Nematicidal Constituents of the Aerial Parts of *Lantana camara*

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Two new constituents, lantanoside (**1**) and lantanone (**2**), and the known compounds linaroside (**3**) and camarinic acid (**4**) were isolated from the aerial parts of *Lantana camara*. Compounds **1**, **3**, and **4** were tested for nematicidal activity against root-knot nematode *Meloidogyne incognita* and showed 90, 85, and 100% mortality, respectively, at 1.0% concentration. The results were comparable to those obtained with the conventional nematicide furadan (100% mortality at 1.0% concentration). Structures of the new compounds were elucidated by spectroscopic and chemical techniques.

Lantana camara L. (Verbenaceae) is a hairy shrub, native to tropical America. It is cultivated as an ornamental or hedge plant. Different parts of the plant are used in the folklore and traditional systems of medicine for treatment of various problems such as itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, tetanus, malaria, tumors, and rheumatism.[1,2] Phytochemical studies undertaken by different groups of workers on different parts of the plant have resulted in the isolation of various terpenoids, steroids, and flavonoids.[3−5] The present studies were undertaken to identify the constituents of this plant showing nematicidal activity. This led to the isolation and structure elucidation of two new compounds, lantanoside (**1**) and lantanone (**2**), along with several known compounds, including linaroside (**3**)[6] and camarinic acid (**4**).[7] The structures of **1** and **2** were elucidated with the help of ^{1}H NMR, ^{13}C NMR (BB, DEPT), and 2D NMR spectral studies including COSY, NOESY, *J*-resolved, HMQC, and HMBC. Compounds **1**, **3**, and **4** were tested for nematicidal activity against the root-knot nematode *Meloidogyne incognita*.

Results and Discussion

The HREIMS of **1** showed a molecular ion at *m*/*z* 518, corresponding to the molecular formula $C_{25}H_{26}O_{12}$, which was confirmed by FABMS. Its FABMS showed a peak at *m*/*z* 315 [(M − 204 + H]$^{+}$, indicating the presence of one acetylated glucose moiety. Its IR spectrum showed hydroxyl and carbonyl bands. The UV spectrum was consistent with a flavonoid.[8] The ^{1}H and ^{13}C NMR data of ring A of the aglycone matched those reported for pubescidin,[9] while rings B and C were comparable with those reported for ladanein.[10] It showed two one-proton singlets at δ 6.91 and 6.97 assigned to H-3[10] and H-8,[9] respectively. UV bathochromic shifts with $AlCl_3$ and $AlCl_3$−HCl and a signal at δ 12.88 in the ^{1}H NMR spectrum indicated the presence of a hydroxyl group at C-5.[8,11] The NMR spectrum indicated two aromatic methoxyl groups (δ_H 3.76/δ_C 60.2; δ_H 3.84/δ_C 55.5) and an acetyl group (δ_H 1.97/δ_C 20.5, 169.5). One OMe was placed at C-4′ due to the presence of two two-proton doublets at δ 8.02 (H-2′, H-6′) and 7.10 (H-3′, H-5′) with the same coupling constants (J = 8.8 Hz). A prominent peak at *m*/*z* 271 (aglycon−CH_3CO) located the second OMe at C-6,[12] which is also in accordance with NMR data (see Experimental Section). For the sugar moiety, NMR chemical shifts were in accord with those reported for 6-*O*-acetyl-β-D-glucoside,[10,13] with a downfield shift of the C-6″ by ca. 2 ppm and an upfield shift of the C-5″ by ca. 3 ppm from expected values of glucose. The HMBC interaction of the anomeric proton (δ 5.14) with C-7 (δ 156.2) led us to place the sugar at 7-OH. Compound **1** formed a tetra acetyl derivative (**1a**) with acetic anhydride/pyridine, identical in all respects with the acetyl derivative (**3a**) obtained from linaroside (**3**) (see Experimental Section). In the present studies, complete ^{1}H and ^{13}C assignments of linaroside (**3**) have also been made with the help of 1D and 2D NMR studies. On the basis of the above discussion, lantanoside (**1**) was deduced to be 7-*O*-(6″-*O*-acetyl-β-D-glucopyranosyl)-6,4′-dimethoxy-5-hydroxy flavone.

Table 1. Nematicidal Activity of Compounds **1**, **3**, and **4** on the Larval Mortality of *Meloidogyne incognita* at 1% Concentration

compound	percent larval mortality/exposure time (h)		
	24	48	72
1	90.0	95.0	95.0
3	85.0	90.0	90.0
4	100.0	100.0	100.0
furadan	100.0	100.0	100.0
control	0.0	2.0	3.0
SD[a]	37.94	37.88	37.48

[a] SD = standard deviation.

The molecular ion for **2** was observed at *m*/*z* 512, corresponding to the molecular formula $C_{32}H_{48}O_5$. Its ^{1}H and ^{13}C NMR data (see Experimental Section) along with the molecular formula revealed that it belongs to the β-amyrin series of triterpenoids. The presence of an acetoxy group was indicated by the IR and NMR spectra (δ_H 2.04/δ_C 21.6, 170.8) and mass fragmentation. It was placed at C-3 on biogenetic grounds and in accordance with NMR data (see Experimental Section). The δ value and coupling constants of H-3 (δ 4.50, 1H, dd, J = 10.2, 5.1 Hz) indicated β-orientation of the acetoxy group.[14] Its UV and IR spectra indicated the presence of an α,β-unsaturated carbonyl function. The significant mass fragments[15] at *m*/*z* 262 and 303 and ^{1}H and ^{13}C NMR data (see Experimental Section) favored a Δ^{12}-11-one functionality. The above fragments, and loss of COOH from both, indicated the presence of a carboxylic group on ring D or E. It was placed at C-17 through comparison of δ values of ^{13}C NMR signals of ring D and E carbons with those of similar compounds.[16,17] On the basis of the above observations, the structure of

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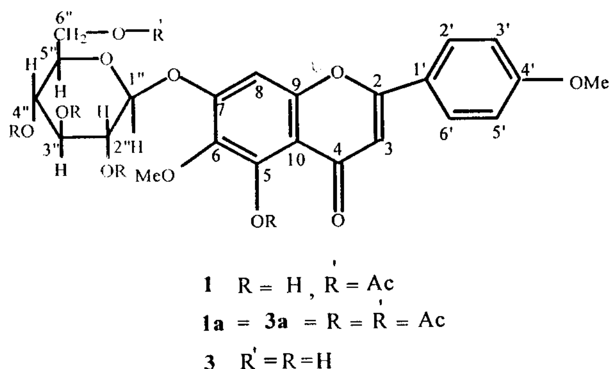
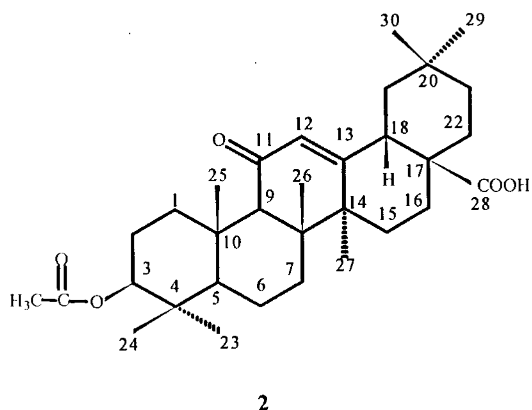
lantanone (**2**) was assigned as 3β-acetoxy-11-oxo-olean-12-en-28-oic acid.

Compounds **1**, **3**, and **4** were tested for nematicidal activity against *Meloidogyne incognita* at different exposure times but at the same concentration (1%). After 24 h, they showed 90, 85, and 100% mortality, respectively, and after 48 h, the mortality increased to 95, 90, and 100%, respectively. These results compared well with the conventional nematicide furadan (Table 1).





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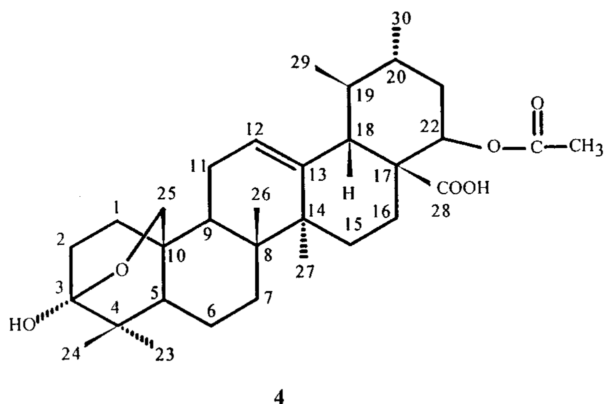

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Experimental Section

General Experimental Procedures. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. UV spectra were obtained on a HITACHI-U-3200 spectrophotometer. IR spectra were recorded on a JASCO A-302 spectrophotometer. The EIMS and HREIMS were recorded on Finnigan MAT-112 and JMS HX-110 spectrometers, respectively. The ^{1}H NMR spectra were recorded on a Bruker AM-500 FT-NMR spectrometer operating at 500 MHz, while the ^{13}C NMR spectra were obtained on the same instrument operating at 125 MHz. Spectra were referenced to the residual solvent signals. The chemical shifts are reported in δ (ppm), and the coupling constants, in Hz. In the ^{13}C NMR spectra various carbons have been identified through BB, DEPT, HMQC, and HMBC and by comparison with related compounds.[9,10,13,14,16,17] Si gel PF_{254} (Merck) and Si gel 9385 (Merck) were used for VLC and flash column chromatography (CC), while Si gel PF_{254} (Merck) was used for TLC.

Plant Material. Aerial parts of *Lantana camara* were collected from the Karachi region. The plant was identified by Mr. Abdul Ghafoor, Senior Taxonomist, Department of Botany, University of Karachi, and a voucher specimen (no. 63482 KUH) is deposited in the herbarium there.

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 extract under reduced pressure showing nematicidal activity, was partitioned between EtOAc and H_2O. The EtOAc phase was treated with 4% aqueous solution of Na_2CO_3 to separate the acidic from the neutral fraction. The EtOAc layer containing the neutral fraction was washed with water, dried (Na_2SO_4), and passed through active C. The charcoal bed was successively washed with EtOAc and MeOH−C_6H_6 (1:1), which were combined on the basis of TLC. The residue obtained on removal of solvent from the charcoal filtrate and washings was divided into hexane-soluble and hexane-insoluble fractions. The hexane-insoluble fraction was again divided into Et_2O-soluble and Et_2O-insoluble portions. The Et_2O-insoluble fraction was again divided into EtOAc-soluble and EtOAc-insoluble portions. The residue (40 g) obtained from the EtOAc-soluble portion was subjected to VLC ($CHCl_3$; $CHCl_3$−MeOH in order of increasing polarity), which ultimately furnished nine fractions (fractions 1−9). Fraction 5, obtained on elution with $CHCl_3$−MeOH (9.6:0.4), afforded pure lantanoside (**1**) (112.8 mg) as colorless crystals. Fraction 8, obtained on elution with $CHCl_3$−MeOH (9.3:0.7), afforded pure linaroside (**3**) (198.0 mg). Fraction 1 (26.0 gm) ($CHCl_3$; $CHCl_3$−MeOH, 9.9:0.1 eluate) was further subjected to VLC (hexane, hexanes−EtOAc, in order of increasing polarity), which ultimately furnished eight fractions (fractions I−VIII). Fraction VI, obtained on elution with hexanes−EtOAc (5:5, 4:6, 3:7), afforded camarinic acid (**4**) (74.0 mg) as colorless crystals on keeping the residue in $CHCl_3$−MeOH at room temperature overnight. The mother liquor of fraction I (200 mg) was subjected to flash CC (hexane, hexanes−EtOAc, in order of increasing polarity), which furnished nine fractions (fractions I-1 to I-9). Fraction I-7, obtained on elution with hexanes−EtOAc (9:1), afforded lantanone (**2**) (10.0 mg).

Lantanoside (1): colorless needles (MeOH); mp 210−212 °C; UV (MeOH) λ_{max} 268, 318 nm; + $AlCl_3$ 274, 378 nm; + $AlCl_3$−HCl 277, 382 nm; IR (KBr) ν_{max} 3450, 2900, 2800, 1730, 1660, 1600−1400, 1080 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.8 (1H, s, 5-OH), 8.02 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.10 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.97 (1H, s, H-8), 6.91 (1H, s, H-3), 5.14 (1H, d, J = 7.1 Hz, H-1″), 4.40 (1H, br d, J = 11.8 Hz, H-6″a), 4.05 (1H, dd, J = 11.8, 7.2 Hz, H-6″b), 3.84 (3H, s, 4′-OCH_3), 3.76 (3H, s, 6-OCH_3), 3.73 (1H, m, H-5″), 3.37 (1H, m, H-2″), 3.36 (1H, m, H-3″), 3.20 (1H, m, H-4″), 1.97 (3H, s, COCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 182.2 (C-4), 169.5 ($COCH_3$), 163.7 (C-2), 162.4 (C-4′), 156.2 (C-7), 152.4 (C-5), 152.0 (C-9), 132.5 (C-6), 128.2 (C-2′, C-6′), 122.6 (C-1′), 114.5 (C-3′, C-5′), 105.8 (C-10), 103.3 (C-3), 99.9 (C-1″), 94.3 (C-8), 76.4 (C-3″), 73.9 (C-5″), 73.0 (C-2″), 69.7 (C-4″), 63.3 (C-6″), 60.2 (6-OCH_3), 55.5 (4′-OCH_3), 20.5 (COCH_3); EIMS m/z 518 $[M]^+$ (**4**), 314 (100), 299 (50), 271 (22), 84 (72), 66 (74); HREIMS m/z 518.1422 (calcd for $C_{25}H_{26}O_{12}$, 518.1423).

Acetylation of 1. Compound **1** (20 mg) was acetylated in pyridine (1 mL) with acetic anhydride (1 mL) at room temperature overnight. Work up as usual afforded the acetate **1a** and, on recrystallization from MeOH−$CHCl_3$ (1:1), formed colorless fine needles: mp 196−198 °C; UV (MeOH) λ_{max} 262, 318 nm; IR (KBr) ν_{max} 1730, 1660, 1600−1400, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.76 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.10 (1H, s, H-8), 6.97 (2H, d, J = 9.0 Hz, H-3′, H-5′), 6.51 (1H, s, H-3), 5.37 (1H, dd, J = 9.5, 7.5 Hz, H-2″), 5.31 (1H, t, J = 9.5 Hz, H-3″), 5.17 (1H, t, J = 9.0 Hz, H-4″), 5.16 (1H, d,

J = 7.5 Hz, H-1″), 4.28 (1H, dd, *J* = 12.0, 5.5 Hz, H-6″a), 4.23 (1H, dd, *J* = 12.0, 2.5 Hz, H-6″), 3.97 (1H, m, H-5″), 3.86 (3H, s, 4′-OCH_3), 3.78 (3H, s, 6-OCH_3), 2.45 (3H, s, 5-$OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.04 (3H, s, $OCOCH_3$), 2.03 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.5 (C-4), 170.4 (5-$OCOCH_3$), 170.1[a] (3″-$OCOCH_3$), 169.4[a] (4″-$OCOCH_3$, 6″-$OCOCH_3$), 169.2[a] (2″-$OCOCH_3$), 162.5 (C-2), 162.3 (C-4′), 154.5 (C-7), 153.2 (C-5), 142.6 (C-9), 140.5 (C-6), 127.8 (C-6′), 127.8 (C-2′), 123.5 (C-1′), 114.5 (C-3′, C-5′), 113.6 (C-10), 103.4 (C-8), 99.4 (C-1″), 72.5 (C-3″), 72.3 (C-5″), 70.8 (C-2″), 68.2 (C-4″), 62.0 (C-6″), 61.8 (6-OCH_3), 55.5 (4′-OCH_3), 21.0 (5-$OCOCH_3$), 20.7[b] (2″-$OCOCH_3$), 20.6[b] (3″-$OCOCH_3$), 20.6[b] (4″-$OCOCH_3$), 20.6[b] (6″-$OCOCH_3$); EIMS *m*/*z* $[M]^+$, not observed, 644 (2), 330 (18), 314 (35), 299 (15), 271 (12), 133 (41), 73 (31); HREIMS *m*/*z* 644.1742 (cald for $C_{31}H_{32}O_{15}$, 644.1740) ([a,b] Values may be interchanged).

Lantanone (2): colorless needles (MeOH), mp 202−204 °C; UV (MeOH) λ_{max} 250 nm; IR (KBr) ν_{max} 3450−2650, 2950, 2850, 1730, 1700, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.65 (1H, s, H-12), 4.50 (1H, dd, *J* = 10.2, 5.1 Hz, H-3α), 2.80 (1H, dd, *J* = 14.4, 4.6 Hz, H-18), 2.04 (3H, s, $OCOCH_3$), 1.12 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.86 (6H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.2 (C-11), 179.7 (C-28), 170.8 ($COCH_3$), 169.8 (C-13), 128.1 (C-12), 81.1 (C-3), 62.2 (C-9), 55.3 (C-5), 46.2 (C-17), 45.3 (C-14) 44.6 (C-19), 44.0 (C-8), 42.4 (C-18), 38.7 (C-1), 37.8 (C-4), 37.0 (C-10), 34.0 (C-22), 33.3 (C-7), 32.9 (C-29), 32.2 (C-21), 30.8 (C-20), 28.3 (C-15), 28.2 (C-23), 23.6 (C-2, C-27), 23.4 (C-16, C-30), 21.6 ($COCH_3$), 19.4 (C-26), 17.9 (C-6), 15.6 (C-25), 15.5 (C-24); EIMS *m*/*z* 512 $[M]^+$ (40), 262 (100), 303 (18), 189 (24), 175 (38); HREIMS *m*/*z* 512.3505 (calcd for $C_{32}H_{48}O_5$, 512.3501).

Linaroside (3): colorless needles (MeOH), ^1H NMR (DMSO-d_6, 500 MHz) δ 12.86 (1H, s, 12-OH), 8.02 (2H, d, *J* = 8.9 Hz, H-2′, H-6′), 7.12 (2H, d, *J* = 8.9 Hz, H-3′, H-5′), 7.02 (1H, s, H-8), 6.91 (1H, s, H-3), 5.11 (1H, d, *J* = 7.3 Hz, H-1″), 3.85 (3H, s, 4′-OCH_3), 3.75 (3H, s, 6-OCH_3), 3.70 (1H, dd, *J* = 10.0, 4.5 Hz, H-6″a), 3.51 (1H, m, H-6″b), 3.49 (1H, m, H-5″), 3.32 (1H, m, H-2″), 3.30 (1H, m, H-3″), 3.21 (1H, m, H-4″); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 182.3 (C-4), 163.8 (C-2), 162.4 (C-4′), 156.5 (C-7), 152.4 (C-5), 152.1 (C-9), 132.6 (C-6), 128.3 (C-2′, C-6′), 122.8 (C-1′), 114.6 (C-3′, C-5′), 105.8 (C-10), 103.3 (C-3), 100.2 (C-1″), 94.4 (C-8), 77.3 (C-5″), 76.7 (C-3″), 73.2 (C-2″), 69.6 (C-4″), 60.7 (C-6″), 60.3 (6-OCH_3), 55.6 (4′-OCH_3).

Acetylation of 3. Compound **3** (10 mg) was acetylated following the method described for the acetylation of **1**. The product (**3a**) was identified as **1a** through mixed mp, mixed TLC, MS, ^1H NMR, and ^{13}C NMR.

Determination of Nematicidal Activity. Experiments were performed under laboratory conditions, 28 ± 2 °C. Fresh egg masses collected from stock culture and maintained on tomato were used for egg hatching. The larvae that emerged within 48 h were used in tests for larval mortality. Compounds **1**, **3**, and **4** were dissolved in distilled water to prepare stock solution. *Meloidogyne incognita* (100 larvae) in 5 mL of distilled water in sterilized Petri dishes were used for each test. Measured amounts of stock solution were added to make the dilutions 1.0%. The standard nematicide furadan was used for comparison, and distilled water was used as the control. Each treatment was replicated twice. The Petri dishes were kept at 28 ± 2 °C for 72 h. After 24 h, one Petri dish was taken from each treatment, and the larvae were examined for mortality under a stereoscopic microscope. In the case of nonmobile larvae, their irreversible immobility was confirmed by transferring them to distilled water. The percent mortality was calculated from an average of three replicates. This procedure was repeated after 48 and 72 h.

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