Iridoids from *Rothmannia macrophylla*

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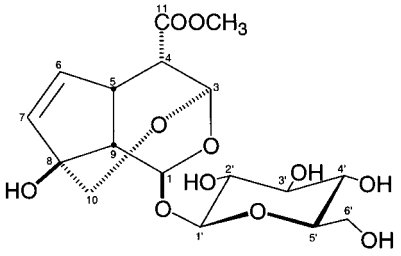
A new iridoid glucoside with an ether linkage between C-3 and C-10 and a novel nonglycosidic iridoid with an ether linkage between C-3 and C-6 and a lactonic linkage at C-1, named macrophylloside (**1**) and macrophyllide (**2**), respectively, were isolated from the leaves of *Rothmannia macrophylla*, along with six known iridoids. Their structures were established by NMR and MS spectroscopies.

The genus *Rothmannia* is represented by about 40 species distributed throughout tropical and subtropical regions of Asia and Africa. Several iridoids, geniposide, mussaenoside, scandoside methyl ester, gardenoside, α- and *β*-gardiol, a dye, and an N-glycoside, were previously isolated from *Rothmannia* species.[1−3]

Rothmannia macrophylla (R. Br. ex Hk. f.) Bremek. (Rubiaceae) is a shrub or small tree found throughout Peninsula Malaysia. It is a dye-producing plant, and the juice has been used by natives for blackening teeth.[4] The root has been used for contraceptive purposes by native communities in the traditional medicinal system.[5] However the chemistry of the plant has not been studied previously. In this paper we describe the isolation and structure elucidation of two new iridoids, macrophylloside (**1**) and macrophyllide (**2**), obtained from the leaves of this plant. Six known iridoids have been isolated: gardenogenin A and B,[1,6] 6α-hydroxygeniposide,[6] 6α-*O*-*cis*-feruloylscandoside methyl ester,[7] 6α-*O*-*trans*-feruloylscandoside methyl ester,[7] and 6α-*O*-*p*-*trans*-coumaroylscandoside methyl ester.[7]



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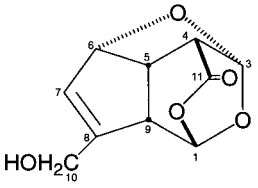

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Compound **1** exhibited the molecular formula $C_{17}H_{24}O_{11}$ (FABMS, *m*/*z* 427 $[M + Na]^+$). The IR spectrum showed absorptions at 3424, 1730, and 1649 cm^{-1}, indicating OH, C=O, and C=C groups, respectively. The ^1H and ^{13}C NMR spectral data suggested the presence of two acetal groups (δ_H 5.69, δ_C 94.0 and δ_H 5.54, δ_C 95.7), one olefin group (δ_H 6.10, δ_C 135.2 and δ_H 5.63, δ_C 137.4), one tertiary hydroxyl group (δ_C 84.5), one methylene (δ_H 3.75 and 3.78, δ_C 67.1), one methoxy (δ_H 3.70, δ_C 52.0), and a *β*-glucopyranosyl moiety. The $^1H-^1H$ COSY experiment gave the gross structure, starting with the acetal proton (δ 5.69), which was coupled with a methine proton (δ 2.52), and in turn coupled to a second methine proton (δ 3.35). The proton at C-5 was further coupled to a methine proton (δ 3.59), which was in turn coupled to an acetal proton (δ 5.54). In the other direction, the proton at C-5 was coupled to an olefinic proton (δ 6.10), which in turn was coupled to another olefinic proton (δ 5.63). These assignments were further confirmed by the $^2J_{CH}$ and $^3J_{CH}$ correlations in the HMBC spectrum giving the cyclopentanopyran ring skeleton and a *β*-glucopyranosyl unit. The point of attachment for the *β*-glucopyranosyl unit (C-1) was confirmed by HMBC correlations between H-1′ and C-1 and between H-1 and C-1′. The C-10 side chain was linked to C-3, forming an ether linkage, based on the downfield shift value of C-10 and the existence of HMBC between C-10 protons with C-3, and vice versa in the HMBC experiment.

The relative configurations at C-1, C-5, and C-9 were determined to be the same as found in other iridoid glucosides based on the small coupling of $J_{1,9}$, (2.0 Hz) and the large coupling of $J_{5,9}$ (8.0 Hz). The stereochemistry of the C-8 OH was determined to be *β* on the basis of the C-9 chemical shift, which is a sensitive probe to establish the configuration of the C-8 OH.[8] It has been observed that the *β* configuration causes the deshielding of C-9 as compared to its α counterpart in the following pairs of C-8 isomers, 10-*des*-cinnamoylglobularimin and 10-*des*-cinnamoylglobularinin,[9] and gardenoside and monotropein methyl ester.[8] The formation of the intramolecular acetal of C-3 with the C-10 oxygen confirmed the C-3 proton to be in *β* configuration. The orientation of the 4-carboxymethoxyl group was assigned from coupling constants among H-3 (*eq*), H-4 (*ax*), and H-5 (*ax*) to be α. Compound **1** was thus elucidated as a rigid tricyclic iridoid. Other iridoids with a similar skeleton have been isolated from *Picrorhiza kurroa*, *Catalpa bignonioides,* and *Rehmannia glutinosa*.[10−12]

The molecular formula of compound **2** was determined as $C_{10}H_{10}O_5$ by HREIMS (*m*/*z* 210.0538, calcd 210.0528), along with ^1H and ^{13}C NMR spectral data. The ^{13}C NMR spectrum showed 10 signals which were characteristic of an iridoid. These signals were shown by the DEPT spectra to consist of seven sp^3 carbons corresponding to two acetals, one methylene, and four methines, and three sp^2 carbons. The gross structure was determined from ^1H NMR and $^1H-^1H$ COSY experiments. The C-3 acetal proton (δ 5.40) was coupled to the methine proton (δ 3.10), which in turn was coupled to a second methine proton (δ 3.25). The C-5 proton

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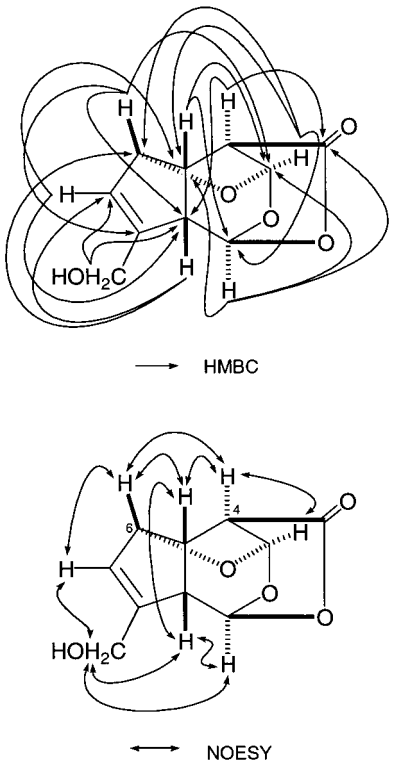

Figure 1. Important HMBC and NOESY of **2**.

was further coupled to two other protons at δ 3.14 and 4.88. The C-6 proton was in turn coupled to the C-7 olefinic proton at δ 5.96. These assignments were confirmed by the $^2J_{CH}$ and $^3J_{CH}$ correlations in the HMBC spectrum (Figure 1). No coupling was observed between H-1 and H-9 since their dihedral angle is nearly 90°. The coupling (8.0 Hz) between H-5 and H-9 indicated a small dihedral angle, demonstrating the *cis* configuration at the ring fusion.

In the ^{13}C NMR spectrum of **2**, the signal of C-6 was significantly shifted downfield and two acetalic carbon signals assignable to C-1 and C-3 were observed. The C-6 proton and carbon were correlated with C-3 and H-3, respectively, in the HMBC experiment. These data suggested that the compound has an ether linkage between C-3 and C-6. The additional HMBC observed between H-1 with C-11 suggested a lactonic linkage between the carbonyl C-11 and C-1. This δ-lactone moiety was confirmed by the IR spectrum (1766 cm^{-1}), and the resulting four-ring structure imposes a rigid conformation. The first report of an iridoid having an ether linkage between C-3 and C-6 was procumboside isolated from *Harpagophytum procumbens* DC.[13] The structure was further confirmed by the NOESY spectrum (Figure 1) in which a cross-peak was observed between H-4 and H-6, arising from the linkage between C-3 and C-6, and C-1 and C-11. To the best of our knowledge, this four-ring skeleton of compound **2** is here described for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded with a JASCO FT/IR-410K spectrometer. Column chromatography was performed with MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries, Ltd.), Chromatorex ODS (Fuji Silysia), and silica gel 60 (0.040−0.063 mm, 0.063−0.200 mm, Merck). MPLC was carried out with prepacked columns, C_{18}-20 and Si-5, equipped with a JASCO PU-986 preparative pump. HPLC was performed on a STR HPLC column (Prep ODS II, ID 20 mm, L 250 mm, Shimadzu Techno Research Inc.) equipped with a JASCO 875-UV detector and a JASCO 880-PU HPLC pump. TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$−CH_3OH−H_2O (9:1:0.1 or 8:2:0.1 or 7:3:0.5 v/v), and spots were detected by UV illumination and by spraying with 10% H_2SO_4 followed by heating. EIMS, positive FABMS, and HREIMS were recorded using a JEOL JMS DX-303 spectrometer, with glycerol as the matrix for FABMS. ^{1}H and ^{13}C NMR spectra were recorded in ppm (δ) in CD_3OD or $CDCl_3$ with TMS as the internal standard, employing Varian Unity plus 500 and Varian Gemini 300 spectrometers operating at 500 and 300 MHz for ^{1}H and 125 and 75 MHz for ^{13}C.

Plant Material. *R. macrophylla* was collected from the forest reserve of Forest Research Institute Malaysia (FRIM), Malaysia, in August 1999. A voucher specimen (FRI 45995) was deposited at the Herbarium of FRIM, Kuala Lumpur, Malaysia.

Extraction and Isolation. Fresh leaves (1.6 kg) were extracted with MeOH by soaking repeatedly (three times). The concentrated extract (98.4 g) was suspended in H_2O and successively partitioned with hexane, ethyl acetate, and butanol. The butanol extract (21.5 g) was chromatographed over MCI gel using H_2O with increasing MeOH (20−100%) to give five fractions. Each fraction was further purified by a combination of column chromatography employing MCI, ODS, and Si, ODS and Si-5 MPLC, and ODS HPLC. Fraction 1 afforded macrophylloside (**1**) (61.6 mg) and 6α-hydroxygeniposide (139.1 mg). Fraction 2 gave an epimeric mixture of gardenogenin A and B (1.7 g). Fraction 3 gave macrophyllide (**2**) (9.3 mg). Fraction 5 afforded 6α-*O*-*cis*-feruloylscandoside methyl ester (5.1 mg), 6α-*O*-*trans*-feruloylscandoside methyl ester (14.0 mg), and 6α-*O*-*p*-*trans*-coumaroylscandoside methyl ester (9.2 mg). The known compounds were identified by comparing their physical and spectral data with the literature values.

Macrophylloside (1): colorless crystals (CH_3OH): $[\alpha]^{22}_D$ +10.52° (*c* 0.5, CH_3OH); IR (dry film) ν_{max} 3424, 1730, 1649 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.10 (1H, dd, J = 3.0, 5.5 Hz, H-6), 5.69 (1H, d, J = 2.0 Hz, H-1), 5.63 (1H, d, J = 5.5 Hz, H-7), 5.54 (1H, d, J = 1.0 Hz, H-3), 4.72 (1H, d, J = 8.0 Hz, H-1′), 3.88 (1H, dd, J = 1.5, 12.0 Hz, H-6a′), 3.78 (1H, d, J = 11.5 Hz, H-10a), 3.75 (1H, d, J = 11.5 Hz, H-10b), 3.70 (3H, s, COOCH_3), 3.67 (1H, dd, J = 5.5, 12.0 Hz, H-6b′), 3.59 (1H, dd, J = 1.0, 8.0 Hz, H-4), 3.35 (1H, ddd, J = 3.0, 8.0, 8.0 Hz, H-5), 3.28−3.40 (3H, m, H-3′, H-4′, H-5′), 3.18 (1H, dd, J = 8.0, 9.0 Hz, H-2′), 2.52 (1H, dd, J = 2.0, 8.0 Hz, H-9); ^{13}C NMR (CD_3OD, 125 MHz) δ 172.4 (s, *C*OOCH$_3$), 137.4 (d, C-7), 135.2 (d, C-6), 99.1 (d, C-1′), 95.7 (d, C-3), 94.0 (d, C-1), 84.5 (s, C-8), 78.2 (d, C-5′), 78.1 (d, C-3′), 74.7 (d, C-2′), 71.6 (d, C-4′), 67.1 (t, C-10), 62.7 (t, C-6′), 53.2 (d, C-9), 52.0 (q, COO*C*H3), 49.6 (d, C-4), 38.0 (d, C-5); positive FABMS *m*/*z* 404 [M + H]$^+$, *m*/*z* 427 [M + Na]$^+$; *anal*. C 49.15%, H 6.26%, calcd for $C_{17}H_{24}O_{11}$·1/2H_2O, C 49.40%, H 6.10%.

Macrophyllide (2): yellow amorphous solid: $[\alpha]^{29}_D$ +0.86° (*c* 0.4, CH_3OH); IR (dry film) ν_{max} 3400, 1766 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.96 (1H, d, J = 1.5 Hz, H-7), 5.72 (1H, s, H-1), 5.40 (1H, d, J = 3.5 Hz, H-3), 4.88 (1H, dd, J = 1.5, 4.5 Hz, H-6), 4.11 (2H, s, H-10), 3.25 (1H, ddd, J = 4.5, 5.5, 8.0 Hz, H-5), 3.14 (1H, d, J = 8.0 Hz, H-9), 3.10 (1H, dd, J = 3.5, 5.5 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz) δ 171.7 (s, C-11), 149.6 (s, C-8), 130.6 (d, C-7), 102.0 (d, C-3), 98.6 (d, C-1), 86.7 (d, C-6), 60.4 (t, C-10), 53.1 (d, C-9), 48.4 (d, C-4), 43.2 (d, C-5); EIMS *m*/*z* 210 [M]$^+$; positive FABMS *m*/*z* 211 [M + H]$^+$; HREIMS *m*/*z* 210.0538 (calcd for $C_{10}H_{10}O_5$, 210.0528).

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