[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Terpenoids. VIII.[1] The Structures of the Cactus Triterpenes Gummosogenin and Longispinogenin[2,3]

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Hydrolysis of the glycosides of the Mexican cactus *Machaerocereus gummosus* yielded a new triterpene, gummosogenin, which was shown to be Δ^{12}-18β-oleanene-3β,16β-diol-28-al (II). Lithium aluminum hydride reduction led to longispinogenin, previously isolated from the Guatemalan cactus *Lemaireocereus longispinus*, thus establishing its structure as that of Δ^{12}-18β-oleanene-3β,16β,28-triol (III). This was confirmed by partial synthesis from echinocystic acid (IV).

In connection with our systematic survey of natural products occurring in giant cacti of the subtribe *Cereanae*,[6-10] it became of considerable interest to investigate the cactus *Machaerocereus gummosus*, one of two species of the genus *Machaerocereus* which grows in the Mexican States of Baja California and Sonora.[11] There existed strong evidence that this cactus contains saponins, since it is reported[11] to be used as a fish poison by the natives and indeed Heyl[12] isolated from this cactus a crude, acidic hemolytic principle which he named cereinic acid. The substance was not characterized further and no reports on cactus saponins have appeared since that time.

Through the kind coöperation of Mr. Howard E. Gates of Corona, California, it has been possible to secure a generous supply of this cactus, which was processed in the customary manner[6,7] by acid hydrolysis of the crude glycosidic fraction. From the neutral[13] portion there was isolated a new triterpene, $C_{30}H_{48}O_2$, which we have named gummosogenin.

The oxygen functions of gummosogenin were characterized easily since the sapogenin formed a diacetate and its infrared spectrum demonstrated the presence of a carbonyl group ($\lambda_{max}^{CHCl_3}$ 5.88 μ) which was confirmed by the preparation of a semicarbazone and of an oxime. Wolff–Kishner reduction[14] afforded in excellent yield the known[15] triterpene maniladiol (I), thus establishing the constitution of gummosogenin with the exception of the nature and position of the carbonyl function. While various oxidation and carbonyl classification tests did not indicate unequivocally whether the carbonyl group was present as an aldehyde or ketone, it was possible to narrow down the various structural possibilities in the following manner. The stability of gummosogenin to bismuth oxide[16] excluded an α-ketol formulation[17] (keto groups at C-2 or C-15), while the stability to base demonstrated the absence of a β-hydroxy ketone grouping (carbonyl at C-1). The reactivity of the carbonyl group automatically excluded positions 6, 11[18] and 19, while the recovery of gummosogenin after treatment with ethyl formate and sodium methoxide eliminated from consideration a reactive methylene group adjacent to the carbonyl function. Mild oxidation of gummosogenin with the chromium trioxide–pyridine complex[19] furnished a tricarbonyl compound which proved to be very sensitive to alkali. This suggested a β-diketone moiety and hence restricted the placement of the carbonyl group to positions 22, 23, 24 and 28. An unequivocal decision in favor of position 28 was arrived at as follows:

Lithium aluminum hydride reduction of gummosogenin proceeded smoothly to yield a triol, which by direct comparison of its physical constants and those of the derived triacetate and triformate was shown to be identical with longispinogenin, a triterpene which was recently[8] isolated in this Laboratory from the Guatemalan cactus *Lemaireocereus longispinus* together with oleanolic acid and erythrodiol. Since the latter two triterpenes are oxygenated at C-28, this suggested on biogenetic grounds that the remaining unassigned hydroxyl group of longispinogenin, and hence the carbonyl group of gummosogenin, should be placed at C-28 and this was proved by partial synthesis from echinocystic acid (IV). Since the C-16 hydroxyl group of maniladiol (I) (and hence of gummosogenin and longispinogenin with which it has been correlated) is equatorial while that of echinocystic acid (IV) is axial,[8,20] it was necessary to start from methyl diketoechinocystate (V)[21] and a generous sample of this sub-

(1) Paper VII, C. Djerassi and C. M. Foltz, THIS JOURNAL, **76**, 4085 (1954).

(2) A preliminary communication has appeared in *Chem. & Ind.*, 161 (1954).

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(4) Organon Predoctorate Research Fellow, 1952-1953.

(5) Postdoctorate Research Fellow, 1953, on funds supplied by the U. S. Public Health Service.

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

(7) C. Djerassi, N. Frick and L. E. Geller, *ibid.*, **75**, 3632 (1953).

(8) C. Djerassi, R. N. McDonald and A. J. Lemin, *ibid.*, **75**, 5940 (1953).

(9) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, *ibid.*, **76**, 2939 (1954).

(10) C. Djerassi, C. R. Smith, S. P. Marfey, R. N. McDonald, A. J. Lemin, S. K. Figdor and H. Estrada, *ibid.*, **76**, 3215 (1954).

(11) H. Bravo, "Las Cactaceas de Mexico," Mexico D.F., 1937, pp. 285–287.

(12) G. Heyl, *Arch. Pharm.*, **239**, 451 (1901).

(13) The acidic triterpenes are now being investigated in this Laboratory by Dr. A. E. Lippman.

(14) Huang-Minlon, THIS JOURNAL, **71**, 3301 (1949).

(15) R. Morice and J. C. E. Simpson, *J. Chem. Soc.*, 795 (1940); O. Jeger, M. Montavon and L. Ruzicka, *Helv. Chim. Acta*, **29**, 1124 (1946); E. Bischof, O. Jeger and L. Ruzicka, *ibid.*, **32**, 1911 (1949).

(16) W. Rigby, *J. Chem. Soc.*, 793 (1951).

(17) Even hindered ketols react with this reagent (*cf.* C. Djerassi, H. J. Ringold and G. Rosenkranz, THIS JOURNAL, **73**, 5513 (1951)).

(18) This position is also excluded by the absence of the typical ultraviolet and infrared maxima corresponding to an unsaturated ketone.

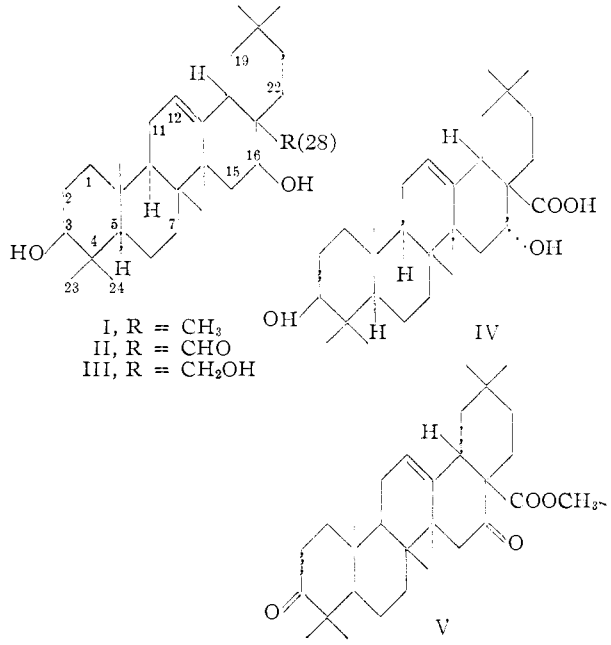
(19) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(20) *Cf.* D. H. R. Barton and N. J. Holness, *J. Chem. Soc.*, 78 (1953).

(21) W. R. White and C. R. Noller, THIS JOURNAL, **61**, 983 (1939).

stance was made available to us through the kind coöperation of Prof. C. R. Noller of Stanford University. Reduction of the diketo ester with lithium in liquid ammonia in the presence of methanol[22] led to longispinogenin, thus establishing structure III for this triterpene and the 28-aldehydo formulation II for gummosogenin. The two other known aldehydo-triterpenes (gypsogenin and quillaic acid[23]) contain this group at C-23.

While a discussion of biogenetic relations among the cactus triterpenes will be deferred until the structures of the various new triterpenes now under investigation in this Laboratory have been elucidated, it is pertinent to point out that the close structural similarity of the two triterpenes gummosogenin (II) and longispinogenin (III) is reflected in the close botanical relationship[24] of the genera *Lemaireocereus* and *Machaerocereus* from which the two triterpenes have been isolated.



Experimental[25]

Isolation of Gummosogenin.—After removal of the spines,[26] the fresh cactus, *Machaerocereus gummosus* (16.5 kg.), was cut into small pieces, dried at 80° to constant weight, powdered in a Wiley mill and the dry material (1.74 kg.) was extracted continuously in a Soxhlet extractor with 9 l. of 95% ethanol for 62 hours. The dark colored solution was evaporated *in vacuo* and the residue (294 g.) was extracted with ether until no more color was removed. This ether extract furnished only traces of oily material when processed in the usual manner for alkaloids or triterpenes. The dark brown, amorphous, ether-insoluble "glycosidic fraction" (280 g.) was refluxed for 4 hours with 1 l. of methanol and 243 cc. of concd. hydrochloric acid, diluted with water and extracted thoroughly with ether. Acidic material[13] was removed by washing repeatedly with 5% potassium hydroxide solution (insoluble potassium salts were filtered); after washing with water until neutral, drying and evaporating there was obtained 46 g. of dark green semi-solid which was chromatographed on 1 kg. of neutral alumina (activity II–III). Elution with benzene–ether (7:3) furnished 13.3 g. (0.76% dry basis; 0.08% based on fresh cactus) of colorless gummosogenin with m.p. 242–245°, which after several recrystallizations from methanol led to the analytical sample with the following constants: m.p. 251–252°, $[\alpha]^{25}D$ +28°; $\lambda_{max}^{CHCl_3}$ 2.80 and 5.88 μ; λ_{max}^{EtOH} 297 mμ, log ϵ 1.95; yellow color with tetranitromethane.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 79.22; H, 10.40.

The diacetate, prepared by the acetic anhydride–pyridine method (15 hours room temperature), crystallized from methanol as needles, m.p. 219–221°, $[\alpha]^{25}D$ +66°, $\lambda_{max}^{CHCl_3}$ 5.87 and 8.0 μ (type A band).[27] Alkaline saponification (10% methanolic potassium hydroxide) regenerated gummosogenin.

Anal. Calcd. for $C_{34}H_{52}O_5$: C, 75.51; H, 9.69; acetyl, 15.90. Found: C, 75.53; H, 9.63; acetyl, 15.25.

Gummosogenin semicarbazone was obtained in nearly quantitative yield after refluxing for 2 hours with an ethanolic solution of semicarbazide; colorless needles from dilute ethanol, m.p. 301–302°.

Anal. Calcd. for $C_{31}H_{51}N_3O_3$: C, 72.47; H, 10.01; N, 8.18. Found: C, 72.65; H, 9.61; N, 8.41.

Gummosogenin diacetate oxime (hydroxylamine hydrochloride, pyridine solution, 1 hour at 90° and 48 hours at room temperature) was recrystallized from aqueous methanol whereupon it showed m.p. 225–227°.

Anal. Calcd. for $C_{34}H_{53}NO_5$: C, 73.47; H, 9.61; N, 2.52. Found: C, 73.55; H, 9.27; N, 2.52.

Wolff–Kishner Reduction of Gummosogenin.—A solution of gummosogenin (0.24 g.) in 9 cc. of ethanol, 8 cc. of diethylene glycol and 1.15 cc. of 85% hydrazine hydrate was refluxed for 30 minutes. Potassium hydroxide (0.75 g.) was added and refluxing was continued for an additional 15 minutes, after which time the condenser was removed and the temperature was allowed to rise to 195°. After refluxing for 2.5 hours, the solution was poured into water, extracted with ether, washed and evaporated yielding 0.235 g. of colorless solid, m.p. 218–220°. Crystallization from ethanol gave **maniladiol** (I), m.p. 220–222°, $[\alpha]^{25}D$ +64°[28]; the infrared absorption spectrum, determined by Dr. G. D. Meakins of the University of Manchester, Manchester, England, using a Perkin–Elmer double beam recording spectrophotometer was identical with that of an authentic sample of maniladiol obtained through the courtesy of Prof. E. R. H. Jones from the collection of the late Dr. J. C. E. Simpson.[14]

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.39. Found: C, 81.58; H, 11.22.

Maniladiol diacetate (acetic anhydride–pyridine, overnight) had m.p. 196–198°, $[\alpha]^{25}D$ +80°.

Anal. Calcd. for $C_{34}H_{54}O_4$: C, 77.82; H, 9.99. Found: C, 78.07; H, 10.20.

Maniladione.—A solution of maniladiol (0.20 g.) in acetone (30 cc.) was treated dropwise with a chromic acid–sulfuric acid solution[29] until a persistent yellow color was

(22) F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi (THIS JOURNAL, **75**, 1282 (1953)) have shown that reduction of even the most hindered ketone by this procedure leads to the corresponding equatorially oriented hydroxyl group. In order to accomplish the simultaneous reduction of the hindered ester function of V, a large amount of methanol was required (*cf.* W. S. Johnson, B. Bannister, B. M. Bloom, A. D. Kemp, R. Pappo, E. R. Rogier and J. Szmuszkovicz, *ibid.*, **75**, 2275, footnote 5 (1953)).

(23) *Cf.* O. Jeger in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Vol. 7, 1950, p. 1.

(24) N. L. Britton and J. N. Rose, "The Cactaceae," Vol. II, Carnegie Institution of Washington, Washington, D. C., 1920, p. 114.

(25) Melting points are corrected (Kofler hot-stage). The infrared absorption spectra were measured with a Baird Associates double beam recording infrared spectrophotometer employing sodium chloride cells of 0.1 mm. thickness. Rotations were determined in chloroform solution. We are indebted to Mr. Joseph F. Alicino (Metuchen, N. J.) and Geller Microanalytical Laboratories (Hackensack, N. J.) for the microanalyses.

(26) In a number of instances where it was difficult to handle the cactus even with thick gloves, it was found convenient to burn off the spines with a blow torch. Control experiments have shown that in view of the thick skin such treatment does not affect the qualitative or quantitative composition of the products isolated.

(27) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951).

(28) Lit. (ref. 15): maniladiol, m.p. 220–221°, $[\alpha]D$ +68°, diacetate, m.p. 193–194°, $[\alpha]D$ +80°, maniladione, m.p. 213°, $[\alpha]D$ +54°, diformate, m.p. 191°, $[\alpha]D$ +84°.

(29) *Cf.* A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2555 (1953).

obtained and allowed to stand at room temperature for 30 minutes. Dilution with water, extraction with ether, washing and evaporation gave 0.19 g. of the crude product, m.p. 190–198°. The analytical sample was crystallized from methanol followed by sublimation at 150° and 0.005 mm. whereupon it exhibited m.p. 211–212.5°, $[\alpha]^{25}D$ +56°.

Anal. Calcd. for $C_{30}H_{46}O_2$: C, 82.13; H, 10.57. Found: C, 82.23; H, 10.46.

Maniladiol diformate prepared by refluxing maniladiol with formic acid in benzene solution for 2 hr. was crystallized from methanol; m.p. 192–194°, undepressed upon admixture with an authentic specimen supplied by Prof. E. R. H. Jones (University of Manchester), $[\alpha]^{25}D$ +80°.

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 77.18; H, 10.04.

Lithium Aluminum Hydride Reduction of Gummosogenin.—Gummosogenin (0.7 g.) in 100 cc. of tetrahydrofuran was stirred with 2.0 g. of powdered lithium aluminum hydride at room temperature for 3 hr., when the excess of reagent was decomposed by addition of dilute sulfuric acid. Extraction with ether, washing and evaporation yielded 0.7 g. of white crystalline material with m.p. 244–247°. Crystallization from acetone gave the analytical sample, m.p. 247–249°, undepressed on admixture with **longispinogenin (VIII)**, $[\alpha]^{25}D$ +51°, infrared absorption spectrum identical with that of authentic longispinogenin.[8]

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.55; H, 11.00.

Longispinogenin triacetate, prepared by acetylation of the above triol (acetic anhydride–pyridine, overnight) had m.p. 218–221°, undepressed on admixture with authentic longispinogenin triacetate,[8] $[\alpha]^{25}D$ + 70°; identity was confirmed by infrared comparison.

Longispinogenin Triformate.—A solution of 0.2 g. of longispinogenin in 12 cc. of benzene and 12 cc. of 98% formic acid was shaken overnight at room temperature, washed with water and evaporated. Crystallization from ethyl acetate–petroleum ether furnished the triformate, m.p. 179–181°, $[\alpha]^{25}D$ +88°.

Anal. Calcd. for $C_{33}H_{50}O_6$: C, 73.03; H, 9.29. Found: C, 72.56; H, 9.30.

The same substance was obtained when longispinogenin, isolated from the cactus,[8] was formylated.

Lithium–Ammonia Reduction of Methyl Diketoechinocystate (V).—To a solution of 0.5 g. of methyl diketoechinocystate[21] in 50 cc. of redistilled liquid ammonia, 20 cc. of methanol and 20 cc. of ether was added with stirring 1.2 g. of lithium in portions over a period of 80 minutes, after which time a permanent blue color was obtained. After addition of 3.5 g. of ammonium chloride, the mixture was allowed to stand at room temperature until the ammonia had evaporated. Water was added and the mixture was extracted with ether, washed with water and evaporated to yield 0.49 g. of colorless solid with m.p. 232–237°. Chromatography on neutral alumina (activity II–III) and elution with benzene–ether (1:1) followed by crystallization from acetone produced **longispinogenin (III)**, m.p. 246–249°, $[\alpha]^{25}D$ +54°; **triacetate**, m.p. 218–220°, $[\alpha]^{25}D$ +69°; *triformate*, m.p. 178–180°, $[\alpha]^{25}D$ +88°. Identity was established in each instance by mixture melting point determination and infrared comparison with the appropriate authentic samples.

Miscellaneous Experiments with Gummosogenin. (a) **Chromium Trioxide–Pyridine Oxidation.**—To a solution of 0.6 g. of chromium trioxide in 20 cc. of pyridine was added at 0° a solution of 0.5 g. of gummosogenin in 10 cc. of the same solvent. After 75 minutes at room temperature, water was added, the mixture was extracted with ether, washed well, dried and evaporated yielding 0.45 g. of the crude colorless oxidation product with m.p. 162–165°. Crystallization from methanol and sublimation at 165° and 0.005 mm. led to the analytical sample of **Δ^{12}-18β-oleanene-3,16-dione-28-al**, m.p. 173–174°, $\lambda_{max}^{CHCl_3}$ 5.80 and 5.88 μ, no pronounced selective absorption in the ultraviolet.

Anal. Calcd. for $C_{30}H_{44}O_3$: C, 79.60; H, 9.80. Found: C, 79.11; H, 10.09.

(b) **Attempted Oxidation with Bismuth Oxide.**—A sample of gummosogenin was refluxed with excess bismuth oxide in acetic acid solution for 14 hours. Acetylation of the crude reaction product yielded over 80% of gummosogenin diacetate.

(c) **Attempted Condensation with Ethyl Formate.**—Over 90% of unreacted starting material was recovered when 0.3 g. of gummosogenin was allowed to stand in benzene (25 cc.) solution with 0.78 g. of sodium methoxide and 0.1 cc. of ethyl formate for 12 hours at room temperature in an atmosphere of nitrogen.

$\Delta^{11,13(18)}$-Oleadiene-3β,16β,28-triol Triacetate.—At an early stage of this investigation it was desirable to demonstrate the presence of the 12–13 double bond in longispinogenin by independent means and this was carried out by the standard[30] selenium dioxide oxidation.

A solution of 0.2 g. of longispinogenin triacetate in 20 cc. of glacial acetic acid was refluxed for 75 minutes with 0.2 g. of selenium dioxide, filtered, diluted with water and the product was extracted with ether. Crystallization of the residue (0.2 g., m.p. 167–169°) after evaporation of the ether from ethanol led to the analytical sample with m.p. 178–179°, $[\alpha]D$ −29°; λ_{max}^{EtOH} 242, 250 and 258 mμ, log ϵ, 4.27, 4.40, 4.23; orange-brown color with tetranitromethane.

Anal. Calcd. for $C_{36}H_{54}O_6$: C, 74.19; H, 9.34. Found: C, 74.10; H, 9.14.

DETROIT, MICHIGAN

(30) L. Ruzicka, G. Müller and H. Schellenberg, *Helv. Chim. Acta*, **22**, 767 (1939).