[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH AND THE DIVISION OF CHEMISTRY OF THE NATIONAL RESEARCH COUNCIL OF CANADA[1]]

Studies in Steroid Metabolism. VI. The Characterization of 11- and 12-Oxygenated Steroids by Infrared Spectrometry

BY R. NORMAN JONES, P. HUMPHRIES AND KONRAD DOBRINER

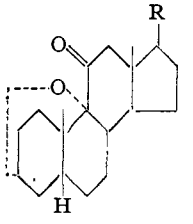
The use of infrared spectrometry for the detection and identification of steroid metabolites in human urine has been reported in a previous publication in this series.[2] It has also been demonstrated that the presence of certain functional groups in the steroid molecule can be recognized by infrared spectrometry,[3] and a distinction between ketosteroids in which the carbonyl group is at the 3, 17 or 20 positions can be made from a determination of the location of the carbon–oxygen stretching vibration in the region between 1660 and 1780 cm.$^{-1}$.

It seemed reasonable to anticipate that a carbonyl group at positions 11 and 12 might also absorb at characteristic frequencies and a systematic investigation of the infrared spectra of a number of steroids containing ketonic and hydroxyl groups at these positions has been carried out.

The recognition of steroids oxygenated at position 11 is of special importance in connection with steroid metabolism, since carbonyl or hydroxyl groups at this position are indicative of steroids originating in the cortex of the adrenal gland. Among the twenty-eight steroids isolated from the adrenal gland[4] fifteen contain a ketone or hydroxyl group at this position.

It has already been demonstrated[5,6] that metabolites of 11-oxygenated adrenocortical hormones are present in human urine. Androstanediol-3α,-11β-one-17, etiocholanol-3α-dione-11,17, and androstanol-3α-dione-11,17 occur in the urine of both normal and diseased persons,[5,6,7] while etiocholanediol-3α,11β-one-17, its transformation product $\Delta^{9,11}$-etiocholenol-3α-one-17 and pregnanol-3α-dione-11, 20 have been observed frequently in the urine of diseased persons and rarely in the urine of healthy individuals.[6]

In addition to these completely characterized compounds, a variety of additional steroids containing a hydroxyl or a carbonyl group at position 11 may be found in human urine, and some of



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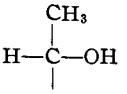
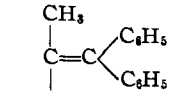
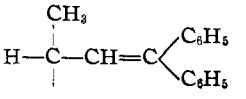
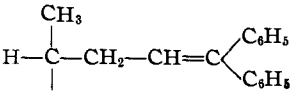
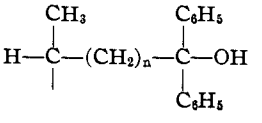
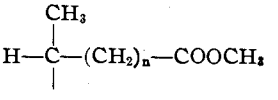
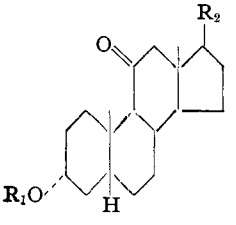
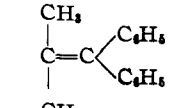
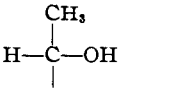
I	
II	
III	
IV	
V (n = 1), VI (n = 2)	
VII (n = 0), VIII (n = 1), IX (n = 2)	



	R_1	R_2
X	—H	
XI	—H	
XII	—H	OH
XIII	—$COCH_3$	O—$COCH_3$
XIV	—H	$COOCH_3$

(1) Published as Contribution No. 1667 from the Laboratories of The National Research Council of Canada.

(2) Dobriner, Lieberman, Rhoads, Jones, Williams and Barnes, *J. Biol. Chem.*, **172**, 297 (1948).

(3) Jones, Williams, Whalen and Dobriner, THIS JOURNAL, **70**, 2024 (1948).

(4) T. Reichstein and C. W. Shoppee, "The Hormones of the Adrenal Cortex," Vitamins and Hormones, Vol. I, Academic Press, Inc., New York, N. Y., 1943, pp. 345–413.

(5) Mason, *J. Biol. Chem.*, **158**, 719 (1945).

(6) Lieberman, Fukushima and Dobriner, Federation of American Societies for Experimental Biology, Proceedings, Vol. VII, 168 (1948). See also Dobriner, Lieberman, Hariton, Sarett and Rhoads, *J. Biol. Chem.*, **169**, 221 (1947).

(7) Lieberman and Dobriner, *J. Biol. Chem.*, **166**, 773 (1946).

TABLE I

CARBON–OXYGEN STRETCHING VIBRATION OF 11-KETOSTEROIDS

Compound	No. structure	Position of max. cm.$^{-1}$ (CS_2)	Source[a]
A. 11-Monoketosteroids			
3α,9α-Epoxypregnanol-20-one-11	I	1713	12
$\Delta^{20,22}$-3α,9α-Epoxy-22,22′-diphenylbisnorcholenone-11	II	1713	12
Δ^{22}-3α,9α-Epoxy-23,23′-diphenylnorcholenone-11	III	1713	12
Δ^{23}-3α,9α-Epoxy-24,24′-diphenylcholenone-11	IV	1713	12
3α,9α-Epoxy-23,23′-diphenylnorcholanol-23-one-11	V	1713	12
3α,9α-Epoxy-24,24′-diphenylcholanol-24-one-11	VI	1713	12
$\Delta^{20,22}$-22,22′-Diphenylbisnorcholanol-3α-one-11	X	1713	12
Pregnanediol-3α,20(*epi*)-one-11[b]	XI	1710, 1742[d]	9
Etiocholanediol-3α,17α-one-11	XII	1716[c]	9
B. 11-Ketosteroid Esters			
3α,9α-Epoxy-11-ketobisnorcholanic acid methyl ester	VII	1742, 1713	12
3α,9α-Epoxy-11-ketonorcholanic acid methyl ester	VIII	1742, 1713	12
3α,9α-Epoxy-11-ketocholanic acid methyl ester	IX	1745, 1713	12
Etiocholanediol-3α,17α-one-11-diacetate	XIII	1742, 1713	9
3α-Hydroxy-11-ketoetiocholanic acid methyl ester	XIV	1742, 1713	12
3α-Hydroxy-11-ketobisnorcholanic acid methyl ester	XV	1742, 1710	12
3α-Hydroxy-11-ketonorcholanic acid methyl ester	XVI	1742, 1710	12
3α-Acetoxy-11-ketonorcholanic acid methyl ester	XVII	1739, 1710	12
3α-Hydroxy-11-ketocholanic acid methyl ester	XVIII	1742, 1710	12
3α-Acetoxy-11-ketocholanic acid methyl ester	XIX	1742, 1710	12
Pregnanediol-3α-20-one-11-diacetate	XX	1739, 1710	9
Pregnanediol-3α-20(*epi*)-one-11-diacetate[b]	XXI	1739, 1710	9
C. 3,11-, 11,20- and 3,11,20-Ketosteroids and Related Esters			
Pregnanol-3α-dione-11,20	XXII	1713	8, 9, 12
Pregnanol-3α-dione-11,20-acetate	XXIII	1739, 1710	12
Uranedione-3,11	XXIV	1713	5
Pregnanetrione-3,11,20	XXV	1713	1, 12
D. 11,17-, 3,11,17-Ketosteroids and Related Esters			
Androstanol-3α-dione-11,17	...	1751, 1713	6
Etiocholanol-3α-dione-11,17	...	1754, 1716	9
Androstanol-3α-dione-11,17-acetate	XXVI	1745[e], 1713	6
Etiocholanol-3α-dione-11,17-acetate	...	1748, 1739, 1713	9
Androstanetrione-3,11,17	...	1751, 1716[e]	4
Etiocholanetrione-3,11,17	XXVII	1751, 1716[e]	4, 9
Δ^4-Androstenetrione-3,11,17 (adrenosterone)	XXVIII	1751, 1719, 1674	2
E. 11,20-Diketo-21-acetoxysteroids			
Pregnanediol-3α-21-dione-11,20-acetate-21	XXIX	1758, 1732, 1713	9
Pregnanol-21-trione-3,11,20-acetate	XXX	1758[f], 1732, 1713	9
Δ^4-Pregnenol-21-trione-3,11,20-acetate (11-dehydrocorticosterone acetate)	XXXI	1758, 1732 1716, 1674	9

[a] (1) T. F. Gallagher, Sloan-Kettering Inst., New York, N. Y. (2) E. C. Kendall, Mayo Clinic, Rochester, Minn. (3) A. Lardon, University of Basel, Basel, Switzerland. (4) S. Lieberman, Sloan-Kettering Inst., New York, N. Y. (5) R. E. Marker, Pennsylvania State College, State College, Penna. (6) Compound prepared or isolated at Memorial Hospital, New York, N. Y. (7) V. Prelog, Eidg, Tech. Hochschule, Zurich, Switzerland. (8) T. Reichstein, University of Basel, Basel, Switzerland. (9) L. H. Sarett, Merck and Co., Rahway, N. J. (10) C. R. Scholz, Ciba Pharmaceutical Products, Inc., Summit, N. J. (11) E. Schwenk, The Schering Corp., Bloomfield, N. J. (12) R. Turner, Harvard University, Cambridge, Mass. (13) A. L. Wilds, University of Wisconsin, Madison, Wis. [b] In these compounds the configuration at position 20 is uncertain and the prefix "epi" is used arbitrarily for convenient reference. [c] Solvent tetrachlorethane. [d] Weak band attributed to an impurity. [e] Broad maximum. [f] Suspension of crystalline material in saturated solution in carbon disulfide.

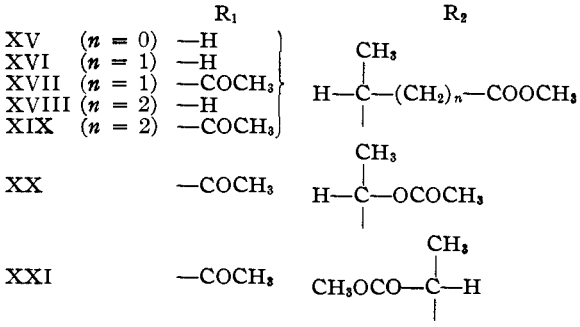
these may be included among compounds which have already been isolated, but which have not, as yet, been fully characterized.[8]

11-Ketosteroids.—The recognition of an absorption band associated with the 11-ketone group could be achieved unequivocally from measurements on 11-monocarbonyl compounds in which there could be no interference from carbonyl groups located elsewhere in the molecule. Unfortunately, the number of such compounds is limited, but through the kind coöperation of Dr. R. Turner and Dr. L. H. Sarett we have obtained

(8) Lieberman, Dobriner, Hill, Fieser and Rhoads, *J. Biol. Chem.*, **172**, 263 (1948).

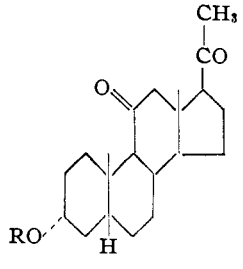
nine 11-ketosteroids which satisfy this criterion.

The infrared absorption spectra of carbon disulfide solutions of eight of these substances (I–VI, X–XI) exhibit one maximum only in the carbonyl region at 1710–1713 cm.$^{-1}$. The ninth compound (XII) gave a maximum at 1716 cm.$^{-1}$ in solution in tetrachlorethane (Table IA). Similar data are also listed in Table IB for twelve 11-ketosteroid esters (VII–IX, XIII–XXI), all of which possess two maxima in the carbonyl region at 1710–1713 and 1739–1742 cm.$^{-1}$, respectively.



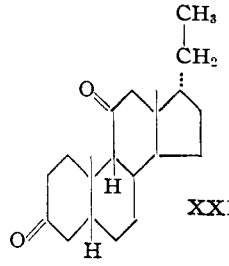
It has been shown previously[3] that both the 3- and 17-acyl esters of steroid alcohols as well as the methyl esters of cholanic acid derivatives possess a maximum at 1739–1742 cm.$^{-1}$. Consequently in these compounds it may be inferred that the absorption band at 1710–1713 cm.$^{-1}$ is associated with the carbonyl group at position 11, and that no interactions occur between the vibrations associated with the ketone group at this position and ester groups at positions 3, 17 or on the side chain.

11,20-Diketones.—In the non-conjugated 20-ketosteroids the carbonyl absorption maximum is at 1706–1710 cm.$^{-1}$ and because of the proximity of the 11-ketone and 20-ketone band maxima a simple spectrometric differentiation of these two important structures is uncertain (*vide* p. 246). Two compounds containing 11,20-diketone grouping have been examined. In XXII only a single band at 1713 cm.$^{-1}$ occurs, while XXIII has two bands at 1710 and 1739 cm.$^{-1}$, the latter due to the ester group at position 3.

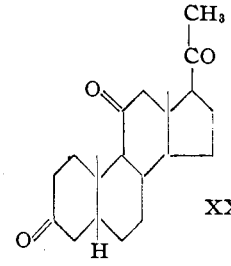


XXII (R = —H)
XXIII (R = —CO—CH3)

3,11-Diketones.—A similar situation arises in the identification of 11-ketosteroids which contain a second (unconjugated) ketone group at position 3, as the non-conjugated 3-ketone group absorbs at 1715–1719 cm.$^{-1}$ which is also very close to the frequency of the 11-ketone band. The only compound of this kind so far examined is uranedione-3,11 (XXIV) which gives a single maximum at 1713 cm.$^{-1}$. Measurements on compounds XXV and XXVII support the evidence from XXIV in indicating that no anomalous displacements of the band positions are associated with the 3,11-diketone structure.



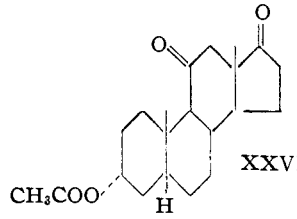
XXIV



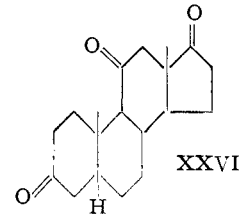
XXV

In 11-ketosteroids containing a conjugated ketone group at position 3, the band at 1674–1677 cm.$^{-1}$ characteristic of this conjugated group occurs at the expected position.

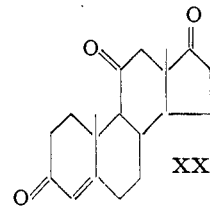
11,17-Diketones.—In 17-ketosteroids in which ring D is saturated, an absorption maximum occurs at 1742–1745 cm.$^{-1}$, but in 11,17-diketones this band is displaced to 1748–1754; the band normal to the 11-ketone at 1710–1716 cm.$^{-1}$ is present also.[9]



XXVI



XXVII



XXVIII

Interaction Effects.—These measurements indicate that interaction occurs between carbonyl groups at positions 11 and 17 which displace the bands from their normal positions. A similar interaction effect has been noted previously between a ketone group at position 20 and an acetoxy group at position 21 in the acetylated derivatives of steroids containing the ketol side chain.[3] Compounds XXIX–XXXI contain both the 11-ketone and the 20-keto-21-acetoxy groupings and are of particular interest as they are derivatives of adrenocortical steroid hormones. The bands at 1732 and at 1756–1758 cm.$^{-1}$,

(9) In compound XXVI which contains an acetate group at position 3 in addition to the 11,17-diketone group, the band maxima are at 1745 and 1713 cm.$^{-1}$. It is presumed that the former band is an unresolved doublet, due to the acetate band at 1739–1742 and a band at 1748–1754 cm.$^{-1}$. In the corresponding compound in the etiocholane stereochemical series this band is resolved (see Table ID).

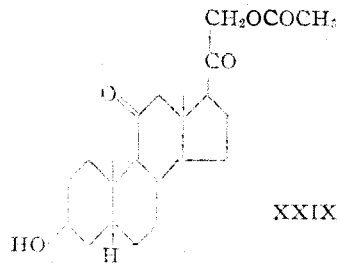
TABLE II

CARBON–OXYGEN STRETCHING VIBRATION OF 12-KETOSTEROIDS

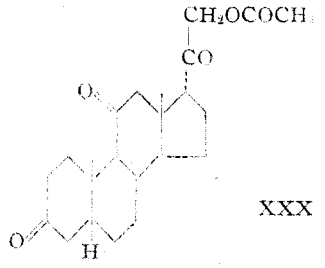
Compound	Structure	Position of max. (cm.$^{-1}$) solvent CS_2	Source[a]
Pregnanone-12	.....	1710	8
3α-Acetoxy-12-ketocholanic acid methyl ester	XXXII	1742, 1710	10, 12
Δ^4-Pregnenol-21-trione-3,12,20-acetate	XXXIII	1758, 1732, 1716, 1677	10
3α,7α-Dihydroxy-12-ketoetiocholanic acid methyl ester	XXXIV	1739, 1723[b]	8
3α-7α-Diacetoxy-12-ketoetiocholanic acid methyl ester	XXXV	1742, 1723	8
3α-Succinoxy-7α-acetoxy-12-ketoetiocholanic acid methyl ester	XXXVI	1742, 1723	8
$\Delta^{9,11}$-3α-Acetoxy-12-ketocholenic acid	.....	1742, 1713, 1684	10
$\Delta^{9,11}$-3α-Acetoxy-12-ketocholenic acid methyl ester	.....	1742, 1680	11
11,12-Diketonorcholanic acid methyl ester	.....	1742, 1726	3
3,11,12-Triketonorcholanic acid methyl ester	.....	1739[c], 1726	12
3,12-Diketocholanic acid methyl ester	.....	1742, 1716	12

[a] See footnote to Table I. [b] See footnote to Table I. [c] Inflection only.

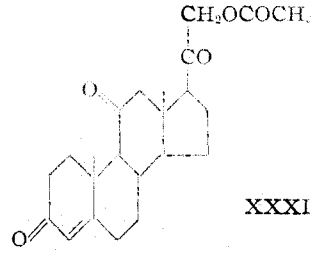
characteristic of the 20-keto-21-acetoxy group in addition to the 11-ketosteroid maximum at 1710–1713 cm.$^{-1}$ occur in all three compounds.[10]



XXIX



XXX

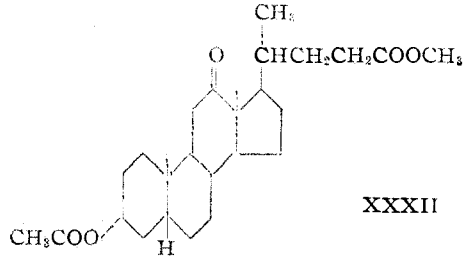


XXXI

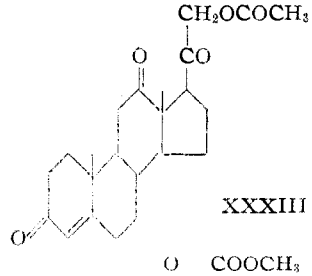
It may be concluded that a band at 1754–1760 cm.$^{-1}$ is suggestive of the presence of either the 11,17-diketone or the 20-keto-21-acetoxy grouping. The former structure also produces a second maximum at 1710–1716 cm.$^{-1}$ whereas in the latter case the second band is near 1730 cm.$^{-1}$. The distinction between these two groupings can be confirmed from measurements in the 1200–1250 cm.$^{-1}$ region since a strong band characteristic of the acetoxy group and lacking in the carbonyl group occurs near 1230 cm.$^{-1}$.

(10) In XXX the non-conjugated 3-ketone group no doubt contributes also to the maximum at 1713 cm.$^{-1}$.

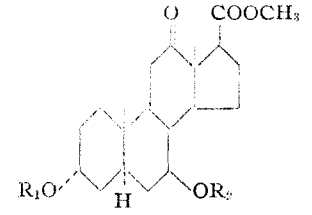
12-Ketosteroids.—Eleven compounds containing a carbonyl group at position 12 have been examined (see Table II). In pregnanone-12 and XXXII the band at 1710 cm.$^{-1}$ can be assigned to the unconjugated 12-ketone grouping. The spectrum of XXXIII, which contains the acetylated ketol side chain and the conjugated ketone group at position 3 exhibits the maxima characteristic of all four carbonyl groups (*cf.* Fig. 1). In compounds XXXIV–XXXVI which contain the 12-keto-17-carbomethoxy grouping, the 12-ketone band is displaced to 1723 cm.$^{-1}$ (*vide p.* 246).



XXXII



XXXIII



XXXIV ($R_1 = R_2 = H$)
XXXV ($R_1 = R_2 = —CO—CH_3$)
XXXVI ($R_1 = —CO—CH_2—CH_2—COOH$; $R_2 = —CO—CH_3$)

In the Δ^9–12 ketones the maximum is at 1680–1684 cm.$^{-1}$. This is significantly higher than the

frequency observed for Δ^4-3-ketosteroids (1674–1677 cm.$^{-1}$) and for Δ^{16}-20-ketosteroids (1666–1669 cm.$^{-1}$). The two 11,12-diketones listed in Table II possess a maximum at 1726 cm.$^{-1}$. The displacement of this band from 1706–1716 to 1726 cm.$^{-1}$ is indicative of interaction in the α-diketone structure.

Other Ketosteroids.—As these investigations were initiated with the primary object of improving techniques for the identification of steroid hormones and hormone metabolites, attention has been chiefly directed to steroids containing functional groups at the positions which are of major importance in relation to the hormonal activity. Up to the present time only a few steroids containing carbonyl groups at other positions have been examined; the results, summarized in Table III, suggest that the non-conjugated 7-ketone group absorbs near 1719 cm.$^{-1}$, the non-conjugated 16-ketone near 1754 cm.$^{-1}$, the 3,6-diketosteroid group at 1723 cm.$^{-1}$ and the $\Delta^{3,5}$-diene-one-7 group at 1663 cm.$^{-1}$. One monoketosteroid of uncertain structure kindly supplied by Dr. Heard[11] and believed to contain a ketone group at position 15 had an absorption maximum at 1751 cm.$^{-1}$. The location of this band is in general agreement with the observation that a carbonyl group in a cyclopentanone ring system absorbs at a high frequency, and would seem to be consistent with the postulated structure of this compound.

11-Hydroxy Compounds.—The position of the hydrogen–oxygen stretching vibration of the hydroxyl group in a steroid molecule cannot be related to the position of substitution in the molecule in a manner similar to that for the carbon–oxygen bond of the carbonyl group.[3] Nevertheless, the presence of an absorption band near 3600 cm.$^{-1}$ in the spectrum of a solution of a steroid is associated with the presence of a hydroxyl group in the molecule.

TABLE III

CARBON–OXYGEN STRETCHING VIBRATION OF KETOSTEROIDS WITH CARBONYL GROUPS AT POSITIONS OTHER THAN 3, 11, 12, 17 AND 20

Compound	Position of max. (cm.$^{-1}$) solvent CS_2	Source[a]
Sitostanedione-3,6	1723	5
Δ^{22}-Sitostenedione-3,6	1723	5
3α,12α-Diacetoxy-7-ketocholanic acid methyl ester	1742 1719	1
3α-Succinoxy-12α-acetoxy-7-ketoetiocholanic acid methyl ester	1742 1719	8
3α-12α-Dihydroxy-7-ketocholanic acid	1719	1
$\Delta^{3,5}$-Cholestadiene-one-7	1663	7
$\Delta^{1,3,5:10,6,8}$-Estrapentaene-one-16	1754	13

[a] See footnote to Table I.

(11) Heard and McKay, *J. Biol. Chem.*, **181**, 371 (1939); **165**, 677 (1946).

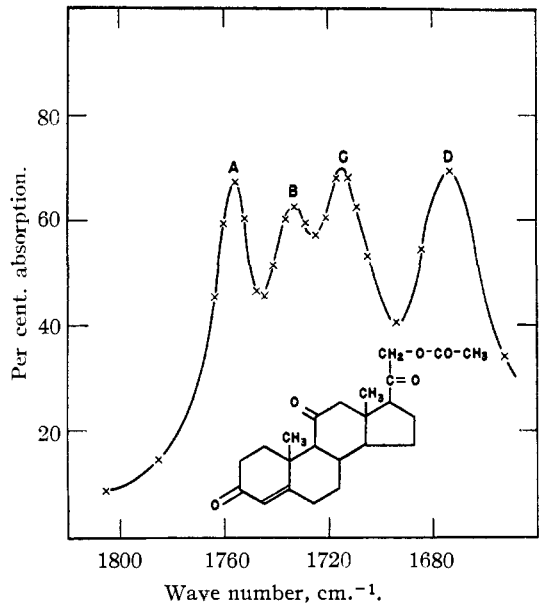


Fig. 1.—Infrared absorption spectrum of 11-dehydrocorticosterone acetate between 1640 and 1800 cm.$^{-1}$. Maxima A and B are associated with the acetylated ketol side chain, maximum C with the ketone group at position 11, and maximum D with the $\alpha\beta$-unsaturated ketone group at position 3 (solvent carbon disulfide).

No simple 11-monohydroxy steroids have been available for measurement, but several dihydroxy steroids in which one of the hydroxyl groups is at position 11 have been examined. In these spectra the normal hydroxyl absorption band near 3600 cm.$^{-1}$ is observed and no behavior specifically relatable to the 11 position is to be noted. In a compound such as androstanediol-3α,11β-one-17-acetate-3, where the hydroxyl group at position 3 is esterified, the absorption band at 3616 cm.$^{-1}$ can be attributed to the 11-hydroxyl group.

Discussion

The relationships which have been observed between the frequencies of the carbonyl band maxima, and the structure for all types of steroids are summarized in Table IV. In evaluating these data, it must be noted that they apply to solutions in carbon disulfide only. The significance which is to be attached to them is dependent on the number of compounds of each type examined, and this is indicated in the third column of the table.

The recognition of the 11-ketone group in a steroid by infrared spectrometry is only partially successful. Such identification is possible provided certain other ketone groupings are known to be absent, but non-conjugated ketone groups at other positions on the hexacyclic rings or in the side chain may absorb quite close to the same frequency. This is certainly true of 3-ketones and 20-ketones, and probably also of ketone groups at positions 6, 7 and 12. With the spec-

TABLE IV

SUMMARY OF POSITIONS OF CARBONYL BAND MAXIMA

Carbon disulfide solutions

Carbonyl type	Position of max. (cm.$^{-1}$)	Compounds examined
Phenolic 3-acetate (naphthalenoid)	1770	1
Phenolic 3-acetate (benzenoid)	1767–1764	4
21-Acetoxy-20-ketone	1758–1756[a]	4
Δ^{14}-17-Ketone	1754	2
16-Ketone	1754	1
$\Delta^{3,5}$-Diene-ol-3-acetate	1754	1
11,17-Diketone	1754–1751[a]	5
17-Ketone	1745–1742	15
Acyl esters of 3-, 11-, 12-, 17- and 20-hydroxysteroids		
Alkyl esters of cholanic, norcholanic, bisnorcholanic acids and related unsaturated acids	1742–1735	60
21-Acetoxy-20-ketone	1732[a]	7
11,12-Diketone	1726	2
Benzoates of steroid alcohols	1724–1717	5
12-Keto-etiocholanic acid alkyl esters	1723[b]	3
3,6-Diketone	1723	2
7-Ketone	1719	3
3-Ketone	1719–1717	14
Δ^{15}-17-Ketone	1716	2
11,17-Diketone	1716–1713[a]	5
11-Ketone	1716–1710	21
12-Ketone	1710	2
20-Ketone	1710–1706	19
Δ^{9}-12-Ketone	1684–1680	2
Δ^{4}-3-Ketone	1677–1674	17
Δ^{16}-20-Ketone	1670–1666	5
$\Delta^{4,6}$-Diene-3-ketone	1669–1666	2
$\Delta^{3,5}$-Diene-7-ketone	1663	1

[a] These structures give rise to two maxima both of which are listed in the table. [b] Second maximum at 1739 cm.$^{-1}$.

trometers at present available and using a sodium chloride prism, an accuracy of ± 3 cm.$^{-1}$ in the determination of the maximum is about the best which can be achieved. If this could be increased to better than ± 1 cm.$^{-1}$ a distinction between 3-, 11- and 20-ketones could be made more certainly. In the case of 3-, 11- and 11,20-diketones an increase in the resolving power of the spectrometer might separate the bands. A careful analysis of the widths and absolute intensities of the absorption bands may also yield further information; such measurements are being made, and will be reported in a subsequent communication.

Certain dicarbonyl groupings, notably the 11,-17-diketones and 11,12-diketones, appear to exhibit characteristic absorption. The band at 1726 cm.$^{-1}$ associated with the 11,12-diketone may be common to other α-diketosteroids, none of which has yet been studied. The absorption specific to the 20-keto-21-acetoxy group is significant in connection with the identification of adrenocortical hormones and their metabolites, as also is the observation that the introduction of the 11-ketone group does not disturb the position of these bands. An example is provided by the spectrum of dehydrocorticosterone acetate (XXXI) in which all of the four carbonyl maxima can be related to distinct carbonyl groups as indicated in Fig. 1.

The interaction effects which occur when two or more carbonyl groups are present are of theoretical interest. Such effects have only been encountered when the two carbonyl groups are in the 1:2, 1:3 or 1:4 positions relative to one another. In the 12-keto-17-carbomethoxy compounds (XXXIV–XXXVI), where the carbonyl groups are 1:4, the 12-ketone maximum is displaced from 1706–1710 cm.$^{-1}$ to 1723 cm.$^{-1}$, but in XIV, which contains the 11-keto-17-carbomethoxy 1:5-grouping, there is a maximum at 1713 cm.$^{-1}$ the location normal to the 11-ketone. In a similar manner, the 1:4 diketone group of 11,17-diketones exhibits an interaction effect while the 1:5-diketone group of 11,20-diketones does not.

The most effective application of infrared spectrometry to the characterization of 11-oxygenated ketosteroids will result from a combination with chemical methods. Advantage might be taken of the inertness of the 11-ketone group to reaction with the common carbonyl reagents, whereby the carbonyl absorption bands of the more reactive 3, 12, 17 and 20 ketone groups could be removed, and the 11-ketone band observed in the spectrum of the derivative. The inertness of the 11-ketone group to reaction with the Girard reagent has already been utilized in this manner. The appearance of an intense band near 1713 cm.$^{-1}$ in the spectrum of a "non-ketonic" urinary steroid fraction led to the isolation and identification of pregnanediol-3α,20-*epi*[b]-one-11.[12]

A similar method might be applied to characterize the unreactive 11β-hydroxyl group, which fails to acetylate under conditions which bring about acetylation of hydroxyl groups at 3, 12, 20, 21, and also at 17 in the androstane and etiocholane series. In the pregnane and allopregnane series the tertiary 17-hydroxyl group is also resistive to acetylation but can be made to react under mildly forcing conditions which still leave the 11β-hydroxyl group unattacked.[13]

The characterization of the readily acetylated 11α-hydroxyl group will be more difficult, but this is of less consequence since it appears that in the naturally occurring steroid hormones and hormone metabolites the 11-hydroxyl group commonly occurs in the β configuration.

Experimental

The spectra were determined in carbon disulfide solution using a Perkin–Elmer model 12a spectrometer with sodium chloride prism and recording galvanometer. Full details of the

(12) Reported by Dr. S. Lieberman at the Laurentian Hormone Conference, St. Adele, Quebec, Sept., 1947.

(13) Long and Gallagher, *J. Biol. Chem.*, **162**, 511 (1946).

experimental techniques have been given in earlier publications.[2,3]

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Summary

A comparative study has been made of the infrared absorption spectra between 1660 and 1780 cm.$^{-1}$ of ketosteroids containing a carbonyl group at positions 11 and 12.

A non-conjugated ketone group at position 11 gives rise to a maximum at 1710–1713 cm.$^{-1}$ in carbon disulfide solution. In 3,11- and 11,20-diketosteroids the maxima associated with the two carbonyl groups are too close to be separated, but there is no evidence of interaction effects causing displacements of the bands. In the 11,17-diketosteroids the band attributed to the 11-ketone group occurs at the normal position (1710–1713 cm.$^{-1}$) but the band attributed to the 17-ketone group is displaced from 1742–1745 to 1748–1754 cm.$^{-1}$.

Similar data are given also for 12-ketosteroids and the significance of these observations in the elucidation of steroid structure is discussed.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

The Dehydration of 22-Phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene

BY F. W. HEYL, M. E. HERR AND A. P. CENTOLELLA

The reaction of 3-acetoxy-bisnor-5-cholenaldehyde with phenylmagnesium bromide, yielding 22-phenyl-3,22-dihydroxy-bisnor-5-cholene (I) as well as the preparation of several analogous 22-phenyl-3-alkoxy-22-hydroxy-bisnor-5-cholenes from stigmasteryl ethers have been reported.[1,2] The problem of introducing a double bond into the C-20,22 position of the side chain involves the difficulties usually encountered in the dehydration of a secondary alcohol.

For the purpose of studying this dehydration reaction, it was decided to use 22-phenyl-3-methoxy-22-hydroxy-bisnor-5-cholene (III) which was prepared from stigmasteryl methyl ether as previously reported,[2] and also by refluxing the monotosylate (II) prepared from 22-phenyl-3,22-dihydroxy-bisnor-5-cholene (I), with methanol, whereby the same methoxy compound (III) is obtained and the position of this tosyl group is established.

The dehydration of this 3-methoxy-22-ol (III) was accomplished by using a variation of the method of Wuyts[3] in which the alcohol was refluxed in toluene in the presence of a catalytic amount of *p*-toluenesulfonic acid and a trace of phenol. The desired 22-phenyl-3-methoxy-bisnor-5,20-choladiene (IV) was obtained in good yields.

The 5,6 dibromo compound of the diene (IV) was ozonized directly to pregnene-3β-ol-20-one methyl ether (V) which was isolated as the semicarbazone in 62% yields. Acid hydrolysis of the semicarbazone resulted in a 92% yield of pregnenolone methyl ether (V). This compound was converted to pregnene-3β-ol-20-one acetate (VI), the immediate precursor of progesterone, according to the recently described method of Huffman and Lott.[4]

Experimental[5]

22-Phenyl-3,22-dihydroxy-bisnor-5-cholene-3-tosylate (II). —To 3.14 g. of diol (I) dissolved in 25 ml. of dry pyridine was added 2.2 g. (1$^1/_2$ moles) of *p*-toluene-sulfonyl chloride. After standing twenty-four hours at 37° it was poured into ice and sodium bicarbonate (1 g.) and the tosyl ester extracted with benzene. The solution was dried over sodium sulfate and upon removing the benzene and remaining pyridine *in vacuo* there was obtained a quantitative yield of crystalline residue. Recrystallization from benzene–hexane gave 2.85 g. of silky needles, m. p. 157–159° (dec.). Working up the mother liquor gave a second crop of slightly lower melting tosyl ester.

Anal. Calcd. for $C_{35}H_{46}O_4S$: C, 74.69; H, 8.24; S, 5.7. Found: C, 74.82; H, 7.98; S, 5.7. $[\alpha]^{25}D$ −38.9 (79.1 mg. made up to 10 ml. in chloroform, $\alpha^{25}D$ −0.308, *l*, 1 dm.).

When the above esterification was carried out at room temperature most of the diol was recovered. When the

(1) Heyl, Centolella and Herr, THIS JOURNAL, **69**, 1957 (1947).
(2) Centolella, Heyl and Herr, *ibid.*, **70**, 2953 (1948).
(3) Wuyts, *Bull. soc. chim. Belg.*, **26**, 304 (1912).
(4) Huffman and Lott, *J. Biol. Chem.*, **172**, 793 (1948).
(5) Analyses and rotations by members of the Upjohn microanalytical group. Melting points are as read on Fisher–Johns melting point apparatus.