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TWO ISOFLAVONE GALACTOSIDES FROM *DALBERGIA SPINOSA*

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Key Word Index—*Dalbergia spinosa*; Leguminosae; leaves; prunetin 4′-*O*-β-D-galactoside; 7-methyltectorigenin 4′-*O*-β-D-galactoside; stem-bark; known isoflavones.

Abstract—The chemical examination of the leaves and stem-bark of *Dalbergia spinosa* has yielded, in addition to a number of known isoflavones, two new isoflavone galactosides, prunetin 4′-*O*-β-D-galactoside and 7-methyltectorigenin 4′-*O*-β-D-galactoside.

INTRODUCTION

Dalbergia spinosa is a stiff erect shrub with numerous short, round, horizontal branchlets ending in pungent spine, leaves crowded from the nodes of the branchlets and small whitish flowers [1]. The roots of *D. spinosa* when powdered and taken in water destroys the effect of alcohol [2]. The occurrence of isoflavonoids from the roots of *D. spinosa* has been reported earlier [3, 4]. In this study we have examined isoflavonoids of the hitherto unexamined leaves and stem-bark of *D. spinosa*.

RESULTS AND DISCUSSION

The benzene extract of the air-dried leaves of *D. spinosa* was column chromatographed over silica gel and yielded prunetin (5,4′-dihdydroxy-7-methoxyisoflavone) and 7-methyltectorigenin (5,4′-dihydroxy-6,7-dimethoxyisoflavone) identified by their mp, UV, ^{1}H NMR and comparison with authentic samples. The benzene extract of the stem-bark on column chromatography over silica gel gave caviunin (5,7-dihydroxy, 6-2′,4′,5′-tetramethoxyisoflavone), dalspinin (5,7-dihydroxy-6-methoxy-3′,4′-methylenedioxyisoflavone) [3] and dalbergin (6-hydroxy-7-methoxy-4-phenylcoumarin). The above compounds were identified by their mp, UV, ^{1}H NMR and by comparison with authentic samples. The alcoholic extract of the leaves of *D. spinosa* on column chromatography over silica gel gave an amorphous powder which was shown to contain two compounds with very close R_f values and they were resolved into pure compounds **1** and **2** by preparatory TLC.

Compound **1**, mp 182–184°, analysed for $C_{22}H_{22}O_{10}$, and appeared from colour reactions to be an isoflavone glycoside with a chelated hydroxyl group at C-5. The UV spectrum had a strong absorption at 264 nm and shifted to 270 nm on the addition of $AlCl_3$ and $AlCl_3$–HCl and to 274 nm on addition of NaOEt. It did not show any shift with NaOAc and NaOAc–H_3BO_3 [5]. These observations indicated the presence of a free hydroxyl at C-5 and no free hydroxyl at C-7 of the isoflavone skeleton. Acid hydrolysis yielded prunetin and galactose. Permethylation and subsequent hydrolysis of the permethylate of **1** by Hakomori's method [6] gave only 2,3,4,6-tetra-*O*-methyl-D-galactose thereby indicating the presence of only one galactose unit in the pyranose form. The glycosidic linkage of the galactoside was shown to be β-by the enzymatic hydrolysis of the compound using β-galactosidase. Hence **1** is prunetin 4′-*O*-β-D-galactopyranoside.

Compound (**2**), mp 171–73°, analysed for $C_{23}H_{24}O_{11}$ gave all the colour reactions give by **1**. The UV spectrum of **2** had its band at 270 nm which was bathochromically shifted only by $AlCl_3$ and $AlCl_3$–HCl to 276 nm and by NaOEt to 279 nm and not by any other diagnostic flavonoid shift reagents. On acid hydrolysis it gave 7-methyltectorigenin and galactose. Permethylation and subsequent hydrolysis of the permethylate yielded only 2,3,4,6-tetra-*O*-methyl-D-galactose. It was also hydrolysed by β-galactosidase. Hence **2** is 7-methyltectorigenin 4′-*O*-β-D-galactopyranoside.

EXPERIMENTAL

Mps: uncorr. PC and TLC systems: (a) *n*-BuOH–HOAc–H_2O (4:1:5, upper), (b) PhOH–H_2O (satd), (c) *t*-BuOH–HOAc–H_2O (3:1:1), (d) EtOAc–Py–H_2O (10:4:3), (e) *n*-BuOH–EtOH–H_2O (4:1:1), (f) *n*-BuOH–EtOH–H_2O (5:1:4), (g) *n*-BuOH–EtOH–NH_3–H_2O (4:1:1:4.9); (h) C_6H_6–EtOAc (9:1), (i) C_6H_6–$CHCl_3$ (4:1) and (j) $CHCl_3$–MeOH (19:1). The TLC spots were visualized in UV light, by $FeCl_3$ spray or by exposure to I_2 vapours. Whatman No. 1 paper was used in PC and the spots were visualized by $FeCl_3$ or aniline hydrogen phthalate spray. For column chromatography, silica gel (60–120 mesh) and for TLC, silica gel G. Galactose was identified in acid

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hydrolysates of **1** and **2** by chromatography and co-chromatography in solvents a–e.

The leaves and stem-bark of *D. spinosa* were collected from the Nagamalai Hills, near the Madurai Kamaraj University campus.

Extraction and isolation. The air-dried leaves (2.6 kg) were extracted with hot C_6H_6 and then with hot alcohol (6 × 6 hr). The benzene extract on concentration gave a semi-solid (3.0 g), which was chromatographed over a column of silica gel (100 g) in petrol (60–80°). The column was eluted with petrol, petrol–C_6H_6 mixtures with increasing amounts of C_6H_6, C_6H_6 and C_6H_6–$CHCl_3$ mixtures with increasing amounts of $CHCl_3$ and finally with $CHCl_3$. Fractions of 100 ml were collected each time, distilled and the homogeneity of the fractions was examined on silica gel TLC plates using systems h, i and j. Only fractions 50–88 and 106–134, both eluted with C_6H_6, gave solids prunetin (300 mg) and 7-methyltectorigenin (120 mg) respectively.

The alcoholic extract of the leaves on concentration gave a semi-solid (3.5 g) which was chromatographed over a column of silica gel (125 g) in $CHCl_3$ and eluted with $CHCl_3$ and $CHCl_3$–MeOH mixtures with increasing amounts of MeOH. Only fractions 72–89, eluted with $CHCl_3$–MeOH (47:3), left a colourless amorphous powder after the removal of the solvent. The amorphous powder (100 mg) when examined on silica gel TLC plates in the solvent system *a* was found to be a mixture of two entities with very close R_f values (0.87 and 0.84). The mixture was resolved into the individual pure components by preparatory TLC using the same solvent system.

The air-dried dark coloured stem-bark (2.5 kg) was extracted with hot C_6H_6 (6 × 6 hr). The C_6H_6 extract gave a semi-solid (2.5 g) which was chromatographed over silica gel column built in petrol. Elution with pure as well as mixtures of the solvents in the order petrol (60–80°), C_6H_6 and EtOAc (4:1) yielded successively the known compounds in the order caviunin (190 mg), dalspinin (95 mg) and dalbergin (35 mg).

Identification of the glycosides. **1** (40 mg), mp 182–84°, analysed for $C_{22}H_{22}O_{10}$ (Found: C, 59.4; H, 5.2. $C_{22}H_{22}O_{10}$ requires: C, 59.2; H, 4.9%), Compound **2** (45 mg), mp 171–173°, analysed for $C_{23}H_{24}O_{11}$ (Found: C, 57.7; H, 5.2. $C_{23}H_{24}O_{11}$ requires: C, 57.9; H, 5.0%).

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