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A TRITERPENE GLYCOSIDE FROM *MOLLUGO SPERGULA*

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Key Word Index—*Mollugo spergula*; Aizoaceae; triterpene glycoside; spergulacin.

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

INTRODUCTION

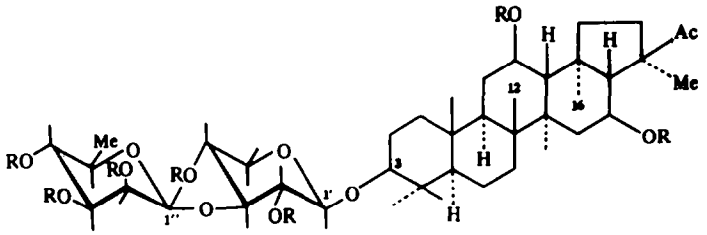
Several new triterpenoid sapogenins called spergulagenic acid [1–3], spergulagenin-A [4–11], spergulagenol and spergulagenin-C [12, 13] have been reported as constituents of *Mollugo spergula*. Kitagawa *et al.* [14] reported a new sapogenin, spergulatriol, from the same source. The present communication reports the isolation from the same plant of a new triterpene glycoside, called spergulacin, the structure of which has been established as spergulagenin-A-3-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-xylopyranoside (**1a**).

RESULTS AND DISCUSSION

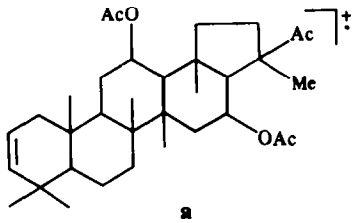
The plant *Mollugo spergula* Linn. was first collected from the suburbs of Calcutta in 1965 and was identified by Dr. S. K. Mukherjee, the then Keeper of National Herbarium, Indian Botanic Garden, Shibpur, Howrah, West Bengal, India, by comparing it with a Herbarium specimen. The plant used for this work was collected during the period June to August when the plant was in full leaf.

From the concentrated ethanolic extract of the defatted powdered plant material (whole plant but excluding the roots) the crude saponin was precipitated by addition of excess diethyl ether. The crude saponin thus obtained was extracted with hot *n*-butanol. The residue obtained from the *n*-butanol extract gave after column chromatography over deactivated silica gel (9% H_2O) a fraction which was found to be a mixture of three compounds by TLC. This mixture on repeated column chromatography over deactivated silica gel and preparative TLC over silica gel G followed by crystallization from ethanol yielded spergulacin (**1a**), $C_{41}H_{68}O_{12}$, mp 282–284° (dec.), $[\alpha]_D$ −13.3° (MeOH). Spergulacin on acetylation by heating with acetic anhydride and pyridine and column chromatography of the product over deactivated silica gel followed by preparative TLC yielded an acetate (**1b**) which was crystallized from aqueous methanol, $C_{55}H_{82}O_{19}$, mp 149–152°. Deacetylation of **1b** by refluxing with methanolic sodium bicarbonate (1%) solution followed by preparative TLC of the product furnished spergulacin (**1a**).

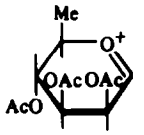
Hydrolysis of **1a** with methanolic hydrochloric acid (5%) yielded spergulagenin-A, L-rhamnose and D-xylose. The sugars were characterized by paper chromatography and GLC (3% SE-30; column temp. 165°; detection temp. 230°; carrier gas N_2; flow rate 60 ml per min). The configurations of the sugars were determined by molecular rotation calculation discussed later. The ^{1}H NMR spectrum ($CDCl_3$) of **1b** clearly showed that the rhamnose, xylose and the aglycone moieties in **1a** must be occurring in 1:1:1 ratio. This was also supported by quantitative acid hydrolysis of **1a**. When hydrolysed with β-xylosidase, in sodium acetate buffer initially at 5° then at room temperature, spergulacin (**1a**) gave spergulagenin-A and a disaccharide which on further acid hydrolysis furnished L-rhamnose and D-xylose. These experiments clearly indicated that in spergulacin the two sugars are occurring as a disaccharide which is attached to the aglycone through a β-xylosido linkage. The above conclusion was further supported by the mass spectral fragmentation pattern of **1b**. The mass spectrum did not show the molecular ion peak at *m/z* 1046 but showed characteristic peaks at *m/z* 540 (44), 489 (5), 273 (86), 217 (14) and 43 (base peak) for the ion species **a**, **b**, **c** and **d**, respectively. The formation of the ion species **c** and **d** confirms the rhamnose–xylose–aglycone sequence in **1a**. The ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of the acetate (**1b**) showed sharp singlets at δ0.73, 0.83, 0.90, 0.98, 1.05, 1.08 and 1.17 for the seven tertiary methyl groups and another at δ2.19 (3H) for the C-21 acetyl group. There were sharp singlets at δ1.93, 1.97, 2.02, 2.04, 2.08, 2.13 and 2.14 for the seven acetoxy groups, five of which were attributed to the acetoxy groups of the disaccharide moiety and two for the C-16 and C-12 acetoxy groups of the aglycone. The signal for the C-5″ secondary methyl group of rhamnose appeared, as expected, as a *d* at δ1.18 (J = 6 Hz). The signal at δ3.94 (1H, *m*, $W_{1/2}$ = 20 Hz) has been assigned to the C-5″ proton because when this signal was irradiated, the signal at 1.18 collapsed to a singlet. The C-1″ anomeric proton appeared at 4.9 (*d*, J = 2.15 Hz) and the low coupling constant indicated *trans* diequatorial coupling between the C-1″ proton and C-2″ proton which requires the presence of an α-L-rhamnose moiety in **1a**. In nature rhamnosides are usually known to occur as α-L-



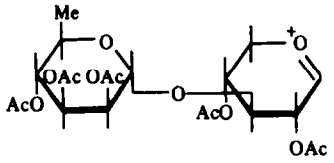
1a R = H
1b R = Ac



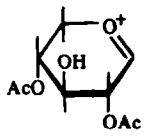
a



c



b



d

rhamnoside in which the rhamnosyl anomeric proton appears at δ4.92–5.00 (*d*, J = *ca* 2 Hz) and the secondary methyl at 1.1–1.3 (J = 6 Hz) [15]. The C-1′ anomeric proton appeared at δ4.41 (*d*, J = 6.9 Hz). The large coupling constant clearly indicated the *trans*-diaxial relationship between the C-1′ and C-2′ protons which necessitates a β-xylosidic linkage between the sugar moiety and the aglycone which has also been confirmed by the hydrolysis of **1a** with β-xylosidase. The signal for the H-5′$_{ax}$ appeared as a *dd* at δ3.24 (J_{gem} = 11.6 Hz; $J_{ax,ax}$ = 8.8 Hz) and the signal for the H-5′$_{eq}$ appeared as a *dd* at 4.11 (J_{gem} = 11.6 Hz; $J_{eq,ax}$ = 4.5 Hz). Irradiation of the signal at 4.11 caused the collapse of the signal at 3.24 to a *d* as expected. The above NMR data indicated that the xylose moiety is present in the expected pyranose form. The signal at δ3.83 (*t*, J = 8.4 Hz) is attributed to the C-3′ 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 far upfield. (ii) The splitting pattern (*t*) and coupling constant (8.4 Hz) indicated that the proton in question is flanked by two axial protons. Thus it could be the C-2′ or C-3′ proton. (iii) When the C-1′ proton signal at δ4.41 (*d*) was irradiated the signal for the proton in question was not affected which eliminates C-2′ as the possible site for linkage. Therefore C-3′ appears to be the only possible point of attachment with the rhamnose moiety.

In the ^{1}H NMR spectrum ($CDCl_3$) of triacetyl spergulagenin-A the signal for the H-3α appeared at δ4.50 (*m*, $W_{1/2}$ = 18 Hz) and at 4.90 (*m*, $W_{1/2}$ = 22 Hz) and 5.24 (*m*, $W_{1/2}$ = 22 Hz) which are assigned to H-16α and H-12α.

In the region δ4.91–5.19 of the ^{1}H NMR spectrum of **1b** there were signals for eight protons which may be attributed to the protons attached to the carbon bearing the acetoxy groups (seven) and the C-1″ proton.

Irradiation of the signal at δ3.08 (*m*, $W_{1/2}$ = 18 Hz) did not affect any signal in the region δ3.18–5.19 typical of the protons of the sugar moiety excluding methyl and the acetoxyls. Therefore this signal must be due to the C-3 proton as the C-12 and C-16 protons are expected to appear further downfield. The appearance of the signal at δ3.08 can only be explained if the disaccharide moiety in **1b** is linked with the aglycone through the C-3 hydroxyl group. With regard to the configuration of the saponin glycosidic linkages it is a general observation that D-sugars occur with β-glycosidic and L-sugars with α-glycosidic linkage [16]. This has been found to be true in spergulacin. Information concerning the pyranose form of the sugars and the configurations of the glycosidic linkages was obtained from the ^{1}H NMR data discussed above. This was further corroborated by molecular rotation measurements [17–19]. The molecular rotation of spergulacin was observed to be $-100°$ showing an acceptable difference of 37.75° from the calculated value

(based on reported [20, 21] molecular rotation [M] values $-111.25°$ and $-107.42°$ for methyl-α-L-rhamnopyranoside and methyl-β-D-xylopyranoside respectively) of $-62.25°$. This showed the sugars to be L-rhamnose and D-xylose.

If the rhamnose were D and the xylose L then the calculated [M] value (based on [M] value of $+111°$ [22] and $+107.42°$* for methyl-α-D-rhamnopyranoside and methyl-β-L-xylopyranoside, respectively) would be $+374.84°$ which is unacceptable. Molecular rotation calculations with a D-rhamnose, D-xylose combination or L-rhamnose, L-xylose combination give high positive values which are unacceptable.

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

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*As the [M] value of methyl-β-L-xylopyranoside could not be traced in the literature, it was considered to have the same magnitude but with opposite sign ($+107.42°$) to that of the corresponding β-L-enantiomer.