Two New Bioactive Monotetrahydrofuran Annonaceous Acetogenins from the Bark of Xylopia aromatica

T. Colman-Saizarbitoria, Z.-M. Gu, and J. L. McLaughlin

J. Nat. Prod., **1994**, 57 (12), 1661-1669• DOI:
10.1021/np50114a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 12, pp. 1661-1669, December 1994

TWO NEW BIOACTIVE MONOTETRAHYDROFURAN ANNONACEOUS ACETOGENINS FROM THE BARK OF *XYLOPIA AROMATICA*

T. COLMAN-SAIZARBITORIA, Z.-M. GU, and J.L. MCLAUGHLIN*

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

ABSTRACT.—Xylopien [**1**] and xylomatenin [**2**], two new bioactive monotetrahydrofuran Annonaceous acetogenins, have been isolated from an EtOH extract of the bark of *Xylopia aromatica*, using bioactivity-directed fractionation employing lethality to brine shrimp. These new compounds each have a double bond in the hydrocarbon chain and have been identified as C-23, C-24 dehydro analogs of xylopiacin and xylomaticin. Their structures were elucidated by spectral analyses of the parent compounds and/or simple chemical derivatives. Their absolute stereochemistries have been established by ^{1}H- and 2D nmr experiments utilizing the production of Mosher esters. These acetogenins showed cytotoxic potencies superior to adriamycin against three human solid tumor cell lines.

The Annonaceae have recently been investigated as potential sources of potent biologically active Annonaceous acetogenins; these compounds have shown a broad spectrum of bioactivities, for example, cytotoxicity, antitumor, T-cell suppression, antimalarial, pesticidal, antiparasitic, and antimicrobial (1,2). The Annonaceous acetogenins act, at least in part, as powerful inhibitors of glutamate-dependent mitochondrial respiration in both mammalian and insect systems, and they specifically inhibit NADH:ubiquinone oxidoreductase activity in complex I (3); all of the acetogenins appear to inhibit this site as a biochemical target (4).

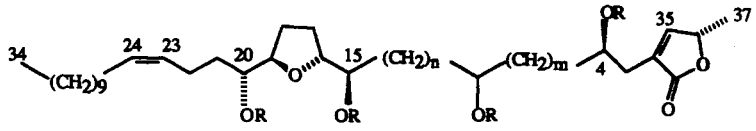
The genus *Xylopia* (Annonaceae) represents 150 species that are distributed in the tropical regions of the Old and New World. *Xylopia aromatica* (Mart.) Lam. is a tree native to tropical America, and this species is common in several regions of Venezuela. Its fruits are used as a substitute spice for *Piper nigrum*, and this and other sister species are used medicinally for several purposes (5). Our earlier discovery of the cytotoxic acetogenins, xylopianin, xylopiacin, xylomaticin, gigantetrocin A, gigantetronenin, annomontacin, and annonacin (6), stimulated our interest in the bioactive components of this species.

Further work using the brine shrimp lethality test (BST) (7,8) to direct the fractionation of the EtOH extract of the bark, has now resulted in the isolation of two additional novel acetogenins, xylopien [**1**] and xylomatenin [**2**]. These compounds belong to the group of monotetrahydrofuran (THF) acetogenins and have an unusual structural feature in that a double bond is present in the hydrocarbon chain.

RESULTS AND DISCUSSION

The dried and pulverized bark was extracted with EtOH. The EtOH residue (F001) was partitioned between H_2O (F002) and $CHCl_3$ (F003), and the residue of F003 was partitioned between hexane (F006) and 10% H_2O in MeOH (F005). The most bioactive extract residue, as evaluated in the brine shrimp test (BST), was F005 (LC_{50}=160 μg/ml). F005 was submitted to successive fractionations by cc, prep. tlc, and hplc, directed by the BST assay at each step, to yield compounds **1** and **2** (Figure 1). Spectral characteristics, including uv, ir, ^{1}H- and ^{13}C-nmr data, suggested that both of the isolated compounds belonged to the mono-THF group of acetogenins and possessed a double bond in the aliphatic chain.

Xylopien [**1**] was a waxy solid with mp 48–49°, $[\alpha]^{23}D$ +15° (c=0.001, MeOH). The molecular ion was indicated by a dominant peak at m/z 623 $[MH]^+$ in the fabms



1 R=H, n=6, m=3 **2** R=H, n=4, m=5
1a R=Ac, n=6, m=3 **2a** R=Ac, n=4, m=5
1b R=Me_3Si, n=6, m=3 **2b** R=Me_3Si, n=4, m=5
1c R=MTPA, n=6, m=3 **2c** R=MPTA, n=4, m=5

FIGURE 1. New acetogenins isolated from *Xylopia aromatica* and their derivatives. Absolute stereochemistries (except for those at C-8 in **1** and C-10 in **2**) were determined by Mosher ester methodology.

(glycerol). The hrfabms (glycerol) gave *m/z* 623.4887 (calcd 623.4887) for the $[MH]^+$, indicating the molecular formula $C_{37}H_{66}O_7$ for **1**. Ir spectra suggested the presence of OH groups (3431 cm^{-1}) and the presence of an α,β-unsaturated γ-lactone (1748 cm^{-1}). The existence of four OH groups was indicated by four successive losses of H_2O (*m/z* 18) from the molecular ion in the fabms and was confirmed by the preparation of a tetraacetate derivative **1a** and a tetra-trimethyl silyl (TMSi) derivative **1b**. A uv λ max 225 nm, log ε 3.16, again suggested the presence of the α,β-unsaturated γ-lactone. The nmr spectra of **1** showed ^{1}H-nmr resonances at δ 7.19 (q, H-35), 5.06 (qq, H-36), 3.86 (m, H-4), 2.53 (H-3a), 2.42 (H-3b), 3.86 (m, H-4), and 1.44 (d, H-37) (Table 1), and six ^{13}C-nmr resonances at δ 174.7 (C-1), 151.9 (C-35), 131.2 (C-2), 78.0 (C-36), 69.8 (C-4), and 19.1 (C-37) (Table 2), which confirmed the presence 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 ^{13}C-nmr spectrum of the C-4 signal, and a peak at *m/z* 213 in the eims of the TMSi derivative [**1b**] (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 3.80 (H-16 and H-19) and 3.42 (H-15 and H-20) (Table 1), and ^{13}C-nmr peaks at δ 82.6 (C-16), 82.6 (C-19), 74.0 (C-15), and 73.5(C-20) (Table 2); similar signals are characteristic of other mono-THF acetogenins having two OH groups adjacent to the ring (1,2). The number of carbons between the unsaturated lactone and the THF ring was established by eims spectral analysis of the fragmentation of the TMSi derivative [**1b**] (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 between C-15 and C-20.

On the basis of the above spectral data, three of the OH groups were assigned at C-4, C-15, and C-20. The remaining OH group was assigned at C-8 by analysis of the eims of **1**, which exhibited ion peaks at *m/z* 213 and 195, and the eims of **1b**, which showed ion peaks at *m/z* 357 and 267. These ions are considered to be formed by cleavage at C-8, C-9 (Figure 2). Confirmation of the OH substitution at the C-8 position was obtained by the measurement of the exact mass and corresponding elemental composition of the fragment at *m/z* 357.1917 for $[C_{11}H_{15}O_4\ (TMS)_2]^+$ (calcd 357.1917) in the hrfabms of **1b**. This assignment was further supported by the analysis of the ^{13}C-nmr chemical shifts of **1** (Table 2). The signal at δ 21.6 for C-6 was shifted upfield from the value expected, and this upfield shift could be explained in terms of the β-effect of two OH groups (14). Hydroxylation at C-8 in the Annonaceous acetogenins has been recently reported in xylopianin and xylopiacin, which we have also isolated from this species (6).

The relative stereochemistries between C-15, C-16, and C-19, C-20 were defined by comparing the ^{13}C-nmr spectrum of **1** for the hydroxylated carbons at C-15 (δ 74.0) and

TABLE 1. ^{1}H-Nmr (500 MHz, $CDCl_3$) Data of Xylopien [**1**], Xylonatenin [**2**], and Their Acetates **1a** and **2a**.

Position	Compound [δ (*J* in Hz)]*			
	1	**1a**	**2**	**2a**
3a	2.53 (dddd, 15.1, 4.0, 1.6, 1.6)	2.56 (dddd, 15.1, 4.0, 1.6, 1.6)	2.53 (dddd, 15.3, 3.3, 1.1, 1.1)	2.56 (m)
3b	2.42 (dddd, 15.1, 8.0, 1.6, 1.6)	2.51 (dddd, 15.1, 8.0, 1.6, 1.6)	2.43 (dddd, 15, 8.6, 1.1, 1.1)	2.51 (m)
4	3.86 (m)	5.10 (m)	3.85 (m)	5.10 (m)
5	1.51 (m)	1.50–1.70 (m)	1.40–1.20 (m)	1.40–1.22 (m)
6	1.40–1.21 (m)	1.40–1.20 (m)	1.40–1.20 (m)	1.40–1.22 (m)
7	1.72–1.50 (m)	1.70–1.50 (m)	1.40–1.20 (m)	1.40–1.22 (m)
8	3.62 (m)	4.87 (m)	1.40–1.20 (m)	1.40–1.22 (m)
9	1.72–1.50 (m)	1.70–1.50 (m)	1.74–1.50 (m)	1.40–1.22 (m)
10	1.40–1.21 (m)	1.40–1.20 (m)	3.60 (m)	4.87 (m)
11–13	1.40–1.21 (m)	1.40–1.20 (m)	1.40–1.20 (m)	1.40–1.22 (m)
14	1.51 (m)	1.50–1.70 (m)	1.51 (m)	1.70–1.50 (m)
15	3.42 (m)	4.85 (m)	3.42 (m)	4.85 (m)
16	3.80 (dt, 12.3, 7.0)	3.98 (m)	3.80 (dt, 12.5, 7.5)	3.98 (m)
17–18	1.98 (m) and 1.68 (m)	1.98 (m) and 1.66 (m)	1.98 (m) and 1.68 (m)	1.98 (m) and 1.66 (m)
19	3.80 (dt, 12.3, 7.0)	3.98 (m)	3.80 (dt, 12.5, 7.5)	3.98 (m)
20	3.42 (m)	4.85 (m)	3.42 (m)	4.85 (m)
21	1.66 (m)	1.66 (m)	1.51 (m)	1.50 (m)
22	2.22 (m)	1.99 (m)	2.22 (m)	1.99 (m)
23	5.35 (ddd, 11.0, 6.5, 7.0)	5.37 (ddd, 11.0, 6.5, 7.0)	5.35 (ddd, 1.0, 7.0,. 7.0)	5.36 (ddd, 11.0, 7.0, 7.0)
24	5.40 (ddd, 11.0, 6.5, 7.0)	5.23 (ddd, 11.0, 6.5, 7.0)	5.40 (ddd, 11.0, 7.0, 7.0)	5.25 (ddd, 11.0, 7.0, 7.0)
25	2.18 (m)	1.96 (m)	2.17 (m)	1.96 (m)
26–33	1.40–1.21 (m)	1.40–1.20 (m)	1.40–1.20 (m)	1.40–1.22 (m)
34	0.88 (t, 7.0)	0.88 (t, 7.0)	0.88 (t, 7.0)	0.88 (t, 7.0)
35	7.19 (q, 1.5)	7.083 (q, 1.5)	7.19 (q, 1.5)	7.085 (q, 1.5)
36	5.06 (qq, 7.0, 1.5)	5.06 (qq, 6.5, 1.5)	5.06 (qq, 6.5, 1.4)	5.10 (qq, 7.0, 1.5)
37	1.44 (d, 7.0)	1.40 (d, 7.0)	1.44 (d, 7.0)	1.40 (d, 7.0)
4-OAc	—	2.026 (s)	—	2.026 (s)
8-OAc	—	2.039 (s)	—	—
10-OAc	—	—	—	2.038 (s)
15-OAc	—	2.079 (s)	—	2.082 (s)
20-OAc	—	2.073 (s)	—	2.073 (s)

*The assignments were made on the basis of COSY ^{1}H-^{1}H and double-relayed COSY.

C-20 (δ 73.5), as well as the ^{1}H-nmr signals of **1** for H-15, H-20 (δ 3.42) and H-16, H-19 (δ 3.80), with those of model compounds of known relative stereochmistry (9); these data suggested that the relative configurations between C-15, C-16 and C-19, C-20 were both threo. The ^{1}H-nmr signals at δ 1.98 and 1.68 corresponding to H-17 and H-18 are typical methylene proton signals of a trans THF ring, while the methylene proton signals for cis THF rings are located at δ 1.94 and 1.82 (10). Thus, the relative configuration for these four chiral centers is threo-trans-threo. The threo assignments were further substantiated by comparing proton resonances of the acetate derivative **1a** at δ 3.98 (H-16 and H-19), 4.85 (H-15 and H-20), and 2.079 and 2.073 (15-OAc and 20-OAc) with those of a group of diacetyl dibutylated bistetrahydrofurans of known stereochemistry (11). Also, the ^{1}H-nmr signals of **1a** at δ 3.98 for H-15 and H-19 substantiated the trans configuration of these two protons.

Two olefinic protons, coupled to each other, were also quickly discerned in the ^{1}H-nmr spectrum at δ 5.40 (ddd, J=11.0, 7.0, and 6.5 Hz) and 5.35 (ddd, J=11.0, 7.0, and 6.5 Hz), suggesting the presence of an isolated cis double bond in **1**; the presence of this

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

Position	**1**	**2**
1	174.7	174.6
2	131.2	131.2
3	33.4	33.4
4	69.8	69.9
5	37.4	37.3
6	21.6	25.5–29.7
7	36.9	25.5–29.7
8	71.7	25.5–29.7
9	37.1	37.4
10	25.5–29.7	71.8
11	25.5–29.7	37.5
12–13	25.5–29.7	25.5–29.7
14	33.5	33.5
15	74.0	74.0
16	82.6	82.6
17	28.7	28.7
18	28.7	28.7
19	82.6	82.6
20	73.5	73.5
21	33.4	33.4
22	23.3	23.3
23	129.0	128.9
24	130.9	130.8
25	27.2	27.2
26–31	25.5–29.7	25.5–29.7
32	31.9	31.9
33	22.6	22.7
34	141.1	141.1
35	151.9	151.9
36	78.0	77.9
37	19.1	19.1

group was substantiated by two ^{13}C-nmr resonances at δ 130.9 and 129.0 (Table 2), and two allylic carbons next to a cis double bond at δ 23.3 and 27.2 (Table 2). The allylic carbons next to a trans double bond normally resonate at δ 30–32. The position of the double bond was determined from the COSY and double-relayed COSY spectra of **1** to be between C-23 and C-24; correlation cross-peaks were seen from H-20 (δ 3.42) to H-21 (δ 1.66) which showed cross-peaks to H-22 (δ 2.22); H-22 then showed cross-peaks to one double-bond proton, H-23 (δ 5.35). Because the H-21 signal was overlapped with some other proton signals, a double-relayed COSY nmr spectrum of **1** was measured to be assured of this assignment; strong cross-peaks between H-20 and H-23 were seen. Recently, related mono-THF acetogenins, with single cis double bonds have been reported, namely giganenin, gigantetronenin, and gigantrionenin from *Goniothalamus giganteus* (12,13), and squamostatin A from *Annona squamosa* (14). Comparative analysis of the ^{1}H- and ^{13}C-nmr spectral data of compound **1** and xylopiacin, which we had found previously in this species (6), showed close similarities in resonances of protons and carbons; however, xylopien [**1**] showed, in both its ^{1}H- and ^{13}C-nmr resonances, some downfield shifts associated with the cis double bond on the aliphatic chain and typical chemical shifts for allylic carbons (C-22 and C-25) near a cis double bond.

The absolute stereochemistries of the carbinol stereocenters in xylopien [**1**], except for that at C-8, have been determined using advanced Mosher ester methodology (15)

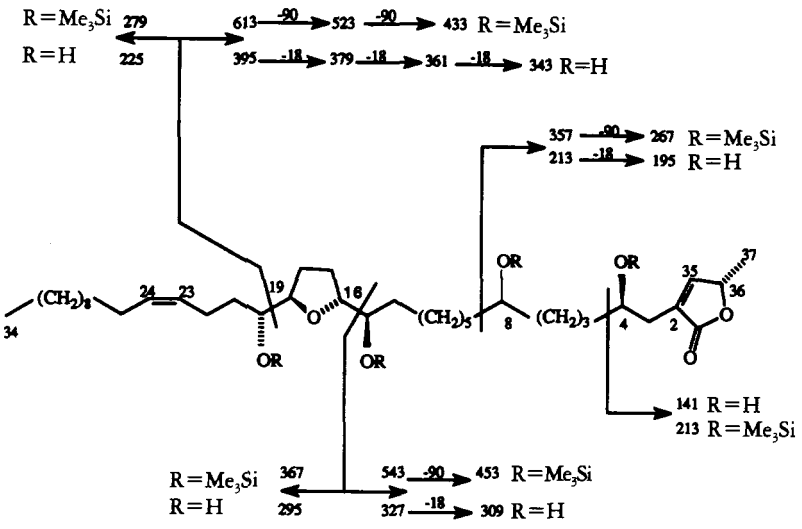


FIGURE 2. Diagnostic eims fragment ions (*m/z*) of xylopien [**1**] (R=H) and its TMSi derivative **1b** (R=Me_3Si).

TABLE 3. ^{1}H-Nmr Data of **1**-*S* and *R*-Per-MTPA and **2**-*S* and *R*-Per-MTPA Esters [δ (*J*=Hz)].

Proton	1-*S*-MTPA[a] **1c**	1-*R*-MTPA[a] **1d**	Δ (δ*S*-δ*R*)	2-*S*-MTPA[a] **2c**	2-*R*-MTPA[a] **2d**	Δ (δ*S*-δ*R*)
3a.....	2.58 (dddd)	2.60 (dddd)	negative	2.60 (dddd)	2.66 (dddd)	negative
3b	2.54 (dddd)	2.56 (dddd)	negative	2.55 (dddd)	2.58 (ddd)	negative
4	5.28 (m)	5.22 (m)	R[b]	5.30 (m)	5.35 (m)	R[b]
5	1.63 (m)	1.56 (m)	positive	1.65 (m)	1.60 (m)	positive
6–7 ...	1.68–1.20	1.15–1.64	—	1.66–1.20	1.64–1.15	—
8	5.01 (m)	5.01 (m)	—	1.66–1.20	1.64–1.15	—
9	1.68–1.20	1.64–1.15	—	1.20–1.66	1.64–1.15	—
10	1.68–1.20	1.64–1.15	—	5.03 (m)	5.05 (m)	—
11	1.68–1.20	1.64–1.15	—	1.66–1.20	1.64–1.15	—
12–13 .	1.68–1.20	1.64–1.15	—	1.66–1.20	1.64–1.15	—
14	1.63 (m)	1.60 (m)	positive	1.62 (m)	1.60 (m)	positive
15	4.94 (m)	5.05 (m)	R[b]	4.90 (m)	5.05 (m)	R[b]
16	3.93 (m)	4.01 (m)	negative	3.92 (m)	4.01 (m)	negative
17	1.64 and 1.48	1.95 and 1.55	negative	1.62 and 1.48	1.90 and 1.52	negative
18	1.64 and 1.48	1.95 and 1.55	negative	1.62 and 1.48	1.90 and 1.52	negative
19	3.93 (m)	4.02 (m)	negative	3.92 (m)	4.01 (m)	negative
20	4.97 (m)	5.03 (m)	R[b]	4.97 (m)	5.05 (m)	R[b]
21	1.65 (m)	1.51 (m)	positive	1.62 (m)	1.50 (m)	positive
22	1.94 (m)	1.90 (m)	positive	2.02 (m)	1.90 (m)	positive
23	5.38 (ddd)	5.35 (ddd)	positive	5.38 (ddd)	5.35 (ddd)	positive
24	5.28 (ddd)	5.28 (dddd)	—	5.27 (ddd)	5.22 (ddd)	positive
25	2.02 (m)	1.90 (m)	positive	1.94 (m)	1.90 (m)	positive
26–33 .	1.68–1.20	1.64–1.15	—	1.66–1.20	1.64–1.15	—
34	0.88 (t, 7.0)	0.88 (t, 7.0)	—	0.88 (t, 7.0)	0.88 (t, 7.0)	—
35	6.72 (d, 1.5)	6.94 (d, 1.5)	negative	6.72 (d, 1.5)	6.93 (d, 1.5)	negative
36	4.86 (qq)	4.91 (qq)	negative	4.86 (qq)	4.90 (qq)	negative
37	1.28 (d, 7.0)	1.30 (d, 7.0)	negative	1.28 (d, 7.0)	1.30 (d, 7.0)	negative
MeO ..	3.514 (s)	3.532 (s)	—	3.504 (s)	3.529 (s)	—
MeO ..	3.459 (s)	3.532 (s)	—	3.459 (s)	3.459 (s)	—
MeO ..	3.387 (s)	3.532 (s)	—	3.387 (s)	3.387 (s)	—
MeO ..	3.353 (s)	3.480 (s)	—	3.353 (s)	3.354 (s)	—
Ar	7.63–7.37	7.65–7.36	—	7.63–7.37	7.65–7.36	—

[a]The assignments were made on the basis of COSY ^{1}H-^{1}H nmr.
[b]Absolute configuration of chiral center.

which analyzes differences between the ^{1}H-nmr chemical shifts of *S*- and *R*-MTPA [methoxy-(trifluoromethyl)-phenylacetate] ester derivatives on both sides of the stereogenic carbinol centers (15–17). The ^{1}H-nmr data for xylopien-*S*-MTPA [**1c**] and xylopien-*R*-MTPA [**1d**] derivatives are summarized in Table 3. Based on Mosher's arguments, C-15 and C-20 were assigned to have the *R* absolute configuration, since the sign of $\Delta\ \delta_H$ (δS-δR) is positive for the chain side showing relatively more shielding for this side in the *S*-MTPA ester. As the relative stereochemistry from C-15 to C-20 of compound **1** is threo-trans-threo, the absolute configuration of C-15 (*R*), C-16 (*R*), C-19 (*R*), and C-20 (*R*) was thus readily concluded. The configuration at C-4 was determined to be *R* according to the Mosher ester data listed in Table 3, and C-36 was assigned as *S* based on the usual 4*R*, 36*S* relationship; the chemical shifts were in good agreement with those of acetogenins with 4*R*, 36*S* stereochemistry (16). The absolute stereochemistry at C-8 was difficult to elucidate because the $\Delta\ \delta_H$ (δS-δR) values of the protons on both sides of the carbinol center (C-8) cannot be determined, i.e., confident assignments could not be made for protons H-7 and H-9. Thus, the structure and absolute configuration of xylopien [**1**] are proposed as illustrated in Figure 1.

Xylomatenin [**2**] was isolated as a waxy solid with mp 52–53°, $[\alpha]^{23}D$ +19° (c=0.001, MeOH). The molecular formula, $C_{37}H_{66}O_7$, was established from the hrfabms (glycerol) of the $[MH]^+$ (found *m/z* 623.4864, calcd 623.4887). The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) exhibited signals characteristic of mono-THF acetogenins, and, as with many other acetogenins and compound **1**, the existence of a methyl substituted α,β-unsaturated γ-lactone fragment with a C-4 OH was indicated. The presence of four OH moieties was determined by four successive losses of H_2O (*m/z* 18) from the molecular ion in the cims and fabms, from preparations of a tetraacetate derivative **2a** (Table 1) and a tetra-TMSi derivative **2b**, and by the ir, uv, and nmr data. The ^{1}H- (Table 1) and ^{13}C-nmr data (Table 2) of **2** showed the existence of a mono-THF ring with two adjacent hydroxyl groups. The skeleton and placement of the THF ring and OH moieties along the aliphatic chain were determined based on the eims analyses of **2** and the TMSi derivative **2b** (Figure 3). The eims fragmentation patterns of **2** and **2b** clearly indicated that the OH groups were positioned at C-4, C-10, C-15, and C-19 (Figure 3).

The ^{1}H-nmr spectrum of **2** showed two signals at δ 5.40 (ddd, J=11.0, 7.0, and 7.0 Hz) and 5.35 (ddd, J=11.0, 7.0, and 7.0 Hz) again suggesting the presence of an isolated

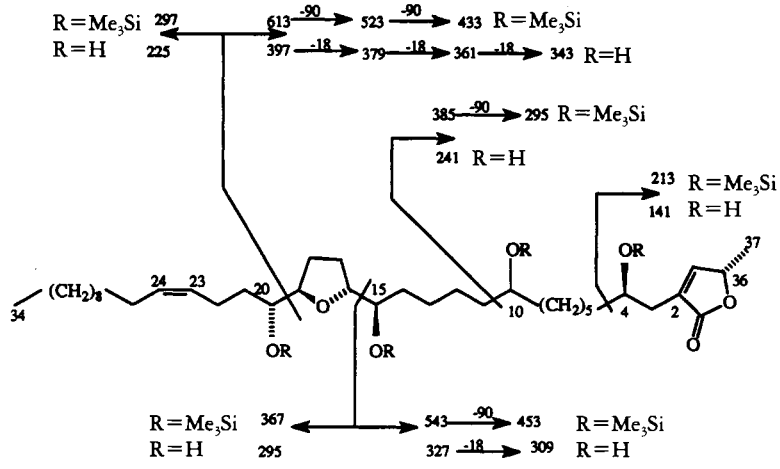


FIGURE 3. Diagnostic eims fragment ions (*m/z*) of xylomatenin [**2**] (R=H) and its TMSi derivative **2b** (R=Me_3Si).

cis double bond; this group was further confirmed by two carbon resonances at δ 130.8 and 128.9 and typical chemical shifts for allylic carbons (C-22 and C-25) near a cis double bond (Table 2). The position of the double bond was determined, from COSY and double-relayed COSY nmr spectra of **2**, to be between C-23 and C-24; strong cross-peaks were observed between H-20 and H-23 in the double-relayed COSY.

As with the previous acetogenin **1**, the relative stereochemistries around the THF ring were determined as threo-trans-threo, for C-15, C-16, C-16, C-19, and C-19, C-20, by comparisons of corresponding nmr data of **2** and its acetate derivative [**2a**] (Table 1) with those of THF model compounds of known relative configuration (9,11). Comparative analyses of the spectral data of **1** vs. **2** revealed close similarities; however, co-tlc separations developed in several solvent systems, showed different behavior, and, in hplc over Si gel, compound **1** showed a shorter retention time than **2**. The absolute stereochemistries of the carbinol stereocenters in **2**, except for that at C-10, were also determined using advanced Mosher ester methodology (14–16), and the absolute configuration of C-4 (*R*), C-15 (*R*), C-16 (*R*), C-19 (*R*), C-20 (*R*), and C-36 (*S*) was concluded.

The absolute stereochemistry at C-10 was difficult to elucidate because the $\Delta\delta_H$ (δS–δR) values of the protons on both sides of the carbinol center (C-10) could not be determined, since confident assignments for protons H-9 and H-11 were not made. Thus, the structure and absolute configuration of xylomatenin [**2**] are proposed as illustrated in Figure 1.

Biological activities of **1** and **2** are summarized in Table 4. These compounds were active in the BST (7,8); the isolated acetogenins showed significant cytotoxicities against A-549 (lung) (18), MCF-7 (breast) (19), and HT-29 (colon) (20) in seven-day human solid tumor cell in vitro tests, using adriamycin as the positive control compound. Compounds **1** and **2** were several times as potent as adriamycin, with **2** showing superior activities against A-549 and HT-29. Compared with the biological activities of xylopiacin and xylomaticin (6), the presence of the double bond in **1** and **2** seems to increase the cytotoxic effectiveness against HT-29 but decrease the effectiveness against MCF-7. The double bond in **1** and **2** is also in a convenient position for the biogenesis and semi-synthesis of corresponding adjacent bis-THF analogues, as has been recently demonstrated (10), and such derivatives would be anticipated to have enhanced bioactivities (4).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mel-Temp apparatus and are uncorrected. Ir spectra (film) were measured on a Perkin-Elmer 1420 ir spectrometer. Optical rotations were

TABLE 4. Bioactivities of Fraction F005 and Compounds **1** and **2** Isolated from *X. 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 (156.8/165.1)	10^{-2}	1.39	10^{-2}
Adriamycin[c,e]	—	1.10×10^{-3}	2.28×10^{-2}	2.70×10^{-3}
Xylopien [**1**][d]	0.871 (0.55/1.27)	3.64×10^{-3}	7.11×10^{-3}	7.26×10^{-3}
Xylomatenin [**2**][d]	0.77 (0.43/1.05)	$<10^{-3}$	6.31×10^{-1}	$<10^{-3}$
Adriamycin[d,e]	—	9.20×10^{-3}	2.3×10^{-1}	3.92×10^{-2}

[a]Brine shrimp lethality, 95% confidence intervals in parentheses (6,7).

[b]Cytotoxicities in human lung (A-549) (17), breast (MCF-7) (18), and colon (HT-29) (19) tumor cell lines.

[c,d]Refers to different runs in the cell culture assay.

[e]Positive standard control.

taken on a Perkin-Elmer 241 polarimeter. Uv spectra were measured on a Beckman DU-7. ^{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 TMSi derivatives were obtained on a Kratos MS50. 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).

PLANT MATERIAL.—Bark of *Xylopia aromatica* was collected at Estado Amazonas, Venezuela. The material was authenticated by Stephen Tillet at the Herbario Ovalles, Universidad Central de Venezuela. A voucher specimen of the bark is deposited in the Hebario 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) (7,8). Cytotoxicities against human solid tumor cells were measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center, in a seven-day MTT assay, for A-549 lung carcinoma (18), MCF-7 breast carcinoma (19), and HT-29 colon adenocarcinoma (20), with adriamycin as a positive control.

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. The active pool P6 (F_1-17–F_1-22) (4 g, BST LC_{50}=23 ppm) was further resolved on another Si gel (160 g) column, eluted with 33% Me_2CO in hexane. Fractions (F_2-1 to F_2-30) were collected into six pools on the basis of similar tlc patterns. The BST-active pool (F_2-8 to F_2-11, 0.200 g, BST LC_{50}=2.62 ppm) was re-chromatographed on hplc over Si gel eluted with a gradient of hexane-THF-MeOH (flow rate 10 ml/min) to afford compounds **1** and **2**.

Xylopien [**1**].—White waxy solid (12 mg); mp 48–49°; $[\alpha]^{23}D$ +15° (c=0.001, MeOH); uv (λ max, MeOH) 225 nm (log ε 3.16); ir ν max (film) 3431, 2924, 2854, 1747, 1652, 1456, 1318, 1076, 668 cm^{-1}; eims see Figure 2; fabms (glycerol) *m/z* $[MH]^+$ 623 (50), $[MH-H_2O]^+$ 605 (5), $[MH-2H_2O]^+$ 587 (12), $[MH-3H_2O]^+$ 569 (4), $[MH-4H_2O]^+$ 551 (7); hrfabms (glycerol) *m/z* 623.4887 $(MH)^+$ (calcd 623.4887 for $C_{35}H_{67}O_7$) 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.

Xylomatenin [**2**].—White waxy solid (6 mg); mp 52–53°; $[\alpha]^{23}D$ +19° (c=0.001, MeOH); uv λ max (MeOH) 222 nm (log ε 2.89); ir ν max (film) 3422, 2923, 2855, 1734, 1650, 1457, 1318, 1079, 668 cm^{-1}; eims see Figure 3; fabms (glycerol) *m/z* $[MH]^+$ 623 (25), $[MH-H_2O]^+$ 605 (1.9), $[MH-2H_2O]^+$ 587 (0.9), $[MH-3H_2O]^+$ 569 (1), $[MH-4H_2O]^+$ 551 (5); hrfabms (glycerol) *m/z* 623.4864 for $C_{37}H_{67}O_7$ (calcd 623.4887); cims (isobutane) *m/z* $[MH]^+$ 623 (78), $[MH-H_2O]^+$ 605 (50), $[MH-2H_2O]^+$ 587 (100), $[MH-3H_2O]^+$ 569 (60), $[MH-4H_2O]^+$ 551 (14); ^{1}H nmr ($CDCl_3$, 500 MHz), see Table 1; ^{13}C nmr ($CDCl_3$, 125 MHz), see Table 2.

TETRACETATE DERIVATIVES.—Compounds **1** or **2** (1 mg) were treated with Ac_2O (0.25 ml), anhydrous pyridine (0.1 ml) at room temperature overnight. The mixture was diluted with CH_2Cl_2 and washed with H_2O. The CH_2Cl_2 layer was dried *in vacuo* to give the tetraacetates **1a** and **2a** as waxes.

TMS DERIVATIVES.—Small amounts (<1 mg) of **1** or **2** 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 (**1b** and **2b**); eims (Figures 2 and 3).

R- AND *S*-MOSHER ESTERS.—To acetogenins **1** or **2** (1 mg, in 0.3 ml of CH_2Cl_2) were sequentially added pyridine (0.2 ml), 4-(dimethylamino)-pyridine (0.5 mg), and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetylchloride. The mixture was stirred at room temperature for 4 h and passed through a disposable pipet (0.6×6 cm) containing Si gel (230–400 mesh) and eluted with 3 ml of CH_2Cl_2. The CH_2Cl_2 residue, dried *in vacuo*, was redissolved in CH_2Cl_2 and washed using 1% $NaHCO_3$ (5 ml) and H_2O (2×5 ml); the CH_2Cl_2 layer was dried *in vacuo* to give the *S*-Mosher esters **1c** and **2c**. Use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the *R*-Mosher esters **1d** and **2d**.

ACKNOWLEDGMENTS

The authors thank the Cell Culture Laboratory, Purdue Cancer Center, for the in-house cytotoxicity data. T. Colman-Saizarborita acknowledges a research fellowship from the Consejo de Desarrollo Cientifico y Humanistico of the Universidad Central de Venezuela and a loan from FUNDAYACUCHO. This investigation was supported in part by R01 grant No. CA-30909, from the National Cancer Institute,

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. K.I. Ahammadsahib, R.M. Hollingworth, J.P. McGovren, Y.-H. Hui, and J.L. McLaughlin, *Life Sci.*, **53**, 1113 (1993), and references cited therein.
4. J.L. Landolt, K.I. Ahammadsahib, R.H. Hollingworth, R. Barr, F.L. Crane, N.L Bueck, G.P. McCabe, and J.L. McLaughlin, *Chem. Biol. Interact.*, in press.
5. C.T. Rizzini and W.B. Mors, "Botanica Economica Brasileira," EPU-EDUSP, Sao Paulo, 1973, p. 61.
6. T. Colman-Saizarbitoria, J. Zambrano, N. Ferrigni, Z.-M. Gu, J.H. Ng, D.L. Smith, and J.L. McLaughlin, *J. Nat. Prod.*, **57**, 486 (1994).
7. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobson, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 463 (1992).
8. J.L. McLaughlin, in: "Methods in Plant Biochemistry." Ed. by K. Hostettmann, Academic Press, London, 1991, Vol. 6, pp. 1–33.
9. L. Born, F. Lieb, J.P. Moescher, H.F. Nonfon, R. Soller, and D. Wendish, *Planta Med.*, **56**, 312 (1990).
10. Z.-H. Gu, X.-P. Fang, L. Zeng, R. Song, J.H. Ng, K.V. Wood, D.L. Smith, and J.L. McLaughlin, *J. Org. Chem.*, **59**, 3472 (1994).
11. T.R. Hoye and Z.-P. Zhuang, *J. Org. Chem.*, **53**, 557 (1988).
12. X.-P. Fang, J.E. Anderson, D.L. Smith, K.V. Wood, and J.L. McLaughlin, *Heterocycles*, **34**, 1075 (1992).
13. X.-P. Fang, J.E. Anderson, D.L. Smith, K.V. Wood, and J.L. McLaughlin, *J. Nat. Prod.*, **55**, 1655 (1992).
14. H. Araya, N. Hara, Y. Fujimoto, A. Srivastava, and M. Sahai, *Chem. Pharm. Bull.*, **42**, 388 (1994).
15. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
16. 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).
17. J.A. Dale and H.S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
18. 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).
19. H.D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennam, *J. Natl. Cancer Inst.*, **51**, 1490 (1973).
20. J. Fogh and G. Trempe, in: "Human Tumor Cells, In Vitro." Ed. by J. Fogh, Plenum Press, New York, 1975, pp. 115–159.

Received 3 June 1994