Two New E-Secoursane Glycosides: Bodiniosides A and B, Isolated from *Elsholtzia bodinieri*

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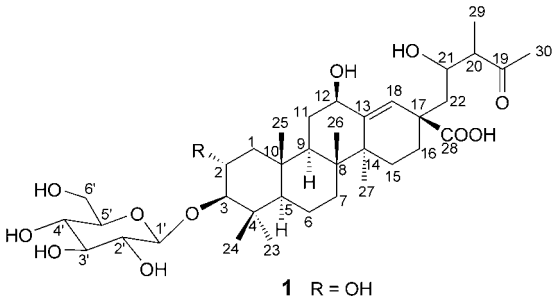
Two unusual 18,19-secoursane glycosides, bodiniosides A (**1**) and B (**2**), and two known triterpenoids, hypadienic acid (**3**) and 2,3,19-trihydroxyurs-12-en-28-oic acid (**4**), were isolated from the whole plants of *Elsholtzia bodinieri*. The structures of **1** and **2** were determined by intensive interpretation of spectral data. This is the first report of E-secoursane glycosides. In addition, the biogenetic relationships among these four triterpenoids are discussed.

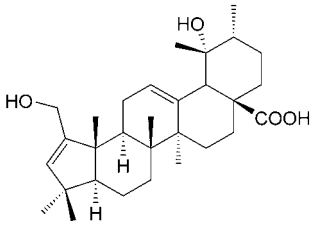
Introduction. – *Elsholtzia bodinieri* (Labiatae), which is distributed in Yunnan and Guizhou Provinces in China, is a traditional Chinese medicine for the treatment of cough, headache, pharyngitis, fever, and hepatitis [1]. A previous study of this plant has resulted in the isolation of two new triterpene glycosides, hederagenin 3-*O*-*β*-D-xylopyranoside and dodecandral 3-*O*-*β*-D-xylopyranoside [2]. In the present study, two new 18,19-secoursane glycosides, bodiniosides A (**1**) and B (**2**), along with two known triterpenoids, hypadienic acid (**3**) [3] and 2,3,19-trihydroxyurs-12-en-28-oic acid (**4**) [4], were isolated from the whole plant. The structure elucidation and a plausible biogenetic pathway of these four compounds are described here.

Results and Discussion. – *Structure Elucidation.* Bodinioside A (**1**), obtained as colorless flake crystals, gave rise to a quasi-molecular-ion peak at *m*/*z* 663.3727 ($[M - H_2O - H]^+$) in the HR-ESI-MS (negative-ion mode), which corresponded to the molecular formula $C_{36}H_{58}O_{12}$. Thus, eight degrees of unsaturation were determined for **1**. Inspection of the 1D and 2D NMR spectra (*Table*) and comparison with those of 2,3,19-trihydroxyurs-12-en-28-oic acid (**4**), allowed us to identify the structure of bodinoside **1** (2*α*,3*β*,12*β*)-3-(*β*-D-glucopyranosyloxy)-2,12,21-trihydroxy-19-oxo-18,19-secours-13(18)-en-28-oic acid, a new 18,19-secoursane glycoside.

The IR spectrum of **1** exhibited absorptions for COOH (3462 cm^{-1}), OH (3385 cm^{-1}), carbonyl (1741 and 1711 cm^{-1}), and olefinic (1640 cm^{-1}) groups. The UV spectrum showed end absorption indicating the absence of a conjugated system. In the ^{1}H-NMR spectrum, the signals of six tertiary Me (*δ* 0.87, 1.00, 1.09, 1.24. 1.39, and 2.12) and one secondary Me group (*δ* 0.92, *d*, *J* = 7.1 Hz) were present. Analysis of the ^{13}C-NMR spectrum revealed that **1** contains 36 C-atoms, including a hexose moiety (*δ* 62.6, 71.6, 75.6, 78.6, 78.7, and 106.6), one C=O (*δ* 209.1), one COOH (*δ* 178.6), and one trisubstituted C=C group (*δ* 116.6 and 149.7), five quaternary C-atoms (*δ* 38.2, 40.8, 41.5, 43.2, and 43.8), and four OCH (*δ* 66.8, 68.9, 77.3, and 95.5), three non-oxygenated CH (*δ* 49.5, 51.9, and 55.8), seven CH_2 (*δ* 18.4, 27.7, 29.1, 33.2, 34.8, 41.6, and 47.6), and seven Me groups (*δ* 11.8, 17.9,



1 R = OH
2 R = H



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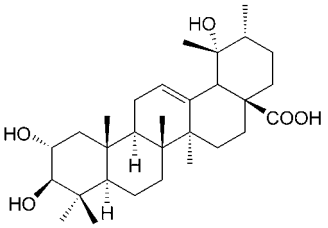

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18.1, 18.5, 22.0, 28.6, and 29.3). The presence of these features suggested that **1** was a triterpenoid derivative. As required by its molecular formula, three OH groups and four carbocyclic rings in the aglycone moiety of **1** should be present.

The ^{13}C-NMR spectrum of **1** showed the characteristic signals for a ursolic acid (=(3β)-3-hydroxyurs-12-en-28-oic acid) triterpenoid, including five tertiary Me groups at δ 18.1 (Me(23)), 28.6 (Me(24)), 17.9 (Me(25)), 18.5 (Me(26)), 22.0 (Me(27)), and a C(28)OOH group at δ 178.6. This, and considering the concomitant isolation of the two ursolic acid triterpenoids **3** and **4** from the same plant, suggested that the aglycone of **1** was derived from ursolic acid. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of **4** suggested similar rings A and B for the two compounds. However, the data for the remaining portion of **1** were quite different from those of known members of the ursane class. Only three non-oxygenated CH (δ 49.5, 51.9, and 55.8) were observed in **1**. Another noticeable feature was the presence of an acetyl group (δ 11.8 and 209.1) directly connected to a MeCH group (δ 51.9 and 29.3), which was confirmed by HMQC and HMBC correlations.

The 2D-NMR data, including ^{1}H,^{1}H COSY and HMQC data, were consistent with the three partial structures **a**–**c** (see *Fig. 1*, bold bonds). The HMBC spectrum (*Fig. 1*) confirmed the above δ(H) assignments and established the connective relationship of the fragments described above. HMBC correlations of Me(26) with C(7), C(8), C(9), and C(14), and of Me(27) with C(8), C(13), C(14), and C(15) required the connection of fragments **a** and **b** through two quaternary C-atoms (C(8) and C(14)). HMBC Cross-peaks CH_2(22)/C(18) and COOH, and CH_2(16)/C(28) established the connectivity of partial structures **b** and **c** through the quaternary C(17). Other correlations, from CH_2(11), CH_2(15), and Me(27) to C(13), and from CH_2(16) to C(18), revealed the position of the C=C bond between C(13) and C(18). This was further confirmed by the observation of HMBC correlations of H–C(18) with C(12), C(14), C(16), and C(17). Moreover, HMBC correlations of Me(30) to C(19) and C(20), and of CH(20) to C(19) indicated the attachment of MeCO at C(20). The additional HMBC correlations from Me(29) to C(19), C(20), and C(21) supported this assignment.

The data of the sugar moiety were consistent with the presence of a β-D-glucopyranose unit in **1** [5]; according to the HMBC cross peaks H–C(3)/C(1′) and H–C(1′)/C(3), it was located at C(3).

The relative configuration of **1** was determined by NOESY analysis (*Fig. 2*) and comparison of the NMR data with those of **4**. As for ursolic acid triterpenoids, Me(24), Me(25), Me(26), and C(28)OOH were β-oriented and H–C(5), H–C(9), Me(23), and Me(27) were α-oriented. NOESY Correlations H–C(3)/

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**[a])

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H_α–C(1)	1.19 (overlap)	47.6 *(t)*	1.16 (*m*)	39.6 (*t*)
H_β–C(1)	2.43 (*dd*, *J* = 4.6, 12.6)	–	1.16 (*m*)	–
H_β–C(2) or CH_2(2)	4.03 (*m*)	66.8 (*d*)	1.42–1.65 (*m*)	26.7 (*t*)
H_α–C(3)	3.31 (*d*, *J* = 9.1)	95.5 (*d*)	3.38 (*dd*, *J* = 4.4, 11.7)	88.8 (*d*)
C(4)	–	40.8 (*s*)	–	39.1 (*s*)
H_α–C(5)	0.91 (overlap)	55.8 (*d*)	0.87 (*m*)	56.1 (*d*)
CH_2(6)	1.49 (*m*, H_α), 1.29 (*m*, H_β)	18.4 (*t*)	1.54 (*m*, H_α), 1.20 (*m*, H_β)	18.4 (*t*)
CH_2(7)	1.44 (*m*)	34.8 (*t*)	1.44 (*m*)	34.9 (*t*)
C(8)	–	43.2 (*s*)	–	43.2 (*s*)
H_α–C(9)	1.66 (*dd*, *J* = 3.5, 11.5)	49.5 (*d*)	1.61 (overlap)	49.6 (*d*)
C(10)	–	38.2 (*s*)	–	37.3 (*s*)
CH_2(11)	2.28 (*m*, H_α), 1.68 (*m*, H_β)	33.2 (*t*)	2.22 (*m*, H_α), 1.63 (overlap, H_β)	33.1 (*t*)
H_α–C(12)	4.62 (*dd*, *J* = 1.5, 10.1)	68.9 (*d*)	4.61 (*m*)	69.1 (*d*)
C(13)	–	149.7 (*s*)	–	149.9 (*s*)
C(14)	–	41.5 (*s*)	–	41.5 (*s*)
CH_2(15)	1.18 (*m*, H_α), 2.87 (*m*, H_β)	27.7 (*t*)	1.20 (*m*, H_α), 2.86 (*m*, H_β)	27.8 (*t*)
CH_2(16)	1.62 (*m*, H_α), 1.93 (*m*, H_β)	29.1 (*t*)	1.54 (*m*, H_α), 1.92 (*m*, H_β)	29.2 (*t*)
C(17)	–	43.8 (*s*)	–	43.8 (*s*)
H–C(18)	6.39 (*s*)	116.6 (*d*)	6.37 (*s*)	116.5 (*d*)
C(19)	–	209.1 (*s*)	–	209.1 (*s*)
H–C(20)	2.74 (*m*)	51.9 (*d*)	2.73 (*m*)	51.9 (*d*)
H–C(21)	4.85 (*m*)	77.3 (*d*)	4.83 (*m*)	77.3 (*d*)
CH_2(22)	1.81 (*t*, *J* = 12.6), 2.07 (*dd*, *J* = 5.6, 12.6)	41.6 (*t*)	1.80 (*m*), 2.06 (*dd*, *J* = 5.7, 12.7)	41.7 (*t*)
Me–C(23)	1.00 (*s*)	18.1 (*q*)	0.94 (*s*)	16.9 (*q*)
Me–C(24)	1.39 (*s*)	28.6 (*q*)	1.30 (*s*)	28.3 (*q*)
Me–C(25)	0.87 (*s*)	17.9 (*q*)	0.79 (*s*)	16.7 (*q*)
Me–C(26)	1.09 (*s*)	18.5 (*q*)	1.08 (*s*)	18.5 (*q*)
Me–C(27)	1.24 (*s*)	22.0 (*q*)	1.25 (*s*)	22.1 (*q*)
C(28)	–	178.6 (*s*)	–	178.6 (*s*)
Me–C(29)	0.92 (*d*, *J* = 7.1)	11.8 (*q*)	0.93 (*d*, *J* = 6.9)	11.8 (*q*)
Me–C(30)	2.12 (*s*)	29.3 (*q*)	2.11 (*s*)	29.3 (*q*)
H–C(1′)	4.95 (*d*, *J* = 7.8)	106.6 (*d*)	4.92 (*d*, *J* = 7.7)	107.0 (*d*)
H–C(2′)	4.09 (overlap)	75.6 (*d*)	4.01 (overlap)	75.8 (*d*)
H–C(3′)	4.23 (*dt*, *J* = 4.8, 8.9)	78.6 (*d*)	4.25 (*dt*, *J* = 4.7, 9.6)	78.4 (*d*)
H–C(4′)	4.19 (*dt*, *J* = 4.8, 8.9)	71.6 (*d*)	4.22 (*dt*, *J* = 4.7, 9.6)	71.9 (*d*)
H–C(5′)	4.08 (overlap)	78.7 (*d*)	3.99 (overlap)	78.8 (*d*)
CH_2(6′)	4.34 (*dd*, *J* = 5.6, 11.6), 4.60 (overlap)	62.6 (*t*)	4.37 (*dd*, *J* = 5.5, 11.7), 4.62 (*dd*, *J* = 2.4, 11.7)	63.1 (*t*)
OH	7.48 (br. *s*), 6.32 (br. *s*), 5.03 (br. *s*)			

[a]) Data were determined at 500 MHz in C_5D_5N, chemical shifts δ in ppm and coupling constant *J* in Hz. Assignments were confirmed by ^{1}H,^{1}H COSY, HMQC, and HMBC.

H–C(5), H–C(3)/Me(23), H–C(2)/Me(24), H–C(2)/Me(25), and H–C(9)/H–C(12) suggested that the configuration of H–C(2) was β, and that of H–C(3) and H–C(12) α. We could not, however, define the relative configurations at C(20) and C(21), on the basis of the NMR data, since the σ-bond between C(17) and C(22) has free rotation.

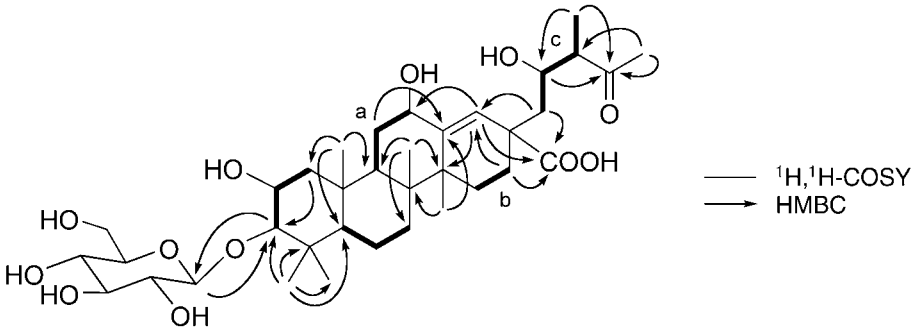


Fig. 1. *Selected 2D NMR correlations for bodinioside A* (**1**)

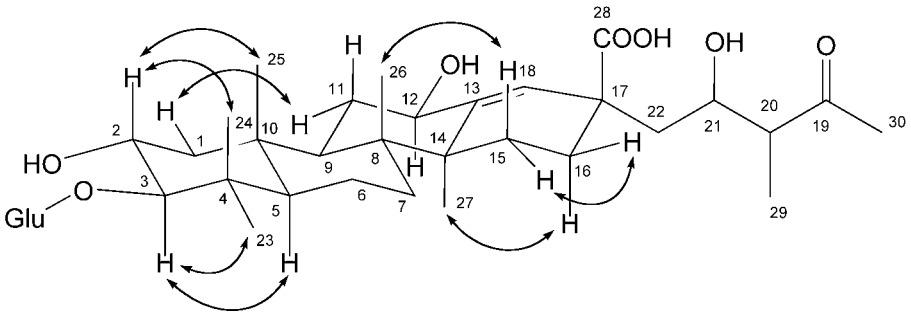
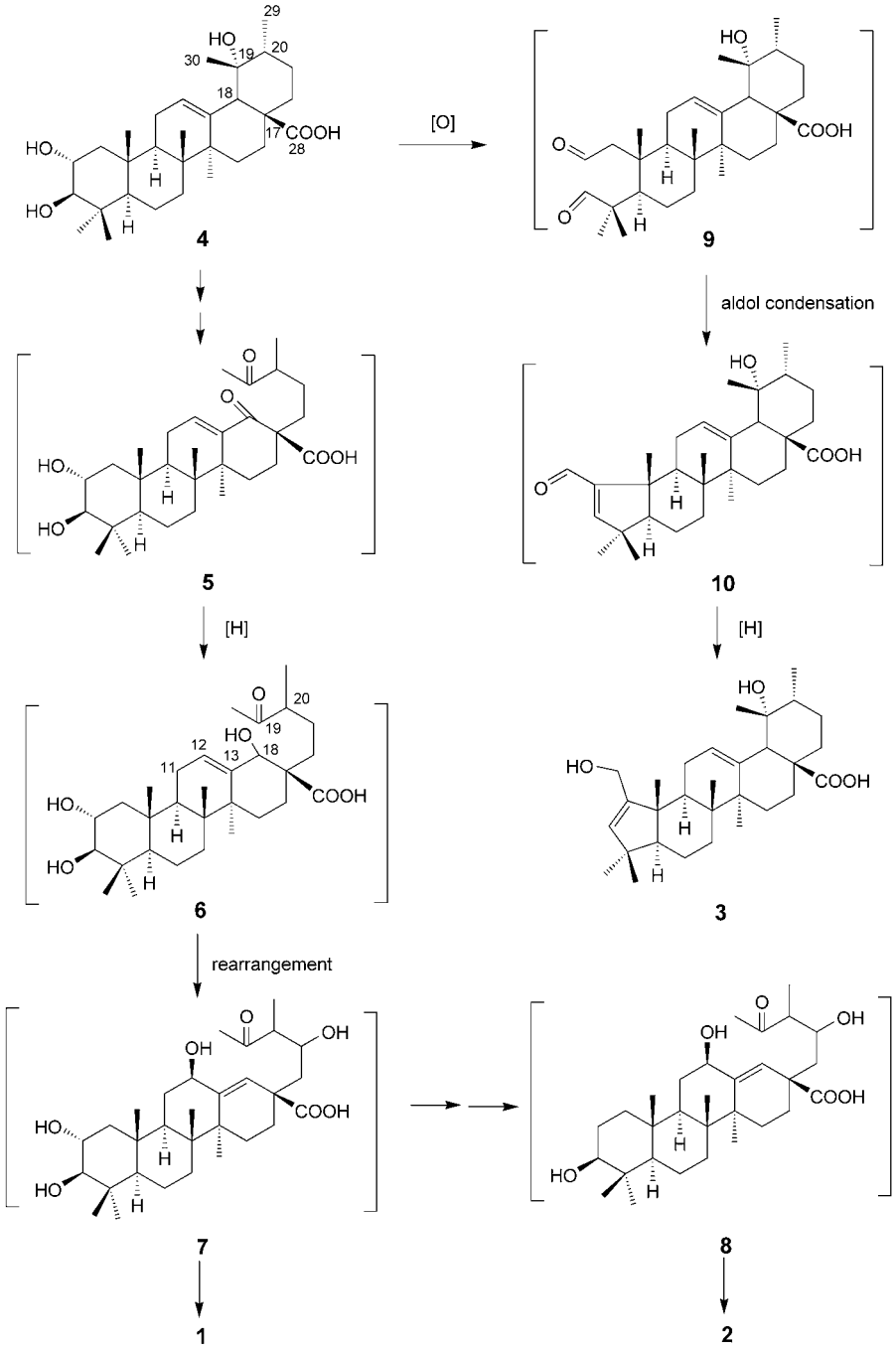


Fig. 2. *Cross-peaks from the NOESY experiment for bodinioside A* (**1**)

The structure of **2** was readily established by analogy with **1**, since the δ(H) and δ(C) of rings B–D and of the C(17) side chain corresponded to each other. The only difference was the lack of OCH (**1**: δ(H) 4.03 (*m* H–C(2)), δ(C) 66.8 (*d*)), which was replaced by a CH_2 signal in **2** (δ(H) 1.42–1.65 (*m* 2 H), δ(C) 26.7 (*t*)). Moreover, the signals of C(1) and C(3) were upfield-shifted in **2** (**1**: δ(C) 47.6 and 95.5; **2**: δ(C) 39.6 and 88.8), suggesting the absence of OH–C(2). The HMBC correlations H–C(2)/C(3), C(4), and C(10) and H–C(3)/C(2) confirmed this assignment. Therefore, the structure of bodinioside B (**2**) was assigned as (3β,12β)-3-(β-D-glucopyranosyloxy)-12,21-dihydroxy-19-oxo-18,19-secours-13(18)-en-28-oic acid.

Besides the two new 18,19-secourane glycosides **1** and **2**, a known A-ring-contracted ursane derivative, hypadienic acid (**3**), and 2,3,19-trihydroxyurs-12-en-28-oic acid (**4**), were isolated from the whole plant of *E. bodinieri*. This is of interest for the biogenetic relationship among these four compounds. Thus compounds **1**–**3** could be derived from **4** *via* the sequence shown in the *Scheme: 1*) Oxidation of **4** would yield 18,19-diketone intermediate **5**, which would be converted to intermediate **6** by reduction of the C(18)=O, and then rearranged to **7**. Dehydration of OH–C(2) of **7**, followed by reduction of the thus produced unsaturation could then yield **8**. Finally, the glycosylation at C(3) of **7** and **8** would produce **1** and **2**. *2*) Oxidation of **4** catalyzed by dioxygenase would give intermediate **9**, which could undergo an aldol condensation to give intermediate **10**. Reduction of the latter would generate **3**.

Scheme. *Plausible Biosynthetic Pathway of Compounds* **1**–**4**



The discovery of the secotriterpenoids as natural products has been useful for the understanding of the biogenetic pathways in organisms. To date, several A-seco-[6–10], D-seco- [11][12], and A-ring-contracted [3][13] ursane derivatives have been found in diverse species. However, only one E-secoursane has been isolated [14], and no E-secoursane glycoside has been reported yet. This is the first report of the occurrence of two unusual 18,19-secoursane glycosides isolated from the plant family of the Labiatae.

Experimental Part

General. CC = Column chromatography. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad FtS-135* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker DRX-500* spectrometers; δ in ppm rel. to $SiMe_4$ as internal standard, J in Hz, in C_5D_5N solns. Mass spectra: *VG Autospec-3000* spectrometer; 70 eV for EI; m/z (rel. %).

Plant Material. The Pharmaceutical Factory of the Yunnan Institute of Materia Medica provided the samples of *E. bodinieri*.

Extraction and Isolation. The powdered air-dried whole plants (6.0 kg) were extracted with 70% aq. Me_2CO (3×26 l) at r. t. overnight. The extract was partitioned between H_2O and AcOEt. The AcOEt extract (610 g) was subjected to CC (*MCI*-gel *CHP 20P*, 90% MeOH/H_2O): *Fractions A* (with 90% MeOH; 500 g) and *B* (with MeOH). *Fr. A* was subjected to CC (silica gel) and eluted with $CHCl_3$ (9 g, *Fr. 1*), $CHCl_3$/MeOH 50 : 1 (15 g, *Fr. 2*), 20 : 1 (18 g, *Fr. 3*), 10 : 1 (56 g, *Fr. 4*; 14 g, *Fr. 5*), 6 : 1 (12 g, *Fr. 6*; 34 g, *Fr. 7*), 4 : 1 (6 g, *Fr. 8*; 16 g, *Fr. 9*), 2 : 1 (23 g, *Fr. 10*; 6 g, *Fr. 11*), 2 : 3 (90.6 g, *Fr. 12*; 23 g, *Fr. 13*; 31 g, *Fr. 14*), and MeOH (*Fr. 15*). *Fr. 4* (56 g) was further subjected to CC (silica gel, $CHCl_3$/Me_2CO 20 : 1 → 10 : 3): **3** (with $CHCl_3$/Me_2CO 4 : 1; R_f 0.59; 8 mg) and **4** (with $CHCl_3$/Me_2CO 4 : 1; R_f = 0.26, 47 mg). *Fr. 7* (34 g) was purified by CC (silica gel, ($CHCl_3$/MeOH/H_2O 8 : 1 : 0.1 → 3 : 1 : 0.1); **2** (with $CHCl_3$/MeOH 9 : 1; R_f 0.38; 1.66 g). *Fr. 8* (6 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 20 : 1 → 4 : 1): **1** (with $CHCl_3$/MeOH 9 : 1, R_f 0.28; 88 mg).

*(2α,3β,12β)-3-(β-*D*-Glucopyranosyloxy)-2,12,21-trihydroxy-19-oxo-18,19-urs-13(18)-en-28-oic Acid* (= *bodinioside A*; **1**): Colorless flake crystals. M.p. 242–243°. $[\alpha]_D^{22.1} = -95.95$ ($c = 0.20$, C_5H_5N). UV: end absorption. IR (KBr): 3462, 3385, 2947, 2879, 1741, 1711, 1640, 1457, 1108, 1073. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 520 (1, [M – glucose]$^+$), 484 (3), 440 (7), 205 (12), 189 (12), 173 (17), 159 (20), 145 (28), 119 (36), 105 (31), 91 (35), 73 (74). ESI-MS: 664 (100, [$M - H_2O$]$^+$). HR-ESI-MS: 663.3744 ([$M - H_2O - H$]$^+$, $C_{36}H_{55}O_{11}^+$, calc. 663.3727).

*(3β,12β)-3-(β-*D*-Glucopyranosyloxy)-12,21-dihydroxy-19-oxo-18,19-secours-13(18)-en-28-oic Acid* (= *Bodinioside B*, **2**): Colorless flake crystals. M.p. 188–190°. $[\alpha]_D^{21.9} = -88.24$ ($c = 0.26$, C_5H_5N). IR (KBr): 3455, 3374, 2941, 2875, 1746, 1713, 1634, 1456, 1358, 1109, 1071, 1031. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 504 (1, [M – glucose]$^+$), 468 (45), 450 (10), 424 (20), 407 (12), 381 (10), 207 (45), 189 (78), 173 (48), 157 (43), 145 (55), 135 (63), 119 (72), 105 (74), 93 (70). ESI-MS: 647 (100, [$M - H_2O - H$]$^+$). HR-ESI-MS: 647.3795 ([$M - H_2O - H$]$^+$, $C_{36}H_{55}O_{10}^+$, calc. 647.3776).

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