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Antimicrobial Biflavone from *Araucaria bidwillii*

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The dichloromethane extract of the leaves of *Araucaria bidwillii* afforded a biflavone, 4", 4"', 7, 7'-tetra-O-methylcupressuflavone by silica gel chromatography. Its structure was elucidated by extensive 1D and 2D NMR and mass spectrometry. Antimicrobial tests on the compound indicated that it inhibited the growth of *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* It was inactive against *Bacillus subtilis, Candida albicans, Trichophyton mentagrophytes,* and *Aspergillus niger.*

Keywords: *Araucaria bidwillii*; biflavone; 4", 4"', 7, 7'-tetra-O-methylcupressuflavone; antimicrobial

INTRODUCTION

Araucaria bidwillii (bunya pine) is a tall Australian forest tree which was an important food source for nearby Aboriginal people, who gathered every few years in the bunya forests for ceremonies and for feasts of roasted bunya nuts. It is now widely planted as an ornamental, and is found throughout the Philippines, particularly at higher altitudes. It has no known medicinal properties. Several studies have been conducted on the isolation and structural elucidation of the constituents of the plant. These works reported the isolation of 7-O-methylagathisflavone, bilobetin, 7-O-methylcupressuflavone and 7,7'-di-O-methylcuppressuflavone from the leaves of *A. bidwillii* [1,2]. Congeners of the plant, *A. cunninghamii, A. cookii* [3], and *A. excelsa* [4] afforded 4", 4"', 7, 7'-tetra-O-methylcuppressuflavone which is of relevance to our present study.

We now report the isolation, structural elucidation and antimicrobial test results of 4", 4"', 7, 7'-tetra-O-methylcuppres-suflavone, **1** from *A. bidwillii*. This is the first report of the isolation of **1** from *A. bidwillii* , and of its antimicrobial properties.

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *A. bidwillii* afforded **1** by silica gel chromatography with acetone/dichloromethane eluent. The structure of **1** was elucidated by extensive 1D and 2D NMR spectrometry as follows.

The ^{1}H NMR spectral data of **1** (Table 1) indicated resonances for two methoxy groups at δ 3.79 (3H, s) and 3.81 (3H, s), para-disubstituted aromatic protons at δ 6.85 (2H, d, J = 8.64

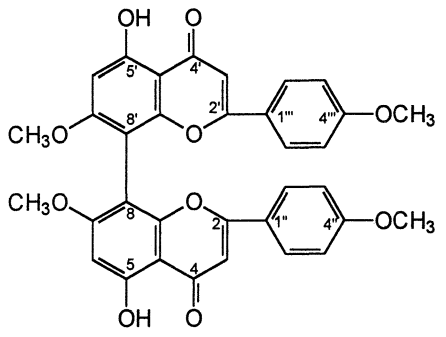

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Table 1. NMR Spectral Data of 1.

Carbon No.	^{13}C, δ	^{1}H, δ (Integration, Multiplicity)	*gHMBC Correlations*
2	163.9	---	H-3
3	103.5	6.58 (1H, s)	---
4	182.9	---	H-3
4a	105.3	---	H-3, H-6, OH
5	162.7[a]	---	OH[b]
6	95.3	6.58 (1H, s)	OH
7	163.3	---	OCH_3
8	99.5	---	H-6
8a	154.7	---	---
1"	123.3	---	H-3, H-3"/5"
2", 6"	127.6	7.42 (2H, d, J = 8.64 Hz)	H-6"/2"
3", 5"	114.6	6.85 (2H, d, J = 8.64 Hz)	H-5"/3"
4"	162.6[a]	---	OCH_3[b]
OCH_3	55.5	3.81 (3H, s)	C-4'
	56.2	3.79 (3H, s)	C-7
OH	---	13.20	C-5, C-6, C-7

[a,b] may be interchanged.

Table 2. Antimicrobial Test Results of 1.

Test Organisms	*Sample*[a]	*Clearing Zone (mm)*[b]	*AI*
E. coli	**1**	13	0.3
	Tetracycline	30	2.0
P. aeruginosa	**1**	13	0.3
	Tetracycline	20	1.0
S. aureus	**1**	16	0.6
	Chloramphenicol	30	2.0
B. subtilis	**1**	---	0
	Chloramphenicol	40	3.0
C. albicans	**1**	---	0
	Chlotrimazole	25	1.5
T. mentagrophytes	**1**	---	0
	Chlotrimazole	38	2.8
A. niger	**1**	---	0
	Cycloheximide	16	0.6

[a]30 µg concentration, [b]Average of three trials.

Hz) and 7.42 (2H, d, J = 8.64 Hz), and further aromatic protons at δ 6.58 (2H, s). A chelated hydroxyl was assigned to the resonance at δ 13.2 (1H, s).

The ^{13}C NMR spectral data of **1** (Table 1) indicated the following functionalities: two methoxy carbons at δ 56.2 and 55.5; six protonated aromatic carbons at δ 95.3, 103.5, 114.6 (2C) and 127.6 (2C); three non-protonated aromatic carbons at δ 99.5, 105.3 and 123.3; five oxygenated aromatic carbons at δ 163.9, 163.4, 162.7, 162,6 and 154.7; and a carbonyl carbon at δ 182.9. Inverse-detected direct bond and long range carbon-proton correlation NMR (Table 1) experiments using gHMQC and gHMBC pulse sequences confirmed **1** as a 5-hydroxy-4',7-dimethoxyflavone, however the identity of the C-8 substituent remained unknown.

The high resolution EIMS of **1** gave a molecular ion at 594.1525; the calculated value for $C_{34}H_{26}O_{10}$ being *m/z* 594.1526. As the molecular ion had twice the number of protons and carbons seen in the ^{1}H and ^{13}C NMR spectra, **1** must therefore be a symmetrical flavone dimer linked through the C-8 carbons. Thus, **1** is identified as 4", 4"', 7, 7'-tetra-O-methylcuppressuflavone, which has been previously isolated from other *Araucaria* species. The NMR data for **1** agree with those reported [3,4] for 4", 4"', 7, 7'-tetra-O-methylcuppressuflavone.

Since flavones are known to have antimicrobial properties, **1** was tested for possible antimicrobial property by the agar cup method. Results of the study (Table 2) indicated that **1** inhibited the growth of the bacteria, *E. coli, P. aeruginosa* and *S. aureus* with activity indices of 0.3, 0.3 and 0.6, respectively. However, it has lower activity compared to the standard antibiotics. It was inactive against *B. subtilis,* and the fungi, *C. albicans, T. mentagrophytes* and *A. niger.*

EXPERIMENTAL

General experimental procedures. The NMR spectra were recorded in $CDCl_3$ with the use of AMX Fourier Transform 300 MHz. The high resolution EIMS were recorded on a Micromass AutoSpec mass spectrometer. Column chromatography was performed with silica gel 60 (70–230 mesh); TLC was performed with plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin-H_2SO_4 and warming.

Sample collection. The sample was collected from Project 4, Quezon City in May 1999. The plant was identified by Maribel Agoo of the Botany Division, National Museum as *A. bidwillii.*

Extraction and isolation. The air-dried leaves of *A. bidwillii* (200 g) were soaked in ethyl acetate for three days, then filtered. The filtrate was concentrated *in vacuo* to afford a crude extract (10 g) which was subjected to gravity column chromatography (dry packing) using increasing proportions of ethyl acetate in petroleum ether (10% increments) as eluents. The 10–20% ethyl acetate in petroleum ether fractions were rechromatographed (2×) in 10% ethyl acetate in petroleum ether to afford 1 (40 mg) after recrystallization from diethyl ether.

Antimicrobial tests. The microorganisms used in these tests were *S. aureus* UPCC 143, *B. subtilis* UPCC 1, *E. coli* UPCC 195, *P. aeruginosa* UPCC 244, *C. albicans* UPCC 2168, *A. niger* UPCC 4063, and *T. mentagrophytes* UPCC 4193. The antimicrobial assay procedure reported in the literature was employed [5]. The test compound was dissolved in 95% ethanol.

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