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Porphyrins and Polyprenol from *Macaranga tanarius.*

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ABSTRACT

The dichloromethane extract of the air-dried leaves of *Macaranga tanarius* afforded chlorophyllide a (**1**), chlorophyll a (**2**), and polyprenol (**3**). The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy, while the structure of **3** was deduced by comparison of its ^{1}H NMR data with those reported in the literature. Antimicrobial tests on **1** indicated that it has low antimicrobial activities against the bacteria: *E. coli, S. aureus,* and *P. aeruginosa* and fungi: *C. albicans* and *T. mentagrophytes.* It was inactive against *B. subtilis* and *A. niger.* Compound **2** was found to exhibit low antimicrobial activities against *P. aeruginosa* and *C. albicans* and inactive against *E. coli, S. aureus, B. subtilis, A. niger,* and *T. mentagrophytes.*

Keywords: *Macaranga tanarius*, Euphorbiaceae, chlorophyll a, chlorophyllide a, polyprenol

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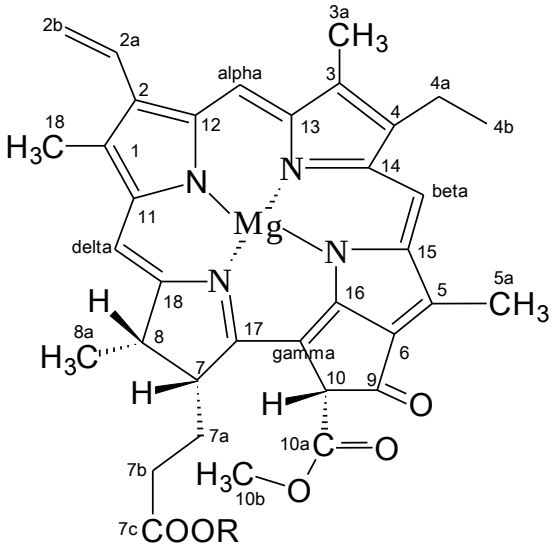
INTRODUCTION

Macaranga tanarius, commonly known as binunga grows in thickets and groves at low and medium altitudes throughout the Philippines. The leaves of the tree are used as an anti-inflammatory [1]. The 70% acetone extract of the leaves of *M. tanarius* was reported to have *in vivo* hepatoprotective activity in mice [2] and *in vitro* antihepatotoxic activity [3]. A recent study reported that *M. tanarius* methanolic leaf extract exhibited antimicrobial property against gram-positive bacteria, but was inactive against gram-negative bacteria [4]. Among four *Macaranga* species tested, *M. tanarius* exhibited the best ferrous-ion chelating (FIC) activity, but showed the lowest total phenolic content (TPC), ascorbic acid equivalent antioxidant activity (AEAC), ferric-ion reducing power (FRAP) and lipid peroxidation inhibition (LPI) activities [4]. Another study reported that the methanolic extract of *M. tanarius* exhibited DPPH radical-scavenging activity [5].

Previous studies on *M. tanarius* reported the isolation of euphorbiasteroid, macarangonol, friedelin, fiedelan-3β-ol, friedel-3-ene, β-amyrenone, and β-amyrin [6], tanarifuranonol, blumenol A and B, and nymphaeol A, B and C [1], roseoside [7], 2003), flavone and tanariflavanone A, B, C and D [8], hyperin, isoquercitrin, macarangioside, and methyl brevifolin carboxylate [5], mallotinic acid, geraniin, tanarinin, corilagin, macaranganin, repanducinic acidphylanthusiin C, 1,4-digalloylglucoside, and 3,4-digalloylglucoside [9, 10].

We report herein the isolation, structure elucidation, and antimicrobial assay of chlorophyll a and chlorophyllide a from the leaves of *Macaranga tanarius*. Polyprenol was also isolated from the leaves of the plant.



1 R = H

2 R = phyltyl

Figure 1: Structures of chlorophyllide a (1) and chlorophyll a (2)

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MATERIALS AND METHODS

General Experimental Procedure

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), while the TLC was performed with plastic backed plates coated with silica gel F_{254}. The plates were visualized by spraying with vanillin-H_2SO_4, followed by warming.

Sample Collection

The plant sample was collected from Anao, Tarlac in June 2006. It was identified as *Macaranga tanarius* (Linn.) Muell.-Arg. at the Botany Department of the National Museum. A voucher specimen # 122 is deposited at the Chemistry Department, De La Salle University-Manila.

Isolation of Secondary Metabolites

The air-dried aerial leaves of *M. tanarius* (377 g) were cut and homogenized, soaked in CH_2Cl_2 for three days, then filtered. The filtrate was concentrated under vacuum to afford the crude extract (88.5 g) which was subjected to silica gel chromatography and eluted with increasing proportions of acetone in CH_2Cl_2 at 10% increment. The pure acetone fraction afforded **1** (429.7 mg, 211-214^{0}C) after washing with petroleum ether, followed by diethyl ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2×) in 15% EtOAc in petroleum ether, followed by rechromatography (2×) in 10% EtOAc in petroleum ether to afford **2** (565.8 mg, mp. 156-158^{0}C) after washing with petroleum ether. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3×) in 7.5% EtOAc in petroleum ether to afford **3** (25 mg).

Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Aspergillus niger* UPCC 4219, *Candida albicans* UPCC 2168, *Bacillus subtilis* UPCC 1295, *Pseudomonas aeruginosa* UPCC 1244, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, and *Trichophyton mentagrophyte* UPCC 4193. The test compound was dissolved in 95% ethanol. The antimicrobial assay procedure reported in the literature [11] was employed. The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *Macaranga tanarius* afforded chlorophyllide a (**1**), chlorophyll a (**2**), and polyprenol by silica gel chromatography.

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Compound **1** was a dark violet crystalline substance isolated from the dichloromethane extract of the air-dried leaves of *Macaranga tanarius*. It gave a dark green spot on TLC when sprayed with vanillin:H_2SO_4 solution. These properties are characteristics of chlorophylls. These green photosynthetic pigments were known to exist in four types, all with the same basic structure, but show variations in the nature of aliphatic side chains attached to the core macrocycle. However, only chlorophylls a and b occur in higher plants, while chlorophylls c and d are found in algae. Literature search revealed that chlorophyll a has a derivative called chlorophyllide a which has a carboxylic acid, instead of phytyl esterified to the carboxylic acid in chlorophyll a. Chlorophyll b on the other hand, has an aldehyde replacing one of the methyl groups in chlorophyll a.

The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **1** (Table 1) indicated allylic methyls at δ 3.19, 3.36 and 3.37, a methyl doublet at δ 1.82, and a methyl triplet at δ 1.68; a methoxy group at δ 3.86; olefinic protons at δ 6.15, 6.26 and 7.41; methylene protons at δ 2.31 and 2.56, 2.28 and 2.61, and 3.62 (2H); aromatic protons at δ 8.55, 9.33 and 9.48; and methine protons at δ 4.19, 4.46 and 6.24.

The ^{13}C NMR spectrum of **1** (Table 1) gave resonances for conjugated olefinic carbons, most of which were deshielded due to the electron withdrawing effect of nitrogen in the macrocycle; a carboxylic acid at δ 177.03; an ester at δ 169.6; and a conjugated carbonyl carbon at δ189.6. The remaining resonances were attributed to methyl, methylene, methine and quaternary carbons in **1**.

Many of the proton resonances were singlets, hence they did not show cross peaks in the COSY spectrum. Thus, the COSY spectrum indicated only three isolated spin systems as follows: H-2a/H_2-2b; H_2-4a/H_3-4b; and H-8/H-7/H_2-7a/H_2-7b.

The ^{1}H and ^{13}C assignments for **1** (Table 1) were verified by HSQC and connectivities were verified by HMBC. Thus, the methoxyl was attached to C-10a on the basis of long-range correlation between the methoxy protons at δ 3.86 and the ester carbonyl at δ 169.62. The conjugated ketone was assigned to C-9 since long-range correlation was observed between H-10 and this carbon. The carboxylic acid was attributed to δ 177.03 (C-7c) due to long-range correlation between the methylene protons at δ 2.28, 2.31, 2.56, and 2.61 and this carbon. The methyl doublet was assigned to C-8a since long-range correlations were observed between H-7, H-8 and this carbon. The methyl singlets were attributed to C-1a, C-3a and C-5a on the basis of long-range correlations between the methyl protons (δ 3.19, 3.37 and 3.66) and the C-1, C-3 and C-5 carbons, respectively. The olefinic protons (H-2a and H_2-2b) were attached to C-2 since long-range correlations were observed between these protons and C-2. The ethyl group was attached to C-4 due to correlations between H_3-4b and H_2-4a and this carbon. All long-range correlations observed were consistent with the structure of **1**. To confirm the structure of **1**, its

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^{1}H and ^{13}C NMR spectral data were compared with those found in the literature [12] for chlorophyllide a. The spectra matched in all essential respects.

Table 1: 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR Spectral Data of 1 and 2 in $CDCl_3$.

Position	δ_C, **1**	δ_H, **1**	δ_C, **2**	δ_H, **2**
1	131.89	3.37	131.78	3.38
1α or 5α	12.06		12.08	
2	136.54		136.45	
2a	129.04	7.94	129.03	7.95
2b	122.78	6.15, 6.26	122.68	6.15, 6.26
3	136.20		136.09	
3a	11.19	3.19	11.12	3.14
4	145.22		145.11	
4a	19.41	3.63	19.35	3.57
4b	17.37	1.68	17.36	1.65
5	128.89		128.97	
5a or 1a	12.06	3.66	12.08	3.68
6 or 17	161.15		161.25	
7	51.02	4.19	51.17	4.23
7a	30.73	2.31, 2.56	31.24	2.24, 2.52
7b	29.65	2.28, 2.61	29.70	2.38, 2.67
7c	177.03		172.97	
8	50.10	4.46	50.14	4.49
8a	23.08	1.82	23.08	1.85
9	189.59		189.66	
10	63.67	6.24	64.74	6.29
10a	169.62		169.64	
10b	52.86	3.86	52.84	3.92
11	142.09		142.83	
12	136.26		136.16	
13	155.67		155.56	
14	150.96		150.92	
15	137.93		137.91	
16	149.67		149.67	
17 or 6	161.15		161.25	
18	172.11		172.21	
α	97.54	9.33	97.44	9.26
β	104.42	9.48	104.32	9.43
γ	105.15		105.24	
δ	93.14	8.55	93.09	8.56
Phytyl				
P1			61.49	4.51
P2			117.76	5.18
P3			142.02	
P3a			16.29	1.60
P4			39.80	1.91
P5			24.99	1.28
P6			36.64	1.02, 1.19
P7			32.62	1.33
P7a			19.66 or 19.72	0.79, 0.81
P8			37.33	1.02, 1.19
P9			24.42	1.33
P10			37.39	1.02, 1.19
P11			32.76	1.25

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P11a			19.66 or 19.72	0.79, 0.81
P12			37.26	1.02, 1.19
P13			24.77	1.25
P14			39.36	1.14
P15			27.96	1.51
P15a			22.62	0.87
P16			22.71	0.87

To determine its relative configuration, the NOESY spectrum of **1** was obtained. There were three asymmetric centers located at C-7 (δ 51.02), C-8 (50.10) and C-10 (64.67) with assigned proton resonances at δ 4.19, 4.46 and 6.24, respectively. A cis relationship existed between the protons at δ4.19 and 4.46 since both of them showed correlation in NOESY. This relation in effect implied that the methyl group anchored at C-8 was in a cis orientation relative to the propionic acid side chain attached to C-7. On the other hand, the proton resonating at δ 6.24 bonded to the third stereocenter at C-10 showed NOESY correlation with H-7. Consequently, the methyl ester group bound to this stereo carbon was oriented in the manner analogous to the bulky substituent of C-7 and C-8. Thus, the relative stereochemistry of **1** is as shown in Fig. 1.

Comparison of the ^{1}H NMR spectral data of **1** and **2** (Table 1) gave similar resonances. The difference was additional resonances were found in the shielded region of **2** which are characteristic resonances for phytol [13]. This was confirmed by the ^{13}C NMR spectrum of **2** which gave twenty additional carbon atoms and the shielding of the carboxylic acid carbonyl in **1** (δ 177.0) to the ester carbonyl (δ 173.0) in **2.** Comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with chlorophyll a [14] indicated that they match in all essential respects.

Chlorophyll and its various derivatives are used in traditional medicine and for therapeutic purposes [15]. Natural chlorophyll and its derivatives have been studied for wound healing [16], anti-inflammatory properties [17], control of calcium oxalate crystals [18], utilization as effective agents in photodynamic cancer therapy [19-21], and chemopreventive effects in humans [22-23]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [24].

The structure of **3** was deduced by comparison of its ^{1}H NMR data with those reported in the literature for polyprenol [25]. Polyprenols act as co-enzymes of membrane active transport systems for polysaccharides, peptidoglycans and carbohydrate containing biopolymers [26]. Polyprenol from *Ginkgo biloba* L exhibited antitumor activity *in vivo* and *in vitro* [27]. The antidyslipidemic activity of polyprenol from *Coccinia grandis* in high-fat diet-fed hamster model was also reported [28].

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, **1** and **2** were tested for antimicrobial activity against seven microorganisms. Results of the study (Table 2) indicated that **1** has low activity against the bacteria: *E. coli, S. aureus,* and *P. aeruginosa* and fungi: *C. albicans* and *T. mentagrophytes.* It was inactive against *B. subtilis* and *A. niger.* Compound **2** was found to exhibit low antimicrobial activity against *P. aeruginosa* and

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C. albicans and inactive against *E. coli*, *S. aureus*, *B. subtilis*, *A. niger*, and *T. mentagrophytes*. The slightly higher antimicrobial activity of **1** as compared to **2** may be due to the presence of the carboxylic acid in **1**.

Table 2: Antimicrobial Test Results on 1 and 2

Organism	Sample (30 µg)	Clearing Zone (mm)[a]	Antimicrobial Index (AI)
E. coli	**1**	11	0.1
	2	-	0
	Chloramphenicol[a]	33	2.8
P. Aeruginosa	**1**	12	0.2
	2	11	0.1
	Chloramphenicol[a]	13	0.3
S. aureus	**1**	11	0.1
	2	-	0
	Chloramphenicol[a]	20	3.2
B. subtilis	**1**	-	0
	2	-	0
	Chloramphenicol[a]	20	2.3
C. albicans	**1**	12	0.2
	2	12	0.2
	Chlotrimazole[b]	17	0.7
T. mentagrophytes	**1**	11	0.1
	2	-	0
	Chlotrimazole[b]	50	7.3
A. niger	**1**	-	0
	2	-	0
	Chlotrimazole[b]	17	0.7

[a] Average of three trials [a]chloramphenicol disc - 6 mm diameter, [b]chlotrimazole conc. 50 µg, disc - 6 mm diameter

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