[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, WESTERN RESERVE UNIVERSITY, AND THE LAKESIDE HOSPITAL

The Characterization of Δ^5-Unsaturated Steroids by Infrared Spectroscopy[1]

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Examination of 55 olefinic and of 13 non-ethenoid steroids in the 12μ region of infrared radiation disclosed that Δ^5-3β-acetoxy compounds with the usual spatial arrangement of the steroid nucleus and without other functional groups in the vicinity of the double bond exhibit two bands near 800 and 812 cm.$^{-1}$. Changes in the configuration or nature of the substituent at C-3 cause shifts in the position of the higher frequency band. At least one of these peaks (800 cm.$^{-1}$) is ascribed to the out-of-plane bending vibrations of the hydrogen at C-6. The twin bands of the Δ^5-3β-acetoxysteroids were not seen in their reduction products or in the acetates of other olefins. Observations on Δ^7-, Δ^{9-11}- and Δ^{14}-unsaturated steroids, while still rather limited in number, suggest that these trisubstituted olefins too can be characterized by measurements in the 800-cm.$^{-1}$ region. Introduction of a substituent or double bond at a carbon atom adjacent to the trialkylated olefin can profoundly alter the spectrum in this range and reduce absorption intensity well below that of the fairly strong peaks that were seen in this region with some dialkylated olefins or with certain non-ethenoid steroids. Spectral changes were smaller if the substituted carbon atom was farther removed from the double bond but slight shifts could still be detected if the two sites were separated by 4 carbon atoms.

During earlier investigations[2] we had occasion to measure the infrared transmission spectra of several 5–6 unsaturated steroids. It was observed that these compounds showed two fairly intense bands near 800 and 812 cm.$^{-1}$ which disappeared upon reduction. Since the out-of-plane bending vibrations of a hydrogen atom attached to a trisubstituted olefin quite generally give rise to a strong absorption band in the region between 800 and 840 cm.$^{-1}$ [3] it seemed probable that at least one of these bands was brought forth by the presence of the 5–6 double bond. To test the diagnostic possibilities of this region these observations have now been extended. The results are presented in the accompanying tables in which all peaks observed in the region between 790 and 840 cm.$^{-1}$ have been recorded regardless of their significance for the characterization of unsaturated steroids.

Δ^5-Steroids.—It is apparent (Table I) that Δ^5-3β-acetoxy steroids with the normal configuration of the nucleus and without other substituents in the vicinity of the double bond show two peaks near 800 and 812 cm.$^{-1}$. The maximum at 800 cm.$^{-1}$ is generally more intense but as indicated in Fig. 1 the relative heights of these peaks can vary considerably. If observations are confined to measurements on solutions (which quite generally give more consistent results than solids) nine 3β-acetoxysteroids, variously substituted at C-17 and in the side chain, absorbed at either 800 or 801 and at 812 or 813 cm.$^{-1}$. The most remote substituents which caused slight shifts of the absorption frequencies were found at C-16. Four 16α-oxygenated substances (compd. 3, 9–11) absorbed at 803 while diosgenin acetate (compd. 13) in which the functional group at C-16 is β-oriented[4] has the lower frequency peak at 797 cm.$^{-1}$. Compounds 15 and 16 which are substituted adjacent to the double bond lack the peak near 800 cm.$^{-1}$. Frequency shifts

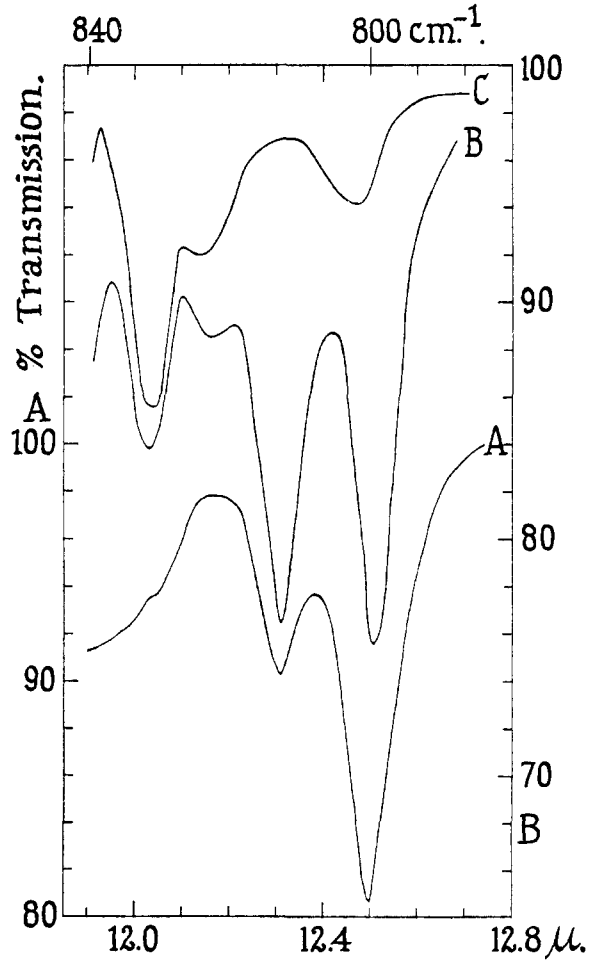

Fig. 1.—Infrared transmission spectra in CS_2: curve A, 3β,20α-diacetoxy-17α-hydroxy-Δ^5-pregnene; curve B, 3β-acetoxy-Δ^5-androstenone-17; curve C, 3β-acetoxyandrostanone-17. Curve A is drawn with respect to the ordinate at the left; the ordinate for curve C is displaced by +2 from that of curve B which is given on the right.

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(2) (a) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **187**, 137 (1950); (b) H. Hirschmann, F. B. Hirschmann and M. A. Daus, THIS JOURNAL, **74**, 539 (1952); and unpublished data from this Laboratory.

(3) D. Barnard, L. Bateman, A. J. Harding, H. P. Koch, N. Sheppard and G. B. B. M. Sutherland, *J. Chem. Soc.*, 915 (1950); *ibid.*, references to the earlier literature.

(4) H. Hirschmann, F. B. Hirschmann and M. A. Daus, *J. Biol. Chem.*, **178**, 751 (1949).

TABLE I

ABSORPTION MAXIMA OF Δ^5-COMPOUNDS

No.	Subst. at C-3	Compound[a]	Maxima in CS_2,[b] cm.$^{-1}$				
		Steroids					
1	β-OAc	3β-Acetoxy-Δ^5-androstenone-17	*800*	813	(822)	831	
		3β-Acetoxy-Δ^5-androstenone-17	N 798	812	(820)	834	846
2	β-OAc	3β,17β-Diacetoxy-Δ^5-androstene	800	812		829	
3	β-OAc	3β,16α,17β-Triacetoxy-Δ^5-androstene	803	813			835
4	β-OAc	3β,20α-Diacetoxy-Δ^5-pregnene	801	812		829 w	
5	β-OAc	3β,20α-Diacetoxy-17α-hydroxy-Δ^5-pregnene	*800*	812		(830) i	
6	β-OAc	3β,20β-Diacetoxy-17α-hydroxy-Δ^5-pregnene	800	813		830	
7	β-OAc	3β-Acetoxy-17α-hydroxy-Δ^5-pregnenone-20	S 801	814		~(829)	837
8	β-OAc	3β,21-Diacetoxy-$\Delta^{5,17}$-pregnadiene[c]	800	812		831 i	~838
9	β-OAc	3β,16α,20α-Triacetoxy-Δ^5-pregnene	803	814			835 w
10	β-OAc	3β,16α,20β-Triacetoxy-Δ^5-pregnene	803	814			835 w
11	β-OAc	3β-Acetoxy-16α-benzyloxy-Δ^5-pregnenone-20	803	814			836
12	β-OAc	Methyl 3β-acetoxy-Δ^5-cholenate	801	812 w		829 w	~841 w
		Methyl 3β-acetoxy-Δ^5-cholenate	N 799	813		825	840
13	β-OAc	3β-Acetoxy-22-iso-Δ^5-spirostene	797	814		(829) i	839
14	β-OAc	3β-Acetoxy-Δ^5-cholestene	801	812 w			~840 w
15	β-OAc	3β,7α-Diacetoxy-Δ^5-cholestene		812			
16	β-OAc	3β,7β-Diacetoxy-Δ^5-cholestene		815 w		832 w	
17	β-OAc	3β-Acetoxy-$\Delta^{5,22}$-stigmastadiene	801	812		829 w	~840 w
18	....	Δ^5-Cholestene[d]	799	809 w		833	
19	β-OH	3β-Hydroxy-Δ^5-androstenone-17	*799*	*807*	(823)	831	
		3β-Hydroxy-Δ^5-androstenone-17	N 800	805 i		835	844
20	β-OH	3β,16α,17β-Trihydroxy-Δ^5-androstene	N 798	808			835
21	β-OH	3β-Hydroxy-20α-acetoxy-Δ^5-pregnene	*800*	806 i	(825) i		840
22	β-OH	Methyl 3β-hydroxy-Δ^5-cholenate	800	(806) i	827 i		839
23	β-OH	3β-Hydroxy-Δ^5-cholestene	801				840
24	β-OMe	3β-Methoxy-16α,17β-dihydroxy-Δ^5-androstene	N 800	808			835
25	β-OMe	3β-Methoxy-16β,17β-dihydroxy-Δ^5-androstene 16,17-acetonide[e]	801	809		829	839
26	β-OMe	3β-Methoxy-Δ^5-cholestene	800	806 i			~837 w
27	β-OBz	3β-Benzoxy-Δ^5-cholestene	801	(809) i			840
28	β-Cl	3β-Chloro-Δ^5-androstenone-17	*800*		*821*		
29	β-Cl	3β-Chloro-20α-acetoxy-Δ^5-pregnene	800		821		
30	β-Cl	3β-Chloro-Δ^5-cholestene	800		*821*		
31	α-OAc	3α-Acetoxy-Δ^5-cholestene	*797*		822		
32	α-OH	3α-Hydroxy-Δ^5-cholestene	*798*			830	
		Modified steroids					
33	β-OAc	17a-Methyl-3β-acetoxy-17aβ-hydroxy-Δ^5-D-homo-androstenone-17	S 800	816			836
34	β-OAc	Dimethyl 3β-acetoxy-Δ^5-etiobilienate	805	817 w		829	
35	β-OAc	Diacetyldihydrojervine	793	*816*		831	

[a] Details concerning the use of the terms α and β have been given previously.[2a,21] The naming of sapogenins as derivatives of spirostane follows suggestions of G. Rosenkranz and C. Djerassi (*Nature*, **166**, 104 (1950)). [b] Data marked as N were obtained on mulls with Nujol; those as S on solids without an embedding medium. Details concerning the use of symbols denoting band intensity are given in the Experimental section. [c] It is possible that the double bond at C-17 which has the *trans* configuration (see footnote *a* to Table II and B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, **26**, 1328 (1943)) makes a contribution to the absorption at 812 cm.$^{-1}$ since the intensity of the band is greater than in compound 4. [d] Data by Bladon, *et al.*,[6] do not list a peak at 809 cm.$^{-1}$. [e] In addition shoulder extending to 792 cm.$^{-1}$.

appear to be associated also with changes in the shape of the steroid nucleus. Departures from the normal pattern were seen if the strain at the juncture of rings C and D is released by ring enlargement (compd. 33) or by ring cleavage (compd. 34) and were larger in the case of diacetyldihydrojervine (compd. 35) for which a 5-membered ring C has been postulated.[5]

Changes in the substituent at C-3 have little effect on the peak near 800 cm.$^{-1}$ but displace the higher frequency band. In 3β-hydroxy and 3β-methoxy compounds this peak appeared near 807 cm.$^{-1}$. It possessed low intensity in several compounds and was absent in one (compd. 23). The shifts were larger in the spectra of the 3α-oxygenated and 3β-chlorinated compounds. The frequencies of the latter (3 examples) were again found to be reproducible (800 and 821 cm.$^{-1}$).

Other Unsaturated Steroids.—The twin bands of the Δ^5-3β-acetoxy compounds have not been observed with the acetates of other unsaturated steroids. A rather close approach to this pattern may be expected for the acetate of compound 48, *cis*-Δ^{17}-pregnenol-3α-one-11, but if this should be the

(5) J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, THIS JOURNAL, **73**, 2970 (1951).

TABLE II

ABSORPTION MAXIMA OF OTHER TRISUBSTITUTED OLEFINS

No.	Δ at	Compound[a]	Maxima in CS_2,[b] cm.$^{-1}$			
36	4–5	3β-Acetoxy-Δ^4-cholestene		810		
37	4–5	3β-Hydroxy-Δ^4-cholestene		805 w		
38	4–5	20α-Acetoxy-Δ^4-pregnenone-3			826	
39	4–5	$\Delta^{2,4}$-Cholestadiene				
40	7–8	3β-Acetoxy-Δ^7-cholestene[c]	800		830	
41	7–8	3β-Acetoxy-Δ^7-ergostene	800		829	~846
42	7–8	3β-Hydroxy-Δ^7-ergostene	796		829	847
43	9–11	3α-Acetoxy-Δ^{9-11}-androstenone-17		(807)	*822*	
44	9–11	Methyl 3α-acetoxy-Δ^{9-11}-etiocholenate			828	
45	9–11	3β-Acetoxy-Δ^{9-11}-22-isoallospirostene[d]	797 w		*821*	
46	14–15	3β-Acetoxy-Δ^{14}-androstenone-17	*801*	808	*825*	
47	14–15	Ethyl 3β,19-diacetoxy-5-hydroxy-Δ^{14}-etiocholenate	800	810	*822*	
48	17–20 *cis*	3α-Hydroxy-Δ^{17}-pregnenone-11	795	813		
49	17–20 *trans*	3α-Hydroxy-Δ^{17}-pregnene		*815*	828	

[a] See footnote *a* to Table I. The configurations of the 17-*cis*- and *trans*-ethylenes are based on those of their main reaction products with osmium tetroxide and are designated in terms of the nomenclature proposed by L. F. Fieser and M. Fieser (*Experientia*, **4**, 285 (1948)). Arguments which confirm their assignments have been presented previously (H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **184**, 270 (1950); W. Klyne, *Chemistry and Industry*, 426 (1951)). The configurations at C-17 and C-20 of the triolone derived from compound 48 in terms of this nomenclature have been established by L. H. Sarett (THIS JOURNAL, **70**, 1690 (1948); **71**, 1169, 1175 (1949)); those of the main hydroxylation product of compound 49 can be deduced from the rotation of its 3,20-diacetate[20a] as being the same as in Reichstein's compound J (17α,20β). [b] See footnote *b* to Table I. [c] No measurements were made above 844 cm.$^{-1}$ (end absorption). [d] Showed also very weak peak near 791 cm.$^{-1}$.

case, hydrolysis to the free steroid should disclose spectrographic dissimilarities between *cis*-Δ^{17}- and Δ^5-compounds.

The results on Δ^4-unsaturated steroids (compd. 36–39) should be compared with those reported[6] for Δ^4-cholestene which shows a strong peak at 810 cm.$^{-1}$. These spectra like those of compounds 15 and 16 demonstrate that the absorption of this region can be greatly affected by structural changes at the carbon adjacent to the double bond. Particularly, the band of Δ^4-cholestenol-3β was weak and $\Delta^{2,4}$-cholestadiene showed, at the concentration studied, no detectable absorption maximum between that at 783 and the end of the tracing at 845 cm.$^{-1}$.[7]

The observations on other trisubstituted olefins are augmented by the findings of Bladon and coworkers[6] who reported two additional examples of Δ^7-unsaturation and one each for the 9–11 and 14–15 double bond. While the spectra studied are not yet numerous enough to permit definite conclusions they indicate that these olefins too possess characteristic absorption frequencies in the 800-cm.$^{-1}$ region. The acetates of Δ^7-unsaturated steroids showed two equally strong peaks near 800 and 829 cm.$^{-1}$. (The one near 846 cm.$^{-1}$ may also be of diagnostic value.) The Δ^{14}-compounds possessed three well defined absorption maxima near 800, 809 and 824 cm.$^{-1}$ while the Δ^{9-11}-steroids displayed in the 800–840 cm.$^{-1}$ range a single major peak between 828–821 cm.$^{-1}$. The last remaining trialkylated olefinic center of the steroid nucleus, the 16–17 double bond has not yet been investigated.

Non-olefinic Compounds.—Comparison of the spectra of the 3β-acetoxy- and 3β-hydroxy-Δ^5-compounds with their reduction products (compds. 56–61, 63, 65, 66) discloses marked differences in the 800–815 cm.$^{-1}$ region. Although bands were seen in several saturated compounds near 800 cm.$^{-1}$, their intensity was always considerably lower than in the olefins. (Curves B and C in Fig. 1 may serve as an illustration.) Only tigogenin acetate (compd. 66) showed a rather intense *single* peak in this region but even this maximum was only about half as high as the corresponding one in the Δ^5-unsaturated sapogenin, diosgenin acetate (compd. 13). It seems justified therefore to conclude that the two bands observed with these Δ^5-unsaturated steroids are brought forth by the presence of the double bond.

Comments.—The greater intensity of absorption bands of trialkylated olefins between 800–840 cm.$^{-1}$ has served well for their distinction from other unsaturated or saturated hydrocarbons. Among steroids sufficient overlap of band intensities has been observed to render such a differentiation more difficult. Lack of a strong peak in this region does not exclude the presence of a trialkylated ethylene group if it is conjugated to another double bond or is part of an allylic system. Conversely the presence of a strong peak does not necessarily prove the presence of such unsaturation. While the bands of most compounds of Table III were quite weak a few substances had maxima (compd. 52 (834 cm.$^{-1}$), 67 and 68) of the same magnitude as those encountered with trisubstituted olefins. It is clear that the possibilities for misinterpretation will be reduced if the various trialkylated olefinic

(6) P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *J. Chem. Soc.*, 2402 (1951). This paper appeared after our experimental work had been completed.

(7) Additional examples for this vicinal effect may be found in the absorption curves of the α-substituted or α-unsaturated Δ^7- and Δ^{9-11}-steroids described by H. Heusser, K. Eichenberger, P. Kurath, H. R. Daellenbach and O. Jeger (*Helv. Chim. Acta*, **34**, 2106 (1951)). These spectra bear little resemblance to those of Δ^7- and Δ^{9-11}-steroids without functional groups at the α-carbons (*vide infra.*). Several of the curves (notably VIII and IX) show rather weak maxima in the 800 cm.$^{-1}$ region. However, not all conjugated dienes with a trialkylated double bond are weak absorbers in this region as ergosterol gave two strong peaks at 801 and 836 cm.$^{-1}$ in carbon disulfide (see aso J. H. Turnbull, D. H. Whiffen and W. Wilson, *Chemistry and Industry*, 626 (1950).

TABLE III

ABSORPTION MAXIMA OF DI- AND TETRASUBSTITUTED OLEFINS AND OF NON-ETHENOID STEROIDS

No.	Δ at	Compound[a]	Maxima in CS_2,[b] cm.$^{-1}$				
50	2–3?	16α,20β-Diacetoxy-Δ²?-allopregnene					
51	6–7	3β-Acetoxy-Δ⁶-cholestene	~(801)			828	
52	11–12	Methyl 3α-acetoxy-Δ¹¹-cholenate	798	(809)			834
53	11–12	3α-Hydroxy-Δ¹¹-cholenic acid	N 794				835
54	8–14	3β-Acetoxy-Δ⁸⁻¹⁴-cholestene	804 w				
55	8–14	3β-Acetoxy-Δ⁸⁻¹⁴-ergostene	~(804)				
56	...	3β-Acetoxyandrostanone-17	802 w		(824)	830	
57	...	3β-Hydroxyandrostanone-17	796 w		823 w	831	~841
		3β-Hydroxyandrostanone-17	N 800 w	~(809)	~819 w	837	842
58	...	3β,16α,17β-Triacetoxyandrostane					836
59	...	3β,16α,17β-Trihydroxyandrostane	N				837
60	...	3β,16α,20α-Triacetoxyallopregnane					
61	...	3β,16α,20β-Triacetoxyallopregnane					
62	...	20α-Acetoxyallopregnanone-3		(809)	(823)		
63	...	Methyl 3β-acetoxyallocholanate	~(801)			~(828)	
64	...	3α-Hydroxycholanic acid	N			835 w	~843 w
65	...	3β-Acetoxycholestane	~(801)				
66	...	3β-Acetoxy-22-isoallospirostane	797			(830) i	~842 w
67	...	3β-Chloroandrostanone-17	*805*		823	831	
68	...	3β-Chlorocholestane	804				

[a,b] See footnotes *a* and *b* to Table I.

centers of the steroid molecule can be characterized by absorption frequencies within rather narrowly defined ranges. Such a characterization appears to be possible for the 5–6 double bond of 3β-acetoxy steroids with the usual configuration of the nucleus and without other substituents or unsaturation in the vicinity of the double bond. The two bands near 800 and 812 cm.$^{-1}$ have been consistently observed with such compounds but not with the acetates of other steroids. While there can be no certainty, of course, that this spectrographic pattern may not be repeated in some other steroid, it is believed that measurements in this region can supplement other indirect methods of structure analysis such as the examination of other spectral ranges,[6,8] the conversion to an α,β-unsaturated ketone with its characteristic spectrum, or the determination of the change in molecular rotation upon reduction. Only the combination of several tests based on analogy rather than a single one taken by itself can give that degree of certainty which is obtained by correlating an unknown steroid through unequivocal reactions with a compound of known structure.

Incidental Observations.—Jones and co-workers[9] recently demonstrated that 3-acetates show a single maximum near 1239 cm.$^{-1}$ if the ester group is *trans* to the hydrogen at C-5, but two or three peaks in this region if a *cis* relationship prevails. It appears that unsaturated steroids such as the acetates of cholesterol, ergosterol or lumisterol which lack the hydrogen at C-5 fit into this general pattern if they are classified according to the polar or equatorial orientation of the ester group. In general, multiple peaks are observed if the 3-acetoxy group is polar in a molecule in which ring A occupies the more stable chair form. The availability of compounds 31 and 36 has permitted the testing of two additional structural types. In the chair conformation the ester group in 3α-acetoxy-Δ⁵-cholestene is polar and its triple peak at 1255, 1243 and 1229 cm.$^{-1}$ is therefore in agreement with expectation. 3β-Acetoxy-Δ⁴-cholestene which cannot be classified unambiguously in these terms showed a single peak at 1243 cm.$^{-1}$. As the multiple peaks of 3α-acetoxy-Δ⁵-cholestene also distinguish it from its 3-epimer, observations in this range can be expected to aid in recognizing the α-configuration in 3-acetates of Δ⁵-stenols and therefore facilitate the interpretation of the 800 cm.$^{-1}$ region.

(8) R. N. Jones, P. Humphries, E. Packard and K. Dobriner, THIS JOURNAL, **72**, 86, 5801 (1950); R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, *ibid.*, **70**, 2024 (1948); P. Bladon, H. B. Henbest and G. W. Wood, *Chemistry and Industry*, 866 (1951).

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

Experimental

Procedures.—The infrared spectra were determined with a single beam Perkin-Elmer spectrometer with sodium chloride prism. The d.c. signals from the thermocouple were fed through a General Motors amplifier to a Brown Electronik recorder. The globar was operated at 250 w. on a separate stabilized power supply. A test signal of 1 μv. gave full scale deflection. The wave length calibration was made with atmospheric water, with ammonia, and with carbon dioxide, and is believed to be accurate within about 0.01 μ. Compounds were examined from 15 to 2.5 μ as far as the transparency of the solvent allowed. Since carbon disulfide has a strong peak at 855 cm.$^{-1}$ [10] measurements on solutions in the upper part of the 800-cm.$^{-1}$ range (above ~ 833 cm.$^{-1}$) were less reliable. (Weak peaks were apt to be overlooked and even stronger ones could be located only approximately. While the situation could have been ameliorated by the use of more concentrated solutions this practice would have eliminated many scarce or sparingly soluble compounds from this study.) At the beginning and end of each tracing a portion of the water vapor spectrum was recorded to permit correction of the drum readings. Within the fluctuations encountered this procedure was found to give adequate corrections in the 12 μ region. In this spectral range a lithium fluoride shutter was employed. The cell for carbon disulfide solutions was of the type described by Colthup[11] and was cemented with glyptal. The cell had a length of 0.8 mm. and a capacity of 0.2 ml.

(10) American Petroleum Institute Project 44, Catalog of Infrared Spectral Data, Serial No. 698, National Bureau of Standards, Washington, D. C.

(11) N. B. Colthup, *Rev. Sci. Instruments*, **18**, 64 (1947).

Compounds were examined in 1% solutions except compounds 48 and 57 which were insufficiently soluble in carbon disulfide. Correction for this was made in judging absorption intensity. Since inconspicuous bands may be located in a region of general absorption or near strong bands they frequently show rather low transmission values. We have, therefore, classified peaks not by comparing actual transmission percentages but by roughly estimating their contribution to the absorption at the maximum. The intensity scale was chosen in relation to the peaks encountered in this region rather than in the complete spectrum. Very weak peaks or inflections are given in the tables in brackets, weak peaks are denoted by w., inflections by i.; very strong peaks are in italics. Solvent blanks were determined at frequent intervals and used as an aid to locating precisely the position of maximal absorption. Absorption intensity data obtained from measurements on solids are difficult to compare with those on solutes since the effective concentration of the solid in the light path can be estimated only very roughly. For this reason only weak or very weak peaks are so described as this information also indicates the accuracy with which the center of the absorption band can be located. Two compounds were examined without embedding medium (7 and 33) as they had to be recovered unchanged. These curves were less satisfactory than those obtained on mulls.

Materials.[12]—Compounds donated by other investigators (see Acknowledgment) were examined as received. The purity of compound 40 (m.p. 110–116°) was stated to conform to that described by Wintersteiner and Moore.[13] Compound 37 which had been in our possession for many years had deteriorated on storage and was repurified. All compounds prepared by members of this Laboratory and those from the Schoenheimer collection were repurified unless their melting points agreed or were very close to those previously recorded. Pertinent data can be found in the following references: compounds 5 and 6,[2a] 9–11, 50 and 61,[2b] 66,[4] 2* and 4,[14] 3, 20, 34*, 58 and 59,[15] 7, 28* and 33,[16] 21, 29, 38 and 62,[17] 22, 64,[18] 39,[19] 49,[20a] 56*[20b] and 60*.[21] Compounds 1 and 23 were purified commercial samples and were used in the preparation of compounds 19 and 57, and of 14 and 27, respectively. Compounds 12, 13, 17, 31, 41, 63 and 65 were prepared by treatment of their parent substances with pyridine and acetic anhydride at room temperature. (In the case of compound 41 there was insufficient material for complete purification, m.p. 152–159°.) Compounds 67 and 68 were prepared from compounds 28 and 30, respectively, by hydrogenation of an alcoholic solution with a palladium-calcium carbonate catalyst.[22]

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(12) A few substances have been examined under various conditions in the 800 cm.$^{-1}$ range by other investigators. These include compounds 18, 23, 42 (Bladon, *et al.*[6]), 19, 28, 57 (R. F. Furchgott, H. Rosenkrantz and E. Shorr, *J. Biol. Chem.*, **163**, 375 (1946)); 17 (Turnbull, *et al.*[7]); 14 (T. D. Fontaine, J. S. Ard and R. M. Ma, THIS JOURNAL, **73**, 878 (1951)); and 26 (S. Bernstein and K. J. Sax, *ibid.*, **73**, 846 (1951)).

(13) O. Wintersteiner and M. Moore, *ibid.*, **65**, 1507 (1943).

(14) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **157**, 601 (1945). Starred compounds were reference specimens.

(15) H. Hirschmann, *ibid.*, **150**, 363 (1943).

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