Two New Triterpenoids and a Steroidal Glycoside from the Aerial Parts of *Ocimum basilicum*

Bina Shaheen SIDDIQUI,* Huma ASLAM, Syed Tariq ALI, Sabira BEGUM, and Nasima KHATOON

International Center for Chemical Sciences, HEJ Research Institute of Chemistry, University of Karachi; Karachi–75270, Pakistan. Received June 2, 2006; accepted November 17, 2006

Studies on the chemical constituents of the aerial parts of *Ocimum basilicum* have led to the isolation of three new compounds, basilol (1), ocimol (2), and basilimoside (3), along with two known constituents betulinic acid and oleanolic acid. The structures of the new constituents have been elucidated through spectral studies including 2D-NMR experiments (HMQC, HMBC, COSY, NOESY, and *J*-resolved) and chemical transformation, as *p*-formylphenyl 3β-hydroxyolean-12-en-28-oate (1), 2-methoxy-4-carbomethoxyphenyl 3β-hydroxy-lup-20(29)-en-28-oate (2), and (22*E*)-24ξ-ethyl-25-methylcholesta-5,22-diene-3β-ol-3-*O*-D-glucopyranoside (3).

Key words *Ocimum basilicum*; Labiatae; oleanane; lupane; steroid

Ocimum basilicum LINN. belongs to the family Labiateae commonly known as Tulsi and is a herbaceous plant (60 cm high). It grows in India, Pakistan, Sri Lanka, and Burma, generally cultivated from Punjab.[1)] The essential oil from the aerial parts, leaves, seeds, flowers, and roots of *Ocimum* spp. is used as medicines. The essential oil has shown *in vitro* antibacterial activity against *Staphylococcus aureus*, *Salmonella enteritidis*, and *Escherichia coli*, antiseptic activity against *Proteus vulgaris*, *Bacillus substilis*, and *Salmonella paratyph*, and antifungal activity against *Candida albicans*, *Penicillium notatum*, and *Microsporeum gyseum*. Oils from *Ocimum* spp. showed insect-repellant properties and larvicidal activity against house flies, bluebottle flies, and mosquitoes.[2)] The present studies undertaken on *O. basilicum* in view of the reported medicinal significance have resulted in the isolation and structure elucidation of three new compounds, basilol (**1**), ocimol (**2**), and basilimoside (**3**), and two known constituents oleanolic acid[3,4)] and betulinic acid.[5,6)] Their structures have been elucidated as *p*-formylphenyl 3β-hydroxyolean-12-en-28-oate (**1**), 2-methoxy-4-carbomethoxyphenyl 3β-hydroxy-lup-20(29)-en-28-oate (**2**), and (22*E*)-24ξ-ethyl-25-methylcholesta-5,22-diene-3β-ol-3-*O*-D-glucopyranoside (**3**). Compound **3** is a rare example of steroids having a *t*-butyl group in the side chain.

The molecular formula of basilol (**1**) was established as $C_{37}H_{52}O_4$ by a positive FAB-MS ion at *m*/*z* 561 $[M+H]^+$. The IR spectrum showed characteristic absorption bands caused by hydroxyl group (3428 cm^{-1}), aldehyde group (2815, 2730, 1685 cm^{-1}), ester carbonyl (1730 cm^{-1}), and benzene ring (1597, 1446 cm^{-1}). The UV spectrum showed absorption maxima at 283.8 nm ($\log\varepsilon=7.6$) and 218.2 nm ($\log\varepsilon=7.5$). In the ^{1}H-NMR spectrum (Table 1) seven C-methyls were indicated: five as three-proton singlets at δ 0.82, 0.84, 0.90, 0.96, and 1.05 and two as a six-proton singlet at δ 0.75. These along with a double doublet at δ 2.81 (1H, dd, J=13.8, 4.3 Hz, H-18), a one-proton triplet at δ 5.22 (1H, t, J=3.4 Hz, H-12), and the ^{13}C-NMR shifts of the skeleton suggested that **1** is an oleanolic acid ester.[3,4)] Furthermore, a one-proton double doublet was present at δ 3.20 (1H, dd, J=10.8, 4.6 Hz, H-3) connected with a carbon at δ 79.2 (C-3) in the HMQC spectrum. EI-MS showed a peak of highest a.m.u. at *m*/*z* 455 due to loss of 105 a.m.u. which in the light of NMR data indicated that **1** is *p*-formylphenyl ester of oleanolic acid. The ^{1}H-NMR spectrum showed a *p*-formylphenyl ester moiety by signals at δ 6.92 (2H, d, J=8.6 Hz, H-2′/6′) and 7.78 (2H, d, J=8.6 Hz, H-3′/5′) and a one-proton singlet at δ 9.81 (CHO) and the corresponding carbon signals at δ 115.9, 132.3, and 190.8. Furthermore, H-2′/6′ showed long-range connectivity in the HMBC spectrum (Fig. 1) with a quaternary carbon at δ 161.4 (C-1′), and CH

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compound **1** (δ in ppm, J in Hz, 400 MHz, $CDCl_3$)

Position	δ_C	δ_H multiplicity (J/Hz)	Position	δ_C	δ_H multiplicity (J/Hz)
1	38.3	0.99, 1.63 m	20	31.0	—
2	28.0	1.05, 1.76 m	21	36.7	1.65, 1.72 m
3	79.2	3.20, dd (10.8, 4.6)	22	34.0	1.24, 1.43 m
4	38.5	—	23	28.1	0.96 s
5	55.1	0.72, dd (11.5, 2.5)	24	15.5	0.90 s
6	18.2	1.46, 1.64 m	25	15.4	0.75 s
7	32.9	1.20, 1.43 m	26	17.0	0.82 s
8	39.1	—	27	23.5	1.05 s
9	47.5	1.49 m	28	170.2	—
10	36.9	—	29	16.9	0.75 s
11	22.8	1.60 m	30	21.1	0.84 s
12	125.8	5.22, t (3.4)	1′	161.4	—
13	143.5	—	2′	115.9	6.92 d (8.6)
14	41.5	—	3′	132.2	7.78 d (8.6)
15	27.1	1.06, 2.23 m	4′	137.9	—
16	23.2	1.76, 2.21 m	5′	132.2	7.78 d (8.6)
17	46.3	—	6′	115.9	6.92 d (8.6)
18	42.0	2.81 dd (13.8, 4.3)	1″	190.8	9.81 s
19	45.8	1.10, 1.79 m			

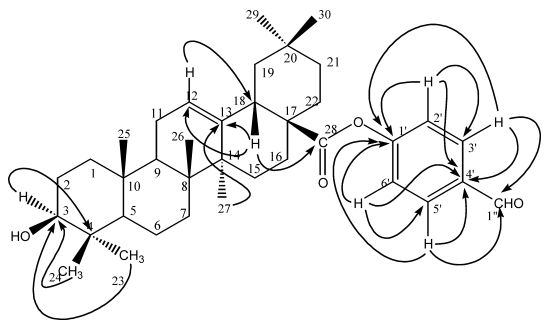

Fig. 1. Selected HMBC Correlations Observed for Compound **1**

* To whom correspondence should be addressed. e-mail: bina@khi.comsats.net.pk

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Table 2. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compound **2** (δ in ppm, J in Hz, 400 MHz, $CDCl_3$)

Position	δ_C	δ_H multiplicity (J/Hz)	Position	δ_C	δ_H multiplicity (J/Hz)
1	38.7	2.18, 1.65 m	21	29.7	1.6 m
2	27.4	1.78 m	22	37.0	1.98, 1.48 m
3	78.9	3.15 dd (10.0, 5.0)	23	27.9	0.91 s
4	38.8	—	24	14.6	0.73 s
5	55.3	0.65, dd (11.5, 2.5)	25	15.3	0.80 s
6	18.3	1.58 m	26	16.0	0.94 s
7	34.3	1.22 m	27	16.1	0.95 s
8	40.7	—	28	170.5	—
9	50.5	1.25 m	29	109.6	*a* 4.71 br s
10	37.2	—			*b* 4.58 br s
11	20.8	1.45 m	30	19.3	1.66 s
12	25.5	1.56 m	1′	150.1	—
13	38.3	0.85 m	2′	145.1	—
14	42.4	—	3′	110.1	7.52 d (1.8)
15	30.5	1.30 m	4′	131.8	—
16	32.1	1.28 m	5′	124.1	7.60 dd (8.3, 1.8)
17	56.2	—	6′	114.0	6.90 d (8.3)
18	46.8	2.66 m	1″	166.8	—
19	49.2	2.99, td (11.0, 5.5)	OMe	55.3	3.86 s
20	150.3	—	OMe	56.1	3.93 s

carbons at δ 132.3 (C-3′/5′). On the other hand, H-3′/5′ showed connectivity in the HMBC spectrum with quaternary carbons at δ 190.8 (C-1″) and 161.4 (C-1′). In light of these observations, the structure of **1** has been elucidated as *p*-formylphenyl 3β-hydroxyolean-12-en-28-oate. The structure was substantiated by various fragment ions observed in the EI-MS spectrum (*vide* structure) and acetylation (Ac_2O/pyr) of **1** to obtain the acetyl derivative **1a** (*vide* Experimental).

The molecular formula of ocimol (**2**) was established as $C_{39}H_{56}O_6$ by an ion in the FAB-MS at *m/z* 621 $[M+H]^+$. In the IR spectrum absorption bands were present caused by hydroxyl group (3431 cm^{-1}), ester carbonyl group (1726 cm^{-1}), and benzene ring (1595—1471 cm^{-1}). The UV spectrum displayed λ_{max} at 252 nm (log ε=5.4). In the ^{1}H-NMR spectrum (Table 2) six singlets were present at δ 0.73, 0.80, 0.91, 0.94, 0.95, and, 1.66 (each 3H, s), which were connected with carbons in the HMQC spectrum at δ 14.6 (C-24), δ 15.3 (C-25), δ 16.0 (C-26), δ 16.1 (C-27), δ 27.9 (C-23), and δ 19.3 (C-30), respectively, and assigned on the basis of 2D NMR analysis. Two one-proton broad singlets were present at δ 4.71 (H-29a) and 4.58 (H-29b) connected with the same carbon in the HMQC spectrum at δ 109.6 (C-29), which along with the vinylic methyl at δ 1.66 were indicative of a lupane skeleton of **2**.[5,6] Furthermore, a double doublet was present at δ 3.15 (1H, dd, J=10.0, 5.0 Hz, H-3), which was connected with a carbon at δ 78.9 (H-3) in the HMQC spectrum. The coupling constants of H-3 were in agreement with its axial (α) configuration and β-disposition of the hydroxyl group at C-3. Beside these, the ^{1}H-NMR data manifested a doublet at δ 6.90 (1H, d, J=8.3 Hz, H-6′, δ_C 114.0), a double doublet at δ 7.60 (H, dd, J=8.3, 1.8 Hz, H-5′, δ_C 124.1), and a doublet at δ 7.52 (1H, d, J=1.8 Hz, H-3′, δ_C 110.1). Furthermore, H-6′ showed long-range connectivity in the HMBC spectrum (Fig. 2) with carbons at δ 150.1 (C-1′) and 145.1 (C-2′) and both H-3′ and H-5′ had cross peaks for a carbon at δ 166.8 (C-1″). A three-proton singlet was present at δ 3.93 (3H, s), which was connected with a carbon at δ 56.1 in the HMQC spectrum and with a carbon at δ 145.1 (C-2′) in the HMBC spectrum. These data and the fragment at *m/z* 165.0549 ($C_9H_9O_3$) a.m.u. indicated that **2** is 2′-methoxy-4′-carbomethoxyphenyl ester of betulinic acid ($\delta_{C\text{-}28}$ 179.0). The ^{13}C-NMR shifts of this compound match well with those reported for similar partial structures.[7,8] From the ^{1}H- and ^{13}C-NMR data[6] as well as the proton–proton and proton–carbon connectivities observed in the COSY, HMQC, and HMBC spectra, the structure of **2** was derived as 2-methoxy-4-carbomethoxyphenyl 3β-hydroxy-lup-20(29)-en-28-oate. The structure was substantiated by acetylation (Ac_2O/pyr) of **2** to obtain the acetyl derivative **2a** (*vide* Experimental).

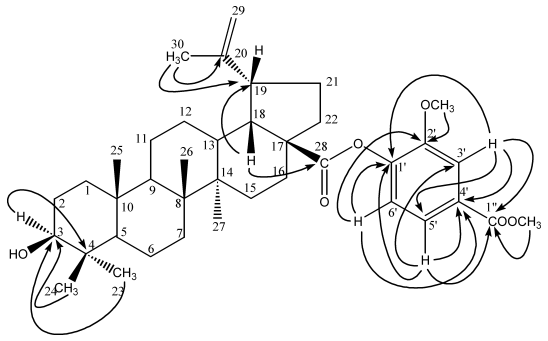

Fig. 2. Selected HMBC Correlations Observed for Compound **2**

The molecular formula of basilimoside (**3**) was established as $C_{36}H_{60}O_6$ by a molecular-related ion in the FAB at *m/z* 589.4450 $[M+H]^+$. The IR spectrum showed characteristic absorption bands caused by hydroxyl groups (3405 cm^{-1}) and C=C (1621 cm^{-1}). In the ^{1}H-NMR spectrum signals for four methyl groups were present at δ 0.66 (3H, s, H-18), δ 0.92 (3H, s, H-19), δ 1.05 (3H, d, J=6.3 Hz, H-21) and δ 0.85 (3H, t, J=7.2 Hz, H-30)], which are comparable with those of corresponding methyl signals of stigmasterol and related compounds.[9,10] A singlet at δ 0.96 (9H, s, H-26/27/28) was due to three methyl signals of *tert*-butyl moiety. A double bond in the side chain at C-22 was evident from two double doublets at δ 5.20 and 5.02 (each 1H, dd, J=15.3, 8.2 Hz, H-22 and H-23 respectively). These protons were connected with carbons at δ 138.8 and δ 129.4, respectively, in the HMQC spectrum identified as CH in DEPT spectrum. A one-proton multiplet due to H-6 was present at δ 5.34 correlated with a carbon at δ 121.8 in the HMQC spectrum. The assignment of these protons and carbons was based on 2D NMR analysis and matched well with the chemical shifts of corresponding nuclei reported in the literature.[9,10] A fragment ion at *m/z* 427 $[M+H-162]^+$ and the NMR data (Table 3) manifested that it is a β-D-glucoside. Thus an anomeric proton was revealed as a doublet at δ 5.06 (J=8.8 Hz), which had connectivity with a carbon at δ 102.5 in the HMQC spectrum. In the HMBC spectrum H-1′ showed long-range connectivity with C-3 (δ 78.0) supporting its attachment with C-3 oxygen (Fig. 3). The NMR values of the glucose protons and carbons (Table 3) are in agreement with those reported in the literature.[11] Acid hydrolysis (*vide* Experimental) of **3** yielded a sugar that was identified as glucose by co-TLC with an authenticated sample. The positive sign of specific rotation ($[\alpha]_D^{25}$ +52) of the glycone revealed that it is D-glucose and hence **3** is a β-D-glucoside.

Table 3. ^{1}H- and ^{13}C-NMR Data for Compound **3** (Pyridine, 300 MHz, δ in ppm)

Position	δ_C	δ_H multiplicity (*J*/Hz)	Position	δ_C	δ_H multiplicity (*J*/Hz)
1	37.4	1.09 m	19	19.1	0.92 s
2	30.2	*a* 1.79 m	20	40.0	1.56 m
2	—	*b* 1.80 m	21	19.9	1.05 d (6.3)
3	78.0	3.96 m	22	138.8	5.20 dd (15.3, 8.2)
4	42.2	2.25	23	129.4	5.02 dd (15.3, 8.2)
5	140.7	—	24	56.2	1.53 m
6	121.8	5.34 m	25	23.0	1.40 m
7	32.0	1.95 m	26	27.0	0.96 s
8	32.1	1.84 m	27	27.0	0.96 s
9	51.2	1.53 m	28	22.0	0.96 s
10	37.0	—	29	24.4	1.23 m
11	21.2	1.51	30	11.9	0.85 t (7.2)
12	39.7	1.90 m	*β*-D-Glu		
13	41.2	—	1′	102.5	5.06 d (8.8)
14	56.8	1.20 m	2′	75.3	4.22 t (8.8)
15	24.4	1.23 m	3′	78.5	4.05 t (8.8)
16	28.5	1.10 m	4′	71.6	3.97 t (8.8)
17	55.9	0.70 m	5′	78.4	4.28 m
18	12.1	0.66 s	6′	62.7	a 4.56 dd (12.1, 2.8)
			6′	—	b 4.38 dd (12.1, 4.7)

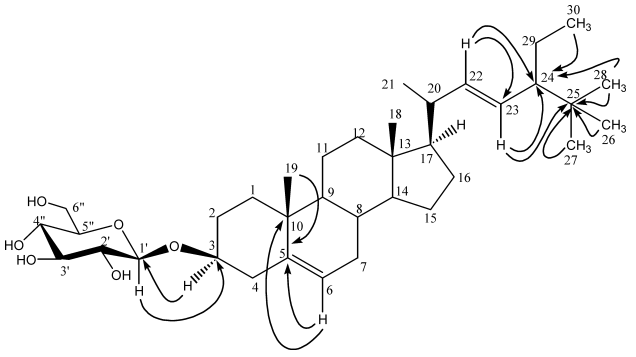

Fig. 3. Selected HMBC Correlations Observed for Compound **3**

On the basis of the data discussed above, **3** was assigned the structure as 24*ξ*-ethyl-25-methylcholesta-5,22-diene-3*β*-ol-3-*O*-D-glucopyranoside. Additional supportive evidence was obtained from the characteristic mass fragments[12] of **3** and its acetylation to obtain the tetra-acetyl derivative (**3a**) (*vide* Experimental).

Experimental

Petroleum ether, bp 60—80°. Column chromatography (CC): silica gel 60 (0.063—0.200 mm, 70—230 mesh; E. Merck); prep. TLC: silica gel 60 PF_{254} (Merck), detection at 254 and 266 nm. UV spectra: Hitachi U-3200 and Secoman-Anthelei-Junior spectrophotometer, λ_{max} in nm (log ε). IR spectra: Bruker vector-22 spectrophotometer; ν_{max} in cm^{-1}; ^{1}H- and ^{13}C-NMR, COSY-45, NOESY, HMQC, HMBC, and *J*-resolved spectra: Avance 400 (400/100 MHz); chemical shifts (δ) in ppm. The spectra were referenced to the residual solvent signal as δ 7.24 ($CHCl_3$) and coupling constants (*J*) in Hz, temp. 25 °C. Optical rotation: Jasco-DIP-360 digital polarimeter (27 °C). EI-MS Varian MAT-312 mass spectrometer; FAB: Jeol JMS-HX110 (70 ev); source at 250°; *m/z* (rel.%) HR-EIMS: Jeol JMS-600H mass spectrometer.

Plant Material The aerial parts of *O. basilicum* were collected from the Karachi region during January 2004 and identified by Sherwali (Herbarium Incharge) Department of Botany, University of Karachi and a voucher specimen (GH. No. S.N. 68316) has been deposited in the Herbarium of the Department of Botany, University of Karachi, Karachi.

Extraction and Isolation Aerial parts (31 kg) of *O. basilicum* were extracted repeatedly (×4) with MeOH at r.t. The combined extract was concentrated under vacuum to give a thick syrup that constituted the crude methanolic extract. This was partitioned between ethyl acetate and water. The ethyl acetate phase was treated with 4% aqueous Na_2CO_3 to separate neutral (NF) and acidic (AF) fractions.

The NF fraction was treated with charcoal and filtered. The filtrate was freed of the solvent under reduced pressure and the residue treated with petroleum ether to give petroleum ether-soluble and insoluble parts. The petroleum ether-soluble part (60 g) was subjected to column chromatography (petroleum ether, petroleum ether–EtOAc, $CHCl_3$, $CHCl_3$–MeOH). As a result 300 fractions were obtained and combined on the basis of TLC to give 20 fractions; of these fraction #5 was further purified through prep. TLC (petroleum ether–EtOAc; 7.5 : 2.5) to afford betulinic acid[5,6] (10 mg, *Rf*=0.72) and oleanolic acid[3,4] (15 mg, *Rf*=0.61). Fraction #14 was a pure compound (**1**, 25 mg, $CHCl_3$–MeOH; 9.5 : 0.5, *Rf*=0.53). Fraction AF (47.4 g) was also subjected to CC (petroleum ether, petroleum ether–EtOAc, $CHCl_3$, $CHCl_3$–MeOH) in increasing order of polarity. As a result 420 fractions were obtained and combined on the basis of TLC ultimately to afford 14 fractions (A—N). Fraction A (100 mg) was purified through prep. TLC (petroleum ether–EtOAc; 7.5 : 2.5) to furnish compound **2** as an amorphous powder (5.0 mg, *Rf*=0.69). Fraction D was purified through prep. TLC ($CHCl_3$) to furnish compound **3** as a white amorphous powder (10 mg, *Rf*=0.22).

p-Formylphenyl 3*β*-hydroxyolean-12-en-28-oate (**1**): White amorphous powder; $[\alpha]_D$=−15° (*c*=0.16, $CHCl_3$, 27 °C); UV (MeOH) λ_{max} (log ε): 283.8 (2.90), 218.2 (2.89) nm; IR ($CHCl_3$) ν_{max}: 3428 (OH), 2815 and 2730 (aldehyde–CH str.), 1730 (ester carbonyl), 1685 (aldehyde C=O), 1597 and 1446 (benzene) cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Table 1; Positive FAB-MS *m/z* 561 $[M+H]^+$; HR-FAB-MS *m/z* 561.3943 $[M+H]^+$ (Calcd for $C_{37}H_{53}O_4$ 561.3945); HR-EI-MS (% int.): *m/z* 455.3520 (3.5, M^+-105), 248.1769 ($C_{16}H_{24}O_2$, 100, *r.D.A.*), 207.1739 ($C_{14}H_{23}O$, 71.3), 203.1789 ($C_{15}H_{23}$, 89.5), 189.1640 ($C_{14}H_{21}$, 31.1), 105.0336 (C_7H_5O, 16.2).

Acetylation of 1 To a solution of **1** (10 mg) in pyridine (0.5 ml) Ac_2O (1 ml) was added and the mixture was left at r.t. overnight. The mixture was poured over crushed ice and extracted with EtOAc. After usual workup of the EtOAc phase and purification by TLC (silica gel, petroleum ether–EtOAc, 8 : 2) the acetyl derivative **1a** was obtained as an amorphous powder (5.0 mg). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 4.47 (dd, 10.8, 4.6 Hz, H-3α), δ 2.02 (3H, s, OAc).

2-Methoxy-4-carbomethoxyphenyl 3*β*-hydroxy-lup-20(29)-en-28-oate (**2**): White amorphous powder; $[\alpha]_D$=−50° (*c*=0.16, $CHCl_3$, 27 °C); UV (MeOH) λ_{max} (log ε): 252.2 (5.4) and 204.3 (5.3) nm; IR ($CHCl_3$) ν_{max}: 3431 (OH), 1726 (C=O), 1595 and 1471 (benzene) cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Table 2; FAB-MS *m/z* 621 $[M+H]^+$; HR-FAB-MS *m/z* 621.4075 $[M+H]^+$ (Calcd for $C_{39}H_{57}O_6$, 621.4078); HR-EI-MS (% int.) *m/z* 455.3519 (10.6, M^+-165), 411.3621 ($C_{29}H_{47}O$, 6.4), 189.1640 ($C_{14}H_{21}$, 100) and 165.0549 ($C_9H_9O_3$, 18.2).

Acetylation of 2 Following the procedure described above for **1** the acetyl derivative (**2a**) of **2** (5 mg) was obtained as an amorphous powder (2 mg). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 4.42 (1H, dd, 10.0, H-3α), 2.02 (3H, s, OAc).

(22*E*)-24*ξ*-Ethyl-25-methylcholesta-5,22-diene-3*β*-ol-3-*O*-D-glucopyranoside (**3**): White amorphous powder; $[\alpha]_D$=67.7 (*c*=0.17, $CHCl_3$, 27 °C); UV (MeOH) λ_{max} (log ε): 203.8 (5.1) nm: IR (KBr) ν_{max}: 3405 (OH), 1621 (C=C) cm^{-1}; ^{1}H- and ^{13}C-NMR data: see Table 3; FAB-MS *m/z* 589 $[M+H]^+$, HR-FAB-MS *m/z* 589.4450 $[M+H]^+$ (Calcd for $C_{36}H_{61}O_6$ 589.4470). HR-EI-MS (% int.): *m/z* 426.3843 (11.8, M^+-162), 411.3623 ($C_{29}H_{47}O$, 9.4), 393.3520 ($C_{29}H_{45}$, 5.9), 273.2209 ($C_{19}H_{29}O$, 100.0) and 255.2101 ($C_{19}H_{27}$, 43.0).

Acetylation of 3 Following the procedure described above for **1** the acetyl derivative (**3a**) of **3** (8 mg) was obtained as an amorphous powder (4.5 mg). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 1.98, 2.01, 2.02, 2.05 (each 3H, s, OAc).

Acid Hydrolysis of 3 Compound **3** (7.0 mg) was dissolved in MeOH (4 ml) containing 2 N HCl (4 ml) and refluxed on boiling water bath for 6 h. After concentration at reduced pressure, the reaction product was diluted with water and extracted with AcOEt. The aqueous phase was neutralized with Ag_2CO_3, filtered, and evaporated *in vacuo* to give a whitish residue. It was identified as glucose by co-TLC with an authenticated sample (*n*-BuOH–AcOH–H_2O; 4 : 1 : 5) and visualizing the spots with aniline phthalate reagent. The observed optical rotation of the glycone [$[\alpha]_D$=+51.9° (*c*=0.1 in water, 27 °C), reported $[\alpha]_D$=+52° (*c*=10 in water, 27 °C)] revealed that it is D-glucose.

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