Kneglomeratanol, Kneglomeratanones A and B, and Related Bioactive Compounds from Knema glomerata

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KNEGLOMERATANOL, KNEGLOMERATANONES A AND B, AND RELATED BIOACTIVE COMPOUNDS FROM *KNEMA GLOMERATA*

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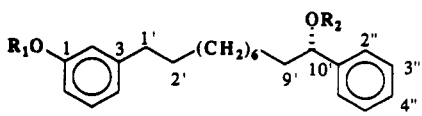
ABSTRACT.—One new phenylalkyl phenol, kneglomeratanol [**1**], and two new acetophenones, kneglomeratanones A [**2**] and B [**3**], together with ten known compounds, 3-(12′-phenyldodecyl)-phenol [**4**], 3-(10′-phenyldecyl)-phenol [**5**], 5-pentadecylresorcinol [**6**], 5-(10′-phenyldecyl)-resorcinol [**7**], 5-(12′-phenyldodecyl)-resorcinol [**8**], 2,4-dihydroxy-6-(10′-phenyldecyl)-acetophenone [**9**], 2-hydroxy-6-(12′-phenyldodecyl)-benzoic acid [**10**], formononetin, biochanin A, and 8-*O*-methylretusin, have been isolated from the stem bark of *Knema glomerata*. Brine shrimp lethality was used for the activity-directed chromatographic fractionations. All of these compounds showed moderate but significant toxicities to three human tumor cell lines and inhibited the growth of crown gall tumors on discs of potato tubers.

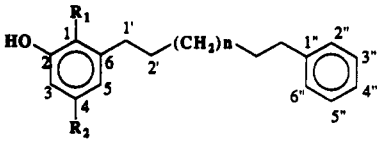
Knema (Myristicaceae) plants are tropical evergreen trees for which some species are described as having medical uses; the stem bark of *K. tenuinervia* is used as a cancer remedy in Thai popular medicine (1), and the dried sap of *K. erratica* or *K. angustifolia* is astringent and used for mouth sores in Assam of India (2,3). A few phytochemical studies have reported the isolation of lignans from *K. attenuata* (4,5), *K. elegans* (6), *K. furfuracea* (7), and *K. austrosiamensis* (8); phenyl phenol derivatives from *K. furfuracea* (7), *K. laurina*, and *K. tenuinervia* (1); and an isocoumarin from *K. tenuinervia* (1). As a part of our continued interest in natural antitumor and pesticidal agents, a Philippine plant, *Knema glomerata* Merrill, was investigated by the use of brine shrimp lethality-directed fractionation. Work on a 95% EtOH extract of the stem bark led to the isolation of one new phenylalkyl phenol, kneglomeratanol [**1**]; two new acetophenone derivatives, kneglomeratanones A [**2**] and B [**3**]; seven known phenolic compounds, 3-(12′-phenyldodecyl)-phenol [**4**] (7), 3-(10′-phenyldecyl)-phenol [**5**] (9), 5-pentadecyl-resorcinol [**6**] (10), 5-(10′-phenyldecyl)-resorcinol [**7**] (9), 5-(12′-phenyldodecyl)-resorcinol [**8**] (9), 2,4-dihydroxy-6-(10′-phenyldecyl)-acetophenone [**9**] (1), 2-hydroxy-6-(12′-phenyldodecyl)-benzoic acid [**10**] (1); and three known isoflavonoids, formononetin (11), biochanin A (12), and 8-*O*-methylretusin (13).

RESULTS AND DISCUSSION

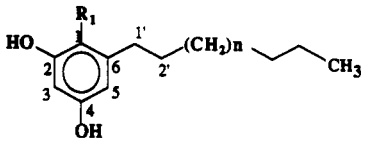
Kneglomeratanol [**1**] was obtained as a colorless oil-like substance with optical activity, $[\alpha]_D$ −8.3°. Its molecular formula, $C_{22}H_{30}O_2$, was indicated by hreims for the $[M]^+$ at *m/z* 326.2265 (calcd 326.2246). Compound **1** formed a diacetate derivative [**1a**]; one substituent of **1a** was a phenoxyl acetyl in which the Me signal appeared at δ 2.29, and the other was an alkyloxyl acetyl in which the Me signal appeared at δ 2.07 in the ^{1}H-nmr spectrum. The presence of 3-alkyl phenol and phenyl moieties was proven



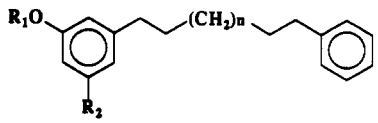
1 $R_1=R_2=H$
1a $R_1=R_2=Ac$
1b $R_1=R_2=S$-MTPA
1c $R_1=R_2=R$-MTPA



2 R_1=$COCH_3$, R_2=OH, n=4
9 R_1=$COCH_3$, R_2=OH, n=6
10 R_1=COOH, R_2=H, n=8



3 R_1=$COCH_3$, n=8
6 R_1=H, n=10



4 R_1=R_2=H, n=8
5 R_1=R_2=H, n=6
7 R_1=H, R_2=OH, n=6
8 R_1=H, R_2=OH, n=8

by comparison of the ^{1}H- and ^{13}C-nmr spectra with those of 3-(12′-phenyldodecyl)-phenol [**4**] and 3-(10′-phenyldecyl)-phenol [**5**], which were isolated from the same plant. The location of the OH group along the chain was determined by mass fragmentation in which the base peak at *m/z* 107 indicated the presence of the phenylmethoxyl unit. The absolute configuration of the chiral center at the benzylic C-10′ position was elucidated as the *S*-form by measurements of the optical rotation and the cd spectrum which showed good agreements with *S*-(−)-1-phenylethanol (14,15).

Evidence that the C-10′ was in the *S*-form was further supported by derivatizations of **1** to the *S*- and *R*-Mosher esters [**1b**, **1c**] using the procedure of Ohtani *et al.* (16). Mosher methodology has proven to be very useful in the determination of the absolute configuration of stereogenic centers bearing an OH group (17,18). The method, using *S*- and *R*-Mosher esters [methoxy-(trifloromethyl)-phenyl acetate or MTPA], introduces more shielded effects or less shielded effects on different substituents of the chiral carbon, and the chemical shifts of the ^{1}H-nmr spectra of these substituents change accordingly. In this case, the chemical shifts of the *S*-MTPA ester [**1b**] and *R*-MTPA ester [**1c**], which were assigned by careful analysis of COSY nmr spectra, showed $\Delta\delta_H(\delta_S-\delta_R)$ changes of the chain side to the phenyl ring side from negative to positive (Table 2), which indicated that the C-10′ position was of the *S*-configuration. It was therefore substantiated that the structure of **1** is 3-[10′(*S*)-hydroxy-phenyldecyl]-phenol.

Kneglomeratanone A [**2**] was obtained as colorless plates. The molecular weight of **2** was given by hreims for the $[M]^+$ at *m/z* 340.2038, corresponding to $C_{22}H_{28}O_3$ (calcd 340.2036). The uv, ^{1}H- and ^{13}C-nmr spectra showed that **2** was an acetophenone derivative in which the carbonyl carbon signal appeared at δ 204.2 and the acetyl methyl signal at δ 32.2 and δ 2.64. The ir spectrum also showed a conjugated carbonyl absorption at 1619 cm^{-1}. The ^{1}H-nmr spectrum showed two singlets, one at δ 5.80 and

TABLE 1. ^{13}C- (125 MHz) and ^{1}H- (500 MHz) Nmr Data for Kneglomeratanol [**1**] and Kneglomeratanones A [**2**] and B [**3**] ($CDCl_3$).

Position	Compound 1			Position	Compound 2			Compound 3		
	δC	δH	*J* (Hz)		δC	δH	*J* (Hz)	δC	δH	*J* (Hz)
1	155.4			1	115.2			115.1		
2	115.3	6.66, br dd	2, 2.5	2	165.8			165.9		
3	144.9			3	101.6	6.24, d	2.5	101.6	6.24, d	2.5
4	120.9	6.64, br dd	2, 8	4	160.8			160.4		
5	129.3	7.14, t	8	5	110.6	6.25, d	2.5	110.4	6.25, d	2.5
6	112.5	6.74, br d	8	6	147.8			147.8		
1′	35.8	2.54, t	8	1′	36.3	2.82, t	8	36.3	2.84, t	8
2′	31.6	1.58, m		2′	31.5	1.60, m		31.9	1.59, m	
3′	29.4	1.23, br s		3′	29.7	1.34, *m*		29.8	1.26, br s	
4′	29.4	1.23, br s		4′	29.2	1.31, br s		29.7	1.26, br s	
5′	29.3	1.23, br s		5′	29.3	1.31, br s		29.7	1.26, br s	
6′	29.3	1.23, br s		6′	29.4	1.31, br s		29.7	1.26, br s	
7′	29.1	1.23, br s		7′	32.2	1.58, m		29.6	1.26, br s	
8′	25.8	1.74, m		8′	36.0	2.60, t	7.5	29.5	1.26, br s	
9′	39.0	1.75, m		9′				29.5	1.26, br s	
10′	74.8	4.68, dd	6, 7.5	10′				29.4	1.26, br s	
1″	142.7			11′				32.3	1.36, m	
2″	125.9	7.35, m		12′				22.7	1.30, m	
3″	128.4	7.35, m		13′				14.2	0.88, t	8
4″	127.5	7.28, m		1″	142.7					
5″	128.4	7.35, m		2″	128.2	7.27, t	8			
6″	125.9	7.35, m		3″	128.3	7.17, m				
1-OH		4.58 br s		4″	125.5	7.17, m				
				5″	128.3	7.17, m				
				6″	128.2	7.27, t	8			
				CO	204.2			204.4		
				$COCH_3$	32.2	2.64, s		32.3	2.65, s	
				2-OH		13.07, s			12.99, s	
				4-OH		5.80, s			5.17, s	

another at δ 13.07, both of which disappeared upon the addition of D_2O, indicating the presence of two OH groups. The presence of a chelated OH group, at δ 13.07, was supported by the measurement of the uv spectrum; the absorption band at 292 nm shifted bathochromically on the addition of $AlCl_3$ and could not be canceled by further addition of HCl. The presence of an alkylphenyl moiety was shown by the ^{13}C-nmr spectral data as listed in Table 1. The substitution pattern of the 2,4-dihydroxy-6-alkylphenyl moiety was determined by analyses of the ^{1}H-^{13}C COSY and COLOC nmr spectra and by calculated chemical shift values of the ring (19). It was, therefore, established that the structure of kneglomeratanone A is 2,4-dihydroxy-6-(8′-phenyloctyl)-acetophenone; **2** is similar to the known compound **9**, differing only in that it contains two fewer carbon atoms in the chain.

Kneglomeratanone B [**3**] was obtained as colorless prisms. Its molecular formula, $C_{21}H_{34}O_3$, was determined by hreims for the $[M]^+$ at *m/z* 334.2510 (calcd 334.2508). The uv, ir, ^{1}H- and ^{13}C-nmr spectra showed that **3** was also an acetophenone derivative with the same substitution pattern as that of **2**, but the alkyl chain lacked a terminal

TABLE 2. ^{1}H-Nmr Data for the Affected Substituents of *S*- and *R*-Di-MTPA Ester Derivatives [**1b, 1c**].

MTPA config.	H-8′	H-8′	H-9′	H-9′	H-10′	H-2″, 6″	H-3″, 5″	H-4″
1b (*S*)	1.14	1.24	1.74	1.94	5.95	7.41	7.36	7.31
1c (*R*)	1.28	1.36	1.81	1.98	5.87	7.35	7.30	7.27
$\Delta\delta H$ (δ_S-δ_R)	−0.14	−0.12	−0.07	−0.04	+0.08	+0.06	+0.06	+0.04

phenyl group. Further analyses of the ^{1}H- and ^{13}C-nmr and ms spectra proved that kneglomeratanone B was 2,4-dihydroxy-6-tridecylacetophenone.

The in-house bioactivities of all the compounds isolated from the title plant are summarized in Table 3. The alkylphenyl phenol compounds **1–10** showed potent activities in the BST bioassay (from 0.18–7.65 μg/ml); whereas the three isoflavonoids were less active in the BST bioassay (from 22.70–106.91 μg/ml). All of the compounds inhibited the growth of crown gall tumors on discs of potato tubers and were moderately cytotoxic to the three human cancer cell lines, but adriamycin, as a positive control, was more active by one to several orders of magnitude.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mettler FP5 hot-stage apparatus and are uncorrected. The optical rotation was determined on a Perkin-Elmer 241 polarimeter. Uv spectra were taken in MeOH on a Beckman DU-7 spectrophotometer. Ir spectra (film) were obtained on a Perkin-Elmer 1600 Ftir spectrophotometer. Low-resolution cims and eims were recorded on Finnigan 4000 and Kratos MS 50 mass spectrometers. High-resolution ms were obtained on the Kratos MS 50 spectrometer through peak matching. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian XL-500S spectrometer using Varian software. Hplc was performed on a Dynamax software sytem and a Si gel (8 μm) column (250×21 mm) equipped with a Rainin UV-1 detector which was set at 284 or 292 nm. Either hexane-Me_2CO (12:1) or hexane-CH_2Cl_2 (5:1) was used as mobile phase. Analytical tlc was carried out on Si gel plates (0.25 mm) developed with either hexane-Me_2CO (3:1) or $CHCl_3$-MeOH (15:1) and visualized with 5% phosphomolybdic acid in EtOH.

PLANT MATERIAL.—The stem bark of the title plant, *Knema glomerata* Merrill (Myristicaceae), was provided under the identification number B-628312 as collected in the Philippines by the USDA, under the auspices of Dr. Robert E. Perdue, for the National Cancer Institute (NCI), National Institutes of Health (NIH).

BIOASSAYS.—The extracts, fractions, and compounds isolated from the title plant were routinely evaluated for lethality to brine shrimp larvae (BST) (20,21). Potato disc (PD) assays (% inhibition of crown gall tumors on potato discs) were performed in-house (21,22), and seven-day in vitro cytotoxicity tests, against three solid tumor cell lines, were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma) (23), MCF-7 (human breast carcinoma) (24), and HT-29 (human colon adenocarcinoma) (25), with adriamycin as a positive control.

TABLE 3. Bioactivities of the Compounds Isolated from *Knema glomerata*.

Compound	BST[a] LC_{50} (μg/ml)	PD[b] % T/C	Human Cancer Cell Lines[c]		
			A-549	MCF-7	HT-29
1	0.21 (0.33-0.13)	29	5.99	2.32	9.33
2	0.37 (0.55-0.25)	38	3.89	3.44	18.84
3	1.92 (3.05-1.21)	38	2.30	1.12	6.56
4	1.37 (2.43-0.80)	39	1.85	2.09	2.62
5	0.45 (0.74-0.28)	54	35.01	7.76	28.59
6	7.65 (26.59-3.76)	41	2.37	2.41	3.25
7	0.47 (0.69-0.31)	62	3.49	2.69	3.24
8	0.33 (0.50-0.22)	45	3.26	1.84	2.87
9	0.18 (0.27-0.11)	30	3.22	2.87	4.47
10	1.24 (1.70-0.86)	61	15.00	3.06	2.98
Formononetin	22.70 (35.9-14.5)	79	2.87	1.96	6.43
Biochanin A	106.91 (166.2-69.2)	54	19.77	5.49	28.38
8-*O*-Methylretusin	56.53 (91.8-36.5)	52	27.15	21.30	26.34
Adriamycin	0.08 (0.069-0.091)	ND[d]	1.48×10^{-3}	1.47×10^{-1}	6.69×10^{-3}

[a]LC_{50}(μg/ml) with 95% confidence interval in parentheses.
[b]Inhibition of crown gall tumors on potato discs; the value for adriamycin was not determined.
[c]ED_{50} values (μg/ml).
[d]ND=not determined.

EXTRACTION AND ISOLATION.—The air-dried stem bark (12 kg) was extracted exhaustively at room temperature with 95% EtOH and evaporated under a vacuum to yield an extract (F001) (795 g, BST LC_{50} 7.1 μg/ml). The EtOH extract (F001) was partitioned between H_2O and CH_2Cl_2 (1:1) to give an H_2O-soluble residue (F002) (510 g, BST LC_{50}>1000 μg/ml), a CH_2Cl_2-soluble residue (F003) (125 g, BST LC_{50} 3.1 μg/ml), and an interface fraction (F004) (160 g, BST LC_{50}>1000μg/ml). The CH_2Cl_2 residue was further partitioned between hexane and 90% aqueous MeOH to yield the MeOH-soluble residue (F005) (55 g, BST LC_{50} 0.87 μg/ml) and the hexane-soluble residue (F006) (70 g, BST LC_{50} 3.8 μg/ml). F005 was chromatographed over Si gel columns repeatedly, monitored by the BST bioassay, to give compounds **1–3** and **6–10**, which were purified by hplc, and the three isoflavonoids, which were purified by recrystallization. F006 was chromatographed over a Si gel column to give compounds **4** and **5**, which were purified by hplc.

Kneglomeratanol [**1**].—Colorless oil (6 mg); $[\alpha]_D$ −8.3° (*c*=0.06); uv λ max (MeOH) (log ε) 284 (3.44) nm; ir (film) ν max 3430 (OH), 2925, 2853, 1589, 1456 cm^{-1}; hreims *m/z* $[M]^+$ 326.2265 (calcd for $C_{22}H_{30}O_2$, 326.2246); eims *m/z* $[M]^+$ 326 (3), 308 (4) 132 (32), 108 (90), 107 (100), 91 (28), 77 (44); cims (isobutane) *m/z* $[M+H]^+$ 327 (55), 309 (90), 107 (100); ^1H and ^{13}C nmr see Table 1; cd (*c*=0.23 mg/ml, MeOH) $[\theta]_{290.0}$ 0, $[\theta]_{278.2}$ +116.9, $[\theta]_{274.3}$ +60.4, $[\theta]_{266.8}$ +560.3, $[\theta]_{263.7}$ +324.4, $[\theta]_{258.8}$ +525.3, $[\theta]_{248.9}$ +185.6, $[\theta]_{246.6}$ +189.0, $[\theta]_{240.3}$ 0, $[\theta]_{238.0}$ −61.66, $[\theta]_{236.5}$ 0.

Kneglomeratanol diacetate [**1a**].—^1H nmr (500 MHz, $CDCl_3$) δ 1.23 (8H, br s, H-4′, -5′, -6′, -7′), 1.28 (4H, m, H-3′, -8′), 1.60 (2H, m, H-2′), 1.75, 1.88 (each 1H, m, H-9′), 2.07 (3H, s, OAc-10′), 2.29 (3H, s, OAc-1), 2.59 (2H, t, *J*=9 Hz, H-1′), 6.89 (1H, d, *J*=2 Hz, H-2), 6.90 (1H, dd, *J*=2 and 8 Hz, H-4), 7.03 (1H, br d, *J*=8 Hz, H-6), 7.30 (1H, t, *J*=8 Hz, H-5), 7.25–7.30 (5H, m, Ar-H).

Kneglomeratanol S-*di-MTPA ester* [**1b**].—^1H nmr (500 MHz, $CDCl_3$) δ 1.14 (1H, m, H-8′), 1.24 (1H, m, H-8′), 1.27 (8H, m, H-4′, -5′, -6′, -7′), 1.29 (2H, m, H-3′), 1.59 (2H, m, H-2′), 1.74 (1H, m, H-9′), 1.94 (1H, m, H-9′), 2.61 (2H, t, *J*=8.5 Hz, H-1′), 3.44 (3H, s, OMe), 3.69 (3H, s, OMe), 5.95 (1H, dd, *J*=6.5 and 9 Hz, H-10′), 6.92 (1H, d, *J*=2 Hz, H-2), 6.94 (1H, br dd, *J*=2 and 8 Hz, H-4), 7.06 (1H, br d, *J*=8 Hz, H-6), 7.30 (1H, t, *J*=8 Hz, H-5), 7.31 (1H, m, H-4″), 7.36 (2H, m, H-3″, -5″), 7.41 (2H, m, H-2″, -6″), 7.46 (3H, m, Ar-H), 7.66 (2H, m, Ar-H).

Kneglomeratanol R-*di-MTPA ester* [**1c**].—^1H nmr (500 MHz, $CDCl_3$) δ 1.27 (8H, m, H-4′, -5′, -6′, -7′), 1.28 (1H, m, H-8′), 1.29 (2H, m, H-3′), 1.36 (1H, m, H-8′), 1.58 (2H, m, H-2′), 1.81 (1H, m, H-9′), 1.98 (1H, m, H-9′), 2.60 (2H, t, *J*=8.5 Hz, H-1′), 3.53 (3H, s, OMe), 3.69 (3H, s, OMe), 5.87 (1H, dd, *J*=6.5, 9 Hz, H-10′), 6.92 (1H, d, *J*=2 Hz, H-2), 6.94 (1H, br dd, *J*=2, 8 Hz, H-4), 7.06 (1H, br d, *J*=8 Hz, H-6), 7.27 (1H, m, H-4″), 7.28 (1H, t, *J*=8 Hz, H-5), 7.30 (2H, m, H-3″, -5″), 7.35 (2H, m, H-2″, -6″), 7.44 (3H, m, Ar-H), 7.66 (2H, m, Ar-H).

Kneglomeratanone A [**2**].—Colorless plates (13 mg); mp 48–49° [hexane-Me_2CO (4:1)], uv λ max (MeOH) (log ε) 292 (3.75) nm, (+$AlCl_3$) 309 (3.95), 370 (3.55) nm; ir (film) ν max 3400 (OH), 2926, 2854, 1619 (C=O), 1590 cm^{-1}; hreims *m/z* $[M]^+$ 340.2038 (calcd for $C_{22}H_{28}O_3$, 340.2036); eims *m/z* $[M]^+$ 340 (47), 325 (92), 322 (47), 175 (69), 165 (43), 151.0392 (41) (calcd for $C_8H_7O_3$ 151.0395), 105 (7), 91.0544 (100) (calcd for C_7H_7, 91.0548); cims (isobutane) *m/z* $[M+H]^+$ 341 (100), 91 (10); ^1H and ^{13}C nmr see Table 1.

Kneglomeratanone B [**3**].—Colorless prisms (5 mg); mp 89–90° ($CHCl_3$) uv λ max (MeOH) (log ε) 292 (3.75) nm, (+$AlCl_3$) 312 (4.00), 368 (3.80) nm; ir (film) ν max 3450 (OH), 2914, 2849, 1620 (C=O), 1588 cm^{-1}; hreims *m/z* $[M]^+$ 334.2510 (calcd for $C_{21}H_{34}O_3$ 334.2508), 151.0397 (calcd for $C_8H_7O_3$ 151.0395); eims *m/z* $[M]^+$ 334 (5), 319 (26), 316 (7), 175 (49), 165 (31), 151 (31), 123 (41), 69 (55), 55 (100); cims (isobutane) *m/z* $[M+H]^+$ 335 (100); ^1H and ^{13}C nmr see Table 1.

IDENTIFICATION OF KNOWN COMPOUNDS.—Compounds **4** (7 mg), **5** (5 mg), **6** (5 mg), **7** (10 mg), **8** (8 mg), **9** (10 mg), and **10** (400 mg), were respectively identified as 3-(12′-phenyldodecyl)-phenol, 3-(10′-phenyldecyl)-phenol, 5-pentadecylresorcinol, 5-(10′-phenyldecyl)-resorcinol, 5-(12′-phenyldodecyl)-resorcinol, 2,4-dihydroxy-6-(10′-phenyldecyl)-acetophenone, and 2-hydroxy-6-(12′-phenyldodecyl)-benzoic acid by the analyses of their uv, ^1H and ^{13}C nmr, and ms spectra, which were compared with literature reports (1, 7, 9, 10). The isoflavonoids, formononetin (300 mg), biochanin A (100 mg), and 8-*O*-methylretusin (15 mg), were identified by analyses of their uv, ^1H-nmr, ^{13}C-nmr, and ms spectra (11-13); the acetate derivative was also prepared for formononetin.

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LITERATURE CITED

1. A. Kijjoa, M.J.T.G. Gonzalez, M.M.M. Pinto, I.-O. Monanondra, and W. Herz, *Planta Med.*, **57**, 575 (1991).
2. S.K. Jain and R.A. DeFilipps, "Medical Plants of India," Reference Publications, Inc., Algonac, Michigan, 1991, Vol. 2, p. 441.
3. R.N. Chopra, I.C. Chopra, and B.S. Varma, "Supplement to Glossary of India Medicinal Plants," Publications & Information, New Delhi, 1969, p. 50.
4. B.S. Joshi, K.R. Ravindranath, and N. Viswanathan, *Experientia*, **34**, 422 (1978).
5. B.S. Joshi, N. Viswanathan, V. Balakrishnan, D.H. Gawad, and K.R. Ravindranath, *Tetrahedron*, **35**, 1665 (1979).
6. G.F. Spencer, L.W. Tjarks, and R. Kleiman, *J. Nat. Prod.*, **43**, 724 (1980).
7. M.M.M. Pinto, A. Kijjoa, I.-O. Monanondra, A.B. Gutierrez, and W. Herz, *Phytochemistry*, **29**, 1985 (1990).
8. M.J.T.G. Gonzalez, M.M.M. Pinto, A. Kijjoa, C. Anantachoke, and W. Herz, *Phytochemistry*, **32**, 433 (1993).
9. Y. Du, R. Oshima, Y. Yamauchi, J. Kumanotani, and T. Miyakoshi, *Phytochemistry*, **25**, 2211 (1986).
10. H. Wagner, W. Breu, F. Willer, M. Wierer, and P. Remiger, *Planta Med.*, **55**, 566 (1989).
11. R.G. Cooke, *Aust. J. Chem.*, **17**, 379 (1964).
12. R.M. Carman, J.K.L. Russell-Maynard, and R.C. Schumann, *Aust. J. Chem.* **38**, 485 (1985).
13. F.B. Albuquerque, F.R. Braz, O.R. Gottlieb, M.T. Magalhaes, J.G.S. Maia, A. B. de Oliveira, G.G. de Oliveria, and V.C. Wilberg, *Phytochemistry*, **20**, 235 (1981).
14. L.P. Fontana and H.E. Smith, *J. Org. Chem.*, **52**, 3386 (1987).
15. R.D. Gillard and P.R. Mitchell, *Trans. Faraday Soc.*, **65**, 2611 (1969).
16. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
17. M.J. Rieser, Y.-H. Hui, J.K. Rupprecht, J.F. Kozlowski, K.V. Wood, J.L. McLaughlin, P.R. Hanson, Z.-P. Zhuang, and T.R. Hoye, *J. Am. Chem. Soc.*, **114**, 10203 (1992).
18. Z.-M. Gu, X.-P. Fang, L. Zeng, K.V. Wood, and J.L. McLaughlin, *Heterocycles*, **36**, 2221 (1993).
19. J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York and London, 1972, p. 197.
20. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J. L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
21. J.L. McLaughlin, in "Methods in Plant Biochemistry." Ed. by K. Hostettmann, Academic Press, London, 1991, Vol. 6, p. 1.
22. N.R. Ferrigni, J.E. Putnam, B. Anderson, L.B. Jacobsen, D.E. Nichols, D.S. Moore, J.L. McLaughlin, R.G. Powell, and C.R. Smith, Jr., *J. Nat. Prod.*, **45**, 679 (1982).
23. S.J. Giard, S.A. Aronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik, and W.P. Parks, *J. Natl. Cancer Inst.*, **51**, 1417 (1973).
24. H.D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennan, *J. Natl. Cancer Inst.*, **51**, 1409 (1973).
25. J. Fogh and G. Trempe, in "Human Tumor Cells in Vitro." Ed. by J. Fogh, Plenum Press, New York, 1975, p. 115.

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