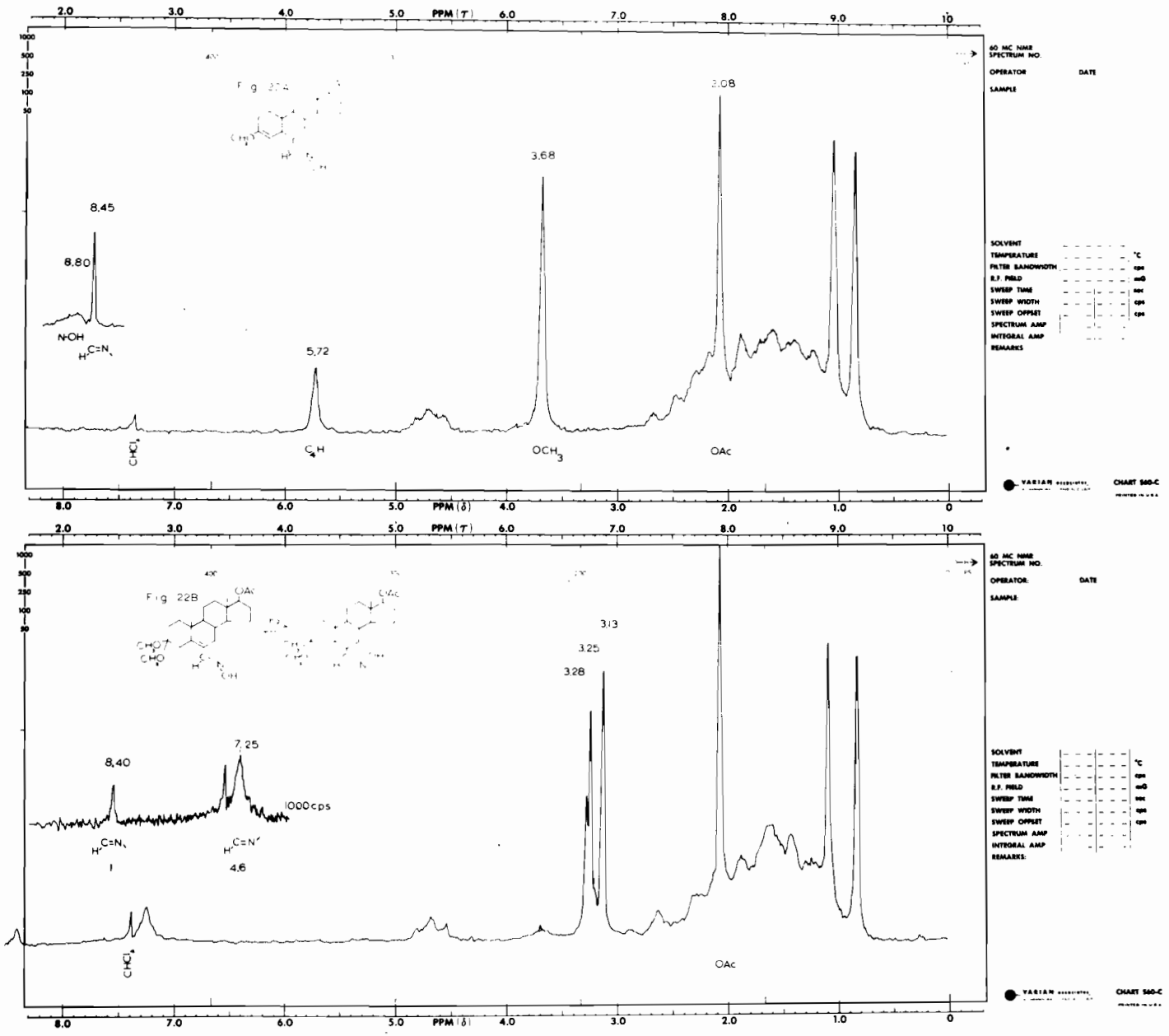

PPM (τ)
PPM (δ)
60 MC NMR SPECTRUM NO.
OPERATOR
DATE
SAMPLE
SOLVENT
TEMPERATURE
FILTER BANDWIDTH
R.F. FIELD
SWEEP TIME
SWEEP WIDTH
SWEEP OFFSET
SPECTRUM AMP
INTEGRAL AMP
REMARKS
CHART 560-C
Fig 22A
8.45
8.80
N-OH
H-C=N
5.72
3.68
3.08
CHCl3
C4H
OCH3
OAc
Fig 22B
8.40
7.25
1000 cps
1
4.6
3.13
3.25
3.28
CHCl3
OAc

Fig. 23

The infrared spectra of

A. syn-oxime enol ether (XXX)

B. syn-oxime ketal (XXXIII).

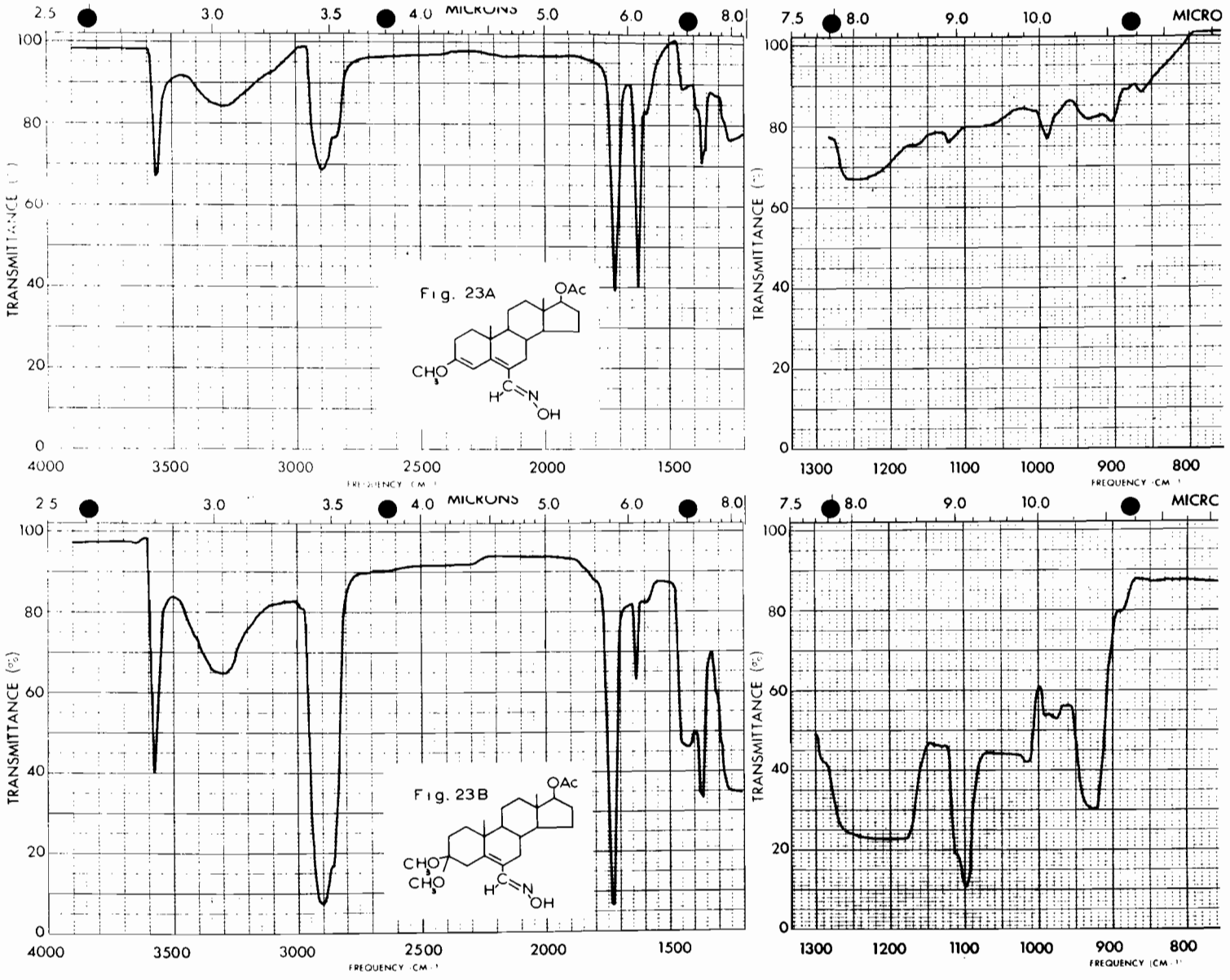

2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
100
80
60
40
20
0
TRANSMITTANCE (%)
4000
3500
3000
2500
2000
1500
FREQUENCY (CM⁻¹)
Fig. 23A
OAc
CHO
H
C
N
OH
7.5
8.0
9.0
10.0
MICRO
1300
1200
1100
1000
900
800
Fig. 23B
CHO
CHO
MICRC

Fig. 24

The nuclear magnetic resonance spectra of

A. 17β -acetoxy-6-cyano-3-methoxyandrosta-3,5-diene (XXXII)

B. 17β -acetoxy-6-cyano-3,3-dimethoxyandrost-5-ene (XXXV)

in carbon tetrachloride.

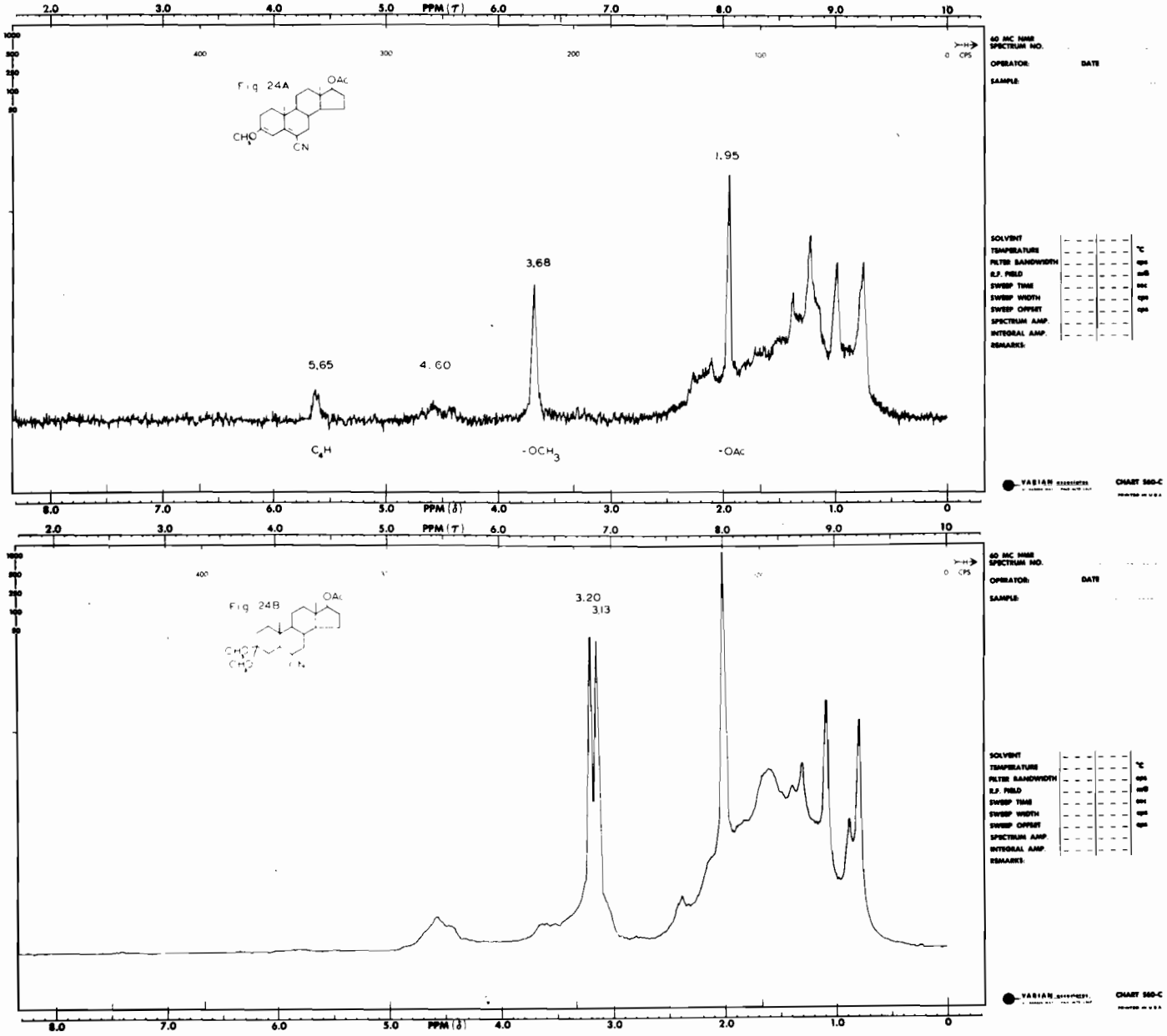

PPM (τ)
Fig. 24A
OAc
CHO
CN
1.95
3.68
5.65
4.60
C4H
-OCH3
-OAc
60 MC NMR SPECTRUM NO.
OPERATOR:
DATE
SAMPLE:
SOLVENT
TEMPERATURE
FILTER BANDWIDTH
R.F. FIELD
SWEEP TIME
SWEEP WIDTH
SWEEP OFFSET
SPECTRUM AMP.
INTEGRAL AMP.
REMARKS:
VARIAN
CHART 560-C
PPM (δ)
Fig. 24B
3.20
3.13

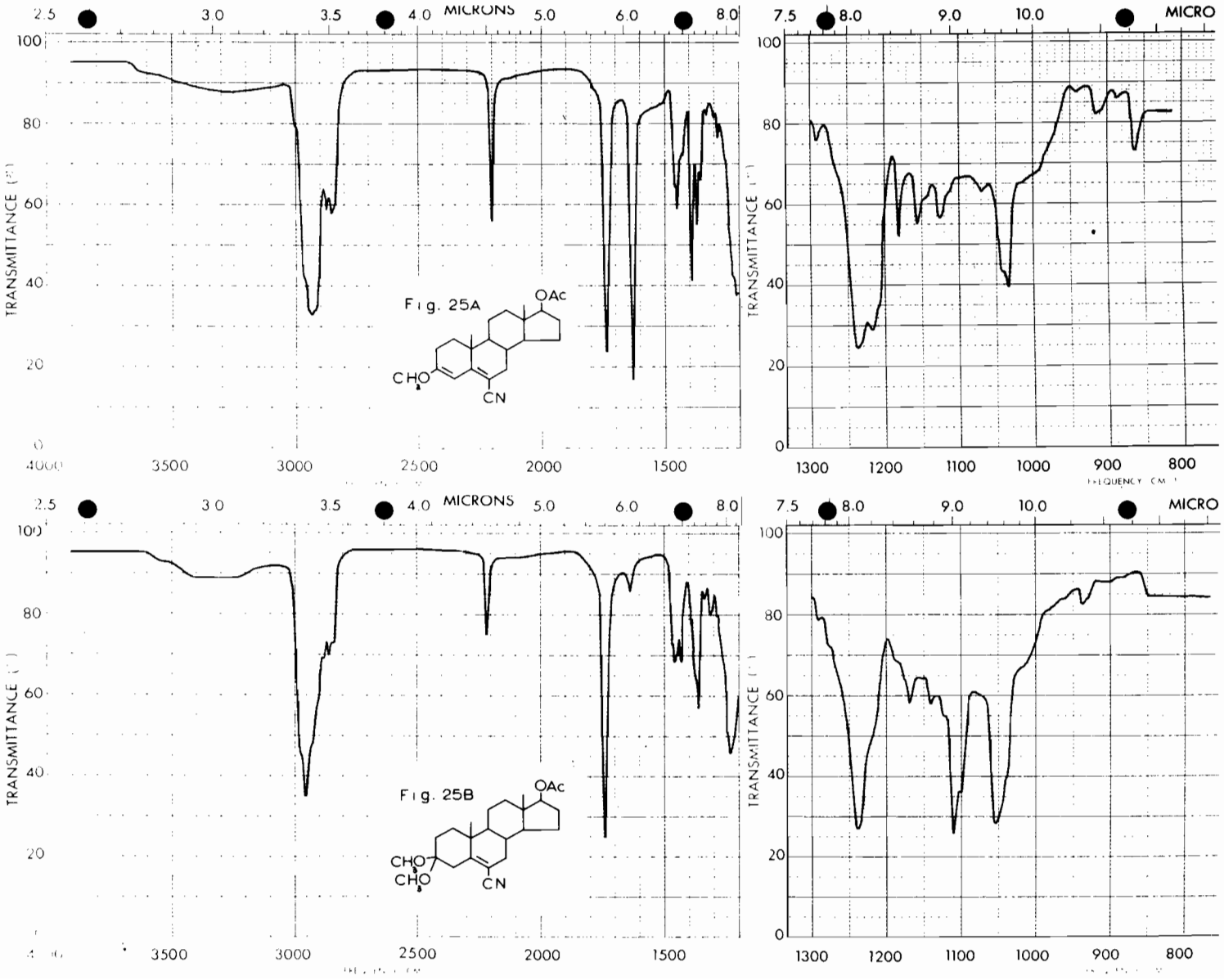
Fig. 25

The infrared spectra of

A. 17β -acetoxy-6-cyano-3-methoxyandrosta-3,5-diene (XXXII)

B. 17β -acetoxy-6-cyano-3,3-dimethoxyandrost-5-ene (XXXV)

in carbon tetrachloride.


2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
100
80
60
40
20
0
TRANSMITTANCE (%)
4000
3500
3000
2500
2000
1500
Fig. 25A
OAc
CHO
CN
7.5
8.0
9.0
10.0
MICRO
1300
1200
1100
1000
900
800
Fig. 25B
CHO
CHO
CN

Oximes

Irradiation of the oxime XXX[33] of 17β-acetoxy-6-formyl-3-methoxyandrosta-3,5-diene with 2537 Å light in absolute methanol gave a mixture, from which were isolated syn-oxime ketal XXXIII, identical with that obtained by oximation of ketal aldehyde (XXII), anti-oxime ketal (XXXIV), enol ether nitrile (XXXII), identical with a sample obtained by dehydration of XXX with acetic anhydride, and ketal nitrile (XXXV). No anti-oxime enol ether XXXI could be detected. However, XXXI could be isolated in low yield when XXX was irradiated with 3500 Å light.

The products were identified as follows: all syn-oximes had n.m.r. signals (H-C=N-) at approximately 8.4 p.p.m., whereas the anti-oximes showed a corresponding signal at higher field[38] at approximately 7.3 p.p.m. (see Table II and Fig. 22). All enol ethers showed a signal around 3.6 p.p.m. due to the presence of one methoxyl group, whereas the ketals absorbed around 3.25 and 3.13 p.p.m. corresponding to two methoxyl groups. The enol ether nitrile (Fig. 25A) and ketal nitrile (Fig. 25B) had a band in the infrared at around 2200 cm^{-1} corresponding to the -CN grouping. Scheme II contains a proposed path in the photochemical transformation of syn-oxime XXX.

Scheme II.

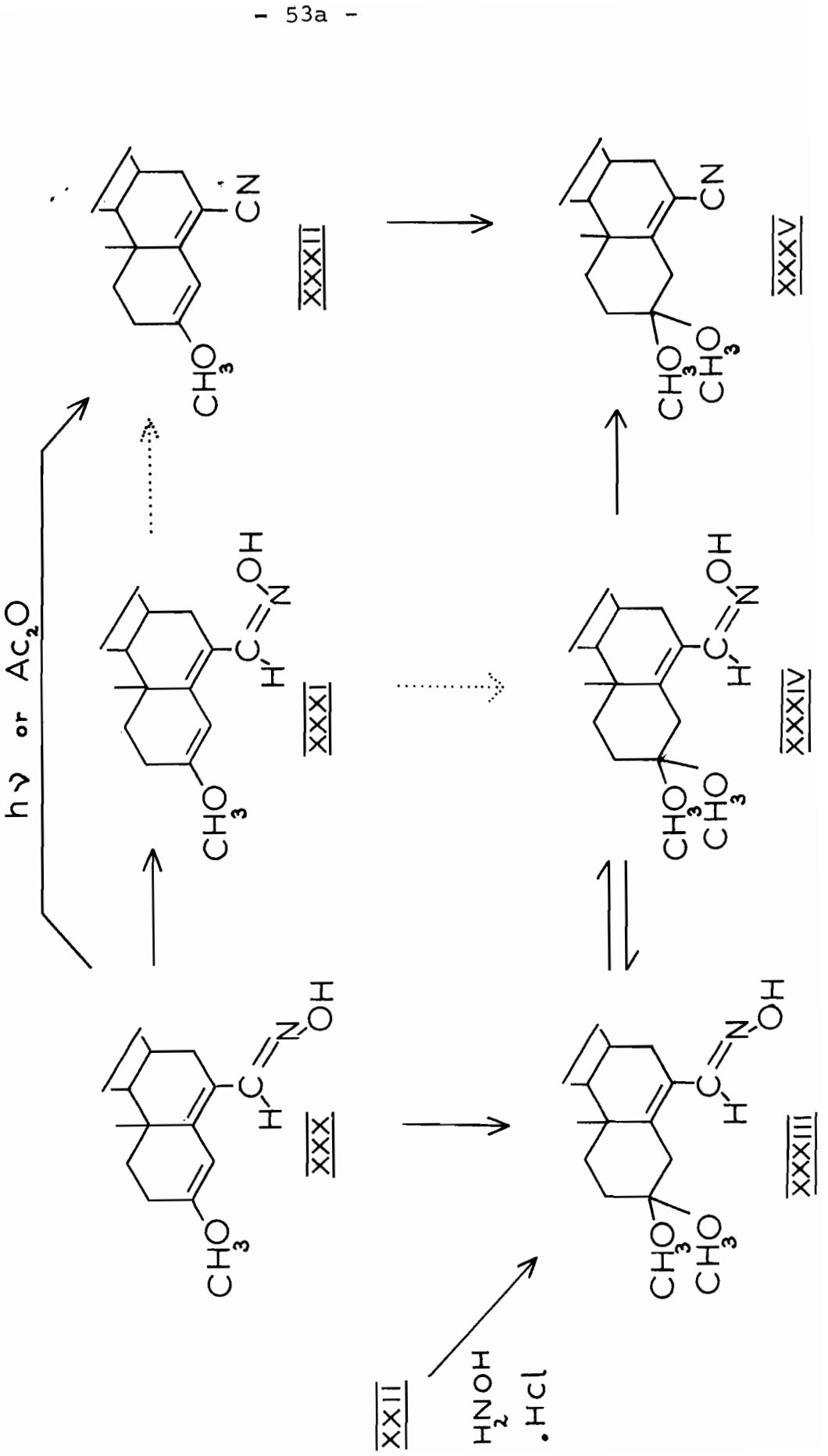


Table II

Some spectral features of the oximes and nitriles.

Compound	H-C=N-R^{+} p.p.m.*	OCH_3 p.p.m.*	λ^{MeOH}_{max} mμ	(ε)
syn-oxime enol ether (XXX)	8.45	3.68 (3H)	296 219	(20,000) (9,800)
syn-oxime ketal (XXXIII)	8.40	3.28 (3H) 3.13 (3H)	247	(17,600)
anti-oxime enol ether (XXXI)	7.30	3.65 (3H)	284 218	
anti-oxime ketal (XXXIV)	7.25	3.25 (3H) 3.13 (3H)	(245)[a]	
enol ether nitrile (XXXII)		3.68 (3H)	282 216	(22,300) (10,000)
ketal nitrile (XXXV)		3.20 (3H) 3.13 (3H)	220	(21,900)

* Tetramethylsilane was used as internal reference = 0 p.p.m.

\+ R = -OH

[a] The spectrum of XXXIV was taken on a mixture containing XXXIII. Irradiation of syn-oxime ketal (XXXIII) produces a rapid photoequilibrium in which the anti- to syn- ketal ratio is 4.6 as determined by the relative area of the HC=N- n.m.r. signals at 7.25 and 8.40 p.p.m. respectively.

The anti-oxime compounds (ketal and enol ether) were converted to their syn- isomers upon crystallization from ether-pyridine. Prolonged irradiation of all oximes resulted in the formation of ketal nitrile, which slowly decomposed upon further irradiation to a mixture of unidentified products.

B. Experimental*

Semicarbazones

Semicarbazone of 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XXVI).

Water was added dropwise to a hot methanolic solution (25 ml.) of 17β -acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XX) (500 mg.) until crystals started to appear. The aqueous methanolic solution was then heated and 500 mg. of semicarbazide hydrochloride and 700 mg. of sodium acetate were added. The contents were swirled until a solution was obtained. Upon cooling, small light yellow needles of XXVI separated. Filtration gave 457 mg., m.p. 237-239° (decomp.) ν_{CHCl_3} (Fig. 17A) 3530, 3410, 3350 cm^{-1} (NH-CO-NH_2), 1725 cm^{-1} (-OAc), 1685 cm^{-1} (N-CO-N), 1630 cm^{-1} (C=C-OCH_3), 1560 cm^{-1} (C=N); δ_{CDCl_3} (Fig. 15A) 10.3 (singlet, 1H, = N-N<u>H</u>-CO), 8.12 (singlet, 1H, H-C=N-), 6.00 (broad singlet, 2H, N-CO-N<u>H</u>$_2$), 5.71 (singlet, 1H, = C_4<u>H</u>), 3.70 (singlet, 3H, -O<u>CH</u>$_3$), 2.08 p.p.m. (singlet, 3H, acetate); λ_{max}^{MeOH} 313 mμ (ϵ 28,000) and 228 mμ (ϵ 8600).

Calc. for $C_{24}H_{35}N_3O_4$: C, 67.10; H, 8.21; N, 9.78.

Found: C, 67.04; H, 7.97; N, 9.59.

Irradiation of XXVI

A solution of 350 mg. of XXVI in 75 ml. absolute methanol was irradiated for three hours at which time the

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* See Chapter I for general procedures.

rate of disappearance of the 313 mμ band in the u.v. decreased appreciably. XXVI (170 mg.) crystallized upon concentration of the photolysis solution. Purification of the mother liquor by preparative t.l.c. using ether-methanol (96%) as the developing solvent, gave 48 mg. of an amorphous compound identified as anti-semicarbazone enol ether (XXVII)*, ν_{CHCl_3} (Fig. 17B), 3534, 3410, 3350 cm^{-1} ($NH-CO-NH_2$), 1725 cm^{-1} (-OAc), 1685 cm^{-1} (N-CO-N), 1641 cm^{-1} ($C=C-OCH_3$), 1560 cm^{-1} (C=N), 1425 cm^{-1}; δ_{CDCl_3} (Fig. 15B) 7.80 (singlet, 1H, N-N<u>H</u>-CO), 6.81 (singlet, 1H, <u>H</u>-C=N), 5.83 (broad singlet, 2H, N-CO-N<u>H</u>$_2$), 4.93 (singlet, 1H, =C_4<u>H</u>), 3.56 (singlet, 3H, -OC<u>H</u>$_3$), 2.05 p.p.m. (singlet, 3H, acetate); λ_{max}^{MeOH} 284 mμ (ε 7600) and 230 mμ (ε 15,000).

Calc. for $C_{24}H_{35}N_3O_4$: C, 67.10; H, 8.21; N, 9.78.
Found: C, 66.31; H, 8.49; N, 10.15.

A further irradiation of 170 mg. XXVI for 13 hours gave, after separation by t.l.c.: 9 mg. of anti-enol ether XXVII; 52 mg. of an amorphous anti-ketal XXIX,* ν_{CHCl_3} (Fig. 18B) 3525, 3405, 3240 cm^{-1} ($NH-CO-NH_2$), 1725 cm^{-1} (-OAc), 1690 cm^{-1} (N-CO-N), 1560 cm^{-1} (C=N), 1425 cm^{-1}, and 1100 cm^{-1} (ketal); ν_{CCl_4} 1105 and 1055 cm^{-1} (ketal); δ_{CDCl_3} (Fig. 16B) 9.15 (singlet, 1H, N-N<u>H</u>-CO), 6.74 (singlet, 1H, <u>H</u>-C=N), 5.61 (broad singlet, 2H, N-CO-N<u>H</u>$_2$), 3.23 (singlet, 6H, ketal), and 2.1 p.p.m. (singlet, 3H, acetate); λ_{max}^{MeOH} 233 mμ (ε 8400);

Calc. for $C_{25}H_{39}N_3O_5$: C, 65.05; H, 8.52; N, 9.10.
Found: C, 64.55; H, 8.69; N, 9.21.

---

* This compound could only be obtained in an amorphous state.

35 mg. of syn-ketal XXVIII, m.p. 223° (decomp.), ν_{CHCl_3} (Fig. 18A) 3525, 3405, 3345 cm^{-1} ($NH-CO-NH_2$), 1725 cm^{-1} (-OAc), 1690 cm^{-1} (N-CO-N), 1560 cm^{-1} (C=N), 1100 cm^{-1} (-ketal); ν_{CCl_4} 1100 and 1050 cm^{-1} (ketal); δ_{CDCl_3} (Fig. 16A) 9.50 (broad singlet, 1H, N-NH-CO), 8.06 (broad singlet, 1H, $\underline{H}$-C=N), 5.75 (broad signal), 2H, N-CO-$N\underline{H}_2$), 3.25 (singlet, 3H, -O$C\underline{H}_3$), 3.10 (singlet, 3H, -O$C\underline{H}_3$), 2.05 (singlet, 3H, acetate); λ_{max}^{MeOH} 272 mμ (ε28,800).

Calc. for $C_{25}H_{39}N_3O_5$: C, 65.05; H, 8.52; N, 9.10
Found: C, 64.84; H, 8.61; N, 9.68.

Preparation of syn-semicarbazone ketal XXVIII from 17β-acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII).

The procedure was the same as that used in the preparation of XXVI. The compound obtained had an infrared spectrum superimposable with that of the photoproduct XXVIII. No depression in melting point was observed when the two compounds were admixed.

Kinetic experiments

Magnetically stirred solutions (200 ml.) of XXVI, XXVII, XXVIII, XXIX (2.5×10^{-5} molar) in absolute methanol were separately irradiated in a helium atmosphere in a quartz cell fitted with a cold water jacket and a cold water condenser. The photochemical reaction was followed by the withdrawal of a 3 ml. aliquot at various time intervals and the recording of the disappearance of the original chromophore on a Beckman DKl recording spectrophotometer. In a separate experiment XXVI was irradiated with 3500 Å light, in which case the spectrum recorded, after 30 seconds of irradiation, displayed a curve almost identical in shape and intensity

with that obtained from a 2.5 x 10^{-5} molar solution of XXVII, indicating an almost complete transformation to this compound.

Oximes

17β -Acetoxy-6-cyano-3-methoxyandrosta-3,5-diene (XXXII).[33]

A mixture of enol ether oxime XXX (104 mg.) in acetic anhydride (4 ml.) was heated at 98° on a water bath for 80 minutes, after which time the acetic anhydride was stripped off in vacuo, giving an oil from which crystals appeared upon addition of methanol. Recrystallization from methanol containing a few drops of pyridine gave 62 mg. of XXXII, m.p. 194.5-195°; ν_{CCl_4} (Fig. 25A) 2200 cm^{-1} (-CN), 1740, 1230, 1050 cm^{-1} (-OAc), 1630 cm^{-1} (CH_3O-C=C), 1180, 1155, 1125 cm^{-1} (ether); δ_{CCl_3} (Fig. 24A) 5.65 (singlet, 1H =C_4-H), 3.68 (singlet, 3H, CH_3O-), 1.95 (singlet, 3H); λ_{max}^{MeOH} 282 mμ (ϵ 22,300) and 216 mμ (ϵ10,000).

Calc. for $C_{23}H_{31}O_3N$: C, 74.76; H, 8.46; N, 3.79.
Found: C, 74.79; H, 8.46; N, 3.66.

17β -Acetoxy-6-cyano-3,3-dimethoxyandrost-5-ene (XXXV).

A solution of 150 mg. of 17β -acetoxy-6-cyano-3-methoxyandrosta-3,5-diene (XXXII) was irradiated in 37 ml. absolute methanol for 15 hours, until no starting material could be detected by t.l.c. using benzene-ether (8:2) as

developing solvent. The photoproduct consisted of one component, as revealed by t.l.c. Evaporation of the solvent gave an oil which was purified by preparative t.l.c. Recrystallization from methanol containing a few drops of pyridine gave XXXV, m.p., 183-184°; ν_{CCl_4} (Fig. 25B) 2210 cm^{-1} (-CN), 1740, 1240 cm^{-1} (-OAc), 1640 cm^{-1} (C=C), 1110, 1050 cm^{-1} (ketal); δ_{CCl_4} 3.20 (singlet, 3H, $-O\underline{CH_3}$), 3.13 (singlet, 3H, $-O\underline{CH_3}$), 2.00 p.p.m. (singlet, 3H); λ_{max}^{MeOH} 220 mμ (ε 21,900).

Calc. for $C_{24}H_{35}NO_4$: C, 71.79; H, 8.79; N, 3.49.

Found: C, 71.54; H, 8.68; N, 3.43.

In a different run, when the photolysis was stopped at an earlier stage, an intermediate could be isolated which decomposed upon standing.

Oxime of 17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXXIII).

A solution of 194 mg. of 17β -acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII) in 30 ml. methanol containing 10 ml. pyridine and 194 mg. hydroxylamine hydrochloride was refluxed for 0.5 hours, followed by an ether extraction and washing of the organic layer with water several times. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness giving a crystalline residue. Two recrystallizations from ether-pyridine gave XXXIII, m.p. 204° (decomp.); λ_{max}^{MeOH} 247 mμ (ε 17,600);

ν_{CHCl_3} (Fig. 23B) 3570, 3300 cm^{-1} (=NOH), 1725 cm^{-1} (-OAc), 1630 cm^{-1} (C=C), 1100 cm^{-1} (ketal); δ_{CDCl_3} (Fig. 22B) 8.40 (singlet, H-C=N), 3.28, 3.13 (ketal), 2.10 p.p.m. (acetate). A decomposition of XXXIII to enol ether XXX took place upon heating in $CDCl_3$.

Calc. for $C_{24}H_{37}NO_5$: C, 68.70; H, 8.89; N, 3.79.
Found: C, 68.45; H, 9.22; N, 3.31.

Irradiation of the oxime of 17β-acetoxy-6-formyl-3-methoxyandrosta-3,5-diene (XXX).

A solution of 171 mg. of XXX in 80 ml. absolute methanol was irradiated in a helium atmosphere. After three hours the u.v. spectrum showed a band at 284 mμ instead of the 294 mμ band present in the starting material XXX. T.l.c. using benzene-ether (8:2) revealed the presence of four compounds. The faster moving product was identified as XXXIII since its i.r. was superimposable with that obtained by dehydration of the syn-oxime enol ether (XXX) described above. No depression in melting point was evident upon admixture of the two compounds. The next component was identified as ketal nitrile XXXV, by its i.r. spectrum and mixture melting point. Elution of the slowest moving compound (Rf. approx. 0.1) gave a semi-crystalline oil which exhibited two spots by t.l.c. (Rf. = 0.1 and 0.2 approx.). Upon recrystallization from ether containing a few drops of pyridine the original compound was converted completely to the higher Rf. component, m.p. 198-199°. Two recrystalliza-

tions from ether-pyridine gave XXXIII, m.p. 204° (decomp.), identical with syn-oxime ketal prepared by oximation of 17β-acetoxy-6-formyl-3,3-dimethoxyandrost-5-ene (XXII), described above. The compound with Rf. = 0.1 (anti-oxime ketal XII)* could also be obtained in a mixture with ketal syn-oxime XXXIII by irradiation of XXXIII.

Irradiation of XXXIII. Photoequilibrium between ketals XXXIII and XXXIV.

Ketal syn-oxime XXXIII (45 mg.) was irradiated in absolute methanol (10 ml.) for 10 minutes after which time t.l.c. (benzene-ether 8:2) revealed two components (Rf = 0.1, 0.2 approx.) and a faint spot (Rf = 0.5 approx.) due to the formation of ketal nitrile (XXXV). The solvent was stripped off, giving a crystalline residue; λ_{max}^{MeOH} 240-245 mμ; δ_{CDCl_3} 8.40 (syn, H-C=N-), 7.25 (anti, HC=N-), 3.28, (syn, $-OCH_3$), 3.13 (syn, $-OCH_3$), 3.25 (anti, $-OCH_3$), 3.13 (anti, $-OCH_3$), 2.08 p.p.m. (acetate). The relative area of the signals at 8.40 and 7.25 p.p.m. indicated a syn:anti ratio of 1:4.6.

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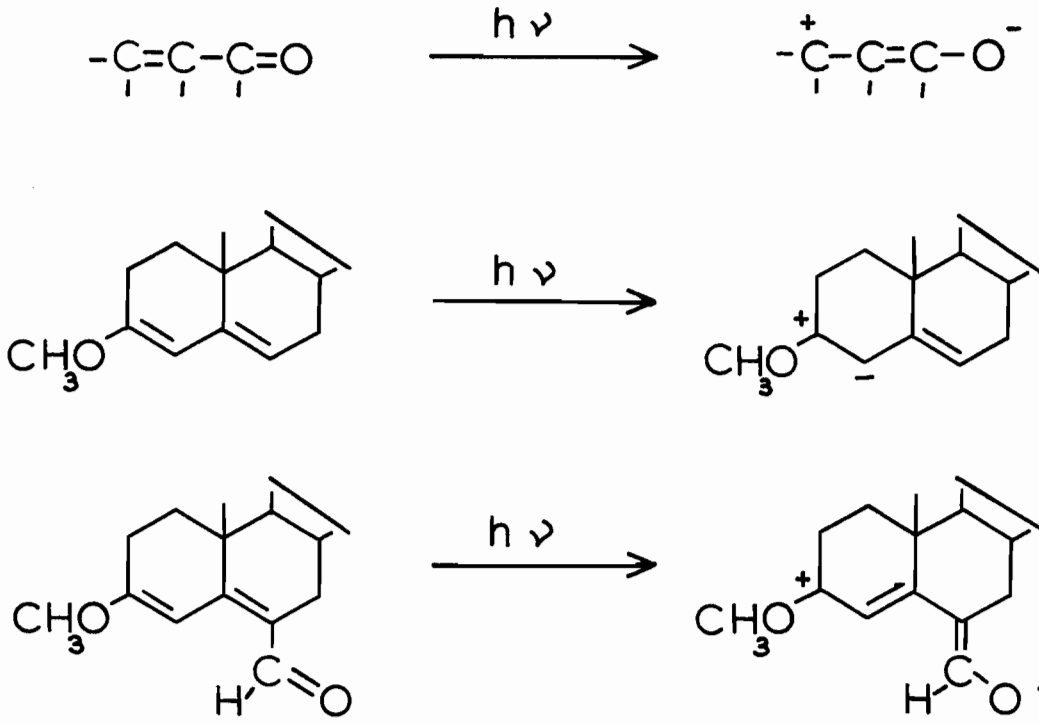
* The ketal anti-oxime (XII) could not be obtained in pure form since t.l.c. converted it to a mixture of syn- and anti-, and any attempt to crystallize the compound led to its conversion to pure syn-oxime ketal. Characterization of the mixture into syn- and anti- was done by n.m.r. spectra.

Discussion

In the previous sections, it was shown that the photolysis of 3-methoxy-6-formylandrost-3,5-diene or its 6-hydroxymethyl, oxime, nitrile, or semicarbazone derivatives in methanol resulted in the formation of the corresponding 3,3-dimethoxy-androst-5-enes. In the case of the oxime and semicarbazone (enol ether or ketal), the corresponding geometrical isomer was also obtained. A photodehydration resulted when the enol ether oxime and ketal oxime were irradiated. When the syn-semicarbazone enol ether was irradiated with 253.7 mμ light, syn- and anti-ketals, and anti-enol ether were obtained; however, when the syn-semicarbazone enol ether was irradiated with 350.0 mμ light, it rapidly isomerized to the anti-semicarbazone enol ether. Therefore it seems likely that excitation of the short wavelength band (228 mμ) of the syn-enol ether leads to ketal formation, whereas the excitation of the longer wavelength band leads to isomerization.

It has previously been stated[23] that photochemical reactions of α,β-unsaturated carbonyl compounds can be rationalized through a Zwitterionic-type representation in which the β-carbon atom bears a positive charge and the oxygen atom a negative charge. If this is so, and if it can

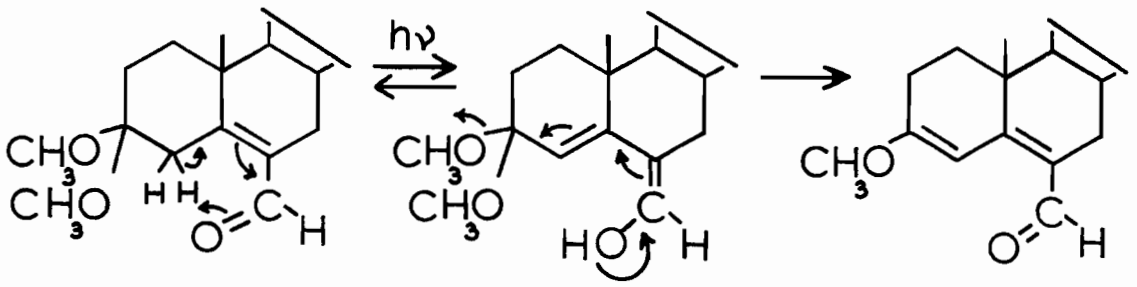


be applied to the enol ether aldehyde chromophore, a positive centre would be set up at the C_3 position. However, Leznoff[40] has already established that an electrophylic centre is set up at C_3, upon irradiation of 3-alkoxycholesta-3,5-dienes. Therefore it would seem that the introduction of a formyl group at C_6 would result in the enhancement of the electrophilicity of the C_3 position and therefore of ketal formation. This has been found to be the case, as only one product (by t.l.c.) was obtained.

A factor contributing to the cleanness of the reaction is probably the small amount of participation of

the C_{5-6} double bond which is now held in conjugation with the 6-formyl group. In 3-methoxycholesta-3,5-diene, the relatively low yield (60%) of ketal was attributed to the probable participation of the Δ^5 double bond, resulting in a photo-induced hydrolysis.

Irradiation of ketal aldehyde XXII has been shown to produce the enol ether aldehyde XX (see Chapter I). This can easily be rationalized as a γ -hydrogen abstraction by a photoexcited carbonyl group[21], with subsequent elimination



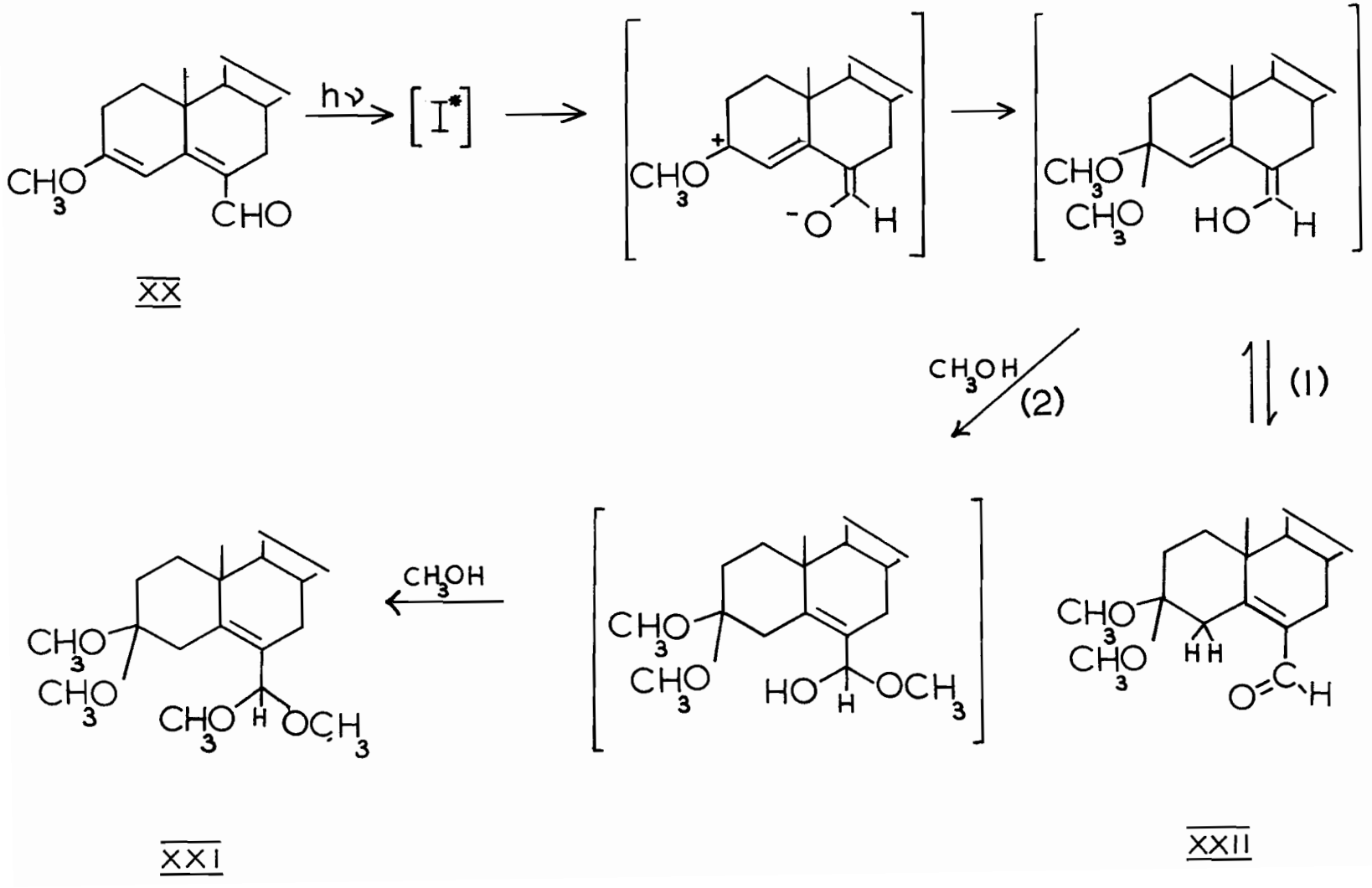
XXII XX

of a methoxyl group from C_3.

Irradiation of XX was shown to produce ketal acetal XXI, with XXII as an intermediate. This probably involves a polar intermediate in which methanol is first incorporated in the C_3 position, followed by two possibilities, (see Scheme I),

(1) either protonation at C_4 initiated by the C_6 enol, leading to compound XXII, which can give back XX by a γ -hydrogen abstraction as indicated above,

Scheme I



XX

XXI

XXII

(2) or, protonation at C_4 initiated by a methoxyl attack at C_6, resulting in a hemiacetal at C_6, followed by acetal formation.

The oxime and semicarbazone gave the respective ketals and their geometrical isomers upon irradiation. In the kinetic approach (Chapter II) towards establishing the reaction path of the semicarbazone photolysis*, it was found that syn-semicarbazone enol ether (λ_{max} 313 mμ, 228 mμ) gave anti-semicarbazone enol ether (λ_{max} 282 mμ , 230 mμ) rapidly within the first few minutes of reaction, together with a slower formation of the two ketals (syn-, λ_{max} 272 mμ and anti-, λ_{max} 233 mμ). However, when the anti-semicarbazone enol ether was irradiated, a very rapid conversion to syn- took place. Similarly when either syn- or anti- semicarbazone ketal was irradiated, a fairly rapid photoequilibrium resulted. (In the case of the ketal oximes, a similar photoequilibrium was also observed, but this was further complicated by a photodehydration to the ketal nitrile).

Thus, a photoequilibrium exists in all the cases, enol ethers and ketals, which seems to be in favour of the anti-isomer in the case of the ketals, and in favour of the syn- with the enol ethers.

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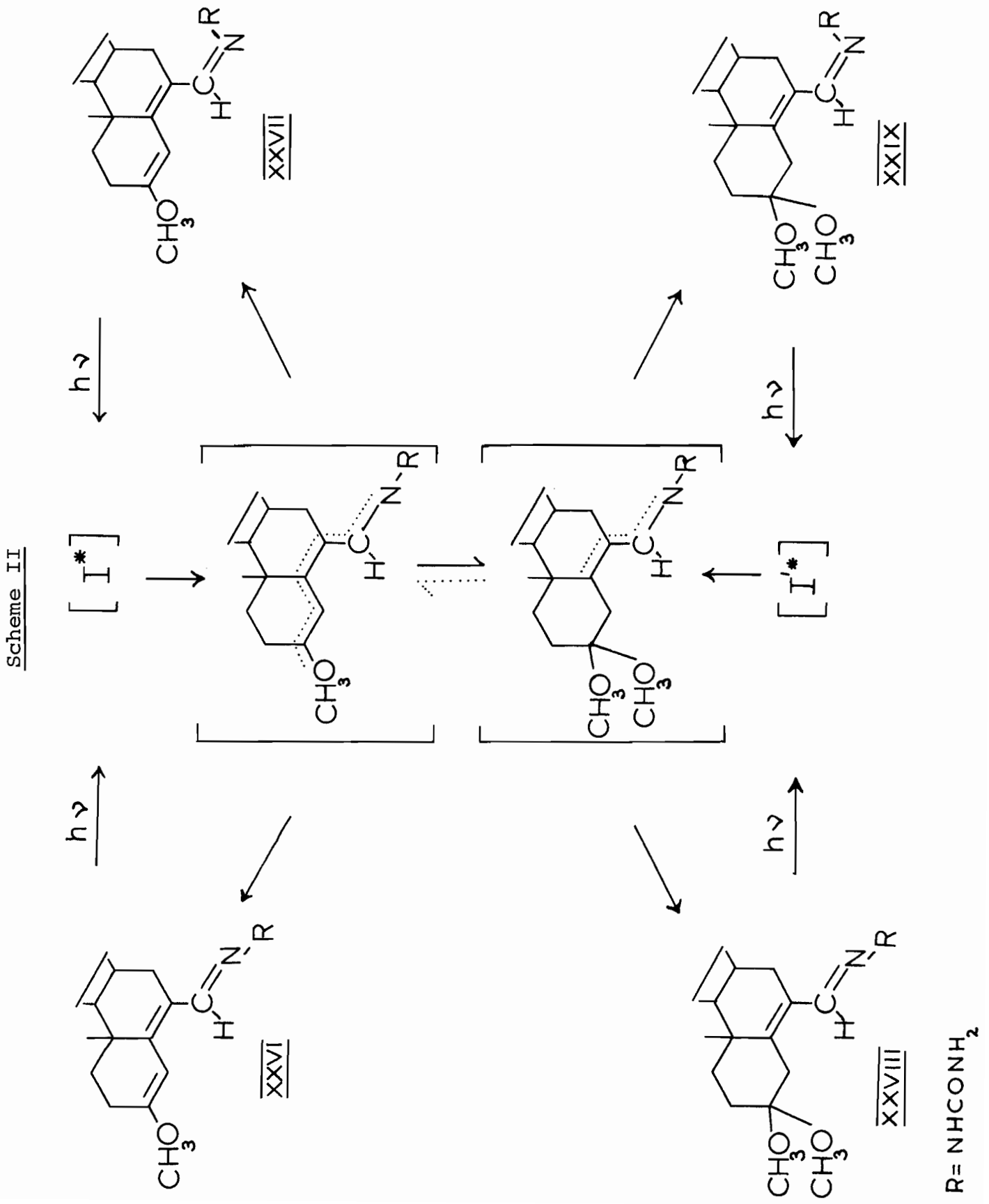
* 2537 Å light was used in the kinetic experiments.

Scheme II



This photoequilibrium can probably be rationalized best by postulating a photoexcited intermediate which must be symmetrical about the C-N bond since the intermediate must be formed from and decompose to either isomer. Furthermore, it must also be such as to be able to react with the solvent to provide ketals. A similar photoexcited intermediate must also be postulated in the case of the ketals.

Scheme II contains a general path for the irradiation of syn-semicarbazone enol ether (XXVI).

The rate of disappearance of a compound depends upon its rate of excitation and therefore upon its absorption maximum and the intensity of the light used at that wavelength. Therefore it seems probable that the enol ether semicarbazone (syn- or anti-) is excited to a symmetrical intermediate at a rate according to its absorption maximum, which then decomposes (in a dark reaction) at a definite ratio to the syn- and anti- isomers. It can also react with the solvent to give another excited intermediate which can similarly decompose in the dark at a definite ratio to give ketals (syn- and anti-). Since the wavelength of the light used is 253.7 mμ, it would therefore be expected that the syn-semicarbazone enol ether (λ_{max} 313 mμ, 228 mμ) would be excited at a rate slower than its anti-isomer (λ_{max} 282 mμ, 230 mμ). This has been

found experimentally. One would not expect to isolate the anti-enol ether during the photolysis of the syn-semicarbazone enol ether, since the former has been shown to disappear faster than the latter compound. The detection of the anti-enol ether can probably be rationalized as follows: The concentration of a compound at any time during photolysis depends mainly upon two factors,

a) its rate of disappearance (through excitation, followed by reaction), and

b) its rate of appearance (through a dark decomposition of the excited intermediate(s) involved).

The symmetrical intermediate obtained from the photolysis of the syn-enol ether decomposes in three ways: back to syn-enol ether, to anti-enol ether, and to ketals. The results obtained indicate the appearance of anti-enol ether at the beginning of the reaction together with the gradual formation of ketals as the reaction proceeds, at the expense of the enol ether isomers. The presence of anti-enol ether can be accounted for if the photoexcited intermediate decomposes in favour of this compound rather than its syn-isomer. As the reaction proceeds a transformation to ketals takes place. Again, a photoexcited symmetrical intermediate exists between the ketals, the decomposition of which (in the dark) determines the ratio of ketal isomers (syn:anti) present. The results

obtained indicate an equilibrium in favour of the anti-isomer in both enol ethers and ketals.

Activation of a chromophore is faster, the closer its absorption maximum lies to that of the light used. The syn-semicarbazone enol ether has absorption maxima at 228 mμ and 313 mμ. Irradiation of this compound with light having a maximum intensity at 253.7 mμ would excite both bands to some extent since there is never a complete cut-off of ultraviolet light in these regions. However, if light with a maximum intensity at 350 mμ is used, then one would expect activation of the longer wavelength band (313 mμ) to occur mainly if not solely. Examination of the product of this photolysis would provide information as to the type of excitation that has taken place. The product of irradiation of syn-semicarbazone enol ether with 3500 Å light gave within 30 seconds an almost complete conversion to anti-semicarbazone enol ether. This probably means that excitation of the longer wavelength u.v. band of the chromophore gives rise to an intermediate in which only geometrical isomerism is favoured. Excitation of this intermediate with shorter wavelength light transforms this intermediate to another one in which ketalization is favoured (as shown with the 253.7 mμ light). When the enol ether aldehyde chromophore was irradiated with 3500 Å light for more than 24 hours no reaction was detected (t.l.c., u.v.) because no geometrical isomerism could occur.

Irradiation of the enol ether oximes similarly gave ketals and their geometric isomers. However a further reaction took place. The oximes were found to undergo a photodehydration to the nitriles. This occurred with both enol ether oxime and ketal oxime. In fact the end product of the photolysis of the syn-oxime enol ether is the ketal nitrile. In agreement with the semicarbazones, an equilibrium exists between the syn- and anti-oxime ketals, in favour of the anti-.

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PART II

SOME CHEMICAL CONSTITUENTS OF C. PARAPSILOSIS AND C. ALBICANS

Materials and Methods

a) Candida parapsilosis 17*

Medium

5% Cerelose	0.05% $MgSO_4 \cdot 7H_2O$
0.5% Proteose-peptone	(Difco)
0.1% KH_2PO_4	0.25% Yeast Extract

100 flasks 250-300 ml. of medium. Ingredients were prepared, distributed into the flasks, and each was autoclaved for 20 minutes at 15 lbs./sq.in. pressure and 121°C.

Inoculum: Ten sabouraud glucose slopes with a two-three day growth of the organism. The growth in each tube was suspended in sterile water, pooled and 2 ml. of the pooled suspension added to each of 24 flasks.

These flasks were incubated on a rotary shaker at 37°C, for 24 hours, after which they were removed and left to stand overnight at room temperature, to allow the organism to sediment. The old medium was then decanted and 250 to 300 ml. of fresh medium added. This process was repeated twice. Following the third transfer the cultures were autoclaved and the yeast cells collected on a Buchner funnel.

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* The author wishes to thank Dr. F. Blank for supplying the data for this section.

b) Candida albicans (Group B)*

The yeast cells were cultured in twenty liter carboys utilizing 2% glucose, 1% neopeptone broth as a growth medium. Sixteen liters of medium were placed in each carboy. 5 ml. of a silicon antifoaming agent (General Electric) were added to each carboy and they were autoclaved for one hour at 15 lbs. pressure (121^{o}C). After the medium had cooled it was inoculated with C. albicans, sterile air bubbled through, and incubated for 48 hours at a temperature of 24-26^{o}C. The growth was harvested in a continuous flow Sorval RC2 refrigerated centrifuge operating at 15,000 rpm. The cells were washed and freeze-dried.

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* The author wishes to thank Dr. H.F. Hasenclever for supplying the information in this section.

Fig. 1

The infrared spectrum of aliphatic amine (A).

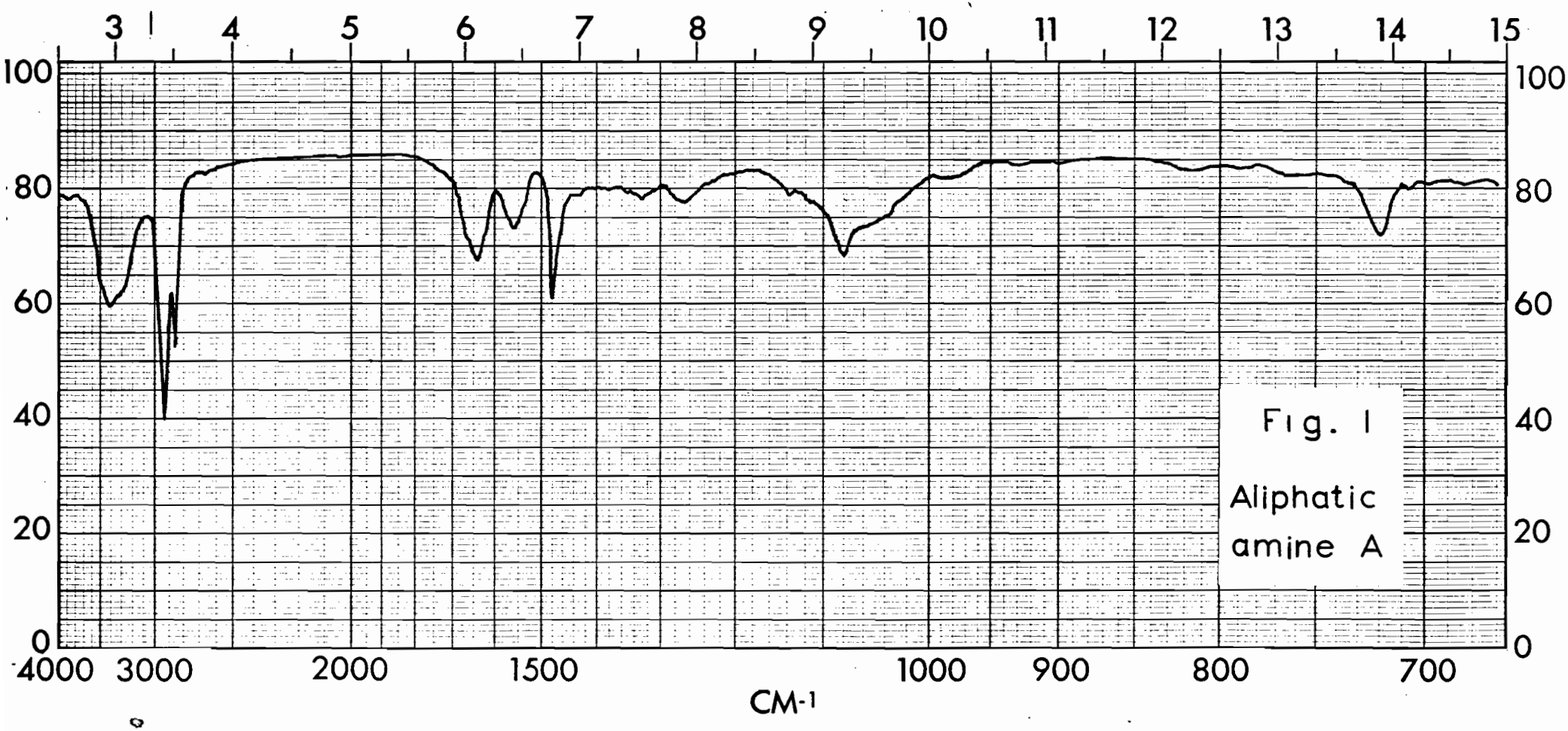

Fig. 1

Aliphatic amine A

Fig. 2.

The infrared spectrum of mixture B.

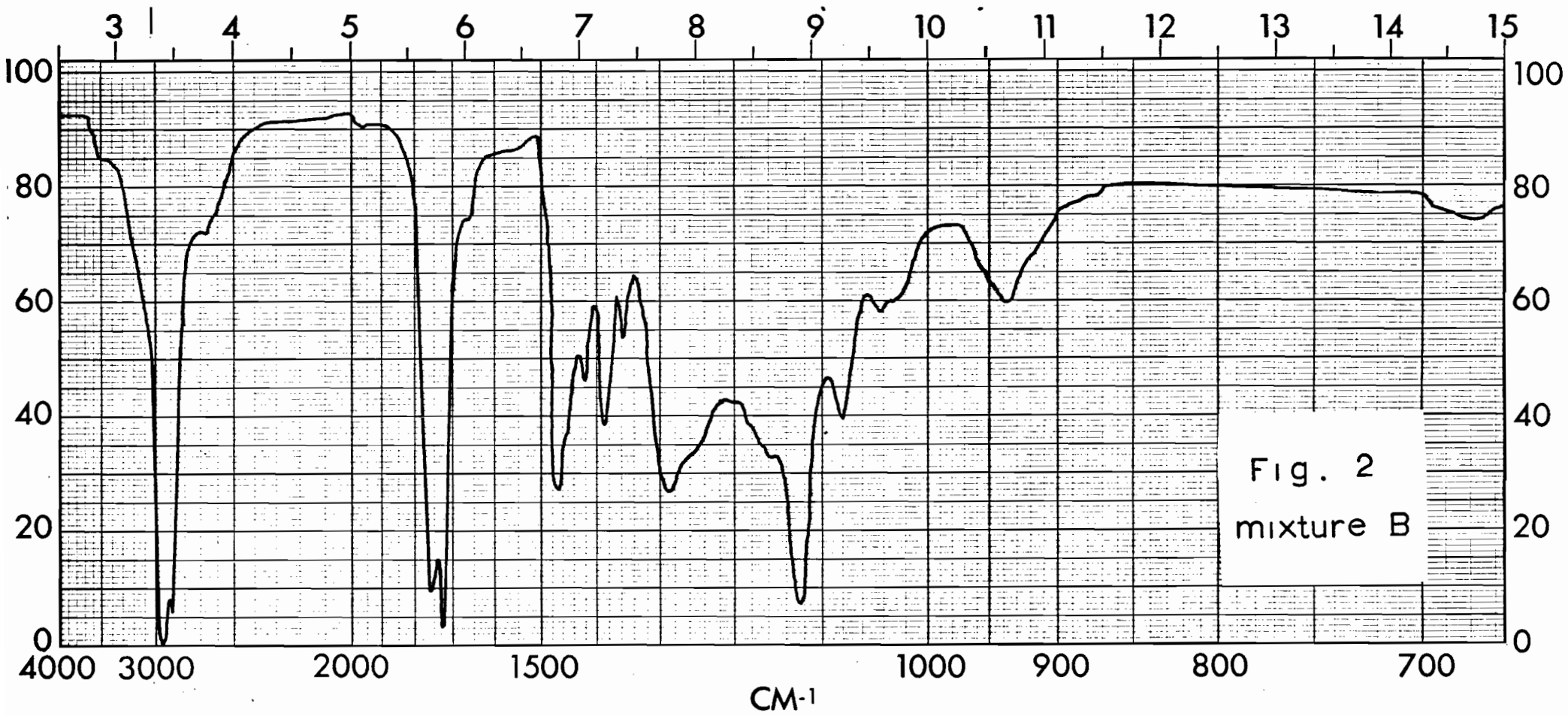

Fig. 2
mixture B

Chapter I

Some Chemical Constitutents of C. parapsilosis

A. Isolation and Identification*

The dried and powdered mycelium was extracted successively with petroleum ether, diethyl ether, benzene, ethyl acetate, acetone and methanol. Extracts were obtained in yields of 0.63%, 0.42%, 0.29%, 0.19%, 0.57% and 7.4% respectively (yields were based on dry weight of mycelium).

1. The diethyl ether extract. The extract was washed with an aqueous solution of sodium bicarbonate and chromatographed on silica gel.

Petroleum ether eluted an oil, the infrared of which was indicative of an ester (ν 1750 cm^{-1}). Hydrolysis gave a mixture of acids which was not investigated further, and 24 mg. of a crystalline material, m.p. 97-100°. Its i.r. (Fig. 1) suggested an aliphatic amine (A).

Benzene-ether (1:1) eluted an oil (B) having strong carbonyl peaks in the i.r. at 1750, 1725 cm^{-1} and peaks in the ether region at 1200-1080 cm^{-1} (Fig. 2). However, no crystalline material could be obtained even after extensive chromatography, indicating the inhomogeneity of the mixture.

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* Only the main constituents that were encountered in this investigation will be mentioned.

Fig. 3

The infrared spectrum of the u.v. fluorescent lactones (fraction C-2).

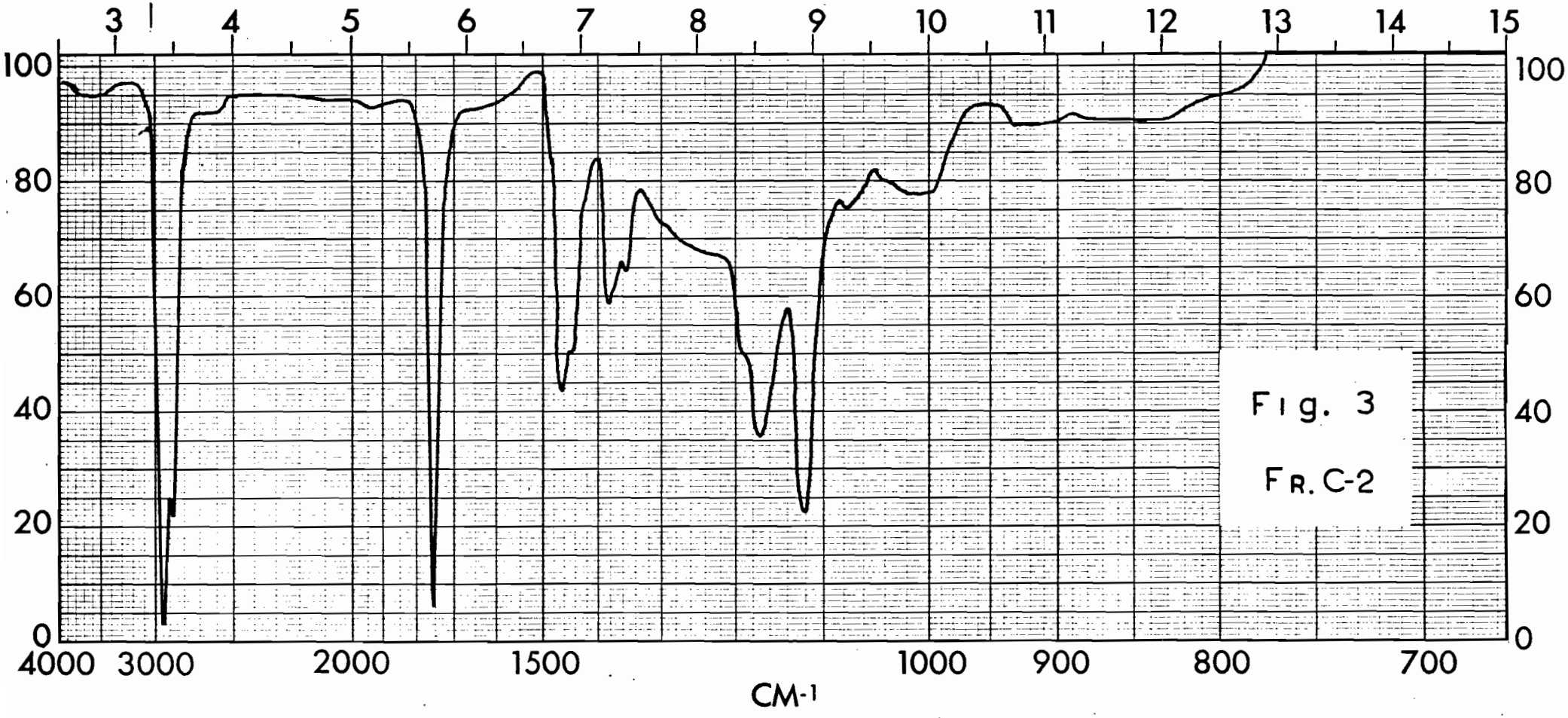

3
4
5
6
7
8
9
10
11
12
13
14
15
100
80
60
40
20
0
4000
3000
2000
1500
1000
900
800
700
CM-1

Fig. 3

FR. C-2

Fig. 4

The nuclear magnetic resonance spectrum of the u.v. fluorescent lactones.

A. fraction C-2

B. fraction C-8.

Fig. 4A

FR. C-2

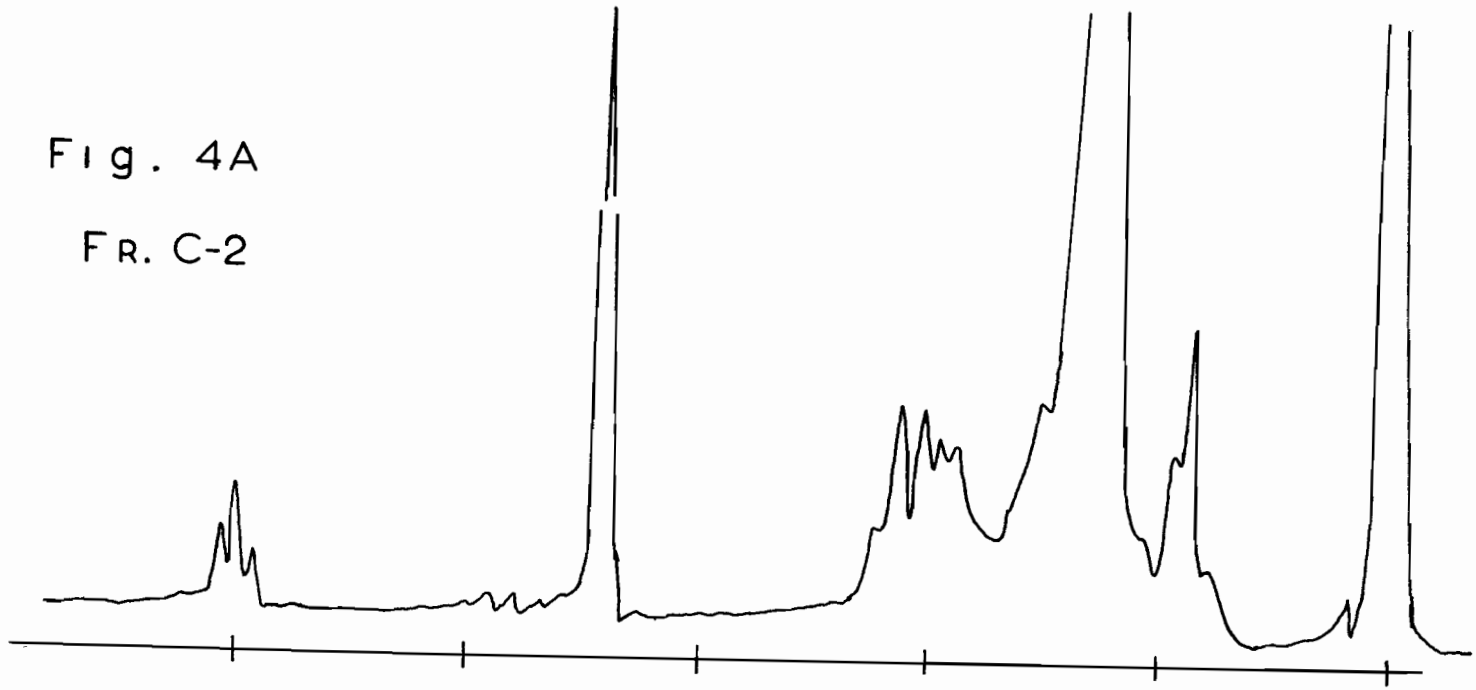


Fig. 4B

FR. C-8

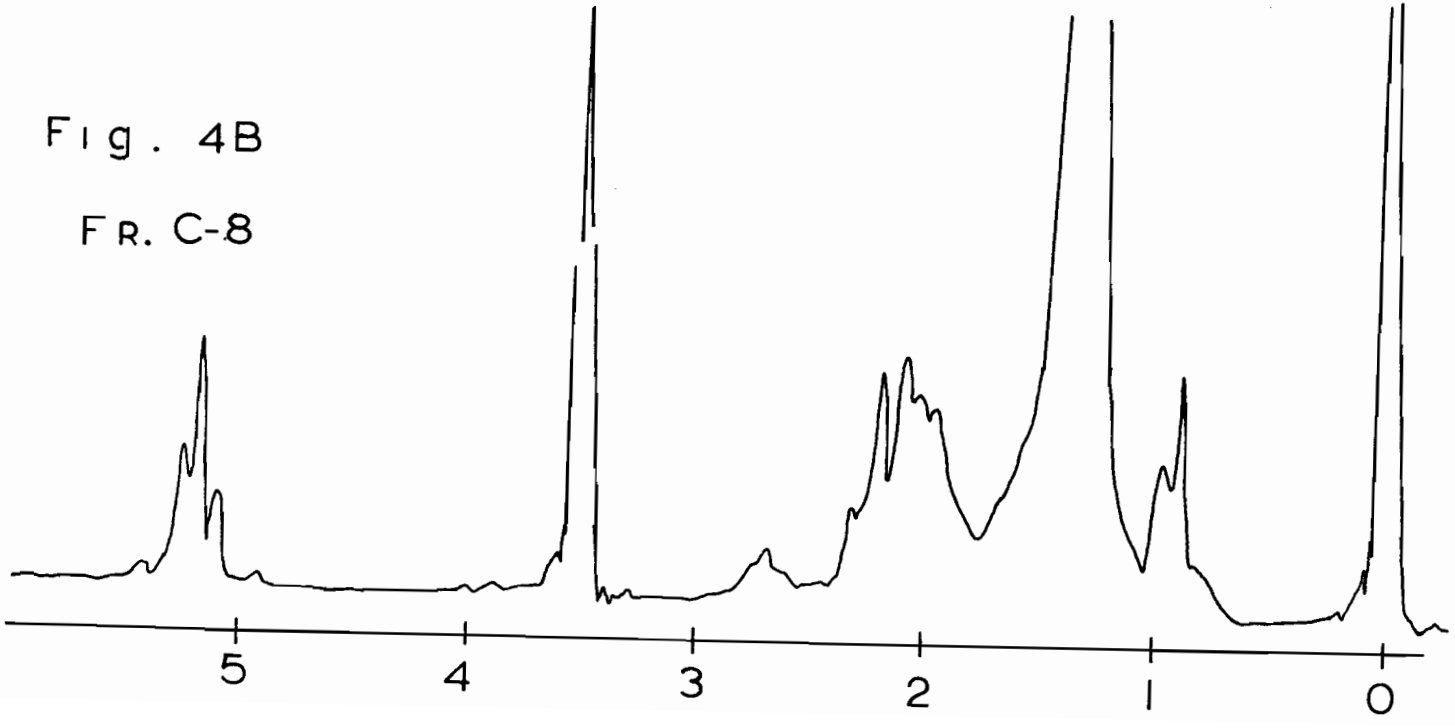
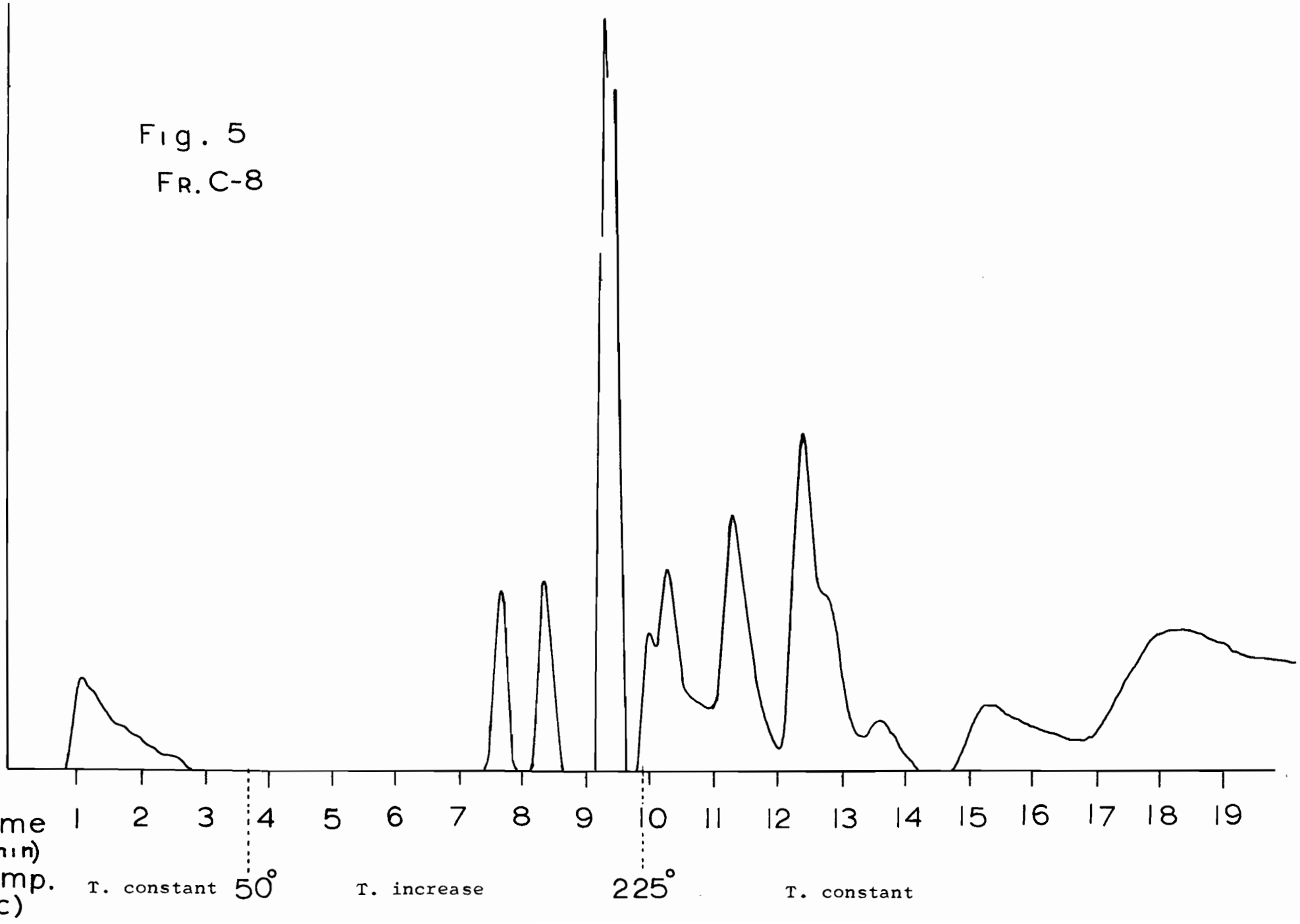


Fig. 5

The vapour phase chromatogram of the u.v. fluorescent lactones (fraction C-8) on ucon 20%.

Fig. 5

FR. C-8


Time (min)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Temp. (°C)
T. constant
50°
T. increase
225°
T. constant

2. The methanol extract (u.v. fluorescent lactones). The extract was dissolved in ether and washed successively with water, aqueous sodium carbonate, aqueous sodium hydroxide, and dilute aqueous hydrochloric acid. The resulting neutral extract was chromatographed on alumina, activity II-III.

Elution with pentane gave a mixture of u.v. fluorescent liquids (C) (fractions 1-10)*. Their i.r. suggested esters or lactones by their absorption at 1750 cm^{-1} and 1170 cm^{-1}. An ether peak at 1120 cm^{-1} was also evident (see Fig. 3). The n.m.r. spectrum (see Fig. 4) showed an intense peak at 1.3 p.p.m. and 0.9 p.p.m., characteristic of a long chain compound. Purification of the liquid mixture could neither be achieved by distillation at reduced pressure nor by vapour phase chromatography, due to extensive decomposition. A single but broak peak was obtained on a column of SE-30 silicone oil due to the poor efficiency of the column. When a more polar column (ucon 20%) was used, the mixture was resolved into at least 12 peaks. Fraction 2, however, showed two main components, whereas fraction 8 had one main constituent (see Fig. 5), indicating that the original mixture was not very inhomogeneous.

When a sample of the liquid mixture was chromatographed on aluminum oxide, activity I, 70% of the material

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* Fractions are numbered 1-10 according to their elution off the column.

Fig. 6

The infrared spectra of

A. carboxylic acid D

B. carboxylic acid E.

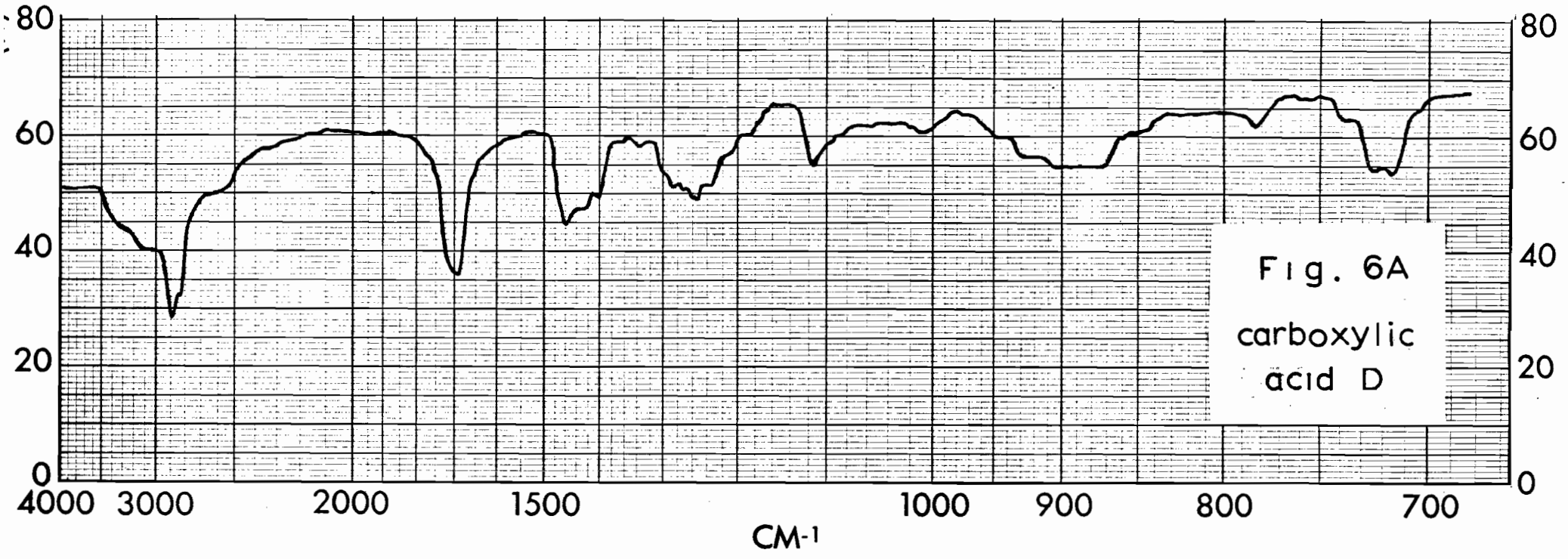

Fig. 6A

carboxylic acid D

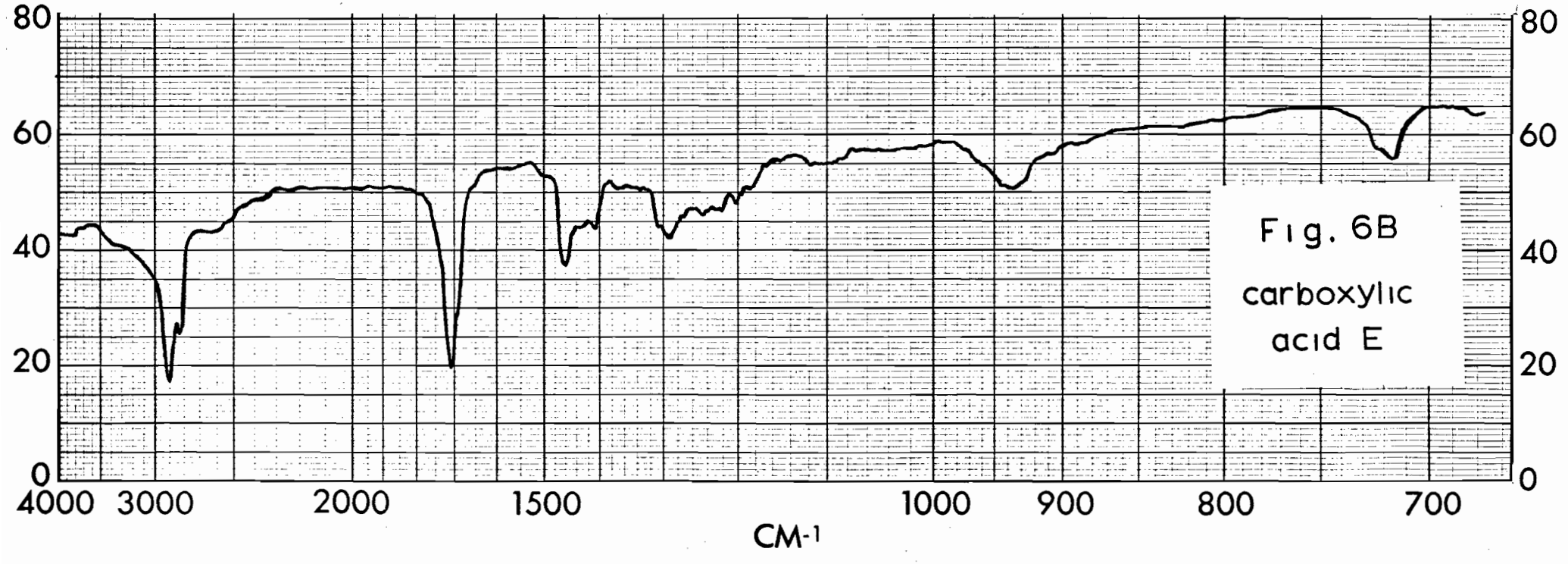

Fig. 6B

carboxylic acid E

was retained on the column; however, elution with methanol-acetic acid (9:1) gave a crystalline fraction, which consisted of two unidentified carboxylic acids, (D) m.p. 53-57° and (E) m.p. 48-50° (for the i.r. spectra see Fig. 6).

Hydrolysis of a sample of the liquid mixture with 5% methanolic potassium hydroxide gave a non-crystalline keto-alcohol, ν 3550 cm^{-1} (hydroxyl), 1725 cm^{-1} (carbonyl) and 1050 cm^{-1} (hydroxyl). Its n.m.r. spectrum indicated the presence of a long alkyl chain. A carboxylic acid and a hydroxy-acid were also obtained, suggesting that the original liquid mixture consisted of fatty esters or lactones. Further investigations had to be postponed until such time as proper equipment was available to purify the mixture into its individual components.

Ergosterol was also isolated from the methanol extract, and a small amount of brassicasterol was detected by t.l.c.

Palmitic acid was isolated from the sodium carbonate and sodium hydroxide washings of the methanol extract.

B. Experimental

All melting points were determined in a Kofler microscope hot plate. The infrared spectra were taken on a Perkin-Elmer 337 grating spectrophotometer and a Perkin-Elmer

Infracord. Nuclear magnetic resonance spectra were recorded on a Varian H.R.60 instrument at 60 megacycles using tetramethylsilane (0 p.p.m.) as internal standard. Optical rotations, ultraviolet absorption spectra and preparative thin layer chromatographic separations were carried out as in Part I. Analyses were provided by Dr. C. Daessle, Montreal, and A. Bernhardt, Mülheim, Germany.

A continuous soxhlet extraction of the dried and powdered mycelium (1700 g.) was carried out with petroleum ether, b.p. 40-70° (11.4 g., 0.63% yield based on mycelium dry weight), diethyl ether (7.5 g., 0.42%), benzene (5.3 g., 0.29%), ethyl acetate (3.4 g., 0.19%) and acetone (10.4 g., 0.57%) respectively for 7 days each, and with methanol (133.6 g., 7.4%) for 14 days.

1. The diethyl ether extract. Free fatty acids were removed from the extract by washing with 5% aqueous sodium carbonate. The resulting extract (4.9 g., 64.4% based on crude extract) was chromatographed on Davidson silica gel (150 g.).

Petroleum ether eluted a semi-crystalline oil (2.86 g., 37.6%) which showed peaks in the i.r. at 3300 cm^{-1} (broad peak, OH or NH), 1750 cm^{-1} (ester), 1650 cm^{-1} (C=C, or NH), 1080-1100 cm^{-1} (C-O stretch), and a strong end

absorption in the u.v. Hydrolysis in 5% methanolic potassium hydroxide for one hour under reflux in a nitrogen atmosphere followed by ether extraction and washing of the organic phase with water gave 486 mg. of an oil which was chromatographed on 25 g. silica gel. Benzene eluted 242 mg. of a hydrocarbon which was not investigated further; acetone eluted 123 mg. of a crystalline material which was recrystallized from acetone to give 24 mg. of an aliphatic amine (A) m.p. 97-100° (for infrared spectrum see Fig. 1). Recrystallization of the mother liquor gave 10 mg. m.p. 146-150°. The sodium hydroxide wash of the hydrolysis reaction was acidified and extracted with ether. The organic phase was washed with water until neutral, dried over anhydrous magnesium sulphate, and evaporated to give 2.68 g. (35.2%) of a semi-crystalline mixture of acids which was not investigated further.

Benzene-ether (1:1) eluted an oil (B) (1.16 g., 15.2%) having strong absorption in the carbonyl region ν_{CCl_4} (Fig. 2) 1750, 1725 cm^{-1}, and in the ether region at 1200-1080 cm^{-1}; however, no crystalline material could be obtained even after several chromatograms, due to the great inhomogeneity of the mixture.

2. The methanol extract. The extract was dissolved in ether and washed with water, 10% aqueous sodium carbonate (25.2 g.,

1.4% based on dry mycelium) 1% aqueous sodium hydroxide (6.5g., 0.39%), and 5% aqueous hydrochloric acid (40 mg., 0.002%), followed by several washings with water. The neutral organic phase was dried over anhydrous magnesium sulphate, and evaporated to give 25 g. (18.4% based on crude) of a brown oil which was chromatographed on 500 g. of aluminum oxide, Woelm, neutral, activity II-III.

Pentane eluted ten fractions C(#1-10)* (10.5 g., 9.3%) of a u.v. fluorescent liquid mixture which crystallized at 0^{o}, in colorless bead-like crystals. However these melted at room temperature; ν_{CCl_4} (Fig. 3) 1750, 1170 cm^{-1} (ester or lactone), 1120 cm^{-1} (ether); δ_{CCl_4} (Fig. 4) 4.8 (triplet, = C<u>H</u>-CH_2), 3.5 (singlet, ether or ester), 2.1 and 2.0 (overlapping quartet and doublet respectively, possibly due to allylic protons), 1.3 (intense singlet due to an aliphatic chain), and 0.9 p.p.m. (triplet <u>CH_3</u>-CH_2-); λ_{max}^{EtOH} 220 mμ. A purification by v.p.c. was attempted.

<u>Vapour phase chromatography</u>

A 2μl sample of liquid was inserted in a Beckman GL-1 v.p.c., fitted with a 6 ft. column containing SE-30 silicone oil, 2% on chromosorb W (60-80 mesh). The temperature

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* The ten fractions of the liquid mixture were labelled from 1 to 10 signifying the order of elution with pentane from the column of alumina.

was programmed over a range of 50° (starting temperature) up to 250° (final temperature) within 6.25 minutes. Single but broad peaks were obtained for an earlier fraction (#2) and a later fraction (#8) at T = 250° and 2.5 minutes after T = 250°. Because of the poor efficiency of the column a more polar one of ucon 20% on chromosorb W (60 - 80 mesh) was attempted at an initial temperature of 50° ranging to 225° in 6.25 minutes. Fraction #2 was resolved into 16 peaks with two main components at T = 169° and at 2 minutes after T = 225°, whereas fraction #8 consisted of 1 main peak at T = 207° and 11 minor components which must be due to decomposition (see Fig. 5 for v.p.c. of C-8).

Decomposition was also observed while attempting to distil a sample of liquid on a spinning band column, at 150° and 3 mm. pressure.

Chromatography of a 4.0 g. sample of the liquid mixture (C) on 100 g. aluminum oxide, Woelm, neutral, activity I, retained 70% of the material; however, methanol-acetic acid (9:1) eluted 554 mg. of a crystalline fraction. Recrystallization from ether-acetone gave two crystalline carboxylic acids (D), m.p. 53-57°; ν_{KBr} (Fig. 6A) 3000 cm^{-1} (broad) 1700 cm^{-1} (carboxylic acid), 1460-1400 cm^{-1} (series of peaks), 1300-1200 cm^{-1} (series of peaks), 1120 cm^{-1}, 900 cm^{-1}, 730-715 cm^{-1} (doublet) and (E) m.p. 48-50.5°; its i.r. was very similar to palmitic acid (see Fig. 6B).

Hydrolysis of a 230 mg. sample of the liquid mixture with 5% methanolic potassium hydroxide under a nitrogen atmosphere for one hour under reflux gave, after the usual work-up, a non-crystalline keto alcohol, ν_{CCl_4} 3550 cm^{-1} (hydroxyl), 1725 cm^{-1} (carbonyl) and 1050 cm^{-1} (hydroxyl); δ_{CCl_4} 8.2 (broad signal, -OH) 5.1 (vinyl proton), 3.8 (triplet), 3.0 (broad signal), 2.2 and 1.9 (overlapping triplet and doublet respectively), 1.3 and 0.8 p.p.m. (alkyl chain); a carboxylic acid, ν_{CCl_4} 1720 cm^{-1}; δ_{CCl_4} 10.2 p.p.m. (broad signal, COOH). Another compound was also obtained having an i.r. suggestive of a hydroxy acid.

Ergosterol was eluted with benzene-ether (7:3), and identified by silica gel t.l.c. (benzene-ethyl acetate 9:1) u.v. and melting point. Brassicasterol was also detected on t.l.c.

Palmitic acid was isolated from the acidified sodium carbonate and sodium hydroxide washes of the crude methanol extract and was identified by having an i.r. superimposable with that of an authentic sample. No depression in mixed melting point was observed.

Fig. 7

The infrared spectra of thiomethyladenosine

A. recrystallized from water

B. recrystallized from methanol.

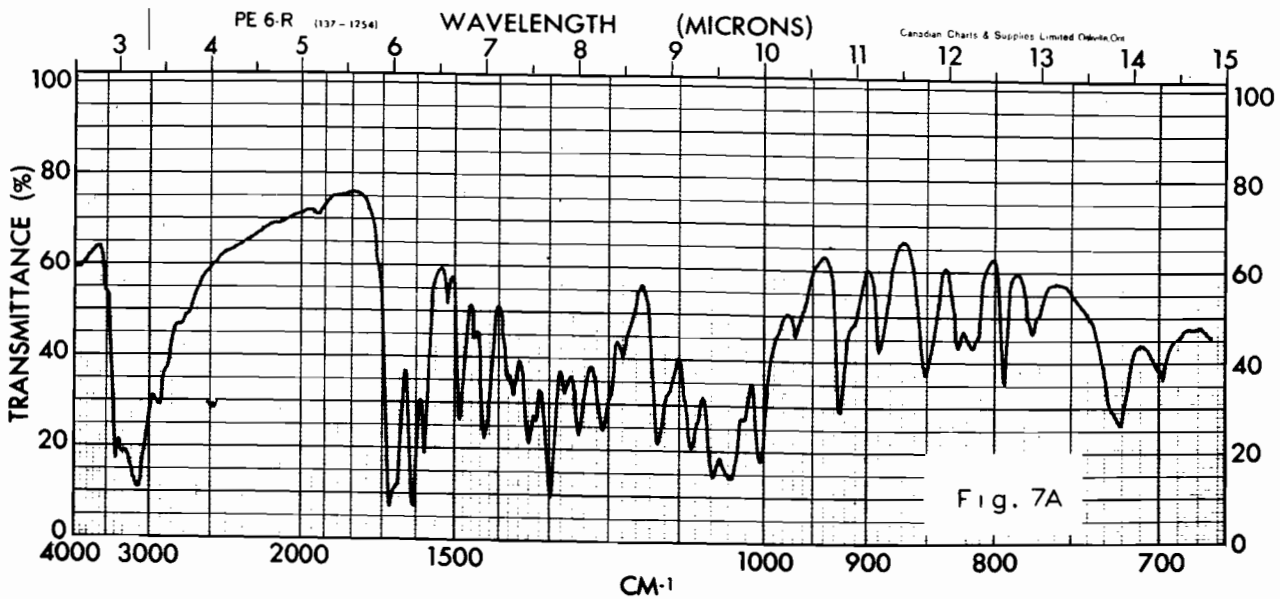

PE 6-R (137 - 1254)
WAVELENGTH (MICRONS)
Canadian Charts & Supplies Limited Oakville, Ont
3 4 5 6 7 8 9 10 11 12 13 14 15
100 80 60 40 20 0
TRANSMITTANCE (%)
4000 3000 2000 1500 1000 900 800 700
CM-1

Fig. 7A

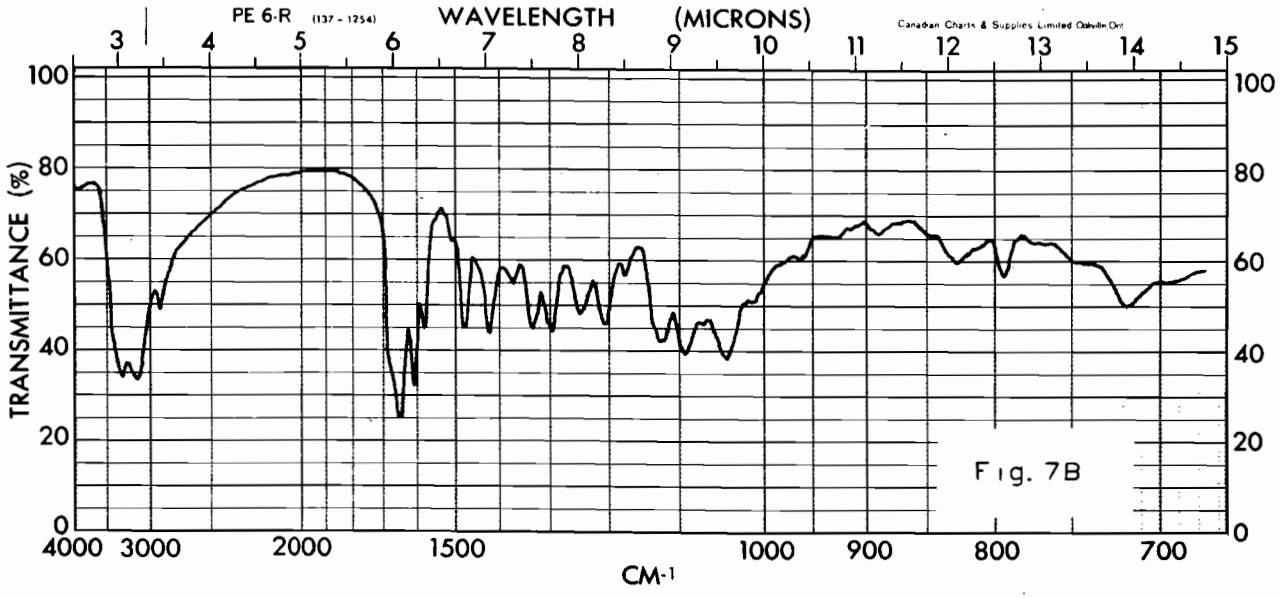

PE 6-R (137 - 1254)
WAVELENGTH (MICRONS)
Canadian Charts & Supplies Limited Oakville, Ont
3 4 5 6 7 8 9 10 11 12 13 14 15
100 80 60 40 20 0
TRANSMITTANCE (%)
4000 3000 2000 1500 1000 900 800 700
CM-1

Fig. 7B

Chapter II

Some Chemical Constituents of C. albicans

A. Isolation and Identification

The mycelium was extracted continuously in a soxhlet extractor with petroleum ether (1.42% yield, based on dry weight of mycelium) for one week, and with chloroform (2.01%) and methanol (16.6%) for two weeks respectively.

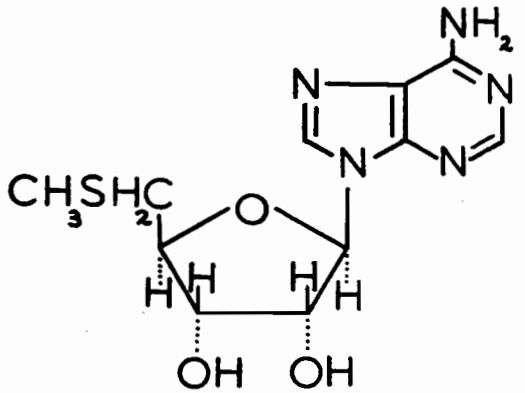
1. The petroleum ether extract. Ergosterol was deposited directly while the solvent was being removed, and the extract was not investigated further.

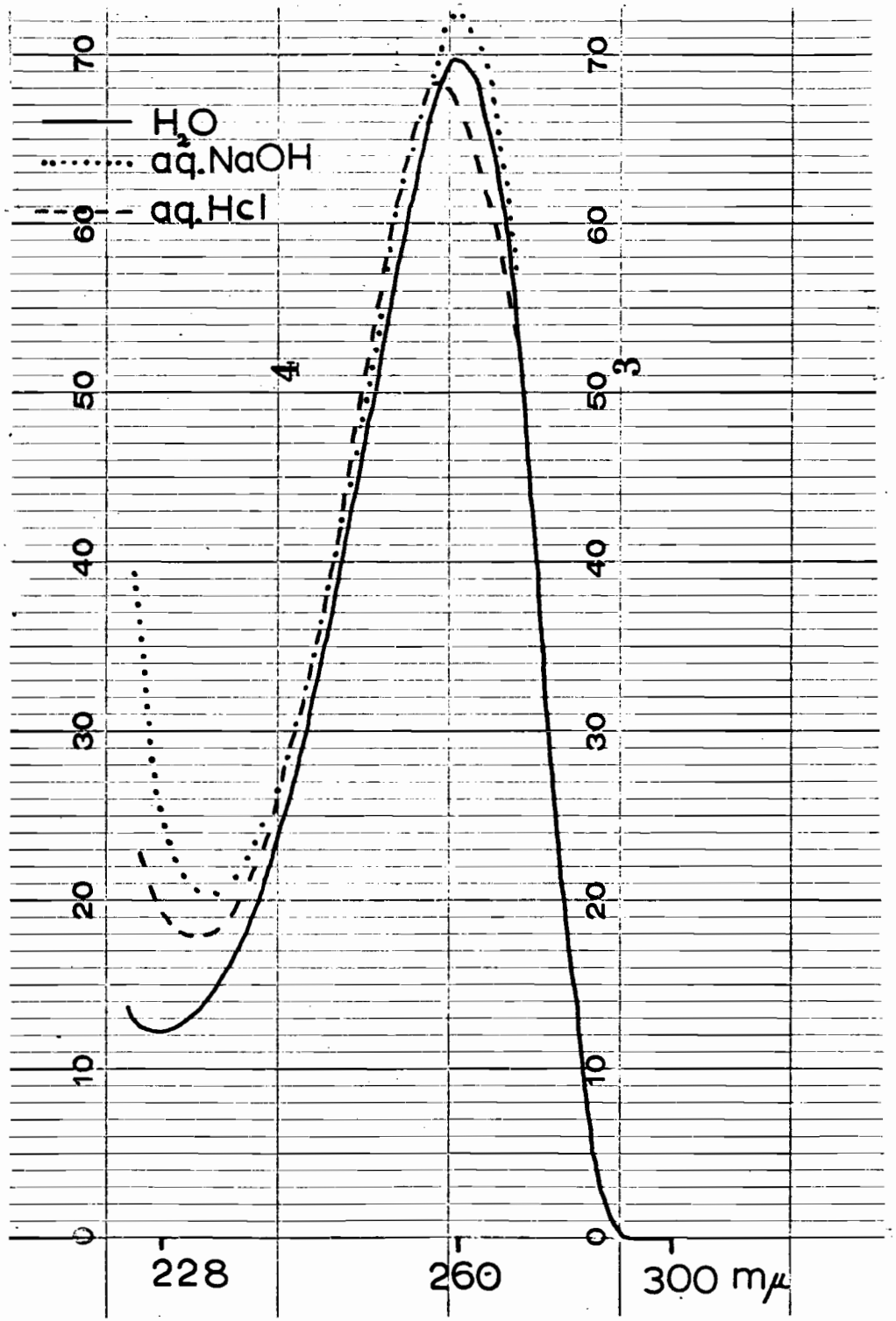
2. The chloroform extract. (Thiomethyladenosine). Concentration of the chloroform extract gave a crystalline compound directly (5.8%, yield based on crude extract), m.p. 200-202°. The compound melted at 206-207.5° when it was recrystallized from methanol, and showed an intense peak in the infrared at 1645 cm^{-1}. It melted at 210.5-212°, when it was recrystallized from water, ν_{KBr} 1660 cm^{-1}. A different pattern was also exhibited in the 3400-3000 cm^{-1} and 900-700 cm^{-1} region for the two recrystallized compounds (see Figs. 7A and 7B). The identity of the two materials was easily established since they could be interconverted by recrystallization from the appropriate solvent.

Fig. 8

The ultraviolet spectrum of thiomethyladenosine in water, alkali and acid.

Fig. 8


NH2
N
N
N
N
CH3SHC2
O
H
H
H
H
OH
OH


H2O
aq.NaOH
aq.HCl
70
60
50
40
30
20
10
0
4
3
228
260
300 mμ

The analysis and mass spectrum (parent peak = 297) of the compound indicated an elemental composition of $C_{11}H_{15}N_5O_3S$. Rast molecular weight determination however, gave a value of 375, probably due to inter-molecular association. The ultraviolet spectrum ($\lambda_{max}^{H_2O}$ 260 mμ; see Fig. 8) together with the high nitrogen content and low carbon to hydrogen ratio suggested a heteroaromatic compound.

The presence of adenine and a terminal thiomethyl group stood out in the mass spectrum which showed peaks at M^+ - 47 (i.e. m/e 250) and M^+ - 162 (i.e. m/e 135) respectively*. Also evident from the mass spectrum was the strong similarity in the fragmentation pattern of the isolated compound with that of adenosine. In particular, the principal peaks B + H, B + 2H and B + 30** are a common feature. The parent peak (M^+) of the isolated compound occurs at m/e 297 instead of m/e 267 as in adenosine. The presence of sulphur in the sugar moiety of the isolated compound is clearly evident by the displacement of the peak at m/e 133 due to the sugar (S) fragment of adenosine by 30 mass units to m/e 163*** The following is a partial degradative scheme of the principal fragments:

---

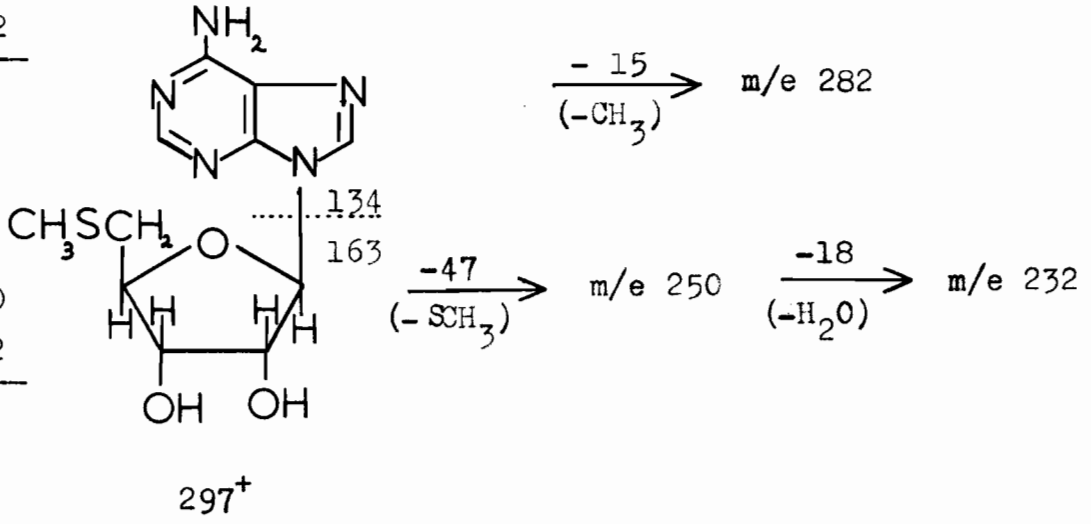
* M^+ signifies the molecular weight of the compound, also labelled molecular ion or parent peak.

** B = molecular weight of purine minus 1 mass unit; also, in this case, the base peak i.e. the most intense peak in the spectrum.

*** S = molecular weight of sugar minus 1 mass unit.

m/e 297

m/e 282

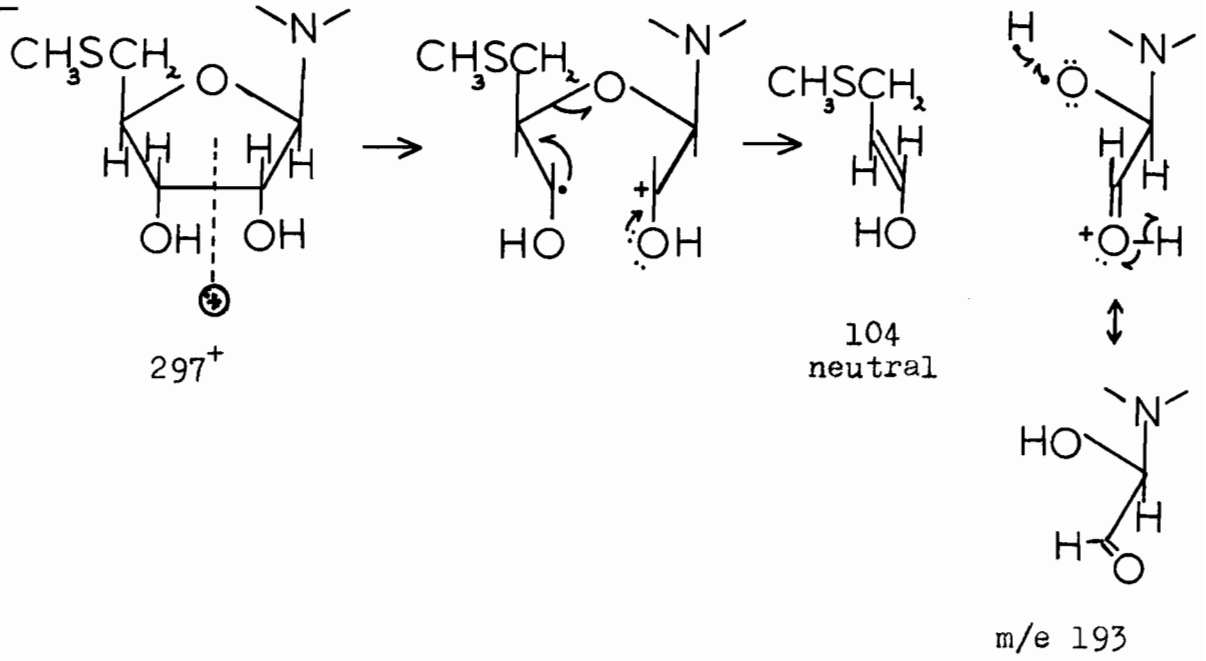


m/e 250

m/e 232

297^+

m/e 193^+ *

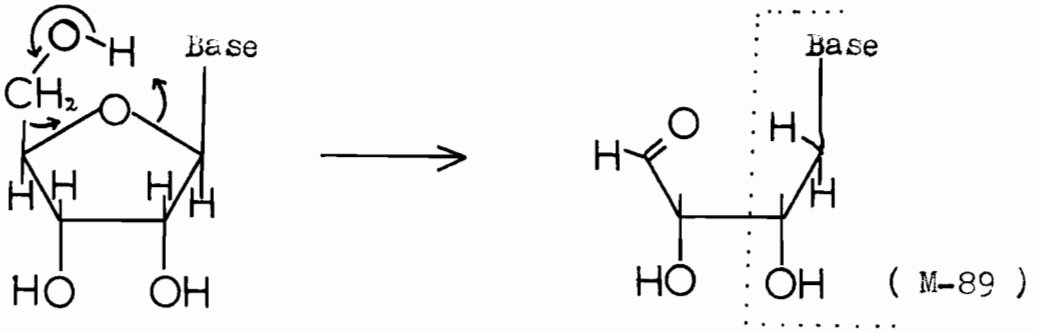


297^+

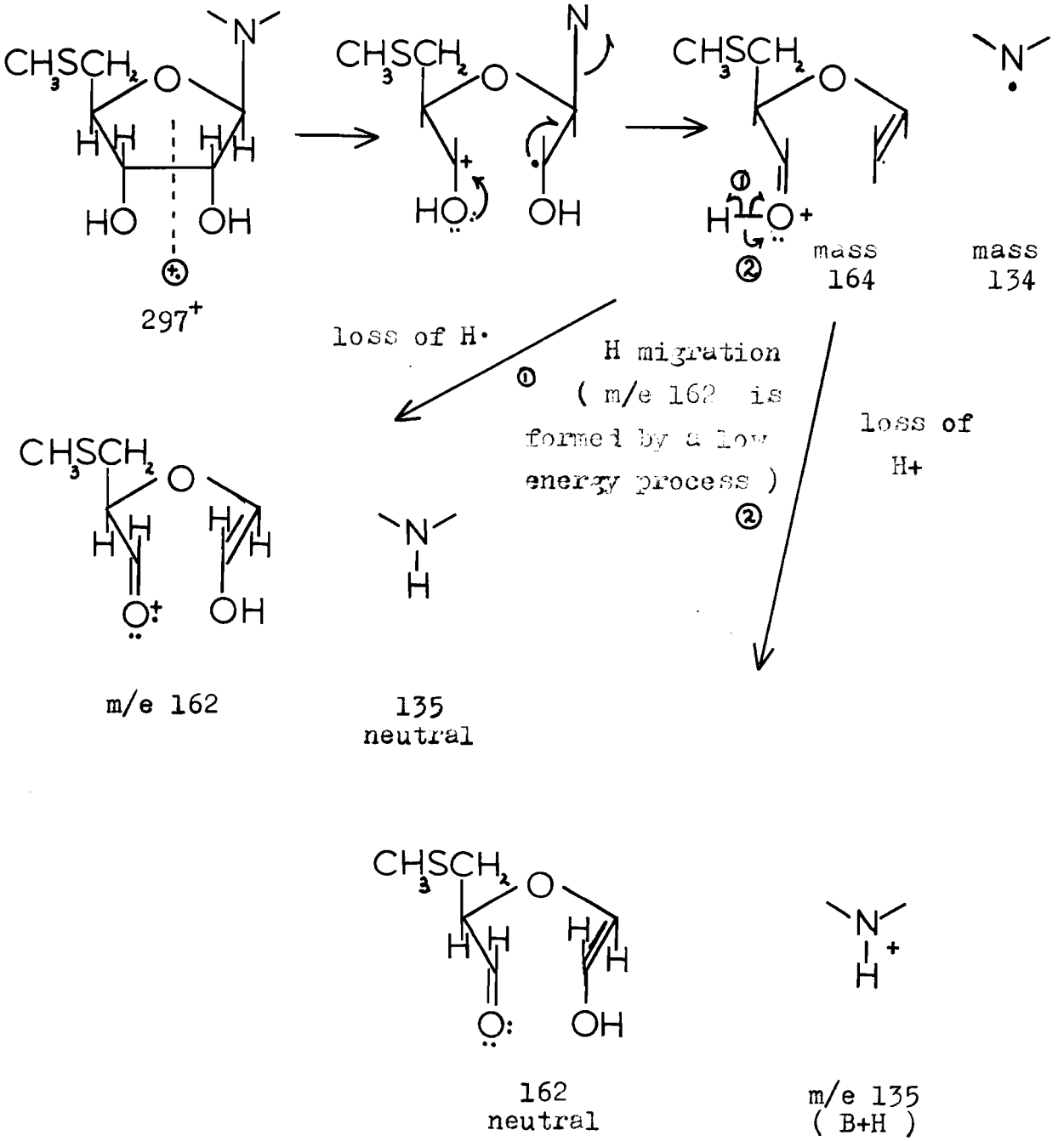
m/e 193

---

* The normal degradation of a riboside[11] is as follows :



m/e 135 (B+H)
m/e 162 (S+H)



The lack of isotopic peaks at m/e 135 indicates the absence of sulfur in this fragment, and confirms the structure assigned.

m/e 164 (B+30) [11]
m/e 136 (B+2H)

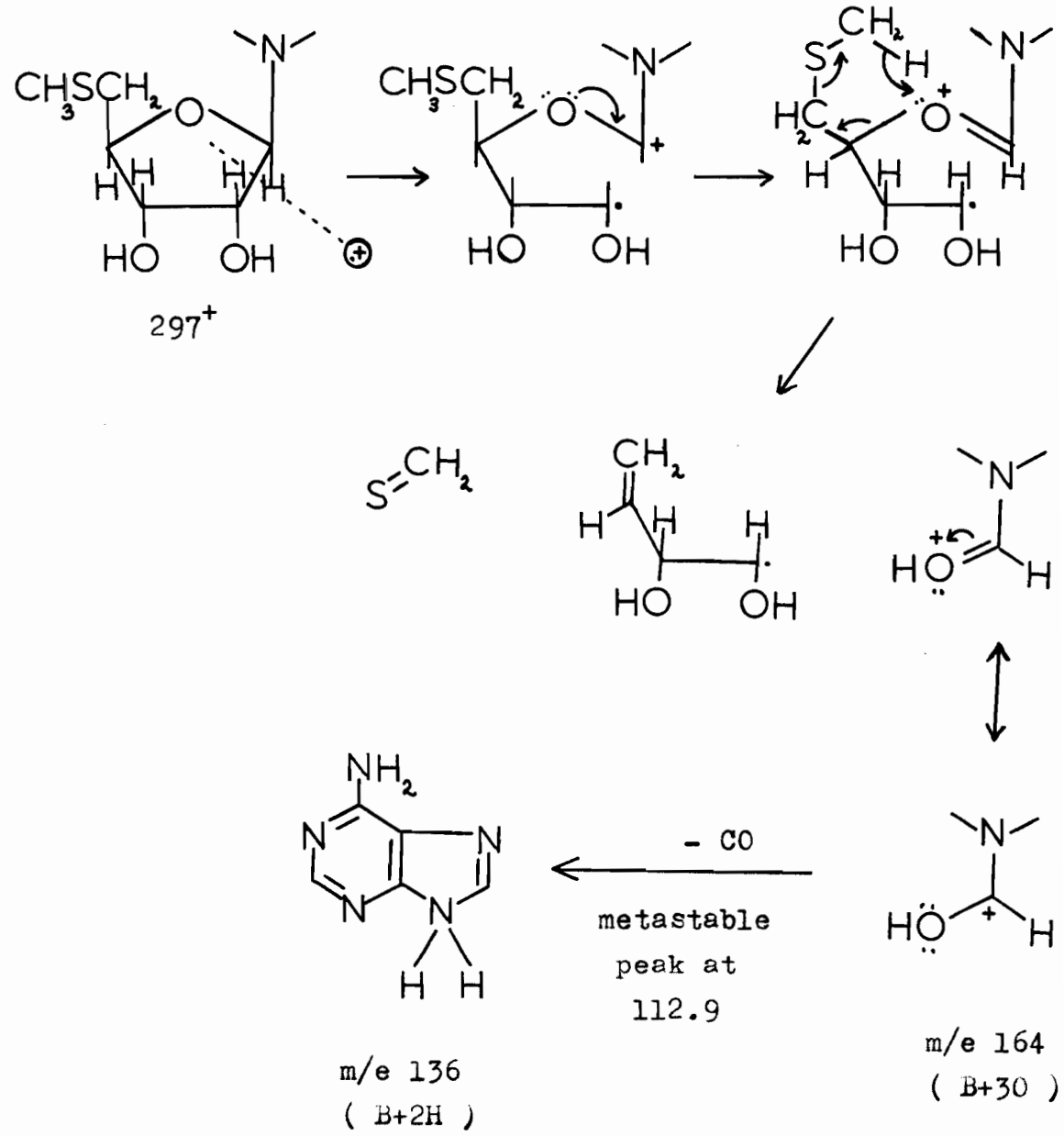


Fig. 9

The nuclear magnetic resonance spectra of thiomethyladenosine in

A. pyridine

B. water-hydrochloric acid

C. trifluoroacetic acid.

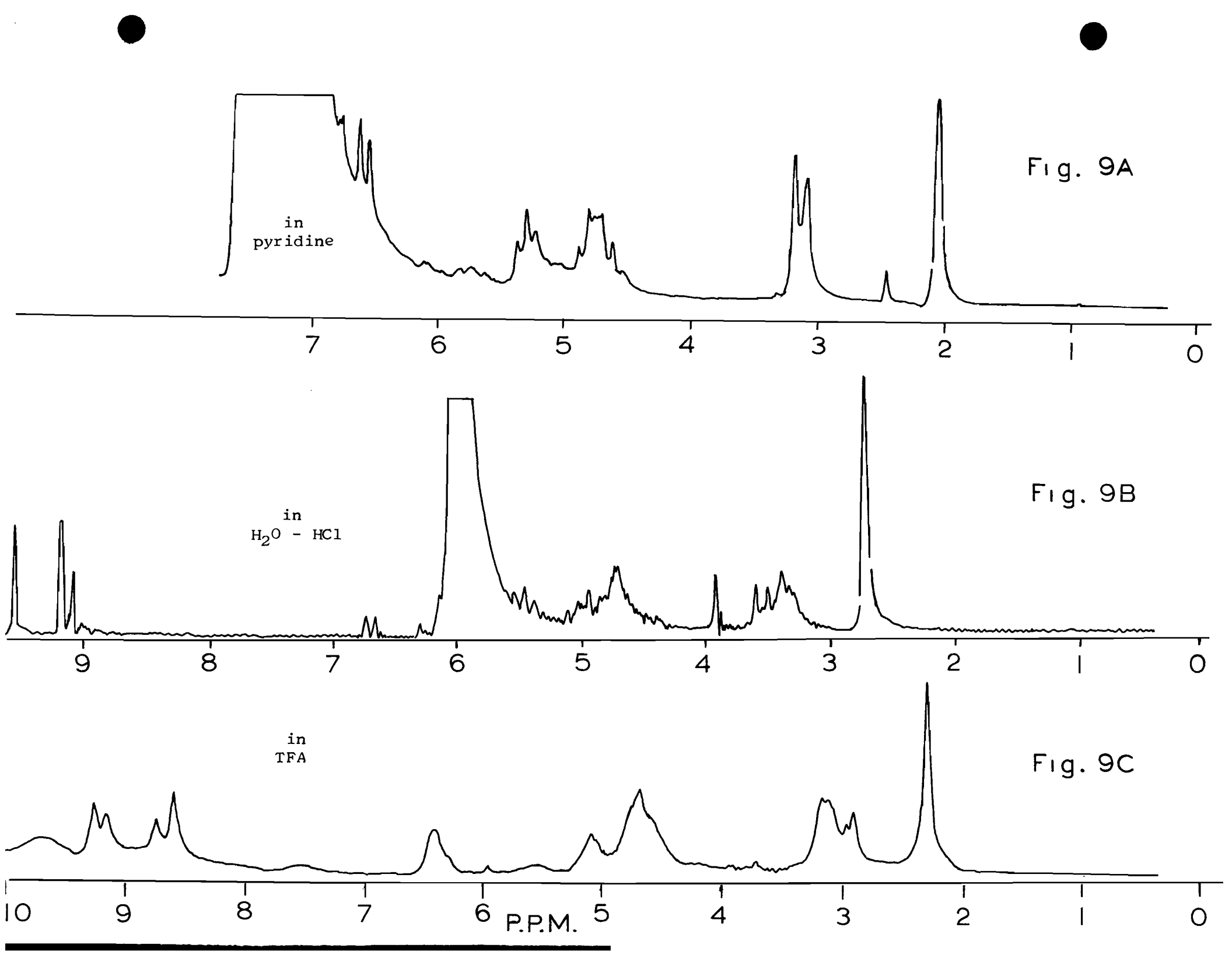

in
pyridine
7
6
5
4
3
2
1
0
Fig. 9A
in
H_2O - HCl
9
8
7
6
5
4
3
2
1
0
Fig. 9B
in
TFA
10
9
8
7
6
P.P.M.
5
4
3
2
1
0
Fig. 9C

Fig. 10

The nuclear magnetic resonance spectra in dimethylsulfoxide of

A. adenosine

B. thiomethyladenosine.

Fig. 10A

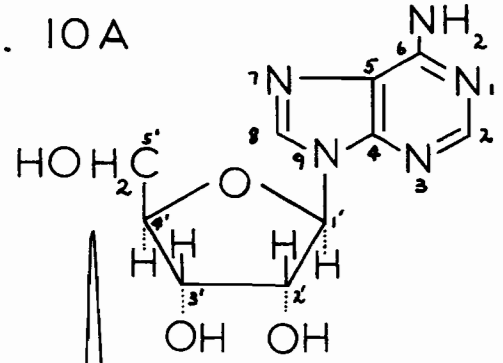
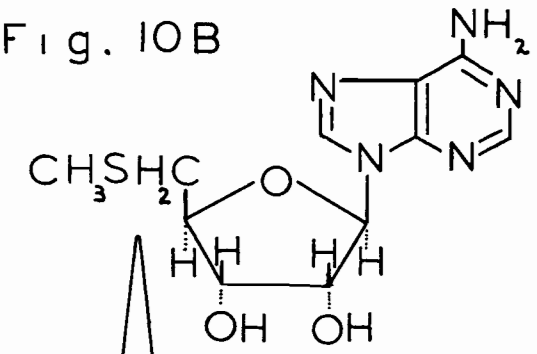


Fig. 10B



Adenine was also obtained by hydrolysis of the isolated compound in formic acid.*

The n.m.r. spectrum of the isolated compound in dimethylsulfoxide (see Table I for actual values), was almost superimposable with that of adenosine (see Fig. 10) suggesting a strong similarity in the structure of the two compounds, having in common, two heteroaromatic protons, an amino group, two secondary hydroxyls, and four tertiary protons. However, unlike the adenosine spectrum, a thiomethyl group was clearly evident in such solvents as trifluoroacetic acid (Fig. 9C), deuterium oxide-hydrochloric acid (Fig. 9B), pyridine (Fig. 9A), as well as dimethylfulfoxide (Fig. 10B). Furthermore, the signal due to the terminal hydroxyl ($\underline{HO}-CH_2-$) of adenosine, was absent in the n.m.r. spectrum of the isolated compound. Evidently, the only difference between the two compounds was the replacement of the terminal hydroxymethyl group of adenosine by a thiomethyl group. This meant that the isolated compound was thiomethyladenosine. Confirmation of this is also obtained from the similarity of the physical constants of the isolated product and thiomethyladenosine tabulated in Table II. Thiomethyladenosine was also found to be present among the water soluble constituents of the chloroform and methanol extracts.

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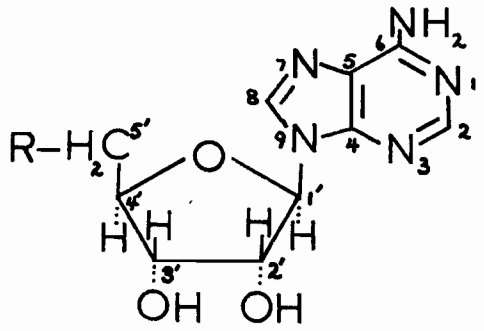
* The author wishes to thank Dr. J. Spencer, McGill University, for carrying out the hydrolysis.

Table I



R = OH Adenosine
R = SCH_3 Thiomethyladenosine (isolated compound)

Nuclear magnetic resonance data of the isolated compound and adenosine in various solvents (Chemical shifts in p.p.m., T.M.S. = 0 p.p.m.)

Solvent	C_2-H	C_8-H	$-NH_2$	C_1'-H[a]	C_3'OH[a]	C_2'-OH[a]	C_2'-H[a]	C_3'-H[a]	C_4'-H[a]	$OCH_2(5')$	$SCH_2(5')$	C_5'-OH	CH_3S
Isolated Compound													
Trifluoro-acetic acid	9.27 9.18	8.77 8.61									3.14 2.95		2.29
Deuterium oxide-hydro-chloric acid	9.53	9.18									3.39[d]		2.71
Pyridine			6.29								3.14[b]		2.05
Dimethyl-sulfoxide	8.23	8.06	7.27	5.83[b]	5.43[b]	5.25[b]	4.7[c]	4.05	4.05	-	3.60	-	3.1
Adenosine													
Dimethyl-sulfoxide	8.24	8.03	7.26	5.86[b]	5.43[b]	5.17[b]	4.6[c]	4.15[c]	4.0[c]	3.67	-	3.41[d]	-

a. In the first three solvents certain signals could not be accurately ascribed to individual protons and hence have been omitted from the table.

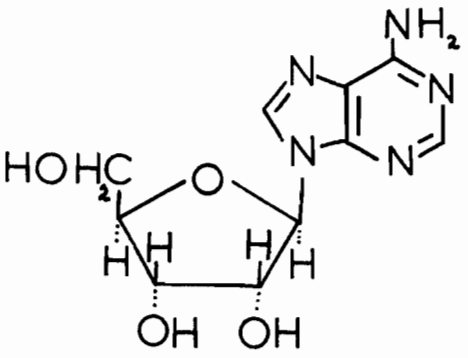
b. Doublets. c. Quartet d. Broad signal

Table II

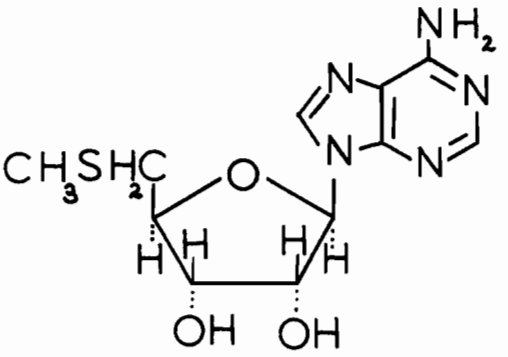
Some physical constants of the isolated compound and thiomethyladenosine

Physical constants	Isolated compound	Thiomethyl-adenosine
Melting point	206-207.5° (MeOH) 210.5-212° (H_2O)	208° (H_2O)[2] 212-213[2] (H_2O)[12]
Analyzed for		
$C_{11}H_{15}N_5O_3S$	Found	Found
C, 44.44	45.14	44.40
H, 5.08	5.36	5.25
N, 23.57	22.80	23.18
O, 16.14	17.19	(16.12)[a]
S, 10.77	10.84	11.05
$\alpha]_D$	+21.25° (c, 1.05 in 1% H_2SO_4)	+12.15° (in 1% H_2SO_4)[12]
$\lambda^{H_2O}_{max}$	260 mμ (ε 10,600)	260 mμ
λ_{min}	227 mμ	230 mμ
Molec wt. (Rast)	375	
(mass spec.)	297	
Picrate		
Melting point	196-7° (decomp.)	183° (decomp.)
Analyzed for		
$(C_{11}H_{15}N_5O_3S)(C_6H_3N_3O_7)$	Found	Found
C, 38.78	38.08	38.57
H, 3.42	4.06	3.68
N, 21.29	21.77	21.47
O, 30.43 S, 6.08 } 36.51	} 36.09[a]	} 36.28[a]

[a] Not analyzed for.



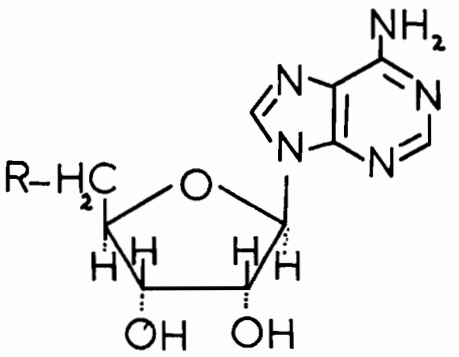
Adenosine



Thiomethyladenosine

There are very few naturally occurring thiosugars known in which the thio group is attached to a carbon atom in other than the reducing position. One of these is thiomethylpentose, a component of an adenine nucleoside (adenylthiomethylpentose, ATMP) occurring in yeast. ATMP has previously been isolated from yeast 'zymin' by Mandel and Dunham[1], from crude oryzanine (Brewer's yeast) by Suzuki et al[2]., and from impure cozymase by Euler[3]. On hydrolysis it yields one mole of adenine and one mole of sugar which was found by Suzuki et al.[2] to contain sulfur. Falconer and Gulland[6] indicated, through ultra-violet studies, an N-9 linkage of the purine-sugar. A point of controversy was the position of the thiomethyl group (i.e. at the 2', 3' or 5' position on the sugar) which couldn't be settled by degradative means because of the scarcity

of the compound. However Satoh[7] settled the matter when he synthesized it from adenosine and determined the structure to be 9-(5'-thiomethyl-β-D-ribofuranosyl) adenine (X), bearing the same β -glycosidic linkage as in adenosine. Another naturally occurring thiosugar[8] of the class mentioned above is S-(5-deoxyadenosyl-5) homocysteine (Y) an analog of ATMP which has the $HO_2CCH(NH_2)CH_2CH_2S-$ moiety instead of the $-SCH_3$ of ATMP. It has been isolated in 1954 by Cantoni and Scaroni[9] and synthesized chemically by Baddiley and Jamieson[10] in 1955. Analogs of ATMP are important in enzymic transmethylations in biological systems, based on the net transfer of the S-methyl group of methionine $[CH_3SCH_2CH_2CH(NH_2)CO_2H]$ to a substrate, in the presence of



(X) R = CH_3S-

(Y) R = $HO_2CCH(NH_2)CH_2CH_2S-$

(Z) R = $^{-}O_2CCH(NH_2)CH_2CH_2S^{+}(CH_3)-$

Fig. 11

The infrared spectra of

A. Amide I

B. Amide J.

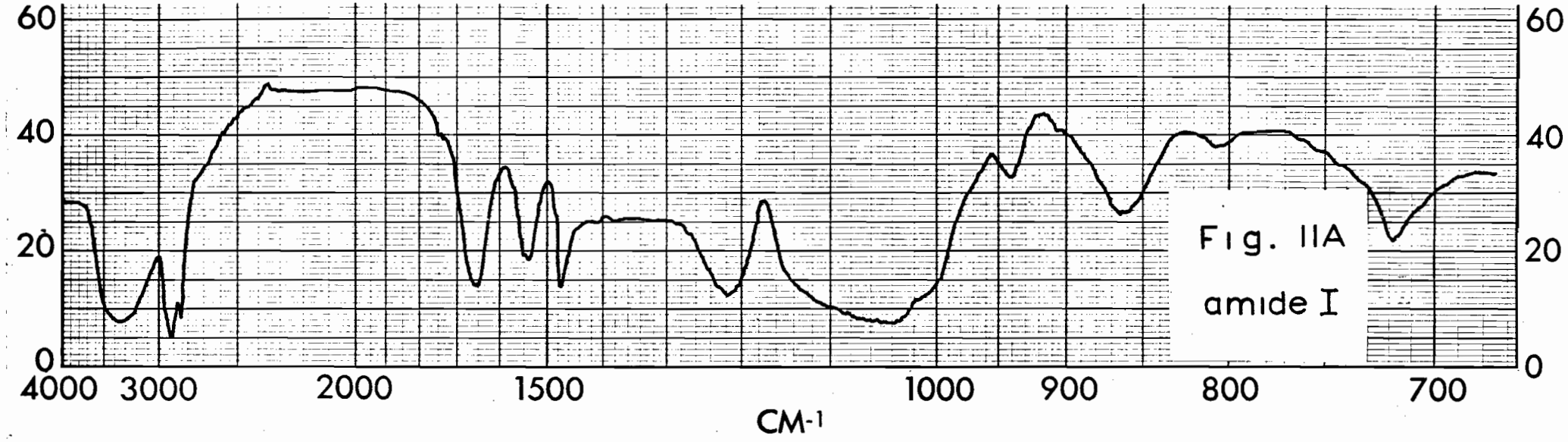


Fig. IIA
amide I

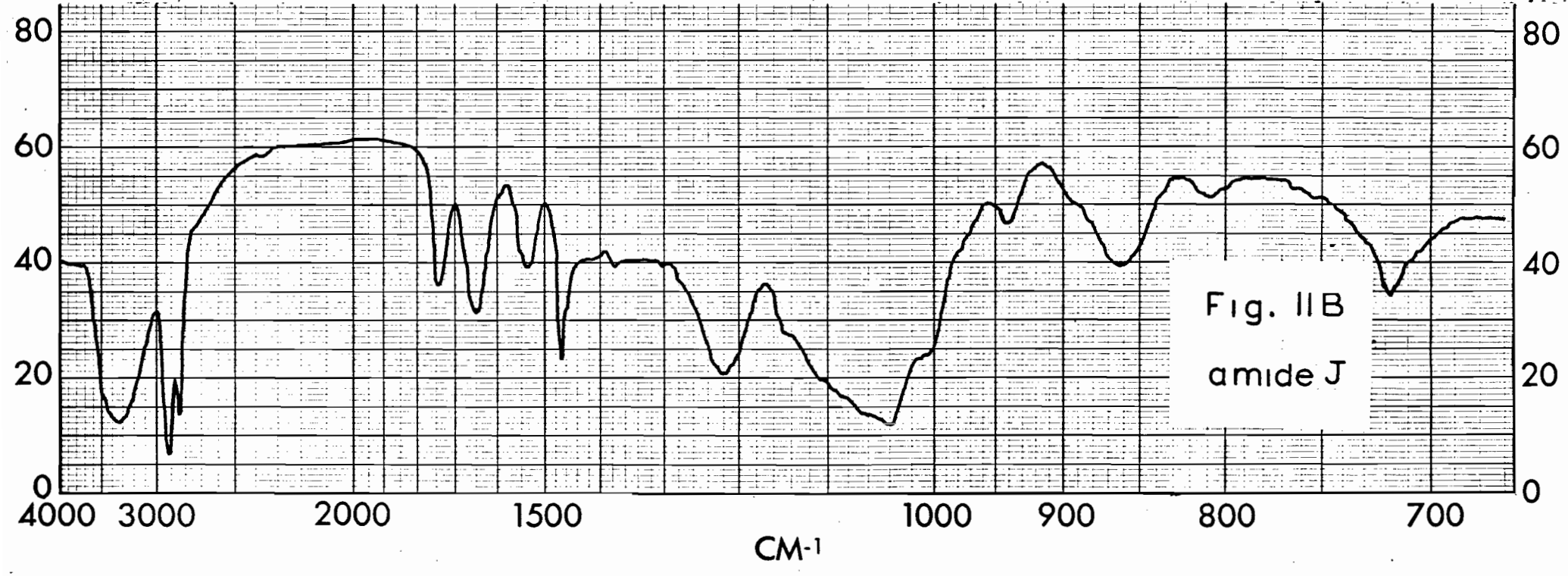


Fig. IIB
amide J

adenosine triphosphate with formation of homocysteine $\left[HSCH_2CH_2CH(NH_2)CO_2H\right]$. The methionine actually reacts as its S-(5-deoxyadenosyl-5) derivative (Z), a compound having the sulfonium structure, and the demethylated product is S-(5-deoxyadenosyl-5) homocysteine (Y).

3. The methanol extract. (Amide I and J). Concentration of the methanol extract gave two micro-crystalline compounds I and J. Their infrared spectrum differed only by the absence (I) (see Fig. 11A) or presence (J) (see Fig. 11B) of a strong peak in the carbonyl region at 1750 cm^{-1}. Strong absorption in the 3300 cm^{-1} region together with intense peaks at 1650 cm^{-1} and 1550 cm^{-1} were suggestive of an amide or lactam; also, broad absorption in the 1100-1000 cm^{-1} region indicated hydroxylation; a peak at 720 cm^{-1} was typical of a long alkyl chain.

(a) Amide I. Recrystallization of compound I from methanol* did not raise the melting point from m.p. 216-218°. Its elemental analysis suggested the empirical formula $C_{33}H_{68}NO_{13}$. It did not form a homogeneous acetate, trimethylsilyl ether, benzoate or picrate. Hydrolysis with 5% methanolic potassium hydroxide gave an inhomogeneous hydroxy amine K (t.l.c.), which absorbed in the infrared (Fig. 12)

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* A soxhlet extraction had to be undertaken due to the material's insolubility in any solvent.

Fig. 12

The infrared spectrum of the hydroxy amine K obtained from the alkaline hydrolysis of I.

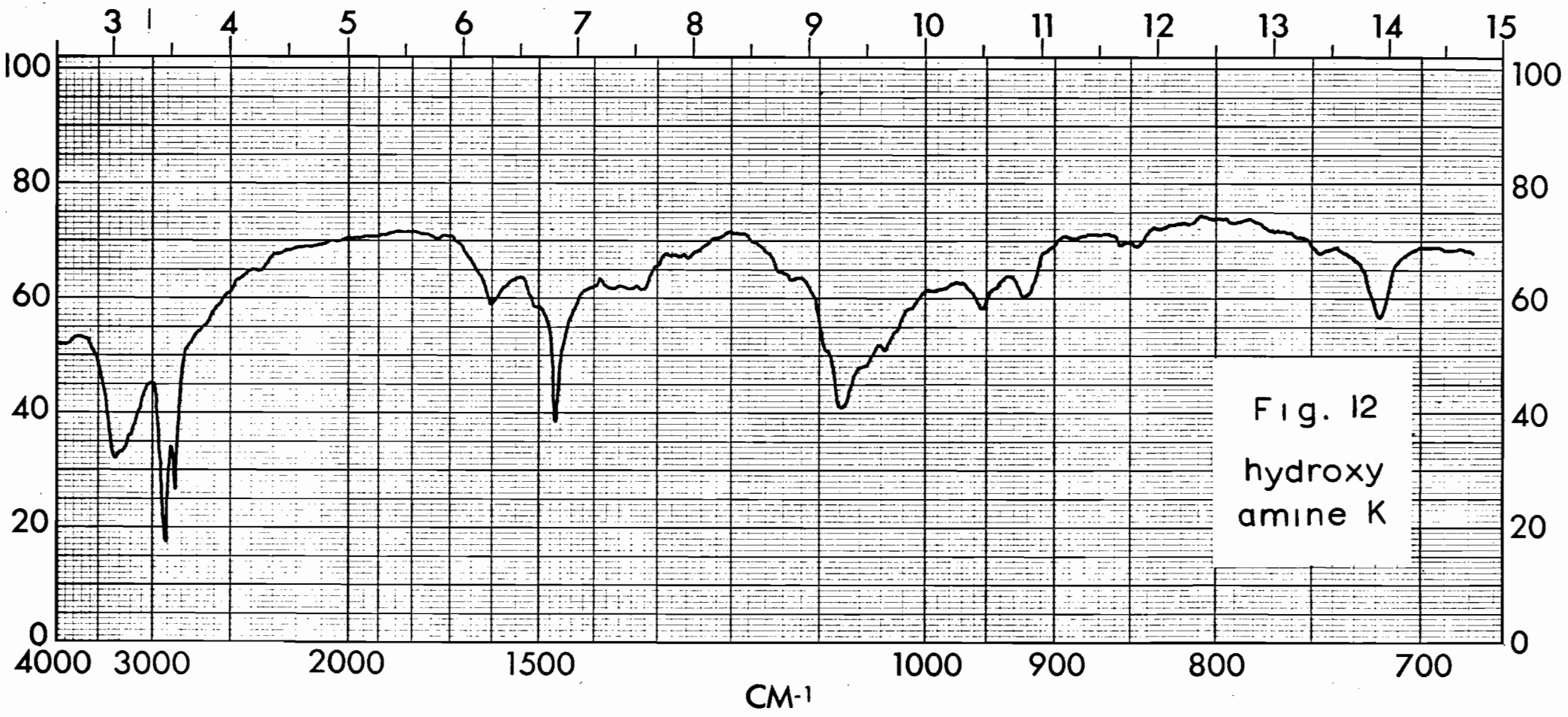


Fig. 12

hydroxy amine K

Fig. 13

The infrared spectra of

A. The hydroxy acid-lactone mixture L obtained from the alkaline hydrolysis of amide I.

B. Lactone M resulting from the acidification of mixture L.

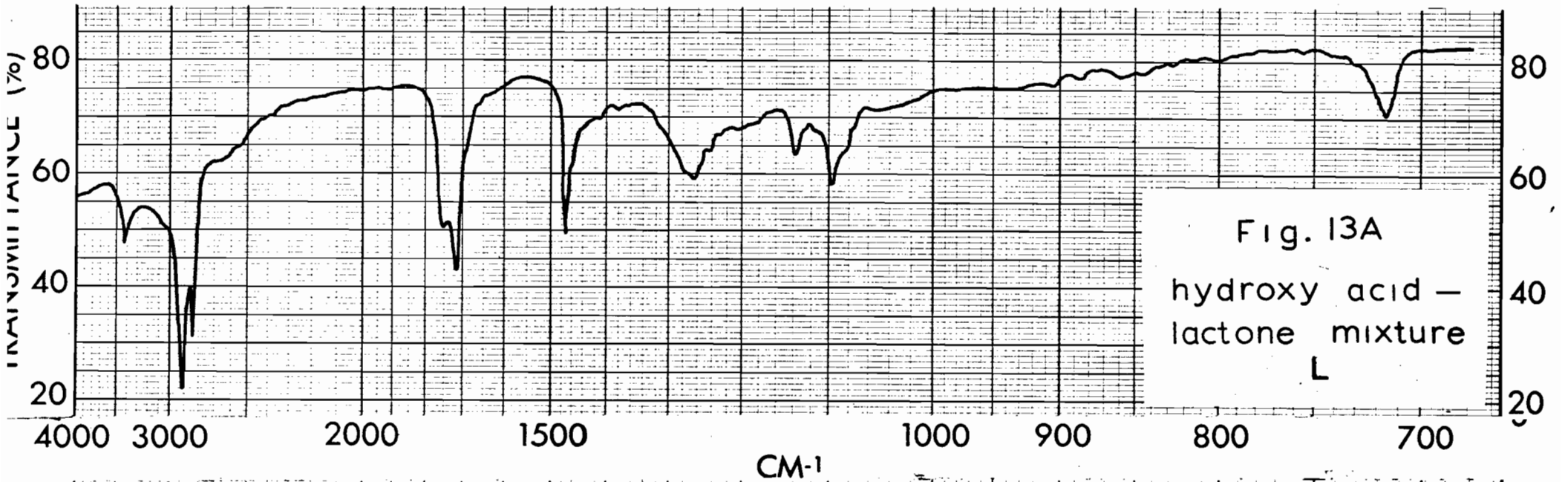


Fig. 13A

hydroxy acid — lactone mixture L

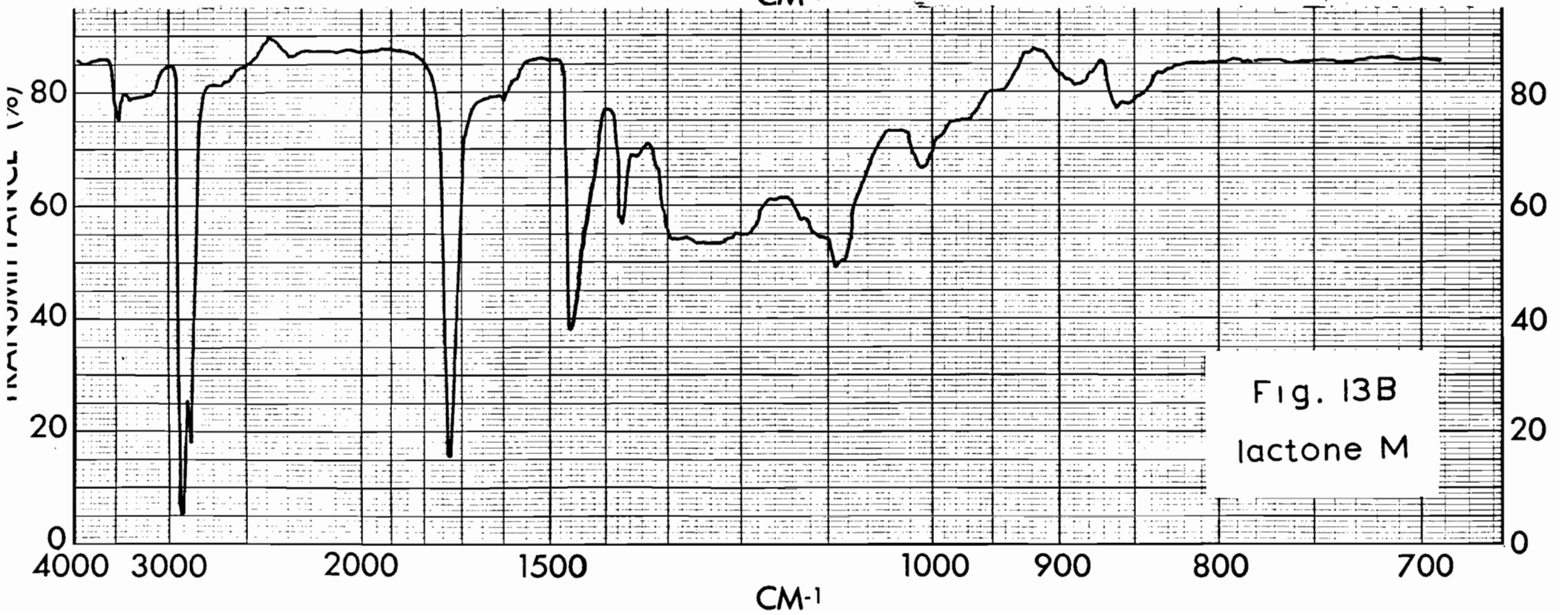
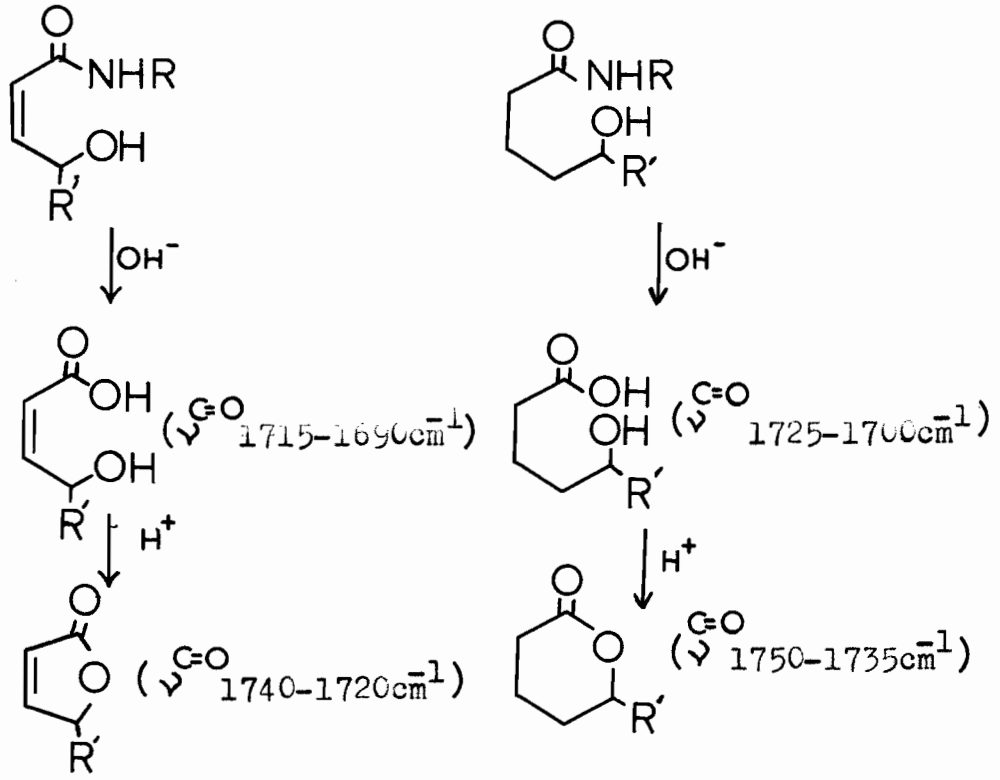


Fig. 13B

lactone M

at 3400, 1600, 1080 cm^{-1}. K gave a positive ninhydrin test. After two recyrstallizations from ethyl acetate, the material melted at 95-98°, but still appeared inhomogeneous (t.l.c.). A purification was therefore attempted by preparative t.l.c. on silica gel using chloroform-formic acid (4:1) as the developing solvent. After recrystallization of the main band from acetone, a compound, m.p. 82-86° was obtained. Recrystallization of the mother liquor gave another compound, m.p. 73-76°. Too little material was available to establish the structure of these compounds. From the hydrolysis, a waxy hydroxy-acid L was also obtained. It seemed to be present as a mixture with a lactone. L absorbed in the i.r. (Fig. 13A) at 3400 cm^{-1} (hydroxyl), 1750 cm^{-1} (lactone carbonyl), 1270 cm^{-1} (C-O-C stretch of lactone), 1100 cm^{-1} (hydroxyl) and 715 cm^{-1} (alkyl chain). In ethanol solution, the acid-lactone mixture displayed a pink coloration. Acidification of the mixture in ethanol-hydrochloric acid gave a waxy solid M showing only one peak in the i.r. (Fig. 13B) in the carbonyl region at 1740 cm^{-1} (lactone). This could either be due to an unsaturated 5-membered ring lactone or a saturated 6-membered ring lactone. However, the absorption of the corresponding hydroxy-acid in the i.r. at 1725 cm^{-1} ruled out the possibility of an α,β-unsaturated carboxylic acid. Therefore the compound is most probably a saturated δ-hydroxyacid. No further work was done to characterize the hydroxyacid since less than 50 mg. was available.



(b) Amide J. Two recrystallizations from a chloroform-methanol mixture gave a product, m.p. 192.5-193.5°. Its n.m.r. spectrum was highly uninformative except to confirm the aliphatic, straight chain, poly-hydroxylated* nature of the compound. Elemental analysis for C, H, N, O, and S could only account for 90% of the elements. No chlorine or phosphorus were detected. Hydrolysis gave too little material to justify further work.

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* Signals due to the presence of hydroxyl groups could not be seen in the spectrum; however an HOD peak was obtained, after mixing the sample with some deuterium oxide, indicating the presence of exchangeable protons.

B. Experimental*

A continuous soxhlet extraction of the dried and powdered mycelium (843 g.) with petroleum ether (b.p. 40-70°) for one week, and with chloroform and methanol for several weeks respectively, gave 12.0 g. (1.42% based on dry weight of mycelium), 17.0 g. (2.0%) and 140 g. (16.6%) respectively.

1. The petroleum ether extract. A crystalline compound m.p. 153-5°, λ_{max} 294, 282, 271, 262 mμ identified as ergosterol separated while the crude petroleum ether extract was being concentrated.

2. The chloroform extract. Upon concentration of the extract to approximately 250 ml., a total of 988 mg. (5.8% based on crude extract, 0.11% based on dry weight of mycelium) of crystals separated, m.p., 200-202°. λ_{max}^{MeOH} 260 mμ. A sample was recrystallized twice from methanol to give m.p. 206-207.5°; ν_{KBr} (Fig. 7B) 3300, 3150 cm^{-1} (OH, NH_2), 2900-2500 cm^{-1} (OH bonded), 1665 cm^{-1} (shoulder), 1645 cm^{-1} (C=N), 1620, 1580 cm^{-1} (heteroaromatic), complex spectrum below 1500 cm^{-1}. For n.m.r. details see Table I, and the corresponding spectra, figures 9 and 10.

Calc. for $C_{11}H_{15}N_5O_3S$: C, 44.44; H, 5.08; N, 23.57; O, 16.14; S, 10.77.

Found: C, 45.14; H, 5.36; N, 22.80; O, 17.19; S, 10.84.

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* See Chapter I for general procedures.

Another sample of the crude crystals were twice recrystallized from water to give m.p. 210.5-212°; ν_{KBr} (Fig. 7A) 3400, 3300, 3100 cm^{-1} (NH , OH), 3000-2500 cm^{-1} (-OH bonded), 1665 cm^{-1} (C=N), 1645 cm^{-1} (shoulder), 1620, 1580 cm^{-1} (heteroaromatic), complex pattern below 1500 cm^{-1}; $[\alpha]_D$ + 21.25° (C, 1.05 in chloroform).

Calc. for $C_{11}H_{15}N_5O_3S$: C, 44.44; H, 5.08; N, 23.57; O, 16.14; S, 10.77.

Found: C, 45.10; H, 5.25; N, 24.46; O, 16.16; S, 10.84.

The methanol-crystallized material was recrystallized from water to give crystals having an i.r. identical with that obtained previously when the crude was directly crystallized from water and an undepressed mixed melting point.

Picrate

A solution of thiomethyladenosine (15 mg.) in water (1 ml.) was treated dropwise with a saturated aqueous solution of picric acid until no further precipitate was obtained. The solution was then boiled until the precipitated picrate had redissolved, and allowed to stand at room temperature overnight. The crystalline picrate (15 mg.) melted at 196-197°; ν_{KBr} 3100 cm^{-1}, 3000-2500 cm^{-1} (OH bonded), 1700, 1630, 1570, 1550, 1510 cm^{-1}.

Calc. for $C_{11}H_{15}N_5O_3S.C_6H_3N_3O_7$: C, 38.78,
H, 3.42; N, 21.29.
Found: C, 38.08; H, 4.06; N, 21.77.

3. The methanol extract. Concentration of the extract in methanol gave two microcrystalline compounds, I (1.5 g., approximately 0.01% based on methanol extract), m.p. 213-215° (decomp.), and J (1.5 g.) m.p. 200-208°.

I was insoluble in all the commonly used organic solvents including dimethylsulfoxide. It was, however, slightly soluble in methanol and was recrystallized from methanol by means of a soxhlet extractor. There was no change in melting point. For the i.r. spectrum, see Fig. 11A.

Calc. for $C_{33}H_{68}NO_{13}$: C, 57.80; H, 9.91 ; N,2.04 .
Found: C, 56.70; H, 9.44; N, 2.40.

Unlike I, compound J was soluble in chloroform. Recrystallization of J from a chloroform-methanol mixture gave a compound, m.p. 192-193° (decomp.); ν_{KBr} (Fig. 11B) 1750 cm^{-1}; δ_{CDCl_3} 1.26 p.p.m. and 0.96 p.p.m. (aliphatic straight chain compound). Its elemental analysis could only account for 90.58%: C, 57.23; H, 9.83; N, 1.75; O, 20.74; S, 1.03; $C-CH_3$, 1.49%. No chlorine and no phosphorus were detected.

I (504 mg.) was hydrolyzed with 100 ml. of 5% methanolic potassium hydroxide solution overnight at reflux temperature. The alkaline hydrolysate was concentrated to a small volume, water added, and extracted with ether. The organic phase was washed with water several times, dried over magnesium sulphate, filtered and the solvent evaporated giving 279 mg. of white solid (K). Its i.r. (Fig. 12) was indicative of a hydroxy amine. (It produced a violet color after a solution of K was boiled with ninhydrin). Recrystallization of K from ethyl acetate gave a compound melting at 95-98°. A second recrystallization did not change the melting point. Since t.l.c. (chloroform - formic acid 4:1) showed the presence of impurities, a purification of K was attempted by t.l.c. When the main band was recrystallized from acetone, two compounds m.p. 82-86° and m.p. 73-76° were obtained. The presence of too little material did not warrant any further investigation.

The alkaline extract of the hydrolysis was acidified with concentrated hydrochloric acid, and extracted with ether. The organic layer was washed several times with water, dried, filtered and the solvent evaporated. A waxy solid (73 mg.) L resulted. Its i.r. (see Fig. 13A) indicated a hydroxy acid. When it was dissolved in ethanol, it displayed a pink

coloration. Acidification of a 6mg. sample of L dissolved in ethanol for 16 hours, followed by evaporation of the solvent, and ether extraction gave, after the usual work-up procedure, 6 mg. of a lactone (see Fig. 13B).

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Claims to Original Research

1. The photolysis of 6-formyltestosterone methol enol ether acetate was investigated, and the structure of the photoproduct proven.
2. Its reaction path was established kinetically.
3. The photolyses of the semicarbazone and oxime derivatives of 6-formyl testosterone methyl enol ether acetate have also been described.
4. Reaction paths for the above reactions have been suggested.
5. A photodehydration has been observed in the irradiation of the oxime derivative.
6. Thiomethyladenosine has been isolated from C. albicans and identified.
7. A lactone fraction, isolated from C. parapsilosis, and an amide fraction, isolated from C. albicans, were isolated and studied.