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Triterpenoid saponins from *Mollugo spergula*

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Abstract

Two novel triterpenoid saponins belonging to a modified hopane group, spergulin A {3-*O*-(β-D-xylopyranosyl 4-sulphate)-spergulagenin A} (**1**) and spergulin B {3-*O*-[α-rhamnopyranosyl (1→2)-β-D-xylopyranosyl]-spergulatriol} (**4**) were isolated from the aerial part of *Mollugo spergula* along with spergulacin (**2**) and spergulacin A (**3**). Their structures and relative stereochemistry were determined by a combination of 2D–NMR (COSY, TOCSY, HETCOR, NOESY and HMBC) and HR-FAB–MS analysis coupled with strategic chemical and enzymatic transformations. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Mollugo spergula*; Molluginaceae; Triterpene saponins; Triterpene; Spergulin A; Spergulin B; 2D NMR; Rearranged hopane; Bis-nor hopane

1. Introduction

Mollugo spergula Linn. (Syn. *Glinus oppositifolia*) (Family: Molluginaceae) is an annual herb which finds application for the treatment of skin diseases in the indigenous system of medicine (Sastri, 1962). Previous chemical investigations of the plant had led to the isolation of a number of genuine and rearranged triterpenes of hopane series (Chakrabarti et al., 1966, 1968; Kitagawa et al., 1974, 1977) and two triterpenoid saponins (Barua et al., 1986a,b). In continuation of our work on chemical studies on naturally occurring saponins (Sahu and Achari, 2001) we decided to reinvestigate the saponin constituents of the plant. The present paper describes the isolation and characterization of two new triterpenoid saponins including a sulfated derivative saponin. As detailed spectral data for the two saponins reported by Barua et al. (1986a,b) are not available, those have also been presented here.

2. Results and discussion

Spergulin A (**1**) was assigned the molecular formula $C_{35}H_{58}O_{11}S$ from the negative high resolution FAB–MS, supported by elemental analysis. The presence of a sulfur atom as part of a sulfate group was also evident from the fragment ions at m/z 97 $[SO_4H]^-$ and 80 $[SO_3]^-$ in the negative low resolution FAB–MS and from the peak at 1383 cm^{-1} for a sulfate group in the IR spectrum. The compound displayed 35 signals in its ^{13}C NMR spectrum of which five could be assigned to a pentose unit. Structure elucidation for spergulin A was mainly accomplished by critical analysis of the 1D and 2D NMR results. HMBC measurement proved to be the method of choice; beginning with assignments for the methyl groups, two and three bond correlations permitted the assignment of most of the signals. The determination of atom connectivities was straightforward and unambiguous. Thus the HMBC correlation involving signals of H-3 with C-23 and C-24; H-3 and H-5 with C-4 and C-25; and 3H-25 with C-1, C-9 and C-10 identified most of the structural elements of rings A and B. This was gradually extended to cover the other rings. Thus the C-9 signal showed cross peaks with 3H-26 and H(β)-11; C-11 with H-13; and C-14 with H-13, 3H-26, 3H-27 and 3H-28. Further observation of HMBC involving C-15 and C-13 with 17-H provided a clear picture of the six membered rings A–D. The cyclopentanyl nature of ring E was ascertained from the following HMBC correlations: H-17/C-21 and C-22, H(β)-19/C-21, H-17/C-18, H(β)-19/C-18, H(α)-20/C-29, H(β)-20/C-18, 3H-29/C-20. That the ketomethyl group is attached to C-21 was established from the observed HMBC between C-22 and 3H-29, H-17, H(α)-20 as also between C-21 and 3H-30.

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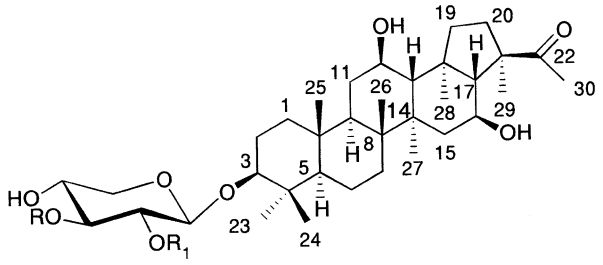
Regarding the substitution pattern, the downfield chemical shifts attributed to 16-H (δ 3.59) and 12-H (δ 3.67) as well as to the corresponding carbon signals suggested the presence of –OH groups at C-16 and C-12. The signals of 2H-11 and H-13 showed HMBC cross peaks with δ 67.3 (C-12) whereas the δ 64.1 (C-16) signal displayed correlations with H-17 and H-15α signals confirming the placement of hydroxy groups. The relative orientations of the aglycone hydrogens and the stereochemistry of the ring system of spergulin A (**1**) were established from identified ^{1}H–^{1}H coupling patterns and cross peaks generated from phase-sensitive NOESY. The β-orientation of the hydroxy groups was evident from the NOESY cross peaks between H(α)-12 and 3H-27, 28 as also between H(α)-16 and 3H-27, 28, 29.



From ^{1}H and ^{13}C chemical shifts, ^{1}H–^{1}H coupling constants and a close examination of TOCSY spectrum, it was obvious that the sugar constituent is D-xylose (The absolute configuration of the monosaccharides were chosen in keeping with those commonly encountered among plant glycosides). Acid hydrolysis of **1** followed by GLC analysis of the hydrolysate after derivatisation gave only one peak corresponding to xylose. The anomeric proton signal appeared as a doublet at δ 4.23 ($J = 7.4$ Hz) confirming its β-configuration ($^4C_1$ conformation). That the sugar constituent was attached to C-3 (δ 87.8) of the aglycone moiety was evident from HMBC and NOESY cross peaks. Thus C-3 showed HMBC with H-1 of xylose, which in turn showed NOE correlation with that of H-3 of the aglycone. Interestingly, the C-3 chemical shift of xylose appeared significantly downfield (δ 82.6) in comparison with that (δ 77.7) of standard methyl β-D-5-xylose, indicating the presence of a substituent. Absence of any NMR signal attributable to a substituent and the IR and mass spectral evidence for a sulfate group as discussed earlier helped to identify the substituent. This was confirmed by solvolysis of the compound to yield sulfuric acid and compound (**5**), characterized mainly by 2D NMR techniques. Acid hydrolysis of **5** furnished the sapogenin spergulagenin A (**6**). Thus the structure of spergulin A is established as 3-*O*-(β-D-xylopyranosyl 4-sulphate)-spergulagenin A (**1**). The same sapogenin had been isolated earlier by Barua et al. (1986 a, b) and Chakrabarti et al. (1966), but only the melting point and some ^{1}H NMR data were reported.

Spergulacin (**2**) and spergulacin A (**3**) proved to be saponins of the same sapogenin **6** and their structures were elucidated following the same method as used for **1**. The monosaccharides for both **2** and **3** were shown to be D-xylose and L-rhamnose, present in 1:1 ratio based on GLC analysis of the acid hydrolysate. β-Configuration ($^4C_1$ conformation) for the xylopyranosyl unit and α-configuration for the rhamnopyranosyl unit were inferred from the large $J_{H1,H2}$ for the xylopyranosyl unit and the singlet like appearance of the H-1 signal for the rhamnopyranosyl unit respectively. The observed HMBC between the H-1 signals of xylose and C-3 signal of the aglycone suggested that in both the saponins xylose was linked directly to the aglycone at C-3. Interglycosidic linkages in **2** and **3** were derived from the observed HMBC of H-1 of rhamnose with C-3 of xylose in **2** and C-2 of xylose in **3**. Compounds **2** and **3** were found to be identical with spergulacin and spergulacin A respectively reported earlier by Barua et al. (1986a,b).

Spergulin B (**4**) was concluded to be a new saponin of another sapogenin spergulatriol (**8**) reported previously by Kitagawa et al. (1977). The structure elucidation was principally based on 2D NMR analysis of the genin (**8**) and the prosapogenin (**7**) obtained by enzymatic hydrolysis of **4.** The monosaccharides were identified as xylose and rhamnose by co-GLC with authentic specimens (vide experimental). Interglycosidic and sugar-aglycone linkages were deduced on the basis of the arguments as described for **2** and **3**. Further, the prosapogenin (**7**) liberated by the enzymatic hydrolysis contained only xylose, linked to C-3 of the aglycone. Thus the structure of spergulin B was established as 3-*O*-[α-rhamnopyranosyl (1→2)-β-D-xylopyranosyl]-spergulatriol (**4**).

It is noteworthy that saponins based on hopane or rearranged hopane skeletons are very few while none have been reported for nor hopane sapogenins (Mahato et al., 1988; Mahato and Nandy, 1991; Mahato and Garai, 1998).

3. Experimental

3.1. General procedures

All melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR were recorded using a Jeol ECP-500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) in DMSO-d_6 or C_5D_5N with TMS as internal standard. LR and HR FAB–MS (negative) were done using KRATOS CONCEPT mass spectrometer with a mixture of glycerol and *m*-nitrobenzyl alcohol for LR–FAB–MS and PEG-600 for HR–FAB–MS as matrices. MALDI–TOF–MS (positive) were performed on a Perspective Biosystems Voyager DE-STR spectrometer with 2,5-dihydroxybenzoic acid as matrix. TLC was carried out on silica gel 60 F_{254} (Merck) [$CHCl_3$:MeOH:H_2O; 30:13:2] and spots were visualized by spraying with 10% H_2SO_4.

3.2. Plant material

The plant material was collected from the suburbs of Calcutta, India and identified at Indian Botanic Garden, Howrah. A voucher specimen was deposited at the Steroids and Terpenoids Chemistry Department.

3.3. Extraction and isolation

The air-dried powdered plant (1 kg) (aerial part of *Mollugo spergula*) was successively extracted with light petrol (60–80 °C), $CHCl_3$ and MeOH under reflux conditions. The residue (15 g) obtained after evaporation of the MeOH under reduced pressure was applied to a column of Diaion HP 20 (500 g) and the column washed with water followed by 30, 40, 60, 80 and 100% of MeOH. Fractions eluted with 60% MeOH showed single spot on TLC over silica gel and the residue was crystallized from methanol-water to furnish 150 mg of spergulin A (**1**) (R_f 0.41). The residue from fractions eluted with 80% methanol was rechromatographed over silica gel. Fractions eluted with $CHCl_3$–MeOH (17:3) showed three spots on TLC and the residue was subjected to fractional crystallization to obtain spergulacin (**2**) (R_f 0.59) (135 mg). The mother liquor was chromatographed repeatedly over silica gel and eluted with various mixtures of chlorform and methanol. Later fractions eluted with $CHCl_3$–MeOH (17: 3) furnished 95 mg of spergulacin A (**3**) (R_f 0.55), while fractions eluted with $CHCl_3$–MeOH (4:1) gave 65 mg of spergulin B (**4**) (R_f 0.49).

3.4. Spergulin A (*1*)

Colorless needles, mp 220–221 °C (dec.); $[\alpha]_D^{25}$ +19.1° (*c* 0.66, DMSO); IR: ν_{max} cm^{-1} (KBr): 3550, 3468, 1698, 1637, 1460, 1383, 1062, 1021, 972, 824; HR FAB–MS (negative): *m*/*z* 685.36164 [M–H]$^-$, (calc. for $C_{35}H_{57}O_{11}$ S, *m*/*z* 685.36216); LR–FAB–MS (negative): *m*/*z* 685.4 [M–H]$^-$, 641.3, 459.1, 352.1, 227.0, 199.0, 97.0 [SO_4H]$^-$ and 80.0 [SO_3]$^-$; MALDI–TOF–MS (positive): 747.03 [M + Na + K–1]$^+$, 731.11 [M + 2Na–1]$^+$, 687.07 [M + H]$^+$. ^{1}H NMR (DMSO-d_6). Sugar moiety: 4.23 (*d*, *J* = 7.4 Hz, H′-1), 3.13 (*m*, H′-2), 3.91 (*t*, *J* = 9 Hz, H′-3), 3.43 (*m*, H′-4), 3.09 (*m*, H′$_a$-5), 3.71 (*dd*, *J* = 5.5, 11.4 Hz, H′$_b$-5); ^{13}C NMR (DMSO-d_6) data: Table 2. (Found: C, 57.91; H, 8.28; S, 4.26. $C_{35}H_{57}O_{11}NaS.H_2O$ requires: C, 57.83; H, 8.18; S, 4.41%.).

3.5. Solvolysis of spergulin A (*1*)

Spergulin A (18 mg) was refluxed with a mixture of pyridine and dioxane (4:1, 5 ml) for 6 h. The reaction mixture was passed through a Sep-pak cartridge (Waters), and eluted successively with water (15 ml) and MeOH (15 ml). The MeOH eluted fraction was chromatographed on silica gel using chloroform–methanol–water (50:10:1) to yield **5** (14.5 mg), crystallized from methanol–acetonitrile to furnish fine needles, mp 238–240 °C (dec.); $[\alpha]_D^{20}$ +16.30° (*c* 0.92, pyridine); IR: ν_{max} cm^{-1} (KBr): 3433, 1696, 1651, 1642, 1097, 1043 and 972; LR–FAB–MS (positive): *m*/*z* 645 [M + K]$^+$, 629 [M + Na]$^+$; ^{1}H NMR (DMSO-d_6): δ 0.74, 0.79, 0.90, 0.91, 0.95, 0.96, 1.25, 2.10 (each 3H *s*, H_3 of C-24, 25, 27, 28, 26, 23, 29, 30), 0.67 (1H, *d*, *J* = 10.7 Hz, H-5), 1.30 (1H, *d*, *J* = 10.5 Hz, H-13), 1.62 (1H, *d*, *J* = 11.4 Hz, H-17), 1.75 (1H, *dd*, *J* = 8.7, 14.0 Hz, H-20β), 2.01 (1H, *dd*, *J* = 7.1, 12.4 Hz, H-19β), 2.96 (*m*, H-3α), 3.58 (*m*, H-16α), 3.67 (*m*, H-12α), Sugar moiety: 4.11 (*d*, *J* = 7.5 Hz, H′-1), 2.95 (*m*, H′-2), 3.07 (*m*, H′-3), 3.26 (*m*, H′-4), 2.96 (*m*, H′$_a$-5), 3.64 (*dd*, *J* = 5.2, 11.2 Hz, H′$_b$-5).; ^{13}C NMR (DMSO-d_6): Table 2. (Found: C, 69.19; H, 9.51; $C_{35}H_{58}O_8$ requires: C, 69.27; H, 9.63%).

The aqueous part was examined by paper chromatography (Advantee, No. 50) employing methanol–water (1:1). Sulfuric acid was detected as a light yellow spot (R_f 0.71) after spraying the paper with a solution of potassium rhodizonate (10 mg in 50 ml of 50% methanol).

3.6. Acid hydrolysis of *5*

Compound **5** (5 mg) was heated in 1 ml of 1 M HCl (dioxane–H_2O, 1:1) at 80 °C for 2 h on a water bath. Dioxane was distilled off under reduced pressure and the solution was extracted with EtOAc (1 ml ×3). The organic layer was washed with water, dried, and the residue was crystallized from EtOAc to give fine needles (3 mg) of spergulagenin A (**6**), mp 276–277 °C; [lit. (Kitagawa

et al., 1975), mp 278–279.5 °C; $[\alpha]_D^{20}$ +35.3° (*c* 0.68, pyridine); LR–FAB–MS (positive): *m/z* 497 $[M+Na]^+$, 513 $[M+K]^+$; ^{1}H NMR (DMSO-d_6): δ 0.67, 0.78, 0.89, 0.91, 0.92, 0.96, 1.25, 2.10 (each 3H *s*, H_3 of C-24, 25, 23, 27, 28, 26, 29, 30), 0.63 (1H, *d*, *J*=9.4 Hz, H-5), 1.30 (1H, *d*, *J*=11 Hz, H-13), 1.62 (1H, *d*, *J*=11.5 Hz, H-17), 1.75 (1H, *dd*, *J*=8.5, 14 Hz, H-20β), 2.01 (1H, *dd*, *J*=7.5, 12.5 Hz, H-19β), 2.98 (1H, *m*, H-3α), 3.68 (1H, *m*, H-12α), 3.58 (1H, *m*, H-16α); ^{13}C NMR: Table 2. (Found: C, 75.86; H, 10.65; Calc. for $C_{30}H_{50}O_4$: C, 75.90; H.10.62%).

The aqueous part was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, then dried under vacuum, and treated with 1-(trimethylsilyl) imidazole at room temperature for 2 h. Excess reagent was decomposed with water and the mixture extracted with *n*-hexane (1 ml ×2). The monosaccharide was identified as xylose (R_t 20.26) by co-GLC analysis of the TMSi derivative with standard sample (R_t 20.56).

3.7. Spergulacin (**2**)

The compound was crystallized from MeOH to yield microneedles, mp 280–282 °C (dec.); $[\alpha]_D^{20}$ −13.7° (*c* 0.76, pyridine) [lit. (Barua et al., 1986b)]; HR–FAB–MS (negative): *m/z* 751.46564 $[M-H]^-$ (calc. for $C_{41}H_{67}O_{12}$: *m/z* 751.46325); LR–FAB–MS (positive): *m/z* 753.4 $[M+H]^+$; ^{1}H NMR (DMSO-d_6): δ 0.75, 0.80, 0.91, 0.92, 0.95, 0.96, 1.26, 2.10 (each 3H, *s*, H_3 of C-24, 25, 27, 28, 23, 26, 29, 30), 0.67 (1H, *d*, *J*=10.8 Hz, H-5), 1.30 (1H, *d*, *J*=10.8 Hz, H-13), 1.62 (1H, *d*, *J*=11.5 Hz, H-17), 1.76 (1H, *dd*, *J*=9, 13.8 Hz, H-20β), 2.01 (1H, *dd*, *J*=7.3, 12.4 Hz, H-19β), 3.02 (1H, *dd*, *J*=4.5, 11.5 Hz, H-3α), 3.60 (*m*, H-16α), 3.67 (*m*, H-12α), Sugar moiety: 4.17 (*d*, *J*=7.5 Hz, H′-1), 3.10 (*m*, H′-2), 3.30 (*m*, H′-3), 3.31 (*m*, H′-4), 3.10 (*m*, H′$_a$-5), 3.70 (*m*, H′$_b$-5); 5.0 (s, H″-1), 3.71 (m, H″-2), 3.48 (m, H″-3), 3.19 (m, H″-4), 3.86 (m, H″-5) and 1.10 (*d*, *J*=6.2 Hz, CH_3); ^{13}C NMR: Table 2. (Found: C, 65.38; H, 9.15; calc. for $C_{41}H_{68}O_{12}$: C, 65.40, H, 9.10%).

3.8. Spergulacin A (**3**)

Crystallized from methanol as colorless needles, mp 260–262 °C (dec.); $[\alpha]_D^{20}$ −16.1° (*c* 0.77, pyridine); [lit., (Barua et al., 1986a)]; HR–FAB–MS (negative): *m/z* 751.46515 $[M-H]^-$ (calc. for $C_{41}H_{68}O_{12}$: *m/z* 751.46325); LR–FAB–MS (positive): 753.5 $[M+H]^+$; ^{1}H NMR (DMSO-d_6): δ 0.74, 0.79, 0.905, 0.910, 0.94, 0.95, 1.25, 2.10 (each 3H, *s*, H_3 of C-24, 25, 27, 28, 23, 26, 29, 30), 0.67 (1H, *d*, *J*=10.8 Hz, H-5), 1.30 (1H, *d*, *J*=10.8 Hz, H-13), 1.61 (1H, *d*, *J*=11.5 Hz, H-17), 1.73 (1H, *dd*, *J*=8.5, 14 Hz, H-20β), 2.01 (1H, *dd*, *J*=7.3, 12.4 Hz, H-19β), 3.02 (*m*, H-3α), 3.59 (*m*, H-16α), 3.71 (*m*, H-12α).

Table 1
^{1}H NMR chemical shifts[a] of spergulin A (**1**)[b] and spergulatriol (**8**)[c]

H	**1** δ (ppm), *J* (Hz)	**8** δ (ppm), *J* (Hz)
1.	1.58 (+, 1α), 0.91 (+, 1β)	1.00 (*m*, 1α), 1.70 (*m*, 1β)
2.	1.68 (*m*, 2α), 1.56 (*m*, 2β)	1.84 (+, 2α and 2β)
3.	3.04 (*dd* like, 3α)	3.45 (*m*, 3α)
5.	0.68 (*d*, 11.5, 5α)	0.82 (*dd*, 2.3, 12.1, 5α)
6.	1.48 (+, 6α), 1.37 (+, 6β)	1.42 (*m*, H-6α), 1.57 (*m*, 6β)
7.	1.17 (+, 7α), 1.41 (+, 7β)	1.50 (*m*, 7α), 1.31 (*m*, 7β)
9.	1.17 (*m*, 9α)	1.42 (*m*, 9α)
11.	1.67 (*m*, 11α), 1.22 (*t*-like, 11β)	2.13 (*m*, 11α), 1.66 (*m*, 11β)
12.	3.67 (*m*, 12α)	4.22 (*m*, 12α)
13.	1.31 (*d*, 10.6, 13β)	1.80 (*d*,, 10.8, 13β)
15.	1.17 (+, 15α), 1.41 (+, 15β)	1.90 (*dd*, 4.4, 12.4, 15α), 1.76 (*m*, 15β)
16.	3.59 (*m*, 16α)	4.32 (*dt*, 4.4, 10.1, 16α)
17.	1.63 (*d*, 11.4, 17β)	2.15 (+,17β)
19.	1.33 (*m*, 19α), 2.01 (*dd*, 7.4, 12.4, H-19β)	2.54 (*ddd*, 1.7, 8.6, 12.5, H-19α), 1.80 (+, 19β)
20.	1.49 (*m*, 20α), 1.76 (*dd*, 7, 14.3, 20β)	2.43 (*m*, 20α), 2.47 (*m*, 20β)
22.	–	5.15 (*t*, 2.1, H$_a$-22), 6.07 (*t*, 2.1, H$_b$-22)
23.	0.98 (*s*, CH_3-23)	1.24 (*s*, CH_3-23)
24.	0.73 (*s*, CH_3-24)	1.04 (*s*, CH_3-24)
25.	0.80 (*s*, CH_3-25)	0.89 (*s*, CH_3-25)
26.	0.96 (*s*, CH_3-26)	1.07 (*s*, CH_3-26)
27.	0.92 (*s*, CH_3-27)	1.16 (*s*, CH_3-27)
28.	0.92 (*s*, *CH*$_3$-28)	1.09 (*s*, *CH*$_3$-28)
29.	1.26 (*s*, CH$_3$-29)	–
30.	2.10 (*s*, CH$_3$-30)	–

[a] Assignments based upon COSY, HOHAHA, TOCSY, HETCOR, HSQC, HMBC and NOESY experiments.
[b] In DMSO-d_6 (30 °C).
[c] In C_5D_5N (30 °C), + overlap.

Table 2
^{13}C NMR chemical shifts[a] [δ_c ($\pm$ 0.1)] of **1, 2**, **3**, **4, 5**, **6**, **7** and **8**

Carbon no.	**1**[b]	**2**[b]	**3**[b]	**4**[c]	**5**[b]	**6**[b]	**7**[c]	**8**[c]
1.	38.2	38.2	38.5	39.1	38.1	38.2	38.9	39.1
2.	25.7	25.8	26.0	27.0	25.8	27.0	26.9	28.3
3.	87.8	87.8	87.5	88.5	87.5	76.6	88.6	78.0
4.	38.8	38.8	38.7	39.6	38.7	38.4	39.7	39.5
5.	54.8	54.8	55.1	56.0	54.7	54.6	55.8	55.7
6.	17.9	17.8	17.9	18.66	17.8	18.0	18.7	19.0
7.	32.8	32.8	32.9	33.6	32.7	32.8	33.6	33.6
8.	44.7	44.7	44.8	45.4	44.6	44.7	45.3	45.37
9.	48.1	48.1	48.1	49.2	48.0	48.1	49.2	49.3
10.	36.2	36.1	36.2	37.0	36.1	36.4	37.0	37.3
11	31.8	31.8	31.8	33.1	31.7	31.8	33.2	33.2
12.	67.3	67.3	67.3	69.3	67.2	67.2	69.3	69.3
13.	54.5	54.5	54.56	53.9	54.4	54.5	53.9	54.0
14.	40.9	40.9	41.0	41.8	40.8	40.8	41.8	41.8
15.	44.4	44.3	44.3	45.1	44.2	44.3	45.1	45.1
16.	64.1	64.0	64.1	67.2	64.0	64.0	67.2	67.2
17.	62.7	62.8	62.9	62.2	62.8	62.8	62.2	62.2
18.	46.0	45.9	46.0	45.4	45.9	45.9	45.38	45.39
19.	44.6	44.6	44.7	43.2	45.5	44.5	43.2	43.2
20.	36.7	36.7	36.8	29.9	36.6	36.6	29.9	29.9
21.	52.2	52.2	52.2	152.0	52.1	52.1	152.2	152.2
22.	213.6	213.6	213.6	106.0	213.6	213.6	106.0	106.0
23.	27.3	27.4	27.3	28.0	27.4	28.0	28.1	28.7
24.	16.1	16.0	16.0	16.9	16.1	15.6	16.8	16.3
25.	15.5	15.5	15.8	16.2	15.5	15.5	16.1	16.1
26.	16.5	16.5	16.6	17.0	16.5	16.5	17.0	17.1
27.	18.5	18.4	18.5	19.1	18.4	18.4	19.1	19.1
28.	17.1	17.1	17.2	16.0	17.1	17.1	16.0	16.0
29.	20.1	20.1	20.1	–	20.0	20.0	–	–
30.	25.7	25.7	25.8	–	25.7	25.7	–	–
Xylose								
C-1′	105.7	105.6	104.6	106.1	106.0		107.7	
C-2′	72.3	73.8	77.8	78.0	73.7		75.6	
C-3′	82.6	81.0	76.6	79.6	76.7		78.7	
C-4′	68.4	68.2	70.0	71.5	69.5		71.3	
C-5′	65.0	65.3	65.5	66.9	65.5		67.1	
Rhamnose								
C-1″		100.5	100.1	101.9				
C-2″		70.5	70.3	72.4				
C-3″		70.6	70.4	72.6				
C-4″		72.1	72.1	74.1				
C-5″		68.0	68.01	69.7				
CH_3		17.8	17.9	18.7				

[a] Assignments based upon COSY, TOCSY, HETCOR, HSQC, HMBC and NOESY experiments.
[b] In DMSO-d_6 (30 °C).
[c] In C_5D_5N (30 °C).

Sugar moiety: 4.23 (*d*, $J=7.0$ Hz, H′-1), 3.23 (*m*, H′-2), 3.19 (*m*, H′-3), 3.27 (*m*, H′-4), 3.02 (*m*, H′$_a$-5), 3.68 (*dd*, $J=5$, 11.3 Hz, H′$_b$-5), 5.22 (*s*, H″-1), 3.71 (*m*, H″-2), 3.48 (*m*, H″-3), 3.14 (*m*, H″-4), 3.78 (*dd*, $J=6.2$, 9.2 Hz, H″-5) and 1.06 (*d*, $J=6.2$ Hz, CH_3); ^{13}C NMR: Table 2. (Found: C, 65.36; H, 9.14; calc. for $C_{41}H_{68}O_{12}$: C, 65.40; H, 9.10%).

3.9. Acid hydrolysis of *2* and *3*

Both of **2** and **3** (5 mg each) was hydrolyzed separately with 1 M HCl in a manner similar to that described for **5**. In both the cases, the genin was identified as spergulagenin A (mp, PMR, CMR and MS) and the monosaccharides were found to be xylose (R_t 13.55) and rhamnose (R_t 20.20) (co-GLC of the TMSi derivatives with standard samples).

3.10. Spergulin B (*4*)

Colorless powder, mp 271–273 °C (dec.); $[\alpha]_D^{20}$ −20.0° (*c* 0.85, pyridine); IR: ν_{max} cm^{-1} (KBr): 3414, 1654, 1454, 1040, 985, 839; HR–FAB–MS (negative): *m*/*z*

707.43757 $[M–H]^-$ (calc. for $C_{39}H_{63}O_{11}$: *m/z* 707.43734); LR–FAB–MS (positive): *m/z* 709.4 $[M+H]^+$; MALDI–TOF–MS (positive): 747.03 $[M+K]^+$, 731.11 $[M+Na]^+$; ^1H NMR (C_5D_5N): δ 0.84, 1.04, 1.07, 1.17, 1.18, 1.25 (each 3H *s*, H_3 of C-25, 26, 28, 27, 24, 23), 0.74 (1H, *d*, $J=11.5$ Hz, H-5), 1.77 (1H, *d*, $J=10.8$ Hz, H-13), 2.12 (1H, *m*, H-17), 3.30 (1H, *dd*, $J=4.1$, 11.7 Hz, H-3α), 4.18 (1H, *t*, $J=8.5$ Hz, H-12α), 4.30 (*m*, H-16α), Sugar moiety: 4.84 (*d*, $J=6.2$ Hz, H′-1), 4.23 (*m*, H′-2), 4.16 (*m*, H′-3), 4.14 (*m*, H′-4), 4.33 (*m*, H'_a-5), 3.71 (*dd*, $J=9.6$, 11.0 Hz, H'_b-5), 6.52 (*brs*, H″-1), 4.86 (*dd*, $J=1.4$, 3.2 Hz, H″-2), 4.67 (*dd*, $J=3.4$, 9.4 Hz, H″-3), 4.33 (*m*, H″-4), 4.76 (*m,* H″-5) and 1.71(*d,* $J=6.2$ Hz, CH_3); ^{13}C NMR (C_5D_5N): Table 2. (Found: C, 66.10; H, 9.05; $C_{39}H_{64}O_{11}$ requires: C, 66.07; H, 9.10%).

3.11. Enzymatic hydrolysis of spergulin B (**4**)

A solution of **4** (9 mg) in acetate buffer (pH 5.0, 1 ml) was treated with naringinase (ca 100 mg) at 37 °C for 6 days to obtain a precipitate which showed two spots on TLC. The mixture was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (5:1:0.1) to yield the prosapogenin **7** (3.1 mg) and the genin **8** (2.2 mg). The aqueous part was worked up as described earlier and the monosaccharides were identified to be xylose and rhamnose by co-GLC analysis of the TMS derivatives with authentic samples.

3.12. Prosapogenin (7)

Amorphous powder, $[\alpha]_D^{20}+34.8°$ (*c* 0.10, MeOH); IR ν_{max} cm^{-1} (KBr): 3428, 1657, 1461, 1042, 971, 890; MALDI–TOF–MS (positive): *m/z* 585 $[M+Na]^+$; ^1H NMR (C_5D_5N): δ 0.83, 1.00, 1.04, 1.08, 1.18, 1.31 (each 3H *s*, 6 x-CH_3), 0.78 (1H, *d*, $J=11.5$ Hz, H-5), 1.79 (1H, *d*, $J=10.8$ Hz, H-13), 2.12 (*m*, H-17), 3.36 (1H, *dd*, $J=4.3$, 11.6 Hz, H-3), 5.15 (1H, *brs*, H_a-22) and 6.07 (1H, *brs*, H_b-22), Sugar moiety: 4.85(*d*, $J=7.6$ Hz, H′-1), 4.03 (*t*, 8 Hz, H′-2), 4.17 (*t*, 9 Hz, H′-3), 4.23 (*dd*, $J=5$, 10 Hz, H′-4), 3.78 (*t*, $J=11.2$ Hz, H'_a-5), 4.39 (*dd*, $J=5.3$, 11.3 Hz, H'_b-5); ^{13}C NMR (C_5D_5N): Table 2. (Found: C, 70.38; H, 9.69; $C_{33}H_{54}O_7$ requires: C, 70.40; H, 9.67%).

3.13. Spergulatriol (**8**)

Crystallized from methanol as fine needles, mp 221–222 °C (dec.), $[\alpha]_D^{20}$ +54.8° (*c* 0.28, $CHCl_3$) [lit. (Kitagawa et al., 1977) mp 224–226 °C (dec.), $[\alpha]_D^{28}$ +60.90° (*c* 0.29, $CHCl_3$)]; IR ν_{max} cm^{-1} (KBr): 3432, 1660, 1081, 1029, 970; MALDI–TOF–MS (positive): *m/z* 453 $[M+Na]^+$, 469 $[M+K]^+$; LR FAB–MS (positive): *m/z* 431 $[M+H]^+$, 429 $[M–H]^+$, 413 $[M–H_2O+H]^+$, 395 $[M–2H_2O+H]^+$, 377 $[M–3H_2O+H]^+$; ^1H and ^{13}C NMR (C_5D_5N): Tables 1 and 2. (Found: C, 78.14; H, 10.70; calc. for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77%).

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