Six Lignans from *Phyllanthus myrtifolius*

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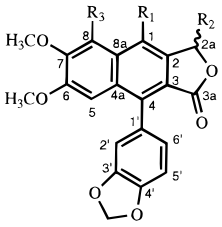
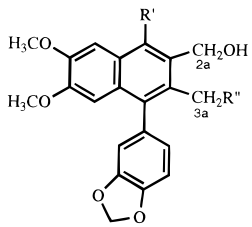
Six lignans comprising phyllamyricins D (**1**), E (**2**), and F (**3**) and phyllamyricosides A (**4**), B (**5**), and C (**6**) were isolated in a continuing study of *Phyllanthus myrtifolius*. Compounds **1** and **2** are 2a-methoxy-4-aryl-2,3-naphthalides. Compounds **3**−**6** belong to the 4-arylnaphthalene class, with compounds **4**−**6** being *O*-*β*-glucosides. Their structures were elucidated on the basis of spectral analysis. Compound **4** increased the activity of HIV-1 reverse transcriptase by 65% at a concentration of 1.89 μM.

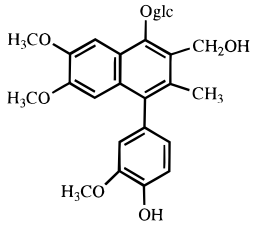
Recently, we reported the isolation of four novel lignans, phyllamyricins A−C and retrojusticidin B, together with the known justicidins A and B from a $CHCl_3$-soluble fraction of an EtOH extract of *Phyllanthus myrtifolius* Moon. (Euphorbiaceae),[1] a small shrub garden plant indigenous to India and Sri Lanka.[2] Among these, phyllamyricin B and retrojusticidin B have been demonstrated to have a strong inhibitory effect on HIV-1 reverse transcriptase (IC_{50} 3.5 and 5.5 μM, respectively) but much less effect on human DNA polymerase α (IC_{50} 289 and 989 μM, respectively).[3] This selective biological activity is of interest in developing potential anti-HIV agents. Continuing with the chemical investigation of this plant material, two additional lignans **1** and **2** were isolated from the same $CHCl_3$-soluble fraction. Repeated separation of the more polar fractions by centrifugal partition chromatography (CPC) and column chromatography over Sephadex LH-20 or Si gel yielded lignan **3** (EtOAc-soluble fraction) and lignan glucosides **4**−**6** (*n*-BuOH-soluble fraction). This paper describes the structural elucidation and biological activity of these six novel compounds.



1 R_1=H, R_2=R_3=OCH_3
2 R_1=R_3=H, R_2=OCH_3
7 R_1=R_2=OCH_3, R_3=H
8 R_3=OCH_3, R_1=R_2=H
9 R_1=R_2=R_3=H



3 R'=R"=H
4 R'=O-β-D-glc, R"=H
5 R'=O-β-D-glc, R"=OH



(glc= β-D-glc)

6

Results and Discussion

Compounds **1** and **2** showed UV absorption maxima around 260, 290, and 355 nm and IR absorption at ca. 1765 cm^{-1}, characteristic for arylnaphthalides.[1] In addition, two singlets at δ 6.34 (1H) and δ 3.65 (3H) in the ^{1}H-NMR spectrum plus a methine (δ_C 101.5) and a methoxy signal (δ_C 56.5) suggested methoxy substitution at either C-2a or C-3a in the lactone ring.

Compound **1** had the molecular formula ($C_{23}H_{20}O_8$) as deduced by HREIMS, having an additional CH_2O unit relative to that of phyllamyricin C (**8**) and the same as that of justicidin P (**7**).[4] The ^{1}H-NMR spectrum of **1** showed a close resemblance to that of **8** (Table 1), with five aromatic protons constituted by two singlets (δ 8.23, 6.90) and an ABX system appearing between δ 6.77 and 6.96, an AB pattern for an OCH_2O group (δ 6.04, 6.08, each d, J = 1.3 Hz), and three MeO singlets (δ 4.07, 3.99, 3.77). Both of these lignans differ only in the type of proton signals in the lactone ring, with two singlets observed in **1** (δ 6.34, 1H; δ 3.66, 3H) compared with a two-proton singlet in **8** (δ 5.39). These data suggested **1** to be the 2a-methoxy analogue of **8**. Analysis of the NOESY spectrum, which displayed the key NOEs of H-1 (δ 8.23) to H-2a (δ 6.34), MeO-2a (δ 3.66) and MeO-8 (δ 4.07); H-2a (δ 6.35) to MeO-2a (δ 3.66); and H-5 (δ 6.90) to MeO-6 (δ 3.77) and H-2′ (δ 6.81), confirmed **1** as 2a-methoxy-2-(hydroxymethyl)-6,7,8-trimethoxy-4-(3,4-methylenedioxyphenyl)-3-naphthoic acid γ-lactone.

Compound **2** possessed a molecular ion at m/z 394.1056 in its HREIMS, corresponding to the formula $C_{22}H_{18}O_7$, being 30 amu less than that of **1**. The ^{1}H-NMR spectrum of **2** was similar to that of **1** except for the absence of one MeO singlet and the presence of an additional aromatic proton singlet at δ 7.20 (Table 1). These data suggested **2** to be the 8-demethoxylated analogue of **1**, that is, 2a-methoxyjusticidin B. The almost superimposable ^{1}H-NMR data of **2** and justicidin B (**9**), except for those proton signals close to C-2a (H-1 and H-2a), supported this suggestion. The NOESY spectrum revealed NOEs of H-1 (δ 7.80) to H-8 (δ 7.20) and H-2a (δ ca. 6.33); H-2a (δ ca. 6.33) to 2a-OMe (δ 3.64); H-8 (δ 7.20) to 7-OMe (δ 4.02); and H-5 (δ 7.09) to 6-OMe (δ 3.79), H-2′ (δ 6.84), and H-6′ (δ 6.81) and thereby confirmed **2** as 2a-methoxy-2-(hydroxymethyl)-6,7-dimethoxy-4-[3,4-(methylenedioxy)phenyl]-3-naphthoic acid γ-lactone.

The ^{13}C-NMR assignments of **1** and **2** (Table 2) were carried out through spectral comparison with those of **8** and **9**,[1] and by analyzing the HMQC and HMBC spectral data of **2**. It is worth noting that the ^{1}H- and

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Table 1. ^{1}H-NMR Data (400.13 MHz) of Compounds **1**−**3** (δ in ppm, J in Hz) in $CDCl_3$

	compound		
proton(s)	**1**	**2**	**3**
H-1	8.23 s	7.80 s	7.69 s
H-2a	6.35 s	6.33 s	4.84 s
	6.34 s	6.32 s	
H-3a			2.18 s
H-5	6.90 s	7.09 s	6.69 s
H-8		7.20 s	7.11 s
H-2′	6.83 d (1.5)	6.85 d (1.5)	6.71 d (1.4)
	6.81 d (1.5)	6.83 d (1.5)	
H-5′	6.95, 6.94 each d (8.0)	6.95, 6.94 each d (8.2)	6.93 d (7.8)
H-6′	6.80, 6.78 each dd (8.0, 1.5)	6.82, 6.80 each dd (8.2, 1, 5)	6.68 dd (7.8, 1.4)
OCH_2O	6.08, 6.04 each d (1.3)	6.07, 6.03 each d (1.3)	6.06, 6.03 each d (1.2)
MeO-6	3.77 s	3.79 s	3.72 s
MeO-7	3.99 s	4.02 s	3.97 s
MeO-8	4.07 s		
MeO-2a	3.664, 3.659 each s	3.643, 3.637 each s	

Table 2. ^{13}C-NMR Data (100.61 MHz) of Compounds **1**, **2** and **4**−**6** (δ in ppm)[a]

	compound				
carbon	**1**	**2**	**4**	**5**	**6**
C-1	116.4 d	120.5 d	150.2 s	150.8 s	150.1 s
C-2	137.2 s	138.1 s	129.9 s	130.5 s	129.9 s
C-3	131.4 s	119.3 s	133.6 s	135.7 s	133.3 s
C-4	139.6 s	139.6 s	135.9 s	137.8 s	136.6 s
C-4a	120.8 s	130.2 s	131.4 s	131.6 s	131.6 s
C-5	102.4 d	106.3 d	106.7 d	107.6 d	106.9 d
C-6	154.0 s	150.6 s	150.8 s	151.3 s	150.7 s
C-7	141.3 s	151.9 s	150.3 s	151.5 s	150.3 s
C-8	147.9 s	106.6 d	103.7 d	104.1 d	103.7 d
C-8a	128.2 s	133.1 s	122.6 s	124.6 s	122.5 s
C-1′	128.2 s	128.2 s	135.4 s	134.2 s	133.6 s
C-2′	110.5 d	110.4 d	111.5 d	112.2 d	114.7 d
		110.5 d	111.7 d		115.0 d
C-3′	147.7 s	147.65 s	149.3 s	149.5 s	149.2 s
C-4′	147.7 s	147.74 s	148.1 s	148.7 s	146.7 s
C-5′	108.3 d	108.20 d	109.4 d	109.3 d	116.4 d
		108.24 d			
C-6′	123.4 d	123.4 d	124.3 d	125.3 d	123.8 d
		123.5 d	124.6 d		124.0 d
MeO-6	55.9 q	55.8 q	55.8 q	56.1 q	55.8 q
MeO-7	61.2 q	56.1 q	56.6 q	57.0 q	56.6 q
MeO-8	61.6 q				
MeO-2a	56.6 q	56.41 q			
		56.44 q			
C-2a	101.9 d	101.5 d	57.9 t	58.0 t	57.9 t
C-3a	167.6 s	167.7 s	17.3 q	61.1 t	17.4 q
$-OCH_2O-$	101.3 t	101.3 t	102.0 t	102.8 t	
MeO-3					56.5 q
C-1″			105.9 d	105.9 d	105.5 d
C-2″			75.7 d	75.9 d	75.7 d
C-3″			78.2 d	78.4 d	78.2 d
C-4″			72.9 d	72.7 d	72.7 d
C-5″			77.9 d	78.1 d	77.9 d
C-6″			63.2 t	63.3 t	63.2 t

[a] Data for **1**, **2** were recorded in $CDCl_3$ and for **4**−**6** in CD_3OD; multiplicities were obtained from DEPT experiments.

^{13}C- NMR spectra of **1** and **2** were split into two sets. The split signals came from the existence of two inseparable atropisomers, resulting from restricted rotation about the C_4-C_1' single bond as that for justicidin P (**7**).[4]

Compounds **3**−**6** showed UV absorption maxima around 245 nm and exhibited no lactone absorption in their IR and ^{13}C-NMR spectra, indicating an arylnaphthalene skeleton for these compounds.[5] In addition, compounds **4**−**6** were deduced to be *O*-β-glucosides from the FABMS spectral observation of a $[M - 162]^+$ fragment ion in each case, arising from the elimination of a glucose unit, and from the analysis of their ^{13}C-NMR data (Table 2), and from their ^{1}H-NMR spectra, which showed an anomeric proton around 4.77 ppm (d, J = 8.0 Hz) (Table 3).

Compound **3** had a molecular formula of $C_{21}H_{20}O_5$, as deduced from its FABMS. Its IR spectrum showed a hydroxyl absorption, but it failed to give a bathochromic shift in the UV spectrum under alkaline conditions, indicating the presence of an alcoholic rather than a phenolic function. In addition to six aromatic proton signals [three singlets (δ 7.69, 7.11, 7.68) and an ABX system between δ 6.67 and 6.94], an AB system for an OCH_2O (δ 6.03, 6.06, J = 1.2 Hz), and two MeO singlets (δ 3.97, 3.72), a CH_2 singlet at δ 4.84 and a Me singlet at δ 2.18 (Table 1) were observed in its ^{1}H-NMR spectrum. These data suggested **3** to be a justicidin B analogue with the lactone being reduced to a hydroxymethyl and a methyl group. The exact locations of these two substituents were determined by NOE experiments. Key NOE observations included the enhancement of H-8 (δ 7.11) and H-2a (δ 4.84) upon irradiation of H-1 (δ 7.69), and the enhancement of H-1 and H-3a (δ 2.18) upon irradiation of H-2a. These results confirmed the structure of **3** as 2-(hydroxymethyl)-3-methyl-6,7-dimethoxy-4-[3,4-(methylenedioxy)phenyl]naphthalene.

Compound **4**, an *O*-β-glucoside as mentioned above, had a molecular formula of $C_{27}H_{30}O_{11}$, assigned from the negative HRFABMS, indicating an additional $C_6H_{10}O_6$ fragment relative to **3**. The ^{1}H-NMR spectrum of **4** was very similar to that of **3** in the aglycon region except for the lack of an aromatic singlet at δ 7.69 for H-1 in **3**. Hence, **4** could be simply the 1-*O*-β-glucoside of **3**. The location of the glucosyl group at C-1 was determined from a NOESY spectrum that revealed the critical NOEs of H-8 (δ 8.14) to the anomeric proton (H-1″, δ 4.77) and 7-OMe (δ 3.96). These results and the HMBC spectrum, which exhibited the three-bond coupling of H-2a (δ 5.23 and 4.61, each d) to C-1 (δ 150.2), and H-3a (δ 2.25) to C-4 (δ 135.9), indicated **4** to be the 1-*O*-β-glucoside of **3**. Analysis of the well-resolved ^{1}H-NMR spectrum and the NOESY spectrum enabled the complete assignment of the ^{1}H-NMR data of **4** (Table 3). With these ^{1}H-NMR data on hand, the complete ^{13}C-NMR assignments for **4** (Table 2) were achieved by analysis of its HMBC spectral data.

Compound **5** has a molecular formula of $C_{27}H_{30}O_{12}$, assigned from both negative- and positive-HRFABMS, having one more oxygen than that of **4**. Comparison of the ^{1}H-NMR spectra of **4** and **5** (Table 3) indicated the only differences were a methyl singlet in **4** (δ 2.25) compared with oxygenated methylene signals in **5** (δ ca. 4.65 and ca. 4.58, each d). These NMR spectral data indicated **5** to be a 3a-hydroxy analogue of **4**. Spectral data from a homo-COSY NMR experiment also showed correlations for two H-2a protons, two H-3a protons, an ABX system in the aryl moiety, and the vicinal protons in the glucose moiety. In addition, the glucose moiety attached to C-1 was confirmed from the observation of the couplings (^3J) of C-1 (δ 150.8) to H-2a (δ ca. 5.32, 4.86, each doublet) and H-1″ (δ ca. 4.78, d) from an HMBC experiment (Table 3). This result also distinguished the chemical shifts of H-2a from those of H-3a. Based on this analysis, **5** was determined as 2,3-

Table 3. ^{1}H-NMR Data (400.13 MHz) for Compounds **4**−**6**, and HMQC and HMBC Data of **5** (δ in ppm, J in Hz) (CD_3OD)

	compound			HMQC of **5**	HMBC of **5**
proton(s)	**4**	**5**	**6**[a]	$\delta_{C(correlated)}$	$\delta_{C(correlated)}$
H-2a	5.23 d, 4.61 d (11.6)	5.31, 5.33 d (11.9) 4.86, 4.85 d (11.9)	5.26, 5.25 d (1H) 4.59 d (1H) (11.6)	58.0	150.8 (C-1), 135.7 (C-3), 130.5 (C-2)
H-3a	2.25 s	4.65, 4.62 d (11.5) 4.62, 4.61 d (8.0)	2.26 s 2.25 s	61.1	137.8 (C-4), 135.7 (C-3), 130.5 (C-2)
H-5	6.67 s	6.76 s	6.70, 6.69 s	107.6	151.5 (C-7), 137.8 (C-4), 124.6 (C-8a)
MeO-6	3.64 s	3.66 s	3.614, 3.607 s	56.1	151.3 (C-6)
MeO-7	3.96 s	3.98 s	3.95 s	57.0	151.5 (C-7)
H-8	8.14 s	8.20 s	8.13 s	104.1	151.5 (C-7), 151.3 (C-6), 131.6 (C-4a)
H-2′	6.663, 6.660 d (1.6)	6.81, 6.79 d (1.6)	6.74 d (1.7)	112.2	148.7 (C-4′), 137.8 (C-4), 125.3 (C-6′)
H-5′	6.96, 6.97 d (7.9)	6.96 d (8.0)	6.933, 6.927 d (7.9)	109.3	149.5 (C-3′), 134.2 (C-1′)
H-6′	6.66, 6.64 each dd (7.9, 1.6)	6.78, 6.75 each dd (8.0, 1.6)	6.62, 6.61 each dd (7.8, 1.7)	125.3	148.7 (C-4′), 112.2 (C-2′), 109.3 (C-5′)
−OCH_2O−	6.04, 6.02 br s	6.05, 6.02 d (0.8)		102.8	149.5 (C-3′), 148.7 (C-4′)
H-1″	4.77 d (8.0)	4.78, 4.77 d (8.0)	4.76 d (8.2)	105.9	150.8 (C-1)
H-2″	3.70 dd (9.0, 8.0)	3.71 dd (9.0, 8.0)	3.70 dd (9.0, 8.2)	75.9	105.9 (C-1″), 78.4 (C-3″)
H-3″	3.47 t (9.0)	3.48 dd (8.0, 9.0)	3.48 t (9.0)	78.4	72.7 (C-4″), 75.9 (C-2″)
H-4″	3.32 t (9.0)	3.32 t (8.0)	3.32 t (9.0)	72.7	78.4 (C-3″), 78.1 (C-5″)
H-5″	3.21 ddd (9.0, 7.8, 2.4)	3.21 ddd (8.0, 7.9, 2.5)	3.21 ddd (9.0, 8.0, 2.4)	78.1	72.7 (C-4″), 63.3 (C-6″)
H-6″a	3.89 dd (11.4, 2.4)	3.88 dd (11.6, 2.5)	3.89 dd (11.5, 2.4)	63.3	78.1 (C-5″)
H-6″b	3.61 dd (11.4, 7.8)	3.62 dd (11.6, 7.9)	3.62 dd (11.5, 8.0)	63.3	

[a] MeO-3′ at δ 3.81 (s).

dihydroxymethyl-6,7-dimethoxy-4-[3,4-(methylenedioxy)phenyl]naphthalene 1-*O*-β-D-glucoside.

The complete ^{1}H- and ^{13}C-NMR assignments of **5** were achieved through analysis of its HMQC and HMBC spectral data. The signals of the nonoxygenated quaternary carbons C-2, C-3, C-4, C-4a, C-8a, and C-1′ were distinguished by their three-bond coupling to H-2a (C-3), H-3a (C-2 and C-4), H-5 (C-4, C-8a), H-8 (C-4a), H-2′ (C-4), and H-5′ (C-1′). The signals of oxygenated quaternary carbons (C-1, C-6, C-7, C-3′, and C-4′) were also assigned from their three-bond coupling to H-2a (C-1), H-5 (C-7), MeO-6 (C-6), MeO-7 (C-7), H-8 (C-6), OCH_2O (C-3′ and C-4′), H-2′ (C-4′), and H-5′ (C-3′). The rest of the ^{13}C-NMR signals were assigned directly from an HMQC spectrum, and further confirmed by the HMBC data (Table 3).

Compound **6** was assigned the molecular formula of $C_{27}H_{32}O_{11}$ as deduced from its HRFABMS data, being two protons more than that of **4**. Comparison of the ^{1}H-NMR spectra of **4** and **6** (Table 3) indicated the presence of an additional MeO singlet (δ 3.81) along with the absence of any methylenedioxy signals (δ 6.02 and 6.04, each broad singlet, **4**) in that of **6**. The aryl proton signals and the ^{13}C-NMR data in the phenyl ring also showed some differences. Based on these observations, the methylenedioxy function in **4** could be replaced by OH and OMe groups in **6**. This additional MeO group was located at C-3′ as determined via an NOE study that enhanced H-2′ (δ 6.74, d, J = 1.7 Hz) upon irradiation of the MeO singlet at δ 3.81. Accordingly, the structure of **6** was elucidated as 2-(hydroxymethyl)-3-methyl-6,7-dimethoxy-4-(3-methoxy-4-hydroxyphenyl)naphthalene 1-*O*-β-D-glucoside. The complete ^{1}H- and ^{13}C- NMR assignments for **6** (Table 2 and 3) were made via correlation with those of **4** and were further confirmed via analysis of homo-COSY and HETCOR (^1J and long-range) spectra.

As discussed for the lignan glucoside phyllanthostatin A,[6] the restricted rotation gives rise to two diastereoisomers, reflected clearly in the ^{1}H-NMR spectrum, which displayed split or doubled signals. This phenomenon also appeared for compounds **4**−**6**. For instance, the methylene protons at both C-2a and C-3a in **5** were observed obviously as two AB systems (Table 3). A solvent effect on the ^{1}H-NMR pattern was also noted. When measured in DMSO-d_6 instead of CD_3OD, the split anomeric proton signals at δ 4.78 and 4.77 were converged to a sharp doublet at δ 4.65, and the two paired signals of H-2a at δ 5.31 (5.33) (each doublet, J = 11.9 Hz) and 4.86 (4.85) (each doublet, J = 11.9 Hz) in CD_3OD appeared as an AB system at δ 6.10 and 5.04 (J = 4.8 Hz). The large difference in shift for one C-2a proton (δ 6.10, $\Delta\delta$ +0.79 ppm) could be ascribed to H-bond formation between OH-2a and the C-1 oxygen in DMSO, locating this proton in a more deshielded zone of the naphthalene ring current.

All of the six compounds isolated are novel natural products and were named phyllamyricins D (**1**), E (**2**), and F (**3**), and phyllamyricosides A (**4**), B (**5**), and C (**6**). To date, only a few lignan glycosides of the arylnaphthalene type have been isolated from natural sources.[6−9] Some of them have been reported to possess cytotoxic activities.[7,9] Therefore, the inhibitory activity against HIV-1 reverse transcriptase (HIV-1 RT) of compounds **2** and **4**−**6** was also investigated. Relative to a positive control, retrojusticidin B (IC_{50} 5.5 μM), the aglycon **2** showed very weak anti-HIV-1 RT activity of 20% inhibition at concentrations higher than 10 μM. The glucosides **5** and **6** were inactive. The glucoside **4**, on the contrary, enhanced HIV-1 RT activity in duplicate experiments. At 1.89 μM, it increased HIV-1 RT activity by 65%, and at 18.9 μM, the activity almost reached the plateau (210% relative to control) (Figure 1). This preliminary study might suggest that hydrophobicity at 4-aryl and C-3a, and sugar moiety at C-1 of the 4-arylnaphthalene lignans would play important roles in determining the enhancing activity toward HIV-1 RT. The detailed mechanism of this action and structure−activity relationship remain to be clarified.

Experimental Section

General Experimental Procedures. The physical data of the isolated compounds were obtained on the same instruments as those used in an earlier paper.[1] The glycosides were fractionated by centrifugal partition chromatography (CPC) on a Sanki CPC instrument,

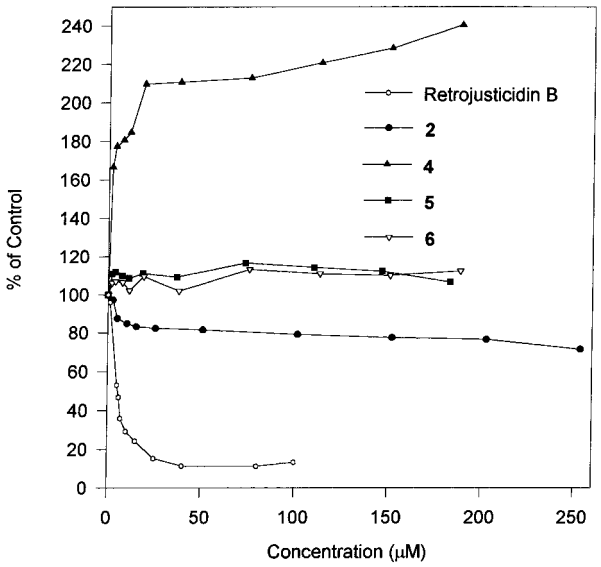

Figure 1. Effects of compounds **2**, **4**−**6**, and retrojusticidin B on HIV-1 reverse transcriptase.

LLN type, with a 1000E cartridge (× 6, volume 410 mL), using the solvent systems A, $CHCl_3$−MeOH−H_2O (10:10:6); B, $CHCl_3$−MeOH−H_2O (13:7:8); C, $CHCl_3$-MeOH−H_2O (10:10:5); and D, EtOAc−EtOH−H_2O (2:1:2), with the organic layer as mobile phase and the aqueous layer as stationary phase, at a flow rate of 3.5 mL/min, a rotation speed of 1000−1100 rpm, and a pressure in the range of 30 to 45 kg/cm^2.

Plant Material. Plant material was described previously.[1]

Extraction and Isolation. The procedure was followed as described in an earlier paper[1] in which an EtOH extract (690.0 g) from the dried and powdered aerial parts (9.7 kg) of *Phyllanthus myrtifolius* was subdivided into fractions soluble in hexane (108 g), $CHCl_3$ (50 g), EtOAc (29 g), *n*-BuOH (250 g), and H_2O (238 g).

Subfraction F (4.6 g) of the first Si gel column of the $CHCl_3$-soluble fraction (50.0 g)[1] was subjected to column chromatography on Si gel (200 g, 230−400 mesh) eluted with 0−20% MeOH in $CHCl_3$ with 15-mL fractions being collected. Following TLC analysis [toluene−Me_2-CO (80:20) and UV 254 nm], the eluates of similar profiles were combined to give seven fractions. Fraction 2 (118.6 mg) was rechromatographed twice over Si gel (230−400 mesh) eluted with 0−1% Me_2CO in toluene to give **1** (ca.1.8 mg) and **2** (18.4 mg).

The EtOAc-soluble fraction (29.0 g) was subjected to CPC (about 6 g each run) using solvent system A with 15-mL fractions being collected. Following the TLC analysis (lower layer of solvent system A) of the eluates, six fractions were obtained. Fraction 1 (8.73 g), which gave a characteristic blue fluorescent spot on the TLC plate under UV light at 254 nm for a lignan, was chromatographed over a Si gel column (350 g, 230−400 mesh), eluted with 1−10% Me_2CO in toluene to gave seven subfractions. Subfractions 2, 4, and 5 contained phyllamyricin A, justicidin B, retrojusticidin B, and compound **2** by TLC comparison [Me_2CO−tolune (1:19)] with authentic samples. Two successive CPC separations of subfraction 6 (271.8 mg) with solvent system B, followed by a Sephadex LH-20 column [$CHCl_3$−MeOH (3:7)] and preparative TLC plates (0.25 mm thick, 20 × 20 cm) developed with hexane−$CHCl_3$−EtOAc (1:2:2) yielded compound **3** (ca. 1 mg).

The *n*-BuOH-soluble fraction (250 g) was fractionated via a Sephadex LH-20 column (4200 mL, 83.0 g × 3) eluted with MeOH to afford 22 fractions (A−V). Among these, fractions I (3.55 g) and L (2.23 g) were found to contain lignans, as recognized from the blue fluorescent spots under UV 254 nm on TLC analysis using the lower layer of the solvent system $CHCl_3$−MeOH−*n*-BuOH−H_2O (10:10:1:6) as eluent. Subsequent CPC separation of fraction I using solvent system B yielded 11 subfractions. Subfraction 4 (1.33 g) was then subjected to successive CPC with solvent system C (once), then with solvent system B (twice) to give **5** (108.1 mg) and **6** (300 mg). Using the CPC technique with solvent system B, fraction L further gave six subfractions. Compound **4** (5 mg) was obtained from subfraction 2 (41.5 mg) after a CPC separation using solvent system D, followed by a Sephadex LH-20 (EtOH) column separation.

Phyllamyricin D (**1**): UV (MeOH) λ max (log ϵ) 231 (4.60), 262 (4.93), 290 (4.11), 360 (3.64) nm; IR (KBr) ν max 2930, 1763, 1620, 1501, 1480, 1435, 1381, 1328, 1265, 1240, 1200, 1160, 1123, 1068, 1040, 938 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 2; NOESY data ($CDCl_3$) MeO-8 ↔ H-1 ↔ MeO-2a ↔ H-2a ↔ H-1, MeO-6 ↔ H-5 ↔ H-2′; EIMS (70 eV) *m*/*z* [M]$^+$ 424 (100), 393 (40), 377 (11), 365 (10), 351 (20), 335 (10), 319 (8), 291 (7), 277 (7); HREIMS *m*/*z* [M]$^+$ 424.1161 (calcd for $C_{23}H_{20}O_8$, 424.1158).

Phyllamyricin E (**2**): colorless amorphous solid; mp 95−99 °C; $[\alpha]^{20}_D$ −1.3° (*c* 0.7, MeOH); UV (MeOH) λ max (log ϵ) 260 (4.62), 291 (3.74) nm; IR (KBr) ν max 2930, 1771, 1620, 1503, 1480, 1438, 1410, 1385, 1330, 1260, 1240, 1220, 1160,1123, 1075, 1040, 940 cm^{-1}; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 2; HMBC data ($CDCl_3$) H-1 (δ 7.80) to C-2a (δ 106.6), C-3 (δ 119.3), C-4a (δ 129.9), C-8 (δ 106.6); H-8 (δ 7.20) to C-1 (δ 120.5), C-4a (δ 129.9), C-6 (δ 150.6), and C-7 (δ 151.9); H-5 (δ 7.09) to C-8a (δ 133.1), C-4 (δ 139.6), C-6 (δ 150.6), and C-7 (δ 151.9); H-5′ (δ 6.94, 6.95) to C-1′ (δ 128.2), C-3′ (δ 147.65); H-2′ (δ 6.83, 6.85) to C-6′ (δ 123.4, 123.5), C-4 (δ 139.6), and C-4′ (δ 147.64); H-6′ (δ 6.80, 6.82) to C-2′ (δ 110.4, 110.5), C-4 (δ 139.6), C-4′ (δ 147.64); −OCH_2O− (δ 6.03, 6.07) to C-4′ (δ 147.64); MeO-7 (δ 4.02) to C-7 (δ 151.9); MeO-6 (δ 3.79) to C-6 (δ 150.6); MeO-2a (δ 3.636, 3.642) to C-2a (δ 101.6); EIMS (70 eV) *m*/*z* [M]$^+$ 394 (100), 363 (42), 350 (6), 335 (13), 321 (25), 291 (7), 277 (19), 261 (5); HREIMS *m*/*z* [M]$^+$ 394.1056 (calcd for $C_{22}H_{18}O_7$, 394.1052).

Phyllamyricin F (**3**): UV (MeOH) λ max (log ϵ) 244 (5.0), 290 (4.54), 313 (3.87), 328 (3.96) nm; IR (KBr) ν max 2950, 1501, 1470, 1437, 1330, 1260, 1240, 1155, 1040, 930 cm^{-1}; ^{1}H-NMR data, see Table 1; NOE data MeO-6 to H-5 (5.4%), MeO-7 to H-8 (6.7%), H-8 to H-1 (16.6%) and MeO-7 (9.6%), H-1 to H-8 (11.1%) and H-2a (5.1%), H-2a to H-1 (3.8%) and H-3a (2.9%); FABMS *m*/*z* [M]$^+$ 352 (55), 335 (100), 323 (30); HRFABMS *m*/*z* [M]$^+$ 352.1316 (calcd for $C_{21}H_{20}O_5$, 352.1311).

Phyllamyricoside A (**4**): colorless amorphous solid; mp 177−182 °C; UV (MeOH) λ max (log ϵ) 245 (4.80), 250 (4.80), 289 (4.04), 330 (3.30) nm; IR (KBr) ν max 3450, 2950, 1502, 1470, 1432, 1340, 1260, 1222, 1175, 1138, 935 cm^{-1}; ^{1}H-NMR data, see Table 4; ^{13}C-NMR data, see Table 2; NOE data (CD_3OD) H-3a to H-2′ and H-6′ (6.7%), MeO-6 to H-5 (5.8%), H-8 to MeO-7 (15.2%);

NOSEY data (CD_3OD) H-6′ ↔ H-5 ↔ MeO-6; MeO-7 ↔ H-8 ↔ H-1″ ↔ H-3″ ↔ H-5″ ↔ H-6″a; H-5″ ↔ H-1″; H-2″ ↔ H-4″ ↔ H-6″b; COSY-45 H-5 ↔ MeO-6, H-8 ↔ MeO-7, H-2′ ↔ H-6′ ↔ H-5′, H-1″ ↔ H-2″ ↔ H-3″ ↔ H-4″ ↔ H-5″ ↔ H-6″a and H-6″b, H-6″a ↔ H-6″b; HMBC data (CD_3OD) H-2a (δ 5.35, 4.61), to C-1 (δ 150.2), C-3 (δ 133.6), C-2 (δ 129.9); H-3a (δ 2.25) to C-4 (δ 135.9), C-3 (δ 133.6), C-2 (δ 129.9); H-5 (δ 6.67) to C-7 (δ 150.3), C-4 (δ 135.9), C-8a (δ 122.6); MeO-6 (δ 3.64) to C-6 (δ 150.8); MeO-7 (δ 3.96) to C-7 (δ 150.3); H-8 (δ 8.14) to C-6 (δ 150.8), C-4a (δ 131.4); H-2′ (δ 6.660, 6.663) to C-4 (δ 148.1), C-6′ (δ 124.4), C-4 (δ 135.9); H-5′ (δ 6.96, 6.97) to C-1′ (δ 135.4), C-4′ (δ 148.1), C-5′ (δ 149.3); H-6′ (δ 6.64, 6.66) to C-2′ (δ 111.7), C-5′ (δ 109.3), C-4 (δ 135.9), C-4′ (δ 148.1); $-OCH_2O-$ (δ 6.02, 6.04) to C-4′ (δ 148.1), C-3′ (δ 149.3); H-2″ (δ 3.70) to C-1″ (δ 105.9), C-3″ (δ 78.2); H-3″ (δ 3.47) to C-2″ (δ 75.7), C-4″ (δ 72.3); H-4″ (δ 3.32) to C-5″ (δ 77.9), C-2″ (δ 78.2), C-6″ (δ 63.2); H-5″ (δ 3.21) to C-4″ (δ 72.3), C-6″ (δ 63.2); H-6″b (δ 3.61) to C-5″ (δ 77.9); negative FABMS *m/z* $[M - H]^-$ 529 (10), $[M - 162 - H]^-$ 367 (100); HRFABMS (negative) *m/z* $[M - H]^-$ 529.1702 (calcd for $C_{27}H_{29}O_{11}$, 529.1710); HRFABMS *m/z* $[M]^+$ 530.1786 (calcd for $C_{27}H_{30}O_{11}$, 530.1788).

Phyllamyricoside B (**5**): colorless amorphous solid; mp 170−175 °C; UV (MeOH) λ max (log ϵ) 243 (4.63), 249 (4.65), 287 (3.86), 331 (3.26) nm; IR (KBr) ν max 3460, 2950, 1501, 1470, 1432, 1260, 1230, 1161, 1070, 1038, 937 cm^{-1}; ^{1}H-NMR data, see Table 3; ^{13}C-NMR data, see Table 2; COSY-45 data (CD_3OD) geminal coupling δ 6.02 ↔ δ 6.05 ($-OCH_2O-$); δ 5.31 and 5.33 ↔ δ 4.86 and 4.85 (H-2a); δ 4.65 ↔ δ 4.58, and δ 4.62 ↔ δ 4.61 (H-3a); H-6″a ↔ H-6″b; vicinal coupling H-2′ ↔ H-6′ ↔ H-5′; H-1″↔ H-2″ ↔ H-3″ ↔ H-4″ ↔ H-5″ ↔ H-6″a and H-6″b; positive FABMS *m/z* $[M + Na]^+$ 569 (100), $[M-162 + Na]^+$ 407 (67), $[M - 162]^+$ 384 (67); negative FABMS *m/z* $[M - H]^-$ 545 (32), 383 (100); HRFABMS (negative) *m/z* $[M - H]^-$ 545.1666 (calcd for $C_{27}H_{29}O_{12}$, 545.1659).

Phyllamyricoside C (**6**): colorless amorphous solid; mp 159−163 °C; UV (MeOH) λ max (log ϵ) 243 (4.75), 247 (4.74), 290 (3.98), 331 (3.32) nm; IR (KBr) ν max 3450, 2950, 1502, 1470, 1458, 1430, 1260, 1170, 1070, 1030 cm^{-1}; ^{1}H-NMR data, see Table 3; ^{13}C-NMR data, see Table 2; NOE data MeO-6 to H-5 (5.5%), MeO-3′ to H-2′ (5.1%); long-range HETCOR data (CD_3OD) C-1 (δ 150.1) to H-2a (δ 5.254, 5.247, 4.59) and H-8 (δ 8.13), C-4 (δ 136.6) to H-3a (δ 2.25, 2.26), C-3 (δ 133.3) to H-3a (δ 2.25, 2.26), C-2 (δ 129.9) to H-3a (δ 2.25, 2.26), C-6 (δ 150.7) to MeO-6 (δ 3.607, 3.614) and H-8 (δ 8.13), C-7 (δ 150.3) to H-5 (δ 6.70, 6.69) and MeO-7 (δ 3.95), C-3′ (δ 149.2) to MeO-3′ (δ 3.81); FABMS (positive) *m/z* $[M + Na]^+$ 555 (35), $[M - 162 + Na]^+$ 393 (66), $[M - 162]^+$ 370 (100); HRFABMS (negative) *m/z* $[M - H]^-$ 531.1870 (calcd for $C_{27}H_{31}O_{11}$, 531.1866).

Reverse Transcriptase Assay. The compounds for assay were prepared to 1 mM stock solution in 50% DMSO. HIV-1 reverse transcriptase was purchased from HT Biotechnology Ltd., Cambridge, U.K. The assay procedure was followed as described in an earlier paper.[3]

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