saline and water. The dried extract was evaporated to afford a white crystalline solid (mixture of IXa and b).

The mixture was dissolved in tetrahydrofuran (250 ml.) and ether (50 ml.). Lithium aluminum hydride (3.0 g.) was added, and the mixture was refluxed for 4 hours (and then allowed to stand at room temperature overnight). The excess hydride was decomposed cautiously with water, ethyl acetate was added, and the inorganic precipitate was removed by filtration. The solid was triturated several times with benzene–ethyl acetate. The extracts were combined, washed with saturated saline and water, dried and evaporated. This afforded a white crystalline solid which was dissolved in pyridine (15 ml.) and acetylated with acetic anhydride (7.5 ml.) (4 days at room temperature). The mixture was poured into ice-water, and was extracted with ethyl acetate. The extract was washed with saturated saline and water, and was dried. Evaporation gave a white crystalline solid which was recrystallized from acetone–petroleum ether; 2.35 g., m.p. 207–227° with previous softening. Further recrystallization did not appreciably alter the wide-range melting point, 209–227° with previous softening. The latter solid together with its evaporated mother liquor was dissolved in benzene (200 ml.), and adsorbed on a silica gel column (120 g., ether washed and re-dried at 110°). The product was eluted with 1 l. of 5% acetone–ether, and was crystallized from acetone–petroleum ether to give practically pure Xb; 0.97 g., m.p. 228–230° with previous softening. Three further crystallizations from acetone–petroleum ether gave pure Xb, m.p. 231–233° with previous softening; infrared: λ_{max}^{KBr} 3530, 1748 and 1100 cm.$^{-1}$; $[\alpha]^{24}D$ +4.1° (24.71 mg., αD +0.05°), $[M]D$ +21.

Anal. Calcd. for $C_{27}H_{42}O_9$ (510.61): C, 63.51; H, 8.29. Found: C, 63.66; H, 8.44.

B.[12]—The 5α,6α-oxide (IXa, about 0.7 g.) of hydrocortisone acetate bis-ketal was dissolved in tetrahydrofuran (90 ml.), and lithium aluminum hydride (1.25 g.) was added. The mixture was refluxed for 3 hours, cooled, and was treated cautiously with water. Ethyl acetate (*ca.* 100 ml.) was added, and the inorganic precipitate was removed by filtration. The product was worked up by extraction with ethyl acetate. Evaporation gave a white powder which was recrystallized from acetone–petroleum ether. This gave 410 mg. of the 5α,11β,17α,21-tetrol-bis-ketal Xa, m.p. 258–261°. Acetylation at room temperature (72 hours) with acetic anhydride (1.5 ml.) and pyridine (4 ml.) followed by the addition of water gave 390 mg. of the 21-acetate Xb, m.p. 227–230°. Its infrared absorption spectrum was practically identical with that of preparation A.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 3,20-Bis-ethylene Ketal (Xa).—The 21-acetate bis-ketal (Xb, 0.50 g.) was saponified by being refluxed for 0.5 hour with 2.5% alcoholic potassium hydroxide (12 ml.). Water was added to the cooled solution and it was allowed to stand overnight at 5°. The crystals were collected and washed with water. In this manner there was obtained 0.40 g. of pure Xa, m.p. 261.5–264° with previous softening. Recrystallization from acetone–petroleum ether did not alter the m.p.; infrared: λ_{max}^{KBr} 3510 and 1100 cm.$^{-1}$; $[\alpha]^{24}D$ +6.5° (15.44 mg., αD +0.05°), $[M]D$ +32.

Anal. Calcd. for $C_{26}H_{40}O_8$ (468.57): C, 64.08; H, 8.60. Found: C, 64.01; H, 8.61.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione (XIa).—The 5α,11β,17α,21-tetrol bis-ketal (Xa, 0.42 g.) was dissolved in methanol (23 ml.), and was hydrolyzed by being refluxed for 10 minutes with 8.5% (v./v.) sulfuric acid (2.3 ml.). Water was added, the solution was neutralized with sodium bicarbonate and the mixture was saturated with salt. Crystals were formed on scratching the flask, and they were collected by filtration. In this manner there was obtained 0.12 g. of crude XIa, m.p. 251.5–255° with previous softening, browning and decomposition. Two crystallizations from acetone improved the m.p., but did not remove the small amount of Δ^4-3-ketone found present in the crude material. Consequently, the crystalline material, mother liquors and a benzene extract of the reaction mixture were combined and evaporated to dryness. The solid residue was dissolved in 50% aqueous methanol (100 ml.), and was extracted ten times with 100-ml. portions of benzene. The aqueous methanol phase was evaporated (the water was distilled azeotropically with benzene). Several crystallizations from acetone gave 59 mg. of pure XIa, m.p. 261–264° with previous softening, browning and decomposition; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{KBr} 3530 and 1710 cm.$^{-1}$; positive Blue Tetrazolium test; $[\alpha]^{24}D$ +75° (9.30 mg., pyridine, αD +0.35°), $[M]D$ +285.

Anal. Calcd. for $C_{21}H_{32}O_6$ (380.47): C, 66.30; H, 8.48. Found: C, 66.29; H, 8.54.

Pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-Acetate (XIb).—The free steroid (XIa, 20 mg.) was dissolved in pyridine (0.5 ml.) and was treated with acetic anhydride (0.5 ml.) at room temperature for 65 hours. Addition of water to the cooled mixture gave 14 mg. of XIb, m.p. 241–244° with previous softening. Recrystallization from acetone–petroleum ether gave 12 mg., m.p. 241–244.5° with previous softening; ultraviolet: $\lambda_{max.}$ none (end absorption only); infrared: λ_{max}^{KBr} 3530, 3440, 1744, 1724 (shoulder) 1710 and 1245 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{34}O_7$ (422.50): C, 65.38; H, 8.11. Found: C, 65.10; H, 8.28.

PEARL RIVER, NEW YORK

[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

Characteristic Infrared Absorption Bands of Steroids with Reduced Ring A. I. Tetrahydro Compounds[1]

BY HARRIS ROSENKRANTZ AND PAUL SKOGSTROM

RECEIVED SEPTEMBER 23, 1954

A band for band analysis has been made of the infrared spectra of 50 3-hydroxy reduced ring A steroids and 57 related acetylated derivatives. The spacial isomers of the 3,5-centers of the free steroids could be differentiated by their absorption as follows: 3α,5α-structures by a band near 1005; 3β,5β- by one near 1035; 3β,5α- by absorption near 1044, 995, 978 and 956 and 3α,5β-arrangements by a band near 1041 cm.$^{-1}$. C-21 *cis* forms also gave rise to a band near 932 while the *trans* orientations absorbed nearer 941 cm.$^{-1}$. In the acetate spectra no relationship between the number of acetate groups and acetate absorption bands could be found. Some possibilities of characteristic acetate absorptions have been discussed. A combination of weak to medium weak bands near 1311, 1265, 1242, 1217 and 1127 cm.$^{-1}$ may aid eventually in the spectroscopic characterization of steroid substances.

A most thorough endeavor has been projected by several investigators in the search for steroid structure–infrared absorption correlations. Dobriner, Katzenellenbogen and Jones[2] have climaxed this

(1) Supported by a grant from the Medical Research and Development Board, Office of the Surgeon General, Department of the Army under Contract No. DA-49-007-MD-310.

(2) K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, "Infrared Absorption Spectra of Steroids, An Atlas," Interscience Publishers, Inc., New York, N. Y., 1953.

endeavor with the publication of 308 infrared spectra in an atlas while Jones and Herling[3] have reviewed the present status of frequency assignment in steroid infrared curves. A practical discussion of infrared techniques and spectra interpretation soon will appear.[4]

The possibility of correlating a particular absorption band in the fingerprint region with a distinct structural unit is difficult since most of these frequencies originate from complex vibrations of the molecule. It might be more fruitful to examine absorption curves for several frequencies which may be related to specific structural configurations. The present study is an attempt to uncover such combinations of bands which then may be useful in establishing structural units of unknown steroids. Since a significant number of reduced ring A steroids have been isolated by members of this Foundation, it was natural to investigate these substances in the infrared. The present study will show that the *cis/trans* arrangements of the 3,5-centers in tetrahydro steroids gave rise to certain characteristics in the fingerprint region which afford a stronger basis for spectroscopically resolving the structures of all 4 spatial isomers. A combination of other bands have been observed in all the steroid spectra investigated and eventually may be useful in characterizing steroid substances. The analysis of dihydro compounds will be the subject of a subsequent report.

Method

The spectra were recorded on a 12C Perkin–Elmer infrared spectrometer between 3700–770 cm.$^{-1}$. The compounds were deposited from suitable solvents as solid films (500 μg.) or studied in carbon disulfide (10 mg./ml. in a one mm. cell or 150 μg. 0.1 ml. in a 3 mm. micro cell). All pertinent spectra in Dobriner's atlas were scrutinized and data from them were included in the tables to be discussed. The large number of infrared spectra make it impractical to publish the curves at this time but fortunately many of the acetate derivatives appear in the steroid atlas.[2]

Analysis of Spectra

In a band for band analysis of this large number of reference compounds some correlations of structure to absorption became apparent. These were related to the *cis/trans* configuration of the 3,5-centers. The earlier findings on steroids (5a, b) have been confirmed and with more samples available the 3β,5β-characteristic absorption has been substantiated. The shift of the pertinent bands to higher frequencies in solid films indicated the effect of hydrogen bonding. The 3α,5α-structures had a consistent absorption of strong intensity between 1009–1001 for solid films (F) and 1001-996 for solutions (S); 3β,5α-, 1054-1038 (F) and 1043-1037 (S); 3α,5β-, 1044-1036 (both solid and solution) and 3β,5β-, 1036-1031 cm.$^{-1}$ (both solid and solution).

Since the possibility existed for confusion in the interpretation of the 3α,5β-, 3β,5α- and 3β,5β-arrangements due to frequency overlap under poor instrument or sample conditions, other bands were examined for correlation with these structural units. A combination of absorption bands of weak to medium weak intensity were found between 1000–950 cm.$^{-1}$ which may be useful in distinguishing between the 3,5-isomers. Table I lists the bands in this region of the spectra and it appears that the 3β,5α-arrangement consistently gives rise to characteristic frequencies near 995, 978 and 956 cm.$^{-1}$. When this finding is combined with the correlations between 1100–1000 cm.$^{-1}$ and the apparent lack of absorption near 947 cm.$^{-1}$, the 3β,5α-isomer can be resolved from the other three possible spatial orientations. The 966 and 969 bands of allopregnane-3β,-20β-diol and allopregnane-3β,20α-diol, respectively, were not included in the averaging of the 956 cm.$^{-1}$ absorption since the former seemed to be displaced and may be characteristic of these non-ketonic steroids.

The necessity for clearly differentiating 3α,5β- and 3β,5β-structures under adverse situations still remained. Some solution to this problem was attained since it was observed in C-21 spectra that 3α,5β- and 3β,5β-structures were apparently responsible for an absorption of medium weak to medium intensity near 932 while the 3β,5α- and 3α,5β-isomers absorbed near 941 cm.$^{-1}$. Pregnane-3α,-17α,20α-triol, pregnane-3α,20α-diol and pregnane-3α,20β-diol which have no carbonyl functions were exceptions. A study of the corresponding region of C-19 infrared curves revealed too much variation in frequency to be useful.

The spectra of a considerable number of acetate derivatives of tetrahydro compounds also were examined and the absorption bands to be used for correlations are given in Table II. Side inflections also have been listed although these were not considered by Jones, *et al.*,[6] in the interpretation of 3-acetoxy spectra. The bands in the region of acetate absorption (1250 cm.$^{-1}$) and those between 1110–1000 cm.$^{-1}$ offered possibilities of assignment to structure. However, the data in Table II clearly demonstrate that no relationship exists between the number of acetate groups on the molecule and the number of absorption bands near 1250 cm.$^{-1}$. The finding of Jones, *et al.*,[6] that 3-acetate *cis* structures have complex bands and the corresponding *trans* compounds have one major band near 1239 cm.$^{-1}$ has been confirmed. In the C-21 series 21-hydroxy di- and triacetates and 21-desoxy monoacetates both with the 3β,5α-arrangement could be differentiated from each other by the 1239 and 1247 bands and from all other compounds by an absorption near 1220 cm.$^{-1}$. The C-19 acetates which gave rise to a side inflection near 1221 could be segregated from the above postulation because of the absence of a band near 1267 cm.$^{-1}$.

A band in the 1110–1000 cm.$^{-1}$ region appeared to be related to the steric orientations of the three acetate and five hydrogen centers. Two monoacetates, allopregnane-3β,17α,21-triol-11,20-dione-21-acetate and pregnane-3β,17α,21-triol-11,20-dione-21-acetate, did not have their group at position three but gave rise to bands in the range seen for the other steroids. Apparently acetates at differ-

(3) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(4) H. Rosenkrantz, "Methods in Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., 1955, Vol. 2, pp. 1–55.

(5) (a) A, R. H. Cole, R. N. Jones and K. Dobriner, THIS JOURNAL, **74**, 5571 (1952); (b) H. Rosenkrantz and L. Zahlow, *ibid.*, **75**, 903 (1953).

(6) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.*, **73**, 3215 (1951).

TABLE I

COMBINATIONS OF CHARACTERISTIC FREQUENCIES IN THE SPECTRA OF REDUCED RING A STEROIDS[1]

Compound[2]	Frequency combinations correlated with the 3,5-centers					
A, 3α,5α						
Allopregnane-3α,17α,21-triol-11,20-dione[F,a]	....	982M	...	947W	...	931M
Allopregnane-3α,11β,17α,21-tetrol-20-one[F,b]	....	983M	...	...	...	935M
Allopregnane-3α,17α,21-triol-20-one[F,c]	997W	980W	955W	...	...	935M
Allopregnan-3α-ol-20-one[F,b]	....	981M	960W	...	...	928M
Cholestan-3α-ol[S]	....	976S	954S	...	...	929M
Androstan-3α-ol[S]	985M	971M	...	951M	939W	...
Androstan-3α-ol-17-one[S,d]	....	974M	964M	...	...	...
Androstan-3α-ol-11,17-dione[F,a]	985W	970M	...	947M	...	931M
Androstane-3α,11β-diol-17-one[S,e]	991W	978M	963M	951W	...	934M
Androstane-3α,17β-diol[F,g]	990W	979W	964M	944W	...	...
B, 3β,5α						
Allopregnane-3β,17α,21-triol-11,20-dione[F,a]	998W	978W	957W	...	941W	927M
Allopregnane-3β,11β,17α,21-tetrol-20-one[F,a]	993W	978W	961W	...	942W	...
Allopregnane-3β,17α,21-triol-20-one[F,h]	998W	978W	955W	...	943M	...
Allopregnane-3β,11β,21-triol-20-one[F,i]	992W	978W	960W	...	943M	...
Allopregnane-3β,11β,17α,20β,21-pentol[F,a]	990W	968M	959M	...	943M	...
Allopregnane-3β,17α-diol-20-one[F,b]	995M	979W	955M	...	943W	934W
Allopregnan-3β-ol-20-one[S,b]	999M	982W	954M	...	937W	...
Allopregnane-3β,20β-diol[F,j]	999M	980W	966W	...	939M	...
Allopregnane-3β,20α-diol[F,c]	990M	979W	969W	...	938W	...
Ergostan-3β-ol[S]	1000W	980W	954M	...	...	935S
Cholestan-3β-ol[S]	992W	978W	953M	...	...	928S
Androstan-3β-ol[S]	996W	980W	957M	...	...	935M
Androstane-3β,17α-diol[F,g]	994W	980W	956M	...	...	...
Androstane-3β,17β-diol[F,g]	994W	979W	956M	...	...	...
Androstan-3β-ol-17-one[S,d]	992W	977W	954M	...	...	932M
C, 3α,5β						
Pregnane-3α,17α,21-triol-11,20-dione[F,d]	995W	...	956W	...	940W	...
Pregnane-3α,11β,17α,21-tetrol-20-one[F,e]	....	983M	954M	...	942M	...
Pregnane-3α,17α,21-triol-20-one[F,e]	....	981M	952M	...	937M	...
Pregnane-3α,17α-diol-11,20-dione[F,e]	988M	...	958M	...	936M	...
Pregnane-3α,17α-diol-20-one[F,b]	989W	...	952M	...	943W	...
Pregnan-3α-ol-11,20-dione[F,k]	....	969W	954W	...	938M	...
Pregnan-3α-ol-20-one[S,d]	1000W	975M	...	...	944M	929W
Pregnane-3α,17α,20α-triol[F,e]	997M	975M	971M	952M	...	...
Pregnane-3α,20α-diol[F,d]	996M	...	962M	950M	...	...
Pregnane-3α,20β-diol[F,e]	999M	972M	962W	945M	...	...
Pregnane-3α,20α-diol-11-one[F,e]	983W	967W	961W	...	941M	...
Pregnane-3α,20β-diol-11-one[S]	988W	971M	960W	0	0	0
Coprostan-3α-ol[S]	992W	...	962M	...	941S	...
Etiocholan-3α-ol[S]	988W	...	951W	...	939W	932W
Etiocholan-3α-ol 17-one[S,m]	989M	...	965W	946W	...	933W
Etiocholan-3α-ol-11,17-dione[S,e]	990W	975W	963W	952W	...	...
Etiocholane-3α,11β-diol-17-one[S,e]	990W	975W	960W	952W	...	935W
Etiocholane-3α,17β-diol[F,e]	....	972M	...	949W	...	...
D, 3β,5β						
Pregnane-3β,17α,21-triol-20-one[F,e]	999W	973M	960M	949M	...	936M
Pregnane-3β,11β,17α,21-tetrol-20-one[F,e]	996W	...	955M	...	942W	933M
Pregnane-3β,17α-diol-20-one[F,d]	991M	974W	...	951M	...	933W
Pregnan-3β-ol[S]	998W	...	961M	946W	...	933W
Coprostan-3β-ol[S]	986M	...	958S	...	...	927W
Etiocholan-3β-ol[S]	982M	...	959M	948M	...	930W
Etiocholan-3β-ol-17-one[S,m]	990W	...	965S	951W	...	934W

[1] The letters following the frequencies denote relative intensity: S = medium strong to strong, M = medium weak to medium and W = weak; 0 = no determination available while B refers to side inflections.

[2] The capital letter superscript following the name of each compound refers to the physical state of the preparation, F = solid film and S = solution, and the small letters to the source of the compound which is given below. Where no reference to source is given, data may be found in references 2 and 6. We wish to thank the following for samples of compounds investigated: [a] E. Caspi. [b] H. Levy. [c] F. Ungar. [d] R. I. Dorfman. [e] S. Burstein. [g] Ciba Pharmaceutical Co. [h] E. Forchielli. [i] L. P. Romanoff. [j] C. Reyneri, [k] B. L. Rubin. [l] K. Savard and [m] D. K. Fukushima.

TABLE II

FREQUENCY CHARACTERISTICS OF ACETATE DERIVATIVES OF REDUCED RING A STRUCTURES[1]

Compound	Frequency correlations Acetate bands			3,5-Centers
A, 3α,5α				
Allopregnane-3α,21-diol-20-one-3,21-diacetate[S,d]	1263M	....	1235S	1021S
Allopregnane-3α,11β,17α,21-tetrol-20-one-3,21-diacetate[F,a]	1266S	....	1238S	1020M
Allopregnane-3α,20α-diol-3,20-diacetate[S]	1256S	1247S	1239S	1018S
Cholestan-3α-ol-3-acetate[S]	1258S	1250S	1241S	1018S
Androstane-3α,17β-diol-3,17-diacetate[S,c]	....	1248S	1239S	1020S
Androstan-3α-ol-3-acetate[S]	1255S	1245S	1233S	1015S
Androstane-3α,16α,17β-triol-3,16,17-triacetate[S]	....	1247S	1239S	1018M
Androstan-3α-ol-17-one-3-acetate[S]	1258S	1248S	1238S	1017S
B, 3β,5α				
Allopregnane-3β,17α,21-triol-11,20-dione-21-acetate[F,n]	1258S	....	1233S	1034S
Allopregnane-3β,17α,21-triol-11,20-dione-3,21-diacetate[F,n]	1263S	1235S	1224B	1033M
Allopregnane-3β,11β,17α,21-tetrol-20-one-3,21-diacetate[F,a]	1264S	1241S	1233S	1030M
Allopregnane-3β,17α,21-triol-20-one-3,21-diacetate[F,h]	1263S	1235S	1222B	1032M
Allopregnane-3β,17α,20β,21-tetrol-11-one-3,20,21-triacetate[F,a]	1271B	1241S	1221B	1034M
Allopregnane-3β,11β,17α,20β,21-pentol-3,20,21-triacetate[F,a]	1266B	1239S	1222B	1035M
Allopregnane-3β,20β,21-triol-3,20,21-triacetate[S]	1272B	1241S	1222B	1022S
Allopregnane-3β,17α,20β,21-tetrol-3,20,21-triacetate[S]	0	0	0	1023S
Allopregnane 3β,21-diol-20-one-3,21-diacetate[S]	0	0	0	1027S
Allopregnane-3β,11β,21-triol-20-one-3,21-diacetate[S]	0	0	0	1028S
Allopregnane-3β,17α,21-triol-20-one-3,21-diacetate[S]	0	0	0	1024S
Allopregnane-3β,21-diol-11,20-dione-3,21-diacetate[S]	0	0	0	1028S
Allopregnane-3β,17α,diol-11,20-dione-3-acetate[F,n]	1267B	1248S	1220S	1032S
Allopregnane-3β,17α-diol-20-one-3-acetate[F,d]	1266S	1248S	1220M	1034S
Allopregnan-3β-ol-20-one-3-acetate[S,i]	1267B	1244S	1220B	1031S
Allopregnane-3β,20α-diol-3,20-diacetate[S]	....	1242S	....	1023S
Allopregnane-3β,20β-diol-3,20-diacetate[S]	....	1244S	....	1023S
Allopregnane-3β,17α,20α-triol-3,20-diacetate[S]	0	0	0	1022S
Allopregnane-3β,17α,20β-triol-3,20-diacetate[S]	0	0	0	1024S
Allopregnan-3β-ol-11,20-dione-3-acetate[S]	0	0	0	1029S
Ergostan-3β-ol-3-acetate[S]	....	1240S	....	1028S
Androstane-3β,17β-diol-3,17-diacetate[S,c]	....	1248S	....	1032S
Androstan-3β-ol-3-acetate[S]	....	1240S	....	1027S
Androstan-3β-ol-17-one-3-acetate[S]	....	1242S	....	1024S
Androstane-3β,11β-diol-17-one-3-acetate[S]	0	0	0	1020S
C, 3α,5β				
Pregnane-3α,17α,21-triol-11,20-dione-3,21-diacetate[F,e]	1261S	1247S	1236S	1024M
Pregnane-3α,11β,17α,21-tetrol-20-one-3,21-diacetate[F,e]	1267S	1248M	1241S	1025M
Pregnane-3α,17α,21-triol-20-one-3,21-diacetate[F,c]	1267S	1255S	1241S	1021M
Pregnane-3α,17α,20α,21-tetrol-11-one-3,20,21-triacetate[S]	0	0	0	1026S
Pregnane-3α,17α-diol-20-one-3-acetate[S,d]	....	1245S	1221B	1031S
Pregnane-3α,20α-diol-11-one-3,20-diacetate[S]	0	0	0	1026S
Pregnane-3α,20β-diol-11-one-3,20-diacetate[S]	....	1241S	....	1030S
Pregnane-3α,6α-diol-20-one-3,6-diacetate[S]	....	1247S	1238S	1028S
Pregnan-3α-ol-11,20-dione-3-acetate[S]	....	1242S	....	1030S
Pregnane-3α,20α-diol-3,20-diacetate[S]	....	1245S	....	1026S
Pregnan-3α-ol-20-one-3-acetate[S]	....	1242S	1221B	1030S
Etiocholan-3α-ol-17-one-3-acetate[S,k]	....	1239S	1218B	1029S
Etiocholan-3α-ol-11,17-dione-3-acetate[S,o]	....	1241S	1222B	1032S
Etiocholane-3α,17β-diol-3,17-diacetate[S,c]	....	1239S	1222B	1032S
Etiocholan-3α-ol-3-acetate[S]	....	1242S	....	1030S
Etiocholane-3α,17β-diol-11-one-3,17-diacetate[S]	....	1241S	1222B	1029S
Etiocholane-3α,11β-diol-17-one-3-acetate[S]	0	0	0	1029S
D, 3β,5β				
Pregnane-3β,17α,21-triol-20-one-3,21-diacetate[F,c]	1263S	....	1238S	1029M
Pregnane-3β,17α,21-triol-11,20-dione-21-acetate[F,e]	1266M	....	1238S	1028M
Pregnane-3β,11β,17α,21-tetrol-20-one-3,21-diacetate[F,e]	1267M	....	1239S	1029S
Pregnane-3β,20β-diol-3,20-diacetate[S]	1252S	1241S	1233S	1021S
Pregnane-3β-ol-20-one-3-acetate[S]	1255S	1244S	1235S	1023S
Pregnane-3β,17α-diol-20-one-3-acetate[S]	1256S	1241S	1232S	1022S
Etiocholan-3β-ol-17-one-3-acetate[S]	1256S	1250S	1239S	1023S

[1] See footnotes of Table I. We wish also to thank [n] A. S. Meyer and [o] J. Davis for samples of compound studied.

ent positions absorb closely to each other in this region. One distinction could be made: 3α,5α-structures absorbed near 1018 (both solid and solution) while the other spatial isomers gave rise to bands at higher frequencies. The 3β,5α-isomer absorbed near 1033 (F) or 1026 (S), the 3α,5β-structures near 1023 (F) or 1029 (S) and the 3β,5β-form near 1028 (F) or 1022 cm.$^{-1}$ (S). It is of interest to note that solid film frequencies of the 3α,5β-form were lower than those of solutions.

The spectra of free and acetylated compounds also were scrutinized for a combination of frequencies which might be associated with steroid structure. This difficult problem of being unequivocally able to establish that a substance is a steroid on the basis of infrared spectrometry remains unsolved. It is empirically known that many bands of varying intensities occur in steroid spectra and the present investigation also was concerned with locating a combination of frequencies which eventually may be an identifying mark for steroid compounds. It was found that weak to medium weak bands occurred near 1311, 1265, 1242, 1217 and 1127 cm.$^{-1}$ in all the spectra of the free steroids. A medium weak absorption near 900 cm.$^{-1}$ appeared in the curves of free 21-hydroxy, 21-desoxy and C-27 compounds but was only present in approximately 75% of C-19 spectra. Furthermore the latter contained a band near 791 which was absent in the other curves while the steroids with more than 19 carbons gave rise to an absorption of weak intensity near 885 cm.$^{-1}$.

Except for the 1311 cm.$^{-1}$ band the relationship seemed to apply to the acetylated derivatives. Naturally the 1266 and 1242 cm.$^{-1}$ bands were obliterated by the acetate group absorption. An interesting observation was that a significant intensification of the 1157 cm.$^{-1}$ band occurred in the spectra of 21-desoxy steroids not having a 17-hydroxyl group (allopregnan-3α-ol-20-one, pregnan-3α-ol-11,20-dione, pregnan-3α-ol-20-one and allopregnan-3β-ol-20-one). It remains to be seen whether the distinctions between C-19 and C-21 molecules and the seemingly characteristic frequencies of tetrahydro steroids will apply to other groups of steroid compounds. It also must be ascertained whether non-steroid spectra will interfere with steroid assignment on the basis of a particular combination of frequencies.

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[CONTRIBUTION FROM RIKER LABORATORIES]

Alkaloids of *Rauwolfia serpentina* Benth. V.[1] Rescinnamine

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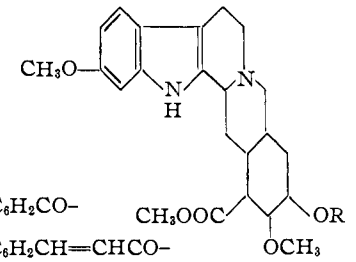
The isolation and characterization of rescinnamine, a new alkaloid from *Rauwolfia serpentina* Benth possessing pronounced hypotensive and sedative activity, is reported. Rescinnamine ($C_{35}H_{42}O_9N_2$) has been shown to be the 3,4,5-trimethoxycinnamic acid ester of methyl reserpate.

The Indian plant *Rauwolfia serpentina* Benth has aroused widespread interest because of its therapeutic value as a hypotensive and sedative agent, and has been the subject of numerous chemical investigations[2] in a search for the components responsible for this physiological activity. The isolation of one of these, reserpine, has been reported recently by Mueller, *et al.*,[3] and independently by this and other laboratories.[4-6]

Extensive pharmacological[7] and clinical[7] comparison of reserpine and an alkaloidal extract[8] of *Rauwolfia serpentina* indicated, however, that the extract possessed a greater degree of activity than could be accounted for by its reserpine content, thus suggesting the presence of other potent alkaloids which our initial chemical investigation had not revealed. On comparing reserpine (I)[9] with the inactive alkaloids present in this species, a significant structural difference relating to its biological activity is manifested by its ester character wherein an aromatic acid is conjugated with an alkaloidal alcohol. The importance of this grouping in potentiating biological activity in this series is shown by the relative inactivity of methyl reserpate (II) when compared with its conjugate



I, R = 3,4,5(OCH_3)$_3C_6H_2CO$-
II, R = H
III, R = 3,4,5(OCH_3)$_3C_6H_2CH$=$CHCO$-

(1) A preliminary report of this investigation appeared in a previous communication; *cf.* M. W. Klohs, M. D. Draper and F. Keller, THIS JOURNAL, **76**, 2843 (1954).

(2) For a comprehensive review of earlier work see Asima Chatterjee in L. Zechmeister "Progress in the Chemistry of Organic Natural Products," Vol. 10, Springer-Verlag, Vienna, Austria, 1953, pp. 390-417. For a summary of more recent work see E. Schlittler, J. A. Schneider and A. J. Plummer, *Angew. Chem.*, **66**, 386 (1954).

(3) J. M. Mueller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952).

(4) M. W. Klohs, M. D. Draper, F. Keller and F. J. Petracek, THIS JOURNAL, **75**, 4867 (1953).

(5) N. Neuss, H. E. Boaz and J. W. Forbes, *ibid.*, **75**, 4870 (1953).

(6) C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, *ibid.*, **75**, 5446 (1953).

(7) This work was carried out by the Biological Sciences and Clinical sections of this Laboratory.

(8) This investigation was carried out on an alkaloidal extract of *Rauwolfia serpentina*, generically designated "alseroxylon," which is available from Riker Laboratories, Inc., Los Angeles, Calif.

(9) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954).