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TWO NEW STERYL GLYCOSIDES FROM *LINDENBERGIA INDICA*

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Key Word Index—*Lindenbergia indica*; Scrophulariaceae; α-L-rhamnopyranosyl (1 → 4)β-D-glucopyranosyl (1 → 3)sitosterol; α-L-rhamnopyranosyl(1 → 5)α-L-arabinofuranosyl(1 → 3)sitosterol; structural determination.

Abstract—Two new saponins, α-L-rhamnopyranosyl (1 → 4) β-D-glucopyranosyl (1 → 3)sitosterol and α-L-rhamnopyranosyl (1 → 5) α-L-arabinofuranosyl (1 → 3)sitosterol, have been reported from *Lindenbergia indica*.

INTRODUCTION

Lindenbergia indica (Scrophulariaceae) is an erect annual herb, 10–30 cm in height, found throughout India. The plant possesses a faint aromatic odour and is slightly bitter. The juice of the plant is given in chronic bronchitis and mixed with that of coriander applied to skin eruptions [1, 2].

RESULTS AND DISCUSSION

Steryl glycoside I

Steryl glycoside I, $C_{41}H_{70}O_{10}$, gave all the tests of saponins [3, 4] and on hydrolysis with 7% H_2SO_4 yielded sitosterol (superimposable IR, mmp and co-TLC), D-glucose and L-rhamnose (co-PPC). The sugars were found to be present in equimolar proportion (1:1), and the genin content was found to be 57.05% (quantitative hydrolysis) against 57.3% calculated for one unit of sitosterol and 2 units of sugars per molecule of steryl glycoside I.

From the structure of sitosterol, it is evident that only the OH at C-3 is available for glycosidic linkage with the sugar residues. The steryl glycoside I, on partial hydrolysis [5], yielded L-rhamnose (co-PPC), indicating it to be the end sugar.

To determine the exact sugar linkages, the glycoside was permethylated [6] and hydrolysed whereupon two methylated sugars, 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose were obtained. These re-results led to the conclusion that C-1 of the D-glucose moiety was linked to C-3 of sitosterol and C-1 of the L-rhamnose was linked to C-4 of D-glucose.

Hydrolysis of the steryl glycoside I with diastase liberated L-rhamnose indicating that L-rhamnose was involved in an α-glycosidic linkage and D-glucose was involved in a β-glycosidic linkage.

The exact configuration of the sugar linkages was established by consideration of the molecular rotation values on the basis of Klyne's rule [7–9]. The possible combinations of both sugar linkages are shown in Table 1.

Table 1. Possible combinations of the sugar linkages in steryl glycoside I

Combination of methyl glycosides	$[M]_D$ values
β-D-Glucose + α-L-rhamnose	−66 − 111 = −177°
β-D-Glucose + β-L-rhamnose	−66 + 168 = +102°

The observed $[M]_D$ value for the glycoside was −321.3° and the $[M]_D$ value of the sapogenin was known to be −148.6°. The difference 172.7° is close to the first combination of sugar linkages. Hence the nature of the linkage was established as rhamnose-α- and glucose-β-.

The foregoing results are in good agreement with the structure of steryl glycoside I as α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 3)sitosterol.

Steryl glycoside II

Steryl glycoside II, $C_{40}H_{68}O_9$, gave an acidic hydrolysis sitosterol, L-rhamnose and L-arabinose. The sugars were found to be present in equimolar proportions (1:1) and the genin content was found to be 59.6% (quantitative hydrolysis) against 59.8% calculated for one unit each of sitosterol, L-arabinose and L-rhamnose.

The sequence of the sugar moieties in the glycoside was determined by partial hydrolysis which yielded L-rhamnose, indicating it to be the end sugar.

The exact sugar linkages were established by permethylation of the glycoside and hydrolysis whereupon two methylated sugars, 2,3-di-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl rhamnose, were obtained. These results led to the conclusion that C-1 of L-arabinose was linked to C-3 of sitosterol and C-1 of L-rhamnose was linked to C-5 of L-arabinose.

The steryl glycoside on hydrolysis with diastase yielded sitosterol, L-arabinose and L-rhamnose indicating that both the sugars were involved in the formation of α-glycosidic linkages. The exact configuration of the sugar linkages was established by a consideration of the molecular rotation values on the basis of Klyne's rule [7–9]. Four possible combinations of both sugar linkages are shown in Table 2.

Table 2. Possible combinations of the sugar linkages in steryl glycoside II

Combination of methyl glycosides	$[M]_D$ values
α-L-Rhamnose + α-L-arabinose	−111 − 205 = −316°
α-L-Rhamnose + β-L-arabinose	−111 − 77 = −188°
β-L-Rhamnose + α-L-arabinose	+168 − 205 = −37°
β-L-Rhamnose + β-L-arabinose	+168 − 77 = +91°

The observed $[M]_D$ value for the glycoside was −460° and the $[M]_D$ value of the sapogenin was known to be −148.6°. The difference 311.4° is close to the first

combination of sugar linkages. Hence the nature of the linkages was established as rhamnose-α- and arabinose-α.

The foregoing results are in good agreement with the structure of steryl glycoside II as α-L-rhamnopyranosyl (1 → 5) α-L-arabinofuranosyl (1 → 3)sitosterol.

EXPERIMENTAL

Extraction and isolation. The defatted powdered plant (5 kg) was exhaustively extracted with EtOH. The EtOH extract (3.5 l.) was concd to a viscous mass *in vacuo*. The residue was extracted with C_6H_6, $CHCl_3$ and Me_2CO successively which yielded oleanolic acid from the C_6H_6 and $CHCl_3$ extracts and 7-hydroxyflavone and quercetin from the Me_2CO extract.

The residue left after extracting with Me_2CO was dissolved in MeOH, filtered and the filtrate was poured into excess Et_2O whereby a brown mass was pptd. The ppt. was again dissolved in a little MeOH and was absorbed over a column of Si gel and eluted with a mixture of Me_2CO and MeOH (1:1) and MeOH. The first fraction gave steryl glycoside I (3.10 g), mp 98° (MeOH). (Found: C, 68.29; H, 9.60. $C_{41}H_{70}O_{10}$ requires: C, 68.14. H, 9.69%). The MeOH fraction gave steryl glycoside II (3.95 g), mp 112° (MeOH). (Found: C, 69.43; H, 9.91. $C_{40}H_{68}O_9$ requires: C, 69.36; H, 9.82%). These compounds were found to be steryl glycosides by the usual tests [3, 4].

Identification of sugars in the hydrolysate and isolation of the genin. The steryl glycosides I and II (600 mg each) were hydrolysed separately by refluxing with 7% H_2SO_4 in EtOH (100 ml) for 5 hr. The products were poured into H_2O (500 ml) and the EtOH was removed by distillation *in vacuo*. The genins were separated from the aq. hydrolysates and crystallized from MeOH into white needles (345 and 35.7 mg, respectively), both melting at 135–137°. Both the genins were found to be the same compound by TLC ($CHCl_3$–C_6H_6, 1:3, spray—30% $SbCl_3$ in $CHCl_3$, R_f 0.42). (Found: C, 83.7; H, 12.4. $C_{29}H_{50}O$ requires: C, 83.05; H, 12.17%). The genin was identified as sitosterol by mmp, co-TLC and superimposable IR. Both hydrolysates were neutralized with $BaCO_3$. The neutral aq. hydrolysate from steryl glycoside I revealed the presence of D-glucose (1) and L-rhamnose (2) and the hydrolysate from steryl glycoside II revealed the presence of L-rhamnose (2) and L-arabinose (3) by PPC (BuOH–HOAc–H_2O, 4:1:5, aniline hydrogen phthalate; R_f 1, 0.18; 2, 0.36, 3, 0.20).

Permethylation of steryl glycosides I and II. The saponins (200 mg each) were treated separately with 0.02 N ethanolic H_2SO_4 (200 ml) and the reaction mixtures were kept at room temp. for 10 days. The mixtures were then poured into excess water and hydrolysates of each saponin revealed the presence of only L-rhamnose (*n*-BAW, 4:1:5, R_f 0.35).

Permethylation of steryl glycosides I and II. The saponins (60 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 mixtures were filtered and the residue washed with a little DMF. The filtrates were evapd to dryness and the residues were taken up in EtOH (30 ml). The syrups obtained after removal of EtOH were hydrolysed with Kiliani's mixture (HOAc–HCl–H_2O, 35:15:50) [10] and the products were worked up in the usual way. The hydrolysate from the permethylated derivative of steryl glycoside I revealed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose (4) and 2,3,6-tri-*O*-methyl-D-glucose (5) and the hydrolysate of the permethylated derivative of steryl glycoside II revealed the presence of 2,3,4-tri-*O*-methyl-L-rhamnose (4) and 2,3-dimethyl arabinose (6) (PPC, BuOH–EtOH–H_2O, 5:1:4; R_g 4, 1.01; 5, 0.83; 6, 0.64 [11].

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