8; **9**, 19689-84-0; **10**, 19689-85-1; 2-nitro-6-methoxybenzyl bromide, 19689-86-2; 2-nitro-6-methoxybenzyl alcohol, 19689-87-3; 2-nitro-6-methoxybenzaldehyde, 19689-88-4.

Acknowledgments.—We wish to thank Messrs. G. S. Abernethy, Jr., and H. L. Taylor for their help in the isolation of alkaloids and Mr. J. B. Thompson for technical assistance.

Thurberin, a New Pentacyclic Triterpene from Organ Pipe Cactus

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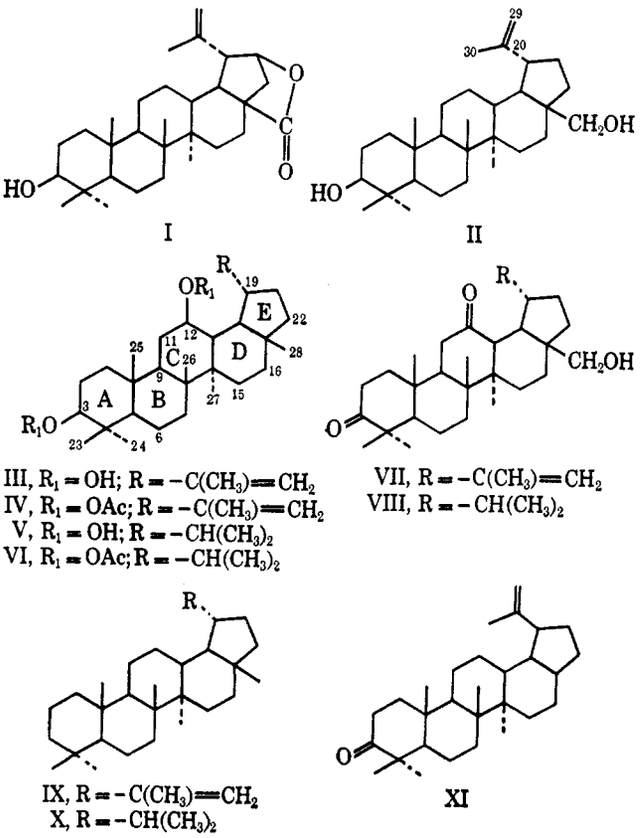
A chemical analysis of the alcoholic extract of the fresh cortical (pulp) portion of the Organ Pipe cactus (*Lemaireocereus thurberi*) affords, besides thurberogenin (I), two more triterpenes, betulin (II) and a new triterpene, thurberin (III), hitherto unidentified in this species. The new triterpene belongs to the lupeol class and is an isomer of betulin. Based on the mass, nmr, spectral, and optical rotatory dispersion (ORD) data, the hydroxyl groups are assigned to positions 3 and 12.

In the course of our studies of the biosynthesis of wound tissue formation in giant cacti,[1,2] we examined the ethanol extract of the Organ Pipe cactus (*Lemaireocereus thurberi*) cortex. Acid hydrolysis of the extract yielded a neutral fraction, which had a very high lipid and steroid content. From this fraction, we isolated three crystalline compounds by column chromatography on alumina. The first was identified as thurberogenin (I), previously found by Djerassi[3] in Organ Pipe. The second compound was betulin (II), hitherto undetected in this species. The third substance (mp 206–208°) had the same molecular formula as betulin $C_{30}H_{50}O_2$, gave a positive tetranitromethane test and had an ir spectrum almost identical with that of betulin. It appeared to be a new triterpene of the lupane class, and was given the trivial name, thurberin (III). Evidence to support the structural assignment (III) is given below.

Thurberin formed a diacetate (IV) and absorbed 1 mol of hydrogen (PtO_2) to yield dihydrothurberin (V), which also could be converted into a diacetate (VI). Oxidation of thurberin with CrO_3–pyridine led to the diketone, thurberindione (VII), which on catalytic reduction (H_2/PtO_2) yielded dihydrothurberindione, VIII. When VII was submitted to Wolff–Kishner conditions, α-lupene (IX) was formed; under identical conditions VIII gave rise to lupane (X).[4]

These transformations established that thurberin is a lupenediol, isomeric with betulin (II). The two hydroxyl groups were assigned the positions C-3 and C-12 on the basis of ir, nmr, mass, and ORD spectral studies, as outlined in the following sections.

Nmr Measurements.—The nature of the two hydroxyl groups was deduced from the 100-Mc CAT nmr spectra of thurberin (III) and its diacetate (IV). The spectrum of III contained a one-proton quartet centered at τ 6.76 with splitting of 10 and 4.5 cps (X component of ABX system), characteristic of the C-3 proton resonance, adjacent to an OH moiety. This



pattern is almost identical in the spectrum of the diacetate, IV, but is displaced downfield to τ 5.6. This evidence also substantiates the equatorial orientation of the C-3 hydroxyl group.

Information regarding the second OH group also was obtained from the above spectra, which showed a C–X proton quartet centered at τ 6.37 (characteristic of an axial proton attached to an OH-substituted carbon atom).[5] Similar results with appropriate downfield shifts (τ 5.2) were observed in the spectrum of the diacetate, IV. Since the ir spectrum of thurberindione, VII, showed only a single band at 5.88 μ, indicating no cyclopentanone moiety[5] or aldehyde moiety, the nmr data confirm the existence of a second OH group in one of the cyclohexane rings.

(1) C. Steelink, M. Young, and R. L. Caldwell, *Phytochem.*, **6**, 1435 (1967).

(2) C. Steelink, E. Riser, and M. J. Onore, *ibid.*, **7**, 1673 (1968).

(3) (a) C. Djerassi, L. E. Geller, and A. J. Lemin, *J. Amer. Chem. Soc.*, **75**, 2254 (1953); (b) C. Djerassi, E. Farkas, L. H. Liu, and G. H. Thomas, *ibid.*, **77**, 5330 (1955); (c) M. Marx, J. Leclercq, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **32**, 3150 (1967).

(4) We are gratefully indebted to Professor C. Djerassi, Department of Chemistry, Stanford University, Stanford, Calif., for his generous supply of these specimens for our work.

(5) N. S. Bhacca and D. H. Williams, "Application of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 47.

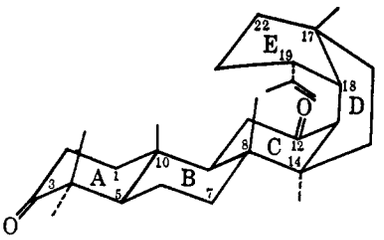


Figure 1.—Conformational model of thurberin-3,12-dione (VII) based on stereochemistry of lupane skeleton.

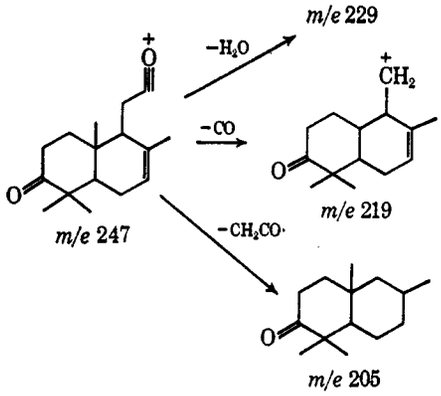
Evidence for the presence of an isopropenyl group was also obtained from the spectra of III and IV, which displayed a three-proton singlet at τ 8.24 (τ 8.34 in IV) due to the C-29 methyl resonances of a side chain isopropenyl group and a doublet centered at τ 5.34 (τ 5.44 in IV), indicative of C-30 protons. Further proof for the presence of the isopropenyl group was afforded by the spectrum of dihydrothurberin diacetate (VI) which exhibited no olefinic proton signal, but rather two three-proton singlets at τ 9.08 and 9.13. This is characteristic of an isopropyl group.[6] All other signals in VI were identical with IV. The location of the isopropenyl group at C-19 was unequivocally established by the previous conversion of VII into lupane and lupene.

Mass Spectral Measurements.—Prominent peaks in the mass spectra of thurberin (III) were *m/e* 442 (25.3), 424 (8.16), 399 (9.12), 234 (34.5), 216 (10.9), 207 (74.1), 189 (100), 147 (81.0), 107 (74.13); diacetate IV *m/e* 526 (3.0), 466 (34.5), 423 (7.15), 276 (7.5), 249 (9.5), 216 (18.5), 189 (100), 135 (60.0), 107 (60.5). These spectra were very similar to those of betulin (II) and its diacetate. This, together with the characteristic peaks at 207 and 189, established the basic structure of the A and B rings as being derived from a 3-hydroxypentacyclic triterpene.[7] A striking feature of the spectrum of III was the *absence* of a strong fragment at 411 (M − 31), which is *prominent* in the spectrum of betulin. This suggests the absence of a $-CH_2OH$ group in III. The low intensity of the M − 43 peak in II and III is consistent with the presence of an isopropenyl moiety.[7]

The possible location of the second OH group was inferred from the mass spectrum of thurberindione (VII): *m/e* 438 (61.5), 395 (6.0), 382 (12.0), 247 (91.5), 229 (47.5), 219 (8.0), 205 (27.5), 23 (100). The spectrum of the dihydro derivative (VIII) also contained prominent peaks at 247, 229, 219 and 205. By assuming carbonyl functions at C-3 and C-12, one can rationalize these results by Scheme I.

ORD Measurements.—Three compounds were used to establish the position of the second OH group: thurberindione (VII), $[\phi]_{300}$ +1408°, lupen-3-one (XI), $[\phi]_{310}$ +3080°, and dihydrothurberindione (VIII), $[\phi]_{310}$ −1412°, all in methanol. Since both VII and XI absorb in essentially the same region of the spectrum and there is no vicinal interaction between the two carbonyl chromophores in VII, the C–X chromophore must have a negative cotton effect. This partially cancels the strong positive cotton effect of the C-3

SCHEME I



ketone group. Such a negative effect will be exhibited by a C-12 carbonyl function and not by a C-11 function, as close examination of a conformational model of the lupane-3,12-dione skeleton (chair–chair–chair–boat–chair)[8] and application of the octant rule reveal[9] (see Figure 1).

Confirmation of this assumption was obtained from the spectrum of dihydrothurberindione (VIII) which exhibited a strong *negative* cotton effect. The isopropenyl moiety at C-19 is able to exert this pronounced effect on the carbonyl function, only if the latter is uniquely situated at C-12 (Figure 1).

Conclusion

Thurberin is shown to be Δ-20,30-lupene-3,12-diol. The presence of an oxygen function at C-12 is unique among cactus triterpenes; its isolation from Organ Pipe represents the first report of its occurrence in nature.

Experimental Section

A. General.—Melting points are uncorrected. Unless otherwise stated, all infrared spectra were recorded on KBr pellets using a Perkin-Elmer Infracord. Model 137 and/or Perkin-Elmer grating Infracord, Model 337. Ultraviolet absorption spectra were determined in methanol using a Cary recording spectrophotometer, Model 14. Nuclear magnetic resonance spectra were run in deuteriochloroform using a Varian HR-100 nmr spectrometer. Chemical shifts are reported in parts per million (τ) from TMS as internal standard. Mass spectra were taken using a Hitachi Perkin-Elmer double-focusing spectrometer (all-glass inlet system), Model RMU-6E. Specific rotations and optical rotatory dispersion measurements were performed in methanol using a Cary recording spectropolarimeter, model 60. Vpc analysis were carried out using 3% QF-1 Chrom W as stationary phase.

Purification of all triterpene derivatives (isolates or synthetates) was routinely carried out by chromatography on dry packed neutral alumina (grade III) followed by repeated crystallization from methanol–chloroform solutions.

B. Extraction of Triterpenes.—The specimens employed in the present investigation were collected on Oct 5, 1967, at the Organ Pipe National Monument, Organ Pipe, Ariz. Wet, cortical (pulp) tissue of mature *Lemaireocereus thurberi* (15 kg) was macerated in a Waring Blendor in hot 95% ethanol. The ethanol extract, after filtration, was concentrated to half volume. Enough concentrated HCl was added to make the solution 0.4 *N*, and the mixture was refluxed for 24 hr. The cooled solution was neutralized with NH_3 to pH, 7; the ethanol was removed under vacuum; and the resulting aqueous layer was repeatedly ex-

(6) A. I. Cohen, D. Rosenthal, G. W. Karkower, and J. Fried, *Tetrahedron*, **21**, 3179 (1965): D. Lavie, Y. Shvo, and E. Glotter, *ibid.*, **19**, 2255 (1963).

(7) H. Budzikiewicz, J. M. Wilsón, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 3688 (1963).

(8) T. G. Halsall, E. R. H. Jones, and G. D. Meakins, *J. Chem. Soc.*, 2862 (1952), and references cited therein.

(9) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 178.

tracted with ether. The ethereal solution was washed with 10% KOH and dried. From this neutral solution, the triterpenes were isolated.

C. Isolation and Identification of Individual Triterpenes—The ethereal solution (above) was concentrated to dark, viscous oil, dissolved in a small amount of chloroform, and chromatographed on dry-packed acid-washed alumina with eluents listed in Table I, in descending order.

TABLE I

CHROMATOGRAPHY OF NEUTRAL FRACTION FROM THE HYDROLYSIS OF ETHANOL EXTRACT OF THE CORTICAL PORTION OF *Lemaireocereus thurberi*

Fractions	Solvent	Compds isolated	Amount, g
A	Benzene–chloroform (4:1 v/v)	Thurberogenin	1.47
B	Benzene–chloroform (2:1 v/v)	Thurberin + betulin	9.82
C	Chloroform	Betulin	0.19
D	Ether	Betulin	0.033

Thurberogenin (I).—The residue from fraction A, after removal of lipids with hexane and extensive rechromatography, yielded colorless needles from methanol: mp 288–290° (acetate mp 247–249°), undepressed by admixture with an authentic sample.[4]

Betulin (II).—The viscous oily material from fractions C and D was rechromatographed on neutral alumina with ligroin–chloroform (3:2 v/v), followed by benzene–chloroform (2:1 v/v) to yield colorless tiny needles from methanol, mp 249–251° (acetate, 217–219°), containing 1 mol of solvent of crystallization. These were identical with authentic samples.[10]

Thurberin (III).—Although we experienced little difficulty in isolating this material betulin was always a minor contaminant. Only thin layer chromatography on silica with benzene–ethyl acetate (8:2 v/v) could separate betulin from thurberin; this could not be duplicated on a preparative scale. For derivatives and other chemical degradations, the crude material (III) was used without purification.

For analysis and spectroscopic measurements, a small sample of pure thurberin was isolated using the invert dry column technique[11] followed by alumina (grade III) column chromatography. Crystallization from methanol yielded colorless needles: mp 206–208°, $[\alpha]^{25}D$ +12° (*c* 0.001, methanol); λ_{max} 2.88, 6.1, 6.99, 7.22 μ; nmr (deuteriochloroform) τ 8.24 (singlet, 3 H), 6.76 (quartet, 1 H), 6.37 (quartet, 1 H), 5.34 (doublet, 2 H). The compound gave a positive tetranitromethane (TNM) test, showed no selective uv absorption, and contained 1 mol of methanol.

Anal. Calcd for $C_{30}H_{50}O_2 \cdot CH_4O$: C, 78.43; H, 11.46; mol wt, 442. Found: C, 78.56; H, 11.48; *m/e* 442.

The diacetate had mp 191–192°; $[\alpha]^{25}D$ +47° (*c* 0.000428, methanol); λ_{max} 5.8, 6.02 μ.

Anal. Calcd for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33; mol wt, 526. Found: C, 77.84; H, 10.29; *m/e* 526.

Dihydrothurberin (V).—A solution of crude thurberin (200 mg) in ethanol (10 ml) was hydrogenated over PtO_2 (15 mg) at room temperature. After the usual purification (part a), 37 mg of product was obtained: mp 259–261°; $[\alpha]^{25}D$ −62° (*c* 0.00122, methanol). No band at 6.0–6.1 μ in the ir spectrum was observed, and a negative TNM test was obtained.

Anal. Calcd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79; mol wt, 444. Found: C, 80.89; H, 11.66; *m/e* 444.

The diacetate had mp 241–243°; $[\alpha]^{25}D$ +9° (*c* 0.0011, methanol); λ_{max} 5.75, 5.80 μ.

Anal. Calcd for $C_{34}H_{56}O_4$: C, 77.22; H, 10.67; mol wt, 528. Found: C, 76.89; H, 10.73; *m/e* 528.

Thurberin-3,12-dione (VII).—A cold solution of crude thurberin (1.2 g) in pyridine (25 ml) was added portionwise to a stirred solution of chromic anhydride (2.0 g) in water (1.2 ml) and pyridine (40 ml) maintained below 0° and left overnight. The reaction was worked up in the usual way, yielding 750 mg of colorless flakes, mp 168–169°, which contained minor impurities. An analytical sample of VII was isolated by rechromatography on alumina and several recrystallizations from methanol–chloroform: mp 182–183°; $[\alpha]^{25}D$ +22° (*c* 0.001, methanol); $\lambda_{max}^{CHCl_3}$ 5.88 and 6.1 μ; λ_{max} 290 mμ; and gave a positive TNM test. It exhibited the following ORD spectrum (*c* 0.001, methanol): $[\phi]_{589}$ +97°, $[\phi]_{305}$ +1364° (shoulder), $[\phi]_{300}$ +1408° (peak), $[\phi]_{270}$ +44 (trough).

Anal. Calcd for $C_{30}H_{46}O_2$: C, 82.14; H, 10.57; mol wt, 438. Found: C, 82.42; H, 10.71; *m/e* 438.

Dihydrothurberin-3,12-dione (VIII).—Hydrogenation of crude VII, as described above, yielded after chromatograpy and crystallization from methanol–chloroform a colorless solid: mp 263–266°; $[\alpha]^{25}D$ −32° (*c* 0.0012, methanol); λ_{max} 5.88 μ; λ_{max} 290 mμ; and gave negative TNM test. ORD data (*c* 0.0012, methanol) were as follows: $[\phi]_{589}$ −18°, $[\phi]_{315}$ −1412° (peak), $[\phi]_{305}$ −1356° (shoulder), $[\phi]_{275}$ −37° (trough).

Anal. Calcd for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98; mol wt, 440. Found: C, 81.69; H, 11.12; *m/e* 440.

Lupane (X) **from Dihydrothurberindione** (VIII).—A mixture of pure dihydrothurberin-3,12-dione (80 mg), diethylene glycol (6 ml), KOH (0.5 g), and hydrazine hydrate (1.0 ml, 99%) was heated under reflux for 20 hr in an atmosphere of nitrogen. Dilution with water, followed by extraction with ether, afforded a solid material. Purification in the usual manner (part A) yielded colorless needles (35 mg), mp 184–186°, of lupane, identical with an authentic sample.[4] In the same manner, α-lupene (IX) was obtained from thurberin-3,12-dione (VII), mp 161–163°, identical with a sample prepared from lupeol[12] by chromic oxidation and Wolff–Kishner reduction.

Registry No.—III, 19769-92-7; III (diacetate), 19769-93-8; V, 19769-94-9; V (diacetate), 19769-95-0; VII, 19806-63-4; VIII, 19769-96-1.

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(10) We are grateful to Dr. J. Knight, Department of Chemistry, Arizona State University, Tempe, Ariz., for the gift of this material.

(11) V. K. Bhalla, U. R. Naik, and S. Dev, *J. Chromatog.*, **26**, 54 (1967).

(12) We are grateful to Professor J. R. Cole, Department of Pharmacy, University of Arizona, Tucson, Ariz., for providing us with this sample.