Acknowledgment.—The authors thank the Graduate Council of the University of Oregon for a grant which helped to further the investigation reported.

Summary

1. 10-Methyl-10-hydroxyanthrone has been prepared by hydrogen peroxide oxidation of 10-methylanthrone. The alcohol has been converted to 10-methyl-10-acetoxyanthrone.

2. Methylmagnesium iodide displaces the acetoxyl group in 10-methyl-10-acetoxyanthrone to give a 10% yield of 9,10,10-trimethyl-9,10-dihydroanthranol, which was synthesized independently from 10,10-dimethylanthrone.

3. The course of the methylation of anthrone to 10,10-dimethylanthrone or to 9-methylanthranyl methyl ether is discussed.

EUGENE, OREGON RECEIVED JUNE 27, 1949

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

Studies in Steroid Metabolism. VIII. The Detection and Location of Ethylenic Double Bonds in Steroids by Infrared Spectrometry

BY R. NORMAN JONES,* P. HUMPHRIES, E. PACKARD AND KONRAD DOBRINER

In preceding papers of this series, the application of infrared spectrometry to the elucidation of steroid structure[2,3] and to the study of steroid metabolism[4] has been discussed. The correlations of molecular structure and spectra have aided in establishing the structure of several metabolites of steroid hormones isolated from urine.[5,6,7]

The work so far described has been concerned mainly with the characterization of the carbonyl group from the position of the maximum of the C=O stretching vibration which occurs between 1660 and 1770 cm.$^{-1}$.

The detection of ethylenic unsaturation and the location of the ethylenic bonds in steroids are also problems of considerable importance. Difficulties arise in this connection if, as frequently occurs, the quantities of the steroid available are insufficient to allow chemical characterization by hydrogenation and by oxidative degradation to compounds of known structure. Conjugated dienes, α,β-unsaturated ketones and the aromatic ring systems of the estrogens can be identified from the intense and characteristic absorption in the medium ultraviolet region of the spectrum,[8,9] but, as yet, no suitable methods have been developed for the location of the non-conjugated ethylenic double bond.

Detection of Ethylenic Double Bonds by Infrared Spectrometry.—Absorption bands characteristic of carbon–carbon unsaturation occur in three regions of the infrared spectrum. Fox and Martin[10] have noted that the *stretching vibration of carbon–hydrogen bond* in the system —C=C—H occurs between 3000 and 3100 cm.$^{-1}$, while in a saturated alkyl system the corresponding bands lie between 2800 and 3000 cm.$^{-1}$. The use of this region of the spectrum in the investigation of unsaturated hydrocarbons has been discussed recently by Saier and Coggeshall.[11]

Absorption associated with the stretching motion of the C=C bond occurs between 1580 and 1680 cm.$^{-1}$, and a third type of double bond absorption involving the *angular deformation of the carbon–hydrogen bond* in —C=C—H is found in the region between 800 and 970 cm.$^{-1}$. The absorption in all three of these regions has been utilized by Sheppard and Sutherland[12] in an investigation of the double bond structure of terpenoid compounds, while the 800–970 and the 1580–1680 cm.$^{-1}$ regions have been employed recently by Gunthard and Ruzicka[13] and by Thompson and Whiffen[14] in similar investigations. Blout, Fields and Karplus have studied the C=C stretching bands in conjugated polyenes.[15]

This paper is concerned with a study of the spectra of various types of unsaturated steroids, principally in the region of the C=C stretching vibrations.

The C=C Stretching Vibrations in Steroids (1580–1680 Cm.$^{-1}$).—Whereas the C=O stretching vibration gives rise to one of the most intense absorption bands in the whole infrared spectrum, the C=C stretching band is weak in compounds containing non-conjugated ethylenic groups, and its observation is complicated by its superposition on water vapor bands which occur in this region of the spectrum. In spite of these experimental difficulties, measure-

* Harvard University Research Fellow 1937–1942.

(1) Published as Contribution No. 2027 from The Laboratories of The National Research Council of Canada.

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

(3) Jones, Humphries and Dobriner, *ibid.*, **71**, 241 (1949).

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

(5) Jones and Dobriner, *Vitamins and Hormones*, **7**, in press (1949).

(6) Lieberman, Fukushima and Dobriner, *J. Biol. Chem.*, in press.

(7) Dobriner and Lieberman, "Symposium on Steroids," University of Wisconsin Press, Madison, Wis.

(8) Dannenberg, *Abhandlungen der Preuss. Akad. Wiss. Math.-naturw. Klasse*, Nr. 21 (1939).

(9) Jones, *Recent Prog. Hormone Research*, **2**, 3 (1948).

(10) Fox and Martin, *Proc. Roy. Soc.* (*London*), **175**, 208 (1940).

(11) Saier and Coggeshall, *Anal. Chem.*, **20**, 812 (1948).

(12) Sheppard and Sutherland, *J. Chem. Soc.*, 1540 (1947).

(13) Gunthard and Ruzicka, *Helv. Chim. Acta*, **31**, 642 (1948).

(14) Thompson and Whiffen, *J. Chem. Soc.*, 1412 (1948).

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

ments of the C=C stretching bands have proved most effective in locating the position of ethylenic linkages in steroids. The frequency of the maximum of this band, like the stretching vibration of the carbonyl group, has been found to be closely dependent on the position of the double bond in the molecule, and is little influenced by other structural features. The relations between the frequency of the absorption maximum and the molecular structure are shown in Tables I–IV and discussed below.

In consequence of the weakness of the band, often it is not possible to recognize the band position from inspection of the instrumental curve as obtained from the potentiometer recorder, and a very careful calculation of the percentage transmission curve must first be carried out to eliminate the water vapor structure before the band may be detected.[16] The data given in Tables I–IV are all derived from percentage transmission curves. The relative intensities of the bands are indicated approximately in column 3 of Tables I and II, and the absorption curves of typical examples from each intensity class are shown in Figs. 1 and 2. Summaries of the tentative assignments of band positions are given in Tables III and IV.

TABLE I

CARBON–CARBON DOUBLE BOND STRETCHING VIBRATIONS IN STEROIDS

Compound[a]	Position of max. cm.$^{-1}$ ($CHCl_3$)	Intensity[b]	Source[c]
A. Monoethylenic Compounds			
Δ^2-Androstenone-17	1657	++	5, 7
Δ^2-Cholestene	1654	++	9
Δ^5-Androstenol-3β	1670	++	11
Δ^5-Androstenol-3β-one-17	1669	+	11
Δ^5-Androstenol-3β-one-17-acetate	1671	+	11, 12
Δ^5-Androstenediol-3β,17α	1669	+	11
Δ^5-Androstenediol-3β,17α-diacetate	1672	+	11
Δ^5-Androstenediol-3β,17β	1671	+	11
Δ^5-Pregnenediol-3β,21-one-20	1670	+	12
Δ^5-3β-Hydroxycholenic acid M. E.	1672	+	4
Δ^5-3β-Acetoxycholenic acid M. E.	1672	+	4
Δ^5-Cholestenol-3β (cholesterol)	1669	++	..
Δ^7-Cholestenol-3β	1664	++	3
$\Delta^{8:14}$-Cholestenol-3β	No max. obsd.		3
$\Delta^{9:11}$-Androstenol-3α-one-17	No max. obsd.		17
$\Delta^{9:11}$-Etiocholenol-3α-one-17	No max. obsd.		17
$\Delta^{9:11}$-3α-Hydroxycholenic acid M. E.	No max. obsd.		4, 6
$\Delta^{9:11}$-3α-Acetoxycholenic acid M. E.	No max. obsd.		11, 13
$\Delta^{9:11}$-3-Ketocholenic acid	No max. obsd.		11
Δ^{11}-3α-Hydroxycholenic acid M. E.	1624	+	4, 6
Δ^{11}-3α-Acetoxycholenic acid M. E.	1625	+	4, 13
Δ^{11}-3β-Acetoxycholenic acid M. E.	1625	+	13
Δ^{11}-3-Ketocholenic acid M. E.	1628	++	13
Δ^{14}-3,5,19-Trihydroxyetiocholenic acid E. E.	1647	++	2
Δ^{14}-Cholestenol-3β	1646	++	3
Δ^{14}-Cholestenol-3β-acetate	1647	+	3
Δ^{16}-Allopregnenediol-3β,20β	1621	++	9
Δ^{16}-Pregnenediol-3β,20β	1621	+	5
Δ^{16}-Pregnenediol-3β,20β-diacetate	1630	+	16
B. Conjugated Dienes			
$\Delta^{3,5}$-Androstadienol-17α-acetate	1618, 1578	+++	15
$\Delta^{3,5}$-Androstadienediol-3,17α-dipropionate(testosterone dipropionate)	1671, 1639	+++	11
$\Delta^{3,5}$-Cholestadienol-3β-acetate	**1670, 1639**	**+++**	**13**
C. Aromatic Compounds			
Etiocholanol-3α-one-17-benzoate	1604, 1586	++	8
Δ^5-Cholestenol-3β-benzoate	1604, 1586	++	8
Δ^{14}-Cholestenol-3β-benzoate	1648, 1603, 1584	++	3
Estradiol-3,17	1613, 1590	+++	5, 11, 12
Estrone acetate	1611, 1589	+++	1,12
1-Methylestradiol diacetate	1596, 1590	+++	1
$\Delta^{1,3,5:10}$-1-Methyl-3-acetoxy-17-carbomethoxyestradiene	1600, 1590	+++	1
$\Delta^{1,3,5:10}$-1-Methyl-17-(1-methylheptyl)-estratrienol-3	1600, 1590 (broad)	+++	1
Equilenin acetate	1625 (broad), 1605, 1573	+++	16

[a] M.E. designates methyl ester; E.E. designates ethyl ester. In the compounds described here, the configuration of the 17-hydroxyl group is designated α if it is the same as that in testosterone. It is recognized that the true configuration is probably the opposite of that designated, but this convention has been retained so that the nomenclature will be consistent with that employed in the earlier papers of this series. [b] See Fig. 1 and p. 89. [c] (1) C. Djerassi and C. R. Scholz, Ciba Pharmaceutical Products, Inc., Summit, N. J. (2) M. Ehrenstein, University of Pennsylvania, Philadelphia, Pa. (3) D. Fukushima, Sloan–Kettering Institute, New York, N. Y. (4) T. F. Gallagher, Sloan–Kettering Institute, New York, N. Y. (5) O. Kamm, Parke, Davis and Co., Detroit, Mich. (6) E. C. Kendall, Mayo Clinic, Rochester, Minn. (7) T. H. Kritchevsky, Sloan–Kettering Institute, New York, N. Y. (8) S. Lieberman, Sloan–Kettering Institute, New York, N. Y. (9) R. E. Marker, Pennsylvania State College, State College, Pa. (10) V. Prelog, Eidg. Tech. Hochschule, Zurich, Switzerland. (11) C. R. Scholz, Ciba Pharmaceutical Products Inc., Summit, N. J. (12) E. Schwenk, The Schering Corp., Bloomfield, N. J. (13) R. Turner, Harvard University, Cambridge, Mass. (14) A. L. Wilds, University of Wisconsin, Madison, Wis. (15) J. Wolfe, Harvard University, Cambridge, Mass. (16) Compound acetylated at the Sloan–Kettering Institute by Dr. Lieberman from alcohol supplied by Dr. Kamm.[5] (17) Compound isolated from urine at Sloan–Kettering Institute, New York, N. Y.

Non-conjugated Ethylenic Double Bonds.—Where the ethylenic group is not conjugated, the bands fall into the weakest intensity class, with the possible exception of the Δ^2-bond. In $\Delta^{8:14}$- and $\Delta^{9:11}$-compounds no C=C stretching band has been detected (see page 90). In certain instances the intensity of the band can be raised, relative to that of the background, by increasing the concentration of the solution. This is particularly effective if the compound possesses no other strong absorption bands between 1500 and

(16) Much of this experimental difficulty could no doubt be avoided by the use of an evacuated or a double-beam spectrometer.

TABLE II

CARBON–CARBON DOUBLE BOND STRETCHING VIBRATIONS IN STEROIDS CONTAINING α,β-UNSATURATED KETONES

Compound[a]	Position of C=C max. ($CHCl_3$)	Intensity[b]	Source[c]
Δ^1-Androstenol-17α-one-3	1606	++	1
Δ^1-Androstenol-17α-one-3-hexahydrobenzoate	1608	+	1
Δ^1-Androstenedione-3,17	1604	+	1
Δ^1-3-Ketoetioallocholenic acid M. E.	1609	+	1
Δ^1-Cholestenone-3	1606	++	1
Δ^4-Androstenol-17α-one-3 (testosterone)	1616	++	11, 12
Δ^4-Androstenedione-3,17	1619	++	11
Δ^4-17-Vinylandrostenol-17α-one-3	1616	++	8
Δ^4-17-Methylandrostenol-17α-one-3	1617	+++	11, 12
Δ^4-Pregnenedione-3,20 (progesterone)	1615	+++	11, 12
Δ^4-Pregnenol-21-dione-3,20	1617	+++	11, 12
Δ^4-Pregnenol-21-dione-3,20-acetate	1616	+++	11
Δ^4-Pregnenol-17α-dione-3,20	1617	+++	11
Δ^4-Cholestenone-3	1615	+++	3, 4, 8
$\Delta^{1,4}$-Androstadienol-17α-one-3	1621, 1606	+++	1, 14
$\Delta^{1,4}$-Androstadienol-17α-one-3-hexahydrobenzoate	1621, 1605	+++	1, 14
$\Delta^{1,4}$-Androstadienedione-3,17	1621, 1604	+++	1, 14
$\Delta^{1,4}$-3-Ketoetiocholadienic acid M. E.	1621, 1605	+++	1, 14
$\Delta^{1,4}$-Cholestadienone-3	1620, 1603	+++	1
$\Delta^{3,5}$-Cholestadienone-7	1627, 1598	++++	10
$\Delta^{4,6}$-Pregnadienedione-3,20	1619, 1587	++++	3
$\Delta^{4,6}$-Cholestadienone-3	1616, 1587	++++	3
$\Delta^{9:11}$-12-Keto-3α-hydroxycholenic acid M. E.	1607	++++	4, 6
Δ^{16}-Pregnenol-3α-one-20	1590	+++	5, 9
Δ^{16}-Pregnenol-3α-one-20-acetate	1590	+++	5
Δ^{16}-Pregnenedione-3,20	1590	+++	5
$\Delta^{5,16}$-Pregnadienol-3β-one-20	1588	+++	5
$\Delta^{5,16}$-Pregnadienol-3β-one-20 acetate	1592	+++	9

[a], [b], [c]. See Footnotes to Table I.

TABLE III

SUMMARY OF POSITIONS OF C=C BAND MAXIMA IN STEROIDS CONTAINING ISOLATED AND CONJUGATED ETHYLENIC DOUBLE BONDS

Structure	Position of max. cm.$^{-1}$ ($CHCl_3$)	Compounds examined
Δ^5	1672–1669	10
$\Delta^{3,5}$ Enol acetate and enol propionate	1671–1670[a]	2
Δ^7	1664	1
Δ^2	1657–1654	2
Δ^{14}	1648–1646	4
$\Delta^{3,5}$Enol acetate and enol propionate	1639[a]	2
Δ^{16}	1630–1621	3
Δ^{11}	1628–1625	4
Equilenin and equilin	1625 (broad)[a]	2
$\Delta^{3,5}$	1618[a]	1
Estrone, estradiol	1613–1611[a]	2
Equilenin,	1605[a]	1
Benzoate ester	1604–1603[a]	3
Estrone, estradiol	1590–1589[a]	2
Benzoate ester	1586–1584[a]	3
$\Delta^{3,5}$	1578[a]	1
Equilenin	1573[a]	1

[a] These structures give rise to two or more maxima all of which are listed in the table.

TABLE IV

SUMMARY OF POSITIONS OF C=C AND C=O BAND MAXIMA IN α,β-UNSATURATED KETOSTEROIDS

Structure	Position of C=C max. cm.$^{-1}$ ($CHCl_3$)	Compounds examined	Position of C=O max. cm.$^{-1}$ (CS_2)
$\Delta^{3,5}$-Dieneone-7	1627[a]	1	1663
$\Delta^{1,4}$-Dieneone-3	1621[a]	5	1666–1663
$\Delta^{4,6}$-Dieneone-3	1619–1616[a]	2	1669–1666
Δ^4-One-3	1619–1615	9	1677–1674
Δ^1-One-3	1609–1604	5	1680–1684
$\Delta^{9:11}$-One-12	1607	1	1680–1684
$\Delta^{1,4}$-Dieneone-3	1606–1603[a]	5	
$\Delta^{3,5}$-Dieneone-7	1598[a]	1	
Δ^{16}-One-20	1592–1588	5	1670–1666
$\Delta^{4,6}$-Dieneone-3	1587[a]	2	

[a] These structures give rise to two maxima both of which are included in the table. The corresponding C=O band maximum is given opposite the higher C=C frequency only.

1750 cm.$^{-1}$. If a carbonyl group is present, the ethylenic band often appears as a small shoulder on the low frequency side of the much more intense carbonyl band, and in these circumstances the resolution cannot be improved by increasing the concentration of the solution. This situation arises most commonly in Δ^5-compounds, as here the C=C stretching vibration occurs at a high frequency (1669–1672 cm.$^{-1}$), very near to the carbonyl maxima (Fig. 1).

Conjugated Dienes.—Although relatively few conjugated dienes have been available for examination, they appear to possess two C=C stretching bands both of which are much more intense than the single band associated with the non-conjugated ethylenic double bond. In the $\Delta^{3,5}$-dienes it will be noted that the introduction of an ester group in the enol esters changes the band positions considerably.

Aromatic Ring Systems.—In estradiol and estrone acetate two high intensity bands occur near 1612 and 1590 cm.$^{-1}$ (Fig. 1). In a series of synthetic estrogen derivatives containing a methyl group at position 1 the 1612 cm.$^{-1}$ band is displaced down to 1595–1600 cm.$^{-1}$. Equilenin acetate shows three bands, one broad near 1625 cm.$^{-1}$ and the others at 1605 and 1573 cm.$^{-1}$ (Fig. 1). The aromatic ring of the benzoate ester group

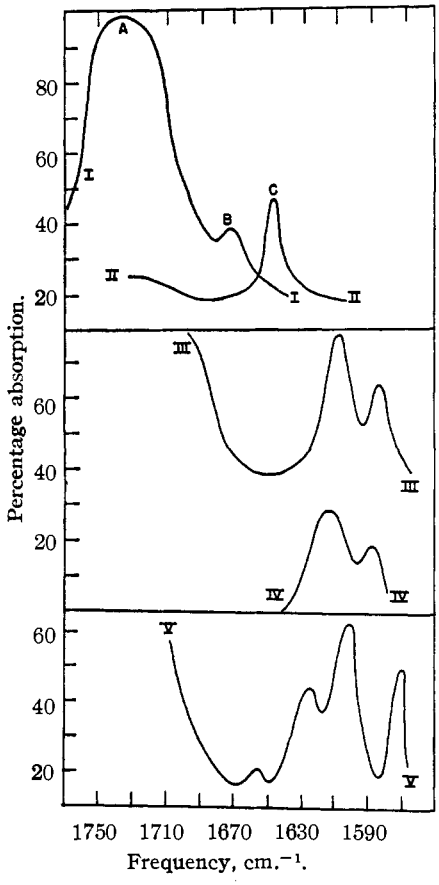


Fig. 1.—Infrared absorption spectra (solvent chloroform): I, Δ^5-androstenol-3β-one-17 (intensity class $^+$); max. A C=O stretching band of 17 ketone group; max. B, C=C stretching band of Δ^5 double bond; II, Δ^{14}-cholestenol-3β (intensity class $^{++}$), max. C, C=C stretching band of Δ^{14}-double bond; III, estrone acetate; IV, estradiol; V, equilenin acetate.

gives two bands in the same region, at 1604 and 1586 cm.$^{-1}$.

α,β-Unsaturated Ketones.—The bands at 1617 cm.$^{-1}$ in the Δ^4-3-ketones and at 1588–1592 cm.$^{-1}$ in the Δ^{16}-20-ketones have been described previously.[2,17] In the Δ^1-3-ketones the band occurs at 1604–1609 cm.$^{-1}$ which is significantly displaced from the position in the two other types of α,β-unsaturated ketones listed above. The $\Delta^{1,4}$-dieneone-3 structure exhibits two strong bands at 1603–1606 and at 1621 cm.$^{-1}$ while in $\Delta^{3,5}$-dieneone-7 compounds the bands are at 1598 and 1627 cm.$^{-1}$. In Table IV the positions of these maxima are listed together with the carbonyl band frequencies of these structures. The identification of the dieneone systems is facilitated by the fact that two bands occur in the C=C region.[18]

(17) Furchgott, Rosenkrantz and Shorr, *J. Biol. Chem.*, **163**, 375 (1946).

(18) Comparison of columns 2 and 4 of Table IV shows that as the frequency of the C=C stretching vibration increases, the C=O vibration moves in the opposite direction. The Δ^{16}-20-ketone is an exception.

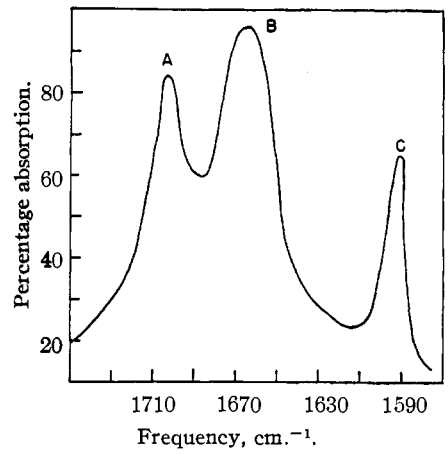


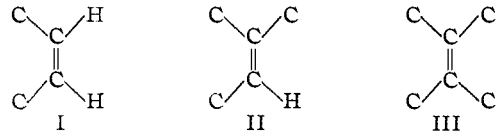
Fig. 2.—Infrared absorption spectra (solvent chloroform) of Δ^{16}-pregnenol-3α-one-20-acetate (intensity class $^{+++}$): max. A, C=O stretching band of acetate carbonyl group; max. B, C=O stretching band of 20-ketone group; max. C, C=C stretching band of Δ^{16}-double bond.

The Carbon–Hydrogen Stretching Vibrations (3000–3100 Cm.$^{-1}$).—Some preliminary observations on the absorption of unsaturated steroids between 3000 and 3100 cm.$^{-1}$ have been reported previously.[2] The presence of an ethylenic double bond in the molecule is usually indicated by the appearance of a small inflection on the high frequency side of the strong group of aliphatic C–H stretching bands which occur between 2800 and 3000 cm.$^{-1}$.

A comparative study of the absorption spectra of steroids between 2800 and 3600 cm.$^{-1}$ is being carried out, and will form the subject of a separate report. It may be observed here, however, that using the high dispersion provided by a lithium fluoride prism the spectrum between 2830 and 2970 cm.$^{-1}$ may often be resolved into six or more bands, which differ greatly in relative intensity in different types of steroids. The first absorption maximum in saturated steroids occurs at 2970 cm.$^{-1}$ and the ethylenic bond maximum in unsaturated steroids is usually at 3030–3040 cm.$^{-1}$ both in non-conjugated ethylenic compounds and in α,β-unsaturated ketones. In the Δ^4-3-ketones the band is usually resolved, but in Δ^5-steroids it may appear as an inflection only. In Δ^2-androstenone-17 it is quite sharp and intense. A weak band also occurs at the same position in estrone acetate and estradiol diacetate. A weak band at 3085 cm.$^{-1}$ in 17-vinyltestosterone can be attributed to the terminal —CH=CH_2 group.[10] In 17-ethynyltestosterone, 17-ethynylestradiol,[19] Δ^5-17-ethynylandrostenediol-3β,17-acetate-3[19] and 17-ethynylestradiol-acetate-3[19] an intense and very sharp band occurs at 3310 cm.$^{-1}$ which can be identified with the carbon–hydrogen stretching vibration of the C≡C—H group.

(19) We wish to thank Drs. Lozinski and Jamieson of Charles E. Frosst and Co., Montreal, for the gift of these compounds.

The Carbon–Hydrogen Bending Region (800–970 cm.$^{-1}$).—In the Δ^{1}-, Δ^{2}-, Δ^{3}-, Δ^{6}-, Δ^{11}- and Δ^{15}-steroids the double bond carries two hydrogen atoms (I). In the Δ^{4}-, Δ^{5}-, Δ^{7}-, $\Delta^{9:11}$-, Δ^{14}-compounds the double bond carries one hydrogen atom only (II), and in Δ^{8}- and $\Delta^{8:14}$-steroids the ethylenic bond is fully substituted (III).



I II III

It has been demonstrated in simpler compounds[12,14,20] that the absorption band associated with the bending of the C–H bond occurs at different frequencies in I (965 cm.$^{-1}$) and II (890 cm.$^{-1}$).

In the Δ^{1}-, Δ^{2}- and Δ^{11}-steroids a band at 955–965 cm.$^{-1}$ is commonly found, and might be attributed to this vibration. However strong bands are also observed in this region of the spectrum in steroids not containing the partial structure I (*i. e.*, Δ^{14}-compounds). The observation is therefore not of particular value in the elucidation of steroid structure; the region characteristic of the partial structure II coincides with an absorption band in carbon disulfide and the compounds have not yet been investigated in other solvents which might be transparent in this region.[21]

Experimental

The spectra were determined in chloroform solution in the region of the C=C stretching vibrations and in carbon tetrachloride in the 3000–3100 cm.$^{-1}$ region. The concentrations employed were not accurately determined. In the C=C stretching region they were of the order of 5–25 mg. per ml. Using a microcell of 1 mm. thickness and 20 cu. mm. capacity a total weight of between 100 and 500 microgram of material is required.

Perkin–Elmer models 12a and 12b spectrometers were used with sodium chloride and lithium fluoride prisms. Full details of the experimental techniques have been given in earlier publications.[2,4]

Comments

In the majority of instances infrared spectrometry provides a method for the detection of ethylenic linkages and their location in the steroid molecule, but in some cases the presence of a double bond has eluded detection. This is mainly a consequence of the low absolute or relative intensity of the absorption associated with the ethylenic bond both in the 3000–3100 and the 1580–1680 cm.$^{-1}$ regions.

Ethylenic unsaturation is most readily recognized by observation of the weak band at 3030 cm.$^{-1}$ in carbon tetrachloride solution, using the high dispersion provided by the lithium fluoride or calcium fluoride prism. This band is generally found in unsaturated steroids with the exception of those containing no C=C—H group (III).[2,5] In the vinyl group the maximum is displaced to 3080 cm.$^{-1}$ and in the ethynyl group it is at 3310 cm.$^{-1}$ and greatly enhanced in intensity.[22]

The location of the double bond in the molecule can generally be determined from a study of the position of the absorption maxima of the C=C stretching vibrations (Tables I–IV). This presents no difficulties in the case of the conjugated dienes, α,β-unsaturated ketones and benzenoid compounds, all of which give bands of moderate-to-high intensity. In the non-conjugated unsaturated steroids the C=C stretching bands are weak and may be occasionally obscured by overlap of carbonyl absorption, or its location rendered uncertain by difficulties in correcting with sufficient accuracy for water vapor absorption.

In the investigation of urinary steroid hormone metabolites, particular importance is attached to the identification of compounds containing the $\Delta^{9:11}$-double bond. These arise from the dehydration of the corresponding 11-hydroxy derivatives excreted in the urine.[6,7] In the case of two compounds isolated from urine ($\Delta^{9:11}$-androstenol-3α-one-17 and $\Delta^{9:11}$-etiocholenol-3α-one-17) as well as the three synthetic compounds included in Table I, an unsuccessful search was made for the band, and it must be concluded that it is either particularly weak or occurs at a sufficiently high frequency as to be totally obscured by carbonyl overlap.

Acknowledgments.—The authors wish to express their indebtedness to the several investigators, listed individually in a footnote to Table I, whose collaboration in supplying many of the compounds made these studies possible. The technical assistance of Mr. R. Lauzon is gratefully acknowledged.

This investigation was aided by grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council), Ayerst, McKenna and Harrison, Ltd., the Jane Coffin Childs Memorial Fund for Medical Research, the Commonwealth Fund, the Anna Fuller Fund, the Lillia Babbitt Hyde Foundation, the Albert and Mary Lasker Foundation, and the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

Summary

The infrared absorption spectra of unsaturated

(20) Rasmussen and Brittain, *J. Chem. Phys.*, **15**, 120, 131, 135 (1947).

(21) There are some indications of other structural correlations in the 900–950 cm.$^{-1}$ region. In the Δ^{1}-compounds a moderately strong band occurs at 933–934 cm.$^{-1}$. In the Δ^{5}-3-hydroxy steroids a band is frequently seen at 902–903 cm.$^{-1}$ and in 11-ketosteroids at 935–942 cm.$^{-1}$. In all of these cases however occasional exceptions have been encountered.

(22) If the concentrations of the solutions are very high, it is possible that overtones of absorption bands in the 1500–1600 cm.$^{-1}$ region may be observed between 3000 and 3200 cm.$^{-1}$. Intense bands in the 1500–1600 cm.$^{-1}$ region are not common but this possibility must be kept in mind.

steroids have been examined in the regions where absorption characteristic of the carbon–carbon double bond is known to occur. The presence of a double bond containing at least one directly linked hydrogen atom can usually be detected from measurements in the neighborhood of 3030 cm.$^{-1}$.

A comparative study of steroids containing ethylenic linkages at different positions has shown that the frequency of the maximum in the region of the C=C stretching vibration (1580–1680 cm.$^{-1}$) is specific for a given location of the bond in the steroid molecule. In conjugated dienes, α,β-unsaturated ketones and the estrogens these bands are intense. In steroids containing nonconjugated ethylenic double bonds the bands, although weak, can generally be observed.

Absorption associated with the carbon–hydrogen bending vibrations in the neighborhood of 800–970 cm.$^{-1}$ is variable in the steroids and so far no unequivocal correlations with structure have been established in this part of the spectrum.

These observations have proved of value in the elucidation of the structure of new steroids isolated from urine.

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Reactions with Heavy Metals and their Bearing on Poisoning and Antidote Action[1]

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The successful use of 2,3-dimercaptopropanol (BAL) as an antidote for poisoning by certain heavy metals climaxes years of investigation in numerous laboratories concerning the mechanism of poisoning by heavy metals. It has been shown that the specific action of the metal is to form derivatives similar to the mercaptides with critical sulfhydryl groups in such a manner as to remove the necessary sulfhydryls from the biochemical systems in which they are functioning. A brief review of the literature on this subject may be found in a publication by Peters, Stocken and Thompson.[4] Our interest in the field stems from investigations which have been under way for a number of years concerning the toxic action of mercurials and other divalent metal derivatives.

The present paper reports our preliminary observations on the chemistry of the displacement reactions which are of interest in connection with heavy metal poisoning. Data on the pharmacological findings will be published elsewhere.

General Principles.—It has been shown that sulfhydryl compounds are essential for the growth of many, and probably all, cells.[5] Recently a large group of enzymes has been shown to contain –SH groups essential for enzymatic activity. This activity is inhibited by mercury and arsenic compounds[6] and is restored by glutathione.[7] The poisoning thus results from metallic combination with essential sulfhydryl groups. *Detoxicant* action is a preliminary combination of a substance with the metallic poison in a compound so firm that the metal is not available for reaction with essential sulfhydryls. *Antidote* action is observed after combination has taken place with the essential sulfhydryls but when the affinity of the antidote for the metal is greater than that of the essential sulfhydryls and displacement takes place. This latter reaction can obviously be favored by the mass action effect of large amounts of antidote but will be most favorable if the affinity of the antidote for the metal is high. For this reason a search for sulfur compounds with high affinity for metals is in order. Peters[4] and his collaborators succeeded in this quest by the use of two sulfhydryl groups in the same compound. An alternative approach is to use a single sulfur atom with substituent groups chosen to give a great increase in chemical affinity for metal. This high chemical affinity may or may not be added to the chelation effect to secure the desired end. The general method of attack is outlined below.

The Poisoning Reaction.—In the equations given below, we have used mercury as the metal but it is our belief that such equations also apply to other heavy metals such as cadmium, lead, and even silver, with appropriate modifications.

Toxic mercurials may be divided into five types: (I) HgX_2, (II) R'HgX, (III) R'_2Hg, (IV) R'-HgSR', and (V) $(R'S)_2Hg$. It is to be expected from the literature that compounds of types I and II would react readily with sulfhydryl compounds. Poisoning by Type III compounds, if it were due to the mechanism suggested, would

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(1) Studies on Heavy Metal Poisoning, paper I. A portion of this paper is from the doctoral dissertation of Iqbal Singh Bhatia, The Johns Hopkins University, 1949. Another portion of this paper was presented before the Div. of Med. Chem. of the Am. Chem. Soc. at the New York Meeting, Sept., 1947.

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(4) Peters, Stocken and Thompson, *Nature*, **156**, 616 (1945).

(5) Volonsky, *Compt. rend. Soc. Biol. Paris*, **109**, 528 (1932), *Compt. rend. Acad. Sci. Paris*, **197**, 712 (1933); *Ann. inst. Pasteur*, **52**, 76 (1934); see also Fildes and Richardson, *Brit. J. Expll. Path.*, **18**, 292 (1937).

(6) Barron and Singer, *Science*, **97**, 356 (1943); *J. Biol. Chem.*, **157**, 221 (1945).

(7) Hellerman, Chinard and Dietz, *J. Biol. Chem.*, **147**, 443 (1943).