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A SAPONIN FROM *ASPARAGUS GONOCLADUS*

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Key Word Index—*Asparagus gonocladus*; Liliaceae; lup-20(29)-en-28-oic-3-*O*-α-L-rhamnopyranosyl-(2 → 1)-*O*-β-D-glucopyranoside.

Abstract—The ethanolic extract of the aerial part of *Asparagus gonocladus* yielded a new saponin. It was identified as lup-20(29)-en-28-oic-3-*O*-α-L-rhamnopyranosyl-(2 → 1)-*O*-β-D-glucopyranoside.

INTRODUCTION

Asparagus gonocladus (Liliaceae) is used in the indigenous system of medicine for various ailments [1]. Previous studies have shown the presence of malvin and asparagin in the flowers of this plant [2]. In the present communication, we report the identification of a new saponin isolated from the aerial parts of *Asparagus gonocladus*.

RESULTS AND DISCUSSION

The compound, $C_{42}H_{68}O_{12}$, responded to colour reactions characteristic of a saponin [3]. It was hydrolysed with 7% sulphuric acid, whereupon the sapogenin was obtained as a precipitate and separated from the hydrolysate by filtration and purified by the usual potassium salt method.

The sapogenin was identified as 3β-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) by comparison of IR, ^{1}H NMR, and MS data of the sapogenin, its acetyl and methyl derivative with those reported in the literature [4–7] and by mmp and co-TLC with an authentic sample.

The hydrosylate on co-PPC examination revealed the presence of D-glucose and L-rhamnose (BuOH–HOAC–H_2O, 4:1:5, spray–aniline hydrogen phthalate, R_f 0.18 and 0.37 respectively). Quantitative hydrolysis of the saponin indicated that the aglycone content was *ca* 59.50% and the sugar moiety 40.5%. A quantitative estimation [8] of the sugars present in the hydrolysate revealed that both sugars were present in equimolecular proportions indicating that the saponin contained 1 mol each of aglycone, D-glucose and L-rhamnose.

In betulinic acid only the OH at C-3 and the COOH at C-17 are available for glycosidic linkage with sugar residues. From the following observations, it was concluded that both sugars were linked as a bioside unit to the C-3 hydroxyl group of the sapogenin. The saponin was not hydrolysed with 5 N NH_4OH, which is a specific reagent [9] for hydrolysis of sugar esters without attacking other glycoside linkages. Thus sugars were not present as an ester in combination with the COOH group of the sapogenin. On methylation with CH_2N_2, the saponin furnished a methyl ester, $C_{43}H_{70}O_{12}$, which on hydrolysis with 7% H_2SO_4 yielded betulinic acid methyl ester, but not betulinic acid, and thus indicating that there was a free COOH group in the genin.

On partial hydrolysis [10] the saponin yielded a prosapogenin, designated PS_1, which was purified on a column of Si gel using methanol as eluting solvent. The hydrolysate on PC examination revealed the presence of D-glucose, only indicating it to be the end sugar in the saponin.

PS_1 on hydrolysis with 7% sulphuric acid yielded betulinic acid and L-rhamnose. The genin content was 75.5% indicating that PS_1 was mono-glycoside and was formed from 1 mol each of betulinic acid and L-rhamnose. Permethylation of PS_1 followed by hydrolysis showed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose (identified by co-PPC with an authentic sample) indicating that C-1 of L-rhamnose was involved in the formation of a glycosidic linkage and also suggesting that L-rhamnose was present as a pyranoside. Thus PS_1 may be assigned the structure lup-20(29)-en-28-oic-3-*O*-α-L-rhamnopyranoside.

Permethylation of the saponin followed by hydrolysis and chromatographic examination of the hydrolysate showed the presence of 3,4-di-*O*-methyl-L-rhamnose and 2,3,4,6-tetra-*O*-methyl-D-glucose (identified by co-PPC). Since the structure of PS_1 had already been established the formation of the above methylated sugars can only be explained by assuming that C-2 of L-rhamnose in the pyranose form was involved in the formation of a glycosidic linkage with C-1 of the D-glucose, and further suggested that the D-glucose was present as pyranoside. The size of the ring and the nature of glycosidic linkages in the saponin were also supported by its periodate oxidation [11]. The saponin on enzymatic hydrolysis with emulsin yielded only D-glucose indicating that D-glucose is linked to L-rhamnose through a β-linkage. The exact configuration of the sugar linkages in the saponin was established by consideration of the molecular rotation value in the light of Klyne's rule [12]. The two possible combinations of the sugar linkages are shown in Table 1.

The observed M_D value [11] for the saponin was −114°. The M_D value of the genin is known to be +63.8°. The difference, −177.8°, is close to the first combination shown

Table 1.

β-D-Glucose + α-L-rhamnose	−66°	−110° = −176°
β-D-Glucose + β-L-rhamnose	−66°	+168° = 102°

in Table 1. Therefore, the exact configuration of the sugar linkage was L-rhamnose-α and D-glucose-β. Hence the saponin is lup-20(29)-en-28-oic-3-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-glucopyranoside.

EXPERIMENTAL

Extraction. The defatted, powdered plant material (5 kg) was exhaustively extracted with EtOH and the EtOH extract (12 l.) was concd *in vacuo*. The residue was washed successively with Et_2O, $CHCl_3$ and Me_2CO and finally dissolved in MeOH, filtrered and the filtrate was poured into excess Et_2O whereby a light brown mass pptd. The ppt. was separated by filtration and purified by repeating the above dissolution process in MeOH and precipitation with Et_2O. It was recrystallized from MeOH to yield micro-crystals (3.85 g) of saponin, mp 242–244° (dec.). Its purity was checked by PPC (BuOH–HOAc–H_2O, 4:1:5; spray 25% CCl_3COOH in Et_2O, yellow spot R_f 0.61). (Found C, 66.00; H, 8.88; $C_{42}H_{68}O_{12}$ requires: C, 65.97; H, 8.90%).

Identification of sugars and study of the sapogenin. Saponin (2.2 g) was hydrolysed by refluxing with 7% H_2SO_4 in EtOH (100 ml) for 5 hr on a steam bath. The products were poured into H_2O (400 ml) and EtOH was removed by distillation *in vacuo*. The sapogenin was separated from the hydrolysate and purified by the K-salt method. It was crystallized from $CHCl_3$ into colourless crystals, mp 315–316° $[\alpha]_D^{27}$ +14° (pyridine). The purity of sapogenin was checked by TLC (C_6H_6–Me_2CO, 1:1; spray $SbCl_3$ in $CHCl_3$, R_f 0.54). (Found: C, 78.96; H, 10.50; MW, 456 (MS). $C_{30}H_{48}O_3$ requires: C, 78.94; H, 10.52%). IR ν_{max}^{KBr} cm^{-1}: 3450, 2935, 2850, 1700, 1640, 1460, 1392, 1381, 1370, 1360, 1295, 1274, 885; MS *m/z*: 456 (M^+), 441, 438, 423, 411, 248, 220, 219, 207 and 189 (base peak). UV λ_{max}^{EtOH} nm: 250. Acetate $C_{30}H_{48}O_2OCOMe$, mp 290°; methyl ester $C_{31}H_{50}O_3$, mp 221–2°; IR ν_{max}^{KBr} cm^{-1}: 3540, 1718, 1641, 1460, 1362, 880; ^1H NMR (in $CDCl_3$): δ 0.75 (3 H), 0.77 (3 H), 0.90 (3 H), 1.00 (3 H), 1.15 (3 H), 1.75 (3 H), 3.62 (3 H), 4.70 (2 H); MS *m/z* 470 (M^+), 455, 452, 437, 411, 262, 233, 220, 207 and 189 (base peak). The hydrolysate was neutralized with $BaCO_3$. The neutral hydrolysate revealed the presence of D-glucose (R_f 0.18) and L-rhamnose (R_f 0.37) by co-PPC with their authentic samples (BuOH–HOAc–H_2O, 4:1:5; spray aniline hydrogen phthalate).

Quantitative estimation of sugars in the saponin hydrolysate. The ratio of sugars in the saponin was determined colorimetically [8] in a Klett–Summerson photoelectric colorimeter using a blue filter (420 nm), with the help of standared curves of authentic sugars. Ten solutions (5, 10, 15, 20, 25 ... 50 μg) in 0.02 ml H_2O of both D-glucose and L-rhamnose were applied on Whatman No. 1 filter paper (50 × 55 cm spot distances 6 cm). The chromatograms were developed by the descending technique with BuOH–HOAc–H_2O (4:1:5) for 24 hr, dried in air, sprayed with aniline hydrogen phthalate and dried at 110° for 15 min. The coloured spots were cut out in equal rectangles eluted by 50% HOAc (10 ml each) and the colour intensity of each eluate was measured. The sugar in the hydrolysate of the saponin was assayed as described above.

Partial hydrolysis of saponin and isolation of PS_1. Saponin (500 mg) was treated with 0.02 N H_2SO_4 and the reaction mixture was kept at room temp. for 8 days. It was then extracted with BuOH. The BuOH extract, after concn was chromatographed over Si gel. Elution with MeOH yielded prosapogenin PS_1, mp 262–264°. (Found: C, 71.71, H, 9.65. $C_{36}H_{58}O_7$ requires: C, 71.76; H, 9.63%).

Permethylation of saponin and PS_1 and hydrolysis of permethylated derivatives. The glycosides (75 mg each) were treated with MeI (2 ml) and Ag_2O in DMF (4 ml) separately for 40 hr at room temp. The mixture were filtered and the residue washed with a little DMF. The filtrate was evapd to dryness and the residue taken up in EtOH (25 ml). The syrup obtained after removal of EtOH was hydrolysed with Killiani's mixture (HOAc–HCl–H_2O, 7:3:10) [13] and the product worked up in the usual way. The products were analysed by PPC (BuOH–HOAc–H_2O, 5:1:4) [14]. The hydrolysate from permethylated PS_1 contained 2,3,4-tri-*O*-methyl-L-rhamnose (R_g 1.01) and permethylated saponin contained 3,4-di-*O*-methyl-L-rhamnose (R_g 0.84) and 2,3,4,6-tetra-*O*-methyl-D-glucose (R_g 1.0).

Periodate oxidation of saponin. Periodate oxidation was carried out by the method of ref. [11]. The glycoside (50 mg) dissolved in 25 ml EtOH and 25 ml 0.15 M sodium metaperiodate soln was added. The oxidation was conducted at room temp. for 60 hr. Aliquots (5 ml) were drawn in duplicate from the reaction mixture at different intervals of time and analysed for periodate and formic acid.

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