New Lignan, Benzofuran, and Sesquiterpene Derivatives from the Roots of *Leontopodium alpinum* and *L. leontopodioides*

by **Michael J. Dobner**[a]), **Ernst P. Ellmerer**[b]), **Stefan Schwaiger**[a]), **Odonchimeg Batsugkh**[a]), **Samdan Narantuya**[c]), **Michaela Stütz**[a]), and **Hermann Stuppner***[a])

[a]) Institut für Pharmazie, Abteilung für Pharmakognosie, Universität Innsbruck, Josef-Möller-Haus, Innrain 52, A-6020 Innsbruck

[b]) Institut für Organische Chemie, Universität Innsbruck, Innrain 52a, A-6020 Innsbruck

[c]) Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia

Three new compounds, including a benzofuran, 1-{(2*R**,3*S**)-3-(β-D-glucopyranosyloxy)-2,3-dihydro-2-[1-(hydroxymethyl)vinyl]-1-benzofuran-5-yl}ethanone (**1**), a lignan, [(2*S*,3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)tetrahydrofuran-3-yl]methyl (2*E*)-2-methylbut-2-enoate (**2**), and a silphiperfolene-type sesquiterpene, [(1*S*,2*Z*,3a*S*,5a*S*,6*R*,8a*R*)-1,3a,4,5,5a,6,7,8-octahydro-1,3a,6-trimethylcyclopenta[*c*]pentalen-2-yl]methyl acetate (**3**), together with the known coumarins obliquin (**4**) and its 5-methoxy derivative **5** were isolated from the roots of *Leontopodium alpinum*. Another known coumarin derivative, 5-hydroxyobliquin (**6**), was isolated from the roots of *L. leontopodioides*. The structures of these compounds were established by spectroscopic studies.

Introduction. – *Leontopodium alpinum* CASS. (Asteraceae) is indigenous to the mountainous regions of Europe. In alpine folk medicine, extracts of this plant are used for the therapy of abdominal aches, angina, bronchitis, cancer, diarrhea, and dysentery [1–5]. *L. leontopodioides* BEAUVERD is distributed in central Asia, and is used in Tibetan medicine together with *L. dedekensii* BEAUVERD, *L. franchetii* BEAUVERD, *L. palibinianum* BEAUVERD, *L. stracheyi* C. B. CLARKE *ex* HEMSL., and various *Anaphalis* species to treat diseases of the lymph nodes and poisoning with minerals and metals [6]. In Mongolian folk medicine, *L. campestre* HAND.-MAZZ., *L. conglobatum* HAND.-MAZZ., *L. leontopodioides*, and *L. ochroleucum* BEAUVERD are used for the therapy of cancer, diarrhoea, dysentery, heart disease, hepatitis, and jaundice [7].

Phytochemical investigations of *L. alpinum* resulted in the identification of flavonoids, phenolic acids, hexahydrofarnesylacetone, and one chromane derivative as well as isocomene, modhephene, and caryophyllene-type sesquiterpenes [8–13]. Caffeic acid and vanillic acid are reported for *L. leontopodioides* [14].

Recently, we reported the isolation of new bisabolane-type sesquiterpenes from the roots of *L. alpinum* [15]. We now describe the isolation and structural elucidation of new lignan, benzofuran, and sesquiterpene derivatives as well as coumarins from *L. alpinum* and *L. leontopodioides.*

Results and Discussion. – ESI-MS of compound **1** showed quasi-molecular-ion peaks at *m*/*z* 419 ($[M+Na]^+$), 414 ($[M+H_2O]^+$), and 397 ($[M+H]^+$). HR-FAB-MS established the molecular formula of $C_{19}H_{24}O_9$. ^{1}H- and ^{13}C-NMR Spectra (*Tables 1* and *2*) suggested the presence of β-D-glucose, a 1-(hydroxymethyl)vinyl group, an ethanone

Table 1. *^{1}H-NMR Spectra* (500 MHz; δ in ppm, J in Hz) *of Compounds* **1**–**3**[1])

1[a])		**2**[b])		**3**[b])	
H–C(2)	5.29 (d, J = 7.0)	H–C(2)	4.87 (d, J = 6.4)	H–C(1)	2.79, (qt, J = 7.5, 1.0)
H–C(3)	5.44 (d, J = 7.0)	H–C(3)	2.60 (m)	H–C(3)	5.28 (br. s)
H–C(4)	8.32 (d, J = 2.0)	H–C(4)	2.75 (m)	CH_2(4)	1.66 (m)[c])
H–C(6)	7.96 (dd, J = 8.5, 2.0)	CH_2(5)	3.77 (dd, J = 8.7, 6.4)		1.15, (m)[c])
H–C(7)	6.92 (d, J = 8.5)		4.08 (dd, J = 8.7, 6.4)	CH_2(5)	1.38 (m)[c])
Me(9)	2.58 (s)	CH_2(6)	4.28 (dd, J = 11.4, 7.1)		1.28 (m)[c])
CH_2(11)	5.42, 5.38 (2s)		4.41 (dd, J = 11.4, 7.1)	H–C(5a)	1.86 (t, J = 7.0)
CH_2(12)	4.18, 4.23	MeO	3.87 (s)	H–C(6)	1.41 (m)[c])
	(each d, J = 13.9)	2 MeO	3.86 (s)[c])	CH_2(7)	1.56 (m)[c])
		MeO	3.85 (s)		1.39 (m)[c])
β-D-Glucose:		Benzyl moiety:		CH_2(8)	1.66 (m)[c])
H–C(1′)	4.57 (d, J = 7.8)	H–C(2′)	6.78 (m)		1.55 (m)[c])
H–C(2′)	3.15 (m)	H–C(5′)	6.68 (m)	CH_2(9)	4.50 (m)
H–C(3′)	3.33 (m)[d])	H–C(6′)	6.72 (m)	MeC(1)	1.03 (d, J = 7.5)
H–C(4′)	3.31 (m)	H–C(7′)	2.57 (d, J = 13.2)	MeC(3a)	0.97 (s)
H–C(5′)	3.36 (m)[d])		2.90 (dd, J = 13.2, 4.6)	MeC(6)	0.97 (d, J = 7.0)
CH_2(6′)	3.76 (dd, J = 11.7, 5.7)	Phenyl moiety:		Acetate moiety:	
	3.97 (dd, J = 11.7, 2.3)	H–C(2″)	6.87 (m)	Me(β)	2.06 (3s)
		H–C(5″)	6.84 (m)		
		H–C(6″)	6.86 (m)		
		2-Methylbut-2-enoate moiety:			
		Me(γ)	1.87 (br. s)		
		H–C(δ)	6.12 (qq)		
		Me(ε)	1.99 (dd, J = 7.1, 1.4)		

[a]) In CD_3OD. [b]) In $CDCl_3$. [c]) Signals overlap. [d]) Signals may be interchanged.

unit, and a dihydrobenzofuran moiety. The linkages of the three partial structures were elucidated by HMBC experiments. Complete assignment of all NMR signals was achieved by application of 2D-NMR techniques (COSY, HMQC, HMBC, HSQC-TOCSY) (*Tables 1* and *2*) and established that **1** is 1-{(2*R**,3*S**)-3-(β-D-glucopyranosyloxy)-2,3-dihydro-2-[1-(hydroxymethyl)vinyl]-1-benzofuran-5-yl}ethanone. A similar compound with *cis* configuration at C(2) and C(3), 3-(angeloyloxy)-12-hydroxytremetone, has been previously isolated from the roots of *Helichrysum stirlingii* [16].

In the HMBC plot of **1**, cross-peaks between the methylene protons at δ(H) 5.38 and 5.42 (CH_2(11)) and at δ(H) 4.18 and 4.23 (CH_2(12)) and the ^{13}C-NMR signal at δ(C) 89.3 of C(2) supported the location of the 1-(hydroxymethyl)vinyl moiety at C(2) of the dihydrobenzofuran back bone[1]). HMBC between CH_3(9) (δ(H) 2.58) and C(5) (δ(C) 132.2), H–C(4) (δ(H) 8.32) and C(8) (δ(C) 199.6) as well as H–C(6) (δ(H) 7.96) and C(8) (δ(C) 199.6) confirmed that the ethanone moiety was attached to C(5) of the dihydrobenzofuran moiety. A HMBC cross-peak due to long-range correlation between the anomeric sugar proton H–C(1′) (δ(H) 4.57) and C(3) (δ(C) 81.1) established that the glucose moiety was linked to C(3) of the aglycone. A ROESY experiment showed coupling between the anomeric proton of the glucose moiety and H–C(2) of the benzofurane which suggested the *trans* configuration of the substituents at C(2) and C(3).

The ESI-MS of compound **2** resulted in quasi-molecular-ion peaks at m/z 493 ($[M+Na]^+$). The molecular formula was deduced to be $C_{27}H_{34}O_7$ from HR-EI-MS.

[1]) Arbitrary numbering; for systematic names, see *Exper. Part.*

Table 2. *^{13}C-NMR Data* (125 MHz; δ in ppm) *of Compounds* **1**–**3**[1])

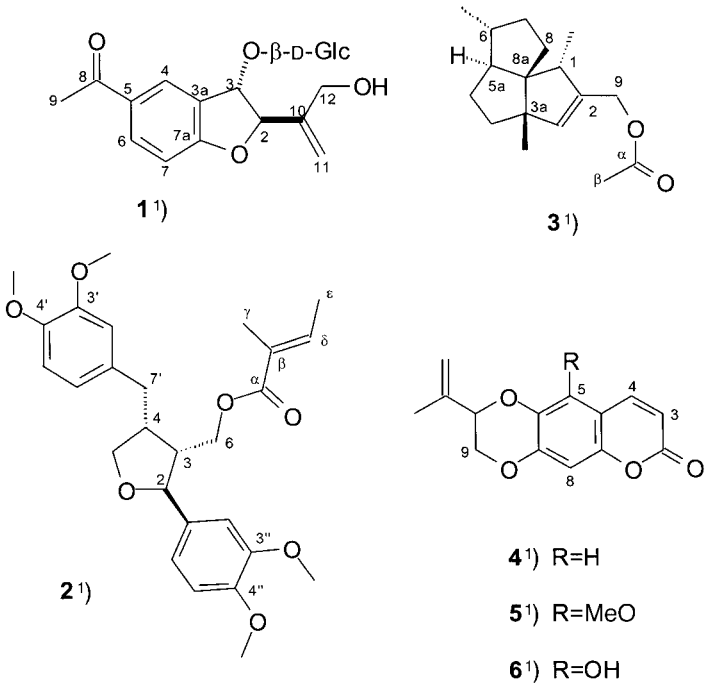
1[a])		**2**[b])		**3**[b])	
CH(2)	89.3	CH(2)	83.0	CH(1)	48.9
CH(3)	81.1	CH(3)	49.3	C(2)	139.4
C(3a)	129.2	CH(4)	42.8	CH(3)	137.4
CH(4)	130.9	CH_2(5)	72.8	C(3a)	57.1
C(5)	132.2	CH_2(6)	62.3	CH_2(4)	36.2
CH(6)	133.0	MeO	56.2	CH_2(5)	30.2
CH(7)	110.9	MeO	56.1	CH(5a)	52.5
C(7a)	165.5	MeO	56.0[c])	CH(6)	42.7
C(8)	199.6	MeO	56.0[c])	CH_2(7)	37.6
Me(9)	26.5	Benzyl moiety:		CH_2(8)	34.8
C(10)	144.8	C(1′)	132.8	C(8a)	65.6
CH_2(11)	114.4	CH(2′)	112.1	CH_2(9)	62.1
CH_2(12)	63.5	C(3′)	148.7	*Me*–C(1)	14.2
β-D-Glucose:		C(4′)	147.7	*Me*–C(3a)	20.2
CH(1′)	105.4	C(5′)	111.3	*Me*–C(6)	19.8
CH(2′)	75.2	CH(6′)	120.6	Acetate moiety:	
CH(3′)[c])	78.1	CH_2(7′)	33.3	C(α)	170.9
CH(4′)	71.5	Phenyl moiety:		Me(β)	21.1
CH(5′)[c])	78.2	C(1″)	135.2		
CH_2(6′)	62.8	CH(2″)	109.1		
		C(3″)	149.3		
		C(4″)	149.2		
		CH(5″)	111.6		
		CH(6″)	118.2		
		2-Methylbut-2-enoate moiety:			
		C(α)	167.8		
		C(β)	127.6		
		Me(γ)	20.7		
		CH(δ)	138.9		
		Me(ε)	15.9		

[a]) In CD_3OD. [b]) In $CDCl_3$. [c]) Signals are interchangeable.

^{1}H- and ^{13}C-NMR signals showed that **2** was a derivative of the lignan lariciresinol dimethyl ether [17–19]. Additional signals indicated a 2-methylbut-2-enoate moiety (for ^{1}H- and ^{13}C-NMR data, see *Tables 1* and *2*) connected to C(6)[1]). HMBC and NOESY Data confirmed that **2** is [(2*S*,3*R*,4*R*)-4-(3,4-dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)tetrahydrofuran-3-yl]methyl (2*E*)-methylbut-2-enoate.

In the HMBC plot of **2**, cross-peaks from CH_2(6) (δ(H) 4.28, 4.41) to the carboxylate C-atom (δ(C) 167.8) established the position of the latter at C(6). The configuration at the furan ring (2,3-*trans*) was confirmed by comparison of the ^{1}H-NMR data with those published for actifolin [18]: accordingly, the signal of H–C(2) was observed at δ(H) 4.87 compared to δ(H) 5.13 for the *cis*-isomer. The (*E*)-configuration of the butenoate moiety, *i.e.*, the presence of a tiglic acid ester, was deduced from a NOESY experiment and the shift value of the olefinic proton (δ(H) 6.12) [15].

The ESI-MS of the irregular sesquiterpene **3** exhibited a quasi-molecular-ion peak at *m*/*z* 285 ($[M+Na]^+$) and a major fragment at *m*/*z* 203 ($[M-60+H]^+$) indicating the presence of an acetate moiety. HR-EI-MS established the molecular formula $C_{17}H_{26}O_2$. ^{1}H- and ^{13}C-NMR signals were similar to those of 7α-silphiperfol-5-ene [20].



The relative configurations of Me–C(1), Me–C(3a), and Me–C(6) of **3** were in agreement with literature data [20][21] and were confirmed by selective 1D-NOESY experiments. Therefore, compound **3** is [(1*S*,2*Z*,3a*S*,5a*S*,6*R*,8a*R*)-1,3a,4,5,5a,6,7,8-octahydro-1,3a,6-trimethylcyclopenta[*c*]pentalen-2-yl]methyl acetate.

Except for the downfield-shifted signals at δ(C) 62.1 and δ(H) 4.50 as well as for additional signals of an acetyl moiety (δ(C) 170.9 and 21.1, δ(H) 2.06) the NMR spectra of **3** were in agreement with those of 7α-silphiperfol-5-ene [20]. The position of the acetoxy group at C(9) was confirmed by the HMBC long-range correlation between H–C(9) (δ(H) 4.50) and the carbonyl C-atom at δ(C) 170.9[1]). Obviously, the Me group at C(2) of 7α-silphiperfol-5-ene is replaced by an acetoxymethyl group, which is further confirmed by HMBC cross-peaks between CH_2(9) (δ(H) 4.50) and C(1) (δ(C) 48.9), C(2) (δ(C) 139.4), and C(3) (δ(C) 137.4). A similar compound has been isolated from an *Osteospermum* species [21].

Compounds **4**–**6** were identified as the known coumarins obliquin, 5-methoxyobliquin, and 5-hydroxyobliquin by ESI-MS and NMR data, which were in agreement with data reported by *Bohlmann* and *Zdero* [22].

This is the first report on the occurrence of coumarins, benzofurans, lignans, and silphiperfolene-type sesquiterpenes in the genus *Leontopodium*. Benzofurans, lignans, and silphiperfolene derivatives are typical constituents of many members of the Asteraceae family. In contrast, C(5)-substituted obliquin derivatives are specific markers for the tribe Gnaphalieae sensu Anderberg [23]. These substances have been detected so far in the genera *Helichrysum* [16][24], *Cassinia* [25], *Myriocephalus* [26], and *Actinobole* [25].

Experimental Part

General. TLC: silica gel *60* F_{254} plates (*Merck*, No. 5554); mobile phase: mixtures of petroleum ether, CH_2Cl_2, AcOEt, or acetone; detection: vanillin/sulfuric acid reagent and subsequent heating. Column chromatography (CC): silica gel *60* (*Merck* 230–400 mesh). *Lobar* chromatography: *Merck-RP-18* column (240 × 10 mm) particle size 40–63 mm; *Perkin-Elmer* series 3 liquid chromatograph pump; *Knauer* UV/VIS filter photometer detector, wavelength 220 nm; solvent: 32% MeOH/H_2O isocratic. M.p.: *Kofler* hot-stage; uncorrected. Optical rotations: *Perkin-Elmer* 341 polarimeter. IR Spectra: in cm^{-1}. 2D and ^1H- and ^{13}C-NMR: *Varian Unity plus 500* spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C); chemical shifts δ in ppm, coupling constants J in Hz; $SiMe_4$ as internal standard. HR-FAB-MS and HR-EI-MS: *Finnigan MAT 95 XL*. ESI-MS: *Finnigan-SSQ-7000* mass spectrometer (pos. mode).

Plant Material. L. alpinum roots were obtained as a gift from *Pentapharm Ltd.*, Switzerland. *L. leontopodioides* was collected in August 2000 near Bayanöndör sum, Orkhon Aimak, Mongolia. Voucher specimens are deposited at the Institute of Pharmacy, University of Innsbruck.

Extraction and Isolation. Air-dried *L. alpinum* roots (804 g) or *L. leontopodioides* roots (227 g), resp., were ground and exhaustively macerated with CH_2Cl_2 and finally with MeOH. The extracts were evaporated and the residues (CH_2Cl_2 extract of *L. alpinum:* 14.7 g; CH_2Cl_2 extract of *L. leontopodioides:* 8.9 g; MeOH extract of *L. alpinum:* 20.8 g), repeatedly submitted to CC (silica gel, gradients of petroleum ether/acetone, CH_2Cl_2/AcOEt, and CH_2Cl_2/MeOH, resp.). Obtained fractions were rechromatographed (*Sephadex LH-20*, MeOH) to give pure **2** (38 mg), **3** (90 mg), obliquin (**4**; 11 mg), 5-methoxyobliquin (**5**; 5 mg), and 5-hydroxyobliquin (**6**; 24.9 mg). Compound **1** had to be purified by *Lobar* chromatography (*RP-18*, 38% MeOH/H_2O): 18 mg of **1**. Compounds **1**–**5** were obtained from *L. alpinum*, **6** from *L. leontopodioides.*

*1-{(2*R**,3*S**)-3-(β-*D*-Glucopyranosyloxy)-2,3-dihydro-2-[1-(hydroxymethyl)ethenyl]-1-benzofuran-5-yl}-ethanone* (**1**). White crystals. M.p. 130° (dec.). $[\alpha]_D^{20} = +55$ (MeOH, $c = 0.002$). FT-IR (microspectrometry; $\tilde{\nu}_{max}^{ZnSe}$): 3474, 2936, 2880, 2360, 1660, 1604, 1489, 1438, 1385, 1302, 1269, 1203, 1154, 1125, 1074, 1028. NMR: *Tables 1* and *2*. FAB-HR-MS (pos.): 397.14590 ($[M+H]^+$, $C_{19}H_{25}O_9^+$; calc. 397.14985), 419.1329 ($[M+Na]^+$, $C_{19}H_{24}O_9^+$; calc. 419.1318).

*{(2*S*,3*R*,4*R*)-2-(3,4-Dimethoxyphenyl)-4-[(3,4-dimethoxyphenyl)methyl]tetrahydrofuran-3-yl]methyl (2*E*)-2-Methylbut-2-enoate* (**2**). Colorless amorphous substance. $[\alpha]_D^{20} = +25$ (CH_2Cl_2, $c = 0.002$). FT-IR (microspectrometry; $\tilde{\nu}_{max}^{ZnSe}$): 2935, 2835, 1715, 1649, 1592, 1516, 1464, 1419, 1354, 1262, 1235, 1158, 1029. NMR: *Tables 1* and *2*. ESI-MS: 963 ($[2M+Na]^+$),493 ($[M+Na]^+$). HR-EI-MS (pos.): 470.2290 (M^+, $C_{27}H_{24}O_7^+$; calc. 470.2304).

*[(1*S*,2*Z*,3a*S*,5a*S*,6*R*,8a*R*)-1,3a,4,5,5a,6,7,8-octahydro-1,3a,6-trimethylcyclopenta[c]pentalen-2-yl]methyl Acetate* (**3**). Yellow oil. $[\alpha]_D^{20} = -80$ (CH_2Cl_2, $c = 0.002$). FT-IR (microspectrometry; $\tilde{\nu}_{max}^{ZnSe}$): 2945, 2864, 1744, 1460, 1374, 1240, 1025. NMR: *Tables 1* and *2*. ESI-MS: 285 ($[M+Na]^+$), 203 ($[M-60+H]^+$). HR-EI-MS (pos.): 262.1934 (M^+, $C_{17}H_{26}O_2^+$; calc. 262.1932).

Obliquin (**4**), *5-Methoxyobliquin* (**5**), *and 5-Hydroxyobliquin* (**6**). Spectra and physical data in accordance with literature data [22].

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