

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, macrophyllside, gardenoside, 6 α -hydroxygeniposide and 6 β -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 macrophyllside (**2**),² gardenoside (**3**),³ 6 α -hydroxygeniposide (**4**)⁴ and 6 β -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 C₁₇H₂₄O₁₁ by HR-FAB MS spectrometry. The ¹³C NMR spectral data revealed the presence of one β -glucopyranosyl unit in addition to eleven carbon signals for the aglycone moiety. DEPT experiments indicated that rothwittioside (**1**) contained one methoxy (δ 53.1), one methylene (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,

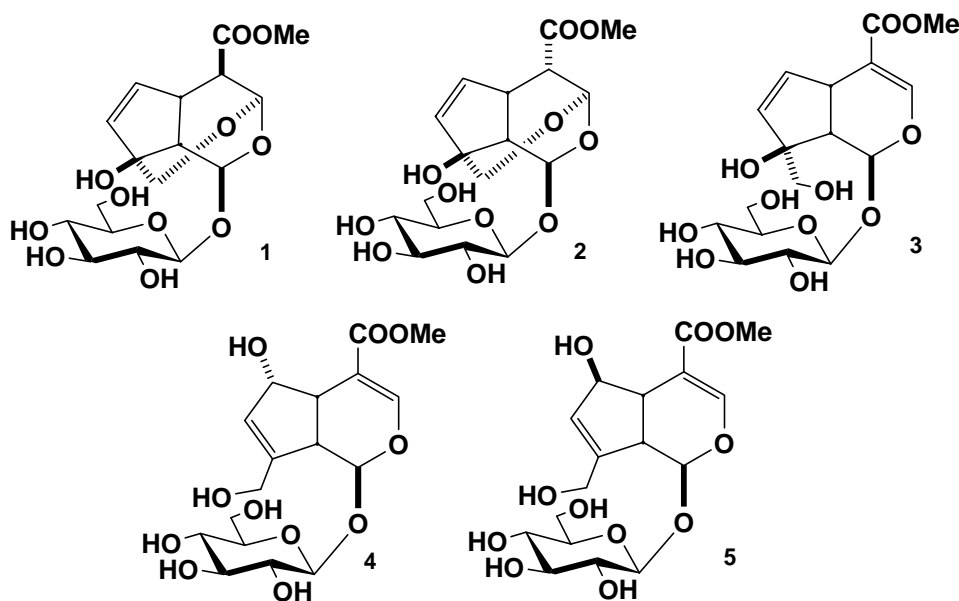


Table 1. ^{13}C NMR spectral data of rothwittioside (**5**) in methanol- d_4 (100 MHz)

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 ^1H 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 macrophyllloside (**2**).

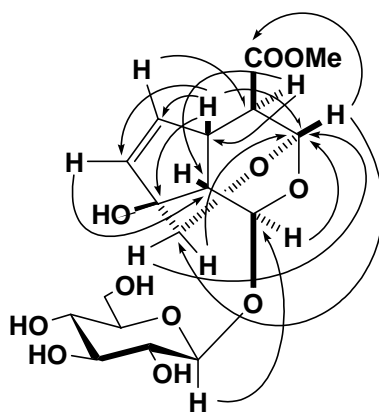


Figure 1. The significant HMBC correlations of rothwittioside (**1**)

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-H₂O (4:1:0.1), (II) EtOAc-MeOH, H₂O (7:3:0.3), (III) EtOAc-MeOH-H₂O (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 Thavinchua, 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 H₂O and defatted with Et₂O. The aqueous layer was applied to a column of Diaion HP-20 and eluted with H₂O,

MeOH and Me₂CO, 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, [α]_D³⁰ -17.8° (MeOH, *c* 0.79); ¹H 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, COOMe), 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); ¹³C NMR (Methanol-*d*₄): see Table 1; Negative HR-FAB-MS, *m/z*: 403.1231 [M-H]⁻ (calcd for C₁₇H₂₃O₁₁, 403.1240).

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Iridoid 2 showed cytotoxicity against the NCI-H187 cancer cell line with an IC₅₀ value of 6.821¹/₄g/mL. In addition, 2 and 5 exhibited weak cytotoxic activity against KB and MCF-7 cell lines, while 4 was active against the NCI-H187 cancer cell line. Download full-text PDF. Source. The first reported study of the isolation and identification of compounds from the bark and fruit of *Rothmannia wittii* yielded two new iridoids, 6¹-hydroxy-10-O-acetylgenipin (1) and 10-O-acetylmacrophyllide (2) together with six known iridoids; 6¹-hydroxygenipin (3), genipin (4), garjamine (5), cerbinal (6), and mixture of 1²-gardiol. The proxy response rate was measured as the ratio of those who completed to those who started the survey. A total of 7285 responses were downloaded from the Qualtrics server on November 25, 2020. The following responses were removed from the dataset: incomplete (those not reaching the end of the survey, n = 2367), no illness onset date (n = 2), onset date before December 2019 (n = 26), 0 days of symptoms (n = 1), duplicate participants (n = 150), symptoms for 28 days or less (n = 401), and. Medicinal plants belong to a big plant group with a great interest due to its pharmaceutical, cosmetic and nutritional application. In addition, they are also an alternative to traditional crop with species in high demand at the current international market. The main purpose of this textbook is that students have an approach to economical importance, uses, botany and harvested processes of the most significant medicinal, aromatic, essential-oil and seasoning species plants. The objectives are The most popular inebriants in society today are wine, beer, and liquor made from the fermentation of fruits and cereals. Wine was first fermented about 6000-8000 years ago in the Middle East, while the first beer was brewed around 5000-6000 BCE by the Babylonians.