Journal of Natural Products
Vol. 58, No. 6, pp. 909-915, June 1995

NEW BIOACTIVE MONOTETRAHYDROFURAN ANNONACEOUS ACETOGENINS, ANNOMURICIN C AND MURICATOCIN C, FROM THE LEAVES OF *ANNONA MURICATA*

FENG-E WU, LU ZENG, ZHE-MING GU, GENG-XIAN ZHAO, YAN ZHANG, JON T. SCHWEDLER, JERRY L. MCLAUGHLIN,*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

and SOELAKSONO SASTRODIHARDJO

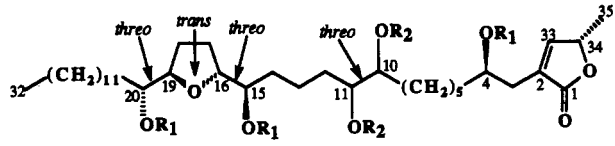
Department of Education and Culture, Center of Interuniversities, Biology Division, Bandung Institute of Technology, 10 Yanesba St., Bandung 40132, Indonesia

ABSTRACT.—The leaves of *Annona muricata* have yielded two additional monotetrahydrofuran Annonaceous acetogenins, annomuricin C [**1**] and muricatocin C [**2**]. Compounds **1** and **2** each possess five hydroxyl groups; two hydroxyl groups are at the C-10/C-11 and C-10/C-12 positions in **1** and **2**, respectively. The absolute configurations of **1** and **2**, except for positions C-10 and C-11 or C-12, were determined by Mosher ester methodology. The C-10/C-11 and C-10/C-12 acetonides (**1c**, **2c**) suggested relative stereochemistry and significantly enhanced the cytotoxicities against the A-549 human lung and the MCF-7 human beast solid tumor cell lines. One known monotetrahydrofuran acetogenin, gigantetronenin, not described previously from this plant, was also found.

Previous studies on the seeds of *Annona muricata* L. (Annonaceae) have resulted in the isolation and characterization of a number of cytotoxic and pesticidal monotetrahydrofuran (mono-THF) acetogenins (1–9). Our previous studies with the leaves have yielded the new bioactive mono-THF acetogenins, annomuricins A and B (10) and muricatocins A and B (11). In the present bioactivity-directed work, the leaves have yielded two new closely related compounds, annomuricin C [**1**] and muricatocin C [**2**]. One known mono-THF acetogenin, gigantetronenin (12), not described previously from this plant, was also isolated from the leaves.

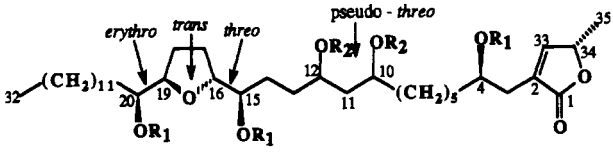
As previously reported (10), the leaves of *A. muricata*, obtained from plantation trees growing in Java, were extracted with EtOH, and the extract residue (F001) was partitioned to furnish the aqueous MeOH residue (F005) which was bioactive in the shrimp lethality test (BST) (13,14). Cc of F005 over Si gel, using gradient elution, gave 154 fractions (10). Two active fractions were further subjected to repeated flash chromatography and hplc to yield compounds **1** and **2**. The known compound, gigantetronenin (12), was isolated from a third active fraction using similar methods.

Annomuricin C [**1**] and muricatocin C [**2**] were obtained as colorless amorphous powders. The ms and nmr spectra indicated that **1** and **2** are adjacent mono-THF ring acetogenins (4–6) (Figures 1 and 2). In the hrfabms both **1** and **2** gave $[MH]^+$ ions at *m/z* 613.4661 (calcd 613.4679) consistent with a molecular formula of $C_{35}H_{64}O_8$. Both **1** and **2** showed a broad OH stretching absorption in the ir spectrum at 3250–3550 cm^{-1}. Five successive losses of H_2O (*m/z* 18) from the $[MH]^+$ from **1** and **2** in the cims (*m/z* 595, 577, 559, 541, and 523) showed the existence of five OH groups, and these were confirmed by the formation of penta-acetates (**1a** and **2a**) and penta-trimethylsilyl (TMSi) ethers (**1b** and **2b**). Compounds **1a** and **2a** gave five singlet proton peaks at δ 2.02–2.08 (Table 1) representing the Me groups of the penta-acetates. The positions of the OH groups in **1** and **2** were assigned, respectively, at C-4, C-10, C-11, C-15, and C-20, and at C-4, C-10, C-12, C-15, and C-



1 R_1, R_2=H
1a R_1, R_2=Ac
1b R_1, R_2=TMSi
1c R_1=H, R=acetonide
1r R_1, R_2=(*R*)-MTPA
1s R_1, R_2=(*S*)-MTPA

FIGURE 1. Structure of annomuricin C [**1**] and its derivatives [**1a**–**1c**, **1r**, and **1s**].



2 R_1, R_2=H
2a R_1, R_2=Ac
2b R_1, R_2=TMSi
2c R_1=H, R_2=acetonide
2r R_1, R_2=(*R*)-MTPA
2s R_1, R_2=(*S*)-MTPA

FIGURE 2. Structure of muricatocin C [**2**] and its derivatives [**2a**–**2c**, **2r**, and **2s**].

20, by careful analysis of the fragments in the eims spectra of the TMSi derivatives (**1b** and **2b**) at *m/z* 701, 631, 487, 385, 271, and 213 (Figure 3) and at *m/z* 701, 631, 503, 573, 385, 341, 271, and 213 (Figure 4). The placement of the mono-THF ring in each compound was determined to be at C-16/C-19 by the diagnostic fragments at *m/z* 631 and 701, 341, 271 (TMSi-eims).

The mono-THF ring, with the usual flanking OH groups on each side, was indicated in **1** and **2** by ^{1}H-nmr chemical shifts (Table 1) at δ 3.41 (H-15), 3.83 (H-16), 3.85 (H-19), and 3.40 (H-20), for **1**, and δ 3.45 (H-15), 3.85 (H-16), 3.80 (H-19), and 3.89 (H-20), for **2**, and by ^{13}C-nmr signals (Table 1) at δ 74.39 (C-15), 82.72 (C-16), 82.56 (C-19), and 74.39 (C-20), for **1**, and at δ 74.28 (C-15), 83.04 (C-16), 82.22 (C-19), and 71.48 (C-20), for **2**. However, the hydroxyls in **1** at C-10/C-11 and in **2** at C-10/C-12 were obviously different. For **1**, the carbinol protons at C-10/C-11 resonated at δ 3.43 and the corresponding ^{13}C-nmr signals were at δ 74.14 (C-10) and 74.16 (C-11); for **2**, the carbinol protons at C-10/C-12 resonated at δ 3.94 and 3.86, respectively, and the corresponding ^{13}C-nmr signals were at δ 69.85 (C-10) and 69.23 (C-12). These upfield positions for the ^{13}C-nmr signals for C-10/C-12 in **2** illustrated a mutual beta effect (15). The chemical shifts of H-4 and C-4 in **1** and **2** were almost identically located at δ 3.81 and δ 69.85, and at δ 3.81 and δ 69.86, respectively, showing identities in this region of the molecules.

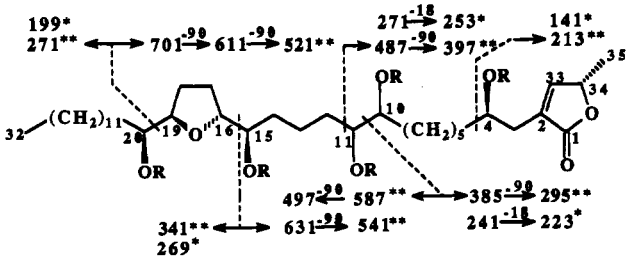
These structural units in **1** and **2** were further confirmed by COSY and single-relayed COSY data in which the proton coupling correlations from (H-3)↔(H-4) and (H-10, H-11, and H-12)↔(H-8, H-9, and H-13), and (H-15 and H-20)↔(H-13, H-14, H-16, H-17, H-18, H-19, H-21, and H-22) (Table 1) could be clearly seen. The assignments of the relative stereochemistries around the mono-THF rings of **1** and **2** were determined using the methodologies of Hoye and co-workers (16,17) and Born *et al.* (18), and by comparison with several acetogenins having both the threo and the erythro configuration at C-19 to C-20

TABLE 1. ^{1}H-Nmr Spectral Data for **1**, **2**, **1a**, **2a**, **1c**, and **2c**, and ^{13}C-Nmr Data for **1** and **2** ($CDCl_3$, δ).

Position	^{1}H Nmr (500 MHz)						^{13}C Nmr (125 MHz)	
	1	**2**	**1a**	**2a**	**1c**	**2c**	**1**	**2**
1	—	—					174.65	174.68
2	—	—					131.12	131.12
3a	2.37 m	2.41 m	2.51 m	2.52 m	2.37 m	2.41 m	33.35	33.33
3b	2.50 m	2.51 m	2.56 m	2.55 m	2.50 m	2.51 m		
4	3.81 m	3.81 m	5.09 m	5.09 m	3.81 m	3.81 m	69.85	69.86
5–9	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	22–34	22–34
10	3.43 m	3.94 m	4.97 m[a]	5.02 m[a]	3.96 m[a]	3.98 m[a]	74.14	69.62
11	3.43 m	2.01 m	4.97 m[b]	1.96 m	2.02 m	2.03 m	74.16	42.67
12		3.86 m		5.08 m[b]	3.99 m[b]	4.06 m[b]		69.23
13–14	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	22–34	22–34
15	3.41 m	3.45 m	4.85 m	4.85 m	3.41 m	3.45 m	74.39	74.28
16	3.83 m	3.85 m	3.95 m	3.95 m	3.86 m	3.88 m	82.72	83.04
17–18	1.57–2.00 m	1.57–2.00 m	1.57–2.00 m	1.57–2.00 m	1.57–2.00 m	1.57–2.00 m	22–34	22–34
19	3.85 m	3.80 m	3.95 m	3.93 m	3.84 m	3.80 m	82.56	82.22
20	3.40 m	3.89 m	4.82 m	4.92 m	3.40 m	3.90 m	74.39	71.48
21–31	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	1.25–1.61 m	22–34	22–34
32	0.88 t	0.88 t	0.88 t	0.88 t	0.88 t	0.88 t	14.11	14.11
33	7.19 d	7.19 d	7.09 d	7.09 d	7.19 d	7.19 d	151.96	151.91
34	5.05 dq	5.07 dq	5.01 dq	5.01 dq	5.06 dq	5.06 dq	78.03	78.06
35	1.42 d	1.43 d	1.39 d	1.39 d	1.43 d	1.43 d	19.18	19.09
OAc-4			2.03 s	2.03 s				
OAc-10			2.08 s	2.05 s[a]				
OAc-11			2.08 s					
OAc-12				2.04 s[b]				
OAc-15			2.08 s	2.08 s				
OAc-20			2.05 s	2.04 s				
Me[c]					1.37 s	1.37 s		

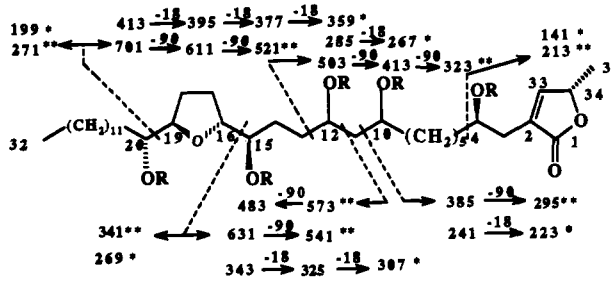
[a,b]Assignments are interchangeable.
[c]Acetonide methyls.

(15, 19–23). In addition, the recent paper by Fujimoto *et al.* (24) describes model mono-THF analogues, with flanking hydroxyls, having all possible relative stereochemistry; **1** matches very well with the threo-trans-threo model and **2** with the threo-trans-erythro model. In **1** and **2**, the OH-substituted CH centers, at C-15



*1 R=H
**1b R=Me_3Si

FIGURE 3. Diagnostic eims fragment ions (*m/z*) of **1** and its penta-TMSi derivative [**1b**].



*2 R=H
**2b R=Me_3Si

FIGURE 4. Diagnostic eims fragment ions (*m/z*) of **2** and its penta-TMSi derivative [**2b**].

and C-20 flanking the ring region (C-16 to C-19), gave very similar chemical shifts in the ^{1}H- and ^{13}C-nmr spectra as with the above models (Table 1); the stereochemistries of C-15/C-16 and C-19/C-20 were concluded to be threo and threo for **1**, and threo and erythro for **2**, respectively, and the stereochemistry was concluded to be trans for the THF ring in each compound. The relative stereochemistries around the mono-THF rings were then confirmed by comparing the ^{1}H-nmr data of the acetates (**1a** and **2a**, Table 1) with those of the model acetylated compounds of known relative stereochemistry (16,18). The proton signals for H-15 at δ 3.41 and H-20 at δ 3.40, in **1**, and, for H-15 at δ 3.45 and H-20 at δ 3.80, in **2**, were shifted downfield in **1a** to δ 4.85 for H-15 and δ 4.82 for H-20 and in **2a** to δ 4.85 for H-15 and δ 4.92 for H-20.

To determine the relative configuration at C-10/C-11 in **1** and at C-10/C-12 in **2**, the acetonide (dioxolane) derivatives, **1c** and **2c**, were prepared. The ^{1}H-nmr signals for H-10 and H-11 of threo and erythro vicinal diols have been previously reported in other Annonaceous acetogenins (10, 11, 23–25). If the configuration of the vicinal diol is threo, the two acetonyl proton Me signals will appear together as a six-proton singlet; and, if the configuration is erythro, the acetonyl Me proton signals will appear as two separated three-proton singlets (15). The acetonyl Me protons in **1c** gave a six-proton singlet at δ 1.37 (Table 1), showing their equivalence and demonstrating that the vicinal diol at C-10/C-11 is threo. Although the acetonide **2c** possesses six carbons in the dioxolane ring, it is, nevertheless, analogous with the C-10/C-11 acetonides that bear five carbons in their dioxolane rings (11). The ^{1}H-nmr signals for the acetonyl methyl protons, between H-10 and H-12 in **2c**, at δ 1.37 (Table 1) also showed a six-proton singlet; consequently a pseudo-threo configuration was suggested for the 1,3-diol group at C-10/C-12 in **2**.

The (*S*)- and (*R*)-methoxytrifluoromethyl phenylacetic acid (MTPA) esters (Mosher esters) of **1** and **2** were prepared (6,26) and numbered **1s**, **1r** and **2s**, **2r**. COSY ^{1}H-nmr analyses of these derivatives were then performed. The ^{1}H-nmr chemical shift data of **1s**, **1r**, **2s**, and **2r** showed that the absolute configuration at C-4 of **1** and **2** is *R* (Table 2). This result is identical to all acetogenins examined so far that possess an OH at C-4.

Similarly, the Mosher ester data (Table 3) allowed the absolute stereochemical assignments of the carbinol centers adjacent to the mono-THF ring as C-15*R* in **1** and **2**, and as C-20*R* in **1** and C-20*S* in **2**. The assignment of the absolute stereochemistries of the asymmetric carbinol centers at C-10/C-11 of **1** and at C-10/C-12 of **2** could not be achieved by direct analyses of the per-Mosher esters.

TABLE 2. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configuration at C-4 of the Penta (*S*)- and (*R*)-MTPA Esters of **1** and **2**.

MTPA ester of	H_2C-5	HC-4	H_2C-3		HC-33	HC-34	H_3C-35
1s δ (*S*)	1.59	5.28	2.57	2.52	6.73	4.86	1.28
1r δ (*R*)	1.58	5.34	2.65	2.56	6.95	4.90	1.30
Δδ	+0.01	4*R*[a]	−0.08	−0.04	−0.24	−0.04	−0.02
2s δ (*S*)	1.57	5.28	2.57	2.52	6.72	4.85	1.28
2r δ (*R*)	1.56	5.34	2.65	2.56	6.95	4.89	1.30
Δδ	+0.02	4*R*[a]	−0.08	−0.04	−0.23	−0.04	−0.02

[a]Absolute configuration of chiral center.

TABLE 3. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configurations at C-15 and C-20 of the Penta (*S*)- and (*R*)-MTPA Esters of **1** and **2**.

MTPA Ester of	H_2C-14	HC-15	HC-16	H_2C-17/18	HC-19	HC-20	H_2C-21
1s δ (*S*)	1.53	5.21	3.92	1.85	3.87	4.85	1.53
	1.46			1.65			1.49
1r δ (*R*)	1.48	5.26	3.98	1.82	3.94	4.92	1.54
	1.42			1.56			1.50
Δδ	pos.	15*R*[a]	neg.	pos.	neg.	20*R*[a]	neg.
2s δ (*S*)	1.53	5.20	3.90	1.86	3.88	4.92	1.53
	1.46			1.65			1.49
2r δ (*R*)	1.48	5.26	3.96	1.83	3.65	4.90	1.52
	1.42			1.57			1.48
Δδ	pos.	15*R*[a]	neg.	pos.	pos.	20*S*[a]	neg.

[a]Absolute configuration of chiral center.

Compound **1** differs from annomuricin A (which is erythro at C-19/C-20) and from annomuricin B (which is erythro both at C-19/C-20 and C-10/C-11) (10); thus, **1** is isomeric with these two compounds and was named annomuricin C. Compound **2** differs from muricatocin A (which is also threo at C-19/C-20 but pseudo-erythro at C-10/C-12), and from muricatocin B (which is erythro at C-19/C-20 and pseudo-erythro at C-10/C-12); thus, **2** is isomeric to these two compounds and was named muricatocin C.

Annomuricin C and muricatocin C (**1** and **2**) were significantly bioactive in the BST and were also cytotoxic (seven-day MTT assays) to human solid tumor cell lines in culture (Table 4). In comparison with annonacin (5), **1** and **2** have one additional OH group, and their cytotoxic effects to these tumor cell lines are notably reduced (5,23). The acetonides (**1c** and **2c**) showed improved bioactivities and were one to threefold more cytotoxic against the human lung cell line (A-549) and the human breast cell line (MCF-7) and three times more toxic in the BST than **1** and **2** (Table 4); this improvement of bioactivity with acetonides has also been observed with other compounds such as annomuricins A and B and muricatocin A and B (10,11). The similar susceptibilities of the cell lines to **1**, **2** and **1c**, **2c**, respectively, (Table 4) suggest that the stereochemistry at C-20 does not play a significant role in controlling selectivity toward specific cell lines and tumor types. All of the Annonaceous acetogenins act, at least in part, as potent inhibitors of complex I in mitochondria (27,28).

TABLE 4. Bioactivities of **1** and **2** and Their Acetonide Derivatives (**1c** and **2c**).

Compounds	BST[a] LC_{50} (μg/ml)	A-549[b] ED_{50} (μg/ml)	MCF-7[c] ED_{50} (μg/ml)	HT-29[d] ED_{50} (μg/ml)
1[e]	6.13×10^{-1}	3.08×10^{-1}	2.28×10^{-1}	1.54
2[e]	6.04×10^{-1}	9.09×10^{-2}	6.45×10^{-2}	1.48
1c[f]	2.35×10^{-1}	6.98×10^{-2}	4.81×10^{-3}	1.41
2c[f]	1.89×10^{-1}	3.76×10^{-3}	3.98×10^{-3}	2.61×10^{-1}
Adriamycin[e,g]	—	2.94×10^{-3}	1.06×10^{-1}	3.48×10^{-2}

[a]Brine shrimp lethality test (13,14).
[b]Human lung carcinoma (29).
[c]Human breast carcinoma (30).
[d]Human colon adenocarcinoma (31).
[e,f]Same cytotoxicity runs; values in different runs were within one order of magnitude of each other.
[g]Positive control standard.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—As described previously (10,11).

PLANT MATERIAL.—As described previously (10,11).

EXTRACTION AND ISOLATION.—The materials and methods employed for isolation and derivatization were as described previously (10,11). A large Si gel column was used to resolve the BST-active fraction F005 into 154 fractions (10). The bioactive fractions Nos. 128–129 [BST, LC_{50}=1.25 μg/ml (No. 128) and 1.80 μg/ml (No. 129)] were subjected to repeated flash chromatography to yield crude compounds **1** and **2**; each was then purified with hplc over Si gel, eluted by hexane-MeOH (90:1, flow rate 10 ml/min), to afford the two colorless, amorphous powders, **1** and **2**. One additional mono-THF acetogenin (8 mg) was isolated from bioactive fraction No. 101 (BST, LC_{50} 0.55 μg/ml) using similar methods and was identified (^{1}H nmr, cims) as gigantetronenin (12).

Annomuricin C [**1**].—White powder (8 mg); $[\alpha]^{22}D$ +57.7° (*c*=0.0005, EtOH); ir ν max (film) 3411 (br OH), 2920, 2851, 1743, 1467, 1321, 1073 cm^{-1}; uv λ max (MeOH) 220 nm ($\epsilon=3.8\times10^3$); hrfabms (glycerol) *m/z* [MH^+] 613.4661 for $C_{35}H_{65}O_8$ (calcd 613.4679); cims (butanol) *m/z* 613 (100), 595 (21), 577 (45), 559 (42), 541 (10), 395 (2), 353 (10), 325 (10), 271 (3), 269 (5), 253 (5), 241 (38), 223 (3), 205 (4), 199 (4), and 141 (3); eims *m/z* 341 (2), 325 (9), 307 (14), 269 (4), 241 (22), 223 (4), 213 (4), 205 (2), 199 (7), and 141 (10); ^{1}H-nmr ($CDCl_3$, 500 MHz) data, see Table 1; ^{13}C-nmr ($CDCl_3$, 125 MHz) data, see Table 1.

Muricatocin C [**2**].—White powder (7 mg); $[\alpha]^{22}D$ +32.5° (*c*=0.001); ir ν max (film) 3410 (br OH), 2920, 2850, 1745, 1466, 1322, 1074 cm^{-1}; uv λ max (MeOH) 225 nm ($\epsilon=9.1\times10^3$); hrfabms (glycerol) *m/z* [MH^+] 613.4661 for $C_{35}H_{65}O_8$ (calcd 613.4679); cims *m/z* [MH^+] 613 (100), 595 (46), 577 (68), 559 (29), 541 (10), 523 (2), 413 (1), 395 (1), 377 (3), 359 (3), 343 (2), 325 (7), 285 (4), 271 (3), 269 (8), 267 (4), 253 (6), 241 (48), 223 (8), 205 (2), 199 (5); eims *m/z* 413 (2), 377 (5), 359 (8), 343 (2), 325 (33), 307 (26), 285 (2), 269 (8), 253 (3), 241 (36), 223 (4), 213 (7), 199 (2), 141 (10); ^{1}H-nmr data ($CDCl_3$, 500 MHz); ^{13}C-nmr data ($CDCl_3$, 125 MHz), see Table 1.

TMSi DERIVATIVES [**1b** AND **2b**].—Eims of **1b** *m/z* 701 (1), 631 (6), 611 (4), 587 (2), 541 (3), 521 (2), 497 (4), 487 (1), 485 (1), 385 (29), 341 (7), 295 (4), 271 (18), 213 (6); eims of **2b** *m/z* 701 (4), 631 (9), 611 (8), 573 (1), 541 (20), 521 (7), 503 (1), 483 (2), 413 (5), 385 (77), 341 (17), 323 (4), 295 (8), 271 (41), 213 (18).

ACKNOWLEDGMENTS

This work was supported, in part, by R01 grant No. CA 30909 from the National Cancer Institute, NIH. One of the authors, F.-E. Wu, Chengdu Institute of Biology, the Chinese Academy of Sciences, People's Republic of China, gratefully acknowledges the support of the K.C. Wang Education Foundation, Hong Kong. The plant material was collected with travel support from the Indonesian University Development Projects, which were financed by the World Bank.

LITERATURE CITED

1. S.H. Myint, A. Laurens, R. Hocquemiller, A. Cavé, D. Davoust, and D. Cortes, *Heterocycles*, **31**, 861 (1990).
2. D. Cortes, S.H. Myint, A. Laurens, R. Hocquemiller, M. Leboeuf, and A. Cavé, *Can. J. Chem.*, **69**, 8 (1991).
3. S.H. Myint, D. Cortes, A. Laurens, R. Hocquemiller, M. Leboeuf, A. Cavé, J. Cotte, and A.-M. Quéro, *Phytochemistry*, **30**, 3335 (1991).

4. M.J. Rieser, F.J. Kozlowski, K.V. Wood, and J.L. McLaughlin, *Tetrahedron Lett.*, **32**, 1137 (1991).
5. M.J. Rieser, X.-P. Fang, J.K. Rupprecht, Y.-H. Hui, D.L. Smith, and J.L. McLaughlin, *Planta Med.*, **59**, 91 (1993).
6. M.J. Rieser, X.-P. Fang, E. Anderson, L.R. Miesbauer, D.L. Smith, and J.L. McLaughlin, *Helv. Chim. Acta*, **76**, 2433 (1993).
7. D. Gromek, B. Figadere, R. Hocquemiller, A. Cavé, and D. Cortes, *Tetrahedron*, **49**, 5247 (1993).
8. F. Roblot, T. Laugel, M. Leboeuf, A. Cavé, and O. Laprévote, *Phytochemistry*, **34**, 281 (1993).
9. A. Hisham, U. Sreekala, L. Pieters, T. de Bruyne, H. Van den Heuvel, and M. Claeys, *Tetrahedron*, **49**, 6913 (1993).
10. F.-E. Wu, Z.-M. Gu, L. Zeng, G.-X. Zhao, Y. Zhang, S. Sastrodihardjo, and J.L. McLaughlin, *J. Nat. Prod.*, in press.
11. F.-E. Wu, L. Zeng, Z.-M. Gu, G.-X. Zhao, Y. Zhang, S. Sastrodihardjo, and J.L. McLaughlin, *J. Nat. Prod.*, **58**, 830 (1995).
12. X.-P. Fang, J.E. Anderson, D.E. Smith, J.L. McLaughlin, and K.V. Wood, *J. Nat. Prod.*, **55**, 1655 (1992).
13. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
14. J.L. McLaughlin, in: "Methods in Plant Biochemistry." Ed. by K. Hostettman, Academic Press, London, 1991, Vol. 6, pp. 1–35.
15. Z.-M. Gu, G.-X Zhao, N.H. Oberlies, L. Zeng, and J.L. McLaughlin, in: "Recent Advances in Phytochemistry." Ed. by J.T. Romeo, Plenum Press, New York, Vol. 27, in press.
16. T.R. Hoye and Z.-P. Zhuang, *J. Org. Chem.*, **53**, 5578 (1988).
17. T.R. Hoye, P.R. Hanson, A.C. Kovelesky, T.D. Ocain, and Z.-P. Zhuang, *J. Am. Chem. Soc.*, **113**, 9369 (1991).
18. L. Born, F. Lieb, J.P. Lorentzen, H. Moeschler, M. Nonfon, R. Sollner, and D. Wendisch, *Planta Med.*, **56**, 312 (1990).
19. F. Lieb, M. Nonfon, U. Wachendorff-Neumann, and D. Wendisch, *Planta Med.*, **56**, 317 (1990).
20. G.-X. Zhao, M.J. Rieser, Y.-H. Hui, L.R. Miesbauser, D.L. Smith, and J.L. McLaughlin, *Phytochemistry*, **33**, 1065 (1993).
21. D. Cortes, S.H. Myint, M. Leboeuf, and A. Cavé, *Tetrahedron Lett.*, **43**, 6133 (1991).
22. J.K. Rupprecht, Y.-H. Hui, and J.L. McLaughlin, *J. Nat. Prod.*, **53**, 237 (1990).
23. X.-P. Fang, M.J. Rieser, Z.-M. Gu, G.-X. Zhao, and J.L. McLaughlin, *Phytochem. Anal.*, **4**, 27 (1993).
24. Y. Fujimoto, C. Murasaki, H. Shimada, S. Nishioka, K. Kakinuma, S. Singh, M. Singh, Y.K. Gupta, and M. Sahai, *Chem. Pharm. Bull.*, **42**, 1175 (1994).
25. Z.M. Gu, X.-P. Fang, L. Zeng, J.F. Kozlowski, and J.L. McLaughlin, *Bioorg. Med. Chem. Lett.*, **4**, 473 (1994).
26. 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).
27. K.I. Ahammadsahib, R.M. Hollingworth, Y.-H. Hui, J.P. McGovren, and J.L. McLaughlin, *Life Sci.*, **53**, 1113 (1993).
28. J.L. Landolt, K.I. Ahammadsahib, R.M. Hollingworth, R.M. Barr, F.L. Crane, N.L. Buerck, G.P. McCabe, and J.L. McLaughlin, *Chem.-Biol. Interact.*, in press.
29. D.J. Giard, S.A. Aronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Doisk, and W.P. Parks, *J. Natl. Cancer Inst.*, **51**, 1417 (1973).
30. H.D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennan, *J. Natl. Cancer Inst.*, **51**, 1409 (1973).
31. J. Fogh and G. Trempe, in: "Human Tumor Cells." Ed. by J. Fogh, Plenum Press, New York, 1975, pp. 115–159.

Received 26 September 1994