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A Relationship between the Stereochemical Configuration of 3-Hydroxysteroids and their Infrared Absorption Spectra[1]

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In the infrared spectra of 3-hydroxysteroids there is a strong band between 995 and 1055 cm.$^{-1}$, the exact position of which is dependent on the stereochemical configurations at C_3 and C_5 in the steroid ring system. The position of this band is not affected by the presence of carbonyl groups at C_7, C_{11}, C_{12} or C_{20} or by unsaturated linkages or hydrocarbon side chains. The 17-ketone group produces two bands in the same region of the spectrum but these can be distinguished by their lower intensity and both the 17-ketone and 3-hydroxyl group vibrations can be sorted out in the spectra of 3-hydroxy-17-keto-steroids. It has been observed, in selected cases, that the infrared spectra of steroids containing two substituents can be approximated by adding together the spectra of the appropriate monosubstituted steroids on a molecular extinction coefficient basis.

In the infrared spectra of 3-hydroxysteroids a band occurs between 995 and 1055 cm.$^{-1}$ which is associated with the hydroxyl group, and is readily recognized in the spectra of simple steroid alcohols by its high intensity (Figs. 1–5). This band can most probably be identified with a stretching vibration of the C–O bond in which the –OH group moves as a whole, and it is related to the strong

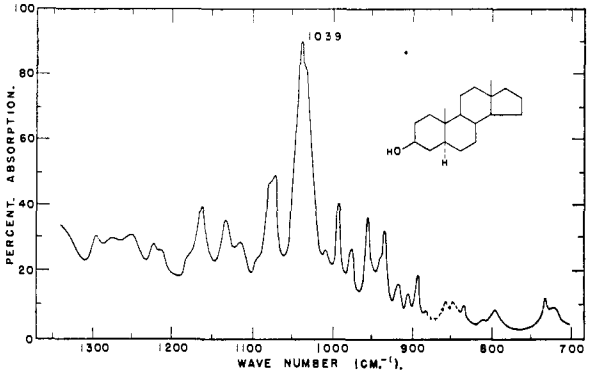

Fig. 1.—Infrared spectrum of androstanol-3β in carbon disulfide solution.

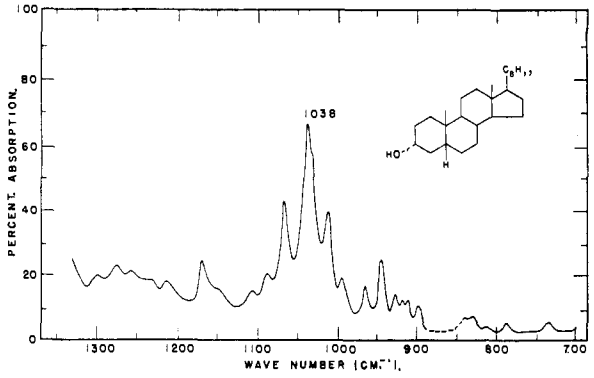

Fig. 2.—Infrared spectrum of coprostanol-3α in carbon disulfide solution.

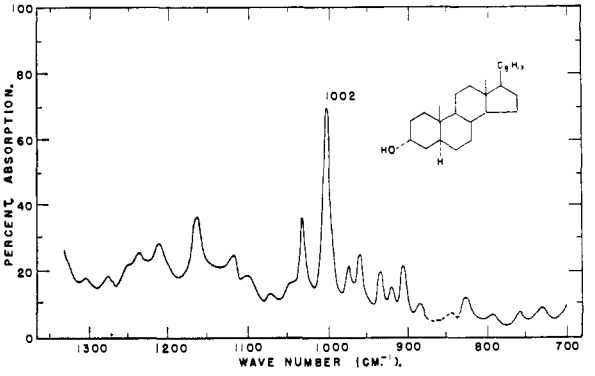

Fig. 3.—Infrared spectrum of cholestanol-3α in carbon disulfide solution.

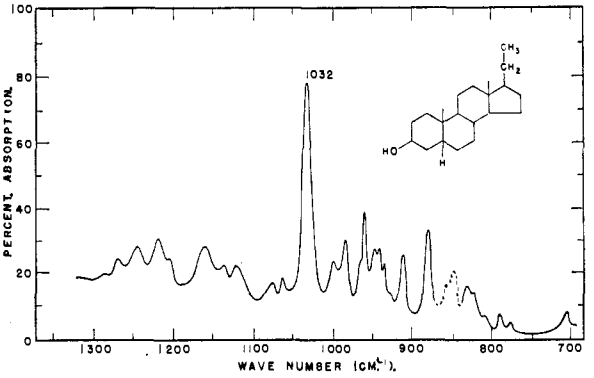

Fig. 4.—Infrared spectrum of pregnanol-3β in carbon disulfide solution.

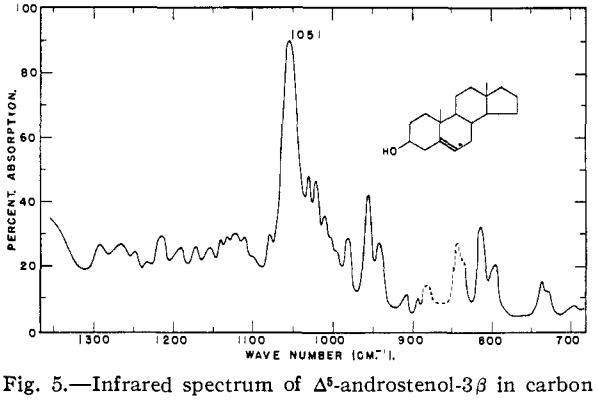

Fig. 5.—Infrared spectrum of Δ^5-androstenol-3β in carbon disulfide solution.

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(3) Died March 10, 1952.

band designated ν_4 by Herzberg,[4,5] which occurs at 1034 cm.$^{-1}$ in the spectrum of methanol.

In 1946 Furchgott, Rosenkrantz and Shorr[6] suggested that the absorption of steroid alcohols between 1000 and 1060 cm.$^{-1}$ was influenced by stereochemical factors; their conclusions were based on the study of solid films of a relatively small number of steroids alcohols. More recent work has shown that correlations between infrared absorption bands and molecular structure hold more precisely for measurements made in solution than in the solid state and it has now been established that the exact position of the strong C–OH maximum in the spectra of 3-hydroxysteroids does indeed depend quite specifically on the stereochemical configuration at C_3 and C_5 for spectra measured in carbon disulfide solution.

Experimental Methods and Results

The spectra were measured on a Perkin–Elmer Model 12C single beam spectrometer with a sodium chloride prism. The compounds were studied in carbon disulfide solution at concentration of approximately 0.030 molar in an absorption cell of 1.0-mm. thickness.

The apparent molecular extinction coefficients were calculated from the equation

$$E_A = \frac{1}{cl} \log_{10} \left(\frac{T_0}{T} \right)_\nu$$

where T_0 and T are the energies incident on, and transmitted by the solution when the spectrometer is set at the frequency ν; c is the concentration of solute in moles per liter of solution and l the cell thickness in cm. With the slit width of approximately 3 cm.$^{-1}$ which was employed, this quantity is about 5% less than the true molecular extinction coefficient for bands of 10–12 cm.$^{-1}$ width at half maximal intensity.[7]

The positions of these C–OH stretching bands for 3-hydroxysteroids containing no other oxygen function are listed in Table I, while in Table II the bands assigned to this absorption in some more complex steroids are reported.

Discussion

Identification of 3-Hydroxysteroid Stereoisomers.—The 3-hydroxysteroids commonly encountered fall into five structural types. These are usually represented by structures I–V, but the stereochemical relationships are illustrated more correctly by the polar-equatorial type structures IA-VA.[8–10] The essential features of these structures are summarized in columns 2–4 of Table III.[11]

In column 5 of Table III the position of the C–OH stretching bands are summarized and it is seen that for each stereochemical type the band falls into a narrow frequency range. For I, II and IV these ranges lie close together in the region between 1032 and 1044 cm.$^{-1}$, but the band positions for III (996–1002 cm.$^{-1}$) and for V (1050–1052 cm.$^{-1}$) fall well outside of the range of the other

TABLE I

POSITION OF C–OH STRETCHING BAND IN THE SPECTRA OF SIMPLE 3-HYDROXYSTEROIDS

Compound	Source[a]	Band position,[b] cm.$^{-1}$
I		
Androstanol-3β	20	1039
Cholestanol-3β	6	1038
$\Delta^{8,14}$-Cholestanol-3β	5	1039
Δ^{14}-Cholestenol-3β	5	1040
Ergostanol-3β	8	1037
Δ^{8}-Ergostenol-3β	2	1039
$\Delta^{8,14}$-Ergostenol-3β	2	1039
Δ^{14}-Ergostenol-3β	2	1039
$\Delta^{7,22}$-Stigmastadienol-3β (*α-spinasterol*)	2	1040
Δ^{22}-Stigmastenol-3β	2	1038
II		
Etiocholanol-3α	7	1037
Δ^{16}-Etiocholenol-3α	17	1040
Coprostanol-3α	6	1038
Δ^{22}-5-Isoergostenol-3α	2	1039
$\Delta^{7,22}$-5-Isoergostadienol-3α	2	1044
Δ^{22}-5-Isostigmastenol-3α	2	1038
III		
Androstanol-3α	20	1001
Δ^{16}-Androstenol-3α	17	1000
Cholestanol-3α	6	1002
IV		
Δ^{16}-Etiocholenol-3β	17	1036
Pregnanol-3β	10	1032
Coprostanol-3β	6	1034
V		
Δ^{5}-Androstenol-3β	20	1050
$\Delta^{5,17}$-Pregnadienol-3β	20	1051
Δ^{5}-Cholestenol-3β (*cholesterol*)	6	1052
Δ^{5}-Stigmastenol-3β (*β-sitosterol*)	1	1051
$\Delta^{5,24(28)}$-Stigmastadienol-3β (*fucosterol*)	22	1052

[a] (1) A. R. Bader, Pittsburgh Plate Glass Co., Milwaukee, Wis.; (2) D. H. R. Barton, Imperial College, London, England; (3) B. J. Brent, Organon, Inc., Orange, N. J.; (4) C. Djerassi and G. Rosenkranz, Syntex S.A., Mexico City, Mexico; (5) D. Fukushima, Sloan-Kettering Institute, New York, N. Y.; (6) T. F. Gallagher, Sloan-Kettering Institute, New York, N. Y.; (7) R. D. H. Heard, McGill University, Montreal, P.Q.; (8) Sir I. M. Heilbron, Imperial College, London, England; (9) W. H. Hoehn, G. A. Breon and Co., Kansas City, Mo.; (10) O. Kamm, Parke, Davis and Co., Detroit, Mich.; (11) E. C. Kendall, Mayo Clinic, Rochester, Minn.; (12) W. Klyne, Postgraduate Med School, London, England; (13) W. Klyne, *vide supra*, and C. W. Shoppee, University College, Swansea, Wales; (14) S. Lieberman, Sloan-Kettering Institute, New York, N. Y.; (15) R. E. Marker, Pennsylvania State College, Pa.; (16) H. L. Mason, The Mayo Clinic, Rochester, Minn.; (17) V. Prelog, Eidg. Tech. Hochschule, Zurich, Switz.; (18) T. Reichstein, University, Basel, Switz.; (19) L. H. Sarett, Merck and Co. Inc., Rahway, N. J.; (20) C. R. Scholz, Ciba Pharmaceutical Products Inc., Summit, N. J.; (21) E. Schwenk, The Schering Corp., Bloomfield, N. J.; (22) D. Thorn, Mt. Allison University, Sackville, N. B.; (23) R. B. Turner, Harvard University, Cambridge, Mass.; (24) R. B. Wagner, Pennsylvania State College, State College, Pa.; (25) compound isolated at the Sloan–Kettering Institute, New York, N. Y. [b] Carbon disulfide solution.

(4) G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., Inc., New York, N. Y., 1945, p. 335.

(5) In CH_3OD the ν_4 vibration occurs at 1040 cm.$^{-1}$ compared with 1034 cm.$^{-1}$ in CH_3OH. In steroid alcohols the band in question is similarly displaced to higher frequency by 6–10 cm.$^{-1}$ on introduction of deuterium into the hydroxyl group.

(6) R. F. Furchgott, H. Rosenkrantz and E. Shorr, *J. Biol. Chem.*, **163**, 375 (1946); **167**, 627 (1947).

(7) D. A. Ramsay, THIS JOURNAL, **74**, 72 (1952).

(8) O. Hassel and H. Viervoll, *Acta Chem. Scand.*, **1**, 149 (1947).

(9) C. W. Beckett, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2488 (1947).

(10) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

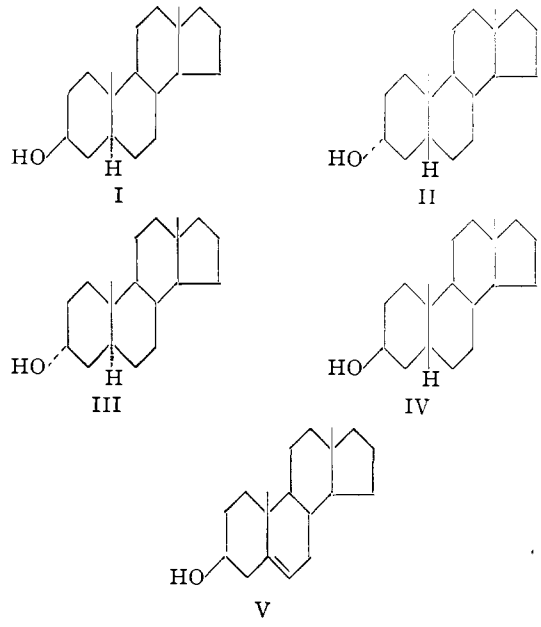
(11) The equatorial conformation of the hydroxyl bond in V is not immediately apparent but can be readily established from the model. It is assumed in all these structures that the A and B ring systems approximate to the chair rather than the boat form of cyclohexane.

TABLE II

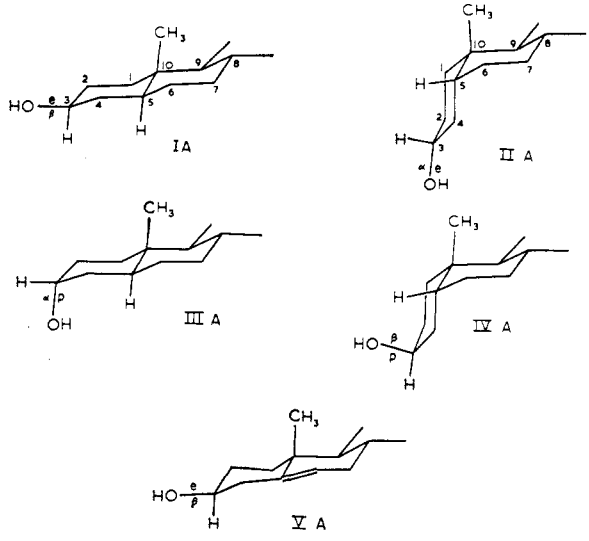
POSITION OF THE C–OH STRETCHING BAND IN THE SPECTRA OF 3-HYDROXYSTEROIDS CONTAINING OTHER OXYGEN FUNCTIONS

Compound[a]	Source[b]	Band position,[c] cm.$^{-1}$
I		
Androstanol-3β-one-17	20	1039
D-Homoandrostanol-3β-one-17a	12	1037
17-Methyl-D-homoandrostanol-3β-one-17a	13	1038
Allopregnanol-3β-one-20	6, 15	1038
16α,17α-Epoxyallopregnanol-3β-one-20	6	1039
$\Delta^{9:11}$-Allopregnenol-3β-one-20	4	1038
Δ^{16}-Allopregnenol-3β-one-20	24	1039
Cholestanol-3β-one-6	12	1060[d]
II		
Etiocholanol-3α-one-17	25	1037
Etiocholanol-3α-dione-11,17	19	1038
$\Delta^{9:11}$-Etiocholenol-3α-one-17	19	1038
3α-Hydroxyetiocholanic acid M.E.	11	1037
3α-Hydroxy-11-ketoetiocholanic acid M.E.	23	1038
Δ^{11}-3α-Hydroxyetiocholenic acid M.E.	11	1037
Pregnanol-3α-one-20	14	1038
17-Isopregnanol-3α-one-20	9	1037
Δ^{11}-Pregnenol-3α-one-20	14	1037
3α-Hydroxybisnorcholanic acid M.E.	11	1039
3α-Hydroxy-11-ketobisnorcholanic acid M.E.	23	1036
3α-Hydroxy-11-ketonorcholanic acid M.E.	23	1036
3α-Hydroxycholanic acid M.E.	6	1036
3α-Hydroxy-6-ketocholanic acid M.E.	6	1054[c]
3α-Hydroxy-11-ketocholanic acid M.E.	23	1036
9α,11α-Epoxy-3α-hydroxycholanic acid M.E.	19	1038
11α,12α-Epoxy-3α-hydroxycholanic acid M.E.	6	1038
$\Delta^{9:11}$-3α-Hydroxy-12-ketocholenic acid M.E.	11	1039
$\Delta^{9:11}$-3α-Hydroxy-12α-chlorocholenic acid M.E.	11	1038
III		
Androstanediol-3α,17β-acetate-17	25	1001
Androstanol-3α-one-17	25	998
Androstanol-3α-dione-11,17	25	1002
9α:11α-Epoxyandrostanol-3α-one-17	16	1000
$\Delta^{9:11}$-Androstenol-3α-one-17	16	996
Allopregnanol-3α-one-20	14	1002
IV		
Etiocholanediol-3β,17β-acetate-17	6	1036
Etiocholanol-3β-one-17	4	1033
Pregnanol-3β-one-20	10	1032
V		
Δ^{5}-Androstenediol-3β,17β	20	1050
Δ^{5}-Androstenediol-3β,17β-acetate-17	10	1050
Δ^{5}-3β-Hydroxyetiocholenic acid M.E.	3	1050
Δ^{5}-Pregnenol-3β-one-20	21	1050
$\Delta^{5,17:20}$-Pregnadienol-3β-one-20	6	1050
Δ^{5}-16α-Methylpregnenol-3β-one-20	4	1052
$\Delta^{5,16}$-Pregnadienol-3β-one-20	10	1052[e]
$\Delta^{5,16}$-16α-Methylpregnadienol-3β-one-20	4	1050
Δ^{5}-3β-Hydroxycholenic acid M.E.	6	1050

[a] M. E. designates methyl ester. [b,c] See footnotes to Table I. [d] The increase in the frequency is attributed tentatively to the effect of the 6-ketone group on the rigidity of the ring system (see page 5574). [e] There is a band of comparable intensity at 1040 cm.$^{-1}$ which is absent from the spectrum of the 16-methyl derivative.



three. The observation of the position of this band can therefore be of considerable help in the determination of the stereochemical configuration of 3-hydroxysteroids, and especially is this so when these data are considered in relation to the character of the absorption near 1240 cm.$^{-1}$ in the spectra of the corresponding acetates.



It has been pointed out previously[12] that for 3-acetoxysteroids in which the acetate group is attached at a polar position (III, IV) a group of two or three intense peaks are observed near 1240 cm.$^{-1}$ but for equatorially oriented acetate groups (I, II, V) there is only a single strong peak in this region (Table III, column 6). The character of this 1240 cm.$^{-1}$ acetate absorption has been used in conjunction with the stereospecificity of the digitonin precipitation reaction of 3-hydroxysteroids to distinguish among the five types of structures. It was not possible to distinguish between I and V, from the acetate spectra and digitonin reaction

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

TABLE III

STEREOCHEMICAL CHARACTERIZATION OF 3-HYDROXYSTEROIDS

Structure	A/B Ring fusion	C-O Bond configuration	C-O Bond conformation	Hydroxyl band frequency,[a] cm.$^{-1}$	Acetate band type[b]	Digitonin reaction
I	*trans*	3β	Equatorial	1037–1040 (18)	A	Ppt.
II	*cis*	3α	Equatorial	1037–1044 (25)	A	No ppt.
III	*trans*	3α	Polar	996–1002 (9)	B	No ppt.
IV	*cis*	3β	Polar	1032–1036 (6)	B	Ppt.
V	Δ^5-C=C	3β	Equatorial	1050–1052 (14)	A	Ppt.

[a] Figures in parentheses indicate number of compounds studied (see Tables I and II). [b] See reference 11.

alone, but the 3-hydroxy C–O stretching band is at 1036–1040 cm.$^{-1}$ for I and at 1050–1052 cm.$^{-1}$ for V, so these two structures may now be characterized. The hydroxyl band can also serve to differentiate between III and IV with peaks at 998–1002 cm.$^{-1}$ and 1032–1034 cm.$^{-1}$, respectively, and recourse to the use of digitonin is necessary only to select between I and II.

Interpretation of the Frequency Differences.—For both the *cis* and *trans* types of A/B ring junctions, the C–O stretching vibration occurs at a slightly higher frequency for the equatorial than for the polar substituent. Any precise interpretation of the small frequency displacements observed must be based on a normal coördinate analysis of the vibrating system; however it can be seen qualitatively from structures Ia–Va that, for the equatorial vibration, the motion of the C_3 atom along the C–O axis will involve an appreciable expansion and contraction of the A ring, whereas for the polar structure the motion is largely normal to the plane of the ring. The restoring force acting on the C_3 atom should therefore be less for the polar than the equatorial motion and this might induce the lower vibration frequency.

In the Δ^5-3-hydroxy system V where the frequency is the highest, the rigidity of the A/B ring system is increased by the Δ^5-double bond. There is some suggestion from the data in Table II that the 6-ketone group and the Δ^7-double bond also raise the frequency of the C–OH vibration slightly and this would be in accord with their effects on the rigidity of the A/B ring system.

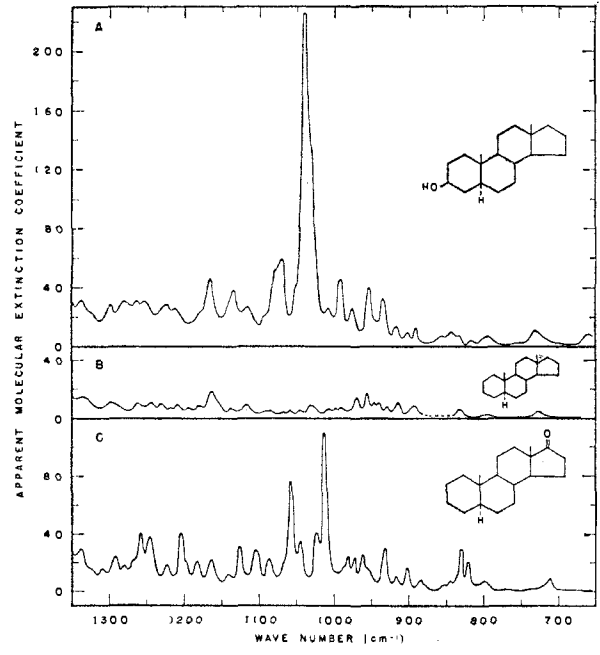


Fig. 6.—Infrared spectra on molecular extinction coefficient intensity scale (carbon disulfide solution): A, androstanol-3β; B, androstane; C, androstanone-17.

Specific Group Vibrations in the "Fingerprint" Region of Steroid Spectra

It is well known that in the spectra of organic compounds the pattern of the absorption at frequencies below 1350 cm.$^{-1}$ is quite sensitive to small changes in molecular structure; this region of the spectrum is often referred to as the "fingerprint" region, with the implication that the spectrum is uniquely characteristic of the given compound.

Certain absorption bands characteristic of specific groups have nevertheless been recognized in this part of the spectrum, such as the C–H deformation vibration near 970 cm.$^{-1}$ in *trans* disubstituted ethylenes, the out-of-plane C–H bending vibrations of aromatic rings and the C–O stretching vibrations in acetates and other esters. In most hydroxysteroids and hydroxyketosteroids strong absorption bands occur near 1000 cm.$^{-1}$, it seems probable that the 3-hydroxy bands discussed above exhibit considerable group specificity in complex steroids and, in favorable cases, can be sorted out from other bands occurring in the same region.

Group vibrations in the region between 650 and 1350 cm.$^{-1}$ in steroid spectra have been a subject of study for some time, and will be discussed in detail in a later publication. It may be noted here, however, that their interpretation is appreciably simplified if consideration is given to the absolute band intensities, as well as to the band positions, and this can be done most conveniently by plotting the apparent molecular extinction coefficients,[7] as in Figs. 6–7.

3-Hydroxy C–OH Bands in More Complex Steroids.—Between 650 and 1350 cm.$^{-1}$ the extinction coefficients of the most intense bands in the spectra of *saturated* steroid hydrocarbons seldom exceed 30, and are usually much below this (*e.g.*, androstane, Fig. 6). On introduction of 3-hydroxyl groups the order of intensity through most of the spectrum is little changed except for the appearance of the strong C–OH stretching bands described above, the intensities of which lie between 200 and 250. Hydroxyl groups at other positions give rise to strong bands in the same region of the spectrum, and the acetates also absorb strongly at frequencies slightly lower than the alcohols. The 3-hydroxyl group absorption therefore may be obscured or displaced in the spectra of dihydroxy steroids or 3-

hydroxyacetoxy steroids. The band associated with the 17β-hydroxyl group is appreciably weaker (E_A = 100–120) and the 3-hydroxyl absorption can be identified without difficulty in 3,17β-dihydroxysteroids and in the corresponding 3,17β-diacetoxy compounds (see Table II).

Carbonyl groups at C_7, C_{11}, C_{12} or C_{20} yield bands of moderate intensity (E_A = 50–100) between 1100 and 1300 cm.$^{-1}$ but do not produce any strong absorption near 1000 cm.$^{-1}$ and the 3-hydroxy band can be recognized without any difficulty in the spectra of 3-hydroxyketosteroids with carbonyl groups at these positions (Table II). The strong absorption bands associated with C–H deformation vibrations of unsaturated linkages occur at frequencies well below 995 cm.$^{-1}$ and do not usually interfere with the identification of the 3-hydroxyl band.

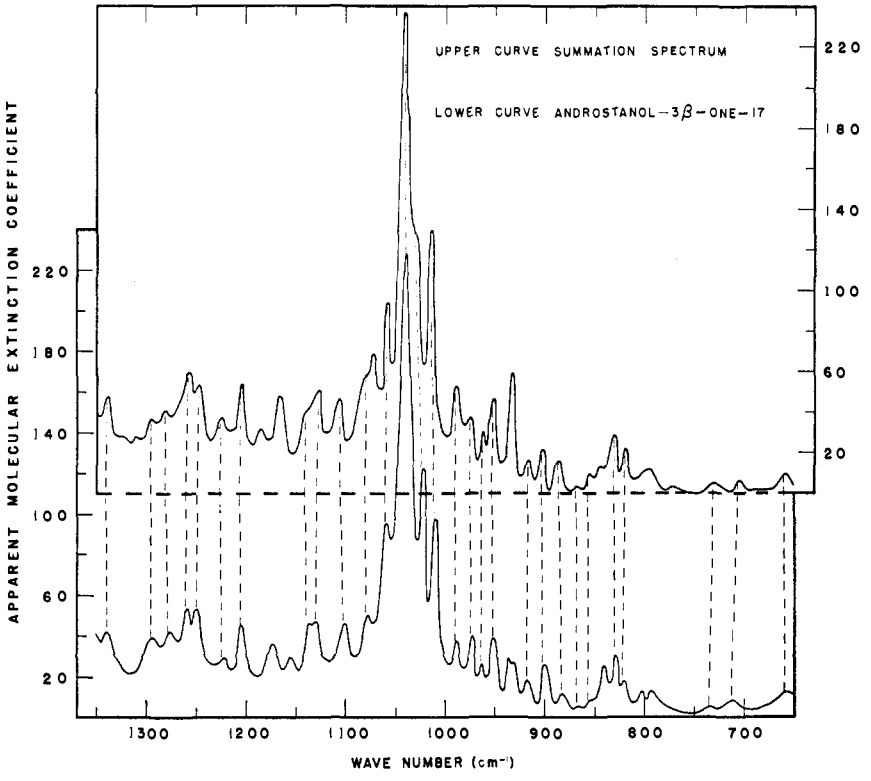


Fig. 7.—Comparison of infrared spectrum of androstanol-3β-one-17 with curve obtained by adding spectra of androstanol-3β and androstanone-17 and subtracting spectrum of androstane.

3-Hydroxy-17-ketosteroids. Summation Spectra.—The recognition of the 3-hydroxy group absorption becomes more difficult when there is a 17-ketone present, as this group introduces two medium-strong bands, one near 1010 cm.$^{-1}$ (E_A = 60–80) and a second near 1055 cm.$^{-1}$ (E_A = 80–100) (see androstanone-17 in Fig. 6). The spectra of the 3-hydroxy-17-ketosteroids are therefore particularly complex between 990 and 1100 cm.$^{-1}$ and indeed have been cited as examples to illustrate the stereochemical specificity of steroid spectra.[13]

It now seems probable that the absorption of 3-hydroxy-17-ketosteroids in this region can be sorted out into the independent vibrations of the 3-hydroxy and 17-ketone groups, as may be illustrated by comparison of the spectrum of androstanol-3β-one-17 (Fig. 7) with the spectra of androstanone-17 and androstanol-3β (Fig. 6).

It is also interesting to observe that *the whole spectrum of androstanol-3β-one-17 between 700 and 1350* cm.$^{-1}$ *can be approximated by a summation of the spectra of androstanol-3β and androstanone-17 on a molecular extinction intensity scale* (Fig. 7). Such a summation procedure involves the assumption that the principal absorption bands in the spectra of androstanol-3β and androstanone-17 are associated with vibrations centered, respectively, in the A and D rings, and that in androstanol-3β-one-17 there is negligible coupling between the strongly infrared active vibrations associated with the hydroxyl and ketone groups at the remote ends of the molecule.

By a simple addition of the two component spectra, the contribution of the hydrocarbon moiety (essentially rings B and C) is taken twice, but an approximate correction may be made by subtracting out the spectrum of androstane. This correction has no appreciable effect on the over-all shape of the computed curve, but it lowers the apparent extinction coefficient by 5–10 units bringing it into better correspondence with the experimental curve for androstanol-3β-one-17, in the regions of low absorption intensity.

Evidence which is at present being accumulated indicates that the spectra of other disubstituted steroids in which the substituent groups are well separated in the molecule may be approximated in a similar way by the summation of the spectra of the relevant monosubstituted steroids. This has been established clearly for the steroid sapogenins[14] and is being investigated also in other types of steroids.

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 to us. The technical assistance of Mrs. M. A. Mackenzie, Mr. D. S. Keir and Mr. R. Lauzon is also gratefully acknowledged. The investigation was aided by grants from the Commonwealth Fund, the American Cancer Society (upon recommendation of the Committee on Growth of the (U. S.) National Research Council), and the National Cancer Institute, U. S. Public Health Service.

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NEW YORK, N. Y.

(13) R. N. Jones, "Recent Progress in Hormone Research," Vol. 2, Academic Press, Inc., New York, N. Y., 1948, p. 3.

(14) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, (in press).