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NOVEL NEMATOCIDAL AGENTS FROM *CURCUMA COMOSA*<sup>1</sup>TANNIS M. JURGENS, EASTER G. FRAZIER, JAMES M. SCHAEFFER, TRACEY E. JONES,  
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ABSTRACT.—*Curcuma comosa* is a member of the economically important plant family, Zingiberaceae. A methanolic extract of *C. comosa* was shown to be nematocidal when tested against the free-living nematode *Caenorhabditis elegans*. Five diphenylheptanoids [1–5], one new and four known, have been isolated and shown to be responsible for the activity. This is the first report of three of these compounds [1, 2, 4] being isolated from a natural source.

The family Zingiberaceae (ginger family), distributed throughout tropical and subtropical regions of the world, especially Indonesia and Malaysia, has been well studied due to its economic importance (1,2). Members of this family, including turmeric (*Curcuma longa* L.), cardamon (*Elettaria cardamomum* (L.) Maton.), and ginger (*Zingiber officinale* Roscoe), have been used for centuries as foods, spices, dyes, perfumes, and in traditional Chinese, Japanese, and Indian medicine (3–5). Medicinally, plants of this family are reputed to have value as antihepatotoxic (6,7), anti-inflammatory (8) and bile-expelling agents (5,9), for their anti-ulcer (4,10,11), antimicrobial (1,12), stomachic (13), insecticidal (14), and antiprotozoal (5) properties, and as an antidote for cobra venom (15).

During the course of a program to screen plants for pharmacological activity it was determined that a methanolic extract of *Curcuma comosa* Roxb. exhibited significant activity in the inhibition of motility of the nematode *Caenorhabditis elegans*. As there have been no reports in the literature concerning the phytochemical investigation of this species, the isolation of compounds responsible for the nematocidal activity was undertaken.

## RESULTS AND DISCUSSION

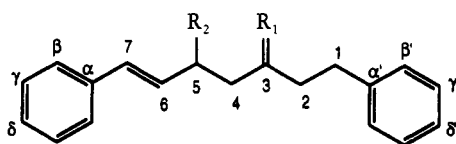
Liquid/liquid partitioning of the methanolic extract of *Curcuma comosa* provided three fractions which were evaluated against *Caenorhabditis elegans*. Both the hexane and CH<sub>2</sub>Cl<sub>2</sub> fractions exhibited comparable activity in the assay (100% inhibition of motility of *C. elegans*), while the aqueous fraction was devoid of activity. Bioassay of subfractions from prep. tlc of the hexane and CH<sub>2</sub>Cl<sub>2</sub> fractions showed that both contained similar regions of activity. Because tlc analysis showed the hexane fraction to be less complicated, it was selected for bioassay-directed fractionation. Column chromatography over Si gel, eluting with Me<sub>2</sub>CO/hexane mixtures, was used to resolve the hexane fraction into four subfractions. Further chromatography, both column and prep. tlc, was utilized to isolate five compounds [1–5].

Compound 3 was the most abundant of the series and thus its structure was elucidated first. The hreims of 3 indicated a molecular formula of C<sub>19</sub>H<sub>22</sub>O. The eims

<sup>1</sup>This work was presented at the International Research Congress on Natural Products, 32nd Annual Meeting of the American Society of Pharmacognosy, 21–26 July 1991, Chicago, IL.

fragmentation showed that the compound contained phenyl moieties and a hydroxyl group. Resonances in the  $^1\text{H}$ -nmr spectrum of **3** accounted for a total of 22 protons (10 aromatic, 2 olefinic, 8 methylene, 1 oxygenated methine and one exchangeable) while the  $^{13}\text{C}$ -nmr spectrum (Table 1) gave signals for 19 carbons (12 aromatics, 2 olefinics, 4 methylene carbons and 1 oxygenated methine carbon). Analysis of the spectral data suggested that **3** was composed of two monosubstituted phenyl rings connected by a seven-carbon chain containing one double bond and one hydroxyl group. A number of diarylheptanoids such as curcumin from *Curcuma* spp. (16,17) and the gingerones from *Zingiber officinale* (3) have been reported from the Zingiberaceae. These compounds are known for their pungent properties (18) and their interesting physiological activities (19). Unlike the compounds reported here, the curcumins and gingerones all contain oxygen substituents in their phenyl rings. Placement of the double bond and the hydroxyl group on the seven-carbon chain connecting the aromatic rings was accomplished based on analysis of COSY spectra and coupling constants. The two olefinic protons showed a  $^3J_{\text{HH}} = 15.8$  Hz indicating a *trans*-configuration. The upfield olefinic proton showed an additional coupling (6.8 Hz) to two methylene protons at 2.32 ppm, while the downfield olefinic proton resonance was somewhat broadened. The signal at 3.67 ppm was assigned to a proton attached to a carbon-bearing oxygen. COSY correlations showed that this proton was coupled to four methylene protons (1.66 and 1.80 ppm). The signal at 1.66 ppm was also coupled to the signal at 2.32 ppm, further coupled to the upfield olefinic proton, while the signal at 1.80 ppm was coupled to the most downfield methylene signal at 2.73 ppm. The structure **3** incorporates the coupling patterns described and was determined to be 1,7-diphenyl-6(*E*)-hepten-3-ol (all figures were named according to Chemical Abstracts), previously isolated as a racemate from *Alpinia katsumadai* Hayata (20). The observed lack of optical activity indicates that **3** was also isolated from *C. comosa* as a racemic mixture. The inavailability of an authentic sample precluded a direct comparison; however, all available spectroscopic data are in accordance with the published data for this compound (20). Similar unsubstituted diarylheptanoids have been reported from two species of *Alnus* (Betulaceae), *Alnus firma* Sieb. et Zucc. (21,22) and *Alnus pendula* Hance (*Alnus multinervis* Callier) (23) as well as from *Alpinia officinarum* Hance (Zingiberaceae) (24).

The spectroscopic characteristics of the remaining four compounds were quite similar to those of **3**, and these compounds were determined to be related diarylheptanoids on the basis of spectral correlations. A carbonyl absorption at  $1733\text{ cm}^{-1}$  in the ir spectrum of **1** provided evidence for the presence of an ester. The  $^1\text{H}$ -nmr spectrum revealed a 3H singlet at 2.02 ppm and the absence of the exchangeable proton seen in **3**. Signals for two additional carbons at 21.34 and 170.95 ppm were present in the  $^{13}\text{C}$ -nmr spectrum, suggesting an acetate. Hreims showed a molecular formula of  $\text{C}_{21}\text{H}_{24}\text{O}_2$ , a gain of 42 mass units with a fragment ion at  $m/z$  248 (strong,  $[\text{M}-\text{AcOH}]^+$ ). Compound **1** was determined to be the previously unreported compound 1,7-diphenyl-



- 1  $\text{R}_1 = \text{H}, \text{OCOCH}_3; \text{R}_2 = \text{H}$
- 2  $\text{R}_1 = \text{O}; \text{R}_2 = \text{H}$
- 3  $\text{R}_1 = \text{H}, \text{OH}; \text{R}_2 = \text{H}$
- 4  $\text{R}_1 = \text{H}, \text{OH}; \text{R}_2 = \text{H}; \Delta^{4,5}$
- 5  $\text{R}_1 = \text{O}; \text{R}_2 = \text{OH}$

TABLE 1.  $^{13}\text{C}$ -Nmr Data for [1-5].<sup>a</sup>

Position	Compound				
	1	2	3	4	5
1 .....	32.00 (2)	29.98 (2)	32.36 (2)	39.38 (2)	29.71 (2)
2 .....	36.25 (2)	44.57 (2)	39.62 (2)	32.07 (2)	45.36 (2)
3 .....	73.51 (1)	209.17 (0)	71.05 (1)	72.23 (1)	210.30 (0)
4 .....	34.13 (2)	42.49 (2)	37.39 (2)	137.21 (1)	49.60 (2)
5 .....	29.22 (2)	27.38 (2)	29.62 (2)	130.98 (1)	68.79 (1)
6 .....	130.49 (1)	130.79 (1)	130.91 (1)	128.76 (1)	130.98 (1)
7 .....	130.27 (1)	129.52 (1)	130.35 (1)	132.84 (1)	130.23 (1)
$\alpha$ .....	138.06 (0)	137.88 (0)	138.11 (0)	137.65 (0)	137.03 (0)
$\beta$ .....	126.23 (1)	126.26 (1)	126.23 (1)	126.69 (1)	126.76 (1)
$\gamma$ .....	128.68 (1) <sup>b</sup>	128.63 (1) <sup>b</sup>	128.74 (1) <sup>b</sup>	128.72 (1) <sup>b</sup>	128.62 (1) <sup>b</sup>
$\delta$ .....	126.17 (1)	126.31 (1)	126.07 (1)	126.15 (1)	126.42 (1)
$\alpha'$ .....	142.21 (0)	141.68 (0)	142.75 (0)	142.52 (0)	141.36 (0)
$\beta'$ .....	128.69 (1) <sup>b</sup>	128.73 (1) <sup>b</sup>	128.68 (1) <sup>b</sup>	128.82 (1) <sup>b</sup>	128.79 (1) <sup>b</sup>
$\gamma'$ .....	128.81 (1) <sup>b</sup>	128.81 (1) <sup>b</sup>	128.81 (1) <sup>b</sup>	128.99 (1) <sup>b</sup>	128.90 (1) <sup>b</sup>
$\delta'$ .....	127.26 (1)	127.35 (1)	127.22 (1)	127.85 (1)	128.00 (1)
1' .....	170.95 (0)				
2' .....	21.34 (3)				

<sup>a</sup>Spectra are recorded in  $\text{CD}_2\text{Cl}_2$ . The numbers of attached protons, as determined by APT, are noted in parentheses.

<sup>b</sup>Assignments within a column may be interchanged.

3-acetoxy-6(*E*)-heptene. Unfortunately, this compound was obtained in insufficient quantity to allow a determination of its optical rotation.

Analysis of the spectral data for compound **2** showed strong similarities to **1**. Like **1**, **2** had a carbonyl absorption in the ir spectrum, but at  $1714\text{ cm}^{-1}$ , this indicated the presence of a ketone. Although there were two fewer protons detected in the nmr spectrum of **2** than in **3**, the carbon count remained at 19 with the signal for the oxygenated methine being replaced by a ketone carbon at 209.17 ppm. Hreims data was consistent with the empirical formula,  $\text{C}_{19}\text{H}_{20}\text{O}$ . Compound **2** was determined to be 1,7-diphenyl-6(*E*)-hepten-3-one, which has been previously reported as a synthetic product (**25**).

Mass spectral data suggested that compound **4** was similar to **3** with the addition of one unit of unsaturation, having an empirical formula of  $\text{C}_{19}\text{H}_{20}\text{O}$  determined by hreims. That four methylene protons were replaced by two olefinic protons in conjugation with the 6,7 double bond was evident in the  $^1\text{H}$ -nmr spectrum. This observation was supported by  $^{13}\text{C}$ -nmr data as well, in which two methylene carbons have been replaced by two olefinic methines. Thus, **4** was determined to be 1,7-diphenyl-4(*E*),6(*E*)-heptadien-3-ol. Unfortunately, **4** was obtained in insufficient quantity to allow determination of its optical rotation. This compound was previously synthesized by Kato *et al.* (19) and was reported to be capable of preventing  $\text{CCl}_4$ -induced hepatotoxicity (6).

A molecular formula of  $\text{C}_{19}\text{H}_{20}\text{O}_2$  was determined by hreims of **5**. The ir and  $^{13}\text{C}$ -nmr spectra indicated the presence of a ketone, while the  $^1\text{H}$  nmr spectrum clearly showed an exchangeable proton. Analysis of COSY nmr spectra established **5** as the known compound 1,7-diphenyl-6(*E*)-hepten-3-one-5-ol, which was previously isolated from *Alpinia katsumadai* (20) and has also been synthesized (6). The observed positive optical rotation of **5** is in agreement with data reported for material isolated from *A. katsumadai*, suggesting an (*R*)-configuration.

TABLE 2. EC<sub>95</sub> Values of 1-5 in the *C. elegans* Nematocidal Assay.

Compound	EC <sub>95</sub>
1 .....	9 µg/ml
2 .....	9 µg/ml
3 .....	0.7 µg/ml
4 .....	>100 µg/ml
5 .....	1 µg/ml

All five diphenylheptanoids were evaluated in the *Caenorhabditis elegans* motility assay. From the results of the assay (Table 2), it was clear that compound **3** was the most potent inhibitor of nematode motility, with an EC<sub>95</sub> of 0.7 µg/ml. Compound **5** was slightly less active, followed by **1** and **2**, which were 10 times less potent, and finally **4**, which was inactive.

### EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—<sup>1</sup>H- (300 MHz) and <sup>13</sup>C- (75 MHz) nmr spectra were obtained on a Varian XL-300 nmr spectrometer in CD<sub>2</sub>Cl<sub>2</sub> with TMS as an internal standard at 0.00 ppm. 2D nmr data were obtained using standard pulse sequences for homo- and heteronuclear correlated spectroscopy (COSY and HETCOR). Mass spectra were recorded on a Finnigan-MAT model 212 spectrometer (electron impact, 90 eV). Exact mass measurements were performed at high resolution using perfluorokerosene (PFK) as internal standard. Uv spectra were determined on a Beckman DU-70 spectrophotometer and ir spectra were obtained using a Perkin-Elmer 1750 Ft-ir spectrometer equipped with a multiple internal reflectance attachment. The optical rotations of **3** and **5** were measured with a Perkin-Elmer 241 polarimeter. Mps were determined using a Haake Buchler mp apparatus and are uncorrected.

PLANT MATERIAL.—Roots of *Curcuma comosa* Roxb. were collected and air-dried in Chiang Mai, Thailand in November 1988. A voucher specimen (Nanakorn 8887) is preserved in the Herbarium of the New York Botanical Garden, Bronx, NY.

EXTRACTION, FRACTIONATION AND ISOLATION.—The dried, ground plant (1 kg) was extracted five times with MeOH at room temperature, for 24 h each time. The filtrates were combined and evaporated to dryness *in vacuo*. The resulting residue was redissolved in 95% MeOH (1 liter) and extracted three times with hexane (each 1 liter). The pooled hexane extracts were freed of solvent to afford 10.4 g of a brown oil. The defatted MeOH extract was freed of solvent, dispersed in 500 ml distilled H<sub>2</sub>O and extracted three times with CH<sub>2</sub>Cl<sub>2</sub> (each 500 ml). The pooled CH<sub>2</sub>Cl<sub>2</sub> extracts were evaporated to dryness to yield 61 g of a brown residue. The residual aqueous fraction was stripped of solvent and lyophilized to afford 12.7 g of a yellow residue.

A portion of the hexane fraction (9 g) was chromatographed over Si gel (1 kg, 230-400 mesh, EM Science) eluting with hexane followed by increasing concentrations of Me<sub>2</sub>CO in hexane. Fractions (20 ml each) were collected and pooled to yield four subfractions: fraction A (833 mg, eluted with 4-8% Me<sub>2</sub>CO/hexane), fraction B (1.89 g, eluted with 10% Me<sub>2</sub>CO/hexane), fraction C (700 mg, eluted with 10% Me<sub>2</sub>CO/hexane) and fraction D (196 mg, eluted with 20% Me<sub>2</sub>CO/hexane).

FRACTION A.—Fraction A was chromatographed over Si gel (100 g) eluting with 0.5-1% Me<sub>2</sub>CO/hexane. The major fraction was rechromatographed over Si gel (100 g) eluting with 1-2% Me<sub>2</sub>CO/hexane followed by prep. tlc over Si gel (Kieselgel 60 F<sub>254</sub>, EM Separations, 20×20 cm plates, 0.5 mm layer, 10% Me<sub>2</sub>CO/hexane). This afforded two subfractions: A-1 and A-2. Fraction A-1 was further purified by prep. tlc (Si gel, 20×20 cm plates, 1.0 mm layer, 50% EtOAc/hexane).

A-1 [**1**] was obtained as a colorless oil: hreims, *m/z* found 308.1771, calcd for C<sub>21</sub>H<sub>24</sub>O<sub>2</sub> 308.1776; eims, *m/z* 308, 248, 157, 144 (100%), 143, 131, 129, 117, 105, 91; ir  $\nu$  max 3454, 3027, 2930, 2363, 1733, 1602, 1496, 1452, 1373, 1241, 1101, 1027, 966, 747, 699 cm<sup>-1</sup>; uv  $\lambda$  max (95% EtOH) ( $\epsilon$ ) 210 (17191) 250 (3679) nm; <sup>1</sup>H nmr  $\delta$  1.75 (2H, m, 4-H<sub>2</sub>), 1.88 (2H, m, 2-H<sub>2</sub>), 2.02 (3H, s, acetate CH<sub>3</sub>), 2.23 (2H, m, 5-H<sub>2</sub>), 2.62 (2H, m, 1-H<sub>2</sub>), 4.95 (1H, m, 3-H), 6.21 (1H, dt, *J* = 15.4, 6.5 Hz, 6-H), 6.38 (1H, bd, *J* = 15.4 Hz, 7-H), 7.1-7.4 (10H, m, 2×phenyl); <sup>13</sup>C nmr, see Table 1.

A-2 [**2**] was obtained as a colorless oil: hreims, found 264.1508, calcd for C<sub>19</sub>H<sub>20</sub>O 264.1514; eims, *m/z* 264, 159, 133, 131, 117, 105, 91 (100%); ir  $\nu$  max 3877, 3434, 3026, 2923, 2364, 1714, 1607, 1495,

1453, 1373, 1249, 1100, 972, 879, 750, 700  $\text{cm}^{-1}$ ; uv  $\lambda$  max (95% EtOH) ( $\epsilon$ ) 210 (10767), 250 (2288) nm;  $^1\text{H}$  nmr, see ref. (25);  $^{13}\text{C}$  nmr, see Table 1.

FRACTION B.—On evaporation, Fraction B spontaneously crystallized and was recrystallized as plates (**3**, 325 mg) from  $\text{Me}_2\text{CO}$ /hexane: mp 44–46°; hreims,  $m/z$  found 266.1670, calcd for  $\text{C}_{19}\text{H}_{22}\text{O}$  266.1670; eims,  $m/z$  266, 248, 144, 117, 115, 105, 91;  $[\alpha]_D^{20}$  ( $c=1.2$ , MeOH); ir  $\nu$  max 3948, 3891, 3391, 3026, 2934, 1601, 1495, 1451, 1185, 1068, 965, 749, 698  $\text{cm}^{-1}$ ; uv  $\lambda$  max (95% EtOH) ( $\epsilon$ ) 210 (4400), 250 (2296) nm;  $^1\text{H}$  nmr, see ref. (20);  $^{13}\text{C}$  nmr see, Table 1.

FRACTION C.—Fraction C was chromatographed over Si gel (230–400 mesh) eluting with 2%  $\text{Me}_2\text{CO}$ /hexane increasing to 10%  $\text{Me}_2\text{CO}$ /hexane. Fractions were pooled to yield two subfractions: fraction C-1, composed primarily of **3**, and fraction C-2.

C-2 was further purified by prep. tlc (Si gel, 20×20 cm plates, 2.0 mm layer, 20%  $\text{Me}_2\text{CO}$ /hexane) to afford **4** as a yellow oil: hreims,  $m/z$  found 264.1508, calcd for  $\text{C}_{19}\text{H}_{20}\text{O}$  264.1514; eims,  $m/z$  264, 246, 133, 117, 105, 91 (100%), 77; ir  $\nu$  max 3425, 3027, 2931, 1717, 1603, 1495, 1452, 1376, 1070, 1032, 971, 751, 699  $\text{cm}^{-1}$ ; uv  $\lambda$  max (95% EtOH) ( $\epsilon$ ) 210 (18280) 250 (5044) nm;  $^1\text{H}$  nmr,  $\delta$  1.60 (1H, exchangeable with  $\text{D}_2\text{O}$ , OH) 1.88 (2H, m, 2- $\text{H}_2$ ), 2.73 (2H, m, 1- $\text{H}_2$ ), 4.20 (1H, m, 3-H), 5.88 (1H, ddt,  $J=15.3, 6.7, 0.8$  Hz, 4-H), 6.41 (1H, dddd,  $J=15.3, 10.4, 1.2, 0.7$  Hz, 5-H), 6.57 (1H, bd,  $J=15.6, 7\text{-H}$ ), 6.81 (1H, ddd,  $J=15.6, 10.4, 0.8$  Hz, 6-H), 7.1–7.4 (10H, m, 2×phenyl);  $^{13}\text{C}$  nmr, see Table 1.

FRACTION D.—Fraction D was purified using prep. tlc (Si gel, 20×20 cm plates, 1.0 mm layer, 20%  $\text{Me}_2\text{CO}$ /hexane) to yield **5** as a yellow oil: hreims,  $m/z$  found 280.1457, calcd for  $\text{C}_{19}\text{H}_{20}\text{O}_2$  280.1463, eims,  $m/z$  280, 262, 175, 133, 105 (100%), 91, 77;  $[\alpha]_D^{20} +16.6$  ( $c=0.06$ , MeOH); ir  $\nu$  max 3402, 3028, 2934, 1708, 1603, 1495, 1451, 1371, 1244, 1068, 1032, 969, 749, 697  $\text{cm}^{-1}$ ; uv  $\lambda$  max (95% EtOH) ( $\epsilon$ ) 210 (28571), 250 (14313), 283 (3002), 292 (2255) nm;  $^1\text{H}$  nmr, see ref. (20);  $^{13}\text{C}$  nmr, see Table 1.

*C. ELEGANS* MOTILITY ASSAY.—*C. elegans*, strain N2, was cultivated on NG agar plates covered with a lawn of *Escherichia coli* as previously described (26). Nematodes were rinsed off the agar plates with HEPES buffer at 22°, washed twice by centrifugation at 1,000× $g$  for two minutes and then resuspended in 25 mM HEPES buffer. Aliquots of the nematode suspension (50  $\mu\text{l}$ , approximately 100 worms) were placed into 13×100 mm glass test tubes. The compounds to be tested were prepared in DMSO and added to the nematodes in a final volume of 500  $\mu\text{l}$  containing 1% DMSO. After 16 h incubation at 22°, the number of worms still motile was determined by examination with a low-power dissecting microscope. More than 90% of the worms continued to swim vigorously in the control tube.  $\text{EC}_{95}$  values represent the concentration of drug which immobilized 95% of the nematodes.

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#### LITERATURE CITED

1. S. Joshi, A.K. Singh, and D.N. Dhar, *Herba Hung.*, **28**, 95 (1989).
2. P.S. Merh, M. Daniel, and S.D. Sabnis, *Curr. Sci.*, **55**, 835 (1986).
3. K. Endo, E. Kanno, and Y. Oshima, *Phytochemistry*, **29**, 797 (1990).
4. C.K. Moon, K.S. Park, S.H. Lee, and Y.P. Yoon, *Arch. Pharmacol. Res.*, **8**, 42 (1985).
5. T.N.B. Shankar and V.S. Murthy, *Indian J. Exp. Biol.*, **17**, 1363 (1979).
6. H. Hikino, Y. Kiso, N. Kata, Y. Hamada, T. Shiori, R. Aiyama, H. Itokawa, F. Kiuchi, and U. Sankawa, *J. Ethnopharmacol.*, **14**, 31 (1985).
7. Y. Kiso, Y. Suzuki, N. Watanabe, Y. Oshima, and H. Hikino, *Planta Med.*, **49**, 185 (1983).
8. H.P.T. Ammon, M.I. Anazodo, H. Safayhi, B.N. Dhawan, and R.C. Srimal, *Planta Med.*, **58**, 226 (1992).
9. N.K. Gorchakova, N.I. Grinkevich, and A.N. Fogel, *Farmatsiya*, **33**, 12 (1984).
10. R. Kuttan, P. Bhanumathy, K. Nirmala, and M.C. George, *Cancer Lett.*, **29**, 197 (1985).
11. K. Watanabe, M. Shibara, S. Yano, Y. Cai, H. Shibuya, and I. Kitagawa, *Yakugaku Zasshi*, **106**, 1137 (1986).
12. T. Sawada, J. Yamahara, S. Shimazu, and T. Ohta, *Shoyakugaku Zasshi*, **25**, 11 (1971).
13. Y. Shiobara, Y. Asakawa, M. Kodama, K. Yasudo, and T. Takemoto, *Phytochemistry*, **24**, 2629 (1985).
14. G. Jilani and H.C.F. Su, *J. Econ. Entomol.*, **76**, 154 (1983).
15. C. Cherdchu and E. Karlsson, *Southeast Asian J. Trop. Med. Public Health*, **14**, 176 (1983).
16. P.J. Roughley and D.A. Whiting, *J. Chem. Soc., Perkin Trans. I*, 2379 (1973).

17. J. Chen, Y. Chen, and J. Yu, *Zhongcaoyao*, **14**, 59 (1983).
18. H. Itokawa, R. Aiyama, and A. Ikuta, *Chem. Pharm. Bull.*, **31**, 2491 (1983).
19. N. Kato, Y. Hamada, and T. Shioiri, *Chem. Pharm. Bull.*, **32**, 3323 (1984).
20. M. Kuroyanagi, T. Noro, S. Fukushima, R. Aiyama, A. Ikuta, H. Itokawa, and M. Morita, *Chem. Pharm. Bull.*, **31**, 1544 (1983).
21. Y. Asakawa, F. Genjida, S. Hayashi, and T. Matsuura, *Tetrahedron Lett.*, 3235 (1969).
22. Y. Asakawa, *Bull. Chem. Soc. Jpn.*, **43**, 2223 (1970).
23. T. Suga, Y. Asakawa, and N. Iwata, *Chem. Ind.*, 766 (1971).
24. H. Itokawa, H. Morita, I. Midorikawa, R. Aiyama, and M. Morita, *Chem. Pharm. Bull.*, **33**, 4889 (1985).
25. J.M. Muchowski, R.J. Greenhouse, J. Ackrell, T.T. Li, and J.R. Pfister, Eur. Pat. Appl. EP 71,399, 09 Feb 1983.
26. J.M. Schaeffer and H.W. Haines, *Biochem. Pharm.*, **38**, 2329 (1989).

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