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Triterpenoids, essential oil and photo-oxidative 28 → 13-lactonization of oleanolic acid from *Lantana camara*[☆]

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Abstract

Two novel triterpenoids have been isolated from the roots of *Lantana camara* L.: 3ß,19α dihydroxy ursan-28-oic acid and 21,22ß- epoxy-3ß-hydroxy olean-12-en-28-oic acid in its methyl ester form. Its leaves have yielded an essential oil which is rich in sesquiterpenes. Oleanolic acid, which is thought to be a hepatoprotective compound, was isolated from *L. camara* roots and converted into its 28 → 13ß lactone by a facile photo-oxidation reaction. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lantana camara; Weed; Verbenaceae; Roots; Triterpenoid; Leaves; Essential oil; Terpenes; Photo-oxidation; Oleanolic acid; Hepatoprotective

1. Introduction

Lantana camara Linn. var. *aculeata* (Verbenaceae), commonly known as "*wild sage*", is a problem weed in India and in parts of the world. It spreads rapidly in sunny environments, in India, threatening native plant species. In order to determine whether economically important chemicals are present in *L. camara*, we have studied its composition in detail and have previously reported the presence of putative hepatoprotective oleanolic acid (**1**) in its roots in high concentrations (Misra et al., 1997). We have also isolated pomonic acid (**6**) whose lactonization followed by a rearrangement under acidic condition has recently been reported (Misra and Laatsch, 1999). In order to derivatize **1** whose hepatoprotective activity has been well proven (Liu, 1995), we have applied photo-oxidative lactonization to obtain 28 → 13ß lactone (**9**) in a single step. The isolation of 3ß,19α-dihydroxy ursan-28-oic acid (**7**) and 21,22ß-epoxy-3ß-hydroxy olean-12-en-28-oic acid in its methyl ester form (**8a**) as new compounds from the roots, and the composition of the essential oil from the leaves along with the photo-oxidation reaction on **1** are presented in this paper.

2. Results and discussion

While the aerial parts of *L. camara* have previously been found to possess several triterpenoids (Siddiqui et al., 1995; Sharma and Sharma, 1989; Hart et al., 1976), the roots have not been extensively searched for biologically active substances. In our present investigation, *L. camara* roots were found to contain oleanolic acid (**1**), oleanolic acid acetate (**2**), oleanonic acid (**3**), lantadene A (**4**), camaric acid (**5**), ß-sitosterol and its glucoside, pomonic acid (**6**) along with two new and several unidentified complex mixture of triterpenoids. The identification of the structures of the new compounds (**7**, **8a**) was achieved by spectral methods.

The ^{1}H-NMR spectrum of **7** showed a double doub-

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let at δ 3.20 ($J = 10.5$, 5.0 Hz) for H-3, a broad singlet at δ 3.00 for H-18 which clearly indicated that C-19 is tetrasubstituted. The absence of a signal due to vinylic proton clearly indicated that the usual double bond at C12–C13, typical for a pentacyclic triterpenoid, is lacking. Also, the presence of a doublet ($J = 7.0$ Hz) for the methyl group at δ 1.08 further supported that the molecule is of the ursane series. When treated with diazomethane, **7** gave **7a** whose EIMS showed $[M]^+$ at m/z 486 and CIMS (NH_3) showed $[M]^+$ at m/z 504, indicating the molecular formula for **7a** as $C_{31}H_{50}O_4$. Further, the ^{1}H-NMR of **7a** showed an additional singlet at δ 3.54 for OMe, in addition to H-3 double doublet ($J = 10.5$, 5.0 Hz) at δ 3.11 and H-18 singlet at δ 2.88. These along with other data further supported that **7** is 12,13-dihydro ursolic acid derivative containing a tertiary hydroxyl group. Its ^{13}C-NMR (APT) showed 7 Me, 10 CH_2, 6 CH, and 7 C, confirming the absence of any double bond in the ursane skeleton. The remaining signals were in agreement with the proposed structure; however, the relative downfield shift of C-19 at δ 87.54 in its ^{13}C-NMR could be explained due to the absence of the C12–C13 double bond. Complete ^{13}C-NMR (Table 1) and ^{1}H-NMR (Section 3), by employing ^{1}H–^{1}H COSY, HMQC, APT and comparison with the literature (Misra and Laatsch, 1999; Misra and Ahmad, 1997; Mahato and Kundu, 1994) clearly established that **7** is 3ß,19α-dihydroxy ursan-28-oic acid (12,13-dihydropomolic acid).

Table 1
^{13}C-NMR spectral data of compounds **7**, **8a** and **9** (75 MHz, δ, $CDCl_3$)

C	**7**	**8a**	**9**
1	38.10	35.22	38.90
2	26.95	26.79	27.12
3	78.77	78.96	78.81
4	38.82	38.19	38.21
5	54.53	54.55	54.71
6	17.54	18.77	17.62
7	34.25	35.22	34.34
8	41.25	38.85	41.36
9	52.72	51.94	53.16
10	36.42	33.79	37.29
11	21.22	23.80	135.85
12	31.42	117.94	126.88
13	50.65	143.00	89.83
14	40.48	41.49	41.56
15	29.68	31.00	25.36
16	26.66	29.09	26.97
17	43.84	50.97	44.02
18	57.06	40.06	50.50
19	87.54	37.28	36.31
20	49.56	36.55	31.11
21	31.05	58.62	33.27
22	37.72	53.52	31.43
23	27.74	27.28	27.73
24	18.82	16.93	14.90
25	15.07	15.49	14.90
26	17.16	20.48	17.62
27	33.17	29.68	18.96
28	179.40	178.12	180.02
29	23.57	32.26	23.54
30	20.02	27.84	23.54
OMe	–	51.84	–

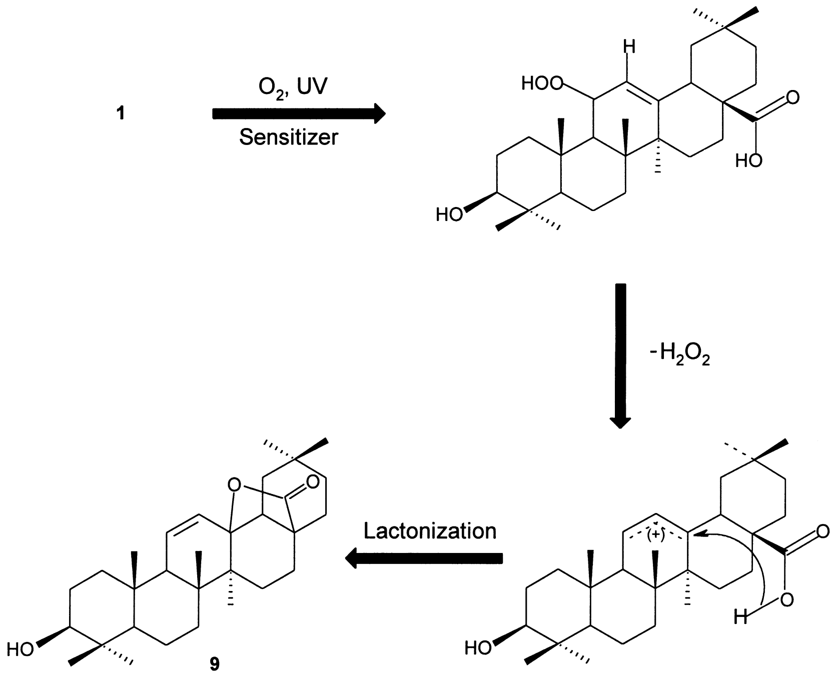
On the other hand, the ^{1}H-NMR of **8a** showed a double doublet at δ 5.43 ($J = 4.0$, 2.0 Hz) which had correlations with the multiplets at δ 1.26 and 1.80 for H-11 in its ^{1}H–^{1}H COSY. In its ^{13}C-NMR, a CH at δ 117.94 and C at 143.0 further supported that the double bond is at the typical position at C12–C13. A doublet ($J = 4.0$ Hz) at δ 2.95 and a doublet doublet ($J = 4.0$, 2.0 Hz) at δ 3.10 suggested the presence of an epoxide ring which was supported by the corresponding (HMQC) signals in ^{13}C-NMR at δ 58.62 and 53.52. In the ^{1}H–^{1}H COSY, these epoxide signals showed corelations with each other, and the double doublet at δ 3.10 also showed correlation with the H-18. These observations were clearly indicative of the epoxide ring at C21–C22 with ß-orientation of epoxide, because 22-epoxide H showed a strong corelation with the H at C-18. Since the oxygenation at C-22 is common in the oleananes from *L. camara* (viz. lantadenes), the epoxide at C22–C21 is also the favored position. The characteristic ßOH at C-3 gave a double doublet ($J = 10.5$, 5.0 Hz) at δ 3.20 which showed corelations in the ^{1}H–^{1}H COSY with H-2 multiplets at δ 1.60. The EIMS of **8a** showed $[M]^+$ at m/z 484 supported by CIMS at m/z 485 for $[M + 1]^+$. The ^{13}C-NMR (Table 1) and ^{1}H-NMR (Section 3) were completely assigned by using APT, HMQC and ^{1}H–^{1}H COSY techniques and by comparison with the literature (Mahato Kundu, 1994).

The aromatic leaves of *L. camara* contain relatively high concentrations of essential oil and are occasionally put to use in the preparation of "*agarbattis*" for generating scented smoke when burnt during religious prayer in the Indian society. Earlier reports concerning the essential oil composition have primarily reported physico-chemical properties without complete information concerning the constituents (Saleh, 1974; Peyron et al., 1971; Singh et al., 1991). Recently, the Brazilian lantana oil has been investigated in detail wherein a comparative study of the major components of Indian lantana oil has also been discussed briefly (Weyerstahl et al., 1999). Our investigation of the essential oil clearly shows that the oil is rich in sesquiterpenes which may be suitable for blending purposes in order to enhance the longevity of the perfumery products. Although none of the components exceeded 10% of the oil composition, the following seven sesquiterpenes constitute about half of the total percentage of the oil: γ-cadinene, α-selinene, ß-gurjunene, eudesma-3,11-diene, δ-cadinene and isolongifolene. Monoterpenes accounted for only 10% of the oil. Because of the high concentration of sesquiterpene hydrocarbons (65%) and sesquiterpene alcohol (20%), the essential oil from *L. camara* leaves could prove useful in the perfumery industry. The identification of these compounds were achieved from retention time in GC and their mass spectra (GC–MS) by comparison with the library spectra available to us and those reported in the literature (Swigar and Silverstein, 1981).

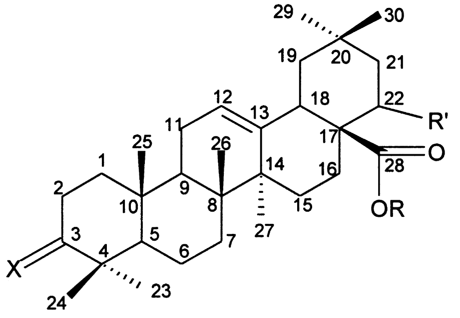
As oleanolic acid (**1**) is otherwise available in many plants, albeit in low concentration, its conversion into the corresponding 28 → 13 lactone (**9**) has earlier been reported by bromination and dehydrobromination reaction (Pereda-Miranda and Delgado, 1990). We have now converted **1** into **9** by a facile photo-oxidation reaction in a single step with 40% yield. The lactone **9** retains a double bond next to the lactone ring at the 11,12 position which may facilitate attack by bionucleophiles. The structure of the reaction product (**9**) was confirmed from its spectral data. Its ^{1}H-NMR showed a double doublet ($J = 5.0$, 10.5 Hz) at δ 3.23 for H-3 and a double doublet ($J = 10.0$, 1.5 Hz) at δ 6.02 along with another double doublet ($J = 10.0$, 3.0 Hz) at δ 5.40 for a CH=CH system. The absence of further downfield signals clearly indicated that after 28 → 13 lactonization the C12–C13 double bond has shifted to C11–C12 position. A general mechanism to rationalize the formation of **10** is given in Scheme 1. However, it is also possible that a photochemical "ene-reaction" has led directly to the 13-hydroxy compound to facilitate the lactonization. The presence of a disubstituted double bond was confirmed by the ^{13}C-NMR which contained doublets at δ 135.85 and 126.88 for C-11 and C-12, respectively. The singlet at δ 89.83 for C-13 also supports the conclusion that lactonization has occurred at C-13. The doublet of C12 at δ 6.02 is slightly downfield as compared to the chemical shift of a normal vinylic proton and could be explained due to the effect of the lactone ring at C13. The remainder of the ^{13}C-NMR (Table 1) and ^{1}H-NMR (Section 3) data were assigned by APT, HMQC, ^{1}H–^{1}H COSY and were comparable to the data reported in the literature (Bellavista et al., 1974; Pereda-Miranda and Delgado, 1990; Bennett et al., 1992). The EIMS also gave base peak at *m*/*z* 454 for $C_{30}H_{46}O_3$, thereby further substantiating the structure of the reaction product.

3. Experimental

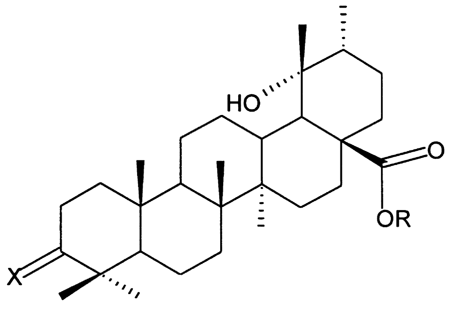
The collection of the roots of *L. camara* and extraction and isolation procedure of **1**, **3**, **4**, **5**, ß-sitosterol and its glucoside, as well as **6** have already been



Scheme 1. Possible mechanism of photo-oxidative lactonization of oleanolic acid (**1**).



1, R= H, X= H, ßOH, R'= H
1a, R= Me, X= H, ßOH, R'= H
2, R= H, X= H, ßOAc, R'= H
2a, R= Me, X= H, ßOAc, R'= H
3, R= H, X= O, R'= H
3a, R= Me, X= O, R'= H
4, R= H, X= O, R'= ß-angeloyloxy
5, R= H, X= ßOH, 3,25-oxy, R'= ß-angeloyloxy
8, R= H, X= H, ßOH, R'= 22,21ßoxy
8a, R= Me, X= H, ßOH, R'= 22,21ßoxy



6, R= H, X= O, delta 12,13
6a, R= Me, X= O, delta 12,13
7, R= H, X= H, ßOH
7a, R= Me, X= H, ßOH

reported (Misra et al., 1997; Misra and Laatsch, 1999). The sixth fraction of the first column after crystallization gave solid crystals containing mainly **1**, whereas the mother liquor after flash CC over Si gel gave **7** (12 mg). A small part of the sixth fraction was treated with diazomethane, and the methyl esters thus produced, after flash CC over Si gel and cyclohexane–EtOAc (4:1), afforded 10 fractions. Fraction 8, which was still a complex mixture after TLC on Si gel (cyclohexane–Et_2O, 4:1) afforded **2a** (30 mg, R_f 0.82), **1a** (250 mg, R_f 0.65), **8a** (15 mg, R_f 0.40) and **7a** (10 mg, 0.25).

3.1. Extraction of essential oil

The leaves (2 kg) of *L. camara* (orange reddish flower type) were procured from the Kukrail forest, Lucknow, India in October 1993. After hydro-distillation, an oil (1.2 ml) was obtained; d^{25} 0.889, n_D^{25} 1.4955, $\alpha_D^{25}-6.3°$. GC was performed on a Perkin–Elmer 3920 B Gas Chromatograph equipped with a 25 m × 0.23 mm Carbowax column under following conditions: from 80°C (5 min) to 200°C (15 min) at 4°/

Table 2
Percentage composition of the essential oil from *L. camara* leaves[a]

S. No.	Component	Identified by	% in total oil
1.	α-Pinene	a, b	3.02
2.	Sabinene	a, b	4.51
3.	α-Phellandrene	a, b	3.52
4.	Thujyl alcohol	a, b	0.66
5.	u.i.	–	t
6.	u.i.	–	t
7.	α-Terpineol	a, b	0.66
8.	u.i.	–	t
9.	Bicyclogermacrene	b	2.46
10.	Germacrene-D	b	3.96
11.	u.i.	–	t
12.	γ-Gurjunene	b	2.23
13.	γ-Cadinene	b	5.59
14.	α-Selinene	b	8.11
15.	ß-Gurjunene	b	6.73
16.	Eudesma-3,11-diene	b	7.12
17.	δ-Cadinene	b	6.47
18.	Isolongifolene	b	7.79
19.	δ-Selinene	b	5.75
20.	Guaiene	b	4.15
21.	u.i.	–	t
22.	u.i.	–	t
23.	u.i.	–	t
24.	α-Elemene	b	1.09
25.	Bisabolene	b	1.62
26.	S.H., $[M]^+$ 204	b	1.31
27.	S.A., $[M]^+$ 222	b	1.30
28.	S.A., $[M]^+$ 220	b	1.77
29.	S.A., $[M]^+$ 222	b	0.66
30.	S.A., $[M]^+$ 222	b	4.03
31.	S.A., $[M]^+$ 222	b	1.02
32.	S.A., $[M]^+$ 218	b	2.31
33.	S.A., $[M]^+$ 222	b	1.21
34.	S.A., $[M]^+$ 220	b	1.88
35.	S.A., $[M]^+$ 222	b	1.74
36.	S.A., $[M]^+$ 222	b	2.22
37.	S.A., $[M]^+$ 222	b	1.54
38.	u.i.	–	t
39.	S.A., $[M]^+$ 220	b	0.46
40.	u.i.	–	t
41.	u.i.	–	t
42.	S.A., $[M]^+$ 220	b	0.22

[a] a = Retention time, b = MS Fragmentation, S.H. = sesquiterpene hydrocarbon, S.A. = sesquiterpene alcohol, t = trace, u.i. = unidentified.

min. Similarly, GC–MS was done on a Kratos MS 80 RFA coupled directly with GC with a 25 m × 0.23 mm Cp sil 19CB column under the same conditions as given in the case of GC. The results of the analysis of the oil are given in Table 2.

3.2. Lactonization of *1*

In a Rayonet Photoreactor, **1** (50 mg) was dissolved in iso-butanol. The oxygen was bubbled at the rate of 2–3 bubbles/s under 254 nm UV light with constant stirring at 38°C for 3 days, using rose bengal as a photosensitizer. The reaction was monitored using TLC and additional iso-butanol was added as required. The product, after removal of the solvent, was chromatographed using a flash column (Si gel, cyclohexane–EtOAc, 4:1) to afford several fractions. Fraction 5 after TLC on Si gel with cyclohexane–Et_2O, 4:1 gave **9** (20 mg, R_f 0.55).

3.2.1. 3ß,19α-Dihydroxy-ursan-28-oic acid (7)

Viscous mass, $[\alpha]_D^{30}$ +22.6° (MeOH: *c* 0.06). $IR_{max}^{CHCl_3}$ cm^{-1}: 3600–3100 (OH, COOH), 1715 (COOH), 1380, 1250; CIMS of **7a** *m/z* (rel. int.): 504 $[M + NH_4]^+$ (79). EIMS of **7a** *m/z* (rel. int.): 486 $[M]^+$ (63), 471 $[M - Me]^+$ (100), 411 (42), 278 (40). ^{1}H-NMR (δ $CDCl_3$): 1.90, 1.30, *m* (H-1), 1.64*, *m* (H-2), 3.20, *dd* (*J* = 10.5, 5.0 Hz) (H-3), 0.70, *m* (H-5), 1.47, 1.57, *m* (H-6), 1.34, 1.28, *m* (H-7), 1.82, *m* (H-9), 1.29, 2.10, *m* (H-11), 1.12, 1.24, *m* [(H-12), 1.52, *m* (H-13), 1.22*, *m* (H-15), 1.64, 1.03, *m* (H-16), 2.88, *s br*, (H-18), 2.29, *m* (H-20), 1.12, 1.24, *m* (H-21), 1.58, 1.81, *m* (H-22), 0.96, *s* (H-23), 0.89, *s* (H-24), 0.76, *s* (H-25), 1.00, *s* (H-26), 0.96, *s* (H-27), 1.23, *s* (H-29) and 1.03, *s* (H-30). *α and ßH have fallen together. ^{13}C-NMR: Table 1.

3.2.2. 21,22ß-Epoxy-ß-hydroxy olean-12-en-28-oic methyl ester (*8a*)

Viscous mass, $[\alpha]_D^{30}$ +6.2° (MeOH: *c* 0.02). IR_{max}^{KBr} cm^{-1}: 3600–3100 (OH, COOMe), 1710 (COOMe), 1645 (C=C), 1385, 1250, 1180. CIMS *m/z* (rel. int.): 485 $[M + 1]^+$ (90). EIMS *m/z* (rel. int.): 484 $[M]^+$ (100), 469 $[M - Me]^+$ (22), 466 $[M - H_2O]^+$ (30), 276 (18), 235 (50), 262 (28), 208 (18). ^{1}H-NMR (δ $CDCl_3$): 1.20, 1.40, *m* (H-1), 1.60*, *m* (H-2), 3.20, *dd* (*J* = 10.5, 5.0 Hz) (H-3), 0.70, *m* (H-5), 1.54, 1.60, *m* (H-6), 1.60, 1.40, *m* (H-7), 1.45, *m* (H-9), 1.26, 1.80, *m* (H-11), 5.43, *dd* (*J* = 2.0, 4.0 Hz) (H-12), 1.20, 1.62, *m* (H-15), 1.90*, *m* (H-16), 2.63 *dd br* (*J* = 10.0, 3.0 Hz) (H-18), 1.98, 1.50, *m* (H-19), 2.95, *d* (*J* = 4.0 Hz) (H-21), 3.10, *dd* (*J* = 4.0, 2.0 Hz) (H-22), 0.98, *s* (H-23), 0.99 *s* (H-24), 0.80, *s* (H-25), 0.83, *s* (H-26), 1.22, *s* (H-27), 1.03, *s* (H-29), 0.91, *s* (H-30), 3.57, *s* (OMe). * α and ßH have fallen together. ^{13}C-NMR: Table 1.

3.2.3. 3ß-Hydroxy olean-11-en-28 → 13 oate (*9*)

Viscous mass, EIMS *m/z* (rel. int.): 454 $[M]^+$ (100), 439 $[M - Me]^+$ (4), 410 $[M - CO_2]^+$ (48). ^{1}H-NMR (δ $CDCl_3$): 0.94*, *m* (H-1), 1.62*, *m* (H-2), 3.23, *dd* (*J* = 10.5, 5.0 Hz) (H-3), 0.72, *m* (H-5), 1.54, 1.60, *m* (H-6), 1.92, 1.16, *m* (H-7), 1.90, *d* (*J* = 2.0 Hz) (H-9), 5.40, *dd* (*J* = 10.0, 3.0 Hz) (H-11), 6.02, *dd* (*J* = 10.0, 1.5 Hz) (H-12), 1.30, 1.36, *m* (H-15), 1.62∗, *m* (H-16), 1.86, *dd* (*J* = 10.0, 3.0 Hz) (H-18), 1.60, 1.82, *m* (H-19), 0.96, 0.88, *m* (H-21), 1.78, 1.35, *m* (H-22), 0.98, *s* (H-23), 0.90, *s* (H-24), 0.78, *s* (H-25), 1.03, *s* (H-26), 0.90, *s* (H-27), 1.03, *s* (H-29), 0.86, *s* (H-30). *α and ßH have fallen together. ^{13}C-NMR: Table 1.

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