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QUERCETIN COUMAROYL GLUCORHAMNOSIDE FROM *GINKGO BILOBA*

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Key Word Index—*Ginkgo biloba*; Ginkgoaceae; flavonol glycoside; quercetin-3-*O*-α-(6‴-*p*-coumaroylglycosyl-β-1,4-rhamnoside).

Abstract—A new flavonoid glycoside was isolated from the leaves of *Ginkgo biloba* (Ginkgoaceae) and its structure elucidated as quercetin-3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside).

INTRODUCTION

We have recently [1] described the isolation and the structural elucidation of an unusual kaempferol derivative, the 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) (**1**) in the leaves of *Ginkgo biloba* L. The extension of the study of this plant gave a homologous compound derived from quercetin (**2**). The structure of this compound was previously reported [2, 3] but without any mention of the isolation method; nor were any structural data included.

RESULTS AND DISCUSSION

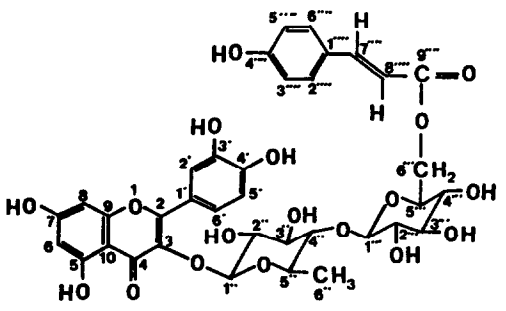
The isolation method of **2** is different from the method described for **1** [1] since the aqueous acetone concentrated was defatted with cyclohexane, then filtered and passed directly through an anion-exchange resin column. The unadsorbed fraction gives the crude flavonoids extract, successively fractioned on silica gel, Sephadex (several columns to obtain a great yield of **2**) and polyamide columns necessary to achieve its purification. Finally, after filtration on Sephadex, the flavonoid **2** was obtained, as a yellow-green amorphous solid.

Upon acid hydrolysis of **2**, quercetin, *p*-coumaric acid, D-glucose and L-rhamnose were identified by TLC. Generally the spectra data of **2** corresponds with those of **1** except for the aglycone part. So in UV light, **2** appeared as a dull brown flavone-like fluorescent spot on silica gel and polyamide plates, indicating the substitution of the C-3 hydroxyl group. This spot changed to orange, after pulverisation with an ethanolic solution of NA and PEG 400, indicating the presence of an *ortho*-dihydroxyl group in the B ring [4]. The UV shift on addition of diagnostic reagents (see Experimental) confirmed the presence of free hydroxyl groups at positions C-3′ and C-4′ and suggested the presence of free hydroxyl groups at positions C-5 and C-7 [5]. Therefore, the position of the linkage between quercetin and the other moiety, occurs at C-3. It is noteworthy that the λ_{max} of band 1, at 316 nm shows a low value; a typical quercetin C-3 linked glycoside shows a corresponding λ_{max} located about 350–360 nm which appears only as a shoulder in the ethanol UV spectrum of **2**. This hypsochromic shift of band 1 is due to the presence of the *p*-coumaroyl group in the side chain.

The MS of **2** exhibited a molecular ion peak at *m/z* 756 in accordance with a *p*-coumaroyl-ester of a quercetin bioside. The fragment ions at *m/z* 449, and 303 showed that glucose was located between rhamnose and *p*-coumaric acid.

The ^{1}H NMR spectrum exhibited two doublets at δ6.24 and 7.45 with large coupling constants (16 Hz) which assigned the *trans* configuration to the *p*-coumaric acid. The anomeric proton (H-1‴) of the glucose appeared as a doublet at 4.28 (J = 8 Hz). This chemical shift confirmed that glucose is not attached to the quercetin nucleus. The diaxial coupling (J = 8 Hz) between H-1 glucose and H-2 glucose indicated that the glucose has a β-configuration. The signal at 5.52 (J = 2 Hz) was assigned to H-1 rhamnose, confirming the position of linkage between the sugar and aglycone at C-3, and the diequatorial coupling (J = 2 Hz) between H-1 rhamnose and H-2 rhamnose indicated the α-configuration [5].

The ^{13}C NMR spectrum of the sugar moiety of **2** shows a downfield shift of C-6 glucose (Δ + 2.1) from the chemical shift values reported for the corresponding carbon resonances of unlinked C-6 glucose (such as flavonol-3-*O*-glucopyranosides) [6]. These shifts are expected from the substituent effect of C-6 glucose acylation [7]. Otherwise, in the ^{13}C NMR spectrum of kaempferol-3-*O*-(rhamno(1–6) glucoside) [8] the glucose C-6″ signal shifts downfield from δ 61.0 to 67.1 due to rhamnosylation at C-6″. This evidence excludes other possible acylation sites in the glucose moiety of **2** and fixes the *trans*-*p*-coumaric acid to C-6 glucose. As for **1**, the chemical shift for C-4 rhamnose in **2** resonates at δ 82.6 indicating that



glucose must be linked to the 4-OH group of the rhamnose moiety. On the basis of these data, we concluded that the structure of **2** is quercetin-3-*O*-α-(6‴-*p*-coumaroylglycosyl-β-1,4-rhamnoside).

EXPERIMENTAL

General techniques. Chromatography columns: Ion exchange resin: Amberlite IRN-78 (Prolabo); Silica gel 60 (Merck); Polyclar AT (Touzart and Matignon) and Sephadex LH-20 (Pharmacia); Precoated silica gel plates 60 F 254 (Merck), cellulose (Merck) and micropolyamide foils F1700 15 × 15 cm (Schleicher & Schüll). The solvent systems were: A, EtOAc–MeCOEt–HCO_2H–H_2O (5:3:1:1); B, MeCOEt–MeOH–HOAc (3:1:1); C, H_2O–MeCOEt–MeOH (4:3:3), D, HOAc 60%. Flavonoids were visualized by UV light and by spraying with an EtOH solution of NA (Naturstoffreagenz-A) 1% and PEG 400 5%. Sugars were visualized by spraying with anisaldehyde soln and heating at 120°.

Isolation of **2**. Ground, dried leaves (10 kg) were extracted with 60% aq Me_2CO in a Soxhlet apparatus. The acetone was evapd. The H_2O extract was defatted with C_6H_6 and chromatographed over an ion resin exchange column (100 g) using H_2O with an increasing ratio of MeOH and then MeOH–0.05 M HCl. The fractions (250 ml) were collected and controlled by TLC. The fraction (42 g) eluted with aq. MeOH contained the flavonoids. It was chromatographed over a silica gel column (1 kg) packed with $CHCl_3$ and eluted with a mixture of $CHCl_3$–MeOH with an increasing ratio of MeOH. The fraction (4.5 g) containing **2** was eluted with MeOH on a Sephadex column (40 g). This operation was repeated × 5. The fraction (250 mg) was chromatographed over a Polyclar column (30 g) packed with $CHCl_3$–MeOH (4:1) and eluted with an increasing ratio of MeOH. The quercetin *p*-coumaroyl glycoside was finally purified on a Sephadex column (15 g) eluted with MeOH. 200 mg of pure glycoside were obtained as an amorphous solid.

Quercetin-3-O-α-(6‴-p-*coumaroylglucosyl*-β-1,4-*rhamnoside*). Mp 231 ± 2°. UV λ_{max}^{EtOH} nm: 360 sh, 316, 300 sh, 268, 258; + NaOAc: 370 sh, 315, 300 sh, 269; + NaOAc–H_3BO_3: 373 sh, 315, 300 sh, 263; + $AlCl_3$: 410 sh, 360 sh, 315, 300 sh, 272; + AlC_3–HCl: 400 sh, 360 sh, 315, 300 sh; 277. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.91 (3H, *d*, *J* = 6 Hz Me rhamnose), 3.03–4.15 (*m*, sugars protons), 4.28 (1H, *d*, *J* = 8 Hz, H-1 glc), 5.52 (1H, *d*, *J* = 2 Hz, H-1rha), 6.16 (1H, *d*, *J* = 2 Hz, H-6), 6.24 (1H, *d*, *J* = 16 Hz, H-8 coum), 6.31 (1H, *d*, *J* = 2 Hz, H-8), 6.70 (2H, *d*, *J* = 8.6 Hz, H-3 coum and H-5 coum), 6.88 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.25 (1H, *dd*, *J* = 2 Hz and 8.4 Hz, H-6'), 7.36 (1H, *d*, *J* = 2 Hz, H-2'), 7.41 (2H, *d*, *J* = 8.6 Hz, H-2 coum and H-6 coum), 7.45 (1H, *d*, *J* = 16 Hz, H-7 coum); CIMS 70 eV *m/z* (rel. int.) 757 [M + H]$^+$ (0.3), 611 (1), 595 (0.5), 472 (6.6), 449 (66.7), 303 (100). ^{13}C NMR (50 MHz, DMSO-d_6): δ 18.3 (C-6 rha), 63.8 (C-6 glc), 70.5 (C-2 rha, C-5 rha), 71.1 (C-4 glc), 72.7 (C-3 rha), 74.7 (C-2 glc, C-5 glc), 76.9 (C-3 glc), 82.6 (C-4 rha), 94.5 (C-8), 99.6 (C-1 rha), 101.6 (C-1 glc), 104.9 (C-10), 107.1 (C-6), 114.8 (C-8 coum, C-2'), 116.5 (C-5', C-3 coum and C-5 coum), 121.5 (C-1'), 121.8 (C-6'), 125.9 (C-1 coum), 130.9 (C-2 coum, C-6 coum), 135.3 (C-3), 145.5 (C-3'), 146.1 (C-4'), 149.5 (C-7 coum), 157.3 (C-9), 157.5 (C-2), 160.6 (C-4 coum), 162.2 (C-5), 165.0 (C-7), 167.3 (C-9 coum), 178.6 (C-4).

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REFERENCES

1. Nasr C., Haag-Berrurier M., Lobstein-Guth A., and Anton R. (1986) *Phytochemistry* **25**, 770.
2. Hänsel, R. and Haas, H. (1984) in *Therapie mit Phytopharmaka* p. 68. Springer, Heidelberg.
3. Haan, J., Reckerman, U., Welter, F. L., Sabin, G. and Müller, E. (1982) *Med. Welt* **33**, 1001.
4. Brasseur, T. and Angenot, L. (1986) **351**, 351.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
6. Markham, K. R. and Chari, V. M. (1982) in *The Flavonoids: Advances in Research* (Harbone, J. B. and Mabry, T. J., eds) pp. 40–44. Chapman & Hall, London.
7. Markham, K. R. (1982) in *Techniques of Flavonoid Identification* p. 82. Academic Press, London.
8. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.