28–38°) was done in order to remove the nitrobenzene completely. A 20.5% (2.74 g.) yield of yellow material melting at 220–222° was obtained. The analytical sample was recrystallized a third time from nitrobenzene to give material melting at 221–222°. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: C, 53.60; H, 3.00; N, 15.61; B, 4.02; neut. equiv., 269.06. Found: C, 53.73, 53.80; H, 3.27, 3.18; N, 15.39, 15.41; B, 4.00, 3.98; neut. equiv., 285.7.

In another experiment where the reaction mass was stirred at 0–5° for 8 hr., an 82.5% yield of crude material melting over the range of 144–170° was obtained. This gave a 17.5% yield of pure material.

For best results, the optimum *p*H was found to be about 8. Both sodium bicarbonate and sodium acetate were used as buffers in some experiments, but they did not seem to be particularly useful. Chromatographic purification was tried without any great success.

2-Hydroxy-5-(*p*-nitrophenylazo)-benzeneboronic Acid Anhydride.—To a stirred solution of 6.00 g. (0.05 mole) of *o*-hydroxybenzeneboronic acid anhydride and 10 g. (0.25 mole) of sodium hydroxide in 50 ml. of water, cooled in an ice–salt-bath to 0–5°, was added slowly a solution of 6.9 g. (0.05 mole) of *p*-nitroaniline diazotized according to the method of Ropp and Coyner.[29] The alkaline reaction mixture (*p*H approximately 8) was stirred at −10 to 0° for 6 hr. and was then filtered. The brick-red filter cake was suspended in 500 ml. of water, and this was acidified by the addition of 10% hydrochloric acid. A 70% (9.35 g.) yield of material melting over the range of 184–200° was obtained. Acidification of the filtrate from the reaction mixture gave an additional 0.51 g. (3.8%) of material having a melting point range of 205–225°. The combined crude materials could not be recrystallized directly from any solvent with much success. The crude was therefore extracted with a small amount of hot acetone and the residue was dissolved in 250 ml. of hot acetone. The insoluble portion was removed by filtration and the dye was reprecipitated from the filtrate by the addition of 900 ml. of warm water. The material, now having a melting point of 207–208°, was recrystallized from nitrobenzene using the procedure described above for the *m*-isomer; 1.4 g. (10.4%) of orange product melting sharply at 241.8–242° was obtained. The analytical sample was recrystallized one additional time from nitrobenzene to give material melting at 242–242.2°. The infrared spectrum supported the expected structure.

Anal. Calcd. for $C_{12}H_8BN_3O_4$: N, 15.61; B, 4.02; neut. equiv., 269.06. Found: N, 15.39, 15.16; B, 3.92, 4.22; neut. equiv., 290.1.

In another experiment an 84% yield of crude material was obtained when an 8-hr. coupling time at 0–5° was used. When the coupling time was increased to 10.5 hr., the amount of crude material did not change, but the product melted at 187° instead of over the range reported above. In this particular experiment 17% of the original *o*-hydroxybenzeneboronic acid anhydride was recovered. The pure yield was not determined exactly in these last two cases. The use of sodium bicarbonate as a buffer did not show any advantages. All of the couplings were run at a *p*H of 8. Chromatographic purification also was attempted on this compound, but it did not work very satisfactorily.

Acknowledgment.—We wish to thank Mr. Robert McCord and Mr. E. Miller Layton of the Ames Laboratory of the Atomic Energy Commission for the infrared spectra. We also wish to acknowledge the support of the Division of Biology and Medicine of the United States Atomic Energy Commission who have made this work possible. The results of the biological testing of these compounds will be reported by Dr. Otho D. Easterday of the Brookhaven National Laboratory.

AMES, IOWA

(29) G. A. Ropp and E. C. Coyner, *Org. Syntheses*, **31**, 80 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Terpenoids. XXVII.[1] The Structure of the Cactus Triterpene Myrtillogenic Acid[2]

BY CARL DJERASSI AND H. G. MONSIMER[3]

RECEIVED JANUARY 9, 1957

Myrtillogenic acid has been shown to be 3β,16β,28-trihydroxy-Δ^{12}-oleanen-29-oic acid (Ia) by various degradation reactions and by conversion to longispinogenin triacetate (XIIb).

During an investigation[4] of the triterpene composition of the genus *Myrtillocactus*, there was isolated from several species in small amounts a new triterpene acid. Since this substance has not been encountered in any other genus of the cactus family, we have named it "myrtillogenic acid" and the present paper is concerned with its structure elucidation.

Myrtillogenic acid was separated from the other triterpenes as the methyl ester ($C_{31}H_{50}O_5$) and then saponified to the free acid ($C_{30}H_{48}O_5$). Mild acetylation with acetic anhydride–pyridine of either the ester or the acid led to the corresponding triacetate, thus accounting for all oxygen functions. The ease of acetylation suggested that the three hydroxyl groups were either equatorially oriented or that one or more were primary. Membership in the β-amyrin class of triterpenes was indicated[5] by the course of the selenium dioxide oxidation of triacetyl methyl myrtillogenate which furnished a $\Delta^{11,13(18)}$-diene (subsequently shown to be II) with the typical[6] triple ultraviolet absorption maxima at 240, 248 and 258 mμ.

All of the triterpene acids which have been encountered[1] so far among the *Cactaceae* contained the carboxyl group at C-17, but two different lines of evidence could be presented that this was not the case with myrtillogenic acid. Oleanolic acid (IIIa)

(1) Paper XXVI, "Cactus Triterpenes," by C. Djerassi in "Festschrift Arthur Stoll." Birkhäuser, Basel, 1957, pp. 330–352.

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

(3) Taken from part of the Ph.D. dissertation of H. G. Monsimer.

(4) C. Djerassi, S. Burstein, H. Estrada, A. J. Lemin, A. E. Lippman, A. Manjarrez and H. G. Monsimer, THIS JOURNAL, in press.

(5) That this is not an unambiguous criterion, as had been believed earlier, was demonstrated recently in the case of the cactus triterpene dumortierigenin (C. Djerassi, C. H. Robinson and D. B. Thomas, *ibid.*, **78**, 5685 (1956)) which belongs to the β-amyrin series but does not react to any appreciable extent with selenium dioxide. However, a positive reaction, as was observed with myrtillogenic acid, appears to be conclusive.

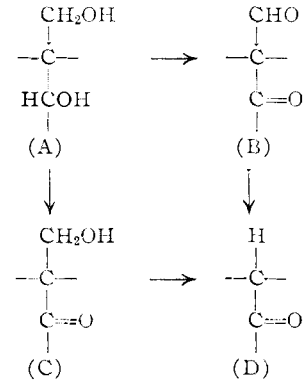
(6) *Cf.* L. Ruzicka, G. Müller and H. Schellenberg, *Helv. Chim. Acta*, **22**, 767 (1939); D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

and related triterpenes are known to form bromolactones[7] and a 16β-acetoxy group does not interfere as shown by the facile formation of the bromolactone IV derived from cochalic acid (IIIc)[8] diacetate. Similar treatment of triacetyl myrtillogenic acid (subsequently shown to be Ic) led only to recovered starting material. Bromolactone formation requires the presence of a carboxyl group at C-17 as well as a double bond at 12–13, and since the presence of the latter was established by the course of the above-mentioned selenium dioxide oxidation as well as by allylic oxidation to an α,β-unsaturated 11-ketone (see Experimental), it was concluded that the carboxyl group of myrtillogenic acid was located elsewhere. Strong support for this supposition was provided by comparative saponification studies.

The methyl esters of oleanolic (IIIb), cochalic (IIId), desoxoglycyrrhetic (IIIf) and myrtillogenic (Ib) acids were refluxed for 8 hr. with 5, 7 and 10% methanolic potassium hydroxide and the extent of saponification determined quantitatively (see Table I, Experimental). The order of ease of saponification was found to be methyl myrtillogenate (Ib) > methyl desoxoglycyrrhetate (IIIf) > methyl cochalate (IIId) > methyl oleanolate (IIIb), the latter being recovered completely unchanged even after treatment with 10% alkali. The facility with which methyl myrtillogenate could be saponified—exceeding even the relatively unhindered methyl desoxoglycyrrhetate (IIIf)—definitely excluded any of the angular positions[9] as sites for the carboxyl group[10] thus leaving only C-4 and C-20 as possible points of attachment.[11]

Attention was next directed toward locating the three hydroxyl groups of myrtillogenic acid. Since the fifteen cactus triterpenes for which structures have been established[1] all possess a 3β-hydroxyl function, it was assumed that this also applied to myrtillogenic acid. The methyl ester did not react with lead tetraacetate nor did it form an acetonide, thus excluding a 1,2-glycol moiety as well as hydroxylation at C-23 or 24.[12] More precise information about the disposition of the hydroxyl groups could be adduced by oxidation experiments.

Oxidation of methyl myrtillogenate ($C_{31}H_{50}O_5$) with chromium trioxide in acetic acid–chloroform[13] led to a neutral substance ($C_{31}H_{44}O_5$), lacking hydroxyl absorption in the infrared, and mild treatment with base resulted in the loss of the elements of CO and the formation of a second neutral product ($C_{30}H_{44}O_4$). When the oxidation was carried out with chromium trioxide in pyridine solution,[14] there was isolated a new compound ($C_{31}H_{46}O_5$) which still exhibited infrared hydroxyl absorption. Treatment with base gave the above mentioned neutral product $C_{30}H_{44}O_4$, which did not show any high, selective ultraviolet absorption, did not give a color with ferric chloride but did possess a 3-keto group since its precursor exhibited a positive Zimmermann test comparable with that of β-amyrone.[15] These observations require the presence of a 1,3-glycol system of type A, chromium trioxide–acetic acid oxidation producing a keto-aldehyde B while chromium trioxide–pyridine apparently did not attack the primary hydroxyl function and led to a keto-alcohol C. Base treatment of B (reverse Claisen condensation)[16] or C(retro-aldol condensation)[17] then furnished the identical nor-ketone D.



With this information at hand it is possible by a process of elimination to reduce the structural alternatives for myrtillogenic acid to two (I or VI). The 1–3 glycol system A cannot involve C-3 since acetonide formation[12] should have been feasible. Furthermore, partial saponification of triacetyl methyl myrtillogenate (Id) under conditions where a 3β-acetoxy group is not attacked[18] gave a 3-monoacetate Ie which was oxidized with chromium trioxide–pyridine[14] to a ketone still showing a hydroxyl band in the infrared. This oxidation product is assigned structure VII since it gave a negative Zimmermann reaction[15] (absence of 3-keto function), exhibited a

(7) See E. J. Corey and J. J. Ursprung, THIS JOURNAL, **78**, 183 (1956), and earlier literature.

(8) C. Djerassi, G. H. Thomas and H. Monsimer, *ibid.*, **77**, 3579 (1955).

(9) Position 27 was eliminated at an early stage since myrtillogenic acid does not suffer decarboxylation upon melting as would be expected from a β,γ-unsaturated acid (*e.g.*, quinovic acid—H. Wieland and M. Erlenbach, *Ann.*, **453**, 83 (1927)).

(10) Suitably situated hydroxyl groups can have an activating effect (Table I—methyl oleanolate (IIIb) *vs.* methyl cochalate (IIId)) but only a carbonyl group (known to be absent in myrtillogenic acid) γ to the carboxyl group will produce a striking effect (*cf.* C. Djerassi and A. E. Lippman, THIS JOURNAL, **77**, 1825 (1955)).

(11) Since adjacent hydroxyl groups can have an effect upon the rate of saponification (ref. 10) and since the location of the hydroxyl substituents in myrtillogenic acid has not yet been discussed, both orientations (29 and 30) at C-20 should be considered. On the other hand, it is known that an axial (β) carboxyl group (24) at C-4 is extremely hindered (*e.g.*, β-boswellic acid—P. Bilham, G. A. R. Kon and W. C. J. Ross, *J. Chem. Soc.*, 35 (1942); J. L. Beton, T. G. Halsall and E. R. H. Jones, *ibid.*, 2904 (1956)), so that here only an equatorial substituent would be compatible with the experimental facts.

(12) A 3β,23-diol (W. Jacobs, *J. Biol. Chem.*, **63**, 631 (1925)) or a 3β,24-diol (J. L. Beton, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 2904 (1956)) is known to form an acetonide, but 1,3-glycols in other locations are not necessarily excluded. For instance, longispinogenin (XIIa) does not condense with acetone under standard conditions (L. E. Geller, M.S. Thesis, Wayne University, 1954; P. R. Leeming, unpublished observation).

(13) A. Zürcher, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **37**, 2145 (1954).

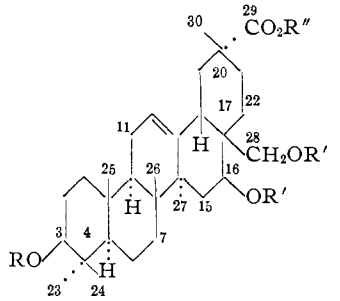
(14) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(15) We are greatly indebted to Drs. W. Klyne and P. M. Jones (Postgraduate Medical School, London) for carrying out the color reactions by the spectrophotometric procedure of I. E. Broadbent and W. Klyne, *Biochem. J.*, **56**, XXX (1954).

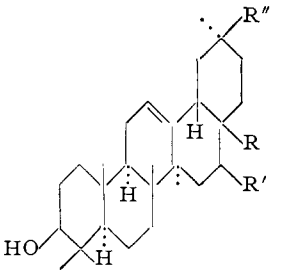
(16) Formyl ketones are very sensitive to base and lose formate under mild conditions (for pertinent examples see A. L. Wilds and C. Djerassi THIS JOURNAL, **68**, 1715 (1946)).

(17) A typical example is represented by icterogenin (D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 887 (1954)).

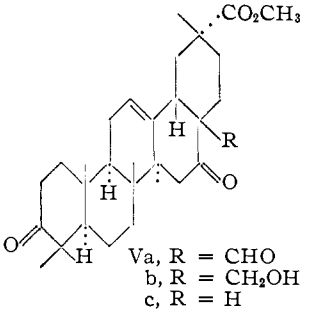
(18) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, THIS JOURNAL, **76**, 1969 (1954).



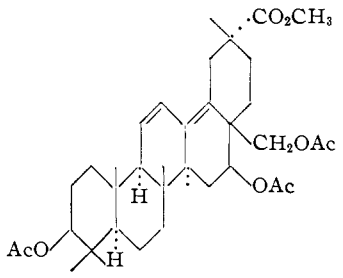
Ia, R = R′ = R″ = H
b, R = R′ = H; R″ = CH_3
c, R = R′ = Ac; R″ = H
d, R = R′ = Ac; R″ = CH_3
e, R = Ac; R′ = H; R″ = CH_3



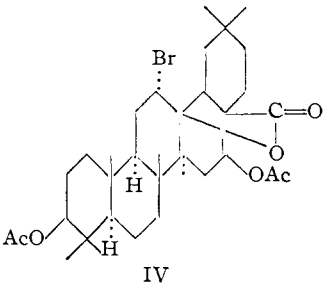
IIIa, R = CO_2H; R′ = H; R″ = CH_3
b, R = CO_2CH_3; R′ = H; R″ = CH_3
c, R = CO_2H; R′ = OH; R″ = CH_3
d, R = CO_2CH_3; R′ = OH; R″ = CH_3
e, R = CH_3; R′ = H; R″ = CO_2H
f, R = CH_3; R′ = H; R″ = CO_2CH_3



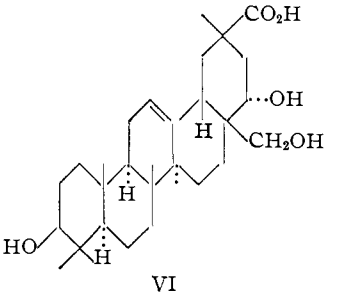
Va, R = CHO
b, R = CH_2OH
c, R = H



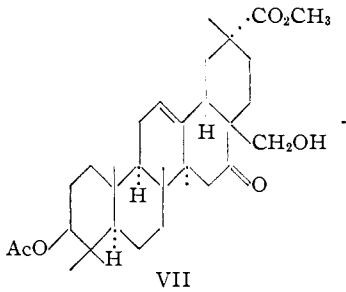
II



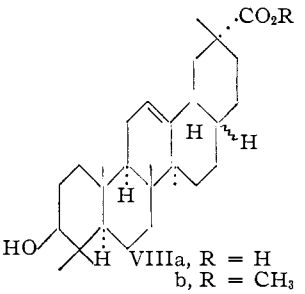
IV



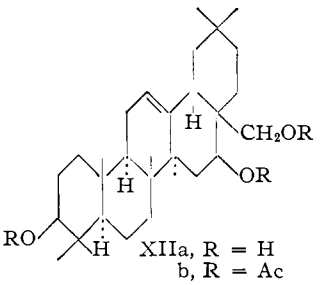
VI



VII



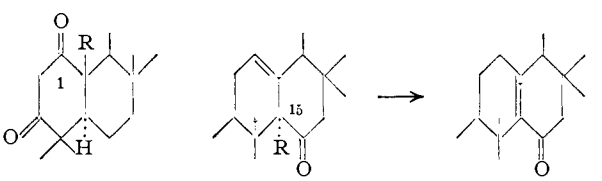
VIIIa, R = H
b, R = CH_3



XIIa, R = H
b, R = Ac

rotatory dispersion curve suggestive of a 16-keto triterpene[19] and upon Wolff–Kishner reduction produced an acid VIIIa, further characterized as a methyl ester VIIIb, in which two of the oxygen functions had been lost. These missing oxygen atoms must have been derived almost certainly from a 1,3-glycol A *via* C followed by retro-aldolization and Wolff–Kishner reduction and cannot have involved C-3, which was protected (up to the Wolff–Kishner stage) by acetylation.

The 1,3-glycol system A cannot be located at C-1 and C-25 since the resulting keto-aldehyde B and keto-alcohol C would have to be represented by IXa and IXb. Base treatment would not only have resulted in expulsion of the angular substituent but the nor-ketone D, still possessing a 1,3-diketone moiety (IXc), would itself have been labile to base; furthermore its structure would not have been consistent with the spectroscopic properties of the product. Positions 7 and 26 were not considered seriously as sites for the glycol system A since no naturally occurring triterpenes are known which are oxygenated at those carbon atoms.[20] Finally C-15 and C-27 could be excluded since the oxidation products (B and C) would have to be represented by **X** (a and b) and base treatment would have led to an α,β-unsaturated nor-ketone **XI**, while the actual product did not show any infrared or ultraviolet maxima corresponding to such a chromophore.



IXa, R = CHO
b, R = CH_2OH
c, R = H

Xa, R = CHO
b, R = CH_2OH

XI

The above analysis indicated that the primary alcohol of the 1,3-glycol would have to be attached at C-17 or C-20. However, the latter (on the basis

(19) The use of rotatory dispersion curves for locating carbonyl groups in triterpenoids will be reported in a future paper.

(20) It was appreciated that rigorous elimination of this alternative would have to be based on an experimental connection with a known triterpene and this was eventually accomplished by conversion to longispinogenin (XIIa).

of the earlier mentioned saponification studies) was one of the only two possible sites for the carboxyl group of myrtillogenic acid and if the primary hydroxyl function were in fact located in ring E, then the carboxyl group would have to be equatorially attached to C-4. An experimental test was now available in the pyridine–chromium trioxide oxidation product (now known to be Vb) of methyl myrtillogenate. As pointed out above, this substance had to possess a 3-keto function (positive Zimmermann test in contrast to VII), and if the carbomethoxyl group were attached to C-4, the substance would have to be a β-keto ester. Alkaline saponification followed by acidification yielded a crude acid which did not decarboxylate thus eliminating C-4 from further consideration and consequently requiring that the carboxyl group be situated at C-20. This in turn implied that the primary alcohol function of the 1,3-glycol moiety of myrtillogenic acid was at C-28, from which it follows that myrtillogenic acid itself should be expressed by structures Ia or VI.

In order to distinguish between these two structural alternatives, triacetylmyrtillogenic acid was converted to the acid chloride and subjected to Rosenmund reduction. The product was difficult to purify, but Wolff–Kishner reduction followed by acetylation furnished longispinogenin triacetate (XIIb)[21] thus locating unambiguously the three hydroxyl groups of myrtillogenic acid. In view of the fact that methyl myrtillogenate is saponified more readily than methyl desoxoglycyrrhetate (IIIf)[22] and since it is doubtful that the C-28 hydroxyl group could exert an activating effect upon the ester function, the latter is assigned the equatorial (α) orientation. Myrtillogenic acid (Ia) can now be given the systematic name 3β,16β,28-trihydroxy-Δ^{12}-oleanen-29-oic acid and represents the first naturally occurring triterpene which is oxygenated at C-29. It has already been pointed out earlier[1] that except for the usual 3β-hydroxyl function, oxygenation among cactus triterpenes has so far been found only in rings D and E. It is interesting to note that myrtillogenic acid falls into that same pattern which may eventually prove to be of some biogenetic significance.

Experimental[23]

Myrtillogenic Acid and Derivatives.—Crude methyl myrtillogenate (Ib), obtained by chromatography of the methylated acidic fraction of various *Myrtillocactus* species,[4] was recrystallized several times from methanol–acetone to yield an analytical sample, m.p. 249–250°,[24] $[\alpha]_D$ +85° (*c* 2.14), λ_{max}^{KBr} 3.0 and 5.79 μ.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.81; H, 10.21.

Triacetyl methyl myrtillogenate (Id) was obtained by acetylation of Ib with acetic anhydride–pyridine at room temperature overnight. The analytical sample was purified by chromatography followed by recrystallization from methanol; m.p. 146.5–148°, $[\alpha]_D$ +77° (*c* 0.75); $\lambda_{max}^{CHCl_3}$ 5.78, 5.81 (inflect.) and 8.0 μ.

Anal. Calcd. for $C_{37}H_{56}O_8$: C, 70.67; H, 8.98; acetyl, 20.27. Found: C, 70.88; H, 9.09; acetyl, 20.57.

Myrtillogenic acid (Ia) was obtained by saponification of its methyl ester with 10% methanolic potassium hydroxide (see Table I) and was recrystallized several times from acetone and then from methanol; m.p. 288–293°, $[\alpha]_D$ +84° (*c* 0.74 in methanol).

Anal. Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.69; H, 9.91.

Acetylation of the acid Ia with acetic anhydride–pyridine and recrystallization from methanol furnished an analytical sample of **myrtillogenic acid triacetate (Ic)**, m.p. 259–263°, $[\alpha]_D$ +65° (*c* 1.03).

Anal. Calcd. for $C_{36}H_{54}O_8$: C, 70.33; H, 8.85; O, 20.81. Found: C, 70.47; H, 9.20; O, 20.42.

TABLE I

RATES OF SAPONIFICATION OF TRITERPENE METHYL ESTERS[a]

Concn. of MeOH–KOH / Methyl ester	5%	7%	10%
	Yield of acid %		
Oleanolate (IIIb)	0	0	0
Cochalate (IIId)	0	3	20
Desoxoglycyrrhetate (IIIf)	5	17(13)[b]	47(40)[b]
Myrtillogenate (Ib)	11	31	85(93)[b]

[a] A 100-mg. sample of the methyl ester was heated under reflux for 8 hr. in 5, 7 or 10% methanolic potassium hydroxide solution, much water was added and the unreacted ester was extracted with ether. The aqueous solution was acidified, extracted with ether, washed, dried and evaporated. Both ester and acid fractions were weighed. [b] Duplicate determination.

Selenium Dioxide Oxidation of Triacetyl Methyl Myrtillogenate (Id).—A solution of 125 mg. of triacetyl methyl myrtillogenate and 125 mg. of selenium dioxide in 10 cc. of glacial acetic acid was heated under reflux for 6 hr., diluted with water and extracted with ether. The residue from the washed and dried ether solution was chromatographed on alumina and recrystallized from methanol to furnish 76 mg. of **methyl 3β,16β,28-triacetoxy-$\Delta^{11,13(18)}$-oleadien-29-oate** (II), m.p. 178–180°; λ_{max}^{EtOH} 240, 248, and 258 mμ; log ϵ 4.38, 4.42 and 4.23.

Anal. Calcd. for $C_{37}H_{54}O_8$: C, 70.90; H, 8.68. Found: C, 71.07; H, 8.71.

Methyl 3β,16β,28-Triacetoxy-Δ^{12}-oleanen-11-one-29-oate.[25]—To a boiling solution of 200 mg. of triacetyl methyl myrtillogenate (Id) in 2 cc. of glacial acetic acid was added 208 mg. of chromium trioxide in 2 cc. of 90% acetic acid over a period of 30 minutes. After heating for an additional hour, the mixture was allowed to stand overnight and then diluted with water. The product was extracted with ether and recrystallized from methanol whereupon it showed a double m.p. at 159–163° and 190–192°. Chromatography and recrystallization gave material which partially melted near 150°, resolidified and melted at 192–195°, λ_{max}^{EtOH} 245 mμ, log ϵ 4.13.

Anal. Calcd. for $C_{37}H_{54}O_9$: C, 69.13; H, 8.47. Found: C, 68.58; H, 8.59.

Chromium Trioxide Oxidation of Methyl Myrtillogenate (Ib). (a) With Chromium Trioxide–Acetic Acid–Chloroform.[13]—To an ice-cooled solution of 250 mg. of methyl myrtillogenate (Ib) in 100 cc. of acetic acid and 10 cc. of chloroform was added over a period of 10 min. 565 mg. of chromium trioxide dissolved in 5 cc. of water and 15 cc. of acetic acid. After 75 min. at room temperature, the excess reagent was destroyed with methanol, water was added and the product was extracted with ether. The ether solution was washed with 5% potassium hydroxide, water, dried and evaporated to afford 240 mg. of semi-solid which crystal-

(21) C. Djerassi, R. N. McDonald and A. J. Lemin, THIS JOURNAL, **75**, 5940 (1953); C. Djerassi, L. E. Geller and A. J. Lemin, *ibid.*, **76**, 4089 (1954).

(22) The axial orientation of the carboxyl group in glycyrrhetic acid (and consequently also in desoxoglycyrrhetic acid (IIIe)) has been established by J. M. Beaton and F. S. Spring, *J. Chem. Soc.*, 3126 (1955).

(23) Melting points were determined on the Kofler block. Unless noted otherwise, all rotations were measured in chloroform solution in 1 dcm. tubes. We are indebted to Mrs. Dolores Phillips for all ultraviolet and infrared spectral measurements. The microanalyses were performed by Dr. A. Bernhardt (Mülheim, Germany) and by Mr. Joseph F. Alicino (Metuchen, N. J.).

(24) The melting point was depressed by over 50° when mixed with the isomeric methyl arjunolate (m.p. 248–250°, $[\alpha]_D$ +68°). We are indebted to Prof. F. E. King (Nottingham) for a sample (F. E. King, T. J. King and J. M. Ross, *J. Chem. Soc.*, 3995 (1954)).

(25) This experiment was carried out by Dr. A. E. Lippman.

ized upon trituration with methanol (m.p. 150–200°). Chromatography on Merck acid-washed alumina and recrystallization from methanol afforded 69 mg. of **methyl Δ^{12}-oleanen-3,16-dione-28-al-29-oate (Va)**, m.p. 202–204°, $\lambda_{max}^{CHCl_3}$ 5.79 and 5.86 μ but no free hydroxyl band.

Anal. Calcd. for $C_{31}H_{44}O_5$: C, 74.96; H, 8.93. Found: C, 74.86; H, 9.14.

(b) **With Chromium Trioxide–Pyridine.**[14]—A solution of 150 mg. of methyl myrtillogenate and 180 mg. of chromium trioxide in 18 cc. of pyridine was allowed to stand at room temperature overnight. After adding much water, the material was extracted with ether, washed well with dilute hydrochloric acid, dried, evaporated and triturated with hexane to give 140 mg. of crystals, m.p. 175–190°. Several recrystallizations from methanol led to the analytical sample of **methyl Δ^{12}-oleanen-3,16-dione-28-ol-29-oate (Vb)**, m.p. 189–192°, $[\alpha]_D$ +47° (*c* 0.32); $\lambda_{max}^{CHCl_3}$ 2.95,[26] 5.80 and 5.87 μ. The rotatory dispersion curve[19] was very similar to that of methyl diketoechinocystate and the Zimmermann reaction over the range 300–700 mμ closely resembled that of β-amyrone.[15]

Anal. Calcd. for $C_{31}H_{46}O_5$: C, 74.66; H, 9.30. Found: C, 74.96; H, 8.90.

When 110 mg. of the oxidation product Vb was heated under reflux for 1 hr. with 25 cc. of 1% methanolic potassium hydroxide and extracted with ether, there was obtained 100 mg. of an oil. Chromatography and elution with benzene gave 90 mg. of solid, m.p. 175–190°, raised after several recrystallizations from methanol to m.p. 203–205°, $[\alpha]_D$ −25° (*c* 0.25), $\lambda_{max}^{CHCl_3}$ 5.78 and 5.83 μ, no strong ultraviolet absorption, negative ferric chloride reaction. The identical substance (mixture melting point and infrared comparison) was obtained when the oxidation product of procedure (a) was heated with 5% methanolic potassium hydroxide solution for 4 hr.

Anal. Calcd. for $C_{30}H_{44}O_4$: C, 76.88; H, 9.46; O, 13.66. Found: C, 77.18; H, 9.70; O, 13.13.

When the oxidation product Vb was heated under reflux for 12 hr. with 10% methanolic potassium hydroxide solution, ether extraction did not remove any material. Acidification followed by ether extraction yielded in nearly quantitative yield an acidic oil which resisted all attempts at crystallization. As pointed out in the discussion, the isolation of an acid eliminates a β-keto ester formulation for Vb and *ipso facto* C-4 as the point of attachment of the carboxyl group of myrtillogenic acid.

Partial Saponification of Triacetyl Methyl Myrtillogenate (Id).—The triacetate (100 mg.) in 34 cc. of methanol was heated for 40 minutes with 2.6 cc. of a solution of 6.3 g. of potassium carbonate in 90 cc. of 1:1 dioxane–water,[18] about 15 cc. of methanol being distilled off during that time. The crude, ether-extracted product was chromatographed on 5 g. of Merck acid-washed alumina. Benzene removed 20 mg. of unreacted triacetate while 1:1 ether–chloroform yielded 56 mg. of the desired **methyl myrtillogenate 3-monoacetate (Ie)**, m.p. 274–280°. Further elution with chloroform–methanol (9:1) furnished 10 mg. of methyl myrtillogenate (Ib). The analytical sample of the monoacetate was obtained from methanol, m.p. 300–302°, $[\alpha]_D$ +77° (*c* 0.90); $\lambda_{max}^{CHCl_3}$ 2.90, 5.78 and 8.00 μ.

Anal. Calcd. for $C_{33}H_{52}O_6$: C, 72.75; H, 9.62; O, 17.63. Found: C, 72.76; H, 9.56; O, 18.01.

Chromium Trioxide–Pyridine Oxidation of Methyl Myrtillogenate 3-Monoacetate (Ie).—The oxidation was carried out exactly as described above for methyl myrtillogenate (Ib) and the crude product (m.p. 225–230°) upon recrystallization from chloroform–methanol yielded the analytical sample of **methyl Δ^{12}-oleanen-3β,28-diol-16-one-29-oate 3-acetate (VII)**, m.p. 237–239°, $[\alpha]_D$ +7° (*c* 0.53), negative Zimmermann reaction,[15] no high selective ultraviolet absorption; λ_{max}^{KBr} 2.90, 5.79, 5.84 and 8.00 μ. The rotatory dispersion curve[19] was very similar to that of methyl 16-ketooleanolate.

Anal. Calcd. for $C_{33}H_{50}O_6$: C, 73.03; H, 9.29; O, 17.69. Found: C, 72.72; H, 9.54; O, 17.74.

Wolff–Kishner reduction of 114 mg. of VII by the Huang-Minlon modification[27] using hydrazine hydrate, ethylene glycol and potassium hydroxide gave 82 mg. of crude acid (m.p. 230–240°) which melted at 280–284° after two recrystallizations from methanol; it is most likely **3β-hydroxy-28-nor-Δ^{12}-oleanen-29-oic acid (VIIIa)**.

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.76; H, 10.78.

The mother liquors from the above recrystallizations were methylated with diazomethane and the resulting **methyl ester VIIIb** was recrystallized from methanol; m.p. 191–193°, $[\alpha]_D$ +60° (*c* 1.37), $\lambda_{max}^{CHCl_3}$ 2.8 and 5.77 μ. The analysis was not satisfactory, but insufficient material was available for a repetition.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.07; H, 11.07.

Conversion of Triacetylmyrtillogenic Acid (Ic) to Longispinogenin Triacetate (XIIb).—Triacetylmyrtillogenic acid (750 mg.) was converted into the acid chloride by heating under reflux for 45 min. with 2 cc. of thionyl chloride. Benzene was added and all volatile material removed *in vacuo* on the steam-bath.

The crude acid chloride, dissolved in 30 cc. of xylene, was stirred at 120° with 0.8 g. of palladium–barium sulfate catalyst and 0.1 cc. of sulfur–quinoline poison, and hydrogen gas was bubbled for 3 hr. through the solution. The crude product, obtained by filtration, washing with dilute sodium hydroxide, water, drying and evaporation was chromatographed on 20 g. of alumina. Benzene elution afforded 500 mg. of solid (m.p. 188–194°), the melting point range of which could not be sharpened even after repeated crystallization from methanol.

A 350-mg. sample of the Rosenmund reduction product was subjected to Wolff–Kishner reduction[27] with 30 cc. of ethylene glycol, 1.5 cc. of hydrazine hydrate and 1.5 g. of potassium hydroxide. The total product was acetylated with acetic anhydride–pyridine and chromatographed on 10 g. of Merck acid-washed alumina. Elution with benzene and recrystallization from methanol yielded 110 mg. of **longispinogenin triacetate**, m.p. 220–222°, $[\alpha]_D$ +68° (*c* 0.64), which was shown to be identical with an authentic sample[21] by mixture melting point determination and infrared spectral comparison (Nujol mull).

Miscellaneous Experiments with Myrtillogenic Acid.—Methyl myrtillogenate was recovered in nearly quantitative yield when subjected to acetonide formation conditions[12] (acetone–hydrochloric acid or acetone–sulfuric acid). Triacetylmyrtillogenic acid was recovered unchanged under conditions where diacetylcochalic acid formed a bromolactone.

Diacetylcochalic Acid Bromolactone (IV).—A methylene chloride solution of 107 mg. of diacetylcochalic acid[8] was treated dropwise with 0.013 cc. of bromine in 1 cc. of methylene chloride, decolorization occurring immediately. Removal of the solvent and crystallization of the solid from methanol–methylene chloride gave 95 mg. of the desired bromolactone IV, m.p. 296–298°, $[\alpha]_D$ +60° (*c* 1.12); $\lambda_{max}^{CHCl_3}$ 5.61, 5.78 and 8.00 μ.

Anal. Calcd. for $C_{34}H_{51}BrO_6$: C, 64.24; H, 8.09; Br, 12.57; O, 15.10. Found: C, 63.70; H, 8.08; Br, 12.95; O, 15.41.

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(26) The presence of a hydroxyl group was confirmed further by acetylation with acetic anhydride–pyridine. The resulting oil could not be crystallized but its infrared spectrum now lacked absorption in the 3 μ region and showed an intense band at 8.0 μ, not present in the starting material.

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