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KAEMPFEROL COUMAROYL GLUCORHAMNOSIDE FROM *GINKGO BILOBA*

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

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

INTRODUCTION

Earlier chemical investigations of the leaves of *Ginkgo biloga* L., the one and only member of the Ginkgoaceae, led to the isolation and characterization of lactonic terpenes proper to this tree [1] and of biflavonoids [2]. The present communication describes the isolation and the structural elucidation of an unusual kaempferol derivative, the 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside. The structure of this compound was previously described [3] but without any mention of the isolation method nor were any structural data included.

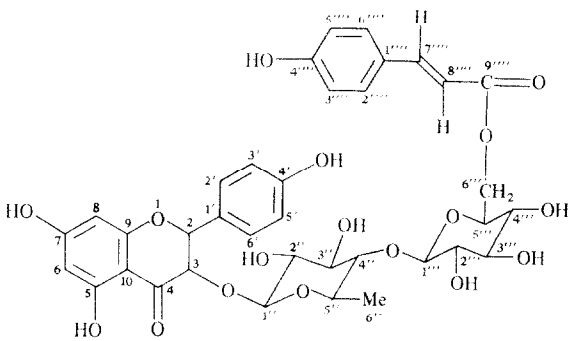
RESULTS AND DISCUSSION

Dried leaves of *Ginkgo biloba* were exhaustively extracted with aqueous acetone. The aqueous acetone residue, defatted first with diethyl ether, was extracted with ethyl acetate. After evaporation, the ethyl acetate extract was passed through an anion exchange resin column. The unadsorbed fraction gives the crude flavonoid extract which was successively refractionated on silica gel and polyamide columns. Finally, the flavonoid **1** was purified on a Sephadex column, and obtained as a pale yellow amorphous solid, mp 337 ± 2°.

Upon acid hydrolysis of **1**, kaempferol, *p*-coumaric acid, D-glucose and L-rhamnose were identified by TLC. Kaempferol and *p*-coumaric acid were also identified by UV and ^{1}H NMR spectroscopy. In UV light, **1** appeared as a dull brown flavone-like fluorescent spot on silica gel and polyamide plates, indicating substitution of the C-3 hydroxyl group. This spot changed to yellow with ammonia and to yellowish-green with NA indicating respectively the presence of free 5- and 4′-hydroxyl groups and the absence of an *ortho*-dihydroxyl group in the B ring. UV maxima in ethanol at 310 and 263 nm and the shifts obtained with diagnostic reagents (see Experimental) suggested the presence of three free hydroxyl groups at positions C-5, C-7 and C-4′ [4]. Therefore, the position of the linkage between kaempferol and the other moiety, occurs at C-3. It is noteworthy that the λ_{max} of band I, at 310 nm shows a very low value (a typical kaempferol C-3 linked glycoside shows a corresponding λ_{max} located about 350–360 nm). This hypsochromic shift of band I is due to the presence of the *p*-coumaroyl group in the side chain.

The MS of **1** exhibited a molecular ion peak at *m/z* 740 in accordance with a *p*-coumaroyl ester of a kaempferol bioside. The fragment ions at *m/z* 595, 433 and 287 showed that glucose was located between rhamnose and *p*-coumaric acid. The MS of peracetylated **1** gave $[M]^+$ at *m/z* 1119, corresponding to the quasi-molecular ion of this derivative. The fragments at *m/z* 707 and 477 suggested that *p*-coumaric acid is in the terminal position. This was confirmed by alkaline hydrolysis, the products of which were identified by TLC as *p*-coumaric acid and a kaempferol bioside. The MS of this kaempferol bioside had fragments at *m/z* 595, 433, and 287, suggesting that rhamnose is directly attached to the kaempferol aglycone.

The ^{1}H NMR spectrum exhibited two doublets at δ6.10 and 7.45 with large coupling constants (16 Hz) which assigned the *trans* configuration to *p*-coumaric acid. The anomeric proton (H-1‴) of the glucose appeared as a doublet at 4.18 (J = 8 Hz). This chemical shift confirmed that glucose is not attached to the kaempferol nucleus. The diaxial coupling (J = 8 Hz) between H-1 Glc and H-2 Glc indicated that the glucose has a β-configuration. The signal at 5.61 (J = 2 Hz) was assigned to H-1 rhamnose, confirming the position of linkage between sugar and aglycone at C-3, and diequatorial coupling (J = 2 Hz)



between H-1 rhamnose and H-2 rhamnose indicated the α-configuration [4].

The ^{13}C NMR spectrum of the sugar moiety of **1** shows a downfield shift of C-6 glc ($\Delta + 2.1$) from the chemical shift values reported, for the corresponding carbon resonances of unlinked C-6 glucose (such as flavonol 3-*O*-glucopyranosides) [5]. These shifts are expected from the substituent effect of C-6 glucose acylation [6]. Otherwise, in the ^{13}C NMR spectrum of kaempferol-3-*O*-(rhamno(1-6) glucoside) [7] the glucose C-6″ signal shift downfield from δ61.0 to 67.1 due to rhamnosylation at C-6″. This evidence excludes other possible acylation sites in the glucose moiety of **1** and shows that *trans-p*-coumaric acid is linked to C-6 glc. Comparison of the rhamnose carbon chemical shifts in the spectrum of **1** and those of unsubstituted methyl-4-*O*-glycosylated-α-L-rhamnoside showed similarities. Thus, the chemical shift for the carbon atom linked to the second sugar in the case of a 2-*O*-glycosylation is at δ79.0, that of a 3-*O*-glycosylation is at 78.8 and in the case of a 4-*O*-glycosylation is at 80.4 [8]. The corresponding atom in **1** resonates at 81.6 indicating that glucose must be linked to the 4-hydroxyl group of the rhamnose moiety. The signal assigned to the rhamnose C-2 (δ71.7) appears to be far downfield compared to the value found for kaempferol 3-*O*-glucorhamnoside (δ70.4) and its monoacetate (δ70.1) [9]. On the basis of these data, we conclude that **1** is kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside). This glycoside is unique in two ways. First, there is the sequence of the sugars in the side-chain in which rhamnose, and not glucose, is directly linked to the C-3 hydroxyl of the aglycone. Secondly, there is the esterification of glucose to *p*-coumaric acid. To our knowledge, only two such glucorhamnosides have been previously reported [9], and a *p*-coumaroyl ester of a flavonol bioside has never been described.

EXPERIMENTAL

General techniques. Chromatography columns employed: ion exchange resin: Amberlite IRN-78 (Prolabo); Silica gel 60 (E. Merck); Polyclar AT (Touzart & Matignon) and Sephadex LH-20 (Pharmacia); Precoated silica gel plates 60 F 254 (E. Merck), cellulose (E. Merck) and micropolyamide foils F 1700 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 NA (Naturstoffreagenz-A) in EtOH. Sugars were visualized by spraying with anisaldehyde soln and after heating at 120°.

Isolation of **1**. Ground, dried leaves (10 kg) were extracted with 60% aq. Me_2CO in a Soxhlet. The extract was evaporated to dryness under red. pres. The residue was then extracted with $CHCl_3$ and EtOAc. The $CHCl_3$ extract was discarded. The concd EtOAc extract (48 g) was chromatographed over an ion resin exchange column 100 g) using H_2O with an increasing ratio of MeOH and then MeOH–0.05 N HCl. The fractions (250 ml) were collected and controlled by TLC. The fraction (22.3 g) eluted with aq. MeOH contained the flavonoids. It was chromatographed over a silica gel column (1.0 kg) packed with EtOAc and eluted with a mixture of EtOAc–MeOH with an increasing ratio of MeOH. The fraction (3 g) containing **1** was chromatographed over a Polyclar column (100 g) packed with $CHCl_3$–MeOH (4:1) and eluted with the same solvent. The kaempferol *p*-coumaroyl glycoside was finally purified on three successive Sephadex columns (15 g) eluted with MeOH. 470 mg of pure glycoside were obtained as an amorphous solid.

The acetate (prepared via Ac_2O–pyridine) recrystallized from EtOH; mp 145°; CIMS 70 eV, *m/z* (rel. int.): 1136 $[M + NH_4]$ (7), 1119 $[M + H]^+$ (42), 1076 $[M - OAc]^+$ (9), 724 (11), 707 (100), 477 (92), 412 (10), 370 (8), 328 (8), 286 (8); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.86 (3H, *d*, *J* = 6 Hz, Me rhamnose), 1.97 (3H), 2.02 (6H), 2.08 (3H), 2.11 (3H), (5 OAc disaccharide), 2.30 (3H), 2.33 (3H), 2.34 (3H), 2.40 (3H), (4 OAc phenolics), 3.2–5.2 (sugar protons), 5.65 (1H, *d*, *J* = 2 Hz, H-1 rha), 6.37 (1H, *d*, *J* = 16 Hz, H-8 coum), 6.81 (1H, *d*, *J* = 2.5 Hz, H-6), 7.05 (2H, *d*, *J* = 9 Hz, H-3′ and H-5′), 7.49 (2H, *d*, *J* = 9 Hz, H-2 coum and H-6 coum), 7.61 (1H, *d*, *J* = 16 Hz, H-7 coum), 7.85 (2H, *d*, *J* = 9H, H-2′ and H-6′).

Kaempferol 3-O-α-(6‴-p-coumaroyl glucosyl-β-1,4-rhamnoside). Mp 337 ± 2°; $[\alpha]_D^{20}$ −60 (EtOH; *c* 1). UV λ_{max}^{EtOH} nm: 355 sh, 310, 263; + NaOAc: 370 sh, 304, 267; + NaOAc–H_3BO_3: 355 sh, 310, 263: + $AlCl_3$: 396 sh, 307 sh, 302, 275, 225; + $AlCl_3$–HCl: 397 sh, 307 sh, 303, 275, 225; ^{1}H NMR (200 MHz, DMSO-d_6): δ0.92 (3H, *d*, *J* = 6 Hz Me rhamnose), 3.06–4.24 (*m*, sugars protons), 4.35 (1H, *d*, *J* = 8 Hz, H-1 glc) 5.61 (1H, *d*, *J* = 2 Hz, H-1 rha), 6.11 (1H, *d*, *J* = 2.5 Hz, H-6), 6.10 (1H, *d*, *J* = 16 Hz, H-8 coum), 6.25 (1H, *d*, *J* = 2.5 Hz, H-8), 6.72 (2H, *d*, *J* = 9 Hz, H-3 coum and H-5 coum), 6.91 (2H, *d*, *J* = 9 Hz, H-3′, and H-6′) 7.37 (2H, *d*, *J* = 9 Hz, H-2 coum and H-6 coum), 7.45 (1H, *d*, *J* = 16 Hz, H-7 coum), 7.72 (2H, *d*, *J* = 9 Hz, H-2′ and H-6′), 8.49 (*s*, OH phenolic), CIMS 70 eV, *m/z* (rel. int.): 741 $[M + H]^+$ (2), 595 (10), 472 (14), 433 (100), 287 (1). ^{13}C NMR (50 MHz, DMSO-d_6): δ17.4 (C-6 rha), 63.1 (C-6 glc), 69.8 (C-5 rha), 70.2 (C-4 glc), 70.4 (C-3 rha), 71.7 (C-2 rha), 73.6 (C-2 glc, C-5 glc), 76.0 (C-3 glc), 81.6 (C-4 rha), 93.9 (C-8), 99.2 (C-6), 100.6 (C-1 rha), 103.3 (C-1 glc), 106.0 (C.10), 113.7 (C-8 coum), 115.4 (C-3′, C-5′), 115.7 (C-3 coum, C-5 coum), 120.3 (C-1′), 124.8 (C-1 coum), 130.0 (C-2′, C-6′), 130.4 (C-2 coum, C-6 coum), 134.2 (C-3), 144.2 (C-7 coum), 156.2 (C-9), 156.5 (C-2), 160.0 (C-4′), 160.2 (C-4 coum), 161.2 (C-5) 165.9 (C-7), 166.4 (C-9 coum), 177.4 (C-4).

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REFERENCES

1. Nakanishi, K. (1967) *Pure Appl. Chem.* **14**, 89.
2. Nakazawa, K. (1941) *J. Pharm. Soc. Japan* **61**, 228.
3. Hänsel, R. and Haas, H. (1984) in *Therapie mit Phytopharmaka*, p. 68. Springer, Heidelberg.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
5. Markham, K. R. and Chari, V. M. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) pp. 40–44. Chapman & Hall, London.
6. Markham, K. R. (1982) in *Techniques of Flavonoid Identification*, p. 82. Academic Press, London.
7. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
8. Liptak, D., Nanasi, P., Neszmelyi, A. and Wagner, H. (1980) *Tetrahedron* **36**, 1261.
9. Yamasaki, K., Kasai, R., Masaki, Y., Okihara, M., Tanaka, O., Oshio, H., Takagi, S., Yamaki, M., Masuda, K., Nonaka, G., Tsuboi, M. and Nishioka, I. (1977) *Tetrahedron Letters* 1231.