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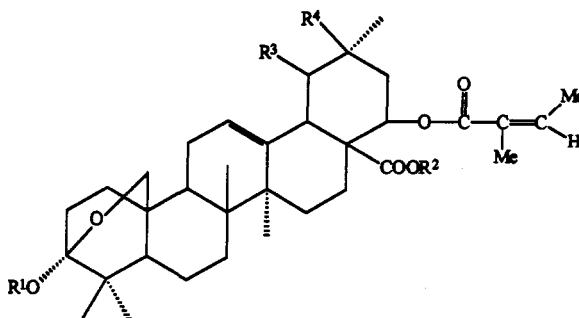
TRITERPENOIDS FROM THE AERIAL PARTS
OF *LANTANA CAMARA*SABIRA BEGUM,* SYED MOHAMMAD RAZA, BINA SHAHEEN SIDDIQUI,
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ABSTRACT.—A new Δ^{12} -oleanane triterpenoid and a new Δ^{12} -ursane type triterpenoid, camarilic acid [**1**] and camaracinic acid [**2**], respectively, have been isolated from the aerial parts of *Lantana camara*, along with five known triterpenes, namely, oleanonic acid, ursonic acid, lantadene A, betulinic acid, and oleanolic acid. The new compounds have been characterized as 3,25-epoxy-3 α -methoxy-22 β -[(Z)-2'-methyl-2'-butenyloxy]-12-oleanen-28-oic acid [**1**] and 3,25-epoxy-3 α -methoxy-22 β -[(Z)-2'-methyl-2'-butenyloxy]-12-ursen-28-oic acid [**2**], respectively, through chemical transformation and spectroscopic studies.

Lantana camara L. (Verbenaceae) is a hairy shrub, native to tropical America and cultivated as an ornamental or hedge plant. Its different parts are reputed to be of use in traditional medicine for the treatment of various human ailments such as ulcers, eczema eruptions, malaria, and rheumatism (1–3). Pharmacological investigations have indicated that extracts of the shoots of *L. camara* exhibit antibacterial properties. Lancamarone, a steroid from the leaves, possesses cardiotonic properties, while lantamine, an alkaloid from the stem and root bark, shows strong antipyretic and antispasmodic properties comparable with those of quinine (2). Phytochemical studies undertaken by different groups of workers have resulted in the isolation of various steroids (4), terpe-

noids (5), and alkaloids (6). In view of the pharmacological properties of *L. camara*, the present studies were undertaken on the chemical constituents of the aerial parts of this plant, which resulted in the isolation and structure elucidation of two new triterpenoids, camarilic acid [**1**] and camaracinic acid [**2**], and five known pentacyclic triterpenoids, oleanonic acid (3-keto-oleanolic acid), ursonic acid (3-keto-ursolic acid), lantadene A, betulinic acid, and oleanolic acid.

The molecular formula ($C_{36}H_{54}O_6$) of **1** was obtained through hrms. Compound **1** showed ir absorptions at 3550–2575 (br, COOH), 2920, 2850 (CH), 1730 (ester C=O), 1715 (acid C=O), 1630 (C=C), and 1060 (br, C–O) cm^{-1} , and a uv maximum at 217 nm. The 1H -



- 1** $R^1=R^4=Me, R^2=R^3=H$
1a $R^1=R^2=R^4=Me, R^3=H$
2 $R^1=R^3=Me, R^2=R^4=H$
2a $R^1=R^2=R^3=Me, R^4=H$
3 $R^1=R^2=R^3=H, R^4=Me$
3a $R^1=R^3=H, R^2=R^4=Me$

nmr spectrum showed six three-proton singlets at δ 0.75, 0.88, 0.95, 0.99, 1.01, and 1.14, attributable to six methyls, and two one-proton double doublets at δ 3.88 ($J=8.4$ and 1.1 Hz, H-25b) and δ 4.21 ($J=8.4$ and 2.3 Hz, H-25a), due to two non-equivalent methylene protons. Two triplets at δ 5.10 ($J=3.0$ Hz) and 5.37 ($J=3.8$ Hz) were ascribed to H-22 α and H-12, respectively. These data showed the close analogy of **1** with lantanilic acid (7), i.e., that the compound possessed a 3,25-epoxy function, a double bond at C-12, and a β -oriented ester function at C-22 in a β -amyrin skeleton. A fragment ion at m/z 246.1628 was also present in the mass spectrum of **1** resulting from retro-Diels-Alder fragmentation and loss of an ester moiety in the form of the corresponding acid. This observation was indicative of a carboxyl function at C-17 (8). Closely related to that of lantanilic acid, the mass spectrum of **1** also showed a peak at m/z 83.0499, corresponding to C_3H_5O , and a peak at m/z 482.3414 ($C_{31}H_{46}O_4$), resulting from the loss of 100 mass units from the molecular ion. These observations were indicative of a dimethyl acrylic acid ester-side-chain at C-22. However, the absence of the $\beta\beta$ -dimethyl acrylic ester group of lantanilic acid was obvious from inspection of the 1H -nmr spectrum of **1**. Thus, it showed a three-proton quintet at δ 1.78 ($J=1.5$ Hz, Me-5'), a three-proton doublet of quartets at δ 1.94 ($J=7.2$ and 1.5 Hz, Me-4') and a one-proton quartet of quartets at δ 5.97 ($J=7.2$ and 1.5 Hz, H-3'), suggesting a 2,3-dimethyl acrylic acid ester with the *Z* configuration (9). An OMe group which appeared at δ 3.25 as a three-proton singlet in the 1H -nmr spectrum was located at C-3 in place of the α -OH group of lantanilic acid, since the compound had no further carbinyl proton in the 1H -nmr spectrum. It may also be noted that the side-chain at C-22 resulted in a downfield shift of H-18, which resonated at δ 3.03 (dd, $J=14.4$

and 4.0 Hz), as against ca. δ 2.8 characteristic for H-18 of a $\Delta^{12}\beta$ -amyrin skeleton (10). On the basis of the data recorded above, compound **1** was characterized as 3,25-epoxy-3 α -methoxy-22 β -[(*Z*)-2'-methyl-2'-butenyloxy]-12-oleanen-28-oic acid. The structure of **1** was conclusively established through its methylation with diazomethane to **1a** which was identical with the product obtained from **3** (11) on ketalization of its methyl ester [**3a**] with MeOH and concentrated H_2SO_4 . ^{13}C -Nmr (broad band and DEPT) and 2D nmr studies (COSY-45, NOESY, *J*-resolved and hetero-COSY) of **1a** were also undertaken, which led to the assignment of all the carbons and protons (Table 1) and also supported the assigned structure of **1**.

The molecular formula of compound **2**, $C_{36}H_{54}O_6$ (M^+ 582.3862), was obtained through hrms. It showed almost identical ir and uv absorptions to those of **1**. The 1H -nmr spectrum showed six methyl signals, four as singlets at δ 0.75, 0.97, 1.00, and 1.02, and two as doublets at δ 0.87 ($J=6.9$ Hz) and δ 0.90 ($J=6.3$ Hz), along with a one-proton doublet at δ 2.45 ($J=11.0$ Hz, H-18), and a one-proton triplet at δ 5.35 (t, $J=3.7$ Hz, H-12) indicating that **2** belongs to the Δ^{12} -ursane series of pentacyclic triterpenes (12). 1H -Nmr (Experimental) and ms analysis revealed that the rest of the molecule is similar to that of **1**, i.e., compound **2** also has a carboxylic group at C-17, a β -oriented α,β -dimethyl acrylic acid ester side-chain with *Z* configuration at C-22, a 3,25-epoxy function, and an α -OMe group at C-3. Compound **2** also formed the methyl derivative **2a** ($COOCH_3$; δ 3.55, 3H, s) on treatment with CH_3N_2 . On the basis of these data compound **2** was characterized as 3,25-epoxy-3 α -methoxy-22 β -[(*Z*)-2'-methyl-2'-butenyloxy]-12-ursen-28-oic acid.

Oleanonic acid (13,14), ursonic acid (13), lantadene A (16), betulinic acid (13,15), and oleanolic acid (13,17) have

TABLE 1. Nmr Data of Compound **1a**.^a

Carbon	δ_c	Proton(s)	δ_H	Multiplicity	J (Hz)
1	34.7	1a	2.11	m	—
		1b	1.22	m	—
2	27.8	2a	1.20	m	—
		2b	1.00	m	—
3	100.4	—	—	—	—
4	38.4	—	—	—	—
5	50.9	5	1.16	m	—
6	19.7	6	1.50	m	—
7	31.2	7	1.32	m	—
8	40.8	—	—	—	—
9	41.8	9	1.67	m	—
10	34.7	—	—	—	—
11	23.8	11a	2.12	m	—
		11b	1.76	m	—
12	122.7	12	5.41	t	3.5
13	143.1	—	—	—	—
14	42.1	—	—	—	—
15	29.4	15a	2.00	m	—
		15b	1.80	m	—
16	24.3	16	1.82	m	—
17	51.0	—	—	—	—
18	39.4	18	3.08	dd	14.0, 4.1
19	45.6	19a	1.75	m	—
		19b	1.27	m	—
20	30.1	—	—	—	—
21	37.1	21a	1.75	m	—
		21b	1.50	m	—
22	75.3	22 α	5.06	t	3.2
23	27.2	23	0.99	s	—
24	17.0	24	0.73	s	—
25	67.7	25a	4.26	dd	8.6, 3.0
		25b	3.87	dd	8.6, 1.0
26	18.4	26	0.94	s	—
27	26.1	27	1.00	s	—
28	178.2	—	—	—	—
29	33.7	29	0.88	s	—
30	25.3	30	1.13	s	—
1'	166.3	—	—	—	—
2'	127.7	—	—	—	—
3'	138.7	3'	6.00	qq	7.2, 1.6
4'	14.1	4'	1.96	dq	7.2, 1.6
5'	20.5	5'	1.79	quintet	1.6
OMe-3	49.3	OMe-3	3.24	s	—
COOMe	51.4	COOMe	3.53	s	—

^aAssignments confirmed by COSY-45, J -resolved, and hetero-COSY nmr methods.

been isolated previously from this source and identified through comparison of their spectral data with published data.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected. Ms data were recorded on a Finnigan MAT 312 double-focusing mass spec-

trometer connected to a PDP 11/34 computer system; optical rotations were measured on a Jasco DIP-360 polarimeter; nmr spectra ($CDCl_3$; 400 MHz for 1H and 100 MHz for ^{13}C), were recorded on a Bruker AM 400 Ft-nmr spectrometer. The chemical shifts are reported in δ (ppm) and the coupling constants are in Hz. The ^{13}C -nmr spectral assignments (Table 1) have been made partly through a comparison of the chemical shifts with

published data for similar compounds (9,18) and partly through the appearance of signals in DEPT and hetero-COSY experiments. Si gel PF₂₅₄ and Si gel E. Merck 9385 have been used for vlc (19) and flash cc (20) (Model Eyela), respectively.

PLANT MATERIAL.—The plant material was collected from the Karachi region in February 1991, and identified as *L. camara* by Mr. Abdul Ghafoor, Department of Botany, University of Karachi. A voucher specimen (No. 63482 KUH) has been deposited in the Herbarium.

EXTRACTION AND ISOLATION.—Air-dried aerial parts of *L. camara* (10 kg) were repeatedly extracted with MeOH at room temperature. The concentrated extract, obtained on removal of the solvent from the combined extracts under reduced pressure, was partitioned between EtOAc and H₂O. The EtOAc phase was treated with 4% aqueous Na₂CO₃ to separate the acidic and neutral fractions. The EtOAc layer containing the neutral fraction was washed with H₂O, dried (Na₂SO₄), and passed over charcoal. The charcoal bed was successively washed with EtOAc and MeOH-C₆H₆ (1:1). The eluates were combined on the basis of tlc and the solvents were removed under reduced pressure. The residue thus obtained was divided into petroleum ether (bp 60–70°)-soluble and petroleum ether-insoluble fractions. The petroleum ether-insoluble fraction was again divided into Et₂O-soluble and Et₂O-insoluble portions. The residue (17.5 g) obtained from the Et₂O-soluble portion on removal of the solvent was subjected to vlc (petroleum ether (bp 60–70°)/EtOAc in order of increasing polarity). On pooling the fractions on the basis of tlc, nine fractions were obtained. Fraction 4 (3.5 g), which eluted with petroleum ether-EtOAc (8:2) was again subjected to vlc [petroleum ether (bp 60–70°)/EtOAc in order of increasing polarity], which ultimately furnished six fractions (a–f).

Fraction b (1.5 g), obtained on elution with petroleum ether-EtOAc (8.75:1.25), was subjected to flash cc (petroleum ether/EtOAc in order of increasing polarity). The major fraction (164 mg) eluted with petroleum ether-EtOAc (9:1) was again subjected to flash cc using petroleum ether and petroleum ether/EtOAc mixtures of increasing polarity. The petroleum ether-EtOAc (9.8:0.2) eluates furnished oleanonic acid (25 mg), ursonic acid (13.5 mg), and lantadene A (10.6 mg) in order of polarity. Similarly, petroleum ether-EtOAc (9.7:0.3) and (9.7:0.3–9.5:0.5) eluates afforded betulinic acid (16.6 mg) and oleanolic acid (69.7 mg), respectively.

Fraction f (1.0 g), obtained from pure EtOAc elution, was also subjected to flash cc (petroleum ether/EtOAc mixtures of increasing polarity). Elution with petroleum ether-EtOAc (9:1) afforded crude **1** and **2** in order of polarity, which were then

purified (**1**, 6.0 mg; **2**, 5.0 mg) by thick-layer chromatography using petroleum ether-EtOAc (7:3, 2 times) as the developing system.

Camarilic acid [1].—Plates (MeOH); mp 287–288°, [α]_D +172° (c =0.1, CHCl₃); uv (MeOH) λ max 217 nm; ir (CHCl₃) ν max 3550–2575, 2920, 2850, 1730, 1715, 1630, 1060 cm⁻¹; hreims m/z [M]⁺ 582.3860 (C₃₆H₅₄O₆ requires [M]⁺ 582.3919, 3), 482.3414 (6), 285.1948 (10), 249.1839 (10), 246.1628 (6), 236.1850 (14), 201.1588 (4), 119.0848 (32), 83.0499 (72), 55.0577 (100); ¹H nmr δ 0.75 (3H, s, Me), 0.88 (3H, s, Me), 0.95 (3H, s, Me), 0.99 (3H, s, Me), 1.01 (3H, s, Me), 1.14 (3H, s, Me), 1.78 (3H, quintet, J =1.5 Hz, Me-5'), 1.94 (3H, dq, J =7.2 and 1.5 Hz, Me-4'), 3.03 (1H, dd, J =14.4 and 4.0 Hz, H-18), 3.25 (3H, s, OMe-3 α), 3.88 (1H, dd, J =8.4 and 1.1 Hz, H-25b), 4.21 (1H, dd, J =8.4 and 2.3 Hz, H-25a), 5.10 (1H, t, J =3.0 Hz, H-22 α), 5.37 (1H, t, J =3.8 Hz, H-12), 5.97 (1H, qq, J =7.2 and 1.5 Hz, H-3'); ¹³C nmr δ 34.6 (C-1), 27.9 (C-2), 100.2 (C-3), 38.5 (C-4), 50.7 (C-5), 19.6 (C-6), 31.0 (C-7), 40.7 (C-8), 42.0 (C-9), 35.0 (C-10), 23.8 (C-11), 122.8 (C-12), 143.4 (C-13), 42.0 (C-14), 29.5 (C-15), 24.3 (C-16), 50.8 (C-17), 39.2 (C-18), 45.9 (C-19), 30.2 (C-20), 37.8 (C-21), 75.1 (C-22), 27.3 (C-23), 17.2 (C-24), 67.6 (C-25), 18.2 (C-26), 26.3 (C-27), 179.7 (weak signal, C-28), 33.7 (C-29), 25.4 (C-30), 166.5 (weak signal, C-1'), 127.8 (C-2'), 138.4 (C-3'), 14.7 (C-4'), 20.5 (C-5'), 49.5 (3-OCH₃).

METHYLATION OF 1.—Methylation of **1** with ethereal CH₃N₂ and the usual workup afforded **1a** as a colorless crystalline solid, mp 215–216°, [α]_D +176° (c =0.12, CHCl₃); ir ν max 2930, 2850, 1735 (br), 1630, 1050 cm⁻¹; hreims m/z [M]⁺ 596.4058 [C₃₇H₅₆O₆ requires M ⁺ 596.4076, 2], 496.3565 [M ⁺-(Z)-2-methyl-2-butenic acid] (C₃₂H₄₈O₄, 70), 437.3451 (C₃₀H₄₅O₂) (12) (m/z 496–COOMe), 299.2002 (C₂₀H₂₇O₂) (39), 260.1734 (C₁-H₃₄O₂) (12), 249.1843 (C₁₆H₂₅O₂) (6), 241.1965 (C₁₈H₂₅) (62), 236.1856 (C₁₅H₂₄O₂) (8), 201.1603 (C₁₅H₂₁) (22), 119.0840 (C₉H₁₁) (43), 83.0482 (C₅H₇O) (84); ¹H-nmr data were identical to those recorded for **1a** prepared from **3** (Table 1).

Camaracinic acid [2].—Plates (MeOH); mp 270–271°, [α]_D +168° (c =0.11, CHCl₃); uv (MeOH) λ max 217 nm; ir (CHCl₃) ν max 3600–2600 br, 2900, 2825, 1730, 1710, 1635, 1050 cm⁻¹; hreims m/z [M]⁺ 582.3862 (C₃₆H₅₄O₆ requires [M]⁺ 582.3919, 2), 482.3412 (5), 285.1946 (2), 249.1842 (12), 246.1631 (8), 236.1853 (8), 201.1595 (4), 119.0852 (30), 83.0496 (70), 55.0575 (100); ¹H nmr δ 0.75 (3H, s, Me), 0.87 (3H, d, J =6.9 Hz, Me-29 or Me-30), 0.90 (3H, d, J =6.3 Hz, Me-29 or Me-30), 0.97 (3H, s, Me), 1.00 (3H, s, Me), 1.02 (3H, s, Me), 1.81 (3H, quintet, J =1.5 Hz, Me-5'), 1.95 (3H, dq, J =7.2

and 1.5 Hz, Me-4'), 2.45 (1H, d, $J=11.0$ Hz, H-18), 3.26 (3H, s, OMe-3 α), 3.85 (1H, dd, $J=8.8$ and 1.1 Hz, H-25b), 4.31 (1H, dd, $J=8.8$ and 2.7 Hz, H-25a), 5.06 (1H, t, $J=3.2$ Hz, H-22 α), 5.35 (1H, t, $J=3.7$ Hz, H-12), 6.00 (1H, qq, $J=7.2$ and 1.5 Hz, H-3'); ^{13}C nmr δ 34.9 (C-1), 27.7 (C-2), 100.4 (C-3), 38.7 (C-4), 50.8 (C-5), 19.7 (C-6), 31.2 (C-7), 40.5 (C-8), 41.9 (C-9), 34.8 (C-10), 23.2 (C-11), 126.1 (C-12), 137.2 (C-13), 42.2 (C-14), 29.6 (C-15), 24.7 (C-16), 51.5 (C-17), 49.3 (C-18), 39.2 (C-19), 38.7 (C-20), 34.8 (C-21), 75.6 (C-22), 27.1 (C-23), 16.9 (C-24), 67.8 (C-25), 18.3 (C-26), 23.2 (C-27), 180.2 (weak signal, C-28), 17.6 (C-29), 21.2 (C-30), 166.4 (weak signal, C-1'), 127.9 (C-2'), 138.2 (C-3'), 14.8 (C-4'), 20.4 (C-5'), 49.4 (3-OCH₃).

METHYLATION OF 2.—Compound **2** formed **2a** as a colorless crystalline substance on treatment with an ethereal solution of diazomethane and the usual workup: $[\alpha]_D^{+179^\circ}$ ($c=0.1$, CHCl₃); ir ν max 2920, 2850, 1730 (br), 1635, 1055 cm⁻¹; hreims m/z 496.4056 [M^+ -(Z)-2-methyl-2-butenic acid] (C₃₂H₄₈O₄, 68), 437.3449 (C₃₀H₄₅O₂) (10) (m/z 496-COOMe), 299.2001 (C₂₀H₂₇O₂) (42), 260.1730 (C₁₇H₂₄O₂) (10), 249.1839 (C₁₆H₂₃O₂) (7), 241.1960 (C₁₈H₂₅) (60), 236.1850 (C₁₅H₂₄O₂) (9), 201.1588 (C₁₅H₂₁) (20), 119.0848 (C₆H₁₁) (40), 83.0499 (C₅H₉O) (86); ^1H nmr δ 0.77 (3H, s, Me), 0.86 (3H, d, $J=6.5$ Hz, Me-29 or Me-30), 0.89 (3H, d, $J=6.4$ Hz, Me-29 or Me-30), 0.98 (3H, s, Me), 1.00 (3H, s, Me), 1.03 (3H, s, Me), 1.80 (3H, quintet, $J=1.5$ Hz, Me-5'), 1.96 (3H, dq, $J=7.2$ and 1.5 Hz, Me-4'), 2.44 (1H, d, $J=11.0$ Hz, H-18), 3.26 (3H, s, OMe-3 α), 3.55 (3H, s, COOCH₃), 3.84 (1H, dd, $J=8.7$ and 1.0 Hz, H-25b), 4.29 (1H, dd, $J=8.7$ and 2.6 Hz, H-25a), 5.07 (1H, t, $J=3.2$ Hz, H-22 α), 5.33 (1H, t, $J=3.5$ Hz, H-12), 6.00 (1H, qq, $J=7.2$ and 1.5 Hz, H-3').

KETALIZATION OF 3a.—Compound **3a** (20 mg) was prepared from **3** according to the method described in the literature (11). It was dissolved in MeOH (5 ml) and refluxed on a steam bath for 2 h with concentrated H₂SO₄ (2 drops). The reaction mixture was diluted with H₂O and extracted with EtOAc. The EtOAc phase on the usual workup afforded **1a** (16 mg). Flowers of needles (MeOH): mp 216–217°; ir (CHCl₃) ν max: data were comparable with those of **1a**; eims data were also similar to those of **1a**. ^1H - and ^{13}C -nmr data, see Table 1.

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Received 20 October 1994