Antimicrobial Sesquiterpenoids and Diarylheptanoid from *Curcuma domestica*

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Abstract
The rhizomes of *Curcuma domestica* afforded curcumin (1), bisacurone (2), a mixture of ar-turmerone (3), β-turmerone (4), and α-turmerone (5), and ar-curcumyl alcohol (6). These compounds were identified by NMR spectroscopy and comparison of their spectroscopic data with those reported in the literature. Antimicrobial tests on 1-6 indicated that 2-6 have moderate antifungal activity against *A. niger*, while 3-6 have moderate antibacterial activity against *P. aeruginosa.*

Keywords: *Curcuma domestica*, sesquiterpenoids, diarylheptanoids, antimicrobial.

Introduction

Curcuma domestica Valet (syn. *Curcuma longa* L.) is widely distributed in the Philippines. The rhizomes are commonly sold in the markets as a condiment, an ingredient of curry powder, and for coloring. In the Philippines, the rhizomes are used as stomachic and vulnerary agents. The juice of the fresh rhizome acts as an anthelmintic. It is given for flatulence and dyspepsia, and administered in intermittent fevers.[1] Earlier studies on *C. longa* reported the isolation of curcumin, dihydrocurcumin, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-hept-1-en-3,5-dione,[2] and sesquiterpenes from the rhizomes of the plant which are of relevance to out present report.[3] We now report the identification of 1-6 from the freeze-dried rhizomes of *C. domestica* colleted in the Philippines. Antimicrobial test results of 1-6 are likewise reported. This is the first report on the antimicrobial activities of 1-6. Previous to this study, antimicrobial activities of turmeric oil have been reported.[4-7]

Results and discussion

The rhizomes of *Curcuma domestica* afforded 1-6 by silica gel chromatography. The structures of 1-4 were identified by NMR spectroscopy and comparison of their spectroscopic data with those reported in the literature. The ^{1}H NMR data of 1 gave characteristic resonances for curcumin as evidenced by similar spectral data to the literature values.[2] The symmetrical nature of the ^{1}H and ^{13}C signals indicates very rapid tautomerization of the two keto/enol forms of curcumin. The ^{1}H and ^{13}C NMR resonances of 2 are typical for a sesquiterpene which was identified as bisacurone based on similar spectral data to literature values[3]. Fraction A is a mixture of 3, 4, and 5 in a 1.0:0.5:0.5 ratio based on integrals and disparity in single hydrogen peaks. Compounds 3, 4, and 5 were identified as ar-turmerone,[8] β-turmerone,[9] and α-turmerone,[8] respectively based on similar spectral data to literature values. Compound 6 was identified as ar-curcumyl alcohol. *C. longa* is known to have antimicrobial properties, thus the isolates from *C. domestica* were tested for possible antimicrobial activities against the Gram positive bacteria (*Bacillus subtillis* and *Staphylococcus aureus*), Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and fungi (*Candida albicans, Trichophyton mentagrophytes,* and *Aspergillus niger*). Results of the study (Table 1) indicated that 2-6 have moderate antifungal activity against *A. niger*, while 1 has low activity against this fungus. 1–6 have low activities against *C. albicans* and *T. mentagrophytes.*

Among the compounds tested 1 has the least antifungal activity. 3-6 have moderate antibacterial activity against *P. aeruginosa*, while 1 and 2 have low activities against this bacterium. 1 has low activity against *S. aureus*, while 3-6 were inactive against this bacterium. 2 were found inactive against *E. coli*, while 1 and 3-6 indicated low activities against this bacterium. All the compounds tested have low activities against *B. subtillis*. The moderate and low antimicrobial activities of the samples compared to the standard antibiotics maybe due to their low concentrations (30 μg).

Curcumin (1): orange crystals, mp 182-183°C; ^{1}H NMR: δ 6.97 (H-2, s), 7.00 (H-5, d, 8.0 Hz), 6.83 (H-6, d, J = 8.0 Hz), 7.48 (H-1', d, J = 16 Hz), 6.38 (H-2', d, J = 16 Hz), 5.72 (H-4', s), 3.84 (OMe, s); ^{13}C NMR: δ 128.0 (C-1), 111.9 (C-2), 150.5 (C-3), 149.2 (C-4), 124.1 (C-5), 116.6 (C-6), 141.8 (C-1'), 122.2 (C-2'), 184.7 (C-3'), 102.0 (C-4'), 56.5 (OMe).

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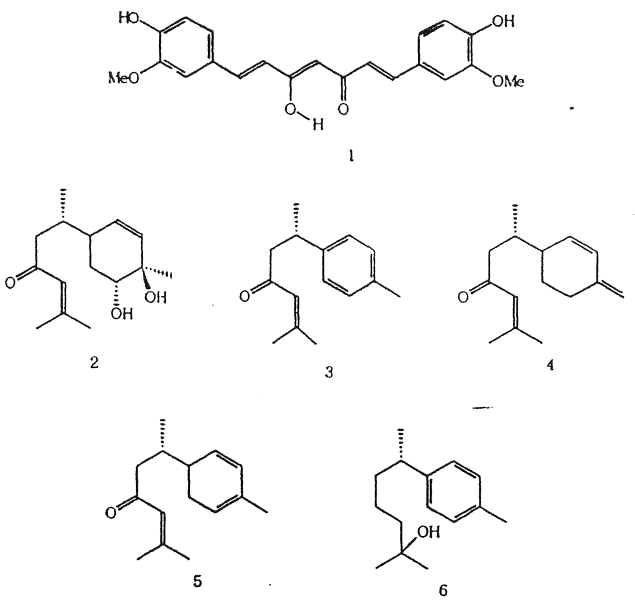
***Bisacurone* (2)**: colorless oil; ^{1}H NMR: δ 2.32 (H-1, m), 5.65 (2H, H-2/3, m), 3.78 (H-5, dd, J = 3.2, 7.2 Hz), 1.71 (H-6a, dt, J = 6.9, 13.8 Hz), 1.83 (H-6b, ddd, J = 3.1 7.5, 13.8 Hz), 2.21 (H-7, m), 2.26 (H-8a, dd, J = 8.3, 14.7 Hz), 2.46 (H-8b, dd, J = 4.7, 14.7 Hz), 6.07 (H-10, septet, J = 1.2 Hz), 2.14 (3H, H-12, d, J = 1.2 Hz), 1.89 (3H, H-13, d, J = 1.2 Hz), 0.91 (3H, H-14, d, J = 6.8 Hz), 1.31 (3H, H-15, s); ^{13}C NMR: δ 36.5 (C-1), 132.1 (C-2), 132.3 (C-3), 70.5 (C-4), 73.4 (C-5), 27.9 (C-6), 33.1 (C-7), 48.7 (C-8), 200.5 (C-9), 124.0 (C-10), 155.6 (C-11), 20.8 (C-12), 27.7 (C-13), 16.9 (C-14), 23.9 (C-15).

Ar-turmerone (3): colorless oil; ^{1}H NMR: δ 7.10 (4H, H-2/3/5/6, m), 3.29 (H-7, m), 2.61 (H-8a, m), 2.71 (H-8b, m), 6.02 (H-10, m), 1.88 (3H, H-12, br s), 2.14 (3H, H-13, br s), 1.24 (3H, H-14, d, J = 7.0 Hz), 2.31 (3H, H-15, br s); ^{13}C NMR: δ 135.5 (C-1), 126.7 (C-2), 129.1 (C-3), 143.7 (C-4), 129.1 (C-5), 126.7 (C-6), 38.0 (C-7), 52.6 (C-8), 199.9 (C-9), 124.1 (C-10), 155.1 (C-11), 20.7 (C-12), 27.7 (C-13), 15.9 (C-14), 22.0 (C-15).

β-turmerone (4): colorless oil; ^{1}H NMR: δ 2.20 (H-1, m), 5.67 (H-2, m), 6.17 (H-3, br d, J = 10.0 Hz), 2.20 (2H, H_2-5, m), 1.40 (2H, H_2-6, m), 1.75 (H-7,m), 2.45 (H-8a, m), 2.48 (H-8b, m), 6.07 (H-10, m), 2.11 (3H, H-12, br s), 1.85 (3H, H-13, br s), 0.89 (3H, H-14, d, 6.8 Hz), 4.76 (2H, H_2-15, br s); ^{13}C NMR: δ 40.5 (C-1), 130.0 (C-2), 133.8 (C-3), 143.4 (C-4), 30.1 (C-5), 24.9 (C-6), 33.3 (C-7), 48.7 (C-8), 200.9 (C-9), 124.1 (C-10), 154.9 (C-11), 20.97 (C-12), 27.7 (C-13), 16.5 (C-14), 110.3 (C-15).

α-turmerone (5): colorless oil; ^{1}H NMR: δ 2.20 (H-1, m), 5.68 (H-2, m), 5.80 (H-3, d, J = 9.4 Hz), 5.43 (H-5, br s), 2.20 (H-6, m), 1.75 (H-7, m), 2.45 (H-8a, m), 2.48 (H-8b, m), 6.06 (H-10, m), 2.11 (3H, H-12, br s), 1.85 (3H, H-13, br s), 0.88 (3H, H-14, d, J = 6.8 Hz), 1.74 (3H, H-15, br s); ^{13}C NMR: δ 35.3 (C-1), 128.4 (C-2), 131.1 (C-3), 143.4 (C-4), 129.5 (C-5), 25.1 (C-6), 33.0 (C-7), 48.6 (C-8), 201.0 (C-9), 124.1 (C-10), 155.1 (C-11), 21.1 (C-12), 27.7 (C-13), 17.1 (C-14), 29.7 (C-15).

Ar-curcumyl alcohol (6): colorless oil; ^{1}H NMR: δ 7.07 (4H, H-2/3/5/6, m), 2.73 (H-7, m), 1.60 (H-8a, m), 1.70 (H-8b, m), 1.40 (H-9a, m), 1.50 (H-9b, m), 1.20 (H-10a, m), 1.30 (H-10b, m), 1.31 (3H, H-12, s) 1.25 (3H, H-13, s), 1.23 (3H, H-14, d, J = 6.8 Hz), 2.31 (3H, H-15, s); ^{13}C NMR: δ 135.5 (C-1), 126.9 (C-2), 128.9 (C-3), 143.7 (C-4), 129.1 (C-5), 126.7 (C-6), 46.1 (C-7), 39.5 (C-8), 40.0 (C-9), 41.1 (C-10), 70.6 (C-11), 21.0 (C-12), 28.4 (C-13), 15.9 (C-14), 21.3 (C-15).



Experimental

General experimental procedures. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer in $CDCl_3$ (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). 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 obtained from Lipa City, Batangas in October and identified as *Curcuma domestica* Valet at the Philippine National Museum. Voucher specimens # 057 was deposited at the Chemistry Department of De La Salle University.

Extraction and isolation. Freeze-dried rhizomes (1 kg) of *C. domestica* were extracted with dichloromethane to afford a crude extract (22 g). The crude extract was washed with acetone and the residue was chromatographed by silica gel chromatography using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9) to afford **1** (50 mg). The filtrate was chromatographed using CH_2Cl_2 as eluent. This was rechromatographed using increasing proportions of ethyl acetate in petroleum ether at 10% increment as eluents. The 10% ethyl acetate in petroleum ether fraction was rechromatographed in 5% ethyl acetate in petroleum ether (2x) to afford **3-5** (20 mg). The 20% ethyl acetate in petroleum ether fraction was rechromatographed in 10% ethyl acetate in petroleum ether to afford **6** (10.5 mg). The 40-50% ethyl acetate in petroleum ether fractions were rechromatographed in CH_3CN:Et_2O:CH_2Cl_2 (2:2:6) to afford **2** (12 mg).

Antimicrobial tests. The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These were *Aspergillus niger* UPCC 4219, *Candida albicans* UPCC 2168, *Bacillus subtillis* 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[10] 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.

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Table 1. Antimicrobial test results on a 1-6

Organism	Sample (30 μg)	Clear Zone, mm			Activity Index (AI)
		Replicate 1	Replicate 2	Replicate 3	
E. coli	1	13	13	13	0.3
	2	-	-	-	0
	3-5	12	12	12	0.2
	6	12	12	12	0.2
	Chloramphenicol				4.0
P. aeruginosa	1	12	12	12	0.2
	2	12	12	12	0.2
	3-5	13	13	13	0.3
	6	13	13	13	0.3
	Chloramphenicol	12			0.2
S. aureus	1	13	13	13	0.3
	2	-	-	-	0
	3-5	-	-	-	0
	6	-	-	-	0
	Chloramphenicol				3.7
B. subtillis	1	18	18	18	0.8
	2	14	14	14	0.4
	3-5	14	14	14	0.4
	6	14	14	14	0.4
	Chloramphenicol				4.8
C. albicans	1	13	13	13	0.3
	2	14	14	14	0.4
	3-5	14	14	14	0.4
	6	14	14	14	0.4
	Clortrimazole				3.0
T. mentagrophytes	1	13	13	13	0.3
	2	14	14	14	0.4
	3-5	15	15	15	0.5
	6	16	16	16	0.6
	Clortrimazole				6.5
A. niger	1	12	12	12	0.2
	2	16	16	16	0.6
	3-5	16	16	16	0.6
	6	18	18	18	0.8
	Clortrimazole				6.5