Two New Leucosesterterpenes from *Leucosceptrum canum*

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Two new sesterterpenes, 14β-methylleucosesterterpenone (**1**) and 17α-hydroxyleucosceptrine (**2**), along with a known triterpene, hypoglauterpenic acid (**6**), were isolated from the *Leucosceptrum canum* of Nepalese origin. The structures were elucidated on the basis of spectroscopy techniques.

Key words: Leucosceptrum canum, Sesterterpenes, Leucosesterterpenes, 14β-Methylleucosesterterpenone, 17α-Hydroxyleucosceptrine

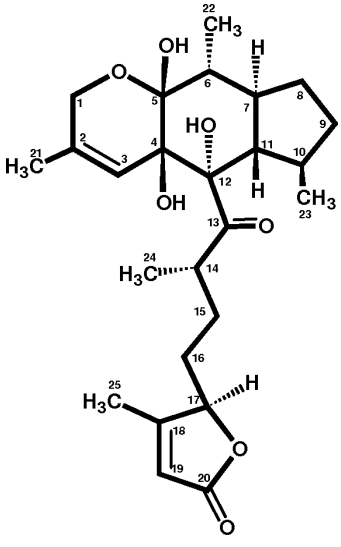
Introduction

Leucoscepturm canum Sm., a small tree, is distributed in the temperate Himalayans regions, Myanmar, and China. The plant is locally known as Bhusure and used as an insecticidal agent in remote areas of Nepal [1, 2]. Our previous study on this plant has yielded three novel sesterterpenes, leucosceptrine (**3**) [3], leucosesterterpenone (**4**), and leucosesterlactone (**5**) [4]. Further phytochemical investigation on this plant lead to the isolation of two more sesterterpenes, **1** and **2**, new members of the leucosesterterpene series, along with a known triterpene, hypoglauterpenic acid (**6**) [5].

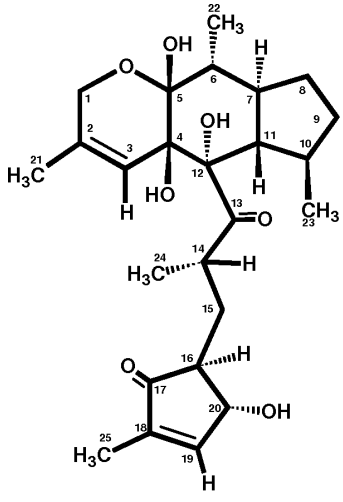
Results and Discussion

The aerial parts of *L. canum* were extracted successively with hexane, $CHCl_3$, EtOAc, and MeOH. The defatted hexane extract was subjected to repeated silica gel column chromatography, which yielded two new leucosesterterpenes **1** and **2**, and a known triterpene **6**.

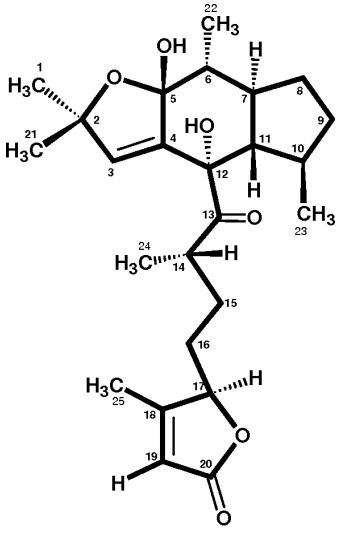
14β-Methylleucosesterterpenone (**1**) was obtained as colorless crystals. The FAB MS of compound **1** showed the $[M–H]^-$ anion at *m/z* = 447 ($C_{25}H_{36}O_7$). The HREI MS of **1** showed an ion at *m/z* = 430 ($C_{25}H_{34}O_6$), representing the loss of H_2O from M^+. The IR absorption at 3404 cm^{-1} indicated the presence of hydroxyl groups. Analysis of the ^1H and ^{13}C NMR and HMQC data revealed the presence of 25 carbon atoms, including seven quaternary, nine methine, four methylene and five methyl carbon atoms characteristic of a leucosesterterpene skeleton. The downfield ^{13}C NMR signals at δ = 73.2 and 82.6 were due to the hydroxyl-bearing methines C-4 and C-12, respectively. A downfield carbon signal at δ = 96.9, the signal for an oxy-methylene carbon atom at δ_C = 64.6 (δ_H = 4.14 and 3.94, d, $J_{1\alpha,1\beta}$ = 16.5 Hz, H_2-1), olefinic carbon atoms at δ_C = 121.5 (δ_H = 4.99) and 137.2, and a methyl carbon atom at δ_C = 17.4 (δ_H = 1.58) have indicated the presence of an unsaturated pyran ring A. A carbon signal at δ = 221.4 and the IR absorption at 1704 cm^{-1} indicated the presence of a ketonic group. Out of five methyl carbon atoms, the secondary methyl groups resonated at δ_C = 10.8, 21.2, and 14.7, correlated with the ^1H NMR doublets at δ = 0.99 ($J_{22,6}$ = 6.7 Hz, H-22), 0.77 ($J_{23,10}$ = 6.1 Hz, H-23), and 1.16 ($J_{24,14}$ = 6.8 Hz, H-24), respectively. The remaining two tertiary methyl carbon atoms appeared at δ = 17.4 (C-21) and 10.1 (C-25), directly correlated with the protons resonating at 1.58 and 1.77, respectively, in the HMQC spectrum. The downfield proton resonance at δ = 7.09, the olefinic carbon signals at δ = 142.3 and 154.9, and a ketonic carbon signal at δ_C = 207.0, all indicated a double bond in conjugation with a ketonic group in a five-membered α,β-unsaturated carbonyl-containing ring with a hydroxyl group at C-19 (δ_C = 75.7). This was also supported by a mass fragment at *m/z* = 111 that resulted from the cleavage of the C-15–C-16 bond (Fig. 1). The COSY 45° interactions between δ_H = 7.09 and 4.72, and δ_H =



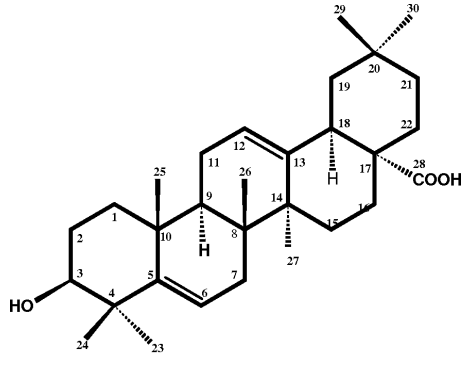
Leucosceptrine (**3**)



Leucosesterterpenone (**4**)



Leucosesterlactone (**5**)



Hypoglauterpenic acid (**6**)

Scheme 1.

4.72 and 2.24, as well as HMBC interactions between δ_H =1.77 (H-25) and δ_C = 207.0 (C-17), and between δ_H = 4.72 (H-20) and δ_C = 142.3 (C-18) and 154.7 (C-19) (Fig. 2) indicated the presence of a leucosesterterpenone-type skeleton [4].

Along with minor differences between the spectroscopic data of compounds **1** and **4**, they also showed clear differences in R_f values (0.39 and 0.30 in 2 % MeOH/$CHCl_3$, respectively) and optical rotations ($-36°$ and $+240°$, respectively, at the same concentrations). This indicated that compound **1** might be an epimer of compound **4**. The upfield shifts in the ^{13}C NMR spectra of compound **1** as compared to compound **4** at C-13 (δ = 221.4), C-14 (41.7), C-15 (27.8), and C-23 (14.7), indicated that the C-14 methyl group has an β-configuration in compound **1**.

17β-Hydroxyleucosceptrine (**2**) was isolated as colorless crystals from the hexane extract. Compound **2** showed strong IR absorptions at 3409 (OH), 1740 (C=O), and 1665 (CH–CH) cm^{-1}. The FAB MS

Position	**1** δ_H (J in Hz)[a]	δ_C[b]	**2** δ_H (J in Hz)[a]	δ_C[b]
1	3.94, 4.14 (d, 16.5)	64.6	4.21, 3.95 (d, 16.4)	64.0
2	–	137.2	–	137.0
3	4.99 (s)	121.5	4.97 (brs)	121.4
4	–	73.2	–	73.2
5	–	96.9	–	96.8
6	2.01 (m)	41.9	1.95 (m)	41.8
7	1.86 (m)	43.3	1.55 (m)	43.1
8	1.37, 1.55 (m)	33.0	1.75, 2.11 (m)	33.9
9	2.15, 1.58 (m)	32.1	1.33, 1.74 (m)	27.8
10	1.28 (m)	31.4	1.90 (m)	31.1
11	1.85 (m)	54.7	1.82 (m)	54.8
12	–	82.6	–	82.5
13	–	221.4	–	220.9
14	3.37 (m)	41.7	3.51 (m)	42.9
15	1.16, 1.12 (m)	27.8	1.15, 2.41 (m)	25.6
16	2.24 (m)	54.1	1.35, 1.95 (m)	32.2
17	–	207.0		107.6
18	–	142.3	–	166.8
19	7.09 (d, 1.7)	154.9	5.79 (m)	117.8
20	4.72 (d, 1.6)	75.7	–	170.3
21	1.58 (s)	17.4	1.75 (brs)	17.2
22	0.99 (d, 6.7)	10.8	0.95 (d, 6.7)	10.8
23	0.77 (d, 6.1)	21.2	0.76 (d, 6.1)	21.0
24	1.16 (d, 6.8)	14.7	1.12 (d, 6.4)	13.7
25	1.77 (s)	10.1	2.04 (brs)	12.5
OH	3.94 (s)	–	–	–
OH	4.93 (s)	–	–	–

Table 1. NMR data of 14β-methylleucosesterterpenone (**1**) and 17α-hydroxyleucosceptrine (**2**) in $CDCl_3$.

[a] ^{1}H NMR data, 300 MHz; [b] ^{13}C NMR data, 100 MHz and 125 MHz for compounds **1** and **2**, respectively.

exhibited the anion $(M–H)^-$ at m/z = 463 ($C_{25}H_{36}O_8$). The EI MS showed an ion at m/z = 446, representing the loss of H_2O from M^+. The HREI MS displayed the M^+ at m/z = 446.2322, ($C_{25}H_{34}O_7$, calcd. 446.2296). The ^{1}H NMR spectrum ($CDCl_3$) showed five methyl signals, including three secondary methyls at δ = 0.95 (d, $J_{22,6}$ = 6.7 Hz), 0.76 (d, $J_{23,10}$ = 6.1 Hz), and 1.12 (d, $J_{24,14}$ = 6.4 Hz), assigned to C-22, C-23, and C-24, respectively, while the two tertiary methyls appeared at δ_H = 1.75 and 2.04 was broad singlets assigned to C-21 and C-25, respectively. A pair of doublets at δ = 4.21 and 3.95 ($J_{1\alpha,1\beta}$ = 16.5 Hz) was due to the C-1 oxymethylene protons. A broad singlet at δ = 4.97 was assigned to the C-3 olefinic proton, whereas another downfield signal at δ = 5.79 was assigned to the C-19 olefinic proton. Twentyfive carbon atoms resonated in the ^{13}C NMR spectrum, including five methyl carbon atoms at δ = 17.2, 10.8, 21.1, 13.7, and 12.5 which were assigned to C-21, C-22, C-23, C-24, and C-25, respectively. The carbon atoms of the two trisubstituted double bonds resonated at δ = 121.4, 137.0, 166.8, and 117.8, and were assigned to C-2, C-3, C-18, and C-19, respectively. Five methylene carbon atoms resonating at δ = 64.7, 33.9, 27.8, 25.6, and 32.2, were assigned to C-8, C-9, C-15, and C-16, respectively. Five methine carbon signals at δ = 41.8, 43.1, 31.1, 54.8, and 42.9 were assigned to C-6, C-7, C-10, C-11, and C-14, respectively. Six quaternary carbon atoms, including two carbonyl carbon atoms, appeared at δ = 73.2, 96.8, 82.5, 220.9, 107.6, and 170.3, were assigned to C-4, C-5, C-12, C-13, C-17, and C-20, respectively. The signals for the oxymethylene protons at δ = 4.21 and 3.95 (δ_C = 64.7), the olefinic proton at δ = 4.97 (δ_C = 121.4), and the tertiary methyl protons at δ_H = 1.75 (δ_C = 17.2), along with the hydroxyl-bearing carbon atoms resonating at δ_C = 96.8 and 73.2, indicated the presence of a six-membered hemiacetal ring, like in leucosceptrine (**3**) [3]. The HMBC spectrum (Fig. 3) of compound **2** further supported a hemiacetal ring by the presence of characteristic carbon signals at δ = 166.8, 117.8, and 170.3. An IR absorption at 1732 cm^{-1} indicated the presence of a five-membered α,β-unsaturated lactone ring.

The main difference between compounds **2** and **3** is an increment of 16 a. m. u. in the M^+ of **2**, suggesting the introduction of a hydroxyl group. The absence of the C-17 oxymethine proton signal in compound **2**, and the presence of a downfield carbon signal at δ_C = 107.6 indicated that the C-17 oxymethine carbon bears the hydroxy group. The loss of an additional wa-

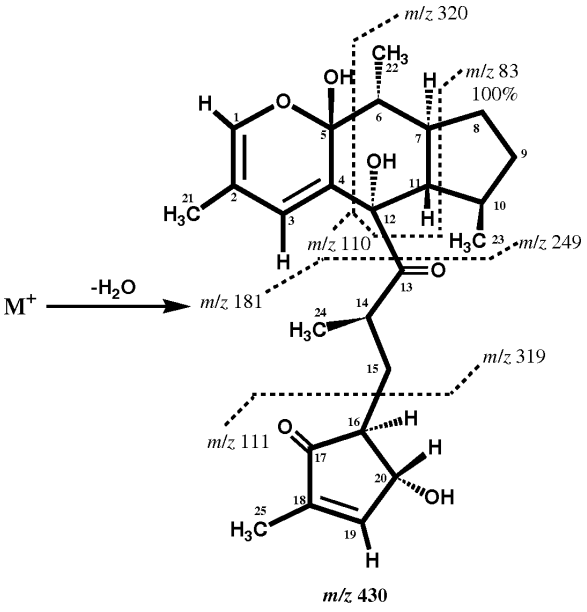


Fig. 1. Key mass fragmentation of **1**.

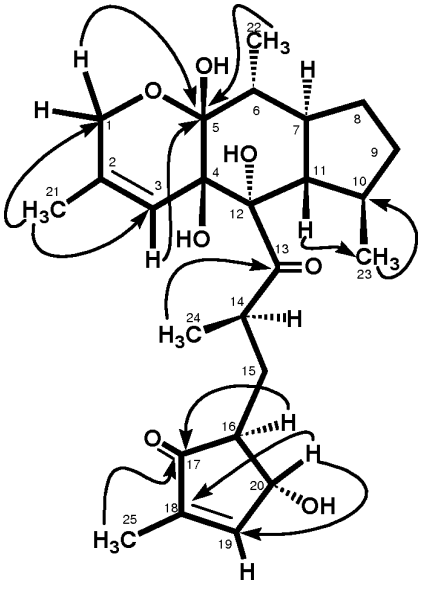


Fig. 2. Key HMBC correlations of **1**.

ter molecule from the M^+ (*m/z* = 428) and mass fragments at *m/z* = 347, 179, 151, and 123 (Fig. 4) also supported the presence of an additional hydroxyl group in **2** at the C-17 position of the lactone ring. Furthermore, in the HMBC spectrum, the C-25 methyl protons (δ = 12.5) were found to be coupled with C-17 (δ = 107.6), C-18 (δ = 166.8), and C-19 (δ = 117.8) of

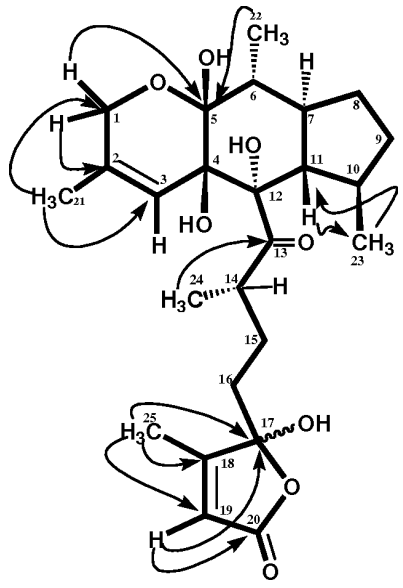


Fig 3. Key HMBC correlations of **2**.

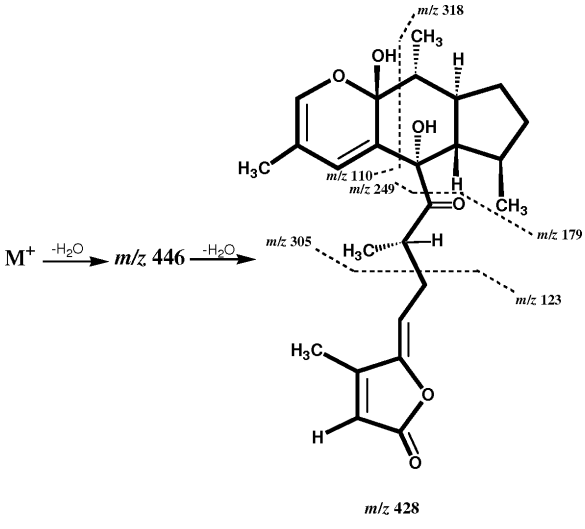
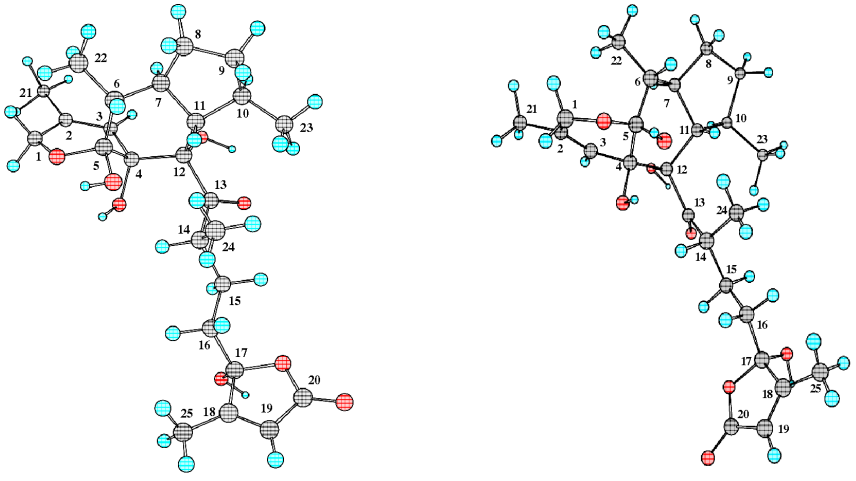


Fig. 4. Key mass fragmentation of **2**.

the five-membered α,β-unsaturated lactone ring. The C-19 olefinic proton (δ = 5.79) also showed heteronuclear couplings with C-17 (δ = 107.6) and carbonyl C-20 (δ = 170.3). The C-16 methylene protons (δ = 1.35) exhibited HMBC interactions with C-17 (δ = 107.6), further indicating the presence of a hydroxyl functionality at C-17.

The stereochemistry at C-17 in compound **2** could not be deduced by NMR techniques or chemical methods due to the quaternary nature of this carbon atom. Thus a computer modeling (CS Chem



(a) α-Configuration (b) β-Configuration

Fig. 5. Molecular mechanics-calculated energy minimized configurations at C-17 in compound **2**.

3D Pro Version 6.0, using MM2 force field calculations) energy minimization of the molecule was carried out to predict the most stable configuration at C-17. The model "a" (Fig. 5) showed a steric energy of 65.942 kcal/mol for the α-configuration, whereas the model "b" showed an steric energy of 69.426 kcal/mol for the β-configuration at C-17. On the basis of these observations, the α-stereochemistry was tentatively proposed. Attempts are continuing to grow crystals of acceptable quality for an X-ray diffraction analysis.

Experimental Section

General

The melting points were recorded on a micro melting point apparatus and are uncorrected. Optical rotations were measured in methanol on a Jasco digital polarimeter (model DIP-3600). Ultraviolet spectra were recorded in methanol on a Hitachi UV 3200 spectrophotometer, infrared spectra on a Jasco A-302 IR spectrophotometer, and mass spectra on a double focusing instrument. Accurate mass measurements were performed with an FAB source using glycerol as the matrix, HREI MS on a Jeol HX 110 mass spectrometer. The ^{1}H NMR spectra were recorded at 300 MHz, while the ^{13}C NMR spectra were recorded on Bruker AMX-400 instruments operating at 100 or 125 MHz using $CDCl_3$ as solvent. Methyl, methylene and methine carbon atoms were distinguished by DEPT experiments. Homonuclear ^{1}H connectivities were determined by COSY experiments, one-bond ^{1}H-^{13}C connectivities with HMQC gradient pulse factor selection. Two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC experiments. Chemical shifts were reported in δ (ppm) and coupling constants (J) were measured in Hz. Precoated TLC plates (silica gel) were used to check the purity of compounds, and ceric sulphate spraying reagent was used for the staining of compounds on TLC. All reagents used were of analytical grades.

Plant Material

The aerial parts of *L. canum* Sm. (Laminaceae) were collected from the Godawari area, Kathmandu, Nepal, on 26th November 2000 at an altitude of 1.550 m. The plant was identified by Mrs. Tirth Maiya Shrestha and a voucher specimen (T037) was deposited at the National Herbarium and Research Laboratory, Department of Plant Resources, Ministry of Forest and Soil Conservation, Godawari, Kathmandu, Nepal.

Extraction and isolation

The aerial parts of *L. canum* were cut into small pieces and dried in the shade. The air-dried and powdered plant material (1.75 kg) was soaked in hexane (10 l) at r. t. for 2 d, filtered and evaporated under reduced pressures. This process was repeated three times to yield 34.03 g of hexane extract. The plant material was subsequently soaked in dichloromethane, ethyl acetate and methanol. The resulting organic extracts were filtered and concentrated under reduced pressures. Some fatty acids were removed from the hexane extract through precipitation with acetone. The filtrate was again concentrated under reduced pressure to afford a semi-dried extract (26.89 g). This hexane extract was repeatedly chromatographed on a silica gel column using various polarities of solvents, starting from hexane, and proceeding with hexane-chloroform, chloroform-methanol, and fi-

nally with methanol to obtain the sub-fractions LCH 207-C, LCH 207-D, and LCH 207-F. Fraction LCH 207-D, obtained on elution with chloroform-methanol (95 : 5), was recrystallized from hexane-chloroform (40 : 60) with a few drops of methanol through slow evaporation. After removing the crystals of leucosesterterpenone (**4**), the solution was again chromatographed on a silica gel column and eluted with 1 % MeOH/$CHCl_3$ to obtain pure compound **1** (6 mg). Similarly, repeated column chromatography of fraction LCH 207-F afforded colorless crystals of compound **2** (5 mg) when eluted with pure chloroform. The purity of the crystals was checked by TLC, visualized under UV light (254 and 366 nm) and by spray with ceric ammonium sulfate reagent.

*Compound **1***

Colorless crystals, m. p. 150 – 153 °C. – R_f = 0.39 (2 % MeOH/$CHCl_3$). – $[\alpha]_D^{25}$ = – 36 (c = 0.04, $CDCl_3$). UV/vis (MeOH): $\lambda_{max}(\log\varepsilon)$ = 389 (3.77), 341 (3.95), 221 (5.24), $\lambda_{min}(\log\varepsilon)$ = 374 (3.62), 339 (3.82), 193 (5.34). – IR ($CDCl_3$): ν_{max} = 3406 (OH), 2929 and 2873 (CH) and 1704 (C=O) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): see Table 1. – ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1. MS (FAB, $M^+ - 1$): *m/z* = 447. – MS (EI, 70 eV): *m/z* (%) = 430 (M^+, 14), 412 (M^+–H_2O, 10), 249 (98), 231 (36), 203 (28), 181 (34), 163 (24), 139 (36), 109 (50), 83 (100), 55 (35). – MS (HREI): *m/z* = 430.2012 (M^+, $C_{25}H_{36}O_7$, calcd. 430.1924).

*Compound **2***

Colorless crystals, m. p. 140 – 146 °C. – R_f = 0.41 (5 % MeOH/$CHCl_3$). – $[\alpha]_D^{25}$ = – 106 (c = 0.03, $CHCl_3$). – UV/vis (MeOH): $\lambda_{max}(\log\varepsilon)$ = 390 (3.52), 343 (3.44), 203 (5.08), $\lambda_{min}(\log\varepsilon)$ = 366 (3.33), 339 (3.26) nm. – IR ($CDCl_3$): ν_{max} = 3409 (OH), 2934 and 2869 (CH), 1740 (C=O) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz): see Table 1. – ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1. – MS (FAB, $M^+ - 1$): *m/z* = 463. – MS (EI, 70 eV): *m/z* (%) = 446 (M^+), 428 (H_2O–27, 20), 346 (M^+–2H_2O, 10), 304 (10), 264 (12), 249 (96), 230 (40), 203 (20), 178 (18), 151 (17), 139 (25), 123 (18) 111 (46), 110 (18), 108 (34), 94 (35), 83 (100), 81 (38). – MS (HREI): *m/z* = 446.2322 (M^+, $C_{25}H_{36}O_7$, calcd. 446.2296).

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