lized to an oily solid. Recrystallization four times from methanol left 1.16 g. of white needles, m.p. 75–76°, λ_{max}^{alc} 304 mμ (ε 9,245). The material showed no mixed melting point depression with authentic diosphenolene prepared from piperitenone oxide. The ferric chloride test was very heavy dark green in color similar to the test with diosphenolene prepared in other ways.

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[JOINT CONTRIBUTION FROM THE INSTITUTO DE QUIMICA DE LA UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO AND THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Terpenoids. XXX.[1] The Structure of the Cactus Triterpene Chichipegenin

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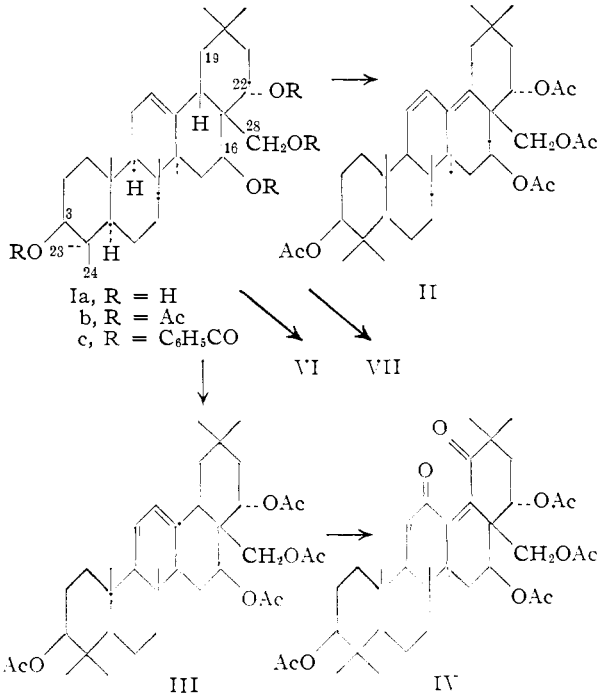
Chichipegenin—isolated from various *Myrtillocactus* species and from *Lemaireocereus chichipe*—has been converted *via* its 28-trityl ether VIa to longispinogenin (Va). Coupled with other degradation experiments, this leads to the conclusion that chichipegenin is Δ^{12}-oleanene-3β,16β,22α,28-tetrol (Ia), thus falling into the earlier established oxygenation pattern established for cactus triterpenes.

As pointed out recently,[4] the most characteristic triterpene of the genus *Myrtillocactus* of the *Cactaceae* family is a new tetrol, $C_{30}H_{50}O_4$. This substance has been named "chichipegenin" since it occurs also in large amounts in a single species of the genus *Lemaireocereus*, *L. chichipe*[5] (see Experimental section), and the taxonomic implications of this observation have already been commented upon.[4,6] We should now like to record the approach employed in the structure elucidation of this interesting triterpene.

Chichipegenin did not exhibit any infrared absorption in the carbonyl region but showed a strong band typical of one or more hydroxyl groups. Acetylation or benzoylation under mild conditions afforded the corresponding tetraacetate Ib and tetrabenzoate Ic, thus accounting for all four oxygen atoms. Furthermore, the ease of acylation indicated that these hydroxyl groups must either be primary and/or equatorially oriented secondary alcoholic functions. Since eventual correlation with a known triterpene was required for a definitive structure proof, it was important to establish to which broad class of triterpenes chichipegenin belonged. This was accomplished by examining the reactivity of chichipegenin tetraacetate toward selenium dioxide.

Oxidation in boiling acetic acid smoothly led to a diene, which on the basis of its characteristic triple ultraviolet absorption maxima[7] could be assigned the heteroannular $\Delta^{11,13(18)}$-formulation II, thus



showing that chichipegenin was a member of the β-amyrin class of triterpenes. In order to eliminate position 19 as the site of one of the hydroxyl groups, the further oxidation of this diene II with selenium dioxide to the well known $\Delta^{9(11),13(18)}$-diene-12,19-dione IV[8] was attempted, but no pure product could be isolated. Reaction of chichipegenin tetraacetate Ib with N-bromosuccinimide did not lead to the desired $\Delta^{9(11),12,18}$-triene[9] but rather to an impure bromine-containing product in accordance with earlier observations in the queretaroic acid series.[10] However, with a limited amount of N-bromosuccinimide, it was possible to isolate

(1) Paper XXIX, C. Djerassi and W. Rittel, THIS JOURNAL, **79**, 8528 (1957).

(2) Postdoctorate research fellow (1956–1957) at Wayne State University on funds supplied by the Division of Research Grants (grant No. RG-3863) of the National Institutes of Health, U. S. Public Health Service.

(3) Postdoctorate research fellow (1955) at Universidad Nacional Autonoma de Mexico under a grant from the Rockefeller Foundation.

(4) C. Djerassi, S. Burstein, H. Estrada, A. J. Lemin, A. E. Lippman, A. Manjarrez and H. G. Monsimer, THIS JOURNAL, **79**, 3225 (1957).

(5) N. L. Britton and J. N. Rose, "The Cactaceae," Vol. II, Carnegie Institution of Washington, Washington, D. C. 1920, p. 89. H. Bravo, "Las Cactaceas de Mexico," Imprenta Universitaria, Mexico, D. F., 1937, p. 258.

(6) C. Djerassi, 'Cactus Triterpenes' in "Festschrift Arthur Stoll," Birkhäuser, A. G., Basel, 1957, pp. 330–352.

(7) 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).

(8) A list of the various rings, C, D and E-substituted precursors leading to the same diene-dione (type IV) is given by D. H. R. Barton, N. J. Holness, K. H. Overton and W. J. Rosenfelder, *ibid.*, 3751 (1952), and by J. M. Beaton, J. D. Johnston, L. C. McKean and F. S. Spring, *ibid.*, 3660 (1953).

(9) *Cf.* L. Ruzicka, O. Jeger and J. Redel, *Helv. Chim. Acta*, **26**, 1235 (1943).

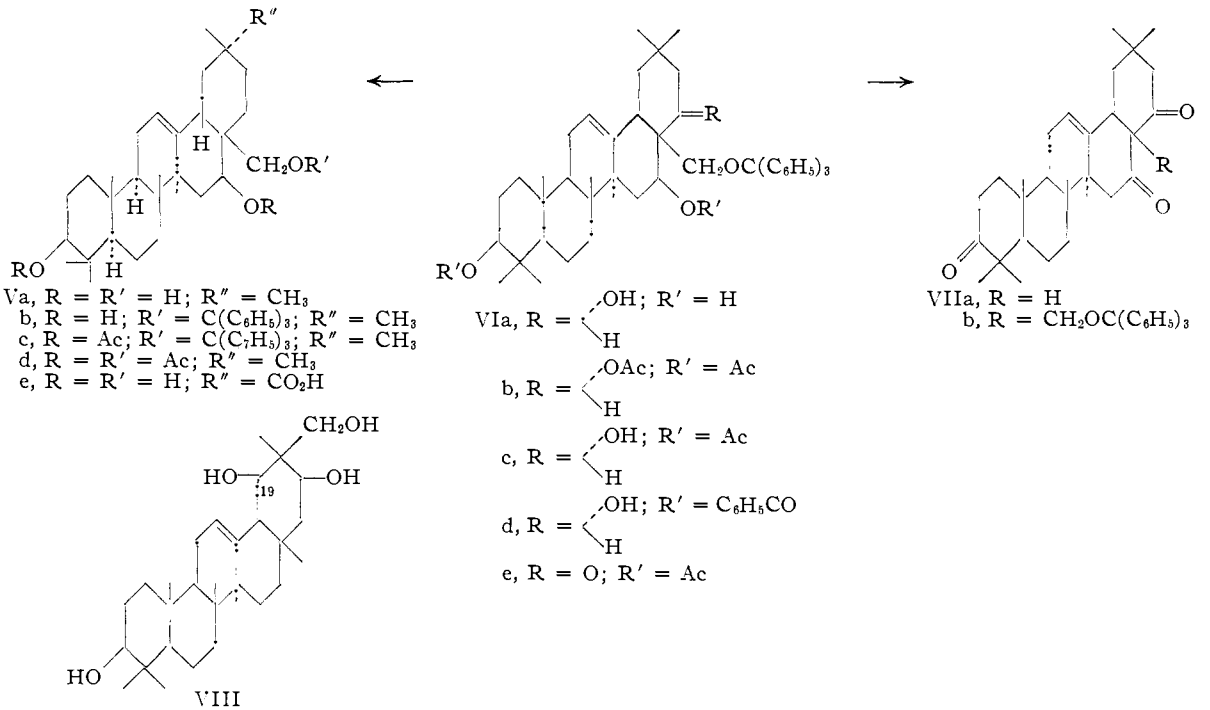
(10) C. Djerassi, J. A. Henry, A. J. Lemin, T. Rios and G. H. Thomas, THIS JOURNAL, **78**, 3783 (1956).

another diene, which must be the homoannular $\Delta^{9(11),12}$-diene III since it exhibited a single ultraviolet absorption maximum at 281 mμ. Selenium dioxide oxidation[8] of the latter (III) gave the required diene-dione IV, demonstrating that C-19 in chichipegenin must have been unsubstituted.

A close companion of chichipegenin in several *Myrtillocactus* species[4] is myrtillogenic acid (Ve),[11] and it seemed attractive to speculate that both triterpenes were oxygenated at identical carbon atoms.[12] This possibility was ruled out by the experimental observation[4] that lithium aluminum hydride reduction of myrtillogenic acid (Ve) produced a tetrol, different from chichipegenin. It was necessary, therefore, to remove one or more hydroxyl groups in chichipegenin in order to achieve a correlation with a known triterpene and attempts at selective manipulation of the hydroxyl groups were initiated.

Chichipegenin did not form an acetonide and assuming the existence of a 3β-hydroxyl group—found in all cactus triterpenes[6]—this observation excluded hydroxyl groups at C-23, C-24,[13] C-2 (β)[14] and presumably also C-1 (β). The presence of a 1,2-glycol moiety was eliminated by the observed stability of chichipegenin toward lead tetraacetate, but the presence of a 1,3-glycol grouping in other parts of the molecule still had to be considered since 16β,28-diols such as longispinogenin (Va) do not form acetonides.

As described in the Experimental portion of this paper, chichipegenin is accompanied in the cactus *L. chichipe* by oleanolic acid and by longispinogenin (Va). This suggested that one of the hydroxyl groups of chichipegenin might be located at C-28, and since a model experiment with longispinogenin (Va) demonstrated the ready formation of a 28-trityl ether Vb, chichipegenin was subjected to similar treatment with triphenylmethyl chloride. The resulting trityl ether VIa yielded a triacetate VIb when heated with acetic anhydride and a triketone VIIb upon oxidation with chromium trioxide–pyridine. This demonstrated the presence of one primary and three secondary hydroxyl groups in chichipegenin. Furthermore, the spectral properties of the triketo-trityl ether VIIb required the absence of an enolizable α- or β-diketone grouping. Detritylation of VIIb by boiling with alcoholic sulfuric acid led in poor yield to a nor-triketone VIIa, which gave a strongly positive ferric chloride reaction and exhibited an ultraviolet absorption maximum (λ_{max}^{EtOH} 290 mμ, λ_{max}^{KOH} 309 mμ) consistent with the presence of a β-diketone function.[15] The identical nor-trione VIIa could also be obtained in one step by direct chromium tri-



oxide–acetic acid oxidation of chichipegenin (Ia), thus requiring a triol moiety of type A in chichipegenin. The direct formation of the nor-trione VIIa (D) from the trityl ether VIIb (B) is due to retro-aldolization of the intermediate keto alcohol C and finds its exact counterpart in a similar reaction sequence described recently with the sesquiterpene iresin.[16]

Partial structure A can be incorporated into an

(11) C. Djerassi and H. G. Monsimer, THIS JOURNAL, **79**, 2901 (1957).

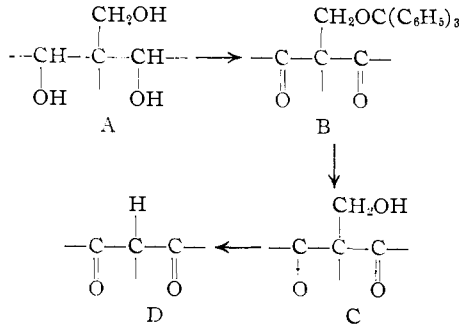
(12) A precedent being the co-occurrence of longispinogenin (Va) and the corresponding acid, cochalic acid, in the same cactus (see Table II in ref. 6).

(13) 3β,23- and 3β-24-acetonides can be formed readily (see J. L. Beton, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 2904 (1956)).

(14) The formation of a 2β,3β-acetonide has recently been demonstrated in the alfalfa sapogenin medicagenic acid (to be published).

(15) No good model appears to be available to predict the exact position of the ultraviolet absorption maximum of such a β-diketone (*cf.* E. R. Blout, V. W. Eager and D. C. Silverman, THIS JOURNAL, **68**, 566 (1946)).

(16) C. Djerassi, W. Rittel, A. L. Nussbaum, F. W. Donovan and J. Herrán, *ibid.*, **76**, 6410 (1954).



intact β-amyrin skeleton only in rings D and E leading to structure I or VIII. However, chichipegenin cannot have a hydroxyl group at C-19 (as in VIII) because of the ease of acetylation, the formation of a tetraacetoxy diene-dione IV and the lack of ultraviolet absorption typical of an α,β-unsaturated ketone[17] shown by the trityl ether trione VIIb in alkaline solution. The above analysis leaves I as the only structural alternative for chichipegenin, but since this is based on a process of elimination which in turn is predicated on a normal triterpene skeleton—so far unproved—it was indispensable to relate chichipegenin to a triterpene of established constitution. This was accomplished in the following manner, starting with chichipegenin 28-trityl ether (VIa).

In contrast to chichipegenin (Ia) itself, which could be acetylated completely at room temperature, its trityl ether VIa under the same conditions furnished an amorphous product still exhibiting hydroxyl absorption in the infrared. This resistance to mild acetylation conditions was believed to be a reflection of the increased steric hindrance produced by the bulky trityl ether grouping and could only exert itself upon C-16 and/or C-22. That C-16 was probably not implicated was demonstrated by the smooth acetylation at room temperature of longispinogenin trityl ether (Vb) to the corresponding 3,16-diacetate Vc, thus suggesting that the amorphous acetylation product of chichipegenin trityl ether (VIa) should be represented as the 3,16-diacetoxy-22-ol-28-trityl ether (VIc).[18] The correctness of this assumption was established by chromium trioxide–pyridine oxidation to the crystalline 3,16-diacetoxy-22-ketone VIe and Wolff–Kishner reduction, which furnished longispinogenin trityl ether (Vb). The identity of the reduction product was confirmed by cleavage of the trityl ether and acetylation, whereupon longispinogenin triacetate (Vd) was obtained. Since the structure and stereochemistry of longispinogenin (Va) has been proved rigorously,[19] chichipegenin can now be given the systematic name Δ^{12}-oleanene-3β,16β-22α,28-tetrol (Ia).[20] Attention already has been called earlier[6] to the fact that the sixteen triterpenes which have so far been isolated in our laboratories from giant cacti all fall into a restricted oxygenation pattern which involves only rings D and E aside from position C-3. The structure of chichipegenin further supports this "rule" which may be of some utility in narrowing down structural alternatives when working with unknown cactus triterpenes.

(17) If structure VIII were the correct expression for chichipegenin, then the triketo trityl ether VIIb would contain a 19-keto function, and such β,γ-unsaturated ketones are readily isomerized with base to the corresponding conjugated ketone (see P. Bilham, G. A. R. Kon and W. C. J. Ross, *J. Chem. Soc.*, 540 (1942); L. Ruzicka, A. Grob, R. Egli and O. Jeger, *Helv. Chim. Acta*, **26**, 1218 (1943)).

(18) Similarly, benzoylation of the trityl ether VIa yielded a 3,16-dibenzoate VId which could be crystallized.

(19) C. Djerassi, L. E. Geller and A. J. Lemin, *Chemistry & Industry*, 161 (1954); THIS JOURNAL, **76**, 4089 (1954).

(20) The equatorial (α) orientation is assigned to the 22-hydroxyl group because of its ease of acetylation and benzoylation.

Experimental[21]

Isolation of Triterpenes from *Lemaireocereus chichipe*.—The fresh cactus (106 kg.) collected near Tehuacán, Puebla, and identified by Dr. Helia Bravo of the Instituto de Biologia (Universidad Nacional Autonoma de Mexico), was cut into small portions and dried for 7 days at 35°. The ground, dried material (32 kg.) was continuously extracted with hot ethanol and the dry extract (9.5 kg.) was washed well with acetone and then ether, leaving 4.5 kg. of "glycosidic" fraction. A 2-kg. portion of it was heated under reflux for 2 hr. with 10 l. of methanol and 2 l. of concd. hydrochloric acid, concentrated to one-half of its volume and diluted with water. Filtration produced 720 g. of solid while basification of the filtrate with ammonia did not reveal the presence of any alkaloids. A chloroform solution of the solid was shaken repeatedly with aqueous potassium hydroxide solution furnishing approximately 400 g. of neutral material and 300 g. of insoluble potassium salts.

The potassium salt was suspended in methanol and acidified with constant stirring yielding 270 g. of acid. A 5.0-g. sample was methylated in the standard manner with diazomethane and purified by chromatography giving 2.6 g. of pure **methyl oleanolate**, m.p. 198–200°, further characterized as the acetate (m.p. 214–216°).

In a typical chromatogram of the neutral fraction, a chloroform solution of 40 g. of solid was placed on a column of 2 kg. of alumina. Elution with the same solvent yielded 10.5 g. of oil while 26.5 g. of **chichipegenin (Ia)** was removed from chloroform–methanol (9:1). Repeated crystallization from chloroform–methanol afforded an analytical sample, m.p. 321–323°, $[\alpha]_D$ +43° (chloroform), +40° (pyridine).

Anal. Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.39; H, 10.40.

Acetylation of chichipegenin could be accomplished at room temperature either with acetic anhydride–pyridine (overnight) or with acetic anhydride containing a trace of perchloric acid (30 min.). Recrystallization from chloroform–methanol led to the **tetraacetate** Ib, m.p. 280–282°, $[\alpha]_D$ + 26°.

Anal. Calcd. for $C_{38}H_{58}O_8$: C, 70.99; H, 9.09. Found: C, 70.96; H, 9.23.

Partial saponification of the tetraacetate (0.5 g.) was carried out by heating a methanolic solution (200 cc.) under reflux for 45 min. with 800 mg. of potassium carbonate and 10 cc. of water. After working up in the customary manner[22] there was isolated *ca.* 100 mg. of **chichipegenin (3 or 22)-monoacetate**, m.p. 280–282°, $[\alpha]_D$ +57°.

Anal. Calcd. for $C_{32}H_{52}O_5$: C, 74.37; H, 10.14. Found: C, 74.35; H, 10.14.

Benzoylation of chichipegenin (benzoyl chloride–pyridine, 14 hr., 23°) followed by chromatography and recrystallization from ether–methanol yielded the **tetrabenzoate Ic**, m.p. 245–247°, $[\alpha]_D$ +62°.

Anal. Calcd. for $C_{58}H_{66}O_8$: C, 78.32; H, 7.65. Found: C, 78.20; H, 7.47.

(21) All melting points were determined on the Kofler block. Unless noted otherwise, rotations were measured in 1-dcm. tubes in chloroform solution. Infrared spectra were obtained in part with a Perkin–Elmer (Instituto de Quimica) and in part with a Baird (Wayne State University) double beam spectrophotometer. The microanalyses were carried out by Geller Laboratories (Hackensack, N. J.), Dr. Franz Pascher (Bonn, Germany) and Dr. Alfred Bernhardt (Mülheim, Germany).

(22) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, THIS JOURNAL, **76**, 2969 (1954).

Similar treatment of the monoacetate led to **chichipegenin tribenzoate monoacetate**, m.p. 221–223°, $[\alpha]_D$ +50°.

Anal. Calcd. for $C_{53}H_{64}O_8$: C, 76.76; H, 7.78. Found: C, 76.95; H, 7.70.

The ether and acetone washings of the "glycosidic" fraction were combined and evaporated to dryness (4.1 kg.) yielding an oily, dark colored mass. A 290-g. portion was dissolved in 200 cc. of dioxane and heated under reflux for 6 hr. with 200 g. of potassium hydroxide dissolved in 2 l. of methanol. After concentrating and diluting with water, the mixture was extracted continuously with ether. Concentration of the washed and dried ether solution gave 77 g. of **longispinogenin (Va)**, m.p. 249–252°, $[\alpha]_D$ +57°, tetraacetate, m.p. 219–222°, $[\alpha]_D$ +76°.

$\Delta^{11,13(18)}$-Oleadiene-3β,16β,22α,28-tetrol Tetraacetate (II).—A solution of 200 mg. of chichipegenin tetraacetate (Ib) in 25 cc. of glacial acetic acid was heated under reflux for 3 hr. with 200 mg. of freshly sublimed selenium dioxide. After working up in the usual way including chromatography and recrystallization from chloroform–methanol, there was obtained 102 mg. of the diene II, m.p. 192–193.5°, $[\alpha]_D$ −114°; λ_{max}^{EtOH} 242, 250 and 260 mμ; log ϵ 4.43, 4.56 and 4.35.

Anal. Calcd. for $C_{38}H_{56}O_8$: C, 71.22; H, 8.81. Found: C, 71.58; H, 9.09.

$\Delta^{9(11),12}$-Oleadiene-3β,16β,22α,28-tetrol Tetraacetate (III).—A mixture of 2.0 g. of chichipegenin tetraacetate (Ib), 1.2 g. of N-bromosuccinimide and 100 cc. of carbon tetrachloride was heated under reflux for 2 hr. After filtering, washing with bicarbonate and water, drying and evaporating, there was obtained an oil which was heated under reflux with 25 cc. of collidine for 3 hr. After processing in the usual manner, there was obtained 0.5 g. of oil which was chromatographed on 20 g. of alumina. Elution with benzene furnished 285 mg. of solid which was recrystallized from chloroform–methanol to give the analytical sample, m.p. 323–325°, λ_{max}^{EtOH} 280 mμ, log ϵ 4.05.

Anal. Calcd. for $C_{38}H_{56}O_8$: C, 71.22; H, 8.81. Found: C, 71.77; H, 9.12.

$\Delta^{9(11),13(18)}$-Oleadiene-3β,16β,22α,28-tetrol-12,19-dione Tetraacetate (IV).—The above diene III (1.0 g.) was heated under reflux for 5 hr. with 1.0 g. of selenium dioxide and 200 cc. of acetic acid. The resulting oil (800 mg.) was chromatographed on 50 g. of alumina, and elution with benzene–ether (1:1) gave 100 mg. of material which crystallized from dilute acetone as light yellow needles, m.p. 171–173°, $[\alpha]_D$ −69°, λ_{max}^{EtOH} 278 mμ, log ϵ 4.15; λ_{max}^{Nujol} 5.75, 6.05 and 6.20 μ.

Anal. Calcd. for $C_{38}H_{52}O_{10}$: C, 68.24; H, 7.84; O, 23.92. Found: C, 68.02; H, 7.87; O, 24.35.

Chichipegenin 28-Trityl Ether (VIa).—A solution of 2.0 g. of chichipegenin (Ia) and 6.0 g. of triphenylmethyl chloride in 160 cc. of 1:1 dioxane–pyridine was heated on the steam-bath for 8 hr. The crude product was isolated with ether and chromatographed on 400 g. of Merck acid-washed alumina. Elution with benzene afforded 4.2 g. of triphenylcarbinol while benzene–chloroform (1:1) eluted the desired trityl ether. Crystallization from benzene–hexane gave 2.3 g. of colorless needles, m.p. 261–270°, which were satisfactory for the next step. The analytical sample exhibited m.p. 263–272°, $[\alpha]_D$ +70°, $\lambda_{max}^{CHCl_3}$ 6.22(w) and 6.70(m) μ.

Anal. Calcd. for $C_{49}H_{64}O_4$: C, 82.08; H, 9.00; O, 8.93. Found: C, 82.20; H, 9.16; O, 8.29.

Complete acetylation could be accomplished by heating a mixture of 300 mg. of the trityl ether, 15 cc. of pyridine and 15 cc. of acetic anhydride for 4 hr. on the steam-bath. Dilution with water and filtration yielded a solid which was chromatographed on 30 g. of Merck acid-washed alumina and eluted with benzene. Recrystallization from ethanol led to 200 mg. of the **triacetoxy trityl ether (VIb)**, m.p. 252–256°, $[\alpha]_D$ +70°, no infrared hydroxyl absorption.

Anal. Calcd. for $C_{55}H_{70}O_7$: C, 78.34; H, 8.37; O, 13.28. Found: C, 78.48; H, 8.29; O, 12.85.

Δ^{12}-Oleanene-3β,16β,28-triol-22-one 3,16-Diacetate 28-Trityl Ether (VIe).—A solution of 1.5 g. of chichipegenin in 100 cc. of pyridine and 50 cc. of acetic anhydride was left at room temperature for 15 hr.[23] After diluting with water, the amorphous precipitate ($\lambda_{max}^{CHCl_3}$ 2.80 and 5.75 μ) was collected, and since it could not be crystallized, even after chromatography, it was oxidized directly with 1 g. of chromium trioxide in 30 cc. of pyridine for 20 hr. at room temperature. The product (62% over-all yield) was isolated with ether and crystallized from methanol; m.p. 287–290°, $[\alpha]_D$ −44°; $\lambda_{max}^{CHCl_3}$ 5.77(broad), 6.20(v.w.), 6.68(m) and 7.95(s).

Anal. Calcd. for $C_{53}H_{66}O_6$: C, 79.64; H, 8.33; O, 12.01. Found: C, 79.66; H, 8.76; O, 11.46.

Longispinogenin 28-Trityl Ether (Vb). (a) From Longispinogenin (Va).—Longispinogenin (Va) (3.0 g.), triphenylmethyl chloride (10.0 g.) and 1:1 dioxane–pyridine (240 cc.) were heated on the steam-bath for 8 hr. Chromatography on 550 g. of Merck acid-washed alumina yielded 8 g. of triphenylcarbinol (benzene), 2.3 g. of the desired trityl ether (benzene–ether 3:1) and 0.7 g. of unchanged longispinogenin (chloroform–methanol 9:1). Crystallization from benzene–hexane afforded colorless needles of the dimorphic trityl ether, m.p. 208–210° and 268–280°, $[\alpha]_D$ +35°.

Anal. Calcd. for $C_{49}H_{64}O_3$: C, 83.95; H, 9.20; O, 6.85. Found: C, 84.38; H, 9.32; O, 6.25.

Acetylation with acetic anhydride–pyridine at room temperature overnight yielded the **diacetoxy trityl ether (Vc)**, m.p. 209–211° and 227–232°, $[\alpha]_D$ +69°.

Anal. Calcd. for $C_{53}H_{68}O_5$: C, 81.09; H, 8.74; O, 10.20. Found: C, 81.05; H, 8.72; O, 9.83.

(b) **By Wolff–Kishner Reduction of VIe.**—A mixture of 1.0 g. of the 22-keto trityl ether VIe, 100 cc. of diethylene glycol and 6 cc. of hydrazine hydrate was heated to reflux until all of the triterpene had dissolved (*ca.* 5 min.). The temperature was then allowed to fall and was kept at 110–130° for 1 hr. Solid potassium hydroxide (5 g.) was added and, after keeping for an additional hr. at that temperature, distillation was commenced until the temperature rose to 215°. After heating under reflux for 5 hr., the solution was poured into ice-water and the solid product was filtered. Chromatography on 50 g. of alumina and elution with benzene–ether (4:1) provided 400 mg. of solid which was recrystallized from benzene–hexane to yield needles of longispinogenin trityl ether (Vb) with m.p. 208–210° and 268–280°, $[\alpha]_D$ +33°. The infrared spectra of the two samples (prepared according to (a) and (b)) were identical.

A 300-mg. sample of longispinogenin trityl ether from the Wolff–Kishner reduction was dissolved in 25 cc. of chloroform, and the solution was saturated with hydrogen chloride gas for 1.5 hr. After 20 hr. at room temperature, water was added, the layers were separated and the acid removed with bicarbonate solution. The residue from the chloroform layer was chromatographed on 15 g. of alumina, and elution with ether–chloroform (3:1) yielded 74 mg. of longispinogenin (Va), m.p. 253–256°, $[\alpha]_D$ +57°. Acetylation at room temperature gave longispinogenin triacetate (Vd), m.p. 226–228°, $[\alpha]_D$ +72°. Identity was confirmed in each case by mixture melting point determination and infrared comparison.

Δ^{12}-Oleanene-3,16,22-trione-28-ol Trityl Ether (VIIb).—Chichipegenin trityl ether (VIa) (0.6 g.) was added to 0.6 g. of chromium trioxide in 25 cc. of pyridine, and the mixture was kept at room temperature overnight. Dilution with water and extraction with ether produced emulsions which were broken up by filtration through a mat of Celite. The solid residue from the ether extract was chromatographed on 30 g. of Merck acid-washed alumina, and elution with benzene–ether (20:1) led to 300 mg. of the triketone. The analytical sample crystallized from benzene–hexane as colorless needles, m.p. 292–295°, $[\alpha]_D$ −53°, λ_{max}^{KBr} 5.80(s), 6.2(w) and 6.68(m) μ. The substance gave no color with ferric chloride and exhibited no high ultraviolet absorption maximum in alcoholic alkali.[17]

Anal. Calcd. for $C_{49}H_{58}O_4$: C, 82.78; H, 8.22; O, 9.00. Found: C, 82.45; H, 8.02; O, 9.39.

28-Nor-Δ^{12}-Oleanene-3,16,22-trione (VIIa). (a) From Chichipegenin (Ia).—Chichipegenin (1.0 g.) was dissolved in 200 cc. of acetic acid, and a solution of 1.0 g. of chromium trioxide in 10 cc. of 90% acetic acid was added. After standing overnight, water was added, the product was extracted with ether and washed with 3% potassium hydroxide to leave 340 mg. of neutral oil. Crystallization from methanol afforded 90 mg. of yellowish needles, m.p. 253–257°, $[\alpha]_D$ −4.5°, λ_{max}^{EtOH} 290 mμ, log ϵ 4.10; λ_{max}^{KOH} 309 mμ,

(23) When the reaction was performed with benzoyl chloride in pyridine, there was obtained after recrystallization from ethanol the 3,16-dibenzoate VId, m.p. 196–198.5°, $[\alpha]_D$ +77°. *Anal.* Calcd. for $C_{61}H_{72}O_6$: C, 81.76; H, 7.85. Found: C, 82.09; H, 7.90.

log ϵ 4.65; $\lambda_{max}^{CHCl_3}$ 5.83, 6.09 and 6.21 (shoulder) μ. The substance gave a red color with ferric chloride.

Anal. Calcd. for $C_{29}H_{42}O_3$: C, 79.40; H, 9.65; O, 10.94. Found: C, 78.97; H, 9.27; O, 11.10.

(b) **From the Trityl Ether VIIb.**—When 1.0 g. of the trityl ether VIIb was heated under reflux for 8 hr. with 50 cc. of ethanol and 0.5 cc. of sulfuric acid and then cooled, there separated approximately 50 mg. of crystals. These were filtered and recrystallized from methanol whereupon they exhibited m.p. 248–251°, undepressed upon admixture with a sample prepared according to (a). The ultraviolet absorption spectrum and the red color with ferric chloride supported the identification of this substance.

Anal. Calcd. for $C_{29}H_{42}O_3$: C, 79.40; H, 9.65. Found: C, 79.41; H, 9.53.

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[CONTRIBUTION FROM THE LABORATORIES OF THE SCHERING CORPORATION]

Long-acting Testosterone Esters. Some Considerations on their Biological Utilization[1]

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A series of testosterone 17-esters was prepared to find those with the best activity and duration. Several potent derivatives were found, including the entire family of aryloxyalkanoates. Injections of these esters in rodents are shown to be efficient and enduring sources of testosterone.

Since testosterone was established as the true androgenic hormone,[2] attempts have been made to potentiate its effect and prevent its rapid metabolic destruction.[3] The program reported here was carried out with this aim, and led to many esters considerably more valuable in potentiating the androgenic effect than testosterone propionate,[4] as well as a group of esters which are outstanding in the assays used with respect to intensity and duration of androgenic action. The results are given in Tables I-IV.

The acids corresponding to the esters, if they were not available commercially, were prepared by methods reported in the literature, mainly from "Beilstein: Handbuch der Organischen Chemie." The acid chlorides were made using thionyl chloride and generally isolated by vacuum distillation, though the residues were used when distillation caused decomposition. Although some of the acid chlorides had not been known previously, they are not reported here, since they were not characterized and analyzed. All of the esters were made according to the standard procedure given below, in which chilling was found to be essential for the ready preparation of pure products. In the case of oily esters, chromatography gave materials with the correct analyses.

The intensity and duration of androgenic action were tested in mice and rats, giving results which are shown qualitatively in the tables according to their relation to the action of testosterone propionate (TP). The effects were measured by weighing the seminal vesicles of castrated animals at various times after subcutaneous injection of the ester. We have considered that compounds effecting a maximum seminal vesicle weight less than $1/2$ of that due to TP are not of interest and such are rated "0." Conversely, compounds with maxima several times that of TP generally last correspondingly longer before falling below our cut-off point.

The original hypothesis on which this study was based stemmed from the maximal activity of the lowest melting members of the straight chain acid series of esters, *e.g.*, heptanoate and nonanoate.[4,5] This suggested that the more oil-soluble esters are most useful; therefore, a group of esters of branched chain acids was prepared (see Table I). The results show that, while none surpasses the heptanoate and nonanoate, several esters containing four to eleven carbons approached them in activity. The variation of the activity from good to poor of intermediate-length esters, all of them low melting solids or oils readily soluble in hydrocarbon solvents, shows the invalidity of the hypothesis. For example, the decreased activity of the 3,3-dimethylpentanoate compared to the 3-methylpentanoate is in disagreement.

It is, however, possible to make some generalizations. (1) All of the esters with dialkyl-substituted chains have poor activity. (2) The 2-alkyl-substituted esters (except isobutyrate) have a low order of activity. These statements suggest that steric hindrance plays an important role in decreasing the activity. Thus, fatty acids, such as trimethylacetic and diethylacetic, which are known to esterify at less than one-tenth the rate of acetic, and, therefore, also to hydrolyze with difficulty, due to hindrance caused by branching,[6] form testosterone esters whose intensity is so reduced that they are therapeutically useless.

Since no trend or advance in activity is observable in the fatty acid series, other types were prepared (Tables II-IV), especially a group of substituted cyclohexane-carboxylic acid esters (Table

(1) Presented in part before the Medicinal and Biological Chemistry Section of the Delaware Valley Regional Meeting of the American Chemical Society, Philadelphia, February 16, 1956.

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