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VENEZENIN: A NEW BIOACTIVE ANNONACEOUS ACETOGENIN FROM THE BARK OF *XYLOPIA AROMATICA*

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ABSTRACT.—Asimicin and a new cytotoxic Annonaceous acetogenin, venezenin [**1**], were isolated from the bark of *Xylopia aromatica* by bioactivity-directed fractionation using lethality to brine shrimp. Compound **1** represents an unusual type of C_{37} Annonaceous acetogenin, lacking either tetrahydrofuran (THF) or epoxide rings and possessing a double bond located two methylenes away from a vicinal diol in the hydrocarbon chain. The structure of **1** was elucidated by ^{1}H- and ^{13}C-nmr, COSY, single-relayed COSY, and by HMBC techniques, and derivatization. Annomontacin 10-one [**6**] and 18/21-*cis*-annomontacin-10-one [**7**], two semi-synthetic mono-THF acetogenins were prepared from **1**. These acetogenins showed cytotoxicity, comparable or superior to adriamycin, against three human solid tumor cell lines. Reduction of the 10-keto of **1** to the racemic OH-10 derivative enhanced the bioactivity, as did the conversion of **1** to **6** and **7**. Venezenin [**1**], like other Annonaceous acetogenins, showed inhibition of oxygen uptake by rat liver mitochondria and demonstrated that the THF ring may not be essential to this mode of action.

Members of the Annonaceae have recently been investigated as potential sources of the Annonaceous acetogenins. These compounds have shown a broad range of potent bioactivities, e.g., antimicrobial, antiparasitic, antitumor, cytotoxic, and pesticidal (1,2). Annonaceous acetogenins are powerful inhibitors of glutamate-dependent mitochondrial respiration in both mammalian and insect systems, and they specifically inhibit NADH:CoQ oxidoreductase activity (3).

Xylopia aromatica (Lam.) Mart. is a tree native to tropical America. Extracts of the bark, collected in Venezuela, showed toxicity in the brine shrimp lethality test (BST) and demonstrated cytotoxicity against three human solid tumor cell lines (4). From the EtOH extract of the bark, nine cytotoxic Annonaceous acetogenins have been isolated and reported to date (4,5), and, among them, three have a double bond in the aliphatic chain; this is a relatively rare feature in the Annonaceous acetogenins (1,2). Further screening, using BST-directed fractionation (6,7) of the EtOH extract of the bark, has now resulted in the isolation of the novel compound, venezenin [**1**], and the known acetogenin, asimicin (8); this is the first time that asimicin or any other bis-tetrahydrofuran (THF) acetogenin has been reported from this genus. Venezenin [**1**] is a keto-acetogenin and is the first example of a C_{37} acetogenin in which neither a THF ring and/or an epoxide are present; this is a rare feature in the Annonaceous acetogenins, with giganenin, a C_{35}-acetogenin being the first of these compounds to be reported (9). The C-21/C-22 double bond in venezenin [**1**] was oxidized with *m*-CPBA acid to give epoxides which then were cyclized using perchloric acid to give a pair of new semi-synthetic mono-THF acetogenins [**6** and **7**]; these reactions have been used previously to mimic the proposed biogenetic pathways leading to the THF rings of Annonaceous acetogenins (10).

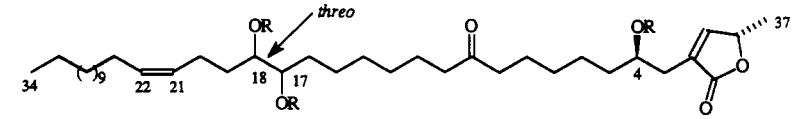
RESULTS AND DISCUSSION

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 extract, as tested by the BST (6,7), was F005 (LC_{50}=160 μg/ml). F005 was submitted to successive fractionations by

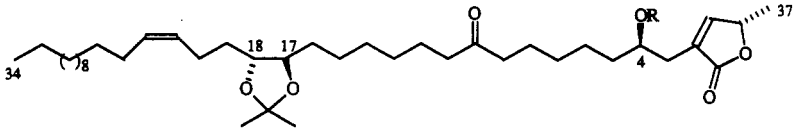
cc, prep. tlc, and hplc, directed by the BST at each step, to yield two compounds, **1** (Figure 1) and asimicin; the latter was identified by comparison with the reference compound (8).

Venezenin [**1**] was isolated as a waxy solid with mp 72–73°, $[\alpha]^{25}D$ +16.8° (c=0.06, MeOH). The cims of **1** gave a MH^+ at m/z 607. The molecular formula was established as $C_{37}H_{66}O_6$ on the basis of hrfabms which gave a molecular ion $(MH)^+$ at m/z 607.4916 (calcd for $C_{35}H_{67}O_6$, 607.4938).

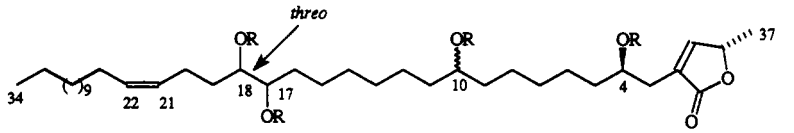
The existence of three OH groups was indicated by an ir hydroxy absorption at 3375 cm^{-1}, three successive losses of H_2O (m/z) from the MH^+ in the fabms, and the preparation of a tri-TMS derivative [**2**]. As with the other acetogenins, the presence of the methyl substituted α,β-unsaturated γ-lactone was suggested by the ir (ν max 1732 cm^{-1}) and uv (λ max 209 nm, log ε 3.28) spectra, and the corresponding resonances in the ^1H- and ^{13}C-nmr spectra (Table 1) of **1**. However, the lack of a THF ring along the aliphatic chain was indicated by the absence of any corresponding THF ether proton and carbon signals in the nmr spectra. Instead, the existence of a double bond was evidenced by olefinic ^1H- and ^{13}C-nmr signals. Two olefinic protons, coupled to each other, were discerned in the ^1H-nmr spectrum at δ 5.40 (ddd, J=11.0, 7.0, and 6.5 Hz) and 5.36 (ddd, J=11.0, 7.0, and 6.6 Hz), suggesting the presence of an isolated cis double bond; this group was further confirmed by two ^{13}C-nmr resonances at δ131.0 and 128.8 (Table 2). The position of the double bond was determined from the COSY and double-relayed



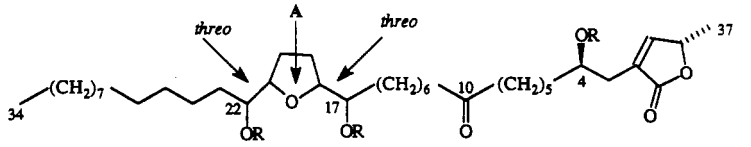
1 R=H
2 R=tri-MeSi



3 R=H (absolute stereochemistry at C-17/C-18 may be reversed)



4 R=H
5 R=tri-MeSi



6 **A**=trans R=H
7 **A**=cis R=H
8 **A**=trans R=tri-MeSi
9 **A**=cis R=tri-MeSi

FIGURE 1. Structures of compounds **1–9**.

TABLE 1. Nmr Spectral Data of Venezenin [**1**], Its Acetonide [**3**], and Racemic Alcohol [**4**].

Position	Compound			
	1 ^{1}H δ (Hz)	**1** ^{13}C δ (Hz)	**3** ^{1}H δ (Hz)	**4** ^{1}H δ (Hz)
1	—	174.6	—	—
2	—	131.1	—	—
3a	2.53 (ddd, 15.0, 4.0, 1.6, 1.6)	33.4	2.53 (ddd, 15.0, 4.0, 1.6, 1.6)	2.53 (ddd, 15.0, 4.0, 1.6, 1.6)
3b	2.40 (ddd, 15.0, 8.0, 1.6, 1.6)	—	2.40 (ddd, 15.0, 8.0, 1.6, 1.6)	2.40 (ddd, 15.0, 8.0, 1.6, 1.6)
4	3.84 (m)	69.8	3.86 (m)	3.86 (m)
5	1.50 (m)	37.1	1.50 (m)	1.50 (m)
6–7	1.40–1.20 (m)	25.3–29.9	1.40–1.20 (m)	1.40–1.20 (m)
8	1.66 (m)	23.7	1.66 (m)	1.40–1.20 (m)
9	2.40 (m)	42.7	2.40 (m)	1.74–1.50 (m)
10	—	211.5	—	3.59 (m)
11	2.40 (m)	42.6	2.40 (m)	1.74–1.50 (m)
12–15	1.60–1.50 (m)	23.6–29.9	1.60–1.50 (m)	1.40–1.20 (m)
16	1.55 (m)	33.5	1.55 (m)	1.55 (m)
17	3.42 (m)	74.4	3.59 (m)	3.44 (m)
18	3.42 (m)	74.2	3.59 (m)	3.44 (m)
19	1.66 (m)	33.4	2.03 (m)	1.66 (m)
20	2.16 (m)	23.5	2.17 (m)	2.16 (m)
21	5.36 (dd, 11.0, 7.0, 6.6)	128.8	5.35 (dd, 11.0, 7.0, 6.6)	5.39 (dd, 11.0, 7.0, 6.6)
22	5.40 (dd, 11.0, 7.0, 6.5)	131.0	5.40 (dd, 11.0, 7.0, 6.5)	5.40 (dd, 11.0, 7.0, 6.5)
23	2.04 (m)	27.3	2.04 (m)	2.04 (m)
24	1.25 (m)	29.9–29.0	1.25 (m)	1.25 (m)
25–31	1.40–1.20 (m)	29.9–29.0	1.40–1.20 (m)	1.40–1.20 (m)
32	1.40–1.20 (m)	31.9	1.40–1.20 (m)	1.40–1.20 (m)
33	1.23 (m)	22.7	1.23 (m)	1.25 (m)
34	0.88 (t, 7.0)	14.1	0.88 (t, 7.0)	0.88 (t, 7.0)
35	7.20 (q, 1.5)	151.9	7.19 (q, 1.5)	7.20 (q, 1.5)
36	5.06 (qq, 7.0, 1.5)	78.0	5.07 (qq, 7.0, 1.5)	5.06 (qq, 7.0, 1.5)
37	1.44 (d, 7.0)	19.1	1.44 (d, 7.0)	1.44 (d, 7.0)
Acetonyl methyls	—	—	1.387 (s) and 1.375 (s)	—

COSY spectra of **1** to be between C-21 and C-22. The COSY spectrum of **1** showed coupling correlations of H-18, H-19, H-20, and H-21. Single-relayed COSY correlation cross-peaks between H-18, H-20 and H-20, H-22 were clearly shown in the spectrum; and ^{1}H-^{13}C correlations in the HMBC spectrum confirmed the assignments (Figure 2). Examination of the ^{1}H-^{1}H COSY spectrum revealed that the double bond and vicinal diol moieties are separated by two methylene units.

Ms fragmentation analyses of the tri-TMSi derivative [**2**] demonstrated that the three OH groups were located at C-4, C-17, and C-18, as shown in Figure 3. The presence of the vicinal diol moiety (C-17/C-18) was also evidenced in the ^{1}H- and ^{13}C-nmr spectra by a ^{1}H-nmr signal at δ 3.42 for two carbinol methine protons and signals for two oxygenated carbons at δ 74.4 and 74.2; these observations strongly supported this conclusion, as the carbons having a single isolated OH group are typically displayed at δ 70–72 in other acetogenins (1,2). The placement of this vicinal diol at C-17/C-18 was made by eims fragmentation analysis of the tri-TMSi derivative [**2**] (Figure 3). To

TABLE 2. ^{1}H-Nmr Data of C-18/C-21-*trans*-Annomontacin-10 one [**6**] and C-18/C-21-*cis*-Annomontacin-10-one [**7**].

Position	Compound	
	6 ^{1}H δ (Hz)	**7** ^{1}H δ (Hz)
3a	2.53 (dddd, 15.0, 4.0, 1.6, 1.6)	2.53 (dddd, 15.0, 4.0, 1.6, 1.6)
3b	2.40 (dddd, 15.0, 8.0, 1.6, 1.6)	2.40 (dddd, 15.0, 8.0, 1.6, 1.6)
4	3.84 (m)	3.84 (m)
5	1.50 (m)	1.50 (m)
6–7	1.20–1.40 (m)	1.20–1.40 (m)
8	1.66 (m)	1.66 (m)
9	2.39 (m)	2.40 (m)
10	—	—
11–15	2.39 (m)	2.40 (m)
16	1.20–1.60	1.80–1.60
12	1.55 (m)	1.55 (m)
17	3.41 (m)	3.42 (m)
18	3.80 (m)	3.84 (m)
19	2.00 and 1.67 (m)	1.94 and 1.75 (m)
20	2.00 and 1.67 (m)	1.94 and 1.75 (m)
21	3.80 (m)	3.84 (m)
22	3.41 (m)	3.42 (m)
23	1.23–1.74 (m)	1.23–1.74 (m)
24–32	1.23–1.74 (m)	1.23–1.74 (m)
33	1.23–1.74 (m)	1.23–1.74 (m)
34	0.88 (t, 7.0)	0.88 (t, 7.0)
35	7.19 (q, 1.0)	7.19 (q, 1.0)
36	5.07 (qd, 6.5, 1.1)	5.07 (qd, 6.5, 1.1)
37	1.44 (d, 6.5)	1.44 (d, 6.5)

determine the relative configuration at C-17/C-18, the acetonide derivative [**3**] of **1** was prepared (Figure 1). The ^{1}H-nmr signals for H-17 and H-18 at δ 3.59 and the signals for the acetonyl methyl protons, showing two separated, but very close singlet peaks at δ 1.375 and 1.387, suggested the trans assignment for the dioxolane ring; the methyl protons of cis dioxolane rings show two well-separated singlet peaks, at δ 1.43 and δ 1.33, and the methine protons of the acetonide are at δ 4.03 and δ 4.00 (11). Thus, the

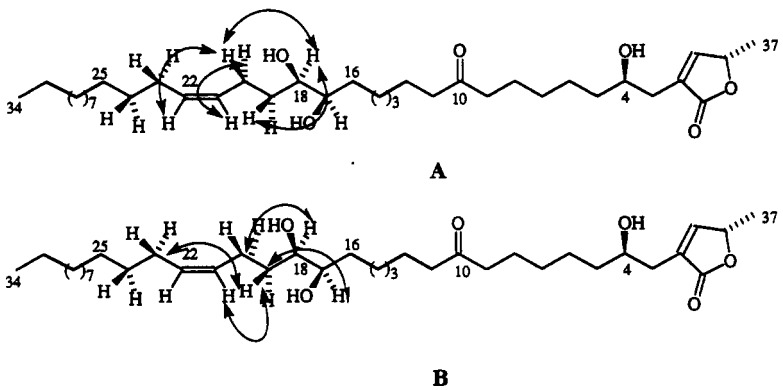


FIGURE 2. **A.** ^{1}H-^{1}H Correlations in the COSY and single-relayed COSY nmr spectra of venezenin [**1**]. **B.** ^{1}H-^{13}C Correlations in the HMBC nmr spectrum of venezenin [**1**].

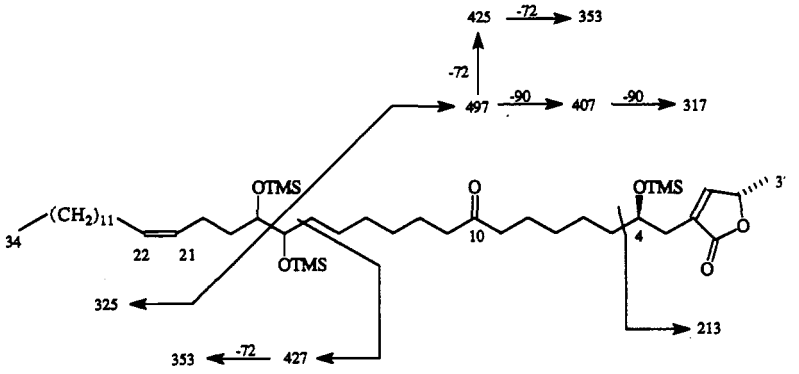


FIGURE 3. Diagnostic eims fragment ions (*m/z*) of the tri-TMSi derivative of venezenin [**1**].

configuration of the diol was determined to be threo, since the trans configuration of C-17/C-18 in **3** could only be derived from a vicinal diol with a threo configuration. The NOESY spectrum of **3** also suggested the trans configuration of the C-17/C-18 dioxolane ring.

The presence of an additional carbonyl signal at 1700 cm^{-1} in the ir spectrum suggested that **1** was a keto-acetogenin compound. The ^{1}H-nmr data also suggested the location of the keto group at C-10 since two additional two-proton triplets (J=7.5 Hz) were seen in the spectrum of **1**, at δ 2.40 and 2.41, consistent with two methylene groups, at C-9 and C-11, flanking the keto group. Confirmation of the structure was achieved by reduction of **1** with $NaBH_4$ which yielded the racemic alcohol [**4**]. The location of the keto group was then confirmed on the basis of eims of the TMSi derivative [**5**] of the alcohol derivative [**4**] (Figure 4).

These functionalities (a double bond and a vicinal diol located two methylenes apart) support the previously proposed role of such groups in the biogenetic pathway leading to the mono-THF acetogenins (2). Thus, venezenin [**1**] might well serve as a precursor of annomontacin (12), which has been previously isolated from this species (4,5). To support this hypothesis and confirm the positions of these functionalities and the relative stereochemistries of the vicinal diol, a biomimetic synthesis of annomontacin-10-one [**6**] was performed. Treatment of **1** with *m*-CPBA gave two epoxides which were then cyclized using perchloric acid to form two mono-THF compounds [**6**,**7**]; **6** and **7** were separated by hplc.

In compounds **6** and **7**, besides the OH-4 γ-lactone moiety, ^{1}H-nmr spectra revealed

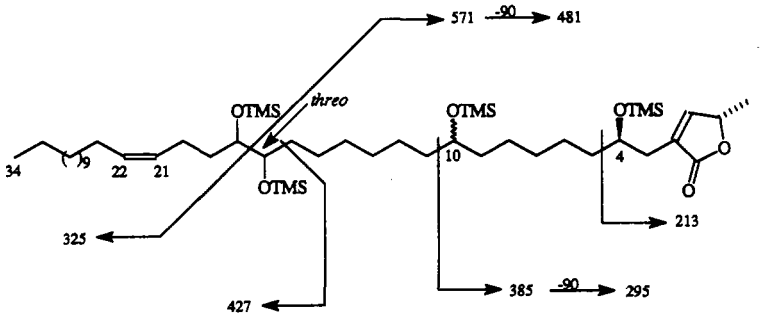


FIGURE 4. Diagnostic eims fragment ions (*m/z*) of the tri-TMSi derivative of the racemic alcoholic derivative [**5**] of venezenin [**1**].

resonances typical for the mono-THF ring, with two OH groups adjacent to the ring at δ 3.80 (H-18 and H-21) and δ 3.42 (H-17 and H-22) (Table 2), characteristic chemical shifts for the long hydrocarbon chain (δ 1.2–1.7) with a terminal Me group (δ 0.88), and a system of four protons adjacent to the carbonyl at δ 2.40. The relative stereochemistries between C-17/C-18 and C-21/C-22 were defined by comparing the ^{1}H-nmr signals of **6** and **7** for H-17, H-22 and H-18, H-21 (Table 2), with those of model compounds of known relative stereochemistry (13,14). Compound **6** showed ^{1}H-nmr signals at δ 2.00 and 1.67, corresponding to H-19 and H-20, which are typical methylene proton signals for a trans THF ring, while compound **7** showed typical methylene proton signals for a cis THF ring at δ 1.94 and 1.75 (10). Thus, the relative configuration for these four chiral centers are threo-trans-threo for **6** and threo-cis-threo for **7**; this result also confirmed the threo relationship of the vicinal diol.

Diagnostic peaks in the eims spectra of TMSi derivatives [**8** and **9**] of **6** and **7**, at *m/z* 567, 497, 341, and 271 (Figure 5) gave confirmatory information on the presence and location of the mono-THF ring in each. The molecular formulas of $C_{37}H_{66}O_7$ for both **6** and **7** were confirmed from hreims of their TMSi derivatives [**8** and **9**] (see Experimental). These results are in agreement with the hypothetical biogenetic pathway of the mono-THF acetogenins, which presumably involves the epoxidation of cis dienes followed by the ring opening of the epoxide moieties and ring closure to form the THF-ring with two flanking hydroxyls (2).

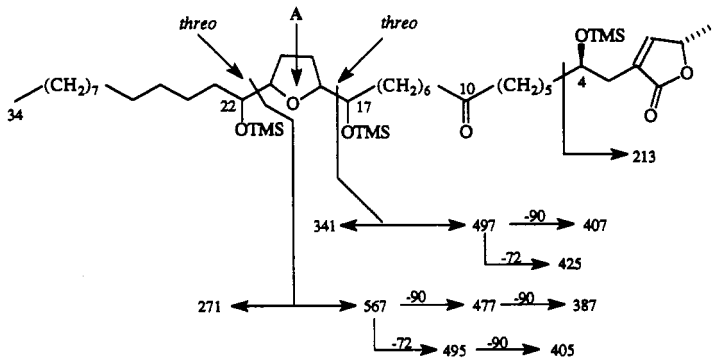


FIGURE 5. Diagnostic eims fragment ions (*m/z*) of the tri-TMSi derivatives **8** (**A**=trans) and **9** (**A**=cis) of venezenin [**1**].

The bioassay data summarized in Table 3 indicate that venezenin [**1**] and the prepared derivatives have cytotoxic activity comparable to adriamycin. The acetonide [**3**] and the newly prepared mono-THF compounds [**6** and **7**] are more active than venezenin [**1**]. Compound **6**, the C-18/C-21-trans isomer, is slightly more active than compound **7**, the C-18/C-21-cis isomer. The IC_{50} value of 1.7 μM/liter/mg protein, obtained at the subcellular level on the rat liver mitochondrial respiration system, demonstrated the inhibitory effect of venezenin [**1**] on mitochondrial respiration; rotenone was used as a positive control (IC_{50} 34 nM/liter/mg protein). Other acetogenins are much more potent as mitochondrial inhibitors (18), but these results clearly demonstrate that the THF ring system is not essential to the inhibition of mitochondria by the Annonaceous acetogenins.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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. ^{1}H- and ^{13}C-nmr

TABLE 3. Bioactivities of F005 and Venezenin [1] and Its Derivatives [3, 4, 6, 7].

Compound	BST[a] LC_{50} μg/ml[c]	A-549[b] ED_{50} μg/ml	MCF-7[b] ED_{50} μg/ml	HT-29[b] ED_{50} μg/ml
F005[d]	160 (156/165)	10^{-2}	1.39	10^{-2}
Venezenin [1]	9.33 (8.95/12.64)	1.08×10^{-2}	$<10^{-2}$	1.58
3	—	$<10^{-3}$	3.47×10^{-1}	9.32×10^{-1}
4	—	$<10^{-3}$	5.72×10^{-2}	2.62
6	0.84 (0.54/1.32)	7.86×10^{-3}	7.47×10^{-5}	3.46×10^{-4}
7	0.98 (0.65/1.29)	4.5×10^{-3}	5.6×10^{-2}	4.7×10^{-2}
Adriamycin	—	9.20×10^{-3}	2.3×10^{-1}	3.92×10^{-2}

[a]Brine shrimp lethality (6,7).

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

[c]95% Confidence intervals are in parentheses.

[d]Partition fraction from which **1** was isolated.

spectra were obtained on a Varian VXR-500S spectrometer. Low-resolution fabms data were collected on a Finnigan 4000 spectrometer. Lreims for TMSi derivatives were obtained on a Kratos MS50. Hrfabms were 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.

BIOASSAYS.—The extracts, fractions, and isolated compounds were evaluated for lethality to brine shrimp larvae (BST) (6,7). Cytotoxicity against human solid tumor cells was measured in 7-day MTT tests at the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the A-549 lung carcinoma (15), MCF-7 breast carcinoma (16), and HT-29 colon adenocarcinoma (17) cell lines, with adriamycin as a positive control. Venezenin [1] was tested in a rat liver mitochondrial assay and gave an IC_{50} value of 1.7 μM/liter/mg protein; standard procedures were followed for mitochondrial isolation (3,18) and protein determination (19); respiratory functions of the rat liver mitochondria were measured polarographically by determination of their rates of oxygen consumption using a small oxygen electrode inserted into a semi-closed reaction cell (3,18); rotenone was used as positive control and gave an IC_{50} value of 34 nM/liter/mg protein.

EXTRACTION AND ISOLATION (10).—The dried and pulverized bark (4.0 kg) was extracted with EtOH and partitioned, as described above, to obtain F005. F005 (60 g) was subjected to cc 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_1-8–F_1-15) (4 g, BST LC_{50}=10 μg/ml) was further resolved on another Si gel (160 g) column, eluted with 33% Me_2CO in hexane. Fractions (F_2-10 to F_2-30) were collected into 6 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}=0.05 μg/ml) was re-chromatographed by hplc over Si gel eluted with a gradient of hexane/THF/MeOH (flow rate 10 ml/min) to afford compound **1** and asimicin.

TMS DERIVATIVES.—Small amounts (<1 mg) of **1**, **4**, **6**, and **7** 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 [**2**, **5**, **8**, and **9**]; eims, see Figures 3–5.

ACETONIDE DERIVATIVE.—To **1** (1.5 mg) was added 0.5 ml of HCl/Me_2CO (0.7 mg HCl in 1 ml Me_2CO), and the solution was left overnight; the mixture was then dried at room temperature *in vacuo* to yield **3**.

REDUCTION.—To 2.5 mg of **1** were sequentially added 0.5 ml of MeOH, 0.05 ml of THF, and 0.025 ml of $NaBH_4$ solution (0.5 M solution in 2-methoxyethyl ether) on an ice-water bath. The mixture was stirred at 0° for 10 min and 1 h at room temperature. CH_2Cl_2 was added to form two distinct layers, and the CH_2Cl_2 layer was dried *in vacuo* to give **4**.

OXIDATION AND CYCLIZATION.—To 5 mg of venezenin [1] (in 2.5 ml of CH_2Cl_2), was added *m*-chloroperbenzoic acid (*m*-CPBA, 4 mg), and the mixture was stirred for 1 h at room temperature. The

mixture was washed using 1% $NaHCO_3$ (5 ml) and H_2O (2×5 ml), and the CH_2Cl_2 layer was dried *in vacuo* to give the C-21/C-22-epoxide of **1**; to the epoxide (in 5 ml of CH_2Cl_2) was added 30% perchloric acid ($HClO_4$, 2.5 μl). The mixture was stirred for another h at room temperature to give a mixture of C-18/-21 *cis*- and *trans*-annomontacin-10-one (**6** and **7**). The mixture was washed using 1% $NaHCO_3$ (5 ml) and H_2O (2×5 ml), and the CH_2Cl_2 layer was dried *in vacuo* and resolved by hplc to give 2.5 mg of **6** and 2.5 mg of **7**.

Venezenin [**1**].—White waxy solid (17 mg); mp 72–73°; $[\alpha]^{23}D$ +16.8° (c=0.001, MeOH); uv λ max (MeOH) 225 (log ε 3.28) nm; ir ν max (film) 3375, 2924, 2854, 1732, 1700, 1642, 1490, 1282, 1074, 669 cm^{-1}; fabms (glycerol) *m/z* $[MH]^+$ 606 (50), $[MH-H_2O]^+$ 588 (5), $[MH-2H_2O]^+$ 570 (12), $[MH-3H_2O]^+$ 552 (4); hrfabms (glycerol) *m/z* 607.4916 $(MH)^+$ (calcd 607.4938 for $C_{35}H_{67}O_7$); ^{1}H-nmr data ($CDCl_3$, 500 MHz), see Table 1; ^{13}C-nmr data ($CDCl_3$, 125 MHz), see Table 2.

Annomontacin-10-one [**6**].—White waxy solid (2.5 mg); mp 44–46°; fabms (glycerol) *m/z* $[MH]^+$ 623 (26), fabms (glycerol) *m/z* [M−Na adduct]$^+$ 645 (50); hrfabms (glycerol) *m/z* 645.4771 (M−Na adduct)$^-$ (calcd 645.4706 for $C_{37}H_{66}O_7Na$); ^{1}H-nmr data ($CDCl_3$, 500 MHz), see Table 1.

C-18/C-21-cis-*Annomontacin-10-one* [**7**].—White waxy solid (2.5 mg); mp 42–44°; fabms (glycerol) *m/z* $[MH]^+$ 623 (60), $[MH-H_2O]^+$ 605 (5), $[MH-2H_2O]^+$ 587 (18), $[MH-3H_2O]^+$ 569 (5), $[MH-4H_2O]^+$ 551 (7); hrfabms (glycerol) *m/z* 623.4887 $(MH)^-$ (calcd 623.4900 for $C_{37}H_{67}O_7$), ^{1}H-nmr data ($CDCl_3$, 500 MHz), see Table 1.

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