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FURTHER ACYLATED SECOIRIDOID GLUCOSIDES FROM *GENTIANA RHODANTHA**

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Key Word Index—*Gentiana rhodantha*; Gentianaceae; secoiridoid glucosides; rhodanthosides.

Abstract—Investigation of the metabolites of *Gentiana rhodantha* by LC–UV and LC–mass spectrometry (MS) resulted in the isolation and identification of two further high molecular weight secoiridoid glycosides, rhodanthosides B and C. These compounds contain four secoiridoid glucoside moieties, two of which were linked with a monoterpene dicarboxylic acid. Their structures were established by LC–UV, TSP LC–MS, CF–FAB LC–MS, ES/LC–MS, FAB–MS and 1D and 2D NMR data in combination with chemical reactions. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

There is an increasing interest in the constituents of gentians as these plants are rich sources of xanthones. These polyphenols possess monoamine oxidase inhibitory activity and may be developed as antidepressive drugs [1–2]. Gentians contain also bitter principles which are generally monoterpene glycosides of the secoiridoid type [3–5]. In the course of our systematic phytochemical studies on species belonging to the Gentianaceae family, we have investigated *Gentiana rhodantha* Fr., a plant used in the traditional medicine of minorities living in Yunnan Province, China. Recently, we reported on the identification of a new type of acylated secoiridoid glucoside named rhodenthoside A [6] which possessed two swerosidic acid moieties linked with a monoterpene dicarboxylic acid (dimethyloctadienedioic acid) through ester groups. Continuation of this work led to the isolation and characterization of two closely related secoiridoids, rhodanthosides B (**2**) and C (**3**), from a methanol extract. This paper deals with the isolation and structural elucidation of these compounds. At the same time, we propose to rename **1** rhodanthoside A, since rhodenthoside ensued from the erroneous name *G. rhodentha* under which the plant was mentioned in a Chinese reference book.

RESULTS

Dried and powdered whole plant of *G. rhodantha* was refluxed in methanol. After evaporation to dryness, the methanol extract was dissolved in H_2O, defatted with petrol followed by chloroform and then partitioned with *n*-butanol. The butanol extract was screened for bitter principles by LC–UV, thermospray (TSP) LC–mass spectrometry and continuous flow–fast atom bombardment (CF–FAB) LC–mass spectrometry using our routine procedure for Gentianaceae [5]. These analyses revealed the presence of common secoiridoid glucosides such as sweroside and swertiamarin, together with less polar high-M_r 'secoiridoid-like' compounds [6]. The latter include rhodanthoside A (**1**) (M_r 914), the characterization of which was reported in our previous paper [6], and the related secoiridoids **2** and **3**. As in the case of rhodanthoside A, compounds **2** and **3** exhibited in their TSP LC–mass spectra a fragment ion at *m/z* 359 corresponding to the swerosidic acid moieties ($[M - H_2O + H]^+$). The M_rs of these two secoiridoids, however, were higher than that of **1**. The CF–FAB mass spectrum of **2** obtained on-line exhibited a pseudomolecular ion at *m/z* 1629 ($[M - H]^-$). The electrospray (ES) LC–mass spectra recorded in both positive {1631.9 ($[M + H]^+$) and negative (1629.8 ($[M - H]^-$)} ion modes confirmed the M_r of **2** to be 1630. Compared with **1**, compound **2** thus appeared to possess two additional swerosidic acid units (2×358). The M_r of **3** was deduced to be 1658 from ES LC–mass spectral analyses {1660.8 ($[M + H]^+$) and 1772.8 ($[M + TFA]^+$)}, which suggested this compound to be a dimethyl derivative of **2**. As **2** and **3** were most probably new derivatives of rhodanthoside A, their targeted isolation was undertaken (see Experimental).

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The ^1H and ^{13}C NMR spectra of **2** and **3** (Tables 1 and 2), together with their mass spectral data, led to the consideration of $C_{74}H_{102}O_{40}$ (calc. 1631.6) and $C_{76}H_{106}O_{40}$ (calc. 1659.6) as their molecular formulae, respectively. As in the case of **1** [6], hydrolysis of **2** and **3** with KOH, followed by acidification, afforded sweroside (**4**) and dimethyloctadienedioic acid (**5**). The ^{13}C NMR data for **2** (Table 1) were quite similar to those for **1**. However, the signals belonging to the swerosidic acid moieties were doubled, and six carbonyl signals [δ 168.6 (×2); 170.6 (×2); 169.4; 168.1] were observed instead of four as in the case of **1**. This was in agreement with the presence of four swerosidic acid units and a monoterpene dicarboxylic acid in the compound. Four carbonyl signals [δ 168.1, 168.6 (×2) and 169.4] were characteristic of esters, while two overlapping carbonyl signals (δ 170.6) were attributed to free carboxylic groups. The ester carbonyls assigned to the monoterpene dicaboxylic acid moiety showed nearly the same resonances [δ 169.4 (C-1′) and 168.1 (C-8′)] as in **1** [δ 169.5 (C-1′) and 168.1 (C-8′)]. The two signals at δ 18.6 (overlapping) suggested the carboxylic groups of the internal swerosidic acid units to be esterified.

Acetylation of **2** yielded the hexadecaacetate **2a** as a white amorphous powder (M_r 2303) (FAB–mass spec-

Table 1. ^{13}C NMR data for compounds **2**, **2a** and **3**

	2 (CD_3OD)	**2a** (CD_3OD)	**2a** ($CDCl_3$)	**3** (CD_3OD)
Aglycone moieties*				
Part 'a'				
1	97.5	97.5	96.1	97.7
3	153.5	2.2	150.9	153.6
4	111.6	112.3	110.9	113.3
5	31.0	28.7, 28.8	27.7	31.1
6	29.8	28.8	27.7	29.9
7	63.5, 63.7	63.8	62.2	63.5, 63.6
8	135.7	134.7	132.6	135.5
9	45.2	44.7	43.0	45.1
10	119.7	120.6	120.1	119.8
COO	168.6	168.2	166.6	168.5, 168.6
Part 'b'				
1″	97.7	98.1	96.1	97.9
3″	153.5	152.4	152.4	153.6
4″	111.6	112.5	110.9	111.5
5″	31.0	29.2, 29.5	27.7	31.3
6″	29.8	28.9	27.7	29.9
7″	64.4	63.1	62.2	63.6, 64.4
8″	135.7	134.8	132.7	135.7
9″	45.2	44.7	42.9	45.1
10″	119.7	120.6	120.2	119.8
COO	170.6	169.5	171.1	169.1
OMe				51.9
Sugar moieties†				
1/1″	100.0	97.6, 98.3	95.8, 96.4	100.1
2/2″	74.5	72.3	72.1	74.5
3/3″	77.7, 77.8	74.0	72.3	77.9
4/4″	71.4	70.0	68.1	71.5
5/5″	78.2	73.3	72.4	78.2
6/6″	62.7	63.1	61.6	62.7
OAc (16 signals)		170.7 – 172.3	169.1–170.6	
		20.4–20.7	20.2–20.7	
Dimethyloctadienedioyl moiety				
1′	169.4	170.0	170.1	169.4
2′	129.5	130.0	128.4	129.6
3′	142.3	142.3	140.8	142.3
4′	27.5	27.8	26.6	27.5
5′	40.3	40.3	39.3	40.3
6′	160.3	160.2	158.6	160.3
7′	117.0	117.2	116.0	117.0
8′	168.1	168.1	167.9	168.1
9′	12.7	12.7	12.3	12.7
10′	19.1	19.2	18.7	19.1

*With a few exceptions, resonances of corresponding carbons in parts 'a1' and 'a2' and in parts 'b1' and 'b2', respectively, were not resolved. When two signals were observed, both values are given.

†Resonances of corresponding carbons unresolved. When two signals were observed, both values are given.

Table 2. ^{1}H NMR data for compounds **2**, **2a** and **3** (coupling constants in Hz)

	2 (CD_3OD)	**2a** CD_3OD)	**2a** ($CDCl_3$)	**3** (CD_3OD)
Aglycone moieties*				
Part 'a'				
1	5.20 (2H, *d*, J = 2.0)	5.54 (1H, *d*, J = 2.0)	5.17 (1H, *d*, J = 1.2)	5.22 (2H, *d*, J = 1.2)
3	7.44 (2H, *br s*)	7.46 (2H, *s*)	7.31 (2H, *s*)	7.43 (1H, *br s*)
5	2.83 (4H, *ddd*, J = 6.0, 5.2, 3.0)	2.90 (2H, *ddd*, J = 5.7, 5.0, 3.4)	2.72 (2H, *ddd*, J = 5.1, 5.2, 3.1)	2.95 (4H, *dd*, J = 6.0, 4.2)
6a	1.80 (4H, *m*)	2.35 (2H, *m*)	1.62 (2H, *m*)	1.82 (4H, *m*)
6b	2.10 (4H, *m*)	1.95 (2H, *m*)	2.13 (2H, *m*)	2.22 (4H, *m*)
7	4.18 (8H, *m*)	4.12 (4H, *m*, H-a, b)	4.13 (4H, *m*, H-a, b)	4.14 (8H, *m*)
8	5.62 (8H, *ddd*, J = 17.2, 8.0, 8.0)	5.79 (2H, *ddd*, J = 16.3, 8.1, 8.1)	5.55 (2H, *ddd*, J = 15.8, 6.1, 17.1)	5.80 (4H, *ddd*, J = 18.0, 9.2, 9.2)
9	2.70 (4H, *dd*, J = 8.2, 3.2)	2.65 (2H, *ddd*, J = 8.0, 5.0, 2.0)	2.60 (2H, *ddd*, J = 8.0, 5.0, 2.0)	2.62 (4H, *dd*, J = 7.6, 4.2)
10a	5.32 (4H, *d*, J = 8.0)	5.31 (2H, *d*, J = 12.0)	5.25 (2H, *d*, J = 10.0)	5.23 (4H, *d*, J = 10.2)
10b	5.42 (4H, *d*, J = 8.0)	5.24 (2H, *d*, J = 4.0)	5.28 (2*H*, *d*, J = 4.0)	5.58 (4H, *d*, J = 10.0)
Part 'b'				
1″	5.33 (2H, *d*, J = 1.8)	5.51 (1H, *d*, J = 2.0)	5.19 (1H, *d*, *J*2.0)	5.39 (2H, *d*, J = 1.0)
3″			7.42 (2H, *br s*)	7.50 (2H, *br s*)
11″-OMe				3.75 (6H, *s*)
Sugar moieties*				
Part 'a'				
1	4.68 (2H, *d*, J = 8.2)	4.75 (1H, *d*, J = 8.1)	4.82 (1H, *d*, J = 8.0)	4.70 (2H, *d*, J = 8.4)
2	3.20 (4H, *dd*, J = 8.0, 7.4)	4.21 (2H, *dd*, J = 8.4, 7.7)	4.13 (2H, *dd*, J = 8.2, 7.2)	3.22 (4H, *dd*, J = 8.2, 7.4)
3	3.36 (4H, unresolved)	5.08 (2H, unresolved)	5.05 (2H, unresolved)	3.38 (4H, unresolved)
4	3.64 (4H, unresolved)	5.01 (2H, unresolved)	5.02 (2H, unresolved)	3.65 (4H, unresolved)
5	3.62 (4H, unresolved)	4.12 (2H, unresolved)	3.99 (2H, unresolved)	3.72 (4H, unresolved)
6a	3.80 (4H, *dd*, J = 10.0, 1.2)	4.32 (2H, *dd*, J = 12.2, 1.1)	4.22 (2H, *dd*, J = 12.4, 1.1)	3.82 (4H, *dd*, J = 10.0, 1.2)
6b	3.95 (4H, *dd*, J = 10.0, 2.2)	4.25 (2H, *dd*, J = 12.2, 2.1)	4.21 (2H, *dd*, J = 12.1, 3.8)	3.70 (4H, *dd*, J = 12.2, 2.4)
Part 'b'				
1″	4.72 *2H, *d*, J = 8.2)	4.94 (1H, *d*, J = 8.1)	4.88 (1H, *d*, J = 8.0)	4.75 (2H, *d*, J = 8.4)
Dimethyloctadienedioyl moiety				
3′	6.70 (1H, *t*, J = 10.0)	6.78 (1H, *t*, J = 8.1, 3.9)	6.68 (1H, *t*, J = 8.1, 3.9)	6.68 (1H, *t*, J = 10.0)
4′	2.22 (2H, *m*)	2.35 (2H, *m*)	2.31 (2H, *m*)	2.21 (2H, *m*)
5′	2.18 (2H, *m*)	2.36 (2H, *m*)	2.25 (2H, *m*)	2.14 (2H, *m*)
7′	5.48 (1H, *br s*)	5.78 (1H, *br s*)	5.65 (1H, *br s*)	5.46 (1H, *br s*)
9′	1.80 (3H, *s*)	1.87 (3H, *s*)	1.78 (3H, *s*)	1.78 (3H, *s*)
10′	2.20 (3H, *s*)	2.20 (3H, *s*)	2.11 (3H, *s*)	2.22 (3H, *s*)
CH_3COO		1.94–2.07 (unresolved, 48H)	1.85–2.05 (unresolved, 48H)	

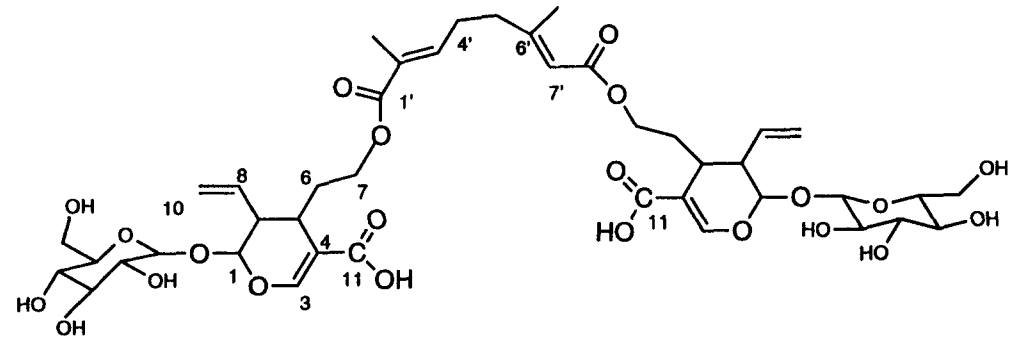
Attributions are supported by double quantum filtered phase sensitive COSY (DQFCOSY) experiments.
*Protons of part 'b' which are not listed have the same resonance as the corresponding ones of part 'a'.

tra: $[M-H]^-$ at m/z 2302 and $[M+Na]^+$ at m/z 2326). These data were in accord with the calculated molecular formula $C_{106}H_{134}O_{56}$ (calc. 2303.1), further confirming the presence of four swerosidic acid units. Moreover, peracetylation of the sugar moieties and consideration of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) excluded the attachment of the terminal swerosidic acid moieties on the glucosyl residues of the internal swerosidic acid units. The ^{1}H and ^{13}C NMR spectra of **2a** recorded in $CDCl_3$ presented better signal resolution than those of **2**. In particular, unambiguous assignment of H-3 and H-3″ of the internal and terminal swerosidic acid units was possible by means of selective INEPT [7] experiments. Irradiation of H-3 (δ 7.31) and H-3″ (δ 7.42), using a delay corresponding to J = 8 Hz, selectively enhanced the carbonyl signals at δ 166.6 (CO-11) and 171.1 (CO-11″), respectively. At the same time, long-range polarization transfers were detected from H-3 and H-3″ to the signals at δ 110.9 (C-4 and C-4″) and 96.1 (C-1 and C-1″). These data were in agreement with the esterification of the carboxylic groups of the internal swerosidic acid moieties. Hence, structure **2** has been assigned to the secoiridoid, which has been named rhodanthoside B.

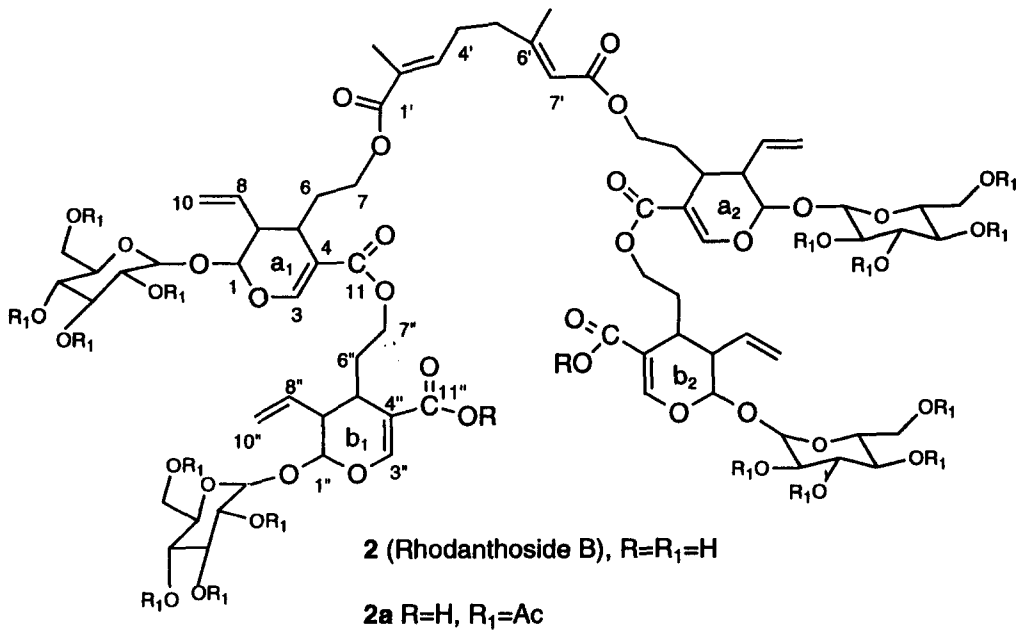
A singlet at δ 3.75 (×2) and a signal at δ 51.9 (×2, *q*) in the ^{1}H and ^{13}C NMR spectra, respectively, confirmed the presence of two methoxyl groups in **3**. Furthermore, the carbonyl signals of the terminal swerosidic acid units were highfield shifted to δ 169.1, indicating esterification of the free carboxylic groups of **2**. Structure **3** was thus assigned to this compound, which has been named rhodanthoside C.

DISCUSSION

Rhodanthosides A, B and C belong to a new type of acylated secoiridoid. Recently, one of us (Prof. C. R. Yang) also isolated rhodanthoside B from *G. k-*



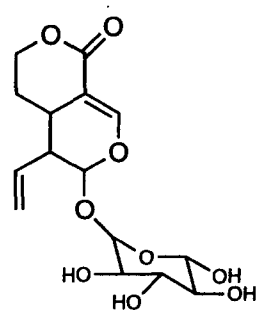
1 (Rhodanthoside A)



2 (Rhodanthoside B), R=R_1=H

2a R=H, R_1=Ac

3 (Rhodanthoside C), R=CH_3, R_1=H



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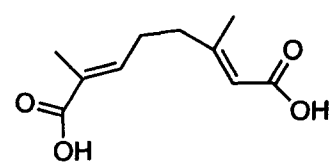

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usnezowii Fr. (unpublished results). Such types of seco-iridoids can thus constitute new markers for further chemotaxonomical investigations of the genus *Gentiana*.

Compound **2** has two more swerosidic acid units than **1**, while **3** is the dimethyl ester derivative of **2**. In order to prove that these compounds were not artefacts resulting from the extraction procedure, methanol, 70% aqueous ethanol and aqueous extracts were prepared at room temperature. All the extracts were submitted to HPLC analyses. Compounds **1–3** were shown to be present in both the methanol and 70% aqueous ethanol extracts. This demonstrated that **1–3** are not artefacts due to extraction under reflux in methanol. On the other

hand, **1–3** were not extracted by water due to their moderate hydrophilic character. Compounds **2** and **3** are bitter in taste. According to a preliminary test, **2** and **3** are, however, less bitter than **1**. The characterization of **2** and **3** provides a new example of the varieties of structures derived from an iridoid skeleton found in Gentianaceae plants [8].

EXPERIMENTAL

General. Mps: uncorr.; TLC: silica gel 60 F_{254} aluminium sheets (Merck) and RP-18 WF HPTLC plates (Merck); CC: silica gel (0.063–0.2 mm; Merck); MPLC: home-packed LiChroprep RP-18 column (25–40 μm, 46 × 2.5 cm i.d.); Semi-prep. HPLC: RP-18 column (7 μm, 16 × 250 mm i.d., Knauer), detection at 254 nm; Analyt. HPLC–UV: Novapak RP-18 column (5 μm, 150 × 3.9 mm i.d.); MeCN-H_2O (1:19 → 1:1; in 30 min, 0.9 ml min^{-1}); ^1H and ^{13}C NMR: 200.06 MHz and 50.3, resp., carbon multiplicities were determined by DEPT experiments. TSP LC–MS, CF–FAB LC–MS, FAB–MS and DCI–MS: triple quadrupole instrument, experimental conditions as previously described [6]. ES/LC–MS (API experiments) were recorded on a Finnigan MAT TSQ 7000 with a capillary transfer tube temp. of 220°.

Plant material. Gentiana rhodantha Fr. was collected in August 1992 in Lu Qien County, Yunnan Province, P.R. China. A voucher specimen (No. 93019) is deposited at the Kunming Institute of Botany, Academia Sinica, Kunming-650204.

Extraction and separation. The powdered dried whole plant (4 kg) was extracted under reflux with MeOH (3 × 5 l). After evapn of the solvent under red. pres., the solid residue was triturated with H_2O. The insoluble material was filtered off through a celite layer. The filtrates were combined and concd under red. pres. to *ca* 800 ml. The soln was defatted successively with petrol and $CHCl_3$, and then extracted with *n*-BuOH (4 × 350 ml). A 55 g portion of the *n*-BuOH extract (200 g) was subjected to CC on silica gel with $CHCl_3$–MeOH mixts of increasing polarity. 10 frs were collected (1–10). Fr. 10 (6.8 g) was submitted to MPLC on RP-18 with MeOH–H_2O (2:3, 8 ml min^{-1}) to give 5 for (A–E). Fr. D was purified on Sephadex LH-20 with MeOH to provide **2** (1.1 g). Sepn of fr. E by MPLC on RP-18 using MeOH–H_2O (1:4, 8 ml min^{-1}) afforded **3** (180 mg).

Acid hydrolysis of **2** *and* **3**. The sample (4 mg) was refluxed in 1 M HCl for 2 hr. The mixt. was extracted with EtOAc. The organic layer was analysed by TLC (silica gel, $CHCl_3$–MeOH, 9:1). The aq. phase was adjusted to pH 6 with $NaHCO_3$. After freeze drying, the residue was extracted with pyridine and analysed for sugars by TLC on silica gel (EtOAC–MeOH–H_2O–HOAc, 13:3:3:4, detection with *p*-anisidine phthalate).

Rhodanthoside B (**2**). Amorphous powder, mp 153–156°. TLC (silica gel, $CHCl_3$–MeOH–H_2O, 14:6:1): R_f 0.12. HPTLC (RP-18, MeOH–H_2O, 13:7): R_f 0.65. $[\alpha]_D$ −111.5° (MeOH, *c* 0.01). UV: λ_{nm}^{MeOH} (log ε): 274.5 (2.40); IR ν^{KBr} cm^{-1}: 3200, 1700, 1600; ^{13}C NMR: Table 1; ^1H NMR: Table 2; FAB–MS (matrix glycerol, negative ion mode): 1629 $[M-H]^-$; CF–FAB–MS (negative ion mode): 1629 $[M-H]^-$; LC/ES–MS (negative ion mode): 1629.8 $[M-H]^-$; LC/ES–MS (positive ion mode): 1631.9 $[M+H]^+$; DCI–MS (NH_3, positive ion mode): 376, 359, 350, 198, 180.

Acetylation. Compound **2** (100 mg) was kept in pyridine–Ac_2O (1:1, 6 ml) at room temp. for 14 hr. The mixt. was poured into ice–H_2O and then extracted with Et_2O. After evapn to dryness, the residue was purified on Sephadex LH-20 with MeOH to give **2a** (118 mg) as a powder, mp 112–114°. TLC (silica gel, $CHCL_3$–MeOH, 19:1): R_f 0.23. HPTLC (RP-18, MeOH-H_2O, 3:1): R_f 0.31 $[\alpha]_D$ −79.0° (MeOH, *c* 0.01). UV: λ^{MeOH} nm (log ε): 274.0 (2.42); IR ν^{KBr} cm^{-1}: 3500, 2950, 1750, 1620, 1420, 1390, 920, 780; ^{13}C NMR: Table 1; ^1H NMR: Table 2; FAB–MS (matrix glycerol, positive ion mode): 2326 $[M+Na]^+$; FAB–MS (matrix glycerol, negative ion mode): 2302 $[M-H]^-$, CF–FAB–MS (positive ion mode): 2326 $[M+Na]^+$.

Alkaline hydrolysis. Compound **2** (40 mg) was dissolved in 0.5 M methanolic KOH (5 ml). The soln was diluted with 3 ml H_2O and kept at room temp. for 18 hr. The aq. phase was adjusted to pH 4 with 1 M HCl and extracted with Et_2O. The organic layer was evapd to dryness and the residue was passed through a Sephadex LH-20 column with MeOH to provide 2,6-dimethyloctadienedioic acid (**5**) (6 mg). The aq. layer was further extracted with *n*-BuOH. The *n*-BuOH extract was submitted to silica gel chromatography ($CHCl_3$–MeOH–H_2O, 14:6:1) followed by gel filtration over Sephadex LH-20 (MeOH) to give sweroside (**4**) (20 mg).

Rhodanthoside C (**3**). Amorphous powder, mp 137–141°. TLC (silica gel, $CHCl_3$–MeOH–H_2O, 14:6:1): R_f 0.80. HPLC (RP-18, MeOH–H_2O, 13:7): R_f 0.22. $[\alpha]_D$ −112.5° (MeOH, *c* 0.01). UV: λ^{MeOH} nm (log ε): 274.5 (2.40); IR ν^{KBr} cm^{-1}: 3500, 2910, 1780, 1620, 1440, 1410, 1280, 920, 780; ^{13}C NMR: Table 1; ^1H NMR: Table 2; LC/ES–MS (positive ion mode): 1660.8 $[M+H]^+$; LC/ES–MS (negative ion mode): 1772.8 $[M+TFA]^-$.

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