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A monoterpene from *Pterocarpus indicus* (Leguminosae)

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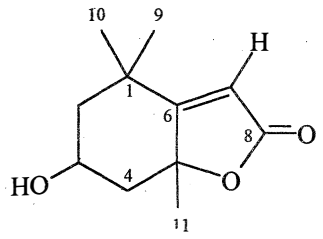
The monoterpene loliolide (**1**), a light yellowish-brown oil, was isolated from the ethyl acetate leaf extracts of *Pterocarpus indicus* by silica gel chromatography. The structure of **1** was deduced by comparison of its ^{1}H- and ^{13}C-NMR chemical shifts with those found in the literature for loliolide. Antimicrobial tests on **1** indicated that it has moderate activity against the fungus, *Candida albicans,* and low activity against the bacteria, *Pseudomonas aeruginosa* and *Escherichia coli,* and the fungus, *Aspergillus niger*. It was found inactive against the bacteria, *Staphylococcus aureus* and *Bacillus subtilis,* and the fungus, *Trichophyton mentagrophytes*.

Keywords: *Pterocarpus indicus*; Leguminosae; loliolide; monoterpene; antimicrobial

INTRODUCTION

Pterocarpus indicus or narra, a common tree in the Philippines known for its valuable wood, also exhibits pharmacological properties. The leaves, wood, bark, and roots, in the form of decoctions and crude extracts, find applications in common diseases like boils, ulcers, prickly heat, stone in the bladder, diarrhea, dysentery, thrush and syphilitic sores [1, 2]. A number of the plant's constituents have been isolated and studied for their bioactivities. Among them are: angolensin, an antifungal component [3]; procycnidin-type tannins having protease inhibitory and antiviral activities [4]; polyphenols with anticancer properties [5, 6]; and, a non-toxic dye as an antibacterial component in shampoos [7].

We now report the isolation, structural elucidation and antimicrobial test results of the monoterpene loliolide (**1**) from *P. indicus*. This is the first report on the isolation of **1** from this source and on its antimicrobial activity.



(1)

EXPERIMENTAL

General experimental procedures. NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Column chromatography was performed with silica gel 60 (70–230 mesh); TLC was performed with plastic-backed plates coated with silica gel F_{254}; and, plates were visualized with vanillin-H_2SO_4 and warming.

Sample collection. Leaves of *P. indicus* were collected from San Pedro, Laguna in October 2000. It was identified as *P. indicus* at the Philippine National Museum.

Isolation. The air-dried leaves (1.5 kg) were ground, soaked in 4 L of ethyl acetate for three days and filtered. The filtrate was concentrated *in vacuo* to afford 43.00 g of crude extract. The extract was dissolved in a minimum volume of anhydrous EtOH and the solution treated with 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components [9]. The treated extract (5.00 g) was fractionated by gravity column chromatography and eluted with ethyl acetate in petroleum ether via gradient elution at 10% increments. The fractions eluted with 40–60% ethyl acetate in petroleum ether were combined and rechromatographed (5×) with 0.5:0.5:9 (acetonitrile:diethyl-ether:DCM) to yield 6.5 mg of compound **1** (light yellowish-brown oil).

Antimicrobial tests. The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *E. coli* UPCC 1195, *P. aeruginosa* UPCC 1244, *S. aureus* UPCC 1143, *B. subtilis* UPCC 1295, *C. albicans* UPCC 2168, *T. mentagrophytes* UPCC 4193, and *A. niger* UPCC 4219.

Microbial suspensions containing approximately 6×10^8 cells/mL (McFarland No. 2) were prepared from 24 h old cultures of the bacteria *E. coli, P. aeruginosa, S. aureus, B. subtilis,* and the yeast *C. albicans* and from 5-day old cultures of the molds *A. niger* and *T. mentagrophytes.* The suspending medium used was 0.1% peptone water. A small amount (0.1 mL) of the suspension of the bacteria, yeast and molds was transferred into pre-poured nutrient agar (NA, DISCO Laboratories, Detroit, Michigan), glucose yeast peptone (GYP) [10] and potato dextrose agar (PDA, DISCO Laboratories, Detroit, Michigan), respectively. About 5 mL of the culture medium, autoclaved and cooled to about 45°C, was poured into a 90-mm petri dish. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. Three 10-mm wells were cut from equidistant points of the seeded agar plates using a sterile cork borer. A solution containing 30 μg of the sample dissolved in 95% EtOH was transferred in each well. For the standard agent, 30 μL of a 1μg of an antibiotic per μL was added.

The NA, GYP, and PDA-based cultures were incubated at 30 ±1°C for 24, 48 and 72 h, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone, in mm. The average diameter of the clear zones was used to calculate an antimicrobial index.

RESULTS AND DISCUSSION

Silica gel chromatography of the ethyl acetate extract from the air-dried leaves of *P. indicus* afforded **1**. Its structure was determined by 1D and 2D NMR spectroscopy as follows.

The ^{1}H-NMR spectral data of **1** (Table 1) indicated chemical shifts at δ 5.68 (1H, s) for a conjugated type olefinic proton; at δ 4.32 (1H, m) for a carbinyl proton of an alcohol; and at δ 1.78 (3H, s), 1.46 (3H, s), and 1.27 (3H, s) for three methyl singlets. In addition, two sets of methylene protons gave resonances at δ 1.97 (1H, td, J = 2.6, 14.0 Hz) with δ 1.66 (1H,m) and δ 2.45 (1H, td, J = 2.5, 14.4 Hz) with δ 1.27 (1H, m).

The ^{13}C-NMR spectral data of **1** (Table 1) point to eleven carbon atoms. A carbonyl carbon gave a resonance at δ 171.9, indicative of a lactone. Two olefinic carbons were attributed to the resonances at δ 182.5 and 112.9. The further deshielding of the carbon in the double bond at δ 182.5 may be due to the steric effect of neighboring methyl groups. Two oxygenated carbons were deduced from the resonances at δ 87.7 and 66.8. Three methyl carbons were assigned to the resonances at δ 30.6, 26.5 and 27.0. Two methylene carbons were also indicated by

Table 1. MHz ^{1}H, ^{13}C and HMBC spectral data of 1 in $CDCl_3$.

Carbon No.	*^{13}C, δ*	*^{1}H, δ*	*HMBC Correlations*
C-1	35.9	–	H-7, H-10, H-9
C-2	47.3	1.97 (1H, Ha, m); 1.66 (1H, Hb, m)	H-4a, H-10, H-9
C-3	66.8	4.32 (1H, m)	H-4a, H-2a, H-4b
C-4	45.6	2.45 (1H, Ha, m); 1.27 (1H, m)	H-2a, H-11, H-2b
C-5	87.7	–	H-7, H-4a, H-11
C-6	182.5	–	H-7, H-4a, H-2a, H-11, H-10, H-9
C-7	112.9	5.68 (1H, s)	–
C-8	171.9	–	H-7
C-9	30.6	1.27 (3H, s)	H-10
C-10	26.5	1.46 (3H, s)	H-2a, H-2b, H-9
C-11	27.0	1.78 (3H, s)	H-4b

Table 2. Comparison of ^{1}H, and ^{13}C spectral data of 1 with loliolide.

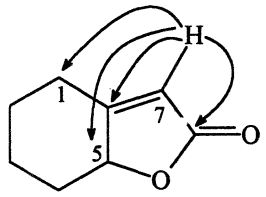
Carbon	**1**		*Loliolide* [8]	
	^{13}C, δ	**^{1}H, δ**	**^{13}C, δ**	**^{1}H, δ**
C-1	35.9	–	35	–
C-2	47.3	1.97, 1.66	50	1.40, 2.05
C-3	66.8	4.32	65	4.10
C-4	45.6	2.45, 1.27	48	1.55, 2.55
C-5	87.7	–	86	–
C-6	182.5	–	181	–
C-7	112.9	5.68	114	5.7
C-8	171.9	–	172	–
C-9	30.6	1.27	30	1.37
C-10	26.5	1.46	25	1.32
C-11	27.0	1.78	26	1.67

the resonances at δ 47.3 and 45.6. A small resonance at δ 35.9 may be due to a quaternary carbon which gave less intense signals than protonated carbons.

The ^{1}H-NMR and ^{13}C-NMR spectral data above are very similar to the NMR spectral data reported for the compound loliolide [8] (Table 2).

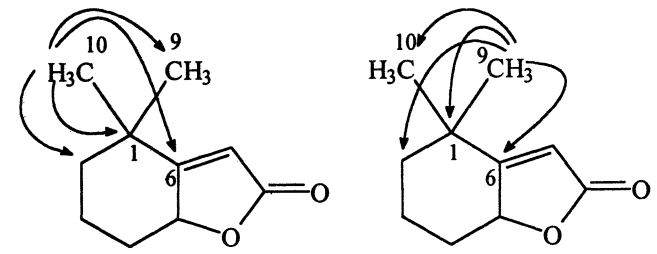
In order to confirm that the oily substance isolated from *P. indicus* is the same as loliolide, HMBC analysis of **1** was conducted. The long-range correlations (Table 1) evident from the HMBC spectrum of the oily substance are the following:

The olefinic proton at δ 5.68 (H-7) is correlated to the carbonyl carbon at δ 171.9 (C-8). This proves the conjugation of the lone double bond to the carbonyl of the lactonic group. This proton also shows correlations to the carbons at δ 87.5 (C-5), 182.5 (C-6) and 35.9 (C-1).

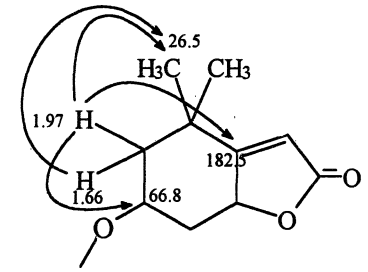


The methyl protons at δ 1.78 (H-11) are correlated to the carbons at δ 46.5 (C-4), 87.7 (C-5), and 182.5 (C-6). These correlations point to the connection of this side group to C-5.

The methyl protons at δ 1.46 (H-10) are long-range correlated to the carbons at δ 35.9 (C-1), 47.3 (C-2), 182.5 (C-6) and 30.6 (C-9). The methyl protons at δ 1.27 (H-9) shows a number of correlations with the carbons at δ 35.9 (C-1), 47.3 (C-2), 182.5 (C-6), and 26.5 (C-10). The HMBC correlations of H-9 and H-10 just described indicate that these two methyl groups are bonded to C-1.



The methylene proton at δ 1.97 (H-2a) exhibits correlations to the carbons at δ 66.8 (C-3), 182.5 (C-6) and 26.5 (C-10). The other methylene proton at δ 1.66 (H-2b) shows correlations to the carbon at δ 26.5 (C-10).



The methylene proton at δ 2.45 (H-4a) is long-range correlated to the carbons at δ 47.3 (C-2), 66.8 (C-3), 87.7 (C-5) and 182.5 (C-6), while the other methylene proton at δ 1.27 (H-4b) shows long-range correlations to the carbons at δ 66.8 (C-3) and 27.0 (C-11).

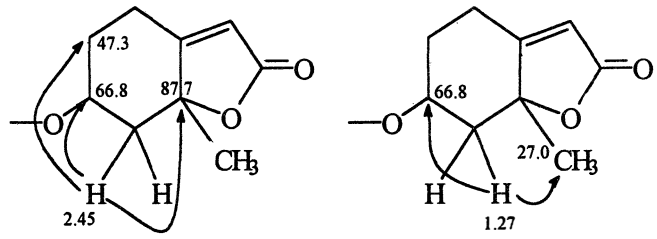


Table 3. Antimicrobial test results on compound 1.

Organism	*Sample* (30 μg)	*Clearing Zone* (mm)			*AI*
		Replicate 1	**Replicate 2**	**Replicate 3**	
E. coli	**1**	12	12	12	0.2
	Chloramphenicol	24			1.4
P. aeruginosa	**1**	12	12	12	0.2
	Chloramphenicol	12			0.2
S. aureus	**1**	–	–	–	0
	Chloramphenicol	28			1.8
B. subtilis	**1**	–	–	–	0
	Chloramphenicol	35			2.5
C. albicans	**1**	15	15	15	0.5
	Clotrimazole	20			1.0
T. mentagrophytes	**1**	–	–	–	0
	Clotrimazole	36			2.6
A. niger	**1**	12	12	12	0.2
	Cycloheximide	13			0.3

All of the above-mentioned HMBC correlations are consistent with the given structure of loliolide.

Some components of *P. indicus* have been reported to have antibacterial [7] and antifungal properties [3], hence, **1** was tested for possible antimicrobial properties. The results of the study (Table 3) indicated that **1** was moderately active against the fungus, *C. albicans,* with antimicrobial index (AI) of 0.5 at a dosage of 30 µg and has slight activity against the fungus, *A. niger,* and the bacteria, *E. coli* and *P. aeruginosa,* with AI of 0.2 at the same dosage. It was inactive against the bacteria, *S. aureus* and *B. subtilis,* and the fungus, *T. mentagrophytes.*

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