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A NEW TRICYCLIC IRIDOID GLUCOSIDE FROM THE THAI MEDICINAL PLANT, *ROTHMANNIA WITTII*

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Abstract-A new tricyclic iridoid glucoside, rothwittioside, was isolated from the aerial part of *Rothmannia wittii*, along with four known iridoid glucosides, macrophylloside, gardenoside, 6α-hydroxygeniposide and 6β-hydroxygeniposide. The structural elucidations were based on analyses of spectroscopic data.

Rothmannia wittii (Craib) Bremek. (Rubiaceae, Thai name: Mak-Mo) is a tree up to 10 m high, distributed in South-East Asia. It is used in Thai traditional medicine for antifever purposes, as well as an anti-inflammatory agent. In the course of our continuing study on Thai rubiaceous plants,[1] the constituents of this plant were investigated. The phytochemical study of this plant has not previously been reported. The present paper deals with the isolation and structural determination of a new tricyclic iridoid glucoside, rothwittioside (**1**), and four known iridoid glucosides (**2-5**) from the leaves and branches of this plant. Four known compounds were identified as macrophylloside (**2**),[2] gardenoside (**3**),[3] 6α-hydroxygeniposide (**4**)[4] and 6β-hydroxygeniposide (scandoside methyl ester, **5**)[5] by comparison of physical data with literature values and from spectroscopic evidence.

Rothwittioside (**1**) was obtained as an amorphous powder. The molecular formula was determined as $C_{17}H_{24}O_{11}$ by HR-FAB MS spectrometry. The ^{13}C NMR spectral data revealed the presence of one β-glucopyranosyl unit in additon to eleven carbon signals for the aglycone moiety. DEPT experiments indicated that rothwittioside (**1**) contained one methoxy (δ 53.1), one methelene (δ 67.9) and seven methines (δ 36.5, 51.3, 52.0, 93.7, 96.0, 136.4 and 137.0), as well as two quaternary carbons (δ 84.4 and 173.7) for an aglycone, consistent with an iridoid skeleton. The chemical shifts at δ 93.7 and 96.0 were characteristic for acetal groups. The signals at δ 137.0 and 136.4 were assigned to a disubstituted olefin group, suggesting to locate at C-6 and C-7. The ^1H NMR spectrum showed signals at δ 5.64 (d, J = 1.9 Hz), 5.53 (dd, J = 2.4, 0.7 Hz), 2.65 (br d , J = 2.4 Hz), 3.43 (ddd, J = 7.8, 3.4, 0.7 Hz), 5.99 (dd, J = 5.7,

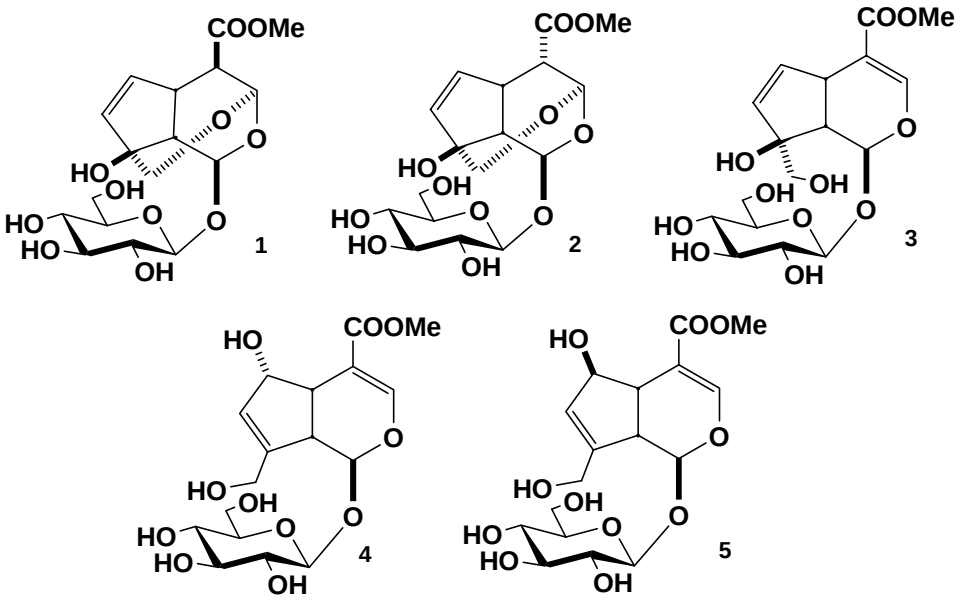

Table 1. ^{13}C NMR spectral data of rothwittioside (**5**) in methanol-d_4 (100 MHz)

C		C	
1	93.7 (CH)	11	173.7 (C)
3	96.0 (CH)	OMe	53.1 (CH_3)
4	51.3 (CH)	1'	99.7 (CH)
5	36.5 (CH)	2'	75.0 (CH)
6	137.0 (CH)	3'	77.3 (CH)
7	136.4 (CH)	4'	71.4 (CH)
8	84.4 (C)	5'	78.3 (CH)
9	52.0 (CH)	6'	62.6 (CH_2)
10	67.9 (CH_2)		

3.4 Hz), 5.54 (d, J = 5.7 Hz) and 2.31 (dd, J = 7.8, 1.9 Hz) assignable for the methine protons H-1, H-3, H-4, H-5, H-6, H-7 and H-9, respectively. Also, it showed an AB type of methylene signals at δ 3.33 and 3.63 (each d, J = 11.7 Hz) attributable to H-10a and 10b. The methyl singlet at δ 3.66 was belonged to a carbomethoxyl functional group at C-4. The doublet signal at δ 4.48 (d, J = 7.8 Hz) of an anomeric proton of a glucosyl moiety indicated the β-configuration. The complete assignments were established by analysing the 2D NMR spectra including COSY, HSQC and HMBC in addition to the coupling constants in the ^{1}H NMR spectrum. In the HMBC spectrum (Figure 1), the long-range correlations were observed between H-10a, 10b and C-3 (δ 96.0), and in turn between H-3 and C-10 (δ 67.9), indicating that the methylene carbon (C-10) linked to C-3 through an ether bond. The glucosyl moiety attached to C-1 from the chemical shift value of this carbon at δ 93.7, and this was confirmed by the HMBC correlations (Figure 1). The coupling constant between H-1 and H-9 (J = 1.9 Hz), H-5 and H-9 (J = 7.8 Hz) led to

conclude the position of protons at C-1, C-5 and C-9 in α, β and β-orientations, respectively. The hydroxyl group at C-8 was determined as β-orientation by the chemical shift found for C-9 at 52.0 ppm,[6] this in turn indicated the orientation of proton at C-3 was β. The appearance of H-4 as a broad doublet (δ 2.65, J = 2.4 Hz) provided a small coupling constant between H-4 and H-5 (J < 1.0 Hz) corresponding to a dihedral angle approximately 90°, showing that the carbomethoxyl group at C-4 was β. Accordingly, the structure of rothwittioside (**1**) was elucidated as the β-epimer at C-4 of macrophylloside (**2**).

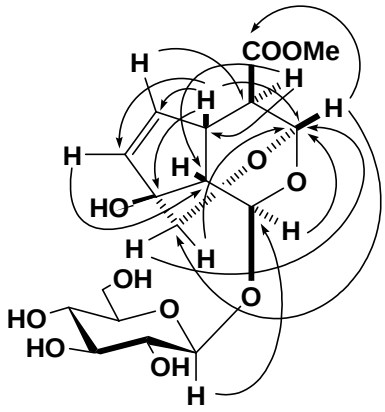

Figure 1. The significant HMBC correlations of rothwittioside (**1**)

EXPERIMENTAL

NMR spectra were recorded in methanol-d_4 using a JEOL JNM α-400 spectrometer. MS spectra were recorded on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on an ODS column (20 x 150 mm i.d., YMC) with a Shimadzu RID-6A refractive index detector. The flow rate was 6 mL/min. For column chromatography, silica gel 60 (Merck), YMC-gel ODS (50 mm, YMC) and Diaion HP-20 (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc-MeOH-H_2O (4:1:0.1), (II) EtOAc-MeOH, H_2O (7:3:0.3), (III) EtOAc-MeOH-H_2O (6:4:1), (IV) 15-50% aq. MeOH, (V) 5% aq. MeCN, (VI) 10% aq. MeCN, and (VII) 15% aq. MeOH.

Plant Material The leaves and branches of *Rothmannia wittii* (Craib) Bremek. was collected in May 2002 from the Botanical Gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The identification was confirmed by Mr. Bamrung Thavinchiua, Department of Pharmaceutical Botany and Pharmacognosy, Khon Kaen University. The voucher specimen is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Extraction and isolation The dried aerial part (2.0 kg) was extracted with MeOH (40 L) under reflux for 20 h. After removal of the solvent *in vacuo*, the resulting residue (166.4 g) was suspended in H_2O and defatted with Et_2O. The aqueous layer was applied to a column of Diaion HP-20 and eluted with H_2O,

MeOH and Me_2CO, successively. The fraction eluted with MeOH (34.6 g) was chromatographed on a column of silica gel (solvent systems I, II and III), affording eight fractions. Fraction 4 (6.2 mg) was applied to a column of ODS using system IV to provide seven fractions. Fraction 4-1 was purified by prep. HPLC-ODS (system V) to give compounds (**2**) (229 mg) and (**4**) (48 mg). Fraction 4-3 was purified by prep. HPLC-ODS (system VI) to give compound (**1**) (12 mg). Fraction 5 (21.2 g) was subjected to a column of ODS (system IV), followed by prep. HPLC-ODS (system V) to afford compound (**3**) (759 mg). Fraction 6 (2.4 g) was similarly purified by a combination of ODS (system IV) and HPLC-ODS (system VII) columns to afford compound (**5**) (27 mg).

Rothwittioside (1) Amorphous powder, $[\alpha]_D^{30}$ −17.8° (MeOH, *c* 0.79); ^{1}H NMR (Methanol-d_4): δ 5.99 (1H, dd, *J* = 5.6, 3.4 Hz, H-6), 5.64 (1H, d, *J* = 1.9 Hz, H-1), 5.54 (1H, d, *J* = 5.7 Hz, H-7), 5.53 (1H, dd, *J* = 2.4, 0.7 Hz, H-3), 4.48 (1H, d, *J* = 7.8 Hz, H-1'), 3.77 (1H, dd, *J* = 12.0, 1.5 Hz, H-6a'), 3.66 (3H, s, COO*Me*), 3.63 (1H, d, *J* = 11.7 Hz, H-10a), 3.57 (1H, dd, *J* = 12.0, 5.4 Hz, H-6b'), 3.43 (1H, ddd, *J* = 7.8, 3.4, 0.7 Hz, H-5), 3.33 (1H, d, *J* = 11.7 Hz, H-10b), 3.28 (1H, dd, *J* = 9.3, 9.0 Hz, H-3'), 3.19-3.22 (2H, m, H-4',5'), 3.07 (1H, dd, *J* = 9.0, 7.8 Hz, H-2'), 2.65 (1H, br d, *J* = 2.4 Hz, H-4), 2.31 (1H, dd, *J* = 7.8, 1.9 Hz, H-9); ^{13}C NMR (Methanol-d_4): see Table 1; Negative HR-FAB-MS, *m/z*: 403.1231 $[M-H]^-$ (cald for $C_{17}H_{23}O_{11}$, 403.1240).

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REFERENCES

1. T. Kanchanapoom, R. Kasai, and K. Yamasaki, *Phytochemistry*, 2002, **59**, 551; T. Kanchanapoom, M. Takanosu, R. Kasai, and K. Yamasaki, *Natural Med.*, 2002, **56**, 20; T. Kanchanapoom, R. Kasai, and K. Yamasaki, *Phytochemistry* (*in press*).
2. S.-K. Ling, T. Tanaka, and I Kouno, *J. Nat. Prod.*, 2001, **64**, 796.
3. F. Bailleul, P. Delaveau, A. Rabaron, M. Plat, and M. Koch, *Phytochemistry*, 1977, **16**, 723.
4. M. Miyagoshi, S. Amagaya, and Y. Ogihara, *Planta Med.*, 1987, **53**, 462.
5. H. Inouye, Y. Takeda, and H. Nishimura, *Phytochemistry*, 1974, **13**, 2219.
6. K. Chaudhuri, F. U. Afifi-Yazar, O. Sticher, and T. Winkler, *Tetrahedron*, 1980, **36**, 2317.