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A NEW TRITERPENE GLYCOSIDE FROM THE STEM OF *ICHNOCARPUS FRUTESCENS*

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Key Word Index—*Ichnocarpus frutescens*; Apocynaceae; α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-amyrin.

INTRODUCTION

Ichnocarpus frutescens (Apocynaceae) is widely distributed throughout India, Ceylon, China and Australia. The plant is used in the treatment of vomiting, fever and blood diseases [1]. Since no work seems to have been done on the chemical analysis of *Ichnocarpus frutescens*, in spite of its medicinal properties, we have examined the stem of this plant. The present paper deals with the structure of a new triterpene glycoside.

RESULTS AND DISCUSSION

The glycosidic compound, $C_{42}H_{70}O_{10}$, mp 178–180° responded to colour reactions of terpenoids [2–4] and gave a pink colour with acetic anhydride and concentrated sulphuric acid. It was hydrolysed with 7% ethanolic H_2SO_4 whereupon the aglycone precipitated. The structure of the glycoside was established by elucidating separately the structures of the aglycone and the sugar moiety and finally establishing the nature and positions of the glycosidic linkages.

Structure of aglycone

The aglycone was characterized as α-amyrin by comparison of IR ^{1}H NMR and MS data of the aglycone and its acetyl derivative [5–7] and by mmp and co-TLC with an authentic sample.

Characterization of the sugar moiety

The presence of D-glucose and L-rhamnose in the hydrolysate was established by co-PPC with authentic sugar samples (*n*-BuOH–AcOH–H_2O, 4:1:5; spray–aniline hydrogen phthalate, R_f 0.18 and 0.37, respectively). Quantitative hydrolysis of the glycoside indicated the aglycone content was *ca* 58% and the sugar moiety 42%. A quantitative estimation [8] of the sugar present in the hydrolysate revealed that the two sugars were present in equimolecular proportions. Therefore, it was concluded that the glycoside contained 1 mol each of triterpene, D-glucose and L-rhamnose.

Sequence of sugars in the glycoside

Partial hydrolysis of the glycoside [9] with 0.02 N H_2SO_4 at room temperature, followed by paper chromatographic examination of the hydrolysate, revealed the presence of only L-rhamnose. This showed L-rhamnose to be the end-sugar in the glycoside.

Permethylation [10] of the glycoside followed by hydrolysis and examination of the sugars in the hydrolysate revealed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose (identified by co-PPC). The release of 2,3,4-tri-*O*-methyl-L-rhamnose indicated the presence of L-rhamnose in the pyranose form and attached at the terminal position with its C-1 position involved in the glycosidic linkage. The formation of 2,3,6-tri-*O*-methyl glucose clearly suggested the presence of glucose in the pyranose form with its C-1 and C-4 positions involved in the glycosidic linkages.

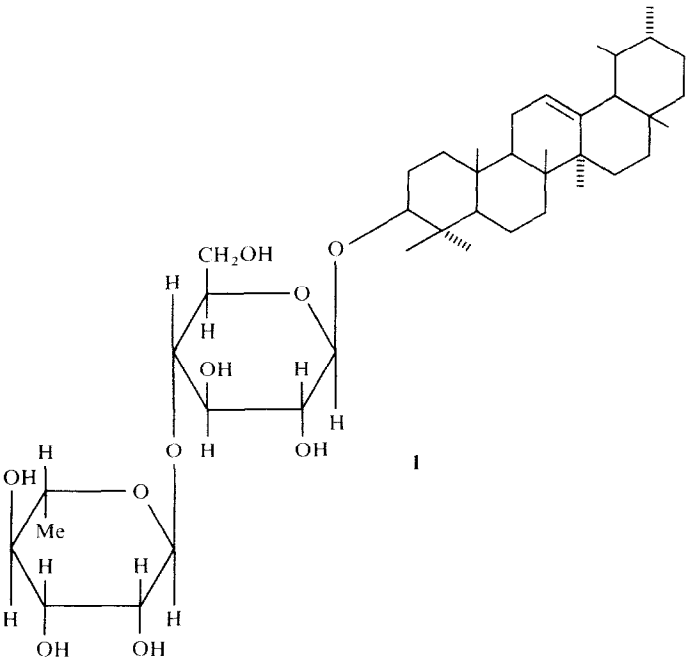
The product obtained after partial hydrolysis of the glycoside on permethylation followed by hydrolysis revealed the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose. This suggested that C-1 of glucose was attached to the hydroxyl group of α-amyrin and C-4 to L-rhamnose.

Hydrolysis of the glycoside with the enzyme diastase produced only L-rhamnose as the free sugar indicating that only L-rhamnose was involved in an α-glycosidic linkage. The exact configuration of the sugar linkages in the glycoside was established by consideration of the molecular-rotation values in the light of Klyne's rule [11] and the two possible combinations of the sugar linkages are shown in Table 1.

Table 1

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

The observed M_D value [11] for the glycoside was +178°. The M_D value of the aglycone is known to be +356°. The difference −178° is close to the first combination of Table 1. Therefore the configuration of the sugar linkages was D-glucose-β and L-rhamnose-α. Hence the glycoside was α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-amyrin (**1**). Periodate oxidation of the glycoside further confirmed the above structure.



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EXPERIMENTAL

Extraction. The defatted powdered plant material (5 kg) was exhaustively extracted with EtOH. The EtOH extract (8 l.) was concd (3.5 l.) and kept overnight in a refrigerator whereby a white ppt. was obtained. The ppt. was separated by filtration and identified as 6,8,8-trimethylpentacosan-7-one [12]. The filtrate was further concd and the residual amount of EtOH was removed *in vacuo*. The residue was successively washed with Et_2O, C_6H_6, $CHCl_3$ and Me_2CO and was finally dissolved in MeOH, filtered and the filtrate poured into excess Et_2O whereby a light brown mass was pptd. The ppt. was separated by filtration and purified by repeating the above dissolution in MeOH and pptn with Et_2O. It was recrystallized from MeOH to yield microcrystals of the glycosidic compound (3.8 g), mp 178–180°. The purity was checked by PPC (BuOH–HOAc–H_2O, 4:1:5, spray 25% CCl_3COOH in Et_2O: yellow spot, R_f 0.44). (Found: C, 68.72; H, 9.51; $C_{42}H_{70}O_{10}$ requires: C, 68.66; H, 9.53%.)

Hydrolysis of glycoside and study of aglycone. Glycoside (1.8 g) was hydrolysed with 200 ml 7% ethanolic H_2SO_4 as usual. The aglycone, obtained as a white ppt., was separated out from the hydrolysate by filtration and washed well to remove acid. It was crystallized from EtOH into colourless crystals, mp 186° $[\alpha]_D^{17}$ 83.5°. (Found: C, 84.67; H, 11.69; MW 426 (MS); $C_{30}H_{50}O$ requires: C, 84.51; H, 11.73%.) IR ν_{max}^{KBr} cm^{-1}: 3630, 2900, 2840, 1654, 1465, 1435, 1390, 1366, 1347, 1325, 1264, 988, 828; ^1H NMR (in $CDCl_3$): δ 5.18 (1 H); 3.28 (1 H), 1.16 (3 H), 1.00 (6 H), 0.93 (6 H), 0.84 (3 H), 0.80 (3 H), 0.75 (3 H); MS *m/e*: 426 (M^+), 411, 408, 257, 218, 207, 203 and 189 (base peak).

Colorimetric estimation of sugars in the hydrolysate of the glycoside. The ratio of sugars in the glycoside was determined colorimetrically [13] 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 D-glucose and L-rhamnose were applied on Whatman No. 1 filter paper (50 × 55 cm, spot distance 4 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 on both sides, and dried at 110° for 15 min. The coloured spots were cut out in equal rectangles, eluted by immersion in 50% HOAc (10 ml each) and the colour intensity of each eluate measured.

Estimation of sugars in the glycoside. The glycoside (60 mg) was hydrolysed by refluxing with 7% H_2SO_4 (30 ml) for 5 hr on a steam bath. The reaction mixture was extracted with $CHCl_3$ to yield the aglycone (33 mg). The hydrolysate was neutralized with $BaCO_3$, filtered and concd to a syrup (2 ml); 0.2 ml of this syrup was dissolved in 2 ml of 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 separately and assayed as described previously.

Partial hydrolysis of the glycoside. The glycoside (450 mg) was treated with 0.02 N H_2SO_4 and the reaction mixture was kept at room temp. for 10 days. It was then extracted with BuOH which on concn yielded a partially hydrolysed substance (320 mg). The hydrolysate contained L-rhamnose only (PPC with an authentic sample).

Permethylation of the glycoside and partially hydrolysed substance and hydrolysis of their permethylated derivatives. The glycosides (60 mg each) were treated with MeI (2 ml) and Ag_2O in DMF (4 ml) separately for 48 hr at room temp. The contents were filtered and the residue washed with a little DMF. The filtrate was evapd to dryness and the residue taken in EtOH (25 ml). The syrup obtained after removal of EtOH was hydrolysed with Kiliani mixture (HOAc–HCl–H_2O, 35:15:50) [14] and the product worked up in the usual way. The hydrolysate from permethylated glycoside contained 2,3,6-tri-*O*-methyl-D-glucose (**2**) and 2,3,4-tri-*O*-methyl-L-rhamnose (**3**) and permethylated derivative of partially hydrolysed substance contained 2,3,4,6-tetra-*O*-methyl-β-D-glucose (**4**) only (PPC, BuOH–EtOH–H_2O, 5:1:4, R_G (**2**) 0.83; (**3**) 1.01; (**4**) 1 [15–17]).

Periodate oxidation of the glycoside. Periodate oxidation of the glycoside was carried out by the method of ref. [18]. Glycoside (50 mg) was dissolved in 25 ml EtOH and 25 ml 0.15 M sodium metaperiodate soln was added. The oxidation was allowed to take place at room temp. for 60 hr. Aliquots (5 ml) were withdrawn in duplicate from the reaction mixture at different intervals of time and analysed for periodate and formic acid.

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