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VOLUBILIN, A NEW ISOFLAVONE-*C*-GLYCOSIDE FROM *DALBERGIA VOLUBILIS* FLOWERS

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Key Word Index—*Dalbergia volubilis*; Leguminosae; flowers; sterols; flavonoids; volubilin; 8-*C*-rhamnopyranosyl-genistein-4′,7-dimethyl ether.

Abstract—*Dalbergia volubilis* flowers after successive extraction with light petroleum, benzene and alcohol yielded β-sitosterol, stigmasterol, biochanin-A and a new isoflavone-*C*-glycoside named volubilin. The glycoside on boiling with HI and phenol gave genistein and on ferric chloride oxidation rhamnose. The compound was identified as 8-*C*-rhamnopyranosyl-genistein-4′,7-dimethyl ether by means of chemical reactions as well as from spectral data.

INTRODUCTION

A NUMBER of isoflavone-*C*-glucosides have been isolated from plants of the Leguminosae, in particular those of the genera Dalbergia and Pueraria. Puerarin, puerarin-*O*-xyloside[1] and di-*O*-acetyl puerarin[2] have been isolated from *Pueraria tuberosa*, whereas *Dalbergia paniculata* has yielded paniculatin[3] (from wood and bark), dalpanin[4] and dalpanitin[5] (from defatted seeds).

The isolation and the characterization of a new isoflavone-*C*-glycoside from *Dalbergia volubilis* flowers is reported in this paper. *Dalbergia volubilis*, closely related to *D. purpurea* and *D. lanceolaria*, is a large yellowish tree commonly found in the foot hills of central and eastern Himalayas. Its wood is used in the construction of buildings and root as a vegetable drug.[6]

RESULTS AND DISCUSSION

The air dried flowers were extracted successively with petroleum ether, benzene and alcohol. The petroleum ether and benzene extracts were similar on TLC and were combined and subjected to column chromatography. Three compounds were isolated and were identified as β-sitosterol, stigmasterol and biochanin-A. The latter was also subsequently isolated from the alcohol extract.

From the alcohol extract, after extensive column chromatography, preparative TLC and preparative paper chromatography, a new light yellow compound was obtained which was named volubilin (m.p. > 300°). It analysed for $C_{23}H_{24}O_9$ and gave Molisch's test, a

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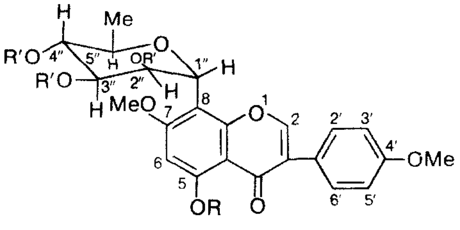
greenish brown ferric reaction and a dark pink colour with sodium amalgam followed by HCl. It gave no colour with Mg/HCl and no gossypetone reaction,[7] indicating the absence of *p*-dihydroxy system. The colour reactions together with the UV spectrum indicated that the compound was probably an isoflavone glycoside. It was stable to acid hydrolysis, which indicated that it is a *C*-glycoside. UV data with diagnostic reagents showed the presence of a free hydroxyl in the 5-position, the other positions being occupied.



(1) R' = R = H
(2) R = Me; R' = H
(3) R' = R = COMe
(4) R = Me; R' = COMe

The sugar generated by ferric chloride oxidation[8] was identified as rhamnose by co-chromatography and the chromotropic acid test.[9] It was also confirmed by subjecting the glycoside to periodate oxidation, followed by reduction of the product with sodium borohydride and hydrolysis with HCl, when propylene 1,2-glycol was identified by paper chromatography.[10] The absence of bands at 1010 and 1035 cm^{-1} in the IR spectrum of volubilin showed that it was not a *C*-glucoside.[11]

Volubilin gave genistein when subjected to simultaneous deglycosidation and demethylation with hydriodic acid and phenol. Since volubilin contained two methoxyls as indicated by micro-Zeisel methoxyl estimation and also by two NMR signals for these groups, the methoxyls should be in the 4' and 7 positions. That the **B** ring is devoid of any substituent except a methoxyl at position 4' is also confirmed by the detection of anisic acid when volubilin methyl ether acetate was oxidized with alkaline H_2O_2. Thus, it was clear that the aglycone is genistein-4',7-dimethyl ether and the rhamnose unit is linked to any of the available nuclear positions of ring A.

The NMR spectrum of volubilin acetate in $CDCl_3$ enabled the location of the rhamnosyl unit. It displayed a typical A_2B_2 system for the **B** ring with two doublets centred at δ 6·8 and δ 7·15 for 3',5' and 2',6' protons respectively. The signals at δ 3·9 and δ 4·2, each integrating for three protons could be attributed to the methoxyls at 4' and 7 positions respectively. There is only one proton that could be attributed to ring A and since it is markedly at low field (δ 6·75), the influence of the neighbouring acetyl group is indicated. This signal could be attributed to the proton at the position 6 which is in conformity with the corresponding proton in the analogous isoflavonoid acetates,[12,13] leaving the 8 pos-

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ition for the sugar residue. The position of the sugar residue is also indicated by the signal at 1·70 for the 2″-acetoxyl which agrees with that of the flavonoid-8-glucosides and moves upfield due to the shielding effect of ring A as contrasted with that of 6-*C*-glucosides ($\delta \geqslant 1{\cdot}8$).[14,15] This conclusion is also supported by a negative Gibbs test. In addition, the NMR spectrum confirms the sugar unit as rhamnose, showing the characteristic C–Me signal at δ 1·25 integrating for three protons.[12]

The pyranose structure of rhamnose was proved by periodate oxidation of the methyl ether of volubilin which consumed 2 mol of HIO_4 yielding 1 mol of formic acid. We therefore conclude that volubilin is 8-*C*-rhamno-pyranosyl genistein-4′,7-dimethyl ether (**1**).

Although a few flavone-*C*-glycosides having sugar units other than glucose have been isolated, characterized[16–18] and synthesized,[19] no isoflavone *C*-glycoside containing a non-glucose sugar unit has been reported to occur in nature so far. Volubilin is the first reported isoflavone derivative with a *C*-linked rhamnose unit.

EXPERIMENTAL

Air-dried flowers of *Dalbergia volubilis* were obtained from Banaras. PC was on Whatman No. 1 MM and 3 MM papers using (1) *n*-BuOH–HOAc–H_2O (4:1:5, upper layer), (2) *n*-BuOH–C_5H_5N–H_2O (6:4:3), (3) EtOAc–C_5H_5N–H_2O (10:4:3) as solvents. TLC was on silica gel using (4) $CHCl_3$–MeOH (7:1), (5) EtOAc–MeOH–H_2O (100:15·5:13·5), (6) EtOAc–MeOH (8:2) and (7) toluene-ethyl formate–HCO_2H (5:4:1) as solvents. The PCs were viewed in UV light and also exposed to NH_3 vapours. For TLC plates in addition to exposure to NH_3 and spraying with alcoholic $FeCl_3$, 10% H_2SO_4 was also used followed by heating at 120°. UV spectra were recorded in MeOH. NMR spectra were recorded on a 60 MHz instrument in $CDCl_3$ using TMS as internal standard and the values are reported using the δ scale.

Extraction and isolation of compounds. Powdered air-dried flowers (200 g) were extracted with petrol. (60–80°), C_6H_6 and EtOH successively. The petrol. and C_6H_6 extracts were combined, concentrated and dried over P_2O_5 under vacuum. The semi-solid mass (5 g) was subjected to column chromatography over silica gel and elution using petrol., C_6H_6 and EtOAc; three compounds A, B and C were isolated in yields of 250, 50 and 30 mg respectively.

The EtOH extract was concentrated under red. pres. and the syrupy liquid mixed with excess Et_2O. A cream coloured solid (10 g) separated which was filtered from the greenish brown ether soln. The Et_2O solution was found to be a complex mixture from which only one compound could be isolated by column chromatography (silica gel) using C_6H_6–EtOAc as the eluant. This compound was found to be identical in all respects with compound C previously isolated. The cream coloured solid gave a reddish brown ferric reaction and Molisch's test and was soluble in MeOH and H_2O. It was dissolved in the minimum amount of MeOH and reprecipitated with Et_2O. This procedure was repeated 3 ×. After column chromatography (silica gel) and elution with $CHCl_3$–MeOH a yellowish solid was obtained which was unresolved by TLC in solvents (4) to (6). On preparative PC on 3 MM paper in solvent (1) it gave a yellow band when exposed to NH_3 vapours. This band was removed from the chromatogram and cut into pieces and extracted with hot EtOH. The EtOH extract after concentration gave on TLC a single spot in all solvents except solvent (5) in which it gave two spots. After preliminary paper chromatography, it was subjected to preparative TLC using (5) as the solvent. The band with higher R_f (observed in UV light) was eluted with EtOH. The compound obtained on evaporation of the eluent was marked D and named volubilin. The compound with lower R_f was present in much smaller amounts and therefore was not analysed in detail. It gave a brown ferric reaction, a positive Molisch's and Na/Hg–HCl tests. The UV and IR data were similar to volubilin.

Compound A (250 *mg*) crystallized from MeOH as white shining leaflets, m.p. 139°. It gave a positive Liebermann–Burchard test and formed an acetate, m.p. 126–7°. It was identified as β-sitosterol (m.m.p., co-TLC and superimposable IR spectrum).

Compound B (50 *mg*) crystallized from $(Me)_2CO$ as colourless shining plates, m.p. 164–5°, gave a positive Liebermann–Burchard test and formed an acetate, m.p. 139°. It was identified as stigmasterol (m.m.p., co-TLC and superimposable IR of the acetate).

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Compound C (45 *mg*) crystallized from EtOH as colourless flakes, m.p. 216°. It gave a red–purple ferric reaction, but no colour with Mg-HCl. A pink colour was developed when it was reduced with Na–Hg followed by acidification. λ_{max}^{MeOH} 263 nm; with NaOAc, 273 nm; with NaOAc + H_3BO_3 272 nm; with $AlCl_3$, 274 nm; with NaOMe, 275 nm. It was identical with biochanin-*A* (m.m.p., TLC, acetate).

Compound D (130 *mg*) *Volubilin* (**1**) formed yellow prismatic crystals from C_6H_6–MeOH, m.p. > 300°, soluble in H_2O, hot MeOH and EtOH and insoluble in C_6H_6, $CHCl_3$ and EtOAc. It gave a brown ferric reaction, no colour with Mg/HCl, a bright pink colour with Na/Hg–HCl and a positive Molisch's test. It contained two methoxyls (micro-Zeisel). (Found: C, 60·5; H, 5·8; OMe, 14·3. $C_{23}H_{24}O_9 . \frac{1}{2}H_2O$ requires C, 60·9; H, 5·5; OMe, 14·6%). λ_{max}^{MeOH} 268, 336 nm; with NaOAc, 238, 268, 343 nm; with NaOAc + H_3BO_3; 268, 335 nm; with $AlCl_3$, 279, 298, 349 nm; with NaOMe 268·5; 395·0 nm; ν_{max}^{KBr} 3420 cm^{-1} (OH), 1652 cm^{-1} (CO).

Hydrolysis. No change was found when volubilin was boiled with H_2SO_4 (10%, 15 hr) or when treated with emulsin.

Acetate. Volubilin (50 mg) was dissolved in dry C_5H_5N (3 ml), $(Ac)_2O$ (2·5 ml) added, the mixture refluxed for 6 hr and poured over crushed ice; the precipitated solid was filtered and crystallized from $CHCl_3$–MeOH as colourless flakes, m.p. 245°. It gave a negative ferric reaction and had λ_{max}^{MeOH} 268 nm. NMR($CDCl_3$) δ: 1·25 (*s*, C–C$\underline{H}_3$); 1·70 (*s*, 2″–O–COC$\underline{H}_3$); 1·90–2·10 (3″,4″–O–COC$\underline{H}_3$); 2·35 (*s*, 5–O–COC$\underline{H}_3$); 3·90 (*s*, 4′–OCH$_3$); 4·20 (*s*, 7–OC$\underline{H}_3$), 4·55 (*s*, 1″–$\underline{H}$); 5·0–5·40 (2″,3″,4″,5″–$\underline{H}$); 6·80 (*d*, *J*9 cps, 3′,5′–H); 7·15 (*d*, *J*9 cps, 2′,6′–H); 8·00 (*s*, 2–H).

Methyl ether (**2**) Volubilin (50 mg) was refluxed in $(Me)_2CO$ (50 ml) with $(Me)_2SO_4$ (0·75 ml) and dry K_2CO_3 (3 g) for 36 hr. The mixture was filtered, and the filtrate conc. under red. pres. and the residue crystallized from $CHCl_3$ as colourless plates m.p. 225–6°, R_f 0·7 in solvent (7) and 0·6 in solvent (1) (Found: C, 62·4; H, 5·8; OMe, 19·8, $C_{24}H_{26}O_9$ requires C, 62·8; H, 5·6; OMe 20·3%). λ_{max}^{MeOH} 267 nm. NMR ($CDCl_3$ + 3 drops MeOH) δ: 6·80 (*d*, *J*8 cps, 2′,6′–$\underline{H}$), 6·2 (*d*, *J*10 cps, 3′,5′–H), 7·15 (*s*, 6–$\underline{H}$).

$FeCl_3$ oxidation of (**1**). Volubilin (40 mg) and $FeCl_3$ (250 mg) in H_2O (1·5 ml) were refluxed at 125° for 6 hr. After cooling and diluting with H_2O, the dark coloured solid was filtered, the filtrate evaporated to dryness and the resultant brown solid passed through a small column of silica gel using H_2O as eluant. The combined H_2O extracts were evaporated to dryness. The product obtained was divided into two. One part was tested for sugars by PC using (1) and (2) as solvents and aniline hydrogen phthalate as spray. Rhamnose was detected by co-chromatography with an authentic sample of *L*-rhamnose. The other portion of the product was diluted with H_2O and identified as rhamnose by the chromotropic acid test.[9]

Periodate reaction. A mixture of volubilin (3 mg) in aq. EtOH (0·2 ml) and $NaIO_4$ (2 mg) was maintained at 20° for 4 hr, $NaBH_4$ (2 mg) in H_2O (0·1 ml) added and reacted for 16 hr at 20°. 1 M HCl (0·2 ml) was then added and the mixture heated at 100° for 15 min. The clear yellow liquid was filtered, evaporated in a vacuum desiccator (3 days) and the product was examined by chromatography in solvent (3) using a mixture of 2% $NaIO_4$ (aq.) (4 parts) and 1% $KMnO_4$ solution in 2% Na_2CO_3 (aq.) (1 part) as spraying reagent, when a yellowish brown spot against a pink background was obtained. Parallel treatment was given to rhamnose and glucose. The R_f value of the oxidation product of volubilin was the same as that of rhamnose. The oxidation product of glucose had a lower R_f value.

Periodate oxidation of volubilin methyl ether. Volubilin methyl ether (15 mg) was dissolved in MeOH (10 ml) and an aq. soln. of $NaIO_4$(0·047 N, 15 ml) added and the mixture allowed to stand at 20° in the dark for 24 hr. Solid $NaHCO_3$ (2 g) was then added followed by the addition of Na_3ASO_3 soln (0·05 N, 25 ml). The resultant mixture was titrated against I_2 soln using soluble starch as indicator. One mol of the methyl ether consumed 1·94 mol of periodate.

Action of HI. To volubilin (50 mg), PhOH (300 mg) and HI (1 ml, S.G. 1·7) were added with cooling. The mixture was refluxed at 137° for 7 hr, cooled, and poured into aq. $NaHSO_3$ with stirring. The separated dark brown mass was then taken up in Et_2O and the Et_2O layer washed with H_2O. Et_2O was removed by evaporation and the gummy mass crystallized from aq. MeOH as colourless plates m.p. 296°. λ_{max}^{MeOH} 262 nm. It was identified as genistein by co-chromatography and mixed m.p. with an authentic sample.

Volubilin methyl ether acetate. Volubilin methyl ether (**2**) (15 mg) was dissolved in C_5H_5N (2·5 ml), $(Ac)_2O$ (2 ml) was added and the mixture kept at 20° for 72 hr. It was then poured over crushed ice and the separated solid filtered and crystallized from EtOAc as colourless plates, m.p. 235°.

Oxidation. The methyl ether acetate (**4**) (10 mg) was treated with 5% KOH (5 ml), followed by H_2O_2 (30%; 0·5 ml) and the mixture was kept at 45° for 2 hr, cooled, poured into ice–H_2O (5 ml), acidified with cold conc. HCl and extracted with Et_2O. The Et_2O soln was washed with H_2O and extracted with aq. $NaHCO_3$. The $NaHCO_3$ extract was acidified with HCl, extracted with Et_2O and the Et_2O evaporated. The residue was identified as anisic acid.