Phytochemistry, 1971, Vol. 10, pp. 621 to 624. Pergamon Press. Printed in England.

STEROIDS AND TRITERPENOIDS FROM THE ROOTS OF *MOLLUGO SPERGULA*

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(*Received* 8 *April* 1970)

Abstract—The structure of mollugo glycoside A, one of the saponin components of the roots of *Mollugo spergula*, has been elucidated as D-xylopyranosyl 1 $\xrightarrow{\beta}$ 4 D-xylopyranosyl 1 $\xrightarrow{\beta}$ OOC (28)—spergulagenic acid (30) methyl ester. Among other components of the roots not reported earlier are α-spinasterol and β-sitosterol-D-glucopyranoside.

Mollugo spergula (Ficoidaceae) a herbaceous plant commonly found at the base of the Eastern Himalayas, is reputed in Indian medicine to have antiseptic and antidermatitic properties. It is rich in saponins.[1] According to earlier work[2–6] hydrolysis of the total glycoside mixture obtained from the entire plant yielded two genins called spergulagenin A and spergulagenic acid, and the structure of the latter was represented as (Ib).

We have now examined the roots of this plant in detail, extracting it with light petroleum, chloroform and alcohol in succession. Chromatography of the petrol extract gave a crystalline substance which was identified as α-spinasterol from its physical properties and i.r. spectrum. The chloroform extract yielded β-sitosterol-D-glucoside which was identified by its properties and those of its acetate and by direct comparison with authentic samples. The alcoholic extract containing the saponins was divided into two portions. One portion was reserved for the examination of saponins as such and the other was hydrolysed to yield the genins. The genin mixture was separated into acidic and neutral fractions. From the acidic fraction three substances were obtained after chromatographic separation. Two of them were identified as oleanolic acid (Ia) and spergulagenic acid (Ib);[3] the third substance, provisionally designated as substance X is described later. The identities of oleanolic acid and spergulagenic acid were established from a consideration of their properties and those of their important derivatives. Chromatography of the neutral fraction yielded a substance which has been identified as spergulagenin A described by Chakrabarti *et al.*[2] by a study of its properties and those of its derivatives.

Substance X, $C_{31}H_{48}O_5$ was a triterpenoid and it contained both acid and methyl ester functions. Complete esterfication with diazomethane gave a substance which was identical with spergulagenic acid dimethyl ester (Ie).[3] This diester when reduced with lithium aluminium hydride yielded a triol which was identified as queratarol (If).[7] Substance X underwent easy saponification with KOH and the product was identical with spergulagenic

[1] J. D. HOOKER, *Flora of British India*, Vol. II, p. 662, L. Reeve, London (1879).
[2] P. CHAKRABARTI and A. K. BARUA, *Indian J. Chem.* **2**, 339 (1964).
[3] P. CHAKRABARTI, D. K. MUKHERJEE, R. CHATTERJEE and A. K. BARUA, *Indian J. Chem.* **3**, 283 (1965).
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[6] P. CHAKRABARTI and A. K. BARUA, *J. Indian Chem. Soc.* **46**, 626 (1969).
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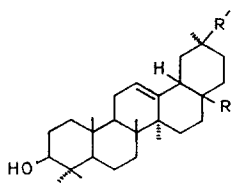
acid.[3] Substance X should therefore be either the C_{28} ester (Ic) or C_{30} ester (Id) of spergulagenic acid. Since the former can be hydrolysed only under relatively drastic conditions, substance X should be the C_{30} ester (Id). Support for this conclusion was furnished by saponification of the diester (Ie) of spergulagenic acid with KOH under mild conditions to effect partial hydrolysis of the C_{30} ester group, leaving the C_{28} ester group intact. The product was different from substance X.

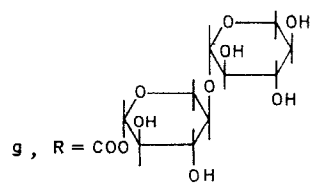
In order to make sure that substance X was not an artefact derived from spergulagenic acid by partial esterification at C_{30} during extraction procedures, the plant material was directly extracted with water (avoiding alcohol) and the extract hydrolysed with hot 7% aq. sulphuric acid. The genin mixture on examination by TLC showed the presence of substance X besides oleanolic acid, spergulagenic acid and spergulagenin A. Further, when spergulagenic acid was refluxed for 4 hr with 7% sulphuric acid in methanol–water, 8:2, it was recovered wholly unchanged.

The reserved portion of the alcohol extract was worked out for the isolation of saponins by the butanol method and the glycosidic mixture chromatographically separated. The major fraction called glycoside A was further purified by preparatory TLC for detailed study.

When hydrolysed with Kiliani mixture[8] the glycoside gave spergulagenic acid C_{30} ester (Id) as the genin and D-xylose as the only sugar. The glycoside did not react with diazomethane. Hydrolysis of the glycoside with 15% aq. KOH for 75 min at 100° yielded spergulagenic acid. These results showed that the sugar should be present in ester linkage with the C_{28} carboxyl. The yield of the genin in a quantitative hydrolysis experiment showed the presence of two sugar units in the molecule (genin found 63·9%; calc. 65·4%). The glycoside did not exhibit reducing properties and hence both the sugar units should have their anomeric hydroxyl groups involved in linkages. In a quantitative periodate oxidation experiment 3 moles of the reagent were consumed. Hydrolysis of the permethylated glycoside yielded 2,3,4-tri-*O*-methyl-D-xylose and 2,3-di-*O*-methyl-D-xylose. These results taken together with the assumption that the middle xylose unit is present in pyranose from, as in all cases where this sugar occurs in combination, point to 1→4 combination between the two sugar units.



I a, R = COOH ; R' = CH_3
b, R = R' = COOH
c, R = $COOCH_3$; R' = COOH
d, R = COOH ; R' = $COOCH_3$
e, R = R' = $COOCH_3$
f, R = R' = CH_2OH



g, R = COO

R' = $COOCH_3$

The configuration at the anomeric carbon atom of the sugar units has been deduced on the basis of Klyne's rule.[9] It is evident from the values given in Table 1 that the rotatory

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[9] W. KLYNE, *Biochem. J.* **47**, xli, 4 (1950).

TABLE 1. ROTATORY VALUES OF GLYCOSIDES

Substance	$[\alpha]_D$	M_D
Observed values		
Mollugo glycoside A	+30°	+229°
Spergulagenic acid C_{30} ester	+100°	+500°
$[M]_D$ Contribution of sugar moiety		−271°
Literature values		
β-Methyl D-xylobioside (β-methyl D-xylpyranosyl $1\xrightarrow{\beta}4$ D-xylopyranoside)[10]	−75°	−222°
β-Methyl D-xylopyranoside[11]	−66°	−108°
Calculated for two such units		−216°
α-Methyl D-xylopyranoside[11]	+154°	+253°
Calculated for two such units		+506°

contribution of the sugar moiety in mollugo glycoside A agrees with the value to be expected if both the sugar units are in β-glycosidic linkages (and not in α-glycosidic linkages). Hence the structure of mollugo glycoside A may be written as D-xylopyranosyl $1\xrightarrow{\beta}4$ D-xylopyranosyl $1\xrightarrow{\beta}$OOC (28)—spergulagenic acid (30) methyl ester (1g).

EXPERIMENTAL

M.ps were recorded on a Kofler Block and are uncorrected. For paper chromatography, Whatmann No. 1 filter paper and the following solvent systems were employed: (A): *n*-BuOH–pyridine–H_2O, 6:4:3; (B): *n*-BuOH–pyridine–H_2O–benzene, 5:3:3:1, upper layer; (C): *n*-BuOH–acetic acid–H_2O, 4:1:5, upper layer; (D): *n*-BuOH–EtOH–H_2O, 5:1:4, upper layer; (E): benzene–EtOH–H_2O, 169:57:15, upper layer.

The residue from the light petroleum extract was chromatographed over neutral alumina. The residue from the benzene–$CHCl_3$ (1:1) eluate crystallised from MeOH as needles, m.p. 169–70°, $[\alpha]_D \pm 0°$ (C, 0·81 in $CHCl_3$); Liebermann–Burchard and TNM tests were +ve, (Found: C, 83·9; H, 11·2. $C_{29}H_{48}O$ requires: C, 84·4; H, 11·6%.) It was identical with authentic α-spinasterol (mixed m.p., TLC and i.r.).

The $CHCl_3$ extract on crystallisation from acetone and then from pyridine yielded β-sitosterol-D-glucoside, colourless plates, $C_{43}H_{68}O_{10}$, m.p. 300–5° (d), $[\alpha]_D$–30° (C, 0·92 in pyridine); acetate m.p. 170–2°, $[\alpha]_D$–40° (C, 0·750 in $CHCl_3$); +ve Liebermann–Burchard, TNM and Molisch tests. Hydrolysis with 7 per cent H_2SO_4 in MeOH yielded β-sitosterol (m.p., mixed m.p., TLC and i.r.) and D-glucose (paper chromatography); tetraacetate m.p. 170–2°, identical with authentic sample (mixed m.p. and TLC).

Neutral genin (*spergulagenin A*). It crystallised from MeOH as needles, m.p. 298–300°, $[\alpha]_D$–37° (C, 1·23 in $CHCl_3$). (Found: C, 76·5; H, 10·3. $C_{30}H_{50}O_4$ requires: C, 75·9; H, 10·5%.) The mass spectrum showed prominent peaks at *m/e* 474, 456, 431, 413 and 207. Liebermann–Burchard test +ve and TNM test −ve. Acetate (Py/Ac_2O at 37°; 24 hr), m.p. 236–8°, $[\alpha]_D$ + 62° (C, 0·900 in $CHCl_3$). The NMR spectrum of the acetate showed signals (δ values) at 1·9, 2·02 (s) (9H, three OCO CH_3), 2·17 (s) (3H, $COCH_3$). On oxidation with Jones' reagent the parent substance gave a ketone, m.p. 277–9°, $[\alpha]_D$ +17° (C, 1·220 in $CHCl_3$). (Found C, 76·8; H, 9·8. $C_{30}H_{44}O_4$ requires: C, 76·9; H, 9·4%.)

Oleanolic acid (*Ia*). Colourless needles from MeOH, m.p. 303–5°, $C_{30}H_{48}O_3$, $[\alpha]_D$ + 85° (C, 1·22 in $CHCl_3$); acetate (Py/Ac_2O), m.p. 268–70°, $[\alpha]_D$ + 99° (C, 0·810 in $CHCl_3$); methylester (CH_2N_2) m.p. 200–2°, $[\alpha]_D$ + 75°) (C, 1·280 in $CHCl_3$) (identical with authentic samples according to mixed m.p., TLC and i.r.).

Spergulagenic acid (*Ib*). It precipitated from MeOH as amorphous powder, m.p. > 310°, $[\alpha]_D$ + 103° (C, 0·81 in pyridine). (Found: C, 75·0; H, 9·6. $C_{30}H_{46}O_5$ requires: C, 74·8; H, 9·4%); dimethyl ester (Ie) (CH_2N_2), m.p. 235–7°, $[\alpha]_D$ + 101° (C, 1·500 in $CHCl_3$) (Found: C, 74·2; H, 10·1. $C_{32}H_{50}O_5$ requires C, 74·7; H, 9·7%); dimethyl ester acetate (Py/Ac_2O) m.p. 285–7°, $[\alpha]_D$ + 90° (C, 0·888 in $CHCl_3$).

[10] *Dictionary of Organic Compounds* (edited by I. HEILBRON, A. H. COOK, H. M. BUNBURY and D. H. HEY), Vol. V, p. 3269, Eyre and Spottiswoode, London (1965).

[11] *Dictionary of Organic Compounds* (edited by I. HEILBRON, A. H. COOK, H. M. BUNBURY and D. H. HEY), Vol. IV, p. 3270, Eyre and Spottiswoode. London (1965).

Compound X (Id). Colourless needles from MeOH m.p. 285–7°, $[\alpha]_D$ + 100° (C, 1·25 in pyridine). (Found: C, 73·8; H, 9·6; OCH_3 6·8. $C_{31}H_{48}O_5$ requires: C, 74·4; H, 9·6; OCH_3 6·2%.) It gave positive Liebermann–Burchard and TNM tests. ν_{max}^{nujol} 3600 (hydroxyl), 1730 (ester), 1700 (acid), 1667, 890, 825 cm^{-1} (triply substituted double bond). The reaction product with CH_2N_2, crystallised from MeOH as feathery needles, m.p. 235–7°, $[\alpha]_D$ + 97° (C, 0·950 in $CHCl_3$). (Found: C, 74·1; H, 10·1. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·7%); identical with spergulagenic acid dimethyl ester (Ie) described above (mixed m.p., TLC and i r.).

Saponification of the diester (Ie) to (Ic). Saponification was carried out by refluxing with 10% KOH in MeOH for 2 hr. The product was acidified and extracted with Et_2O and worked up. The residue crystallised from methanol as needles m.p. 292–4°, $[\alpha]_D$ + 93° (C, 1·120 in pyridine). (Found: OCH_3, 6·0. $C_{31}H_{48}O_5$ requires: OCH_3 6·2%) 3750 (hydroxyl), 1733 (ester), 1712 (acid), 1667, 890, 825 cm^{-1} (triply substituted double bond). TLC behaviour and i.r. were very different from those of substance X.

$LiAlH_4$ reduction of diester (Ie) to queratarol (If). The diester was refluxed with $LiAlH_4$ in dry tetrahydrofuran for 10 hr. The excess of reagent was destroyed with moist ethyl acetate, the product worked up and crystallised from MeOH as needles, m.p. 272–3°, $[\alpha]_D$ + 91° (C, 1·230 in $CHCl_3$). It was identical with authentic queratarol.[7] (Mixed m.p., TLC and i.r.)

Saponification of compound X. Compound X was refluxed with 10% KOH in MeOH for 2 hr. The product was worked up as before and crystallised from MeOH, m.p. > 310°, $[\alpha]_D$ + 98° (C, 0·930 in pyridine). It was identical with spergulagenic acid (Ib) (TLC and i.r.).

Isolation of Mollugo glycoside A. The *n*-BuOH extract containing saponins was washed (H_2O) and evaporated under reduced pressure. The brown syrup was taken in the minimum MeOH and precipitated with Et_2O and this process was repeated till an almost colourless solid was obtained (yield 0·7%). On TLC it showed a streak with 8 spots It did not react with CH_2N_2, It was chromatographed over a column of SiO_2 gel. Most fractions consisted of mixtures (TLC). The fractions eluted by $CHCl_3$–MeOH, 84·16, contained only two compounds, a major and minor one (TLC). The first one called mollugo glycoside A was separated in required quantities by means of preparative TLC (solvent system, chloroform–methanol–water, 10:4 1; yield, 0·0025%)

Mollugo glycoside A. m.p. 220–5°, $[\alpha]_D$ + 30°. (C, 0·61 in pyridine). (Found: C, 65·0; H, 8 8. $C_{41}H_{64}O_{13}$ requires: C, 64·4; H, 8·4%.) Liebermann–Burchard and Molish tests were +ve, as also the test for saponins.

Hydrolysis. The glycoside was heated with Kiliani mixture (acetic acid–HCl–H_2O, 35:15:50) in a sealed tube at 100° for 3 hr. The product was diluted with H_2O and extracted with $CHCl_3$. The aqueous part was examined by paper chromatography in solvent systems A, B and C and it showed the presence of D-xylose only. The $CHCl_3$ extract yielded spergulagenic acid C_{30} ester (Id) (m.p., mixed m.p., TLC and i.r.).

Hydrolysis with 15% KOH. The glycoside was heated with 15% aq. KOH solution at 100° for 75 min. The reaction product isolated in usual manner crystallised from MeOH as colourless powder, m p. > 310°, $[\alpha]_D$ + 99° (C, 1·230 in pyridine). It was identical with spergulagenic acid (Ib) (analysis, TLC and i.r.).

Permethylation of glycoside A and hydrolysis (Hakomori's method).[12] A mixture of 50% NaH dispersion in oil (20 mg) and dimethyl sulphoxide (2 ml) was kept at 80° for 1 hr. A solution of mollugo glycoside A (20 mg) in dimethyl sulphoxide (2 ml) was added and the mixture was kept for 1 hr at 80°, cooled in ice and MeI (0·5 ml) added in drops and the mixture kept at room temp. for 24 hr. The product was poured into ice water, extracted with $CHCl_3$ and solvent evaporated. The syrupy residue obtained was permethylated as above three times more.

The product was refluxed with 7% H_2SO_4 in MeOH for 4 hr, and the resulting mixture processed as usual and the sugar fragments examined by paper chromatography in solvent systems D and E using authentic tetra-*O*-methyl-glucose as the reference. In the literature the R_G values of 2,3 di-*O*-methyl xylose and 2,3,4 tri-*O*-methyl xylose in system D are given as 0·74 and 0·94 respectively[13] and those of 2,3 di-*O*-methyl xylose and 3,4-di-*O*-methyl xylose in system E as 0·48 and 0·55 respectively.[14] The methylated sugar mixture under study had R_G values 0·74 and 0·93 in system D, and 0 49 and 0 99 in system E.

Acknowledgements—The authors are grateful to Professor T. R. Seshadri for his kind interest and the Indian Council of Medical Research for financial assistance.

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