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A TRITERPENOIDAL SAPONIN FROM ROOTS OF *ACANTHUS ILLICIFOLIUS*

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Key Word Index—*Acanthus illicifolius*; Acanthaceae; saponin; [α-L-arabinofuranosyl-(1 → 4)-β-D-glucuronopyranosyl(1 → 3)]-3β-hydroxy-lup-20(29)-ene.

Abstract—A new triterpenoidal saponin has been isolated from the ethanolic extract of *Acanthus illicifolius*. It was shown to be [α-L-arabinofuranosyl-(1 → 4)-β-D-glucuronopyranosyl(1 → 3)]-3β-hydroxy-lup-20(29)-ene.

INTRODUCTION

Acanthus illicifolius Linn (N.O. Acanthaceae) is an evergreen spiny herb found in the mangroves of the Indian peninsula, Bengal and Burma. The plant is useful in paralysis and asthma [1]. Since no work seems to have been done previously on the chemical analysis of *A. illicifolius* we have examined the roots of this plant in order to provide a detailed study of its constituents.

RESULTS AND DISCUSSION

Saponin, $C_{41}H_{66}O_{11}$, mp 262–264° (d) gave the colour reactions of terpenoids and produced a pink colour with acetic anhydride and concentrated H_2SO_4. It was hydrolysed with 7% H_2SO_4 whereupon the aglycone precipitated. The aglycone was identified as lup-20(29)en-3β-ol (lupeol) by comparison of IR, ^{1}H NMR and mass spectral data of the aglycone and acetyl derivative [2–4] and by mmp and co-TLC with an authentic sample.

Characterization of the sugar moiety

The hydrolysate on paper chromatography examination revealed the presence of D-glucuronic acid and L-arabinose (*n*-BuOH–HOAc–H_2O, 4:1:5; spray, aniline hydrogen phthalate, R_f 0.12 and 0.21, respectively). Quantitative hydrolysis of the saponin indicated the alycone content to be *ca* 58% and the sugar moiety *ca* 42%. A quantitative estimation [5] of the sugars present in the hydrolysate revealed that the two sugars were present in equimolecular proportions indicating that the saponin contained 1 molecule each of aglycone D-glucuronic acid and L-arabinose.

Sequence of sugars in the saponin

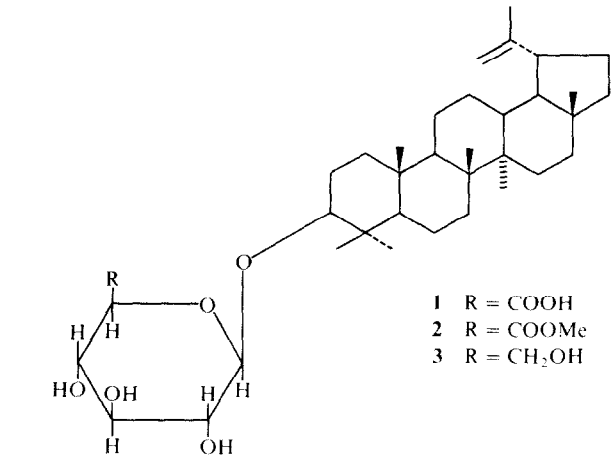
The saponin on partial hydrolysis (see Experimental) [6] yielded a prosapogenin, designated as PS_1, which was purified by chromatography on Si gel using methanol as solvent. The hydrolysate on paper chromatography revealed the presence of only L-arabinose indicating it to be the terminal sugar in the saponin.

Study of prosapogenin PS_1

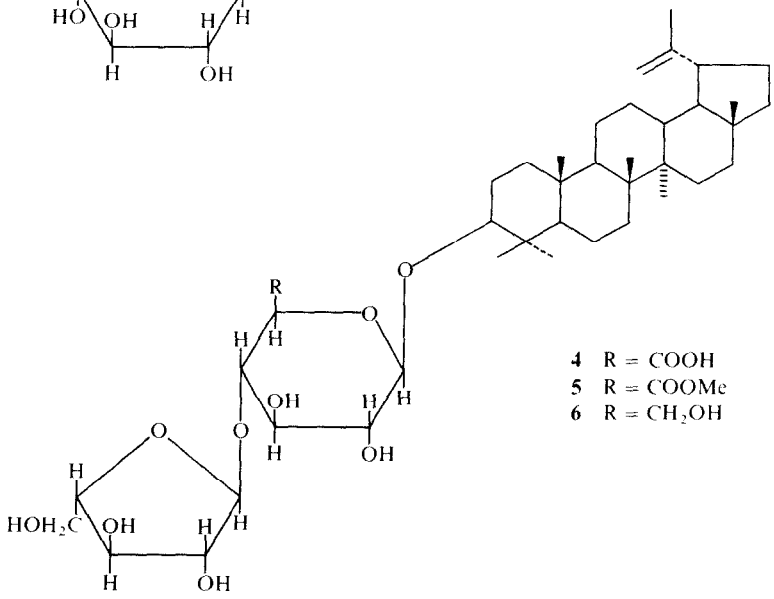
On hydrolysis with 7% H_2SO_4 PS_1 yielded lupeol and D-glucuronic acid. The genin content was found to be 68.5% indicating that PS_1 was a monoglucoside and was formed from 1 mole each of lupeol and D-glucuronic acid. PS_1 on treatment with CH_2N_2 formed a methyl ester (**2**) which on reduction with $LiAlH_4$ gave a glycoside (**3**). Hydrolysis of **3** with 7% H_2SO_4 produced lupeol and D-glucose and permethylation [7] followed by hydrolysis furnished 2,3,4,6-tetra-*O*-methyl glucose indicating that C-1 of glucuronic acid was glycosidically linked to prosapogenin PS_1 (**1**).

Nature and position of the glycosidic linkages in saponin

Saponin methyl ester (**5**) on $LiAlH_4$ reduction yielded another glycoside (**6**) which on hydrolysis yielded lupeol, D-glucose and L-arabinose. The genin content was *ca* 59% and the ratio of sugars in **6** was found to be 1:1 by colorimetric estimation [5], suggesting that **6**, and hence saponin **4**, was a bioside of lupeol. Permethylation of **6** followed by hydrolysis and chromatographic examination of the hydrolysate showed the presence of 2,3,6-tri-*O*-methyl-D-glucose and 2,3,5-tri-*O*-methyl-L-arabinose. Release of 2,3,5-tri-*O*-methyl-L-arabinose clearly suggested that the arabinose unit in the saponin was present as a furanoside leaving only its anomeric OH for glycoside linkage with the D-glucuronic acid unit. Since the structure for PS_1 had already been established, the formation of 2,3,6-tri-*O*-methyl-D-glucose (from **6**) can only be explained by assuming that C-4 of the glucuronic acid moiety in the pyranose form was involved in the formation of a glycosidic linkage with C-1 of L-arabinose. The size of the rings and the nature of the glycosidic linkages in the saponin were also supported by periodate oxidation [8] of **6** and **3**. The saponin on enzymatic hydrolysis with diastase yielded only L-arabinose indicating that L-arabinose was linked to glucuronic acid through an α-linkage and glucuronic acid was linked to the aglycone through a β-linkage. The above assignments of the glycoside linkages in the saponin were also supported by the calculated molecular rotation (M_D) value [9] for structure **6** which was in good agreement with the experimental value (Table 1). Therefore, the exact configurations of the sugar linkages in the saponin were D-glucuronic acid-β and L-arabinose-α. Hence the



1 R = COOH
2 R = COOMe
3 R = CH_2OH



4 R = COOH
5 R = COOMe
6 R = CH_2OH

Table 1

Substance	M_D
β-Methyl-D-glucoside	−66
α-Methyl-arabinoside	−205
Lupeol	+140.58
Observed value for structure **6**	−132
Calculated value for **6** (+140.58 − 66 − 205)	−130.42

saponin was [α-L-arabinofuranosyl-(1 → 4)-β-D-glucuronopyranosyl(1 → 3)]-3β-hydroxy-lup-20(29)-ene (**4**).

EXPERIMENTAL

Extraction. The defatted powdered plant material (5 kg) was exhaustively extracted with EtOH. The EtOH extract (10 l.) was concd (3 l.) under red. pres. and kept in a refrigerator for 3 days when a white ppt. was obtained. It was separated by filtration and identified as pentacosane-5-one [10]. The filtrate was further concd and the residual amount of EtOH was removed *in vacuo*. The residue obtained was successively washed with Et_2O, $CHCl_3$ and Me_2CO, and was finally dissolved in MeOH, filtered, and the filtrate poured into excess Et_2O whereby a light brown mass pptd. The ppt. was separated by filtration and purified by repeating the above process of dissolving in MeOH and pptn with Et_2O. It was recrystallized from MeOH to yield microcrystals (4.15 g) of saponin, mp 262–264° (d). Its purity was checked by PPC (*n*-BuOH–HOAc–H_2O, 4:1:5, spray 25% CCl_3.COOH in Et_2O, yellow spot, R_f 0.33). (Found: C, 67.11; H, 9.04. $C_{41}H_{66}O_{11}$ requires: C, 67.03; H, 8.99%).

Isolation of the sapogenin. Saponin (**4**) (500 mg) was hydrolysed by refluxing with 7% H_2SO_4 for 5 hr on a steam bath. The aglycone part was separated from the aq. hydrolysate and crystallized from $CHCl_3$ to give colourless crystals (285 mg), mp 215–217°, $[\alpha]_D^{30}$ + 33°. The purity of the sapogenin was established by TLC (C_6H_6–$CHCl_3$, 1:2; spray 30% $SbCl_3$ in $CHCl_3$, R_f 0.69). (Found: C, 84.58; H, 11.70. MW 426 (MS). $C_{30}H_{50}O$ requires: C, 84.50; H, 11.73%). IR ν_{max}^{KBr} cm^{-1}: 3325, 2941, 1639, 1449, 1380, 1300, 1190, 1110, 1042, 1015, 948 and 885. MS *m/e*: 426 (M^+), 411, 393, 383, 370, 315, 220, 218, 207, 205, 191, 189, 187. ^{1}H NMR (in $CDCl_3$): δ 0.76, 0.80, 0.86, (*t*, 9H), 0.95, 1.04 (*d*, 6H), 1.28, 1.40, 1.47 (*t*, 2H), 1.70 (*s*, 3H), 3.06, 3.15, 3.27, 3.35 (*q*, 1H), 4.58, 4.70 (*d*, 2H). Acetate: $C_{30}H_{49}OCOMe$, mp 213–214°.

Quantitative estimation of sugars in the saponin hydrolysate. The sugar ratio in the saponin (**4**) was determined colorimetrically (**5**) in a Klett–Summerson photoelectric colorimeter using a blue filter (420 nm) with the help of standard curves of authentic

sugars. Ten solns (5, 10, 15.... 50 μg in 0.03 ml H_2O) of both sugars, D-glucuronic acid and L-arabinose, were applied on Whatman No. 1 filter papers (50 × 55 cm, spot distance 5 cm). The chromatograms were developed by the descending technique with *n*-BuOH–HOAc–H_2O (4:1:1) for 20 hr, dried in air, sprayed with aniline hydrogen phthalate on both sides and dried at 100 for 20 min. The coloured spots were cut out in equal rectangles, eluted by immersion in 50% HOAc (5 ml each), and the colour intensity of each eluate measured.

Estimation of sugars in saponin hydrolysate. The saponin (**4**) (70 mg) was hydrolysed by refluxing with 7% H_2SO_4 for 5 hr on a steam bath. The reaction mixture was extracted with $CHCl_3$ to give the sapogenin (40 mg). The hydrolysate was neutralized with $BaCO_3$, filtered and concd. to a syrup (1 ml). A very small portion of the syrup was dissolved in 1 ml H_2O and aliquots applied on Whatman No. 1 filter paper. The chromatograms were developed, sprayed , dried and the coloured spots were cut out in equal rectangles, eluted and assayed as described above.

Partial hydrolysis of saponin (**4**) *and isolation of PS*$_1$ (**1**). Saponin (1.5 g) and 1% H_2SO_4 in MeOH (50 ml) were kept for 5 days at 35°. MeOH was removed and H_2O (20 ml) was added. the aq. soln was extracted with *n*-BuOH. The *n*-BuOH extract of the acid hydrolysate on concn to a syrup and CC over Si gel, solvent MeOH, yielded prosapogenin PS_1 (**1**), mp 242–244°. (Found: C, 71.80; H, 9.60. $C_{36}H_{58}O_7$ requires: C, 71.76; H, 9.63%).

Methyl ester of saponin **4** *and prosapogenin* **1**. Glycosides **1** and **4** (500 mg each) in MeOH were treated separately with an Et_2O soln of CH_2N_2 until a permanent colour was obtained. The reaction mixtures were kept overnight at 0 . The methyl ester of saponin **5** was crystallized from MeOH to give light brown crystals, mp 271–273°. (Found: C, 67.44; H, 9.07. $C_{42}H_{68}O_{11}$ requires: C, 67.34; H, 9.09%). The methyl ester of PS_1 (**2**) was crystallized from $CHCl_3$–MeOH (1:2) to give colourless plates, mp 256–258°. (Found: C, 72.00; H, 9.70. $C_{37}H_{60}O_7$ requires: C, 72.08; H, 9.74%).

Reduction of methyl ester of PS$_1$ (**2**) and saponin methyl ester (**5**). 100 mg each of **2** and **5** were reduced under reflux with $LiAlH_4$ in Et_2O–THF for 10 hr. The excess $LiAlH_4$ was decomposed with moist EtOAc. The products obtained were acidified with HCl and extracted with *n*-BuOH. The solvent was removed and the residues were separately crystallized from MeOH.

Permethylation of the reduced methyl ester of saponin (**6**) *and reduced methyl ester of PS*$_1$ (**3**) *and the hydrolysis of permethyl derivatives.* The glycosides (70 mg each) were treated with MeI (2 ml) and Ag_2O (1 g) in DMF (4 ml) separately for 48 hr at room temp. The contents were filtered and the residue washed with a small vol. of DMF. The filtrate was evapd to dryness and the residue taken up in EtOH (25 ml). The syrups obtained after removal of EtOH were hydrolysed with Kiliani's mixture (HOAc–HCl–H_2O, 7:3:10) [11] and the product worked up in the usual way. The products were analysed by PPC (*n*-BuOH–EtOH–H_2O, 5:1:4) [12]. The hydrolysate from **3** contained 2,3,4,6-tetra-*O*-methyl-D-glucose (R_G 1.00) and the hydrolysate from **6** contained 2,3,6-tri-*O*-methyl-D-glucose (R_G 0.83) and 2,3,5-tri-*O*-methyl-L-arabinose (R_G 0.95).

Periodate oxidation of **6** *and* **3**. Periodate oxidation was carried out by the method of ref. [8]. Compounds **6** and **3** (40 mg of each) were dissolved in 20 ml EtOH and 20 ml 0.25 M sodium metaperiodate soln added. The oxidation was allowed to take place at room temp. for 60 hr. Aliquots (6 ml) were withdrawn in duplicate from the reaction mixture at different time intervals and analysed for periodate and formic acid.

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