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FLAVONOL 3-GLYCOSIDES FROM THE LEAVES OF *FLEMINGIA STRICTA*

C. P. RAO, T. HANUMAIAH, V. S. S. VEMURI and K. V. JAGANNADHA RAO

Department of Chemistry, Nagarjuna University, Nagarjunanagar 522 510, India

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Key Word Index—*Flemingia stricta*; Leguminosae; flavonoids; tamarixetin 3-rhamnoside; kaempferol 3-rhamnoside; mearnsetin 3-rhamnoside; quercetin 3-rhamnoside; myricetin 3-rhamnoside.

Abstract—A new glycoside, tamarixetin 3-rhamnoside together with kaempferol 3-rhamnoside, mearnsetin 3-rhamnoside, quercetin 3-rhamnoside, myricetin 3-rhamnoside and sitosterol glucoside, was identified from the leaves of *Flemingia stricta*.

INTRODUCTION

The previous chemical work on *Flemingia stricta* Roxb. [1–5] revealed the presence of flemiflavanones A–D, sitosterol, naringenin, genistein, 5,7,2′,4′-tetrahydroxyisoflavone, genistin and sitosterol glucoside in the roots and six chalcones (flemistrictins A–F) in the leaves. The present chemical investigation reports the isolation and identification of a new flavonol glycoside, tamarixetin 3-rhamnoside, together with kaempferol 3-rhamnoside, mearnsetin 3-rhamnoside, quercetin 3-rhamnoside, myricetin 3-rhamnoside and sitosterol glucoside from a methanolic leaf extract of *F. stricta*.

RESULTS AND DISCUSSION

From a methanolic leaf extract of *F. stricta* six compounds (**1**–**6**) were isolated. Compound **1** was identified as sitosterol glucoside. The remaining five glycosides responded individually to ferric reaction, Molisch and Shinoda (Mg–HCl) tests. They developed an orange-red colour slowly when zinc was used instead of magnesium (Pew's modification) indicating that they were flavonol 3-glycosides [6]. R_f values on PC in BAW were: 0.94, 0.91, 0.89, 0.87 and 0.83, respectively. They all appeared purple in UV light changing to yellow with ammonia vapour. The UV, IR and NMR data of **3**–**6** were in close agreement with those described for kaempferol 3-rhamnoside, mearnsetin 3-rhamnoside, quercetin 3-rhamnoside and myricetin 3-rhamnoside, respectively, and their identities were confirmed by co-PC and co-TLC with authentic samples. Mearnsetin 3-rhamnoside is a rare flavonoid first reported by Mackenzie from *Acacia mearnsii* [7]. The aglycone, mearnsetin, was detected later by Ray, *et al.* in the free state in leaves of *Elaeocarpus lanceofolius* [8]. The present paper documents the second report of mearnsetin 3-rhamnoside in nature.

Compound **2** is a new flavonol glycoside. Elemental analysis indicated its molecular formula as $C_{22}H_{22}O_{11}$. Its IR spectrum displayed a strong absorption band at 1640 cm^{-1} for a chelated carbonyl and a broad band at 3300 cm^{-1} for a chelated hydroxyl. The UV spectrum of **2** in methanol showed stronger absorption at 255 nm (band II) than at 345 nm (band I) indicating that the sugar residue is at C-3. The bathochromic shift of 46 nm of band I with aluminium chloride–hydrochloric acid is a characteristic feature of 5-hydroxy-3-substituted flavonols [9]. The bathochromic shift of 18 nm with sodium acetate indicated the presence of an unsubstituted 7-hydroxyl. In the presence of sodium acetate, band I exhibited a bathochromic shift of only 35 nm with a decrease in intensity in accord with a 3,4′-substituted quercetin derivative. Acid hydrolysis of the glycoside gave an aglycone and rhamnose in equimolecular ratio. The UV, NMR data and mp of the aglycone were in agreement with those of tamarixetin (quercetin 4′-methyl ether). The UV spectrum of the aglycone in methanol showed absorption maxima at 256 (band II) and 366 nm (band I). The NMR spectrum exhibited signals typical for quercetin 4′-methyl ether: a three proton signal at δ 3.93 for one methoxy group; aromatic signals at δ 7.86 (J = 9, 2.5 Hz), 7.71 (J = 2.5 Hz) and 7.07 (J = 9.0 Hz) attributable to H-6′, H-2′ and H-5′, respectively, and *meta*-coupled doublets at 6.51 (J = 2.5 Hz) and 6.24 (J = 2.5 Hz) for H-8 and H-6. Thus the new glycoside was identified as tamarixetin 3-rhamnoside.

EXPERIMENTAL

Plant material was collected from Dehra Dun, India in Nov. 1980. TLC was performed on plates coated with Si gel G impregnated with 2% oxalic acid (to allow clear separation of compounds) using $CHCl_3$–MeOH (4:1) for glycosides and $CHCl_3$–MeOH (9:1) for aglycones. Spots were visualized by a 10% MeOH H_2SO_4 spray followed by heating the plates at 100° for 10 min. Whatman No. 1 paper was used for PC in BAW (4:1:5) and the spots were viewed in UV light and after exposure to NH_3 vapour. Si gel (100–200 mesh) was employed for CC and Si gel G for TLC ($CHCl_3$ MeOH, 4:1).

Isolation of glycosides. Dried and powdered leaves (1.6 kg) were extracted successively in a Soxhlet with *n*-C_6H_{14}, Me_2CO and MeOH. The concd MeOH extract (90 g) was taken-up in H_2O and filtered. From the H_2O-insoluble residue sitosterol glucoside was obtained by repeated crystallization from MeOH. The concd filtrate was extracted with Et_2O (4 × 250 ml) and then *n*-BuOH (4 × 250 ml). The *n*-BuOH concentrate (12 g) was mixed with H_2O (200 ml), refrigerated overnight, the upper layer decanted and the inorganic residue rejected. The aq. soln was evaporated below 40° to dryness under red. pres. The residue (8 g) gave five yellow compounds when examined by TLC which were separated by CC over Si gel (200 g). Elution was carried out with $CHCl_3$–MeOH (9:1) followed by increasing amounts of MeOH. Fractions were collected by monitoring by TLC in

$CHCl_3$–MeOH (4:1) on Si gel G. Fractions containing more than one compound were further separated by prep. TLC in $CHCl_3$–MeOH (4:1) on Si gel G and eluted with MeOH.

Compound **2** was obtained in small quantity (20 mg) and resisted crystallization from the usual solvents. R_f values for **2** were 0.8 (TLC in $CHCl_3$–MeOH, 4:1, on Si gel G) and 0.94 (PC in BAW). UV λ_{max}^{MeOH} nm (log ε): 255 (4.36), 264 (sh), 345 (4.10); λ_{max}^{NaOMe} nm: 270, 380; $\lambda_{max}^{AlCl_3}$ nm: 265, 271 (sh), 298 (sh), 360, 390; $\lambda_{max}^{AlCl_3-HCl}$ nm: 265, 271 (sh), 298 (sh), 360, 391; λ_{max}^{NaOAc} nm: 273, 365; $\lambda_{max}^{NaOAc-H_3BO_3}$ nm: 268, 350. IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*), 1640. Acid hydrolysis of **2** (10 mg) with 6% HCl (10 ml) at 100° for 45 min afforded tamarixetin and rhamnose in a 1:1 ratio. The sugar residue was identified by co-PC with an authentic sample (R_f 0.41 in BAW). The aglycone crystallized from EtOAc *n*-C_6H_{14} as pale yellow crystals mp 258–260° (lit. 259–260° [10]) TLC, R_f 0.8 ($CHCl_3$–MeOH, 9:1) on Si gel G. UV λ_{max}^{MeOH} nm (log ε): 256 (3.99), 366 (3.94). NMR (Me_2CO-d_6, TMS, δ-scale): 3.93 (*s*, –OMe), 6.24 (1H, *d*, J = 2.5 Hz), 6.51 (1H, *d*, J = 2.5 Hz), 7.07 (1H, *d*, J = 9.0 Hz), 7.71 (1H, *d*, J = 2.5 Hz) and 7.86 (1H, *dd*, J = 9, 2.5 Hz).

Methylation of **2** (5 mg) followed by acid hydrolysis of the product gave quercetin 5,7,3',4'-tetramethyl ether, mp 194–195° (lit. 195–198° [11]). When demethylated with excess pyridinium chloride at 140° for 2 hr tamarixetin (2 mg) gave quercetin which was identified by direct comparison with an authentic sample (mmp and co-TLC).

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TRISUBSTITUTED FLAVONOL GLYCOSIDES IN *CORONILLA EMERUS* FLOWERS

JEFFREY B. HARBORNE and MARIAN BOARDLEY

Phytochemical Unit, Plant Science Laboratories, The University, Whiteknights, Reading, RG6 2AS, U.K.

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Key Word Index—*Coronilla emerus*; Leguminosae; flowers; leaves; kaempferol 3-glucoside-7,4'-dirhamnoside; flavonol glycosides; UV patterning.

Abstract—A novel trisubstituted kaempferol glycoside has been isolated from leaves and flowers of *Coronilla emerus* and identified as the 3-glucoside-7,4'-dirhamnoside. It co-occurs in the flowers with the 3-glucosides and 3-glucoside-7-rhamnosides of kaempferol and quercetin. A second kaempferol triglycoside based on glucose and xylose is also present. All six glycosides contribute to the UV patterning present in the wings of the flowers. This is the first report of kaempferol triglycosides with monosaccharide units substituting hydroxyl groups at the 3-, 7- and 4'-positions.

INTRODUCTION

While studying the flavonol glycosides of yellow flowered legume species in relation to their role as UV patterning guides to insect pollinators [1], the presence of a novel flavonol glycoside with unusual colour reactions and UV spectrum was discovered in the wings of *Coronilla emerus*. This plant is pollinated by bumble bees, who are attracted to the flowers solely by the pollen, since there are no nectaries [2]. The UV absorbing flavonols, present mainly in the wings, presumably guide the insect in its approach