33. Corniculoside, a New Biosidic Ester Secoiridoid from *Halenia corniculata*

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Chemical screening of the secondary metabolites from *Halenia corniculata* L. (CORNAZ), by LC/UV, LC/TSP-MS (thermospray), and LC/ES-MS (electrospray) was used for the targeted isolation of corniculoside (**1**), a new biosidic ester secoiridoid. The structure was established as 7-β-[(*E*)-4′-*O*-(β-D-glucopyranosyl)caffeoyloxy]-sweroside by 1D- and 2D-NMR, LC/UV, LC/MS, and FAB-MS data, in combination with chemical reactions.

1. Introduction. – In the course of our phytochemical investigations of the Gentianaceae, more than 50 crude extracts of species of this family were analysed by liquid chromatography with photodiode array detection (LC/DAD-UV) and thermospray mass spectrometry detection (LC/TSP-MS). These hyphenated techniques have shown their efficiency for the chemical screening of crude plants extracts [1–5] and permit, when used with an in-house UV-spectral library, an early recognition of known compounds and a targeted isolation of natural products which show interesting spectroscopic features.

Halenia corniculata is a Gentianaceae species used in Mongolian traditional medicine for its stomachic properties and as internal anti-inflammatory and hepatoprotective agent. The analysis of both MeOH and CH_2Cl_2 extracts by LC/UV and LC/TSP-MS led to the identification of 26 xanthones, secoiridoids, and flavonoids [3]. Nevertheless, some UV-active compounds were not ionized under the thermospray conditions. To undertake a complementary chemical screening, electrospray analyses (LC/ES-MS) of the extracts of *H. corniculata* were performed [6]. This type of atmospheric-pressure ionization (API) technique allowed the detection of corniculoside (**1**), a new biosidic ester secoiridoid. Its targeted isolation and structure elucidation is reported here.

2. Results. – Dried and powdered *H. corniculata* whole plant was extracted at room temperature with solvents of increasing polarity (CH_2Cl_2 and MeOH), and both extracts were analysed by LC/DAD-UV and LC/TSP-MS. As reported in [3], most xanthones, secoiridoids, and flavonoids present in the extracts were identified. To obtain molecular-weight information for compounds which were not ionized in the TSP mode and to evaluate the potential of the electrospray interface in plant-metabolite detection, the same extracts were analysed by LC/ES-MS. As shown in *Fig. 1*, ES (negative-ion mode) permitted the ionization of all glycosides found in the extract. The ES spectra of flavonoid, secoiridoid, and xanthone glycosides exhibited strong trifluoroacetate adducts

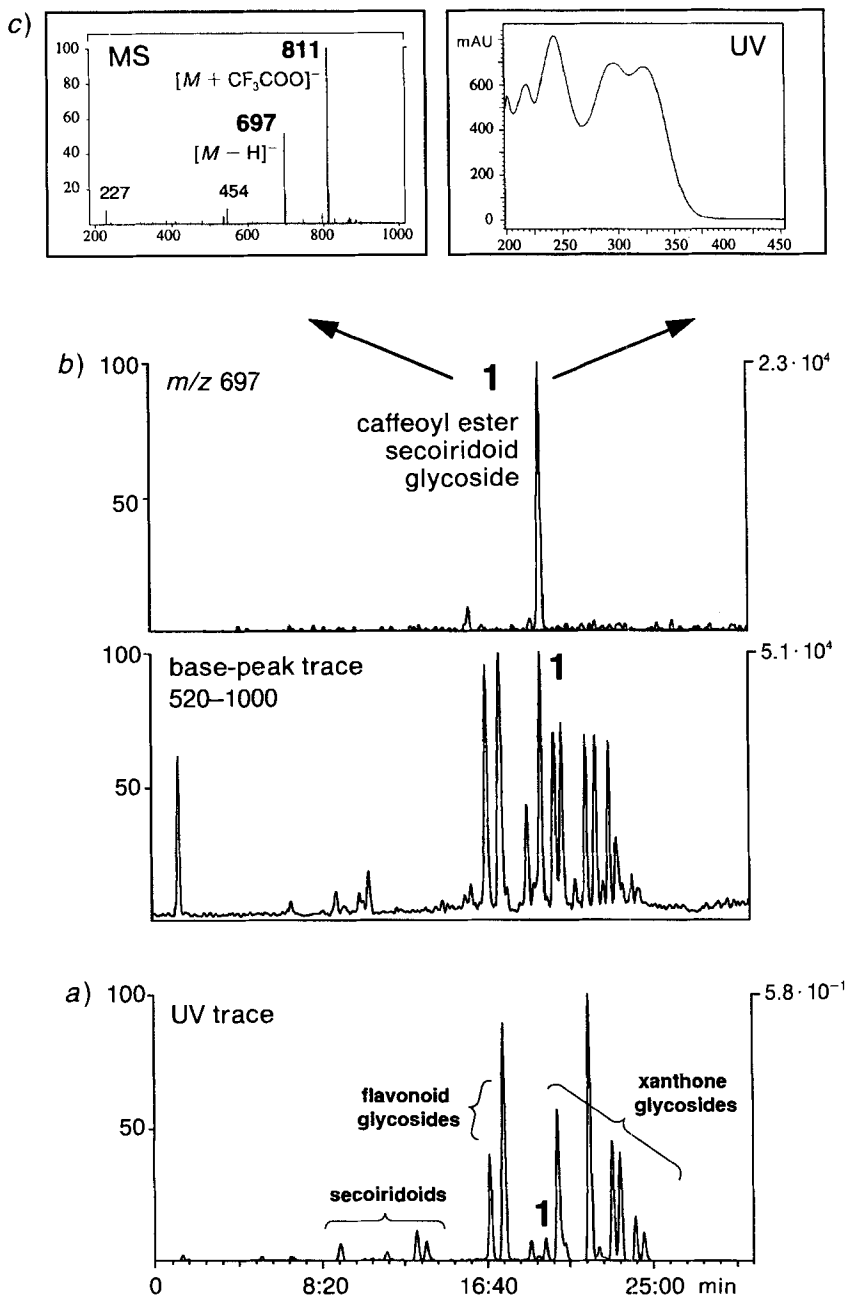
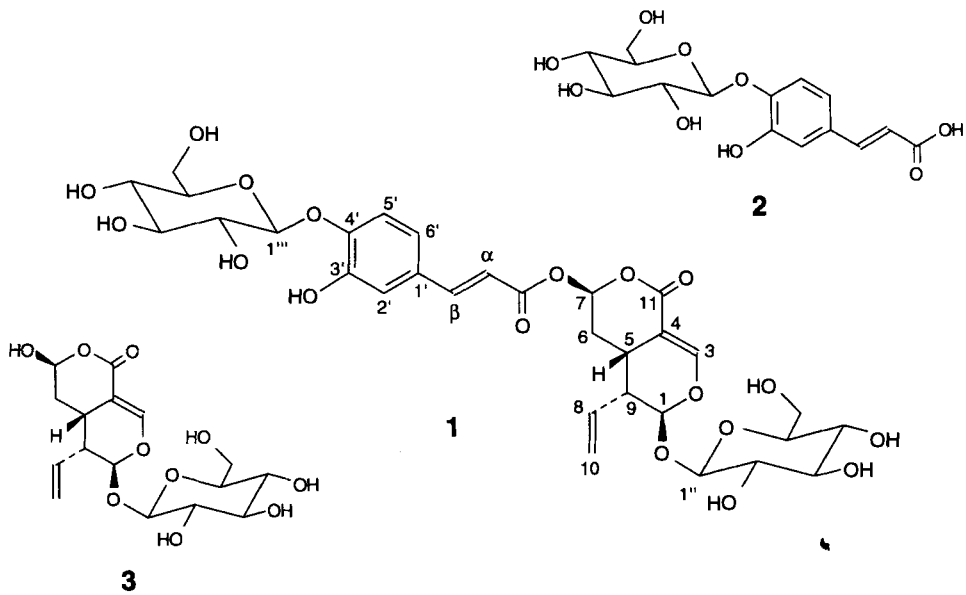


Fig. 1. a) *LC/DAD-UV and* b) *LC/ES-MS analysis of the MeOH extract of* H. corniculata. c) *UV and MS spectra of the minor polar compound* **1**. HPLC: *Novapak RP-18* column (4 μm, 150 × 3.9 mm i.d.), $MeCN/H_2O$ gradient 5:95 → 65:35 containing 0.05% CF_3COOH in 50 min, flow rate 1 ml/min. LC/ES-MS: negative-ion mode, capillary transfer tube temperature 220°, rf potential of the source octapole 10 eV.

$[M + CF_3COO]^-$, together with deprotonated $[M - H]^-$ ions. Compound **1**, a minor polar constituent of the MeOH extract, gave a large molecular ion $[M - H]^-$ at m/z 697 and a trifluoroacetate adduct $[M + CF_3COO]^-$ at m/z 811, which indicated a molecular weight of 698 amu. This compound was not ionized in the TSP mode, and its UV spectrum (maxima at 219, 244, 297, and 321 nm) differed from those usually encountered in Gentianaceae-species extracts. The two UV maxima at 219 and 244 nm, together with the retention time (*ca.* 16 min) suggested that **1** was an iridoid-type compound. The two other maxima at 297 and 321 nm indicated the presence of an aromatic part. No previous references were found in the literature for such a compound corresponding to this molecular weight. Thus, the isolation of **1** was undertaken by a combination of gel filtration on *Sephadex LH-20* (MeOH) and medium-pressure liquid chromatography (MPLC) on *RP-18*.



Acid (2N HCl) and enzymatic (β-glucosidase) hydrolyses of **1** afforded glucose, while the aglycone apparently decomposed. However, the presence of caffeic acid in the organic phase (see *Exper. Part*) was revealed by HPLC/UV analysis and comparison with standards. An alkaline hydrolysis was then performed and followed by HPLC/UV (injection after 5, 60, 120 min). Two hours after the addition of NaOH (see *Exper. Part*), **1** was fully degraded, and the formation of compounds **2** and **3** was observed (*Fig. 2*). The UV spectrum of **2** was identical to that of caffeic acid, but its retention time was 3 min shorter. This suggested that **2** was a glucosylcaffeic acid. The single absorption band at 230–240 nm observed in the UV spectrum of **3** is characteristic for a secoiridoid. Moreover, this hydrolysis product eluted between sweroside and swertiamarin, two well known secoiridoids. Thus, the alkaline hydrolysis indicated that compound **1** consisted of a secoiridoid part combined to a glucosylcaffeoyl moiety by an ester linkage. The FAB-MS spectra (negative-ion mode) confirmed this hypothesis by presenting a strong deproto-

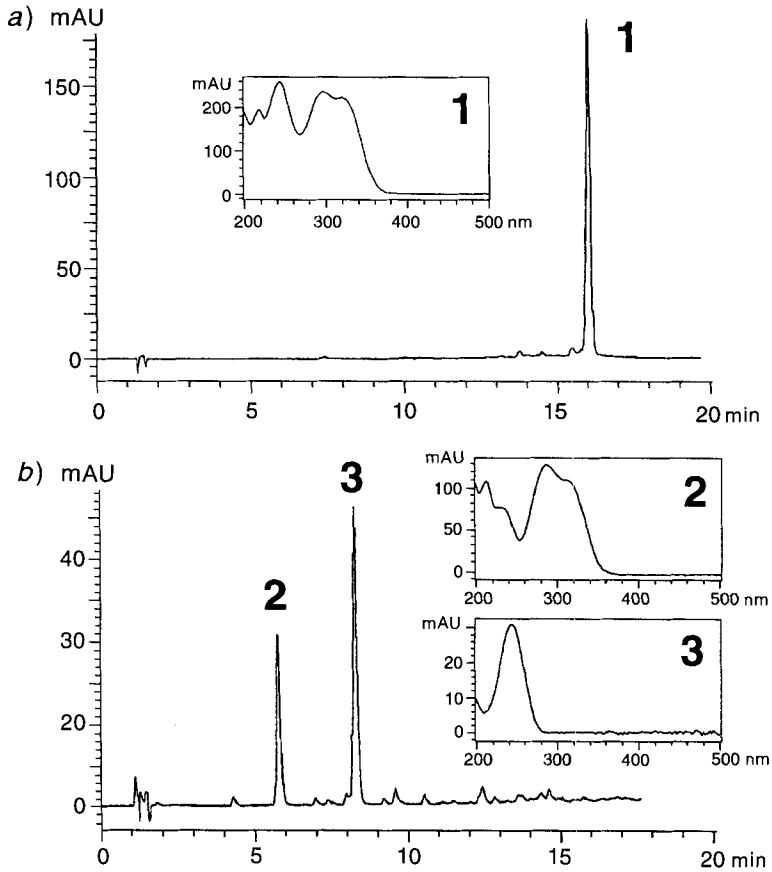


Fig. 2. *LC/DAD-UV analyses of corniculoside* (**1**) a) *before and* b) *after alkaline hydrolysis, indicating the presence of a secoiridoid* (see **3**) *and a glucosylcaffeoyl moiety* (see **2**) *in* **1**

nated molecular ion at *m/z* 697 and fragment ions at *m/z* 535, 373, and 161, characteristic of the loss of glucosyl or caffeoyl units. Comparison of ^{1}H- and ^{13}C-NMR data of **1** (*Table*[1])) with literature values confirmed the presence of a caffeoyl, a secoiridoid, and two hexosyl moieties.

The broad-band-decoupled ^{13}C-NMR spectrum and DEPT spectra of **1** showed 31 distinct resonances (*Table*): 2 ester carbonyls (166.04 and 166.36 ppm), 2 acetal C-atoms (98.61 and 93.36 ppm), a vinylic side chain (133.10 and 121.41 ppm), 2 sp^3 CH groups (22.99 and 43.41 ppm), an sp^3 CH_2 group (28.98 ppm), a quaternary olefinic C-atom (104.64 ppm), a tertiary olefinic C-atom (155.21 ppm), 2 sp^2 CH groups (118.01, 148.04 ppm), 3 quaternary aromatic C-atoms (130.77, 149.24, 148.54 ppm), 3 aromatic sp^2 CH groups (116.12, 122.73, 116.12 ppm) together with 12 signals attributable to two hexosyl moieties. These data and the molecular weight of 698 amu suggested $C_{31}H_{38}O_{18}$ as molecular formula for compound **1**.

In the ^{1}H-NMR spectrum, the three aromatic protons (7.1–7.2 ppm) forming an *ABM* system and the two *trans*-olefinic protons at δ 6.43 (*d*, J = 16 Hz, 1 H) and 7.67 (*d*, J = 16 Hz, 1 H) of the caffeoyl unit were clearly

[1]) For convenience, the 'biogenetic numbering' generally used for secoiridoids is employed throughout the text and *Table* (see *Formula* **1**); the systematic name is given in the *Exper. Part.*

Table. *^{13}C- and ^{1}H-NMR Data of Compound* **1**. Coupling constants *J* in Hz.

C-Atom[a])	δ [ppm][b])	H-Atom[a])	δ [ppm][b])	Multiplicity
Secoiridoid moiety				
C(1)	98.61	H–C(1)	5.61	*d* (*J* = 1.7)
C(3)	155.21	H–C(3)	7.68	*d* (*J* = 2.6)
C(4)	104.64	H–C(5)	hidden	
C(5)	22.99	H_a–C(6)	2.05	*ddd* (*J* = 2.6, 5.8, 13.8)
C(6)	28.98	H_b–C(6)	1.92	*dt* (*J* = 2.2, 13.8, 13.8)
C(7)	93.36	H–C(7)	6.71	*t* (*J* = 2.2)
C(8)	133.10	H–C(8)	5.51	unresolved
C(9)	43.41	H–C(9)	2.75	*ddd* (*J* = 1.7, 5.8, 9.1)
C(10)	121.41	H_a–C(10)	5.29	unresolved
C(11)	166.04	H_b–C(10)	5.34	*dd* (*J* = 2.3, 8.6)
Caffeoyl moiety				
C=O	166.36			
C(α)	118.01	H–C(α)	6.43	*d* (*J* = 16)
C(β)	148.04	H–C(β)	7.67	*d* (*J* = 16)
C(1′)	130.77			
C(2′)	116.12[c])	H–C(2′)	7.16	*d* (*J* = 1.8)
C(3′)	149.29[d])			
C(4′)	148.54[d])			
C(5′)	116.12[c])	H–C(5′)	7.21	*d* (*J* = 8.4)
C(6′)	122.73	H–C(6′)	7.09	*dd* (*J* = 1.8, 8.4)
Glucosidic moieties				
C(1″)	100.31	H–C(1″)	4.57	*d* (*J* = 7.4)
C(2″)	74.69[e])			
C(3″)	77.72[f])			
C(4″)	71.46[g])			
C(5″)	78.41[h])			
C(6″)	62.63[i])			
C(1‴)	103.40	H–C(1‴)	4.81	*d* (*J* = 7.0)
C(2‴)	75.13[e])			
C(3‴)	78.41[f])			
C(4‴)	71.46[g])			
C(5‴)	78.09[h])			
C(6‴)	62.52[i])			

[a]) 'Biogenetic numbering'[1]). [b]) In CD_3OD. [c])–[i]) Assignments interchangeable.

discernible. The signals of H–C(1) at δ 5.61 (*d*, *J* = 1.8 Hz, 1 H) and H–C(3) at δ 7.68 (*d*, *J* = 2.4 Hz, 1 H), typical of a secoiridoid moiety, together with four other *m*'s were in good agreement with those reported for sweroside [7][1]). The signal of H–C(5), normally at δ 3.1 (*m*, 1 H), was hidden by the sugar signals. The ^{1}H-NMR signal of H–C(7) (6.71 ppm, *t*, *J* = 2.2 Hz, 1 H) and ^{13}C-NMR signal of C(7) (93.36 ppm) were shifted downfield by *ca.* 1.2 and 2.7 ppm, respectively, suggesting an esterification at C(7). Such an effect was in accord with that reported for menthiafolin [6] and vogeloside [7]. The configuration of the two anomeric centres was readily established as β by ^{1}H- (*J*(1,2) = 7.8 and 7.4 Hz) and ^{13}C-NMR (C(1″) and C(1‴) at 100.31 and 103.40 ppm). The remaining C-signals were in accord with data reported for β-D-glucopyranosides [8].

The configuration at the C(7) atom could not be determined by NOE difference experiments. However, in the ^{1}H-NMR spectrum, the signal of H–C(7) appeared as a *t* and both equatorial-axial and equatorial-equatorial couplings were observed between H–C(7) and H_{eq}–C(6) (*J* = 2.2 Hz) and H–C(7) and H_{ax}–C(6) (*J* = 2.2 Hz). In the case

of an α-position of the ester group, a *d* with a *trans*-diaxial ($J = 9$–14 Hz) and an equatorial-axial coupling should be observed. These results were in accord with those reported for 7-methoxysweroside for which both α- and β-positions of the MeO group were described in vogeloside and epi-vogeloside [9]. These two secoiridoids were present in similar amounts in the MeOH extract of *H. corniculata* [3].

Attachment of the first glucosyl unit at position C(1) was confirmed by performing NOE experiments: presaturation of H–C(1″) in compound **1** gave enhancement of the H–C(1) signal. Due to overlapping of the aromatic H–C(2′) and H–C(5′) signals of the caffeoyl moiety, it was not possible to identify the OH group carrying the second glucosyl residue by means of NMR experiments. Thus, the free OH group of compound **1** was methylated with diazomethane. The monomethoxy derivative was then hydrolysed with HCl, and the residue was analysed by HPLC/UV after purification on *Sephadex LH-20* (MeOH). Pure standards of ferulic and isoferulic acids were run under the same chromatographic conditions. Comparison of the UV spectra and the retention times showed that the methylation followed by the acid hydrolysis of **1** gave ferulic acid. This demonstrated that the second glucosyl moiety was attached in *para* position. Compound **1** was thus identified as 7-β-[(*E*)-4′-*O*-(β-D-glucopyranosyl)caffeoyloxy]sweroside, called corniculoside.

3. Discussion. – Bitter principles of Gentianaceae constitute an important class of compounds from a pharmacological viewpoint and explain the use of most species of this family in traditional medicine or for the preparation of bitter tonic. Most bitter principles encountered in the Gentianaceae are simple monoterpene glycosides of the sweroside type. Corniculoside (**1**) is a rare type of a secoiridoid because of its ester linkage at position C(7) and its terminal glucosyl moiety on the caffeoyl group. Indeed, the aromatic part is generally connected to the secoiridoid by a sugar unit [11] [12]. On the other hand, the literature only reports secoiridoid glycosides linked with monoterpene units (neroloyl, foliamenthoyl, menthiafoloyl) [10–13]. Moreover, the 4-*O*-(β-D-glucopyranosyl)caffeoyl function is quite unusual and is only described in a few iridoids [10] [11] [14].

The electrospray interface appeared to be well suited for the ionization of minor compounds of high molecular weight in mass spectrometry and is thus a useful complementary tool to thermospray MS for the analysis of crude plant extracts.

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Experimental Part

General. TLC: Silica gel *60* F_{254} sheets (*Merck*); detection at 254 and 366 nm and with *Godin* reagent. Column chromatography (CC): *Sephadex LH-20* (650 × 65 mm i.d., *Pharmacia*). Medium-pressure liquid chromatography (MPLC): home-packed *LiChroprep-RP-18* column (15–25 μm, 460 × 36 mm i.d., *Merck*). M.p.: *Mettler-PF-80/82* hot-stage apparatus; uncorrected. ^{1}H- and ^{13}C-NMR: *Varian VXR 200* at 200.06 and 50.3 MHz, resp.; chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz. FAB-MS: *70-BioProbe* accessory (*Finnigan MAT*); source temp. r.t., probe tip 50°, matrix glycerol, FAB gun 4 kV and 1.2 mA, Xe gas used for bombardment, negative-ion mode.

LC/UV Analysis. The crude extracts were submitted to reversed-phase HPLC: *Waters 600-MS* solvent delivery system, on-line UV *Hewlett-Packard-1050* photodiode array detector (DAD), *Waters-NovaPak-RP-18*

column (4 μm, 150 × 3.9 mm i.d.), MeCN/H_2O gradient 5:95 → 65:35 containing 0.05% CF_3COOH in 50 min, flow rate 1 ml/min. The UV trace was observed at 254 nm, and UV spectra were recorded between 190 and 600 nm.

LC/MS Analyses. For LC/TSP-MS analyses, an aq. buffer of 0.5M NH_4OAc was added post-column (0.2 ml/min) to induce ionization (*Waters-590-MS* pump). TSP-MS: *Thermospray 2* (*Finnigan MAT*) interface, source temp. 280°, vaporizer 100°, aerosol 280–360°, electron-multiplier voltage 1800 V, dynode 15 kV, filament off and positive-ion mode, detection on a *Finnigan-MAT-TSQ-700* triple quadrupole instrument; spectra (150–900 amu) were recorded every 3 s. LC/ES-MS (API experiments): *Finnigan MAT TSQ 7000* with a capillary transfer tube temp. of 220°; suppression of solvent clustering by setting the rf potential of the source octapole to 10 eV; spectra (150–1500 amu) were recorded every 3 s.

Plant Material. Whole plants of *Halenia corniculata* L. (CORNAZ) were collected in 1992 in the vicinity of Ulaanbaatar and identified by Dr. *Sanchir* (Herbarium of the Botanical Institute, Ulaanbaatar, Republic of Mongolia). A voucher specimen (No. 92012) is deposited at the Institute of Pharmacognosy and Phytochemistry (University of Lausanne, Switzerland).

Extraction and Isolation. At r.t., 205 g of dry powdered whole plant were extracted successively with CH_2Cl_2 (3 × 1000 ml) and MeOH (3 × 1000 ml) to afford 9 and 34 g of extract, resp. A portion (10 g) of the MeOH extract was fractionated by gel filtration on *Sephadex LH-20* with MeOH as eluent. Twelve fractions were collected (*1–12*). *Fr. 2*, enriched in xanthone glycosides (3.3 g) was subjected to MPLC (*RP-18*, MeOH/H_2O 40:60, flow rate 9 ml/min) in three batches of 600 mg and yielded 42 mg of **1**. Purification was then realized on *Sephadex LH-20* with MeOH.

Acid Hydrolysis of Compound **1**. The sample (2 mg) was refluxed in 1N HCl (10 ml) for 4 h. The mixture was extracted with AcOEt, the org. layer analysed by TLC (SiO_2, $CHCl_3$/MeOH 7:3) and HPLC (*RP-18*, MeCN/H_2O 5:95 → 65:35 in 50 min) and the aq. phase adjusted to pH 7 with $NaHCO_3$. After freeze drying, the residue was extracted with pyridine and analysed for sugar by TLC (SiO_2, AcOEt/H_2O/MeOH/AcOH (13:3:3:4), detection with *p*-anisidine phthalate) [15].

Enzymatic Hydrolysis of **1**. A soln. of **1** (1 mg) and β-glucosidase (8 mg, *Sigma*) in 0.5M NaOAc buffer (3 ml, pH 5) was incubated overnight at 37°. The hydrolysate was extracted with $CHCl_3$ and the org. phase analysed by HPLC.

Alkaline Hydrolysis of **1**. The sample (1 mg) was dissolved in 0.5 ml of MeOH with a drop of 1N NaOH and stirred at 40° for 2 h. The reaction was followed by HPLC (same system as above, injection without purification) and afforded *(E)-4-O-(β-D-glucopyranosyl)caffeic acid* (**2**) and *7-hydroxysweroside* (**3**).

Methylation and Acid Hydrolysis of **1**. A MeOH soln. of **1** (3 mg in 0.5 ml) was treated with excess of freshly prepared CH_2N_2/Et_2O (1 ml, 35 mM) and stirrred overnight at 0°. After evaporation, the residue was submitted to acid hydrolysis (same as above) and purified on *Sephadex LH-20* with MeOH. HPLC Analyses with standards of ferulic and isoferulic acids were then performed.

7-β-[(E)-4′-O-(β-D-Glucopyranosyl)caffeoyloxy]sweroside[1] (= *(3S,4aS,5R,6S)-5-Ethenyl-6-(β-D-glucopyranosyloxy)-4,4a,5,6-tetrahydro-1-oxo-1H,3H-pyrano[3,4-c]pyran-3-yl (E)-3-[4-(β-D-glucopyranosyloxy)-3-hydroxyphenyl]prop-2-enoate* = *Corniculoside*; **1**): Yellow powder. TLC (SiO_2, $CHCl_3$/MeOH/H_2O 65:35:5): R_f 0.41, red-brown with *Godin* reagent. HPLC (*Novapak RP-18*, MeCN/H_2O gradient 5:95 → 65:35): t_R 16.5 min. M.p. 136–138°. $[\alpha]_D^{20} = -176$ ($c = 2.1$, MeOH). UV (MeOH): 219, 244, 297, 321. ^{1}H- and ^{13}C-NMR ($CDCl_3$; see *Table*). ^{13}C-NMR ((D_6)DMSO[2]): 96.25 (C(1)); 153.12 (C(3)); 103.16 (C(4)); 21.34 (C(5)); 27.16 (C(6)); 91.69 (C(7)); 131.95 (C(8)); 40.97 (C(9)); 120.83 (C(10)); 162.84 (C(11)); 164.51 (C(=O)); 116.19 (C(α)); 146.80 (C(β)); 128.36 (C(1′)); 114.88 (C(2′)); 147.78 (C(3′)); 146.50 (C(4′)); 115.15 (C(5′)); 121.31 (C(6′)); 101.46 (C(1″)); 72.96 (C(2″)); 77.25 (C(3″)); 69.96 (C(4″)); 76.36 (C(5″)); 60.94 (C(6″)); 98.63 (C(1‴)); 73.10 (C(2‴)); 77.14 (C(3‴)); 69.74 (C(4‴)); 76.64 (C(5‴)); 60.66 (C(6‴)). ES-MS (negative-ion mode): 811 ($[M + CF_3COO]^-$), 697 ($[M - H]^-$). FAB-MS (negative-ion mode): 697 ($[M - H]^-$), 535 ($[M - H - 162]^-$), 373 ($[M - H - 162 - 162]^-$), 161 ($[M - H - 162 - 162 - 162]^-$). TSP-MS (positive-ion mode): 375 ($[M + H - 162 - 162]^+$), 537 ($[M + H - 162]^+$, weak).

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[2]) The assignments of corresponding C-atoms of the glucose moieties are interchangeable, except for C(1″) and C(1‴).

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