Pergamon

Tetrahedron Letters 40 (1999) 6861–6864

TETRAHEDRON LETTERS

New Pentasaccharide Macrolactone from the European Convolvulaceae *Calystegia soldanella.*

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Received 14 June 1999; accepted 13 July 1999

Abstract: A new nonlinear pentasaccharide resin glycoside Soldanelline A (**1**) has been isolated from a Portuguese Convolvulaceae *Calystegia soldanella*. The structure of compound **1** was elucidated using high-field NMR spectroscopy, mass spectrometry and chemical studies.

Extracts from *Calystegia soldanella* (Convolvulaceae) have been used in Portuguese traditional medicine to cure hydropsy, paralysis, rheumatism and scurvy.[1] However there have been no reports on the chemical constituents of this plant nor any other European species.

The most common constituents of the Convolvulaceae family are alkaloids[2] and resin glycosides.[3] The unique structural features of Convolvulaceae glycosides and their multiple pharmacological properties[4] and allelopathic interference[5,6] encouraged us to initiate a detailed chemical investigation of European species in the hope of isolating and identifying pharmacologically and/or agrochemically useful chemical models. We began with a Portuguese plant of the genus *Calystegia*, whose chloroform extract showed both cytotoxic (UISO, ED_{50} 2 μg/ml) and antibacterial (*Bacillus subtilis*, MIC 14.7 μg/ml) activities.

The chloroform extract (270 g) obtained from the lyophilized roots of the plant (2.9 Kg) was subjected successively to column chromatography (flash chromatography, $CHCl_3$:MeOH, 4:1), reverse-phase chromatography (methanol as eluent) and recycling HPLC on a C_{18} μ-Bondapack column (MeOH, 9:1), to give compound **1** (40 mg). Compound **1**, white powder m.p.166-169° C (dec.), $[\alpha]_D$ – 60° (c = $3x10^{-4}$, MeOH) was then submitted to alkaline hydrolysis with a 5% KOH aqueous solution, and the resulting mixture was neutralized with 1M HCl solution. An aliquot was acidified to pH 1, extracted with Et_2O, and the Et_2O extract methylated and ethylated with CH_2N_2 and $CH_3CH_2N_2$ respectively. By GC-MS, the mass spectra and retention times for the three major peaks corresponded to those of the methyl and ethyl 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). GC analysis using a cyclodextrin column (chiral column) allowed identification of the mba as (*S*) isomer. Nilic acid, by optical rotation $[\alpha]_D$ +17.2 ° (c = 4.3, MeOH), showed to be (2*S*, 3*R*) isomer.[7]

On acidic hydrolysis (4M HCl, reflux 24 h) the aqueous solution yielded a hydroxy fatty acid and a monosaccharide mixture. The methyl ester of the acid (CH_2N_2 in Et_2O) was subjected to GC-MS analysis and it was identified as (11*S*)-methyl jalapinolate.[8,9] The monosaccharide fraction was converted into TMS ethers of methylthiazolidine 4(*R*)-carboxylate derivatives[10,11] and the mixture was analysed by GC (DB-5

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0040-4039/99/$ - see front matter
PII: S0040-4039(99)01385-4

fused silica capillary column). Retention times were identical with those of authentic samples derived from D-quinovose, L-rhamnose and D-glucose in the approximate ratio of 1:1:3 respectively.

The ^{1}H-NMR spectrum of **1** showed five anomeric proton signals, at δ 4.696 ppm (quinovose), δ 5.094 ppm (glucose''' (C)), δ 5.32 ppm (glucose'' (B)), δ 5.54 ppm (glucose' (A)) and δ 5.875 ppm (rhamnose). The observation of five methine ^{13}C peaks in the region expected for anomeric carbons, δ 105.25 ppm (glucose C), δ 103.39 (quinovose), δ 101.77 (glucose A), δ 99.57 (glucose B) and δ 96.98 ppm (rhamnose), confirmed that the compound was a pentasaccharide. Also, three methylene carbons were observed in the sugar region, δ 63.03, δ 62.82 and δ 61.54 ppm, which confirmed the presence of three glucose units.

The positive FABMS of **1** exhibited a peak at m/z 1320 $[M+Na]^{+}$ and in the negative ion FABMS (figure 1) Soldanelline A (**1**) showed major ions at m/z 1297 $[M-1]^{-}$, m/z 1253, 1138, 1070, 807, 723, 579, 417 and m/z 271. The m/z 271 ion for compound **1** confirmed the presence of an 11-hydroxyhexadecanoic acid (11-hydroxypalmitic or jalapinolic acid) moiety. This acid should be glycosidically linked to a pentasaccharide consisting of two deoxyhexose (1 x qui and 1 x rha) and three hexose (glc) units and one mole each tiglic, nilic and 2-methylbutyric acids, which are combined with the hydroxy groups of the sugar moiety. Also, the carboxy group of the aglycone (jalapinolic acid) should be intramolecularly linked with a hydroxyl group of the sugar moiety as a macrocyclic lactone. This is confirmed by fragment ion peaks at m/z 417 (271 + 146; methylpentose unit)$^{-}$, m/z 579 (271 + 146 + 161; hexose unit)$^{-}$ and by peaks at m/z 807, 1070 and m/z 1138; the three latter suggest that the pentasaccharide moiety is nonlinear, having two terminal glucose units (figure 1).

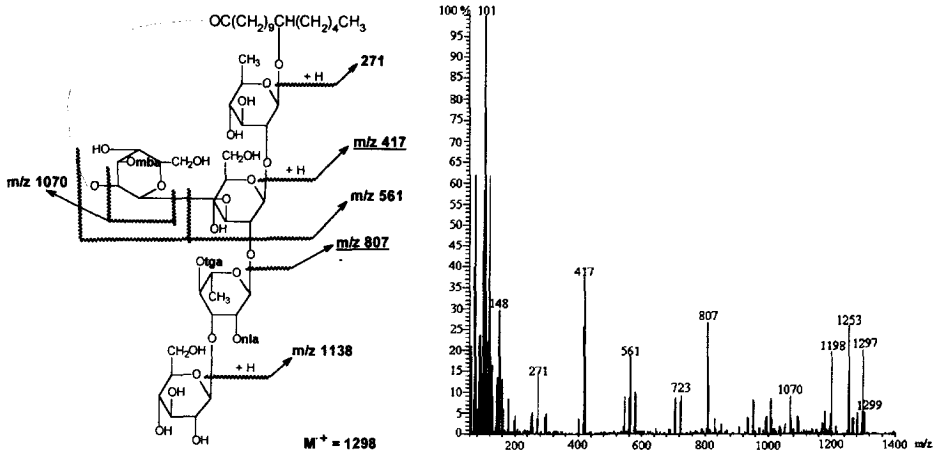

Figure 1. Fragmentation pattern and negative ion FABMS spectrum of Soldanelline A (**1**).

A combination of ^{1}H, ^{13}C and DEPT-NMR spectra in conjunction with the COSY, TOCSY, HMQC, HMBC and ROESY NMR techniques was used to identify the positions of the individual hexoses in the chain and the stereochemistry of the sugar linkages. COSY and TOCSY spectra were particularly useful for identifying and assigning ^{1}H-NMR spectra for individual monosaccharides and short-chain fatty acids (table 1). HMQC then allowed assignment of the corresponding carbons via one-bond ^{13}C-^{1}H correlation (table 1) while HMBC spectra was used to assign the linkages sites within the pentasaccharide core as well as the sites of esterification by short-chain fatty acids. The former were determined from observed three-bond ^{1}H-C-O-^{13}C-H correlation between different monosaccharide units while the latter were determined from three-

bond ^{1}H-C-O-^{13}C=O correlation between sugar protons and ester carbonyl groups. In HMBC spectrum (figure 2) we could observe correlation between C-1 of the aglycone (δ 172.97 ppm) and glucosyl$_B$ H-2 (δ 5.54 ppm), C-1 of the mba acid at δ 175.45 ppm and glucosyl$_B$ H-3 (δ 5.96 ppm), C-1 of the nilic acid at δ 175.35 ppm and rhamnosyl H-2 (δ 6.12 ppm), C-1 of the tiglic acid (δ 167.61 ppm) and the H-4 proton of the rhamnose unit (δ 5.98 ppm). Correlation was also observed between H-3 proton of the rhamnose unit at δ 5.21 ppm and C-1 of the glucose C unit (δ 105.25 ppm), aglycone C-11 at δ 81.84 ppm and quinovosyl H-1 at δ 4.70 ppm, glucosyl$_C$ H-1 (δ 5.09 ppm) and C-3 (δ 71.52 ppm) of the rhamnose unit, and C-1 of the glucose A unit (δ 101.77 ppm) and the quinovosyl H-2 proton (δ 4.15 ppm).

The linkage sites within the pentasaccharide core were confirmed by ROESY cross-peaks between the

Table 1. NMR Assignments for Resin Glycoside Soldanelline A (**1**) (in pyridine d_5).

sugar	position	δ^{1}H	δ^{13}C	acids	position	δ^{1}H	δ^{13}C
agl	1		172.97	mba	1		175.451$^{\bullet}$
	2	2.67, m	33.73		2	2.41, tq (7.0, 7.0)	41.49
	11	3.72, m	81.84		4	0.88, t (7.0)	11.58
	16	0.86, t (7.5)	14.18		CH_3	1.10, d (7.0)	16.64
qui	1	4.70, d (7.5)	103.39	nla	1		175.35$^{\bullet}$
	2	4.15, dd (9.0, 9.0)	80.69		2	3.01, dq (7.0, 7.0)	48.14
	3	4.31, dd (8.5, 8.5)	79.04		3	4.63, dq, (6.0, 11.75)	68.99
	4	3.54, dd (9.0, 9.0)	76.69		4	1.44, d (6.0)	20.42
	5	3.61-3.65†	72.22		$2CH_3$	1.54, d (7.0)	13.10
	6	1.60, d (6.0)	18.31	tga	1		167.61
glc$_A$	1	5.54, d (8.0)	101.77		2		128.94
	2	4.14, dd (7.5, 7.5)	75.62		3	7.47, dq (1.5, 6.5)	138.56
	3	4.15, dd (8.0, 8.0)	85.10		4	1.76, dd (1.0, 6.0)	14.42
	4	3.78 dd (9.0, 9.0)	70.10		CH_3	2.06, s	12.70
	5	3.89-3.92††	78.14				
	6	4.04-4.1*	62.82				
		4.40, dd (1, 10.5)					
rha	1	5.87, s	96.98				
	2	6.12, dd (1.0, 3.5)	72.78				
	3	5.21, dd (3.5, 10.5)	75.37				
	4	5.98, dd (10.0, 10.0)	73.63				
	5	5.30, dd (6.5, 10.0)	67.17				
	6	1.63, d (6.5)	17.73				
glc$_B$	1	5.32, d (8.0)	99.57				
	2	5.54, dd (7.5, 13.75)	72.83				
	3	5.96, dd (8.5, 8.5)	75.83				
	4	4.22, dd (9.0, 9.0)	70.15				
	5	4.18, ddd (2.0, 4.75, 9.5)	78.30				
	6	4.32-4.34**	61.54				
		4.44, dd (1.0, 10.5)					
glc$_C$	1	5.09, d (8.0)	105.25				
	2	3.82-3.85$^{\bullet}$	78.14				
	3	3.89-3.92††	71.52				
	4	3.82-3.85$^{\bullet}$	74.79				
	5	3.61-3.65†	76.99				
	6	4.04-4.1*	61.54				
		4.32-4.34**					

Spectra recorded at 500 MHz (J values in Hz). All assignments are based on ^{1}H-^{1}H COSY. ^{13}C chemical shifts from HMQC and HMBC. Agl = aglycone, qui = quinovose, glc = glucose, rha = rhamnose; mba = 2-methylbutanoic acid; nla = nilic acid; tga = tiglic acid. $^{\bullet}$Assignments could be interchanged. $^{\dagger, \dagger\dagger, *, **, \bullet}$Signals are overlapped.

pairs of protons on the carbons forming C-O-C linkages (figure 2). In fact, a throughspace connectivity was observed between the proton at δ 3.72 ppm (H-11 on the fatty acid) and the H-1 proton of the quinovose unit (δ 4.70 ppm), between the H-3 proton of the nilic acid (δ 4.63 ppm) and the proton at δ 6.12 ppm (rhamnosyl H-2), also between the H-1 proton of the glucose C unit (δ 5.09 ppm) and the proton H-3 of the rhamnose unit (δ 5.21 ppm) and between rhamnosyl H-1 (δ 5.87 ppm) and H-1 proton of the glucose A unit (δ 5.54 ppm).

The conformation of the sugars could be deduced from the chemical shifts and coupling constants for the anomeric protons of each of the sugars.[9,12] The assignments for each of the sugars were characteristic of β-glucopyranosyl, β-quinovopyranosyl and α-rhamnopyranosyl units by comparison with values reported for corresponding residues in oligosaccharides. Thus the structure of Soldanelline A, was elucidated as 11*S*-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→3)-*O*-(2-*O*-(3(*R*)-hydroxy-2(*S*)-methylbutyryl)-4-*O*-tiglyl)-α-L-rhamnopyranosyl-(1→2)-*O*-[3-*O*-2(*S*)-methylbutyryl-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular-1,2'' ester. It should be noted that Soldanelline A is a new branched pentasaccharide resin glycoside, being the first one to be isolated and identified from an European Convolvulaceae plant.

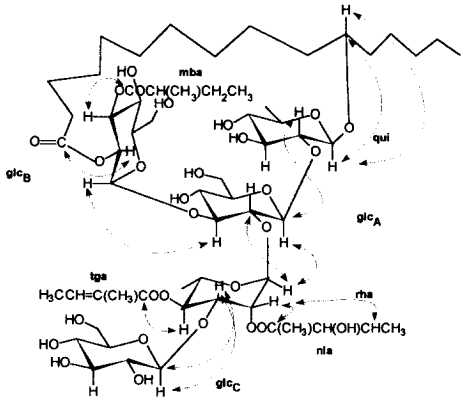

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Figure 2. ROESY (H-H) and HMBC (H-C) correlations for establishing sugar linkages.

Acknowledgments: The author wish to express her grateful recognition to M.Sc. Isabel Chavez, Laboratorio de R.M.N., Instituto de Quimica, UNAM, Mexico for 500 MHz NMR data. I thank Dr. Adriano Teixeira and Mr. Luis Ramalho, INETI, Portugal, for FAB$^+$MS data. Financial support from FCT-PRAXIS XXI, is gratefully acknowledged. Finally my thanks to Professor J. Barroso, F.C.- U. L., Portugal, for botanical classification of the plant.

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