

Arundinoside A – a New Spirostane Saponin from *Chlorophytum arundinaceum*^ψ

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Chlorophytum arundinaceum Baker (Liliaceae) is reported to be used as tonic and is the ingredient of Ayurvedic preparations. Commonly known as *Safed Musli* the root extract of this plant has displayed significant adaptogenic activity (details will be published elsewhere). Recently, we have reported a novel disubstituted tetrahydrofuran¹, a bibenzyl xyloside², and several steroidal sapogenins³. This communication describes the isolation and characterisation of a new tokorogenin-based saponin arundinoside A.

Results and Discussion

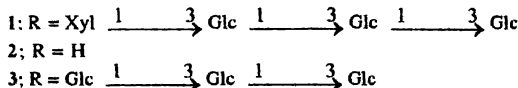
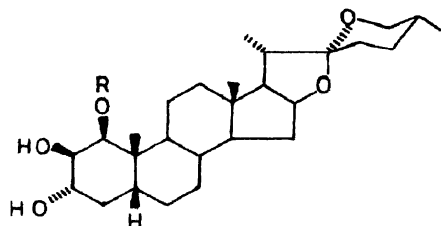
Saponin 1, m.p. 258–60° (dec), C₅₀H₈₂O₂₄ (M⁺ 1066), [α]_D²⁵ -54.2° was obtained by repeated column chromatographic purifications of the n-BuOH fraction followed by preparative tlc. The ir spectrum exhibited broad absorption bands at 3 400 and 1 050 cm⁻¹ for OH groups indicating its glycosidic nature. The other bands at 980, 920, 900 and 860 cm⁻¹ with the intensity of 900 > 920 indicated it to be a spirostane saponin with 25 R configuration⁴.

Saponin 1 on acid hydrolysis yielded tokorogenin (2), m.p. 260–62° as the aglycone which was identified by comparison of physical and spectral properties^{5,6}. A direct com-

[M + Na-(132 + 162)]⁺, 633 [M + Na-(132 + 2 × 162)]⁺ and 471 [M + Na-(132 + 3 × 162)]⁺ were attributed to the loss of pentosylhexose, pentosyldihexosyl and pentosyltrihexosyl units respectively. The results obtained from FAB-MS indicated the sugar sequence in 1. Interglycosidic linkages in 1 were suggested by permethylation followed by acid hydrolysis which liberated as methylated sugars identified as 2,4,6-tri-O-methyl-D-glucose (3 mol) and 2,3,4-tri-O-methyl-D-xylose (1 mol) by paper chromatography and direct comparison with the authentic methylated sugars. The presence of xylose as the terminal sugar was confirmed by its detection on partial hydrolysis of 1 on tlc in a HCl atmosphere⁸. Partial hydrolysis of 1 with methanolic HCl also yielded xylose and a prosaponin (3) which displayed an [M + Na]⁺ ion at m/z 957 in FAB-MS.

The proton noise decoupled ¹³C nmr spectrum of 1 displayed 50 carbon resonances. The number of attached hydrogens to each carbon was determined by the DEPT technique⁹ which suggested the presence of 13×CH₂, 30×CH, 4×CH₃ and 3 quaternary C atoms, indicating the presence of a tetrasaccharide moiety in a C₂₇ spirostane. The appearance of the four anomeric carbon signals at δ 105.35, 105.24, 104.88 and 103.19 was in accord with the presence of a tetrasaccharide moiety in 1. On the basis of analysis of DEPT spectrum the molecular formula of 1 could be assigned as C₅₀H₈₂O₂₄. A comparison of ¹³C nmr spectral data of the aglycone moiety of 1 with those of tokorogenin⁶ further confirmed the identity of aglycone as tokorogenin.

The interglycosidation assignments were further confirmed by the chemical shifts of the glycosylated carbon atoms. The C-3 signals of the three glucose units were observed at δ 84.6, 83.6 and 83.9, revealing deshielding of ca 6, 5 and 5 ppm for these carbon resonances; hence, C-3 was concluded to be the glycosidation site of all the glucose. The ¹H nmr spectrum showed 4 anomeric protons as doublets at δ 5.58, 5.50, 5.20 and 4.96 (J 7–8 Hz) for three glucose and one xylose units respectively. The chemical shifts and coupling constants of these signals suggested the β-anomeric configuration of the sugar moieties when compared with the reported values¹⁰. The tetrasaccharide moiety in 1 was linked at C-1 of the aglycone as the signals of 1 and 2 were almost superimposable except for those of C-1 and C-2 which were different due to glycosidation shifts. The C-1 signal was found downfield by δ 9.40, while C-2 signal was observed upfield by δ 0.75 in case of 1 relative



parison with authentic sample (m.m.p., co-tlc) also established the identity. The sugar components in the hydrolysate were identified as D-glucose and D-xylose in the ratio of 3 : 1 indicating 1 to be a tokorogenin tetraglycoside. The ratio of the sugars was estimated by colorimetric method⁷. The positive ion FAB-MS showed a pseudomolecular ion peak at m/z 1089 [M + Na]⁺ indicating a molecular mass of 1066 which is in good agreement with the molecular formula C₅₀H₈₂O₂₄. The fragment at m/z 957 was consistent with the loss of a terminal pentose unit from the molecular ion, whereas the other fragments at m/z 795

^ψ CMAP Publication no. 93-61.

to 2, demonstrating the monodesmosidic nature of 1 and that C-1 was the site of sugar linkage⁶. The ¹³C nmr spectral data of 1 are in close agreement with those of related saponins¹¹.

In the light of the above observations the structure of 1 was established as 1 β -O-[β -D-xylopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranosyl]-25R,5 β -spirostan-1 β ,2 β ,3 α -triol.

Experimental

M.ps. were determined in a H₂SO₄-bath and are uncorrected. Optical rotation was measured on a Jasco DIP-181 polarimeter. ¹H and ¹³C nmr spectra (C₅D₅N) were recorded on a Varian spectrometer operating at 400 and 100 MHz, respectively. All the nmr spectra were recorded using TMS as internal standard, ir spectra (KBr) disc on a Perkin-Elmer 1710 spectrophotometer, and EI-MS on a Jeol-JMSD-3000 spectrometer (70 eV) while the positive-ion mode FAB-MS on a Jeol SX 102/DA-6000 spectrometer using argon as FAB gas and accelerating voltage of 10 kV with nitrobenzyl alcohol as matrix. Column chromatography was carried out on silica gel (B.D.H.; 60–120 mesh), tlc and prep.-tlc on silica gel 60 precoated plates, F-254 (Merck). Spots were visualized by exposure to iodine vapour and by spraying with 10% H₂SO₄, followed by heating at 110°. Paper chromatography of sugars was performed on Whatman no. 1 paper using the descending mode in n-BuOH-AcOH-H₂O (4 : 1 : 5) and developed with aniline hydrogen phthalate while tlc in CHCl₃-MeOH-Me₂CO-H₂O (3 : 3 : 3 : 1).

Roots of *C. arundinaceum* were collected from Madhya Pradesh and identified in our Botany Department, where a voucher specimen is maintained. The air-dried and powdered roots (3.5 kg) were extracted with MeOH (11 : 3.5 l) and the combined extract was concentrated to 500 ml and H₂O (500 ml) was added. The aqueous methanolic extract was then fractionated successively with n-hexane (5 × 500 ml, 35 g) and n-BuOH (10 × 250 ml, 40 g). A portion of n-BuOH fraction (18 g) was chromatographed over silica gel (550 g) eluting with varying proportions of CHCl₃ and MeOH. Fractions (200 ml each) were collected and monitored by tlc. Elution with CHCl₃-MeOH (8 : 2) yielded a residue which was purified by rechromatography followed by prep.-tlc in CHCl₃-MeOH-EtOAc-H₂O (8 : 10 : 32 : 5) to yield arundinoside A.

Arundinoside A: Colourless crystals (60 mg), m.p. 258–60°; [α]_D –54.2° (C₅H₅N); $\nu_{\max}$ 3400 (OH), 2950 (CH), 1450 (CH₂), 1380 (CH₃), 1050, 980, 920, 900, 860 cm^{–1} (intensity 900 > 920, 25 R spiroketal); ¹H nmr δ 5.58 (1H, d, *J* 7 Hz, H-1 glc), 5.50 (1H, d, *J* 7 Hz, H-1 glc), 5.50 (1H, d, *J* 7.2 Hz, H-1 glc), 4.96 (1H, d, *J* 7 Hz, H-1 Xyl), 0.64 (3H, s, 18-CH₃), 0.82 (3H, s, 19-CH₃), 1.20 (3H, d, *J* 6 Hz, 21-CH₃), 0.68 (3H, d, 6 Hz, 27-CH₃); FAB-MS *m/z* 1089 [*M* + Na]⁺, 957, 795, 633, 471.

Acid hydrolysis of 1: Saponin 1 (27 mg) was refluxed with 5% HCl (10 ml) on a boiling water bath for 6 h. The usual work-up of the reaction mixture afforded tokorogenin m.p. 260–62°; [α]_D –39° (C₅H₅N); MS *m/z* 448 [*M*]⁺, 433, 430, 415, 389, 379, 376, 334, 333, 316, 302, 198, 168, 139, 115.

Identification of sugar moieties of 1: The aqueous layer separated after the removal of tokorogenin was neutralised with silver carbonate, filtered and concentrated under reduced pressure. The residue obtained was compared with standard sugars on tlc and paper chromatography indicating them to be D-glucose and D-xylose.

Partial hydrolysis of 1 on tlc: Saponin 1 was applied on tlc plate and left in an HCl atmosphere at room temperature for 30 min. The tlc plate was freed of the acid vapour by blowing air and authentic glucose and xylose were applied to it. The chromatoplate was developed in CHCl₃-MeOH-Me₂CO-H₂O (3 : 3 : 3 : 1) which showed the presence of xylose only.

Partial hydrolysis of 1: Saponin 1 (11 mg) was hydrolysed with 5% methanolic HCl (5 ml) for 5 min. The neutralised and concentrated aqueous hydrolysate showed the presence of xylose while the organic layer provided a prosaponin (3), m.p. 195–200°; FAB-MS *m/z* 957 [*M* + Na]⁺, 795, 633, 471.

Permethylation of 1 and acid hydrolysis of the product: A solution of 1 (15 mg) in hexamethyl phosphoric triamide (5 ml) was treated with NaH (300 mg) and CH₃I (5 ml) at

TABLE 1—¹³C NMR CHEMICAL SHIFTS OF 1 AND TOKOROGENIN (2)

Carbon	1	2	Carbon	1
1	86.00	76.60	Glc 1	103.19
2	73.65	74.40	2	75.74
3	71.50	71.40	3	84.60
4	35.00	35.30	4	70.14
5	36.00	35.92	5	78.30
6	26.10	26.40	6	62.58
7	26.40	26.52	Glc 1	105.35
8	35.80	35.65	2	75.65
9	42.50	42.35	3	83.60
10	42.00	41.84	4	70.78
11	21.82	21.48	5	77.26
12	40.80	40.25	6	62.00
13	40.90	40.75	Glc 1	105.25
14	56.50	56.34	2	75.05
15	31.60	31.65	3	83.90
16	81.09	81.10	4	70.52
17	63.00	63.10	5	77.58
18	16.28	16.35	6	63.02
19	19.28	19.14	Xyl 1	104.88
20	42.60	42.50	2	76.10
21	14.68	14.52	3	78.50
22	110.12	109.28	4	72.52
23	31.56	31.50	5	67.29
24	29.30	28.50		
25	30.60	30.38		
26	67.29	66.88		
27	17.39	17.18		

Glc = Glucose; Xyl = xylose.

room temperature for 3 h. The usual work-up of the reaction mixture yielded a residue which was purified by prep. tlc in petroleum ether-EtOAc (1 : 1). Hydrolysis of permethylated **1** was performed by refluxing with 1 M HCl MeOH (1 : 1, 5 ml). Paper chromatography of the neutralised and concentrated hydrolysate in n-BuOH-EtOH-H₂O (5 : 1 : 4) showed the presence of 2,3,4-tri-*O*-methyl-D-xylose and 2,4,6-tri-*O*-methyl-D-glucose (co-pc).

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