ene chloride and was then recrystallized from acetone-hexane, yielding 25.0 mg. of colorless platelets, m.p. 176–179°. This sample was used for the determination of the optical rotation and of the ultraviolet absorption spectrum $[\alpha]_D^{20}$ −20.5°; M_D^{20} −71° (12.82 mg., α −0.26°); λ_{max}^{alc} 243 mμ, ε 15,970.

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.43): C, 73.23; H, 8.19. Found: C, 72.80; H, 8.44.

PHILADELPHIA 4, PA.

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

Steroids and Related Natural Products. VI. The Structure of α-Apoallobetulin[1,2]

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The allobetulin dehydration product, α-apoallobetulin, has been shown to be represented by structure IV. Initial support for this assignment was obtained by oxidizing α-apoallobetulin to a diketone (VII). Direct oxidation of α-apoallobetulin to diketone VII was unexpectedly achieved employing a modified osmium tetroxide-pyridine procedure. The same oxidation product was prepared by ozonization of α-apoallobetulin and by lead tetraacetate oxidation of its glycol derivative (VI). Substantial evidence favoring the proposed endocyclic olefin (IV) formulation was provided by the facile isomerization of δ-apoallobetulin (III) to α-apoallobetulin. The ketone (VIII) obtained following ozonization of δ-apoallobetulin was assigned an A/B *cis*-configuration on the basis of optical rotatory dispersion measurements.

Conversion of betulin (Ia) to the formate of an isomeric substance designated allobetulin (IIa), in the presence of hot 90–95% formic acid, was reported by Schulze and Pieroh in 1922.[3] Allobetulin was subsequently found to readily lose one molecular equivalent of water, upon treatment with phosphorus pentachloride or pentoxide in chloroform solution, giving rise to a new compound termed apo-allobetulin.[3] Several years later Dischendorfer and Juvan noted that apo-allobetulin could also be prepared by heating betulin with palladium-charcoal or with fuller's earth suspended in refluxing xylene.[4]

A concise structural and mechanistic interpretation of the interesting transformation of betulin to allobetulin was unavailable until 1951.[5] More recently, Simonsen and Ross[6] suggested that apo-allobetulin, now known as α-apoallobetulin,[7] might be represented by formulations III or IV. As α-apoallobetulin, if indeed represented by structure III, was needed as starting material for another investigation, it became necessary to determine exactly the position of unsaturation.

The exocyclic olefin III appeared to most accurately represent α-apoallobetulin, as it is well known that reagents such as phosphorus pentachloride favor this type of Wagner-Meerwein rearrangement product.[8] However, Ruzicka and colleagues were able to prove, for example, that fuller's earth dehydration of 18-isooleanolic acid lactone (V) in refluxing xylene solution yields the endocyclic olefin analogous to IV, while dehydration in petroleum ether solution with phosphorus pentachloride afforded the exocyclic isomer.[9]

The most direct procedure for establishing or eliminating structure III for α-apoallobetulin appeared to simply involve studying the reaction of ozone with this substance. Consequently, α-apoallobetulin, prepared by fuller's earth dehydration of betulin essentially as described by Dischendorfer,[4] was treated in chloroform solution at −30° with excess ozone. Following zinc dust-acetic acid reduction of the ozonides, two discrete products were isolated. The first substance, obtained by fractional recrystallization of the crude reduction product, was an oxidation product melting at 240–242°.[10] Further recrystallization of the residual

(1) Consult G. R. Pettit, U. R. Ghatak, B. Green, T. R. Kasturi, and D. M. Piatak, *J. Org. Chem.*, 26, 1685 (1961) for the preceding contribution.

(2) This investigation was supported by PHS Research Grants CY-4074(C1) and CY-4074(C1S1) from the National Cancer Institute, Public Health Service.

(3) H. Schulze and K. Pieroh, *Ber.*, 55, 2332 (1922).

(4) O. Dischendorfer and H. Juvan, *Monatsh.*, 56, 272 (1930).

(5) G. S. Davy, T. G. Halsall, E. R. H. Jones, and G. D. Meakins, *J. Chem. Soc.*, 2702 (1951).

(6) J. Simonsen and W. C. J. Ross, *The Terpenes*, Vol. IV, Cambridge University Press, New York, 1957, p. 305.

(7) L. Ruzicka, H. Brungger, and E. L. Gustus, *Helv. Chim. Acta*, 15, 634 (1932).

(8) For example, consult: (a) L. Ruzicka, M. Montavon, and O. Jeger, *Helv. Chim. Acta*, 31, 819 (1948); (b) D. H. R. Barton, J. S. Fawcett, and B. R. Thomas, *J. Chem. Soc.*, 3147 (1951); (c) W. Voser, D. E. White, H. Heusser, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 35, 830 (1952); (d) D. H. R. Barton and K. H. Overton, *J. Chem. Soc.*, 2639 (1955); and (e) D. H. R. Barton and R. C. Cookson, *Quart. Rev.*, 10, 44 (1956).

(9) L. Ruzicka, A. Rudowski, J. Norymberski, and O. Jeger, *Helv. Chim. Acta*, 29, 210 (1946). An interesting application of the fuller's earth dehydration reaction has recently been described by K. Schaffner, L. Caglioti, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, 41, 152 (1958).

(10) A stable ozonide structure was excluded when this product (m.p. 240–242°) proved to be unaffected by hydrogenation (in ethyl acetate solution during 12 hr.) over 5% palladium-barium carbonate. *Cf.* L. Ruzicka, E. Volli, and O. Jeger, *Helv. Chim. Acta*, 28, 1628 (1945). The structure of this substance was not pursued further.

mixture afforded a second pure compound, m.p. 113–114°. The latter substance, on the basis of elemental analyses and infrared spectrum (λ_{max} 5.80 and 5.85 μ) appeared to represent the diketone which could arise from endocyclic olefin IV.

At first, isolation of the higher melting (240–242°) product suggested that α-apoallobetulin might actually be a mixture or that partial isomerization of the olefin might have occurred during ozonolysis. For this reason, it was considered desirable to convert α-apoallobetulin to a glycol[11] derivative (VI) and then further oxidize to the ketone. The modified osmium tetroxide-pyridine hydroxylation technique, recently described by Baran,[12] was selected for preparation of the glycol.

Allowing α-apoallobetulin to react with osmium tetroxide in pyridine solution, followed by treatment of the osmium intermediate in aqueous pyridine with sodium bisulfite,[12] yielded directly a nonhydroxylated ketone melting at 113–114°. The product was identical with the diketone (VII) obtained by ozonization of α-apoallobetulin. That this hydroxylation procedure did in fact cause the direct oxidation of an olefin (IV) to a diketone (VII) was substantiated by the following experiments. The hydroxylation step was repeated using osmium tetroxide in benzene solution, and hydrogen sulfide[13] was used to cleave the osmate ester. Lead tetraacetate oxidation of the resulting glycol[14] (VI, m.p. 230°) gave a diketone identical with the product (VII) previously obtained from both ozonization and attempted hydroxylation of α-apoallobetulin. The endocyclic olefin structure IV was therefore tentatively assigned to α-apoallobetulin.

A suitable reaction sequence that would interrelate allobetulin (IIb) and α-apoallobetulin seemed the most expeditious manner by which to conclusively prove the structure of α-apoallobetulin. Reinvestigation of the phosphorus pentachloride dehydration[3] of allobetulin was necessary, as the original conversion of this compound to α-apoallobetulin could have included a double bond migration (III → IV).[15]

Betulin diacetate (Ib) was converted to allobetulin in an ethanolic solution of dry hydrogen chloride[16] and the product (IIb) dehydrated in benzene solution at 0° with phosphorus pentachloride. The resulting olefin was apparently not the "apo-allobetulin" (m.p. 198–200°) arising from the earlier study,[3] but instead, an isomeric substance melting at 216–218°. In order to conveniently describe this new olefin (III) derived from allobetulin, the name δ-apoallobetulin is suggested.[17] Isomerization (III → IV) of δ-apoallobetulin in ethanol-carbon tetrachloride solution containing concentrated hydrochloric acid did indeed yield α-apoallobetulin. Treating δ-apoallobetulin with excess ozone at −60° in methylene chloride solution gave the expected cyclopentyl ketone derivative VIII, m.p. 215–216°.

In order to determine whether the ozonolysis product (VIII) was an A/B *cis* or *trans* ketone,[18] it was first subjected to treatment with sodium ethoxide in hot ethanol. Since unchanged starting material was recovered in almost quantitative yield, it appeared that compound VIII contained the stable A/B *cis*-configuration typical of the A-nor-3-oxo steroids.[19] Compelling support for the A/B *cis* assignment was provided by optical rotatory dispersion measurements. The positive multiple Cotton effect curve exhibited by substance VIII was analogous to the positive Cotton effect curves displayed by several A-nor-3-oxo steroids of known A/B *cis* stereochemistry.[20,21]

Isomerization of δ-apoallobetulin to α-apoallobetulin and oxidation of the latter substance to diketone VII clearly establishes that α-apoallo-

(11) A recent review of hydroxylation procedures has been prepared by F. D. Gunstone, *Advances in Organic Chemistry: Methods and Results*, Vol. I, R. A. Raphael, E. C. Taylor, and H. Wynberg, eds., Interscience, New York, 1960, p. 103.

(12) J. S. Baran, *J. Org. Chem.*, **25**, 257 (1960).

(13) *Cf.*, D. H. R. Barton and D. Elad, *J. Chem. Soc.*, 2085 (1956).

(14) The α-*cis*-glycol isomer which would arise from attack by osmium tetroxide at the less hindered α-side of IV would be expected to represent the major hydroxylation product. For example, refer to the studies of S. J. Angyal and R. J. Young, *J. Am. Chem. Soc.*, **81**, 5251 (1959), and J. A. Zderic, H. Carpio, and C. Djerassi, *J. Org. Chem.*, **24**, 909 (1959).

(15) Several closely related examples are described by L. Ruzicka, H. Silbermann, and M. Furter, *Helv. Chim. Acta*, **15**, 482 (1932); and R. Nowak, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **32**, 323 (1949). Consult also ref. 8b and 9. It is interesting to note that A. C. Cope, D. Ambros, E. Ciganek, C. F. Howell, and Z. Jacura, *J. Am. Chem. Soc.*, **82**, 1750 (1960) have reported an equilibrium constant of 1144 for the system 1-methylcyclopentene/methylenecyclopentane in acetic acid solution containing *p*-toluenesulfonic acid.

(16) This convenient procedure for the preparation of allobetulin was developed by Dr. U. R. Ghatak of this laboratory during a related study.

(17) Two other compounds isomeric with α-apoallobetulin have already been designated β- and γ-apoallobetulin (ref. 7).

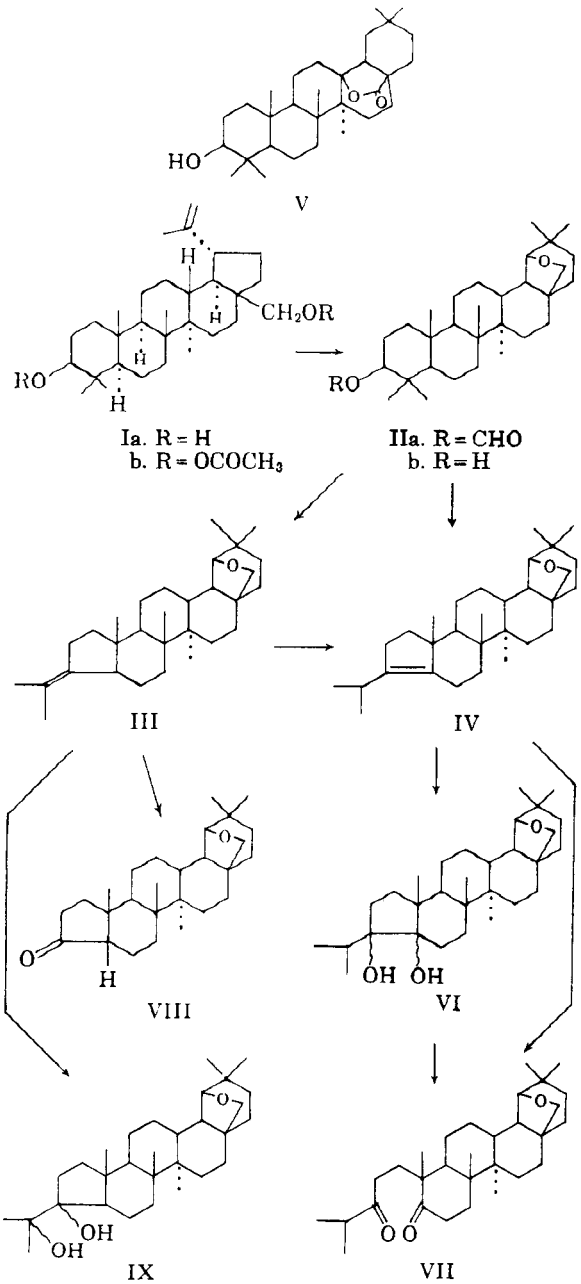
(18) A number of A-nor 3-ketones, prepared by dehydration of 3β-hydroxy-5α-triterpenoids followed by an osmium tetroxide-lead tetraacetate oxidation sequence, have been assigned the A/B *cis*-configuration on the basis of their large positive $[M]_D$ values: W. Klyne, *J. Chem. Soc.*, 2927 (1952).

(19) The following references provide a summary of recent experiments pertinent to this conclusion: (a) N. L. Allinger, R. B. Hermann, and C. Djerassi, *J. Org. Chem.*, **25**, 922 (1960); (b) N. L. Allinger and J. L. Coke, *J. Am. Chem. Soc.*, **82**, 2553 (1960); (c) N. W. Atwater, *J. Am. Chem. Soc.*, **82**, 2847 (1960); (d) W. G. Dauben, *Bull. soc. chim. France*, 1338 (1960).

(20) The epimeric 5α-isomers usually give a negative Cotton effect curve. See ref. (19a) and C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry*, McGraw Hill, New York, 1960, p. 97.

betulin is represented by the endocyclic olefin structure IV.

In view of the novel oxidation of α-apoallobetulin to diketone VII, using the modified osmium tetroxide-pyridine procedure,[12] it was of interest to determine the course of this reaction with the somewhat analogous olefin system of δ-apoallobetulin. However, glycol IX was the only well defined product isolated.



(21) While this study was in progress, J. F. Biellmann and G. Ourisson, *Bull. soc. chim. France*, 348 (1960), reported several physical constants for the A/B *trans* isomer of VIII (m.p. 230°). More recently, we have received a sample of the A/B *cis* ketone (VIII) prepared in the same laboratory. The sample of compound VIII prepared during this investigation was identical with the substance kindly provided by Professor G. Ourisson.

EXPERIMENTAL[22]

Betulin diacetate (Ib). In a typical experiment, the bark of Betula papyrifera (white birch), collected in various sections of Northeastern Maine, was cut into shavings (495 g.) and extracted with boiling benzene.[23] Following removal of solvent, the straw colored crystalline residue weighed 97 g. (20%).[24] A 28.5-g. sample of the crude extract in 50 ml. of acetic anhydride was heated at reflux for 2 hr. The product, which crystallized from the reaction mixture upon cooling, was collected, washed with several small portions of cold ethanol, and dried. The resulting impure diacetate (22.5 g.) was chromatographed in petroleum ether (b.p. 30–60°) on activated alumina. Elution with the same solvent, followed by 1:1 petroleum ether–benzene, gave 17 g. of colorless crystalline betulin diacetate, m.p. 219–222°. Recrystallization from petroleum ether gave material melting at 220–222° (lit.,[3] m.p. 223–224°).

α-Apoallobetulin (IV). The following procedure is a modification of the original method employed by Dischendorfer.[4] Fuller's earth (12 g. activated prior to use by heating at 300° for 2 hr. *in vacuo*) was added to a solution of betulin (Ia, 25 g.), prepared[25] from the diacetate derivative, in dry xylene (450 ml.) and the resulting mixture heated at reflux. After 3 hr. another 12 g. of activated fuller's earth was added and heating at reflux, with separation of water (distilling trap), was continued for a total of 6 hr. The hot mixture was filtered and the filtrate concentrated to a pale yellow residue *in vacuo*. Two recrystallizations from 95% ethanol gave colorless needles; yield 12.5 g., m.p. 200–201°, $[\alpha]_D^{23}$ +81° (*c*, 1.23), (lit.,[3,4] m.p. 198–200° and 200–201°, $[\alpha]_D$ +74.7°).

Anal. Calcd. for $C_{30}H_{48}O$: C, 84.84; H, 11.39; O, 3.77. Found: C, 84.58; H, 11.02; O, 4.36.

Ozonization of α-apoallobetulin (IV). A solution of α-apoallobetulin (3 g.) in 150 ml. of chloroform, cooled to −30°, was treated with a stream of ozone in oxygen. After excess ozone (indicated by potassium iodide solution) was detected (*ca.*, 40 min.) the flow of ozone was stopped. Acetic acid (30 ml.) was added, and the solution stirred at 0° for 1 hr. with zinc dust (9 g.). Following filtration the solution was washed successively with aqueous sodium bicarbonate and water. Removing the dry (sodium sulfate) solvent yielded a yellow gum which crystallized on trituration with methanol. Recrystallization from chloroform-methanol gave colorless needles (0.7 g.), m.p. 238–241°. Two additional recrystallizations from the same solvent mixture gave an analytical sample melting at 240–242°, $[\alpha]_D^{18}$ +67.7° (*c*, 1.34).

Anal. Found: C, 81.20; H, 10.85; O, 7.98. Two other sam-

(22) Melting points are uncorrected and were observed using open Kimble glass capillaries. Merck aluminum oxide, "suitable for chromatography," was employed in all the column adsorption chromatographic procedures. The infrared spectra were recorded by Dr. R. A. Hill and Mr. D. M. Piatak of this laboratory. RD measurements were determined in 0.1 dm. annealed quartz cells using the optical rotatory dispersion accessory (θ = 79°44′) for the Perkin-Elmer model 4000 A spectrophotometer. Several elemental analyses and all the optical rotation (chloroform solution) measurements were provided by Drs. Weiler and Strauss, Oxford, England. Microanalyses were also provided by Dr. A. Bernhardt, Mulheim, Germany.

(23) A similar procedure has been summarized by L. Ruzicka and O. Isler, *Helv. Chim. Acta*, 19, 506 (1936).

(24) We wish to thank Dr. I. B. Douglass, and Messrs. R. H. Young and T. M. Liu of this department for providing part of the crude extract necessary for this study.

(25) Betulin was readily prepared by lithium aluminum hydride reduction of the corresponding diacetate in ether solution. However, saponification of the diacetate in aqueous methanol, using potassium hydroxide, was a more convenient procedure.

ples analyzed for: C, 79.92; H, 10.62; and C, 80.75; H, 10.72.

Concentrating the methanol washings afforded a second crop (0.8 g.) which crystallized from chloroform-methanol as colorless prisms (0.55 g.) and colorless needles (0.05 g.) melting at 238–241°. Recrystallizing the product obtained in the form of prisms from chloroform-methanol gave 0.4 g. m.p. 108–110°. One additional recrystallization from the same solvent raised the melting point to 109–110.5°. A pure specimen of *diketone VII* was obtained in the form of plates following recrystallization from ethanol-water, m.p. 113–114°, $[\alpha]_D^{22}$ +35.2° (*c*, 1.32).

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 78.89; H, 10.57; O, 10.51. Found: C, 78.60; H, 10.61; O, 10.72.

Osmium tetroxide oxidation of α-apoallobetulin (IV). A solution composed of α-apoallobetulin (2.5 g.), osmium tetroxide (1.53 g.) and pyridine (40 ml.) was stirred at room temperature for 2 hr. The solution, rapidly became warm and brown. After standing for *ca.* 20 hr. at room temperature, the reaction mixture was diluted with a solution composed of sodium bisulfite (3.1 g.), water (45 ml.), and pyridine (20 ml.), and stirred for 20 min. The orange solution was extracted with chloroform and the combined extract was washed with dilute hydrochloric acid and water. Removal of solvent yielded a dark purple oil which was dissolved in benzene and chromatographed on activated alumina (60 g.). Elution with ether afforded oily fractions (1.59 g. total) which crystallized on standing, and melted from 100–109°. Recrystallization from methanol gave 0.58 g. of colorless plates, m.p. 109–111°. A pure sample of *diketone VII* recrystallized from the same solvent, m.p. 113–114°. The product was found (mixture melting point and infrared spectral comparison) to be identical with the ketone (VII, m.p. 113–114°) obtained by ozonization of α-apoallobetulin.

Osmium tetroxide hydroxylation of α-apoallobetulin (IV). To a solution of α-apoallobetulin (1.6 g.) dissolved in dry benzene (75 ml.) was added, with stirring, a solution of osmium tetroxide (1 g.) in benzene (25 ml.). After a 2-hr. period, stirring was stopped, and the brown mixture was allowed to remain at room temperature for 3 days. The dark brown solution was saturated with hydrogen sulfide and the resulting black precipitate was removed. Evaporation of the filtrate yielded a colorless gum, which solidified upon trituration with methanol. Colorless prisms (0.64 g.), m.p. 223–227°, were obtained by recrystallizing the product from methanol. Concentrating the methanol filtrate afforded 0.57 g. of lower melting (210–215°) material. An analytical sample of *glycol VI* was obtained by recrystallizing the higher melting material from ethyl acetate–methanol. The colorless prisms melted at 230°, $[\alpha]_D^{22}$ +44.9°, (*c*, 1.26), λ_{max}^{KBr} 2.85 and 2.95 μ.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 77.96; H, 11.00. Found: C, 78.46; H, 10.94.

Repeated recrystallization of the lower melting (210–215°) fraction from ethyl acetate–methanol afforded an additional quantity of pure (m.p. 230°) glycol.

Lead tetraacetate oxidation of the glycol (VI) *from α-apoallobetulin.* Part (0.12 g.) of the glycol prepared in the preceding experiment was dissolved in chloroform (5 ml.)–glacial acetic acid (12 ml.) and treated with 0.16 g. of lead tetraacetate. Following a 24-hr. period at room temperature, the reaction mixture was successively diluted with ether, washed with water, aqueous sodium carbonate, and again with water.

The nonaqueous layer was dried over sodium sulfate and concentrated to a yellow oil. A solution of the crude product in petroleum ether was chromatographed on activated alumina (5 g.). Elution with 1:3 petroleum ether–benzene gave a series of fractions (0.08 g. total) melting from 96–110°. The intermediate fractions (0.05 g.), m.p. 108–110°, were combined and further purified by three recrystallizations from methanol. Mixture melting point determination and infrared spectral comparison (chloroform solution) of the resulting colorless plates, m.p. 110–113°, with the diketone (VII, m.p. 113–114°) obtained by ozonization of α-apoallobetulin established that both were identical.

Allobetulin (IIb).[16] A saturated solution of dry hydrogen chloride in absolute ethanol (100 ml.) was added to a solution of betulin diacetate (12 g.) in chloroform (100 ml.). The resulting solution gradually darkened. After 5 days at room temperature the solution was transferred to a container of crushed ice. Following separation, the aqueous layer was extracted with chloroform and the combined organic extract washed successively with water, sodium bicarbonate solution and water. Removal of dry (sodium sulfate) solvent yielded a dark residue which crystallized upon trituration with ethyl acetate. Two recrystallizations from ethyl acetate gave almost colorless crystals (3.5 g.) melting at 261–263° (lit.,[3] m.p. 260–261°).

Passing a benzene solution of this material through a column of activated alumina gave a pure colorless specimen of allobetulin, without change in melting point, $[\alpha]_D^{22}$ +48.7°, (*c*, 1.23), (lit.,[3] $[\alpha]_D$ +48.3°).

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.42; H, 11.41.

The structure of this substance was confirmed by mixture melting point determination and infrared spectral comparison (chloroform solution) with an authentic specimen prepared from betulin diacetate according to the procedure described by Vystrcil and colleagues.[26]

Phosphorus pentachloride dehydration of allobetulin (IIb). A mixture composed of phosphorus pentachloride (0.75 g.) and a solution of allobetulin (1.1 g.) in dry benzene (200 ml.) was cooled (0°) and stirred for 2 hr. The reaction mixture was then diluted with ether and washed with water, aqueous sodium bicarbonate and again with water. After drying over sodium sulfate, the solvent was evaporated to a light brown oily residue which crystallized from chloroform-methanol as colorless plates (0.8 g.), m.p. 200–205°. Two recrystallizations from the same solvent mixture afforded a pure specimen of *δ-apoallobetulin* (*III*) melting at 216–218°, $[\alpha]_D^{22}$ +48.2°, (*c*, 1.31).

Anal. Calcd. for $C_{30}H_{48}O$: C, 84.84; H, 11.39; O, 3.77. Found: C, 84.85; H, 11.35; O, 4.18.

The mixture melting point of δ-apoallobetulin with α-apoallobetulin was 170–180°. Comparison of the infrared spectra (chloroform solution) indicated minor differences.

Conversion of δ-apoallobetulin (III) *to α-apoallobetulin* (IV). Concentrated hydrochloric acid (4 ml.) was added to a solution of δ-apoallobetulin (0.1 g.) dissolved in ethanol (100 ml.)–carbon tetrachloride (8 ml.). The solution was heated at reflux for 3 hr. and then concentrated to *ca.* 20 ml. *in vacuo*. The remaining warm solution was diluted with water and the product (0.09 g.), m.p. 201°, which crystallized, was further purified by recrystallization from chloroform-methanol. The colorless needles displayed a melting point and a mixture melting point with α-apoallobetulin of 210–211°. Infrared spectral comparison (potassium bromide) of this compound with α-apoallobetulin confirmed that both substances were identical.

Ozonization of δ-apoallobetulin (III). Reaction of δ-apoallobetulin (0.76 g.) in methylene chloride (100 ml.) solution (cooled to −60°) with ozone was carried out according to the procedure described for the ozonization of α-apoallobetulin. The crude oily product crystallized from chloroform-methanol as colorless needles (0.3 g.), m.p. 160–200°. Further purification was achieved as follows. A 1:1 petroleum ether–benzene solution of the crystalline product was chromatographed on 20 g. of activated alumina. Elution with the same solvent and recrystallization of the resulting material from ethanol led to 0.22 g. of pure *ketone VIII;* m.p. 215–216° $[\alpha]_D^{22}$ +151.6°, (*c*, 1.22), λ_{max}^{KBr} 5.78 μ. RD in dioxane (*c*, 1.07), 24–25°; $[\alpha]_{400}$ +748°, $[\alpha]_{323}$ +3430°, $[\alpha]_{316}$ +2310°, $[\alpha]_{312}$ +2981°, $[\alpha]_{305}$ +962° (shoulder), $[\alpha]_{283}$ −252°.

(26) A. Vystrcil, E. Stejskalová-Vondraskova, and J. Cerný, *Coll. Czechoslov. Chem. Commun.*, **24**, 3279 (1959).

Anal. Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 80.90; H, 10.62.

The product (VIII) was found to be identical by mixture melting point and infrared spectral comparison (chloroform solution) with a sample provided by Professor G. Ourisson.[21]

The ketone (VIII, 0.10 g.) from δ-apoallobetulin was heated during 1.5 hr. in a refluxing solution prepared from ethanol (25 ml.) and sodium (1 g.). After cooling, and dilution with dilute hydrochloric acid, starting material was recovered in almost quantitative yield.

Osmium tetroxide hydroxylation of δ-apoallobetulin (III). Hydroxylation of δ-apoallobetulin (0.6 g.) with osmium tetroxide (0.37 g.) in pyridine (10 ml.) solution was accomplished as described previously for the direct oxidation of α-apoallobetulin to diketone VII. The crude dark purple oil was dissolved in 1:1 petroleum ether–benzene and chromatographed on activated alumina (20 g.). Elution with chloroform, after first using a series of petroleum ether–benzene mixtures, benzene and ether respectively, gave a solid which recrystallized from methanol-water as almost colorless crystals; yield 0.2 g., m.p. 220–225°. Another recrystallization from the same solvent raised the melting point to 230–235° and yielded 0.16 g. A pure specimen was obtained from 95% ethanol as colorless plates, m.p. 242–244°, $[\alpha]_D^{22}$ +75.6°, (*c*, 1.23), λ_{max}^{KBr} 2.85 and 2.98 μ.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.29; H, 10.97.

ORONO, ME.

[CONTRIBUTION FROM THE MEDICAL RESEARCH LABORATORY, DEPARTMENT OF MEDICINE, VETERANS ADMINISTRATION HOSPITAL, INDIANAPOLIS, AND THE DEPARTMENT OF BIOCHEMISTRY, INDIANA UNIVERSITY SCHOOL OF MEDICINE]

Some Reactions of Methyl 3α-Hydroxy-12α-methoxy-9(11)-cholenate

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The tosylate (**2**) of methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate reacts with chloride ion, collidine, dimethylformamide and sodium methoxide to give the corresponding 3β-chloro (**3**), Δ⁸- (**4**), 3β-formoxy (**15**) and 3β-methoxy (**20**) derivatives. Compounds **2**, **3**, and **4** are readily chlorinated in the 12-position with hydrogen chloride; under the same conditions **15** loses the 3β-formate group. The tosylate (**9**) of methyl 12α-acetoxy-3α-hydroxy-9(11)-cholenate also was converted to the corresponding 3β-chloro (**10**), Δ⁸ (**11**), and 3β-formoxy (**12**) derivatives. Three compounds (**3**, **6**, and **7**) exhibit high activity in a seroflocculation reaction.

Bile acid derivatives containing two active groups (halogen, ester, unsaturation) in the ring system have been shown to be active seroflocculants.[1] Compounds containing three such active groups have not been examined, and methyl 3α-hydroxy-12α-methoxy-9(11)-cholenate seemed to be a convenient starting material for the synthesis of such a series.

As outlined in Fig. 1, the key intermediate for the preparation of five of the desired derivatives was methyl 12α-methoxy-3α-tosyloxy-9(11)-cholenate (**2**). It was prepared by room temperature tosylation of **1** under standard conditions,[1c] but resisted our attempts at crystallization.[2] The reaction of the tosylate **2** with pyridinium chloride[1c] gave a crystalline 3β-chloro derivative (**3**), and dehydrotosylation[1d] of **2** gave an oily diene (**4**).[3] Chlorination of compounds **2**, **3** and **4** following the

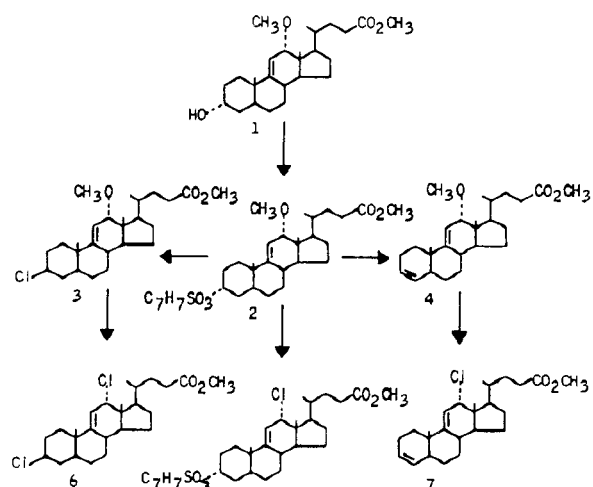

Figure 1

(1) (a) F. C. Chang and D. H. Sprunt, *J. Am. Chem. Soc.*, **76**, 3213 (1954); (b) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2161 (1957); (c) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2164 (1957); (d) F. C. Chang *et al.*, *J. Am. Chem. Soc.*, **79**, 2167 (1957); (e) R. T. Blickenstaff and F. C. Chang, *J. Am. Chem. Soc.*, **80**, 2726 (1958); (f) R. T. Blickenstaff and F. C. Chang, *J. Am. Chem. Soc.*, **81**, 2835 (1959); (g) R. T. Blickenstaff, *J. Am. Chem. Soc.*, **82**, 3673 (1960).

(2) The same compound had been obtained by Sarett [*J. Biol. Chem.*, **162**, 591 (1946)] in the form of an oil. It was dehydrotosylated to a diene which was not isolated, but was hydrolyzed to the acid. The acid was hydrogenated to 12α-methoxy-9(11)-cholenic acid.

(3) Issidorides, Fieser, and Fieser recently have reported [*J. Am. Chem. Soc.*, **82**, 2002(1960)] that dehydrotosylation of methyl 3α-tosyloxycholanate in refluxing lutidine produces an olefin mixture estimated to comprise 25% of the Δ²- and 75% of the Δ³-isomer. Methyl 2-cholenate is evidenced in the mixture by an infrared absorption band at 15.05 μ, which is in addition to the 14.70 μ band of pure methyl 3-cholenate. Our dehydrotosylation products **4** and **11** exhibit a weak band at 14.85 μ. An infrared curve of compound 4 determined on a Beckman IR5 contains only the single maximum below 15 μ, and an absorption minimum at 15.05 μ. We believe these products to be predominantly Δ⁸ and have so named them, but we cannot exclude the possibility of Δ² contamination.