1.5 ml/min. The total amount of anthocyanins was calculated as cyanidin-3-galactoside from a standard curve.

TLC. TLC of pigments was carried out on cellulose plastic sheets (Merck, 5565) with BAW (*n*-BuOH–HOAc–H_2O, 6:1:2), HW (conc. HCl–H_2O, 3:97), AW (HOAc–H_2O, 15:85) and FHW (HCO_2H–conc. HCl–H_2O, 6:1:5). R_f values for cyanidin-3-arabinoside, pelargonidin-3-arabinoside, cyanidin-3-galactoside and pelargonidin-3-galactoside were 0.26, 0.41, 0.14 and 0.24 in BAW; 0.04, 0.08, 0.05 and 0.09 in HW; 0.37, 0.49, 0.37 and 0.49 in AW; 0.57, 0.69, 0.56 and 0.69 in FHW.

Degradation procedures. Acid hydrolysis, deacylation with alkali and H_2O_2 oxidation were according to standard procedures [14].

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REFERENCES

1. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, p. 196. Academic Press, London.
2. Ballinger, W. E., Galetta, G. J. and Maness, E. P. (1979) *J. Am. Soc. Hort. Sci.* **104**, 554.
3. Troyan, A. V. and Borukh, I. F. (1968) *Tr. Vses. Semin. Biol. Activ. (Lech.). Veshchestvam Plodov. Yagod.* 3rd., 224; *Chem. Abstr.* (1970) **73**, 119394.
4. Andersen, Ø. M. (1985) *J. Food Sci.* **50**, 1230.
5. Baj, A., Bombardelli, E., Gabetta, B. and Martinelli, E. M. (1983) *J. Chromatogr.* **279**, 365.
6. Lowry, J. B. (1971) *Phytochemistry* **10**, 689.
7. Jarman, S. J. and Crowden, R. K. (1976) *Phytochemistry* **15**, 1796.
8. Jarman, S. J. and Crowden, R. K. (1974) *Phytochemistry* **13**, 743.
9. Kitamura, S. and Okamoto, S. (1975) *Trees and Shrubs of Japan*, p. 196. Hoikusha, Osaka.
10. Fernald, M. L. (1950) *Gray's Manual of Botany*, p. 1135. American Book Company, New York.
11. Fuleki, T. and Francis, F. J. (1967) *Phytochemistry* **6**, 1705.
12. Du, C. T. and Francis, F. J. (1973) *Phytochemistry* **12**, 2487.
13. Fuleki, T. and Francis, F. J. (1968) *J. Food Sci.* **33**, 266.
14. Francis, F. J. (1982) in *Anthocyanins as Food Colors* (Markakis, P., ed.) p. 181. Academic Press, New York.

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FLAVONOL GLYCOSIDES FROM *CALLITRIS GLAUCA*

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Key Word Index—*Callitris glauca*; Cupressaceae; myricetin 7-arabinoside; kaempferol 5-rhamnoside; flavonol glycosides.

Abstract—From the leaves of *Callistris glauca* myricetin 7-arabinoside, quercitrin, kaempferol 5-rhamnoside, a quercetin arabinoside, quercetin, kaempferol, galangin and shikimic acid were isolated. The natural occurrence of myricetin 7-arabinoside has not previously been reported.

INTRODUCTION

A reinvestigation of the leaves of *Callitris glauca* R.Br. has resulted in the isolation of a new glycoside, myricetin 7-*O*-arabinoside (**1**), together with the known substances quercetin 3-*O*-rhamnoside (**2**), a quercetin arabinoside (**3**), kaempferol 5-rhamnoside (**4**), quercetin, kaempferol, galangin and shikimic acid. Kaempferol 5-rhamnoside was recently reported from this plant for the first time with other flavonoid constituents [1].

RESULTS AND DISCUSSION

The aqueous layer of a methanolic leaf extract of *C. glauca* was extracted with ethyl acetate and concentrated to give yellow crystals of shikimic acid (co-TLC, mmp and spectral data). The concentrated filtrate upon repeated CC and TLC on silica gel followed by crystallization afforded four compounds (**1**–**4**). Myricetin 7-arabinoside (**1**) on acid hydrolysis gave myricetin [2, 3] and arabinose (PC). A comparative study of the ^{1}H NMR spectral data of the acetate of **1** and acetylated myricetin indicated glycosylation at C-7. The two doublets (J = 2.5 Hz) at δ6.46 and 6.75 due to C-6 and C-8 protons of acetylated **1** were shifted downfield to δ6.50 and 6.90 in the sugar-free myricetin acetate. This downfield shifting in ring A and the greater shift of the C-8 proton than that of C-6 showed that the change of electron density was in the vicinity of C-8. The mass spectrum of **1** showed no molecular ion peak but a peak of low intensity at m/z 391 (2) $[(M-60)+H]^+$ was obtained, indicating that the sugar involved in glycosylation was an aldopentose. The UV spectral data for **1** and its partially methylated aglycone in different diagnostic reagents (see

Experimental) were in agreement with a structure in which the sugar moiety is linked to the 7-hydroxyl of myricetin.

Quercetin 3-rhamnoside (2) was identified by standard procedures and quercetin arabinoside (3) was present in too small an amount to be characterized. Kaempferol 5-rhamnoside (4) on acid hydrolysis rapidly gave kaempferol [2, 3] and rhamnose (PC). The UV absorption spectra of 4 showed appreciable shifts in bands II and I with sodium acetate and a large shift of band I with aluminium chloride, suggesting free hydroxyls at C-7, C-4′ and C-3. The position of glycosylation at C-5 was further supported by UV and ^{1}H NMR spectral studies of 5-hydroxy-7,3,4′-trimethoxyflavone obtained from permethylation of 4. A shift of 49 nm in band I with aluminium chloride showed that the 3-hydroxyl is not free [2]. In the acetate of 4, the acetoxyl signal at δ2.46 is in agreement with the 5-acetate group, distinct from other acetoxyls which absorb near δ2.34 [4, 5].

Flavonoid constituents from the water-insoluble fraction are described in the Experimental.

EXPERIMENTAL

Extraction. Dried and powdered leaves of *Callitris glauca* (4 kg) (obtained from the Forest Research Institute, Dehradun (U.P.), India) were extracted with MeOH and the extract concentrated under red. pres. The residue after exhaustive digestion with petrol (bp 60–80°), C_6H_6 and $CHCl_3$ was treated with H_2O. The aq. layer was extracted with EtOAc and concentrated to give yellow crystals of shikimic acid, mp 179°. The filtrate was concentrated to a semisolid mass (6 g) which on CC (silica gel, C_6H_6–EtOAc as eluant) and TLC (silica gel, EtOAc–xylene–HCOOH–H_2O, 35:1:2:2) followed by crystallization from MeOH–EtOAc yielded: **1** (R_f 0.56, 150 mg), **2** (R_f 0.64, 160 mg), **3** (R_f 0.64, 15 mg) and **4** (R_f 0.77, 100 mg).

Myricetin 7-O-arabinoside (**1**). Yellow cubes, mp 208°. MS *m/z* (rel. int.): 391 (2), 318 (11), 317 (47), 290 (10), 167 (17), 166 (8), 153 (7), 152 (5), 145 (5), 133 (9), 132 (26), 125 (8), 73 (100), 60 (67), 32 (79). UV λ_{max}^{MeOH} nm: 255 sh, 274, 300 sh, 340; +NaOAc 268, 275 sh, 335, 400; +NaOAc/H_3BO_3 264, 310 sh, 370; +$AlCl_3$ 275, 315, 435; +$AlCl_3$/HCl 265 sh, 280, 310, 360, 420 sh. Acetate, mp 162°; ^{1}H NMR ($CDCl_3$): δ6.46, 6.75 (*d*, *J* = 2.5 Hz, H-6, H-8), 7.55 (*s*, H-2′, 6′), 2.02 (9H, three alc. acetoxyls), 2.30–2.38 (15 H, five phenolic acetoxyls), 5.20 (*br*, H-1″), 4.10–4.35 (H-2″, 3″, 4″), 3.45–3.65 (H-5″, 5″). Aglycone, myricetin 5,3,3′,4′,5′-pentamethyl ether (from permethylation of **1**), mp 248°, UV λ_{max}^{MeOH} nm: 255, 265, 370; +NaOAc 272, 370; +NaOAc/H_3BO_3 265, 368; +$AlCl_3$ 250, 268, 365; +$AlCl_3$/HCl 250, 265, 370. Hydrolysis of **1** (50 mg) in alcohol with 8% HCl provided an aglycone and a sugar. The aglycone, mp 355°, on acetylation (Ac_2O–pyridine) gave colourless cubes ($CHCl_3$–MeOH) of myricetin hexaacetate, mp 218°; ^{1}H NMR ($CDCl_3$): δ6.50, 6.90 (*d*, *J* = 2.5 Hz, H-6, H-8), 7.60 (*s*, H-2′,6′), 2.30–2.40 (18 H, six phenolic acetoxyls). The sugar was identified as arabinose by PC (R_f, co-PC and colour developed by aniline hydrogen phthalate, identical with authentic sugar).

Kaempferol 5-O-rhamnoside (**4**). Yellow needles, mp 198°; UV λ_{max}^{MeOH} nm: 253, 270 sh, 355; +NaOAc 270, 315 sh, 380; +NaOAc/H_3BO_3 255, 270 sh, 290 sh, 354; +$AlCl_3$ 272, 305 sh, 430; +$AlCl_3$/HCl 269, 300 sh, 428. Aglycone, kaempferol 7,3,4′-trimethyl ether, mp 149°; UV λ_{max}^{MeOH} nm: 270, 305, 325 sh, 350; +NaOAc 268, 305 sh, 349; +NaOAc/H_3BO_3 269, 300 sh, 350; +$AlCl_3$ 279, 300 sh, 308, 348, 399; +$AlCl_3$/HCl 278, 300 sh, 308, 346, 398. Its acetate, mp 135°; ^{1}H NMR ($CDCl_3$): δ6.60, 6.86 (*d*, *J* = 2.5 Hz, H-6, H-8), 8.24, 7.18 (*d*, *J* = 9 Hz, H-2′,6′, H-3′,5′), 2.46 (3H, OAc-5), 3.90–3.86 (9H, OMe-7,3,4′). Hydrolysis of **4** (20 mg) in alcohol with 5% HCl for 20 min afforded kaempferol and rhamnose (PC).

The water-insoluble residue after CC (silica gel) followed by prep. TLC (silica gel, C_6H_6–pyridine–HCOOH, 36:9:5) yielded hypolaetin, quercetin, kaempferol, galangin, amentoflavone, sequoiaflavone, hinokiflavone and di-*O*-methylamentoflavone. These were identified by UV, MS and ^{1}H NMR spectral studies and also by co-TLC, mp and mmp with authentic specimens.

REFERENCES

1. Ansari, F. R., Ansari, W. H. and Rahman, W. (1981) *Indian J. Chem.* **20B**, 724.
2. Geissman, T. A. (1962) *The Chemistry of Flavonoid Compounds.* Pergamon Press, Oxford.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, New York.
4. Henrick, C. A. and Jefferies, P. R. (1964) *Aust. J. Chem.* **17**, 934.
5. Jefferies, P. R. and Payne, T. G. (1965) *Aust. J. Chem.* **18**, 1441.