SHORT COMMUNICATION

Rotenoids from the flowers of *Millettia brandisiana*

Orasa Pancharoen[1], Soros Petveroj[2] and Souwalak Phongpaichit[3]

Abstract

Pancharoen, O., Petveroj, S. and Phongpaichit, S.
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Songklanakarin J. Sci. Technol., 2007, 29(1) : 151-156

From the hexane extract of the flowers of *Millettia brandisiana* (Leguminosae), four rotenoids, α-toxicarol (1), 12a-hydroxy-α-toxicarol (2), 6-deoxyclitoriacetal (3) and 6a,12a-dehydro-α-toxicarol (4) were isolated. Their structures were determined by spectroscopic methods. In addition, rotenoids 2 and 3 were evaluated for antimicrobial activity and found to be inactive at 128 μg/ml.

Key words : *Millettia brandisiana*, Leguminosae, rotenoids, flowers, antimicrobial activity

[1]Ph.D.(Organic Chemistry), Asst. Prof., [2]B.Sc. Student, Department of Chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom 73000 Thailand. [3]Dr.Sc.hum (Microbiology), Assoc. Prof., Natural Products Research Unit, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkla 90112 Thailand.
Corresponding e-mail: orasa@su.ac.th
Received, 15 March 2006 Accepted, 15 June 2006

บทคัดย่อ

อรษา ปานเจริญ[1] โสพส เพชรเวโรจน์[1] และ เสาวลักษณ์ พงษ์ไพจิตร[2]

สารโรทีนอยด์จากดอกพี้จั่น (*Millettia brandisiana*)

ว. สงขลานครินทร์ วทท. 2550 29(1) : 151-156

ในการแยกสารจากส่วนสกัดด้วยเฮกเซนของดอกพี้จั่น ได้สารกลุ่มโรทีนอยด์ 4 สาร คือ α-toxicarol (1), 12a-hydroxy-α-toxicarol (2), 6-deoxyclitoriacetal (3) และ 6a,12a-dehydro-α-toxicarol (4) การศึกษาหาสูตรโครงสร้างของสารที่แยกได้อาศัยเทคนิคและข้อมูลทางสเปกโตรสโคปี ได้นำสาร 2 และ 3 ทดสอบฤทธิ์ยับยั้งการเจริญเติบโตของเชื้อจุลินทรีย์ ซึ่งพบว่าสารทั้งสองไม่มีฤทธิ์ต่อต้านเชื้อดังกล่าวที่ความเข้มข้น 128 μg/ml

[1]ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศิลปากร วิทยาเขตพระราชวังสนามจันทร์ จังหวัดนครปฐม 73000 [2]หน่วยวิจัยผลิตภัณฑ์ธรรมชาติ ภาควิชาจุลชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Millettia brandisiana Kurz (Leguminosae), known in Thai as "Pee-jan", is a medium tree which is widely distributed in Thailand (Veesommai and Kaewduengtian, 2004). The genus *Millettia* is a rich source of flavonoids (Dewick, 1994). Phytochemical studies of some plants of this genus have resulted in the isolation of a number of isoflavones, rotenoids and chalcones (Yenesew *et al*., 1998: Phrutivorapongkul *et al*., 2003.). To dates, a few species of *Millettia* in Thailand have been investigated. It was found that *M. decipiens* contained chalcones and flavones (Pattanaprateeb and Ruangrungsi, 2003). Chalcones and flavones with antiviral and anti-inflammatory activities were isolated from the stem bark of *M. leucantha* (Phrutivorapongkul *et al*., 2003). Studies of the leaves, stem bark, roots and pods of *M. erythrocalyx* yielded a number of flavones and flavanones (Sritularak *et al*., 2002a and 2002b; Likhitwitayawuid *et al*.,2005; Sritularak and Likhitwitayawuid, 2006). *M. brandisiana* has never been used in traditional medicine. Since there is no report on chemical and biological investigation of *M. brandisiana*, we have undertaken the research on this plant. We report here the isolation and characterization of four rotenoids from the hexane extract of the flowers of *M. brandisiana* as well as the antimicrobial activity of two isolated compounds.

Experimental

General procedure: Melting points were determined using a Kofler hot stage apparatus. UV spectra were obtained on a Hewlett Packard 8453 UV-VIS spectrophotometer. IR spectra were recorded on a Perkin-Elmer GX FT-IR spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AVANCE (300 MHz) spectrometer using TMS as internal standard. HMQC and HMBC spectra were acquired using the standard Bruker software. Optical rotations were measured on a JASCO P1010 digital polarimeter. EIMS were obtained using a Hewlett Packard 5989 B.Vacuum liquid chromatography (VLC) and column chromatography were performed on silica gel (70-230 mesh, Merck).TLC and preparative TLC (PLC) were conducted on silica gel plates (60 F_{254}, Merck).

Plant material: Dried flowers of *M. brandisiana* were collected from Silpakorn University, Sanamchandra Palace campus, Nakorn Pathom in April 2004. The material was identified by Assoc. Prof. Nijsiri Ruangrungsi. A voucher specimen has been deposited at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and isolation: The dried flowers of *M. brandisiana* (690g) were extracted with hexane, EtOAc and MeOH at room temperature to yield the hexane extract (8.0g), EtOAc extract (6.2 g) and MeOH extract (108.3 g), respectively. The hexane extract was fractionated by vacuum liquid chromatography (hexane-EtOAc, gradient) to give 11 fractions. Fraction V (669 mg) was purified by PLC (hexane-acetone, 3:1) to afford 12a-hydroxy-

α-toxicarol **(2)** (34.4 mg). Column chromatography of fraction VI (2.43g) (hexane-acetone, gradient) yielded 8 fractions (VIa-VIh). Fraction VIc was purified by PLC (hexane-acetone, 4:1) and further crystallized from acetone to give 6a,12a-dehydro-α-toxicarol **(4)** (1.8 mg). Purification of fraction VIe by PLC (hexane-CH_2Cl_2-acetone, 8:1:2, multiple runs) gave **2** (135.2mg). Fractions VIf and VIg were purified by PLC (hexane-CH_2Cl_2-acetone, 8:1:2, multiple runs) to yield 6-deoxyclitoriacetal **(3)** (28.6 mg). Fraction VId was purified by PLC (hexane-CH_2Cl_2-EtOAc, 6:1:1, multiple runs) to furnish **1** (17.6 mg), **2** (26.9 mg) and **4** (7.1 mg).

The structures of the isolated compounds were identified by physical properties (m.p., $[\alpha]_D$), spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR and MS) and by comparison with published values. The ^{1}H and ^{13}C-NMR data are shown in Table 1.

Antibacterial assays: Minimum inhibitory concentrations (MICs) were determined by the agar microdilution method (Lorian, 1996) against bacteria (*Staphylococcus aureus* ATCC25923, methicillin-resistant *S. aureus* SK1 (MRSA), *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922) and yeasts (*Candida albicans* NCPF3153, *Cryptococcus neoformans* ATCC90112 and a clinical isolate of *C. neoformans* SH-MU-1). The test substances were dissolved in DMSO (Merck).Serial 2-fold dilutions of the test substances were mixed with melted Mueller-Hinton agar or Sabouraud's dextrose agar (Difco, Becton Dickinson and Company) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc). Final concentrations of the substances in agar ranged from 0.03-128 μg/ml. Inoculum suspensions (10 μl, 10^4 CFU) were spotted on agar-filled wells. The inoculated plates were incubated at 35°C for 18 h for bacteria and *C. albicans* and 48 h for *C. neoformans*. MICs were recorded by reading the lowest concentration that inhibited visible growth. DMSO was used as negative control, vancomycin and gentamycin were used as the standard antibacterial drugs, and amphotericin B was used as the standard antifungal drug.

Results and discussion

The separation of the hexane extract of the flowers of *M. brandisiana* using chromatographic techniques resulted in the isolation of four known rotenoids (Figure 1) including α-toxicarol **(1),** m.p. 88-90°C, $[\alpha]_D$ +43.5° (m.p. 102-104°C, [α]D +34.4°, Jang *et al*., 2003; Andrei *et al*., 1997), 12a-hydroxy-α-toxicarol **(2)**, m.p. 107-110 °C, $[\alpha]_D$ +21.2° (m.p. 226 °C, $[\alpha]_D$ -1.7°, Prashant and Krupadanam., 1993; Andrei *et al*., 1997), 6-deoxyclitoriacetal **(3)**, m.p. 86-90°C, $[\alpha]_D$ +222.5° (m.p. 130-131°C, $[\alpha]_D$ +233°, Lin *et al*., 1992; Mathias *et al*., 1998) and 6a,12a-dehydro-α-toxicarol **(4)**, m.p. 232-234°C (m.p. 261-263°C, Lin and Kuo, 1993; Andrei *et al*., 1997). These four rotenoids were not obtained from other *Millettia sp*. but previously isolated from different leguminous plants, *Tephrosia sp*. and *Clitoria sp*. (Andrei *et al*., 1997; Jang *et al*., 2003; Lin *et al*., 1992; Mathias *et al*., 1998).

The IR spectra of four rotenoids showed hydroxyl and carbonyl absorption bands (3400 and 1640 cm^{-1}). Compounds **1** and **2** have very similar ^{1}H NMR spectra (Table 1). The dimethylchromene system is well characterized by two doublets for the vinyl protons (H-4' and H-5') and a singlet integrating for 6 protons (7'-Me and 8'-Me). The spectra showed three singlets for aromatic protons (H-1, H-4 and H-10) and two singlets for methoxyl groups (2-OMe and 3-OMe). The doublets between δ 4.18-4.89 were assigned to H-6eq, H-6ax and H-6a. The difference in the NMR spectra could be observed, the spectra of **1** showed a doublet of H-12a (δ 3.86) whereas that of **2** showed a broad singlet of 12a-OH (δ 4.19). Compound **1** and **2** were then identified as α-toxicarol and 12a-hydroxy derivative of α-toxicarol, respectively. The ^{1}H NMR spectrum of **3** is similar to that of **2** except for the absence of the dimethylchromene ring. Consequently, **3** was 12a-hydroxyrotenoid (6-deoxyclitoriacetal). The ^{1}H NMR spectrum of **4** displayed a different pattern compared to those of **1, 2** and **3**. The low field signals of H-1 (δ 8.26) and H-6 (δ 5.01) typically represent the protons for a

Table 1. ^{1}H and ^{13}C NMR data for compounds 1,2,3 and 4; δ in ppm and couling constants in parentheses

Position	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	6.88(*s*)	110.3	6.73(*s*)	109.3	6.70(*s*)	109.2	8.26(*s*)	109.7
1a	-	104.4	-	108.4	-	108.3	-	109.9
2	-	144.0	-	144.1	-	144.1	-	144.2
3	-	149.7	-	151.3	-	151.4	-	149.2
4	6.47(*s*)	101.0	6.50(*s*)	101.1	6.50(*s*)	101.2	6.57(*s*)	100.6
4a	-	147.3	-	148.3	-	148.4	-	146.3
6eq	4.18(*d*.12.0)	66.0	4.48(*dd*,0.9,12.0)	63.6	4.47(*d*,11.7)	63.7	5.01(*s*)	64.8
6ax	4.63(*dd*,3.0,12.0)		4.62(*dd*,2.4,12.0)		4.60(*dd*,2.4,11.7)		-	
6a	4.89(dd,3.0,4.2)	71.9	4.55(*dd*,0.9,2.4)	75.7	4.57(*d*,2.4)	75.6	-	156.8
7a	-	155.9	-	155.5	-	161.6	-	150.9
8	-	101.8	-	102.0	6.00(*d*,2.4)	94.6	-	101.1
9	-	162.8	-	164.0	-	169.1	-	159.3
10	5.97(*s*)	97.5	6.00(*s*)	98.0	6.06(*d*,2.4)	95.6	6.30(*s*)	100.7
11	-	164.5	-	163.6	-	164.3		162.4
11a	-	101.2	-	99.9	-	100.2		106.0
12	-	194.2	-	194.8	-	195.0		179.3
12a	3.86(*d*,4.2)	43.5	-	66.8	-	67.0		110.9
4'	6.57(*d*,10.2)	115.4	6.53(*d*,10.2)	115.1	-	-	6.62(*d*,10.0)	114.4
5'	5.48(*d*.10.2)	126.4	5.48(*d*,10.2)	126.6	-	-	5.61(*d*,10.0)	127.8
6'	-	78.3	-	78.6	-	-	-	78.1
7'Me/8'Me	1.45(*s*)/1.38(*s*)*	28.6/28.3*	1.33(*s*)/1.45(*s*)*	28.6/28.4*	-	-	1.50(*s*)	28.2
2OMe	3.81(*s*)	56.3	3.77(*s*)	56.4	3.76(*s*)	55.8	3.95(*s*)	56.4
3OMe	3.83(*s*)	55.9	3.84(*s*)	55.9	3.83(*s*)	56.4	3.90(*s*)	55.9
9OMe	-	-	-	-	3.78(*s*)	55.9	-	-
11OH	12.21(*s*)	-	11.65(*s*)	-	11.54(*s*)	-	13.02(*s*)	-
12aOH	-	-	4.19(*s*)	-	4.21(*s*)	-	-	-

*** interchangeable values**

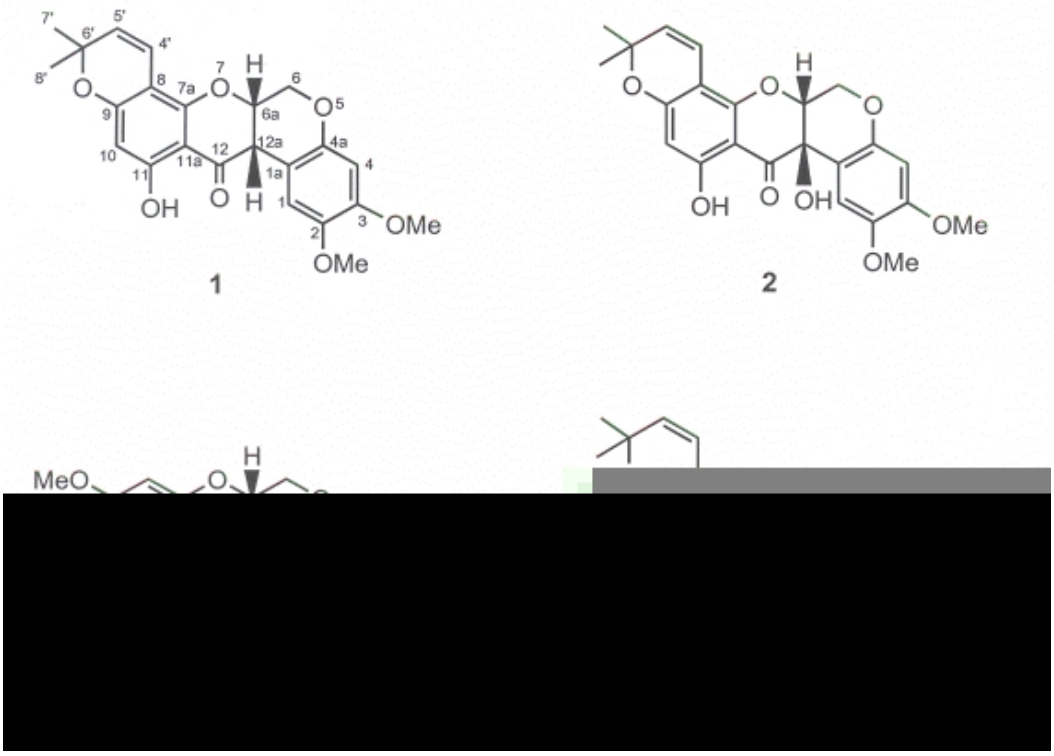

Figure 1. Compounds isolated from the flowers of *M. brandisiana*

dehydrorotenoid having a 6a-12a double bond (Andrei *et al*., 1997). Compound **4** was deduced to be 6a,12a-dehydro-α-toxicarol. The assignments of protons and carbons of the four compounds were confirmed by HMBC correlations.

Compounds **1, 2** and **4** were previously evaluated for their potential chemopreventive properties in a mouse mammary organ culture (MMOC) model,only α-toxicarol (**1**) was found to exhibit an 80% inhibition of DMBA-induced pre-neoplastic lesions at a dose of 10 μg/ml (Jang *et al*.,2003).Rotenoid **3** was reported to possess strong cytotoxic activity against culture **P**-338 lymphocytic leukemia cells (Lin *et al*., 1992).Compounds **2** and **3** were tested for antimicrobial activity in this investigation. Neither of them showed activity against the tested bacteria and yeasts at the highest concentration tested (128 μg/ml). However, compounds **1** and **4** were isolated in small amounts and have not been tested for antimicrobial activity.

Since several chemical studies of *Millettia sp*. in Thailand yielded only flavones and chalcones (Pattanaprateeb and Ruangrungsi, 2003; Phrutivorapongkul *et al*.,2003; Likhitwitayawuid *et al*., 2005; Sritularak and Likhitwitayawuid, 2006).It is interesting to note that this paper is the first report of Thai *Millettia* which produces rotenoids. Further investigation of the EtOAc extract of the flowers is in progress.

Acknowledgements

We are grateful to Ms. Panit Vedchakanchana and Mr. Vitoon Ngow, Department of Chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom, for the low resolution EIMS data. We also thank Assoc. Prof. Nijsiri Ruangrungsi, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, for identification of the plant material.

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