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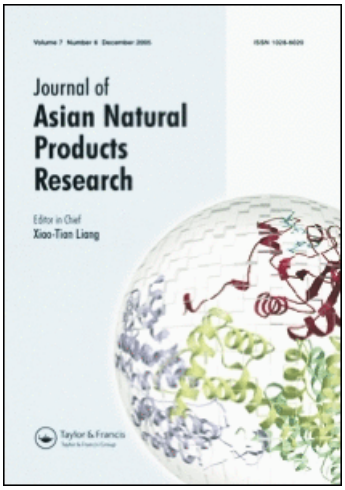

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A TRITERPENE FROM *FICUS PUMILA*

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The leaves of *Ficus pumila* afforded a new neohopane (**1**) by silica gel chromatography. The structure of **1** was elucidated by 1D and 2D NMR and IR spectroscopy and mass spectrometry. It showed antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* with an average antimicrobial index of 0.5, 0.3, 0.3 and 0.7, respectively, at a concentration of 30 μg.

Keywords: *Ficus pumila*; Moraceae; Creeping fig; Neohopane; Triterpene; Antimicrobial

INTRODUCTION

Ficus pumila or creeping fig, an ornamental plant of the family Moraceae grows vigorously in adobe and concrete walls throughout the Philippines. Its leaves are used in the treatment of dysentery and haematuria, while the juice is employed to treat skin diseases [1]. Previous studies on *Ficus pumila* reported the isolation of 1,4-polyisoprenes [2], amyrin acetate, mesoinositol, rutin, sitosterol and taraxenyl acetate [3] and the flavonoids, bergapten and oxypeucedanin hydrate [4].

We now report the isolation, structure elucidation and antimicrobial test results of a new neohopane triterpene (**1**) from the chloroform extract of *Ficus pumila*.

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RESULTS AND DISCUSSION

The chloroform extract of the air-dried leaves of *Ficus pumila* afforded **1**. Its structure was elucidated by extensive 1D and 2D NMR spectroscopy as follows:

The ^{1}H NMR spectrum of **1** (Table I) indicated resonances for five methyl singlets at δ 1.23, 1.09, 0.791, 0.83 and 0.785 and two isopropyl methyl doublets at δ 0.89 ($J = 6.6$ Hz) and 0.93 ($J = 6.6$ Hz). Two geminal carbinyl protons were deduced from the resonances at δ 3.33 (1H, d, $J = 11.1$ Hz) and 4.17 (1H, d, $J = 11.1$ Hz). This was supported by the IR spectrum at 3254 cm^{-1} (OH stretch) and 1004 cm^{-1} (C–O stretch). Another carbinyl proton was attributed to the resonance at δ 3.44 (1H, dd, $J = 11$,

TABLE I Three hundred MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectral Data of 1

Carbon no.	^{13}C, δ	^{1}H, δ
C-1	38.8	0.97 (1H, m), 1.75 (1H, m)
C-2	29.4	1.30 (1H, m), 1.90 (1H, m)
C-3	80.9	3.44 (1H, dd, $J = 11$, 3.5 Hz)
C-4	43.1	—
C-5	56.0	0.83 (1H, m)
C-6	18.6	1.32 (1H, m), 1.65 (1H, m)
C-7	34.9	1.40 (1H, m), 1.65 (1H, m)
C-8	41.5	—
C-9	52.0	1.40 (1H, m)
C-10	43.05	—
C-11	21.9	1.22 (1H, m), 1.50 (1H, m)
C-12	27.9	1.40 (1H, m), 1.70 (1H, m)
C-13	132.0	
C-14	43.0	—
C-15	26.6	1.90 (1H, m), 2.40 (1H, m)
C-16	26.5	1.20 (1H, m), 2.40 (1H, m)
C-17	38.5	—
C-18	142.0	
C-19	37.9	1.28 (1H, m), 1.80 (1H, m)
C-20	27.5	1.40 (1H, m), 1.85 (1H, m)
C-21	59.0	1.02 (1H, m)
C-22	29.8	1.60 (1H, m)
C-23	23.0	0.89 (3H, d, $J = 6.6$ Hz)
C-24	22.9	0.93 (3H, d, $J = 6.6$ Hz)
C-25	17.8	0.785 (3H, s)
C-26	26.6	1.09 (3H, s)
C-27	18.7	0.83 (3H, s)
C-28	17.2	0.791 (3H, s)
C-29	22.3	1.23 (3H, s)
C-30	64.5	3.33 (1H, d, $J = 11.1$ Hz), 4.17 (1H, d, $J = 11.1$ Hz)

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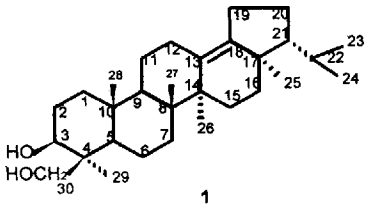
3.5 Hz). This is supported by the IR spectrum at 3254 cm^{-1} (OH stretch) and 1038 cm^{-1} (C–O stretch). The rest of the ^1H NMR signals were overlapping resonances in the relatively shielded region (δ 0.80–2.4). The aliphatic nature of the compound was supported by the IR spectrum at 2934 and 2880 cm^{-1} (aliphatic C–H stretch) and 1455 and 1378 cm^{-1} (aliphatic C–H bending). The seven shielded methyl groups and the relatively shielded proton resonances are characteristics of a triterpene.

The ^{13}C NMR spectrum of **1** (Table I) indicated thirty carbons with the following functionalities: a carbinyl methine carbon at δ 80.9; a methylene carbinyl carbon at δ 64.5; and two non-protonated olefinic carbons at δ 132 and 142; ten methylene, four methine, seven methyl and five quaternary carbons.

The mass spectrum of **1** gave a molecular weight of 442.3821, while the calculated molecular weight for $C_{30}H_{50}O_2$ is 442.3811. From the molecular



1

formula, the index of hydrogen deficiency is six, with only one C=C indicated by the ^{13}C NMR spectrum, the rest of the hydrogen deficiency could be accounted for by a pentacylic system.

The ^1H and ^{13}C assignments (Table I) were verified by HMQC and connectivity was verified by inverse long-range heteronuclear experiment HMBC (Table II) optimized for $J = 10$ Hz. All long-range correlations observed were consistent with the structure of **1**. The position of the double bond was supported by the HMBC which showed long-range correlations between C-13 and the methyl protons at C-26; and C-18 and the methyl protons at C-25. The position of the isopropyl is also supported by HMBC which showed long-range correlation between the C-21 and the methyl protons at C-23, C-24 and C-25. The assignment of the methylene carbinyl (C-30) was confirmed by its long-range correlation with the protons attached to C-5 and C-29. The assignment of the methine carbinyl (C-3) was confirmed by its long-range correlation with the protons at C-29 and C-30.

Compound **1** was tested for its antimicrobial potential, and the results of the study are presented in Table III. Among the seven microorganisms

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TABLE II Three hundred MHz HMBC Spectral Data of **1** in $CDCl_3$

Carbon no.	^1H, δ
C-1	H-2, H-28
C-2	H-1
C-3	H-29, H-30
C-4	H-5, H-29
C-5	H-28, H-29
C-6	H-5, H-7
C-7	H-27
C-8	H-26, H-27
C-9	H-1, H-1′, H-27, H-28
C-10	H-1, H-28
C-11	H-9, H-12
C-12	H-11
C-13	H-12, H-26
C-14	H-15, H-15′, H-26
C-15	H-16, H-26
C-17	H-25
C-18	H-19, H-20, H-25
C-21	H-23, H-24, H-25
C-22	H-21, H-23, H-24
C-23	H-21, H-22, H-24
C-24	H-21, H-22, H-23
C-25	H-16, H-16′
C-26	H-15, H-15′
C-27	H-7, H-9
C-28	H-1, H-9
C-29	H-6
C-30	H-5, H-29

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tested, **1** was found to be active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* with an average antimicrobial index of 0.5, 0.3, 0.3 and 0.7, respectively, at a concentration of 30 μg. For the antibiotics chloramphenicol for *B. subtilis*, tetracycline for *P. aeruginosa* and *E. coli* and chlotrimazole for *C. albicans*, the average antimicrobial index are 1.0, 1.5 and 2.0, respectively, at a concentraton of 30 μg. Thus, **1** has a moderate antimicrobial activity.

EXPERIMENTAL SECTION

General Experimental Procedures

The melting point was obtained by the use of Fischer–Johns melting point apparatus. The polarimeter used was Optical Activity Ltd. The IR analysis

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TABLE III Antimicrobial test results on **1**

Sample	*Concn.* (μg)	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus niger*		*Trichophyton mentagrophytes*	
		C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z.	A.I.
1	30	11, 11	0.1	15, 15	0.5	12, 12	0.3	15, 11	0.3	17, 17	0.7	12, 12	0.2	—	0
Standard antibiotic	30	20 Chloramphenicol	1.0	25 Tetracycline	1.5	25 Tetracycline	1.5	20 Chloramphenicol	1.0	30 Chlotrimazole	2.0	30 Chlotrimazole	2.0	30 Chlotrimazole	2.0

was carried out on a Perkin Elmer 1600 Fourier Transform IR spectrometer. NMR spectra were recorded in $CDCl_3$ with the use of Bruker AMX Fourier Transform 300 MHz NMR spectrometer. The high resolution ESIMS were recorded on a Bruker BioApex 47e (FTMS) mass spectrometer. Silica gel 60 (70–230 mesh); CC plastic backed plates coated with silica gel F_{254} TLC. Fractions were monitored by TLC and spots were visualized by spraying with vanillin/H_2SO_4 then warming.

Plant Material

The plant was collected in December 1995 in Kalookan City. It was identified at the National Museum as *Ficus pumila* and deposited at the Philippine National Herbarium (PNH # 14540).

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Extraction and Isolation

Air dried leaves (889 g) were soaked in chloroform (3.5 L), then filtered. The filtrate was concentrated under vacuum to afford a crude extract which was treated with 4% aqueous $Pb(OAc)_2$ solution to precipitate the pigments [5]. The treated extract (12.8 g) was subjected to gravity column chromatography packed with silica gel (60–230 mesh) and eluted with increasing proportions of acetone in chloroform (10% increment). The fractions eluted with 20–30% acetone in chloroform were rechromatographed with 20% acetone in chloroform to afford **1**, 10 mg, colorless crystal, m.p. = 228–230°C, $\alpha_D = +96.2$ (C 0.026, $CHCl_3$); ^1H NMR and ^{13}C NMR data, see Table I; IR (neat) ν_{max} 3254 (OH), 1004, 1038 (C–O), 2934, 2880, 1455, 1378 (CH); ESIMS *m/z* [MH] + 442 (14), 318 (3), 229+ (17), 218 (9), 205 (23), 203 (14), 189 (26), 187 (18), 175 (46), 173 (21), 161 (54), 159 (36), 149 (24), 147 (54), 145 (51), 135 (28), 133 (68), 131 (40), 121 (68), 119 (92), 109 (48), 107 (97), 105 (100).

Antimicrobial Test

Microbial suspension containing approximately 6×10^8 cells/mL was prepared for each test organism for 24 h culture of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* and from a 5 day old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water.

One-tenth (0.1) mL of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* were transferred into pre-poured nutrient agar (NA) plates. While

A. niger and *T. mentagrophytes* were transferred into pre-poured potato dextrose agar (PDA) plates. About 5 mL of corresponding medium, melted and cooled to about 45°C was poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. One cm wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Thirty (30) μg of samples and standard agent were used. The clearing zones (CZ) were measured and the activity index (AI) was calculated by subtracting the diameter of the well from the clearing zone and dividing by the diameter of the well.

Acknowledgment

The antimicrobial tests were conducted at the University of the Philippines-Natural Sciences Research Institute (UP-NSRI).

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References

[1] Quisumbing, E. *Medicinal Plants of the Philippines* 1951, Bureau of Printing: Manila.
[2] Nwadinigwe, C.A. *Phytochemistry* 1988, **27**(7), 2135–2136.
[3] Ts'eng, K. *Yao Hsueh Hsueh Pao* 1965, **12**(9), 577; *Chemical Abstr.* 1965, **64**: 17353b.
[4] Juan, E.A., Ragasa, C.Y. and Rideout, J.A. *Philippine Journal of Science* 1997, **26**(2), 143–153.
[5] Padolina, W.G., Yoshioka, H., Nakatani, N., Mabry, T.J., Monti, S.A., Davis, R.E., Cox, P.J., Sim, G.A., Watson, W.H. and Wu, I.B. *Tetrahedron* 1974, **30**, 1161.