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Studies in Steroid Metabolism. X. The Effects of Stereochemical Configuration at Positions 3 and 5 on the Infrared Spectra of 3-Acetoxy Steroids

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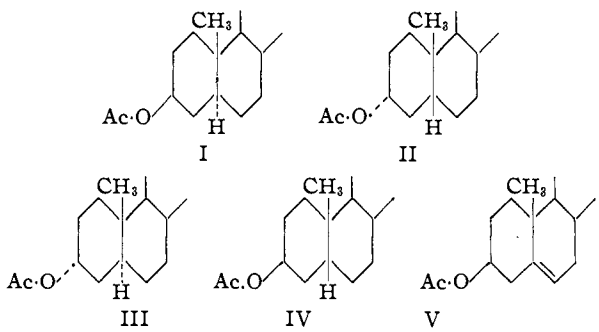
Steroid acetates exhibit strong absorption bands between 1200 and 1260 cm.$^{-1}$. In 3-acetoxy steroids in carbon disulfide solution, only a single strong band is observed in this region if the hydrogen at position 5 bears a *trans* relationship to the 3-substituent. If the 3-acetate group is *cis* to the 5-hydrogen atom two or three strong bands occur in this region. This observation aids in the determination of the stereochemical configuration of 3-acetoxy steroids, and when considered in relation to the precipitation reaction of the 3-hydroxy steroid with digitonin it permits the stereochemical configurations at positions 3 and 5 to be assigned. This has been applied in the identification of new steroid metabolites isolated from urine. From a consideration of the band intensities and of the alteration of band contours with temperature it is suggested that in 3-acetates exhibiting multiple bands an equilibrium may exist among labile stereoisomers; these may be rotational isomers resulting from hindrance to the rotation about the C–O bonds of the acetate group.

The establishment of a comprehensive and precise series of correlations between the stereochemical configuration of a steroid and the infrared absorption spectrum can only be regarded as a remote possibility. Nevertheless, correlations of a limited scope may be expected, and it is the principal purpose of this paper to draw attention to certain features of the spectra of 3-acetoxy steroids which provide information about the steric configuration of the substituent at position 3 and the hydrogen atom at position 5.

Spectra of 3-Acetoxy Steroids.—Two bands of outstanding intensity are observed in the spectra of alkyl acetates; in carbon disulfide solution one of these occurs near 1735 cm.$^{-1}$ and is associated with a C=O stretching vibration. The second band is between 1200 and 1250 cm.$^{-1}$ and has been attributed to a stretching vibration of a C–O linkage.[2]

In many steroid acetates the strong band which occurs between 1200 and 1250 cm.$^{-1}$ is single, but a comparative study of a large number of 3-acetoxy steroids in carbon disulfide solution has shown that in several instances two or three separate peaks of comparable intensity occur, or there is a principal peak accompanied by strong subsidiary maxima. In Fig. 1 the absorption spectra between 1190 and 1290 cm.$^{-1}$ of four stereoisomeric 3-acetoxy steroids are shown. These contain the partial structures I–IV. The acetates of structure I and II give a band of simple contour which we shall



designate *Type A*. The stereoisomers of structures III and IV give a complex band, or group of bands designated *Type B*. The simple Type A band is given also by Δ^5-3β-acetoxy steroids (V).

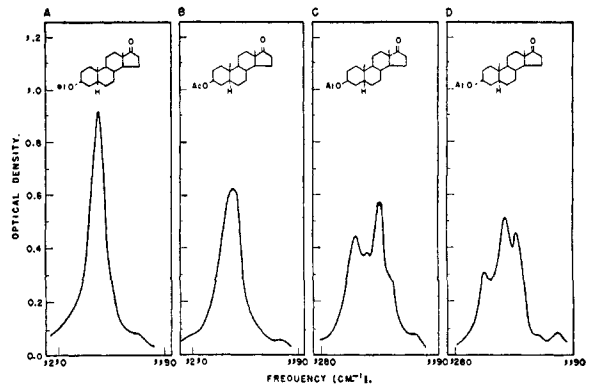


Fig. 1.—Characteristic acetate bands of stereoisomeric 3-acetoxy C_{19} steroids (CS_2 solution): A, etiocholanol-3α-one-17; B, androstanol-3β-one-17; C, etiocholanol-3β-one-17; D, androstanol-3α-one-17.

It is observed quite generally that 3-acetoxy steroids of structure I, II or V give a Type A band and those of structure III and IV a band of Type B. Additional examples of stereoisomeric 3-acetoxy steroids of the C_{21} and C_{27} series are shown in Figs. 2 and 3. In Table I the measurements on some one hundred steroid acetates which provide the basis of these correlations are summarized.

Application to the Determination of Steric Configuration.—Observation of the shape of this absorption band in the spectrum of the acetoxy derivative has been most useful as an aid to the elucidation of the stereochemistry of the A and B rings of 3-hydroxy steroids. Provided other acetoxy groups are absent from the molecule, the 3-acetoxy absorption band between 1200 and 1250 cm.$^{-1}$ can readily be characterized as Type A or Type B.[3] Further differentiation among the 3-hydroxy stereoisomers is possible if the reactions of the free alcohols with digitonin are taken into consideration. In Table II the 3-acetoxy band type and the reaction with digitonin are listed[4]

(1) Published as Contribution No. 2440 from the Laboratories of The National Research Council of Canada. Presented at a Symposium on Molecular Spectroscopy held at The Ohio State University, Columbus, Ohio, June, 1950.

(2) Thompson and Torkington, *J. Chem. Soc.*, 640 (1945).

(3) A few of the compounds of structure I, II or V do show weak inflections on the sides of the strong band (*cf.* curve A of Fig. 2), but these are clearly distinguishable from the well defined multiple curves of Type B.

(4) In this discussion the isomeric Δ^5-3α-acetoxy structure is not considered since it has not been identified in any naturally occurring steroid and no compounds of this type have been available to us for spectrographic examination.

TABLE I

CHARACTERISTIC ABSORPTION BANDS OF 3-ACETOXY STEROIDS BETWEEN 1190 AND 1290 CM.$^{-1}$

All measurements were made in carbon disulfide solution. The configuration of the 17-hydroxyl group is designated β if it is the same as that of testosterone. The source of the individual compounds are indicated by superscripts and given in footnotes at the end of the table. In some cases the compound was acetylated at the Sloan–Kettering Institute, and this is indicated by an asterisk following the reference to the donor. "M.E." designates methyl ester.

A. STEROIDS OF STRUCTURE I.—The 3-acetoxy derivatives of the following steroids gave a single absorption maximum between 1236 and 1242 cm.$^{-1}$: androstanol-3β[26]; 9,11-epoxyandrostanol-3β[16]; androstanol-3β-one-17[16]; allopregnanol-3β-one-20[7,17]; allopregnanediol-3β,17α-one-20[23,24]; cholestanol-3β[6,22]; $\Delta^{6,14}$-cholestanol-3β[6]; Δ^{14}-cholestenol-3β[6]; $\Delta^{6,8}$-cholestadienol-3β[1]; 8,14-epoxycholestanol-3β-one-15[31]; ergostanol-3β[9]; Δ^{7}-ergostenol-3β[1]; $\Delta^{8:14}$-ergostadienol-3β[9]; stigmastanol (fucostanol)-3β[9]; $\Delta^{7,21}$-stigmastadienol-3β.[1]

B. STEROIDS OF STRUCTURE II.—The 3-acetoxy derivatives of the following steroids gave a single absorption maximum between 1237 and 1242 cm.$^{-1}$: Δ^{16}-etiocholenol-3α[22*]; etiocholanetriol-3α,11β,17β[25]; etiocholanol-3α-one-17[16]; Δ^{9}-etiocholenol-3α-one-17[25]; etiocholanol-3α-dione-11,17[25]; etiocholanediol-3α,11β-one-17[25*]; pregnanol-3α-one-20[16]; 17-iso-pregnanol-3α-one-20[11]; Δ^{11}-pregnenol-3α-one-20[7,23]; pregnanol-3α-dione-11,20[30]; pregnanediol-3α,-11α-one-20[23]; pregnanediol-3α,11β-one-20[23*]; pregnanediol-3α,17α-one-20[7,16]; pregnanediol-3α,12-one-20-methanesulfonate-12[7]; 12-bromopregnanol-3α-dione-11,20[29]; coprostanol-3α[7]; 23-chloronorcholanol-3α-one-22.[7]

The methyl esters of the following steroid acids gave a single band between 1238 and 1242 cm.$^{-1}$: 3α-acetoxy-12-ketoetiocholanic[7]; 3α-acetoxy-11-keto-12α-bromoetiocholanic[7]; 3α-acetoxy-11-ketonorcholanic[30]; 3α-acetoxycholanic[7]; $\Delta^{9:11}$-3α-acetoxycholenic[26,30]; 3α-acetoxy-11α,-12α-epoxycholanic[30]; 3α-acetoxy-11β-hydroxycholanic[6]; 3α-acetoxy-11-ketocholanic[30]; 3α-acetoxy-12-ketocholanic[26,30]; $\Delta^{9:11}$-3α-acetoxy-12-ketocholenic.[27]

The following compound gave two absorption bands at 1232 and 1242 cm.$^{-1}$: 3α-acetoxy-6-ketocholanic acid methyl ester.[11]

C. STEROIDS OF STRUCTURE III.—The 3-acetoxy derivatives of the following steroids gave three strong bands which are listed in the order of diminishing intensity: androstanol-3α (1241, 1234, 1256)[26]; Δ^{16}-androstenol-3α (1239, 1234, 1254)[8*,22*]; androstanediol-3α,11β-one-17 (1239, 1233, 1255)[4,18*]; androstanediol-3α,17β (1233, 1244, 1255)[26]; androstanol-3α-one-17 (1239, 1234, 1256)[16,26]; $\Delta^{9:11}$-androstenol-3α-one-17 (1234, 1250, 1212)[4]; Δ^{11}-androstenol-3α-one-17 (1232, 1246, 1212)[5]; allopregnanol-3α-one-20 (1239, 1249, 1258)[7]; cholestanol-3α (1236, 1246, 1256).[7]

3α-Acetoxy-6-ketoallocholanic acid methyl ester gave bands at 1240, 1255, 1220.[8]

D. STEROIDS OF STRUCTURE IV.—The 3-acetoxy derivatives of the following steroids gave two or three strong bands which are listed in the order of diminishing intensity: Δ^{16}-etiocholenol-3β (1236, 1252, 1229)[22*]; etiocholanol-3β-one-17 (1236, 1252, 1244)[24]; pregnanol-3β (1236, 1230, 1249)[15*]; pregnanol-3β-one-20 (1230, 1238, 1252)[15]; pregnanediol-3β,11α-one-20 (1230, 1251, 1239)[23]; pregnanediol-3β,17α-one-20 (1228, 1236, 1251)[7,24]; pregnanol-3β-dione-11,20 (1236, 1219, 1251)[23]; coprostanol-3β (1238, 1253, 1228)[7]; digitoxigenin (1232, 1252)[23]; dihydrodigitoxigenin (1232, 1252)[23]; dihydro-17-isodigitoxigenin (1237, 1253).[23]

The methyl ester of Δ^{11}-3β-acetoxycholenic acid gave bands at 1236, 1250, 1222.[30]

E. STEROIDS OF STRUCTURE V.—The 3-acetoxy derivatives of the following steroids gave a single absorption maximum between 1236 and 1242 cm.$^{-1}$; Δ^{5}-androstenol 3β[26]; Δ^{5}-androstenediol-3β,17β[26]; Δ^{5}-androstenol-3β-one-17[17,27]; $\Delta^{5,17}$-methylandrostenediol-3β,17β[27]; Δ^{5}-17-ethynylandrostenediol-3β,17β[12]; Δ^{5}-pregnenol-3β-one-20[26]; $\Delta^{5,16}$-pregnadienol-3β-one-20[15,17,26]; $\Delta^{5,17:20}$-pregnadienol-3β[26]; $\Delta^{5,17}$-isopregnenediol-3β,17β[26]; Δ^{5}-pregnenediol-3β,-17α-one-20[14]; Δ^{5}-16-methoxypregnenol-3β-one-20[6]; Δ^{5}-16-ethoxypregnenol-3β-one-20[6]; $\Delta^{5,16}$-16-methylpregnadienol-3β-one-20[24]; Δ^{5}-norcholestenol-3β-one-25[2,10]; Δ^{5}-cholestenol-3β[16]; Δ^{5}-cholestenediol-3β,4β[6,16]; Δ^{5}-cholestenol-3β-one-7[7]; fucosterol.[17]

The methyl esters of the following acids gave a single absorption maximum at 1239 cm.$^{-1}$: Δ^{5}-3β-acetoxyetiocholenic[28]; Δ^{5}-3β-acetoxycholenic.[2,7]

F. OTHER 3-ACETOXY STEROIDS. *Phenolic 3-acetate:* Estrone acetate (1206)[16,24]; equilenin acetate (1202)[16,26]; $\Delta^{1,3,5:10,6}$-estratetraenol-3-one-17 (1206)[3,31]; 17-ethynylestradiol-3,17β-acetate-3.[13]

5,6-Epoxy-3-acetate: 5α,6α-Epoxyetiocholanol-3β-one-17-acetate (1240)[7,16]; 5β,6β-epoxyetiocholanol-3β-one-17-acetate (1240)[7,16]; 5,6-epoxycholestanol-3β-one-4-acetate (1228).[16]

5,6-Dibromo-3-acetate: 5,6-Dibromocholestanol-3β-acetate (1240).[6]

$\Delta^{5,7}$-*3-Acetate:* $\Delta^{5,7}$-Cholestadienol-3β-acetate (1240)[6,28]; ergosterol acetate (1239).[13]

10-Iso-3-acetate: Lumistanol-3β-acetate (1246, 1228)[9]; lumisterol acetate (1236, 1250, 1222).[9]

G. OTHER ESTERS OF 3-HYDROXY STEROIDS. *Formate:* Δ^{5}-Cholestenol-3β-formate (1180)[7,16]; pregnanediol-3α,-17α-dione-11,20-formate-3 (1180).[7]

Propionate: Δ^{5}-Androstenol-3β-one-17-propionate (1188).[26]

Benzoate: Etiocholanol-3α-one-17-benzoate (1270)[16]; Δ^{5}-androstenol-3β-one-17-benzoate (1270)[16]; Δ^{5}-cholestenol-3β-benzoate (1271)[6]; $\Delta^{8:14}$-cholestenol-3β-benzoate (1271)[6]; Δ^{14}-cholestenol-3β-benzoate (1271).[6]

Phenolic Benzoate: Estrone benzoate (1257, 1241, 1221, 1208)[20]; equilin benzoate (1257, 1243, 1212)[20]; equilinin benzoate (1257, 1243, 1224, 1206).[20]

(1) D. H. R. Barton, Imperial College, London, England. (2) B. J. Brent, Organon, Inc., Orange, N. J. (3) C. Djerassi and C. R. Scholz, Ciba Pharmaceutical Products, Inc., Summit, N. J. (4) R. I. Dorfman, Western Reserve University, Cleveland, Ohio. (5) L. F. Fieser and J. Wolfe, Harvard University, Cambridge, Mass. (6) D. Fukushima, Sloan–Kettering Inst., New York, N. Y. (7) T. F. Gallagher, Sloan–Kettering Inst., New York, N. Y. (8) G. A. D. Haslewood, Guy's Hospital Medical School, London, England. (9) Sir I. M. Heilbron, Imperial College, London, England. (10) E. B. Hershberg, The Schering Corp., Bloomfield, N. J. (11) W. H. Hoehn, G. A. Breon and Co., Kansas City, Mo. (12) J. R. Jamieson and E. Lozinski, Charles E. Frosst and Co., Montreal, P. Q. (13) E. R. H. Jones, Manchester University, Manchester, England. (14) P. L. Julian, The Glidden Co., Chicago, Ill. (15) O. Kamm, Parke, Davis and Co., Detroit, Mich. (16) S. Lieberman, Sloan–Kettering Inst., New York, N. Y. (17) R. E. Marker, Pennsylvania State College, State College, Pa. (18) H. L. Mason, The Mayo Clinic, Rochester, Minn. (19) H. B. MacPhillamy, Ciba Pharmaceutical Products, Inc., Summit, N. J. (20) G. Papineau-Couture, Ayerst, McKenna and Harrison, Ltd., Montreal, P. Q. (21) P. A. Plattner, Eidg. Tech. Hochschule, Zurich, Switz. (22) V. Prelog, Eidg. Tech. Hochschule, Zurich, Switz. (23) T. Reichstein, University, Basel, Switz. (24) G. Rosenkranz, Syntex, S. A., Mexico City, Mexico. (25) L. H. Sarett, Merck and Co., Inc., Rahway, N. J. (26) C. R. Scholz, Ciba Pharmaceutical Products, Inc., Summit, N. J. (27) E. Schwenk, The Schering Corp., Bloomfield, N. J. (28) M. Tainter, Winthrop Chemical Co., Rensselaer, N. Y. (29) M. Tishler, Merck and Co., Inc., Rahway, N. J. (30) R. B. Turner, Harvard University, Cambridge, Mass. (31) O. Wintersteiner, Squibb Inst. for Medical Research, New Brunswick, N. J.

for the structures I–V. A unique assignment of the stereochemical configuration can be made for the saturated structures I–IV by taking account of

TABLE II

CHARACTERIZATION OF STEREOCHEMICAL CONFIGURATION FROM SPECTRUM OF 3-ACETATE AND REACTION OF 3-HYDROXIDE WITH DIGITONIN

Structure	I	II	III	IV	V
Acetate spectrum type	A	A	B	B	A
Reaction of 3-hydroxide with digitonin	Ppt.	No ppt.	No ppt.	Ppt.	Ppt.

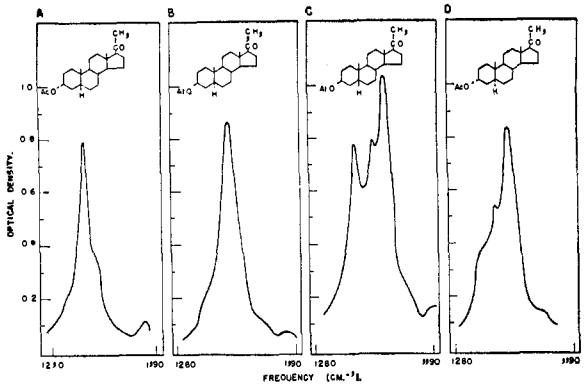


Fig. 2.—Characteristic acetate bands of stereoisomeric 3-acetoxy C_{21} steroids (CS_2 solution): A, pregnanol-3α-one-20; B, allopregnanol-3β-one-20; C, pregnanol-3β-one-20; D, allopregnanol-3α-one-20.

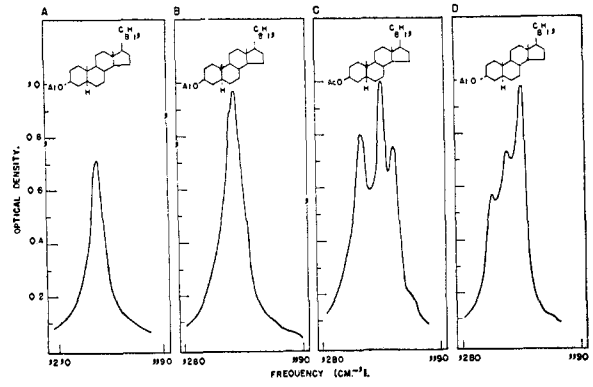


Fig. 3.—Characteristic acetate bands of stereoisomeric 3-acetoxy C_{27} steroids (CS_2 solution): A, coprostanol-3α; B, cholestanol-3β; C, coprostanol-3β; D, cholestanol-3α.

both criteria. Ambiguity between I and V may exist, but the presence of the Δ^5 bond can be recognized both chemically[5] and spectrographically.[6]

Discussion of Results.—Among the 3-acetoxy steroids included in these studies only rare exceptions to these correlations have been observed. It may be noted that the presence of a side-chain carbomethoxy group (as in 3-acetoxycholanic acid methyl ester) does not introduce any complication, nor does the presence of a ketonic carbonyl with the possible exception of the 6-ketone group discussed below.

The introduction of substituents at position 5 has been observed to modify the spectrum from Type B to Type A (Table I section F). Thus the Type B band of etiocholanol-3β-one-17-acetate changes to Type A on introduction of either a 5α,6α- or a 5β,6β-epoxy group; cholestanol-3β-acetate gives the expected Type B band, but this changes to Type A in the 5,6-dibromo derivative.

Special consideration must be given also to the spectra of 3-acetoxylumistane derivatives in which the configuration of the C_{10} methyl group is inverted (VI). The spectra of ergostanyl and lumistanyl acetates are shown in Fig. 4. While ergostanyl acetate gives a "normal" Type A band, that

(5) Munson, Jones, McCall and Gallagher, *J. Biol. Chem.*, **176**, 73 (1948).

(6) Jones, Humphries, Packard and Dobriner, THIS JOURNAL, **71**, 86 (1950).

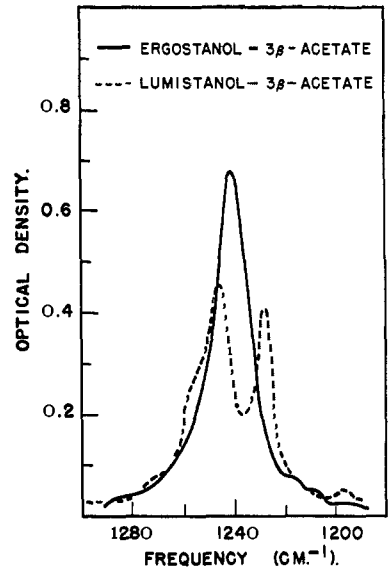
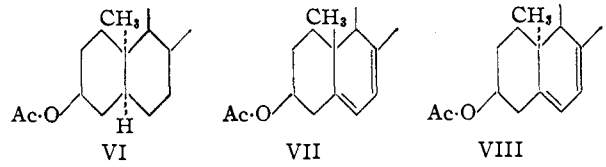


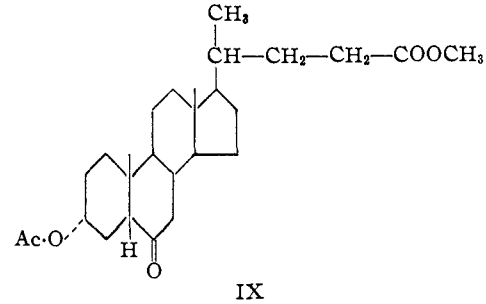
Fig. 4.—Characteristic acetate bands of normal and 10-iso-3-acetoxy steroids (CS_2 solution).

of lumistanyl acetate is Type B. That the stereochemical inversion of the C_{10} methyl group may be a factor in causing this effect is supported by the fact



VI VII VIII

that both 7-dehydrocholesteryl acetate and ergosteryl acetate (VII) give a Type A band while in lumisteryl acetate (VIII) the band is Type B.[7]



IX

The only other compound in Table I which calls for comment is the methyl ester of 3α-acetoxy-6-ketocholanic acid (IX) which gives a type B spectrum instead of the single band expected of structure II.[9]

(7) The stereochemical configuration of lumistanyl acetate has not been established completely, as the possibility of the C_5 hydrogen having a β configuration has not been excluded. Lumistanol has been prepared by hydrogenation of lumisterol (reference 8); if the reduction proceeded analogously to that of ergosterol a 5α hydrogen atom would be introduced. However the inversion of the C_{10} methyl group could lead to introduction of the 5-hydrogen atom in a β position and this also would account satisfactorily for the observed spectrum.

(8) Dimroth, *Ber.*, **69**, 1123 (1936).

(9) The configuration of the hydrogen atom at position 5 has been observed to influence the frequency of the C=O stretching vibration in 6-ketosteroids (see reference 10) so that a reverse effect may not be out of place. The occurrence of abnormalities in the spectra of 3-acetoxy-4-ketosteroids might also be anticipated, but no compounds of this structure have been available for study.

(10) Jones, Humphries and Dobriner, THIS JOURNAL, **72**, 956 (1950).

The phenolic 3-acetate constitutes an additional category of 3-acetoxy steroid and a strong single band is observed at 1206 cm.$^{-1}$ in compounds containing this group. The position of this band is characteristic for the phenolic acetate group.

Acetoxy Groups at Other Positions.—In Table III the frequencies of the intense bands between 1190 and 1290 cm.$^{-1}$ are listed for steroids acetylated at positions other than 3. In most of these

TABLE III

PRINCIPAL ABSORPTION BANDS BETWEEN 1180 AND 1290 CM.$^{-1}$ IN THE SPECTRA OF STEROIDS ESTERIFIED AT POSITIONS OTHER THAN THREE[a]

A. 4-ACETATE: Δ^5-cholestenediol-3β,4β-acetate-4 (1236, 1228).[6,16]

B. 7-ACETATE: 7α-acetoxy-3α,12α-dihydroxyetiocholanic acid M.E. (1237)[23]; 7α-acetoxy-3,12-diketoetiocholanic acid M.E. (1232, 1206).[23]

C. 12-ACETATE: pregnanediol-3α,12β-one-20-acetate-12 (1235)[23]; 12α-acetoxy-3-ketocholanic acid M.E. (1243)[3]; 12α-acetoxy-3-keto-4-bromocholanic acid M.E. (1240)[3]; 12α-acetoxy-3-hydroxy-7-ketocholanic acid M.E. (1242)[7]; 12β-acetoxy-3,11-diketocholanic acid M.E. (1232)[7]; Δ^9-12α-acetoxy-3α-hydroxycholanic acid M.E. (1242).[30]

D. 17-ACETATE: androstanol-17β-acetate (1242, 1215[d])[17,26]; $\Delta^{3,5}$-androstadienol-17β-acetate (1242, 1216[d])[5]; Δ^5-androstenediol-3β,17β-acetate-17 (1239, 1219[d])[16,17]; androstanol-17α-one-3-acetate (1244, 1236[d])[26]; androstanol-17β-one-3-acetate (1242, 1215[d])[26]; Δ^4-androstenol-17α-one-3-acetate (1243, 1221[d])[24,26]; Δ^4-androstenol-17β-one-3-acetate (1243, 1221[d])[26]; Δ^4-androstenol-17β-dione-3,6-acetate (1239, 1218[d])[7]; 2,2-dibromoandrostanol-17β-one-3-acetate (1242, 1215[d])[3]; 2,4-dibromoandrostanol-17β-one-3-acetate (1242, 1216[d])[3]; etiocholanediol-3α,17α-acetate-17 (1242)[7]; Δ^4-pregnenol-17β-dione-3,20 (1242, 1228).[10,21]

E. 20-ACETATE: pregnanol-20α-acetate (1244)[15*]; allopregnanol-20α-one-3-acetate (1244)[15]; pregnanediol-3α, 20α-acetate-20 (1242)[7]; allopregnanediol-17α,20-one-3-acetate-20 (1236)[23*]; Δ^4-pregnenediol-17α,20-one-3-acetate-20 (1236).[26*]

F. $\Delta^{17:20}$-ACETATE-21: $\Delta^{4,17:20}$-pregnanediol-21-one-3-acetate (1228).[26]

G. $\Delta^{17:20}$-20-(ENOL) ACETATE: $\Delta^{17:20}$-allopregnanediol 3β-20-diacetate (*trans* isomer) (1239[b], 1219)[7]; $\Delta^{17:20}$-allopregnanediol-3β-20-diacetate (*cis* isomer) (1239,[b] 1221).[7]

H. 21-ACETATE: Δ^4-pregnenetriol-17α,20,21-one-3-acetate-21 (1239, 1282[d], 1216[d]).[26]

I. 21-ACETOXY-20-KETONE: pregnanediol-3α,21-one-20-acetate-21 (1228, 1267, 1188)[23]; pregnanetriol-3α,12α-21-one-20-acetate-21 (1230, 1265, 1188)[26]; Δ^4-pregnenol-21-trione-3,11,20-acetate (1226, 1270, 1192)[17]; Δ^4-pregnenediol-11β,21-dione-3,20-acetate-21 (1228, 1270, 1208, 1188)[30]; Δ^1-allopregnenol-21-dione-3,20-acetate (1225, 1196, 1256, 1269)[3]; $\Delta^{1,3,5,10}$-3-methoxy-17(β-acetoxyacetyl)-estratriene (1228, 1251, 1276, 1200).[3,19]

J. DIACETATES. *17-Hydroxy-20,21-diacetate:* Δ^4-pregnenetriol-17β,20,21-one-3-diacetate-20, 21 (1236, 1220)[14,15,26]; Δ^4-pregnenetriol-17α,20,21-one-3-diacetate-20,21 (1222, 1236).[26*,27*]

2,3-Diacetates: gitogenin diacetate (1244, 1228)[17,24]; kammogenin diacetate (1236, 1228)[17]; 12-dihydromannogenin diacetate (1242, 1228)[17]; mexogenin diacetate (1242, 1219)[17]; smilagenin diacetate (1242, 1219)[17]; yuccagenin diacetate (1233–1242,[c] 1249).[17]

3,4 - Diacetate: Δ^5 - cholestenediol - 3β,4α - diacetate (1236)[6,16]

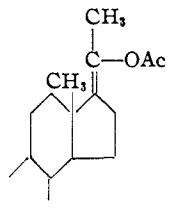
K. OTHER ESTERS: Δ^4-androstenol-17β-one-3-propionate (1188)[27]; Δ^5-androstenediol-3β,17β-acetate-3-benzoate-17 (1240,[b] 1275)[26]; Δ^4-androstenol-17β-one-3-benzoate (1271)[26]; Δ^4-6-bromandrostenol-17β-one-3-benzoate (1271).[3]

[a] For identification of source see Table I. [b] This band attributed to the 3-acetate group. [c] Broad band. [d] Weak band.

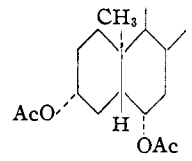
there is a sharp peak near 1240 cm.$^{-1}$ similar to the type A band of the 3-acetates. In the 12α-acetates this band occurs at 1240–1242 cm.$^{-1}$ (4 examples) and in the 12β-acetates at 1232–1235 cm.$^{-1}$ (2 examples). The 17β-acetates possess a strong band at 1242 cm.$^{-1}$ usually accompanied by a weak band or inflection at 1215–1225 cm.$^{-1}$.

The 21-acetoxy-20-ketones possess a strong band at 1228–1230 cm.$^{-1}$ accompanied by weak subsidiaries near 1192 and 1265–1270 cm.$^{-1}$ (Fig. 5). The $\Delta^{17:20}$-enol-acetates (X) show a strong band at 1219–1225 cm.$^{-1}$.

In diacetates in which the two acetate groups are well separated in the molecule the individual acetate groups appear to absorb independently (*e.g.*, estradiol diacetate with maxima at 1242 and 1207 cm.$^{-1}$). The 2,3-diacetate group present in certain sapogenin acetates gives two bands of comparable intensity at 1228 and 1236–1248 cm.$^{-1}$. In 3α,6α-diacetoxycholanic acid methyl ester (XI) there are two peaks at 1246 and 1234 cm.$^{-1}$.



X



XI

Other esters of steroid alcohols may show bands of comparable intensity in the same general region of the spectrum. Steroid formates possess a band at 1180–1188 cm.$^{-1}$ in agreement with observations of Thompson and Torkington[2] on simple alkyl formates. Steroid benzoates give a single band at 1270 cm.$^{-1}$. The *p*-toluenesulfonate esters (which contain no C–O linkage) give strong bands at 1100 and 1178–1180 cm.$^{-1}$. The carbomethoxy group in the methyl esters of cholanic, norcholanic, bisnorcholanic and etiocholanic acids also has a strong band near 1180 cm.$^{-1}$.

Nature of the Multiple Bands of Type B Spectra.—In seeking for common structural features as between I and II on the one hand, and III and IV on the other, it may be significant that in III and IV both the acetate and the 5-hydrogen atom project from the same side of the ring system, whereas in I and II they are *trans* related. Some interaction involving the vibration of the 5-hydrogen atom and the 3-acetoxy group may play a part in giving rise to the Type B spectrum.

That these groups are closer in III and IV than in I and II is indicated by a consideration of their conformations in terms of the concept of equatorial and polar bonds.[11] In the four structures I–IV the conformation of the 5-hydrogen atom is polar with respect to ring A; in I and II the 3-acetate bond is equatorial, and in III and IV the 3-acetate bond is polar. In the chair form of the cyclohexane ring system, polar bonds are more hindered than equatorial bonds,[11] so that the polar–polar conformations at positions 3 and 5 in III and IV

(11) Barton, *Experientia*, **6**, 316 (1950); see particularly structures XIII and XIV.

will be more hindered than the equatorial–polar conformations in I and II.[11a]

The question arises as to whether the several bands of the Type B spectrum are all associated with the same intrinsic vibration of the acetoxy group or whether the secondary bands are associated with other centers in the molecule and are superimposed on a single acetate band common to both the Type A and the Type B spectra. Some information concerning this can be derived from a study of the band intensities.

Measurements of the intensities of both Type A and Type B acetate bands have been carried out for a selected group of compounds. The apparent molecular extinction[12] ($E^{s}_{a_{max.}}$) of the most intense maximum of the group has been measured and also the apparent integrated absorption intensity (B) over the range 1190 to 1290 cm.$^{-1}$. The values are listed in Table IV.

TABLE IV

INTENSITIES OF ACETATE BANDS

Carbon disulfide solution

Compound	Max. apparent molecular ext. coeff.[a]	Apparent integrated absorption intensity[b]
A. Acetates of structures I, II, V giving single bands		
Androstanol-3β-one-17-acetate	620	3.90
Allopregnanol-3β-one-20-acetate	760	4.44
Cholestanol-3β-acetate	690	4.12
Ergostanol-3β-acetate	750	4.19
Etiocholanol-3α-acetate	910	4.42
Δ^5-Androstenol-3β-one-17-acetate	720	4.33
Δ^5-17-Ethynylandrostenediol-3β,17β-acetate-3	710	4.40
Δ^5-Pregnenol-3β-one-20-acetate	790	4.68
B. Acetates of structures III, IV giving multiple bands		
Androstanol-3α-one-17-acetate	540	4.04[c]
Androstanol-3α-one-17-acetate	520	3.93[c]
Allopregnanol-3α-one-20-acetate	570	4.41
Cholestanol-3α-acetate	560	4.10
Etiocholanol-3β-one-17-acetate	580	4.53
C. 17-Acetate		
Δ^4-Androstenediol-3β-17β-acetate-17	730	4.57
D. 3-Acetate of uncertain stereochemical structure[d]		
Lumistanol-3β-acetate	550	4.13

[a] Slit width 5 cm.$^{-1}$. [b] Integration limits 1190–1290 cm.$^{-1}$. One intensity unit equals 10^4 mole^{-1} liter cm. [c] Independent measurements on different samples. [d] See footnote 7 to text.

It will be observed that for the Type A band, the extinction coefficient at the maximum lies between 620 and 910 while for the most intense peak of the Type B spectra the value is lowered to 540–580. This suggests that in the complex band groups of Type B spectra the secondary bands arise from a splitting of the single band into a number of components. If the secondary bands represented additional structure superimposed onto a single "acetate band" common to Type A and Type B spectra they would be expected to enhance the peak intensity and not to diminish it.

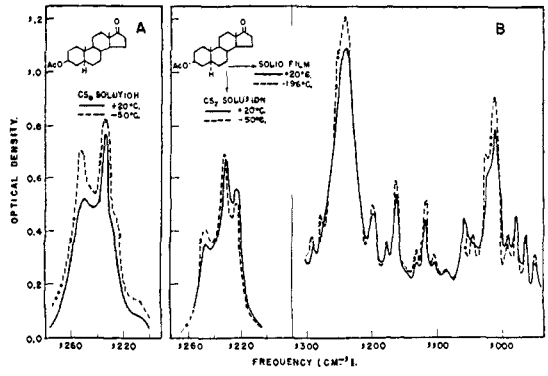
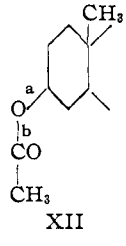


Fig. 5.—Effect of temperature change on characteristic acetate bands: A, etiocholanol-3β-one-17; B, androstanol-3α-one-17.

Consideration of the integrated absorption intensities supports the same view. For the Type A band the intensities lie between 3.90 and 4.66 intensity units and the Type B band intensities fall closely in the same range (3.96–4.53). Superposition of additional bands onto a common "acetate band" in the Type B spectra would be expected to increase the integrated absorption intensity.

If the bands in the Type B spectra have a common origin in one of the two C–O stretching vibrations, two hypotheses come under consideration. The multiple band system can result from the presence, in the solution, of a number of different molecular species, each of which gives rise to one of the bands of the group. Such differing molecular species might be rotational isomers in which the barriers to free rotation about bonds a or b of XII are such as to permit two or more equilibrium positions of comparable but slightly differing energy.



XII

Alternatively, if such an equilibrium among labile isomers is excluded, the multiple bands must be assumed to be intrinsic to the spectrum of each individual molecule.

The spectrum of an equilibrium mixture of labile isomers would be expected to be more sensitive to temperature changes than a single molecular species, and in an endeavor to distinguish between these two hypotheses, two acetates giving Type B spectra have been examined in carbon disulfide solution at +20° and −50°. The curves, shown in Fig. 5 do exhibit significant changes in relative intensities of the peaks for a temperature change of 70°. While these results are not considered con-

(11a) In III and IV the steric effect of the polar hydrogen at position 1 must also be considered, but this is probably small, since in the Δ^5-3β-acetoxy compounds the spectrum reverts to type A.

(12) The factors which have to be considered in the evaluation of the intensities of infrared absorption bands have been discussed elsewhere (see references 13, 14), and these papers should also be consulted for definitions of the apparent molecular extinction coefficient and the apparent integrated absorption intensity.

(13) Marion, Ramsay and Jones, THIS JOURNAL, **73**, 305 (1951).

(14) Jones, and Dobriner, *Vitamins and Hormones*, **7**, 293 (1949).

clusive, they tend to support the hypothesis of an equilibrium mixture of labile isomers.[15]

Experimental.—The spectra were determined on Perkin–Elmer model 12c spectrometers using sodium chloride prisms and carbon disulfide solutions. For the low temperature measurements in solution a cell similar to that described by Bernstein[16] was employed, and for measurements of cooled solid films a Hornig type cell was employed.[17]

For the evaluation of the integrated absorption intensities the curves were plotted as apparent optical density ($\log_{10}(T_0/T)_\nu$) against the frequency in wave numbers on mm. graph paper. The area under the curve between 1190 and 1290 cm.$^{-1}$ was computed by counting squares and the apparent integrated absorption intensity (B) evaluated from

$$B = \frac{e}{cl}\int_{1190}^{1290} \log_{10}\left(\frac{T_0}{T}\right)_\nu d\nu \text{ mole}^{-1}/\text{liter/cm.}^{-2}$$

the equation in which T_0 and T are the intensities of the incident and transmitted radiation when the spectrometer is set at a frequency ν, c is the concentration of the solute in moles per liter, l the cell length in cm. and e the base of the natural logarithms. The intensities were measured at a slit width of 5 cm.$^{-1}$.

Concluding Remarks.—The recognition of the principal *functional groups* in steroids by infrared spectrometry, and the correlation of the position of the group in the molecule with the frequency of certain absorption bands has been reported,[3,7,10] and recently summarized.[14] Although the suggestion has been made previously[18] that the *stereochemical configuration* of a steroid may be associated with characteristic absorption bands in the "fingerprint" region, the studies described here provide the first instance in which we have observed such a correlation to hold over a wide variety of compounds. The use of this correlation, in association with the digitonin precipitation reaction, for the unique assignment of the stereochemical configuration at positions 3 and 5 provides an additional example of the importance of considering spectrographic and chemical evidence together in the elucidation of molecular structure.

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 Miss L. Groth, 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), Ayerst, McKenna and Harrison, Ltd., the Jane Coffin Childs Memorial Fund for Medical Research, The Commonwealth Fund, the National Cancer Institute of the National Institutes for Medical Research, U. S. Public Health Service and the Anna Fuller Fund.

(15) In an attempt to achieve a greater temperature difference, the spectrum of a solid film of androsterone acetate was measured at room temperature and when cooled with liquid nitrogen. The curve, which is included in Fig. 5, indicated that at room temperature the acetate band system is less well resolved than in carbon disulfide solution. On cooling the acetate bands are not appreciably affected, although neighboring bands sharpen considerably.

(16) Powling and Bernstein, THIS JOURNAL, **73**, 1815 (1951). The assistance of Dr. Bernstein in making these measurements is gratefully acknowledged.

(17) Wagner and Hornig, *J. Chem. Phys.*, **18**, 296 (1950).

(18) Furchgott, Rosenkrantz and Shorr, *J. Biol. Chem.*, **163**, 375 (1946); *ibid.*, **167**, 627 (1947).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Study of Protein–Ion Interaction by the Moving Boundary Method. The Combination of Bovine Serum Albumin with Chloride Ion[1]

BY ROBERT A. ALBERTY AND H. H. MARVIN, JR.[2]

The binding of chloride ions by crystallized bovine serum albumin in 0.15 molar sodium chloride at 0° has been determined by the moving boundary method. This method involves the accurate measurement of the chloride and sodium constituent mobilities in a protein solution and interpretation of the results in terms of the moving boundary equation for weak electrolytes. The measurements at *p*H 7.00, 5.40 and 3.20 indicate the binding of 8, 9 and 29 chloride ions per molecule of albumin, in excellent agreement with the values obtained by the membrane equilibrium and electromotive force methods.

Evidence for the binding of chloride ion by serum albumin has been obtained by a variety of physical measurements: these include osmotic pressure,[3] membrane equilibrium[4] electromotive force[4] and shift in isoelectric[5,6] and isoionic[7] points. In view of the importance of this particular interaction in determining the properties of serum albumin under physiological conditions we have undertaken a study of the binding of chloride ion by crystallized bovine serum albumin in 0.15 molar sodium chloride by the moving boundary method. This method has recently been described[8,9] and has been applied by Smith and Briggs[9] to the study of the interaction of bovine serum albumin with methyl orange. The method depends upon the measurement of the constituent mobility of the interacting ion and of the protein in a solution of known composition and is applica-

(1) This material is taken from the Ph.D. thesis of H. H. Marvin, Jr., University of Wisconsin, August, 1950.

(2) United States Rubber Company Fellow, 1949–1950. Research Laboratory, General Electric Co., Schenectady, N. Y.

(3) G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

(4) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., *ibid.*, **72**, 535 (1950).

(5) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, **53**, 126 (1949).

(6) R. A. Alberty, *ibid.*, **53**, 114 (1949).

(7) G. Scatchard and E. S. Black, *ibid.*, **53**, 88 (1949).

(8) R. A. Alberty and H. H. Marvin, Jr., *ibid.*, **54**, 47 (1950).

(9) R. F. Smith and D. R. Briggs, *ibid.*, **54**, 33 (1950).