Eudesmane-Type Sesquiterpene Derivatives from Saussurea conica

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Four new eudesmane-type sesquiterpene derivatives, 3β -[(β -D-glucopyranosyl)oxy]-11 α H-eudesm-4(14)en-12,8 β -olide (1), (3 β)-eudesma-4(14),11(13)-diene-3,12-diol (2), 3 β -[(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 (3 β)-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.



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

¹³C-NMR (DEPT) spectral data. The signals of a β -D-glucopyranosyl (Glc) moiety were observed in the ¹H- and ¹³C-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 CH₂ (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 ¹³C-NMR (DEPT) and ¹H-NMR spectral data. Thus, compound **1** was identified as 3β -[(β -D-glucopyranosyl)oxy]-11 α H-eudesm-4(14)-en-12,8 β -olide.

In the HMBC spectrum of **1**, the signal at $\delta(H) 2.83 (H-C(11))$ correlated with both $\delta(C) 9.59 (d, Me(13))$ and $\delta(C) 179.28 (s, C(12)=O)$. The signal at $\delta(H) 4.50 (dd, J=4.7, 11.4 Hz, H-C(3))$ correlated with the anomeric C-atom of the Glc moiety at $\delta(C) 103.26 (C(1'))$, as well as with the olefinic signals at $\delta(C) 105.70 (CH_2(14))$ 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 $\delta(H) 0.79 (s, Me(15))$ correlated with $\delta(C) 34.87 (s, C(10))$. The resonance at $\delta(H) 4.38 (br. s, H-C(8))$ showed a cross-peak with the C=O signal, indicating that the lactone group was between C(8) and C(12), as in many sequiterpenes of the eudesmane type. The final assignment of all atoms was based on a combination of ¹H- and ¹³C-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 ¹H- and ¹³C-NMR spectral data (*Tables 1* and 2) inferred that compound **2** was a sesquiterpene. Fifteen C-atom signals were observed: one Me, eight CH₂ (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 CH₂(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 ¹³C-NMR (DEPT) experiments. The presence of a β -D-glucopyranosyl moiety was

1	2	3	4	3α -Hydroxycostol ^a)
1.05 (dt, J = 2.8, 10.8)	1.37, 1.50 (<i>m</i>)	1.38, 1.48 (<i>m</i>)	1.37, 1.47 (<i>m</i>)	-
1.70, 2.05 (<i>m</i>)	1.51, 1.99 (<i>m</i>)	1.61, 1.97 (<i>m</i>)	1.60, 1.97 (<i>m</i>)	-
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)
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. $d, J = 12.5$)
1.45 (dd, J = 5.4, 13.4), 1.21*	1.39, 1.62 (<i>m</i>)	1.42, 1.58 (<i>m</i>)	1.25, 1.63 (<i>m</i>)	-
2.25 (<i>m</i>)	2.04 (<i>m</i>)	2.03 (<i>m</i>)	1.35 (<i>m</i>)	-
4.38 (br. s)	1.46, 1.63 (<i>m</i>)	1.45, 1.63 (<i>m</i>)	1.32, 1.63 (<i>m</i>)	-
2.02 (br. $d, J = 15.5$), 1.21*	1.24 (dt, J = 4.2, 13.8),	1.25, 1.60 (m)	1.21, 1.55 (<i>m</i>)	-
	1.58 (<i>m</i>)			
2.83 (<i>m</i>)	_	_	-	-
_	4.12 (s)	4.06 (s)	1.16 (s)	4.08(s)
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)
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)
0.79 (s)	0.73 (s)	0.74(s)	0.71 (s)	0.66(s)
5.08 (d, J = 7.9)	_	4.37 (d, J = 7.8)	4.38 (d, J = 7.7)	-
4.12 (t-like, $J = 7.3$)	_	3.23 (<i>m</i>)	3.25 (<i>m</i>)	-
4.25 (<i>m</i>)	_	3.33 (<i>m</i>)	3.25 (<i>m</i>)	-
4.23 (<i>m</i>)	_	3.28 (<i>m</i>)	3.27 (<i>m</i>)	-
4.00 (t-like, $J = 7.0$)	_	3.23 (<i>m</i>)	3.23 (<i>m</i>)	-
4.62 (br. $d, J = 11.5$),	_	3.88 (dd, J = 2.2, 11.9),	3.86 (dd, J = 2.1, 11.9),	-
4.40 (dd, J = 5.7, 11.5)		3.68 (dd, J = 5.8, 11.9)	3.64 (<i>dd</i> , <i>J</i> = 5.7, 11.9)	

Table 1. 400-MHz ¹H-NMR Data of the New Compounds 1-4 and of the Reference Substance 3a-Hydroxycostol [3]. Solvents: (D₅)pyridine (1), CDCl₃ (2 and 3ahydroxycostol), CD₃OD (**3** and **4**). δ in ppm, J in Hz. Asterisks mark overlapping signals.

^a) Diagnostic signals from [3]. ^b) $H_a - C(8)$ in the case of 1. ^c) Me groups in the case of 4.

H-Atom

 $CH_{2}(1)$

 $CH_2(2)$

H-C(3) $H_a - C(5)$

 $CH_2(6)$

 $H_a - C(7)$

 $CH_{2}(8)^{b}$

 $H_a - C(11)$

 $CH_2(12)$ °)

 $CH_2(13)$ °)

 $CH_{2}(14)$ Me(15) H-C(1')H-C(2')

H-C(3')H-C(4')H - C(5')CH₂(6')

 $CH_2(9)$

inferred from the ¹H- and ¹³C-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, ¹H-NMR, and EI-MS. In the HMBC spectrum of **3**, an oxygenated CH group at $\delta(C)$ 80.39 (C(3)) showed correlations with the CH₂(14) resonances at $\delta(H)$ 4.60 (br. *s*) and 5.39 (br. s), and with the anomeric H-atom of the Glc moiety at $\delta(H)$ 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 ¹³C-NMR Data of Compounds 1-5. Solvents: (D₅)pyridine (1), CDCl₃ (2 and 5), CD₃OD (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 ¹³C-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 ¹³C-NMR spectral data (*Table 2*) revealed that C(3) at $\delta(C)$ 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 CH₂(14) ($\delta(H)$ 4.63, 5.39 (2d, J = 1.1 Hz)), as well as between C(3) and C(1') of the Glc moiety at $\delta(H)$ 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-GF₂₅₄ plates (Qingdao Haiyang Chemical Plant). Column chromatography (CC): Silica gel (200–300 mesh) or MCI GEL CHP20P (75–150 μ m; Mitsubishi Chemical Industries); reverse-phase (RP) CC: C₁₈ 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 (¹³C) MHz; δ in ppm rel to SiMe₄ (=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 H₂O (1500 ml) and extracted with CHCl₃ and BuOH to afford water-soluble (*W*; 46 g), CHCl₃-soluble (*CL*; 47 g), and BuOH-soluble (*BU*; 32 g) fractions, respectively. The BuOH extract (30 g) was subjected to CC (*MCI CHP20P*; H₂O (1500 ml)¹), then MeOH/H₂O 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 (SiO₂; CHCl₃/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/H₂O 70:30). *BU-4c* was further purified by CC (*Sephadex LH-20*; EtOH/H₂O 70:30). *BU-4c* was subjected to CC (SiO₂; CHCl₃/MeOH 3:1, 6:1, and 4:1 (4500 ml each)): fractions (*L-1 – 7. CL-5*) (3.03 g) was subjected to CC (SiO₂; petroleum ether/acetone 4:1, 3:1, and 2:1 (1200 ml each)) to afford fractions *CL-5c*, **a** (*CL-5d*, among other mixtures. From *CL-5c*, **5** (120 mg) was isolated by RP-CC (*C₁₈* SiO₂; MeOH/H₂O 70:30 (250 ml each)). *CL-5d* was purified by CC (*Sephadex LH-20*, EtOH) to yield **2** (85 mg).

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

 (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 ¹³C-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. [a]₂₀²⁰ = -31.9 (c = 0.500, MeOH). IR (KBr): 3405, 2927, 1650, 1450, 1379, 1161, 1079, 1028, 899. ¹H- and ¹³C-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. $[\alpha]_{20}^{20} = -17.6$ (c = 0.560, MeOH). IR (KBr): 3394, 2937, 1631, 1452, 1379, 1159, 1079, 1024, 910. ¹H- and ¹³C-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. NaHCO₃ 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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