This was distilled to afford 6.66 g. (82%) of colorless ester **14**, b.p. 74–79° (0.1 mm.). The analytical sample distilled at 87° (1 mm.); $n^{27}D$ 1.4769; $\lambda_{max}^{CHCl_3}$ 5.85 μ; n.m.r. (CCl_4) 5.89 and 5.97 (m),[20] 8.75 (t), 9.13 (s), 9.28 τ (s).

Anal. Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 11.00. Found: C, 75.8; H, 11.0.

1,1-Dimethyl-*trans*-decalin-10-carboxylic Acid (15). (A) By Lithium Iodide Cleavage.—This experiment is based on the procedure of Elsinger, Schreiber, and Eschenmoser.[11] A solution of 1.3395 g. of 10-carbethoxy-1,1-dimethyl-*trans*-decalin (**14**), b.p. 74–79° (0.1 mm.), in 100 ml. of *sym*-collidine (freshly distilled from potassium hydroxide) containing 7.0969 g. of lithium iodide[32] was refluxed under nitrogen for 72 hr. The cooled golden yellow solution was diluted with 100 ml. of ether and 50 ml. of chloroform and was washed thoroughly with 7% hydrochloric acid. The washings were combined, acidified with 30 ml. of concentrated hydrochloric acid, and extracted with a 2:1 ether–chloroform mixture. The organic extracts were combined, washed with saturated salt solution, and extracted with 5% potassium hydroxide.[33] The basic extract was acidified with concentrated hydrochloric acid and extracted with chloroform, which was dried over magnesium sulfate and concentrated *in vacuo* to 1.1020 g. (94%) of white plates, m.p. 89–90°; λ_{max}^{KBr} 3.2–3.9 (broad), 5.9 μ. Recrystallization from aqueous methanol gave 0.9893 g. of the acid **15** as white prisms, m.p. 93–97°, and then 0.0423 g. as white plates, m.p. 88.5–91°. Infrared spectra of potassium bromide pellets or chloroform solutions of both forms were identical. Further recrystallization from aqueous methanol gave white prisms with m.p. 96–98° and after cooling and resolidification, m.p. 92–92.5°; $\lambda_{max}^{CHCl_3}$ 3.2–3.8 (broad), 5.92 μ; n.m.r. (CCl_4) −2.31 (s), 9.12 (s), 9.20 τ (s).

Anal. Calcd. for $C_{13}H_{22}O_2$: C, 74.24; H, 10.54. Found: C, 74.4; H, 10.6.

(B) By "Reductive Hydrolysis".—This experiment was based on the procedure of Wenkert and Jackson.[25] A solution of 0.203 g. of 10-carbethoxy-1,1-dimethyl-*trans*-decalin (**14**), b.p. 74–79° (0.1 mm.), in 25 ml. of tetrahydrofuran was added to *ca.* 75 ml. of liquid ammonia, and small bits of lithium were added over a 3-hr. period to maintain a blue color. The solution was allowed to evaporate at room temperature, the residue was diluted with 25 ml. of chloroform and 35 ml. of 10% hydrochloric acid, and the products were isolated by chloroform extraction. The chloroform solution was extracted with 5% potassium hydroxide, dried over magnesium sulfate, and concentrated *in vacuo* to 0.1203 g. of yellow amorphous material, λ_{max}^{KBr} 3.0 μ. Chromatography on Woelm neutral alumina (activity grade III) gave 60 mg. (30%) of crystalline carbinol, m.p. 78–79°, the melting point of which was undepressed on admixture with authentic 1,1-dimethyl-*trans*-10-decalylcarbinol.

The basic aqueous phase was acidified with 10 ml. of concentrated hydrochloric acid and the acid **15** was isolated by chloroform extraction, drying over magnesium sulfate, and concentration *in vacuo*, which gave 0.0711 g. (40%) of yellow plates, m.p. 86–88°, having an infrared spectrum identical with that of the analytical sample previously described.

The yield of acid was reproducible to within a few per cent, but the neutral fraction contained varying proportions of alcohol and starting ester.

1,1-Dimethyl-*trans*-10-decalylcarbinol.—To a slurry of 0.38 g. of lithium aluminum hydride in 15 ml. of dry tetrahydrofuran was added a solution of 0.238 g. of 10-carbethoxy-1,1-d methyl-*trans*-decalin (**14**), b.p. 74–79° (0.1 mm.), in 5 ml. of dry tetrahydrofuran. The slurry was stirred and refluxed for 18 hr., while protected with a calcium sulfate drying tube. A few drops of water and then 2 ml. of 20% sodium hydroxide were added to the cooled mixture. The mixture was filtered, the precipitate was washed with ether, and the filtrate was concentrated *in vacuo* to 0.182 g. of waxy solid. Recrystallization from aqueous methanol gave 0.144 g. (73%) of the carbinol as white fibrous crystals, m.p. 75–77°. The analytical sample (from aqueous ethanol) had m.p. 79–80°; $\lambda_{max}^{CHCl_3}$ 2.76 (sharp), 2.90 μ (broad); n.m.r. (CCl_4) 6.30 (s) 9.17 (s), 9.27 τ (s).

Anal. Calcd. for $C_{13}H_{24}O$: C, 79.53; H, 12.32. Found: C, 79.4; H, 12.15.

Acknowledgment.—We express thanks to the U. S. Public Health Service, National Institutes of Health, for a grant (A 4215) in support of this work. The n.m.r. instruments were provided by grants from the U. S. Public Health Service and the National Science Foundation, to whom we are grateful.

(32) Mallinckrodt Chemical Co. lithium iodide trihydrate was heated with a free flame at *ca.* 0.5 mm. for 2 hr. immediately before use.

(33) In one experiment with 3.00 g. of **14**, 5% sodium hydroxide was employed for this extraction, but the sodium salt precipitated from the aqueous phase.

Stereoisomeric 3β,17β-Dihydroxyandrostan-16-ylacetic Acids

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A careful study of the catalytic hydrogenation of 3β,17β-diacetoxy-5-androsten-16-ylidenacetic acid afforded an improved yield of the selective hydrogenation product, 3β,17β-diacetoxy-5-androsten-16β-ylacetic acid, and permitted stereochemical assignments to the four possible tetrahydro products.

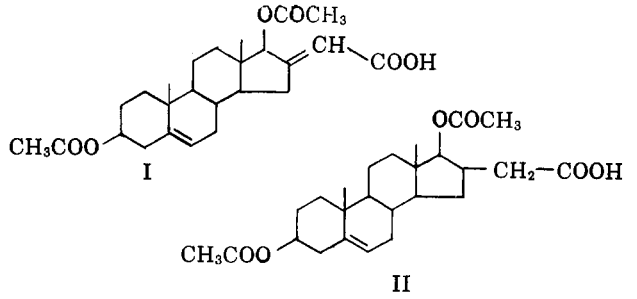
Partial hydrogenation of 3β,17β-diacetoxy-5-androsten-16-ylidenacetic acid (I) in glacial acetic acid in the presence of a 2% ratio of platinum oxide gave a 48% yield of the desired 3β,17β-diacetoxy-5-androsten-16β-ylacetic acid (II) when the reaction was stopped after 110–120% of the calculated amount of hydrogen was absorbed.[1] In an attempt to improve this yield, palladium-on-charcoal as well as rhodium-on-alumina catalysts were used in the reductions. The results with these catalysts were less satisfactory. Best results were finally obtained when the reduction of I was carried out with platinum in a solution of methanol containing 5% of water to yield 63% of the desired compound II.

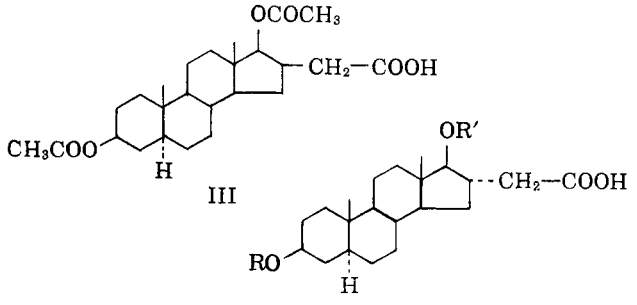
(1) P. Kurath and W. Cole, *J. Org. Chem.*, **26**, 1939 (1961).

The complete reduction of I over a 10% ratio of platinum oxide in acetic acid solution gave 3β,17β-diacetoxy-5α-androstan-16β-ylacetic acid (III) in 65% yield.[1] Since this yield appeared to be maximum, an investigation was made of the by-products. During the catalytic hydrogenation of I, two new asymmetric centers at C-5 and C-16 were introduced. Thus, we could expect the possible formation of the main product III and three similar structures isomeric at C-5 and/or C-16. Chromatography of the mixture obtained from the above mother liquors resulted in the isolation of a small additional amount of III; however, a considerable amount of material remained still unidentified.

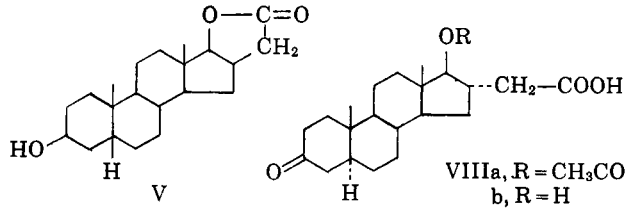
In a later experiment, a corresponding mixture was hydrolyzed in the presence of potassium hydroxide and

subsequently treated with hydrochloric acid. A new compound, 3β,17β-dihydroxy-5α-androstan-16α-ylacetic acid (IVa), was isolated by direct crystallization in 15% yield. Subsequent chromatographic separation of the remaining material gave 2.8% of 3β,17β-dihydroxy-5β-androstan-16β-ylacetic acid lactone (V). Later fractions afforded the known[1] 3β,17β-dihydroxy-5α-androstan-16β-ylacetic acid lactone (VI), and finally about 1% of 3β,17β-dihydroxy-5β-androstan-16α-ylacetic acid (VIIa) was obtained.

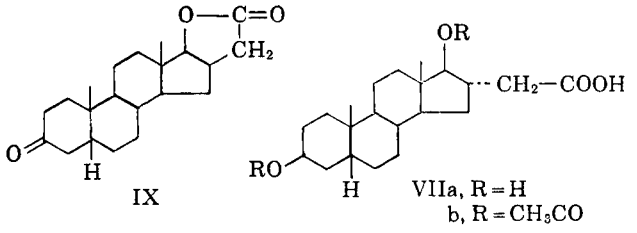




IVa, R = R' = H
b, R = R' = CH_3CO
c, R = H; R' = CH_3CO



VIIIa, R = CH_3CO
b, R = H



VIIa, R = H
b, R = CH_3CO

The assignment of the *trans*-configuration (16α) to the acetic acid side chain with respect to the 17β-hydroxyl in the new 3β,17β-dihydroxy-5α-androstan-16α-ylacetic acid (IVa) was based on the observation that this compound formed no lactone when treated with acid. Failure of such a system to lactonize was first recorded for *trans*-cyclopentanol-2-acetic acid[2] and subsequently confirmed in systems with closer relationships to our own.[3] The assignment of the 5α-configuration at the A/B ring junction in IVa was based on the optical rotation differences observed with the several derivatives prepared from the compound. Its diacetate IVb was partially hydrolyzed to give 17β-acetoxy-3β-hydroxy-5α-androstan-16α-ylacetic acid (IVc). The small negative shift observed in the comparison of the molecular rotations of IVc and IVb is consistent with the expected shift for the acetylation of a 3β-hydroxy-5α-system.[4] The monoacetate IVc was oxidized to give the 3-ketone VIIIa, which was hydrolyzed to yield 17β - hydroxy - 3 - oxo - 5α - androstan - 16α - ylacetic acid (VIIIb). The molecular rotation differences found in the comparisons of the 3β-ols (IVa and IVc) with the corresponding 3-ketones (VIIIb and VIIIa) are consistent with the ketonization of a 3β-hydroxy-5α-system[4] and thus support these formulations. The molecular rotation differences discussed previously are recorded in Table I.

TABLE I
MOLECULAR ROTATION DIFFERENCES

Compound	M_D°	ΔM_D, deg. Observed	ΔM_D, deg. Expected[a]
17β-Acetoxy-3β-hydroxy-5α-androstan-16α-ylacetic acid (IVc)	−177	−40	−29
3β,17β-Diacetoxy-5α-androstan-16α-ylacetic acid (IVb)	−217		
3β,17β-Dihydroxy-5α-androstan-16α-ylacetic acid (IVa)	−88		
17β-Hydroxy-3-oxo-5α-androstan-16α-ylacetic acid (VIIIb)	−38	+50	+73
17β-Acetoxy-3β-hydroxy-5α-androstan-16α-ylacetic acid (IVc)	−177	+68	+73
17β-Acetoxy-3-oxo-5α-androstan-16α-ylacetic acid (VIIIa)	−109		
3β,17β-Dihydroxy-5β-androstan-16β-ylacetic acid lactone (V)	+80		
17β-Hydroxy-3-oxo-5β-androstan-16β-ylacetic acid lactone (IX)	+119	+39	+36

[a] See ref 4.

The fact that the optical rotation of 3β,17β-diacetoxy-5α-androstan-16α-ylacetic acid (IVb) was found to be more negative than that[1] of the corresponding 16β-isomer III adds further support[5] to the configurational assignments of the side chains at C-16.

The following evidence permits the assignment of the 3β,17β-dihydroxy-5β-androstan-16β-ylacetic acid lactone structure to the new compound V. Chromic acid oxidation of V led to the isolation of 17β-hydroxy-3-oxo-5β-androstan-16β-ylacetic acid lactone (IX), a compound clearly different from the previously prepared 5α-isomer.[1] The observed molecular rotation difference resulting from the conversion of V to IX compares well with that reported[4] for the ketonization of a 3β-hydroxy-5β-system (see Table I). The addition of one mole of bromine to IX followed by the elimination of the elements of hydrogen bromide gave the known 17β-hydroxy-3-oxo-4-androsten-16β-ylacetic acid lactone (X), thus confirming the assigned *cis* A/B-ring

(2) W. Hückel and W. Gelmroth, *Ann.*, **514**, 233 (1934); R. P. Linstead and E. M. Meade, *J. Chem. Soc.*, 935 (1934); W. E. Grigsby, J. Hind, J. Chanley, and F. H. Westheimer, *J. Am. Chem. Soc.*, **64**, 2606 (1942).

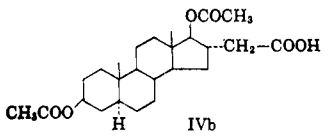
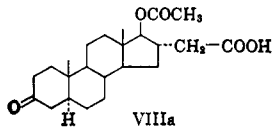
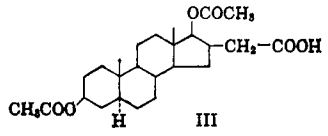
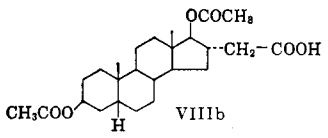
(3) A. Bowers, T. G. Halsall, and G. C. Sayer, *J. Chem. Soc.*, 3070 (1954); Y. Mazur, N. Danieli, and F. Sondheimer, *J. Am. Chem. Soc.*, **82**, 5889 (1960); L. J. Chinn, E. A. Brown, R. A. Mikulec, and R. B. Garland, *J. Org. Chem.*, **27**, 1733 (1962).

(4) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 180.

(5) *Cf.* K. Brückner, K. Irmscher, F. v. Werder, K.-H. Bork, and H. Metz, *Chem. Ber.*, **94**, 2897 (1961).

TABLE II

PROTON MAGNETIC RESONANCE SPECTRA[a]

Structure	17α-H	17α-H Half-width[b]	3α-H	3α-H Half-width[b]	C-13 Methyl	C-10 Methyl	Acetate Methyl
 IVb	275	11	287[c]	15–20[c]	50	50	122, 124
 VIIIa	275	10	...	...	51	61	123
 III	287, 296[d]	12	285[c]	15–20[c]	46	50	122, 123
 VIIIb	276	10	308	8	49	58	123

[a] The spectra were recorded with a Varian A-60 n.m.r. spectrometer at 60 Mc.; 5–10% solutions in deuteriochloroform were employed using tetramethylsilane as an internal reference. Chemical shifts are reported in c.p.s. from tetramethylsilane (0 c.p.s.) in the direction of decreasing field. [b] Half-width indicates the width (in c.p.s.) of the band at half its height. [c] Estimated. [d] Doublet.

junction of V and IX, as well as the 16β,17β-*cis*-fusion[2,3] of the lactone structure with ring D. In view of the careful reduction work of Hershberg, *et. al.*,[6] in the cholesterol series, the isolation of a small amount of the 5β-compound is not surprising.

By a process of elimination, the dihydroxy acid VIIa was regarded as the last of the four possible compounds stereoisomeric at C-5 and C-16, and was, therefore, formulated as 3β,17β - dihydroxy - 5β - androstan-16α-ylacetic acid. The assignment of the 16α,17β-*trans* structure was based on the fact that the compound did not form a lactone.[2,3] The new dihydroxy acid VIIa is clearly different from the 3β,17β-dihydroxy-5α-androstan-16α-ylacetic acid (IVa) discussed earlier, and it is, therefore, reasonable to assign the 5β-configuration to VIIa. The similarity of the optical rotations of IVa and VIIa is consistent with the recognized closeness of $[\alpha]_D$ values for 3β-hydroxy-5α- and 3β-hydroxy-5β-systems.[7] Acetylation of VIIa furnished 3β,17β-diacetoxy-5β-androstan-16α-ylacetic acid (VIIb). The observation that the optical rotation of this compound was more positive[4] than that of the 5α-isomer IVb further supports the stereochemical assignment of VIIa and VIIb.

Additional support for the structures of the stereoisomeric 3β,17β-diacetoxyandrostan-16-ylacetic acids (IVb, III and VIIb) as well as the 3-oxo analog (VIIIa) was obtained by examination of their n.m.r. spectra. The relevant data are recorded in Table II.

The n.m.r. spectra of IVb and VIIIa demonstrated that the 17β-acetoxy group had remained intact during the partial hydrolysis of IVb. The 17α-proton absorptions of both IVb and VIIIa appeared at 275 c.p.s. as narrow, but complex multiplets with half-widths of 11 and 10 c.p.s., respectively. These chemical shifts are close to that of the 17α-proton of 17β-acetoxy-3-oxo-5α-androstane (277.2 c.p.s.) which appears as a triplet absorption (half-width approximately 20 c.p.s.) with peaks showing secondary splittings.[8] In the region between 200 and 400 c.p.s., the spectrum of the keto acetate VIIIa showed only the narrow absorption envelope of the 17α-proton, while the spectrum of IVb showed partial overlapping of the 17α-proton absorption by the broad, unresolved 3α-axial proton absorption, centered at approximately 287 c.p.s.

Since the 17α-proton in both IVb and VIIIa is directly coupled only to the 16β-proton, the complexity of the 17α-proton absorption must be a consequence of virtual long-range coupling[9] due to coupling of the 16β-proton with the methylene protons at C-15 as well as those of the 16α-acetic acid side chain. It is of interest to note that the absorption of the 17α-proton of the 16β-acetic acid III appears as a well resolved doublet with sharp and well defined component peaks (287 and 296 c.p.s.). The magnitude of the observed splitting of this multiplet (9 c.p.s.) is that predicted by the Karplus relationship for the coupling constant for eclipsed vicinal protons.[10]

A change in the A-ring should have a greater effect on the chemical shift of the C-10 methyl proton absorption than on that of the C-13 methyl proton absorption. Similarly, substituents in the D-ring should have a negligible effect on the absorption of the C-10 methyl protons. The angular methyl protons of IVb give rise to only a single peak at 50 c.p.s. due to their overlapping absorptions. This value is in good agreement

(6) E. B. Hershberg, E. Oliveto, M. Rubin, H. Staeudle, and L. Kuhlen, *J. Am. Chem. Soc.*, **73**, 1144 (1951); T. Reichstein and A. Lardon, *Helv. Chim. Acta*, **24**, 955 (1941).

(7) W. Klyne, "The Chemistry of the Steroids," John Wiley and Sons, Inc., New York, N. Y., 1957, p. 54.

(8) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "N.M.R. Spectra Catalog," Varian Associates, 1962, Spectrum no. 353.

(9) J. I. Musher and E. J. Corey, *Tetrahedron*, **18**, 791 (1962).

(10) *Cf.* H. Conroy, "Nuclear Magnetic Resonance in Organic Structural Elucidation" in "Advances in Organic Chemistry: Methods and Results," Vol. 2, Interscience Publishers, New York, N. Y., 1960, pp. 308–311.

with the chemical shifts reported by Zürcher[11] for the C-10 methyl proton absorptions of four 17-substituted 3β-acetoxy-5α-steroids, which occur between 49.0 and 50.7 c.p.s. In the keto acetate VIIIa the angular methyl protons absorb at 61 and 51 c.p.s. The low field (61 c.p.s.) absorption is attributed to the C-10 methyl protons, based on consideration of the change in functionality at C-3 in VIIIa as compared with IVb. This is consistent with the values (60.4 to 62.2 c.p.s.) reported for the C-10 methyl protons of three 17-substituted 3-keto-5α-steroids.[8,11] In the case of the 16β-acetic acid III the angular methyl proton absorptions at 46 c.p.s. and 50 c.p.s. are assigned to the C-13 and C-10 methyls, respectively, on the basis of the previously established[1] 3β-acetoxy-5α-structure.

Comparison of the C-13 methyl proton absorptions of III, IVb, and VIIIa indicates that the 16β-acetic acid side chain effects a small diamagnetic shift of the C-13 methyl proton absorptions, relative to the 16α-epimers.

The spectrum of the diacetate VIIb, like the spectra of the isomeric diacetate IVb and the keto acetate VIIIa, shows a narrow but complex multiplet at 275 c.p.s. (half-width 10 c.p.s.) attributed to the 17α-proton. The chemical shifts and half-widths of these absorptions, as well as the chemical shifts of the C-13 methyl proton absorptions (49 to 51 c.p.s.), strongly suggest that the three isomers must have identical D-ring stereochemistry.

In contrast to the broad absorptions of the axial C-3 protons of III and IVb, the C-3 proton absorption of VIIb occurs at 308 c.p.s. as a narrow, unresolved multiplet (half-width 8 c.p.s.). The magnitude of the half-width, which is essentially equal to that of the C-1 proton of *cis*-4-*t*-butylcyclohexyl acetate,[12] demonstrates that the C-3 proton of VIIb must be equatorial. Of the two possible structures having a 3-equatorial proton, 3β-acetoxy-5β-H and 3α-acetoxy-5α-H, only the former is consistent with the C-10 methyl proton absorption (58 c.p.s.) of VIIb. Zürcher[11] reported values of 57.8 and 59 c.p.s. for two 17-substituted 3β-acetoxy-5β steroids. The spectral data thus support the 3β,17β-diacetoxy-5β-androstan-16α-ylacetic acid structure for compound VIIb.

Experimental[13]

3β,17β-Diacetoxy-5-androsten-16β-ylacetic Acid (II).—A solution of 4.54 g. of 3β,17β-diacetoxy-5-androsten-16-ylidenacetic acid (I) in 100 ml. of methanol and 5 ml. of water was reduced in the presence of a 2% ratio (0.09 g.) of platinum oxide.[14] The reduction stopped after 1.05 equivalents of hydrogen was absorbed. The methanol solution was filtered, the residue was washed with glacial acetic acid, and the clear filtrate was evaporated under reduced pressure. The residue was twice recrystallized from acetone–petroleum ether to give 2.837 g. (63%) of the desired compound II; m.p. 251–253°. On admixture of this product with a reference sample[1] of II, no melting point depression was observed. Upon concentration of the mother liquors a second crop of 0.773 g., m.p. 240–242°, was isolated.

Complete Reduction of 3β,17β-Diacetoxy-5-androsten-16-ylidenacetic Acid (I).—A total of 40 g. of I in glacial acetic acid was hydrogenated over a 10% ratio (4.0 g.) of platinum oxide until the uptake of hydrogen ceased and worked up as previously described.[1] The reduction mixture was recrystallized twice from methanol–water to yield 22.38 g. (55%)[15] of **3β,17β-diacetoxy-5α-androstan-16β-ylacetic acid (III)**; m.p. 243–244°. Admixture of this compound with a reference sample[1] of III did not depress the melting point; the two samples had superimposable infrared spectra.

Evaporation of the mother liquors left a solid residue of 17.5 g. This was dissolved in acetic acid and shaken in an atmosphere of hydrogen in the presence of platinum catalyst; no gas absorption was observed. The catalyst was removed by filtration and the resulting solution was evaporated to dryness under reduced pressure to leave a residue of 17.6 g. The material was dissolved in 750 ml. of methanol and refluxed with 20.5 g. of potassium hydroxide in 75 ml. of water for 2 hr. The reaction mixture was diluted with 2400 ml. of water and evaporated to about 2000 ml. The resulting slurry was acidified with 500 ml. of 10% hydrochloric acid, warmed on the steam bath for 20 min., and allowed to cool. The resulting aqueous suspension was extracted with two 3000-ml. and three 1500-ml. portions of ethyl acetate. The organic solution was washed to neutrality with water, dried, and concentrated to about 500 ml. The solution was allowed to cool; the crystals were collected on a filter and dried to give 5.089 g. (15% based on I) of **3β,17β-dihydroxy-5α-androstan-16α-ylacetic acid (IVa)**; m.p. 270–271°. A sample was recrystallized from methanol for analysis; m.p. 275–276°; $[\alpha]^{26}D$ −25° (*c* 0.616, in dioxane); λ_{max}^{KBr} 2.91, 3.05, 3.75–4.0, 5.78, 5.88 μ; $\lambda_{max}^{pyridine}$ 5.81 μ.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.18; H, 10.12.

This ethyl acetate mother liquor was evaporated to dryness. The residue of 8.405 g. was chromatographed on 600 g. of silica gel. The early ether–acetone (95:5) eluates gave, after evaporation of the solvent, 1.482 g. of a solid residue. This yielded, after several recrystallizations from acetone–petroleum ether, 0.888 g. (2.8% based on I) of **3β,17β-dihydroxy-5β-androstan-16β-ylacetic acid lactone (V)**; m.p. 196–197°. A second crop amounted to 0.384 g.; m.p. 143–168°.

A part of the previous first crop of V was recrystallized for analysis; m.p. 196–197°; $[\alpha]^{21}D$ +24° (*c* 1.20, in chloroform); $\lambda_{max}^{CHCl_3}$ 2.76, 2.86, 5.65, 8.51 μ. The fingerprint region of the infrared spectrum of the 5β-isomer V is clearly different from that of the 5α-isomer VI.[1]

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.71. Found: C, 75.78; H, 9.76.

The residues of the later ether–acetone (95:5) and ether–acetone (90:10, 80:20) eluates amounted to 4.958 g. After two recrystallizations from acetone, 2.198 g. (7% based on I) of **3β,17β-dihydroxy-5α-androstan-16β-ylacetic acid lactone (VI)**, m.p. 236–238°, was obtained. Mixture melting point determination and the comparison of infrared spectra established the identity of this compound with a reference sample[1] of VI. Concentration of these mother liquors gave rise to a second crop of 0.991 g. of less pure VI; m.p. 224–229°.

This chromatogram was further eluted with acetone and acetone–methanol (90:10, 80:20) to yield 0.901 g. of a new compound. Several recrystallizations from acetone gave 0.166 g. of **3β,17β-dihydroxy-5β-androstan-16α-ylacetic acid (VIIa)**; m.p. 259–260°. Concentration of the mother liquors afforded an additional 0.212 g. of less pure VIIa, m.p. 252–253°, thus bringing the yield of VIIa to 1.2% (based on I).

A sample of the first crop was dried for analysis; $[\alpha]^{21}D$ −25° (*c* 0.562, in dioxane); λ_{max}^{KBr} 2.98, 3.75–4.0, 5.82 μ. The fingerprint region of the infrared spectrum of VIIa is clearly different from that of IVa; on admixture of the two compounds a melting point depression is observed.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.80; H, 9.90.

3β,17β-Diacetoxy-5α-androstan-16α-ylacetic Acid (IVb).—A solution of 4.784 g. of 3β,17β-dihydroxy-5α-androstan-16α-ylacetic acid (IVa) in 56 ml. of pyridine and 28 ml. of acetic anhydride was allowed to stand at room temperature overnight. Following the addition of 26 ml. of water to the reaction mixture, the latter was warmed on the steam bath for 2 hr. The cooled

(11) R. F. Zürcher, *Helv. Chim. Acta*, **44**, 1380 (1961).

(12) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).

(13) The melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus. The optical rotation values have a limit of error of ±2°. The infrared spectra were recorded on a Perkin-Elmer Model 21 infrared spectrophotometer. The petroleum ether fraction boiling in the range 90–100° was used in this work.

(14) Strict adherence to the weight–volume ratio in this experiment is important.

(15) The yield of carefully purified material is recorded without regard to crude and second crops.

solution was poured into 560 ml. of ice-cold water. The resulting precipitate was collected on a filter and washed with several small amounts of water. The compound was dried and recrystallized twice from acetone–petroleum ether to give 4.180 g. (70%) of the desired compound IVb; m.p. 203–205°. Upon concentration of the mother liquors an additional 1.713 g. of crystalline material, m.p. 183–190°, was obtained.

An analytical sample of IVb had the following physical constants: m.p. 208–209°; $[\alpha]^{25}D$ −55° (*c* 1.115, in chloroform); $[\alpha]^{22}D$ −50° (*c* 1.152, in dioxane); $\lambda_{max}^{CHCl_3}$ 3.75–4.25, 5.85, 8.05 μ.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.82. Found: C, 69.03; H, 8.67.

17β-Acetoxy-3β-hydroxy-5α-androstan-16α-ylacetic Acid (IVc).—The mixture of 4.180 g. of the diacetate IVb, 2.59 g. of potassium carbonate, 26 ml. of water, and 212 ml. of methyl alcohol was allowed to stand at room temperature for 4 days. The addition of 240 ml. of water was followed by evaporation of most of the methanol at about 40° under reduced pressure. The reaction mixture was made acidic with 150 ml. of 2 *N* hydrochloric acid; the precipitate was collected on a filter and washed with several small amounts of water. The compound was recrystallized from methanol–water to give 3.566 g. (95%) of the monoacetate IVc; the compound sintered at 238° and melted from 243–246°.

A sample was recrystallized for analysis. It sintered at 238° and melted at 244–246°; $[\alpha]^{27}D$ −45° (*c* 0.968, in dioxane); λ_{max}^{KBr} 2.95, 3.75–4.25, 5.79, 5.92, 8.1 μ.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.60; H, 9.47.

Some experiments gave a higher-melting form (m.p. 258–259°) of the monoacetate IVc. A sample of this material was recrystallized from methanol–water for analysis; m.p. 263–264°; $[\alpha]^{28}D$ −48° (*c* 0.975, in dioxane); λ_{max}^{KBr} 2.95, 3.75–4.25, 5.76, 5.90, 8.1 μ.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.66; H, 9.37.

Both crystal forms furnished the same ketone VIIIa; thus their structural identity was demonstrated.

17β-Acetoxy-3-oxo-5α-androstan-16α-ylacetic Acid (VIIIa).—To a cold (5°) solution of 2.267 g. of 17β-acetoxy-3β-hydroxy-5α-androstan-16α-ylacetic acid (IVc) in 290 ml. of acetone was added while swirling 2.9 ml. of chromium trioxide reagent[16] in an atmosphere of nitrogen. After 10 min. the reaction mixture was diluted with 1200 ml. of water; the precipitate was collected on a filter, washed with several small amounts of water, and dried at 60° under reduced pressure. The product was further purified by chromatography on 200 g. of silica gel. From the benzene–ether (1:1) and ether eluates a total of 1.555 g. of a solid residue was obtained. The compound was recrystallized from acetone–petroleum ether to give 1.102 g. of VIIIa; m.p. 200–202°. A second crop amounted to 0.199 g., m.p. 199–201°, thus bringing the yield to 58%. A third crop of 0.162 g., m.p. 188–192°, was not satisfactory.

A part of the first crop was recrystallized for analysis; m.p. 201–202°; $[\alpha]^{24}D$ −31° (*c* 1.095, in chloroform); $[\alpha]^{22}D$ −28° (*c* 0.934, in dioxane); $\lambda_{max}^{CHCl_3}$ 3.75–4.25, 5.79, 5.87, 8.05 μ.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.99; H, 8.96.

The compound remained unchanged on sublimation at 200–220° under high vacuum.

17β-Hydroxy-3-oxo-5α-androstan-16α-ylacetic Acid (VIIIb).—A solution of 1.26 g. of VIIIa and 1.33 g. of potassium hydroxide pellets in 133 ml. of methanol and 13 ml. of water was heated under reflux for 90 min. The solution was diluted with 165 ml. of water and most of the methanol was removed under reduced pressure. The mixture was made acidic by adding 50 ml. of 10% hydrochloric acid. The resulting slurry was warmed on the steam bath for 10 min. and allowed to cool. The precipitate was collected on a filter, washed with several small amounts of water, and dried under reduced pressure at 75°. The product was recrystallized from acetone to give 1.012 g. (90%) of VIIIb; m.p. 253–254°. A second crop amounted to 0.046 g.; m.p. 243–246°.

An analytical sample was prepared; m.p. 254–255°; $[\alpha]^{25}D$ −11° (*c* 0.765, in dioxane); λ_{max}^{KBr} 3.06, 3.75–4.25, 5.87, 5.95 μ.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.55; H, 9.25.

The compound was not changed on sublimation at 220–250° under high vacuum.

17β-Hydroxy-3-oxo-5β-androstan-16β-ylacetic Acid Lactone (IX).—To the cooled and stirred mixture of 0.76 g. of sodium dichromate, 1.03 ml. of concentrated sulfuric acid, 0.57 ml. of acetic acid, 3.3 ml. of water, and 26 ml. of benzene was added 0.478 g. of the hydroxy lactone V. The reaction mixture was kept immersed in an ice bath for 20 min. and then stirred at room temperature for 20 hr.[17] The mixture was diluted with 75 ml. of benzene; the water layer was separated and extracted with two 100-ml. portions of benzene. The benzene extract was washed with water, dried, and evaporated to leave a residue of 0.465 g. of partly oily material. This was purified by chromatography on 50 g. of silica gel. The residue from the eluates with benzene–ether (80:20) gave, after recrystallization from acetone–petroleum ether, 0.324 g. (68%) of the desired lactone IX; m.p. 145–146°. A second crop amounted to 0.052 g.; m.p. 143–144°.

A part of the first crop was recrystallized for analysis; m.p. 146–147°; $[\alpha]^{22}D$ +36° (*c* 1.032, in chloroform); $\lambda_{max}^{CHCl_3}$ 5.66, 5.83, 8.54 μ.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 76.38; H, 9.07.

17β-Hydroxy-3-oxo-4-androsten-16β-ylacetic Acid Lactone (X).—To 0.20 g. of 17β-hydroxy-3-oxo-5β-androstan-16β-ylacetic acid lactone (IX) and 0.006 g. of *p*-toluenesulfonic acid in 5 ml. of dimethylformamide was added 0.107 g. of bromine in 3 ml. of dimethylformamide over a period of 1 hr. with occasional gentle warming.[18] The reaction mixture was allowed to stand at room temperature for 1 hr., then was diluted with 70 ml. of water, and extracted with methylene chloride. The extract was washed with water, dried, and evaporated, at the end under high vacuum, to leave a residue of 0.27 g. of white foam.

This crude bromo derivative in 13.5 ml. of methylene chloride was added to a suspension of 0.136 g. of semicarbazide base in 22.5 ml. of *t*-butyl alcohol and stirred for 1 hr. in an atmosphere of nitrogen. The reaction mixture was diluted with 0.4 ml. of pyruvic acid, 0.7 ml. of water, and 3.5 ml. of acetic acid. Stirring was continued for 30 min. The resulting solution was allowed to stand at room temperature overnight in an atmosphere of nitrogen.[19] The reaction mixture was concentrated under reduced pressure, diluted with water, and extracted with three portions of methylene chloride. The methylene chloride solution was washed with water, dried, and evaporated to leave 0.205 g. of crude solid which was further purified by chromatography on 25 g. of silica gel. From the eluates with benzene–ether (1:1) a residue of 0.138 g. was obtained after the evaporation of the solvent. Recrystallization gave 0.079 g. (40%) of 17β-hydroxy-3-oxo-4-androsten-16β-ylacetic acid lactone (X); m.p. 244–246°. A second crop amounted to 0.008 g.; m.p. 233–239°. The product was sublimed at 210° under high vacuum and recrystallized from acetone to give 0.044 g. of a sample with a constant melting point of 251–252°. Admixture of this lactone with a reference sample[1] of X did not depress the melting point. Its identity with the reference sample X was further demonstrated by the comparison of the infrared and ultraviolet spectra.

3β,17β-Diacetoxy-5β-androstan-16α-ylacetic Acid (VIIb).—A solution of 0.105 g. of 3β,17β-dihydroxy-5β-androstan-16α-ylacetic acid (VIIa) in 2 ml. of pyridine and 1 ml. of acetic anhydride was allowed to stand at room temperature overnight and worked up as described in the case of the 5α-isomer IVb to leave a crystalline residue of 0.136 g. Chromatographic purification of this compound gave 0.100 g. of the desired diacetate VIIb, which was recrystallized from acetone–petroleum ether to give 0.059 g. of an analytical sample melting at 109–111°; $[\alpha]^{18}D$ −41° (*c* 0.822, in chloroform); $\lambda_{max}^{CHCl_3}$ 3.5–4.0, 5.78, 5.83, 7.97 μ.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.82. Found: C, 69.38; H, 8.65.

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(16) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956); K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(17) W. F. Bruce, "Organic Syntheses" Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 139.

(18) R. E. Beyler, A. E. Oberster, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, **82**, 170 (1960).

(19) W. F. McGuckin and E. C. Kendall, *ibid.*, **74**, 5811 (1952); J. F. Kerwin, M. E. Wolff, F. F. Owings, B. B. Lewis, B. Blank, A. Magnani, C. Karash, and V. Georgian, *J. Org. Chem.*, **27**, 3628 (1962).

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The Alkaloids of *Cephalotaxus drupacea* and *Cephalotaxus fortunei*

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A new alkaloid, cephalotaxine, $C_{18}H_{21}NO_4$, has been isolated from *Cephalotaxus fortunei* and *Cephalotaxus drupacea*, and a partial structure VIII–IX has been proposed for it.

The presence of alkaloids in *Cephalotaxus drupacea*,[2] *C. henryi*,[3] *C. wilsonia*,[4] and *C. fortunei*[5] has been demonstrated, but these alkaloids have not been chemically investigated. We now wish to report on the separation and partial identification of the major alkaloid of *Cephalotaxus fortunei* and *drupacea*.

Although the botanical classification of these plants is not yet clear, *Cephalotaxus* generally is listed as a genus of the family *Taxaceae* and a member of the *Taxeae*.[6] The Taxaceae belong to the order *Coniferae*. Many species have been listed for *Cephalotaxus*,[6-8] but the genus is now considered to contain only four pure ones: *C. pedunculata*, a native of Japan; *C. oliveri*, a Native of China; *C. drupacea;* and *C. fortunei*.[9] *C. drupacea*, a small tree which is found predominantly in China and Japan, is commonly known as Cow's Tail Pine or Japanese plum-yew. *C. fortunei*, known as the Chinese plum-yew, is found in North China.

The powdered leaves and stems of *C. fortunei* and *C. drupacea*[10] yielded 0.39% and 0.35%, respectively, of crude alkaloidal material by a conventional acid–base extraction of the concentrated alcohol extracts. A comparison of paper chromatograms indicated the presence of at least four different alkaloids in *C. fortunei* and at least five different alkaloids in *C. drupacea*. Alumina-column chromatography yielded one crystalline alkaloid from each species. The identity of these two alkaloids was established by a comparison of their infrared, ultraviolet, and n.m.r. spectra, as well as by the nondepression of their melting points when mixed with each other. This alkaloid which was named cephalotaxine, was present to the extent of 50 and 54%, respectively, in the crude alkaloidal mixtures of *C. fortunei* and *C. drupacea*.

The molecular formula of cephalotaxine, $C_{18}H_{21}NO_4$, was determined by duplicate analysis of two different samples recrystallized from two different solvents, and by a molecular weight determination in benzene. Cephalotaxine is moderately basic (pK_a 8.95) and is optically active ($[\alpha]^{25}D$ −204°). It does not contain a C–CH_3 or N–CH_3 grouping. The presence of one methoxyl grouping was established by a Zeisel methoxyl determination. The existence of an absorption peak in the infrared spectrum of the alkaloid at 3500 cm.$^{-1}$ (in chloroform) indicates the presence of a –NH or hydrogen bonded –OH function.[11] The alkaloid does not give a ferric chloride test, suggesting the absence of a phenolic hydroxyl group. Cephalotaxine can be acetylated to a monoacetyl derivative ($C_{20}H_{23}NO_5$), which is still basic (pK_a 7.97) and whose infrared spectrum is transparent in the 3200–3600-cm.$^{-1}$ region, while showing the expected absorption peak (1735 cm.$^{-1}$) for the acetyl function. The 3500-cm.$^{-1}$ peak is consequently due to one –OH group. Since this peak does not shift upon dilution, the hydrogen bonding must be *intramolecular*. The possibility that the acetylcephalotaxine arises by a molecular rearrangement was eliminated by the isolation of cephalotaxine from a lithium aluminum hydride reduction of the acetyl compound. The absence of any absorption peaks between 3200–3600 cm.$^{-1}$ in acetylcephalotaxine also excludes the possibility that the nitrogen atom in cephalotaxine is either primary or secondary. That it is not a secondary amine is further established by the negative test obtained with the nickel chloride–carbon disulfide reagent for secondary amines.[12]

The presence of a strong infrared absorption peak at 1650 cm.$^{-1}$ could indicate the presence of a —C=N— linkage.[11] This peak should be altered upon protonation of the nitrogen atom. The observation that this peak is still present in the perchlorate and hydrochloride salt of cephalotaxine permits one to eliminate this possibility. The nitrogen atom in cephalotaxine is consequently tertiary.

Some insight into the relative steric relationships between the nitrogen atom and the hydroxyl group is gained by the observation (see previous discussion) that the hydroxyl group is strongly hydrogen bonded and

(1) Participant of an National Science Foundation Undergraduate Research Participation Program Fellowship, 1962.

(2) T. Kariyone, M. Takahashi, A. Nitta, and Yoshinao Tsunehisa, *J. Pharm. Soc. Japan*, **76**, 611 (1956).

(3) M. E. Wall, *J. Am. Pharm. Assoc.*, **43**, 505 (1954).

(4) I. H. Y. Hsu, *J. Taiwan Pharm. Assoc.*, **9**, 5 (1957).

(5) Smith, Kline and French Laboratories, private communication.

(6) W. Dallimore and A. B. Jackson, "Handbook of Coniferae," Longmans Green and Co., New York, N. Y., 1923, pp. 20–23.

(7) C. Coltman-Rogers, "Conifers and Their Characteristics," The MacMillan Co., New York, N. Y., 1920, p. 239.

(8) L. H. Bailey, "The Cultivated Conifers," The Macmillan Co., New York, N. Y., 1933, p. 33.

(9) P. Greguss, "Identification of Living Gynosperms on the Basis of Xylotomy," Akademiai Kiado, Budapest, 1955, p. 84.

(10) We wish to express our gratitude to the Smith, Kline and French Laboratories of Philadelphia, Pa., for suggesting these plants to us as interesting, alkaloid-containing materials and for supplying us with the initial plant extracts and powdered plant materials.

(11) L. C. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1959.

(12) W. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," Academic Press, Inc., New York, N. Y., 1955, p. 124.