Iridoid Glycosides from the Leaves of *Sambucus ebulus*

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Six new iridoid glycosides (**1**−**6**) of the "*Valeriana* type" were isolated from leaves of *Sambucus ebulus*. The structures were elucidated by 1D- and 2D-NMR spectroscopy, mass spectrometry, and chemical degradation methods as 10-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-*β*-D-ribohexo-3-ulopyranoside] (**1**), 7-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-*β*-D-ribohexo-3-ulopyranoside] (**2**), 10-*O*-acetylpatrinoside-aglycone-11-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-ribohexo-3-ulopyranoside] (**3**), patrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-*β*-D-ribohexo-3-ulopyranoside] (**4**), 10-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside] (**5**), and patrinoside-aglycone-11-*O*-2′-deoxy-*β*-D-glucopyranoside (**6**). Compounds **1**−**4** represent the first examples of acylated iridoid diglycosides bearing the uncommon D-ribohexo-3-ulopyranosyl sugar moiety. Compound **6** is the first iridoid glycoside with a 2-deoxy-D-glucopyranosyl sugar moiety.

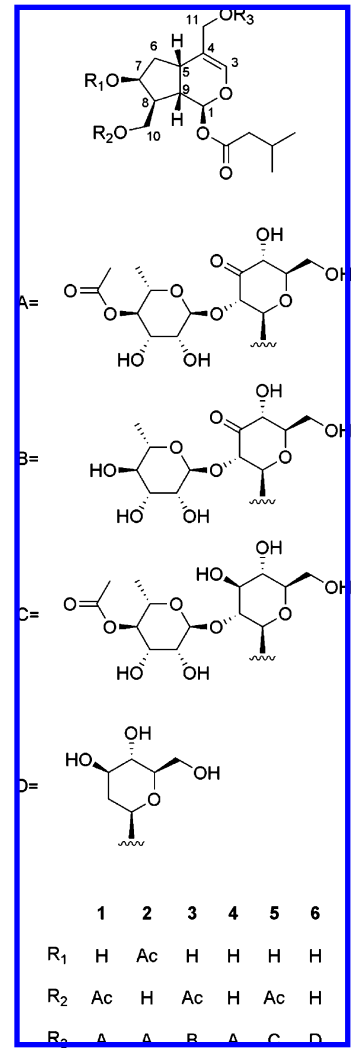
Sambucus ebulus L. (Adoxaceae), commonly referred to as "dwarf elder", is a perennial herbaceous plant distributed in most of Europe, northern Africa, and western Asia.[1] Various parts of the plant are used in traditional medicine as a remedy for several pathological processes related to inflammation and as a diuretic.[1,2] Extracts obtained from the plant have shown anti-inflammatory,[2−6] antinociceptive,[2] diuretic,[7] hypotensive,[8] anti-*Helicobacter pylori*,[9] and antioxidative[10,11] properties.

Hitherto, phytochemical investigations have been focused on the root material of *S. ebulus*, resulting in the isolation of four iridoid glycosides, namely, ebuloside,[12] 7,7-*O*-dihydroebuloside,[13] 6′-*O*-apiosylebuloside,[13] and morroniside,[14] together with the secoiridoid glycoside isosweroside.[14] Despite wide use of the leaves as a traditional remedy, there have been no reports on the isolation of secondary metabolites from the leaves of *S. ebulus*. This study deals with the isolation and structure elucidation of six new iridoid glycosides of the "*Valeriana* type" (**1**−**6**) from leaves of *S. ebulus*.

Results and Discussion

Air-dried leaves of *S. ebulus* were ground and extracted with EtOH. Removal of the solvent under reduced pressure yielded a crude extract, which was suspended in H_2O and sequentially partitioned with petroleum ether, diethyl ether, EtOAc, and *n*-BuOH. The EtOAc solubles were fractionated by a combination of column chromatography (CC), high-speed counter-current chromatography (HSCCC), and semipreparative HPLC to yield compounds **1**−**6**.

Compound **1** was isolated as a white, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m*/*z* 691.28 ($[M + H]^+$), consistent with the chemical formula $C_{31}H_{46}O_{17}$. Analysis of the ^{1}H, HSQC, and HMBC NMR spectra revealed the presence of five methyl, five methylene, 16 methine, and five quaternary carbons. In the ^{13}C NMR spectrum, resonances of an isovaleryl moiety (*δ* 22.4, 25.9, 43.4, 172.0), two acetyl residues (*δ* 20.9, 171.0; *δ* 21.3, 171.0), two anomeric carbons (*δ* 100.3, 103.0), and a ketonic function (*δ* 206.3) were observed (Table 1). The downfield-shifted ^{1}H NMR signals at *δ* 6.51 (d, $J = 4.9$ Hz) and 6.62 (br s) were assigned to H-1 and H-3 of an iridoid backbone, respectively. Extensive analysis of the COSY spectrum enabled the unambiguous assignment of four further methine (CH-5, CH-7, CH-8, CH-9) and three methylene groups (CH_2-6, CH_2-10, CH_2-11) to the iridoid backbone. HMBC correlations between H-1 (*δ* 6.51) and the carbonyl carbon of the isovaleryl group (*δ* 172.0) and between H_2-10 (δ_a 4.83, δ_b 4.68) and the carbonyl carbon of one acetyl residue (*δ* 171.0) indicated esterification of O-1 and O-10



	1	2	3	4	5	6
R_1	H	Ac	H	H	H	H
R_2	Ac	H	Ac	H	Ac	H
R_3	A	A	B	A	C	D

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**3** (C_5D_5N)

	1		**2**		**3**	
position	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]
aglycon						
1	6.51, d (4.9)	92.4	6.78, d (4.0)	92.0	6.34, d (6.0)	93.2
3	6.62, br s	139.8	6.57, br s	139.9	6.56, br s	139.7
4		115.3		114.2		115.9
5	3.48, q-like	33.3	3.40, q-like	32.2	3.61, q-like	33.5
6α	2.14, m[c]	40.7	2.32, m[c]	36.7	1.90, m	41.1
6β	2.52, ddd (13.2, 7.3, 3.0)		2.40, m[c]		2.56, ddd (13.2, 7.2, 2.5)	
7	4.67, m[c]	71.0	5.70, q-like	75.2	4.61, m[c]	71.2
8	2.42, m	45.6	2.64, m	46.6	2.38, m[c]	46.1
9	2.67, td (8.7, 4.9)	42.8	2.83, td (8.7, 4.0)	43.3	2.61, td (8.4, 6.0)	42.6
10a	4.83, dd (10.9, 8.0)	64.5	4.21, dd (10.6, 6.9)	61.1	4.76, dd (10.7, 8.7)	64.3
10b	4.68, m[c]		4.11, dd (10.6, 6.9)		4.64, dd (10.7, 6.5)	
11a	4.52, d (11.1)	69.7	4.49, d (11.2)	69.5	4.52, d (11.1)	69.4
11b	4.28, d (11.1)		4.25, d (11.2)		4.24, d (11.1)	
isovaleryl						
−COO−		172.0		171.7		172.0
$-CH_2-$	2.36, m	43.4	2.30, m	43.4	2.37, m[c]	43.4
−CH<	2.23−2.15, m[c]	25.9	2.20 - 2.10, m[c]	25.8	2.24 - 2.13, m	25.9
$(-CH_3)$a	0.95, d (6.8)	22.4	0.91, d (6.7)	22.3	0.94, d (6.8)	22.5
$(-CH_3)$b	0.93, d (6.8)		0.91, d (6.7)		0.93, d (6.8)	
sugar						
1′	4.95, d (8.0)	103.0	4.95, m[c,d]	103.1	4.94, d (8.0)	103.0
2′	4.92, dd (8.0, 1.4)	79.2	4.92, m[c,d]	79.1	4.97, dd (8.0, 1.5)	79.1
3′		206.3		206.2		206.7
4′	4.93, dd (10.1, 1.4)	74.0	4.92, m[c]	74.0	4.93, dd (10.0, 1.5)	74.1
5′	3.87, ddd (10.1, 4.3, 1.9)	78.6	3.88, ddd (10.1, 4.3, 1.9)	78.6	3.86, ddd (10.0, 4.4, 2.0)	78.6
6′a	4.51, dd (12.1, 1.9)	62.2	4.52, dd (12.1, 1.9)	62.2	4.51, dd (12.2, 2.0)	62.2
6′b	4.41, dd (12.1, 4.3)		4.41, dd (12.1, 4.3)		4.41, dd (12.2, 4.4)	
1″	5.78, d (1.3)	100.3	5.78, d (1.3)	100.3	5.82, d (1.3)	100.5
2″	4.86, dd (3.4, 1.3)	71.8	4.85, dd (3.4, 1.3)	71.8	4.88, dd (3.4, 1.3)	72.0
3″	4.64, m[c]	70.0	4.56, dd (9.8, 3.4)	70.1	4.58, m[c]	72.4
4″	5.84, t (9.8)	75.7	5.83, t (9.8)	75.6	4.29, t (9.5)	74.1
5″	4.65, m[c]	67.5	4.62, dq (9.8, 6.3)	67.4	4.58, m[c]	70.0
6″	1.48, d (6.3)	18.1	1.48, d (6.3)	18.0	1.65, d (6.2)	18.6
acetyl						
7-COO−				170.7[e]		
$7\text{-}CH_3$			2.08, s[e]	21.0[f]		
10-COO−		171.0[e]				170.9
$10\text{-}CH_3$	2.02, s[e]	20.9[f]			2.00, s	21.0
4″-COO−		171.0[e]		170.8[e]		
$4''\text{-}CH_3$	2.21, s[e]	21.3[f]	2.22, s[e]	21.3[f]		

[a] Recorded at 600 MHz. [b] Assignments based on 2D HSQC and HMBC spectra recorded at 75 MHz. [c] Overlapping signals. [d] Signal resolved in the ^{1}H NMR spectrum measured in DMSO-d_6 (Table 4, Supporting Information). [e,f] Signals in the same column may be interchanged.

with isovaleric and acetic acid, respectively. A further HMBC correlation was found between H_2-11 (δ_a 4.52, δ_b 4.28) and the anomeric carbon (δ 103.0) of a sugar moiety, showing glycosylation of O-11. The ^{1}H and ^{13}C NMR data of the sugar residue (Tables 1 and 3) were in good agreement with those reported for D-ribohexo-3-ulopyranose.[15−18] In particular, the COSY and HMBC NMR spectra showed correlations identical to those described for the sugar moiety of 1*H*-indol-3-yl-β-D-ribohex-3′-ulopyranoside[18] and were consistent with the presence of a keto group in position 3′. Briefly, COSY correlations were found between H-1′ and H-2′, H_2-6′ and H-5′, and H-5′ and H-4′. HMBC correlations were found between C-3′ and H-1′, H-2′, H-4′, and H-5′. The β glycosidic linkage of the sugar moiety was deduced from the coupling constant $J_{1',2'}$ (8.0 Hz).[18] Further signals were assigned to a rhamnopyranosyl residue on the basis of comparison with ^{13}C and ^{1}H NMR literature data[19] and by extensive analysis of the COSY spectrum. The HMBC correlation between H-4″ (δ 5.84) of the rhamnopyranosyl moiety and the carbonyl carbon (δ 171.0) of an acetyl residue indicated acetylation of the rhamnopyranosyl residue at O-4″. The HMBC spectrum also revealed a cross-peak between the anomeric proton of 4″-*O*-acetylrhamnopyranose (δ 5.78) and C-2′ (δ 79.2) of ribohexo-3-ulopyranose, thus indicating a 4″-*O*-acetylrhamnopyranosyl-(1→2)-ribohexo-3-ulopyranosyl linkage. The coupling constant $J_{1'',2''} = 1.3$ Hz suggested an α glycosidic linkage of the 4″-*O*-acetylrhamnopyranosyl residue.[19] Acid hydrolysis followed by reduction with $NaBH_4$[20] provided D-allitol and D-sorbitol as determined by GC analysis and thus proved the absolute configuration of D-ribohexo-3-ulose. The absolute configuration of L-rhamnose was confirmed by hydrolysis followed by GC analysis of its thiazolidine derivative.[21] The relative configurations of C-1, C-5, C-7, C-8, and C-9 of the aglycon part of the molecule were established on the basis of ^{1}H and ROESY NMR spectra. The coupling constants $J_{1,9}$ (4.9 Hz) and $J_{5,9}$ (8.7 Hz) were consistent with H-1α, H-5β, and H-9β orientations. The ROESY spectrum showed ROE correlations between H-1 (δ 6.51) and H-8 (δ 2.42), H-9 (δ 2.67), and H_2-10 (δ_a 4.83, δ_b 4.68) and thus indicated the α orientation of H-8. Furthermore, a strong correlation was observed between H-7 (δ 4.67) and H-8, which, however, was not conclusive for the α orientation of H-7, as NOE contacts between vicinal protons of an iridoid backbone with a *trans*-relationship may be observed.[22] Therefore, in order to determine the orientation of H-7, the ROE contacts between H-7 and H_2-6 (δ_α 2.14, δ_β 2.52) were considered. In particular, a stronger correlation between H-7 and H-6$_\alpha$ compared to the correlation between H-7 and H-6$_\beta$ was found, which indicated the α orientation of H-7. The validity of the ^{1}H NMR signal assignments for H-6$_\alpha$ and H-6$_\beta$ was proved by observing the ROE contacts between H-5 (δ 3.48) and H-6$_\beta$, H-5, and H-6$_\alpha$, which respectively showed strong and weak correlations. The α orientation of H-7 was also supported by the absence of interactions between H-7 and H-5 and between H-7 and H-9. Thus,

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **4**−**6** (C_5D_5N)

	4		**5**		**6**	
position	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]
aglycon						
1	6.68, d (4.5)	92.6	6.49, d (4.9)	92.5	6.552, d (5.3)	93.0
3	6.59, br s	139.7	6.63, br s	139.7	6.560, br s	138.9
4		115.0		115.6		116.0
5	3.49, q-like	33.0	3.49, q-like	33.5	3.39, q-like	33.8
6α	2.22, m[c]	40.6	2.14, m[c]	40.8	2.00, m	41.2
6β	2.51, ddd (13.1, 7.3, 3.9)		2.54, ddd (13.2, 7.1, 2.9)		2.43, m (13.0, 7.3, 3.1)	
7	4.81, q-like	72.7	4.65, m[c]	71.0	4.76, q-like	72.8
8	2.42, dq (8.7, 6.1)	48.1	2.41, m	45.7	2.39, m	48.7
9	2.91, td (8.7, 4.5)	42.3	2.65, td (8.6, 4.9)	42.9	2.76, td (8.6, 5.3)	42.3
10a	4.41, m[c]	62.0	4.81, dd (10.9, 8.1)	64.5	4.37, m[c]	62.1
10b	4.33, dd (10.8, 5.6)		4.67, m[c]		4.30, dd (10.8, 5.4)	
11a	4.52, d (11.1)	69.8	4.53, m[c]	69.2	4.46, d (11.7)	68.6
11b	4.26, d (11.1)		4.25, m[c]		4.20, d (11.7)	
isovaleryl						
−COO−		171.9		172.0		171.9
−CH_2−	2.29, m	43.4	2.36, m	43.4	2.23, m	43.4
−CH<	2.18−2.09, m[c]	25.9	2.22−2.12, m[c]	25.9	2.18−2.05, m[c]	25.8
(−CH_3)a	0.910, d (6.7)	22.4	0.93, d (6.8)	22.4	0.88, d (6.7)	22.4
(−CH_3)b	0.909, d (6.7)		0.92, d (6.8)		0.88, d (6.7)	
sugar						
1′	4.95, m[c,d]	103.0	4.85, m[c,d]	102.4	4.90, dd (9.7, 1.8)	100.1
2′a	4.91, m[c,d]	79.1	4.23, m[c,d]	77.5	2.56, ddd (12.3, 4.8, 1.8)	40.8
2′b					2.11, m[c]	
3′		206.2	4.24, m[c]	79.7	4.18, ddd (11.8, 8.6, 4.8)	72.4
4′	4.93, m[c]	74.0	4.12, t (9.0)	71.9	4.04, t (9.0)	73.5
5′	3.87, ddd (10.1, 4.0, 1.8)	78.6	3.88, ddd (9.4, 5.4, 2.2)	78.5	3.81, ddd (9.4, 5.2, 2.4)	78.7
6′a	4.51, m[c]	62.2	4.51, m[c]	62.7	4.54, dd (11.8, 2.4)	63.0
6′b	4.41, m[c]		4.32, dd (11.9, 5.4)		4.39, m[c]	
1″	5.78, d (1.3)	100.3	6.42, d (1.3)	101.6		
2″	4.87, m[c]	71.9	4.75, m (3.4, 1.3)	72.5		
3″	4.64, m[c]	70.0	4.66, m[c]	70.4		
4″	5.85, t (9.8)	75.7	5.87, t (9.8)	76.1		
5″	4.66, m[c]	67.5	4.84, m[c]	67.0		
6″	1.48, d (6.3)	18.1	1.53, d (6.2)	18.3		
acetyl						
10-COO−				171.0[e]		
10-CH_3			2.01, s[e]	21.0[f]		
4″-COO−		171.0		171.0[e]		
4″-CH_3	2.21, s	21.3	2.21, s[e]	21.3[f]		

[a] Recorded at 600 MHz. [b] Assignments based on 2D HSQC and HMBC spectra recorded at 75 MHz. [c] Overlapping signals. [d] Signal resolved in the ^{1}H NMR spectrum measured in DMSO-d_6 (Tables 3 and 4, Supporting Information). [e,f] Signals in the same column may be interchanged.

the configurations of C-1, C-5, C-7, C-8, and C-9 were proved to be identical to those of patrinoside.[23] The structure of **1** was therefore elucidated as 10-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside].

Compound **2** was isolated as a white, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m/z* 691.28 ([M + H]$^+$), consistent with the chemical formula $C_{31}H_{46}O_{17}$. Compounds **1** and **2** were thus isomers. Comparison of ^{13}C and ^{1}H spectroscopic data of compounds **1** and **2** showed almost identical signals for the sugar moieties, suggesting differences only in the aglycon part of the molecules. In particular, the downfield values for CH-7 (δ_C 75.2, δ_H 5.70) and the upfield values for CH_2-10 (δ_C 61.1, δ_H 4.21, 4.11) compared to those of **1** indicated the presence of an acetoxy group at CH-7 and a free OH group at CH_2-10. Analysis of the HMBC spectrum showed the expected correlation between H-7 (δ 5.70) and the quaternary carbon of the acetyl group (δ 170.7). The relative configurations of C-1, C-5, C-7, C-8, and C-9 of the aglycon part were deduced on the basis of ^{1}H and ROESY NMR spectra and were found to be identical to those of **1**. Acid hydrolysis followed by derivatization and GC analysis confirmed NMR signal assignments in Table 1. Therefore, the structure of **2** was elucidated as 7-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside].

Compound **3** was isolated as a white, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m/z* 649.27 ([M + H]$^+$), consistent with the chemical formula $C_{29}H_{44}O_{16}$ and thus suggesting the lack of one acetyl group compared to **1**. As expected, comparison of ^{13}C and ^{1}H spectroscopic data of **3** and **1** showed the lack of one methyl group and one quaternary carbon. Moreover, it was found that the signal of H″-4 of the rhamnopyranosyl residue was shifted upfield (δ 4.29) compared to **1** (δ 5.84), thus indicating the presence of a free OH group at this position. No correlation between H″-4 and quaternary carbons was observed in the HMBC spectrum. The β glycosidic linkage of the ribohexo-3-ulopyranosyl sugar moiety and the α glycosidic linkage of the rhamnopyranosyl residue were deduced from the anomeric coupling constants ($J_{1',2'}$ = 8.0 Hz and $J_{1'',2''}$ = 1.3 Hz, respectively).[18,19] Acid hydrolysis followed by derivatization and GC analysis provided results identical to those of compound **1**. The ^{13}C and ^{1}H spectroscopic data for the aglycon part of **3** were almost identical to those of **1**, indicating that the two compounds possessed the same aglycon. The relative configurations of C-1, C-5, C-7, C-8, and C-9 were also found to be identical to those of **1**, as deduced from ^{1}H and ROESY NMR data. Therefore, the structure of **3** was elucidated as 10-*O*-acetylpatrinoside-aglycone-11-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside].

Compound **4** was isolated as a light yellow, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m/z*

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **1** and **5** (DMSO-d_6)

	1		**5**	
position	δ_H (J in Hz)[a]	δ_C[b]	δ_H (J in Hz)[a]	δ_C[b]
aglycon				
1	5.93, d (4.5)	90.6	5.90, d (4.6)	90.7
3	6.40, br s	138.1	6.36, br s	138.1
4		113.8		113.7
5	2.85, q-like	31.3	2.85, q-like	31.5
6α	1.81, m	38.5	1.79, m	38.6
6β	1.91, m[c]		1.91, m[c]	
7	4.13, m[c]	69.5	4.12, m[c]	69.5
8	1.95, m[c]	43.4	1.95, m[c]	43.6
9	2.14, td (8.5, 4.5)	41.4	2.13, td (8.5, 4.6)	41.4
10a	4.20, m[c]	63.2	4.20, dd (10.9, 7.5)	63.2
10b	4.08, m[c]		4.08, dd (10.9, 7.0)	
11a	4.22, d (11.1)	68.0	4.15, d (11.1)	67.5
11b	4.00, d (11.1)		3.89, d (11.1)	
isovaleryl				
−COO−		171.0		171.0
$-CH_2-$	2.20, m	42.3	2.20, m	42.3
−CH<	1.97, m[c]	24.8	1.98, m[c]	24.8
$(-CH_3)$a	0.891, d (6.6)	20.8	0.891, d (6.6)	20.8
$(-CH_3)$b	0.890, d (6.6)		0.890, d (6.6)	
sugar				
1′	4.53, d (8.0)	100.8	4.27, d (7.9)	100.3
2′	4.19, dd (8.0, 1.6)	77.6	3.24, dd (8.9, 7.9)	75.4
3′		204.6	3.34, m[c]	77.5
4′	4.10, m[c]	72.2	3.07, m[c]	70.0
5′	3.29, ddd (10.0, 5.2, 1.9)	76.2	3.10, m[c]	76.5
6′a	3.74, m	60.5	3.67, m	60.7
6′b	3.59, m		3.45, m	
1″	4.78, d (1.3)	98.1	5.20, d (1.3)	99.0
2″	3.83, m	69.6	3.72, m	70.0
3″	3.66, m	67.7	3.62, m	67.8
4″	4.77, t (9.8)	73.4	4.75, t (9.8)	73.7
5″	3.90, dq (9.8, 6.3)	65.7	4.01, dq (9.9, 6.3)	65.1
6″	0.99, d (6.3)	17.2	0.96, d (6.3)	17.2
acetyl				
10-COO−		170.4		170.3
10-CH_3	1.99, s[d]	20.6[d]	1.99, s[d]	20.6[d]
4″-COO−		169.9		170.1
4″-CH_3	2.00, s[d]	20.6[d]	2.00, s[d]	20.6[d]

[a] Recorded at 600 MHz. [b] Assignments based on 2D HSQC and HMBC spectra recorded at 150 MHz. [c] Overlapping signals. [d] Signals in the same column may be interchanged.

649.27 ($[M + H]^+$), consistent with the chemical formula $C_{29}H_{44}O_{16}$. Thus, compounds **4** and **3** were isomers. The ^{13}C and ^{1}H spectroscopic data of the sugar moieties (Table 2) showed very good correspondence with those of **1** and confirmed the presence of the same disaccharide moiety, linked to C-11, as demonstrated by the HMBC spectrum. Also, acid hydrolysis followed by derivatization and GC analysis provided results identical to compound **1**. Concerning the aglycon part of the molecule, the upfield values for CH_2-10 (δ_C 62.0, δ_H 4.41, 4.33), compared to **1** (δ_C 64.5, δ_H 4.83, 4.68), suggested the absence of an acetyl group at this position. This was confirmed by analysis of the HMBC spectrum, which showed no correlations between H_2-10 (δ_a 4.41, δ_b 4.33) and quaternary carbons. ^{1}H and ROESY NMR data were in agreement with the relative configurations of C-1, C-5, C-7, C-8, and C-9 of the aglycon part as assigned for compound **1**. Therefore, the structure of **4** was elucidated as patrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside].

Compound **5** was isolated as a white, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m/z* 693.30 ($[M + H]^+$), consistent with the chemical formula $C_{31}H_{48}O_{17}$ and thus revealed one degree of unsaturation lower compared to **1**. Except for an additional oxymethine signal (δ_C 79.7, δ_H 4.24) and the lack of a ketone resonance, ^{13}C and ^{1}H NMR signals as well as ROESY correlations of compounds **1** and **5** were almost identical. Accordingly, β-D-ribohexo-3-ulopyranose had been replaced by another sugar moiety. Corresponding ^{1}H and ^{13}C signals of this sugar moiety were assigned to glucopyranose, which was in complete agreement with literature data.[16,17,24] The glycosylation position was determined to be at C-11 of the aglycon by analysis of the HMBC spectrum, which showed a correlation between H_2-11 (δ 4.53, 4.25) and C-1′ (δ 102.4) of glucopyranose. A further HMBC correlation between the anomeric proton of 4″-*O*-acetylrhamnopyranose (δ 6.42) and C-2′ (δ 77.5) of the glucopyranosyl residue confirmed the 4″-*O*-acetylrhamnopyranosyl-(1→2)-glucopyranosyl linkage. The coupling constants $J_{1',2'} = 7.9$ Hz and $J_{1'',2''} = 1.3$ Hz indicated a β glycosidic linkage of the glucopyranosyl sugar moiety and an α glycosidic linkage of the 4″-*O*-acetylrhamnopyranosyl residue.[16,19] Acid hydrolysis followed by derivatization with L-cysteine methyl ester hydrochloride confirmed the absolute configurations of the sugar moieties as D-glucose and L-rhamnose as determined by GC analysis. Thus, **5** is 10-*O*-acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside].

Compound **6** was isolated as a white, amorphous solid. The HRFABMS spectrum displayed a quasimolecular ion at *m/z* 447.22 ($[M + H]^+$), consistent with the chemical formula $C_{21}H_{34}O_{10}$. ^{1}H, COSY, HSQC, HMBC, and ROESY NMR spectra showed that the aglycon part of the molecule was identical to that of compound **4**. The HSQC spectrum also exhibited one anomeric signal (δ_C 100.1, δ_H 4.90), revealing the presence of a sugar moiety at C-11 (δ 68.6) as determined by analysis of the HMBC spectrum. Interestingly, the COSY spectrum showed correlations between the anomeric proton (δ 4.90) and two protons (δ_a 2.56, δ_b 2.11) of a methylene group, which was assigned to C′-2 of the sugar moiety. Extensive analysis of the COSY spectrum enabled determination of the connectivity of the sugar residue, which was consistent with a 2-deoxypyranose. The ^{13}C and ^{1}H spectroscopic data were comparable to those of 2-deoxy-β-D-glucopyranose (see Supporting Information). The β glycosidic linkage was deduced from the coupling constants of the anomeric proton (9.7, 1.8 Hz).[24] Acid hydrolysis followed by derivatization with L-cysteine methyl ester hydrochloride and GC analysis proved the absolute configuration of the sugar moiety to be 2-deoxy-D-glucose. Therefore, the structure of **6** was elucidated as patrinoside-aglycone-11-*O*-2′-deoxy-β-D-glucopyranoside.

Compounds **1**−**4** represent the first examples of acylated iridoid diglycosides bearing the D-ribohexo-3-ulopyranosyl sugar moiety. The occurrence of D-ribohexo-3-ulose as a sugar moiety in glycosides isolated from natural sources is relatively rare and has been hitherto reported for five iridoid glycosides,[15,25−27] one acyclic monoterpene glycoside,[28] one dihydrochalcone glycoside,[16] three cardenolide glycosides,[17,29] and two indoxyl glycosides.[18] To the best of our knowledge, compound **6** is the first example of an iridoid glycoside bearing the 2-deoxy-D-glucopyranosyl sugar moiety. Compounds **1**−**6** were also identified in leaves of *S. ebulus* collected in July 2009 by means of HPLC-MS (see Supporting Information).

Experimental Section

General Experimental Procedures. All reagents used were of analytical grade and were purchased from Sigma Aldrich (Vienna, Austria). HPLC solvents were of gradient grade. Technical-grade solvents were distilled before use. Water was produced by reverse osmosis followed by distillation. Optical rotations were measured using a Perkin-Elmer (Wellesley, MA) 341 polarimeter. FTIR spectra were recorded on a Bruker (Bruker Optics, Ettlingen, Germany) IFS 25 FTIR spectrometer in transmission mode (4000−600 cm^{-1}) using ZnSe disks of 2 mm thickness. NMR spectra were acquired using Bruker (Bruker Biospin, Rheinstetten, Germany) DRX 300 and Avance II 600 spectrometers using C_5D_5N or DMSO-d_6 (containing 0.03% TMS) (Euriso-Top, Saint-Aubin, France). Chemical shift values were referenced to the residual solvent signals. HRFABMS spectra were recorded in positive mode using a Finnigan (San Jose, CA) MAT 95-S spectrometer (Cs-Gun, 20 keV, matrix: glycerine). LC analyses were carried out using an HP 1100 system (Agilent, Waldbronn, Germany) equipped with autosampler, DAD, and column thermostat. Separations

were performed on a Phenomenex (Torrance, CA) Synergi Max-RP 80A column (150 × 4.60 mm i.d., 4 μm) and a Merck (VWR, Darmstadt, Germany) LiChroCART 4-4 guard column with LiChrospher 100 RP_{18} (5 μm) packing. A mobile phase consisting of 0.025% TFA in H_2O (v/v) (solvent A) and a mixture MeCN−MeOH (1:1; v/v) (solvent B) was employed with gradient elution (0 min, 70:30 (A:B); 50 min, 45:55; 55 min, 2:98; 60 min, 2:98). The detection wavelength was 205 nm, and the thermostat was set at 35 °C. The injection volume was 10 μL; the flow rate was 0.6 mL/min. For LC-ESIMS experiments, the HPLC system was coupled to a Bruker (Bruker Daltonics, Bremen, Germany) Esquire 3000^{plus} iontrap, replacing solvent A with a solution of 0.1% formic acid in H_2O (v/v). The MS parameters were as follows: split 1:5; ESI positive mode; spray voltage +4 kV; nebulizer gas 30 psi; drying gas flow rate 8.00 L/min; *m/z* range 100−1200. For HSCCC separations a P.C. Inc. (Potomac, MD) series 690 multilayer (triple) coil HSCCC instrument with a Gilson (Villiers-le-Bel, France) pump system (model 302/803 C) was used. Semipreparative HPLC separations were carried out on a Dionex (Dionex Softron, Germering, Germany) system fitted with a P580 pump, a ASI-100 autosampler, a UVD 170U detector, a Gilson 206 fraction collector, and a Waters (Milford, MA) X-Terra Prep MS C18 column (100 × 7.8 mm i.d., 5 μm) or Phenomenex AQUA 125A column (250 × 10.0 mm i.d., 5 μm). GC analyses were carried out on a Perkin-Elmer autosystem GC fitted with a FID detector. A PERMABOND SE-54 fused silica capillary column (Macherey-Nagel, Düren, Germany) (50 m × 0.32 mm i.d., 0.25 μm film thickness) was used with helium as carrier gas (1 mL/min). The injector and detector temperatures were kept at 280 and 300 °C, respectively. The oven temperature program was set as follows: injection at 65 °C; isothermal hold for 2 min; temperature increase of 6 °C/min to 300 °C; isothermal hold for 15 min. The injection volume was 2.5 μL. Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) and silica gel (VWR, Darmstadt, Germany) were used as stationary phases for CC. TLC was carried out on silica gel 60 F_{254} plates (VWR, Darmstadt, Germany) using $CHCl_3$−MeOH−H_2O (100:30:3; v/v/v) as mobile phase, and detection was performed with vanillin/H_2SO_4 (1% w/v and 5% v/v methanolic solutions, respectively).

Plant Material. Leaves of *Sambucus ebulus* were collected near Magdalensberg, Carinthia, Austria, in September 2003. A voucher specimen (CS-09200301) was deposited at the Herbarium of the Institut für Pharmazie/Pharmakognosie, Leopold-Franzens-Universität Innsbruck, Austria.

Extraction and Isolation. Air-dried leaves of *S. ebulus* (401.5 g) were ground and extracted with 1.5 L of 96% EtOH at room temperature (8 times). After removal of the solvent under reduced pressure, the resulting crude extract (104.1 g) was suspended in 1 L of H_2O and successively extracted with petroleum ether (1 L × 8), diethyl ether (1 L × 8), EtOAc (1 L × 5), and *n*-BuOH (1 L × 5). The resulting extracts were concentrated under reduced pressure, affording petroleum ether (27.60 g), diethyl ether (4.69 g), EtOAc (8.90 g), *n*-BuOH (21.77 g), and H_2O (44.33 g) extracts. The EtOAc extract (8.53 g) was subjected to CC over Sephadex LH-20 (90 × 3.5 cm) eluting with MeOH to yield nine fractions. Fraction 3 (3.045 g) was divided into three equal parts of approximately 1 g each and separately subjected to HSCCC using petroleum ether−EtOAc−96% EtOH−H_2O (1:2:1:1; v/v/v/v) as solvent system in "tail-to-head" mode with the upper layer as mobile phase (coil volume 325 mL; coil rotation 800 rpm; flow rate 1 mL/min). Thirty, 27, and 22 fractions were obtained, respectively. Fractions containing compounds **1**−**6** were combined according to TLC and HPLC chromatograms to yield six enriched fractions (A−F). Fraction A (218.9 mg) was subjected to CC over silica gel (90 × 2 cm), affording seven subfractions. Subfractions 4 (23.4 mg), 5 (65.2 mg), and 7 (27.0 mg) were separately fractionated by semipreparative HPLC (X-Terra column; isocratic elution, H_2O−MeCN (73:27; v/v); flow rate 3 mL/min; column temperature 35 °C) to yield compound **1** (7.3, 12.8, and 12.4 mg, respectively). Fraction B (113.57 mg) was chromatographed by CC over Sephadex LH-20 (90 × 2 cm) using acetone as mobile phase, affording 11 subfractions. Compound **2** (11.4 mg) was purified from subfraction 2 (34.9 mg) by semipreparative HPLC (X-Terra column; isocratic elution, H_2O−MeCN (75:25; v/v); flow rate 3 mL/min; column temperature 35 °C). Fraction C (104.4 mg) was subjected to CC over Sephadex LH-20 (90 × 2 cm), affording 11 subfractions. Subfraction 4 (42.6 mg) was subjected to semipreparative HPLC (AQUA column; isocratic elution, H_2O−MeCN (70:30; v/v); flow rate 3 mL/min; column temperature 20 °C) to yield compounds **3** (5.0 mg) and **5** (8.8 mg). Fractions D (82.1 mg) and E (95.0 mg) were separately subjected to CC over Sephadex LH-20 (90 × 2 cm) using acetone as mobile phase, affording 15 and 5 subfractions, respectively. Subfraction 10 derived from fraction D contained compound **4** (9.2 mg). Further amounts (17.6 mg) of compound **4** were obtained from subfraction 2 derived from fraction E by semipreparative HPLC (X-Terra column; isocratic elution, H_2O−MeCN (75:25; v/v); flow rate 3 mL/min; column temperature 35 °C). Fraction F (57.8 mg) was subjected to HSCCC separation using EtOAc−2-propanol−H_2O (1:0.2:1; v/v/v) as solvent system in "tail-to-head" mode with the upper layer as mobile phase (coil volume 325 mL; coil rotation 800 rpm; flow rate 1 mL/min) to yield 26 subfractions. Compound **6** (6.9 mg) was found in subfractions 18−20.

10-*O*-Acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside] (1): white, amorphous solid; $[\alpha]^{20}_D$ −103.9 (*c* 0.31, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}) 3423, 2960, 2934, 2875, 1739, 1668, 1600, 1575; ^1H NMR (C_5D_5N and DMSO-d_6, 600 MHz) see Tables 1 and 3; ^{13}C NMR (C_5D_5N and DMSO-d_6, 75 and 150 MHz) see Tables 1 and 3; ESIMS *m/z* 709 $[M + H_2O + H]^+$; HRFABMS *m/z* 691.28 $[M + H]^+$ (calcd for $C_{31}H_{47}O_{17}$, 691.28).

7-*O*-Acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside] (2): white, amorphous solid; $[\alpha]^{20}_D$ −86.1 (*c* 0.30, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}) 3382, 2961, 2935, 2875, 1738, 1668, 1602, 1573; ^1H NMR (C_5D_5N and DMSO-d_6, 600 MHz) see Tables 1 and 4 (Supporting Information); ^{13}C NMR (C_5D_5N, 75 MHz) see Table 1; ESIMS *m/z* 709 $[M + H_2O + H]^+$; HRFABMS *m/z* 691.28 $[M + H]^+$ (calcd for $C_{31}H_{47}O_{17}$, 691.28).

10-*O*-Acetylpatrinoside-aglycone-11-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside] (3): white, amorphous solid; $[\alpha]^{20}_D$ −67.8 (*c* 0.29, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}) 3381, 2960, 2932, 2875, 1738, 1667, 1600, 1575; ^1H NMR (C_5D_5N, 600 MHz) see Table 1; ^{13}C NMR (C_5D_5N 75 MHz) see Table 1; ESIMS *m/z* 667 $[M + H_2O + H]^+$; HRFABMS *m/z* 649.27 $[M + H]^+$ (calcd for $C_{29}H_{45}O_{16}$, 649.27).

Patrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-ribohexo-3-ulopyranoside] (4): light yellow, amorphous solid; $[\alpha]^{20}_D$ −111.1 (*c* 0.32, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}): 3374, 2960, 2933, 2875, 1739, 1668, 1600, 1575; ^1H NMR (C_5D_5N and DMSO-d_6, 600 MHz) see Tables 2 and 4 (Supporting Information); ^{13}C NMR (C_5D_5N, 75 MHz) see Table 2; ESIMS *m/z* 671 $[M + Na]^+$; HRFABMS *m/z* 649.27 $[M + H]^+$ (calcd for $C_{29}H_{45}O_{16}$, 649.27).

10-*O*-Acetylpatrinoside-aglycone-11-*O*-[4″-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside] (5): white, amorphous solid; $[\alpha]^{20}_D$ −70.2 (*c* 0.24, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}) 3395, 2960, 2933, 2875, 1739, 1667, 1580; ^1H NMR (C_5D_5N and DMSO-d_6, 600 MHz) see Tables 2 and 3; ^{13}C NMR (C_5D_5N and DMSO-d_6, 75 and 150 MHz) see Tables 2 and 3; ESIMS *m/z* 711 $[M + H_2O + H]^+$; HRFABMS *m/z* 693.30 $[M + H]^+$ (calcd for $C_{31}H_{49}O_{17}$, 693.30).

Patrinoside-aglycone-11-*O*-2′-deoxy-β-D-glucopyranoside (6): white, amorphous solid; $[\alpha]^{20}_D$ −63.3 (*c* 0.28, MeOH); HPLC-online UV λ_{max} 205; FTIR ν_{max} (cm^{-1}) 3374, 2958, 2929, 2873, 1746, 1666, 1593; ^1H NMR (C_5D_5N, 600 MHz) see Table 2; ^{13}C NMR (C_5D_5N, 75 MHz) see Table 2; ESIMS *m/z* 469 $[M + Na]^+$; HRFABMS *m/z* 447.22 ($[M + H]^+$ (calcd for $C_{21}H_{35}O_{10}$, 447.22) and *m/z* 469.20 $[M + Na]^+$ (calcd for $C_{21}H_{34}NaO_{10}$, 469.20).

Acid Hydrolysis of 1−6. Compounds **1**−**6** (1 mg each) were subjected to hydrolysis with 1.5 mL of 1 N HCl (90 °C, 3 h). After cooling, the reaction mixture was neutralized with NaOH and partitioned three times with $CHCl_3$. The H_2O phase was then evaporated to dryness and reconstituted in 2 mL of H_2O.

Determination of L-Rhamnose, D-Glucose, and 2-Deoxy-D-glucose (1−6). Solutions (1 mL each) derived from acid hydrolysis of **1**−**6** were evaporated to dryness and the residues extracted with 300 μL of pyridine. L-Cysteine methyl ester hydrochloride (1 mg) was added, and the resulting mixture was stirred at 60 °C for 1 h.[21] Then, 150 μL of a mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)-trimethylchlorosilane (TMCS) (99:1) was added, and the solution was stirred at 60 °C for 30 min. After centrifugation, 2.5 μL of the supernatant was subjected to GC analysis. Standards of L-rhamnose (0.2 mg), D-glucose (0.2 mg), and 2-deoxy-D-glucose (0.2 mg) were derivatized using the same procedure, showing chromatographic peaks at 36.44,

38.63, and 37.37 min, respectively. For identification purposes, the analytical samples were co-injected with the derivatized standards, showing matching peaks corresponding to L-rhamnose for compounds **1**−**4**, L-rhamnose and D-glucose for compound **5**, and 2-deoxy-D-glucose for compound **6**.

Determination of D-Ribohexo-3-ulose. To 1 mL of the solution derived from acid hydrolysis of **1**−**4**, 1 mg of $NaBH_4$[30] was added while stirring. After 1 h, the reagent excess was eliminated by dropwise addition of concentrated CH_3COOH. The mixture was then concentrated under reduced pressure. The excess boric acid was eliminated by repeated addition of 10% CH_3COOH in MeOH followed by concentration under reduced pressure (five times).[31,32] The residue was then extracted with 300 μL of pyridine, to which 150 μL of a mixture BSTFA−TMCS (99:1) was added under stirring (60 °C, 30 min). After centrifugation, 2.5 μL of the supernatant was subjected to GC analysis. Standards of D-allose (0.2 mg) and D-glucose (0.2 mg) were derivatized using the same procedure, showing chromatographic peaks at 30.42 min (D-allitol) and 30.56 min (D-sorbitol), respectively. For identification purposes, the analytical samples for compounds **1**−**4** were co-injected with the derivatized standards, showing matching peaks corresponding to D-allitol and D-sorbitol.

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Supporting Information Available: 1D and 2D NMR spectra of compounds **1**−**6**, ^{1}H NMR data for the D-ribohexo-3-ulopyranosyl sugar moiety in compounds **2** and **4** (DMSO-d_6, 600 MHz), ^{1}H and ^{13}C NMR data for 2-deoxy-β-D-glucose (C_5D_5N, 600 MHz), HPLC-MS comparison with plant material collected in July 2009, GC chromatograms of the analysis of the sugar residues. This material is available free of charge via the Internet at http://pubs.acs.org.

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