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ADDITIONAL BIOACTIVE COMPOUNDS AND TRILOBACIN, A NOVEL HIGHLY CYTOTOXIC ACETOGENIN, FROM THE BARK OF *ASIMINA TRILOBA*

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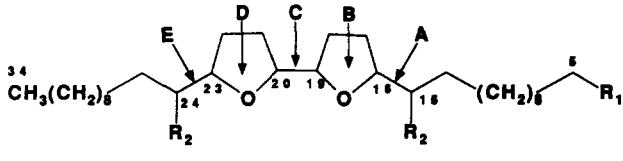
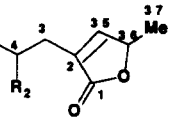
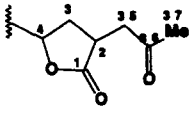
ABSTRACT.—Fractionation of the EtOH extract of the bark of *Asimina triloba*, monitoring by brine shrimp lethality, has led to the isolation and structural elucidation of a novel highly cytotoxic Annonaceous acetogenin, trilobacin [**1**], in addition to six known compounds: asimicin [**2**], bullatacin [**3**], bullatacinone [**4**], *N-p*-coumaroyltyramine [**5**], *N-trans*-feruloyltyramine [**6**], and (+)-syringaresinol [**7**]. Acetogenin **1** was identified as a diastereomer of asimicin [**2**] by spectral and chemical methods, and both **1** and **2** showed potent and selective cytotoxicities in the NCI human tumor cell line screen.

Asimina triloba (L.) Dunal (Annonaceae), commonly known as the pawpaw tree, is native to the United States and, since before the first European settlers arrived, has been prized for its delicious, custard-like fruit. The distribution of *A. triloba* covers the eastern portion of the United States. It occurs as far north as southern Michigan, south to Florida, east to New York, and as far west as eastern Nebraska and Oklahoma (1). Previous phytochemical studies of *A. triloba* have led to the isolation of oil, lipids, fatty acids and proteins from its fruits and seeds (2–5), tannins (6), sitosterol (3), caffeic acid (7), some flavonoids [procyanidin, quercetin (7), quercetin 3-glycoside, quercetin 3-rutinoside, and quercetin 3-glycoside-7-glucoside (8)], a number of alkaloids [asiminine, which was reported to be emetic, analobine, which was once used as a medicine (9), coreximine, anolobine, asimilobine, isocorydine, liriodenine (10), and norushinsunine (11)]. The discovery of asimicin [**2**], a novel acetogenin which is highly cytotoxic and has promising antitumor and pesticidal activities, was reported by Rupprecht *et al.* in our earlier work (12). To continue our search for new potential anticancer and pesticidal constituents in this plant, we further investigated the EtOH extract of the stem bark, using brine shrimp lethality (BST) (13) to direct the fractionation. This



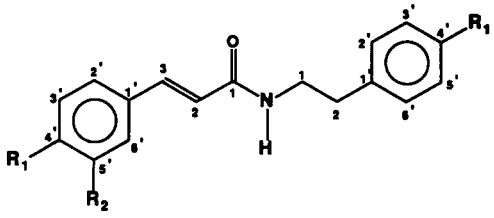
1, 2, 3 R_1=  4 R_1= 

compounds	A	B	C	D	E	R_2
1	threo	trans	erythro	trans	threo	OH
1 triacetate	threo	trans	erythro	trans	threo	OAc
2	threo	trans	threo	trans	threo	OH
3	erythro	trans	threo	trans	threo	OH
4	erythro	trans	threo	trans	threo	OH

work has resulted in the isolation of a new highly bioactive Annonaceous acetogenin named trilobacin [**1**], as well as six known compounds **2–7**, all of which are bioactive.

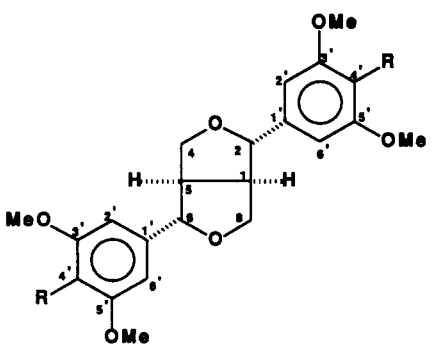
RESULTS AND DISCUSSION

The residue of the EtOH extract of the ground stem bark of *A. triloba* was partitioned following a standard scheme (see Experimental). The most bioactive fraction, F005 (BST $LC_{50} = 7.151 \times 10^{-1}$ μg/ml), was subjected to repeated Si gel cc and subsequent radial chromatographic (Chromatotron) separations, directed by the BST (13) bioassay at each step, to yield compounds **1–7**. Compound **1** is a new waxy Annonaceous acetogenin named trilobacin, while **2** is the previously reported acetogenin, asimicin (12); **3** was identified as bullatacin, another potent acetogenin, and was accompanied by fine needles of bullatacinone [**4**] (14); two amides, *N-p*-coumaroyltyramine [**5**] and *N-trans*-feruloyltyramine [**6**] (15), and a lignan, (+)-syringaresinol [**7**] (16), were also isolated as bioactive constituents.



5	R_1=OH, R_2=H
5 diacetate	R_1=OAc, R_2=H
6	R_1=OH, R_2=OMe

The acetogenin compounds **2–4** were identified by direct comparisons of spectral data with those of the original isolated compounds available in our group (12,14). As previously noted (14), both **3** and **4** were extremely cytotoxic against certain human tumor cell lines, with ED_{50} values as low as 10^{-12} to 10^{-18} μg/ml. In tests reported herein, asimicin [**2**] exhibited potent activity in the three-day NCI in vitro human cancer cell line screen, with an average GI_{50} (concentration giving 50% growth inhibition) value of 7.58×10^{-6} μg/ml; **2** was most active, selectively, against certain cell lines of leukemia (K-562), non-small-cell lung cancer (NCI-H23), small cell lung cancer (DMS-114), and melanoma (SK-MEL-5), with GI_{50} values less than 1.0×10^{-8} μg/ml. In seven-day human cancer cell culture tests performed at the Purdue Cancer



7	R=OH
7 diacetate	R=OAc

Center (PCC) Cell Culture Laboratory, **2** showed selective cytotoxicities, with an ED_{50} value of less than 1.0×10^{-15} μg/ml against the human colon cancer HT-29 cell line.

The structures of compounds **5–7** were established by ms, ^{1}H-nmr, and ^{13}C-nmr spectral data analysis and comparison with the reported data of authentic samples (15,16). This is the first report of these compounds occurring in the Annonaceae family. Compounds **5–7** showed biological activities in the BST bioassay (13) and inhibition of crown-gall tumors at ca. 22.2%–27.5% in the potato disc bioassay (PD) (17). Testing in the PCC human cancer cell line panel indicated that compounds **5–7** have selective cytotoxicities against the MCF-7 (human breast carcinoma) cell line with ED_{50} values of 7.16×10^{-1}, 4.76, and 3.69 μg/ml respectively. Compound **5** was also active against the A-549 (human lung cancer) ($ED_{50} = 3.24$ μg/ml) and HT-29 (human colon cancer) ($ED_{50} = 6.74 \times 10^{-1}$ μg/ml) cell lines.

The novel compound **1** was obtained as a colorless waxy gum. Its mol wt of 622 was established from cims (isobutane), *m/z* 623 $[MH]^+$ (100%), and confirmed by hrcims (isobutane) with *m/z* $[MH]^+$ 623.4868 corresponding to the molecular formula

TABLE 1. Comparison of ^{1}H-Nmr Spectral Data of Trilobacin [**1**], Trilobacin Triacetate, and Asimicin [**2**].

Proton	Compound					
	1		**1** triacetate		**2**	
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
H_a-3	2.49 (dddd),	3a,3b (15.1) 3a,4 (3.5) 3a,35 (1.4) 3a,36 (1.4)	2.57 (dddd),	3a,3b (15.0) 3a,4 (4.3) 3a,35 (1.4) 3a,36 (1.4)	2.45 (dddd),	3a,3b (15.1) 3a,4 (3.6) 3a,35 (1.4) 3a,36 (1.4)
H_b-3	2.37 (dddd),	3a,3b (15.1) 3b,4 (8.3) 3b,35 (1.4) 3b,36 (1.4)	2.51 (dddd),	3a,3b (15.0) 3b,4 (8.0) 3b,35 (1.4) 3b,36 (1.4)	2.34 (dddd),	3a,3b (15.1) 3b,4 (8.2) 3b,35 (1.4) 3b,36 (1.4)
H-4	3.80 (m)		5.13 (m)		3.79 (m)	
H-5	1.36–1.44 (m)		1.5–1.62 (m)		1.30–1.44 (m)	
H-6–H-13	1.23 (brs)		1.25 (brs)		1.25 (brs)	
H-14	1.36–1.44 (m)		1.5–1.62 (m)		1.30–1.44 (m)	
H-15	3.34 (m)		4.85 (m)		3.33 (m)	
H-16	3.80 (m)		3.96 (m)		3.79 (m)	
H-17, -18	1.65–2.03 (m)		1.7–2.1 (m)		1.58–1.91 (m)	
H-19[a]	4.01 (m)		3.82 (m)		3.79 (m)	
H-20[a]	3.93 (m)		3.82 (m)		3.79 (m)	
H-21, -22	1.65–2.03 (m)		1.7–2.1 (m)		1.58–1.91 (m)	
H-23	3.80 (m)		3.96 (m)		3.79 (m)	
H-24	3.34 (m)		4.85 (m)		3.33 (m)	
H-25	1.36–1.44 (m)		1.5–1.62 (m)		1.30–1.44 (m)	
H-26–H-33	1.23 (brs)		1.25 (brs)		1.20 (brs)	
H-34	0.84 (t)	34,33 (7.1)	0.88 (t)	34,33 (7.1)	0.82 (t)	34,33 (7.1)
H-35	7.16 (ddd),	35,36 (1.4) 35,3a (1.4) 35,3b (1.4)	7.085 (ddd),	35,36 (1.4) 35,3a (1.5) 35,3b (1.4)	7.16 (ddd),	35,36 (1.4) 35,3a (1.4) 35,3b (1.4)
H-36	5.03 (qddd),	36,37 (6.8) 36,3a (1.4) 36,3b (1.4) 36,35 (1.4)	5.01 (qddd),	36,37 (6.8) 36,3a (1.4) 36,3b (1.4) 36,35 (1.4)	5.00 (qddd),	36,37 (6.8) 36,3a (1.4) 36,3b (1.4) 36,35 (1.4)
H-37	1.4 (d),	37,36 (6.8)	1.4 (d)	37,36 (6.8)	1.37 (d),	37,36 (6.8)
-Ac (C-4)			2.03 (s)			
-Ac (C-15)			2.084 (s)			
-Ac (C-24)			2.079 (s)			

[a]Assignments may be interchangeable.

$C_{37}H_{67}O_7$ (calcd 623.4887). The ms and nmr spectra clearly indicated that **1** is one of the familar adjacent bis-THF acetogenins (18). A strong hydroxyl group absorption at 3445 cm^{-1} in the ir spectrum and a series of peaks at *m/z* 604, 586, and 568, arising from sequential losses of three molecules of H_2O, in the eims, suggested the presence of at least three hydroxyl groups. This was confirmed by the cims (isobutane) of the triacetate derivative of **1** which gave the exact molecular ion $[MH]^+$ at *m/z* 749.

A strong ir absorption at 1748 cm^{-1} and a uv band at 223 nm ($\epsilon = 2653$) for **1** indicated the presence of an α,β-unsaturated lactone ring. The lactone ring moiety with an

TABLE 2. Comparison of Proton-Decoupled ^{13}C-nmr Data (δ) of Trilobacin [**1**] and Asimicin [**2**].

Carbon	Compound	
	1	**2**
C-1	174.51	174.51
C-2	131.13	130.94
C-3	34.25	33.29
C-4	69.98	69.80
C-5	37.44	37.30
C-6[a]	25.60	25.60
C-7[a]	28.17	28.33
C-8[a]	29.37	29.29
C-9[a]	29.54	29.48
C-10[a]	29.72	29.62
C-11[a]	29.53	29.56
C-12[a]	28.95	29.48
C-13[a]	25.82	25.54
C-14[a]	33.58	33.17
C-15[b]	73.88	73.91
C-16[c]	82.62	83.08
C-17[a]	29.55	28.34
C-18[a]	29.66	28.96
C-19[d]	80.87	81.73
C-20[d]	81.59	81.73
C-21[a]	29.66	28.96
C-22[a]	29.55	28.34
C-23[c]	83.25	83.08
C-24[b]	74.56	73.93
C-25[a]	33.36	33.17
C-26[a]	25.67	25.58
C-27[a]	28.28	29.44
C-28[a]	29.59	29.48
C-29[a]	29.62	29.56
C-30[a]	29.65	29.59
C-31[a]	29.77	29.68
C-32[a]	31.94	31.85
C-33[a]	22.73	22.64
C-34	14.17	14.09
C-35	151.69	151.74
C-36	77.96	77.92
C-37	19.16	19.05

[a]Assignments of methylene groups may be interchanged.

[b–d]Assignments of methine groups may be interchanged.

alkyl substitutent at the α position to the lactone carbonyl was confirmed by the ^{1}H-nmr and ^{13}C-nmr spectra. The ^{1}H-nmr signals at δ 7.16 (H-35, q), 5.03 (H-36, qq), and 1.4 (H-37, d) and the coupling constants of these protons clearly showed the connectivities of H-37 to H-36 (J = 6.8 Hz), H-36 to H-35 (J = 1.4 Hz), H-35 to H-3 (J = 1.4 Hz, allylic coupling), and H-36 to H-3 (J = 1.4 Hz, homo-allylic coupling). In the ^{13}C-nmr spectrum, the peaks corresponding to the lactone ring were shown at δ 174.51 (C-1), 131.13 (C-2), 151.69 (C-35), and 77.96 (C-36). In the ^{1}H-nmr spectrum, the peaks at δ 2.49 (H_a-3, dddd, J = 15.1, 3.5, 1.4, 1.4 Hz), 2.37 (H_b-3, dddd, J = 15.1, 8.3, 1.4, 1.4 Hz), and 3.80 (H-4, m), and the ^{13}C-nmr chemical shifts at δ 33.356 (C-3) and 69.975 (C-4) clearly indicated the presence of an hydroxyl at C-4 (18). Therefore, this subunit was established as an asimicin type of acetogenin (12).

Comparative analysis of the spectral data of compound **1** and asimicin [**2**] showed the same mol wt and fragmentation patterns in the cims and close similarities of resonances of protons and carbons in the nmr spectra, clearly suggesting that **1** was a diastereomer of **2**. The major nmr differences caused by oxygen-associated protons and carbons were concentrated in the lower field region. In addition to H-36 and C-36, there were seven oxymethine multiplet protons recognized in the ^{1}H-nmr spectrum at chemical shifts between δ 3 and 4.5, which were assigned to H-15 and H-24 (δ 3.34, m, 2H), H-4, H-16, and H-23 (δ 3.80, m, 3H); H-19 (δ 4.01, m, 1H), and H-20 (δ 3.93, m, 1H). These assignments are, perhaps, interchangeable. The related seven oxygen-bearing methine carbons in the ^{13}C-nmr spectrum appeared at lower field from δ 70 to 84. Interchangeable assignments are made for these three pairs of carbons: C-15 and C-24 for δ 73.88 and 74.56; C-19 and C-20 for δ 80.87 and 81.59; C-16 and C-23 for δ 82.62 and 83.25. The peak at δ 69.98 clearly belonged to C-4 (18).

TABLE 3. In-House Bioactivities of Compounds **1–7** from *Asimina triloba*.

Compound	BST[a]	PD[b]	Human Cancer Cell Lines[c]		
	LC_{50} (μg/ml) (95% Confidence interval)	Inhibition (%)	A-549 ED_{50} (μg/ml)	MCF-7 ED_{50} (μg/ml)	HT-29 ED_{50} (μg/ml)
Trilobacin [**1**]	8.7×10^{-3} (5.2×10^{-3}–14.4×10^{-3})	46.2	8.02×10^{-4}	3.29×10^{-1}	$<10^{-15}$
Asimicin [**2**]	2.56×10^{-2} (1.54×10^{-2}–4.25×10^{-2})	70	8.43×10^{-4}	8.52×10^{-1}	$<10^{-15}$
Bullatacin [**3**][d]	1.59×10^{-3} (0.8×10^{-3}–12.4×10^{-3})	53	1.25×10^{-13}	9.7×10^{-18}	10^{-12}
Bullatacinone [**4**][d]	3×10^{-3} (0.000–9.0×10^{-3})	15	10^{-3}	5.4×10^{-18}	5×10^{-12}
N-p-Coumaroyl-tyramine [**5**]	6.7 (3.80–11.19)	27.5	3.24	7.16×10^{-1}	6.74×10^{-1}
N-trans-Feruloyl-tyramine [**6**]	6.7 (3.80–11.19)	26.4	13.35	4.76	23.58
(+)-Syringa-resinol [**7**]	13.6 (7.31–26.12)	22.2	14.9	3.69	15.5
Adriamycin	NT[e]	NT[e]	1.48×10^{-3}	1.47×10^{-1}	6.69×10^{-3}

[a]From Meyer *et al.* (13).
[b]From Ferrigni *et al.* (17).
[c]From Giard *et al.* (21), Soule *et al.* (22), and Fogh (23).
[d]Data from Hui *et al.* (14).
[e]NT = not tested.

The determination of the relative stereochemical relationships among the six stereogenic carbon atoms in the adjacent bis-tetrahydrofuran dihydroxy moiety was made by following the method described by Hoye and co-workers (19,20). Careful

TABLE 4. NCI Human Cancer Cell Line In Vitro Test[a] Results of Trilobacin [1] and Asimicin [2].

Panel	Cell Line	Compound	
		[1] GI_{50} (μg/ml)[b]	[2] GI_{50} (μg/ml)
Leukemia	CCRF-CEM	1.70×10^{-6}	1.50×10^{-6}
	HL-60(TB)	$<1.00 \times 10^{-8}$	
	K-562	$<1.00 \times 10^{-8}$	$<1.00 \times 10^{-8}$
	MOLT-4	1.16×10^{-6}	1.21×10^{-6}
	RPMI-8226	3.93×10^{-7}	6.15×10^{-7}
	SR	1.09×10^{-6}	1.62×10^{-6}
Non-Small-Cell-Lung Cancer	A549/ATCC	1.64×10^{-6}	2.96×10^{-6}
	EKVX	2.00×10^{-6}	2.09×10^{-6}
	HOP-62	2.34×10^{-6}	1.93×10^{-6}
	HOP-92	2.76×10^{-6}	2.93×10^{-6}
	NCI-H226	8.21×10^{-8}	1.27×10^{-6}
	NCI-H23	$<1.00 \times 10^{-8}$	$<1.00 \times 10^{-8}$
	NCI-H322M	4.06×10^{-6}	2.86×10^{-6}
	NCI-H460	2.93×10^{-6}	2.16×10^{-6}
	NCI-H522	8.63×10^{-8}	1.46×10^{-6}
	LXFL 529	2.17×10^{-6}	2.54×10^{-6}
Small Cell Lung Cancer	DMS 114	$<1.00 \times 10^{-8}$	$<1.00 \times 10^{-8}$
	DMS 273	1.77×10^{-6}	1.60×10^{-6}
Colon Cancer	COLO 205	3.49×10^{-6}	2.17×10^{-6}
	DLD-1	2.70×10^{-6}	3.68×10^{-6}
	HCC-2998	2.92×10^{-6}	3.59×10^{-6}
	HCT-116	2.21×10^{-8}	2.24×10^{-6}
	HCT-15		1.23×10^{-6}
	HT29	2.16×10^{-6}	1.66×10^{-6}
	KM20L2	3.75×10^{-6}	3.33×10^{-6}
	SW-620	2.64×10^{-6}	2.51×10^{-6}
CNS Cancer	SF-268	1.69×10^{-6}	1.11×10^{-6}
	SF-295		2.90×10^{-7}
	SF-539	1.38×10^{-6}	1.55×10^{-6}
	SNB-19		2.05×10^{-6}
	SNB-75	3.19×10^{-6}	2.25×10^{-6}
	SNB-78	2.05×10^{-6}	2.32×10^{-6}
	U251	4.33×10^{-6}	3.58×10^{-6}
	XF 498	2.03×10^{-6}	2.21×10^{-6}
Melanoma	LOX IMVI	1.20×10^{-6}	1.18×10^{-6}
	MALME-3M	1.44×10^{-6}	1.39×10^{-6}
	M14	1.04×10^{-5}	7.00×10^{-6}
	M19-MEL	2.63×10^{-6}	2.22×10^{-6}
	SK-MEL-2	2.28×10^{-6}	1.76×10^{-6}
	SK-MEL-28	2.01×10^{-6}	1.88×10^{-6}
	SK-MEL-5	$<1.00 \times 10^{-8}$	$<1.00 \times 10^{-8}$
	UACC-257	$<1.00 \times 10^{-8}$	1.20×10^{-6}
	UACC-62	2.67×10^{-6}	1.97×10^{-6}
Ovarian Cancer	IGROVL	4.81×10^{-6}	1.73×10^{-6}
	OVCAR-3	1.09×10^{-6}	6.74×10^{-7}
	OVCAR-4	$<1.00 \times 10^{-8}$	2.75×10^{-6}
	OVCAR-5	4.33×10^{-6}	2.58×10^{-6}
	OVCAR-8	2.93×10^{-6}	2.29×10^{-6}
	SK-OV-3	8.16×10^{-6}	3.17×10^{-6}

TABLE 4. Continued.

Panel	Cell Line	Compound	
		[1] GI_{50} (μg/ml)[b]	[2] GI_{50} (μg/ml)
Renal Cancer	786-0	2.20×10^{-6}	2.68×10^{-6}
	A498	6.86×10^{-6}	3.15×10^{-6}
	CAKI-1	8.83×10^{-6}	4.09×10^{-6}
	RXF-631	1.79×10^{-6}	2.02×10^{-6}
	SN12C	1.64×10^{-6}	1.76×10^{-6}
	TK-10	6.96×10^{-6}	4.05×10^{-6}
	UO-31	6.48×10^{-8}	1.12×10^{-6}
Average Log GE_{50}		−6.04	−5.88
Delta		1.906	2.12
Range		3.02	2.85

[a]By the method of Monks *et al.* (24).
[b]GI_{50} = concentration needed for 50% growth inhibition in three-day tests.

comparison of ^{1}H-nmr chemical shifts of the diagnostic protons indicated that the chemical shifts of **1** triacetate matched the th/t/er/t/th model revealing the relative stereochemical relationships between the chiral centers of C-15/C-16, C-16/C-19, C-19/C-20, C-20/C-23, and C-23/C-24. Thus, **1** was concluded to be a novel diastereomer of asimicin [**2**] and was given the name trilobacin [**1**].

Tables 3 and 4 summarize the biological activities of all of the isolated compounds. Trilobacin [**1**] was very active, similar to asimicin [**2**] (12), in the BST (LC_{50} 8.7×10^{-3} μg/ml) (13) and potato disc bioassay (PD, 46.2% inhibition) (17), and was similarly extremely active in the three-day NCI human cancer cell line in vitro screen, with an average GI_{50} value of 1.10×10^{-6} μg/ml. It was most active, selectively, against certain cell lines of leukemia [K-562 and HL-60 (TB)], non-small-cell lung cancer (NCI-H23, NCI-H226, and NCI-H522), small cell lung cancer (DMS-114), colon cancer (HCT-116), melanoma (SK-MEL-5 and UACC-257), ovarian cancer (OVCAR-4), and renal cancer (UO-31), with GI_{50} values $< 1.0 \times 10^{-8}$ μg/ml. In the seven-day human cancer cell line tests performed at the PCC Cell Culture Laboratory, it showed potent cytotoxicities, with ED_{50} values as low as $< 1.0 \times 10^{-15}$ μg/ml against the human colon cancer (HT-29) cell line and lesser, but still significant, activities against the A-549 (human lung carcinoma, $ED_{50} = 8.02 \times 10^{-4}$ μg/ml) and MCF-7 (human breast carcinoma, $ED_{50} = 3.29 \times 10^{-1}$ μg/ml) cell lines. These remarkably potent cytotoxicities and the very high sensitivity of certain human cancer cell lines suggest that trilobacin [**1**] should be further evaluated for future application in cancer chemotherapy.

EXPERIMENTAL

PLANT MATERIAL.—The bark of *A. triloba* was collected from stands growing wild at the Purdue Horticultural Research Farm. The identification was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the bark is on file at the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

BIOASSAYS.—The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) (13) and in the PD bioassay (% inhibition of crown gall tumors on potato discs) (17). Seven-day in vitro cytotoxicity tests against human tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma) (21), MCF-7 (human breast carcinoma) (22), and HT-29 (human colon carcinoma) (23) with adriamycin as a positive control. Cytotoxicity results of the NCI three-day human tumor cell line screen (24) were obtained through the cooperation of Dr. Matthew Suffness at NCI.

INSTRUMENTATION.—Mp determinations were made on a Mel-Temp apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. Ir spectra were obtained on a Perkin-Elmer 1600 FTIR spectrometer. Uv spectra were measured on a Beckman DU-7 uv spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer in $CDCl_3$ with proton signals referenced to TMS. Low resolution cims and eims data were collected on a Finnigan 4000 spectrometer. Exact masses on ms measurements were obtained on a Kratos MS50 spectrometer through peak matching. Tlc separations were made on Si gel 60 F-254 (EM5717) glass plates (0.25 mm) and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Chromatotron plates were prepared with Si gel 650 PF 254 containing gypsum (1 or 2 mm) and dried at 70° overnight.

EXTRACTION AND ISOLATION.—The air-dried stem bark (15 kg) was pulverized and extracted exhaustively at room temperature with 95% EtOH and evaporated under vacuum to yield extract F001 (1645 g). The EtOH extract (F001) was partitioned between H_2O and CH_2Cl_2 (1:1). The partitioned fractions were evaporated to leave a dark brown H_2O-soluble residue (F002) (5 g), the CH_2Cl_2-soluble residue (F003) (1560 g), and an insoluble interface fraction (F004) (80 g). The syrupy CH_2Cl_2 residue (F003) was further partitioned between hexane and 90% aqueous MeOH to yield the MeOH-soluble residue (F005) (650 g) and the hexane-soluble residue (F006) (905 g). The bioactivities were concentrated into the MeOH fraction (F005) (BST $LC_{50} = 7.151 \times 10^{-1}$ μg/ml). The active, syrupy, MeOH residue (F005) (200 g) was adsorbed onto 260 g of Celite and placed on a chromatographic column of Si gel 400 g (60–200 mesh) packed in a slurry of hexane and eluted first with hexane-EtOAc (2:1, 1:2, and 100% EtOAc), then eluted with MeOH-EtOAc (1:9, 1:7, 1:5, 1:2, 1:1, and 100% MeOH). Collected fractions were combined into 12 pools according to their tlc patterns, weighed, and evaluated by the BST bioassay.

The very active pools 5–7 (35 g total) were combined and applied to another column (Si gel, 1000 g, 230–400 mesh). A gradient of hexane/Me_2CO was used to elute the column, and 88 fractions of 200 ml each were collected. Bioactive fractions 49–71 were subjected to further separation on a Chromatotron plate (Si gel, 2 mm), eluted with $CHCl_3$-MeOH (98.5:1.5) to yield fine needle-like crystals of bullatacinone [**4**] and *N-p*-coumaroyltyramine [**5**] as a white powder. A white precipitate was removed from bioactive fractions 72–88 with a solvent mixture of hexane-EtOAc (3:1) to give the major component, asimicin [**2**], as a white amorphous powder. The mother liquor was concentrated and applied to repeated Chromatotron separations (Si gel, 1 mm), eluted with $CHCl_3$/MeOH gradients, to obtain the white waxy compound named trilobacin [**1**], a white amorphous powder, bullatacin [**3**] mixed with a trace of asimicin, and crude crystals which gave colorless crystals of *N-trans*-feruloyltyramine [**6**] after purification with Me_2CO and hexane. The remaining residue was subjected to further Chromatotron purification (Si gel, 1 mm) with C_6H_6-Me_2CO (85:15), and recrystallization from hexane/$CHCl_3$ yielded compound **7** [(+)-syringaresinol].

Trilobacin [**1**].—Colorless wax (30 mg): $[\alpha]^{22}D + 10°$ ($c = 0.0005$, $CHCl_3$), uv λ max (EtOH) 223 nm ($\epsilon = 2653$); ir (film from $CHCl_3$) ν max cm^{-1} 3445 (OH), 2920, 2855, 1748 (C=O), 1643 (C=C), 1456, 1370, 1314, 1074, 720; cims (isobutane) *m/z* (%) $[MH]^+$ 623.4868 (100) (calcd for $C_{37}H_{67}O_7$, 623.4887), $[MH - H_2O]^+$ 605 (36), $[MH - 2H_2O]^+$ 587 (14), 363 (9), 311 (29); eims *m/z* (%) $[M]^+$ 622 (3), $[M - H_2O]^+$ 604, $[M - 2H_2O]^+$ 586, $[M - 3H_2O]^+$ 568, 381, 311 (34), 241, 113 (10), 109 (13), 97 (23), 81 (40), 55 (100); ^{1}H nmr (500 MHz, $CDCl_3$) see Table 1; ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2.

Trilobacin triacetate.—A sample of compound **1** (5 mg) was treated with Ac_2O-pyridine (1:1) at room temperature overnight. Workup in the usual way afforded **1** triacetate as a colorless wax: ir (film from $CHCl_3$) ν max cm^{-1} 2925, 2855, 1754 (C=O), 1734 (C=O), 1654 (C=C), 1453, 1368, 1318, 1238, 1077, 951, 724; ^{1}H nmr (500 MHz, $CDCl_3$) see Table 1.

IDENTIFICATION OF KNOWN COMPOUNDS.—The ^{1}H-nmr, ^{13}C-nmr, ir, and ms spectra of compound **2** (135 mg) were identical with those of asimicin as previously isolated (12), and compounds **3** (9 mg) and **4** (34 mg) showed identical ^{1}H-nmr and ^{13}C-nmr spectra with those of bullatacin and bullatacinone previously isolated from *Annona bullata* (14).

Compounds **5**–**7** were identified by ir, ms, ^{1}H-nmr, and ^{13}C-nmr spectral data analysis and comparison with the reported data of the known compounds (15,16). The identifications were also confirmed by examination of the spectral data of the acetate derivatives of **5** and **7**: **5** diacetate and **7** diacetate.

N-p-*Coumaroyltyramine* [**5**].—White powder (32 mg): ir (KBr) ν max cm^{-1} 3433 (NH), 3312 (br, OH), 2940, 1661, 1602, 1535, 1514, 1448, 1382, 1342, 1241, 1174, 981, 832, 522; hrcims (isobutane) *m/z* (%) $[MH]^+$ 284.1267 (100) (calcd for $C_{17}H_{18}NO_3$, 284.1287), eims *m/z* (%) $[M]^+$ 283 (4), 164 (58), 147 (100), 120 (56), 119 (27), 107 (17), 91 (36), 77 (19), 65 (38); ^{1}H nmr (500 MHz, CD_3OD) δ tyramine moiety 2.74 (2H, t, $J = 7.4$ Hz, H-2), 3.44 (2H, t, $J = 7.4$ Hz, H-1), 6.78 (2H, dd,

J = 8.6, 2.1 Hz, H-3', -5'), 7.39 (2H, dd, J = 8.6, 2.1 Hz, H-2', -6'), coumaroyl moiety 6.38 (1H, d, J = 15.7 Hz, H-2), 6.71 (2H, dd, J = 8.6, 2.1 Hz, H-3', -5'), 7.04 (2H, dd, J = 8.6, 2.1 Hz, H-2', -6'), 7.44 (1H, d, J = 15.7 Hz, H-3); ^{13}C nmr (125 MHz, pyridine-d_5) δ tyramine moiety 35.82 (C-2), 42.06 (C-2), 116.80 (C-3', -5'), 130.53 (C-2', -6'), 130.59 (C-1'), 157.41 (C-4'), coumaroyl moiety 116.38 (C-3', -5'), 119.48 (C-2), 127.10 (C-1'), 130.08 (C-2', -6'), 140.27 (C-3), 160.51 (C-4'), 166.83 (C-1).

N-p-*Coumaroyltyramine diacetate*.—A sample of **5** (8 mg) was treated wtih Ac_2O-pyridine (1:1) at room temperature overnight. Workup in the usual way afforded **5** diacetate as a white powder: ^{1}H nmr (500 MHz, pyridine-d_5) δ tyramine moiety 2.19 (3H, s, CH_3-CO-), 2.98 (2H, t, J = 7.0 Hz, H-2), 4.99 (2H, dt, J = 7.0, 5.9 Hz, H-1), 7.17 (2H, dd, J = 8.6, 2.1 Hz, H-3', -5'), 7.27 (2H, dd, J = 8.6, 2.1 Hz, H-2', -6'), 8.97 (1H, t, J = 5.9 Hz, -NH), coumaroyl moiety 2.21 (3H, s, MeCO), 6.86 (1H, d, J = 15.7 Hz, H-2), 7.20 (2H, dd, J = 8.6, 2.1 Hz, H-3', -5'), 7.54 (2H, dd, J = 8.6, 2.1 Hz, H-2', -6'), 8.05 (1H, d, J = 15.7 Hz, H-3).

N-trans-*Feruloyltyramine* [**6**].—Colorless crystals (40 mg): ir (KBr) ν max cm^{-1} 3345 (OH), 1702 (C=O), 1651, 1591, 1515, 1452, 1429, 1366, 1270, 1125, 1032, 978, 820, 756; hrcims (isobutane) *m/z* (%) $[MH]^+$ 314.1383 (100) (calcd for $C_{18}H_{20}NO_4$, 314.1392), eims *m/z* (%) $[MH]^+$ 313 (13.6), 192 (53), 193 (41), 177 (83), 145 (36), 120 (35), 107 (38), 89 (40), 77 (60), 63 (23), 51 (46), 43 (100); ^{1}H nmr (500 MHz, Me_2CO-d_6) δ tyramine moiety 2.77 (2H, t, J = 7.5 Hz, H-2), 3.52 (2H, dt, J = 7.5, 5.5 Hz, H-1), 6.78 (2H, dd, J = 8.6, 2.1 Hz, H-3', -5'), 7.07 (2H, dd, J = 8.6, 2.1 Hz, H-2', -6'), feruloyl moiety 3.85 (3H, s, MeO-), 5.6 (1H, d, J = 15.6 Hz, H-2), 6.85 (1H, d, J = 8.2 Hz, H-5'), 7.05 (1H, dd, J = 8.2, 2.0 Hz, H-6'), 7.15 (1H, d, J = 2.0 Hz, H-2'), 7.5 (1H, d, J = 15.6 Hz, H-3); ^{13}C nmr (125 MHz, Me_2CO-d_6) δ tyramine moiety 35.61 (C-2), 41.96 (C-1), 115.92 (C-3', -5'), 130.34 (C-2', -6'), 130.77 (C-1'), 156.53 (C-4'), feruloyl moiety 56.07 (MeO-), 111.12 (C-3), 119.57 (C-2'), 122.45 (C-6'), 127.93 (C-3'), 140.53 (C-2), 148.41 (C-4'), 148.99 (C-1'), 166.69 (C-1).

(+)-*Syringaresinol* [**7**].—Colorless crystals (46 mg): ir (KBr) ν max cm^{-1} 3415 (OH), 2939, 2844, 1614, 1518, 1462, 1427, 1323, 1215, 1114, 753; hreims (isobutane), *m/z* $[M]^+$ 418.1623 (calcd for $C_{22}H_{26}O_8$, 418.1628), cims (isobutane) *m/z* (%) $[MH]^+$ 419 (66), $[MH-H_2O]^+$ 401 (28), $[MH-2H_2O]^+$ 383 (3), 265 (100), 235 (29), 205 (13), 167 (11); eims *m/z* (%) $[M]^+$ 418 (63), $[M-MeO]^+$ 387, 235 (19), 210 (19), 193 (34), 181 (100), 167 (85), 161 (35), 154 (23), 77 (22); ^{1}H nmr (500 MHz, $CDCl_3$) δ 2.98 (2H, ddd, J = 6.9, 4.3, 3.7 Hz, H-1, -5), 3.77 (12H, s, MeO), 3.79 (2H, dd, J = 9.2, 3.7 Hz, H_a-4, H_a-8), 4.17 (2H, dd, J = 9.2, 6.9 Hz, H_b-4, H_b-8), 4.62 (2H, d, J = 4.3 Hz, H-2, -6), 6.47 (4H, s, H-2', -6'); ^{13}C nmr (125 MHz, $CDCl_3$) δ 54.27 (2C, C-1, -5), 56.30 (4C, MeO), 71.72 (2C, C-4, -8), 85.98 (2H, C-2, -6), 102.54 (4C, C-2', -6'), 131.92 (2C, C-1'), 134.11 (2C, C-4'), 147.0 (4C, C-3', -5').

(+)-*Syringaresinol diacetate*.—Compound **7** (10 mg) was treated with Ac_2O-pyridine (1:1) at room temperature overnight. Workup in the usual way afforded **7** diacetate as colorless crystals: ^{1}H nmr (500 MHz, $CDCl_3$) δ 2.33 (6H, s, MeCO), 3.07 (2H, ddd, J = 6.9, 4.3, 3.7 Hz, H-1, -5), 3.81 (12H, s, MeO), 3.93 (2H, dd, J = 9.2, 3.7 Hz, H_a-4, H_a-8), 4.29 (2H, dd, J = 9.2, 6.9 Hz, H_b-4, H_b-8), 4.75 (2H, d, J = 4.3 Hz, H-2, -6), 6.57 (4H, s, H-2', -6').

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