Pergamon

PII: S0031-9422(98)00172-1

Phytochemistry, Vol. 49, No. 2, pp. 565–571, 1998

Printed in Great Britain
0031-9422/98/$-see front matter

MURICOREACIN AND MURIHEXOCIN C, MONO-TETRAHYDROFURAN ACETOGENINS, FROM THE LEAVES OF *ANNONA MURICATA*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

GEUM-SOOG KIM,*† LU ZENG,† FERAS ALALI,† LINGLING L. ROGERS,† FENG-E. WU,† SOELAKSONO SASTRODIHARDJO‡ and JERRY L. MCLAUGHLIN†§

†Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47906, U.S.A
‡Department of Education and Culture, Center of Interuniversities, Biology Division, Bandung Institute of Technology, 10 Yanesba St., Bandung 40132, Indonesia

(*Received* 25 *November* 1997; *in revised form* 24 *February* 1998)

Key Word Index—*Annona muricata* ; Annonaceae ; Annonaceous acetogenins ; leaves ; muricoreacin ; murihexocin C, brine shrimp lethality test ; cytotoxicities.

Abstract—Bioactivity-directed fractionation of the leaves of *Annona muricata* L. (Annonaceae) resulted in the isolation of two new Annonaceous acetogenins, muricoreacin (**1**) and murihexocin C (**2**). Compounds **1** and **2** showed significant cytotoxicities among six human tumor cell lines with selectivities to the prostate adenocarinoma (PC-3) and pancreatic carcinoma (PACA-2) cell lines.

INTRODUCTION

The Annonaceous acetogenins are a series of C_{35}/C_{37} fatty acid derivatives. Since the first acetogenin, uvaricin, was reported in 1982, research on these acetogenins has expanded greatly due to their diverse bioactivities, including *in vivo* antitumor, cytotoxic, pesticidal, antibacterial, antiparasitic and immunosuppressive effects [1–5].

The Annonaceous acetogenins usually have two to five hydroxyls, with one or two of them generally flanking a system of one to three tetrahydrofuran (THF) rings, and the others located at different positions along the fatty acid chain. Acetogenins with six hydroxyl and even seven hydroxyl groups have been reported recently [6–8]. The Annonaceous acetogenins are classified into five main types, non-THF, mono-THF, adjacent bis-THF, non-adjacent bis-THF and tri-THF, according to the number and the positions of THF rings. Recently, research on their structure-activity relationships (SAR) [9, 10] and syntheses [11, 12] have been actively performed as interest in their optimum structural characteristics and potent biological activities has increased. Screening for the acetogenins, by the rapid and relatively uncomplicated selected ionization procedure of liquid chromatography/mass spectrometry (LC/MS), has recently simplified their detection [13], but, for the determination of their stereochemistries and their biological properties, they must be individually isolated and evaluated.

Annona muricata, known as sour sop or guanabana, is grown commercially in many regions of the tropical world. This plant has been studied extensively for new Annonaceous acetogenins from its seeds, bark, and leaves [6, 7, 14–25], and, to date, more than 50 acetogenins with diverse biological activities have been reported from *A. muricata* [3]. Our previous studies on the leaves of *A. muricata* has led to the isolation of 19 cytotoxic acetogenins [6, 7, 14–20]. This paper reports two new bioactive Annonaceous acetogenins, muricoreacin (**1**) and murihexocin C (**2**), isolated from the leaves.

As previously reported, the leaves of *A. muricata* were extracted with 95% EtOH, and the extracted residue (F001) was partitioned by a standard extraction scheme [19]. The aqueous MeOH residue (F005), which was the most bioactive fraction in the brine

* Visiting Scientist from the Department of Field Crops, Medicinal Crops Division, Yeongnam Agricultural Experiment Station, Rural Development Administration, Milyang, 617-130, Republic of Korea.

§Author to whom correspondence should be addressed. Phone: 317 494 1455; Fax: 317 494 6790

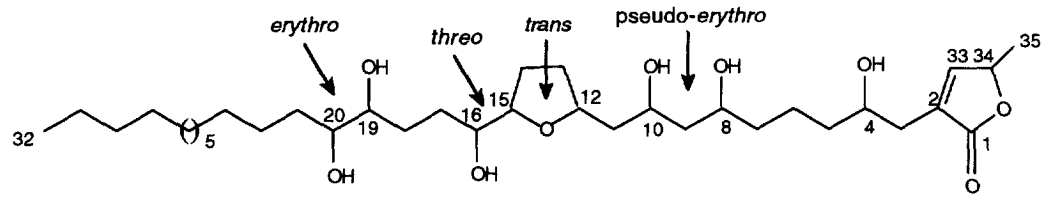

Fig. 1. The structure of muricoreacin (**1**).

shrimp lethality test (BST LC_{50} 1.6 ppm), was subjected to column chromatography on Amberlite XAD-2 and HPLC on reverse phase and normal phase. From the more polar fractions, **1** and **2** were isolated. The determination of their structures was achieved through MS, ^1H and ^{13}C NMR, COSY and single-relayed COSY experiments.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white solid. The molecular formula of **1** was determined to be $C_{35}H_{64}O_9$ from a peak at *m/z* 629 $[MH^+]$ in the CIMS; the HR-FABMS gave the $[M+Li]^+$ ion at 635.4694 (calcd. 635.4710). In the ^1H-NMR spectrum, the ^1H signals at δ 7.22 (H-33), 5.06 (H-34), 3.86 (H-4), 2.47 (H-3) and 1.43 (H-35), as well as the ^{13}C signals at δ 52.1 (C-33), 130.9 (C-2), 78.1 (C-34), 69.2 (C-4), 33.2 (C-3) and 19.0 (C-35) in the ^{13}C-NMR spectrum, indicated that **1** has a typical methylated α,β-unsaturated γ-lactone fragment with a 4-OH group (Fig. 1). This moiety was confirmed by a peak at *m/z* 141 in the EIMS (Fig. 2).

Eight ^1H NMR signals, including that for H-4 between δ 3.49–4.15, as well as eight ^{13}C signals at δ 82.3, 79.1, 74.8, 74.6, 73.8, 72.2, 71.6 and 69.2, except that for C-34 at δ 78.1, suggested the presence of the mono-THF ring with one flanking OH group and a total of six hydroxyl groups in **1** (Table 1). The successive losses of six *m/z* 18 (H_2O) units in the CIMS confirmed the presence of the six OH groups. The mono-THF ring was determined to be of the gigantetrocin A-type from the correlation between two ^1H signals at δ 3.90 (H-15) and 3.49 (H-16), corresponding to ^{13}C signals at δ 82.3 (C-15) and 73.8 (C-16), in the ^1H-^1H COSY spectrum for **1** [1]. The placement of the mono-THF ring was determined to be at C-12/C-15 by the EIMS fragment ions at *m/z* 341 and 287 (Fig. 2). The relative stereochemistry around the mono-THF ring was determined to be *threo/trans* by comparison of their ^1H NMR signals with several model compounds of known relative stereochemistry [7, 19, 26–29].

In the ^{13}C-NMR spectrum of **1**, the ^{13}C signals at δ 74.8 and 74.6, corresponding to two ^1H resonances at δ 3.59 in the ^1H-NMR spectrum, indicated the existence of one 1,2-vicinal diol with an *erythro* configuration, based on the direct comparison of the spectral data with those of model compounds bearing vicinal diols with the same relative stereochemistry [15, 19, 30]. The position of the vicinal diol was assigned at C-19/C-20 from the fragment ions at *m/z* 429 and 199 resulting from the cleavage between C-19 and C-20 in the EIMS (Fig. 2).

In the ^{13}C-NMR spectrum of **1**, two ^{13}C signals appeared at δ 43.5 and 42.9, which indicated the possibility of the presence of at least one 1,3-diol in **1**. To establish the placement of the two OH groups related to the ^1H signals at δ 4.13 and 4.06, corresponding to the ^{13}C signals at δ 72.2 and 71.6, single-relayed COSY experiments were performed. Correlations between δ 3.86 and 4.06 (H-8↔H-10), δ 4.06 and 4.15 (H-10↔H-12) and δ 1.97 and 1.54 and 4.15 (H-14a, H-14b↔H-12) were observed in the single-relayed COSY (Fig. 3). These observations clearly showed that the 1,3-diol was located at C-8/C-10 and was separated by a

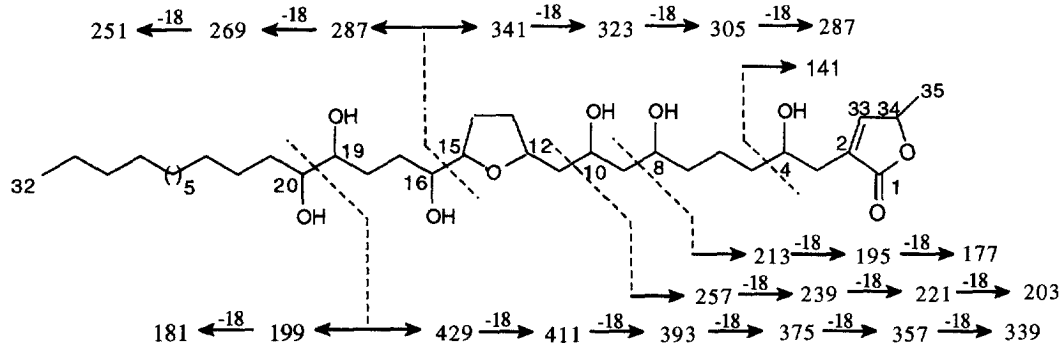

Fig. 2. Diagnostic EIMS fragment ions (*m/z*) of muricoreacin (**1**).

Table 1. ^{13}C-NMR (125 MHz, $CDCl_3$) and ^{1}H-NMR (500 MHz, $CDCl_3$) Spectra Data for **1**

Carbon	**1**	
	δ C	δ H (J on Hz)
1	174.8	
2	130.9	
3a,3b	33.2[a]	2.40 m, 2.48 m
	69.2	3.86 m
5	37.1[b]	1.52–1.68 m
6	21.3[c]	1.26–1.68 m
7	36.9[d]	1.52–1.68 m
8	71.6[c]	3.86 m
9	43.5[d]	1.52–1.68 m
10	72.2	4.06 m
11	42.9	1.52–1.68 m
12	79.1	4.15 m
13	31.9	2.11 m, 1.54 m
14	27.9	1.97 m, 1.66 m
15	82.3	3.90 m
16	73.8	3.49 m
17	29.3–29.9	1.26–1.68 m
18	29.3–29.9	1.43–1.56 m
19	74.8[e]	3.59 m
20	74.6[e]	3.59 m
21	33.1[a]	1.43–1.56 m
22–29	29.3–29.9	1.26–1.68 m
30	31.9	1.26–1.58 m
31	22.7	1.26–1.58 m
32	14.1	0.88t(7.0)
33	152.1	7.22d(1.5)
34	78.1	5.06dq(1, 6.5)
35	19.0	1.43d(6.5)

[a,b,c,d,e] Assignments are interchangeable within the same column.

methylene group from the mono-THF ring. Considering that the hydrocarbon chain methylenes normally resonate at *ca.* δ 29, the ^{13}C signal at δ 21.3 in **1** indicated the presence of a double β-effect on the methylene carbon, by two hydroxylated carbons at C-4 and C-8 [3, 4], and further illustrated that the 1,3-diol was located at C-8/C-10. This placement was clearly confirmed by the EIMS fragment ions at m/z 213 and m/z 257 which showed cleavage between C-8 and C-9 and between C-10 and C-11, respectively (Fig. 2).

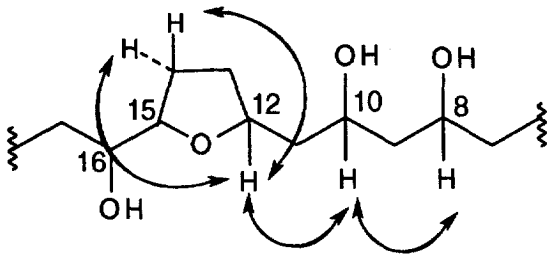

Fig. 3. Single-relayed COSY experiment of **1** (the arrows showing single-relayed COSY correlations).

Annohexocin bearing a 1,3,5-triol (H-8/H-10/H-12) was recently reported to be a mono-THF acetogenin with six OH groups isolated from leaves of *A. muricata* [6]. While unique ^{1}H signals for H-10 at H-8/H-10/H-12 appeared at δ 4.13 in annohexocin, **1** showed two ^{1}H signals at δ 4.06 and 4.15 beyond δ 3.95. The relative placement of three oxygenated carbons from C-8 to C-12 was considered to result in downfield shifts of the ^{1}H signals for H-10 and H-12 at δ 4.06 and 4.15, respectively (Table 1). Compound **1** is distinguished from the annopentocin-type acetogenins by having one OH group separated by a methylene group from the mono-THF ring on the lactone side and by one additional OH group at C-8, forming a 1,3-diol with an OH group at C-10 [9]; thus, **1** is a new and different type of acetogenin. The relative stereochemistry of the 1,3-diol was indicated to be pseudo-*erythro* by spectral comparisons with known model compounds bearing the same configuration [6, 8, 16, 17]. Compound **1** was named muricoreacin.

Compound **2** was also obtained as a white solid. The molecular weight was determined by a peak at m/z 629 [MH$^+$] in the CIMS, corresponding to the molecular formula, $C_{35}H_{64}O_9$, which was confirmed by an HR-FABMS peak at m/z 635.4717 [M+Li]$^+$ (calcd. 635.4710). The IR spectrum of **2** showed strong absorption at 1743 cm^{-1} for a γ-lactone carbonyl. Signals in the ^{13}C-NMR spectrum were observed at δ 175.0 (C-1), 152.4 (C-34), 135.6 (C-2), 78.2 (C-35) and 69.2 (C-4) as well as ^{1}H signals at δ 7.22 (H-34), 5.07 (H-35) and 3.87 (H-4) (Table 2), which indicated that **2**, like **1**, also has an α,β-unsaturated γ-lactone fragment with an OH group at C-4 (Fig. 4). The fragment ion at m/z 141 in the EIMS confirmed the presence of this moiety (Fig. 5).

Compound **2** was observed to possess a mono-THF ring with one flanking OH group from the ^{13}C signals at δ 81.7 (C-15), 79.3 (C-12), and 74.0 (C-16) (Table 2). An additional four ^{13}C signals were observed at ca. δ 73.9–74.5 due to hydroxylated carbons, which indicated the presence of a total of six OH groups in **2**. The presence of six OH groups was also shown by the successive losses of six m/z 18 (H_2O) units in the CIMS.

The placements of the mono-THF ring and the OH groups were suggested by diagnostic analyses of the EIMS of **2** (Fig. 5). A series of intense fragment ions were observed at m/z 341, 323, 305 and 287, due to the cleavage between C-15 and C-16. Since the same strong series of fragment ions was shown also in the EIMS of **1**, the mono-THF ring was assumed to be located at the same position, C-12/C-15, and also flanked by a single OH group at C-16 in **2**. The relative stereochemistry around the mono-THF ring from C-12 to C-16 was similarly determined to be of the *trans/threo* configuration, since the correlation between δ 3.86 and 3.47 was observed in the ^{1}H-^{1}H COSY spectrum and the ^{13}C signals at δ 31.6 and 28.1, as well as

Table 2. ^{13}C-NMR (125 MHz, $CDCl_3+CD_3OD$) and ^{1}H-NMR (500 MHz, $CDCl_3$) Spectra Data for **2**

Carbon	**2**	
	δ C	δ H (*J* on Hz)
1	175.0	
2	135.6	
3a,3b	22.3–35.0	2.45 m, 2.54 m
4	69.2	3.87 m[a]
5	22.3–35.0	1.60 m
6	22.3–35.0	1.26–1.72 m
7	73.9[a]	3.43 m[b]
8	74.0	3.43 m[b]
9–11	22.3–35.0	1.26–1.72 m
12	79.3	3.94 m
13	32.3–35.0	2.04 m, 1.56 m
14	22.3–35.0	1.97 m, 1.68 m
15	81.7	3.86 m[a]
16	74.0[a]	3.47 m[b]
17	22.3–35.0	1.26–1.72 m
18	22.3–35.0	1.43–1.60 m
19	74.5[a]	3.60 m
20	74.5[a]	3.60 m
21	22.3–35.0	1.43–1.60 m
22–31	22.3–39.0	1.26–1.72 m
32	13.8	0.88*t*(7.5)
33	152.4	7.22*d*(1.0)
34	78.2	5.07*dq*(1.0, 6.5)
35	18.6	1.44*d*(6.5)

[a,b,c] Assignments are interchangeable within the same column.

the ^{1}H signals at δ 2.04 and 1.56 (H-13), δ 1.97 and 1.68 (H-14), were assigned to C-13 and C-14, respectively (Table 2) [7, 19, 26–29].

In the ^{13}C-NMR spectrum of **2**, the four ^{13}C signals at δ 73.9–74.5 for hydroxylated carbons, as well as the four ^{1}H signals at δ 3.43 (2H) and 3.60 (2H) in the ^{1}H-NMR spectrum, clearly indicated the existence of two vicinal diol groups in **2** based on comparisons with known and model acetogenins [15, 17, 19, 27, 30]. One was assigned at C-7/C-8 by the fragment ions at *m/z* 299 and 199 (Fig. 5), and this was further confirmed by the upfield shift to δ 22.1, in the ^{13}C-NMR spectrum, due to the double β-effect on the carbon at C-10 [6, 19, 31]. The remaining vicinal diol group was assigned at C-19/C-20 from two strong fragment ions series at *m/z* 429, 411, 393, 375, 357 and 339 and *m/z* 199 and 181 due to the cleavage between C-19 and C-20 (Fig. 5). The same strong fragment ion series, due to cleavages between C-15/C-16 and C-19/C-20, also appeared in **1**; these observations indicated that **1** and **2** have the same structural moiety from C-12 of the mono-THF ring down the hydrocarbon chain.

Consequently, **2** was elucidated to be a hexahydroxylated mono-THF ring acetogenin with two 1,2-vicinal diols. This type of acetogenin has previously been reported as murihexocins A and B [7]. The ^{13}C-NMR spectum of **2** was obtained in a mixture of $CDCl_3$ and CD_3OD to improve solubility. Since the small quantity of CD_3OD did not appreciately change the chemical shift, it was possible to compare directly the NMR spectra; almost all of the chemical shifts in the ^{13}C-NMR spectrum of **2** matched well with those of murihexocins A and B. However, murihexocins A and B bear two *threo* vicinal diols. In the

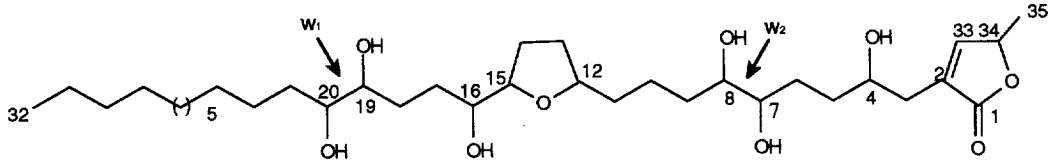

Fig. 4. The structure of murihexocin C (**2**) (W_1:*erythro*, W_2:*threo* or W_1:*threo*, W_2:*erythro*).

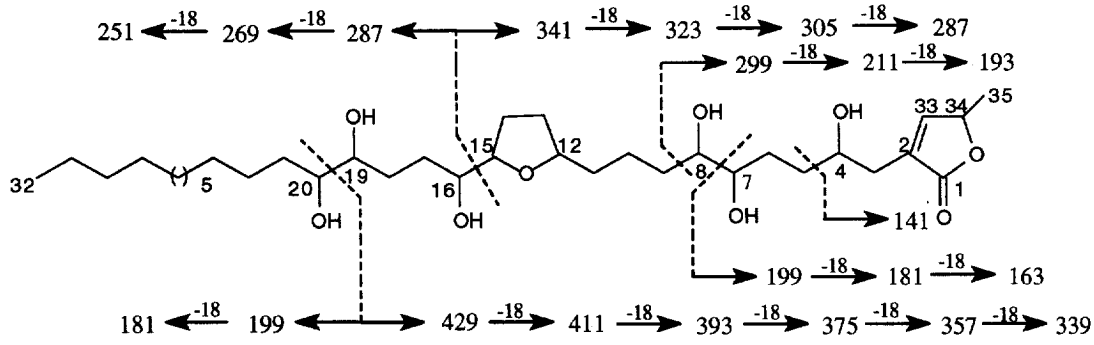

Fig. 5. Diagnostic EIMS fragment ions (*m/z*) of murihexocin C (**2**).

Table 3. Bioactivities of **1** and **2** from *Annona muricata* (ED_{50}:μg/ml)

Compounds	BST[a]	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PACA-2[g]
1	19	0.23	1.3	0.57	0.71	0.025	2.3
2	10	1.1	3.8	1.3	2.5	0.86	0.49
Adriamycin[h]		0.013	0.21	0.055	0.027	0.11	0.0042

[a] The brine shrimp(*Artemia salina* Leach) test (LD_{50}:μg/ml), [b] Human lung carcinoma, [c] Human breast carcinoma, [d] Human colon adenocarcinoma, [e] Human kidney carcinoma, [f] Human prostate adenocarcinoma, [g] Human pancreatic carcinoma, [h] A positive antitumor control.

^{1}H-NMR spectrum of **2**, two ^{1}H signals were observed at δ 3.60, which clearly showed the presence of an *erythro* configuration in one of its 1,2-vicinal diols. The *threo* configuration in the other vicinal diol was indicated by the two ^{1}H signals at δ 3.43 in **2**. Thus, **2** was indicated to have one *erythro* vicinal diol and one *threo* vicinal diol, but the respective placements of each could not be solved. Compound **2** was named murihexocin C, considering that it had the same planar structure as murihexocins A and B.

The bioactivities of muricoreacin (**1**) and murihexocin C (**2**) are summarized in Table 3. Compounds **1** and **2** showed significant bioactivities in the BST and among the six human solid tumor cell lines. Particularly, **1** was selectively cytotoxic against the prostate adenocarcinoma (PC-3) cell line at about five times the potency of adriamycin. Compound **2** showed selective cytotoxicity against the prostate adenocarcinoma (PC-3) and the pancreatic carcinoma (PACA-2) cell lines, with a lower level of potency. These potencies are typical of penta- and hexahydroxylated Annonaceous acetogenins which show lower activities than the tetra- or tri-hydroxylated compounds in SAR studies [9, 10].

EXPERIMENTAL

Instrumentation

HPLC was carried out with Rainin HPLC pumps, a Rainin Model UV-1 detector at 220 nm using the Dynamax software system and a C-18 column (250 × 21 mm, 8 μm, 60 Å). Optical rotations were determined by using a Perkin–Elmer 241 polarimeter. IR spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrophotometer. UV spectra were made on a Beckman DU 640 spectrophotometer in MeOH. ^{1}H-NMR, ^{1}H-^{1}H COSY, and ^{13}C-NMR spectra were obtained on a Varian VXR-500S spectrometer. Low resolution EIMS and CIMS were taken on a Finnigan 4000 spectrometer. High resolution FABMS were performed on a MicroMass AutoSpec. spectrometer.

Bioassays

The brine shrimp (*Artemia salina* Leach) lethality test (BST) was routinely employed for evaluating the crude extracts, fractions, and isolated compounds [34, 35]. Cytotoxicities to human solid-tumor cell lines were evaluated at the Purdue Cancer Center, Cell Culture Laboratory, using the standard seven-day MTT assays for A-549 (human lung carcinoma) [34], MCF-7 (human breast carcinoma) [35], HT-29 (human colon adenocarcinoma) [36], A-498 (human kidney carcinoma) [34], PC-3 (human prostate adenocarcinoma) [37], and PACA-2 (human pancreatic carcinoma) [38]. Adriamycin was used as a positive antitumor control.

Plant material

The leaves (2.0 kg) of *Annona muricata* L. were obtained from fruit-producing trees growing in the experimental orchard of Bandung Institute of Technology, Bandung, Indonesia, and were dried and pulverized through an 8 mm sieve in an electric mill.

Extraction and Isolation

The leaves (2.0 kg) were percolated by 95% EtOH to give 386 g of an extract (F001, BST LC_{50} 30.5 ppm). The EtOH extract was partitioned between CH_2Cl_2 and H_2O (1 : 1). The H_2O-soluble fraction (F002) was freeze-dried to yield a sticky yellow mass (260 g), while the CH_2Cl_2-soluble fraction was concentrated by rotary evaporation to yield a residue of 126 g (F003, BST LC_{50}19.6 ppm). F003 was then partitioned between 90% aqueous MeOH and hexane (1 : 1). The two phases were dried by rotary evaporation to yield the hexane-soluble fraction (F006, BST $LC_{50} > 100$ ppm) (11 g) and an aqueous MeOH-soluble fraction (F005, BST LC_{50} 1.6 ppm) (115 g). Open column chromatography of F005 (55 g) was performed on 250 g of Amberlite XAD-2 resin eluted by hexane, hexane-Me_2CO (1 : 1), and then Me_2CO. Flash column chromatography of the hexane-Me_2CO (1 : 1) residue (10.2 g, BST LC_{50} 0.98 ppm) from the Amberlite column, with 0–25% MeOH in CH_2Cl_2, eluted over Baker 40 μm silica gel, separated mixtures of active compounds from inert materials as determined by the BST. Active fractions were pooled by activity and subjected to repeated chromatography, by open columns over silica gel using gradient solvent systems of $CHCl_3$-MeOH or CH_2Cl_2-MeOH. From

the more polar of these active impure fractions, muricoreacin **1** and murihexocin C **2** were isolated by repeated reversed phase HPLC using gradient solvent systems of 60–90% acetonitrile in H_2O for 90 min(flow rate:10 ml/min).

Muricoreacin (**1**)

White solid (7.7 mg); $[\alpha]_D^{23}+1.56°$ (MeOH; *c* 0.06); UV (MeOH) λ_{max}nm (log ϵ): 220 nm (3.65); IR $\nu^{film}cm^{-1}$: 3347 (br OH), 2916, 2849, 1743, 1470; ^1H and ^{13}C NMR, Table 1; CIMS *m/z* (rel. int.): 629 ($[MH]^+$, 100), 611 ($[MH\text{-}H_2O]^+$, 15), 593 ($[MH\text{-}2H_2O]^+$, 14), 575 ($[MH\text{-}3H_2O]^+$, 14), 557 ($[MH\text{-}4H_2O]^+$, 4), 539 ($[MH\text{-}5H_2O]^+$, 1), 521 ($[MH\text{-}6H_2O]^+$, 1); HR-FABMS $[M+Li]^+$ *m/z* 635.4694 (calcd 635.4710), corresponding to $C_{35}H_{65}O_9^7Li^+$; EIMS *m/z* (rel. int.): 429 (1), 411 (8), 393 (25), 375 (25), 357 (14), 341 (8), 339 (14), 323 (70), 305 (31), 287 (8), 269 (23), 257 (2), 251 (4), 239 (42), 221 (10), 213 (71), 203 (3), 199 (8), 195 (18), 181 (27), 177 (12), 141 (20), 123 (15).

Murihexocin C (**2**)

White solid (3.0 mg); $[\alpha]_D^{23}+37.5°$ (MeOH; *c* 0.04), UV (MeOH) λ_{max}nm (log ϵ): 220 nm (3.69); IR $\nu^{film}cm^{-1}$:3367 (br OH), 2915, 2849, 1743, 1474; ^1H and ^{13}C NMR, Table 2; CIMS *m/z* (rel. int.) 629 ($[MH]^+$, 100), 611 ($[MH\text{-}H_2O]^+$, 26), 593 ($[MH\text{-}2H_2O]^+$, 26), 575 ($[MH\text{-}3H_2O]^+$, 28), 557 ($[MH\text{-}4H_2O]^+$, 25), 539 ($[MH\text{-}5H_2O]^+$, 7), 521 ($[MH\text{-}6H_2O]^+$, 7); HR-FABMS $[M+Li]^+$ *m/z* 635.4717 (calcd 635.4710), corresponding to $C_{35}H_{65}O_9^7Li^+$; EIMS *m/z* (rel. int.): 429 (1), 411 (7), 393 (26), 375 (65), 357 (12), 341 (2), 339 (3), 323 (77), 305 (24), 299 (10), 287 (9), 281 (52), 269 (11), 263 (22), 251 (2), 245 (4), 211 (17), 199 (12), 193 (19), 181 (17), 163 (6), 141 (20), 123 (20).

Acknowledgements—This investigation was supported by RO1 Grant no. CA 30909 from the National Cancer Institute, National Institutes of Health. G.-S. Kim gratefully acknowledges the support of the Ministry of Government Administration, Republic of Korea. We are also grateful to the Cell Culture Laboratory, Purdue Cancer Center. Thanks are due to the Nebraska Center for Mass Specrotrometry for the HR-FABMS measurement.

REFERENCES

1. Zeng, L., Ye, Q., Oberlies, N. H., Shi, G., Gu, Z. M., He, K. and McLaughlin, J. L., *Natural Product Reports*, 1996, **13**, 275.
2. McLaughlin, J. L., Zeng, L., Oberlies, N. H., Alfonso, D., Johnson, H. A. and Cumming, B. A., in *Phytochemicals for Pest Control*, eds. P. A. Hedin, R. M. Hollingworth, E. P. Masler, J. Miyamoto, D. G. Thompson. American Chemical Society, ACS Symposium Series, Washington D.C., 1997, p. 117.
3. Cavé, A., Figadère, B., Laurens, A., and Cortes, D., in *Progress in the Chemistry of Organic Natural Products*, Vol. 70, eds. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, Ch. Tamm. Springer–Verlag, Wien New York, 1997, p. 81.
4. Oberlies, N. H., Croy, V. L., Harrison, M. L. and McLaughlin, J. L., *Cancer Letters*, 1997, **115**, 73.
5. Oberlies, N. H., Jones, J. L., Corbett, T. H., Fotopoulos, S. S. and McLaughlin, J. L., *Cancer letters*, 1995, **96**, 55.
6. Zeng, L., Wu, F. E. and McLaughlin, J. L., *Bioorganic and Medicinal Chemistry Letters*, 1995, **5**, 1865.
7. Zeng, L., Wu, F. E., Gu, Z. M. and McLaughlin, J. L., *Tetrahedron Letters*, 1995, **36**, 5291.
8. Silva, E. L. M., Roblot, F., Laprévote, O., Sérani, L. and Cavé, A., *Journal of Natural Products*, 1997, **60**, 162.
9. He, K., Zeng, L., Ye, Q., She, G., Oberlies, N. H., Zhao, G. X., Njoku, C. J. and McLaughlin, J. L., *Pesticide Science*, 1997, **49**, 372.
10. Oberlies, N. H., Chang, C. J. and McLaughlin, J. L., *Journal of Medicinal Chemistry*, 1997, **40**, 2102.
11. Marshall, J. A. and Hinkle, K. W., *Journal of Organic Chemisry*, 1997, **62**, 5989.
12. Marshall, J. A. and Hinkle, K. W., *Journal of Organic Chemisry*, 1997, **62**, 5996.
13. Gu, Z. M., Zhou, D., Wu, J., Shi, G., Zeng, L. and McLaughlin, J. L., *Journal of Natural Products*, 1997, **60**, 242.
14. Rieser, M. J., Fang, X. P., Rupprecht, J. K., Hui, Y. H., Smith, D. L. and McLaughlin, J. L., *Planta Medica*, 1993, **59**, 91.
15. Wu, F. E., Gu, Z. M., Zeng, L., Zhao, G. X., Zhang, Y., McLaughlin, J. L. and Sastrodihardjo, S., *Journal of Natural Products*, 1995, **58**, 830.
16. Wu, F. E., Zeng, L., Gu, Z. M., Zhao, G. X., Zang, Y., Schwedler, J. T., McLaughlin, J. L. and Sastrodihardjo, S., *Journal of Natural Products*, 1995, **58**, 902.
17. Wu, F. E., Zeng, L., Gu, Z. M., Zhao, G. X., Zang, Y., Schwedler, J. T., McLaughlin, J. L. and Sastrodihardjo, S., *Journal of Natural Products*, 1995, **58**, 909.
18. Wu, F. E., Zhao, G. X., Zeng, L., Zhang, Y., Schewedler, J. T., McLaughlin, J. L. and Sastrodihardjo, S., *Journal of Natural Products*, 1995, **58**, 1430.
19. Zeng, L., Wu, F. E., Oberlies, N. H. and McLaughlin, J. L., *Journal of Natural Products*, 1996, **59**, 1035.
20. Kim, G. S., Zeng L., Rogers, L. L., Alali, F., Wu. F. E., McLaughlin, J. L., and Sastrodihardjo, S., *Journal of Natural Products*, 1998, **61**, 732.
21. Rieser, M. J., Gu, Z. M., Fang, X. P., Zeng, L., Wood, K. V. and McLaughlin, J. L., *Journal of Natural Products*, 1996, **59**, 100.

22. Roblot, F., Laugel, T., Lebæuf, M., Cavé, A. and Laprévote, O., *Phytochemistry*, 1993, **34**, 281.
23. Rieser, M. J., Kozlowski, J. F., Wood, K. V. and McLaughlin, J. L., *Tetrahedron Letters*, 1991, **32**, 1137.
24. Hisham, A., Sreekala, U., Pieters, L., De Bruyne, T., Van den Heuvel, H. and Claeys, M., *Tetrahedron*, 1993, **49**, 6913.
25. Myint, S. H., Cortes, D. C., Laurens, A., Hocquemiller, R., Lebæuf, M., Cavé, A., Cotte, J. and Quéro, A. M., *Phytochemistry*, 1991, **30**, 3335.
26. Fujimoto, Y., Murasaki, C., Shimada, H., Nishioka, S., Kakinuma, K., Singh, S., Singh, M., Gupta, Y. K. and Sahai, M., *Chemical and Pharmaceutical Bulletin*, 1994, **42**, 1175.
27. Yu, J. G., Ho, D. K., Cassady, J. M., Xu, L. and Chang, C. J., *Journal of Organic Chemistry*, 1992, **57**, 6198.
28. Gu, Z. M., Zeng, L., Fang, X. P., Colman-Saizarbitoria, T., Huo, M. and McLaughlin, J. L., *Journal of Organic Chemistry*, 1994, **59**, 5162.
29. Zhao, G. X., Rieser, Y. H., Miesbauer, L. R., Smith, D. L. and McLaughlin, J. L., *Phytochemistry*, 1993, **33**, 1065.
30. Shi, G., MacDougal, J. M. and McLaughlin, J. L., *Phytochemistry*, 1997, **45**, 719.
31. Jossang, A., Douaele, B. and Cavé, A., *Tetrahedron Letters*, 1990, **31**, 1861.
32. McLaughlin, J. L., in *Methods in Plant Biochemistry*, Vol. 6, ed. K. Hostettmann. Academic, London, 1991, p. 1.
33. McLaughlin, J. L., Rogers, L. L., and Anderson, J. E., *Drug Information Journal* (in press).
34. Giard, D. J., Aronson, S. A., Todaro, G. J., Arnstein, P., Kersey, H. J., Dosik, H. and Parks, W. P., *Journal of the National Cancer Institute*, 1973, **51**, 1417.
35. Soule, H. D., Vazquez, J., Long, A., Albert, S. and Brennam, M., *Journal of the National Cancer Institute*, 1973, **51**, 1409.
36. Fogh, J. and Trempe, G., in *Human Tumor Cells*, ed. J. Fogh. Plenum Press, New York, 1975, p. 115–159.
37. Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F. and Jones, L. W., *Investigative Urology*, 1979, **17**, 16.
38. Yunis, A. A., Arimura, G. K. and Russian, D., *International Journal of Cancer*, 1997, **19**, 128.