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# A NEW FLAVONOL GLYCOSIDE FROM *CERBERA MANGHAS*

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**Key Word Index**—*Cerbera manghas*; Apocynaceae; quercetin tetraglycoside; 3-*O*-(2$^G$-rhamnosylrutinosyl)-7-*O*-β-glucosylquercetin.

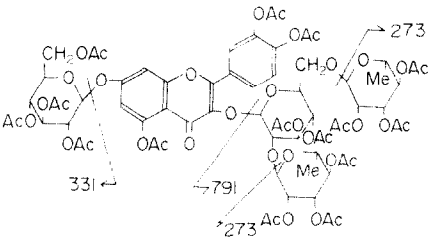
In previous papers [1, 2] the structures of four flavonol glycosides from the leaves of *Cerbera manghas* L. were elucidated. In the present paper a new flavonol tetraglycoside, 3-*O*-(2$^G$-rhamnosylrutinosyl)-7-*O*-β-glucosylquercetin (**1a**) was isolated. The only known flavonol having four sugars is kaempferol-3-sophorotrioside-7-rhamnoside from *Solanum tuberosum* seed.

Acid hydrolysis of **1a** gave quercetin, L-rhamnose and D-glucose. The behavior of the peaks at 258, 268 (shoulder) and 357 nm in the UV spectrum of **1a**, in the presence of NaOAc, NaOMe and $AlCl_3$, respectively, were in agreement with those of quercetin 3-*O*-glucoside-7-*O*-rhamnoside [3], thus indicating the absence of free phenolic groups at C-3 and C-7. **1a** was treated with acetic anhydride and pyridine at room temperature to give the acetylated derivative **1b**. The $^1$H NMR spectral data of **1b** showed the presence of two rhamnose methyl protons as a multiplet, aromatic acetoxyl protons as three singlets and four glycose protons. The glycosylation of the 7-hydroxyl group was indicated by signals at 6.70 ppm (6-H, *d*) and 7.03 (8-H, *d*) to shift downfield [4]. The mass spectrum of **1b** (see Experimental) showed that the parent glucoside contained two rhamnose and two glucose units (Fig. 1.) [5]. Similar mass spectral fragments for sugar and aglycone moieties are reported for eudiposide [6] and 3-*O*-(2$^G$-rhamnosylrutinosyl)-quercetin [2]. Therefore, **1a** is either 3-*O*-(2$^G$-rhamnosylrutinosyl)-7-*O*-β-glucosylquercetin or 7-(2$^G$-rhamnosylrutinosyl)isoquercitrin. The position of the glucosidic and 2$^G$-rhamnosylrutinosidic linkages was determined by enzymatic hydrolysis of **1a** with β-glucosidase to give 3-*O*-2$^G$-rhamnosylrutinosylquercetin and D-glucose. This also proves the β-linkage of the glucose moiety at the 7-position of the molecule. Therefore, **1a** is 3-*O*-(2$^G$-rhamnosylrutinosyl)-7-*O*-β-glucosylquercetin.

## EXPERIMENTAL

The compounds were detected by TLC on precoated Si gel (F-254). MS were obtained by direct inlet, electron energy 20 eV, ion source temp. 290°.

*Isolation of* **1a**. Dried leaves of *Cerbera manghas* L. collected in Okinawa Island, were extracted ×3 with MeOH. The concd extract plus $H_2O$ was extracted successively with $Et_2O$, $CHCl_3$, EtOAc and *n*-BuOH. The *n*-BuOH extract



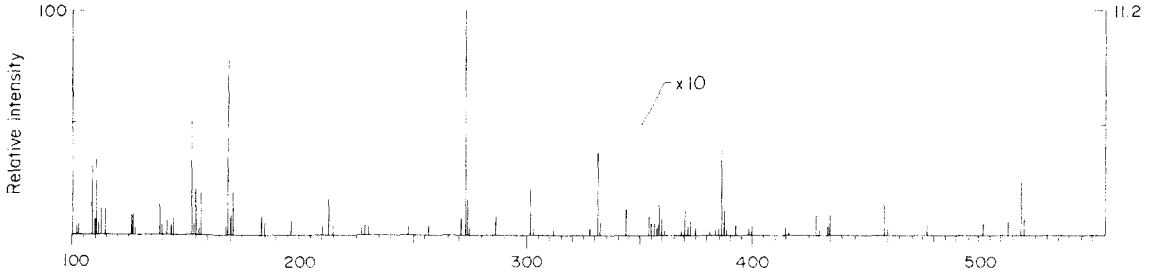




Fig. 1. Mass spectrum of 3-*O*-(2$^G$-rhamnopyranosyl)rutinosyl-7-*O*-β-glucopyranosylquercetin peracetate (**1b**).

was chromatographed on a Si gel column with a $CHCl_3$–MeOH gradient, collecting those fractions which gave a positive HCl–Mg reaction. These fractions were chromatographed on a Si gel column with $CHCl_3$–MeOH (13:7) to give a yellow powder (**1a**).

*3-O-(2$^G$-Rhamnosylrutinosyl)-7-O-β-glucosylquercetin* (**1a**). 60 mg (from abs. MeOH), mp 197–203°, $R_f$ 0.17 on TLC, EtOAc-MeCOEt-HCOOH–$H_2O$ (5:3:1:1) 269 (sh), 357; +$AlCl_3$: 274, 300 (sh), 411.5; +$AlCl_3$/HCl: 270, 300 (sh), 402; +NaOAc: 261.5, 294 (sh), 378, 430 (sh); +NaOMe: 245 (sh), 267, 396.5. IR: KBr—3380 (OH) 2920 (CH), 1660 (C=O), 1600 (C=C), 1200, 1070 (C—O) (Found: C, 48.56; H, 5.61. $C_{39}H_{50}O_{25}\cdot 2\frac{1}{2}H_2O$, requires: C 48.60; H, 5.75%).

*3-O-(2$^G$-Rhamnosylrutinosyl)-7-O-β-glucosylquercetin peracetate* (**1b**). **1a** was treated with $Ac_2O$ and $C_4H_5N$ at room temp. for 7 days to give the acetate (**1b**). $^1H$ NMR (60 MHz, $CDCl_3$): δ 0.85–1.10 (6H, *m*, rhamnose-$CH_3\times 2$) 1.19–2.16 (36H, *m*, glycose-$COCH_3\times 12$), 2.30, 2.35, 2.50 (9H, each *s*, 5, 3′, 4′-$OCOCH_3\times 3$), 3.45–5.70 (30H, *m* glycose-H), 6.70 (1H, *d*, $J=3$ Hz, 6-H), 7.03 (1H, *d*, $J=3$ Hz, 8-H), 7.35 (1H, *br*, 5′-H), 7.93–8.03 (2H, *m*, 2′,6-H) MS *m/e* (rel. int.): 791, 759, 717, 519 (2.3), 428 (1.0) 386 (4.7), 344 (11.0), 331 (38.0), 302 (20.6), 273 (100) 213 (16.5), 169 (78.0), 153 (51.2), 139 (13.1), 111 (34.5) 109 (31.1).

*Enzymatic hydrolysis of* **1a**. **1a** was treated with β-glucosidase (Miles laboratories) at room temp. for 2 weeks, 3-O-(2$^G$-rhamnosylrutinosyl)quercetin ($R_f$ 0.30) was identified by TLC (EtOAc–MeCOEt–HCOOH–$H_2O$, 5:3:1:1, with an authentic sample. D-glucose was identified by GLC.

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# QUERCETAGETIN 5-METHYL ETHER FROM THE PETALS OF *TAGETES PATULA*

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*Tagetes patula* has been examined extensively for its chemical components [1]. The present communication describes the isolation and characterization of a new flavone, allopatuletin, from the petals. Air-dried petals (4 kg) of *T. patula* were extracted successively with petrol, $C_6H_6$ and EtOH. The EtOH extract was extracted with $Et_2O$ and then EtOAc to separate the glycosidic and non-glycosidic fractions. The glycosidic fractions ($Et_2O$ and EtOAc insoluble) yielded patulitrin and quercetagitrin.

The non-glycosidic fraction was chromatographed over a Si gel column using several solvent systems. Elutions with $C_6H_6$–MeOH (93:7; 9:1) gave compounds A and B, $C_6H_6$–MeOH (17:3; 41:9) yielded compounds C and D whereas $C_6H_6$–MeOH (7:3) gave D only. Since these mixtures could not be further resolved by column chromatography the fractions A+B and C+D were acetylated ($Ac_2O$/Py) separately and the resulting acetate mixtures ($A_1+B_1$ and $C_1+D_1$) were separated and isolated by PLC on Si gel ($C_6H_6$–MeOH; 9:1). $A_1$–$D_1$ were deacetylated to obtain A–D using EtOH–HCl (19:1) at 100° for 30 min. On direct comparison with authentic samples, A, B, D and their acetates were identified as luteolin, patuletin, quercetagetin and their acetates, respectively (mp, mmp, TLC, UV, NMR and co-IR). C, a new flavone, allopatuletin was characterized as 3,6,7,3′,4′-pentahydroxy-5-methoxyflavone (**1**).

Allopatuletin (**1**) analysed for $C_{16}H_{12}O_8$, gave a pentamethyl ether (**1a**), a pentaacetate (**1b**), a pentaethyl ether (**1c**) and positive ferric and Mg/HCl tests. Colour reactions, spectral (IR and UV) data and derivatives indicated **1** to be a pentahydroxy flavone. Moreover, the acetate (**1b**) was shown by its NMR