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SPERGULACIN-A: A TRITERPENOID SAPONIN FROM *MOLLUGO SPERGULA*

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Key Word Index—*Mollugo spergula*; Aizoaceae; new triterpenoid saponin; spergulacin-A.

Abstract—From *Mollugo spergula* a new triterpenoid saponin, spergulacin-A, has been isolated and its structure established as spergulagenin-A-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranoside.

INTRODUCTION

The plant *Mollugo spergula* Linn. is widely distributed in India and is used as a green vegetable. It is bitter in taste and is used in the indigenous system of medicine as an antiseptic [1]. The bitter principles have been characterized as triterpenoid saponins. The isolation of several new sapogenins, spergulagenic acid [2–4], spergulagenin-A [5–12], spergulagenin-C and spergulagenol [13, 14], has been reported from this laboratory. Kitagawa *et al.* [15] reported a new sapogenin, spergulatriol, from the same plant. The present paper reports the isolation of a new triterpenoid saponin called spergulacin-A, the structure of which has been elucidated as spergulagenin-A-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranoside (**1a**).

RESULTS AND DISCUSSION

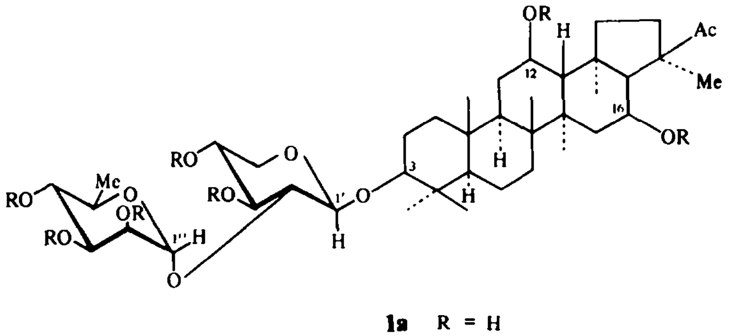
Spergulacin-A (**1a**), $C_{41}H_{68}O_{12}$, obtained in a crystalline state from an ethanolic extract of *M. spergula*, was found to be homogeneous by TLC. On acetylation with acetic anhydride and pyridine, **1a** yields an acetate, $C_{55}H_{82}O_{19}$ (**1b**). Deacetylation of **1b** by refluxing with methanolic sodium bicarbonate gives back **1a**.

When hydrolysed with ethanolic hydrochloric acid, **1a** yields spergulagenin-A, L-rhamnose and D-xylose. The sugars were characterized by paper chromatography, GLC and from molecular rotation calculations. The ^{1}H NMR spectrum of **1b** indicates that rhamnose, xylose and aglycone moieties in **1a** are in the ratio 1 : 1 : 1 which is also supported by quantitative acid hydrolysis of **1a**.

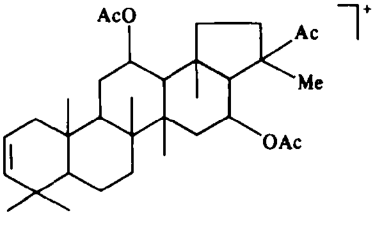
Hydrolysis of **1a** with β-xylosidase yields spergulagenin-A and a disaccharide which, on acid hydrolysis, yields rhamnose and xylose. This experiment shows that the two sugars exist as a disaccharide, which is attached to the aglycone through a β-xylosido linkage. The above conclusion was further corroborated by the mass spectral (Jeol-JMS-D-300, 70 eV, EIMS) fragmentation pattern of **1b**. The mass spectrum of **1b** does not show $[M]^+$ at m/z 1046 but shows characteristic peaks at m/z 540 (38), 489 (4), 273 (80) and 217 (8) for the ion species *a*, *b*, *c* and *d*, respectively, besides the base peak at m/z 43. The formation of the ion species *c* and *d* clearly shows the rhamnose–xylose–aglycone sequence in **1a**.

The ^{1}H NMR spectrum ($CDCl_3$, Varian XL-200) of **1b** shows sharp singlets at δ0.79, 0.85, 0.99 (6H), 1.05, 1.09, 1.18 for the seven tertiary methyl groups and another at δ2.19 for the C-21 acetyl group (–CO–C$\underline{H}_3$). There are also sharp singlets at δ1.93, 1.97, 2.02, 2.03, 2.06, 2.08 and 2.14 for the seven other acetoxy groups (–O–CO–C$\underline{H}_3$), of which five are attributed to the acetoxy groups of the disaccharide moiety and the other two to the C-16 and C-12 acetoxy groups.

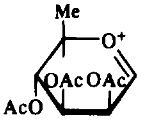
The signal for the C-5″ secondary methyl group of rhamnose appears, as expected, at δ1.18 (d, $J = 6$ Hz). The signal for the C-5″ proton appears at δ4.17 (1H, m, $W_{1/2} = 20$ Hz). It overlaps partly with the signal for the C-5′ Heq. Irradiation of the signal at δ4.17 causes collapse of the signal (δ1.18, d, $J = 6$ Hz) for the secondary methyl group of rhamnose to a singlet. The C-1″ anomeric proton appears at δ5.03 (d, $J = 2$ Hz) and the low coupling constant indicates *trans* diequatorial coupling between the C-1″ proton and the C-2″ proton, which indicates the presence of an α-L-rhamnose moiety in **1a**. Rhamnosides are known to occur as α-L-rhamnosides and the anomeric proton appears at δ4.92–5.00 (d, J *ca* 2 Hz) and the secondary methyl at δ1.1–1.3 ($J = 6$ Hz) [16]. The C-1′ anomeric proton appears at δ4.62 (d, $J = 5.25$ Hz). The large coupling constant indicates the *trans* diaxial relationship between the C-1′ and C-2′ protons which requires a β-xylosido linkage between the sugar moiety and the aglycone. This has been confirmed by the hydrolysis of **1a** with β-xylosidase. The signal for the C-5′ H_{ax} appears at δ3.42 (dd, $J_{gem} = 11.9$ Hz; $J_{ax,ax} = 7.5$ Hz) and that for the C-5′ H_{eq} at δ4.10 (dd, $J_{gem} = 11.9$ Hz; $J_{eq,ax} = 4.5$ Hz). Irradiation of the above signal (δ4.10) causes collapse of the signal at δ3.42 to a doublet, as expected. The above NMR data indicate that the xylose moiety in **1a** occurs in the expected pyranose form. The signal at δ3.73 (dd, $J_{ax,ax} = 7.30$ Hz, $J_{ax,ax} = 5.25$ Hz) is attributed to the C-2′ proton on the basis of the following arguments: (i) the signal of any proton attached to a carbon bearing a secondary acetoxy group in a sugar moiety is not expected to appear so upfield; (ii) the splitting pattern (dd) and the coupling constants ($J = 7.3$, 5.25 Hz) indicate that the proton in question is flanked by two axial protons, thus it could be either the C-2′ or C-3′



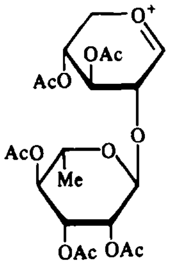
1a R = H
1b R = Ac



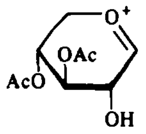
a



c



b



d

proton; (iii) when the C-1' proton signal (δ4.62) is irradiated, the signal at δ3.73 collapses to a doublet, as expected. Similarly, when the signal at δ 3.73 is irradiated, the doublet at δ 4.62 collapses to a singlet. So the signal at δ3.73 must be due to the C-2' proton and hence the rhamnosyl moiety must be linked to the xylose through the C-2' hydroxyl group.

In ^{1}H NMR spectrum ($CDCl_3$) of triacetyl spergulagenin-A, the signal for the C-3 αH appears at δ4.50 (*m*, $W_{1/2}$ = 18 Hz) and the signals at δ4.90 (*m*, $W_{1/2}$ = 22 Hz) and 5.24 (*m*, $W_{1/2}$ = 25 Hz) were assigned to H-16α and H-12α, respectively.

In the region δ4.81–5.31 of the ^{1}H NMR spectrum of **1b** there are signals for eight protons which may be attributed to those attached to the carbon bearing the secondary acetoxy groups (seven) and the C-1'' proton.

Irradiation of the signal at δ3.12 (*m*, $W_{1/2}$ = 18 Hz) does not affect any signal in the region δ3.42–5.31, which is typical of the protons of the sugar moiety, excluding those of the methyl and acetoxy groups. So this signal must be due to the C-3 proton as the C-12 and C-16 protons are expected to appear further downfield. Hence the disaccharide moiety must be linked to the aglycone through the C-3 hydroxyl group.

It is a general observation that in the case of triterpenoid saponins the D-sugars occur with β-glycosidic and L-sugars with α-glycosidic linkages [17] and this has been found to be true in the case of spergulacin-A also.

Information concerning the pyranose form of the sugars and the configurations of the glycosidic linkages in spergulacin-A acetate were obtained from the ^{1}H NMR data discussed earlier. This was further corroborated by molecular rotation calculations [18–20]. The molecular rotation of spergulacin-A was observed to be −85.35°, showing an acceptable difference of 22.44° from the calculated value (based on [M] values of −111.25 and −108.08 for methyl-α-L-rhamnoside [21] and methyl-β-D-xyloside [21], respectively) of −62.91°. This showed the sugars to be L-rhamnose and D-xylose.

If, by any chance, the rhamnose was D and the xylose L then the calculated [M] value (based on [M] values of +111.25° and +108.08° for methyl-α-D-rhamnopyranoside and methyl-β-D-L-xylopyranoside, respectively) would be +375.75°, which is unacceptable. Molecular rotation calculations with either combinations of D-rhamnose and D-xylose or L-rhamnose and L-xylose give high positive values, which are unacceptable.

On the basis of the data presented the structure of the saponin may be represented as spergulagenin-A-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-xylopyranoside (**1a**).

EXPERIMENTAL

The mps are uncorrected and were recorded in an electrothermal apparatus. The petrol used had bp 60–80°. Silica gel and silica gel G (BDH, India) were used for chromatography. *Mollugo spergula* was collected from the suburbs of Calcutta during June–August when the plant is in full leaf and was identified by the Keeper of the National Herbarium, Indian Botanic Garden, Shibpur, Howrah, West Bengal, by comparison with the herbarium specimen.

Isolation of spergulacin-A (**1a**). Air-dried, defatted, powdered aerial parts of the plants (1.5 kg) were extracted in Soxhlet apparatus with EtOH (90%). The crude saponin was precipitated by the addition of excess Et_2O to the concd ethanolic extract. The precipitate was taken up in hot *n*-BuOH and the residue left, after distillation of the solvent, was chromatographed over deactivated (9% water) silica gel. Elution with $CHCl_3$–MeOH (24:1) yielded a fraction which was purified by repeated CC and crystallized from EtOH mp 258–262° (dec.), $[\alpha]_D^{23}$ − 11.35° (MeOH), single spot by TLC [0.35 mm silica gel G: (i) EtOAc–*n*-BuOH–MeOH, 35:17:3; (ii) EtOAc–MeOH–AcOH, 45:5:2: detection Liebermann–Burchard reagent and/or I_2 vapour]. The yield of spergulacin-A was 65 mg.

Spergulacin-A acetate (**1b**). Spergulacin-A (30 mg) was heated with Ac_2O (1 ml) and C_5H_5N (0.5 ml) on a steam bath for 2 hr. The product was worked up in the usual way and was purified by prep. TLC (0.35 mm silica gel G, $CHCl_3$–MeOH, 49:1: detection by exposure to I_2) to give 11 mg of pure acetate mp 177–181° (MeOH).

Spergulacin-A acetate (**1b**, 10 mg) was refluxed with methanolic $NaHCO_3$ (1%, 2 ml) for 2 hr. After removal of the solvent, the residue was subjected to prep. TLC. The product was identified as spergulacin-A by comparison of mp and TLC.

Acid hydrolysis of spergulacin-A. Spergulacin-A (23 mg) was refluxed with EtOH (5 ml) and HCl (1 ml) on a steam bath for 3 hr. The product was diluted with H_2O and the ppt. filtered, washed with H_2O and dried (13.9 mg). The residue, crystallized from $CHCl_3$–MeOH, had mp 278–282° (dec.), $[\alpha]_D^{23}$ + 33° (EtOH). It was identified as spergulagenin-A by comparison of mp, mmp and IR with those of an authentic sample available in the laboratory.

The aq. filtrate was neutralized with $AgCO_3$, filtered and evaporated. The resultant syrup was subjected to PC along with standard sugars (*n*-BuOH–HOAc–H_2O, 4:1:1; spray reagent: aniline hydrogen phthalate).

The mixture of sugars obtained by acid hydrolysis of **1a** were silylated in the usual way with trimethylchlorosilane and hexamethyldisilazane in C_5H_5N (Trisil-Z, Pears Chemical Co., Rockford, IL, U.S.A.) and subjected to GC (3% SE-30; column temp. 165°; detection temp. 230°; N_2, 60 ml/min) along with silyl derivaties of standard sugars. The sugars were identified as rhamnose and xylose (ratio 1:1).

Enzymatic hydrolysis of spergulacin-A. Spergulacin-A (10 mg) was dissolved in EtOH (0.6 ml) and NaOAc buffer (pH 5, 0.1 M, 5 ml) was added to it, followed by β-xylosidase (Sigma Chemicals, EC 3.2.1.37) in $(NH_4)_2SO_4$ and NaOAc suspension (0.2 ml); the mixture was stirred at 25° for 9 hr. The ppt. was filtered off and characterized as spergulagenin-A. The filtrate was evaporated under vacuum and the residue subjected to PC. The chromatogram showed a single spot which was more polar than those of xylose and rhamnose. The sugar was hydrolysed by refluxing with ethanolic-HCl, the product worked up in the usual way and examined by PC; two spots for xylose and rhamnose were observed.

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