JOURNAL OF NATURAL PRODUCTS

Subscriber access provided by ISTANBUL TEKNIK UNIV

Bioactive Annonaceous Acetogenins from the Bark of Xylopia aromatica

T. Colman-Saizarbitoria, J. Zambrano, N. R. Ferrigni, Z.-M. Gu, J. H. Ng, D. L. Smith, and J. L. McLaughlin

J. Nat. Prod., **1994**, 57 (4), 486-493• DOI: 10.1021/np50106a007 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50106a007 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 57, No. 4, pp. 486-493, April 1994

BIOACTIVE ANNONACEOUS ACETOGENINS FROM THE BARK OF *XYLOPIA AROMATICA*

T. COLMAN-SAIZARBITORIA, J. ZAMBRANO, N.R. FERRIGNI,

Facultad de Farmacia, Universidad Central de Venezuela, Apartado-40109, Caracas, Venezuela

Z.-M. GU, J.H. NG, D.L. SMITH, and J.L. MCLAUGHLIN*

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

ABSTRACT.—Bioactive Annonaceous acetogenins have been isolated from the EtOH extract of the bark of *Xylopia aromatica* by bioactivity-directed fractionation using lethality to brine shrimp. These acetogenins include xylopianin [**1**], xylopiacin [**2**], and xylomaticin [**3**], which are three new mono-tetrahydrofuran ring type acetogenins, in addition to the known compounds, annomontacin, gigantetronenin, gigantetrocin A, and annonacin. Compounds **1** and **2** are unusual in having hydroxylation at C-8; **3** has the same functionalities as annonacin but with 37 carbons instead of 35 carbons. The structures were elucidated by spectral analysis of the parent compounds and/or simple chemical derivatives. These acetogenins showed cytotoxicities, comparable to adriamycin, against three human solid tumor cell lines.

The Annonaceae is a moderate-sized family of flowering plants with approximately 130 genera and 2,300 species. Phytogeographically it is almost entirely tropical. The plants in this family have recently been investigated as potential sources of potent biologically active Annonaceous acetogenins; these compounds, so far, have been reported from the genera *Uvaria, Asimina, Goniothalamus, Rollinia,* and *Annona.* In this paper, acetogenins are reported for the first time from the genus *Xylopia* (1,2).

Xylopia aromatica (Mart.) Lam. is a tree native to tropical America; the bark was collected in Estado Amazonas (Venezuela), and, using the brine shrimp lethality test (BST) for activity-directed isolation (3,4), three new acetogenins, named xylopianin [**1**], xylopiacin [**2**], and xylomaticin [**3**], in addition to four known bioactive acetogenins, annomontacin (5), gigantetronenin (6), gigantetrocin A (7,12) and annonacin (8,9), have been isolated and identified. These compounds belong to the group of mono-tetrahydrofuran (THF) acetogenins, which have become more common in recent years (8,9). Other new mono-THF acetogenins recently published include giganenin (10), cis- and trans-gigantetrocinones (11), muricatetrocins A and B (12), and *cis-* and *trans*-annonacin-A-one (11).

RESULTS AND DISCUSSION

The bark was extracted with EtOH. The EtOH residue (F001) was partitioned between H_2O (F002) and $CHCl_3$ (F003), and F003 was partitioned between hexane (F006) and 10% H_2O in MeOH (F005). The most bioactive fraction, as tested by the BST (3,4), was F005 (LC_{50}=160 μg/ml). F005 was submitted to successive purifications by cc, prep. tlc, and hplc, directed by the BST assay at each step, to yield compounds **1–3** (Figure 1), and four known compounds. Spectral characteristics, including uv, ir, ^{1}H- and ^{13}C-nmr data, suggested that all of the isolated compounds belonged to the mono-THF group of acetogenins; the known compounds were identified as annomontacin (5), gigantetronenin (6), gigantetrocin A (7,12), and annonacin (8,9) by co-tlc in two different solvent systems and comparison of their spectral characteristics, including ms and ^{1}H- and ^{13}C-nmr data, with those of the original compounds available in our group.

Xylopianin [**1**] was a waxy solid with mp 78–79°, $[\alpha]^{25}$D +23.3° (MeOH, c=0.008). The fabms gave $[MH]^+$ at m/z 597 indicating an M^+ of 596, and hrfabms gave

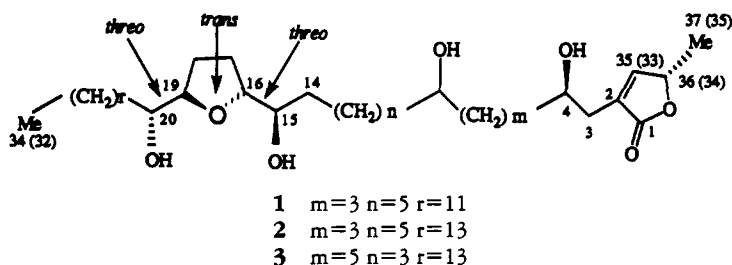

FIGURE 1. New acetogenins isolated from *Xylopia aromatica*. Absolute stereochemistries [except for those at positions C-8 and C-10] are proposed based on the close similarities to annonacin, whose absolute stereochemistry is known [**15**].

m/z 597.4725 for the $[MH]^+$ (calcd 597.4730), corresponding to the molecular formula $C_{35}H_{65}O_7$. The ir spectrum suggested the presence of OH groups (3426 cm^{-1}) and the presence of an α,β-unsaturated γ-lactone (1737 cm^{-1}). The nmr spectrum of **1** showed ^{1}H resonances at δ 7.15 (q, H-33), 5.06 (qd, H-34), 2.53 (H-3a), 2.42 (H-3b), 3.86 (m, H-4) and 1.44 (d, H-35) (Table 1), and six ^{13}C-nmr resonances at δ 174.6 (C-1), 151.9 (C-33), 131.0 (C-2), 78.0 (C-34), 69.7 (C-4), and 19.1 (C-35) (Table 2), which are characteristic spectral features of an α,β-unsaturated γ-lactone with a 4-OH moiety as is common in many of the Annonaceous acetogenins (1,2). The absence of equivalence between the protons at C-3, the downfield chemical shift in the nmr of the C-4, and a peak at *m/z* 213 in the eims of the TMSi derivative [**4**] (Figure 2) substantiated the presence of this terminal moiety.

The presence in **1** of a mono-THF ring, with two OH groups adjacent to the ring, was suggested by ^{1}H-nmr resonances at δ 3.41 (H-15 and H-20) and 3.80 (H-16 and H-19), and ^{13}C-nmr peaks at δ 82.7 (C-16), 82.6 (C-19), 74.1 (C-15) and 74.0 (C-20); similar peaks are characteristic of other mono-THF acetogenins having two OH groups adjacent to the ring, such as annonacin (8,9) and annomontacin (5). Determination of the

TABLE 1. ^{1}H-Nmr (500 MHz, $CDCl_3$) Data of Xylopianin [**1**], Xylopiacin [**2**], and Xylomaticin [**3**].

Position	Compound δ, (*J* in Hz)[a]		
	1	**2**	**3**
3a	2.53 (dddd, 15; 3.3; 1.0; 1.0)	2.53 (dddd, 15; 3.5; 1.0; 1.0)	2.53 (dddd, 15; 3.3; 1.1; 1.1)
3b	2.42 (dddd, 15; 8.6; 1.0; 1.0)	2.42 (dddd, 15, 8.6, 1.0, 1.0)	2.42 (dddd, 15; 8.6; 1.1; 1.1)
4	3.86 (m)	3.86 (m)	3.85 (m)
5–7	1.23–1.74 (m)	1.24–1.74 (m)	1.22–1.74 (m)
8	3.60 (m)	3.60 (m)	1.22–1.74 (m)
9	1.23–1.74 (m)	1.24–1.74 (m)	1.22–1.74 (m)
10	1.23–1.74 (m)	1.24–1.74 (m)	3.60 (m)
11–14	1.23–1.74 (m)	1.24–1.74 (m)	1.22–1.74 (m)
15	3.41 (m)	3.41 (m)	3.41 (m)
16	3.80 (dt, 12.3; 7.0)	3.80 (dt, 12.3; 7.5)	3.80 (dt, 12.5; 7.5)
17–18	1.67 (m) and 1.98 (m)	1.67 (m) and 1.95 (m)	1.68 (m) and 1.95 (m)
19	3.80 (dt, 12.3; 7.0)	3.80 (dt, 12.3; 7.5)	3.80 (dt, 12.5; 7.5)
20	3.41 (m)	3.41 (m)	3.41 (m)
21–31	1.23–1.74 (m)	1.24–1.74 (m)	1.22–1.74 (m)
32	0.88 (t, 7.0)	1.24–1.74 (m)	1.22–1.74 (m)
33	7.15 (q, 1.5)	1.24–1.74 (m)	1.22–1.74 (m)
34	5.06 (qd, 6.5; 1.5)	0.88 (t, 7.0)	0.88 (t, 7.0)
35	1.44 (d, 6.5)	7.19 (q, 1.0)	7.19 (q, 1.5)
36	—	5.07 (qd, 6.5; 1.1)	5.06 (qd, 6.5; 1.4)
37	—	1.44 (d, 6.5)	1.44 (d, 6.5)

[a]All of the assignments were made by comparisons with those of annonacin (8,9,15).

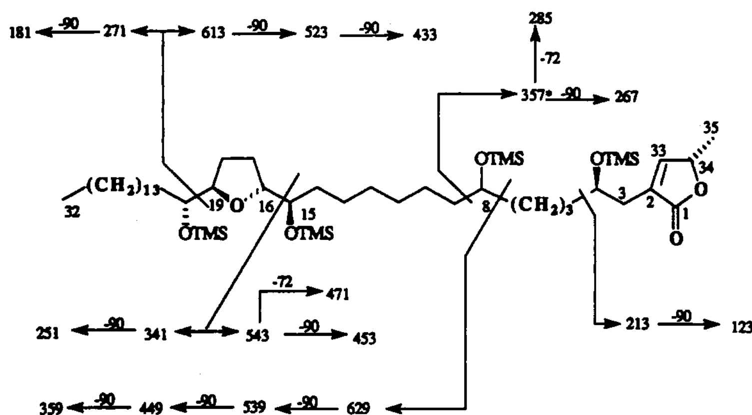

FIGURE 2. Diagnostic eims fragmentation ions of xylopianin-TMSi derivative [**4**]. Numbers above the arrows represent loss of TMSiOH (*m/z* 90) and loss of TMSi (*m/z* 72). The elemental composition of the fragment marked with an asterisk was confirmed through exact mass measurement.

relative stereochemistry around the mono-THF moiety was done by Born's technique (13) and by comparison with reported spectral data of a series of synthetic mono-THF diol compounds of known relative configuration (14). The arrangement was assigned as threo between C-15/16 and C-19/20 because of the proton signals of compound **1** at δ 3.41 for H-15 and H-20 and carbon signals at δ 74.1 for C-15 and 74.0 for C-20; the trans relationship between C-16/19 was determined by comparison with reported spectral data of model compounds (14), suggesting that the relative configuration for these four chiral centers was threo-trans-threo (Figure 1). The absolute stereochemistries shown in Figure 1, are proposed, for purpose of illustration, to be identical to those of natural annonacin (15); however, the mirror images of the mono-THF portion of crossolone give identical spectral signals (16), and the absolute configurations need to be confirmed by Mosher ester preparation (15).

The number of the carbons between the unsaturated lactone and the THF ring was established by eims spectral analysis of the fragmentation of the TMSi-derivative [**4**] (Figure 2). From the abundant ion signals at *m/z* 613 and 543, both of which contain the unsaturated lactone ring, it was obvious that the THF ring was located from C-15 to C-20.

The existence of four OH groups in **1** was indicated by four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the fabms and the preparation of the tetra-trimethylsilyl (TMSi) derivative [**4**], which showed four successive losses of TMSiOH (*m/z* 90) in the eims. Furthermore, the ^{13}C-nmr spectra of **1** confirmed four hydroxylated carbons with signals at δ 74.1 (C-15), 74.0 (C-20), 71.7 (C-8) and 69.7 (C-4). The eims fragmentation patterns of **4** clearly indicated that the OH groups were positioned at C-4, C-8, C-15, and C-20 (Figure 2). The assignment of the OH group at C-8 was firmly confirmed by the measurement of exact mass and corresponding elemental composition of the key fragment at *m/z* 357.1917 for $[C_{11}H_{15}O_4(TMS)_2]^+$ (calcd 357.1917). Hydroxylation of C-8 is unusual in the Annonaceous acetogenins but has previously been reported in montanacin, annomonicin (17), and 8-hydroxyannonacin (18).

Xylopiacin [**2**] was isolated as a waxy solid with mp 90–91°, $[\alpha]^{25}$D +24° (MeOH, c=0.006). The fabms displayed $[MH]^+$ at *m/z* 625 allowing a molecular weight of 624. The hrfabms gave *m/z* 625.5024 for the $[MH]^+$ (calcd 625.5043) corresponding to the molecular formula $C_{37}H_{69}O_7$. The ir spectrum of **2** suggested the presence of OH (3426 cm^{-1}) and the usual α,β-unsaturated γ-lactone (1737 cm^{-1}). The ^{1}H-nmr spectrum

showed resonances at δ 7.19 (H-35), 5.07 (H-36), 2.53 (H-3a), 2.42 (H-3b), 3.86 (H-4) and 1.44 (H-37), and six resonances appeared in the ^{13}C-nmr spectrum at δ 174.6 (C-1), 151.9 (C-35), 131.0 (C-2), 78.0 (C-36), 69.8 (C-4), and 19.2 (C-37) (Table 2) which, as in **1**, are all characteristic spectral features of Annonaceous acetogenins having α,β-unsaturated γ-lactones with a 4-OH moiety (1,2).

TABLE 2. ^{13}C-Nmr (125 MHz, $CDCl_3$) Data of Xylopianin [**1**], Xylopiacin [**2**], and Xylomaticin [**3**].

Position	Compound δ		
	1	**2**	**3**
1	174.6	174.6	174.6
2	131.0	131.0	131.1
3	33.4–33.5	33.4–33.5	33.4–33.5
4	69.7	69.8	69.9
5	37.5	37.5	37.4
6	21.7	21.7	25.5–32.0
7	37.2	37.2	25.5–32.0
8	71.7	71.7	25.5–32.0
9	36.9	36.9	37.3
10	25.5–31.9	25.5–32.0	71.8
11	25.5–31.9	25.5–32.0	37.3
12–13	25.5–31.9	25.5–32.0	25.5–32.0
14	33.4–33.5	33.4–33.5	33.4–33.5
15	74.1	74.1	74.1
16	82.7	82.7	82.6
17	28.8	28.8	28.9[a]
18	28.8	28.8	28.8[a]
19	82.6	82.6	82.6
20	74.0	74.0	74.0
21	33.4–33.5	33.4–33.5	33.4–33.5
22–30	25.5–31.9	25.5–32.0	25.5–32.0
31	22.7	25.5–32.0	25.5–32.0
32	14.2	25.5–32.0	25.5–32.0
33	151.9	22.7	22.7
34	78.0	14.2	14.2
35	19.1	151.9	151.8
36	—	78.0	78.0
37	—	19.2	19.2

[a]Signals may be interchanged.

The presence in **2** of a mono-THF ring with two OH groups adjacent to the ring was suggested by proton resonances at δ 3.41 (H-15 and H-20) and 3.80 (H-16 and H-19) and carbon peaks at δ 82.7 (C-16), 74.1 (C-15), 74.0 (C-20) and 82.6 (C-19); these were analogous to similar peaks of other mono-THF ring acetogenins, such as **1**, with two OH groups adjacent to the ring. Comparison of the spectral data of **1** and **2** revealed close similarities, although co-tlc performed in several solvent systems showed different behavior, and by hplc over Si gel, compound **2** showed a shorter R_t than **1**.

The determination of the relative stereochemistry around the mono-THF moiety again was determined by Born's technique (13) and by comparison with spectral data of the mono-THF model compounds of known relative configuration (14); the relative configuration for these four chiral centers is threo-trans-threo as in **1**.

The carbon skeleton and placement of the THF ring in **2** were determined based on eims spectral analysis of the TMSi derivative [**5**] (Figure 3); the fragment ions at *m/z* 613 and 543, indicated that the THF ring was between C-15 and C-20.

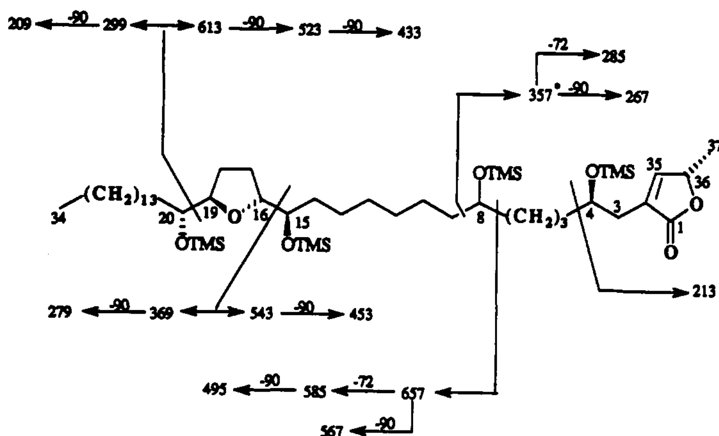

FIGURE 3. Diagnostic eims fragmentation ions of xylopiacin-TMSi derivative [**5**]. Numbers above the arrows represent loss of TMSiOH (*m/z* 90) and loss of TMSi (*m/z* 72). The elemental composition of the fragment marked with an asterisk was confirmed through exact mass measurement.

The existence of four OH groups in **2** was suggested by four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the fabms and the preparation of the tetra-trimethylsilyl (TMSi) derivative [**5**], which showed four successive losses of TMSiOH (*m/z* 90) in the eims. Furthermore, the ^{13}C-nmr spectrum of **2** showed four hydroxylated carbons with signals at δ 71.7 (C-8), 74.1 (C-15), 74.0 (C-20), and 69.8 (C-4). The eims fragmentation patterns of **5** clearly indicated that the OH groups were positioned at C-4, C-8, C-15, and C-19 (Figure 3). The assignment of the OH group at C-8 was, furthermore, supported by the exact mass and corresponding elemental composition of the key fragment at *m/z* 357.1915 for $[C_{11}H_{15}O_4\,(TMSi)_2]^+$ (calcd 357.1917).

Xylomaticin [**3**] was isolated as a waxy solid with mp 67–68°, $[\alpha]^{25}D$ +5.3° (MeOH, *c*=0.006). Its mol wt of 624 was established from fabms (glycerol) $[MH]^+$ at *m/z* 625 and confirmed by hrfabms (glycerol) with $[MH]^+$ at *m/z* 625.5032 for $C_{37}H_{69}O_7$ (calcd 625.5043). The ir spectrum suggested the presence of OH groups (3408 cm^{-1}) and the presence of an α,β-unsaturated γ-lactone (1744 cm^{-1}). In the ^1H-nmr spectrum (Table 1) and in the ^{13}C-nmr spectrum, the resonances of the expected α,β-unsaturated γ-lactone, as in **1** and **2**, were observed.

The presence of a mono-THF ring with two OH groups adjacent to the ring was suggested by the nmr spectra with proton resonances at δ 3.41 (H-15 and H-20) and 3.80 (H-16 and H-19) and carbon peaks at δ 82.6 (C-16 and C-19) and 74.0 (C-15 and C-20). The relative configuration in this THF ring moiety, once again was established as threo-trans-threo, by comparison with the reported ^1H- and ^{13}C-nmr chemical shifts of mono-THF model compounds (13,14).

The carbon skeleton and placement of the THF ring of **3** were determined based on the eims spectral analysis of the TMSi derivative [**6**] (Figure 4); the fragment ions at *m/z* 613, 543, 629, and 385 determined the chain length between the α,β-unsaturated γ-lactone and the THF ring moieties and placed the THF ring between C-15 and C-20.

The existence of four OH groups in **3** was indicated by four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the fabms and the preparation of the tetra-TMSi derivative [**6**]. The eims of **6** clearly showed that the OH groups were located at C-4, C-10, C-15, and C-19 (Figure 4). Examination of the published nmr spectral data of annonacin (8,9) and those of **3** indicated very close similarities although tlc analysis and hplc showed that

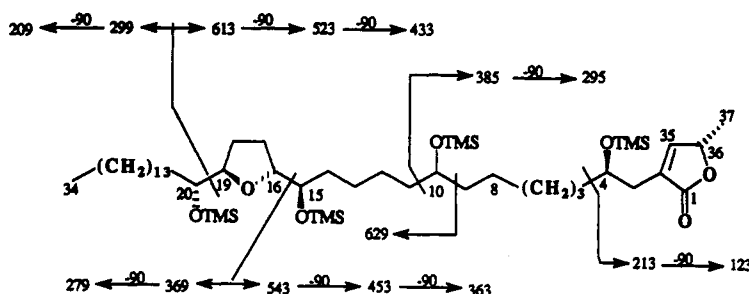

FIGURE 4. Diagnostic eims fragmentation ions of xylomaticin-TMSi derivative [**6**]. Numbers above the arrows represent loss of TMSiOH (*m/z* 90) and loss of TMSi (*m/z* 72).

3 was less polar than annonacin. Thus, xylomaticin [**3**] is identical to annonacin (8,9), but with two extra methylene groups in the terminal aliphatic chain.

Biological activities of **1**–**3** and the four previously known compounds are summarized in Table 3. These compounds are active in the BST (3,4); the isolated acetogenins showed significant cytotoxicities against A-549 (lung) (19), MCF-7 (breast) (20), and HT-29 (colon) (21) in seven-day human solid tumor cell in vitro tests, using adriamycin as the positive control compound. The acetogenins act as inhibitors of mitochondrial electron transport systems, via blocking complex I, and they show promising in vivo antitumor effects (22).

TABLE 3. Cytotoxicities of Fraction F005 and the Seven Acetogenin Compounds Isolated from *Xylopia aromatica*.

Compound	BST[a] LC_{50} μg/ml	A-549[b] ED_{50} μg/ml	MCF-7[b] ED_{50} μg/ml	HT-29[b] ED_{50} μg/ml
F005[c]	160	10^{-2}	1.39	10^{-2}
Xylopiacin [**2**][c]	4.9×10^{-1}	1.36×10^{-6}	1.39×10^{-7}	1.11×10^{-1}
Xylomaticin [**3**][c]	1.1×10^{-1}	1.54×10^{-4}	1.47×10^{-6}	3.04×10^{-1}
Annomontacin[c]	12.5	7.72×10^{-3}	1.65×10^{-1}	2.58×10^{-3}
Gigantetronenin[c]	9.4	4.7×10^{-3}	6.03×10^{-1}	5.37×10^{-2}
Gigantetrocin[c]	1.4	2.5×10^{-1}	6.3×10^{-1}	4.1×10^{-5}
Annonacin[c]	2.3×10^{-1}	$<10^{-7}$	2.4×10^{-3}	$<10^{-7}$
Adriamycin[c]	—	1.10×10^{-3}	2.28×10^{-2}	2.79×10^{-3}
Xylopianin [**1**][d]	3.3×10^{-1}	4.26×10^{-2}	18.47	6.63
Adriamycin[d]	—	3.89×10^{-2}	4.43×10^{-1}	4.15×10^{-2}

[a]Brine shrimp lethality (3,4).
[b]Cytotoxicities in human lung (A-549) (19), breast (MCF-7) (20), and colon (HT-29) (21) tumor cell lines.
[c,d]Refer to different runs in the cell culture assay.

EXPERIMENTAL

PLANT MATERIAL.—Bark of *Xylopia aromatica* was collected at Estado Amazonas (Venezuela). The material was authenticated by Stephen Tillet at the Herbario Ovalles. A voucher specimen of the bark is deposited in the Herbario Ovalles, Universidad Central de Venezuela. The dried bark was pulverized.

BIOASSAYS.—The extracts, fractions, and isolated compounds were evaluated for lethality to brine shrimp larvae (BST) (3,4). Cytotoxicities against human solid tumor cells were measured at the Cell Culture Laboratory, Purdue Cancer Center, for the A-549 lung carcinoma (19), MCF-7 breast carcinoma (20), and HT-29 colon adenocarcinoma (21), with adriamycin as a positive control, in seven day assays.

INSTRUMENTATION.—Mp determinations were on a Mel-Temp apparatus and are uncorrected. Ir spectra (film) were measured on a Perkin-Elmer 1420 ir spectrometer. Optical rotations were taken on a Perkin Elmer 241 polarimeter. Uv spectra were taken on a Beckman DU-7 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a Varian VXR-500S spectrometer. Low-resolution fabms data were collected on a Finnigan 4000 spectrometer. Low-resolution eims for TMS derivatives were obtained on a Kratos MS50 spectrometer. Hrfabms was obtained on the Kratos MS50 spectrometer through peak matching. Hplc was carried out using a Dynamax software system and a Si gel (8 mm) column (250×21 mm) equipped with a Rainin uv-1 detector. Analytical tlc was performed on Si gel plates developed with $CHCl_3$-MeOH (8:2) and hexane-Me_2CO (8:2) and visualized with 5% phosphomolybdic acid in EtOH (1).

EXTRACTION AND ISOLATION.—The pulverized bark (4.0 kg) was extracted with EtOH and partitioned, as described above, to obtain F005. F005 (60 g) was subjected to column chromatography over Si gel (2 kg) eluted with a gradient of hexane/EtOAc/MeOH. Fractions (F_1-1 to F_1-92) were collected and pooled according to their similar tlc patterns; bioactivities in the BST showed two active pools (P4 and P5). P5 (5 g, BST LC_{50}=7.9 ppm) was subjected to Si gel (200 g) column chromatography, eluted by 1.5% MeOH in $CHCl_3$. Fractions were collected and combined into 5 pools on the basis of similar tlc patterns, and BST results were obtained. P5-2 (F_2-5 to F_2-15; BST, LC_{50}=1.88 ppm) was re-chromatographed on an hplc column of Si gel, eluted with a gradient of hexane/THF/MeOH (flow rate 10 ml/min); this procedure yielded, in order of increasing polarity, **1–3**.

The active pool P4 (8 g, BST LC_{50}=10.49 ppm) was further resolved on another Si gel (300 g) column, eluted with 1.5% MeOH in $CHCl_3$. Fractions (F_3-1 to F_3-64) were collected into 6 pools on the basis of similar tlc patterns. The BST-active pool (F_3-40 to F_3-56, 0.120 g, BST LC_{50}=3.38 ppm) was re-chromatographed using hplc over Si gel eluted with a gradient of hexane/10% MeOH in THF (flow rate 10 ml/min) to afford the four white powders containing the four previously known compounds: annomontacin, gigantetronenin, gigantetrocin A, and annonacin.

Xylopianin [**1**].—White waxy solid (7 mg); mp 78–79°; $[\alpha]^{25}$D +23.3° (MeOH, c=0.008); uv λ max, (MeOH), 217 nm; fabms (glycerol) m/z $[MH]^+$ 597 (99), $[MH-H_2O]^+$ 579 (5), $[MH-2H_2O]^+$ 561 (10), $[MH-3H_2O]^+$ 543 (5.8), $[MH-4H_2O]^+$ 525 (11); hrfabms (glycerol) m/z 597.4730 $[MH]^+$ (calcd 597.4725 for $C_{35}H_{65}O_7$) and 357.1917 (calcd 357.1917 for $[C_{11}H_{15}O_4(TMSi)_2]^+$); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125 MHz) see Table 2; ir ν max (film) 3426, 2920, 2837, 1737, 1455, 1314, 1073, 667 cm^{-1}.

Xylopiacin [**2**].—White waxy solid (5 mg); mp 90–91°; $[\alpha]^{25}$D +24° (MeOH, c=0.006); uv λ max (MeOH) 220 nm; fabms (glycerol) m/z $[MH]^+$ 625 (76), $[MH-H_2O)]^+$ 607 (12), $[MH-2H_2O]^+$ 589 (11), $[MH-3H_2O]^+$ 571 (14), $[MH-4H_2O]^+$ 553 (27); hrfabms (glycerol) m/z 625.5024 $[MH]^+$ (calcd 625.5043 for $C_{37}H_{69}O_7$), and 357.1915 for $[C_{11}H_{15}O_4(TMSi)_2]^+$ (calcd 357.1917); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125 MHz) see Table 2; ir ν max (film) 3426 (OH), 2920, 2847, 1737, 1314, 1071 cm^{-1}.

Xylomaticin [**3**].—White waxy solid (6 mg); mp 67–68°; $[\alpha]^{25}$D +5.3° (MeOH, c=0.006); uv λ max (MeOH) 219 nm; fabms (glycerol) m/z $[MH]^+$ 625 (25), $[MH-H_2O]^+$ 607 (1.9), $[MH-2H_2O]^+$ 589 (0.9), $[MH-3H_2O]^+$ 571 (1), $[MH-4H_2O]^+$ 553 (5); hrfabms (glycerol) m/z 625.5032 for $C_{37}H_{69}O_7$ (calcd 625.5043); ^{1}H nmr ($CDCl_3$, 500 MHz) see Table 1; ^{13}C nmr ($CDCl_3$, 125 MHz) see Table 2; ir ν max (film) 3408, 2934, 2841, 1744, 1460, 1315, 1067, 845 cm^{-1}.

IDENTIFICATION OF KNOWN COMPOUNDS.—The four known compounds, annomontacin, gigantetronenin, gigantetrocin A, and annonacin, were identified by ir, ms, ^{1}H- and ^{13}C-nmr spectral data analysis and comparison with those previously reported (5–9).

TMS DERIVATIVATIONS.—Small amounts (<1 mg) of **1–3** were treated with 20 μl of *N,O-bis*-(trimethylsilyl)-acetamide and 2 μl of pyridine and heated at 70° for 30 min to yield the respective tetra-TMSi derivatives [**4–6**]; eims (Figures 2–4).

ACKNOWLEDGMENTS

The authors thank the Cell Culture Laboratory, Purdue Cancer Center, for the in-house cytotoxicity data. T.C.S. acknowledges sabbatical support from the Consejo de Desarrollo Cientifico y Humanistico of the Universidad Central de Venezuela. This investigation was supported in part by R01 grant No. CA30909, from the NCI, National Institutes of Health, and by the Facultad de Farmacia of the Universidad Central de Venezuela. Thanks are expressed to Dr. M. Hasegawa for the plant material and to Dr. S. Tillett for the identification of the material.

LITERATURE CITED

1. J.K. Rupprecht, Y.-H. Hui, and J.L. McLaughlin, *J. Nat. Prod.,* **43**, 947 (1990).

2. X.-P. Fang, M. Reiser, Z.-M. Gu, G.-X. Zhao, and J.L. McLaughlin, *Phytochem. Anal.*, **4**, 27 and 69 (1993).
3. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobson, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 463 (1992).
4. J.L. McLaughlin, in: "Methods in Plant Biochemistry." Ed. by K. Hostettmann, Academic Press, London, 1991, Vol. 6, p. 1.
5. A. Jossang, B. Dubaele, A. Cavé, M. Bartoli, and H.J. Beriel, *J. Nat. Prod.*, **54**, 967 (1991).
6. X.-P. Fang, J.E. Anderson, D.L. Smith, K.V. Wood, and J.L. McLaughlin, *J. Nat. Prod.*, **55**, 1655 (1992).
7. X.-P. Fang, J.K. Rupprecht, A. Alkofahi, Y.-H. Hui, Y.-M. Lui, D.L. Smith, K.V. Wood, and J.L. McLaughlin, *Heterocycles*, **32**, 11 (1992).
8. T.G. McCloud, D.L. Smith, C.-J. Chang, and J.M. Cassady, *Experientia*, **43**, 947 (1987).
9. A. Alkofahi, J.K. Rupprecht, D.L. Smith, C.-J. Chang, and J.L. McLaughlin, *Experientia*, **44**, 83 (1988).
10. X.-P. Fang, J.E. Anderson, D.L. Smith, K.V. Wood, and J.L. McLaughlin, *Heterocycles*, **34**, 1075 (1992).
11. G.-X. Zhao, M.J. Reiser, Y.-H. Hui, L.R. Miesbauser, D.L. Smith, and J.L. McLaughlin, *Phytochemistry*, **33**, 1065 (1993).
12. M.J. Reiser, X-P. Fang, J.E. Anderson, L.R. Miesbauer, D.L. Smith, and J.L. McLaughlin, *Helv. Chim. Acta*, **76**, 2433 (1993).
13. L. Born, F. Lieb, J.P. Moescher, H.F. Nonfon, R. Soller, and D. Wendish, *Planta Med.*, **56**, 312 (1990).
14. J.C. Harmange, B. Figadere, and A. Cavé, *Tetrahedron Lett.*, **33**, 5749 (1992).
15. M.T. Rieser, Y.-H. Hui, J.K. Rupprecht, J.F. Kozlowski, K.V. Wood, J.L. McLaughlin, P.R. Hanson, Z. Zhuang, and T.R. Hoye, *J. Am. Chem. Soc.*, **114**, 10203 (1992).
16. D. Gromek, B. Figadere, R. Hocquemiller, and A. Cavé, *Tetrahedron*, **49**, 5247 (1993).
17. A. Jossang, B. Dubaele, and A. Cavé, *Tetrahedron Lett.*, **13**, 1861 (1990).
18. J.G. Yu, D.K. Ho, L. Xu, and J.M. Cassady, *J. Org. Chem.*, **57**, 6198 (1992).
19. D.J. Giard, S.A. Aaronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik, and W.P. Parks, *J. Natl. Cancer Inst.*, **55**, 1417 (1973).
20. H.D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennam, *J. Natl. Cancer Inst.*, **51**, 1490 (1973).
21. J. Fogh and G. Trempe, in: "Human Tumor Cells, In Vitro." Ed. by J. Fogh, Plenum Press, New York, 1975, pp. 115–159.
22. K.I. Ahammadsahib, R.M. Hollingworth, J.P. McGovern, Y.-H. Hui, and J.L. McLaughlin, *Life Sci.*, **53**, 1113 (1993).

Received 6 October 1993