Soldanelline B − The First Acylated Nonlinear Tetrasaccharide Macrolactone from the European Convolvulaceae *Calystegia soldanella*

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The first acylated nonlinear tetrasaccharide resin glycoside, soldanelline B (**1**), and its fatty acid derivative soldanellic acid B (**2**), have been isolated from a Portuguese Convolvulaceae *Calystegia soldanella*. The structures were elucidated using high-field NMR spectroscopy, FAB mass spectrometry and chemical studies.

Introduction

The most common constituent compounds of the Convolvulaceae family are alkaloids[1] and resin glycosides.[2] Plants of this family are widely used in folk medicine all over the world, particularly as purgatives.[3] Pharmacological studies on extracts of these plants have reported antimicrobial, analgesic, spasmogenic, spasmolytic, hypotensive, psychotomimetic and anticancer effects.[4] Some species also exhibit very aggressive competitive effects, due to their strong propagative power, complemented with a high allelopathic interference.[5,6]

The unique structural features of Convolvulaceae glycosides and their multiple pharmacological properties encouraged us to initiate a study of European species. We began with a Portuguese plant of the genus *Calystegia*, described as having purgative properties and used to cure hydropsy, paralysis, rheumatism and scurvy.[7] A chloroform extract of this plant had shown interesting cytotoxic activity (UISO, ED_{50} 2 μg/ml; KB, ED_{50} 7 μg/mL).

This paper deals with the isolation and structural elucidation of the major component of the chloroform extract: soldanelline B (**1**), together with its fatty acid derivative, soldanellic acid B (**2**). Their chemical structures were determined by extensive application of high resolution 2D NMR techniques and FABMS. Soldanelline B (**1**) is different from all resin glycosides reported in the literature to date: it is the second branched tetrasaccharide reported,[8] but is the first nonlinear tetrasaccharide to contain short-chain fatty acids attached by ester linkages to the sugar units.

Results and Discussion

Compound **1**, a white amorphous solid, was obtained from the chloroform extract of the lyophilised roots of *Calystegia soldanella* after column chromatography (flash chromatography, $CHCl_3$/MeOH, 4:1; and reversed-phase chromatography, methanol) and recycling HPLC on a C_{18} μ-Bondapack column (MeOH/H_2O, 9:1). A sample of product **1** was then submitted to alkaline hydrolysis with 5% aqueous KOH, and the resulting mixture was neutralized with 1 M HCl solution. An aliquot was acidified to pH = 1, extracted with Et_2O, and the Et_2O extract methylated with CH_2N_2. By GC-MS, the mass spectra and retention times for the three major peaks corresponded to those of the methyl esters of 3-hydroxy-2-methylbutanoic acid (hmba or nilic acid), 2-methylbutanoic acid (mba) and (*Z*)-2-methyl-2-butenoic acid (tiglic acid, tga) in the approximate ratio of 1:1:1. GC analysis using a chiral column permitted the assignment of the 2-methylbutanoic acid as its (*S*) isomer. The 3-hydroxy-2-methylbutanoic acid was shown by optical rotation to be the (2*S*,3*R*) isomer.

The neutral aqueous phase from the alkaline hydrolysis was purified by HPLC, using a C_{18} μ-Bondapack column (MeOH/H_2O, 7:3), to give compound **2** as a colourless solid [m.p. 157−160°C, $[\alpha]_D^{20} = -11.24$ (c = 0.125, MeOH)]. The observation of four methine ^{13}C peaks in the region expected for anomeric carbon atoms (δ = 100−105) suggested that the compound was a tetrasaccharide. Also, the signals of the two methylene carbon atoms were observed in the sugar region. The negative FABMS of **2** showed major ion peaks at m/z = 887 $[M - 1]^-$, 741, 725, 581, 439, 417, 271. The ion peak at m/z = 271 for compound **2** suggested the presence of a hydroxyhexadecanoic acid (probably 11-hydroxyhexadecanoic, or jalapinolic acid − the aglycone most frequently obtained from Convolvulaceous glycosides) moiety, conjectured to be glycosidically linked to a tetrasaccharide consisting of two deoxyhexose (1 × qui and 1 × rha) and two hexose (glc) units, $[M - 1]^-$ at m/z = 887. This was confirmed by fragment ion peaks at m/z = 417 [271 + 146; methylpentose unit]$^-$, 581 [271 + 146 + 161; hexose unit + H]$^-$ and by peaks at m/z = 741, 725, which were, respectively, assigned to $[M - H - 146]^-$ and $[M - H - 161]^-$. The latter suggested that the hydroxyhexadecanoic acid was directly attached to a branched saccharide possessing terminal rhamnose or quinovose and glucose units; it could also be concluded, since the linkage had sur-

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vived alkaline hydrolysis, that the tetrasaccharide was bound to the hydroxy group, and not the carboxylate group, of the hydroxy fatty acid.

On acidic hydrolysis (4 M HCl, reflux 24 h), the aqueous solution yielded a hydroxy fatty acid and a monosaccharide mixture. The methyl ester (CH_2N_2 in Et_2O) of the acid was subjected to GC-MS analysis and was identified as the methyl derivative of (11*S*)-hydroxyhexadecanoic acid.[9,10] Its mass spectrum showed diagnostic α-cleavage ions at *m*/*z* = 215, 101.[11,12] The monosaccharide fraction was converted into tris(trimethylsilyl) (TMS) ethers of methyl (4*R*)-thiazolidine-4-carboxylate derivatives[13,14] and the mixture was analysed by GC. Retention times were identical with those of authentic samples derived, respectively, from D-quinovose, L-rhamnose and D-glucose, in the approximate ratio of 1:1:2.

To ascertain the positions of the individual deoxyhexoses in the chain, and also the stereochemistry of the sugar linkages, it was necessary to use high-field ^1H and ^{13}C NMR spectroscopy, together with 2D NMR techniques (COSY, HMQC, HMBC, TOCSY and ROESY).

Proton assignments in the aglycone moiety were determined from ^1H NMR and COSY and TOCSY spectra. The corresponding carbon assignments (Table 1) were then determined from one-bond HMQC spectra.

Table 1. ^1H and ^{13}C NMR spectroscopic data of soldanellic acid (**2**) in [D_5]pyridine (spectra recorded at 500 MHz; agl = aglycone, qui = quinovose, glc = glucose, rha = rhamnose)

Sugar	Position	δ_H (multiplicity, ^1J [Hz], intensity)	δ_C
agl	1		181.70
	2	2.61 (t, 7.5, 2 H)	39.12
	3	1.94 (m, 2 H)	27.44
	11	3.86 (m, 1 H)	81.27
	15	1.28 (m, 2 H)	23.11
	16	0.86 (t, 7.0, 3 H)	14.44
qui	1	4.84 (d, 8.0, 1 H)	102.86
	2	4.30 (dd, 8.5, 8.5, 1 H)	79.94
	3	4.39 (dd, 9.0, 9.0, 1 H)	79.05
	4	3.56 (dd, 9.0, 9.0, 1 H)	77.22
	5	3.71 (dq, 9.0, 9.0, 1 H)	72.64[a]
	6	1.57 (d, 6.0, 3 H)	18.69
glc_A	1′	5.73 (d, 7.5, 1 H)	101.93
	2′	4.16 (dd, 8.5, 8.5, 1 H)	78.26
	3′	3.9−4.0[b]	89.68
	4′	4.09 (dd, 9.0, 9.0, 1 H)	71.63
	5′	3.9−4.0[b]	78.61
	6′	4.51 (dd, 2.0, 11.5, 1 H)	62.48
		4.22 (dd, 6.5, 11.7, 1 H)	
rha	1	6.17 (s, 1 H)	102.56
	2	4.86 (dd, 1.5, 3.0, 1 H)	72.17
	3	4.62 (dd, 3.0, 9.25, 1 H)	72.61[a]
	4	4.27 (dd, 9.5, 9.5, 1 H)	74.32
	5	4.96 (dd, 9.0, 6.0, 1 H)	69.70
	6	1.78 (d, 6.0, 3 H)	19.13
glc_B	1″	4.93 (d, 8.0, 1 H)	104.96
	2″	4.06 (dd, 8.0, 8.0, 1 H)	74.88
	3″	4.15 (dd, 9.0, 9.0, 1 H)	78.87
	4″	3.9−4.0[b]	70.58
	5″	3.77 (m, 1 H)	77.39
	6″	4.36 (dd, 2.5, 11.8, 1 H)	63.21
		4.14 (dd, 6.0, 11.5, 1 H)	

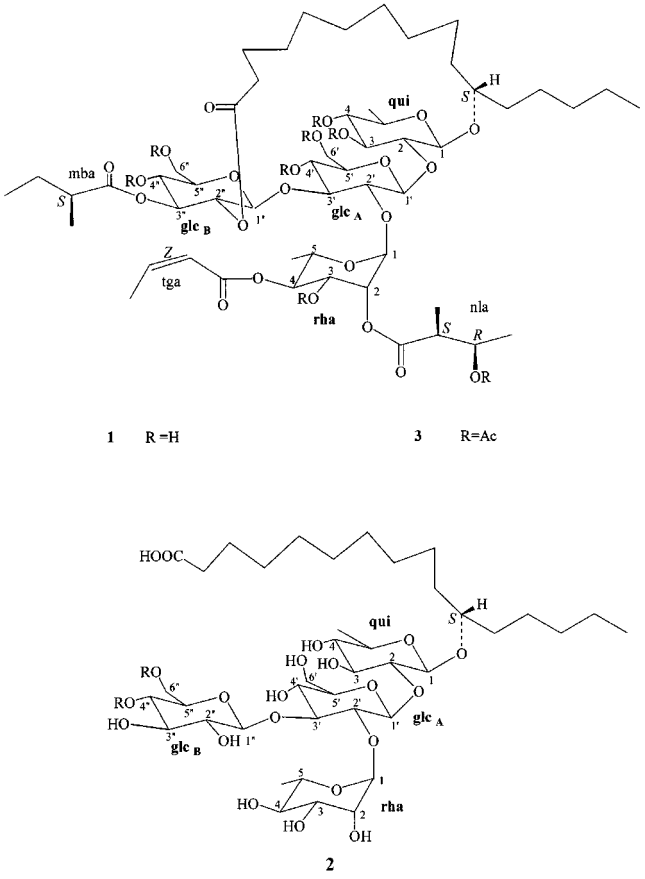
[a] Assignments might be interchangeable. − [b] Signals overlapping.

The ^1H NMR assignments for the individual monosaccharides were also determined with the aid of COSY and TOCSY spectra and the corresponding carbon assignments made with the assistance of HMQC spectra. The conformations of the sugars could be deduced from the chemical shifts and coupling constants for each of their anomeric protons.[10,15] The assignments for each of the sugars, by comparison with values reported for corresponding residues in oligosaccharides, were characteristic of β-glucopyranosyl, β-quinovopyranosyl and α-rhamnopyranosyl units.

Confirmation of the earlier mass-spectrometric finding that the glycoside was bound through the hydroxy group on C-11 came in the form of a through-space connectivity observed in the ROESY spectrum between the proton signal at δ = 3.86 (11-H on the fatty acid) and the anomeric proton signal at δ = 4.84 on the attached quinovosyl moiety. A correlation was also observed between the C-11 signal of the fatty acid at δ = 81.27 and the quinovosyl anomeric proton signal at δ = 4.84 in the HMBC spectrum. ROESY and HMBC correlations were also used to determine the linkages within the tetrasaccharide. Connectivities were observed between the rhamnose unit anomeric proton (signal at δ = 6.17) and 2′-H and C-2′ on glucose A (signals at δ = 4.16 and 78.26). Correlations were also observed between the glucose A unit anomeric proton (signal at δ = 5.73) and 2-H and C-2 of the quinovose unit (signals at δ = 4.30 and 79.94). In the HMBC spectrum, a connectivity was also observed between C-3′ of the glucose A (signal at δ = 89.68) and the anomeric proton of the glucose B unit (signal at δ = 4.93). The structure of soldanellic acid B (**2**), the alkaline hydrolysis product, was hence established as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside (Scheme 1).

Natural product soldanelline B (**1**), a white powder of m.p. 160−163°C (dec.), exhibited a peak at *m*/*z* = 1159 $[M + Na]^+$ in the positive-ion FAB-MS. This suggested that compound **1** consists of one unit of glycosidic acid **2**, with one unit each of (*Z*)-2-methyl-2-butenoic (tga), 3-hydroxy-2-methylbutanoic (nla) and 2-methylbutanoic (mba) acids attached at the hydroxy groups of the sugar moiety of **2**, and with the carboxy group of the aglycone [(11*S*)-hydroxyhexadecanoic acid] linked intramolecularly with a further sugar moiety hydroxy group, forming a macrocyclic lactone. The ^1H NMR spectrum of **1** showed four anomeric proton signals − at δ = 4.69 (quinovose), 5.24 [glucose″ (B)], 5.67 [glucose′ (A)], 5.86 (rhamnose) − together with the signals attributable to 1 mol each of 2-methylbutanoic, 3-hydroxy-2-methylbutanoic and (*Z*)-2-methyl-2-butenoic acids (Table 2). The existence of three acylated sites was also confirmed by acetylation of **1** to give the octaacetyl derivative **3**.

The remarkable downfield shifts, relative to their counterparts in compound **2**, of the signals of 2-H and 4-H of rhamnose and 2-H and 3-H of glucose B are attributable to acylation. Confirmation was obtained in the form of a through-space connectivity observed in the ROESY spectrum (Figure 1) between the proton signal at δ = 2.72 (2-H on the fatty acid) and the 3-H proton signal of the glucose B unit (δ = 5.88). Moreover, in the HMBC spectrum, cor-



Scheme 1

relation was observed between rhamnosyl 2-H (signal at δ = 5.92) and C-1 of the 3-hydroxy-2-methylbutanoic acid (signal at δ = 176.41), rhamnosyl 4-H (signal at δ = 5.76) and C-1 of the (*Z*)-2-methyl-2-butenoic acid (signal at δ = 167.58) and between glucosyl$_B$ 3-H (signal at δ = 5.88) and C-1 of the 2-methylbutanoic acid (signal at δ = 176.38). Because, in the natural product (soldanelline B, **1**), the ^{13}C NMR chemical shifts of the signals of C-1 of the 2-methylbutanoic acid unit and C-1 of the 3-hydroxy-2-methylbutanoic acid moiety are very close (δ = 176.38 and 173.41, respectively), there were some doubts about their relative positions in the compound; they might have been interchangeable. However, this was definitively cleared up in the spectra of acetyl derivative **3** (signals at δ = 175.31 and 173.48, respectively).

As well as this, a correlation between C-11 of the fatty acid (signal at δ = 82.01) and the quinovosyl anomeric proton (signal at δ = 4.69) was observed in the HMBC spectrum of **1**, confirming that quinovose is the monosaccharide unit directly linked to hydroxyhexadecanoic acid.

The structure **1** of soldanelline B was hence established as (11*S*)-hydroxyhexadecanoic acid 11-*O*-{3-*O*-[(2*S*)-methylbutanoyl]-β-D-glucopyranosyl}-(1→3)-*O*-{2-*O*-[(2*S*,3*R*)-3-hydroxy-2-methylbutanoyl]}-4-*O*-[(*Z*)-2-methyl-2-butenoyl]-α-L-rhamnosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2′′-ester.

It should be noted that soldanelline B is the second new natural product we have isolated from this plant,[16] the first being an acylated, branched tetrasaccharide macrolactone found in a Convolvulaceae plant.

Soldanelline B (**1**), like the chloroform extract mentioned above, was subjected to a cytotoxic bio-screening assay, using cultured cancer cells representing nasopharyngeal carcinoma (KB), colon carcinoma (HCT-15) and squamous cell cervix carcinoma (SQC-1, UISO). Soldanelline B exhibited interesting activities against squamous cell cervix carcinoma (UISO, ED_{50} 1.8 μg/mL) and nasopharyngeal carcinoma (KB, ED_{50} 6 μg/mL), while inactive (ED_{50} > 15 μg/mL) against colon carcinoma. These results contrast, in terms of their selectivities, with those reported in a previous work.[17] In the latter, the macrocyclic lactones were linear tetrasaccharides of the same degree of esterification as the oligosaccharide core of soldanelline B (**1**) (three short-chain fatty acids). ED_{50} values were 1.5, 1.5 and 3.2 μg/mL for KB, HCT-15 (colon) and UISO, respectively. The observation of different selectivities in cytotoxic activity for structurally different tetrasaccharides highlights the potential value of additional structure-activity research into such compounds.

Experimental Section

General Remarks: All melting points were measured with a Leica hot-stage microscope, and are uncorrected. − Optical rotations (MeOH): Perkin−Elmer 241 MC polarimeter. − LR FAB-MS and HR FAB-MS were recorded using triethanolamine (TEA), glycerol or 3-nitrobenzyl alcohol (NBA) as matrix with a Jeol SX 102A spectrometer. − Chromatography was performed with a Carlo Erba Vega series model 5300 chromatograph, equipped with a split-splitless injector and FID, and fitted with a DB-17 (J&W) 50 m × 0.25 mm i.d. × 0.25 μm film or a DB-5 (J&W) 30 m × 0.25 mm i.d. × 0.25 μm film. The oven temperature was linearly programmed from 150 to 300 °C at 2.5 °C min^{-1} (DB-17) and from 40 to 150 °C at 2 °C min^{-1} (DB-5). Hydrogen (P_i = 70 kPa) was used as carrier gas. − GC-MS measurements were performed under identical chromatographic conditions, with a Finnigan Mat ion trap detector (ITD). − ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were conducted using a Bruker AMX-500 spectrometer, with internal TMS as reference. Methyl, methylene and methine carbon atoms were distinguished by DEPT experiments. Homonuclear ^1H connectivities were determined by performing COSY experiments. The inverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded by using field gradients to suppress the undesired signal of protons bound to ^{12}C; the inter-pulse delays were adjusted for an average $^1J_{CH}$ = 145 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiment was optimised for an $^nJ_{CH}$ of 8.5 Hz. − Silica gel (230−400 mesh; 30% deactivated) and LiChroprep RP-18 (40−63 μm) were used for CC. HPLC separations were performed with a Waters 501 apparatus equipped with a refractometer detector and with μBondapack C_{18} columns.

Collection, Extraction and Isolation: Fresh roots of *Calystegia soldanella* (10 kg) were collected by hand along the dunes of Costa da Caparica beach, and identified by Prof. J. Barroso (Universidade de Lisboa). The lyophilised roots (2.15 kg) were extracted success-

Table 2. ^{1}H and ^{13}C NMR spectroscopic data of soldanelline B (**1**) and its acetyl derivative (**3**) in [D_5]pyridine (agl = aglycone, qui = quinovose, glc = glucose, rha = rhamnose, mba = 2-methylbutanoic acid, nla = 3-hydroxy-2-methylbutanoic acid, tga = (*Z*)-2-methyl-2-butenoic acid)

		1		**3**	
	Position	δ_H [mult.,^1J in Hz, int.]	δ_C	δ_H [mult.,^1J in Hz, int.]	δ_C
agl	1		172.84		172.27
	2	2.72 (m, 2 H)	33.90	2.72 (m, 2 H)	34.06
	11	3.72 (m, 1 H)	82.01	3.72 (m, 1 H)	81.63
	12	1.28 (m, 2 H)	22.83		
	16	0.85 (t, 7.5, 3 H)	14.16	0.88 (t, 6.5, 3 H)	14.10
qui	1	4.69 (d, 7.0, 1 H)	103.63	4.66 (d, 7.5, 1 H)	102.25
	2	4.22 (dd, 9.0, 9.0, 1 H)	80.34	4.20 (dd, 8.0, 9.8, 1 H)	77.36[a]
	3	4.25 (dd, 8.5, 8.5, 1 H)	79.13	5.52 (dd, 9.0, 9.0, 1 H)	76.27
	4	3.55 (dd, 9.0, 9.0, 1 H)	76.71	5.00 (dd, 10.0, 10.0, 1 H)	74.61
	5	3.59 (dq, 5.5, 9.3, 1 H)	72.28	3.66 (dq, 6.5, 9.8, 1 H)	69.39
	6	1.58 (d, 6.5, 3 H)	18.32	1.56 (d, 6.5, 3 H)	17.50
glc_A	1′	5.67 (t, 8.0, 1 H)	101.54	5.11 (t, 8.0, 1 H)	101.26
	2′	4.12 (dd, 8.2, 8.2 1 H)	76.43	4.10 (dd, 8.0, 9.3, 1 H)	77.29[a]
	3′	4.28 (dd, 8.5, 8.5, 1 H)	84.85	4.82 (dd, 9.0, 9.0, 1 H)	80.41
	4′	3.84 (dd, 9.5, 9.5, 1 H)	70.07	5.33 (dd, 9.5, 9.5, 1 H)	69.15
	5′	3.71 (ddd, 2.5, 6.0, 9.5, 1 H)	77.16	4.16 (ddd, 2.5, 5.0, 10.0, 1 H)	72.00
	6′	4.42 (dd, 5.5, 11.5, 1 H)	62.98	4.70 (dd, 4.5, 12.0, 1 H)	62.88
		4.32-4.37[b]		4.41 (dd, 2.5, 12.3, 1 H)	
rha	1	5.86 (s, 1 H)	97.62	5.77 (br. s, 1 H)	98.27
	2	5.92 (d, 4.0, 1 H)	72.88	6.21 (dd, 1.5, 3.5, 1 H)	70.20
	3	4.79 (dd, 3.5, 9.8, 1 H)	68.41	5.86 (dd, 3.5, 10.5, 1 H)	70.52
	4	5.76 (dd, 9.5, 9.5, 1 H)	75.49	5.80 (dd, 10.0, 10.0, 1 H)	70.75
	5	5.22 (dd, 6.5, 12.5, 1 H)	66.92	4.89 (dd, 1 H, 6.5, 10.0, 1 H)	67.76
	6	1.56 (d, 6.5, 3 H)	17.64	1.55 (d, 6.5, 3 H)	17.34
glc_B	1″	5.24 (d, 8.5, 1 H)	99.83	5.08 (d, 7.5, 1 H)	99.99
	2″	5.55 (dd, 8.0, 9.8, 1 H)	72.80	5.52 (dd, 9.0, 9.0, 1 H)	73.04
	3″	5.88 (dd, 10.0, 10.0, 1 H)	70.04	5.49 (dd, 9.5, 9.5, 1 H)	72.00
	4″	4.20 (dd, 9.5, 9.5, 1 H)	69.99	5.44 (dd, 9.5, 9.5, 1 H)	68.96
	5″	4.04 (ddd, 9.5, 4.0, 2.0, 1 H)	78.25	3.89 (ddd, 3.0, 2.8, 9.6, 1 H)	72.45
	6″	4.34 (dd, 2.0, 11.0, 1 H)	61.48	4.51 (dd, 3.5, 12.5, 1 H)	61.70
		4.10 (dd, 4.0, 11.0, 1 H)		3.88 (dd, 2.5, 12.3, 1 H)	
mba	1		176.38[a]		175.31
	2	2.41 (tq, 7.0, 7.0, 1 H)	41.51	2.36 (tq, 7.0, 7.0, 1 H)	41.33
	4	0.90 (t, 7.5, 3 H)	11.65	0.82 (t, 7.5, 3 H)	11.52
	CH_3	1.12 (d, 7.5, 3 H)	16.65	1.09 (d, 7.0, 3 H)	16.49
nla	1		176.41[a]		173.48
	2	2.99 (dq, 7.0, 7.0, 1 H)	48.01	3.31 (dq, 7.0, 6.0 1 H)	43.97
	3	4.37 (dq, 6.5, 7.8, 1 H)	70.36	5.63 (dq, 6.0, 6.0, 1 H)	71.30
	4	1.48 (d, 6.5, 3 H)	21.38	1.54 (d, 6.5, 3 H)	16.49
	$2CH_3$	1.52 (d, 6.5, 3 H)	13.47	1.46 (d, 7.0, 3 H)	11.86
tga	1		167.58		167.44
	2	7.29 (dq, 1.5, 6.5, 1 H)	128.20	7.45 (dq, 1.0, 6.5, 1 H)	128.90
	3	1.69 (d, 7.0, 3 H)	138.30	1.77 (dd, 1.0, 7.5, 3 H)	138.85
	4	1.87 (s, 3 H)	14.33	2.02 (s, 3 H)	14.45
	CH_3		17.64		12.21

[a] Assignments might be interchangeable. – [b] Signals are overlapping.

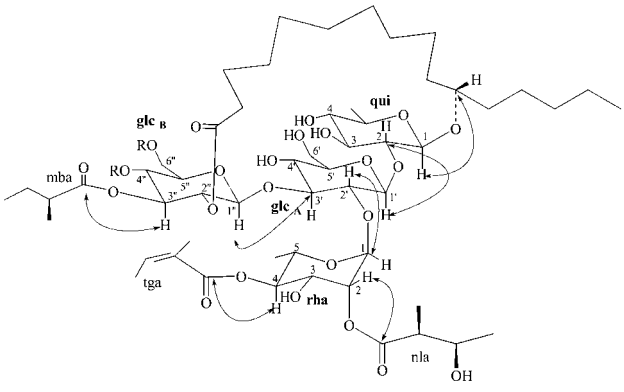


Figure 1. Key ROESY (H-H) (---) and HMBC (H−C) (−) correlations for establishing sugar linkages in compound 1

ively with *n*-hexane (6 × 3 L), $CHCl_3$ (10 × 4 L) and MeOH (5 × 4 L). The chloroform extract, a dark brown syrup (237.46 g; UISO: ED_{50} 2μg/mL, KB: ED_{50} 7 μg/mL), was fractionated 3 times on silica gel (230−400 mesh), eluting with a solvent gradient from $CHCl_3$ to MeOH. Fractions eluted with $CHCl_3$/MeOH (8:2) were combined and further purified on a LiChroprep RP-18 column (MeOH) to give a pale yellow solid. A pooled sample was subjected to preparative HPLC on a μBondapack C_{18} column (19 × 300 mm, 10 μm). The elution was isocratic with MeOH/H_2O (9:1), using a flow rate of 6 mL/min. The eluate was collected across the peak with t_R = 60 min (ca. 100 mg). The preparative HPLC system was then operated in recycle mode[18] to separate further and to guarantee the maximum purity of the major constituent soldanelline B (**1**) (80 mg; UISO: ED_{50} 1.8 μg/mL, KB: ED_{50} 6 μg/mL).

Soldanelline B (1): White, amorphous solid, m.p. 160−163 °C (dec.). − $[\alpha]_D^{20}$ = −1.7 (*c* = 0.001 in MeOH). − IR (KBr): $\tilde{\nu}_{max}$ = 3420, 2929, 1733, 1269, 1154, 1079 cm^{-1}. − ^{1}H and ^{13}C NMR

([D5]pyridine): see Table 2. − FAB-MS (positive ions, NBA matrix); *m/z*: 1159 [M + Na]$^+$. − FAB-MS (negative ions, TEA matrix); *m/z*: 1290 [M + TEA + 3 H]$^-$, 1136 [M]$^-$, 1118 [M − H_2O]$^-$, 1036 [M − nla]$^-$, 908 [M − glucose unit − mba + H_2O]$^-$, 808 [M − glucose unit − mba − tga]$^-$, 561 [M − rha − gluc − mba − nla − tga]$^-$, 417 [hydroxyhexadecanoic acid + qui − H]$^-$, 271 [hydroxyhexadecanoic acid − H]$^-$. − HR FAB-MS (negative ions); *m/z*: 1135.5873 [M − H]$^-$; calcd. for $C_{55}H_{91}O_{24}$: 1135.5900; *m/z* = 1091.5836 [M − H − tga + Na + H_2O]$^-$; calcd. for $C_{50}H_{84}NaO_{24}$: 1091.5250.

Soldanellic Acid B (2): White, amorphous solid, m.p. 157−160 °C. − $[\alpha]_D^{20}$ = −11.24 (*c* = 0.125 in MeOH). − FAB-MS (negative ions, triethanolamine matrix); *m/z*: 887 [M − H]$^-$, 741 [M − H − methylpentose unit]$^-$, 725 [M − H − hexose unit]$^-$, 581 [hydroxyhexadecanoic acid + methylpentose + hexose units]$^-$, 417 [hydroxyhexadecanoic acid + methylpentose unit]$^-$, 271 [hydroxyhexadecanoic acid − H]$^-$. − HR FAB-MS (negative ions); *m/z*: 888.4371 [M − H]$^-$; calcd. for $C_{40}H_{71}O_{21}$: 887.4488. − ^{1}H and ^{13}C NMR ([D5]pyridine): see Table 1.

Alkaline Hydrolysis: A solution of compound **1** (50 mg) in 5% KOH (10 mL) was refluxed for 3 h. The reaction mixture was acidified to pH = 1 and extracted with Et_2O (10 mL). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated under an N_2 stream. The residue was treated with CH_2N_2 and analysed by GC-MS. The three major peaks had the same retention time and mass spectra as methyl 2-methylbutyrate (mba) (t_R = 4.3 min), methyl 3-hydroxy-2-methylbutyrate (nla) (t_R = 21.8 min) and methyl tiglate (tga) (t_R = 6.5 min) standards. Identification of methyl (2*S*)-methylbutyrate was accomplished by comparison of its retention time with that of a standard, on a cyclodextrin B (J&W) column (40−150 °C, Δ 1 °C min^{-1}): t_R = 6.3 min. 3-Hydroxy-2-methylbutanoic acid (nla), isolated by preparative RP-HPLC [MeOH/H_2O (7:3), flow rate 3.5 mL/min, t_R = 8 min], was shown by optical rotation {$[\alpha]_D^{20}$ = + 17.2 (*c* = 4.3, MeOH)} to be the (2*S*,3*R*) isomer.[19] The aqueous phase was extracted with *n*BuOH (3 × 10 mL). The organic layer was then concentrated to give crude compound **2**. Pure Soldanellic acid B (**2**) (60 mg) was obtained by preparative RP-HPLC [MeOH/H_2O (7:3), flow rate 3.5 mL/min].

Acid Hydrolysis: Soldanellic acid B (50 mg) in 4 M HCl (10 mL) was refluxed for 24 h. The reaction mixture was extracted with Et_2O. The organic layer was evaporated and chromatographed on Si gel [TLC: $CHCl_3$/MeOH (8:2); R_f = 0.6]. The methyl ester of the acid (excess CH_2N_2 in Et_2O) was subjected to GC-MS analysis. The acid was identified as (11*S*)-hydroxyhexadecanoic acid.[9,10] The aqueous phase (monosaccharide fraction) was neutralized with 1 M KOH, dried under vacuum, converted into TMS ethers of methyl (4*R*)-thiazolidine-4-carboxylate derivatives[13,14] and the mixture was analysed by GC (DB-17). Retention times were identical with those of authentic samples respectively derived from D-quinovose, L-rhamnose and D-glucose, in the approximate ratio 1:1:2.

Cytotoxic Activity: Squamous cell cervix carcinoma (SQC-1, UISO), nasopharyngeal carcinoma (KB), and colon cancer (HCT-15) cell lines were maintained in RMPI 1640 (10×) medium supplemented with 10% fetal bovine serum. All cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at log phase of their growth cycle were treated in triplicate at various concentrations of the $CHCl_3$ extract (0.15−20 μg/mL), and incubated for 72 h. The cell concentration was determined by the sulforhodamine method.[20] Ellipticine was included as positive control: ED_{50} = 0.42 (HCT-15), 0.04 (SQC-1, UISO) and 0.15 (KB).

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