# TABLE I NEW COMPOLINDS

Compounds	B. p., °C.	t, °C.	du	#24D	Fluor Calcd.	ine, % Found	Chior Calcd.	ine, % Found	Mol Calcd.	. wt. Found
CF3CHClCCl3	125.1	24	1.6757	1.4180	24.1	24.8	60.2	60.3	• • •	·
CF <sub>8</sub> CHClCCl <sub>2</sub> F	87.0-87.3	23	1.6174	1.3699	34.6	35.4	48.5	48.8	•••	•••
CF <sub>8</sub> CHClCClF <sub>2</sub>	50.4	22	1.5564	1.3208	46.7	47.7	34.9	35.4	203	199
CF3CHCICF3	14.5-15.0	4	1.5415		61.1	61.4	19.1	20.6	186	183

In another experiment, a 2-liter, monel lined autoclave was charged with CF2CCl=CCl2 (3.3 moles) and anhydrous hydrogen fluoride (12 moles) and heated at 240° for sixty-seven hours. A pressure of 1225 lb./sq. in. was observed. The product was isolated in the usual manner and rectified. Five-hundred and sixty-six grams of the starting material, CF3CCl=CCl2, was recovered, indicating that substantially no fluorination had occurred. Synthesis of CF<sub>3</sub>CH=CF<sub>2</sub> and CF<sub>3</sub>CH<sub>2</sub>CF<sub>3</sub>.—A 5-

liter, 3-necked flask was fitted with a dropping funnel, a mercury sealed stirrer and a reflux condenser through which ice water was circulated. A receiver cooled by solid carbon dioxide was connected in series with the condenser. The flask was charged with zinc dust (7 moles) and absolute ethanol (1500 ml.). This mixture was heated to the temperature of refluxing alcohol and CF:CHClCClF<sub>2</sub> (5.8 moles) was added dropwise to the criscitic Criscitics (5.5 moles) was added utopwise to the zinc-alcohol suspension over a period of six hours. Upon rectification of the product, 655 g. CF<sub>3</sub>CH=CF<sub>2</sub> (5.0 moles) and 122 g. CF<sub>3</sub>CHClCClF<sub>2</sub> (0.6 mole) were obtained representing a conversion of 86% and a yield of 96%. CF<sub>3</sub>CH=CF<sub>2</sub> was converted to CF<sub>3</sub>CH<sub>2</sub>CF<sub>3</sub> by addition of hydrogen fluoride.<sup>5</sup>

Synthesis of CF<sub>3</sub>CHClCCl<sub>3</sub>.—A Carius tube was charged with CF<sub>3</sub>CH=CCl<sub>2</sub> (0.4 mole), liquid chlorine (0.75 mole) and antimony (V) chloride (0.04 mole) and the mixture heated at 140° for twenty-four hours. The product was washed with aqueous alkali, dried and purified by recti-fication. Fifty grams of CF<sub>3</sub>CHClCCl<sub>3</sub> (0.21 mole) was obtained representing a yield and conversion of 53%. Synthesis of CF<sub>3</sub>CHClCCl<sub>2</sub>F.—A mixture of CF<sub>3</sub>-

CHClCCl<sub>2</sub> (0.18 mole), antimony(III) fluoride (0.13 mole)

and antimony(V) chloride (0.06 mole) was refluxed for one hour. The product was steam distilled and purified by rectification. Twenty-three grams (0.10 mole) of CF<sub>1</sub>CHClCCl<sub>2</sub>F was obtained representing a yield and

CF<sub>4</sub>CHClCCl<sub>2</sub>F was obtained representing a yield and conversion of 56%. Synthesis of CF<sub>3</sub>CHBrCBrF<sub>2</sub>.—Two Carius tubes were charged with CF<sub>4</sub>CH=CF<sub>2</sub> (0.7 mole) and bromine (0.72 mole) and heated at 140° for twenty-four hours. The products were combined, washed free of bromine with aqueous Na<sub>2</sub>SO<sub>4</sub>, dried and rectified. There was ob-tained 42 g. of CF<sub>3</sub>CBr=CF<sub>3</sub>, b. p. 24.7-25.0°, 216 g. of CF<sub>3</sub>CHBrCBrF<sub>2</sub>, b. p. 88.0°,  $d^{23}_4$  2.1637,  $n^{25}_5$  1.3780. Dehydrobromination of CF<sub>3</sub>CBr=CF<sub>2</sub> caused the formation of CF<sub>3</sub>CBr=CF<sub>4</sub>. caused the formation of CF3CBr=CF.

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## Summary

A new series of reactions for the preparation of CF<sub>2</sub>CH<sub>2</sub>CF<sub>3</sub> is described. The fluorination of CF<sub>3</sub>CCl=CCl<sub>2</sub> with anhydrous hydrogen fluoride and antimony(V) chloride gave CF3CHClCCl2F, CF3CHClCClF2, CF3CHClCF3, CF3CCl2CCl2F and CF<sub>3</sub>CCl<sub>2</sub>CClF<sub>2</sub>. Several new compounds are reported.

LAFAYETTE, INDIANA

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[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH, NEW YORK, THE NATIONAL RESEARCH COUNCIL OF CANADA, AND THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID CO.]

# Studies in Steroid Metabolism. IV. The Characterization of Carbonyl and Other Functional Groups in Steroids by Infrared Spectrometry<sup>1</sup>

# By R. Norman Jones, V. Z. Williams, M. J. Whalen and Konrad Dobriner

Many organic compounds can be identified by the direct comparison of their ultraviolet, visible or infrared absorption spectra with the spectra of known substances measured under comparable experimental conditions. Such a procedure is strictly empirical and involves no premises as to the nature of the processes concerned in the absorption of the radiation. However, much more insight into the molecular structure of the compound can be derived from the spectrometric measurements if the location and the intensities of the absorption bands can be related to specific molecular structure. In the case of a new compound it is only through such correlations that information about the molecular structure can be derived from the spectrometric measurements.

The high specificity of the infrared absorption spectra of organic compounds has recently become generally appreciated,<sup>2,3</sup> and infrared spectrometry is now applied quite extensively for the qualitative and quantitative analysis of organic compounds. Thus infrared spectrometry was used as an aid in the establishment of the identity of synthetic folic acid with that isolated from natural sources,<sup>4</sup> and the application of infrared spectrometry to the analysis of the steroid constituents of human urine has been described in an

<sup>(1)</sup> Presented in part at The Laurentian Hormone Conference, St. Adele, Quebec, September, 1946, and at a Meeting of the Optical Society of America, New York, February, 1947. Published as contribution No. 1546 from the Laboratories of the National Research Council of Canada.

<sup>(2) &</sup>quot;The Application of Infrared Spectra to Chemical Problems. A General Discussion," Trans. Faraday Soc., 41, 171 (1945).

<sup>(3)</sup> R. B. Barnes, R. C. Gore, U. Liddel and V. Z. Williams. "Infrared Spectroscopy, Industrial Applications and Bibliography," Reinhold Publishing Corp., New York, N. Y., 1944.

<sup>(4)</sup> Angier, et al., Science, 102, 227 (1945).



Fig. 1.—Infrared absorption spectrum of androsterone in carbon disulfide solution: A, O-H stretching motion of hydroxyl group; B, carbonyl overtone band; C, C-H stretching maxima; D, C=O stretching maximum of carbonyl group.

earlier paper of this series.<sup>5</sup> In such investigations the spectrometric curves have been used in an empirical manner as "molecular fingerprints."

The dependence of the infrared absorption spectrum on the molecular structure has been investigated very thoroughly for simple symmetrical molecules which are susceptible to mathematical treatment<sup>6</sup> and numerous studies of more complex molecules have been made by many observers following up the pioneer work of Coblenz.<sup>7</sup> In spite of this, comparatively little use has yet been made of infrared absorption data in the elucidation of molecular structure, although the possibilities of the method have been demonstrated by recent work on the structure of the penicillins.<sup>8</sup>

Infrared Absorption Spectra of Steroids.—In this paper an attempt is made to correlate the position of certain infrared absorption bands with the presence of specific molecular groupings in steroids. The infrared spectrum of a typical steroid, androsterone, is shown in Fig. 1. For the purposes of subsequent discussion it is convenient to consider this spectrum in two parts; a high frequency region from 4000 to about 1200 cm.<sup>-1</sup>, and a lower frequency region extending down from 1200 cm.<sup>-1</sup> to the lower limit of measurement. In the lower frequency region all steroids exhibit very complex absorption. This part of the spectrum is exceedingly sensitive to minor changes in chemical structure or steric configuration and has been utilized principally for empirical identification purposes.<sup>5</sup>

In the higher frequency region a smaller number of bands is observed. These are associated with hydrogen motions, or the stretching vibrations of doubly or triply bonded atoms from the second row of the periodic table. Such motions usually give rise to absorption bands in specific frequency ranges since they are little subject to perturbing interactions. For example, in the spectrum in Fig. 1, the band at 1742 cm.<sup>-1</sup> can be correlated with the stretching vibration of the carbon-oxygen bond of the carbonyl group. It is this higher region of the spectrum which offers the most encouraging prospect of yielding information concerning molecular structure.

The investigation described here was carried out in an endeavor to supplement the chemical methods available for the elucidation of the structure of new steroids isolated from biological material. In such compounds interest is centered mainly on the carbonyl group, the hydroxyl group and the double bond. Information is desired as to the presence or absence of these functional groups in the molecule, and if present it is important to locate their position in the ring system or on the side chain. It has been shown by Lecomte<sup>9</sup> and

(9) J. Lecomte, "Spectres dans l'Infra-rouge," Traîté de Chimie Organique, sous le direction de V. Grignard, Secrétaire General, Paul Baud, Tome II, Fascicule 1, Masson et Cie, Paris, 1936, p. 143.

<sup>(5)</sup> Dobriner, Lieberman, Rhoads, Jones, Williams and Barnes, J. Biol. Chem., 172, 297 (1948).

<sup>(6)</sup> Gerhard Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," Van Nostrand Co., New York, N. Y., 1945.

<sup>(7)</sup> Coblenz, Publ. Carnegie Inst. of Washington, No. 35, Part 1, 1905.

<sup>(8)</sup> Fowler and Randall, Symposium on Molecular Structure and Spectroscopy, Ohio State University, June, 1946.



Fig. 2.—Diagram illustrating the relation between the frequency at the maximum of the carbon-oxygen stretching vibration and the location of the carbonyl group in steroid molecules (solvent carbon disulfide). The figures in parentheses indicate the number of individual compounds on which the frequency assignment is based.

others<sup>3</sup> that in certain types of organic compounds all of these groups may give rise to characteristic infrared absorption bands, and Furchgott, Rosenkrantz and Shorr<sup>10,11,12</sup> have demonstrated the presence of these absorption bands in the spectra of certain crystalline steroids.

The Carbonyl Group.—The carbon-oxygen stretching vibration of the carbonyl group may occur between 1550 and 2150 cm.<sup>-1</sup> and it has been observed in the spectra of ketones, aldehydes, carboxylic esters, acids, and ions, acid anhydrides, lactones and amides. The range of frequencies encompassed by this band varies somewhat for the different types of carbonyl groups.<sup>3</sup>

A comparison has been made of the infrared absorption spectra of carbon disulfide solutions of some one hundred and thirty steroids, and the positions of the maxima in the region between 1660 and 1780 cm.<sup>-1</sup> are listed for the monocarbonyl compounds in Table I, and for di- and polycarbonyl compounds in Table II. It is to be noted that an absorption band is seen only in the spectra of those steroids which contain a carbonyl group, so that the appearance of a strong band in this region of the spectrum of an unknown steroid is indicative of the presence of a carbonyl group in the molecule.

The carboxylic esters included in Tables I and II are mainly the acetates or propionates of steroid alcohols, although a few methyl esters of bile acid derivatives and some benzoates are also included. These esters give rise to a single absorption maximum between 1719 and 1771 cm.<sup>-1</sup>. The position of the carbonyl maximum in the ketosteroids may be between 1666 and 1754 cm.<sup>-1</sup> so that it is not always possible to distinguish with certainty between a ketosteroid and a steroid ester from measurements in this region of the spectrum as some overlap in the band positions can occur.<sup>13a</sup>

A more precise consideration of the position of this carbonyl absorption band shows it to be closely dependent on the molecular environment of the carbonyl group in the molecule. The monoketosteroids and mono-steroid esters in Table I are grouped according to the position of the substituent, and the characteristic frequency positions derived from this analysis are summarized diagrammatically in Fig. 2.

For a given position of substitution, the carbonyl stretching vibration occurs at a sharply defined frequency. The frequencies of the maxima in steroids containing more than one carbonyl group are listed in a similar manner in Table II, and here, with certain exceptions to be considered later, the two carbonyl groups do not appear to exert any significant interaction effects, and the bands occur at the same positions as in the monocarbonyl compounds. The four ketonic positions of main significance in the steroid hormones are at carbon atoms 3, 11, 17 and 20 (I). In eleven steroids containing a non-conjugated ketonic carbonyl group at position 3 there is a maximum at (13a) Vide page 2029.

<sup>(10)</sup> Furchgott, Rosenkrantz and Shorr, J. Biol. Chem., 163, 375 (1946).

<sup>(11)</sup> Furchgott, Rosenkrantz and Shorr, ibid., 164, 621 (1946).

<sup>(12)</sup> Furchgott, Rosenkrantz and Shorr, ibid., 167, 627 (1947).

June, 1948 CARBONYL AND OTHER GROUPS IN STEROIDS BY INFRARED SPECTROMETRY

TABLE I			G. Conjugated 17-keto	nes	
CARBON-OXYGEN STRETCHING VIER.	ATION IN	STEROIDS	$\Delta^{1,3,5:10,6,8,15}$ -Estrahexaene-one-17	1716	g
CONTAINING NOT MORE THAN ONE	CARBONYL	GROUP	<b>3-Methoxy-</b> Δ <sup>1,3,5:10,6,8,15</sup> -estrahexa-		5
	Max.w		ene-one-17	1716	ø
Compound	(cm. <sup>-1</sup> ) CS:	Source	H Non conjugated 20 ke	tones	a
A. Non-carbonyl comt	ounds		TI. Non-conjugated 20-ke	tones	
The following showed no obcomption		hotmoon	Pregnanol- $3\alpha$ -one-20	1706	l
1660 and 1780 am =1 (Source of co	m maxima	indicated	Allopregnanol- $3\alpha$ -one-20	1710	l
1000 and 1780 cm (Source of co	mpound is	(d) prog	Allopregnanol-3 $\beta$ -one-20	1706	l
in parentneses): androstane (d), et		(u), preg-		(1706)	_
nane (d), allopregnane (d), cho	lestane (c)	$\Delta^{-1}$	$17$ -Iso-pregnanol- $3\alpha$ -one- $20$	1706	f
cholestene (j), cholestanol- $3\alpha$ (l),	cnolestand	01-30 <sup>(1)</sup> ,	$\Delta^{\mathfrak{s}}$ -Pregnenol-3 $\beta$ -one-20	1707	Þ
and rost and $-3\alpha$ (0), and rost and $-3\beta$	(o), and rost	$\tan 0 - 17\alpha$		(1702)	
(o), $\Delta^{\mathfrak{d}}$ -androstenol-3 $\beta$ (o), $\Delta^{\mathfrak{d}}$ -andros	stenediol-3/	$3,17\alpha$ (0),	$\Delta^{11}$ -Pregnenol-3 $\beta$ -one-20	1706	с
$\Delta^{\mathfrak{s}}$ -androstenediol-3 $\beta$ ,17 $\beta$ (o),cholest	enetriol-3 $\beta$ ,	$5\beta, 6\alpha$ (1).	Pregnanediol- $3\alpha$ , $11\alpha$ -one- $20$	1706"	С
B. Non-conjugated 3-k	etones		Pregnanediol- $3\beta$ , $12\beta$ -one- $20$	1706"	0
Audrostanone-3	1719	0	$\Delta^{5}$ -Pregnenediol-3 $\beta$ ,21-one-20	1706	Þ
Etiocholanone-3	1719	d	$\Delta^{2(\text{ or } 3)}$ -Allopregnenone-20	1706	1
Cholestanone-3	1719	c	I Conjugated 20-ketor	nes	
Coprostanone-3	1718	l	Alf Deserved 2, and 20	1000	
Androstanol-17%-one-3 (trans-dihydro-		•	$\Delta^{10}$ -Pregnenol-3 $\alpha$ -one-20	1000	J
testosterone)	1718	0	$\Delta^{\circ,10}$ -Pregnanedienoi-3 $\beta$ -one-20	1669.	n
Androstanol-178-one-3 (cis-dihydro-	1110	U	J. 3-Acyl esters		
tostostorono)	1718		Cholestanol-3a-acetate	1739	с
	1718	v	Cholestanol-38-acetate	1739	l
C. Conjugated 3-ket	ones		$\Lambda^{5}$ -Cholestanol-38-acetate	1739	t
$\Delta^4$ -Androstenone-3	1677	0	$\Lambda^{8:14}$ -Cholestanol-38-acetate	1739	+
$\Delta^4$ -Cholestenone-3	1674	l	A <sup>14</sup> -Cholestenol-38-acetate	1730	*
	(1656) <sup>w</sup>		At 7-Cholestadienol-38-acetate	1730	, ,
$\Delta^4$ -Androstenol-17 $\alpha$ -one-3	1675	Þ	Androstonodiol 2 n 17 n sostate 2	1720	
(testosterone)	(1656) <sup>w</sup>	-	Androstaneolol-30,170-acetate-3	1739	0
$\Delta^4$ -Androstenol-17 $\beta$ -one-3	1674	0	$\Delta^{\circ}$ -Androstenedioi- $3\beta$ , 17 $\alpha$ -acetate- $3$	1739	0
(cis-testosterone)	(1652)		K. 3-Aryl esters		
$\Delta^4$ -17-Methylandrostenol-17 $\alpha$ -one-3	1675	0	$\Delta^{8:14}$ -Cholestenol-3 $\beta$ -benzoate	1719	t
	(1663)	•	$\Delta^{14}$ -Cholestenol-3 $\beta$ -benzoate	1719	t
$\Delta_{2}^{4}$ 17-Ethylandrostenol-17 $\alpha$ -one-3	1675	0	I 17-Aoul ester		
A4-17-Vinylandrostenol-17a-one-3	1675	0	D. 17-Acylester	1 100	
A -11-Villylandrostenor 174 one o	(1660)	Ũ	$\Delta^{\bullet}$ -Androstendiol-3 $\beta$ ,17 $\alpha$ -acetate-17	1739	J
A4 20 21 Epoyypreguenol 17 group 3	1674"	0	M. Cholanate and cholenate me	thyl esters	r
	1014	U	$3\alpha$ -Hydroxycholanic acid m. e. <sup>*</sup>	1742	c
D. $\Delta^{*,\circ}$ -Diene-one-	-3			(1730)	•
$\Delta^{4,6}$ -Cholestadiene-one-3	1666	l	3a-Hydroxy- A <sup>9:11</sup> -cholenic acid m e *	1742	c
E. Non-conjugated 17-1	cetones			(1732)	U
Andrestanona 17	1745	<u>^</u>	3a-Hydrovy- All-cholenic acid m e *	1742	c
Alla Androstadiona ana 17	1740	6	Su-Hydroxy-A -cholenic acid in. c.	(1739)	i
2 Oblaman destances 17	1742	<i>.</i>	2 . Hydrovy 11 . 12 . epoyycholania	(1102)	
3-Chloroandrostanone-17	1740	<i>J</i>	acid m o <sup>2</sup>	1749	-
Androstanol-3 $\alpha$ -one-17 (androsterone)	1740	ł	2. Hudrows 198 mothews Abili	1742	í T
1 1 (	(1737)	_	sa-Hyuroxy-12p-methoxy-2,	(1720)	r
Androstanol-38-one-17	1745	0	cholenic acid	(1732)	~
(isoandrosterone)	(1735)		38,128-Dinydroxycholanic acid m. e."	1742	Ĵ
Etiocholanol- $3\alpha$ -one-17	1743	1	$3\alpha$ , 12\beta-Dihydroxy- $\Delta^{g,11}$ -cholenic acid	1742	r
	(1735)		m. e.*	(1732)	
Etiocholanol- $3\beta$ -one-17	1742	q	<sup>a</sup> R. I. Dorfman, Western Reserve U	., Clevelan	d, Ohio.
Δ <sup>5</sup> -Androstenol-3β-one-17 (dehydro-			<sup>o</sup> L. Engel, Mass. General Hosp., Bost	on, Mass.	T.F.
isoandrosterone	1745	0	<sup>4</sup> R D H Heard McGill U Montrea	ew YORK,	N. Y. Hersh-
$\Delta^{9:11}$ -Androstenol-3 $\alpha$ -one-17	1742	k	berg, The Schering Corp., Bloomfield	I. N. J.	W. H.
$\Delta^{9:11}$ -Etiocholenol- $3\alpha$ -one-17	1745	n	Hoehn, G. A. Breon and Co., Kansas (	Čity, Mo.	• W. S.
$\Delta^{11:12}$ -Etiocholenol- $3\alpha$ -one-17	1742	ı	Johnson, U. of Wisconsin. <sup>h</sup> O. Kamr	n, Parke, l	Davis &
Androstanediol- $3\alpha$ , $11\beta$ -one- $17$	1742	a, k	Rochester Minn i R F. Marber I	uall, Mayo	ia State
F. By-Unsaturated 17-1	cetones		College, Penna, <sup>k</sup> H. L. Mason, Mayo	Clinic. Re	chester.
A1, 3, 5:10, 6, 8, 14 Detro have one 17	1754	a	Minn. <sup>1</sup> S. Lieberman, Memorial H	losp., Nev	v York,
2 Methown Alis 5:10.6.8.14 octrohemo	1104	£	N. Y. T. Reichstein, U. of Basel,	Switzerlan	d. "H.
one one 17	1754	a	P. Sarett, Merck and Co., Rahway	', N. J.	°C. R.
EIIC-011C-17	1104	б	Genoiz, Ciba Fharm. Frou. Inc., Su		1 tr

Schwenk, The Schering Corp., Bloomfield, N. J. <sup>e</sup> H. Selye, U. Montreal. <sup>r</sup> R. Turner, Harvard U., Cam-bridge, Mass., E. C. Kendall, Mayo Clinic, Rochester, Minn. <sup>e</sup> Compound acetylated at Memorial Hosp., by Dr. S. Lieberman from alcohol supplied by H. P. Sarett. <sup>f</sup> D. K. Fukushima, Memorial Hosp., New York, N. Y. <sup>w</sup> Suspension of crystalline material in saturated solution in carbon disulfide. <sup>w</sup> Weak absorption band at 1719 cm.<sup>-1</sup> attributed to trace of impurity. <sup>w</sup> Figures in paren-theses refer to maximum in chloroform solution. <sup>\*</sup> In the names of these compounds "m. e." is used as an abbreviation for "methyl ester."

## TABLE II

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Carbon-Oxygen	STRETCHING	VIBRATION	IN	STEROIDS
CONTAINING	Two or Mor	E CARBONYL	GR	OUPS

Compound	Max. in		Sauraa
A. 3.17-Diketo	nes (cm.	,	Source-
Androstonedione-2 17	1745	1710	,
Etiocholonedione 2 17	1745	1710	,
At Androstonediana 2.17	1740	1674	•
A-Androstaneurone-5,17	(1720)	10/4	0
	(1739)	(1003)	
B. 3,20-Diketo	ones		
Allopregnanedione-3,20	1719	1710	h
Pregnanedione-3,20	1719	1710	l
Δ <sup>*</sup> -Pregnenedione-3,20	1719	1710	l
<b>Δ<sup>4</sup>-Pregnenedione-3,20</b>	1708	1677	Þ
(progesterone)	(1706)	(1669)	
$\Delta^4$ -Pregnenol-17 $\alpha$ -dione-3,20	1710	1674	0
$\Delta^4$ -Pregnenol-21-dione-3,20			
(desoxycorticosterone)	1710	1677	0
$\Delta^{4,6}$ -Pregnenediene-dione-3,20	1710	1669	l
$\Delta^{16}$ -Pregnenedione-3,20	1717	1669	h
	(1710)	(1666)	
C. Diacyl est	ers		
Androstanediol-3~ 17~-diacetate	1742		,
Al-Androstenediol-38 17~	11 14		•
discetate	1749		•
A \$.6. Androstadienediol-3 17~-	1/74		U
dipropionate	1754	1749	
Dregnanedial 2x 12x amina 20	1704	1744	0
hydrochloride diagetete	17496		
Prograndial 2 = 20 = disastata	1790		,
Programadial 2 × 200 diagetate	1720		
Programedial 38 208 disastate	1790		j
2 A Acatory Agill cholonic acid	1109		J
sa-Aceroxy-Δ <sup>*</sup> cholemc acid	1720		
111. C. Al. 3.5.10 Entrotwice diel 9 17.	1198		0
$\Delta$ -instate (astradiol dispetate)	1767	1740	,
diacetate (estradior diacetate)	1707	1/44	1
D. 3-Ketoeste	ers		
Androstanol-17 $\alpha$ -one-3-acetate	1742	1719	1
Androstanol-17β-one-3-acetate	1739	1719	1
$\Delta^4$ -Androstenol-17 $\alpha$ -one-3-acetate			
(testosterone acetate)	1742	1677	0
Δ <sup>4</sup> -Androstenol-17α-one-3-propio-			
<b>nate</b> (testosterone propionate)	1742	1677	Þ
$\Delta^4$ -Androstenol-17 $\beta$ -one-3-acetate	1739	1677	1
E. 17-Ketoeste	ers		
Androstanol-3 $\alpha$ -one-17-acetate			
(androsterone acetate)	1742		1
Androstanol- $3\beta$ -one-17-acetate			
(isoandrosterone acetate)	1742		l
Etiocholanol- $3\alpha$ -one-17-acetate	1742		1

Etiocholanol-3 $\beta$ -one-17-acetate	1742		h
$\Delta^{s}$ -Androstenol-3 $\beta$ -one-17-acetate (dehydroisoandrosterone acetate) $\Delta^{s}$ -Androstenol-3 $\beta$ -one-17-propio-	1742		1
propionate)	1742		0
$\Delta^{3-41}$ -Androstenol- $3\alpha$ -one-17- acetate	1742		a
$\Delta^{9:11}$ -Etiocholenol-3 $\alpha$ -one-17- acetate	1742		5
5α,6α-Epoxyetiocholanol-3β-one- 17-acetate	1742		с
5β,6β-Epoxyetiocholanol-3β-one- 17-acetate	1742		c
Δ <sup>1.3,5:10</sup> -Estratrienol-3-one-17-			
acetate (estrone acetate) $\Delta^{1,3,5:10,7}$ -Estratetraenol-3-one-17-	1764	1742	1
acetate (equilin acetate) ∆ <sup>1,3,5:10,6,8</sup> -Estrapentaenol-3-one-	1764	1742	1
17-acetate (equilenin acetate)	1770	1742	l
Androstanoi-3a-one-17-benzoate			
(androsterone benzoate)	1745	1723	1
Etiocholanol- $3\alpha$ -one-17-benzoate	1745	1719	1
Δ-Anurostenoi-3β-one-17-benzo-			
ate (dehydroisoandrosterone			
Denzoate)	1745	1719	l
$\Delta$ -Androstenol-1/ $\alpha$ -one-3-benzo-	1504		
ate (testosterone benzoate)	1724	1674	0
F. 20-Ketoeste	rs		
Allopregnanol-38-one-20-acetate	1739	1708	i
$17$ -Iso-pregnanol- $3\alpha$ -one- $20$ -		2100	5
acetate	1730	1706	f
A <sup>5</sup> -Pregnenol-38-one-20-acetate	1730	1706	,
A <sup>11</sup> -Pregnenol-3 <i>c</i> -one-20-acetate	1720	1706	0
Pregnanediol- $3\alpha$ , $17\alpha$ -one-20-	1709	1700	c
acetate-3	1735	1710	l
$\Delta^{16}$ -Pregnenol-3 $\alpha$ -one-20-acetate	1742	1670	i
Δ <sup>5,16</sup> -Pregnadienol-3β-one-20-			5
acetate	1739	1669	i
Δ <sup>5</sup> -Pregnenediol-38.21-one-20-	2.00	2000	,
acetate-21	1758	1732	•
Pregnanetriol-3 a 128 21-one-20-	1100	1102	Ű
acetate_21	1756	1729	~
	1100	1102	U
G. Polyesters			
Δ <sup>8</sup> -Androstenetriol-3β,16,17-			
triacetate	1740		k
$\Delta^{1,3.5:10}$ -Estratrienetriol-3,16,17-			
triacetate (estriol triacetate)	1767	1742	1
triacetate	1758 <sup>d</sup>	1740	~
U Dalm hateast		1110	v
H. Poly-ketoeste	ers		
Pregnanediol- $3\alpha$ , $11\alpha$ -one-20-			
diacetate	1739	1710	C
∆ <sup>4</sup> -Pregnenol-21-dione-3,20-	1758	1732	0
acetate (desoxycorticosterone			
acetate)	1674		
Pregnanediol-128,21-dione-3,20-	1758	1732	0
acetate-21	1703		
• See footnotes to Table I. • "m	. e." i	s abbrevia	atio

m for "methyl ester." • Measurements made on a suspen-sion of crystalline material in a saturated solution in car-bon disulfide. • Inflection only. 1717-1719 cm.<sup>-1</sup>. Twenty-one ketosteroids with a non-conjugated carbonyl group at position 20 have a maximum at 1706-1710 cm.<sup>-1</sup>, while in fourteen non-conjugated 17-ketosteroids the maximum is at 1742-1745 cm.<sup>-1</sup>.



The introduction of a double bond in the  $\alpha,\beta$ position to the ketonic carbonyl group shifts the maximum by about 40 cm.<sup>-1</sup> to lower frequencies. In seventeen  $\Delta^{4}$ -3-ketosteroids (II) there is a band at 1674–1677 cm.<sup>-1</sup> while in five  $\Delta^{16}$ -20ketosteroids (III) the maximum occurs at 1666– 1670 cm.<sup>-1</sup>. Two compounds containing the  $\Delta^{4,6}$ -diene-one-3 system (IV) also possess a maximum at 1666–1669 cm.<sup>-1</sup>. Data on the effect of ethylenic unsaturation in ring D on the 17-carbonyl group is at present limited to the four synthetic equilenin derivatives V–VIII.<sup>13</sup> It is curious to note that in V and VI the introduction of the  $\beta\gamma$ - double bond shifts the position of the



carbonyl maximum about 10 cm.<sup>-1</sup> to higher frequencies from its position in the 17-ketosteroids in which ring D is saturated. A change in the force constants in the carbon-oxygen bond may be brought about by the shortening of the bond between carbon atoms 14 and 15 in the unsaturated

(13) Johnson, Petersen and Gutsche, THIS JOURNAL, **69**, 2942 (1947).

compound with a resultant strain effect on the valence angles at carbon atom  $17.^{14}$ 

Few data are yet available concerning the location of the carbonyl band in steroids containing a ketone group at positions other than 3, 17 or 20. The effects of carbonyl groups at 11 and 12, which are of considerable interest in connection with the adrenocortical steroids will form the subject of a separate publication.

In the non-conjugated 3,17-diketosteroids two maxima occur at 1745 and 1719 cm.<sup>-1</sup>, positions normal to the corresponding monocarbonyl compounds. The same is true also of the 3,20-diketones which have two maxima at 1719 and 1710 cm.<sup>-1</sup>. In the latter case the separation between the maxima is about the limit of resolution possible under the experimental conditions employed.<sup>15</sup>



The carbonyl group of the non-conjugated carboxylic ester tends to be less sensitive than the ketonic carbonyl group to the position of substitution in the sterol ring system, and the acetates and propionates of 3-, 17- and 20-hydroxysteroids all absorb at 1739-1742 cm.<sup>-1</sup>. The methyl esters of cholanic acid derivatives also absorb at the same position. This lies quite close to the position of the maximum in non-conjugated 17-ketosteroids so that it is not possible to distinguish 17-ketosteroids from certain steroid esters by this criterion alone. However, the acetate esters possess a strong band near 1240 cm.<sup>-1</sup> (see Table III) which is lacking from the spectra of ketosteroids, so that a supplementary examination of the absorption in the region near 1240 cm.<sup>-1</sup> permits a

(14) The possibility that this shift to higher frequency results from effects of the aromatic system in rings A and B cannot be excluded on the basis of this evidence. However, the interpretation given above seems most probable when the data on the band positions in the acetates of estrone, equilin and equilenin are also taken into consideration. These three compounds all possess a band at 1742 cm.<sup>-1</sup> which can be attributed most reasonably to the unperturbed 17-ketosteroid absorption. These compounds contain a second band at 1764–1771 cm.<sup>-1</sup> which is attributed to the phenolic acetate group. In estradiol diacetate two maxima are observed at 1767 and 1742 cm.<sup>-1</sup> indicating clearly that the carbonyl of the phenolic ester.

(15) Better resolution of these absorption maxima might be achieved by the use of higher dispersion, as may result from the substitution of a calcium fluoride prism or a grating in place of the sodium chloride prism used in these measurements. Measurements of the band intensity for standard conditions of concentration and sample thickness, or determination of the band width at half the maximum intensity may also yield more information about the nature of the absorbing group, and make possible a distinction between absorption caused by one or two carbonyl groups at the same frequency. distinction to be made between a 17-ketosteroid and a steroid acetate ester. Some significance may be attached to the observation that in compounds containing the partial structure XI this maximum is displaced by 10-12 cm.<sup>-1</sup> to lower frequencies as indicated by the last four compounds in Table III.

## TABLE III

POSITION OF STRONG "ACETATE BAND" IN REPRESENTA-TIVE GROUP OF STEROID ACETATE ESTERS

	Max. (cm. <sup>-1</sup> )	
Compound	ČS <sub>1</sub>	Source <sup>a</sup>
Androstanol- $3\alpha$ -one-17-acetate	1240	1
Androstanol-3 $\beta$ -one-17-acetate	1240	1
Androstanol-17 $\alpha$ -one-3-acetate	1245	l
Androstanol-17 $\beta$ -one-3-acetate	$1238 \ 1245$	l
$\Delta^4$ -Androstenol-17 $\alpha$ -one-3-acetate	1242	0
$\Delta^4$ -Androstenol-17 $\beta$ -one-3-acetate	1245	l
$\Delta^{\delta}$ -Androstenol-3 $\beta$ -one-17-acetate	1240	l
Androstanediol- $3\alpha$ , $17\alpha$ -acetate- $3$	1238	0
Androstanediol- $3\alpha$ , $17\alpha$ -diacetate	1242	l
$\Delta^{5}$ -Androstenediol- $3\beta$ , $17\alpha$ -acetate- $3$	1242	0
Etiocholanol- $3\alpha$ -one-17-acetate	1240	ı
Pregnanol- $3\alpha$ -one-20-acetate	1240	l
Allopregnanol- $3\beta$ -one-20-acetate	1240	j
$17$ -Iso-pregnanol- $3\alpha$ -one- $20$ -acetate	1242	f
$\Delta^{16}$ -Pregnenol-3 $\alpha$ -one-20-acetate	$1235 \ 1255$	j
$\Delta^{5}$ -Pregnenediol-3 $\beta$ ,21-one-20-acetate-		
21	1228	0
$\Delta^4$ -Pregnenol-21-dione-3,20-acetate	1230	0
Pregnenediol- $3\alpha$ , 21-dione-11, 20-		
acetate-21	1228	n
Pregnanol-21-trione-3,11,20-acetate	1228	n

<sup>a</sup> See footnotes to Table I.

Where the esterified hydroxyl group is phenolic in character, as in the 3-acetates of compounds in the estrone and equilenin series, the carbonyl ester absorption maximum is shifted to higher frequencies (Fig. 2). This is true also when the esterified hydroxyl group is associated with a conjugated system as in the enol ester X. It is inter-



esting to observe that this type of attachment of the ester carbonyl group to an ethylenic double bond through the oxygen atom, shifts the carbonyl maximum in the opposite direction from that brought about by direct conjugation with the carbonyl bond, as is seen in the steroid benzoates and the conjugated ketosteroids (Fig. 2).

The above observations are all based on measurements in carbon disulfide solution. The positions of the carbonyl stretching maxima have been determined also in a few cases using chloroform as solvent, and the results are listed in Tables I and II. In chloroform the band maxima tend to be displaced to lower frequencies; the effect is least  $(0-5 \text{ cm}.^{-1})$  for non-conjugated 20-ketosteroids, somewhat greater for carboxylic esters and 17-ketosteroids  $(7-10 \text{ cm}.^{-1})$  and greatest for conjugated 3-ketosteroids  $(12-20 \text{ cm}.^{-1})$ .

Interaction Effects.—In dicarbonyl compounds it might be anticipated that some interactions involving the vibrations of the two carbonyl groups would be encountered and result in the displacement of the bands from the positions observed in comparable monocarbonyl compounds.

As has been shown above, such interactions have not been detected between carbonyl groups at positions 3 and 17 and 3 and 20. An example of such a displacement is seen in the spectra of the 21-acetoxy-20-ketosteroids which contain the partial structure XI. Such compounds would be ex-



pected to possess a band at 1706-1710 cm.<sup>-1</sup> for the 20-ketone group and at 1739-1742 cm.<sup>-1</sup> for the acetate ester. Actually all of the four such compounds examined possess bands at 1732 cm.<sup>-1</sup> and 1756-1758 cm.<sup>-1</sup>. Four compounds containing the partial structures XII or XIII on the contrary show the normal 20-ketosteroid band at 1706-1710 cm.<sup>-1</sup> from which it may be argued that the displacements associated with structure XI involve carbonyl group interactions, and are not due merely to steric or other non-specific effects of the large oxygen atoms adjacent to the 20-ketone.

Other evidence of the influence of the molecular environment on the frequency of the carbonoxygen stretching vibration in cyclic compounds has been reported in the literature. Biquard<sup>16</sup> has measured the position of the analogous band in the Raman spectra of a series of mono- and dicyclic ketones and noted quite similar displacements of the Thus in the Raman spectrum of band positions. cyclopentanone there is a band at  $\Delta \nu = 1744$ cm.<sup>-1</sup> which is displaced to  $\Delta \nu = 1714$  cm.<sup>-1</sup> in cyclohexanone. Furchgott, Rosenkrantz and Shorr<sup>12</sup> have reported that in the crystalline state non-conjugated 3-ketosteroids absorb at 1739 cm.<sup>-1</sup>, 17-ketosteroids at 1738-1740 cm.<sup>-1</sup> and 20-ketosteroids at 1703-1715 cm.<sup>-1</sup> and that a 17-ketosteroid could not be distinguished from a 3-ketosteroid. This latter observation is contrary to our experience, based on measurements in solu-

(16) Biquard, Bull. soc. chim. France, 7, 894 (1940); 8, 55, 725 (1941).

tion. Measurements made on solutions would also appear preferable for the purposes of structural identification since the spread of the absorption maximum for a given substituent position seems to be less than for crystalline films or powder suspensions.

In addition to the carbon-oxygen stretching vibration, discussed above, the ketosteroids containing a double bond in the  $\alpha\beta$ -position to the carbonyl group possess another strong absorption band between 1580 and 1615 cm.<sup>-1</sup>. Both carbon disulfide and carbon tetrachloride absorb appreciably in this region but satisfactory measurements can be made in chloroform solution as has been shown recently by Blout, Fields and Karplus.<sup>17</sup> Measurements on a few unsaturated ketosteroids in this region are summarized in Table IV. These are in agreement with the observation of Furchgott, Rosenkrantz and Shorr<sup>12</sup> who have suggested that the  $\Delta^4$ -3-ketosteroid and the  $\Delta^{16}$ -20-ketosteroid systems might be distinguished on the basis of the difference in the position of this band in the two types of  $\alpha\beta$ -unsaturated compounds.

#### TABLE IV

Absorption at 1580–1620 Cm.<sup>-1</sup> Associated with the Conjugated Carbonyl Group

	Max, (cm)		
Substance	CHCla soln.	or mull	Sources
A. $\Delta^4$ -3-Ketosteroid	ds		
$\Delta^4$ -Androstanol-17 $\alpha$ -one-3			
(testosterone)	1615	1615	Þ
$\Delta^4$ -Androstanol-17 $\beta$ -one-3- (cis-			
testosterone)	1613	1615	0
$\Delta^4$ -Androstenol-17 $\alpha$ -one-3-propionate	• •	1615	Þ
$\Delta^4$ -17-Methylandrostanol-17 $\alpha$ -one-3	1615	1620	0
$\Delta^4$ -17-Vinylandrostanol-17 $\alpha$ -one-3	1615	••	l
$\Delta^4$ -Androstenedione-3,17	1615	1620	0
$\Delta^4$ -Pregnenedione-3,20 (progesterone)	1615	1618	Þ
∆⁴-Cholestenone-3	1612	1615	1
B. $\Delta^{16}$ -20-Ketostero	ids		
$\Delta^{16}$ -Pregnenol-3 $\alpha$ -one-20		1588	·j
$\Delta^{6,16}$ -Pregnadienol-3 $\beta$ -one-20-acetate		1585	j
Δ <sup>i6</sup> -Pregnenedione-3,20	1588		h
<sup>a</sup> See footnotes to Table I.			

Carbonyl Harmonic Band.—In addition to the fundamental carbonyl stretching vibration discussed above, another weak absorption band attributed to the carbonyl group occurs between 3300 and 3475 cm.<sup>-1</sup>. This is an overtone of the fundamental carbon–oxygen stretching vibration. It should occur at twice the frequency of the main carbonyl maximum or at a frequency slightly lower than this. The position of this band in the spectrum of androsterone is indicated as B in Fig. 1. This carbonyl harmonic is not likely to be of appreciable value in the elucidation of sterol structure, since it is much weaker than the fundamental band. However its occurrence should be noted,

(17) Blout, Fields and Karplus, THIS JOURNAL, 70, 194 (1948).

since in certain instances it may be confused with the hydrogen–oxygen stretching vibration of the hydroxyl group (*vide infra*).

The Non-Conjugated Double Bond.-The presence or absence of a non-conjugated ethylenic double bond in a steroid cannot always be determined with certainty by infrared measurements. Several investigators<sup>3,17,18</sup> have shown that in many organic compounds there is an absorption band near 1650 cm.<sup>-1</sup> associated with a longitudinal vibration of the carbon-carbon double bond. It is difficult to observe this band in the spectra of carbon disulfide solutions since the solvent absorbs appreciably in this region. In only a few instances of solutions in carbon tetrachloride or in crystalline films and suspensions has this band been seen in the spectra of unsaturated steroids. It may be seen more readily in chloroform solutions, but when present it is of low intensity and its detection is rendered more difficult by the fact that atmospheric water vapor produces considerable background absorption in this region of the spectrum. Its detection would be facilitated by the use of a double beam instrument.

At higher frequencies, near 3000 cm.<sup>-1</sup> there is a group of strong bands associated with the stretching vibration of the carbon-hydrogen bond. Several observers have reported<sup>19</sup> that when the C-H bond forms part of an unsaturated system, this band occurs at a frequency greater than 3000 cm.<sup>-1</sup> whereas in a saturated group it is normally below 3000 cm.<sup>-1</sup>. In all steroids there are strong bands in the region between 3000 and 2800 cm.<sup>-1</sup> due to the --C--H vibrations of the ring and side chain aliphatic system. In carbon disulfide solutions of several unsaturated steroids, a band or inflection is seen also on the high frequency side of this band group. A particularly favorable example is illustrated in curves A and B of Fig. 3,



Fig. 3.—Infrared absorption spectra in carbon disulfide solution: A, —, etiocholanol- $3\alpha$ -one-17 (XIV); B, ---,  $\Delta^{11}$ -etiocholenol- $3\alpha$ -one-17 (XV); C, ----, etiocholanol- $3\alpha$ -one-17-acetate.

(18) Thompson and Torkington, Trans. Faraday Soc., 41, 246 (1945).

(19) See Fox and Martin, Proc. Roy. Soc. (London), A175, 208 (1940).

where the spectrum of etiocholanol- $3\alpha$ -one-17 (XIV) is compared with that of the unsaturated derivative with a double bond at the 11,12 position (XV).



In column A of Table V the positions of this band in the spectra of some 47 steroids in carbon disulfide solution are recorded. Among these compounds are nineteen containing the -C-H group and a band or inflection near 3020 cm.<sup>-1</sup> is observed in the spectra of fourteen of them. No band was detected in this neighborhood in the spectra of any of the saturated steroids included in this survey. In these compounds the band attributed to the =-C-H group is weak and as in many cases it occurs on the rising slope of the -C-H band group, the frequency at the maximum cannot be determined with much certainty. In many cases it fails to appear unless the concentration of the solution is increased considerably above the 10 mg. per ml. which is adequate for the determination of the remainder of the spectrum in a 1 mm. cell. In some instances the concentration cannot be increased sufficiently because of the low solubility of the steroid and this may account

#### TABLE V

Absorption between 3000 and 4000 Cm.<sup>-1</sup> Associated with C=C-H and C-O-H Groups

	I	<b>Maxim</b> a	٥ (cm	-1)	
	C=I	с—н	CS.	0—н	
	soln.	Solid	soln.	Solid	
Compound	A	в	С	D	Source

A. Steroids Containing Neither C=C-H Nor C-O-H Groups

The following showed no bands at positions attributable to the above groups (source of compounds is indicated in footnotes): androstane,<sup>d</sup> etiocholane,<sup>d</sup> allopregname,<sup>d</sup> pregname,<sup>d</sup> allopregnamedione-3,20,<sup>h</sup> pregnanedione-3,20,<sup>l</sup> androstanol-3α-one-17-acetate,<sup>l</sup> 3choloroandrostanone-17,<sup>l</sup> cholestanone-3,<sup>c</sup> coprostanone-3,<sup>s</sup> sitostanedione-3,6.<sup>l</sup>

В.	Steroids	Containing	-C=C-H	but not	-c-	-0-H	Group
				-	~	-	

	A	в	С	D	
$\Delta^4$ -Androstenedione-3,17	3020	3028	a	a	0
Δ4-Cholestenone-3	n. o.	3020	a	8.	r
$\Delta^{5}$ -Androstenol-3 $\beta$ -one-17- acetate (dehydroisoandro- sterone acetate)	3050	n. o.	a	a	1
Δ <sup>5</sup> -Androstenol-3β-one-17- propionate	3000	3050	a	a	0
$\Delta^{5}$ -Androstenol-3 $\beta$ -one-17- benzoate	3025	3080, 3050	a	a	ı
$\Delta^{5}$ -Androstenediol- $3\beta$ , $17\alpha$ - dipropionate	n. o.	3010	a	a	0
Δ <sup>5</sup> -Pregnenol-3β-one-20- acetate	3040	3035	a	a	o
Δ <sup>4</sup> -Pregnenedione-3,20 (progesterone)	3020	3025	a	a	1
Δ <sup>5+16</sup> -Pregnanedienol-3β-one- 20-acetate	3020	3065, 3040	a	a	ţ

	A	Б	C		D	
Androstanol-17	a	a	3570	3200	, 3270ь	j
Androstanediol-3 <i>a</i> ,17 <i>a</i>	a	8	8575	3375	b	0
Androstanediol- $3\beta$ , $17\beta$	8.		3575			0
Cholestanol-38	8.	a	3600	3200 34	), 3350, 50	L
Cholestanediol-3,4	a	8	3600	3270 34	), 3370, 35	3
Etiocholanediol-3α,17α	a	a	3600	3290	), 3370	3
Pregnanediol-3α,20α	8	a	3610	3260	), 3310Ь	j
Pregnanediol-3\$,20\$	a	a	3610	3355	ib	j
Androstanol-3 <i>a</i> -one-17 (androsterone)	8	a	3610	3500	)s	ı
Androstanol-38-one-17	a		3610			0
Etiocholanol-3 <i>a</i> -one-17	a		3610			1
Etiocholanol-3β-one-17	a	a	3620	3425	is	q
Allopregnanol- $3\alpha$ -one-17	a	a	3620	3280	ь	ĩ
<b>17-Iso-</b> pregnanol-3α-one-20	a	—	3605			f
Cholestanol-3β-one-6	a	a	3600	3420	), 3490b	j
D. Steroids Containing both	h —	c—(	0—Н а	nd	с=с-н	Groups
	4	ł	в	С	D	
Δ <sup>5</sup> -Androstenediol-3,17	in	s.	3030	ins.	3310ь	0
Δ <sup>5</sup> -Androstenediol-3,17	n.	0.	n. o.	3560	3350	0
					3200ь	1
Δ <sup>6</sup> -Cholestanol-3	30	20	3040	3600	3360	
					31755	
$\Delta^{1, \$, 5:10}$ -Estratrienediol-	in	<b>s</b> .	3055	ins.	3450	Þ
$3,17-\alpha(\alpha-estradiol)$			3020		3235b	
Δ4-Androstenol-3-one-17	30	45	3020	3600	3430	0
(dehydroisoandrosterone)					3370	
Δ <sup>4</sup> -Androstenol-17β-one-3 (testosterone)	30	20	3000	3610	3300Ъ	Þ
Δ <sup>4</sup> -Androstenol-17β-one-3 (cis-testosterone)	30	10	3010	3610	3410b	0
Δ4-17-Methylandrostenol- 17α-one-3	30	10	3020	3600	3450Ь	0
Δ <sup>5</sup> -Pregnenol-3β-one-20	30	20	3030	3605	3455	
<b>G F  - - - - - - - -</b>					34205	Þ
$\Delta^{16}$ -Pregnenol-3 $\alpha$ -one-20	30	40	3050	3600	3365b	j

C. Steroids Containing -C-O-H but not C=C-

<sup>a</sup> See footnotes to Table I. <sup>b</sup> b = broad band; s = sharp band; a = band absent; — = spectrum not measured; n. o. = spectrum measured but band not observed; ins. = compound insoluble in carbon disulfide.

for the failure to detect this band in certain of the compounds in Table V. In column B of Table V similar data are listed for crystalline films or suspensions of these steroids. The possibility of introducing a thicker sample layer into the radiation beam in the form of a crystalline film or suspension in a "Nujol" mull favors the use of films rather than solutions for the investigation of this particular band.

In a molecule containing the partial structure XVI in which there are no hydrogen atoms attached to the unsaturated carbon atoms, this band would not be expected to appear. An example is given in Fig. 4 where the spectra of  $\Delta^{8:14}$ -cholestenol-3 $\beta$  (XVII) and  $\Delta^{14}$ -cholestenol- $3\beta$  (XVIII) are compared over the region between 2500 and 4000 cm.<sup>-1</sup>. The absence of the band near 3000 cm.<sup>-1</sup> from the spectrum of XVII is clearly in evidence.

The Conjugated Diene Systems.—The number of compounds containing this system which have been measured is comparatively small. Absorption associated with the conjugated diene group occurs between 1580 and 1620 cm.<sup>-1</sup> and the steroids must be examined as crystalline films,

H Group



powder suspensions, or in chloroform solution. In view of the fact that a system of conjugated

ethylenic bonds can be detected and characterized with facility by ultraviolet spectrometry<sup>20,21</sup> the identification of this group from infrared measurements is of comparatively small importance. The same is true also of the aromatic systems present in the estrogens.

The Hydroxyl Group.—The longitudinal vibration of the hydrogenoxygen bond of the hydroxyl group is responsible for an absorption band at the high frequency end of the spectrum. The position of this band may vary considerably with the experimental conditions under which the measurements are made, thus it may shift as a result of change in solvent or change in concentration, and may appear quite different (usually broadened, intensified and displaced to lower frequencies) when examined in a crystalline state.

Some data on the frequencies of the

hydroxyl band in carbon disulfide solutions and in films of a number of steroids are listed in columns C and D of Table V. Displacement of the absorption band to lower frequency with increase in intensity and band width is associated with a lowering of the strength of the oxygen-hydrogen bond occasioned by inter- or intra-molecular hydrogen bonding. Where the hydroxyl group is not subject to such effects a maximum is observed near  $3600 \text{ cm.}^{-1}$ , a condition most favored in dilute solution.

It has not proved possible to associate the exact

- (20) Woodward, THIS JOURNAL, 64, 72 (1942).
- (21) Booker, Evans and Gillam, J. Chem. Soc., 1453 (1940).

frequency of this absorption maximum with the position of the hydroxyl group in the steroid molecule. However, the presence or absence of a hydroxyl group in a steroid can be determined with certainty by examination of this region of the spectrum. This is illustrated in curves A and C of Fig. 3 where the spectra of etiocholanol- $3\alpha$ -one-17 and that of its acetate are compared. Twentyfive of the compounds included in Table V contain a hydroxyl group. When examined as crystalline films, most of these showed absorption bands of varied width between 3200 and 3500 cm.<sup>-1</sup>. In carbon disulfide or carbon tetrachloride solution they showed a much weaker narrow absorption band between 3575 and 3610 cm.<sup>-1</sup>. The two hydroxy steroids which failed to exhibit hydroxyl absorption in solution were poorly soluble and it is most probable that in these cases insufficient of the solute was present in the radiation path for the band to be manifested. Under conditions where association takes place, the spectrum of a compound containing a single hydroxyl group may exhibit two or more broad bands between 3200 and 3500 cm.<sup>-1</sup> and the relative intensities



to lower frequencies) when examined Fig. 4.—Infrared absorption spectra in carbon disulfide solution: —, in a crystalline state.  $\Delta^{14}$ -cholestenol-3 $\beta$ ; —————,  $\Delta^{14}$ -cholestenol-3 $\beta$ .

of these maxima may vary with the conditions of sample treatment. This behavior is suggestive of the presence of two or more types of hydrogen bonding or different modes of packing of the hydroxyl group in two or more crystalline forms.

# **General** Discussion

While the absorption associated with the carbonyl group is influenced by the molecular environment in such a manner as to permit of the close correlation of absorption frequency with molecular structure, this would appear not to be the case with regard to absorption associated with the double bond and hydroxyl group. The analytical treatment developed in this paper has been concerned almost exclusively with the high frequency region of the infrared spectrum, but it must be kept in mind that structural variations such as the introduction of a double bond also give rise to very large changes in the appearance of the absorption curves at frequencies less than 1200 cm.-1. Similar modifications are produced also by stereochemical inversions; studies in the higher frequency region have given no indication of spectral variations which can be related to the stereochemical structure, and this is an important factor in the elucidation of steroid structure. It is reasonable to assume that the variations in the spectra in the lower frequency region should also be subject to regulation and correlatable with specific differences in molecular structure, and it is probably in this region that spectral features relatable to specific stereochemical configurations will be observed. Furchgott, Rosenkrantz and Shorr<sup>10,11,12</sup> have drawn attention to this and pointed out certain relationships, between the stereochemical configurations at positions 3 and 5 and absorption in the neighborhood 1000 cm. $^{-1}$ . While we also have noted that absorption near 1000 cm.<sup>-1</sup> is highly susceptible to structural and stereochemical changes involving the 3 and 5 positions, we have as yet been unable to establish any specific correlations between structure and spectra in this region which are of general application.

## Experimental

The data given in this work were obtained at the Sloan-Kettering Institute and at the Stamford Research Laboratories of the American Cyanamid Co. using Perkin-Elmer instruments with either galvanometer or electronic systems of automatic recording; both sodium chloride and lithium fluoride prisms were employed.

Because of the correlations for the carbonyl groups, the region between 1660 and 1780 cm.<sup>-1</sup> was subjected to special study at the Sloan-Kettering Institute. The positions of the absorption maxima in the carbonyl region were obtained directly by measurement of the displacement of the bands from the water vapor absorption bands at 1830 and 1637 cm.<sup>-1</sup> which appeared on every curve and were used as external standards of frequency. The estimated accuracy of the frequency measurements in this region is  $\pm 3$  cm.<sup>-1</sup>. Most of the measurements were made

in a cell of approximately 1 mm. thickness, although a 3 mm. cell was also used in certain instances. The solutions were made up to an initial concentration of 10 mg. per ml. and then diluted so as to obtain an absorption of about 50-75% in the region of the maximum. Under these conditions no appreciable error in the position of the maximum is caused by the slope of the background radiation over the width of the band.

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#### Summary

A comparative study has been made of the infrared absorption spectra of one hundred and thirty steroids in solution in carbon disulfide, or chloroform. Particular attention has been given to the region between 1660 and 1780 cm.<sup>-1</sup> where prominent absorption bands occur which are associated with the presence of carbonyl groups in the molecule. The exact location of the maxima of these bands can be employed to locate the position of the carbonyl group in ketosteroids and to distinguish conjugated from non-conjugated ketosteroids. Absorption bands in other regions of the spectrum associated with the presence of ethylenic double bonds and hydroxyl groups have been similarly investigated.

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