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A NEW PENTACYCLIC TRITERPENE ACID FROM *LANTANA INDICA*

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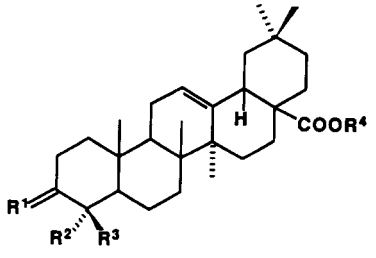
ABSTRACT.—A new pentacyclic triterpene acid **1** has been isolated from the roots of *Lantana indica*, and its structure has been established as 24-formyl-3-oxoolean-12-en-28-oic acid by chemical and spectroscopic methods.

Lantana indica Roxb. (Verbenaceae), a shrub native to India, is used as a bechic, sudorific, intestinal antiseptic, and diaphoretic and in the treatment of tetanus, rheumatism, and malaria in the Indian ayurvedic system of medicine (1). Previous chemical reports on the genus *Lantana* include the isolation of triterpenes of the oleanane, ursane, and lupane skeletons from *Lantana camara* (2–6), *Lantana tiliaefolia* (7), and *L. indica* (8,9). Here we report the isolation and structure elucidation of a new oleanane pentacyclic triterpene acid, 24-formyl-3-oxoolean-12-en-28-oic acid [**1**], from *L. indica*.

From the $CHCl_3$ extracts of air-dried, ground roots of *L. indica*, compound **1**, $C_{30}H_{44}O_4$ ($[M]^+$ 468), was obtained as white crystals crystallized from MeOH. Compound **1** was recognized as a triterpene acid from its positive Liebermann-Burchardt color reaction (red), the appearance of ir absorption bands at 1720, 1710, and 1690 cm^{-1}, and its methylation to a monomethyl ester **2**, ir ν max 1725–1720 and 1720 cm^{-1}, by treatment with ethereal CH_2N_2. Analysis of the ^{13}C-nmr spectrum (6 × Me, 10 × CH_2, 4 × CH, 5 × C, 1 × C = CH, 2 × CO, 1 × CHO) not only confirmed the molecular formula of the compound but also supported a triterpene structure with six methyl groups, one trisubstituted double bond, and three carbonyl groups. Additional information about the molecule came from an analysis of its 90 MHz ^1H-nmr spectrum in $CDCl_3$ (TMS) which showed signals for six tertiary *C*-methyl groups (3H singlets at δ 0.82, 0.90, 0.93, 1.06, 1.13, and 1.26), a ketomethylene group (2H multiplet at δ 2.84), an isolated olefinic hydrogen (1H triplet at δ 5.26), and an aldehyde group adjacent to an asymmetric center (1H singlet at δ 9.68).

This spectral data and the eims fragmentation pattern of the free acid (mass peaks at *m/z* 248, 203, 189, and 133) and its derivatives were indicative of the presence of an olean-12-ene skeleton with a carboxylic group at the C-17 position (10,11). It is also



1 R^1=O, R^2=Me, R^3=CHO, R^4=H
2 R^1=O, R^2=R^4=Me, R^3=CHO
3 R^1=O, R^2=R^4=Me, R^3=H
4 R^1=OH and H, R^2=R^4=Me, R^3=CH_2OH
5 R^1=O, R^2=Me, R^3=CH_2OH, R^4=H

evident from the eims that the keto and formyl groups were located somewhere in rings A/B and not in rings C, D, and E, as the fragment ion at *m/z* 248 would not then have been formed.

The presence of a keto group at the C-3 position was highly probable on a biogenetic basis. The formyl group would occupy any of four positions: C-23, C-24, C-25, or C-26. When the methyl ester **2** was treated with methanolic KOH solution, **2** underwent a reaction typical of aldehydes having no α-hydrogen, and the product was readily recognized as methyl-3-oxonorolean-12-en-28-oate [**3**] from an analysis of its physical properties and spectral data (2,7). The loss of one carbon atom by this reaction clearly indicated the formation of a β-keto acid which undergoes decarboxylation to give rise to **3**. From the above observations, the formyl group was assumed to be located either at the C-23 or at the C-24 position, and the problem remained to settle the exact position of this group. A solution to this problem came from the spectral analysis (^{1}H and ^{13}C nmr) of the major constituent **4** from $NaBH_4$ reduction of **1**. The ^{1}H-nmr signals at δ 3.23 and 4.12, $J = 11.6$ Hz as an AB quartet ($-CH_2-O$) and the ^{13}C-nmr signal at δ_c 23.0 (C-23) in **4** fixed the position of the formyl group at C-24 (12–14). Also, the ^{13}C-nmr spectrum of **1** showed thirty carbon atoms possessing complexity and chemical shifts in perfect agreement with the proposed structure (Table 1). Finally, on treatment

TABLE 1. ^{13}C-nmr Data for Compounds **1**, **2** and **4** [δC values (ppm) in $CDCl_3$ at 22.49 MHz].

Carbon	Compound		
	1	**2**	**4**
C-1	39.1 (t)	39.2 (t)	38.2 (t)
C-2	22.8 (t)	23.0 (t)	27.5 (t)
C-3	209.7 (s)	209.7 (s)	80.7 (d)
C-4	37.1 (s)	37.2 (s)	41.6 (s)
C-5	57.6 (d)	57.7 (d)	55.7 (d)
C-6	19.6 (t)	19.6 (t)	18.5 (t)
C-7	32.6 (t)	32.7 (t)	32.2 (t)
C-8	39.4 (s)	39.6 (s)	39.3 (s)
C-9	46.1 (d)	46.1 (d)	47.6 (d)
C-10	35.9 (s)	36.0 (s)	36.6 (s)
C-11	23.5 (t)	23.6 (t)	23.0 (t)
C-12	122.1 (d)	122.1 (d)	122.2 (d)
C-13	143.7 (s)	143.6 (s)	143.6 (s)
C-14	41.0 (s)	41.4 (s)	41.3 (s)
C-15	27.6 (t)	27.7 (t)	27.5 (t)
C-16	23.8 (t)	23.9 (t)	23.6 (t)
C-17	46.5 (s)	46.7 (s)	46.6 (s)
C-18	41.7 (d)	41.8 (d)	41.3 (d)
C-19	45.8 (t)	45.8 (t)	45.8 (t)
C-20	30.6 (s)	30.7 (s)	30.6 (s)
C-21	33.8 (t)	33.9 (t)	33.7 (t)
C-22	32.3 (t)	32.3 (t)	32.8 (t)
C-23	14.5 (q)	14.4 (q)	22.5 (q)
C-24	201.2 (d)	201.3 (d)	64.4 (t)
C-25	17.0 (q)	17.0 (q)	16.6 (q)
C-26	17.2 (q)	17.2 (q)	22.4 (q)
C-27	25.7 (q)	25.8 (q)	25.8 (q)
C-28	183.9 (s)	178.9 (s)	178.1 (s)
C-29	32.6 (q)	33.1 (q)	33.1 (q)
C-30	23.5 (q)	23.5 (q)	23.6 (q)
Others	—	51.6 (q)	51.6 (q)

with CrO_3 and pyridine (Sarett oxidation), 24-hydroxy-3-oxoolean-12-en-28-oic acid [**5**], a known compound (2,7) previously isolated from the same plant, afforded **1**. Since the formyl group was assumed to be formed in the reaction by oxidation of the $-CH_2OH$ group of **5**, it might be expected that the -CHO group will have the same location and orientation as the $-CH_2OH$ group has in **5**. Therefore, the formyl group in compound **1** located the position at C-24 (β orientation).

The foregoing evidence led to the characterization of compound **1** as 24-formyl-3-oxoolean-12-en-28-oic acid, which might be expected as an important biogenetic intermediate derived from the analogous alcohol during the biogenetic oxidations in the plant. From the chemotaxonomic point of view, it is interesting to point out that triterpenoids possessing a formyl group in rings A/B of the amyrin group were reported previously only from a few species (15,16).

EXPERIMENTAL

ANALYTICAL TECHNIQUES.—All mp's were determined using a Perfit mp apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer model 783 spectrophotometer in Nujol. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL FT-NMR spectrometer (90 MHz) in $CDCl_3$ using TMS as an internal standard, and the chemical shifts were determined in ppm relative to TMS. The eims were recorded on a JEOL JMS D 300 spectrometer. Glindia's Si gel was used for all chromatographic purposes, and tlc plates were visualized by spraying with Liebermann Burchardt reagent.

PLANT MATERIALS.—The roots of the plant were collected from the suburb of Varanasi, India, dried in the shade, and powdered. A voucher specimen was identified at the Center of Advanced Study in Botany, B.H.U., Varanasi, and deposited in the Department of Dravya Guna, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

EXTRACTION AND ISOLATION.—The shade-dried and powdered roots of *L. indica* (4.0 kg) were extracted exhaustively with $CHCl_3$ in a Soxhlet extractor, and the extract was concentrated under reduced pressure. The extract (50 g) was chromatographed over Si gel using petroleum ether (60–80°) C_6H_6, $CHCl_3$, EtOAc, and EtOAc-MeOH (3:2) as eluents. The EtOAc eluates were further chromatographed over Si gel using C_6H_6, $CHCl_3$, and $CHCl_3$-MeOH (3:1) as eluents. Three successive fractions from the $CHCl_3$-MeOH (3:1) elution were collected on the basis of the tlc patterns, and fraction 1 was rechromatographed over Si gel eluting with $CHCl_3$. Preparative layer chromatography of the $CHCl_3$ eluates yielded a solid material which was crystallized from MeOH to afford white needles (100 mg).

COMPOUND **1**.—Mp 246–248°; ir see text. Found C 76.8, H 9.7; $C_{30}H_{44}O_4$ requires C 76.9, H 9.4%. Eims *m/z* (%) $[M]^+$ 468 (88), 454 (20), 440 (43), 424 (36), 262 (10), base peak 248 (100), 203 (93), 191 (10), 189 (11), 133 (25); ^{1}H nmr see text; ^{13}C nmr see Table 1.

METHYL ESTER **2**.—Mp 156–158°; ir see text. Found C 77.4, H 9.8; $C_{31}H_{46}O_4$ requires C 77.1, H 9.5%. Eims *m/z* (%) $[M]^+$ 482 (73), $[M+1]^+$ 483 (28), 454 (19), 424 (9), 423 (33), 422 (20), 262 (55), 248 (20), 204 (18), base peak 203 (100), 189 (30), 187 (10), 133 (18); ^{1}H nmr δ 0.80, 0.90, 0.93, 1.07, 1.14, and 1.26 (each 3H, s, Me-26, -29, -30, -25, -23, -30), 2.46 (2H, m, H-2), 2.76 and 2.88 (1H, dd, H-18), 3.65 (3H, s, COOMe), 5.28 (1H, t, H-12), 9.68 (1H, s, H-24); ^{13}C nmr see Table 1.

METHYL-3-OXONOROLEAN-12-EN-28-OATE [**3**].—Mp 202–205° [lit. (2) mp 203–205°]. Found C 79.2, H 9.7; $C_{30}H_{46}O_3$ requires C 79.3, H 10.1%. Ir, ^{1}H nmr, ^{13}C nmr, and eims identical with methyl-3-oxonorolean-12-en-28-oate (2,7).

$NaBH_4$ REDUCTION OF **1** TO DIOL **4** AS METHYL ESTER.—Mp 205–206°; ir (Nujol) ν max 3500, 1725 cm^{-1}. Found C 76.3, H 10.25; $C_{31}H_{50}O_4$ ($[M]^+$ 486) requires C 76.54, H 10.2%. ^{1}H nmr δ 0.70, 0.85, 0.90, 0.93, 1.12, and 1.23 (each 3H, s, Me-26, -29, -30, -25, -23, -27), 2.61 (2H, bs, -OH), 2.78 and 2.90 (1H, dd, H-18), 3.42 (1H, m, H-3), 3.23 and 4.12 (each 1H, pair of AB doublets, J = 11.6 Hz, H_2-24), 3.61 (3H, s, COOMe), 5.25 (1H, t, H-12, J = 2.9 Hz); ^{13}C nmr see Table 1.

SARETT OXIDATION OF **5** TO **1**.—Compound **5** (100 mg) dissolved in dry C_5H_5N (1 ml) was added to 120 mg CrO_3 and 4 ml dry pyridine. Usual workup followed by crystallization from MeOH afforded a compound which was found essentially identical (mp, ir, ^{1}H nmr, ^{13}C nmr, and ms) to compound **1**.

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