Journal of Natural Products
Vol. 52, No. 2, pp. 325-331, Mar-Apr 1989

ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF ROTTLERIN-TYPE COMPOUNDS FROM *HYPERICUM DRUMMONDII*[1]

HIRANTHI JAYASURIYA, JAMES D. MCCHESNEY,*

Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

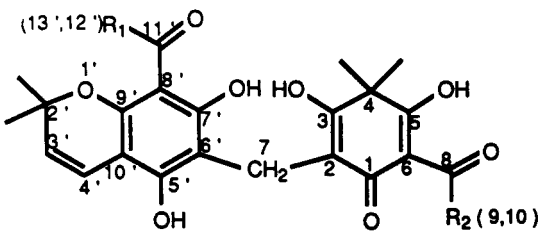
STEVEN M. SWANSON, and JOHN M. PEZZUTO*

Program for Collaborative Research in the Pharmaceutical Sciences, and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, and Division of Surgical Oncology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT.—Hexane extracts of *Hypericum drummondii* showed significant activity against the Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis,* and the acid-fast bacterium *Mycobacterium smegmatis* in an agar well diffusion assay. Employing bioassay-directed fractionation procedures, four new rottlerin-type compounds (drummondins A, B, C [**1–3**], and F [**4**]) were isolated and identified by spectral and physical characterization. The antimicrobial activity of these compounds was comparable to or greater than that demonstrated by streptomycin and generally correlated with cytotoxic activity determined with cultured P-388, KB, or human cancer cell lines (breast, colon, lung, melanoma). No cell-type selectivity was observed. In addition, two known compounds, albaspidins A-A [**5**] and P-P [**6**], were isolated and structurally characterized. Neither demonstrated appreciable antimicrobial or cytotoxic activity.

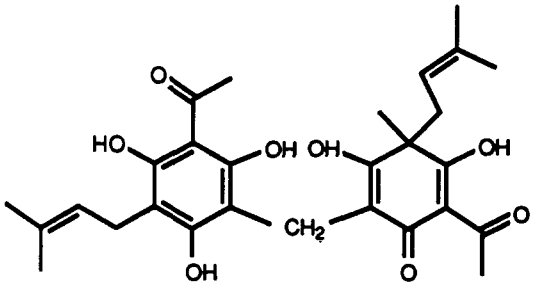
Plants of the genus *Hypericum* have been used in folk medicine (1–3) and for the treatment of tumors (4). During the course of evaluating the biological potential of plants indigenous to Mississippi, it was found that hexane extracts derived from the roots and the above-ground parts (leaves + stems) of *Hypericum drummondii* (Grev. & Hook.) T.&G. (Hypericaceae) showed significant activity against the Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis,* and the acid-fast bacterium *Mycobacterium smegmatis,* in an agar well diffusion assay. Bearing in mind that this genus has previously been used for the isolation of antibiotics (1–7) and an antifungal chromene (8), bioassay (qualitative antimicrobial)-directed fractionation procedures were undertaken with this species. As a result, four drummondins (drummondins A [**1**], B [**2**], C [**3**], and F [**4**]), and two albaspidins (albaspidins A-A [**5**] and P-P [**6**]) were isolated and characterized.

Drummondins are new rottlerin-type compounds. Drummondins A, B, and C pos-

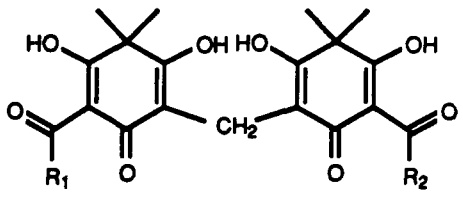


1 $R_1=R_2=Et$
2 $R_1=Me$, $R_2=Et$
3 $R_1=R_2=Me$

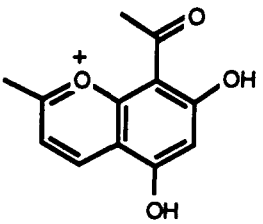
[1]Some of the results contained herein were presented at the Twenty-eighth Annual Meeting of the American Society of Pharmacognosy, July 19–22, 1987, University of Rhode Island, Kingston, RI.



4



5 $R_1=R_2=Et$
6 $R_1=R_2=Me$



7

sess a 3-acylfilicinic acid moiety connected to a 2,2-dimethyl chromene via a methylene bridge. Drummondin F possesses a modified filicinic acid residue connected to a prenylated phloroacetophenone via a methylene bridge. The isolation and the structure elucidation of drummondin F will be reported elsewhere with other structurally similar compounds (9). The albaspidins are dimers of 3-acylfilicinic acid and are known compounds (10).

The ir spectra of drummondins A, B, and C and the albaspidins showed broad absorption in the 3000 cm^{-1} region. Together with intense peaks at 1600–1650 cm^{-1}, this suggested the presence of an enolic 1,3-diketo system or a 2-hydroxyaryl ketone (11,12). The very low field (18 ppm exchangeable proton) signal in the ^{1}H-nmr spectra of these compounds further supports the presence of an enolizable β-triketone system. This correlation, together with signals for methyl groups absorbing at 1.5 ppm in the ^{1}H-nmr spectrum, strongly indicated the presence of a 3-acyl filicinic acid moiety in the compounds. Both the ^{1}H- and ^{13}C-nmr of these compounds show certain anomalies which can be explained by the expected tautomerization of the acylfilicinic acid system (12).

The ^{1}H-nmr spectral data of drummondins A, B, and C suggested a close resemblance to the rottlerin-type phloroglucinols, uliginosin B (7) and sarothralen B (2), isolated from *Hypericum uliginosum* HBK. and *Hypericum japonicum* Thunb., respectively. Compared with uliginosin B, the only differences in the ^{1}H-nmr spectra were in the region of 1–4 ppm, indicating different acyl groups in our compounds. The ^{1}H-nmr spectra did not show any evidence for the presence of branched alkyl groups which are present in the acyl moieties of all other rottlerin-type phloroglucinols reported from the genus *Hypericum* to date (1,2,4,5).

Drummondin A [**1**] showed two quartets centered at δ 3.25 (q, $J=7.5$) and δ 3.12 (q, $J=7.5$) in the ^{1}H-nmr spectrum. These were coupled with two almost overlapping

triplets at δ 1.18 (t, $J = 7.5$) and δ 1.20 (t, $J = 7.5$). Taken in conjunction, these facts strongly suggested that an *n*-propionyl substituent was attached to each carbocyclic ring of the compound. The ^{13}C-nmr signals for the ethyl moieties were seen at δ 34.8 (methylene), δ 8.6 (methyl), and δ 37.1 (methylene), δ 8.9 (methyl).

Drummondin B [**2**] showed in the ^{1}H-nmr spectrum only one quartet centered at δ 3.25 (q, $J = 7.5$) coupled to a triplet at δ 1.18 (t, $J = 7.5$) which suggested an *n*-propionyl moiety attached to one of the carbocyclic rings. There was an additional singlet at δ 2.7 integrating for three protons which indicated an acetyl moiety in the system. The ^{13}C-nmr spectrum provided additional information since there were signals for the acetyl moiety at δ 32.6 (methyl) and for the ethyl moiety at δ 34.8 (methylene) and δ 8.6 (methyl). Based on the fragment ion **7** at *m/z* 219 (100%) in the mass spectrum, the *n*-propionyl group was placed in the filicinic acid moiety and the acetyl group in the other carbocyclic ring.

Drummondin C [**3**] showed two singlets in the ^{1}H-nmr spectrum at δ 2.74 and δ 2.69, each integrating for three protons, thus suggesting two acetyl groups in the compound. This was confirmed by the ^{13}C-nmr signals at δ 29.3 (methyl) and δ 32.2 (methyl) for the two acetyl methyl groups. Complete carbon and proton signal assignments for the compounds were performed by extensive SINEPT experiments (13) and are reported in Tables 1 and 2.

TABLE 1. ^{1}H-nmr Chemical Shifts (ppm) and Coupling Constants (Hz) of Drummondins **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
4-Me	1.49 br s	1.49 br s	1.49 br s
H-7	3.53 br s	3.53 br s	3.52 br s
H-9	3.25 q (7.5)	3.25 q (7.5)	
9-Me			2.74 s
10-Me	1.18 t (7.5)	1.18 t (7.5)	
2′-Me	1.49 br s	1.49 br s	1.49 br s
H-3′	5.44 d (9.9)	5.44 d (9.9)	5.44 d (9.9)
H-4′	6.69 d (9.9)	6.68 d (9.9)	6.68 d (9.9)
H-12′	3.12 q (7.5)		
12′-Me		2.69 s	2.69 s
13′-Me	1.20 t (7.5)		
3-OH	9.95 s	9.90 s	9.94 s
5-OH	18.49 s	18.49 s	18.42 s
5′-OH	11.44 s	11.47 s	11.47 s
7′-OH	16.01 s	15.86 s	15.88 s

[a]Recorded at 300 MHz in $CDCl_3$.

Differences in the albaspidins were also apparent in their acyl substituents. Here again, similar observations were seen in both the ^{1}H- and ^{13}C-nmr spectra of these compounds (as in the analogous spectra of drummondins C and A), and the isolates were identified as albaspidin A-A [**5**] and albaspidin P-P [**6**].

The minimum inhibitory concentrations of compounds **1–6** (Table 3) were determined with representative Gram-positive bacteria and an acid-fast bacterium. With all three tester strains, the activities of compounds **1**, **2**, and **4** were equivalent to or higher than those demonstrated by streptomycin. Relative to this positive comparative stan-

TABLE 2. ^{13}C-nmr Spectral Data of Drummondins **1–3**.[a]

Carbon	Compound		
	1	**2**	**3**
1	198.0 (0)[b]	198.0 (0)	198.7 (0)
2	108.0 (0)	108.0 (0)	108.5 (0)
3	171.6 (0)	171.6 (0)	171.8 (0)
4	44.3 (0)	44.2 (0)	44.4 (0)
4-Me	24.8 (3)	24.8 (3)	24.8 (3)
5	187.3 (0)	187.3 (0)	187.3 (0)
6	111.2 (0)	111.2 (0)	111.2 (0)
7	16.8 (2)	16.8 (2)	16.8 (2)
8	207.1 (0)	207.1 (0)	207.1 (0)
9	34.8 (2)	34.8 (2)	29.3 (3)
10	8.6 (3)	8.6 (3)	
2′	78.2 (0)	78.2 (0)	78.2 (0)
2′-Me	28.1 (3)	28.2 (3)	28.2 (3)
3′	124.6 (1)	124.7 (1)	124.7 (1)
4′	117.3 (1)	117.2 (1)	117.2 (1)
5′	159.1 (0)	159.4 (0)	159.4 (0)
6′	106.1 (0)	106.0 (0)	106.0 (0)
7′	161.4 (0)	161.2 (0)	161.2 (0)
8′	104.4 (0)	104.8 (0)	104.8 (0)
9′	155.7 (0)	155.9 (0)	155.9 (0)
10′	103.6 (0)	103.6 (0)	103.6 (0)
11′	206.9 (0)	203.5 (0)	203.5 (0)
12′	37.1 (2)	32.6 (3)	32.2 (3)
13′	8.9 (3)		

[a]Recorded at 75 MHz in $CDCl_3$.

[b]The numbers in parentheses indicate number of attached protons as determined by APT.

dard, the activity demonstrated by compound **3** did not differ by more than a factor of two. On the other hand, albaspidins **5** and **6** demonstrated relatively weak activity (Table 3). All of the compounds were also tested for activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), but none was active (MIC > 100 μg/ml).

Compounds **1–6** were also tested for cytotoxic potential with a variety of cultured

TABLE 3. Evaluation of the Antimicrobial Potential of Compounds **1–6** with *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*.[a]

Compound	Microorganism tested		
	S. aureus	*B. subtilis*	*M. smegmatis*
1	1.56	0.78	1.56
2	3.12	0.39	1.56
3	3.12	1.56	6.25
4	0.78	0.78	1.56
5	50.0	12.5	50.0
6	100.0	50.0	100.0
Streptomycin	6.25	0.78	3.12

[a]The data are presented as the minimum inhibitory concentration (in μg/ml) that prevented growth of the microorganism.

cells (Table 4). Drummondins A–C [**1–3**] induced the strongest cytotoxic response, but no apparent selectivity toward specific cell type was noted. Demonstrable ED_{50} values were also obtained with compound **4** in all of the cell lines except melanoma, and compounds **5** and **6** were inactive. Thus, with the notable exception of compound **4**, there was a positive correlation between the antibacterial and cytotoxic activity of these compounds.

TABLE 4. Evaluation of the Cytotoxic Potential of Compounds **1–6** with P-388, KB, and a Variety of Human Tumor Cell Lines.[a]

Compound	Cell Line Tested					
	P-388	KB	Breast	Colon	Lung	Melanoma
1	2.1	4.5	3.4	6.6	4.5	8.0
2	1.9	3.1	NT[b]	6.3	7.0	5.0
3	2.8	8.6	5.1	11	8.7	12
4	21	18	21	42	30	>50
5	>50	>50	>50	>50	>50	>50
6	>50	>50	>50	>50	>50	>50

[a]The data are presented as ED_{50} values (μg/ml).
[b]NT, not tested.

Examination of the structures of these compounds suggests a number of potential toxic mechanisms, e.g., generation of free radical species, chelation of intracellular cations, and binding to various macromolecules or nucleophilic centers within the cell. It is obvious from our data that the toxic response is not specific for bacteria (e.g., inhibition of cell wall synthesis) and is not affected by placement of Me or Et groups at the designated R-group positions. Rottlerin-type compounds that are structurally similar to drummondins A–D, isolated from *Mallotus japonicus* Muell. Arg. (Euphorbiaceae), have previously been reported to induce cytotoxic effects (14–16) of similar magnitude to those shown in Table 4 for compounds **1–3**. It appears from the results of Arisawa and co-workers that a dimeric structure is required for activity, and it seems likely that the conjugation system of compounds **1–4** (versus that of compounds **5** and **6**) contributes to the demonstrated biological activity. The mechanism of toxicity mediated by these compounds, as well as their role in their native habitat, remains the subject of future investigation. Due to their generalized activity, however, they are unlikely candidates for additional development as antitumor or antibiotic agents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns digital melting point analyzer (Model 355) and were not corrected. Ir spectra (KBr) were taken on a Perkin-Elmer 281B spectrometer. Uv spectra were recorded on a Varian Cary 2200 spectrophotometer using EtOH as the solvent. Low resolution mass spectra were obtained on a Hewlett-Packard spectrometer (Model 5985) operating in electron impact mode at 70 eV.

Nmr spectra were recorded on a Varian VXR (300 MHz). For both ^1H and ^{13}C nmr, chemical shifts were expressed as ppm relative to TMS as internal standard (δ units).

Si gel cc employed Si gel HF_{254} type 60. Due to the instability, high polarity, and difficulty of separating these closely-related compounds, no attempts were made to calculate yields as a percentage.

PLANT MATERIAL.—The plant *H. drummondii* was collected at a farm near Oxford, Mississippi, in August 1986. A voucher specimen is lodged in the Herbarium, Department of Pharmacognosy, School of Pharmacy, The University of Mississippi. The plant material was air-dried, separated into roots, stems, and leaves, and ground in a Wiley mill.

EXTRACTION AND ISOLATION.—The air-dried, finely ground roots of the plant material (3.5 kg)

were exhaustively percolated with hexane at room temperature. The combined extracts were concentrated under reduced pressure to produce a waxy residue (20 g).

The first phases of the isolation of the antimicrobially active materials from the crude extract were followed by bioautography. This was performed by developing a sample of the extract on tlc plates with 5% Et_2O in hexane with 0.25% of HCO_2H added. After development, the tlc plate was air-dried and placed on agar medium in a 150 mm Petri dish streaked with the test microorganism, *B. subtilis*. After 30 min, the tlc plate was removed, and the Petri dish was incubated at 30°. A clear elongated zone appeared at $R_f = 0.16$–0.53. Subsequent isolation work was followed by tlc using R_f values as guides.

The residue of the hexane extract (15 g) was partially purified by a Si gel HF_{254} type column prepared and developed with 2% Et_2O in hexane with 0.25% HCO_2H added. Three light yellow bands were collected separately to yield three fractions (A, B, and C) totalling 1.33 g.

The fractions A, B, and C were chromatographed on Si gel HF_{254} columns using 1:100 sample/Si gel ratios. The developing solvent was 2% Et_2O in hexane with 0.25% HCO_2H added. Fraction A (264.2 mg) yielded compound **1** (14.6 mg) and compound **5** (42 mg). Fraction B (572.6 mg) also yielded compound **5** (23.8 mg) and compound **2** (45.1 mg). Fraction C (496.0 mg) yielded compound **6** (42.1 mg) and compound **3** (14.8 mg).

Drummondin A [**1**].—Yellow crystals from hexane (14.6 mg); mp 130–132°; uv (EtOH) λ max (log ε) 285 (4.6), 358 nm (4.5); ir (KBr) ν max 3300–2500, 2980, 2930, 1640, 1605, 1430, 1365, 1130 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 470 (30), 455 (30), 261 (15), 245 (32), 233 (100), 227 (13), 215 (19); ^1H nmr see Table 1; ^{13}C nmr see Table 2; high resolution mass measurement found 470.1950, calcd for $C_{26}H_{30}O_8$, 470.1939.

Drummondin B [**2**].—Yellow crystals from hexane (45.1 mg); mp 136–138°; uv (EtOH) λ max (log ε) 356 (4.5), 282 (4.8), sh 270 nm (4.8); ir (KBr) ν max 3300–2500, 2980, 2930, 1635, 1595, 1555, 1365, 1125 cm^{-1}; eims *m/z* (rel. int. %) $[M]^+$ 456 (31%), 441 (26), 247 (16), 234 (20), 233 (11), 231 (35), 219 (100), 213 (12); ^1H nmr see Table 1; ^{13}C nmr see Table 2; high resolution mass measurement found 456.1786, calcd for $C_{25}H_{28}O_8$, 456.1782.

Drummondin C [**3**].—Yellow crystals from EtOAc (14.8 mg); mp 184–187°; uv (EtOH) λ max (log ε) 355 (4.2), 284 nm (4.6); ir (KBr) ν max 3300–2500, 2980, 2930, 1640, 1605, 1430, 1365, 1130 cm^{-1}; eims *m/z* (rel. int. %) $[M]^+$ 442 (27), 427 (29), 247 (12), 234 (17), 231 (37), 219 (100), 213 (12), 201 (16); ^1H nmr see Table 1; ^{13}C nmr see Table 2; high resolution mass measurement found 498.2247, calcd for $C_{28}H_{34}O_8$, 498.2252.

Drummondin F [**4**].—Yellow crystals from hexane (66.7 mg); mp 106–107°; $[\alpha]_D$ +56.4 ($c = 2.5$, MeOH); ir (KBr) ν max 3600–2500 (broad), 3520 (w, br), 3150 (m, br), 2930 (m), 1650 (s), 1610 (s, br), 1460 (m), 1440 (m), 1370 (s) cm^{-1}; uv (MeOH) λ max (log ε) 350 (4.09), 300 (4.27); ms *m/z* (rel. int. %) 165 (13), 182 (15), 193 (29), 195 (25), 236 (27), 355 (10), 498 (10); high resolution mass measurement found 498.2247, calcd for $C_{28}H_{34}O_8$, 498.2252.

Albaspidin P-P [**5**].—Colorless crystals from hexane (42 mg); mp 136–137° [lit. (10) 135–137°]; uv (EtOH) λ max (log ε) 264 (4.6), 294 (4.5), 344 (4.4); ir (KBr) ν max 3150–2300, 2990, 2940, 1635, 1560, 1385, 1205, 850; eims *m/z* (rel. int. %) $[M]^+$ 432 (100), 417 (10), 223 (89), 210 (87), 195 (68), 193 (59), 182 (38), 167 (37).

Albasipidin A-A [**6**].—Colorless crystals from hexane (42.1 mg); mp 164–166° [lit. (10) 170–171°]; uv (EtOH) λ max (log ε) 366 (4.6), 339 (4.8), 294 (4.7); ir (KBr) ν max 3150–2200, 3000, 2945, 1635, 1575, 1560, 1540, 1435, 1385, 1205, 850; eims *m/z* (rel. int. %) $[M]^+$ 404 (68), 209 (57), 208 (29), 196 (100), 193 (27), 181 (21), 168 (40), 153 (39), 137 (18).

MICROORGANISMS.—*S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), and *M. smegmatis* (ATCC 607) strains are deposited in the University of Mississippi, Department of Pharmacognosy Culture Collection and were originally obtained from the American Type Culture Collection (ATCC), Rockville, Maryland.

QUALITATIVE ANTIMICROBIAL ASSAY.—The qualitative, antimicrobial assay employed was an agar well diffusion assay. Plates for the assay were prepared by dispensing 25 ml of sterile agar medium into 100 × 15 mm sterile Petri dishes. The solidified sterile agar plates were streaked uniformly with a dilution of the test organism by using the quadrant streak method. Wells with a diameter of approximately 11 mm were produced by using a sterile cork borer, and 100 μl of the sample solution was then added to each well. Streptomycin sulfate (100 μl of a 1 mg/ml solution) was used as standard control antibacterial agent for the assay. Plates with *B. subtilis* were incubated at 30° while the others were incubated at 37°. The radii of the inhibition zones were measured after incubation for 24 h (*S. aureus*, *B. subtilis*) or 48 h (*M. smegmatis*).

QUALITATIVE ANTIMICROBIAL ASSAY.—The MIC values were determined using a twofold serial di-

lution technique previously described (17). Tubes inoculated with *B. subtilis* were incubated at 30° while tubes inoculated with *M. smegmatis* and *S. aureus* were incubated at 37°. The MIC values were determined after 24 h (*S. aureus* and *B. subtilis*) or 48 h (*M. smegmatis*) of incubation. Streptomycin sulfate was used as a positive control in the assay system, and all compounds were tested within the range of 0.20–100 μg/ml.

EVALUATION OF CYTOTOXIC POTENTIAL.—Compounds **1–6** were evaluated for cytotoxic potential essentially by procedures established by the National Cancer Institute (18) as described previously (19,20). In brief, samples were dissolved in DMSO and added to the culture media (final DMSO concentration, 0.5%). Five concentrations of test materials were employed, ranging from 0.08 to 50 μg/ml. After an incubation period of 48 h (P388 cells) or 72 h (epithelial cell-types), the resulting cell number was enumerated (P388 cells) or analyzed by protein determination (epithelial cell-types). The results were then expressed as a percentage relative to solvent-treated controls, after correcting for the cell number at the start of the experiment. All procedures were performed in duplicate, and ED_{50} values were obtained from semilogarithmic dose–response curves. The human tumor cell lines (breast, colon, lung, melanoma) were established in the Division of Surgical Oncology, University of Illinois College of Medicine, from primary human tumor specimens. Their characteristics will be described at a later date. In each case, the assay procedure paralleled the procedure routinely preformed with cultured KB cells.

ACKNOWLEDGMENTS

The authors are grateful to Dr. E.M. Croom, Jr., of the Research Institute of Pharmaceutical Sciences, University of Mississippi, for the identification of the plant material, and Professors S. Takagi and K. Ishiguro, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Japan, for the provision of the spectra of sarothralen B and albaspidin iB-iB. J.M.P. is the recipient of a Research Career Development Award from the National Cancer Institute, NIH (1984–1989).

LITERATURE CITED

1. H.L. Taylor and R.M. Brooker, *Lloydia*, **32**, 217 (1969).
2. K. Ishiguro, M. Yamaki, M. Kashihara, and S. Takagi, *Planta Med.*, **4**, 288 (1986).
3. K.N. Gaind and T.N. Granjoo, *Indian J. Pharm.*, **21**, 172 (1959).
4. K. Ishiguro, M. Yamaki, S. Takagi, Y. Yamagata, and K. Tomita, *J. Chem. Soc., Chem. Commun.*, 26 (1985).
5. K. Ishiguro, M. Yamaki, M. Kashihara, and S. Takagi, *Planta Med.*, **5**, 415 (1987).
6. M. Nagai and M. Tada, *Chem. Lett.*, 1337 (1987).
7. W.L. Parker and F. Johnson, *J. Am. Chem. Soc.*, **90**, 4716 (1968).
8. L. Décosterd, H. Stoeckli-Evans, J.D. Msonthi, and K. Hostettmann, *Planta Med.*, 429 (1986).
9. H. Jayasuriya and J.D. McChesney, *J. Nat. Prod.*, in press.
10. A. Penttila and J. Sundman, *Acta Chem. Scand.*, **18**, 344 (1964).
11. S. Forsen, F. Merenyi, and M. Nilsson, *Acta Chem. Scand.*, **18**, 1208 (1964).
12. S. Forsen, M. Nilsson, and C.A. Wachtmeister, *Acta Chem. Scand.*, **16**, 583 (1962).
13. H. Jayasuriya, N.P.D. Nanayakkara, and J.D. McChesney, submitted for publication in *Aus. J. Chem.*
14. M. Arisawa, A. Fujita, R. Suzuki, T. Hayashi, N. Morita, N. Kawano, and S. Koshimura, *J. Nat. Prod.*, **48**, 455 (1985).
15. M. Arisawa, A. Fujita, M. Saga, T. Hayashi, and N. Morita, *J. Nat. Prod.*, **49**, 298 (1986).
16. A. Fujita, T. Hayashi, M. Arisawa, M. Shimizu, N. Morita, T. Kikuchi, and Y. Tezuka, *J. Nat. Prod.*, **51**, 708 (1988).
17. C.D. Hufford, M.J. Funderburk, J.M. Morgan, and L.W. Robertson, *J. Pharm. Sci.*, **64**, 789 (1975).
18. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**(3), 1 (1972).
19. M. Arisawa, J.M. Pezzuto, C. Bevelle, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).
20. J.M. Pezzuto, S.K. Antosiak, W.M. Messmer, M.B. Slaytor, and G.R. Honig, *Chem. Biol. Interact.*, **43**, 323 (1983).

Received 14 October 1988