Eudesmane-Type Sesquiterpene Derivatives from *Saussurea conica*

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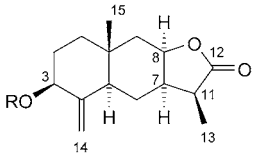
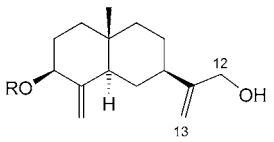
Four new eudesmane-type sesquiterpene derivatives, 3β-[(β-D-glucopyranosyl)oxy]-11αH-eudesm-4(14)-en-12,8β-olide (**1**), (3β)-eudesma-4(14),11(13)-diene-3,12-diol (**2**), 3β-[(β-D-glucopyranosyl)oxy]eudesma-4(14),11(13)-dien-12-ol (**3**), and 3β-[(β-D-glucopyranosyl)oxy]eudesm-4(14)-en-11-ol (**4**), together with the known (3β)-eudesm-4(14)-ene-3,11-diol (**5**) were isolated from *Saussurea conica*, and their structures were elucidated both spectroscopically and by chemical methods.

Introduction. – The whole plants of the *Saussurea genus* (Asteraceae), precious herb medicines in both traditional Chinese and Tibet folklore medicine, are being used to cure rheumatic arthritis, dysmenorrhea and gynopathy [1]. A variety of sesquiterpene derivatives from this genus have been reported to possess interesting biological activities [2]. The plant *Saussurea conica* C. B. CLARKE has not been investigated chemically so far. In preliminary investigations, we found that *S. conica* contains several sesquiterpene derivatives. Herein, we present the isolation and structural identification of four new and one known eudesmane-type sesquiterpene derivatives, *i.e.*, **1**–**4** and **5**, respectively.



1 R = Glc



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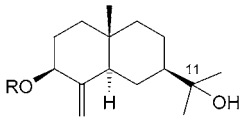
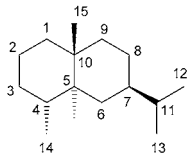
3 R = Glc

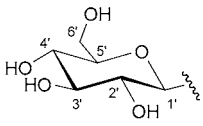


4 R = Glc
5 R = H

Eudesmane



Glc =



Results and Discussion. – Compound **1** was obtained as a white amorphous powder. Its molecular formula ($C_{21}H_{32}O_8$) was deduced by positive- and negative-mode ESI-MS (*m/z* 435.2 ($[M+Na]^+$) and 411.4 ($[M-H]^-$), respectively) in combination with

^{13}C-NMR (DEPT) spectral data. The signals of a β-D-glucopyranosyl (Glc) moiety were observed in the ^{1}H- and ^{13}C-NMR spectra of **1** (see *Tables 1* and *2*, resp.). The remaining 15 C-atoms of the aglycone were identified as a *singlet* Me, a *doublet* Me, five CH_2 (one olefinic), and five CH (two oxygenated) groups, as well as three quaternary C-atoms (including one olefinic and one C=O group). The quaternary C-atom at δ(C) 149.06 and the signal for an olefinic secondary C-atom at δ(C) 105.70 indicated the presence of an exocyclic C=C bond. The aforementioned spectral data suggested that compound **1** was a glucoside of a tricyclic sesquiterpene. The aglycone moiety was further identified as a sesquiterpene lactone of the eudesmane type (see chemical formulae) by extensive analysis of ^{13}C-NMR (DEPT) and ^{1}H-NMR spectral data. Thus, compound **1** was identified as 3β-[(β-D-glucopyranosyl)oxy]-11αH-eudesm-4(14)-en-12,8β-olide.

In the HMBC spectrum of **1**, the signal at δ(H) 2.83 (H–C(11)) correlated with both δ(C) 9.59 (*d*, Me(13)) and δ(C) 179.28 (*s*, C(12)=O). The signal at δ(H) 4.50 (*dd*, J = 4.7, 11.4 Hz, H–C(3)) correlated with the anomeric C-atom of the Glc moiety at δ(C) 103.26 (C(1′)), as well as with the olefinic signals at δ(C) 105.70 (CH_2(14)) and 149.06 (C(4)) in the HMBC spectrum, indicating the presence of a 3-*O*-Glc moiety and $\Delta^{4(14)}$ unsaturation. The signal at δ(H) 0.79 (*s*, Me(15)) correlated with δ(C) 34.87 (*s*, C(10)). The resonance at δ(H) 4.38 (br. *s*, H–C(8)) showed a cross-peak with the C=O signal, indicating that the lactone group was between C(8) and C(12), as in many sesquiterpenes of the eudesmane type. The final assignment of all atoms was based on a combination of ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*, resp.), HMQC, HMBC, and NOESY spectra. From the NOESY experiment (*Figure*), the relative configuration of **1** could also be determined.

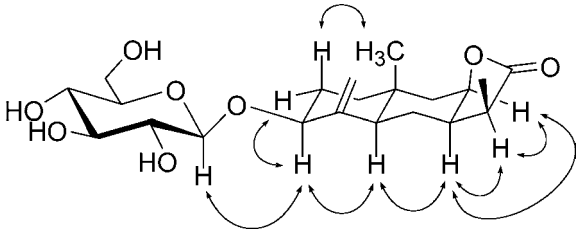


Fig. 1. *Key NOESY correlations for the eudesmenolide* **1**

The molecular formula of **2** was found to be $C_{15}H_{24}O_2$, as deduced by HR-EI-MS (*m/z* 236.1774 (M^+; calc. 236.1776)). The ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*) inferred that compound **2** was a sesquiterpene. Fifteen C-atom signals were observed: one Me, eight CH_2 (two olefinic and one oxygenated), three CH (one oxygenated) groups, and three quaternary C-atoms (two olefinic), thus, indicating the presence of two terminal (exocyclic) C=C bonds. The mass-spectrum fragments and NMR data of **2** were very similar to 3α-hydroxycostol [3] (see *Table 1*), except for the coupling constants for H–C(3) at δ(H) 4.02 (*dd*, J = 5.6, 11.4 Hz) in **2** (the coupling constant of H–C(3) of 3α-hydroxycostol was only 2.8 Hz), indicating the presence of a 3β-OH group in **2**. The slightly altered chemical shifts for H–C(3), H–C(5), and CH_2(14) in **2** relative to those of 3α-hydroxycostol (*Table 1*) could be rationalized by a different configuration at C(3). Thus, compound **2** was identified as (3β)-eudesma-4(14),11(13)-diene-3,12-diol.

Compound **3** showed a molecular formula of $C_{21}H_{34}O_7$, as deduced by positive- and negative-mode ESI-MS (*m/z* 421.2 ($[M + Na]^+$) and 397.3 ($[M - H]^-$)) in combination with ^{13}C-NMR (DEPT) experiments. The presence of a β-D-glucopyranosyl moiety was

Table 1. *400-MHz ^{1}H-NMR Data of the New Compounds* **1**–**4** *and of the Reference Substance 3α-Hydroxycostol* [3]. Solvents: (D_5)pyridine (**1**), $CDCl_3$ (**2** and 3α-hydroxycostol), CD_3OD (**3** and **4**). δ in ppm, *J* in Hz. Asterisks mark overlapping signals.

H-Atom	**1**	**2**	**3**	**4**	3α-Hydroxycostol[a])
CH_2(1)	1.05 (*dt*, *J* = 2.8, 10.8)	1.37, 1.50 (*m*)	1.38, 1.48 (*m*)	1.37, 1.47 (*m*)	–
CH_2(2)	1.70, 2.05 (*m*)	1.51, 1.99 (*m*)	1.61, 1.97 (*m*)	1.60, 1.97 (*m*)	–
H–C(3)	4.50 (*dd*, *J* = 4.7, 11.4)	4.02 (*dd*, *J* = 5.6, 11.4)	4.20 (*dd*, *J* = 5.4, 11.6)	4.19 (*dd*, *J* = 5.4, 11.5)	4.23 (*t*, *J* = 2.8)
$H_α$–C(5)	1.53 (br. *d*, *J* = 12.2)	1.77 (br. *d*, *J* = 12.7)	1.77 (br. *d*, *J* = 10.8)	1.69 (br. *d*, *J* = 11.1)	2.30 (br. *d*, *J* = 12.5)
CH_2(6)	1.45 (*dd*, *J* = 5.4, 13.4), 1.21*	1.39, 1.62 (*m*)	1.42, 1.58 (*m*)	1.25, 1.63 (*m*)	–
$H_α$–C(7)	2.25 (*m*)	2.04 (*m*)	2.03 (*m*)	1.35 (*m*)	–
CH_2(8) [b])	4.38 (br. *s*)	1.46, 1.63 (*m*)	1.45, 1.63 (*m*)	1.32, 1.63 (*m*)	–
CH_2(9)	2.02 (br. *d*, *J* = 15.5), 1.21*	1.24 (*dt*, *J* = 4.2, 13.8), 1.58 (*m*)	1.25, 1.60 (*m*)	1.21, 1.55 (*m*)	–
$H_α$–C(11)	2.83 (*m*)	–	–	–	–
CH_2(12) [c])	–	4.12 (*s*)	4.06 (*s*)	1.16 (*s*)	4.08 (*s*)
CH_2(13) [c])	1.18 (*d*, *J* = 7.1)	4.94, 5.06 (br. *s*)	4.91, 5.03 (br. *s*)	1.16 (*s*)	4.87, 4.99 (*s*)
CH_2(14)	4.78, 6.13 (br. *s*)	4.62, 5.05 (br. *s*)	4.60, 5.39 (br. *s*)	4.63, 5.39 (br. *s*)	4.51, 4.87 (*s*)
Me(15)	0.79 (*s*)	0.73 (*s*)	0.74 (*s*)	0.71 (*s*)	0.66 (*s*)
H–C(1′)	5.08 (*d*, *J* = 7.9)	–	4.37 (*d*, *J* = 7.8)	4.38 (*d*, *J* = 7.7)	–
H–C(2′)	4.12 (*t*-like, *J* = 7.3)	–	3.23 (*m*)	3.25 (*m*)	–
H–C(3′)	4.25 (*m*)	–	3.33 (*m*)	3.25 (*m*)	–
H–C(4′)	4.23 (*m*)	–	3.28 (*m*)	3.27 (*m*)	–
H–C(5′)	4.00 (*t*-like, *J* = 7.0)	–	3.23 (*m*)	3.23 (*m*)	–
CH_2(6′)	4.62 (br. *d*, *J* = 11.5), 4.40 (*dd*, *J* = 5.7, 11.5)	–	3.88 (*dd*, *J* = 2.2, 11.9), 3.68 (*dd*, *J* = 5.8, 11.9)	3.86 (*dd*, *J* = 2.1, 11.9), 3.64 (*dd*, *J* = 5.7, 11.9)	–

[a]) Diagnostic signals from [3]. [b]) $H_α$–C(8) in the case of **1**. [c]) Me groups in the case of **4**.

inferred from the ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*, resp.). Except for the sugar moiety, the NMR spectral data of the aglycone of **3** were very similar to those of **2**. Acid hydrolysis of **3** afforded D-glucose (Glc) and an aglycone, the latter being identical with that of **2** according to TLC, optical rotation, ^{1}H-NMR, and EI-MS. In the HMBC spectrum of **3**, an oxygenated CH group at δ(C) 80.39 (C(3)) showed correlations with the CH_2(14) resonances at δ(H) 4.60 (br. *s*) and 5.39 (br. s), and with the anomeric H-atom of the Glc moiety at δ(H) 4.37 (*d*, $J = 7.8$ Hz, H–C(1′)), indicating that the glucopyranosyloxy moiety was at C(3). From these results and 2D-NMR experiments (HMQC and HMBC), the structure of **3** was elucidated as 3β-[(β-D-glucopyranosyl)oxy]eudesma-4(14),11(13)-dien-12-ol.

Table 2. *100-MHz ^{13}C-NMR Data of Compounds* **1**–**5**. Solvents: (D_5)pyridine (**1**), $CDCl_3$ (**2** and **5**), CD_3OD (**3** and **4**). δ in ppm, J in Hz.

Position	**1**	**2**	**3**	**4**	**5**
1	40.38	39.64	41.34	41.32	39.67
2	31.32	32.52	32.73	32.77	32.82
3	78.54	73.11	80.39	80.43	73.32
4	149.06	152.53	150.90	151.17	153.04
5	45.16	48.01	49.93	49.82	47.99
6	21.86	29.78	31.59	26.62	25.01
7	40.31	41.01	42.86	50.84	49.18
8	77.83	27.07	28.83	23.86	22.31
9	41.47	40.61	42.31	42.33	40.67
10	34.87	35.58	37.09	37.01	35.58
11	41.92	153.62	155.63	73.62	72.85
12	179.28	64.86	65.60	27.18	27.02
13	9.59	107.79	108.51	27.60	27.29
14	105.70	102.36	105.01	104.92	102.23
15	18.07	16.28	17.12	17.11	16.31
1′	103.26		103.35	103.33	
2′	75.74		75.82	75.83	
3′	78.82		78.50	78.50	
4′	72.02		72.11	72.12	
5′	78.82		78.23	78.23	
6′	63.10		63.15	63.15	

Compound **4** was also found to be a sesquiterpene glycoside, as judged from its spectral data. A molecular formula of $C_{21}H_{36}O_7$ was deduced by positive- and negative-mode ESI-MS (*m/z* 423.1 ($[M+Na]^+$) and 399.4 ($[M-H]^-$)) in combination with ^{13}C-NMR (DEPT) experiments. Acid hydrolysis of **4** yielded D-glucose (Glc) and an aglycone identical to (3β)-eudesm-4(14)-ene-3,11-diol (**5**), isolated previously from this plant [4]. Comparison of ^{13}C-NMR spectral data (*Table 2*) revealed that C(3) at δ(C) 80.43 of **4** was shifted downfield by *ca.* 7.1 ppm relative to **5** due to glucosylation, indicating that the sugar moiety was at C(3). This was corroborated by HMBC correlations between C(3) and CH_2(14) (δ(H) 4.63, 5.39 (2*d*, $J = 1.1$ Hz)), as well as between C(3) and C(1′) of the Glc moiety at δ(H) 4.38 (*d*, $J = 7.7$ Hz). The Glc moiety was assigned the β-configuration according to the large coupling constant ($J = 7.7$ Hz) of the anomeric H-atom. Compound **4** was, thus, identified as 3β-[(β-D-glucopyranosyl)oxy]eudesm-4(14)-en-11-ol.

Experimental Part

General. Solvents were of anal. grade (*Shanghai Chemical Plant*). Thin-layer chromatography (TLC): precoated silica-gel-GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): Silica gel (200–300 mesh) or *MCI GEL CHP20P* (75–150 μm; *Mitsubishi Chemical Industries*); reverse-phase (RP) CC: C_{18} silica gel (150–200 mesh; *Merck*). Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* and *Varian Mercury-400* spectrometers; at 400 (^1H) and 100 (^{13}C) MHz; δ in ppm rel to $SiMe_4$ (= 0 ppm), J in Hz. EI-MS (70 eV): *Finnigan MAT-95* mass spectrometer, in m/z (rel. %).

Plant Material. The whole plant of *Saussurea conica* was collected in September 2000 in the Tibet Autonomous Region of China, and was identified by *H. L.* A voucher specimen (Access No. Sc-2000-2Y) was deposited at the Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. Dried and powdered *Saussurea conica* (whole plant; 1.5 kg) was extracted with 95% aq. EtOH at r.t. to give, after evaporation, a crude extract (128 g). The residue was suspended in H_2O (1500 ml) and extracted with $CHCl_3$ and BuOH to afford water-soluble (*W*; 46 g), $CHCl_3$-soluble (*CL*; 47 g), and BuOH-soluble (*BU*; 32 g) fractions, respectively. The BuOH extract (30 g) was subjected to CC (*MCI CHP20P*; H_2O (1500 ml)[1]), then MeOH/H_2O 20:80, 40:60, 60:40, and 100:0 (1000 ml each)) to give fraction *BU-4* (1.57 g), which contained mainly sesquiterpene glycosides (TLC). *BU-4* was subjected to CC (SiO_2; $CHCl_3$/MeOH 8:1, 6:1, and 4:1 (800 ml each)): fractions *BU-4a–e*. Compound **1** (9 mg) was obtained from *BU-4b* by CC (*Sephadex LH-20*; EtOH/H_2O 70:30). *BU-4c* was further purified by CC (*Sephadex LH-20*; EtOH/H_2O 70:30) and RP-CC (C_{18} SiO_2; column: 1.5 × 30 cm; MeOH/H_2O 55:45 → 60:40 (180 ml each)) to give compounds **3** (14 mg) and **4** (17 mg). The *CL* fraction (45 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0, 20:1, 15:1, 9:1, 6:1, and 4:1 (4500 ml each)): fractions *CL-1–7*. *CL-5* (3.03 g) was subjected to CC (SiO_2; petroleum ether/acetone 4:1, 3:1, and 2:1 (1200 ml each)) to afford fractions *CL-5c* and *CL-5d*, among other mixtures. From *CL-5c*, **5** (120 mg) was isolated by RP-CC (C_{18} SiO_2; MeOH/H_2O 70:30 (250 ml each)). *CL-5d* was purified by CC (*Sephadex LH-20*, EtOH) to yield **2** (85 mg).

*3β-[(β-*D*-Glucopyranosyl)oxy]-11αH-eudesm-4(14)-en-12,8β-olide* (**1**). White amorphous powder. $[\alpha]_D^{20} = -61.2$ ($c = 1.10$, pyridine). IR (KBr): 3507, 3423, 3302, 2946, 2861, 1747, 1647, 1454, 1355, 1180, 1125, 1077, 1022, 964, 939. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 847.3 (82, $[2M + Na]^+$), 435.2 (100, $[M + Na]^+$); ESI-MS (neg.): 823.7 (100, $[2M - H]^-$), 411.4 (53, $[M - H]^-$).

(3β)-Eudesma-4(14),11(13)-diene-3,12-diol (**2**). White amorphous powder. $[\alpha]_D^{20} = +34.5$ ($c = 0.290$, MeOH). IR (KBr): 3280, 2919, 2848, 1650, 1637, 1446, 1433, 1047, 1022, 899, 891. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 236 (24, M^+), 218 (25), 203 (22), 133 (81), 107 (81), 105 (93), 101 (100), 91 (98), 79 (85). HR-EI-MS: 236.1774 (M^+, $C_{15}H_{24}O_2^+$; calc. 236.1776).

*3β-[(β-*D*-Glucopyranosyl)oxy]eudesma-4(14),11(13)-dien-12-ol* (**3**). Colorless gum. $[\alpha]_D^{20} = -31.9$ ($c = 0.500$, MeOH). IR (KBr): 3405, 2927, 1650, 1450, 1379, 1161, 1079, 1028, 899. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 819.3 (97, $[2M + Na]^+$), 421.2 (100, $[M + Na]^+$). ESI-MS (neg.): 795.5 ([90, $[2M - H]^-$), 397.3 (100, $[M - H]^-$).

*3β-[(β-*D*-Glucopyranosyl)oxy]eudesm-4(14)-en-11-ol* (**4**). Colorless gum. $[\alpha]_D^{20} = -17.6$ ($c = 0.560$, MeOH). IR (KBr): 3394, 2937, 1631, 1452, 1379, 1159, 1079, 1024, 910. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 823.5 (100, $[2M + Na]^+$), 423.1 (61, $[M + Na]^+$). ESI-MS (neg.): 799.3 (53, $[2M - H]^-$), 399.4 (100, $[M - H]^-$).

Acid Hydrolyses of Compounds **3** *and* **4**. Compound **4** (5 mg) was dissolved in 4 ml of a 3% aq. H_2SO_4/MeOH 1:1 soln., which was refluxed for 3 h. Then, the mixture was neutralized with 5% aq. $NaHCO_3$ soln. After workup, the crude product was purified by CC (*Sephadex LH-20*; EtOH) to give D-glucose (Glc), as identified by TLC and optical rotation, together with **5** (1.8 mg) as the aglycone. Compound **3** (4 mg) was subjected to the same procedure, affording Glc and **2** (1.1 mg).

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[1]) To remove small polar molecules such as sugars and amino acids.

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