Journal of Natural Products
Vol. 58, No. 3, pp. 392-400, March 1995

ANTI-AIDS AGENTS, 18.[1] SODIUM AND POTASSIUM SALTS OF CAFFEIC ACID TETRAMERS FROM *ARNEBIA EUCHROMA* AS ANTI-HIV AGENTS

YOSHIKI KASHIWADA,

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

MAKOTO NISHIZAWA, TAKASHI YAMAGISHI,

Natural Products Laboratory, Sumitomo Metal Industries, Ltd., Souraku, Kyoto 619-02, Japan

TAKASHI TANAKA,[2] GEN-ICHIRO NONAKA,

Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

L. MARK COSENTINO, JAMES V. SNIDER,

Biotech Research Laboratories, 3 Taft Court, Rockville, Maryland 20850

and KUO-HSIUNG LEE*

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599

ABSTRACT.—Monosodium and monopotassium salts [**2–4**] of isomeric caffeic acid tetramers were isolated from *Arnebia euchroma* as anti-HIV agents. Mixtures of dipotassium and disodium salts [**1**] of a caffeic acid tetramer and dipotassium and potassium-sodium salts [**5**] of a caffeic acid tetramer glucoside were also isolated from the active fraction. The structures of **1–5** were characterized by chemical and spectral evidence. Compounds **2–4** demonstrated potent anti-HIV activity with EC_{50} values of 2.8, 4.0, and 1.5 μg/ml, respectively. Treatment of **1–4** with dilute HCl yielded known caffeic acid tetramers [**8** and **9**], which were found to be less active, indicating the importance of the sodium and potassium salts to the enhanced anti-HIV activity.

Since the spread of AIDS is worldwide, efforts have been made to discover therapeutic agents to arrest the replication of its causative agent, the human immunodeficiency virus (HIV). Because HIV-1 reverse transcriptase (RT) and HIV-1 protease are essential enzymes in the HIV replication cycle, the investigation has focused on the search for inhibitors of these enzymes. Although the anti-AIDS drugs, AZT, ddI, ddC, and D4T, which are nucleoside RT inhibitors, are the only approved drugs at present, adverse side effects and the development of drug-resistant viral strains have been reported (2–5). Therefore, the search for compounds with novel structures and different mechanisms of action from those of the known nucleoside analogues is of current interest.

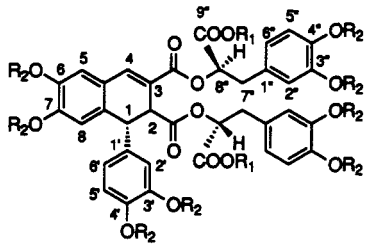
In the course of our preliminary screening of Chinese crude drugs as anti-HIV agents, an aqueous Me_2CO extract of *Arnebia euchroma* (Royle) Johnst. (Boraginaceae) showed potent inhibitory activity (≤20 μg/ml) against HIV replication in acutely infected H9 cells. The roots of *A. euchroma* have been used in traditional Chinese prescriptions for antiinflammatory, antipyretic, and antibacterial purposes (6,7). They have also been used in the treatment of epidemic hepatitis in the People's Republic of China (6). This paper describes the isolation and structure elucidation of anti-HIV phenolics from *A. euchroma*.

[1]For part 17, see Xie *et al.* (1).

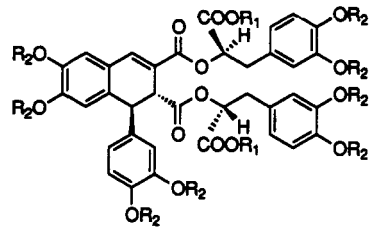
[2]Present Address: Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi, Nagasaki 852, Japan.

RESULTS AND DISCUSSION

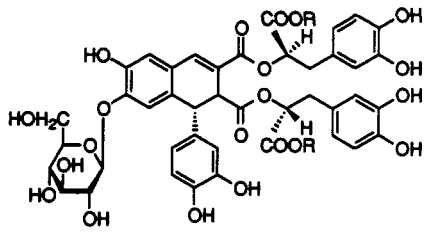
Primary bioactivity-guided solvent extraction of *A. euchroma* successively with hexane and 70% aqueous Me_2CO, followed by solvent fractionation of the aqueous Me_2CO extract with EtOAc and *n*-BuOH revealed that the aqueous fraction possessed the most potent anti-HIV activity. Subsequent bioassay-directed fractionation of this fraction by MCI-gel CHP20P, Sephadex LH-20, ODS, and Toyopearl HW40F chromatography led to the isolation of compounds **1–5**.



	R_1	R_2
1	K	H
2	Na; H	H
3	K(Na); H	H
6	CH_3	CH_3
8	H	H



	R_1	R_2
4	K(Na); H	H
7	CH_3	CH_3
9	H	H



5 R=K or K; Na

Compound **1** was clearly separated by MCI-gel CHP20P cc of the aqueous fraction, while compounds **2–4** possessed similar chromatographic properties on MCI-gel CHP20P and ODS, and were initially regarded as homogeneous. Although the hplc analysis (ODS; solvent $CH_3CN/H_2O/H_3PO_4$) of a mixture of **2–4** also showed a single peak, the ^1H- and ^{13}C-nmr spectra suggested the presence of several structurally related compounds. The mixture was separated by chromatography on Toyopearl HW40F giving compounds **2–4**.

Compounds **1–4** showed a dark-blue coloration with $FeCl_3$ reagent, indicative of their phenolic nature. They gave quite similar ^1H-nmr spectra (Table 1) in spite of their different chromatographic character. The ^1H-nmr spectra of **1–4** exhibited signals ascribable to three catechol rings, three one-proton aromatic or olefinic singlets, two oxygen-bearing methines, two aliphatic methines, and two methylenes. The ^{13}C-nmr spectra of these compounds were also essentially indiscernible from one another (Table 2), showing signals due to four carboxyl carbons, four aromatic rings, a double bond, two oxygen-bearing carbons, and four aliphatic carbons. On methylation with Me_2SO_4/K_2CO_3 in Me_2CO, **1–3** yielded the same decamethylate [**6**], while **4** furnished a different

TABLE 1. ^{1}H-Nmr Spectral Data for Compounds **1–5**, **7**, and **8** (300 MHz, *J* values in Hz).

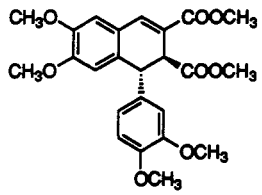
Proton	Compound						
	1[a]	**2**[a]	**3**[a]	**4**[a]	**7**[b]	**8**[b]	**5**[a]
1	[c]	[c]	[c]	[c]	4.55 (br s)	4.18 (br s)	4.66 (br s)
2	4.01 (d, *J*=1.5)	4.04 (d, *J*=1.5)	4.06 (br s)	3.98 (d, *J*=1.5)	3.93 (d, *J*=1.5)	3.94 (d, *J*=1.5)	4.07 (br s)
4	7.64 (s)	7.62 (s)	7.62 (s)	7.78 (s)	7.65 (s)	7.64 (s)	7.67 (s)
5	6.93 (s)	6.92 (s)	6.93 (s)	7.01 (s)	6.95 (s)	6.93 (s)	7.10 (s)
8	6.72 (s)	6.68 (s)	6.67 (s)	6.33 (s)	6.60 (s)	6.47 (s)	6.95 (s)
2′	6.63 (d, *J*=2)	6.59 (d, *J*=2)	6.59 (d, *J*=2)	6.60 (d, *J*=2)	6.46 (d, *J*=2)	6.51 (d, *J*=2)	6.71 (d, *J*=2)
5′	6.69 (d, *J*=8)	6.68 (d, *J*=8)[c]	6.68 (d, *J*=8)	6.63 (d, *J*=8)	6.68 (d, *J*=8)	6.65 (d, *J*=8)	6.69 (d, *J*=8)
6′	6.42 (dd, *J*=2 and 8)	6.37 (dd, *J*=2 and 8)	6.36 (dd, *J*=2 and 8)	6.18 (dd, *J*=2 and 8)	6.30 (dd, *J*=2 and 8)	6.32 (dd, *J*=2 and 8)	6.50 (dd, *J*=2 and 8)
2″	6.79, 6.90 (d, *J*=2)	6.77, 6.88 (d, *J*=2)	6.75, 6.87 (d, *J*=2)	6.89, 6.93 (d, *J*=2)	6.80, 6.86 (d, *J*=2)	6.81, 6.82 (d, *J*=2)	6.80, 6.91 (d, *J*=2)
5″	6.73, 6.76 (d, *J*=8)	6.70, 6.72 (d, *J*=8)	6.69, 6.71 (d, *J*=8)	6.76, 6.90 (d, *J*=8)	6.75, 6.76 (d, *J*=8)	6.73, 6.80 (d, *J*=8)	6.71, 6.75 (d, *J*=8)
6″	6.53, 6.64 (dd, *J*=2 and 8)	6.44, 6.54 (dd, *J*=2 and 8)	6.41, 6.52 (dd, *J*=2 and 8)	6.70, 6.78 (dd, *J*=2 and 8)	6.59, 6.63 (dd, *J*=2 and 8)	6.60, 6.63 (dd, *J*=2 and 8)	6.54, 6.64 (dd, *J*=2 and 8)
7″	2.82–3.15 (m)	2.75–3.35 (m)	2.72–3.15 (m)	2.89–3.21 (m)	2.98–3.06 (m)	2.87–3.04 (m)	2.80–3.15 (m)
8″	4.84, 4.98 (dd, *J*=3 and 9.5)	4.81, 4.98 (dd, *J*=3 and 9.5)	4.83, 5.02 (br d, *J*=10)	4.77, 4.86 (br d, *J*=10.5)	5.00 (t, *J*=6.5) 5.04 (dd, *J*=5 and 7.5)	4.97 (dd, *J*=4 and 9) 5.09 (t, *J*=6)	4.87, 5.00 (dd, *J*=3 and 10)
anomeric-H							5.09 (d, *J*=8)

[a]Measured in Me_2CO-d_6+D_2O.
[b]Measured in Me_2CO-d_6.
[c]Overlapped with DOH.

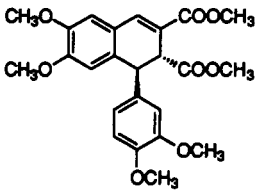
TABLE 2. ^{13}C-Nmr Data for Compounds **1–5**, **7**, and **8** (75 MHz).

Carbon	Compound						
	1[a]	**2**[a]	**3**[a]	**4**[a]	**7**[b]	**8**[b]	**5**[a]
1	44.4	45.1	44.8	45.0	45.4	45.8	40.5
2	46.7	47.4	47.0	47.0	47.7	47.6	46.0
3	131.1	131.3	130.9	129.9	130.7	130.7	131.2
4	139.6	140.0	139.7	140.5	140.1	140.2	138.9
4a	135.9	136.3	135.9	135.7	136.5	136.2	136.0
5	116.9	117.3	116.9	116.7	116.9	116.8	117.7
6	143.9	144.5	144.1	144.2	144.9	144.9	144.7
7	149.3	148.1	147.7	147.7	148.4	148.3	147.0
8	117.1	117.4	117.0	117.2	117.1	117.0	116.9
8a	123.8	124.3	123.9	124.0	124.1	124.2	126.2
1′	121.6	121.9	121.6	120.7	121.0	121.3	123.8
2′	115.1	115.5	115.2	115.1	115.2	115.3	114.9
3′	143.4	143.8	143.5	143.5	144.3	144.4	143.6
4′	144.3	145.1	144.4	144.5	145.5	145.6	145.3
5′	115.9	116.3	116.0	116.2	115.9	116.1	115.9
6′	119.4	119.7	119.3	118.9	119.3	119.3	119.2
1″	130.5	130.8	130.4	130.5	128.7	128.7	130.6
	130.6	130.9	130.5	130.8	128.8	129.0	130.7
				115.8			115.9
2″	116.0 (2C)	116.3 (2C)	115.9 (2C)		116.0 (2C)	115.9 (2C)	
				115.9			116.0
	143.2	143.7	143.3		144.5		143.3
3″				143.3 (2C)		144.6 (2C)	
	143.3	144.0	143.4		144.6		143.4
	144.5	144.9	144.6	144.7		145.4	144.5
4″					145.4 (2C)		
	144.6	145.3	144.7	144.8		145.3	144.6
	116.8					117.2	
5″		117.2 (2C)	116.8 (2C)	116.9 (2C)	117.3 (2C)		117.1 (2C)
	116.9					117.3	
		121.8				121.6	121.4
6″	121.5 (2C)		121.4 (2C)	121.4 (2C)	121.6 (2C)		
		122.0				121.7	121.6
	37.2	37.7	37.3	37.3	37.1	37.3	37.3
7″							
	37.5	38.0	37.6	37.4	37.4	37.4	37.6
	77.6	78.1		77.7	74.2	74.1	
8″			77.8 (2C)				77.8 (2C)
	77.8	78.3		77.8	74.3	74.4	
	174.4	176.5	176.8	176.8	171.6	171.0	175.8
9″							
	176.2	176.7	178.0	177.4	171.5	171.4	178.5
2-COO-	173.2	173.8	173.4	173.5	172.0	172.0	173.0
3-COO-	167.9	168.4	168.0	167.9	166.7	166.6	167.6
Glucosyl							
1							101.5
2							73.5
3							76.5
4							70.1
5							76.0
6							61.2

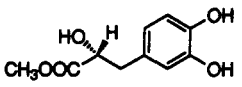
[a]Measured in Me_2CO-d_6+D_2O.
[b]Measured in Me_2CO-d_6.



10

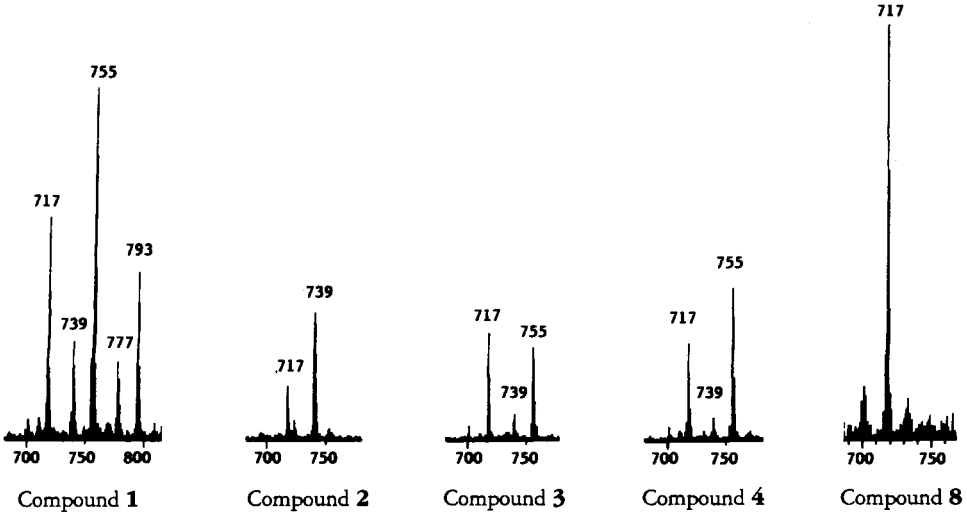


11



12

decamethylate [7]. Subsequent alkaline methanolysis of **6** and **7** with NaOMe-MeOH yielded (−)-[**10**] (8,9) and (+)-dimethyl-1,2-dihydro-1-(3,4-dimethoxyphenyl)-6,7-dimethoxynaphthalene-2,3-dicarboxylate [**11**] (9), respectively, together with methyl 3-(3′,4′-dimethoxyphenyl)-(*R*)-lactate [**12**] (8). Therefore, compounds **6** and **7** were shown to be identical with rabdosiin decamethylate and its stereoisomer, respectively, by comparison of their physical and spectral data with those described in the literature (8,9). Because **1**–**4** were almost insoluble in most organic solvents but were freely soluble in H_2O, these compounds were considered to be salts of the caffeic acid tetramers **8** and **9**. On treatment with dilute HCl, followed by extraction with EtOAc, **1**–**3** furnished rabdosiin [**8**], while **4** yielded compound **9**, thus confirming this assumption. Furthermore, negative fabms showed the presence of alkaline metals (Figure 1) (10). Using negative fabms, rabdosiin [**8**] and its isomer [**9**] gave identical spectra, which both exhibited a $[M-H]^-$ peak at *m/z* 717; there was no peak higher than the $[M-H]^-$ peak. In contrast, the negative fabms of **2** exhibited, together with a peak at *m/z* 717, a peak at *m/z* 739, which coincided with the molecular mass for the monosodium salt of **8**. Accordingly, compound **2** was concluded to be rabdosiin sodium salt. Using negative fabms, **3** and **4** gave similar spectra to each other and both showed a peak at *m/z* 755, corresponding to the molecular mass of the monopotassium salts of **8** and **9**, respectively. A small peak at *m/z* 739 was also observed in each case, but these compounds were considered to be mainly the potassium salts. Consequently, compounds **3** and **4** were concluded to be potassium salts of rabdosiin [**8**] and its stereoisomer [**9**], respectively, although they are contaminated with a small amount of the corresponding sodium salts. On the other hand, the negative fabms of **1** gave peaks at *m/z* 793 and 755, which

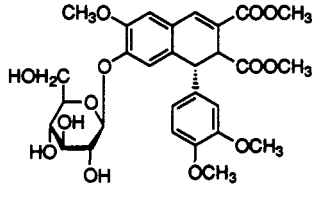


Compound **1** Compound **2** Compound **3** Compound **4** Compound **8**

FIGURE 1. Negative-ion fabms data of compounds **1**–**4** and **8**. (Figures indicate *m/z* values).

coincided with the molecular masses of the di- and monopotassium salts of **8**, respectively. It also showed ions at *m/z* 777 and 739, corresponding to the di- and monosodium salts of **8**. Therefore, compound **1** was concluded to be a mixture of the dipotassium and disodium salts of **8**, although the proportion of the dipotassium salt seems to be predominant. The positions of the sodium and potassium salts in **1** were concluded to be at the C-8″ carboxyl groups, since the C-8″ and C-9″ signals in the ^{13}C-nmr spectrum were shifted downfield compared to those of **8**. Downfield shifts of C-8″ and C-9″ were also observed in **2–4** relative to those in **8** and **9**. However, which carboxyl group is bound to the potassium or sodium ion still remains to be determined.

The ^{1}H-nmr spectrum of **5** was similar to those of **1–4**, showing signals due to an olefinic singlet, three catechol rings, two aromatic singlets, two oxygen-carrying methines, two aliphatic methines, and two methylenes. The existence of a sugar moiety in **5** was easily deduced from an anomeric proton resonance (δ 5.09) and by six aliphatic carbon resonances (Table 2). The negative fabms exhibited a peak at *m/z* 879, which coincided with the $[M-H]^-$ ion peak for the hexoside of compounds **8** and **9**. Acid hydrolysis of **5** yielded glucose, thus confirming its sugar moiety. Methylation of **5**, followed by methanolysis with NaOMe/MeOH furnished 3-(3′,4′-dimethoxyphenyl)-(*R*)-lactate [**12**] (8) and a hydrolysate [**13**]. The ^{1}H-nmr spectrum of **13** showed signals due to a catechol ring [δ 6.66 (1H, d, J=8 Hz), 6.64 (1H, d, J=2 Hz) and 6.44 (1H, dd, J=2 and 8 Hz)], an olefinic singlet (δ 7.64), two aromatic singlets (δ 6.84 and 6.81), and two aliphatic doublets [δ 4.57 and 4.03 (each 1H, d, J=3 Hz)], which were similar to those of dimethyl-1,2-dihydro-1-(3,4-dimethoxyphenyl)-6,7-dimethoxy-6,7-dimethoxynaphthalene-2,3-dicarboxylate (**10** and **11**). Compound **13** also showed an anomeric proton signal [δ 4.78 (1H, d, J=8 Hz)], together with five methoxy singlets [δ 3.78, 3.77, 3.76, 3.65, and 3.64 (each 3H)], suggesting that **13** is a glucoside of **10** or **11**. This was further supported by the positive fabms of **13**, which showed a quasimolecular ion peak at *m/z* 613 $(M+Na)^+$. The location of the glucosyl moiety in **13** was concluded to be at the C-7 hydroxy group, based on a nOe difference experiment; irradiation of the methoxy signals at δ 3.76–3.78 resulted in the observation of nOes at H-5 (δ 6.84), H-2′ (δ 6.64), and H-5′ (δ 6.66). An nOe was also observed at H-4 (δ 7.64), which was considered to be an nOe transferred through H-5. The mode of the sugar linkage was concluded to be β from the anomeric coupling constant value (d, J=8 Hz). The configurations at C-1 and C-2 in **5** were concluded to be *R* and *S*, respectively, since the cd spectrum of **13** exhibited peaks (Δε) at 241 (−2), 250 (−3), and 308 (−10) nm, and troughs at 220 (−9), 276 (−5), and 355 (−13) nm, similar to those seen in **10** (8), although the Δε values were shifted to the negative side, probably due to the contribution of the sugar moiety. The negative fabms of **5** exhibited, along with a peak at *m/z* 879, peaks at *m/z* 901, 917, 939, and 955, which corresponded to sodium, potassium, sodium-potassium, and dipotassium salts of rabdosiin 7-*O*-β-D-glucoside, respectively. Since downfield shifts of C-8″ and C-9″ were also observed in **5**, these salts were bound to the C-8″ carboxyl groups. Consequently, the structure of **5** was concluded



13

to be a mixture of dipotassium and sodium-potassium salts of rabdosiin 7-*O*-β-D-glucoside.

Previously, compounds **8** and **9** were isolated from the same plant source (7,8). However, they were isolated under acidic conditions (prep. hplc; CH_3CN/H_2O/HCOOH as eluent). This suggested that they are present in their salt forms in the plant material.

Compounds **2–4** demonstrated relatively potent anti-HIV activity in acutely infected H9 cells, with EC_{50} values of 2.8, 4.0, and 1.5 μg/ml, respectively. Their therapeutic index values were, in turn, 19.6, 12.5, and 33.3. Compounds **1** and **5** were less active (IC_{50} values of 16 and 30 μg/ml, respectively; therapeutic index values of 3 and 2, respectively) (Table 3). Compounds **8** and **9**, which are the free acid analogues of **2–4**, were found to be less active (IC_{50} values of 15 and 13 μg/ml, respectively) than **2–4**, although they showed similar levels of toxicity. This indicated that a monopotassium or monosodium salt at the C-8″ carboxyl group is important for enhanced anti-HIV activity.

It is also of note that uridine was also isolated from the aqueous fraction, and was shown to exhibit a weak anti-HIV activity with an EC_{50} value of 10 μg/ml and a therapeutic index value of >10.

TABLE 3. HIV Inhibitory Effects (μg/ml) for Compounds **1–5**, **8**, and **9**.[a]

Compound	EC_{50}[b]	IC_{50}[c]	T.I.[d]
1	16	50	3.1
2	2.8	55	19.6
3	4.0	50	12.5
4	1.5	50	33.3
5	30	60	2
8	15	50	3.3
9	13	45	3.5

[a]Data represent mean values for three (for **2–4**) or two (for **1**, **5**, **8**, and **9**) experiments.

[b]Concentration which inhibits virus replication by 50%.

[c]Concentration which inhibits H9 cell growth by 50%.

[d]Therapeutic index.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker AC-300 spectrometer with TMS as internal standard. Fabms measurements were carried out on JEOL JMS-HX100 and JMS-AX505H mass spectrometers. The cd spectrum was measured with a Jasco J-720 spectropolarimeter. Hplc was conducted on a Shimadzu LC-6A apparatus equipped with a SPD-6AV spectrometer.

PLANT MATERIAL.—The dried root of *Arnebia euchroma* [=*Macrotomia euchroma* (Royle.) Pauls] (Boraginaceae) (7), grown in Mengku Province, People's Republic of China, was obtained from Mikuni Co., Ltd. A voucher specimen of this material is available for inspection at Mikuni Co., Japan.

EXTRACTION AND ISOLATION.—The dried roots of *Arnebia euchroma* (4.5 kg) were extracted with hexane (2×7 liters), and the extract was concentrated to afford a hexane fraction (165 g). The residue was subsequently extracted with 70% aqueous Me_2CO (4×5 liters). The aqueous Me_2CO extract was concentrated to yield an aqueous solution, which was extracted successively with EtOAc and *n*-BuOH, giving EtOAc (96 g), *n*-BuOH (15 g), and H_2O (125 g) fractions. The H_2O fraction was chromatographed over MCI-gel CHP20P with H_2O containing an increasing amount of MeOH to give six fractions: Fractions I (106 g), II (2.4 g), III (1.8 g), IV (6.5 g), V (2.4 g), and VI (3.3 g). Fraction III was subsequently chromatographed over Sephadex LH-20 (H_2O) and Bondapak C_{18} [H_2O-MeOH (1:0→3:7)] to furnish compound **1** (468 mg). Repeated chromatography of fraction IV on Sephadex LH-20 [H_2O-MeOH

(1:0→1:1)], Bondapak C_{18} [H_2O-MeOH (1:0→3:7)], and Toyopearl HW40F [H_2O-MeOH (1:0→3:7)], afforded compounds **2** (328 mg), **3** (368 mg), and **4** (338 mg). Fraction II was chromatographed on Sephadex LH-20 (H_2O) and then Bondapak C_{18} [H_2O-MeOH (1:0→3:7)] to yield compound **5** (42 mg) and uridine (50 mg). Uridine was identified by comparison with an authentic sample.

Compound **1**.—A tan amorphous powder; $[\alpha]^{24}D$ −113.5° [*c*=0.77, Me_2CO-H_2O (1:1)]; negative fabms *m/z* 793 (27), 777 (12), 755 (56), 739 (16), 717 (35); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Compound **2**.—An off-white amorphous powder; $[\alpha]^{24}D$ −95.1° [*c*=0.68, Me_2CO-H_2O (1:1)]; negative fabms *m/z* 739 (20), 717 (9); ^{1}H-nmr data, see Table 1; ^{13}C-nmr, see Table 2; *anal.* found C 50.80%, H 4.79%, calcd for $C_{36}H_{29}O_{16}Na{\cdot}6H_2O$, C 50.95%, H 4.87%.

Compound **3**.—An off-white amorphous powder; $[\alpha]^{24}D$ −102.4° [*c*=0.63, Me_2CO-H_2O (1:1)]; negative fabms *m/z* 755 (15), 739 (3), 717 (17); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* found C 51.50%, H 4.96%, calcd for $C_{36}H_{29}O_{16}K{\cdot}5H_2O$, C 51.50%, H 4.67%.

Methylation of **1–3**.—A mixture of each sample (50–70 mg), Me_2SO_4 (0.3–0.4 ml), and anhydrous K_2CO_3 (500–600 mg) in dry Me_2CO (10–15 ml) was stirred at room temperature for 2 h, and then refluxed for 5 h with stirring. After removal of the inorganic salt by filtration, the filtrate was concentrated under reduced pressure and chromatographed on a Si gel column. Elution with C_6H_6-EtOAc (3:1) gave the decamethylate [**6**] (14–18 mg) as a white amorphous powder, $[\alpha]^{24}D$ −89.1° (*c*=0.2, $CHCl_3$); ^{1}H nmr δ 3.04, 3.10 (2H each, d, *J*=6 Hz), 3.70, 3.80, 3.81, 3.83, 3.86, 3.91 (30H in total, each s), 4.00 (1H, d, *J*=2 Hz), 4.61 (1H, d, *J*=2 Hz), 5.13 (1H, t, *J*=6.5 Hz), 5.21 (1H, t, *J*=6.5 Hz), 6.40 (1H, dd *J*=2 and 8 Hz), 6.53 (1H, d, *J*=2 Hz), 6.61 (1H, s), 6.63 (1H, dd, *J*=2 and 8 Hz), 6.65 (1H, dd, *J*=2 and 8 Hz), 6.66 (1H, d, *J*=8 Hz), 6.70 (1H, s), 6.75 (1H, d, *J*=8 Hz), 6.78 (1H, d, *J*=8 Hz), 7.62 (1H, s), 7.70 (1H, d, *J*=2 Hz), 7.73 (1H, d, *J*=2 Hz). Compound **6** was found to be rabdosiin octamethylate by comparison of physical and spectral data with those described in the literature (8,9).

Methanolysis of **6**.—A solution of **6** (52 mg) in 2% NaOMe/MeOH (5 ml) was kept at room temperature for 2 h. The reaction mixture was neutralized with IR-120B resin, filtered, and concentrated. The residue was chromatographed over Si gel [C_6H_6-Me_2CO (20:1→15:1)] to yield **12** (11 mg) as a colorless syrup, $[\alpha]^{24}D$ −4.0° (*c*=0.2, $CHCl_3$); ^{1}H nmr ($CDCl_3$) δ 2.70 (1H, d, *J*=6 Hz, OH), 2.90 (1H, dd, *J*=6 and 14 Hz, H-3), 3.09 (1H, dd, *J*=4 and 13 Hz, H-3), 3.77, 3.86, 3.88 (3H each, s, OCH_3), 4.45 (1H, dt, *J*=4 and 6.5 Hz, H-2), 6.83–6.70 (3H, m), and **10** (14 mg), as a white amorphous powder, $[\alpha]^{24}D$ −89.5° (*c*=0.1, $CHCl_3$); ^{1}H nmr ($CDCl_3$) δ 3.65, 3.77, 3.80, 3.82, 3.83, 3.90 (3H each, s, OCH_3), 4.01 (1H, d, *J*=3 Hz, H-2), 4.63 (1H, d, *J*=3 Hz, H-1), 6.41 (1H, dd, *J*=2 and 8 Hz, H-6′), 6.66 (1H, d, *J*=2 Hz, H-2′), 6.65 (1H, s, H-5), 6.68 (1H, d, *J*=8 Hz, H-5′), 6.88 (1H, s, H-8), 7.66 (1H, s, H-4). Compounds **10** and **12** were shown to be identical with (−)-dimethyl-1,2-dihydro-1-(3,4-dimethoxyphenyl)-6,7-dimethoxynaphthalene-2,3-dicarboxylate and methyl 3-(3′,4′-dimethoxyphenyl)-(*R*)-lactate, respectively, by comparison of physical and spectral data with those described in the literature (8).

Acid treatment of **1–3**.—Compounds **1** (75 mg), **2** (32 mg), and **3** (31 mg) were separately treated with 0.5 N HCl (2–5 ml). The solution was diluted with H_2O and extracted with EtOAc giving **8** (67 mg from **1**; 31 mg from **2**; 29 mg from **3**) as an off-white amorphous powder, $[\alpha]^{24}D$ −61.5° (*c*=0.3, MeOH), which was shown to be identical with rabdosiin by comparison of physical and spectral data with those described in the literature (8,9); ^{1}H nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Compound **4**.—An off-white amorphous powder; $[\alpha]^{24}D$ +132.8° [*c*=0.94, Me_2CO-H_2O (1:1)]; negative fabms *m/z* 755 (24), 739 (4), 717 (9); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* found C 52.36%, H 4.50%, calcd for $C_{36}H_{29}O_{16}K{\cdot}4H_2O$, C 52.17%, H 4.50%.

Methylation of **4**.—A mixture of **4** (75 mg), Me_2SO_4 (0.5 ml), and anhydrous K_2CO_3 (700 mg) in dry Me_2CO (10 ml) was stirred at room temperature for 2 h, and then refluxed for 5 h with stirring. The reaction mixture was worked up as for **1–3** to give the decamethylate [**7**] (24 mg) as a white amorphous powder, $[\alpha]^{24}D$ +115.1° (*c*=0.2, $CHCl_3$); ^{1}H nmr δ 3.04, 3.10 (each 2H, m), 3.50, 3.60, 3.75, 3.80, 3.84, 3.91 (30H in total, each s), 4.02 (1H, d, *J*=2 Hz), 4.41 (1H, d, *J*=2 Hz), 5.10 (1H, dd, *J*=5 and 8.5 Hz), 5.21 (1H, t, *J*=6.5 Hz), 6.40 (1H, dd, *J*=2 and 8 Hz), 6.50 (1H, s), 6.57 (1H, d, *J*=2 Hz), 6.63–6.78 (7H in total, m), 6.80 (1H, s), 7.70 (1H, s). The structure was assigned by comparison of physical and spectral data with those described in the literature (8).

Methanolysis of **7**.—A solution of **7** (10 mg) in 2% NaOMe/MeOH (3 ml) was kept standing at room temperature for 3 h. The reaction mixture was worked up as for **6** to yield **12** (2 mg), and **11** (3 mg), as a white amorphous powder, $[\alpha]^{24}D$ +90.5° (*c*=0.1, $CHCl_3$). The ^{1}H-nmr ($CDCl_3$) data were the same as those of **10**. Compound **11** was shown to be identical with (+)-dimethyl-1,2-dihydro-1-(3,4-dimethoxyphenyl)-

6,7-dimethoxynaphthalene-2,3-dicarboxylate by comparison of physical and spectral data with those described in the literature (8).

Acid treatment of **4**.—Compound **4** (25 mg) was treated with 0.5 N HCl (2 ml). The solution was diluted with H_2O and extracted with EtOAc giving **9** (24 mg) as an off-white amorphous powder, $[\alpha]^{24}D$ +132.4° (c=0.2, MeOH), which was shown to be identical with the rabdosiin isomer by comparison of physical and spectral data with those described in the literature (8); ^{1}H-nmr data, see Table 1, ^{13}C-nmr data, see Table 2.

Compound **5**.—An off-white amorphous powder; $[\alpha]^{24}D$ −125.4° [c=0.46, Me_2CO-H_2O (1:1)]; negative fabms m/z 955 (8), 939 (8), 917 (28), 901 (13), 879 (16); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* found C 47.85%, H 4.87%, calcd for $C_{42}H_{38}O_{21}K_2 \cdot 6H_2O$, C 47.36%, H 4.73%.

Methylation followed by methanolysis of **5**.—A solution of **5** (15 mg) in MeOH (5 ml) was treated with ethereal CH_2N_2 for 3 h. The reaction mixture was concentrated and was subsequently treated with Me_2SO_4 (0.5 ml) and anhydrous K_2CO_3 (700 mg) in dry Me_2CO (10 ml) at reflux for 5 h with stirring. The reaction mixture was worked up as for **1**–**4** to give a methylate (11 mg) as a white amorphous powder, $[\alpha]^{24}D$ −88.1° (c=0.48, $CHCl_3$), which was further treated with 2% NaOMe/MeOH (5 ml) for 5 h at room temperature. Workup as described above and Si gel chromatography with C_6H_6-Me_2CO (3:1→1:1) furnished **12** (3.5 mg) and **5a** (3.7 mg). **5a**: A white amorphous powder; $[\alpha]^{20}D$ −88.1° (c=0.18, $CHCl_3$); positive fabms m/z 613 $[M+Na]^+$; ^{1}H nmr ($CDCl_3$) δ 3.64, 3.65, 3.76, 3.77, 3.78 (each 3H, s, OCH_3), 4.03 (1H, d, J=3 Hz, H-2), 4.57 (1H, d, J=3 Hz, H-1), 4.78 (1H, d, J=8 Hz, anomeric-H), 6.44 (1H, dd, J=2 and 8 Hz, H-6′), 6.64 (1H, s, J=2 Hz, H-2′), 6.66 (1H, d, J=8 Hz, H-5′), 6.81 (1H, s, H-8), 6.84 (1H, s, H-5), 7.64 (1H, s, H-4).

HIV GROWTH INHIBITION ASSAY.—The H9 T-cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37° and was used in experiments only when in the log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/ml, at a multiplicity of infection of 0.1–0.001 IU/cell) for 1 h at 37° and 5% CO_2. The cells were then washed thoroughly to remove unadsorbed virions and resuspended at 4×10^5 cells/ml in complete medium. Aliquots (1 ml) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a four-day incubation at 37°, the cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus related in the medium of the HIV-infected cultures. The p24 antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 is used to tag any p24 "captured" onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples was then revealed by addition of substrate. The cut-off for the p24 ELISA assay is 12.5 pg/ml. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

ACKNOWLEDGMENTS

This investigation was supported by grant AI-33066 from the National Institute of Allergy and Infectious Diseases awarded to K.-H. Lee.

LITERATURE CITED

1. L. Xie, J.X. Xie, Y. Kashiwada, L.M. Cosentino, S.H. Liu, R.B. Pai, Y.C. Cheng, and K.-H. Lee, *J. Med. Chem.*, in press.
2. R.F. Schinazi, J.R. Mead, and P.M. Feorino, *AIDS Res. Human Retrovir.*, **8**, 963 (1992).
3. T. Shirahata, R. Yarchoan, M.C. O'Brien, R.N. Husson, B.D. Anderson, E. Kojima, T. Shimoda, S. Broder, and H. Mitsuya, *Proc. Natl. Acad. Sci. USA*, **91**, 562 (1993).
4. M.I. Johnson and D.F. Horth, *Science*, **260**, 1286 (1993).
5. D.D. Ho, T. Moudgil, and M. Alam, *N. Engl. J. Med.*, **321**, 1622 (1989).
6. "Encyclopedia of Chinese Materia Medica (Zhon Yao Dai Zi Ten)," Ed. by Jian Su New Medical College, Shanghai Science and Technology Publishers, Shanghai, 1977, p. 2342.
7. "Zhon Yao Zhi, Vol. 1," Chinese Academy of Medical Sciences, People's Hygenic Publishers, Shanghai, 1979, p. 596.
8. M. Nishizawa, M. Tsuda, and K. Hayashi, *Phytochemistry*, **29**, 2645 (1990).
9. I. Agata, T. Hatano, S. Nishibe, and T. Okuda, *Phytochemistry*, **28**, 2447 (1989).
10. T. Tanaka, S. Morimoto, G. Nonaka, I. Nishioka, T. Yokozawa, H.Y. Chung, and H. Oura, *Chem. Pharm. Bull.*, **37**, 340 (1989).

Received 27 June 1994