[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Terpenoids. XXIII.[1] Interconversion of Thurberogenin and Betulinic Acid[2]

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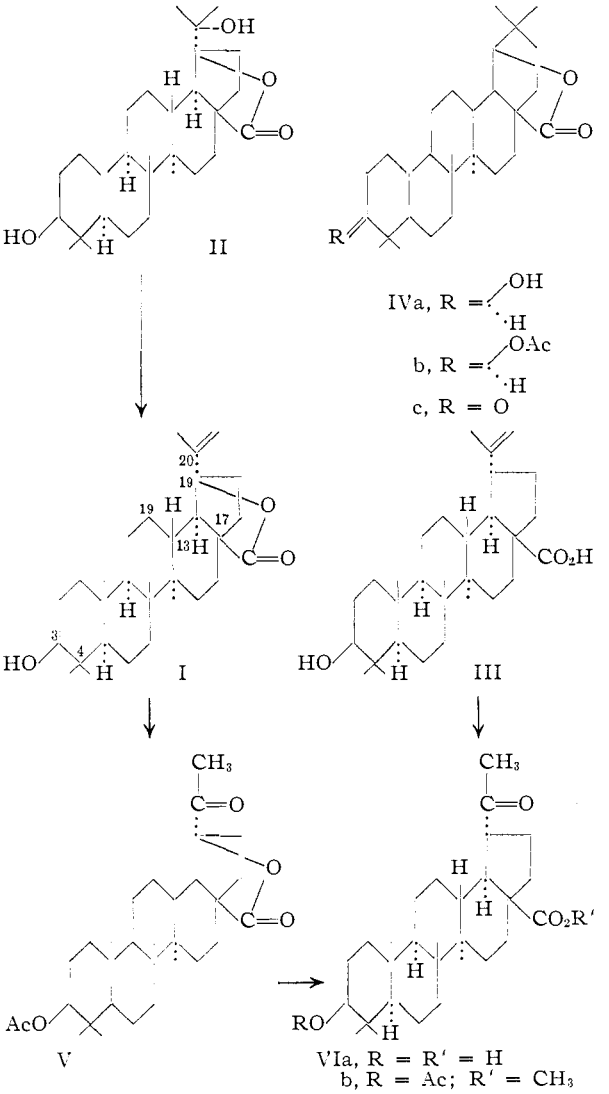
Reduction of the 30-nor-20-ketone V of thurberogenin (I) with calcium in liquid ammonia leads in poor yield to the corresponding derivative (VI) of betulinic acid. Coupled with earlier evidence, this interconversion establishes the structure and stereochemistry of the cactus triterpenes thurberogenin (I) and stellatogenin (II). Some miscellaneous reactions involving the side chain and ring E of thurberogenin are also recorded.

Thurberogenin (I)[3] and especially stellatogenin (II)[1,4] are widely distributed among various cactus species and they represent triterpenes of unusual interest. Together with dumortierigenin[5] they are the only naturally occurring triterpenoid lactones,[6] and furthermore, thurberogenin and stellatogenin belong to the rare class of lupeol triterpenes of which only three members (lupeol, betulin and betulinic acid) were known[7] prior to our investigations of cactus triterpenes. The structure assignment[8] of these two substances, which had not been converted to a triterpene of known structure, rested on circumstantial evidence of the following type:

The presence in thurberogenin (I) of the 3β-hydroxy-4,4-dimethyl moiety in ring A, of an isopropenyl side chain and of a five-membered lactone ring involving a tertiary hydroxyl group was established[8] rigorously. Since stellatogenin (II) can be dehydrated[4] to thurberogenin (I) without rearrangement and possesses an additional tertiary hydroxyl group, the relationship between the two triterpenes implicit in structures I and II is unequivocal. The co-occurrence[4] of stellatogenin with oleanolic acid and especially with betulinic acid (III) and oxyallobetulin (IV)[9] suggests on biogenetic grounds that the carbonyl group of the lactone ring originates at C-17. Assuming a normal triterpene skeleton, thurberogenin and stellatogenin must then be represented by structures I and II or the corresponding isomers with the lactone ring terminating at C-13. The reasons for our preference for I (and II) are given in detail in our earlier paper[8] and are based to a large extent upon the base-catalyzed E-homo rearrangement of the 30-nor-20-ketone V derived from thurberogenin (I), a reaction which would not have occurred if the lactone terminated at C-13.



Any rigorous structure proof of a new triterpene must ultimately involve conversion to a known member of this class and initial attempts to accomplish this were unsuccessful. Some of these abortive experiments are described below since they are of some intrinsic interest, particularly when compared with analogous reactions in the

(1) Paper XXII, C. Djerassi, A. Bowers, S. Burstein, H. Estrada, J. Grossman, J. Herran, A. J. Lemin, A. Manjarrez and S. C. Pakrashi, THIS JOURNAL, **76**, 2312 (1956).

(2) This investigation was supported by a research grant (No. G-3863) from the Division of Research Grants of the National Institutes of Health, U. S. Public Health Service.

(3) C. Djerassi, L. E. Geller and A. J. Lemin, THIS JOURNAL, **75**, 2254 (1953).

(4) C. Djerassi, L. H. Liu, E. Farkas, A. E. Lippman, A. J. Lemin, L. E. Geller, R. N. McDonald and B. J. Taylor, *ibid.*, **77**, 1200 (1955).

(5) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, *ibid.*, **78**, 2969 (1954).

(6) The crude glycosides of *Lemaireocereus dumortieri* (reference 5) and *L. stellatus* (reference 4) show an infrared band (Nujol mull) at 5.65 μ attributable to a five-membered lactone. Coupled with the evidence presented earlier (footnote 7 in reference 8) this would indicate that the lactone ring was present in the plant material rather than produced during the acid hydrolysis of the glycoside.

(7) For reviews see O. Jeger in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Vol. VII, Springer, Vienna, p. 1, and D. H. R. Barton in E. H. Rodd's "Chemistry of Carbon Compounds," Vol. IIB, Elsevier Press, Houston, Texas, 1953, p. 726.

(8) C. Djerassi, E. Farkas, L. H. Liu and G. H. Thomas, THIS JOURNAL, **77**, 5330 (1955).

(9) The isolation of oxyallobetulin is described in the Experimental portion. It is not certain whether this substance is present in the cactus or formed as an artifact from betulinic acid during the acid hydrolysis of the glycosides.

steroid series. The correlation of thurberogenin (I) (and hence also of stellatogenin (II)) with a triterpene of established structure has now been accomplished by a two-step sequence involving ozonization[8] to the 30-nor-20-ketone V followed by reduction with calcium in liquid ammonia.[10] The major product was a tetrol, characterized as the triacetate VIIb and most likely represents the C-20 epimer of the tetrol VIIa obtained earlier[8] by lithium aluminum hydride reduction of the nor-ketone V. The minor portion of the calcium–ammonia reduction was acidic and after methylation and reacetylation yielded the known[11] methyl 3-acetoxy-30-nor-20-ketobetulinate (VIb); identity with an authentic specimen was established by mixture melting point determination, infrared comparison and close similarity of the rotatory dispersion curves[12] (*Fig. 1*).

The stereochemistry of betulinic acid (III) has been established[13] and since only C-19 is involved in the calcium reduction, all other asymmetric centers of thurberogenin (I) must be stereochemically identical with those of betulinic acid (III). In particular, this requires a D/E *trans* juncture with the potential C-17 carboxyl group β-oriented, which in turn, permits lactone formation only *via* a 19β-oxygen bridge. The termination point of the lactone ring at C-19 follows from the mechanism of the calcium–liquid ammonia reduction and of the base-catalyzed E-homo rearrangement[8] of the nor-ketone V. The correctness of structures I and II for thurberogenin and stellatogenin is thus confirmed.

Most of our earlier attempts to transform thurberogenin (I) into a degradation product of a known triterpene were centered at the conversion to a trisnor-19-ketone. Trisnorketones of the lupeol series are known,[14] and the corresponding betulin derivative VIII has now been prepared by the same procedure as described in the Experimental section. However, attempts[8] to obtain this ketone VIII by glycol cleavage (lead tetraacetate, periodic acid or sodium bismuthate) of the tetrols VIIa or VIIc failed and this surprising resistance must be ascribed to steric hindrance[15] of the glycol moiety produced by the C-12 methylene group and the axial substituent at C-17. An alternative route to this trisnor ketone VIII was based on the reported[16] oxidation of steroidal 17α-hydroxyetianic acids (XIIa) to 17-ketosteroids (XIII). The appropriate acid XIa in the thurberogenin series could be prepared by ozonolysis of the unsaturated aldehyde X, which in turn had been obtained earlier[8] by selenium dioxide oxidation of the triol diacetate derived from thurberogenin, but all attempts to oxidize it to a ketone failed, only unchanged acid XIa being recovered.

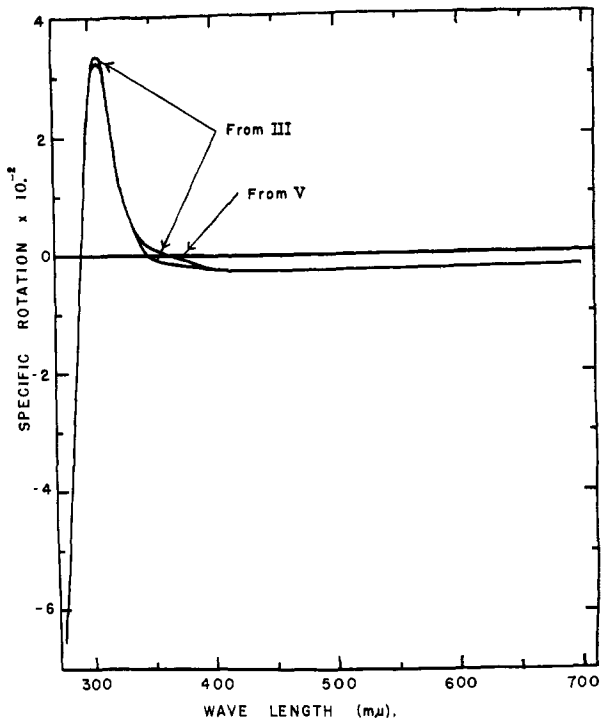
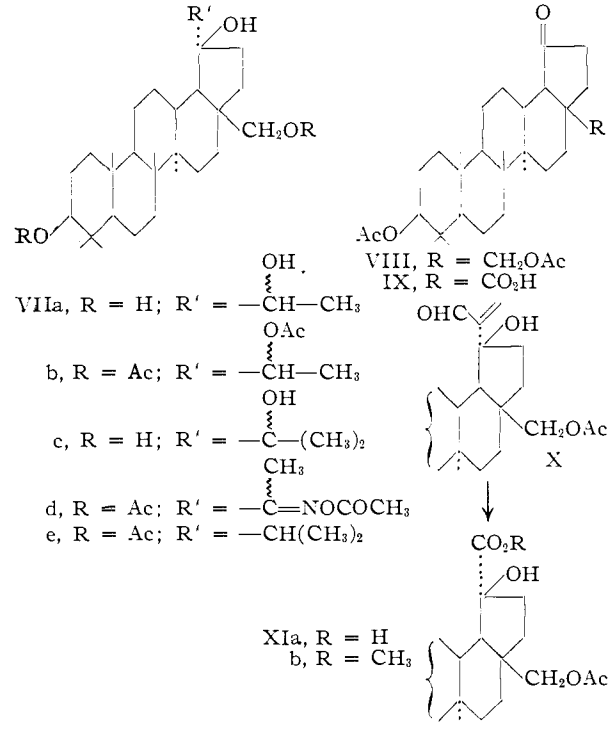


Fig. 1.—Rotatory dispersion (dioxane solution) of methyl 3-acetoxy-30-nor-20-ketobetulinate (VIb).



Attention was next directed at converting thurberogenin to the trisnorketone IX, derivable from

(10) *Cf.* J. H. Chapman, J. Elks and L. J. Wyman, *Chemistry and Industry*, 603 (1955).

(11) L. Ruzicka and E. Rey, *Helv. Chim. Acta*, **26**, 2143 (1943).

(12) The application of rotatory dispersion measurements to structural problems in the triterpene field will be covered in a future paper. For general discussion of rotatory dispersion of polycyclic (steroid) ketones and experimental procedure, see C. Djerassi, W. Closson and A. E. Lippman, THIS JOURNAL, **78**, 3163 (1956), and earlier papers cited therein.

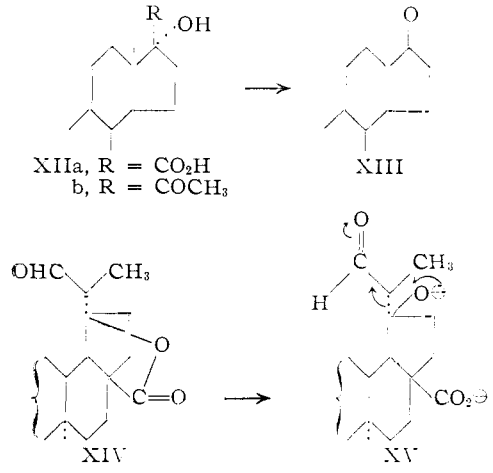
(13) J. M. Guider, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 3024 (1953).

(14) L. Ruzicka, W. Huber and O. Jeger, *Helv. Chim. Acta*, **28**, 195 (1945); G. S. Davy, E. R. H. Jones and T. G. Halsall, *Rec. trav. chim.*, **69**, 368 (1950).

(15) *Cf.* T. R. Ames, J. L. Beton, A. Bowers, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 1912 (1954).

(16) *Cf.* J. v. Euw and T. Reichstein, *Helv. Chim. Acta*, **25**, 988 (1942); H. L. Mason, W. M. Hoehn and E. C. Kendall, *J. Biol. Chem.*, **124**, 459 (1938).

betulinic acid. Since 17α-hydroxy-20-ketosteroids XIIb can be degraded to 17-ketosteroids XIII by either perbenzoic acid oxidation[17] or Beckmann rearrangement of the oxime,[18] these conditions were applied to the nor-ketone V but without success. The failure of these reactions appears to be a further reflection of the considerable degree of steric hindrance at C-19 and C-20. Finally, the saturated aldehyde XIV, readily obtainable[8] in two steps from thurberogenin acetate, was subjected to treatment with base in the hope that a reverse aldol condensation (XV, arrows) would lead to propionaldehyde and the desired trisnor-ketone IX. Both the saturated aldehyde XIV as well as its α,β-unsaturated precursor[8] were affected by base to yield, after acidification, predominantly acidic material,[19,20] but neither propionaldehyde nor any crystalline degradation product could be isolated.



Other miscellaneous attempts to correlate thurberogenin with a known triterpene (*e.g.*, taraxastane series) are described in the Experimental portion and were not pursued further once the successful calcium–ammonia reduction had been accomplished.

Experimental[21]

Calcium–Ammonia Reduction of 3-Acetoxy-30-nor-20-ketothurberogenin (V).—A solution of 1.1 g. of the nor-ketone V[8] (purified chromatographically at the stellatogenin acetate, thurberogenin acetate and nor-ketone stages) in 125 cc. of dry toluene was added to 2 g. of calcium metal dissolved in 500 cc. of liquid ammonia (distilled from sodium). The mixture was stirred for 5 hr. at Dry Ice temperature while adding 0.5-g. portions of calcium every 30 minutes. At the end of the reaction, 25 g. of ammonium chloride was added, the ammonia was allowed to evaporate, the residue was diluted with water containing 20 cc. of acetic acid and extracted thoroughly with chloroform.

The chloroform was extracted with *cold* 5% aqueous potassium hydroxide (in which lactones of the thurberogenin type are insoluble), the extracts were acidified with acetic acid and the product again removed with chloroform. This procedure was repeated in order to ensure that only acidic material was removed and yielded 96 mg. of crude acid VIa. Brief methylation with ethereal diazomethane followed by acetylation with acetic anhydride–pyridine and chromatography on 20 g. of alumina (deactivated with 3% of 10% acetic acid) yielded from the benzene eluates after crystallization from methanol–chloroform 21 mg. of **methyl 3-acetoxy-30-nor-20-ketobetulinate (VIb)**, m.p. 215–218°, $[\alpha]_D$ −22° (*c* 0.24), rotatory dispersion[12] (Fig. 1) in dioxane: $[\alpha]_{700}$ −17°, $[\alpha]_{589}$ −23°, $[\alpha]_{275}$ −623°, "max." $[\alpha]_{307.5}$ +324°. Identity with an authentic specimen (see below) was confirmed by mixture melting point determination and identity of the infrared spectra.

Anal. Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.82; H, 10.07.

The original chloroform solution (after alkaline extraction) was evaporated to dryness, and the residue was refluxed for 3 hr. with 2.5% methanolic potassium hydroxide in order to convert any unreacted lactone V to the acidic E-homo rearrangement product.[8] The mixture was poured into water and extracted with chloroform to furnish 805 mg. of neutral material. Acetylation with acetic anhydride followed by chromatography over deactivated alumina and elution with benzene–ether (4:1) yielded a crystalline residue which was recrystallized several times from methanol–chloroform; yield, 105 mg., m.p. 280–283° (with sublimation), $[\alpha]_D$ +26.6°. This product is apparently the triacetate VIIb of the tetrol VIIa and is probably epimeric at C-20 with the corresponding derivative (m.p. 220–222°)[8] resulting from the lithium aluminum hydride reduction of V.

Anal. Calcd. for $C_{35}H_{56}O_7$: C, 71.39; H, 9.59. Found: C, 70.95; H, 9.34.

A 45 mg. sample of the triacetate VIIb was refluxed for 5 hr. with 5% methanolic potassium hydroxide and the resulting triol VIIa was purified by recrystallization from dilute methanol and by sublimation at 230° and 0.01 mm., m.p. 275–280° (with sublimation), $[\alpha]_D$ +15° (methanol).

Anal. Calcd. for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 75.23; H, 10.95.

Conversion of Betulin to Methyl 3-Acetoxy-30-nor-20-ketobetulinate (VIb).—The required betulinic acid (III) methyl ester acetate was prepared by a slight modification of the literature directions[22] from the readily available betulin which was obtained from birch bark.

A mixture of 2 g. of betulin diacetate and 0.2 g. of potassium hydroxide in 1:1 methanol–dioxane (400 cc.) was kept for 24 hr. at room temperature. The crude product was chromatographed on deactivated alumina and after removing 105 mg. of unreacted betulin diacetate with benzene–hexane (4:1), the desired betulin 3-monoacetate (1.6 g., m.p. 260–263°) was eluted with benzene–ether (4:1). A small amount of betulin (210 mg.) could be recovered from the column by washing with ether.

The betulin 3-monoacetate (1.5 g.) was oxidized with 230 mg. of chromium trioxide in 25 cc. of glacial acetic acid for 10 minutes at 25° and 15 minutes on the steam-bath. The crude acid was methylated with diazomethane and purified by chromatography leading to 400 mg. of methyl 3-acetoxybetulinate, m.p. 197–201°.

The above acetate methyl ester (370 mg.) was left standing with 185 mg. of osmium tetroxide in 50 cc. of dioxane for 5 days at 0° and the osmate ester was cleaved by the sodium sulfite technique. The crude product (305 mg.) was oxidized (24 hr., room temperature) without purification with periodic acid in methanol and the crude solid was chromatographed. Elution with benzene and recrystallization from methanol–chloroform furnished the desired nor-ketone VIb.[23] m.p. 214–216°, $[\alpha]_D$ −18° (*c* 1.0); rotatory dispersion[24]

(17) N. S. Leeds, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **76**, 2265 (1954).

(18) J. Schmidt-Thomé, *Angew. Chem.*, **67**, 715 (1955); *Ber.*, **88**, 895 (1955).

(19) It should be remembered (reference 8) that thurberogenin itself is not affected by alkali except for opening of the lactone ring and that acidification results in immediate relactonization.

(20) While the course of this reaction, especially with the unsaturated aldehyde, is obscure, it nevertheless must be associated in some manner with the 19-hydroxyl substituent. As demonstrated in the Experimental portion of this paper, the unsaturated aldehyde derived from betulin diacetate is not affected by base except for loss of the acetoxyl groups.

(21) Melting points were determined on the Kofler block. Unless noted otherwise, rotations were measured in chloroform solution. All spectroscopic measurements were carried out by Mrs. Dolores Phillips. The microanalyses were performed by Spang Microanalytical Laboratory, Plymouth, Michigan.

(22) L. Ruzicka, A. H. Lamberton and E. W. Christie, *Helv. Chim. Acta*, **21**, 1706 (1938).

(23) The literature constants (reference 11) for this substance prepared by another route are: m.p. 235°, $[\alpha]_D$ −16°.

(24) We are grateful to Mrs. R. Riniker for these measurements.

(Fig. 1) in dioxane: $[\alpha]_{700}$ −26°, $[\alpha]_{589}$ −26°, $[\alpha]_{275}$ −654°, "max." $[\alpha]_{307.5}$ + 336°.

Anal. Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.63; H, 9.87.

Isolation of Oxyallobetulin (IV).—The thurberogenin for the present work was prepared[4] by dehydration of crude stellatogenin 3-monoacetate, which in turn had been obtained from a new lot of *Lemaireocereus stellatus*. In one instance, the resulting thurberogenin acetate was found to be contaminated by another substance which could not be separated by chromatography but which on repeated (10–15) recrystallization was obtained in a pure state. Its infrared carbonyl bands at 5.65 and 5.78 μ indicated the presence of lactone and acetoxyl groups, and the substance was identified as oxyallobetulin acetate (IVb) (m.p. *ca.* 360°, $[\alpha]_D$ +55°) by direct comparison with an authentic specimen[25] and by conversion to oxyallobetulone (IVc) (m.p. 330–333°) which is the most suitable derivative.[26]

Oxyallobetulin acetate (IVb) can be removed very readily by chromatography *prior* to dehydration since stellatogenin acetate is considerably more polar.

19-Ketotrisnorbetulin Diacetate (VIII).[27]—A solution of 900 mg. of 3β,28-diacetoxy-19α-methoxycarbonyltrisnorlupane[13] in 200 cc. of benzene was added to a boiling solution of phenylmagnesium bromide prepared from 2 g. of magnesium, 6 cc. of bromobenzene and 20 cc. of ether. The ether was removed and the benzene solution was refluxed for 6 hr. After decomposition with ammonium chloride, the crude product was passed through a column of alumina. The solid (440 mg., m.p. 195–199°) apparently represented a mixture of the diphenylcarbinol and the diphenylethylene since it possessed λ_{max}^{EtOH} 250 mμ, log ϵ 3.97.

A 195-mg. sample was dehydrated completely by refluxing for 3 hr. with 25 cc. of acetic anhydride and 4 cc. of pyridine, and the product was purified by chromatography (m.p. 247–251°, λ_{max}^{EtOH} 248 mμ, log ϵ 4.3), but no analytical sample of the diphenylethylene was prepared. The total material was ozonized in chloroform solution at 25° and the ozonide decomposed in acetic acid solution with zinc dust. Chromatography on alumina removed benzophenone in the benzene–hexane fractions while elution with ether–benzene furnished the desired trisnorketone VIII, which was recrystallized from methanol–chloroform; m.p. 246–250°, $[\alpha]_D$ +37.5°, $\lambda_{max}^{CHCl_3}$ 5.79 and 8.01 μ.

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.35; H, 9.65.

3β,28-Diacetoxy-19β-hydroxy-19α-methoxycarbonyltrisnorlupane (XIb).—A stream of ozonized oxygen was passed through a solution of 450 mg. of the triol-diacetoxy-aldehyde X[8] in 50 cc. of acetic acid for 40 minutes at 20°. Water (50 cc.) was added, the solution was heated on the steam-bath for 5 minutes and the crystalline material (330 mg., m.p. 199–207°) was collected. This appeared to be the intermediate ozonide and an analytical sample was obtained by dissolving in ether, washing with dilute potassium bicarbonate and recrystallizing rapidly from dilute acetic acid; colorless crystals (bright yellow when hot), m.p. 208–212°, $[\alpha]_D$ +1°, no high selective absorption in the ultraviolet, $\lambda_{max}^{CHCl_3}$ 2.90 and broad band at 5.78–5.81 μ.

Anal. Calcd. for $C_{34}H_{52}O_9$: C, 67.52; H, 8.67. Found: C, 68.01; H, 8.86.

The mother liquors of the ozonide were evaporated to dryness and crystallized from dilute acetic acid to furnish 80 mg. of the desired bisnor acid XIa, m.p. 251–260°. The same acid was produced in nearly quantitative yield when the crystalline ozonide was warmed for 5 minutes with a 5% solution of 30% hydrogen peroxide in acetic acid. For characterization purposes, a specimen of the acid was methylated with diazomethane, passed through an alumina column and recrystallized from methanol–chloroform. The methyl ester XIb exhibited m.p. 171.5–173.5°, $[\alpha]_D$ +19°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.68; H, 9.35. Found: C, 70.55; H, 9.25.

(25) Prepared from betulin *via* allobetulin formate according to H. Schulze and K. Pieroh, *Ber.*, **55**, 2332 (1922).

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

(27) This experiment was carried out by Dr. L. H. Liu and Mr. R. N. McDonald using the procedure employed earlier (reference 14) in the lupeol series.

The bisnor acid XIa (45 mg.) was recovered unchanged after standing at room temperature for 12 hr. with 50 mg. of chromium trioxide[16] in 5 cc. of glacial acetic acid. No neutral material was formed when 40 mg. of the acid XIa was heated at 50–60° for 1.5 hr. with 60 mg. of lead tetraacetate[16,28] in 10 cc. of acetic acid.

Miscellaneous Experiments. (a) **Reaction of 30-Aldehydo Derivatives with Base.**—When the unsaturated aldehyde (diacetoxylupenal), obtained by selenium dioxide oxidation[29] of betulin diacetate, is refluxed for 2 hr. with 2% potassium carbonate solution in 1:1 dioxane–water, there is obtained the free dihydroxy aldehyde[29] accompanied by some 3-monoacetate, m.p. 240–245°, $\lambda_{max}^{CHCl_3}$ 2.90, 5.80 and 5.90 μ. When the reaction time is extended beyond 4 hours, only the free diol is isolated.

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 77.57; H, 10.22.

Under comparable conditions (or refluxing with 2% potassium hydroxide for 8–12 hr.), the unsaturated aldehyde[8] derived from thurberogenin yields over 90% of acidic material[19] but no crystals were obtained, even after chromatography of the methylated and acetylated product. The same applied to the saturated aldehyde XIV[8] and to the unsaturated aldehyde X.

(b) **Reaction of 30-Nor-20-ketothurberogenin Acetate (V) with Perbenzoic Acid.**—A solution of 220 mg. of the norketone V and 20 mg. of *p*-toluenesulfonic acid monohydrate in 15 cc. of ethyl acetate was kept at 0° for 4 days with 1.1 equivalents of perbenzoic acid in 1 cc. of chloroform. Over 95% of unreacted starting material was recovered. The same results were observed in chloroform solution in the absence of *p*-toluenesulfonic acid.

(c) **Reactions of 30-Nor-20-ketothurberogenin Acetate (V) Oxime.**—The Beckmann rearrangement was carried out with phosphorus oxychloride in pyridine solution,[18] but less than 5% of crude, acidic material was produced.

Acetylation of the 3-acetoxy oxime[8] with acetic anhydride–pyridine followed by recrystallization from methanol–chloroform led to plates of the 3-acetoxy oxime acetate, m.p. 245–249° dec., $\lambda_{max}^{CHCl_3}$ 5.80 and 6.05 μ.

Anal. Calcd. for $C_{33}H_{49}NO_6$: C, 71.32; H, 8.89. Found: C, 71.20; H, 9.12.

In an attempt to prepare a 19-hydroxy-20-amine which might be useful for certain ring E enlargement reactions, 95 mg. of the oxime[8] was refluxed for 2 hr. with an excess of lithium aluminum hydride in ether. Acetylation, chromatography and recrystallization from methanol–chloroform produced 30 mg. of the oxime acetate VIId, m.p. 222–232° dec., $\lambda_{max}^{CHCl_3}$ 5.80 and 6.07 μ.

Anal. Calcd. for $C_{35}H_{55}NO_7$: C, 69.83; H, 9.21; N, 2.33. Found: C, 69.87; H, 9.11; N, 2.39.

(d) **Attempted Dehydration of Dihydrothurberogenin Triol Diacetate (VIIe).**—The resistance of the tertiary hydroxyl group in thurberogenin triol diacetate has already been noted[8] and several experiments were now carried out in order to determine whether dehydration would be more facile in the dihydro analog VIIe. An uncrystallizable gum was obtained when the diacetate was refluxed for 2 hr. with boron trifluoride etherate in benzene while the following experimental conditions resulted in 80–90% recovery of starting material; refluxing for 2 hr. in benzene solution with thionyl chloride, refluxing for 30 minutes with phosphorous oxychloride in pyridine, refluxing for 1 hour with acetic anhydride and sodium acetate or shaking with an excess of phosphorus pentachloride in petroleum ether for 30 minutes.

(e) **Reactions of the E-Homo Rearrangement Product of V.**—Any correlation of thurberogenin with a member of the taraxastane group would require elimination of the 19α-hydroxy group of the E-homo rearrangement product (as the 3-acetoxy methyl ester).[8] The hydroxyl group was not removed by zinc dust in refluxing acetic acid and since ketol acetates react more readily under those conditions,[30] an attempt was make to acetylate the 19α-hydroxy group under

(28) *Cf.* E. Rohrmann, R. G. Jones and H. A. Shonle, THIS JOURNAL, **66**, 1856 (1944).

(29) L. Ruzicka, M. Brenner and E. Rey, *Helv. Chim. Acta*, **25**, 161 (1942).

(30) *Cf.* R. S. Rosenfeld and T. F. Gallagher, THIS JOURNAL, **77**, 4367 (1955), and references cited.

acid-catalyzed conditions which have proved effective with 17α-hydroxy-20-keto steroids,[31] but only unreacted starting material was recovered.

(31) *Cf.* Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, THIS JOURNAL, **74**, 5394 (1952); R. B. Turner, *ibid.*, **75**, 3489 (1953).

Acknowledgment.—We should like to acknowledge the benefit of a stimulating discussion with Prof. Gilbert Stork of Columbia University.

DETROIT, MICHIGAN

COMMUNICATIONS TO THE EDITOR

THE RESOLUTION OF O-ETHYL ETHYLPHOSPHONOTHIOLIC ACID

Sir:

We wish to record the successful resolution of O-ethyl ethylphosphonothiolic acid ($C_2H_5(C_2H_5O)P(O)SH$).[1] The compound was resolved by fractional recrystallization of its quinine salt (I) from acetone–ether. The more insoluble diastereoisomeric salt (Ia) crystallized as a monohydrate: prisms, m.p. 151–153° (with loss of its water of hydration), $[\alpha]^{26}D$ −96.6 ± 0.8° (α_{obs} −1.990 ± 0.015°, acetone, 2-dcm., c = 1.130), equiv. wt., 492 (calcd. 497 for the monohydrate). When vacuum dried over phosphorus pentoxide for three hours at 100°, Ia gave rise to anhydrous product: m.p. 158–160°, $[\alpha]^{33}D$ −97.6 ± 0.6° (α_{obs} −1.070 ± 0.007°, acetone, 1-dcm., c = 1.096), equiv. wt., 469 (calcd. 479).

The acid was separated from Ia as its sodium salt in an essentially aqueous solution by treating Ia in methanol with an equivalent amount of aqueous base. That the phosphorus atom maintains its tetrahedral configuration in the anion is demonstrated by the optical activity of the product recovered from the sodium salt. The acid was recovered by the addition of an equivalent amount of dilute hydrochloric acid to the sodium salt solution and extraction of the product from the resulting solution with ether. The acid was characterized as its dicyclohexylamine salt: m.p. 159–160.5° $[\alpha]^{25}D$ −7.11 ± 0.23° (α_{obs} −0.153 ± 0.005°, methanol, 1-dcm., c = 2.150), found: C, 57.38; H, 10.00 (calcd. for $C_{16}H_{34}O_3NP$: C, 57.28; H, 10.22).

After the removal of a mixed middle crop of I, the more soluble diastereoisomeric salt (Ib) crystallized as soft anhydrous needles: m.p. 166–168°, $[\alpha]^{25}D$ −81.7 ± 0.6° (α_{obs} −1.613 ± 0.012, acetone, 2-dcm., c = 0.9868); equiv. wt., 475 (calcd. 479). The dicyclohexylamine salt of this enantiomorph of the acid gave m.p. 158–160°, $(\alpha)^{25}D$ +6.85 ± 0.25° (α_{obs} + 0.221 ± 0.008°, methanol, 1-dcm., c = 3.230), found: C, 57.30, H 10.02, mixed melting point with the enantiomorphic dicyclohexylamine salt, above: 163–165°. Racemic O-ethyl ethylphosphonothiolic acid forms a dicyclohexylamine salt, m.p. 166–168°.

This communication represents the first reported resolution of a phosphorus acid, the optical activity of which is due solely to the presence of an asymmetric phosphorus atom. Moreover, the presence of a reactive group directly attached to phosphorus in a resolved compound of this type provides one with a convenient tool applicable to a study of the reactions and stereochemistry of the asymmetric phosphorus atom. Detailed reports on the resolution, reactions and stereochemistry of this and similar compounds will be published at a later date.

(1) The preparation of alkylphosphonothiolic acids will be described in a forthcoming paper by F. W. Hoffmann and co-workers.

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ELECTROPHORETIC DEMONSTRATION OF THE ISOMERIZATION OF BOVINE PLASMA ALBUMIN AT LOW *p*H

Sir:

Recently much interest has been exhibited in a pronounced conformational change which takes place in bovine plasma albumin at *p*H values acid to the isoelectric point. It was first suggested by Tanford[1] that expansion of the protein molecule results upon titration with acid. Gutfreund and Sturtevant[2] demonstrated a slow thermal effect upon adding acid to this protein. Yang and Foster[3] demonstrated a parallel and reversible enhancement of the optical rotation and intrinsic viscosity acid to *p*H 4, and suggested that there exists an all-or-none equilibrium between two forms of the protein molecule. Tanford[4] has recently shown evidence for an intermediate which he terms the "expandable" form.

We have recently been successful in attaining excellent resolution of two boundaries in the electrophoretic patterns of this protein over the *p*H range 4.6 to 3.5. Heterogeneity of plasma albumins in this *p*H range has been reported previously.[5-8] However, we can now demonstrate that this heterogeneity is due in the main to a *p*H dependent transition of the normal form of the protein into a faster migrating form, presumably of higher positive charge. Results summarized in

(1) C. Tanford, *Proc. Iowa Acad. Sci.*, **59**, 205 (1952).

(2) H. Gutfreund and J. Sturtevant, THIS JOURNAL, **75**, 5447 (1953).

(3) J. T. Yang and J. F. Foster, *ibid.*, **76**, 1588 (1954); **77**, 2374, 3895 (1955).

(4) C. Tanford, J. Buzzell, D. Rands and S. Swanson, *ibid.*, **77**, 6421 (1955).

(5) J. Luetscher, *ibid.*, **61**, 2888 (1939).

(6) D. Sharp, G. Cooper, J. Erickson and H. Neurath, *J. Biol. Chem.*, **144**, 139 (1942). In this paper it was also shown that heterogeneity disappears below *p*H 3.5, in accord with our own results.

(7) R. Alberty, *J. Phys. Chem.*, **53**, 114 (1949).

(8) L. Longsworth and C. Jacobsen, *ibid.*, **53**, 126 (1949).