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NEW LIGNANS FROM *ANOGEISSUS ACUMINATA* WITH HIV-1 REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY

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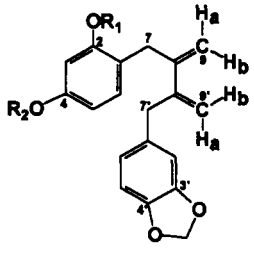
ABSTRACT.—Anolignan A [**1**] and anolignan B [**3**] are new dibenzylbutadiene lignans isolated from *Anogeissus acuminata*. Compounds **1** and **3** were identified as the active HIV-1 reverse transcriptase (RT) inhibitory constituents of this plant obtained by bioassay-guided fractionation. Compound **3**, which was very weakly active when tested alone, showed high activity when combined with **1**. The activity of **1** was likewise enhanced in the presence of **3**. A concave isobole obtained from a plot of data derived from assays with **1** and **3** in combination indicated their synergistic effects. Another new lignan, anolignan C [**5**], and a known lignan, (−)-secoisolariciresinol [**10**], were also isolated from this plant. Compounds **5** and **10** did not have activity against HIV-1 RT. Compounds **1**, **3** and **5** were either weakly cytotoxic or noncytotoxic when tested in various cancer cell lines. The structures of **1**–**5** and **10** were established by spectroscopic methods, especially by 1D and 2D nmr experiments.

Anogeissus acuminata (Roxb. ex DC.) Guill. & Perr. var. *lanceolata* Wall ex C.B. Clarke (Combretaceae) is one of two varieties of *A. acuminata* (1). This variety is indigenous in Bangladesh, Burma, Cambodia, India, Thailand, and Vietnam. The plant material used for this study was collected in Thailand. There are no known folkloric uses nor are there any reports on biological activity of extracts of this species. However, in a general screening of several plant extracts for activity against HIV-1 RT, the MeOH extract of the stems of this variety showed high activity. A detailed bioassay-directed fractionation was therefore pursued to isolate the active constituent(s). After a series of Si gel chromatographic separations and a final prep. tlc step, **1** and **3** were identified as the HIV-1 RT inhibitory constituents of *A. acuminata* var. *lanceolata* stems.

RESULTS AND DISCUSSION

The hreims of **1** established the molecular formula as $C_{19}H_{18}O_4$. The 19 carbon resonances displayed in the ^{13}C-nmr spectrum of **1** were determined from APT and HETCOR methods to consist of eight C, six CH, and five CH_2 carbons. The ^{1}H-nmr spectrum of **1** indicated the presence of six aliphatic, four vinyl, and six aromatic protons. The resonances at δ 3.43 and 3.47 (each 2H, s) which, in the HETCOR spectrum, showed correlation peaks with CH_2 carbons at δ 34.10 and 41.29, respectively, were typical resonances for benzylic methylene protons (H-7 and H-7′, respectively) (Table 1). The proton signal at δ 5.81 (2H, s), which showed a correlation peak with a CH_2 carbon at δ 101.87, indicated the presence of methylenedioxy protons. The resonances at δ 5.27, 5.15, 4.83, and 4.79 (each 1H, s) represented vinyl protons. In the HETCOR spectrum, the proton resonances at δ 4.79 and 5.15 showed correlation peaks with a CH_2 carbon at δ 114.90, while the proton resonances at δ 4.83 and 5.27 had correlation peaks with a CH_2 carbon at δ 115.30. These four vinyl protons were determined to be terminal methylene protons. The two upfield vinyl proton signals (δ 4.79 and 4.83, H-9a and H-



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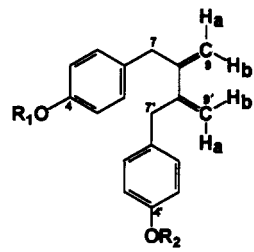
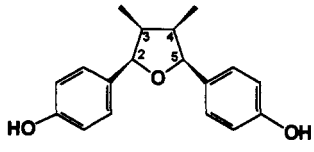
2 $R_1=R_2=CH_3$



3 $R_1=R_2=H$
4 $R_1=R_2=CH_3$



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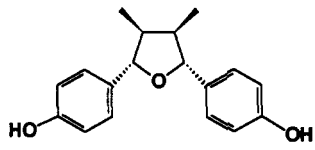

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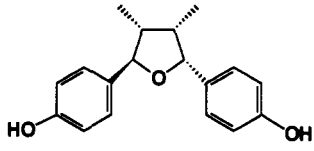

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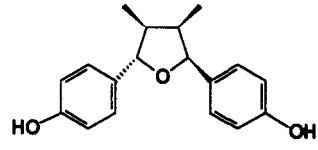

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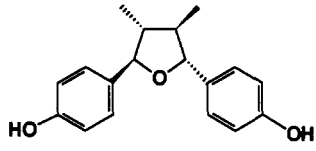

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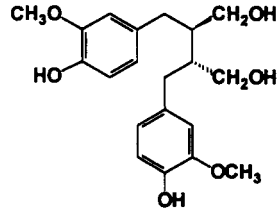


10

9′a, respectively) were cis while the two downfield signals (δ 5.15 and 5.27, H-9b and H-9′b, respectively) were trans to the benzyl moiety. These assignments were confirmed from a 2D-nOe experiment that showed the proton at δ 3.43 (H-7) to have cross-peaks with the protons at δ 4.79 (H-9a) and 5.27 (H-9′b); and the proton at δ 3.47 (H-7′) to exhibit cross-peaks with the protons at δ 4.83 (H-9′a) and 5.15 (H-9b) (Figure 1). In the aromatic region, the peaks at δ 6.70 (1H, d, J=8.2 Hz), 6.29 (1H, d, J=2.4 Hz), and 6.19 (1H, dd, J=8.2 and 2.4 Hz) demonstrated an ABX system (H-3, H-5, and H-6, respectively). Another cluster of peaks, which integrated for 3 protons, was also observed in the aromatic region. This consisted of a doublet at δ 6.65 (H-5′), a singlet at δ 6.60 (H-2′), and what seemed to be a doublet at δ 6.58–6.60 (H-6′), which was overlapped by the singlet. On methylation of **1** with CH_2N_2, this cluster of aromatic proton signals was shown to be due to another ABX system, i.e., the ^{1}H-nmr spectrum of the methylated product **2** showed resonances at δ 6.72 (1H, d, J=7.8 Hz, H-5′), 6.66 (1H, d, J=1.6 Hz, H-2′), and 6.63 (1H, dd, J=7.8 and 1.6 Hz, H-6′). The ^{1}H-nmr spectrum of **2** also showed two aromatic methoxy peaks at δ 3.79 and 3.80 (each 3H, s), which

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for Anoligans A [**1**] and B [**3**].

Position	Anolignan A			Anolignan B		
	δ ^{13}C[a]	δ ^{1}H[b] (*J* in Hz)	FLOCK[a]	δ ^{13}C[a,c]	δ ^{1}H[a,c] (*J* in Hz)	FLOCK[a]
1	118.57			132.01		
2	156.45			130.72	6.92 (2H, d, 8.5)	C-4, C-6
3	103.18	6.29 (1H, d, 2.4)	C-1, C-5	115.90	6.67 (2H, d, 8.5)	C-1, C-4, C-5
4	157.28			156.43		
5	107.37	6.19 (1H, dd, 8.2 and 2.4)	C-1, C-3	115.90	6.67 (2H, d, 8.5)	C-1, C-3
6	131.53	6.70 (1H, d, 8.2)	C-2, C-4	130.72	6.92 (2H, d, 8.5)	C-2, C-4
7	34.10	3.43 (2H, s)	C-1, C-2, C-6, C-9, C-8′	40.87	3.47 (2H, s)	C-1, C-2, C-6, C-9, C-8′
8	147.18			147.83		
9	114.90	4.79 (1H, s, 9a) 5.15 (1H, s, 9b)	C-7, C-8′	115.44	4.85 (1H, s, 9a) 5.22 (1H, s, 9b)	C-7, C-8′
1′.....	135.20					
2′.....	110.02	6.59 (1H, br s)	C-4′, C-6′			C-4′, C-6′
3′.....	148.79					C-1′, C-4′, C-5′
4′.....	146.98					
5′.....	108.72	6.57 (1H, d, 7.7)	C-1′, C-3′			C-1′, C-3′
6′.....	122.64	6.58–6.59 (1H, m)	C-2′, C-4′			C-2′, C-4′
7′.....	41.29	3.47 (2H, s)	C-8, C-1′, C-2′, C-6′, C-9′			C-1′, C-2′, C-6′, C-9′, C-8
8′.....	147.53					
9′.....	115.30	4.83 (1H, s, 9′a) 5.27 (1H, s, 9′b)	C-8, C-7′			C-7′, C-8
OCH_2O	101.87	5.81 (2H, s)	C-3′, C-4′			

[a]Recorded at 300 MHz in CD_3OD.
[b]Recorded at 360 MHz in CD_3OD.
[c]^{1}H- and ^{13}C-nmr signals of 1′–9′ are the same as those of 1–9, respectively.

indicated the presence of two phenolic hydroxy groups in **1**. Compound **1** was thus determined as 2-(2′,4′-dihydroxybenzyl)-3-(3″,4″-methylenedioxybenzyl)-1,3-butadiene. The structure of **1** was confirmed by a FLOCK 2D long-range heteronuclear shift correlation nmr experiment (2) (Table 1). Compound **1** is a novel dibenzylbutane lignan bearing a conjugated double bond at positions 9 and 8′ and has been named anolignan A.

Very close to **1** on tlc was **3**, but these two compounds were separated by prep. tlc. The ^{1}H-nmr spectrum of **3** displayed two doublets in the aromatic region at δ 6.92 and 6.67, two vinyl singlets at δ 5.22 and 4.85, and an aliphatic singlet at δ 3.47 (Table 1). The ^{13}C-nmr spectrum of **3** exhibited only seven resonances, but two peaks (δ 130.72 and 115.90) were significantly more intense than the rest of the peaks, and represented two carbons each. Therefore, there appeared to be nine carbons in **3**. The HETCOR spectrum of **3** showed a correlation peak between the intense carbon peak at δ 130.72 (C-2/C-6) and the aromatic doublet at δ 6.92 (H-2/H-6), and between the other intense carbon at δ 115.90 (C-3/C-5) and the aromatic doublet at δ 6.67 (H-3/H-5). These correlations indicated an AA′BB′ system in a para-disubstituted benzene ring. The benzylic singlet at δ 3.47 showed a correlation with the CH_2 (determined from APT) carbon at δ 40.87

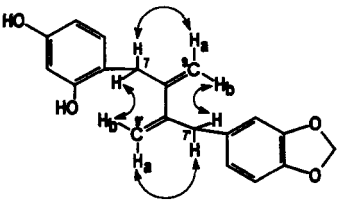


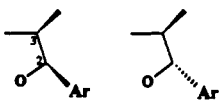
FIGURE 1. Significant NOe Correlations of **1**.

in the HETCOR spectrum. The two vinyl singlets at δ 5.22 and 4.85 showed a correlation peak with the methylene carbon at δ 115.44. As in **1**, the upfield vinyl proton δ 4.85 (H-9a) was cis, while the downfield proton δ 5.22 (H-9b) was trans to the benzyl moiety. On methylation of **3**, an aromatic methoxy resonance at δ 3.77 (3 or 6H, s) appeared in the ^{1}H-nmr spectrum of the methylated product [**4**], and indicated that **3** had one or more phenolic hydroxy substituents. From the eims of **3**, which displayed a molecular ion peak at *m/z* 266, it was deduced that **3** was a dimer. The hrms of **3** established the molecular formula of $C_{18}H_{18}O_2$. The structure of **3** was confirmed from a FLOCK (2) experiment (Table 1). Compound **3**, 2,3-*bis*-(4-hydroxybenzyl)-1,3-butadiene, is also a new dibenzylbutadiene lignan. It is structurally similar to **1**, and has been named anolignan B.

Compound **5** was isolated from a Si gel cc fraction (fraction C22-C24) containing **1** and **3** (see Experimental). Analysis of the ^{1}H- and ^{13}C-nmr spectra of **5**, and a molecular ion peak at *m/z* 284 obtained from eims, indicated **5** to have a symmetrical molecular structure. Five signals were observed in its ^{1}H-nmr spectrum: a methyl doublet at δ 0.56 (J=7.0 Hz), a methine multiplet at δ 2.60 (H-3, H-4), an oxymethine doublet at δ 5.06 (J=6.6 Hz, H-2/H-5), and two doublets in the aromatic region at δ 6.79 (J=8.6 Hz; H-3′/H-5′, H-3″/H-5″) and 7.23 (J=8.6 Hz, H-2′/H-6′, H-2″/H-6″). The ^{13}C-nmr spectrum of **5** displayed seven resonances, two of which (δ 115.73 and 128.68) represented two (four) carbons each as inferred from their peak intensities. The HETCOR spectrum revealed a correlation of the intense signal at δ 115.73 (C-3′/C-5′, C-3″/C-5″) with the aromatic doublet at δ 6.79, and a correlation of the intense signal at δ 128.68 (C-2′/C-6′, C-2″/C-6″) with the doublet at δ 7.23, which suggested an AA′BB′ system in a para-disubstituted benzene ring. Compound **5** was determined to be a 2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran lignan. The structure of **5** was confirmed from FLOCK and selective INEPT experiments, and the molecular formula $C_{18}H_{20}O_3$ was established from hreims.

The symmetry shown by the ^{1}H- and ^{13}C-nmr spectra, and the optical inactivity of **5**, suggested that **5** is a meso isomer. There are two meso isomers of 2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran lignan (**5** and **6**). These two isomers can be distinguished from each other because of the difference in the orientation of the methyl and aryl substituents of the tetrahydrofuran ring. The chemical shift values of the oxymethine (H-2/H-5) and methyl protons, and of the carbons bearing the oxymethine protons (C-2/C-5) are diagnostic in determining the cis/trans relationship between the methyl and the aryl substituents (Table 2) (3–8). The methyl and the oxymethine protons of **5** showed signals at δ 0.56 and 5.06, respectively, which indicated that the methyl and aryl groups were in a cis relationship. The resonance of C-2/C-5 of **5** at δ 84.2 also gave evidence to the cis-orientation of the methyl and aryl groups. The chemical shift

TABLE 2. Comparison of the Chemical Shifts of H-2, CH_3, and C-2 in 2,3-*cis*- and 2,3-*trans*-2-Aryl-3-methyltetrahydrofuran.[a]



Position	2,3-*cis*-	2,3-*trans*-	Obtained
H-2	5.0–5.5 ppm	4.4–4.7 ppm	5.06 ppm
CH_3	0.5 ppm	1.0 ppm	0.56 ppm
C-2	82–83 ppm	87–88 ppm	84.2 ppm

[a]Data are from various authors (3–8).

of C-3/C-4 at 42.7 ppm was also in agreement with a cis-orientation of the methyl groups; C-3/C-4 resonate at δ 41–44 ppm in cis- and at δ 47–50 ppm in trans-oriented methyl groups (3,9,10). That the optical inactivity may be due to a racemic mixture of isomers not having an all-cis substitution has been ruled out on the basis of the ^{1}H- and ^{13}C-nmr chemical shift values obtained for **5**. Compound **5** was therefore determined to be the all-cis mesoisomer 2,3-*cis*-3,4-*cis*-4,5-*cis*-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran lignan. Four isomers of this group of lignans have been isolated previously from plants, namely, (2α,3β,4β,5α)-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran [**6**] (11); 2,3-*trans*-3,4-*cis*-4,5-*cis*-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran [**7**] (12–14) and its enantiomer [**8**] (11,15); and 2,3-*trans*-3,4-*trans*-4,5-*trans*-2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran [**9**] (12,13). This is, however, the first isolation of the all-cis isomer of 2,5-*bis*-(4-hydroxyphenyl)-3,4-dimethyltetrahydrofuran, which has been named anolignan C.

Compound **10** was also isolated from fractions C22 through C24. The spectral data of **10** were in agreement with literature data on (−)-secoisolariciresinol (16). The optical activity of **10**, $[\alpha]_D$ −9.5° (*c*=0.20, MeOH), was similar to that reported for (−)-secoisolariciresinol isolated from *Salvia plebeia*, $[\alpha]_D$ −7° (*c*=0.12, Me_2CO) (17). However, earlier publications reported higher optical activity for the same compound, viz., $[\alpha]_D$ −30.8° (*c*=1.0, Me_2CO) (18), and $[\alpha]_D$ −35.6° (*c*=1.07, Me_2CO) (19).

Compounds **1** and **3** were identified as the HIV-1 RT inhibitory constituents through a bioassay-directed fractionation procedure. Compounds **1** and **3** were found to have potentiating effects for each other from assays consisting of a combination of **1** and **3** in varying proportions (100:0, 75:25, 50:50, 25:75, 0:100) (Table 3). The kind of interaction existing between **1** and **3** was shown by constructing an isobologram for 33% inhibition of enzyme activity, viz., 33% inhibition was obtained with ca. 40 μg/ml of pure **1**, ca. 48 μg/ml of a 75:25, ca. 50 μg/ml of a 50:50, and ca. 58 μg/ml of a 25:75 mixture (which consisted of 36:12, 25:25, and 14.5:43.5 μg/ml of **1:3**, respectively), and ca. 200 μg/ml of pure **3**. A plot of these points gave a concave isobole (Figure 2) which indicated synergism (20,21). An isobologram constructed for 50% inhibition (IC_{50}) of enzyme activity (Table 4) also showed a concave isobole (Figure 3), which furthermore demonstrated synergism between **1** and **3**.

Methylation of **1** caused a significant decrease in HIV-1 RT inhibitory activity, while methylation seemed to enhance the activity of **3** (data not presented). These observations cannot be taken as conclusive, however, because insufficient data are

TABLE 3. Percent Inhibition of HIV-1 Reverse Transcriptase Activity by Anolignan A [**1**] and Anolignan B [**3**] (Alone and in Combination).[a]

Conc. (μg/ml)	Ratio of **1:3**				
	Anolignan A [**1**]	75:25	50:50	25:75	Anolignan B [**3**][b]
250	97.4±1.6	98.8±1.2	94.1±2.7	68.3±1.0	—
200	92.8±4.2	96.1±0.6	85.7±0.1	62.1±2.1	32.9±2.5
150	80.8±7.8	92.1±2.6	78.8±7.5	50.2±2.7	31.2±5.4
100	73.9±5.1	73.7±5.1	62.1±6.3	41.4±4.3	27.4±6.1
80	68.3±4.9	58.3±3.4	50.5±3.1	40.7±4.8	20.9±4.2
60	51.5±4.1	43.5±4.4	38.7±2.0	34.2±2.3	25.5±3.6
40	33.8±2.9	26.4±1.6	27.2±3.6	17.1±3.0	17.7±3.8
20	4.1±3.7	10.2±2.8	9.9±1.8	5.6±2.0	10.0±2.7

[a]Average of three experiments, each experiment in duplicate.

[b]At 250 μg/ml, anolignan B caused precipitation in the assay mixture, and results obtained could not be considered reliable.

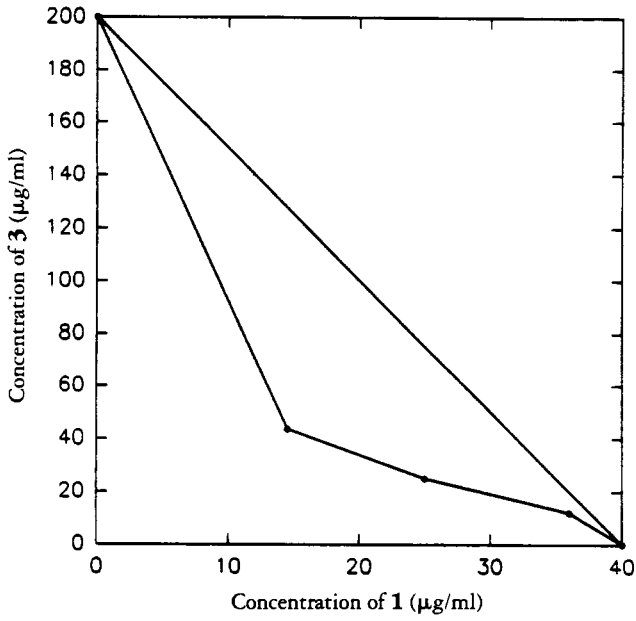


FIGURE 2. Isobologram Showing Synergism between **1** and **3** in Inhibiting HIV-1 RT Activity by 33%.

available to draw a definite structure-activity relationship. Compounds **5** and **10** did not show HIV-1 RT activity.

The activities of **1–5** and **10** against HIV-2 RT were also tested; only **1** showed activity (IC_{50} 156.9 μg/ml). Compound **1** also showed moderate inhibitory activity against a drug-resistant form of HIV-1 RT (IC_{50} 106.0 μg/ml).

Compounds **1**, **3**, **5**, and **10** were also tested for in vitro cytotoxic activity against a variety of cancer cell lines. Compound **10** did not show any cytotoxic activity. Compound **5** showed specific, but only moderate, cytotoxicity against hormone-dependent breast cancer cells. Compounds **1** and **3** did not show significant cytotoxicity in any of the cell lines, with an ED_{50} value ≤ 4 μg/ml being taken as a significant cytotoxic activity (Table 5).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were measured on a Kofler hot-stage apparatus. Uv absorption spectra were obtained using a Beckman DU-7 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Ir spectra were measured in a Midac Collegian Ft-ir Spectrometer. Eims (70 eV) and cims (CH_4 reagent gas) were recorded with a Varian MAT 112S instrument. Hreims spectra were obtained on a Finnigan MAT-90 instrument. 1D-^{1}H- and ^{13}C-nmr experiments were run on either a Varian XL-300 or a Nicolet NT-360 spectrometer. All 2D nmr experiments were conducted on a Varian XL-300 spectrometer using standard pulse sequences.

TABLE 4. HIV-1 RT 50% Inhibitory Concentrations (μg/ml) of Anolignans A [**1**] and B [**3**] (Alone and in Combination).

Compound	Alone	Ratio of **1:3**		
		75:25	50:50	25:75
1	60.4	46.7	36.2	32.1
3	1,072	15.6	36.2	96.3

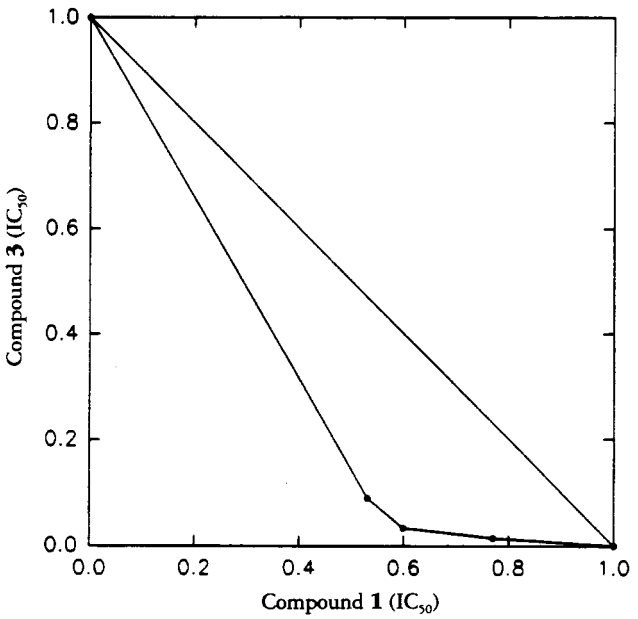


FIGURE 3. Isobologram Showing Synergism Between **1** and **3** in Inhibiting HIV-1 RT Activity by 50%. IC_{50} Values of **1** and **3** Alone Were Normalized to One Unit of IC_{50}. In Each Combination, IC_{50} Values Were Plotted as Fractional IC_{50}s.

PLANT MATERIAL.—*A. acuminata* var. *lanceolata* stems were collected in Thailand. Plant identification was provided by one of us (T.S.). Voucher specimens were deposited at the Forest Herbarium of the Royal Forest Department, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—Ground stems of *A. acuminata* var. *lanceolata* (17 kg) were extracted three times with MeOH (80 liters×3). The dried MeOH extract was partitioned between $CHCl_3$ and H_2O.

TABLE 5. Cytotoxic Activity of New Lignans Isolated from *Anogeissus acuminata*.

Cell Line[a]	Compound (ED_{50} μg/ml)		
	Anolignan A [**1**]	Anolignan B [**3**]	Anolignan C [**5**]
BC-1	4.3	5.1	>20
HT-1080	13.1	4.7	>20
LU-1	15.8	11.1	>20
MEL-2	7.6	19.0	>20
COL-1	16.8	18.0	>20
KB	>20	>20	>20
KB-V1 (+VLB)	>20	>20	>20
KB-V1 (−VLB)	>20	>20	>20
P388	>20	1.0	>20
A431	>20	>20	>20
LNCaP	10.8	8.7	>20
ZR-75-1	6.1	>20	9.5
U373	13.5	5.7	>20

[a]BC-1=human breast cancer, HT-1080=human fibrosarcoma, LU-1=human lung cancer, MEL-2=human melanoma, COL-1=human colon cancer, KB=human oral epidermoid carcinoma, KB-V1=vinblastine (VLB)-resistant KB (+=with, −=without VLB in the assay mixture), P388=murine lymphoid neoplasm, A431=human epidermoid carcinoma, LNCaP=hormone-dependent human prostatic cancer, ZR-75-1=hormone-dependent breast cancer, U373=human glioblastoma.

The HIV-1 RT-inhibitory activity was found only in the $CHCl_3$ fraction (99.4% inhibition at 400 μg/ml). This fraction was subjected to Si gel cc separation ($CHCl_3$→MeOH gradient) affording 60 fractions. Every third fraction was tested for activity, and highest activity was found in fraction C21 (98% inhibition at 400 μg/ml). This fraction was further purified by Si gel cc (hexane/Me_2CO/EtOAc gradient) giving 150 fractions. Fractions 15–50, which were active fractions, were pooled. The subsequent Si gel cc of this pooled fraction (hexane/2-propanol gradient) resulted in 32 column fractions, and activity was found in fractions 24–28. Prep. tlc of combined fractions 24–28 [hexane-ethyl formate HCO_2H (6:5:0.05)] yielded the closely related compounds **1** and **3** (R_f values 0.32 and 0.41, respectively).

Fractions C22–C24 which also showed activity, and contained tlc spots corresponding to **1** and **3**, were combined and subjected to the same silica cc separation used for fraction C21 to isolate more of **1** and **3**. A total of 9 mg of **1** and 22 mg of **3** were obtained pure. Compound **5** was very close to **3** on tlc and was separated by prep. tlc [$CHCl_3$-hexane-MeOH (8:4:1); R_f values 0.54 and 0.48 for **5** and **3**, respectively]. C22 through C24 also contained **11**, which was purified by medium-pressure cc using a Lobar™ column (310 mm length×25 mm i.d.; prepacked with reversed-phase Si gel Lichroprep™ RP-8, 40–63 μm), eluted with MeOH-H_2O (1:1) (flow rate 2 ml/min).

Anolignan A [**1**].—Off-white amorphous powder: uv λ max (MeOH) (log ε) 226 (4.34), 284 (3.94) nm; ir ν max (film) 3398, 2926, 1602, 1489, 1454, 1244, 1040, 976, 895 cm^{-1}; cims *m/z* $[M+1]^+$ 311 (38), $[M]^+$ 310 (82), 201 (34), 187 (47), 175 (53), 135 (20), 123 (100); hreims 310.1209 (calcd for $C_{19}H_{18}O_4$, 310.1205); ^1H- and ^{13}C-nmr data, see Table 1.

2,4-Di-O-methylanolignan A [**2**].—Methylation of **1** with CH_2N_2 by standard procedures yielded a dimethylated off-white amorphous solid: uv λ max (MeOH) (log ε) 243 (4.00), 286 (3.76) nm; ir ν max (film) 2918, 1614, 1503, 1246, 1209, 1040, 933, 904, 833 cm^{-1}; eims *m/z* $[M]^+$ 338 (43), 203 (27), 187 (40), 151 (100), 135 (20), 121 (21); ^1H nmr (300 MHz, $CDCl_3$) δ 6.94 (1H, d, J=8.2 Hz, H-6), 6.72 (1H, d, J=7.8 Hz, H-5′), 6.66 (1H, d, J=1.6 Hz, H-2′), 6.63 (1H, dd, J=7.8 and 1.6 Hz, H-6′), 6.45 (1H, d, J=2.4 Hz, H-3), 6.40 (1H, dd, J=8.2 and 2.4 Hz, H-5), 5.93 (2H, s, OCH_2O), 5.29 (1H, s, H-9′b), 5.21 (1H, s, H-9b), 4.98 (1H, s, H-9′a), 4.90 (1H, s, H-9a), 3.80 (3H, s, 4-OCH_3), 3.78 (3H, s, 3-OCH_3), 3.54 (2H, s, H-7′), 3.52 (2H, s, H-7) ppm; ^{13}C nmr (75.4 MHz, $CDCl_3$) δ 159.19 (C-4), 157.95 (C-2), 147.43 (C-3′), 145.79 (C-8′), 145.60 (C-8), 145.43 (C-4′), 133.96 (C-1′), 130.20 (C-6), 121.55 (C-6′), 120.55 (C-1), 115.02 (C-9′), 114.54 (C-9), 109.19 (C-2′), 107.94 (C-5′), 103.82 (C-5), 100.72 (OCH_2O), 98.30 (C-3), 55.36 (OCH_3-4), 55.29 (OCH_3-2), 40.34 (C-7′), 33.17 (C-7) ppm.

Anolignan B [**3**].—White crystals: mp 147°; uv λ max (MeOH) (log ε) 280 (3.57), 230 (4.25) nm; ir ν max (film) 3308, 2939, 1604, 1614, 1514, 1352, 1099, 895, 833 cm^{-1}; eims *m/z* $[M]^+$ 266 (27), 159 (100), 107 (39); hreims 266.1306 (calcd $C_{18}H_{18}O_2$, 266.1307); ^1H and ^{13}C nmr, see Table 1.

4,4′-Di-O-methylanolignan B [**4**].—Methylation of **3** with CH_2N_2 by standard procedures yielded **4**: uv λ max (MeOH) (log ε) 244 (3.28), 278 (3.58), 283 (3.52) nm; ir ν max (film) 2918, 2835, 1614, 1604, 1514, 1246, 1032, 905, 833, 814 cm^{-1}; eims *m/z* $[M^+]$ 294 (19), 173 (100), 121 (61); ^1H nmr (300 MHz, $CDCl_3$) δ 7.05 (4H, d, J=8.5 Hz, H-2,6,2′,6′), 6.80 (4H, d, J=8.5 Hz, H-3,5,3′,5′), 5.25 (2H, s, H-9b,9′b), 4.85 (2H, s, H-9a,9′a), 3.77 (6H, s, OCH_3-4,4′), 3.47 (4H, s, H-7,7′) ppm; ^{13}C nmr (75.4 MHz, $CDCl_3$) δ 157.78 (C-4,4′), 145.77 (C-8,8′), 131.90 (C-1,1′), 129.60 (C-2,6,2′,6′), 115.28 (C-9,9′), 113.56 (C-3,5,3′,5′), 55.17 (OCH_3-4,4′), 39.76 (C-7,7′) ppm.

Anolignan C [**5**].—White crystals: mp 158°, optically inactive; uv λ max (MeOH) (log ε) 277 (3.57), 228 (4.21), 209 (3.93) nm; ir ν max (film) 3345, 2974, 2940, 1615, 1460, 1237, 1001 cm^{-1}; eims *m/z* $[M]^+$ 284 (3), 162 (100), 147 (63), 121 (17), 107 (16); hreims 284.1410 (calcd for $C_{18}H_{20}O_3$, 284.1412); ^1H nmr, see text; ^{13}C nmr (75.4 MHz, CD_3OD) δ 157.43 (C-4′,4″), 132.34 (C-1′,1″), 128.68 (C-2′,6′,2″,6″), 115.73 (C-3′,5′,3″,5″), 84.23 (C-2,5), 42.70 (C-3,4), 12.26 (CH_3-3,4) ppm.

HIV-1 RT ASSAYS.—HIV-1 RT inhibitory activity testing was performed using an assay procedure previously described (22). For the combination of **1** and **3**, 75:25, 50:50, and 25:75 refer to percent compositions of **1**:**3** in each mixture at a given concentration, e.g., a 250 μg/ml sample of 75:25, 50:50, and 25:75 mixture contained 187.5:62.5, 125:125, and 62.5:187.5 μg/ml of **1**:**3**, respectively.

CYTOTOXICITY ASSAYS.—In vitro cytotoxicity of the isolated compounds was determined using a procedure described in detail previously (23).

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