# Three New Pentacyclic Triterpenoids from *Lantana camara*

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Three new pentacyclic triterpenes ursoxy acid (**1**), methyl ursoxylate (**2**), and ursangilic acid (**3**), along with three known compounds dotriacontanoic acid, oleanolic acid acetate, and tetracosanoic acid, were isolated from the aerial parts of *Lantana camara* LINN. Structures of the new compounds were elucidated by chemical transformation and spectral studies including 1D ($^1$H- and $^{13}$C-NMR) and 2D (COSY-45, NOESY, *J*-resolved, HMQC, and HMBC) NMR spectroscopy.

**1. Introduction.** – *Lantana camara* L. (Verbenaceae), commonly known as lantana, is a hairy shrub, native to tropical America and cultivated as an ornamental or hedge plant. Its different parts are reputed in traditional medicine for the treatment of various human ailments such as ulcers, eczema eruptions, malaria, and rheumatism [1]. 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 [1][2]. Phytochemical studies undertaken by different research groups on different parts of the plant have resulted in the isolation of various steroids, terpenoids, and flavonoids [3–5]. In view of the reputed pharmacological properties, the present studies were undertaken on the chemical constituents of the aerial parts of *L. camara* and have resulted in the isolation and structure elucidation of three new pentacyclic triterpenoids, ursoxy acid (**1**), methyl ursoxylate (**2**), and ursangilic acid (**3**), and three known compounds dotriacontanoic acid [6], oleanolic acid acetate [7], and tetracosanoic acid [8].

**2. Results and Discussion.** – The $M^+$ ion of compound **1** was observed at *m*/*z* 484 and 484.3541 in the EI-MS and HR-EI-MS, respectively, leading to the molecular formula as $C_{31}H_{48}O_4$. Its IR spectrum showed absorption bands at 3450–2610 (br., COOH), 2925, 2850 (CH), 1700 (acid C=O), 1620 (C=C), and 1120 cm$^{-1}$ (C–O), and the UV spectrum exhibited an absorption maximum at 204 nm, indicating the lack of conjugation. The $^1$H-NMR spectrum of **1** (*Exper. Part*) showed six Me signals, four as *singlets* at 0.73, 0.94, 0.99, and 1.08 ppm, and two as *doublets* at 0.88 ($J = 6.4$ Hz) and 0.91 ppm ($J = 6.5$ Hz) assigned to Me(29) and Me(30), respectively. The $^1$H-NMR spectrum also showed resonance for an olefinic H-atom at 5.24 ppm (*t*, $J = 3.5$ Hz, H–C(12)) and a characteristic [9] CH H-atom at 2.19 ppm (*d*, $J = 11.2$ Hz, H–C(18)). These data along with the characteristic $^{13}$C-NMR chemical shifts of C(12) and C(13) at 126.0 and 138.0 ppm (*Table*), respectively [9], indicated that **1** belongs to the $\Delta^{12}$-$\alpha$-

amyrin series of pentacyclic triterpenoids. The $^1$H-NMR spectrum further exhibited two 1-H *dd*s at 4.26 ($J = 8.5$, 2.9 Hz) and 3.88 ppm ($J = 8.5$, 1.1 Hz) due to two nonequivalent $CH_2$ H-atoms ($\delta$(C) 67.9; DEPT; HMQC), which were assigned to $H_a-C(25)$ and $H_b-C(25)$, respectively, indicating that the compound possesses a 3,25-epoxy function [10]. Connectivities of $H_a-C(25)$ with C(1), and $H_b-C(25)$ with C(1), C(3) and C(5) in the HMBC spectrum supported this assignment. A MeO group, the signal of which appeared at 3.24 ppm as a 3-H *singlet* in the $^1$H-NMR spectrum ($\delta$(C) 49.4; DEPT; HMQC) was located at C(3) with $\alpha$-dispositon in analogy with compounds having similar structures [11]. The interaction of this MeO, $H_a-C(25)$, and $H_b-C(25)$ with quaternary the C-atom C(3) ($\delta$(C) 100.5 ppm; br. band) in the HMBC spectrum confirmed these functionalities of ring A. A fragment ion at *m/z* 248.1788 (*Fig. 1*) appeared in the mass spectrum of **1**, resulting from *retro-Diels-Alder* fragmentation [12]. It also formed methyl ester ($\delta$(OMe) 3.64 ppm) on treatment with $CH_2N_2$. These observations were indicative of a COOH group (IR: 3450–2610, 1700 cm$^{-1}$) at C(14) or C(17) [12] of **1**. Comparison of $^{13}$C-NMR chemical-shift data of rings D and E with the published values of the compounds having similar structures [13] and connectivities of H–C(18) with C(17) and C(28) in the HMBC spectrum confirmed its position at C(17). On the basis of the above data, the structure of **1** was assigned as *3,25-epoxy-3α-methoxyurs-12-en-28-oic acid*.

Compound **2** had a molecular formula of $C_{32}H_{50}O_4$ (EI-MS; HR-EI-MS). Its IR spectrum showed absorption bands at 2920, 2850 (CH), 1730 (ester C=O), and 1620 cm$^{-1}$ (C=C), and the UV spectrum displayed an absorption at 205 nm. Its $^1$H- and $^{13}$C-NMR spectra (*Exper. Part* and the *Table*) indicated that **2** also belongs to $\Delta^{12}$-$\alpha$-

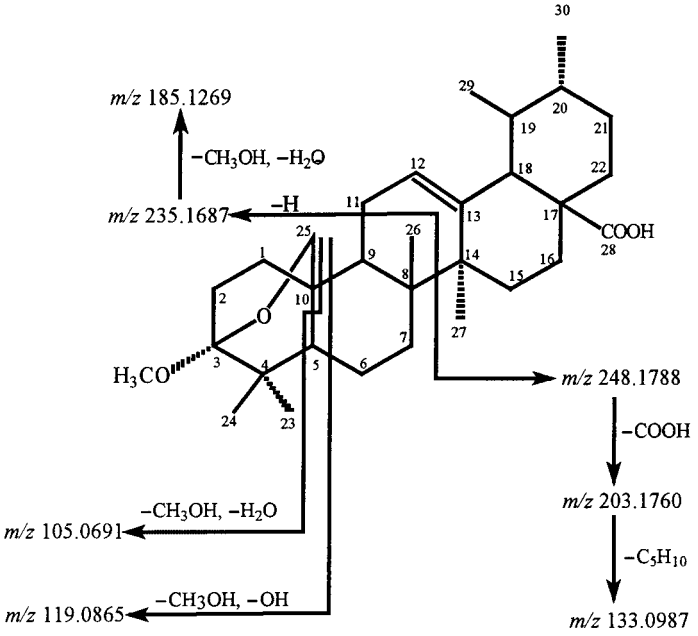


Fig. 1. *Mass fragments of* **1**

Table. *$^{13}$C-NMR Data* ($CDCl_3$) *of Triterpenes* **1**–**3**

| | δ(C) | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| C(1) | 35.0 | 35.1 | 35.0 |
| C(2) | 27.8 | 27.7 | 27.7 |
| C(3) | 100.5 | 100.4 | 100.4 |
| C(4) | 38.8 | 38.3 | 38.5 |
| C(5) | 50.5 | 50.9 | 50.9 |
| C(6) | 19.5 | 19.6 | 19.6 |
| C(7) | 31.2 | 31.4 | 31.4 |
| C(8) | 40.8 | 40.7 | 40.7 |
| C(9) | 42.0 | 41.8 | 41.9 |
| C(10) | 34.7 | 34.7 | 34.6 |
| C(11) | 23.8 | 23.7 | 23.4 |
| C(12) | 126.0 | 125.9 | 126.0 |
| C(13) | 138.0 | 138.2 | 137.2 |
| C(14) | 42.1 | 41.9 | 42.1 |
| C(15) | 29.7 | 29.7 | 29.7 |
| C(16) | 24.1 | 24.2 | 24.2 |
| C(17) | 48.2 | 48.2 | 51.5 |
| C(18) | 53.0 | 53.2 | 49.3 |
| C(19) | 39.1 | 39.1 | 39.2 |
| C(20) | 38.9 | 38.8 | 38.7 |
| C(21) | 30.6 | 30.7 | 34.7 |
| C(22) | 36.8 | 36.6 | 75.8 |
| C(23) | 27.3 | 27.3 | 27.3 |
| C(24) | 16.9 | 17.1 | 16.9 |
| C(25) | 67.9 | 67.8 | 67.8 |
| C(26) | 18.3 | 18.4 | 18.4 |
| C(27) | 23.5 | 23.2 | 23.1 |
| C(28) | 180.6 | 179.1 | 180.1 |
| C(29) | 17.0 | 16.9 | 17.7 |
| C(30) | 21.2 | 21.1 | 21.0 |
| C(1′) | – | – | 166.4 |
| C(2′) | – | – | 128.0 |
| C(3′) | – | – | 138.6 |
| C(4′) | – | – | 15.8[a]) |
| C(5′) | – | – | 20.4 |
| C(1″) | – | – | 64.8 |
| C(2″) | – | – | 15.6[a]) |
| MeO–C(3) | 49.4 | 49.3 | – |
| MeO–C(28) | – | 51.5 | – |

[a]) Values may be interchanged.

amyrin series of triterpenoids with same A and B rings as in compound **1**. The mass spectrum showed a fragment at 262.1557 ($C_{17}H_{26}O_2$) resulting from *retro-Diels-Alder* fragmentation (*Fig. 2*), which indicated the presence of a methyl ester function (IR: 1730 $cm^{-1}$; MeO δ(H) 3.65; δ(C); 51.5; HMQC) at C(14) or C(17) [12]. It was placed at C(17) on the basis of comparison of $^{13}$C-NMR chemical-shift values of ring D and E with compounds having COOMe at this position [9][13]. These observations led to the elucidation of the structure of **2** as *methyl 3,25-epoxy-3α-methoxyurs-12-en-28-oate*,

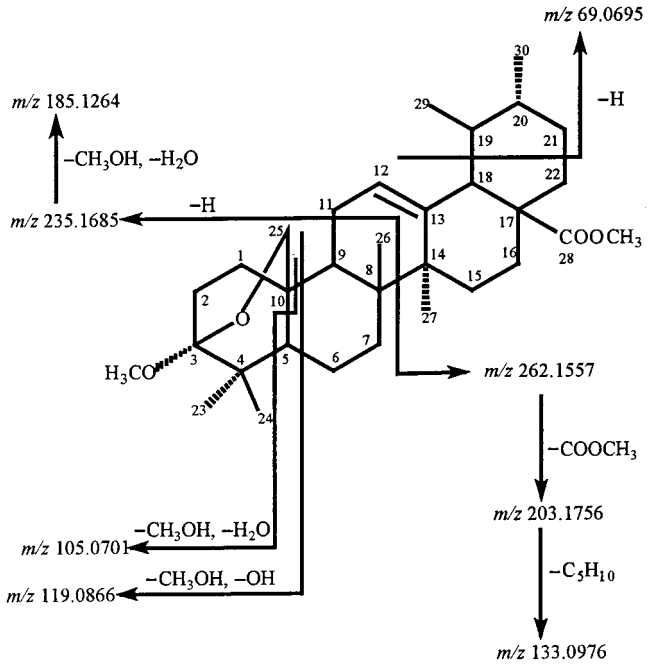


Fig. 2. *Mass fragments of* **2**

which was confirmed by various $^1$H, $^1$H-COSY, HMQC, and HMBC connectivities. Compound **2** is a new natural product; however, its synthesis from lantic acid has been reported earlier [14]. It is also comparable with the methyl ester prepared from **1**.

Compound **3** exhibited $M^+$ peak at *m/z* 596 and 596.4068 in the EI-MS and HR-EI-MS, respectively, corresponding to the molecular formula $C_{37}H_{56}O_6$. It showed IR absorption bands at 3428–2650 (br., COOH), 2920, 2855 (CH), 1730 (ester C=O), 1710 (acid C=O), 1635 (C=C), and 1150 cm$^{-1}$ (C–O), and UV absorption maximum at 217 nm. The $^1$H- and $^{13}$C-NMR spectra (*Exper. Part* and *Table*) of **3** shows resemblance to those of **1** in that **3** also possesses a 3,25-epoxy function, a C=C bond at C(12), and a COO group at C(17) in the ursane skeleton. Further, its $^1$H-NMR spectrum showed a 2,3-dimethylbut-2-enyloxy function with (*Z*)-configuration by the presence of a 3-H *quintet* at 1.78 ppm ($J = 1.5$ Hz, Me(5′)), a 3-H *doublet* of *quartets* at 1.94 ppm ($J = 7.0$, 1.5 Hz, Me(4′)), and a 1-H *quadruplet* of *quadruplets* at 6.00 ppm ($J = 7.0$, 1.5 Hz, H–C(3′)) [15]. This was supported by the $^{13}$C-NMR chemical shifts of the ester C-atoms (*Table*) and the HR-EI-MS fragments at *m/z* 496.3840 ($C_{32}H_{48}O_4$, $[M-100]^+$) and 83.0422 ($C_5H_7O$). This ester functionality was placed at C(22) with $\beta$-orientation due to the appearance of a 1-H *triplet* at 5.06 ppm with $J = 3.5$ Hz along with a quaternary C-atom signal at 75.8 ppm in the DEPT spectrum [11] and their HMQC connectivity. This assignment was confirmed by the relatively downfield resonance of H–C(18) at 2.43 instead of *ca.* 2.2 ppm [9]. A fragment ion at *m/z* 246.1610 (*Fig. 3*) was also present in the mass spectrum of **3**, resulting from *retro-Diels-Alder* fragmentation and loss of 2,3-dimethylbut-2-enoic acid. The $^1$H-NMR spectrum of **3** further showed

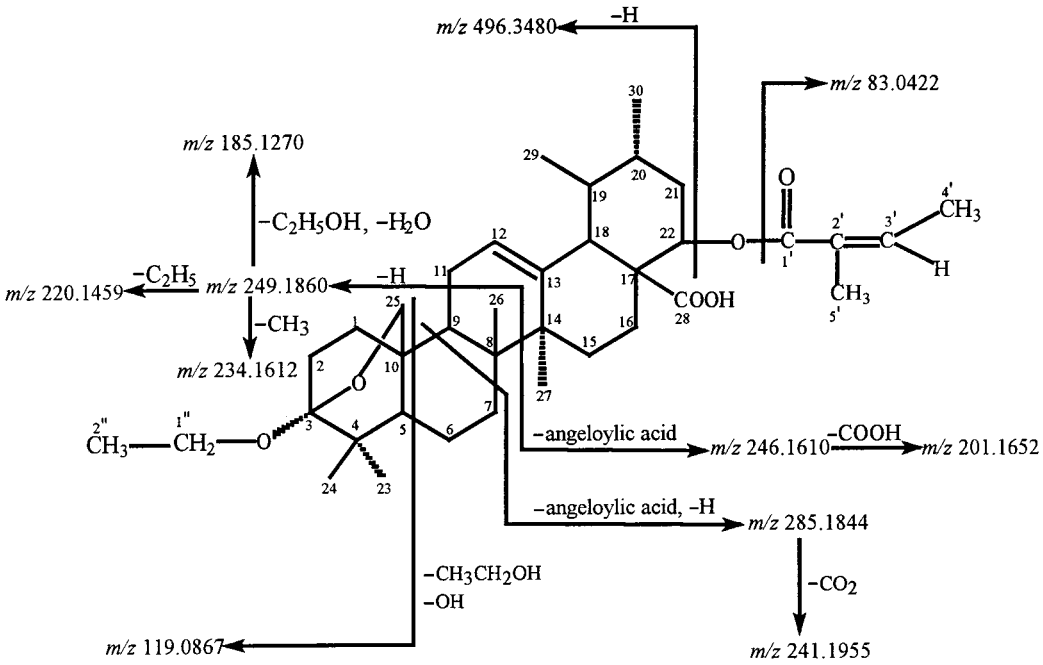


Fig. 3. *Mass fragments of* **3**

the presence of an EtO group (3.70 ppm (*q*, $J = 7.0$ Hz, 2 H–C(1″))) and (1.10 ppm (*t*, $J = 7.0$ Hz, Me(2″))), which was confirmed by $^{13}$C-NMR chemical shifts (*Table*) and HMQC connectivities. It was located at C(3), since the compound had no further signal of a quaternary C-atom in the $^{1}$H-NMR spectrum and was supported by fragments at *m*/*z* 249.1860, 220.1459, and 185.1270 in the mass spectrum (*Fig. 3*) and by interaction of H–C(1″) with C(3) at 100.4 ppm in the HMBC spectrum. In the light of these observations, the structure of **3** has been established as *3,25-epoxy-3α-ethoxy-22β-[(Z)-2′-methylbut-2-enyloxy]urs-12-en-28-oic acid*.

**Experimental Part**

*General*. Vacuum-liquid chromatography (VLC): silica gel *60PF$_{254}$* (*Merck*). Flash column chromatography (FCC): *Eyela Flash Column EF-10* chromatograph; silica gel *9385* (*Merck*, 0.040–0.063 mm). Prep. TLC: silica gel *60PF$_{254}$* (*Merck*); detection with $I_2$ spray. UV Spectra: *Hitachi U-3200* spectrophotometer; $\lambda_{max}$ in nm. IR Spectra: *Jasco A-302* spectrophotometer; $\tilde{\nu}$ in cm$^{-1}$. $^{1}$H-NMR (COSY, NOESY, and *J*-resolved): *Bruker* spectrometers, at 300 and 500 MHz; chemical shifts $\delta$ in ppm. rel. to $Me_4Si$ as internal standard; coupling constants *J* in Hz. $^{13}$C-NMR: *Bruker* spectrometer, at 75 and 125 MHz. EI-MS: *Finnigan-MAT 311A* mass spectrometer; source at 250° and 70 eV; *m*/*z* (rel. %). HR-EI-MS: *Jeol JMS-HX-110* mass spectrometer; EI, source at 250° and 70 eV; *m*/*z* (rel. %).

*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.

*Extraction and Isolation*. Air-dried aerial parts of *L. camara* (10 kg) were repeatedly extracted with MeOH at r.t. The concentrated extract, obtained on removal of the solvent from the combined extract under reduced pressure, was partitioned between AcOEt and $H_2O$. The AcOEt phase was treated with 4% aq. $Na_2CO_3$ soln. to

separate the acidic from the neutral fraction. The AcOEt layer containing the neutral fraction was washed with $H_2O$, dried ($Na_2SO_4$), and passed through active charcoal. The charcoal bed was successively washed with AcOEt and MeOH/benzene 1:1, which were combined on the basis of TLC. The residue obtained on removal of the solvent from the charcoal filtrate and washings was divided into petroleum ether-soluble and petroleum ether-insoluble fractions. The petroleum ether-insoluble fraction was again divided into $Et_2O$-soluble and $Et_2O$-insoluble portions. The $Et_2O$-insoluble fraction was further divided into AcOEt-soluble and AcOEt-insoluble portions. The residue (40 g) obtained from the AcOEt-soluble portion was subjected to VLC ($CHCl_3 \rightarrow CHCl_3$/MeOH $\rightarrow$ MeOH): *Fr. 1 – Fr. 9. Fr. 1* (26.0 g; eluted with $CHCl_3 \rightarrow CHCl_3$/MeOH 9.9:0.1) was resubmitted to VLC (petroleum ether $\rightarrow$ petroleum ether/AcOEt $\rightarrow$ AcOEt): *Fr. 1 – Fr. VIII. Fr. 1* (eluted with petroleum ether/AcOEt 9:1) afforded 16.5 mg of dotriacontanoic acid [8]. The mother liquor of *Fr. 1* (200 mg) was subjected to FCC (petroleum ether $\rightarrow$ petroleum ether/AcOEt $\rightarrow$ AcOEt): *Fr. I-1 – Fr. I-9. Fr. I-1* (eluted with 100% petroleum ether), gave two major spots on TLC, which, on purification by TLC ($CHCl_3$/MeOH 9.5:0.5), gave 32.9 mg of oleanolic acid acetate [10] and 27.9 mg of tetracosanoic acid [11].

*Fr. II* (2.6 g; eluted with petroleum ether/AcOEt 8:2) was further subjected to FCC (petroleum ether $\rightarrow$ petroleum ether/AcOEt $\rightarrow$ AcOEt): *Fr. II-1 – Fr. II-18. Fr. II-9* (eluted with petroleum ether/AcOEt 9:1) afforded 8.0 mg of **2**. *Fr. II-11* (eluted with petroleum ether/AcOEt 9:1) gave two major spots on TLC, which, on separation by TLC ($CHCl_3$/MeOH 9.5:0.5), gave 4.7 mg of **1** and 3.6 mg of **3**.

*Ursoxy Acid* (= *3,25-Epoxy-3α-methoxyurs-12-en-28-oic acid*; **1**). Amorphous powder. UV (MeOH): 204. IR ($CHCl_3$): 3450–2610, 2925, 2850, 1700, 1620, 1120. $^1$H-NMR ($CDCl_3$): 5.24 (*t*, *J* = 3.5, H–C(12)); 4.26 (*dd*, *J* = 8.5, 2.9 $H_a$–C(25)); 3.88 (*dd*, *J* = 8.5, 1.1, $H_b$–C(25)); 3.24 (*s*, MeO–C(3)); 2.19 (*d*, *J* = 11.2, H–C(18)); 1.08 (*s*, Me(27)); 0.99 (*s*, Me(23)); 0.94 (*s*, Me(26)); 0.91 (*d*, *J* = 6.5, Me(30)); 0.88 (*d*, *J* = 6.4, Me(29)); 0.73 (*s*, Me(24)). $^{13}$C-NMR: *Table.* EI-MS: 484 (38, $M^+$), 452 (28), 248 (100), 235 (16), 203 (66), 185 (20), 133 (41), 119 (32), 105 (38), 69 (70). HR-EI-MS: 484.3541 ($M^+$, $C_{31}H_{48}O_4^+$; calc. 484.3552), 452.3289 ($C_{30}H_{44}O_3^+$), 248.1788 ($C_{16}H_{24}O_2^+$), 235.1687 ($C_{15}H_{23}O_2^+$), 203.1760 ($C_{15}H_{23}^+$), 185.1269 ($C_{14}H_{17}^+$), 133.0987 ($C_{10}H_{13}^+$), 119.0851 ($C_9H_{11}^+$), 105.0691 ($C_8H_9^+$).

*Methylation of* **1**. Compound **1** (3 mg) formed the methyl derivative (2.5 mg) on treatment with an ethereal solution of $CH_2N_2$ and the usual workup. $^1$H-NMR ($CDCl_3$): 3.64 (COOMe). EI-MS: 498 ($M^+$).

*Methyl Ursoxylate* (= *Methyl 3,25-Epoxy-3α-methoxyurs-12-en-28-oate*; **2**). Amorphous powder. UV (MeOH): 205. IR ($CHCl_3$): 2920, 2850, 1730, 1620, 1150. $^1$H-NMR ($CDCl_3$): 5.25 (*t*, *J* = 3.4, H–C(12)); 4.25 (*dd*, *J* = 8.6, 3.0, $H_a$–C(25)); 3.85 (*dd*, *J* = 8.6, 1.0, $H_b$–C(25)); 3.65 (*s*, MeO–C(28)); 3.24 (*s*, MeO–C(3)); 2.17 (*d*, *J* = 11.4, H–C(18)); 2.06 (*m*, H–C(11)); 1.06 (*s*, Me(27)); 0.99 (s, Me(23)); 0.94 (*s*, Me(26)); 0.90 (*d*, *J* = 6.0, Me(30)); 0.87 (*d*, *J* = 6.0, Me(29)); 0.73 (*s*, Me(24)). $^{13}$C-NMR: *Table.* EI-MS: 498 (13, $M^+$), 262 (100), 235(19), 203 (87), 185 (15), 133 (38), 119 (32), 105 (17), 69 (25). HR-EI-MS: 498.3665 ($M^+$, $C_{32}H_{50}O_4^+$; calc. 498.3708), 262.1557 ($C_{17}H_{26}O_2^+$), 235.1685 ($C_{15}H_{23}O_2^+$), 203.1756 ($C_{15}H_{23}^+$), 185.1264 ($C_{14}H_{17}^+$), 133.0976 ($C_{10}H_{13}^+$), 119.0866 ($C_9H_{11}^+$), 105.0701 ($C_{10}H_9^+$), 69.0695 ($C_5H_9^+$).

*Ursangilic Acid* (= *3,25-Epoxy-3α-ethoxy-22β-[(Z)-2′-methylbut-2-enyloxy]urs-12-en-28-oic Acid*; **3**). Amorphous powder. UV (MeOH): 217. IR ($CHCl_3$): 3428–2650 br., 2920, 2855, 1730, 1710, 1635, 1150. $^1$H-NMR ($CDCl_3$): 6.00 (*qq*, *J* = 7.0, 1.5, H–C(3′)); 5.38 (*t*, *J* = 3.5, H–C(12)); 5.06 (*t*, *J* = 3.5, H–C(22α)); 4.25 (*dd*, *J* = 8.8, 2.5, $H_a$–C(25)); 3.86 (*dd*, *J* = 8.8, 1.0, $H_b$–C(25)); 3.70 (*q*, *J* = 7.0, H–C(1″)); 2.43 (*d*, *J* = 11.2, H–C(18)); 1.94 (*dq*, *J* = 7.0, 1.5, Me(4′)); 1.78 (*quint.*, *J* = 1.5, Me(5′)); 1.10 (*t*, *J* = 7.0, Me(2″)); 1.05 (*s*, Me(27)); 0.99 (*s*, Me(23)); 0.95 (*s*, Me(26)); 0.91 (*d*, *J* = 6.5, Me(30)); 0.84 (*d*, *J* = 6.0, Me(29)); 0.74 (*s*, Me(24)). $^{13}$C-NMR: *Table.* EI-MS: 596 (21, $M^+$), 496 (32), 452 (29), 285 (54), 249 (18), 246 (19), 241 (92), 234 (8), 220 (7), 201 (45), 185 (16), 119(8), 83 (100). HR-EI-MS: 596.4068 ($M^+$, $C_{37}H_{56}O_6^+$; calc. 596.4076), 496.3480 ($C_{32}H_{48}O_4$, [*M* – angeloylic acid]$^+$), 452.3605 ($C_{31}H_{48}O_2$, [496 – $CO_2$]$^+$), 285.1844 ($C_{19}H_{25}O_2^+$), 249.1860 ($C_{16}H_{25}O_2^+$), 246.1610 ($C_{16}H_{22}O_2^+$), 241.1955 ($C_{18}H_{25}^+$), 234. 1612 ($C_{15}H_{22}O_2^+$), 220.1459 ($C_{14}H_{20}O_2^+$), 201.1652 ($C_{15}H_{21}^+$), 185.1270 ($C_{14}H_{17}^+$), 119.0867 ($C_9H_{11}^+$), 83.0422 ($C_5H_7O^+$).

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