[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. XII. The Determination of the Structure of the Side Chains of C-21 Steroids by Infrared Spectrometry

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

Infrared absorption bands associated with the C=O and C=C stretching vibrations of functional groups in the side chains of C-21 steroids are described and discussed. Examples are given illustrating the use of these spectra in the elucidation of the structure of the metabolites of adrenocortical hormones isolated from urine, and of related steroids prepared synthetically.

The determination of the structural and stereochemical relationships among the carbonyl, ethylene and hydroxyl groups in the side chains of C-21 steroids often presents a difficult problem. This communication describes a number of correlations between the infrared absorption spectra and the side chain structure which have proved of considerable help in the identification of the side chains of newly isolated or synthesized C-21 steroids. These correlations principally involve the frequency of the absorption maxima of the carbonyl stretching bands.

Experimental Methods and Results

The spectra were measured on Perkin-Elmer single beam spectrometers, and the frequencies of the absorption maxima of the C=O and C=C stretching bands are listed in Table I. Many of the steroids included in this survey contained carbonyl groups and double bonds in the ring system as well as on the side chain, and the absorption maxima associated with these are italicized in the table.

For the majority of the measurements a sodium chloride prism was used, the band positions were determined as described in a previous publication[2] and the estimated accuracy is ± 3 cm.$^{-1}$. Representative compounds of the structural types of major interest were measured also with a calcium fluoride prism and the spectra corrected for water vapor and solvent absorption. The bands so measured are indicated in the table by an asterisk and their estimated accuracy is ±1 cm.$^{-1}$.

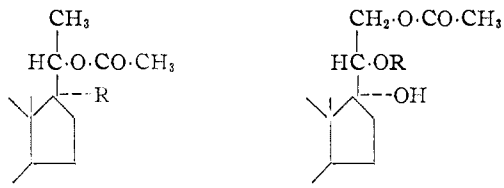
Solvent Effects.—When the spectra are measured in carbon disulfide or carbon tetrachloride solution, the positions of the carbonyl stretching bands are the same. The characteristic band positions for different types of steroid carbonyl compounds have been discussed[3] and summarized.[4] Many of the more highly hydroxylated adrenocortical steroid hormones and metabolites are not sufficiently soluble in either of these two solvents, even after acetylation. They may be studied in chloroform solution[5] but unfortunately the carbonyl frequencies, although still characteristic of the individual carbonyl groups are all displaced to lower frequencies by varying amounts; the bands are broader, and the separate carbonyl maxima in polycarbonyl compounds are less well resolved. The conjugated carbonyl bands in particular tend to exhibit an asymmetry in chloroform solution which makes a precise evaluation of the position of the maximum more difficult. In the following discussion the importance of these solvent effects must be kept in mind.

Discussion

The carbonyl bands associated with simple side chain acetates and ketones are discussed first. The displacements in the carbonyl band positions associated with hydroxyl–carbonyl and carbonyl–carbonyl interactions of certain types of side chain groups are next considered, and finally the effects of introducing unsaturated linkages are noted.

20- and 21-Acetates.—The absorption of the 20-acetate group (I) is at 1734–1736 cm.$^{-1}$ (CS_2) and is in the same range as that of the 3-, 6-, 7-, 11-, 12- and 17-acetates (1734–1742 cm.$^{-1}$ (CS_2, CCl_4), 1719–1722 cm.$^{-1}$ ($CHCl_3$)). No comparisons between 20α- and 20β-acetates have been made but it would seem unlikely that significant differences would occur. The introduction of the 17α-hydroxyl group has no effect on the carbonyl maximum (II).

The data for simple 21-acetates are not available, but for structures III and IV a single carbonyl band is observed at 1749 cm.$^{-1}$ (CS_2), 1736–1739 cm.$^{-1}$ ($CHCl_3$). This value is significantly high, but whether it is characteristic of the 21-acetate *per se*, or involves interaction with substituents at the 17 and 20 positions remains to be determined.



I, R = H
II, R = OH

III, R = H
IV, R = O·CO·CH₃

20-Keto-21-methyl Group.—This group (V) absorbs at 1706–1710 cm.$^{-1}$ in CS_2 and CCl_4 and at 1698–1702 cm.$^{-1}$ in $CHCl_3$. The band positions for the 3-ketone group are 1715–1719 cm.$^{-1}$ (CS_2, CCl_4) and 1702–1705 cm.$^{-1}$ ($CHCl_3$), so that the 3- and 20-ketosteroids can be differentiated in carbon disulfide or carbon tetrachloride but not in chloroform solution.[6]

Several types of substituents (VI–X, XII) may be introduced at C-16, C-17 or C-21 without displacing the frequency of the 20-ketone maximum, and the band position is also unaffected by stereochemical inversion at C-17 (XIII, XIV). Bromination at the 17α-position leaves the band position unchanged (VII) but on bromination at C-21 (XI) the maximum is displaced to 1716–1722 cm.$^{-1}$ ($CHCl_3$).

In the spectra of 16,17-methylene-20-ketones (XV) the carbonyl band is at 1685 cm.$^{-1}$ (CCl_4,

(1) Published as Contribution No. 2683 from the Laboratories of The National Research Council of Canada, and presented, in part, before the Division of Biochemistry of the American Chemical Society, April 11th, 1950, at Philadelphia, Penna.

(2) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

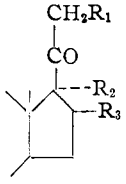
(3) R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **71**, 241 (1949); **72**, 956 (1950).

(4) R. N. Jones and K. Dobriner, *Vitamins and Hormones*, **7**, 293 (1949).

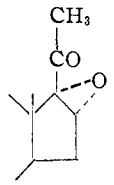
(5) Recently methylene chloride has also been employed to dissolve the more highly hydroxylated steroids; it has the advantage of greater chemical stability.

(6) The 4-, 6-, 7-, 11- and 12-ketosteroids all absorb between 1706 and 1719 cm.$^{-1}$ in CS_2 and CCl_4 (see reference 3) and the 11- and 12-ketosteroids at 1701–1706 cm.$^{-1}$ $CHCl_3$.

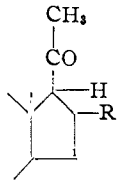
CS_2) indicating that the cyclopropanyl group is exercising a conjugation effect on the 20-ketone similar to that of the cyclopropane ring in *i*-cholestanone-6 and other *i*-ketosteroids.[7]



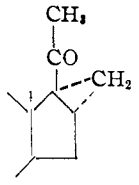
V, R_1 = H R_2 = H R_3 = H
VI, R_1 = OH R_2 = H R_3 = H
VII, R_1 = H R_2 = Br R_3 = H
VIII, R_1 = H R_2 = H R_3 = CH_3
IX, R_1 = H R_2 = H R_3 = OCH_3
X, R_1 = H R_2 = H R_3 = OC_2H_5
XI, R_1 = Br R_2 = OH R_3 = H



XII



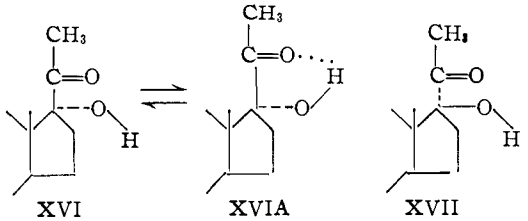
XIII, R = H
XIV, R = CH_3



XV

Hydroxy–Carbonyl Interactions

The 17α-Hydroxy-20-ketone Group.—The introduction of a 17α-hydroxy group (XVI) alters the 20-ketone carbonyl absorption in a characteristic manner. In Fig. 1 the spectrum of pregnanol-3β-one-20-acetate is compared with the spectrum of the 17α-hydroxy derivative in CCl_4 solution. A small band is observed in the latter at 1693 cm.$^{-1}$ in addition to the bands at 1735 and 1708 cm.$^{-1}$ expected for the 3-acetate and the 20-ketone groups. In the hydroxyl stretching region the absorption of the 17α-hydroxyl group is also atypical; two bands occur, one at 3620 cm.$^{-1}$ due to a free hydroxyl and a second band at 3500 cm.$^{-1}$ due to an associated hydroxyl group. The two hydroxyl bands are of about equal intensity and their relative intensities are not significantly altered by threefold dilution (Fig. 1). In dilute solution most monohydroxysteroids show a strong free hydroxyl band near 3600 cm.$^{-1}$ and a weak second band at 3500 cm.$^{-1}$ the relative intensity of which increases with concentration. To explain these effects an equilibrium between XVI and XVIA is proposed, intramolecular bonding between the hydroxylic hydrogen atom and the ketonic oxygen atom accounting for both the anomalous hydroxyl and carbonyl absorption. In chloroform solution the carbonyl bands are lowered by about 5 cm.$^{-1}$ but the positions and relative intensities of the hydroxyl bands are not changed (Fig. 2a). On stereochemical inversion at C-17 the carbonyl band is still observed at 1690 cm.$^{-1}$ (Fig. 2b) (XVII) but the intensity is lower and the relative intensities of the free and associated hydroxyl bonds change considerably on threefold dilution. Thus it



XVI XVIA XVII

(7) M.-L. Josien, N. Fuson and A. S. Cary, THIS JOURNAL, 73, 4445 (1951).

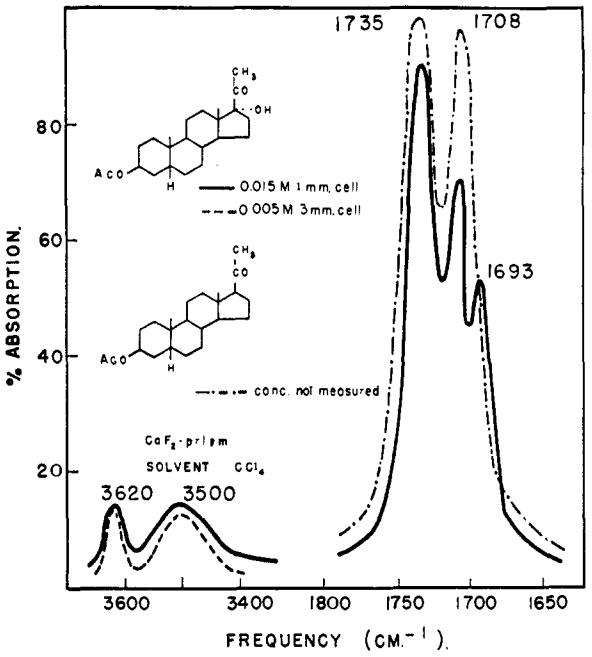
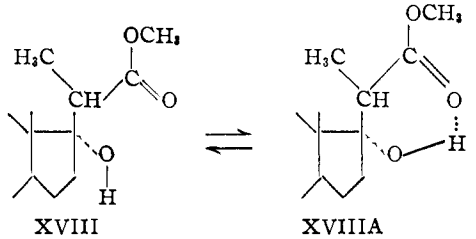


Fig. 1.

would appear that the bonding is weaker in the 17β-hydroxy-20-ketone than in the 17α-hydroxy isomer. The effect is lost on bromination at C-21 (XI), and it is not shown by the 20-keto-21-methylol compounds (VI), Fig. 3.

Other Hydroxy–Carbonyl Interactions.—Other examples of carbonyl band displacements to lower frequencies, and anomalous hydroxyl absorption attributable to intramolecular hydrogen bonding have been noted in structures involving steroid side chains.

One of these (Fig. 4) concerns the 17α-hydroxy group and the carbomethoxy group of the bisnorcholanic acid methyl ester side chain (XVIII). A band would normally be expected for the methyl ester at 1738–1742 cm.$^{-1}$ (CS_2, CCl_4) but the principal band actually occurs at 1719 cm.$^{-1}$ (CCl_4) with only a shoulder at 1740 cm.$^{-1}$. Two hydroxyl bands are present, their relative intensities not sensitive to concentration. An equilibrium with XVIIIA may account for both of these effects.



XVIII XVIIIA

Another example of hydroxy–carbonyl interaction involves the 12α-acetoxy group and the 20β-hydroxy group (Fig. 5) (XIX–XIXA), where the 12α-acetoxy band is displaced from 1733–1740 cm.$^{-1}$ (CCl_4) to 1718 cm.$^{-1}$ with a shoulder persisting at 1740 cm.$^{-1}$, again two hydroxyl bands are present, relatively insensitive to concentration. No analogous interaction occurs in XX where the positions of the acetate and hydroxyl groups are

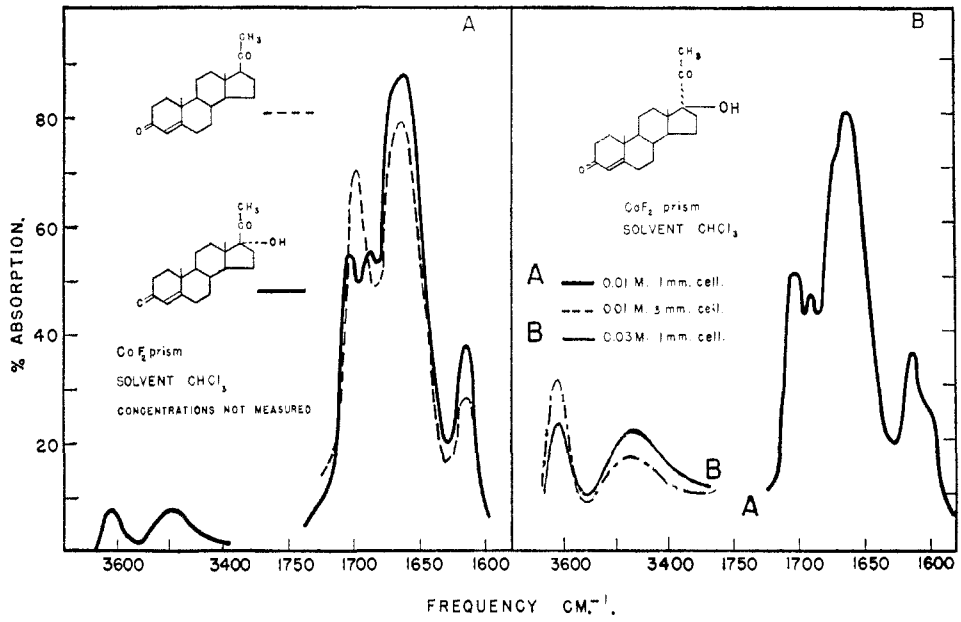


Fig. 2.

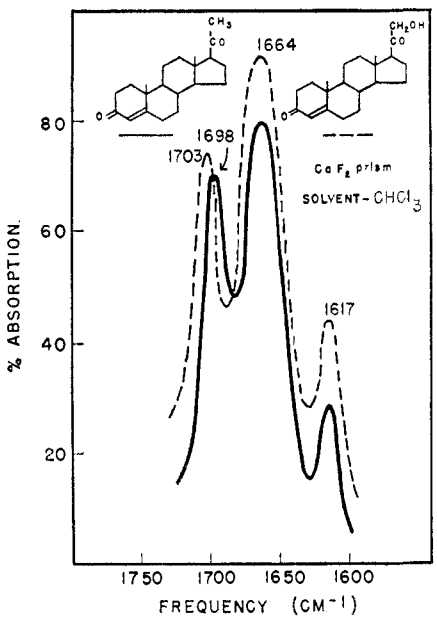
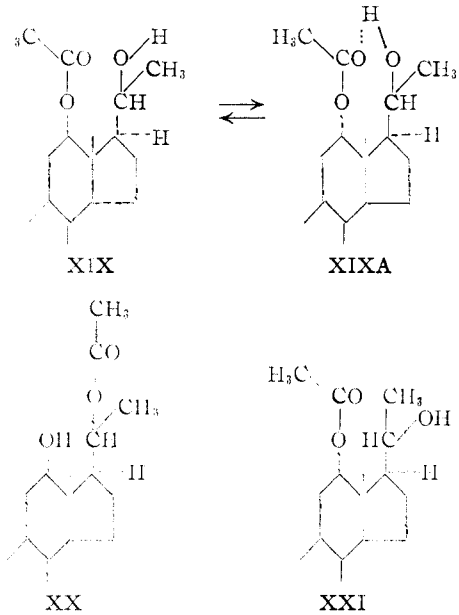


Fig. 3.



interchanged, or in XXI where the hydroxyl group at C-20 has the α-configuration.[8]

Dicarbonyl Interactions

When two carbonyl groups are present in the molecule they usually absorb independently both as to frequency[3] and intensity.[10] Exceptions arise where the two carbonyl groups are close together[3,4] and the resulting dicarbonyl interaction effect differs from the hydroxy-carbonyl interaction discussed above in that the carbonyl maxima are displaced to higher instead of to lower frequencies.

The 21-Acetoxy-20-ketone Group.—This group (XXII) provides an important example of this effect (Fig. 6). In chloroform solution two carbonyl maxima occur at 1745–1750 cm.$^{-1}$ and 1720–1727 cm.$^{-1}$, the normal unperturbed frequencies being 1725–1736 cm.$^{-1}$ for the acetate group and 1698–1702 cm.$^{-1}$ for the 20-ketone.[11,12] These two

(8) The effect of hydrogen bonding in the 12α-acetoxy-20β-hydroxy system is also apparent on the C–O stretching band in the 1200–1250 cm.$^{-1}$ region of the spectrum (see reference 9). In compounds containing XIX two bands occur, at 1251 and 1256 cm.$^{-1}$ (CS_2). The normal position for the 12α-acetoxy group is 1240–1242 cm.$^{-1}$ and the direction of this shift is in accord with the general rule that structural changes which displace the C=O stretching vibration of acetates to lower frequencies displace the 1200–1250 cm.$^{-1}$ acetate band in the opposite direction.

(9) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951).

(10) R. N. Jones, D. A. Ramsay, D. S. Keir and K. Dobriner, *ibid.*, **74**, 80 (1952).

(11) The band positions for 21-acetoxy-20-ketones in CS_2 and CCl_4 are at 1755–1758 and 1724–1736 cm.$^{-1}$.

(12) They have been discussed previously (reference 3).

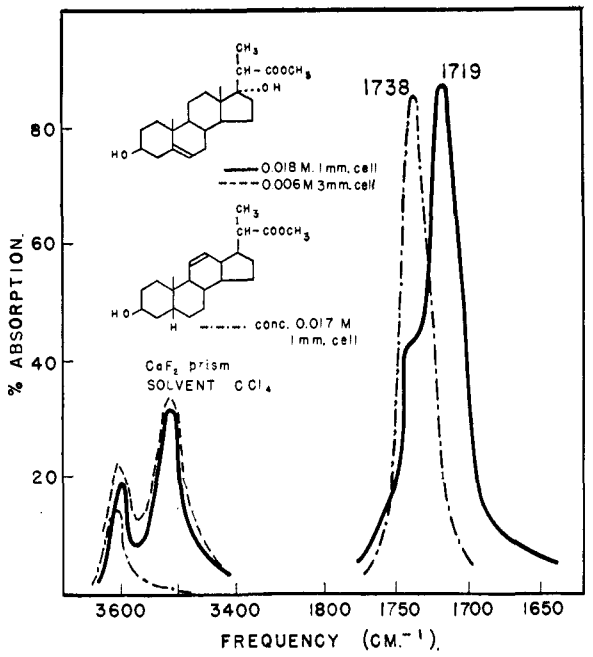


Fig. 4.

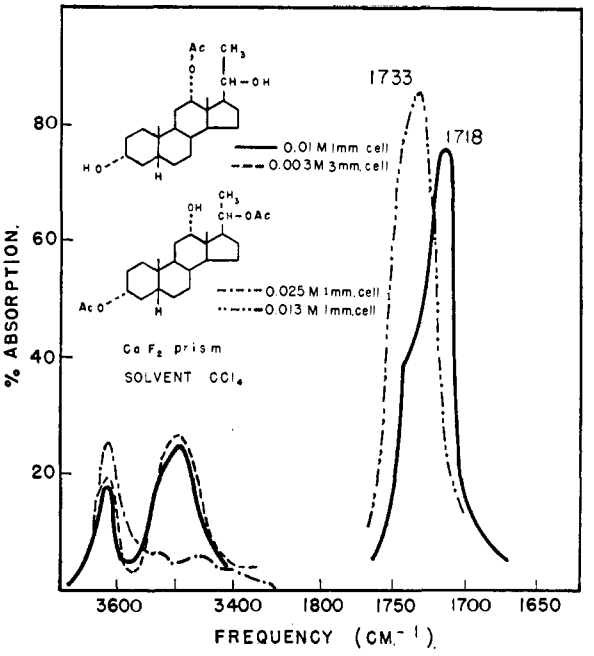
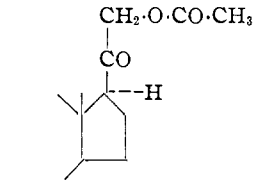
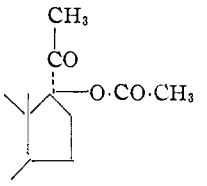


Fig. 5.

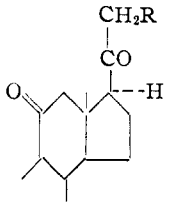
bands are highly characteristic of the 21-acetoxy-20-ketone group.



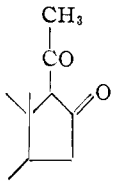
XXII



XXIII



XXIV, R = H
XXV, R = OH



XXVI

Other Dicarbonyl Interactions.—Several other structures containing two carbonyl groups at nearby positions are included in Table I. A small but significant band shift occurs in the low frequency band of 17β-acetoxy-20-ketones (XXIII) where the maxima are at 1742 and 1715–1716 cm.$^{-1}$ (CS_2), the normal positions being 1735–1742 and 1706–1710 cm.$^{-1}$. The 11,20- and 16,20-diketones (XXIV–XXVI) absorb normally.

The reasons for these upward frequency displacements in dicarbonyl compounds are not yet clear. A simple resonance coupling between the carbonyl vibrations is ruled out, since this would displace the bands in opposite directions. A mutual effect in which the dipole field of each carbonyl diminishes the polarization of the other carbonyl bond may be involved. An effect of this kind would have a vector character; its magnitude would depend on the angle between the carbonyl bonds, being negligible if the bonds were perpendicular. It would also depend on the spacial distance between the two oxygen atoms.

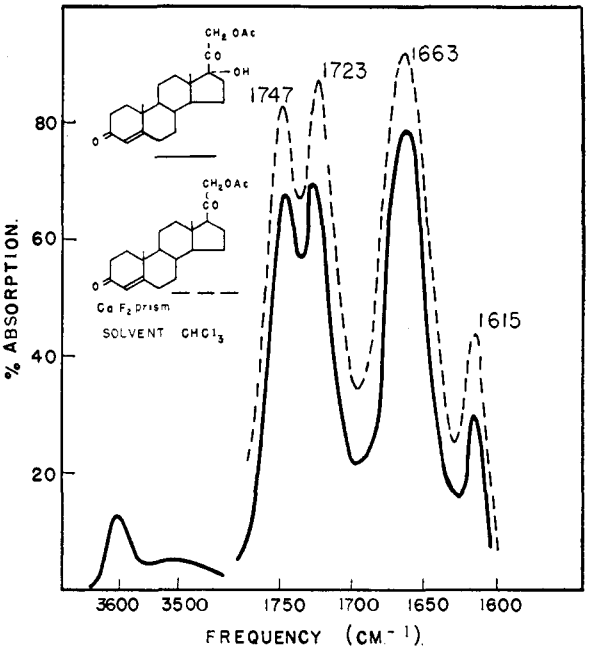
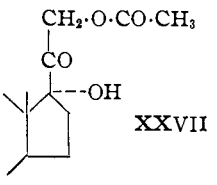


Fig. 6.

The 17α-Hydroxy-20-keto-21-acetoxy Group.—The introduction of the 17α-hydroxy group into the 21-acetoxy-20-ketone group yields a structure (XXVII) which might be expected to combine the spectrographic characteristics of XVI and XXII.



XXVII

TABLE I

POSITIONS OF C=O AND C=C STRETCHING BANDS IN THE INFRARED SPECTRA OF C-21 STEROIDS

The compounds are listed in order of the structural formulas given in the text. The absorption bands associated with the carbonyl groups of the ring system, as opposed to the side chain are italicized unless they are superimposed on side chain bands. Measurements made with a calcium fluoride prism are indicated by an asterisk (see Experimental section). The donors of the compounds are indicated by superscripts to footnotes at the end of the table.

Structure	Compound	Solvent	Maxima, cm.$^{-1}$		
I	Allopregnanol-20α-one-3-acetate[l]	CS_2	1736	*1719*	
	Pregnanol-20α-acetate[l]	CS_2	1734		
II	Allopregnanediol-17α,20-one-3-acetate-20[r]	CS_2	1738	*1719*	
	Pregnanetriol-3,17α,20-diacetate-3,20[n]	CS_2	1739		
	Δ^5-Pregnenetriol-3β,17α,20-diacetate-3,20	CS_2	1736		
III	Δ^4-Pregnenetriol-17α,20,21-one-3-acetate-21[t]	$CHCl_3$	1736	*1666*	*1617*
IV	Δ^4-Pregnenetriol-17α,20,21-one-3-diacetate-20,21[s,k,t]	CS_2	1749[a]	*1678*[a]	
		$CHCl_3$	1739[a]	*1662*[a]	*1615*[a]
V[b]	Allopregnanol-3α-one-20[f]	$CHCl_3$	1700		
	Allopregnanediol-3α,6α-one-20[y]	$CHCl_3$	1700		
	Pregnanediol-3α,6α-one-20[y]	$CHCl_3$	1703		
	Δ^5-Pregnenol-3β-one-20[w]	$CHCl_3$	1702		
	$\Delta^{1,3,5(10)}$-17-Acetylestratrienol-3[d]	$CHCl_3$	1700		
	Δ^4-Pregnenedione-3,20 (*progesterone*)[w]	$CHCl_3$	1698[a]	*1663*[a]	*1615*[a]
	$\Delta^{4,11}$-Pregnadienedione-3,20[p]	$CHCl_3$	1702[a]	*1663*[a]	*1618*[a]
VI	Δ^4-Pregnenol-21-dione-3,20 (*desoxycorticosterone*)[t]	CS_2	1710	*1677*	
		$CHCl_3$	1703[a]	*1664*[a]	*1617*[a]
	Δ^4-Pregnenediol-11β,21-dione-3,20 (*corticosterone*)[w]	$CHCl_3$	1704	*1663*	
VII	17-Bromoallopregnanone-20[o]	CS_2	1705		
VIII	Δ^5-16α-Methylpregnenol-3β-one-20[e]	CS_2	1706		
	Δ^4-16α-Methylpregnenedione-3,20[e]	CS_2	1709	*1676*	
IX	Δ^5-16α-Methoxypregnenol-3β-one-20-acetate[f]	CS_2	*1736*	1706	
	Δ^4-16α-Methoxypregnenedione-3,20[e]	CS_2	1706	*1679*	
X	Δ^5-16α-Ethoxypregnenol-3β-one-20-acetate[f]	CS_2	*1736*	1709	
XI	21-Bromopregnanediol-3α,17α-one-20[f]	$CHCl_3$	1720		
	21-Bromopregnanediol-3α,17α-one-20-formate-3[f]	CCl_4	1725[a]		
		$CHCl_3$	1717		
	21-Bromopregnanediol-3α,17α-one-20-acetate-3[f]	$CHCl_3$	*1730*	1722	
	21-Bromopregnandiol-17α-dione-3,20[f]	$CHCl_3$	1716	*1700*	
XII	16α,17α-Epoxyallopregnanol-3β-one-20[f]	$CHCl_3$	1704		
	16α,17α-Epoxyallopregnanol-3β-one-20-acetate[f]	CS_2	*1736*	1709	
	Δ^5-16α,17α-Epoxypregnenol-3β-one-20-acetate[k]	CS_2	*1736*	1706	
		$CHCl_3$	*1724*	1704	
XIII	17-Isopregnanol-3α-one-20[i]	CS_2	1706		
	17-Isopregnanol-3α-one-20-acetate[i]	CS_2	*1735*[a]	1704[a]	
XIV	Δ^4-16-Methyl-17-isopregnenedione-3,20[e]	CS_2	1703	*1676*	
XV	Δ^4-16,17-Methylenepregnenedione-3,20[e]	CS_2	1685		
	Δ^5-16,17-Methylenepregnenol-3β-one-20[e]	CCl_4	1685[a]		
XVI	Allopregnenediol-3β,17α-one-20-acetate-3[c]	CCl_4	*1733*[a]	1710[a]	1690[a]
	(*Reichstein's Compound L acetate*)	$CHCl_3$	*1720*[a]	1710[a]	1688[a]
	Δ^5-Allopregnenediol-3β,17α-one-20-acetate-3	$CHCl_3$	*1720*[a]	1706[a]	1688[a]
	Pregnanediol-3α,17α-one-20[f]	$CHCl_3$	1702	1685	
	Pregnanediol-3β,17α-one-20[f]	$CHCl_3$	1700	1685	
	Pregnanediol-3α,17α-one-20-formate-3[f]	CCl_4	*1725*[a]	1710[a]	1694[a]
	Pregnanediol-3α,17α-one-20-acetate-3[f]	CS_2	*1736*	1707	1696
	Pregnanediol-3β,17α-one-20-acetate-3[c,f]	CS_2	*1735*[a]	1708[a]	1693[a]
	Δ^5-Pregnenediol-3β,17α-one-20-acetate-3[k]	CS_2	*1738*	1710	1697
	Δ^4-Pregnenol-17α-dione-3,20[k]	$CHCl_3$	1704[a]	1687[a]	*1661*[a] *1615*[a]
	Pregnanol-17α-trione-3,11,20[f]	$CHCl_3$	1705	1685	
XVII	Δ^4-17-Isopregnenol-17β-dione-3,20[f]	$CHCl_3$	1703[a]	1690[a]	*1663*[a] *1613*[a]
XVIII	Δ^5-3β,17α-Dihydroxybisnorcholenic acid methyl ester[h]	CCl_4	1740[a]	1719[a]	
XIX	Pregnanetriol-3α,12α,20β-acetate-12[f]	CCl_4	1718[a]		
	Pregnanetriol-3α,12α,20β-diacetate-3,12[f]	CCl_4	*1735*[a]	1718[a]	
XX	Pregnanetriol-3α,12α,20β-diacetate-3,20[f]	CCl_4	1733[a]		
XXI	Pregnanetriol-3α,12α,20α-acetate-12[f]	CS_2	1736		
	Pregnanetriol-3α,12α,20α-acetate-20[f]	CS_2	1736		

TABLE I (*Continued*)

Structure	Compound	Solvent	Maxima, cm.$^{-1}$		
XXII[b]	Allopregnanediol-3β-21-one-20-acetate-21[r]	$CHCl_3$	1746	1723	
	Allopregnanol-21-dione-3,20-acetate[e]	CS_2	1755	1732	*1719*
	Δ^1-Allopregnenol-21-dione-3,20-acetate[d]	CS_2	1756	1733	*1683*
		$CHCl_3$	1749	1727	*1670*
	Pregnanediol-3α,21-one-20-acetate-21[r]	$CHCl_3$	1750	1723	
	Pregnanetriol-3α,11α,21-one-20-diacetate-11,21[f]	CS_2	1755	1736	
	Pregnanetriol-3α,12α,21-one-20-acetate-21[t]	CS_2	1756[a]	1724[a]	
		$CHCl_3$	1746	1720	
	Pregnanol-21-dione-3,20-acetate[d]	CS_2	1755	1732	*1719*
	Δ^5-Pregnenediol-3β,21-one-20-acetate-21[c,t]	$CHCl_3$	1745	1721	
	Δ^4-Pregnenol-21-dione-3,20-acetate[t]	$CHCl_3$	1747[a]	1723[a]	*1663*[a]
	(*desoxycorticosterone acetate*)				*1615*[a]
	Δ^4-Pregnenediol-11β,21-dione-3,20-acetate-21[w]	CS_2	1754	1732	*1675*
	(*corticosterone*)	$CHCl_3$	1745	1724	*1663*
					1620
	Δ^4-Pregnenol-21-trione-3,11,20-acetate[s]	CS_2	1759[a]	1733[a]	*1709*[a]
	(*dehydrocorticosterone acetate*)				*1678*[a]
XXIII	Allopregnanediol-3β,17α-one-20-diacetate[c]	CS_2	1736	1716	
	Δ^5-Pregnenediol-3β,17β-one-20-diacetate[q]	CS_2	1742	*1736*	1716
		$CHCl_3$	1733	*1722*	1712
	Δ^4-Pregnenol-17β-dione-3,20-acetate[h,g]	CS_2	1742[a]	1715[a]	1677[a]
		$CHCl_3$	1730	1714	*1666*
					1617
XXIV[b]	Pregnanol-3α-dione-11,20[r]	CS_2	1709[a]		
	Pregnanol-3α-dione-11,20-acetate[r]	CS_2	*1737*[a]	1709[a]	
	Δ^4-Pregnentrione-3,11,20[s]	CS_2	1709	*1676*	
XXV	Allopregnanediol-3β,21-dione-11,20[w]	CS_2	*1713*	1703	
	Δ^4-Pregnenol-21-trione-3,11,20[m]	$CHCl_3$	1707	*1663*	*1620*
XXVI	Pregnanol-3β-dione-16,20-acetate[o]	CS_2	*1739*	1706	
XXVII	Allopregnanetriol-3β,17α-21-one-20-diacetate-3,21[c]	$CHCl_3$	1745[a]	1724[a]	
	Allopregnanetetrol-3β,11β,17α,21-one-20-diacetate-3,21[r]	$CHCl_3$	1745	*1736*	1724
	Allopregnanetriol-3β,17α,21-dione-11,20-diacetate-3,21[s]	$CHCl_3$	1746[a]	1724[a]	*1705*[a]
	Pregnanetriol-3α,17α,21-one-20-diacetate-3,21[f]	CS_2	1753	1736	
	Pregnanetriol-3β,17α,21-one-20-diacetate-3,21[f]	CS_2	1752	1732	
	Pregnanetetrol-3α,11β,17α,21-one-20-diacetate-3,21[v]	$CHCl_3$	1750	*1733*	1720
	Pregnanediol-17α,21-trione-3,11,20-acetate-21[s]	$CHCl_3$	1748[a]	1725[a]	1708[a]
	Pregnanetriol-4,17α,21-trione-3,11,20-diacetate-4,21[s]	$CHCl_3$	1744[a]	1725[a]	*1706*[a]
	Δ^4-Pregnenediol-17α,21-dione-3,20-acetate-21[k]	$CHCl_3$	1746[a]	1728[a]	*1660*[a]
	(*Reichstein's Compound S acetate*)				*1615*[a]
	Δ^4-Pregnenediol-17α,21-trione-3,11,20-acetate-21[s]	$CHCl_3$	1748[a]	1728[a]	*1705*[a]
	(*cortisone acetate*)			*1668*[a]	*1617*[a]
	Δ^4-Pregnenetriol-11β,17α,21-dione-3,20-acetate-21[u]	$CHCl_3$	1748	1728	*1665*
	(*Kendall's Compound F acetate*)				*1615*
XXVIII	$\Delta^{4,17:20}$-Pregnadienol-21-one-3-acetate[t]	CS_2	1742	*1676*	
XXIX	Δ^{16}-Allopregnenediol-3β,20β-diacetate[o]	CS_2	1739		
	Δ^{16}-Pregnenediol-3β,20β-diacetate[o]	CS_2	1742		
XXX	$\Delta^{17:20}$-Allopregnenediol-3β,20-diacetate (*cis* and *trans*-isomers)[f]	CS_2	1750	*1739*	
	$\Delta^{17:20}$-Pregnenediol-3α,20-diacetate[f]	CS_2	1750	*1742*	
	$\Delta^{5,17:20}$-Pregnadienediol-3β,20-diacetate[f]	CS_2	1749	*1736*	
	$\Delta^{17:20}$-Pregnenetriol-3β,11β,20-triacetate[f]	CS_2	1753	*1739*	
	$\Delta^{17:20}$-Pregnenetriol-3α,12α,20-triacetate[f]	CS_2	1756	*1739*	
XXXI	$\Delta^{17:20}$-21-Benzalpregnenetriol-3α,12α,20-triacetate[f]	CS_2	1762	*1738*	
XXXII	Δ^{16}-Allopregnenol-3β-one-20-acetate[x]	CS_2	*1736*	1668	
	Δ^{16}-Pregnenol-3α-20[i,o]	$CHCl_3$	1656		
	Δ^{16}-Pregnenediol-3β,12β-one-20-diacetate[d]	CS_2	*1740*	1671	
	$\Delta^{5,16}$-Pregnadienediol-2,3β-one-20-diacetate[o]	CS_2	*1743*	1671	
	$\Delta^{4,16}$-Pregnadienedione-3,20[c]	$CHCl_3$	1662[a]	*1617*[a]	1587[a]
XXXIII	$\Delta^{5,16}$-16-Methylpregnadienol-3β-one-20-acetate[c]	CS_2	*1736*	1661	
		$CHCl_3$	*1724*	1652	
XXXIV	Δ^4-17α-Vinylandrostenol-17β-one-3[t]	CCl_4	3083[z]		
XXXV	Δ^4-17α-Ethynylandrostenol-17β-one-3[t]	$CHCl_3$	3308[az]		
	Δ^5-17-Ethynylandrostenediol-3β,17-acetate-3[j]	CCl_4	3310[az]		
	$\Delta^{1,3,5:10}$17-Ethynylestratrienediol-3,17-acetate-3[j]	$CHCl_3$	3310[az]		

[a] Determined with calcium fluoride prism. [b] For additional examples in CS_2 solution see references 2 and 3. [c] C. Djerassi

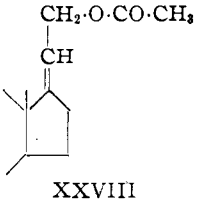
and G. Rosenkranz, Syntex, S. A. Mexico City, Mexico. [d] C. Djerassi and C. R. Scholz, Ciba Pharmaceutical Products Inc., Summit, N. J. [e] L. F. Fieser and M. Fields, Harvard University, Cambridge, Mass. [f] T. F. Gallagher, Sloan-Kettering Institute, New York, N. Y. [g] G. A. Grant, Ayerst, McKenna and Harrison Ltd., Montreal, P. Q. [h] E. B. Hershberg, The Schering Corp., Bloomfield, N. J. [i] W. H. Hoehn, G. A. Breon and Co., Kansas City, Mo. [j] J. R. Jamieson and E. Lozinski, Charles E. Frosst and Co., Montreal, P. Q. [k] P. L. Julian, The Glidden Co., Chicago, Ill. [l] O. Kamm, Parke, Davis and Co., Detroit, Mich. [m] M. H. Kuizenga, The Upjohn Co., Kalamazoo, Mich., [n] H. L. Mason, Mayo Clinic, Rochester, Minn. [o] R. E. Marker, Pennsylvania State College, State College, Pa. [p] H. B. MacPhillamy, Ciba Pharmaceutical Products, Inc., Summit, N. J. [q] P. A. Plattner, Eidg. Tech. Hochschule, Zurich, Switz. [r] T. Reichstein, University of Basel, Basel, Switz. [s] L. H. Sarett, Merck and Co., Inc., Rahway, N. J. [t] C. R. Scholz, Ciba Pharmaceutical Products Inc., Summit, N. J. [u] E. Schwenk, The Schering Corp., Bloomfield, N. J. [v] C. M. Suter, Sterling-Winthrop Research Inst., Rensselaer, N. Y. [w] R. Turner, Harvard University, Cambridge, Mass. [x] R. B. Wagner, Pennsylvania State College, State College, Pa. [y] Compound isolated at the Sloan-Kettering Institute. [z] C=C—H or C≡C—H carbon-hydrogen stretching vibration.

In fact, the introduction of the 17α-hydroxy group into XXII does not give rise to any new band analogous to the 1685–1695 cm.$^{-1}$ band of XVI (Fig. 6). In the hydroxyl stretching region compounds containing XXVII possess a strong free hydroxyl band at 3600–3625 cm.$^{-1}$, a weaker associated hydroxyl band at 3500 cm.$^{-1}$ and, in some cases, a third band[13] between 3400 and 3450 cm.$^{-1}$.

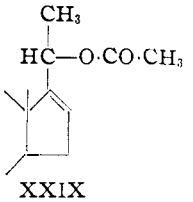
Such hydroxyl bands in the spectra of acetylated adrenocortical steroids or their metabolites are highly suggestive of the presence of the 17α-hydroxyl group. In these compounds positions 11β- and 17α- are the only ones at which hydroxyl groups resistant to mild acetylation are normally encountered, and these two positions can be readily distinguished, since mild oxidation converts the 11β-hydroxyl group to an 11-ketone and a new maximum appears at 1710–1716 cm.$^{-1}$ (CS_2, CCl_4), 1702 cm.$^{-1}$ ($CHCl_3$).

Unsaturated Groups

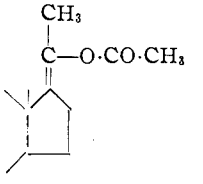
Unsaturated Acetates.—In the unsaturated acetates XXVIII and XXIX the β,γ-double bonds have no effect on the carbonyl frequency, but in the enol acetate XXX the band is displaced from 1735–1742 cm.$^{-1}$ to 1749–1756 cm.$^{-1}$ (CS_2) and a further displacement to 1762 cm.$^{-1}$ (CS_2) is noted in the cross conjugated system XXXI. An absorption maximum between 1750 and 1765 cm.$^{-1}$ (CS_2, CCl_4) is seen in many compounds containing the —C=C—O—C=O structure (*e.g.*, phenolic acetates[3]).



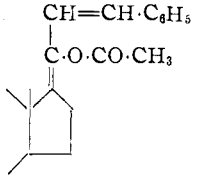
XXVIII



XXIX

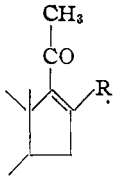


XXX



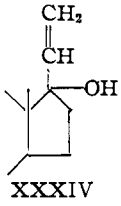
XXXI

The Δ^{16}-20-ketone Group.—In this group XXXII the carbonyl maximum position differs from that in other conjugated ketones.[14] In chloroform solution the differentiation among these conjugated ketones is rendered more difficult by the broadening of the band, but the C=C stretching bands of conjugated ketones are readily observed[15] and serve to identify the Δ^{16}-20-ketone group.[16] The carbonyl frequency is not altered on introduction of a 16-methyl group XXXIII.

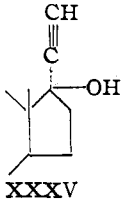


XXXII, R = H
XXXIII, R = CH_3

17-Vinyl and 17-Ethynyl Groups.—The 17α-vinyl group in XXXIV gives a weak =C—H stretching vibration at 3085 cm.$^{-1}$ but the C=C stretching band has not yet been detected. The ethynyl group in XXXV is easily recognized by the strong narrow C≡C—H carbon-hydrogen stretching band at 3340 cm.$^{-1}$. Typical curves illustrating these characteristic ethylenic and acetylenic bands in steroid spectra have been published elsewhere.[17] In the ethynyl compounds a C≡C stretching band should occur between 2080 and 2180 cm.$^{-1}$ but has not yet been detected. The wide variation in the intensity of this band in acetylenic compounds has been commented on by Wotiz and Miller.[18]



XXXIV



XXXV

Elucidation of Structure

These correlations have been applied to several problems concerned with the identification of naturally occurring and synthetic steroids, and a few examples may be briefly noted.

(13) This third band is not necessarily associated with an hydroxyl vibration; it may be a harmonic carbonyl band, since most of the compounds containing XXVII possess several ring carbonyl groups and the carbonyl intensity will be strong.

(14) Δ^{16}-*20-ketone, 1666–1670 cm.*$^{-1}$; Δ^4-3-ketone, 1674–1678 cm.$^{-1}$; Δ^5-7-ketone, 1677 cm.$^{-1}$; Δ^1-3-ketone, 1680–1684 cm.$^{-1}$; $\Delta^{9:11}$-12-ketone, 1680–1684 cm.$^{-1}$; $\Delta^{8:14}$-15-ketone, 1705 cm.$^{-1}$; Δ^{15}-17-ketone, 1716 cm.$^{-1}$ (all in CS_2 or CCl_4 solution). Δ^{16}-*20-ketone, 1652–1662 cm.*$^{-1}$; Δ^4-3-ketone, 1660–1668 cm.$^{-1}$; Δ^1-3-ketone, 1670 cm.$^{-1}$ ($CHCl_3$ solution).

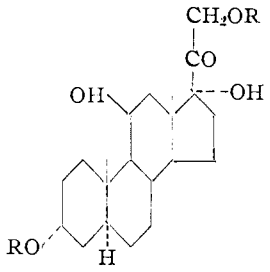
(15) R. N. Jones, P. Humphries, E. Packard and K. Dobriner, THIS JOURNAL, **72**, 86 (1950).

(16) Δ^{16}-*20-ketone, 1585–1590 cm.*$^{-1}$; Δ^1-3-ketone, 1604–1609 cm.$^{-1}$; $\Delta^{9:11}$-12-ketone, 1607 cm.$^{-1}$; Δ^4-3-ketone, 1615–1619 cm.$^{-1}$ (all in $CHCl_3$ solution).

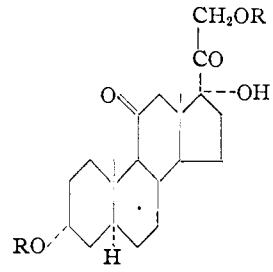
(17) R. N. Jones, *Chemistry in Canada*, **2**, 26 (94) (1950).

(18) J. H. Wotiz and F. A. Miller, THIS JOURNAL, **71**, 3441 (1949).

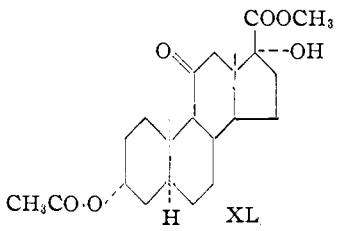
(**A**).—During an investigation of the steroid hormones and metabolites excreted in the urine of patients receiving cortisone or ACTH,[19] Δ^4-pregnenediol-17α,21-trione-3,11,20-acetate-21 (cortisone acetate) and Δ^4-pregnenetriol-11β,17α,21-dione-3,20-acetate-21 (Kendall's Compound F acetate) were isolated from acetylated ketonic fractions. Those substances were readily identified by comparison with the infrared spectra of authentic specimens between 950 and 1150 cm.$^{-1}$. It was anticipated that metabolites of these hormones would also occur in which ring A is reduced. As yet few model compounds of this type are available for direct spectral comparison, but the presence of compounds XXXVI and XXXVIII in human urine was inferred from the spectra of the hydroxyl and carbonyl stretching regions of the spectra of two acetylated steroid metabolites which were isolated (XXXVII, XXXIX).



XXXVI, R = H
XXXVII, R = CO·CH$_3$



XXXVIII, R = H
XXXIX, R = CO·CH$_3$



XL

The spectrum of one of these compounds (XXXIX) possessed maxima in chloroform solution at 1752, 1724 and 1708 cm.$^{-1}$. The center band was more intense than either of the other two (Fig. 7), and bands were also present in the hydroxyl region at 3610 and 3460 cm.$^{-1}$. The bands at 1752 and 1724 strongly suggested the presence of the 21-acetoxy-20-ketone group (XII), and the absence of any carbonyl absorption below 1700 cm.$^{-1}$ showed that the Δ^4-3-ketone group of the hormone had been reduced. The band at 1708 cm.$^{-1}$ suggested a ketone group at either position 3, or 11 (positions 4, 6, 7 and 12) being excluded from consideration on biochemical grounds. It was considered that the reduction of the Δ^4-3-ketone would more probably yield a 3-hydroxyl than a 3-ketone group and the 1708 cm.$^{-1}$ band was tentatively assigned to the 11-ketone. An acetylated 3-hydroxyl group would be required by this hypothesis; this would absorb in chloroform solution near 1720 cm.$^{-1}$ and the likelihood of two carbonyl groups contributing to the 1724 cm.$^{-1}$ maximum seemed reasonable in view of its high relative intensity. If the 1708 cm.$^{-1}$ band were associated with the 11-ketone group, then the hydroxyl group present in the acetylated compound is almost certainly at the 17α-position. Thus the tentative structure XXXIX was arrived at for this acetylated metabolite and this was subsequently confirmed by oxidation to XL and comparison with the spectrum of the authentic compound which was available.

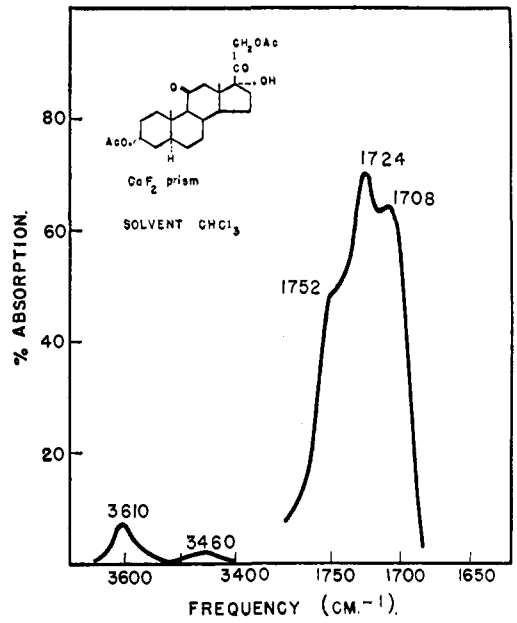
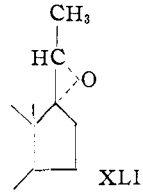


Fig. 7.

(**B**).—In the course of an investigation of the action of perbenzoic acid on the enol acetate XXX a product was obtained by Gallagher and Kritchevsky[20] which exhibited the characteristic absorption of the 17-hydroxy-20-keto-21-methyl group (XVI) in both the C=O and O—H regions. An intermediate product, obtained prior to hydrolysis possessed no hydroxyl band and the carbonyl absorption of a normal saturated 3-acetate consistent with the epoxide structure XLI.



XLI

(**C**).—In a reinvestigation by Gallagher and Fukushima[21] of a reaction described by Marker[22] for the introduction of the 17α-hydroxyl group, the product obtained did not exhibit the characteristic absorption of the 17α-hydroxy-20-keto-21 methyl group, and this observation led subsequently to the correct identification of the compound as a 16-methoxy-20-keto-21-methyl derivative by chemical means.

(**D**).—During the synthesis of progesterone labeled at position 21 with C^{14}, Heard and Yates[23]

(19) S. Lieberman, L. B. Hariton, M. B. Stokem, P. E. Studer and K. Dobriner, *Federation Proc.*, **10**, 216 (1951).

(20) T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951).

(21) D. K. Fukushima and T. F. Gallagher, *ibid.*, **72**, 2306 (1950).

(22) R. E. Marker, *ibid.*, **71**, 4149 (1949).

(23) R. D. H. Heard and C. Yates, private communication.

utilized the reaction between Δ^5-3β-acetoxyetiocholenyl chloride and dimethylcadmium to introduce the 20-keto-21-methyl group. The course of this reaction is sensitive to changes in reagent concentration, and Δ^5-3β-acetoxyetiocholenic acid methyl ester may be obtained instead of the desired Δ^5-pregnenol-3β-one-20-acetate. These two compounds possess identical melting points and optical rotations, and show no mixed melting point depression. The required 20-ketone absorbs at 1706 cm.$^{-1}$ and the undesired methyl ester at 1735 cm.$^{-1}$ so that measurement of the carbonyl region of the infrared spectrum provided a rapid method for the identification of the product.

Concluding Remarks

For convenience in presentation, the infrared absorption characteristic of the side chain structures have been treated here independently of absorption within the ring system. This separation is of course an artificial one, and in the evaluation of the infrared absorption of any individual compound the absorption of the whole molecule must be considered, as was done in the case of XXXIX above.

Acknowledgments.—The authors wish to thank the several investigators listed individually in a footnote to Table I, who kindly made available many of the compounds. The technical assistance of Mr. D. S. Keir and Mr. R. Lauzon at the National Research Council and Miss B. Boland and Miss R. Connolly at the Sloan-Kettering Institute is also gratefully acknowledged.

This investigation was aided by grants from the American Cancer Society (upon recommendation of the Committee on Growth of the (U. S.) National Research Council), the Commonwealth Fund, the Anna Fuller Fund, the Albert and Mary Lasker Foundation, the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

NEW YORK, N. Y.
OTTAWA, CANADA

RECEIVED OCTOBER 26, 1951

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

The Infrared Spectra of α-Brominated Ketosteroids

BY R. NORMAN JONES, D. A. RAMSAY, F. HERLING AND KONRAD DOBRINER[1a]

RECEIVED NOVEMBER 29, 1951

The effect of bromination at the α-carbon atom on the carbonyl stretching bands in the infrared spectra of keto steroids is shown to depend on the stereochemical configuration of the carbon-bromine bond. It is suggested that if the bromine atom enters at an equatorial position on the cyclohexanone ring, in the chair configuration the band is displaced by about 20 cm.$^{-1}$ to higher frequency while bromination at a polar position causes little displacement. The 20-ketone band is similarly displaced by about 20 cm.$^{-1}$ on bromination at C-21 but is hardly affected by bromination at the 17α-position. A diminution in the integrated adsorption intensity accompanies a positive frequency shift on bromination. These observations aid in the determination of the steric configuration and structure of brominated ketosteroids.

In the course of a systematic study of the infrared spectra of steroids, it has been observed that, in certain instances, the spectra may be influenced in a characteristic manner by stereochemical as well as by structural differences. One example of such stereochemical specificity involving the C–O stretching vibrations at 1200–1250 cm.$^{-1}$ in the spectra of 3α- and 3β-acetoxy steroids has been discussed previously.[2] The frequency[3] and intensity[4] of the carbonyl stretching bands in ketosteroids are influenced by bromination at an adjacent methylene group, and it is the purpose of this communication to show that the effect of such α-bromination depends on the stereochemical configuration of the carbon–bromine bond.

Experimental Methods and Results

The spectra were determined on a Perkin–Elmer Model 12C spectrometer, using a calcium fluoride prism. The frequencies of the carbonyl maxima, which are given in Table I, were determined after correction for water vapor and solvent absorption, and the estimated accuracy is ±1 cm.$^{-1}$. A few of the measurements, indicated in the table by an asterisk, were made with a sodium chloride prism; these are accurate to ±3 cm.$^{-1}$. The integrated absorption intensities were determined by the modification of the Wilson and Wells method described previously[4] for polycarbonyl compounds.

Discussion

In the *3-ketones*, the introduction of a single α-bromine atom increases the frequency of the carbonyl maximum by 13–19 cm.$^{-1}$ and depresses the integrated absorption intensity[4] by about 25%. The introduction of a second bromine atom at *the same α-carbon atom* to form a gem dibromide produces little further change in either the carbonyl frequency or intensity, but if a second bromine atom is introduced on the α'-methylene group, to yield a 2,4-dibromo-3-ketone, an additional increase of about 20 cm.$^{-1}$ occurs in the carbonyl frequency and the intensity is further depressed. A positive frequency shift is observed also in a *6-bromo-7-ketone* (see Table I). In some of these compounds, *e.g.*, 2-bromoandrostanol-17-one-3-hexahydrobenzoate, the brominated carbonyl band overlaps ester carbonyl absorption at 1735–1740 cm.$^{-1}$.

In the *11-bromo-12-ketones* series, the two com-

(1) Published as Contribution No. 2696 from the Laboratories of The National Research Council of Canada, and No. XIII in the series "Studies in Steroid Metabolism."

(1a) Died March 10, 1952.

(2) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951).

(3) R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **72**, 956 (1950).

(4) R. N. Jones, D. A. Ramsay, D. S. Keir and K. Dobriner, *ibid.*, **74**, 80 (1952).