Phytochemistry, Vol. 22, No. 4, pp. 1063–1064, 1983.
Printed in Great Britain.

0031–8422/83/041063–02$03.00/0

AN ISOFLAVONE DIGLYCOSIDE FROM THE SEEDS OF *DOLICHOS BIFLORUS*

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(*Revised received* 15 *August* 1982)

Key Word Index—*Dolichos biflours*; Leguminosae; 5-hydroxy-7,3′,4′-trimethoxy-8-methylisoflavone; 5-*O*-α-L-rhamnopyranosyl(1 → 2)-*O*-β-D-glucopyranoside.

Abstract—5-Hydroxy-7,3′,4′-trimethoxy-8-methylisoflavone 5-neohesperidoside has been identified from the seeds of *Dolichos biflorus*.

From the ethyl acetate fraction of the ethanolic extract of the seeds of *D. biflorus* a new isoflavone diglycoside has been isolated and characterized by spectral and chemical studies. The compound (**1**), molecular formula $C_{31}H_{38}O_{15}$, mp 184°(dec.) gave a light pink colour with sodium amalgam and hydrochloric acid [1, 2] and yellow colour with magnesium–hydrochloric acid [3] and concentrated sulphuric acid and aqueous sodium hydroxide [4] showing its isoflavonoid nature which was further confirmed by its UV and ^{1}H NMR spectra. The compound was found to be glycosidic in nature. Hydrolysis with 7% sulphuric acid gave rhamnose, glucose (co-chromatography with authentic samples) and an aglycone (**1a**) $C_{19}H_{18}O_6$ mp 198° analysed for one hydroxyl group (acetate and IR ν_{max} 3221 cm^{-1}) one *C*-methyl group (NMR signal at δ 2.4 corresponding to 3H of C–Me) and three methoxyl groups (Ziesel, IR ν_{max} 2881, 1137 cm^{-1} and multiplet at δ 3.8–4.0 in the ^{1}H NMR spectra corresponding to 9H of three O–Me). Spectral studies of (**1a**) [λ_{max} 248, 300(sh) nm] and colour reactions indicated the presence of one hydroxyl group at position 5 (bathochromic shift of 15 and 17 nm of band II with aluminium chloride and aluminium chloride–hydrochloric acid, respectively). In order to assign the positions of methyl and methoxyl groups, the aglycone was methylated with diazomethane and the resulting methyl ether was subjected to alkali fission. One of the products was identified as the 2,4-dimethyl ether of 6-hydroxytoluene, mp 60° (lit. 61°) [5]. Formation of this product confirmed the position of one methyl group at position 8 and methoxyls at both the 5 and 7 positions of ring A. The other product of alkaline fission was identified as homoveratric acid mp 81° (lit. 82°) which showed the presence of two methoxyl groups at the 3′ and 4′ positions of ring B. This was further confirmed when **1a** and its methyl ether gave veratric acid mp 180° (lit. 181°) on potassium permanganate oxidation. The structure is further confirmed by a multiplet between δ 6.7 and 6.9 corresponding to the protons at positions 2′, 5′, and 6′ in the ^{1}H NMR spectra which also showed a singlet at δ 6.3 (C-6 proton of ring A) and a sharp singlet at δ 7.5 corresponding to the C-2 proton (specific to isoflavones) [6]. Thus the aglycone is 5-hydroxy-7,3′,4′-trimethoxy-8-methylisoflavone. The sugar moieties were found to be attached at position 5 from comparison of the UV spectra of the aglycone and glycoside, when the aglycone gave a distinct bathochromic shift with aluminium chloride and aluminium chloride–hydrochloric acid and the glycoside did not. Both the sugars were in the pyranose form since periodate oxidation consumed 3 mol periodate with the liberation of 1 mol formic acid/mol glycoside.

Mild acid hydrolysis with 1% aqueous sulphuric acid showed rhamnose to be the terminal sugar. The intersugar linkage was confirmed as the neohesperidoside (1 → 2) type by its ^{1}H NMR (doublet at δ 1.3 due to the rhamnose methyl group) [7, 8]. It was further confirmed when hydrolysis of the permethylated glycoside gave two methylated sugars identified as 3,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose using 2,3,4,6-tetra-*O*-methyl-D-glucose as reference. Hydrolysis of the glycoside with takadiastase liberated free rhamnose indicating its α-nature. After complete hydrolysis with takadiastase the glycoside was hydrolysed with almond emulsin; glucose being observed in the hydrolysate showed its β-nature.

EXPERIMENTAL

The seeds were identified by the Allahabad Branch of Botanical Survey of India. The crushed seeds were extracted with boiling EtOH and concd extract (150 ml) fractionated into petrol, C_6H_6 and EtOAc soluble portions. Compound **1** was ppted by petrol from the EtOAc fraction using fractional pption to remove impurities. Its purity was checked by PC and TLC.

Isoflavone diglycoside (**1**). (Found C, 48.15; H, 5.51. $C_{31}H_{38}O_{15}$ requires C, 49.60; H, 5.06%.) UV λ_{max}^{MeOH} nm: 260, 318(sh); $\lambda_{max}^{AlCl_3}$ nm: 261, 316 (sh); $\lambda_{max}^{AlCl_3-HCl}$ nm: 260, 316(sh), premethylated glycoside (10% NaOH + 10 ml/Me_2SO_4) mp 168°. (Found C, 76.16; H, 9.10; OMe, 13.18. $C_{38}H_{52}O_{15}$ requires C, 77.62; H, 8.39; OMe, 13.98%.) ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.8 (1H, *s*, C-2), 6.5 (1H, *s*, C-6), 6.8–6.9 (3H, *m*, C-2′, C-5′, C-6′), 3.9–4.1 (9H, *m*, OMe, C-7, C-3′, C-4′), 2.6 (3H, *s*, Me-8), 1.3 (3H, *d*, J = 6 Hz, Rha – Me), 4.2 (1H, *s*, H-1″ rhamnosyl), 5.0 (1H, *br*, H-1″ glycosyl), 3.6 (*br*, sugar protons).

Aglycone (**1a**). (Found C, 65.91; H, 5.81, $C_{19}H_{18}O_6$ requires C, 66.66; H, 5.26%.) UV λ_{max}^{MeOH} nm: 248, 300 (sh); $\lambda_{max}^{AlCl_3}$ nm: 263, 303 (sh); $\lambda_{max}^{AlCl_3-HCl}$ nm: 265, 305 (sh). Acetate (pyridine–Ac_2O; 24 hr at room temp.) mp 159°. (Found C, 64.81; H, 5.91; acetyl,

10.98%. $C_{21}H_{20}O_7$ requires C, 65.62; H, 5.28; acetyl 11.19%.) ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.5 (1H, *s*, C-2), 6.3 (1H, *s*, C-6), 6.7–6.9 (3H, *m*, C-2′, C-5′, C-6′), 3.8–4.0 (9H, *m*, OMe, C-7, C-3′, C-4′), 2.4 (3H, *s*, Me-8).

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Phytochemistry, Vol. 22, No. 4, pp. 1064–1065, 1983.
Printed in Great Britain.

0031-9422/83/041064-02$03.00/0
Pergamon Press Ltd.

GLYCOZOLININE, A CARBAZOLE DERIVATIVE FROM *GLYCOSMIS PENTAPHYLLA*

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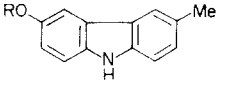
(*Received* 9 *August* 1982)

Key Word Index—*Glycosmis pentaphylla*; Rutaceae; seeds; alkaloids; glycozolinine; carbazole.

Abstract—A new carbazole derivative, glycozolinine, was isolated from the seeds of *Glycosmis pentaphylla*. From physical and chemical evidence its structure is 6-hydroxy-3-methylcarbazole.

In continuation of our investigations on the chemistry of carbazole alkaloids [1–4], we wish to report the isolation and structure elucidation of a new carbazole derivative from the seeds of *Glycosmis pentaphylla*.

Glycozolinine (**1**), $C_{13}H_{11}NO$ (M^+ 197, determined by MS), mp 231–232° was isolated from the benzene extract of the defatted seeds of the plant. The homogeneity of glycozolinine was confirmed by TLC using various solvent systems. Glycozolinine gave a red colour with ferric chloride indicating the presence of a phenolic hydroxyl in the molecule. The UV spectrum showed absorption at λ_{max} nm (log ε): 224 (3.92), 254 (4.02), 269 (3.96) and 298 (4.04). The IR spectrum showed absorption peaks at ν_{max}^{KBr} cm^{-1}: 3440 (–NH– function), 3390 (phenolic hydroxyl), 1630, 1570, 1480 (aromatic residue), 1390 (aromatic C–Me) and 790 (substituted benzene derivative). The ^{1}H NMR spectrum showed signals for one indolic proton (broad singlet at δ 7.65, confirmed by D_2O exchange), two aromatic protons (doublet around δ 7.8), four aromatic protons (multiplet at δ 7.20 6.85), three protons of an aromatic C–Me group (singlet at δ 2.38) and a phenolic hydroxyl (broad singlet at δ 11.04, confirmed by D_2O exchange).



1 R=H
2 R=COMe
3 R=Me

Glycozolinine on treatment with acetic anhydride and pyridine at room temperature for 16 hr gave acetate **2**, which crystallized from benzene, mp 210°. The UV spectrum of the acetate showed absorption maxima at λ_{max} nm (log ε): 230 (4.62), 239 (4.14), 266 (4.26), 299 (4.20) and 332 (3.48). The IR spectrum showed absorption at ν_{max}^{KBr} cm^{-1}: 3438 (–NH– function), 1746 (acetyl function), 1628, 1590, 1445 (aromatic residue), 1390 (C–Me group) and 778, 730 (substituted aromatic system). The UV spectrum was very similar to that of 3-methylcarbazole suggesting that the methyl group in glycozolinine is in either the 3- or 6-position in the carbazole skeleton. That the methyl group of glycozolinine is in the 3-position was confirmed by the fact that on zinc dust distillation of glycozolinine 3-methylcarbazole was obtained. On treatment with diazomethane, glycozolinine furnished a carbazole derivative **3**, $C_{14}H_{13}NO$, mp 182° which was identical with glycozoline [5]. The above data, therefore, lead to the formulation of glycozolinine as 3-methyl-6-hydroxycarbazole (**1**).

EXPERIMENTAL

All mps were uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively. ^{1}H NMR was measured at