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CYTOTOXIC CONSTITUENTS OF THE BARK OF *PLUMERIA RUBRA* COLLECTED IN INDONESIA[1]

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ABSTRACT.—By bioactivity-directed fractionation, six cytotoxic constituents have been characterized from the bark of *Plumeria rubra* collected in Indonesia. Three iridoids, fulvoplumierin [**1**], allamcin [**2**], and allamandin [**3**], as well as 2,5-dimethoxy-*p*-benzoquinone [**4**], were found to be active constituents of the *P. rubra* petroleum-ether- and $CHCl_3$-soluble extracts. Cytotoxic compounds isolated from the H_2O-soluble extract of the bark were the iridoid plumericin [**5**], and the lignan liriodendrin [**6**]. Each of these substances was found to demonstrate general cytotoxic activity when evaluated with a panel of cell lines composed of murine lymphocytic leukemia (P-388) and a number of human cancer cell-types (breast, colon, fibrosarcoma, lung, melanoma, KB). Five additional iridoids, 15-demethylplumieride [**7**], plumieride [**8**], α-allamcidin [**9**], β-allamcidin [**10**], and 13-*O-trans-p*-coumaroylplumieride [**11**], were obtained as inactive constituents. Compound **7** was found to be a novel natural product, and its structure was determined by spectroscopic methods and by conversion to plumieride [**8**]. The configuration of the C-4 stereocenter was unambiguously assigned for compounds **9** and **10**, and certain nmr reassignments have been provided for compound **1**.

As part of our collaborative search for naturally occurring antineoplastic agents, the bark of *Plumeria rubra* L. (syn. *Plumeria acuminata* Ait.; *Plumeria acutifolia* Poir.) (Apocynaceae), of Indonesian origin, was selected for investigation. In Indonesia, a decoction of *P. rubra* bark is used to treat gonorrhea, while in the Philippines bark extracts of this plant are employed for their purgative, emmenagogue, and febrifuge effects (2). Extensive previous phytochemical work on this species has afforded many iridoids, including fulvoplumierin [**1**], plumericin [**5**], plumieride [**8**], and plumieride coumarate [**11**] (3–12), in addition to the quinolizidine alkaloid, plumerinine (13). Several of the iridoids previously isolated from *P. rubra* have been shown to exhibit algicidal (11), antibacterial (15,16), cytotoxic (17), and/or plant growth inhibitory activity (18,19). Plumericin [**5**] has been obtained by total synthesis (20,21).

In our previous work, a novel flavan-3-ol glucoside was isolated from the bark of *Plumeria rubra* (1), and we now report six cytotoxic components from three extracts of this plant part. The cytotoxic constituents comprised compounds in three structural classes: the iridoids fulvoplumierin [**1**], allamcin [**2**], allamandin [**3**], and plumericin [**5**]; the quinone 2,5-dimethoxy-*p*-benzoquinone [**4**]; and the lignan liriodendrin [**6**]. Among an additional five iridoids **7–11** isolated that were not cytotoxic was the new natural product 15-demethylplumieride [**7**]. A stereochemical uncertainty in the structure of the known compounds α-allamcidin [**9**] and β-allamcidin [**10**] was resolved in the present investigation.

RESULTS AND DISCUSSION

In preliminary studies, the petroleum-ether- and $CHCl_3$-soluble extracts of *P.*

[1]Paper No. II in the series, "Studies on Indonesian Medicinal Plants." For part I, see Kardono *et al.* (1).

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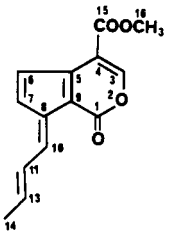

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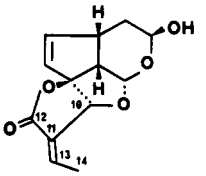

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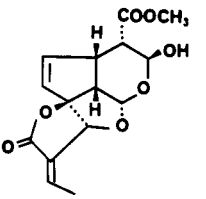

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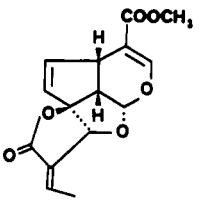

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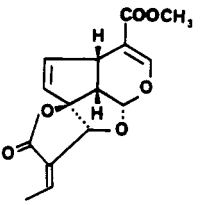
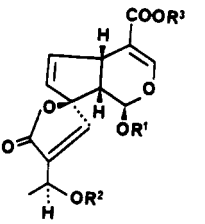
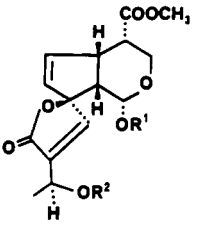


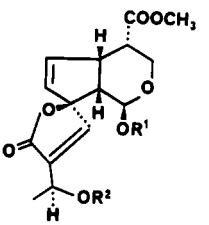
5a



7 R^1=Glc, R^2=R^3=H
8 R^1=Glc, R^2=H, R^3=Me
11 R^1=Glc, R^2=*trans*-*p*-coumaroyl, R^3=Me
Glc = glucose



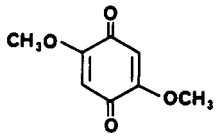
9 R^1=R^2=H
9a R^1=R^2=Ac



10 R^1=R^2=H
10a R^1=R^2=Ac

rubra bark were found to be active against the P-388 murine lymphocytic leukemia assay in vitro. As a result of bioactivity-guided fractionation experiments, four cytotoxic compounds were obtained, namely, fulvoplumierin [**1**], from the petroleum ether extract, and allamcin [**2**], allamandin [**3**], and 2,5-dimethoxy-*p*-benzoquinone [**4**] from the $CHCl_3$ extract. Of these, only compound **1** has so far been isolated from *P. rubra*.

Fulvoplumierin [**1**] was obtained in the form of red crystals, and its identity was confirmed by comparison with published data (5,9,16,22,23) and with an authentic sample. Unambiguous ^{1}H- and ^{13}C-nmr assignments for compound **1** are shown in Tables 1 and 2, respectively. In the present study, revisions are proposed for the chemical shifts of protons H-6 (δ 7.22) and H-7 (δ 7.34) of **1** when compared with published data (16), based on an observation of long range-coupling between H-7 and H-10 in the ^{1}H-^{1}H-COSY nmr spectrum of this compound. The performance of ^{1}H-^{13}C-HETCOR and selective INEPT nmr experiments permitted the assignments of the ^{13}C-nmr spectrum of compound **1** at carbons C-6, C-7, C-10, and C-13, as well as at its four quaternary carbon sites (C-4, C-5, C-8, C-9). Of significance were observations obtained during a selective INEPT study, wherein irradiation of H-3 (δ 8.28), H-6 (δ 7.22), H-7 (δ 7.34), and H-10 (δ 7.95) ($^3J_{CH}$ = 8 Hz) led to enhancements, respectively, of C-1, C-5, and C-15; C-4, C-8, and C-9; C-5, C-9, and C-10; and C-7, C-9, and C-13. In this



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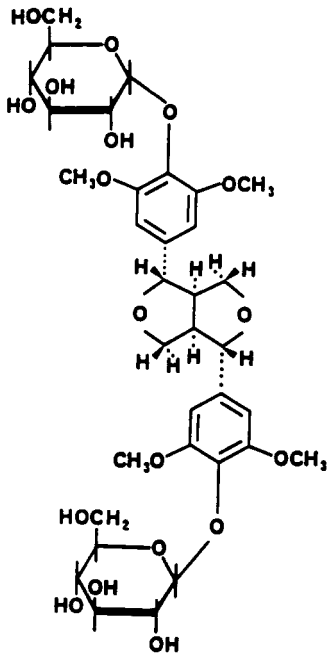

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manner, the published ^{13}C-nmr data for fulvoplumierin (16) have been modified in Table 2 for carbons C-4 through C-7, C-9 through C-11, and C-13.

Compounds **2–4** from the *P. rubra* bark $CHCl_3$ extract were established in turn as allamcin, allamandin, and 2,5-dimethoxy-*p*-benzoquinone, by comparison with published data in each case (22, 24–26). Compounds **2** and **3** were also compared with authentic samples. Allamandin [**3**] has previously been reported to exhibit cytotoxicity against the KB test system (17,24) and to possess significant P-388 antileukemic activity in vivo (24). Compound **4** is the first benzoquinone to have been isolated from this genus, and its cytotoxic activity does not appear to have been reported before. However, this compound has recently been demonstrated to inhibit cyclic adenosine 3′,5′-monophosphate phosphodiesterase (26).

Although the H_2O-soluble extract of the bark of *P. rubra* was found to be inactive when tested against the P-388 test system in vitro (ED_{50}>100 μg/ml), chromatographic fractionation of this extract led to column cuts with significant activity for P-388 cells, and, of seven isolates characterized, two known compounds were found to be cytotoxic, namely the iridoid, plumericin [**5**], and the lignan, liriodendrin [**6**]. Compound **5**, which has been previously isolated as a *P. rubra* constituent (8), was identified by data comparison with published values (8,22,27). The stereochemistry of the ethylidine group attached to the lactone ring of plumericin (and hence a distinction from its stereoisomer, isoplumericin [**5a**]), was confirmed by a ^{1}H-^{1}H-NOESY nmr experiment, in which the proximity of the H-10 proton and the Me-14 group was established. Compound **6** was identified as the symmetrically aryl-substituted furofuranoid lignan, liriodendrin, by comparison with published physical and spectroscopic data (28,29). Liriodendrin [**6**] was first isolated from *Liriodendron tulipifera* L. (28), and the ^{1}H- and ^{13}C-nmr assignments, complete stereochemistry, and in vivo P-388 antileukemic activity of this compound, were later described by Jolad *et al.* (29).

Among the constituents of the *P. rubra* H_2O-soluble extract that were not signifi-

TABLE 1. ^{1}H-nmr Assignments for Compounds **1**, **7**, **9**, **9a**, **10**, and **10a**.[a]

Proton	Compound					
	1	**7**	**9**[b]	**9a**	**10**[b]	**10a**
H-1	—	5.64 (d, 8)	5.92 (d, 3)	6.75 (d, 3)	5.32 (d, 8)	6.29 (d, 8)
H-3	8.28 (s)	7.80 (s)	4.45 (dd, 9, 4) 3.88 (dd, 9, 4)	4.06 (dd, 9, 4) 3.70 (dd, 14, 4)	3.90 (dd, 14, 4)	3.90 (dd, 14, 4)
H-5	—	4.20 (m)	3.60 (m)	3.58 (m)	3.48 (m)	3.39 (m)
H-6	7.22 (d, 5.5)	6.72 (dd, 6, 3)	6.48 (dd, 6, 3)	6.46 (dd, 6, 3)	6.25 (dd, 6, 3)	6.33 (dd, 6, 3)
H-7	7.34 (dd, 5.5, 1.5)	5.42 (d, 6)	5.65 (d, 6)	5.70 (d, 6)	5.60 (d, 6)	5.67 (d, 6)
H-9	—	3.12 (m)	2.80 (m)	3.06 (m)	2.75 (m)	2.98 (m)
H-10	7.95 (dd, 12, 1.5)	8.00 (s)	7.86 (s)	7.89 (s)	7.78 (s)	7.91 (s)
H-11	6.84 (dd, 15, 12)	—	—	—	—	—
H-13	6.67 (dq, 15, 7)	5.02 (q, 6)	5.14 (q, 6)	5.80 (q, 6)	5.05 (q, 6)	5.91 (q, 6)
H-14	2.00 (d, 7)	1.66 (d, 6)	1.75 (d, 6)	1.57 (d, 6)	1.70 (d, 6)	1.53 (d, 6)
OMe	3.81	—	3.70	3.64	3.75	3.67
OAc	—	—	—	2.04	—	1.96
	—	—	—	1.95	—	1.95
H-1′	—	5.42 (d, 8)				
H-4′, -6′	—	4.42 (m)				
H-5′	—	4.28 (m)				
H-2′	—	4.18 (m)				
H-3′	—	3.92 (m)				

[a]Recorded in $CDCl_3$ for **1** and in pyridine-d_5 for **7**, **9**, **9a**, **10**, and **10a**.
[b]Plus two drops of D_2O.

cantly cytotoxic, the novel natural product compound **7** was assigned a molecular formula of $C_{20}H_{24}O_{12}$, based on the occurrence of its protonated molecular ion at *m/z* 457 in the low resolution fab mass spectrum. This assignment was confirmed using high resolution fabms. With the exception of the lack of a methoxy group signal, the ^{1}H-nmr spectrum of **7** showed a close similarity to that of the known compound plumieride [**8**], which was obtained in this investigation as the major iridoid constituent of the *P. rubra* aqueous extract. The fact that compound **7** is the 15-demethyl derivative of **8** was suggested by analysis of its spectral data, and the ^{1}H- and ^{13}C-nmr assignments for **7** shown in Tables 1 and 2, respectively, were obtained with recourse to ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HETCOR, and selective INEPT nmr experiments. Final proof of the structure of this compound was obtained by its conversion to plumieride [**8**] by methylation with CH_2N_2 in 100% yield. Although 15-demethylplumieride ("plumieride acid") [**7**] has been produced by hydrolysis of plumieride [**8**] with

TABLE 2. ^{13}C-nmr Assignments for Compounds **1**, **7**, **9a**, and **10a**.[a]

Carbon	Compound			
	1	**7**	**9a**	**10a**
C-1	164.20	94.03	89.60	93.65
C-3	156.48	151.36	57.20	63.06
C-4	113.28	110.74	44.40	44.76
C-5	150.20	40.65	38.38	44.59
C-6	130.35	141.70	141.43	139.54
C-7	127.34	128.69	131.50	131.47
C-8	136.83	96.68	97.48	97.27
C-9	109.93	50.12	48.34	49.83
C-10	143.34	149.70	149.28	149.16
C-11	129.57	138.53	135.82	135.07
C-12	—	171.41	170.59	171.59
C-13	145.45	62.68	65.50	60.00
C-14	19.52	23.02	19.56	19.41
C-15	157.48	168.81	170.34	170.45
OMe	52.02	—	52.05	52.18
Ac	—	—	20.72	20.50
	—	—	20.64	20.43
	—	—	169.80	169.20
	—	—	169.25	169.23
C-1′	—	100.83	—	—
C-2′	—	74.73	—	—
C-3′	—	78.72	—	—
C-4′	—	70.78	—	—
C-5′	—	78.16	—	—
C-6′	—	62.10	—	—

[a]Recorded in $CDCl_3$ for **1** and in pyridine-d_5 for **7**, **9a**, and **10a**.

$Ba(OH)_2$ solution (4), it has not so far been isolated as a natural product. The only other iridoid with a free carboxylic acid group attached to C-4 that has previously been isolated from *P. rubra* is the compound β-dihydroplumericinic acid (8,30). Plumieride [**8**], a bitter-tasting iridoid that is well-known as a constituent of *P. rubra* (4, 7, 10–12), was obtained in high yield in this present investigation and identified by analysis of its physical and spectral parameters (4, 7, 10–12, 19, 22, 25). When the melting

TABLE 3. Evaluation of the Cytotoxic Potential of Isolates.[a]

Compound	Cell Lines[b]						
	A	B	C	D	E	F	G
Fulvoplumierin [**1**]	3.4	0.7	3.5	3.0	1.3	4.6	2.5
Allamcin [**2**]	3.1	0.9	0.1	1.2	0.3	0.3	0.2
Allamandin [**3**]	0.8	0.3	0.4	0.7	0.3	0.4	0.2
2,5-Dimethoxy-*p*-benzoquinone [**4**]	3.4	2.7	5.2	1.3	1.4	3.8	2.5
Plumericin [**5**]	0.2	2.8	0.4	0.2	0.1	0.3	1.2
Liriodendrin [**6**]	8.9	19	30	6.0	16	6.0	2.4

[a]Results are expressed as ED_{50} values (μg/ml).

[b]Key: A, Fibrosarcoma; B, Melanoma; C, Breast Cancer; D, Lung Cancer; E, Colon Cancer; F, KB; G, P-388.

point, optical rotation, and spectroscopic data obtained for compound **11** were compared with literature values (11,12,14,19), this known *P. rubra* constituent with algicidal and plant growth inhibitory activities (11,14,18,19), was identified as 13-*O*-*trans*-*p*-coumaroylplumieride.

The epimeric α-allamcidin [**9**] and β-allamcidin [**10**] were separated from a chromatographic column cut by preparative tlc, and all of the usual physical and spectroscopic parameters were measured with the exception of ^{13}C-nmr data, which was not possible because of the inherent instability of these isolates. Compound **9** and **10** have previously been isolated only as a mixture, and were then separated and individually characterized on acetylation (25). In the present study, spectroscopic data of the acetylated products of **9** and **10** were compared with published values for α- and β-allamcidin diacetates (**9a** and **10a**, respectively), and α-allamcidin diacetate [**9a**] was confirmed by direct comparison with an authentic sample. However, no definite stereochemistry has been proposed in the past for the C-15 carboxymethyl group of either α-allamcidin [**9**] or β-allamcidin [**10**] (25). In the ^{1}H-nmr spectrum of α-allamcidin diacetate [**9a**], the H-1 proton appeared at δ 6.75 (J = 3 Hz), confirming that H-1 and H-9 are cis oriented. Cross peaks of H-1 and H-9, H-9 and H-5, and H-5 and H-4 were observed in the ^{1}H-^{1}H NOESY spectrum, suggesting that the C-15 carboxymethyl group is in the α orientation in compound **9**. Compound **10** was confirmed as β-allamcidin, by comparison of the ^{1}H- and ^{13}C-nmr spectra of its diacetate with literature values for compound **10a** (25). The H-1 proton resonating at δ 6.29 (J = 8 Hz) suggested that H-1 and H-9 were positioned with a trans relationship. Similarly, when a ^{1}H-^{1}H NOESY experiment was performed for compound **10a**, cross-peak correlations were observed for H-9 and H-5 and for H-5 and H-4, again confirming that C-15 carboxymethyl group at C-4 was present in the α configuration. In this manner, unambiguous structural assignments have been made for the first time for α-allamcidin [**9**] and β-allamcidin [**10**].

The cytotoxic activity of compounds **1–6** against a number of human cancer cell lines (fibrosarcoma, melanoma, breast cancer, lung cancer, colon, and KB) is shown in Table 3. Also shown in this table, for the purposes of comparison, are the activities of these compounds against P-388 cells in culture. All of these compounds **1–6** demonstrated general cytotoxic activity; no cell type specificity was discernible. Compounds **7–11** were also evaluated for cytotoxic potential with this battery of cell lines and found to be inactive (ED_{50}>50 μg/ml). Based on this information, some speculation is possible concerning structure-activity relationships. For example, it appears likely that the exo-ethylidene group attached to the C-11 position of active compounds **2**, **3**, and **5** is required for activity, given the otherwise structural homology of inactive compounds **7–11**. However, other structural dissimilarity (e.g., the double bond between C-10 and C-11, the oxo-bridge linking C-1 and C-10) may also modulate activity. Neither the carbomethoxy nor the hydroxy substituent (located at positions 3 or 4) appears to be required for activity, since there are examples of equipotent compounds that do not bear these groups. Compound **4** also demonstrated non-specific cytotoxic activity, but this may be contrasted with other structurally uncomplicated benzoquinones capable of demonstrating specificity with cultured melanoma cells (31).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points, optical rotations, and uv, ir, nmr and low-resolution mass spectra were obtained as described previously (32). For selective INEPT and CSCM-1D nmr experiments (Nicolet NT-360 spectrometer), C-H long-range coupling parameters were 8 Hz (olefinic protons) or 6 Hz (methine protons). High-resolution fab mass spectra were obtained using a Finnegan MAT 90 instrument.

PLANT MATERIAL.—The stem bark of *P. rubra* (2.5 kg) was collected in Bandung, Indonesia, and identified by one of us (K.P.). A voucher specimen (No. 9273) has been deposited in the Herbarium of Department of Biology, Bandung Institute of Technology, Bandung, Indonesia.

EXTRACTION AND FRACTIONATION.—The air-dried, milled plant material (2.5 kg) was defatted with petroleum ether (3 × 5 liters) to afford 40 g of a petroleum-ether-soluble extract on drying (P-388, ED_{50} 18 μg/ml). The marc was extracted with MeOH (4 × 5 liters) to afford an initial MeOH extract (350 g) on removal of solvent in vacuo. A portion (295 g) of the dried MeOH extract was partitioned between $CHCl_3$ and H_2O (5 liters of each) and yielded, on drying, 70 g of an $CHCl_3$-soluble extract (P-388, ED_{50} 5 μg/ml), 225 g of an H_2O-soluble extract (P-388, ED_{50} >100 μg/ml), and 40 g of interfacial material (P-388, ED_{50} >50 μg/ml).

ISOLATION AND CHARACTERIZATION OF ISOLATES.—A portion of the petroleum-ether-soluble extract (30 g) was subjected to flash cc over Si gel (750 g, 230–400 mesh), using $CHCl_3$-petroleum ether (1:1) as solvent. Altogether, 9 fractions (F001–F009) were collected and combined on the basis of similar tlc profiles. Fraction F002 (200 mg) was further purified over Si gel (120 g), with $CHCl_3$-MeOH (99:1) as solvent, to afford fulvoplumierin [**1**], which was recrystallized from petroleum ether-$CHCl_3$ (1:1) as red crystals (25 mg, 0.001% w/w), and exhibited mp 150–151° [lit. (5) 151–152°] and spectroscopic data comparable to literature values (5,9,16,22,23). The identity of this isolate was confirmed by direct comparison (mmp, ^{1}H nmr, ms, co-tlc) with an authentic sample donated by Dr. M. Alam. Unambiguous ^{1}H- and ^{13}C-nmr values for fulvoplumierin [**1**] are shown in Tables 1 and 2, respectively.

A portion of the $CHCl_3$-soluble extract (60 g) was subjected to gravity-column cc over Si gel (1.5 kg), using $CHCl_3$ and $CHCl_3$-MeOH mixtures of increasing polarity as solvent. A total of 8 combined fractions were collected, with cytotoxic activity concentrated in F012 (P-388, ED_{50} 12 μg/ml) and F014 (P-388, ED_{50} 8 μg/ml). Further purification of F012 (800 mg) by cc over Si gel (600 g), with petroleum ether-$CHCl_3$-EtOAc (1:3:1) as solvent, afforded allamandin [**3**] and 2,5-dimethoxy-*p*-benzoquinone [**4**], in fractions F066 and F075, respectively. Allamandin [**3**] was recrystallized as white crystals from $CHCl_3$ (12 mg, 0.00048% w/w), and exhibited mp 233–235°, $[\alpha]^{25}D$ +20° (*c* = 0.06, pyridine) [lit. (24) mp 212–215°, $[\alpha]^{21}D$ +15° (*c* = 0.06, MeOH)], and spectroscopic data comparable to literature values (22,24). 2,5-Dimethoxy-*p*-benzoquinone [**4**] was recrystallized as flat yellow crystals from $CHCl_3$ (12 mg, 0.00048% w/w), and exhibited mp 222–223° [lit. (26) 225° (dec)], and was identified by interpretation of its spectroscopic data. Fraction F014 (1.2 g) was further purified by cc over Si gel (400 g), using as solvent system EtOAc-MeOH (97:3), to afford allamcin [**2**], which was recrystallized as white needle crystals from $CHCl_3$ (9 mg, 0.00036% w/w) and exhibited mp 208–210°, $[\alpha]^{25}D$ +66° (pyridine, *c* = 0.06) [lit. (25) mp 198–200°, $[\alpha]^{20}D$ +65.6° (*c* = 0.06, pyridine)], and spectroscopic data comparable to published values (25). Confirmation of the identities of **2** and **3** as allamcin and allamandin, respectively, was obtained by direct comparison (mmp, ^{1}H-nmr, ms, co-tlc) with authentic samples provided by Prof. T. Yamauchi.

A portion of the H_2O-soluble extract (200 g) was subjected to gravity-column cc over Si gel (2 kg), using $CHCl_3$ and mixtures of $CHCl_3$ and MeOH of increasing polarity as solvents, to afford a total of 12 combined fractions (F018–F029). The fractions that were determined active were F018 (P-388, ED_{50} 12 μg/ml), F019 (P-388, ED_{50} 14 μg/ml), and F022 (P-388, ED_{50} 18 μg/ml). Further purification of F018 (800 mg) over Si gel (400 g), using EtOAc and EtOAc/MeOH mixtures of increasing polarity as solvents, afforded plumericin [**5**] in EtOAc-MeOH (95:5); it was recrystallized as needle crystals in EtOAc (18 mg, 0.0007% w/w) and exhibited mp 195°, $[\alpha]^{25}D$ +200° (*c* = 0.1, $CHCl_3$) [lit. (8,22) mp 211.5–212.5°, [α] +197.5° (*c* = 0.982, $CHCl_3$)] and spectroscopic data comparable to published values (8,22,27). F019 was separated by additional cc over Si gel (250 g), with EtOAc-$CHCl_3$-MeOH (6:6:1) as solvent system, to afford an unstable cytotoxic iridoid aldehyde, which has not been completely characterized, as well as a mixture of α- and β-allamcidin, which was resolved by preparative tlc on Si gel G plates (20 × 20 cm, 250 μm, Merck, Darmstadt, W. Germany), using EtOAc-$CHCl_3$-MeOH (3:3:1) as solvent. Pure α-allamcidin [**9**] (R_f 0.6, 12 mg, 0.00048% w/w) and β-allamcidin [**10**] (R_f 0.5, 16 mg, 0.00064% w/w) were obtained on treatment with $CHCl_3$ as prism crystals and as a powder, respectively. Plumieride [**8**] (55 g, 2.20% w/w) was crystallized directly as prisms from F022, using MeOH with a few drops of $CHCl_3$, and exhibited mp 234° $[\alpha]^{25}D$ −80° (*c* = 0.2, MeOH) [lit. (4,22) mp 224–225°, $[\alpha]^{16}D$ −114° (*c* = 0.54, H_2O)] and spectroscopic data comparable to literature values (4, 7, 10–12, 19, 22, 25). Further purification of F022 (29 g) by gravity cc over Si gel, using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity, afforded the active compound liriodendrin [**6**], the new natural product 15-demethylplumieride [**7**], and 13-*O*-*trans*-*p*-coumaroylplumieride [**11**], which were eluted with $CHCl_3$-MeOH (9:1), $CHCl_3$-MeOH (8:2), and $CHCl_3$-MeOH (7.5:2.5), respectively. Liriodendrin [**6**] was crystallized from MeOH as white crystals (50 mg, 0.02% w/w) and exhibited mp 270°, $[\alpha]^{25}D$ −12.0° [*c* = 0.1, $CHCl_3$-MeOH (1:1)] [lit. (29) mp 265–266°, $[\alpha]^{25}D$ −12.1° (*c* = 0.596, pyridine)] and spectroscopic data comparable to literature values (29). 13-*O*-*trans*-*p*-Coumaroylplumieride [**11**] was obtained as an amorphous solid (3 g,

0.12% w/w), and exhibited $[\alpha]^{25}D$ −57° (*c* = 1.0, MeOH) [lit. (11) $[\alpha]D$ −58.3° (*c* = 1.0, MeOH)] and spectroscopic data comparable to literature values (11,12,14,19).

15-Demethylplumieride [**7**] was crystallized as white cubic crystals from MeOH-$CHCl_3$ (1:1) and exhibited the following data: mp 230–232°; $[\alpha]^{25}D$ −120° (*c* = 0.1, H_2O); uv λ max (log ε) (MeOH) 219 nm (3.5); ir ν max (KBr) 3435–3545 (broad), 1769, 1689, 1117, 1708, 1039, 1006 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms (glycerol) *m/z* $[M+1]^+$ 457, hr fabms mass measurement found 457.1347, calcd for $C_{20}H_{25}O_{12}$, 457.1346. When 20 mg of compound **7** was methylated with CH_2N_2 using the Diazald Kit®, 18 mg of a product was obtained that was identical (mmp, ^1H nmr, ms, co-tlc) with plumieride [**8**].

α-Allamcidin [**9**] was crystallized from $CHCl_3$ as prisms (12 mg, 0.00048% w/w) and exhibited mp 180°, $[\alpha]^{25}D$ +36° (*c* = 0.1, MeOH); uv λ max (log ε) (MeOH) 211 nm (4.25); ir ν max (KBr) 3400, 1756, 1731, 1231, 1125, 1120, 1023, 877 cm^{-1}; ^1H nmr see Table 1; eims (70 eV) *m/z* $[M]^+$ 310 (4%), 292 (5), 279 (8), 246 (40), 233 (35), 187 (70), 160 (100), 98 (18). These eims data are closely comparable to analogous values obtained for an unresolved mixture of α-allamcidin [**9**] and β-allamcidin [**10**] from *Allamanda neriifolia* Hook. (25). On conversion to α-allamcidin 1,13-diacetate [**9a**] (8 mg) by treatment of 9 mg of **9** with pyridine-Ac_2O (4:1) (1 ml), a product was obtained, mp 120°, $[\alpha]^{25}D$ +36 (*c* = 0.1, $CHCl_3$), that exhibited spectroscopic values (uv, ir, ^1H-nmr, ^{13}C-nmr, ms) closely comparable to published values (25) for this compound, as well as to an authentic sample donated from Prof. T. Yamauchi. Certain ^1H- and ^{13}C-nmr reassignments for compound **9a** are shown in Tables 1 and 2, respectively.

β-Allamcidin [**10**] was obtained from $CHCl_3$ as a powder (16 mg, 0.00064% w/w) and exhibited mp 165°; $[\alpha]^{25}D$ +38° (*c* = 0.1, MeOH); uv λ max (log ε) (MeOH) 211 nm (4.05); ir ν max (KBr) 3400, 1758, 1731; 1129, 1120, 1007, 787 cm^{-1}; ^1H nmr see Table 1; eims (70 eV) *m/z* $[M]^+$ 310 (1%), 292 (1), 279 (2), 247 (11), 219 (4), 201 (7), 187 (100), 160 (4), 115 (16). On conversion to β-allamcidin 1,13-diacetate [**10a**] (10 mg) by treatment of 12 mg of **10** with pyridine-Ac_2O (4:1) (1 ml), a product was obtained, mp 160°, $[\alpha]^{25}D$ +40° (*c* = 0.1, $CHCl_3$), that exhibited spectroscopic values (uv, ir, ^1H-nmr, ^{13}C-nmr, ms) closely comparable to published data for this compound (25). Some ^1H- and ^{13}C-nmr reassignments for compound **10a** are shown in Tables 1 and 2, respectively. These reassignments were made after performing ^1H-^1H COSY, ^1H-^{13}C HETCOR, and CSCM-1D experiments.

CYTOTOXIC ASSAY.—Extracts, fractions and compounds were evaluated for cytotoxic potential essentially by established procedures (33) as described previously (34–36). In addition to the standard KB and P-388 cell lines, the fibrosarcoma line used in this study was obtained from the ATCC (HT-1080). The remaining cell lines were established from primary human tumors in the Division of Surgical Oncology, University of Illinois at Chicago. A complete description of these cell lines and their responsiveness to a wide range of natural products will be presented elsewhere.

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