150. Rhodenthoside A, a New Type of Acylated Secoiridoid Glycoside from *Gentiana rhodentha*

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Investigation of the metabolites from *Gentiana rhodentha* FR. (Gentianaceae) by LC-UV and LC-MS resulted in the isolation and identification of a new type of secoiridoid glycoside, rhodenthoside A (**1**), together with the known secoiridoids sweroside (**2**) and swertiamarin (**3**), as well as the known iridoids kingiside (**4**) and 8-epi-kingiside (**5**). The structures were established by 1D- and 2D-NMR, EI-MS, D/CI-MS, FAB-MS, CF-FAB, LC-MS, TSP, LC-MS, and LD-MS data in combination with chemical reactions.

Introduction. – In the course of our systematic studies on secondary metabolites typical of the Gentianaceae family [1–4], a phytochemical investigation of *Gentiana rhodentha* FR. was undertaken. This is a small herb growing in south-western China, especially in the Yunnan province. Traditional and ethnic healers in Yunnan use this medicinal plant to treat pneumonia, bronchitis, tuberculosis, and inflammations of the gallbladder and liver [5].

Preliminary LC-UV and LC-MS investigations (LC = liquid chromatography) of the methanolic extract of the whole plant of *G. rhodentha* suggested the presence of common secoiridoids, iridoids, and xanthones. There was also evidence for less polar high-molecular-weight secoiridoids. This paper reports the isolation and structure elucidation of rhodenthoside A (**1**), a new acylated secoiridoid, together with sweroside (**2**), swertiamarin (**3**), kingiside (**4**), and 8-epikingiside (**5**).

Results. – Dried and powdered plant material was refluxed three times with MeOH. The MeOH extract was successively defatted with petroleum ether and $CHCl_3$ and then partitioned with BuOH. This extract was screened by LC-MS and LC-UV using our routine procedure for the Gentianaceae [3] in order to obtain rapid and precise information on its composition.

HPLC-UV Analysis of the extract on a *RP-18* column exhibited different peaks with retention times less than 10 min arising from compounds **2**–**5** (*Fig. 1*). Their UV spectra showed only one maximum at *ca.* 240 nm (see **2** in *Fig. 1a*) which was characteristic for a chromophore containing an α,β-unsaturated ketone function, attributable to a secoiridoid glycoside [1]. These bitter principles are widespread in the Gentianaceae family. A very predominant component **6** (t_R 9 min 40 s) was identified as the xanthone *C*-glycoside mangiferin (= 2-(β-D-glycopyranosyl)-1,3,6,7-tetrahydroxy-9*H*-xanthen-9-one; for-

mula not shown) by comparison with an authentic sample [6] [7]. The slower-running peaks of compounds **1** and **7–9** (t_R 20–25 min; *Fig. 1*) also exhibited the same characteristic UV spectra of secoiridoids (one band at *ca.* 240 nm; see **1** in *Fig. 1b*). These compounds, which were less polar than the common secoiridoids, were studied in more detail. A thermospray (TSP) LC-MS analysis [8] with NH_4OAc as buffer (positive-ion mode) was carried out on a fraction enriched in the less polar components (*Fig. 1a*). Under these conditions, this technique usually gives intense pseudomolecular $[M + H]^+$ or $[M + NH_4]^+$ ions for this type of compounds [1]. The analysis revealed the presence of sweroside (**2**) in trace amount (t_R 9 min 50 s). This compound exhibited a characteristic TSP-MS with an intense pseudomolecular ion at *m/z* 359. The display of the *m/z* 359 single-ion trace allowed the specific assignment of peak **2** (*Fig. 1a*), but it also showed important signals corresponding to the less polar 'secoiridoid-like' compounds **1** and **7–9**. Indeed, the TSP spectra recorded for **1** and **7–9** were identical, and all exhibited an intense ion at *m/z* 359 and no ion at higher masses. These first results, obtained on-line with the crude extract of *G. rhodentha*, showed that these compounds exhibited the same UV

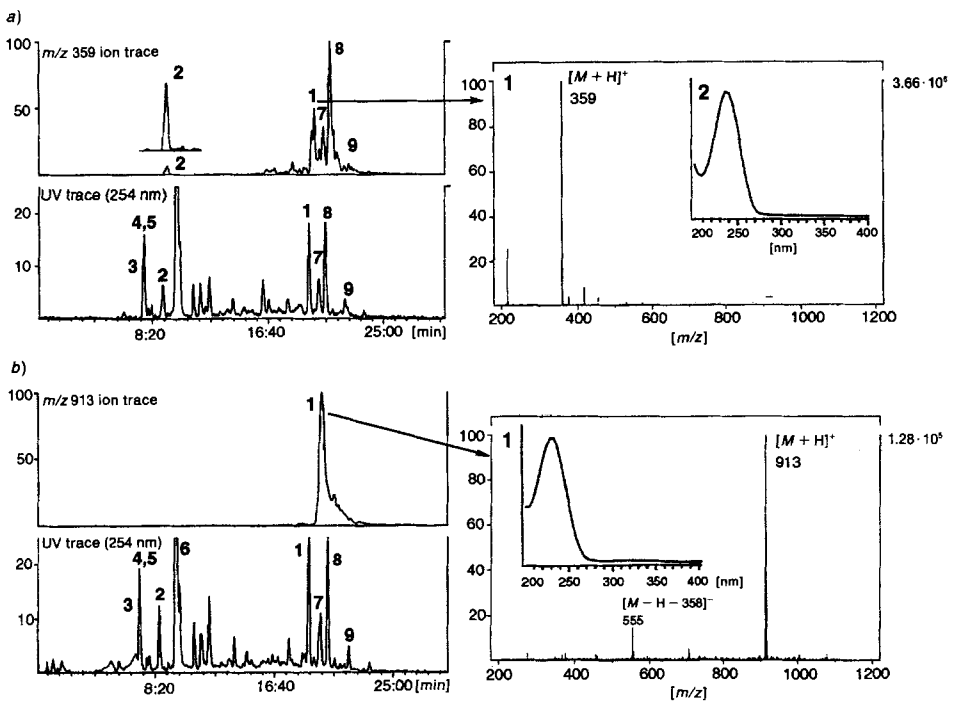


Fig. 1. *LC-MS and LC-UV analysis of the methanolic extract of* G. rhodentha a) *TSP LC-MS analysis of the enriched BuOH fraction of the MeOH extract*; b) *CF-FAB- LC-MS analysis of the crude BuOH fraction of the MeOH extract.* TSP: 0.5M NH_4OAc added post-column (0.2 ml/min), positive-ion mode, source 230°, vapourizer 90°, filament off; for HPLC, *Novapak RP-18* columnn (4 μm, 150 × 3.9 mm i.d.), $MeCN/H_2O$ gradient 5:95→50:50 (flow rate 0.9 ml/min, in 30 min) containing 0.05% CF_3COOH. CF-FAB: glycerol added post-column (0.15 ml/min, 50% *v/v*), negative-ion mode, source 100°, probe tip 50°, flow rate 10 μl/min after splitting (1/100); for HPLC, same LC conditions as for the TSP LC-MS analyses.

spectra and TSP-MS as sweroside (**2**), but their chromatographic behaviour was quite different. A second LC-MS analysis, using continuous-flow fast-atom bombardment (CF-FAB) [9], a softer ionisation technique than TSP LC-MS, was achieved to confirm the molecular weights of **1** and **7–9**. The crude MeOH extract of *G. rhodentha* was analysed in the negative-ion mode with post-column addition of glycerol. Virtually the same HPLC conditions as in the case of the TSP LC-MS analysis were used (*Fig. 1b*), and efficient splitting of the LC eluent (1/100) was performed to obtain a flow rate compatible with CF-FAB operation (10 µl/min). The total ion current recorded for the whole chromatogram showed a very important MS response for compounds **1** and **7–9**, while the more polar metabolites were only weakly ionised. The CF-FAB spectrum of **1** recorded on-line exhibited a very intense pseudomolecular ions $[M - H]^-$ at *m/z* 913. This complementary information indicated clearly that the molecular weight of **1** is 914 amu. According to the different results obtained on-line for **1** in the HPLC screening of the extract of *G. rhodentha*, it could be concluded that **1** is probably a type of moderately polar compound containing at least one unit very similar to sweroside (**2**). The CF-FAB spectra of compounds **7–9** exhibited pseudomolecular ion $[M - H]^-$ at *m/z* 1271, 1629, and 1643, respectively. Thus, these compounds have even higher molecular weights than **1**, and they all should have at least a common sweroside-type unit.

On the basis of the data provided by the HPLC analyses, the isolation of compound **1** was undertaken. The BuOH extract was fractionated by open-column chromatography on silica gel, MPLC (*RP-18*), and filtration on *Sephadex LH-20*, affording compounds **1–5**.

Acid hydrolysis of compound **1** afforded glucose, while the aglycone apparently decomposed. As already observed on-line in the extract, the FAB-MS of **1** revealed peaks at *m/z* 913 $[M - H]^-$ (negative-ion mode). These data indicated that the molecular weight of **1** was 914 and, together with the ^{1}H- and ^{13}C-NMR, suggested $C_{42}H_{58}O_{22}$ as molecular formula. Confirmation of the molecular weight was achieved by FAB-MS (positive-ion mode, signals at *m/z* 937 ($[M + Na]^+$) or 953 ($[M + K]^+$)) and laser-desorption (LD) MS (*m/z* 921.9 ($[M + Li]^+$) or 937.9 ($[M + Na]^+$)) of **1**. Moreover, the FAB-MS (negative-ion mode) exhibited a fragment ion at *m/z* 375, and EI-MS showed signals at *m/z* 375 and 198. These cleavages of the molecule indicated that **1** consisted of a secoiridoid glycoside moiety *m/z* 375 and a monoterpene moiety *m/z* 198. As already observed in the on-line TSP-MS of **1**, the D/CI-MS (positive-ion mode, NH_3) exhibited intense fragment ions at *m/z* 359 and 376 corresponding to the protonated sweroside pseudomolecular ion and its ammonium adduct, respectively. Other fragment ions (*m/z* 250 and 196) were characteristic for sweroside and *m/z* 180 and 198 for the glucose moiety. No molecular-weight ions were observed. The ^{13}C-NMR spectra (*Table 1*) of **1** showed 34 distinct resonances. However, the occurrence of eight pairs of signals implied the presence of two secoiridoid moieties. In addition, a set of signals attributable to the monoterpene moiety were readily apparent. Comparison with literature data [10–14] suggested that **1** was composed of two secoiridoid moieties linked with a monoterpene unit through two ester groups. Careful studies of the 1D- and 2D-NMR of **1**, its chemical transformation, and spectroscopic identification of the formed derivatives confirmed that **1** was 7,7′-*O*-[(2*E*,6*E*)-2,6-dimethylocta-2,6-dienedioyl]bis[swerosidic-acid 1-(β-D-glucopyranoside)] which we call rhodenthoside A.

Table 1. *^{13}C-NMR Data of Compounds* **1**, **1a**, **1b**, *and* **2**–**5**[a])

	1	**1a**	**1b**	**2**	**3**	**4**	**5**
Aglycone[b]), part 'a'							
C(1)	97.1	98.2	97.4	97.9	99.0	94.3	96.2
C(3)	149.2	151.9	153.2	153.9	154.8	154.2	154.4
C(4)	116.2	112.5	111.3	105.9	108.8	111.3	109.4
C(5)	31.5	29.1	31.2	28.3	64.2	27.9	28.2
C(6)	29.9	28.8	29.9	25.9	33.7	34.3	34.6
C(7)	64.5	63.8	63.9	69.2	65.9	174.4	174.8
C(8)	136.2	134.8	135.3	133.3	133.8	76.7	75.7
C(9)	45.3	44.7	27.9	43.7	51.9	39.8	41.8
C(10)	119.2	120.4	119.1	120.8	121.2	18.4	21.7
COO	168.1	170.0	167.7	168.5	167.9	168.2	168.3
MeO			51.3				
part 'b'							
C(1)	97.2	98.3	97.5				
C(3)	149.1	151.9	153.1				
C(4)	116.1	112.5	111.3				
C(5)	31.0	29.4	31.1				
C(6)	29.8	28.7	29.8				
C(7)	63.4	62.8	63.1				
C(8)	136.3	134.8	135.2				
C(9)	45.2	44.7	27.9				
C(10)	119.3	120.4	119.1				
COO	168.1	169.9	167.7				
MeO			51.2				
Sugar moiety, part 'a' and 'b'							
C(1)	99.7	98.0	99.9	99.6	100.2	100.0	100.6
C(2)	74.6	72.3	74.4	74.6	74.4	74.7	74.6
C(3)	77.8	74.0	77.7	77.8	77.7	77.9	77.8
C(4)	71.5	69.9	71.3	71.5	71.4	71.6	71.6
C(5)	78.3	73.2	78.0	78.3	78.5	78.4	78.5
C(6)	62.7	62.7	62.5	62.6	62.5	62.8	62.8
Dimethyloctadienedioyl moiety[b])							
C(1′)	169.5	169.5	169.1				
C(2′)	129.7	129.6	129.3				
C(3′)	142.1	142.3	141.7				
C(4′)	26.9	27.7	27.1				
C(5′)	40.2	40.2	39.9				
C(6′)	160.0	160.0	159.6				
C(7′)	117.3	117.2	116.8				
C(8′)	168.1	168.2	168.8				
C(9′)	12.7	12.6	12.1				
C(10′)	18.8	19.1	18.5				

[a]) Spectra were measured in MeOD; δ in ppm rel. to $SiMe_4$. [b]) Arbitrary numbering, see *Fig. 2*.

Table 2. *^{1}H-NMR Data of Compounds* **1**, **1a**, **1b**, *and* **2**–**5**[a])

	1	**1a**	**1b**	**2**	**3**	**4**	**5**
Aglycone[b]), part 'a'							
H–C(1)	5.40 (*d*, *J* = 2.0, 1 H)	5.31 (*d*, *J* = 2.0, 1 H)	5.54 (*d*, *J* = 2.0, 1 H)	5.55 (*d*, *J* = 1.6, 1 H)	5.73 (*d*, *J* = 1.4, 1 H)	5.66 (*d*, *J* = 6.1, 1 H)	5.49 (*d*, *J* = 8.0, 1 H)
H–C(3)	7.23 (*d*, *J* = 0.8, 1 H)	7.41 (*s*, 2 H)	7.46 (*s*, 2 H)	7.60 (*d*, *J* = 2.3, 1 H)	7.64 (*s*, 1 H)	7.52 (*s*, 1 H)	7.59 (*d*, *J* = 0.4, 1 H)
H–C(5)	2.90 (*ddd*, *J* = 5.8, 5.0, 3.4, 2 H)	2.80 (*ddd*, *J* = 5.7, 5.0, 3.4, 2 H)	2.90 (*ddd*, *J* = 5.7, 5.0, 3.4, 2 H)	3.10–3.45 (*m*, 1 H)		3.25 (*m*, 1 H)	3.05 (*dddd*, *J* = 11.5, 7.6, 4.7, 0.4, 1 H)
H_a–C(6)	2.15 (*m*, 2 H)	2.38 (*m*, 2 H)	2.35 (*m*, 2 H)	1.75 (*m*, 1 H)	1.90 (*dddd*, *J* = 10.2, 8.8, 10.2, 6.8, 1 H)	2.61 (*dd*, *J* = 17.1, 6.1, 1 H)	2.50 (*dd*, *J* = 16.7, 12.1, 1 H)
H_b–C(6)	1.75 (*m*, 2 H)	1.65 (*m*, 2 H)	1.95 (*m*, 2 H)	1.62 (*m*, 1 H)	1.74 (*dddd*, *J* = 16.2, 12.0, 16.2, 6.8, 1 H)	3.02 (*dd*, *J* = 17.1, 7.5, 1 H)	2.85 (*dd*, *J* = 16.7, 4.8, 1 H)
H_a–C(7)	4.15 (*m*, 4 H, H_a and H_b)	4.15 (*m*, 4 H, H_a and H_b)	4.12 (*m*, 4 H, H_a and H_b)	4.34 (*dd*, *J* = 9.8, 3.4, 1 H)	4.31 (*dd*, *J* = 5.6, 1.8, 1 H)		
H_b–C(7)				4.44 (*m*, 1 H)	4.38 (*dd*, *J* = 5.6, 1.8, 1 H)		
H–C(8)	5.81 (*ddd*, *J* = 18.2, 9.4, 9.4, 2 H)	5.65 (*dd*, *J* = 14.2, 8.1, 2 H)	5.79 (*ddd*, *J* = 16.3, 8.1, 8.1, 2 H)	5.55 (*ddd*, *J* = 16.5, 9.5, 9.5, 1 H)	5.45 (*ddd*, *J* = 16.8, 8.5, 8.6, 1 H)	4.78 (*dd*, *J* = 7.2, 2.7, 1 H)	4.49 (*quint*. *J* = 6.45, 1 H)
H–C(9)	2.60 (*ddd*, *J* = 7.6, 5.0, 2.0, 2 H)	2.68 (*ddd*, *J* = 8.2, 5.0, 2.0, 2 H)	2.65 (*ddd*, *J* = 8.0, 5.0, 2.0, 2 H)	2.70 (*ddd*, *J* = 9.7, 5.5, 1.6, 1 H)	2.92 (*dd*, *J* = 8.0, 1.4, 1 H)	2.42 (*ddd*, *J* = 7.8, 5.4, 3.2, 1 H)	2.13 (*dd*, *J* = 8.1, 7.9, 1 H)
H_a–C(10)	5.28 (*d*, *J* = 14.0, 2 H)	5.35 (*d*, *J* = 12.0, 2 H)	5.31 (*d*, *J* = 12.0, 2 H)	5.25 (*m*, 1 H)	5.29 (*m*, 1 H)	1.50 (*d*, *J* = 6.8, 3 H)	1.50 (*d*, *J* = 7.6, 3 H)
H_b–C(10)	5.19 (*d*, *J* = 10.0, 2 H)	5.25 (*d*, *J* = 10.0, 2 H)	5.24 (*d*, *J* = 4.0, 2 H)	5.30 (*m*, 1 H)	5.40 (*m*, 1 H)		
MeO–C(11)			3.65 (*s*, 6 H)			3.70 (*s*, 3 H)	3.72 (*s*, 3 H)
part 'b'[c])							
H–C(1)	5.38 (*d*, *J* = 2.0, 1 H)	5.27 (*d*, *J* = 2.0, 1 H)	5.51 (*d*, *J* = 2.0, 1 H)				
H–C(3)	7.24 (*d*, *J* = 1.2, 1 H)						

Table 2 (cont.)

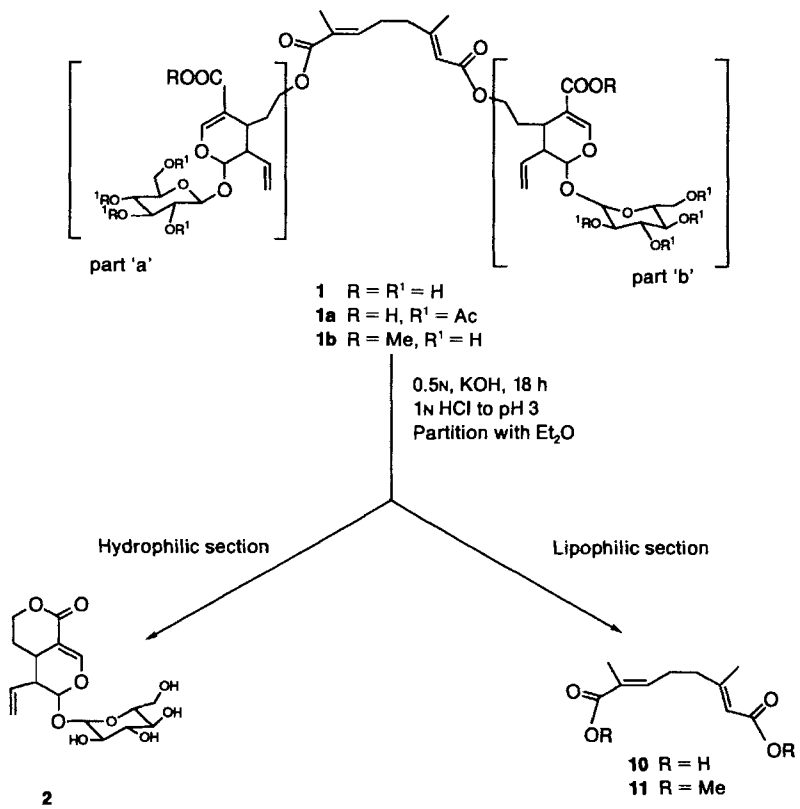
	1	1a	1b	2	3	4	5
Sugar moiety, part 'a'							
H–C(1)	4.68 (*d*, *J* = 8.0, 1 H)	4.91 (*d*, *J* = 8.4, 1 H)	4.71 (*d*, *J* = 8.1, 1 H)	4.68 (*d*, *J* = 7.8, 1 H)	4.65 (*d*, *J* = 8.1, 1 H)	4.69 (*d*, *J* = 8.1, 1 H)	4.70 (*d*, *J* = 8.0, 1 H)
H–C(2)	3.22 (*dd*, *J* = 8.8, 7.6, 2 H)	4.94 (*dd*, *J* = 8.0, 8.1, 2 H)	3.21 (*dd*, *J* = 8.4, 7.7, 2 H)	3.10–3.45 (*m*, 4 H, H–C(2) to H–C(5))	3.10–3.40 (*m*, 4 H, H–C(2) to H–C(5))	3.27–3.45 (*m*, 4 H, H–C(2) to H–C(5))	3.15–3.45 (*m*, 4 H, H–C(2) to H–C(5))
H–C(3)	3.31 (unresolved, 2 H)	5.04 (unresolved, 2 H)	3.30 (unresolved, 2 H)				
H–C(4)	3.30 (unresolved, 2 H)	5.08 (unresolved, 2 H)	3.29 (unresolved, 2 H)				
H–C(5)	3.89 (unresolved, 2 H)	5.01 (*dd*, *J* = 7.6, 0.8, 2 H)	3.31 (unresolved, 2 H)				
H_a–C(6)	3.87 (*dd*, *J* = 11.5, 0.4, 2 H)	4.15 (*dd*, *J* = 10.2, 6.0, 2 H)	3.90 (*dd*, *J* = 12.4, 1.1, 2 H)	3.89 (*dd*, *J* = 12.2, 1.2, 1 H)	3.89 (*dd*, *J* = 14.2, 1.2, 1 H)	3.89 (*dd*, *J* = 12.0, 1.8, 1 H)	3.90 (*dd*, *J* = 12.2, 1.5, 1 H)
H_b–C(6)	3.68 (*dd*, *J* = 11.5, 4.3, 2 H)	3.91 (*dd*, *J* = 10.2, 0.4, 2 H)	3.65 (*dd*, *J* = 12.4, 4.1, 2 H)	3.65 (*dd*, *J* = 12.2, 6.8, 1 H)	3.66 (*dd*, *J* = 14.2, 7.0, 1 H)	3.66 (*dd*, *J* = 12.0, 6.4, 1 H)	3.62 (*dd*, *J* = 12.2, 7.3, 1 H)
part 'b'[c])							
H–C(1)	4.67 (*d*, *J* = 8.0, 1 H)	5.06 (*d*, *J* = 7.8, 1 H)	4.69 (*d*, *J* = 8.1, 1 H)				
Dimethyloctadiendioyl moiety[b])							
H–C(3′)	6.65 (*t*, *J* = 8.2, 4.1, 1 H)	6.75 (*t*, *J* = 8.2, 4.1, 1 H)	6.71 (*t*, *J* = 8.1, 3.9, 1 H)				
H–C(4′)	2.42 (*m*, 2 H)	2.35 (*m*, 2 H)	2.40 (*m*, 2 H)				
H–C(5′)	2.35 (*m*, 2 H)	2.36 (*m*, 2 H)	2.35 (*m*, 2 H)				
H–C(7′)	5.62 (br. *s*, 1 H)	5.72 (br. *s*, 1 H)	5.69 (br. *s*, 1 H)				
Me(9)	1.81 (br. *s*, 3 H)	1.85 (*s*, 3 H)	1.85 (*s*, 3 H)				
Me(10)	2.15 (br. *s*, 3 H)	2.18 (*s*, 3 H)	2.17 (*s*, 3 H)				
Ac		1.94, 1.95, 1.98, 2.04 (each 6 H)					

[a]) Spectra were measured in MeOD; δ in ppm rel. to $SiMe_4$, *J* in Hz. [b]) Arbitrary numbering, see *Fig. 2*. [c]) Omitted data are the same as that for part 'a'.

The broad-band decoupled ^{13}C-NMR and DEPT spectra of **1** (*Table 1*) showed signals of acid carbonyl C-atoms (168.1 ppm (2 CO)), 2 acetal C-atoms (97.1, 97.2 ppm), 2 vinylic side chains (136.2, 119.2, 136.3, 119.3 ppm), 2 O-bearing CH_2 groups (63.4, 64.5 ppm), 2 quaternary olefinic C-atoms (116.1, 116.2 ppm), 2 tertiary olefinic C-atoms (149.1, 149.2 ppm), 2 additional CH_2 groups (29.8, 29.9 ppm), and 2 CH groups (31.5, 31.0 ppm), together with signals attributable to 2 hexosyl moieties. These signals were in agreement with the ^{13}C-NMR data of the secoiridoid moiety of some secoiridoid glycosides [4] [14] [15]. The fragment ion at m/z 375 in the FAB- and EI-MS was in accord with the molecular weight of a swerosidic-acid glucoside [16]. Furthermore, in the ^{1}H-NMR (*Table 2*), the typical resonance of H–C(1) of the secoiridoid units at δ 5.40 (*d*, J = 2.0, Hz, 1 H) and 5.38 (*d*, J = 2.0 Hz, 1 H) and the resonance of the sugar anomeric protons at δ 4.68 (*d*, J = 8.0 Hz, 1 H) and 4.67 (*d*, J = 8.0 Hz, 1 H) confirmed that **1** possessed two identical secoiridoid moieties (for numbering, see *Fig. 2*). However, the signal of C(7) of the swerosidic-acid moiety was shifted downfield by *ca.* 3.5 ppm in the ^{13}C-NMR, suggesting that the OH group at C(7) was esterified. This α-effect (+3.5 ppm) produced by esterification at C(7) was also in accord with that reported in [4]. Resonances of the monoterpene moiety which appeared at 169.5, 168.1 (carbonyl), 129.7, 117.3 (quaternary olefinic C-atoms), 142.1 160.0 (tertiary olefinic C-atoms), 26.9, 40.2 (CH_2 groups), 12.7 and 18.8 (Me groups) were quite similar to the ^{13}C-NMR data of (2*E*,6*E*)-2,6-dimethyl-8-oxoocta-2,6-dienoate [10]. However, the absence of the resonances of an aldehyde group in the ^{1}H- and ^{13}C-NMR of **1** indicated that C(8′) could be an ester carbonyl. The fragment ion at m/z 198 in the EI-MS, corresponding to a fragment due to the loss of two secoiridoid units, suggested that the monoterpene was probably a dicarboxylic acid.

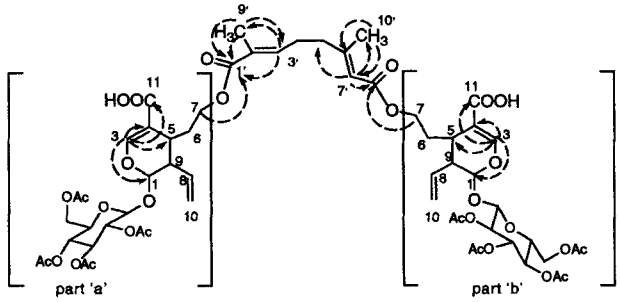
Acetylation of **1** readily yielded octaacetate **1a** as a colourless amorphous powder. The FAB-MS (negative- and positive-ion modes) exhibited the molecular ions of **1a** at m/z 1249 ($[M - H]^-$) and 1273 ($[M + Na]^+$), respectively, while the LD-MS showed a strong pseudomolecular ion at m/z 1274.6 ($[M + H + Na]^+$). All these data were in

Scheme



accord with the molecular formula $C_{58}H_{74}O_{30}$. $^1H,^1H$ Connectivities were confirmed by a phase-sensitive COSY [17] [18]. ^{13}C-NMR Assignments were obtained by 2D $^{13}C,^1H$ HETCOR [19] shift correlation *via* $^1J(C,H)$, while two-dimensional heteronuclear correlation *via* long-range coupling (FLOCK) [20] enabled complete assignment of the quaternary C-atoms of **1** and **1a** (*Table 1*). Selective INEPT [21] experiments on **1a** were performed to confirm the esterification of the monoterpene moiety at C(7) of the swerosidic-acid moiety (*Fig. 2*). Methylation of **1** with CH_2N_2/Et_2O [22] yielded the dimethyl ester **1b** as a white amorphous powder.

Irradiation of *H*–C(7) of **1a**, using a delay corresponding to $J = 7$ Hz, selectively enhanced the CO signals at δ 169.5 and 168.2 (*Fig. 2*). At the same time, long-range polarization transfer from *H*–C(3′) and CH_3(9′) enhanced the signal for the quaternary C-atom at δ 169.5 and that from *H*–C(7′) enhanced the C-atom at δ 168.2, confirming the esterification at C(7). The FAB-MS of **1b** (*m/z* 941 ($[M - H]^-$, negative-ion mode) and 965 ($[M + Na]^+$, positive-ion mode)) showed the compound to have the molecular formula $C_{44}H_{62}O_{22}$. In the NMR, ^{13}C-resonances arising from the 2 MeO groups appeared at 51.2 and 51.3 ppm, and one ^1H-signal at 3.65 ppm (6 H, 2 MeO) confirmed the presence of 2 free COOH groups in compound **1**.



1a rhodenthoside A octaacetate (arbitrary numbering)

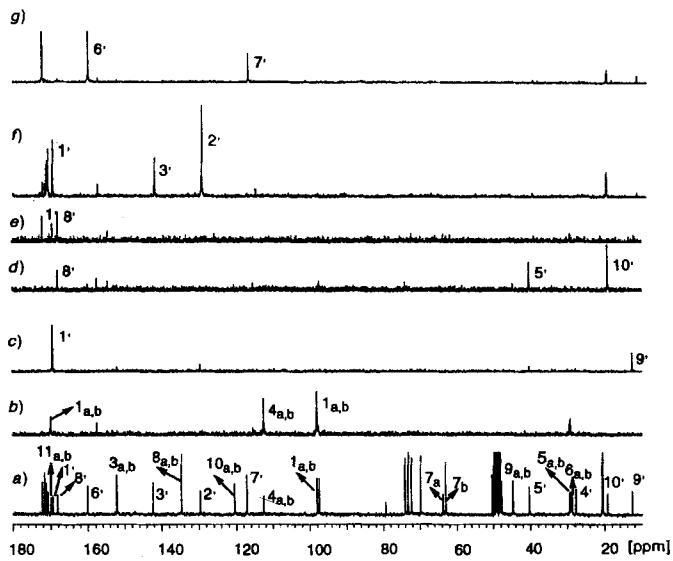


Fig. 2. *Selective INEPT experiments of* **1a**. *a*) Broad-band decoupled spectrum; *b*) irradiation of H–C(3) (part 'a') and H–C(3) (part 'b'), $J = 7$ Hz; *c*) irradiation of *H*–C(3′), $J = 7$ Hz; *d*) irradiation of *H*–C(7′), $J = 7$ Hz; *e*) irradiation of H–C(7) (part 'a') and H–C(7) (part 'b'), $J = 7$ Hz; *f*) irradiation of CH_3(9′), $J = 7$ Hz; *g*) irradiation of CH_3(10′), $J = 7$ Hz.

Alkaline hydrolysis of **1** with KOH (0.5M) followed by acidification [23] yielded, after liquid-liquid extraction (see *Exper. Part*), compounds **10** as a colourless viscous oil and **2** as an amorphous powder. The ^{1}H-NMR of **10** displayed resonances (see *Exper. Part*) which were similar to the monoterpene moieties (2*E*,6*E*)-8-hydroxy-2,8-dimethylocta-2,6-dienoic acid (= foliamenthic acid) and (2*E*,6*E*)-2,6-dimethyl-8-oxoocta-2,6-dienoyl connected with the iridoid glycosides [10]. The spectral data suggested that **10** was a monoterpene dicarboxylic acid. The presence of the two COOH groups was confirmed by methylation with CH_2N_2/Et_2O affording the colourless viscous oily diester **11**. Compound **2** was identified as sweroside by its D/CI-MS and ^{1}H- and ^{13}C-NMR data and by comparison with literature data [1] [4] [15] [24] [25]. Sweroside is the product of the lactonization between the OH at C(7) and the acid group at C(11) of the swerosidic-acid moiety which was generated by acidification after the alkaline hydrolysis of **1** [16]. Apparently, the same reaction occurred during the TSP ionization of **1**, since a strong fragment ion at *m/z* 359 was observed (*Fig. 1a*).

The D/CI-MS of **10** exhibited a pseudomolecular ion at *m/z* 216 ($[M + NH_4]^+$), and the EI-MS showed fragment ions at *m/z* 180 ($[M - H_2O]^+$), 162 ($[M - 2H_2O]^+$), 152 ($[M - H_2O - CO]^+$), and 134 ($[M - 2H_2O - CO]^+$).

The D/CI-MS of **11** provided a pseudomolecular ion at *m/z* 244 ($[M + NH_4]^+$). The EI-MS exhibited a molecular ion at *m/z* 226 together with fragment ions at *m/z* 194 ($[M - MeOH]^+$), 166 ($[M - MeOH - CO]^+$), and 134 ($[M - 2MeOH - CO]^+$). The ^{1}H-NMR of **11** showed a signal for 2 MeO groups at 3.61 ppm. Moreover, the ^{13}C-NMR displayed 12 resonances: 2 MeO groups appeared at 51.0 and 51.9 ppm, while the other signals were similar to those reported for monoterpene moieties in iridoid glycosides [10]. A selective INEPT experiment was carried out for **11**. Irradiation of *H*–C(3), *H*–C(7), CH_3(10) and CH_3(9), using a delay corresponding to $J = 4$ Hz, allowed the complete assignment of the ^{13}C-NMR data (*Fig. 3*).

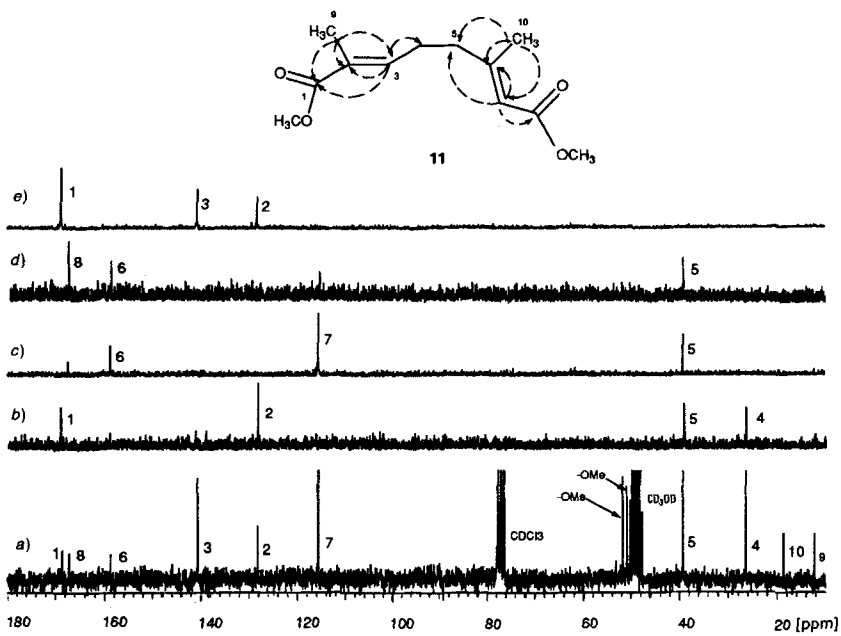


Fig. 3. *Selective INEPT experiment of* **1e**. *a*) Broad-band decoupled spectrum; *b*) irradiation of *H*–C(3), $J = 4$ Hz; *c*) irradiation of CH_3(10), $J = 4$ Hz; *d*) irradiation of *H*–C(7), $J = 4$, Hz; *e*) irradiation of CH_3(9), $J = 4$ Hz.

The ^{1}H-NMR data of rhodenthoside A (**1**) are characteristic for a *cis*-diaxial relationship of an α-configurated H–C(1) and an α-configurated H–C(9) ($J(1,9) = 2$ Hz). As in the ^{1}H-NMR spectrum of **1** overlapping of the signals prevented the determination of the relative configuration at C(9) and C(5), careful study of the ^{1}H-NMR data of sweroside (**2**) was carried out, indicating a small dihedral angle (*ca.* 30°) between H–C(9) and α-configurated H–C(5) ($J(5,9) = 5$ Hz), thus establishing the conformation of the dihydropyran ring of **1**.

Based on chemical transformations (including acid hydrolysis) and spectroscopic data (EI-MS and ^{1}H- and ^{13}C-NMR *Tables 1* and *2*), as well as on comparison with literature data [1] [4] [15] [24–31], compounds **2**–**5**, isolated from the extract, were identified as sweroside (**2**), swertiamarin (**3**), kingiside (**4**), and 8-epikingiside (**5**). The other 'secoiridoid-like' high-molecular-weight compounds **7**–**9** (*Fig. 1*) were also isolated, but their structures are currently under investigation and will be presented separately.

Discussion. – Iridoid glycosides are a main class of compounds distributed widely in Gentianaceae plants. Varieties of structures derived from the simple iridoid-glycoside moieties were described [4]. However, it is noteworthy that, until now, secoiridoid glycosides connected with a 'nerol-type' or 'foliamenthic-type' monoterpene acid by esterification were only isolated from plants belonging to the Menyanthaceae, Scrophulariaceae, Bignoniaceae, and Verbenaceae [10–13] [32–34]. Rhodenthoside A (**1**) is the first representative of a new type of secoiridoid glycoside linked with a monoterpene unit from the Gentianaceae family.

As most of the secoiridoid glycosides contained in gentianaceous plants are bitter in taste, rhodenthoside A is also a bitter principle. According to a approximative preliminary test, the taste of **1** is more bitter than that of sweroside (**2**). This bitterness, as for other secoiridoids, is probably due to the high number of oxygenated functions.

The investigation of further iridoid glycosides in *Gentiana rhodentha* FR. is in progress.

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Experimental Part

General. TLC: Silica gel *60* F_{254} Al sheets (*Merck*); *RP-18-WF* HPTLC plates (*Merck*); detection at 254 and 366 nm and with *Godin* reagent. Column chromatography (CC): silica gel (0.063–0.2 mm; *Merck*) and *Sephadex LH-20* (*Pharmacia*). Medium-pressure liquid chromatography (MPLC): home-packed *LiChroprep-RP-18* column (25–40 μm, 46 × 2.5 cm i.d.). Semiprep. HPLC: *Shimadzu-LC-8A* pump, *Knauer-LiChrosorb-RP-18* column (7 μm, 16 × 250 mm i.d.), detection at 254 nm. Anal. HPLC-UV: *Hewlett-Packard-1090-II* instrument equipped with a photodiode array detector, *Novapak-RP-18* column (4 μm, 150 × 3.9 mm i.d.); MeCN/H_2O gradient 5:95→50:50 (flow rate 0.9 ml/min, in 30 min) containing 0.05% CF_3COOH. M.p.: *Mettler-FP-80/82* hot-stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV: *Perkin-Elmer-Lambda-3* spectrophotometer. IR: *Perkin-Elmer-781* spectrophotometer. ^{1}H- and ^{13}C-NMR: *Varian VXR 200* at 200.06 and 50.3 MHz, resp. TSP LC-MS, CF-FAB LC-MS, FAB-MS, EI-MS, and D/CI-MS: *Finnigan-MAT-TSQ-700* triple-stage quadrupole instrument. LC-MS: HPLC configuration using *Waters-600MS* solvent-delivery system, on-line UV *Waters 490MS* multiwavelength detector, and *Waters 590MS* pump for post-column addition of reagents. TSP LC-MS: chromatographic conditions identical with those for HPLC-UV; 0.5M aq. NH_4OAc was added post-column (0.2 ml/min) to induce ionization; *Thermospray 2* (*Finnigan MAT*) interface, source temp. 230°, vaporizer 90°, filament-off and

positive-ion mode. CF-FAB LC-MS: chromatographic conditions identical with those for HPLC-UV; an aq. soln. of glycerol was added post-column (0.15 min/min, 50% *v/v*); the HPLC eluent 1.0 min/min was split by a *Dual Split Accurate* (*LC* Packings) (1/100) and one part, 10 µl/min, introduced in the CF-FAB *70-BioProbe Accessory* (*Finnigan Mat*); CF-FAB source temp. 100°, probe tip 50°, negative-ion mode. LD-MS: time of flight mass spectrometer (TOF-MS) *Mass Monitor LDI-1700* (*Linear Scientific Inc.*) using various salts (NaCl, KCl, or LiCl) in the matrix.

Plant Material. Gentiana rhodentha FR. was collected in August 1992 in Lu Qien county, Yunnan Province, P.R. China. A voucher specimen is deposited at the Kunming Institute of Botany, Academia Sinica, Kunming-650204, P. R. China.

Extraction and Separation. The powder of dried whole plants (4 kg) was extracted 3 times with hot MeOH, and the residue obtained by removal of the solvent *in vacuo* was triturated with H_2O. The insoluble material was filtered off through a *Celite* layer which was washed with H_2O, and the filtrate and washings were combined and concentrated *in vacuo* to *ca.* 800 ml; the aq. soln. was defatted successively with petroleum ether and $CHCl_3$ and then extracted with BuOH (4 × 350 ml). The BuOH layer was evaporated to give a residue (200 g). Part of the residue (55 g) was subjected to CC (silica gel, $CHCl_3$/MeOH mixtures of increasing MeOH content) and afforded 10 fractions. *Fr. 10* (6.8 g) was submitted to MPLC (*RP-18*, MeOH/H_2O 40:60, flow rate 8 ml/min) and gave 5 fractions (*A–E*). *Fr. C* was repurified on *Sephadex LH-20* with MeOH and provided compound **1** (300 mg). *Fr. 5* was separated by MPLC (*RP-18*, MeOH/H_2O 20:80, flow rate 8 ml/min) affording compounds **2** (120 mg), **3** (340 mg), **4** (120 mg), and **5** (60 mg).

Acid Hydrolysis of Compounds **1–5**. The sample (2 mg) was refluxed in 1N HCl for 2 h. The mixture was extracted with AcOEt, the org. layer analyzed by TLC (SiO_2, $CHCl_3$/MeOH 9:1), and the aq. phase adjusted to pH 6 with $NaHCO_3$. After freeze drying, the residue was extracted with pyridine and analyzed for sugar by TLC on silica gel (AcOEt/MeOH/H_2O/AcOH 65:15:15:20, detection with *p*-anisidine phthalate). Glucose as sugar moiety in compounds **1–5** was confirmed.

7,7′-O-[(2E,6E)-2,6-Dimethylocta-2,6-dienedioyl]bis[sweroside-acid 1-(β-D-Glucopyranoside)] (= *4,4′-{[(2E,6E)-2,6-Dimethylocta-2,6-dienedioyloxy]bis(ethane-2,1-diyl)}-5,5′-ethenyl-6,6′-(β-D-glucopyranosyloxy)-5,5′,6,6′-tetrahydrobis(4H-pyran-3-carboxylic Acid)* = *Rhodenthoside A*; **1**). White, amorphous powder. M.p. 187–192°. TLC (SiO_2, $CHCl_3$/MeOH/H_2O 60:40:8): R_f 0.60. HPTLC (*RP-18 WF*, MeOH/H_2O 1:1): R_f 0.47. $[\alpha]_D^{25} = -48.5$ (MeOH, $c = 20$). UV (MeOH): 274.5 (2.40). IR (KBr): 3400, 2910, 1780, 1640, 1530, 1400, 1340, 1150, 1070, 940, 870, 800. ^{13}C-NMR: *Table 1*. ^{1}H-NMR: *Table 2*. FAB-MS (neg.-ion mode): 913 ($[M - H]^-$), 375 ($[M - 539]^-$), 577 ($[M - H - 375 + K]^-$). FAB-MS (pos.-ion mode): 937 ($[M + Na]^+$), 953 ($[M + K]^+$). LD-MS: 921.9 ($[M + Li]^+$), 937.9 ($[M + Na]^+$). EI-MS: 375 ($[M - 539]^+$), 231 ($[375 - Glc]^+$), 198 ($[M - 716]^+$).

Acetylation of **1**. For 10 h, **1** (40 mg) was kept in pyridine/Ac_2O 1:1 (6 ml) at r.t. The mixture was poured into ice-H_2O and then partitioned with Et_2O. The residue, after evaporation of the Et_2O, was purified on *Sephadex LH-20* with MeOH: octaacetate **1a** (45 mg). White powder. M.p. 96–99°. TLC (SiO_2, $CHCl_3$/MeOH 90:10): R_f 0.47. HPTLC (*RP-18 WF*, MeOH/H_2O 75:25): R_f 0.24. $[\alpha]_D^{25} = -172.7$ (MeOH, $c = 16.7$). UV (MeOH): 274.0 (2.42). IR (KBr): 3610, 3150, 1850, 1730, 1520, 1480, 1340, 1150, 1110, 980, 890. ^{13}C-NMR: *Table 1*. ^{1}H-NMR: *Table 2*. FAB-MS: 1249 ($[M - H]^-$), 1273 ($[M + Na]^+$). LD-MS: 1274.6 ($[M + H + Na]^+$).

Methylation of **1**. A MeOH soln. of **1** (70 mg in 8 ml) was treated with excess CH_2N_2/Et_2O as described in [22] and the yellowish residue was redissolved in MeOH (10 ml). The MeOH-soluble fraction was purified by semi-prep. HPLC (*RP-18*, MeOH/H_2O 65:35): dimethyl ester **1b** (7 mg). White powder. M.p. 167–170°. TLC (SiO_2, $CHCl_3$/MeOH 80:20): R_f 0.32. HPTLC: (*RP-18*, MeOH/H_2O 75:25): R_f 0.40. $[\alpha]_D^{25} = -266.6$ (MeOH, $c = 3$). UV (MeOH): 274.5 (2.73). IR (KBr): 3600, 3110, 1790, 1740, 1550, 1400, 1180, 1150, 1050, 980, 880. ^{13}C-NMR: *Table 1*. ^{1}H-NMR: *Table 2*. FAB-MS: 941 ($[M - H]^-$), 569 ($[M - 373]^-$), 965 ($[M + Na]^+$).

Alkaline Hydrolysis of **1**. The soln. of **1** (25 mg) in 1N KOH/MeOH (3 ml) was diluted with H_2O (3 ml) and placed at r.t. for 18 h. The aq. phase was adjusted to pH 3 with 1N HCl and then partitioned with Et_2O. The org. layer was evaporated and the white residue passed through *Sephadex LH-20* with MeOH: *(2E,6E)-2,6-dimethyl-octa-2,6-dienedioic acid* (**10**; 7 mg). The aq. layer was partitioned with BuOH. The BuOH extract was submitted to CC (1. silica gel, $CHCl_3$/MeOH/H_2O 70:30:5; 2. *Sephadex LH-20*, MeOH): *sweroside* (**2**; 10 mg).

10: Colourless viscous oil. ^{1}H-NMR ($CDCl_3$): 6.73 (*t*, $J = 8.0$, H–C(3)); 2.36 (*m*, 2 H–C(4), 2 H–C(5)); 5.69 (br. *s*; H–C(7)); 1.82 (*s*, Me–C(2)); 2.15 (*s*, Me–C(6)). D/CI-MS: 216 ($[M + NH_4]^+$). EI-MS: 180 ($[M - H_2O]^+$), 162 ($[M - 2H_2O]^+$), 152 ($[M - H_2O - CO]^+$), 134 ($[M - 2H_2O - CO]^+$).

2: White powder.M.p. 106–109°. $[\alpha]_D^{25} = -157.64$ ($c = 8.5$, MeOH). UV (MeOH): 277.0 (1.62). ^{1}H-NMR: 5.54 (*d*, $J = 1.6$, H–C(1)); 7.59 (*d*, $J = 2.2$, H–C(3)); 3.2–3.5 (*m*, H–C(5)); 1.75 (*m*, H_a–C(6)); 1.60 (*m*, H_b–C(6));

4.44 (*dd*, J = 9.6, 3.6, H_a–C(7)); 4.32 (*m*, H_b–C(7)); 5.56 (*ddd*, J = 16.4, 9.2, 9.2, H–C(8)); 2.71 (*ddd*, J = 9.4, 5.3, 1.2, H–C(9)); 5.20 (*m*, H_a–C(10)); 5.15 (*m*, H_b–C(10)); 4.66 (*d*, J = 8.0, H–C(1), Glc); 3.0–3.5 (*m*, H–C(2), H–C(5), Glc); 3.90 (*dd*, J = 12.0, 1.4, H_a–C(6), Glc); 3.55 (*dd*, J = 12.0, 6.7, H_b–C(6)), Glc). ^{13}C-NMR: 98.0 (C(1)); 153.5 (C(3)); 106.0 (C(4)); 28.3 (C(5)); 25.9 (C(6)); 69.5 (C(7)); 133.2 (C(8)); 43.1 (C(9)); 120.7 (C(10)); 168.2 (C(11)); 99.9 (C(1), Glc); 74.8 (C(2), Glc); 77.9 (C(3), Glc); 71.8 (C(4), Glc); 78.0 (C(5), Glc); 62.3 (C(6), Glc). D/CI-MS: 376 ($[M + NH_4]^+$), 196 ($[M - Glc]^+$).

Methylation of **10**. Acid **10** (7 mg) was treated with an excess of CH_2N_2/Et_2O. The product was purified on *Sephadex LH-20* with MeOH: diester **11** (10 mg). Colourless viscous oil. UV (MeOH): 271.0 (2.13). IR (KBr): 2810, 1700, 1650, 1500, 1430, 1350, 1040, 940, 880, 840. ^{1}H-NMR ($CDCl_3$): 6.58 (*t*, J = 8.1, H–C(3)); 2.21 (*m*, 2H–C(4)); 2 H–C(5)); 5.58 (br. *s*, H–C(7)); 1.70 (*s*, Me–C(2)); 2.05 (*s*, Me–C(6)); 3.61 (*s*, 2 MeO). ^{13}C-NMR: 168.2 (C(1)); 128.1 (C(2)); 140.1 (C(3)); 26.2 (C(4)); 39.2 (C(5)); 158.2 (C(6)); 115.8 (C(7)); 167.1 (C(8)); 12.2 (*Me*–C(2)); 18.9 (*Me*–C(6)); 51.9, 51.0 (2 MeO). D/CI-MS: 244 ($[M + NH_4]^+$). EI-MS: 266 (M^+).

Sweroside (**2**), *swertiamarin* (**3**), *kingiside* (**4**), and *8-epi-kingiside* (**5**) were identified by comparison of their ^{1}H-NMR, ^{13}C-NMR, EI- and D/Cl-MS with literature data [1] [4] [15] [24–31].

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