Phytogrowth-Inhibitory and Antifungal Constituents of *Helianthella quinquenervis*[†]

Perla Castañeda, Leda Gómez, and Rachel Mata*

Departamento de Farmacia, Facultad de Química and Unidad Conjunta de Plantas Medicinales, Instituto de Química and Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, México D.F., Mexico

Blas Lotina-Hennsen

Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, Mexico D.F., Mexico

Ana Luisa Anaya

Instituto de Fisiología Celular, Universidad Nacional Autónoma de Mexico, Apartado Postal 70-265, 04511, México D.F., Mexico

Robert Bye

Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, México D.F., Mexico
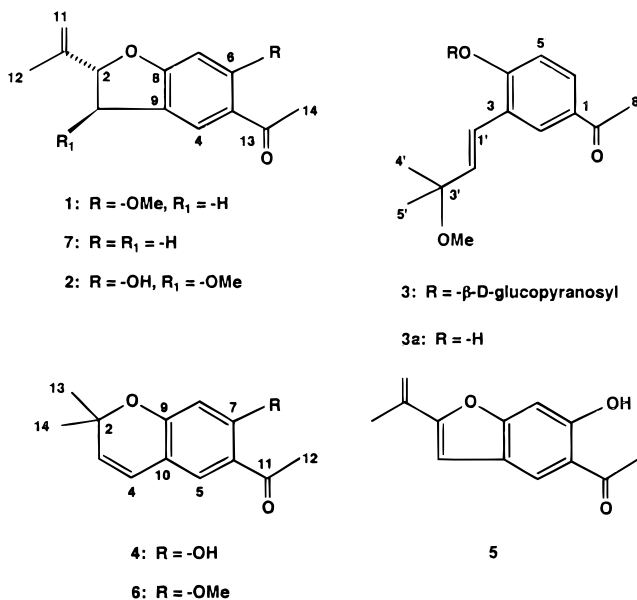
Received April 21, 1995[®]

Investigation on the roots of *Helianthella quinquenervis* (Hook.) A. Gray (Asteraceae), led to the isolation of one new benzofuran (6-methoxy-tremetone (**1**)) and a new prenylacetophenone (4-β-D-(glucopyranosyloxy)-3-[3-methoxy-*trans*-isopenten-1-yl]acetophenone (**3**)). In addition, 6-hydroxy-3-methoxytremetone (**2**), encecalin (**6**), euparin (**5**), demethylencecalin (**4**), and angelic acid were obtained. Structural assignments of the isolated compounds were based on spectroscopic and spectrometric analysis. Natural products **1**–**4** showed marginal cytotoxicity against three human tumor cell lines [MCF-7, A-549, and HT-29]. Compounds **4** and **6** inhibited the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli*. Furthermore, substances **4**–**6** exhibited antifungal activity against *Trichophyton mentagrophytes*.

Helianthella quinquenervis (Hook.) A. Gray (Asteraceae), commonly known as "little sunflower", is a perennial herb with large yellow flowering heads and grows in the subalpine coniferous forests of the Rocky Mountains of the western United States from Montana to New Mexico. Isolated populations extended into the pine–oak forests of the extreme northern Sierra Madre Occidental and Sierra Madre Oriental of Mexico. It is known to the Tarahumara Indians of Chihuahua, Mexico, as "rarésoa", and its thick, vertical roots are used medicinally.[1] The powdered roots are applied topically to the body of humans and domestic animals in order to eliminate ectoparasites. The decoction can be employed for the same purpose as well as to improve the quality of the hair. Taken internally, the decoction is said to be good for treating gastrointestinal ailments and ulcers.

A previous phytochemical study on the aerial parts of this plant led to the isolation and characterization of one prenylhydroxyphenone and several known acetyl-chromenes, including demethylencecalin (**4**) and encecalin (**6**).[2]

In the course of our continuing search for antitumor, antimicrobial, and herbicidal agents from Mexican medicinal plants,[3–5] we describe in this investigation the isolation and structural elucidation of the major phytotoxic, cytotoxic, and antifungal principles from the roots of *H. quinquenervis.*

1: R = -OMe, R₁ = -H → 1: R = -OMe, R$_1$ = -H

7: R = R$_1$ = -H

2: R = -OH, R$_1$ = -OMe

3: R = -β-D-glucopyranosyl

3a: R = -H

4: R = -OH

6: R = -OMe

5

Results and Discussion

The roots of *H. quinquenervis* were extracted with MeOH. The MeOH extract and the isolated compounds were evaluated for their potential cytotoxic, phyto-growth-inhibitory, and antifungal properties. Their lethality to brine shrimp larvae (BST) was also investigated.[6] The cytotoxic activity was determined against three human solid tumor cell lines.[6] The results are summarized in Table 1. The initial phytogrowth-inhibitory activity (Table 2) was evaluated on seedlings of *Amaranthus hypochondriacus* L. and *Echinochloa*

Table 1. Cytotoxicity and Brine Shrimp Lethality of the MeOH extract and Compounds **1**−**6** from *H. quinquenervis*

	ED$_{50}$ μg/mL			LC$_{50}$ μg/mL
compd	MCF-7[a]	HT-29[a]	A-549[a]	BST
1	33	16	1	8
2	34	16	31	68
3	44	61	6	>1000
4	2	2	10	31
5	6	6	22	44
6	29	18	25	31
extract	3	>100	5	9
Adriamycin[b]	2×10^{-2}	4×10^{-2}	2×10^{-3}	ND

[a] Cytotoxicities in human breast (MCF-7),[18] colon (HT-29),[19] and lung (A-549)[20] tumor cell lines. Activity criteria: extracts, ED$_{50}$ < 20 μg/mL; pure compounds, ED$_{50}$ < 4 μg/mL. [b] Positive standard control.

Table 2. Phytogrowth-Inhibitory Activity of the MeOH Extract and Compounds **1**−**6** from *H. quinquenervis*

	seedling growth IC$_{50}$ (μg/mL)	
compd	*E. crusgalli*	*A. hypochondriacus*
1	68	11
2	323	61
3	231	148
4	79	27
5	252	111
6	6	7
MeOH extract	53	25
tricolorin A[a]	12	37

[a] Positive standard control.

crusgalli (L.) Beauv. by using the Petri dish bioassay (PDPIB).[7] Finally, the antifungal bioassay was performed by the agar dilution procedure[8] against *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger*. The minimum inhibitory concentrations (MIC) were >1000, 300, and >1000 μg/mL, respectively. According to the results of the biological evaluations, the MeOH extract of *H. quinquenervis* was bioactive in the BST (Table 1) and in the phytogrowth-inhibitory bioassay (Table 2). It also showed antifungal properties against *T. mentagrophytes*.

The active extract was fractionated by column chromatography over Si gel, using the BST at each step for activity-directed fractionation, to yield three toxic primary fractions (F-1, F-3, and F-4, see Experimental Section). F-1, F-3, and F-4 also showed inhibitory activity when tested by the bioautographic phytogrowth-inhibitory bioassay [BPIB].[9] Repeated column chromatography on Si gel of fraction F-1 (BST LC$_{50}$ = 35 μg/mL) and F-4 (BST LC$_{50}$ = 65 μg/mL) afforded the known compounds **4**−**6** and **2**, respectively. Their spectral characteristics, including UV, IR, and ^{1}H NMR data, were identical to those previously described for 6-hydroxy-3-methoxytremetone (**2**),[10] encecalin (**6**), euparin (**5**), and demethylencecalin (**4**).[11,12] Full assignment of the ^{13}C-NMR (not previously described) of compounds **2**, **4**, and **6** was carried out.

Extensive chromatographic separation of active fraction F-3 (BST LC$_{50}$ = 22 μg/mL) allowed the isolation of the novel compound **1**. Finally, the new glycoside **3** and angelic acid were isolated from the inactive fraction F-6.

6-Methoxytremetone (**1**) was a yellow oil, optically active, and analyzed for C$_{14}$H$_{16}$O$_3$. The UV, IR, and NMR spectra were very similar to those of tremetone (**7**) and related benzofurans.[13–15] When the ^{1}H NMR data of **1** and **7** were compared, the main differences

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **3** and **3a**[a]

	3		**3a**	
position	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1		125.9		126.9
2	8.06 d (2.0)	126.5	7.61 d (2.0)	126.8
3		130.9		132.0
4		157.9		160.0
5	7.20 d (8.8)	114.9	7.21 d (9.0)	115.3
6	7.81 dd (2.1, 8.6)	129.1	7.74 dd (2.0, 8.2)	129.2
1'	6.85 d (16.6)	122.5	6.79 d (16.5)	122.9
2'	6.35 d (16.6)	137.3	6.35 d (16.5)	136.9
3'		74.9		74.6
4'	1.30 s	25.8	1.35 s	25.6
5'	1.30 s	25.8	1.35 s	25.6
3'-OMe	3.09 s	49.8	3.10 s	49.0
7		196.9		197.0
8	2.58 s	26.8	2.60 s	26.9
1''	5.01 d (7)	100.0		
2''	3.32 dd (8.5, 7)	73.3		
3''	3.29 dd (8.5, 8.5)	76.6		
4''	3.19 m	69.6		
5''	3.37 m	77.1		
6''	3.46 dd (11.2, 5), 3.68 dd (12, 3.7)	60.6		
OH	5.45, 5.12, 5.0, 4.56		3.20 brs	

[a] Spectra are recorded in DMSO-d_6. Chemical shifts are in ppm with TMS as internal standard. Assignments were supported by COSY, DEPT, HETCOR, and NOESY experiments. Coupling constants in Hz are given in parentheses.

were the presence of a singlet at δ 3.87 due to the methoxyl group at C-6 and the aromatic region, which exhibited two *para* related protons at δ 6.42 (s, H-7) and 7.68 (t, J_{3-4} = 1.2 Hz, H-4). The chemical shift values of H-4 and H-7 as well as the UV absorption maxima at 279 and 320 nm were in agreement with the placement of the ketone moiety and the methoxyl group at C-5 and C-6, respectively.[16]

4-β-D-(Glucopyranosyloxy)-3-[3-methoxy-*trans*-isopenten-1-yl]acetophenone (**3**), was an amorphous powder. The molecular ion was indicated by a peak at *m/z* 397 [M + 1] in the FABMS (nba), and it was consistent with the molecular formula C$_{20}$H$_{28}$O$_8$ established by elemental analysis. The IR spectrum exhibited characteristic absorptions for an aromatic compound bearing hydroxyl and conjugated carbonyl groups. Acid or enzymatic hydrolysis of **3** gave the aglycon **3a** and glucose. The ^{1}H NMR data of the aglycon turned out to be identical to that previously described for 4-hydroxy-3-[3-methoxy-*trans*-isopenten-1-yl]acetophenone (**3a**), isolated from *H. uniflora*.[11] The ^{13}C NMR data of **3** (Table 3) supported the assignment of β-D-glucopyranose as the sugar unit [δ_C 100.02, 77.12, 76.64, 73.27, 69.55, 60.56].[4,17] The β configuration of the sugar residue was inferred from the enzymatic hydrolysis (β-glucosidase) and the chemical shift and coupling constant values observed for the anomeric proton (δ 5.01, d, J = 7 Hz). The linkage of the β-D-glucopyranosyl moiety to the phenolic group at C-4 was clearly indicated in the ^{13}C NMR spectrum (Table 3) by the significant upfield displacement of the *ipso* carbon (Δ = −2.15) when compared with aglycon **3a**.[4] The strong interaction observed between the resonances at δ 7.20 (H-5) and δ 5.01 (H-1'') in the 2D NOESY NMR spectrum of **3** provided additional evidence for the attachment of the glucopyranosyloxy moiety at C-4. The NOESY spectrum also exhibited a cross-peak between the signals at δ 6.35 (H-2') and δ 8.06 (H-2); this correlation was consistent with the disposition of the isoprenyl moiety at C-3 and with the

closer proximity of H-2′ to H-2 (H-1′ did not show correlation with H-2).

Biological activities of compounds **1**−**6** are summarized in Tables 1 and 2. All of these compounds but **1** were inactive in the BST, and only **1** and **4** showed weak cytotoxicity against MCF-7 (breast)[18] and HT-29 (colon)[19] human solid tumor cells in 7-day *in vitro* tests, using adriamycin as the positive control compound.

The six compounds were also evaluated for their ability to inhibit the radical growth of *Echinochloa crusgalli* and *Amaranthus hypochondriacus*.[7] According to the results summarized in Table 2, these products reduced the radical growth of both target species in a concentration dependent manner with higher activity at increasing concentrations. *E. crusgalli* was less sensitive to compounds **2**, **3**, and **5**.

The phytotoxic effects displayed by compounds **4** and **6** were similar to the results previously described by Merrill,[21] who found that both compounds reduce the growth of the radicle and hypocotyl of *Panicum miliaceum*, *E. crusgalli*, *Medicago sativa*, and *Lolium perenne* at concentrations ranging from 1 to 200 μg/mL.

Compounds **4** and **5** showed antifungal activity against *T. mentagrophytes* with MIC values of 58 and 29 μg/mL, respectively. Griseofulvin (MIC of 2.6 μg/mL) was used as the reference antibiotic. None of the isolated compounds was active against *C. albicans* and *A. niger*.

The results of this study showed that the isolated compounds possess promising phytogrowth-inhibitory activity. Therefore, they could be developed into environmentally safe herbicide agents. To our knowledge, this is the first report of benzofurans as plant growth regulators and these compounds, together with the isolated chromenes, might be involved in the allelopathic interactions of *H. quinquenervis*.

It has been reported that encecalin and related chromenes possess insecticidal and repellent properties.[22] Thus, the presence of this type of compounds in *H. quinquenervis* might be related to the ethnomedical application of the roots to eliminate ectoparasites in humans and domestic animals.

Free angelic acid has been previously found in the carabid beetle acting as a defensive substance.[23−25] To the best of our knowledge this is the first report of free angelic acid in plants.

Experimental Section

General Experimental Procedures. IR spectra were obtained in KBr on a Perkin-Elmer 599 B spectrophotometer. NMR spectra were recorded on a Varian VXR-300 S or on a Varian VXR-500 S spectrometers. Mass spectra were taken on a Hewlett-Packard 5985 apparatus. Melting points were determined in a Fisher Johns apparatus and are uncorrected. Optical rotations were taken on a digital polarimeter JASCO Dip 360. UV spectra were registered on spectrophotometer Perkin-Elmer 202. The FAB-MS spectrum (positive mode) of compound **3** was recorded in a JEOL DX 300 with JMA-3500 system. The target was bombarded with 10-keV Xe atoms. The sample was suspended in nba.

Plant Material. The plant material (roots) was collected in Bocoyna, Chihuahua, Mexico, in November 1991. A voucher specimen (R. Bye 18058) has been deposited at the Ethnobotanical collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation. The air-dried plant material (3 kg) was ground into powder and extracted exhaustively by maceration at room temperature with MeOH. After filtration, the extract was concentrated *in vacuo* to yield 735 g of residue. The active extract was subjected to column chromatography (CC) over Si-gel (1.25 kg) and eluted with a gradient of hexane/CHCl$_3$/MeOH. One hundred fifteen fractions (1 L each) were collected and pooled on the basis of their TLC profiles to yield six major fractions (F-1−F-6); bioactivities in the BST showed three active pools (F-1, F-3, and F-4). F-1 (23 g, BST LC$_{50}$ = 35 μg/mL), eluted with hexane−CHCl$_3$ (9:1), was further chromatographed on a Si-gel column (165 g) using benzene with increasing amounts of CHCl$_3$ to yield **4** (100 mg), **5** (45 mg), and **6** (300 mg) which were further purified by preparative TLC over Si-gel using hexane−EtOAc (9:1) as the eluant. Further CC of fraction F-3 (5g, BST LC$_{50}$ = 22 μg/mL, eluted with CHCl$_3$) on Si-gel (100 g), using hexane with increasing amounts of EtOAc, followed by preparative TLC (hexane−EtOAc (9:1), three developments) yielded **1** (48 mg). Fraction F-4 [53 g, BST LC$_{50}$ = 65 μg/mL, eluted with hexane−CHCl$_3$, 1:1)] was subjected to CC on Si-gel (940 g) using hexane−EtOAc (9:1) to yield **2** (42 mg). Finally, the BST-inactive fraction F-6 [6.6 g eluted with CHCl$_3$−MeOH (1:1)] was further chromatographed on a column of silica gel (120 g, using CHCl$_3$ with increasing amounts of MeOH) to yield impure compound **3** which was purified by preparative TLC (CHCl$_3$−MeOH, 9:1) to afford **3** (72 mg).

6-Methoxytremetone (1): yellow oil; [α]$_D$ = −42.27 (c = 1.1, CHCl$_3$); IR ν_{max} (KBr) 2920, 1634, 1484, 1400, 1370, 1262 cm^{-1}; UV λ (CHCl$_3$) nm 245, 279, 320; EIMS m/z (rel int) 232 (M$^+$, 47.4), 217 (100), 189 (28), 175 (9), 174 (28), 91 (24), 46 (18), 43 (98), 39 (28); ^{1}H-NMR (CDCl$_3$) δ 1.75 (s, CH$_3$-12), 2.56 (s, CH$_3$-14), 2.96 (ddd, J = 15.3, 8.0, 1.2 Hz, H-3), 3.30 (dd, J = 15.3, 9.5, 0.6 Hz, H-3′), 3.87 (s, 6-OMe), 4.92 and 5.07 (m, CH$_2$-11), 5.25 (m, H-2), 6.42 (s, H-7), 7.68 (dd, J = 1.2 Hz, H-4); ^{13}C-NMR (CDCl$_3$) δ 16.9 (C-12), 31.8 (C-14), 33.3 (C-3), 55.6 (6-OMe), 87.6 (C-2), 93.2 (C-7), 112.5 (C-11), 118.9 (C-10), 120.7 (C-9), 127.1 (C-4), 143.5 (C-5), 161.7 (C-8), 165.1 (C-6), 197.9 (C-13). Anal. Calcd for C$_{14}$H$_6$O$_3$: C, 72.41; H, 6.89. Found: C, 72.37; H 6.80.

6-Hydroxy-3-methoxytremetone (2): yellow oil; ^{13}C-NMR (CDCl$_3$) δ 17.5 (C-12), 29.7 (C-14), 55.3 (OMe), 83.3 (C-2), 92.2 (C-3), 92.9 (C-7), 113.2 (C-11), 114.4 (C-9), 117.8 (C-10), 129.2 (C-4), 141.1 (C-5), 167.1 (C-6, C-8), 202.2 (C-13).

4-β-D-(Glucopyranosyloxy)-3-(3-methoxy-*trans*-isopenten-1-yl]acetophenone (3): amorphous powder; [α]$_D$ = +32.5 (c = 1.4, MeOH); mp 150−154 °C; IR ν_{max} 3378, 1664, 1604, 1576, 1492, 1364, 1074 cm^{-1}; ^{1}H and ^{13}C-NMR data (see Table 3); FAB-MS 397 [M + H]$^+$. Anal. Calcd for C$_{20}$H$_{28}$O$_8$: C, 60.60; H, 7.07. Found: C, 60.99; H, 6.99.

Enzymatic Hydrolysis of 3 with β-Glucosidase. To 5 mg of **3** were added 1 mL of H$_2$O and 5 mg of β-glucosidase (Sigma Type 1). The mixture was incubated at 36 °C for 72 h. After the usual workup, the aglycon **3a** (2 mg) and glucose (TLC) were obtained.

Acid Hydrolysis of 3. The glycoside **3** (20 mg) was refluxed for 30 min with 1 mL of 1 N HCl. The acid solution was diluted with water (13 mL) and then extracted with CHCl$_3$ (3 × 15 mL); the organic phase

was successively washed with $NaHCO_3$ (10% aqueous) and water and then dried over Na_2SO_4. After removal of the solvent, 3 mg of **3a** was obtained.

Demethylencecalin (4): ^{13}C-NMR (CDCl$_3$) δ 26.2 (C-13, C-14), 28.5 (C-12), 77.8 (C-2), 104.4 (C-8), 113.9 (C-10), 120.9 (C-3), 122.9 (C-6), 128.5 (C-4), 128.8 (C-5), 160.4 (C-9), 165.1 (C-7), 202.3 (C-11).

Euparin (5): Yellow crystals; mp 97−100 °C.

Encecalin (6): ^{13}C-NMR (CDCl$_3$) δ 28.3 (C-12), 31.8 (C-13, C-14), 55.5 (7-OMe), 77.8 (C-2), 99.5 (C-8), 113.8 (C-10), 120.5 (C-3), 121.2 (C-6), 128.3 (C-4), 128.9 (C-5), 158.3 (C-9), 161.0 (C-7), 197.4 (C-11).

Phytogrowth-Inhibitory Bioassays. The phytogrowth-inhibitory activity of the MeOH extract, primary fractions, and pure compounds were evaluated on seeds of *A. hypochondriacus* and *E. crusgalli* by using a Petri dish bioassay.[7] In addition, a direct bioautographic bioassay system[9] was employed to guide secondary fractionation. The seeds of *E. crusgalli* were purchased from Valley Seed Service, Fresno, CA, and those of *A. hypochondriacus* from Mercado de Tulyehualco, D.F., Mexico. The data were analyzed by ANOVA ($p < 0.05$), and IC$_{50}$ values were calculated by Probit analysis based on percent inhibition obtained. The extract was evaluated at 10, 20, 50, 100, 150, and 200 μg/mL. Compounds **1**−**3** were evaluated at 50, 100, 200, and 400 μg/mL. Finally, compounds **4**−**6** were evaluated at 10, 20, 40, 100, and 200 μg/mL. Tricolorin A was used as positive control.[26] The direct bioautographic assay was carried out as previously described.[9] The concentration threshold required for most of the natural phytogrowth-inhibitors tested in similar experimental designs is often in the 100−1000 μg/mL range.[26]

Antimicrobial Assay. The microorganisms used in the present study were obtained from ATCC: *C. albicans* (ATCC 10231), *A. niger* (ATCC 16888), and *T. mentagrophytes* (ATCC 9129). For each microorganism a positive control was employed: amphotericin B (Sigma) for *C. albicans* and *A. niger* and griseofulvin (Sigma) for *T. mentagrophytes.* To test the quantitative antimicrobial activity against *T. mentagrophytes*, the agar dilution method was used.[8]

Cytotoxicity Assays. The extracts, fractions, and isolated compounds were evaluated for lethality to brine shrimp larvae (BST). Criteria of activity: LC$_{50}$ values of <1000 μg/mL for extracts and <20 μg/mL for pure compounds.[6] Cytotoxicities against human solid tumor cells were measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center, in a 7 day MTT assay, for MCF-7 breast carcinoma,[18] HT-29 colon adenocarcinoma,[19] and A-549 lung carcinoma,[20] with adriamycin as the positive control. Criteria of activity: ED$_{50}$ values of <20 μg/mL for extracts and <4 μg/mL for pure compounds.

References and Notes

(1) Bye, R. In *Two Mummies from Chihuahua: A Multidisciplinary Study*; Tyson, R. A., Elñerick, D. V., Eds.; San Diego Museum Papers: San Diego, 1985; Vol. 19, pp 77−104.
(2) Herz, W.; Kulanthaivel, P. *Phytochemistry* **1984**, *23*, 435.
(3) Castañeda, P.; García, M. R.; Hernández, B. E.; Torres, B. A.; Anaya, A. L.; Mata, R. *J. Chem Ecol.* **1992**, *18*,1025.
(4) Jiménez, A.; Pereda-Miranda, R.; Bye, R.; Linares, E.; Mata, R. *Phytochemistry* **1993**, *34*, 1079.
(5) Segura-Correa, R.; Mata, R.; Anaya, A. L.; Hernández, B. E.; Villena, R.; Soriano-García, M.; Bye, R.; Linares, E. *J. Nat. Prod.* **1993**, *56*, 1567.
(6) Anderson, J. E.; Goetz, C. M.; McLaughlin, J. L.; Suffness, M. *Phytochem. Anal.* **1991**, *2*, 107.
(7) Anaya, A. L.; Calera, M. R.; Mata, R.; Pereda-Miranda, R. *J. Chem. Ecol.* **1990**, *16*, 2145.
(8) Hufford, C. D.; Funderburck, M. J.; Morgan, J. M.; Robertson, L. W. *J. Pharm. Sci.* **1975**, *64*, 789.
(9) Li, H-H.; Nishimura, H.; Hasegawa, K.; Mizutani, J. *J. Chem. Ecol.* **1992**, *18*, 1785.
(10) Bohlmann, F.; Jakupovic, J.; Schuster, A.; King R. M.; Robinson, H. *Phytochemistry* **1982**, *21*, 161.
(11) Bohlmann, F.; Grenz, M. *Chem. Ber.* **1970**, *103*, 90.
(12) Bjeldanes, L. F.; Geissman, T. A. *Phytochemistry* **1969**, *8*, 1293.
(13) Bohlmann, F.; Zdero, C.; Grenz, M. *Chem. Ber.* **1977**, *110*, 1034.
(14) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1980**, *19*, 1141.
(15) Bohlmann, F.; Mahanta, P. K.; Natu, A. A.; King, R. M.; Robinson, H. *Phytochemistry* **1978**, *17*, 471.
(16) Siebertz, R.; Proksch, P.; Wray, V.; Witte, L. *Phytochemistry* **1989**, *28*, 789.
(17) Warashina, T.; Ishino, M.; Miyase, T.; Ueno, A. *Phytochemistry* **1990**, *29*, 3217.
(18) Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnestein, P.; Kersey, J. H.; Dosik, H.; Parks., W. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.
(19) Soule, H. D.; Vázquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.
(20) Fogh, J.; Trempe, G. In *Human Tumor Cells*; Fogh, J., Ed.; Plenum Press: New York, 1975; pp 115−119.
(21) Merrill, G. B., *J. Chem. Ecol.* **1989**, *15*, 2073.
(22) Kanehisa, K.; Kawazu, K. *Appl. Ent. Zool.* **1982**, *17*, 460.
(23) Attygalle, A. B; Meingwald, J.; Liebherk, J. K.; Eisner, T. *Experientia* **1991**, *47*, 296.
(24) Davidson, B. S.; Eisner, T.; Witz, B.; Meinwald, J. *J. Chem Ecol.* **1989**, *15*, 1689.
(25) Inoue, M.; Nishimura, H.; Li, H.-H.; Mizutani, J. *J. Chem. Ecol.* **1992**, *18*, 1833.
(26) Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Mahinda Wickramaratne, D. B.; Pezzuto, J. M.; Kinghorn, D. *J. Nat. Prod.* **1993**, *56*, 571.

NP960199M