Cyclobutane Dimers from the Colombian Medicinal Plant *Achyrocline bogotensis*

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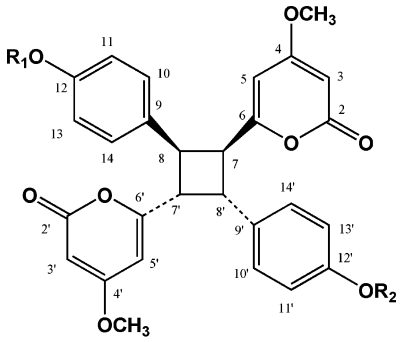
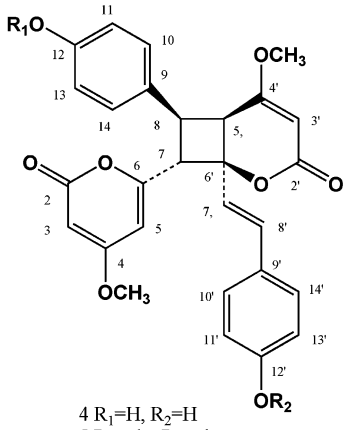
Five cyclobutane dimers, achyrodimers A−E (**1**−**5**), along with 11 known compounds were isolated from the methanol extract of the Colombian medicinal plant *Achyrocline bogotensis*. Their structures were elucidated by spectroscopy. Several of these compounds were screened for cytokine-inducing activity in human peripheral blood mononuclear cells.

Achyrocline bogotensis (HBK.) DC. (Asteraceae) is a folk medicinal herb grown in Colombia. This plant, known by the vernacular name "vira-vira", "cenizo", or "suso", is used for treatment of skin disease or prostatitis.[1] The only reported constituents of *A. bogotensis* have been flavonoids.[2] Recently, achyrofuran from a plant of the same genus, *A. satureioides*, was found to have significant antihyperglycemic activity in a mouse model of type 2 diabetes.[3] Some additional constituents from the same genus have also been reported.[4,5] In our search for pharmacologically active compounds from Colombian crude drugs of plant origin,[6] we studied the chemical constituents of *A. bogotensis*. This paper deals with the isolation and structural elucidation of five new cyclobutane dimers, achyrodimers A−E (**1**−**5**), and 11 known compounds, from the methanol extracts of *A. bogotensis*. The cytokine-inducing activity of some of the isolated compounds is also reported.



1 R_1=H, R_2=H
2 R_1=glu, R_2=H
3 R_1=glu, R_2=glu



4 R_1=H, R_2=H
5 R_1=glu, R_2=glu

Results and Discussion

Repeated column chromatography of an ethyl acetate soluble fraction from the methanol extract of the aerial parts of *A. bogotensis* yielded five new compounds (**1**−**5**) and 11 known compounds.

Achyrodimer A (**1**), obtained as an amorphous powder, showed hydroxy and carbonyl bands at 3394 and 1689 cm^{-1} in the IR spectrum, and the UV spectrum indicated the presence of an aromatic moiety (285 nm). The ^1H NMR spectrum showed signals due to a disubstituted benzene ring [δ_H 7.16, 7.47 (each 2H, d, J = 8.4 Hz)], four methines [δ_H 4.43 and 4.63 (each 1H, dd, J = 7.3, 9.5 Hz), 5.38 and 6.28 (each 1H, d, J = 2.0 Hz)], and one methoxyl [δ_H 3.39 (3H, s)]. The ^{13}C NMR spectrum (Table 1) showed 12 signals due to a disubstituted benzene ring (δ_C 116.0, 128.8, 129.3, 157.8), two methines (δ_C 43.2 and 46.0), one methoxyl (δ_C 55.4), and a γ-pyrone ring (δ_C 87.8, 101.1, 163.6, 163.9, and 170.6), which was assigned by comparing these with the chemical shifts of the known compound *p*-hydroxy-5,6-dehydrokawanin.[7] From these results, the partial structures of disubstituted benzene, γ-pyrone, and >CH−CH< were estimated for compound **1**. In the HMBC spectrum, the proton signals at δ_H 7.47 (H-10 and H-14) were correlated with the carbon signal at δ_C 43.2 (C-8), indicating the connection of partial structures, disubstituted benzene and γ-pyrone. The proton signal at δ_H 4.43 (H-7) was correlated with the carbon signals at δ_C 101.1 (C-5) and 163.9 (C-6), indicating the connection of partial structures, γ-pyrone and >CH−CH<, and the methoxyl proton at δ_H 3.39 was correlated with the carbon signal at δ_C 170.6 (C-4). The $^1H-^1H$ COSY and other HMBC data

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Table 1. ^{13}C NMR Data (δ) for **1**–**5**

C	1	2	3	4	5
2	163.6	167.1*	167.0	163.5	166.8
3	87.8	88.2	88.3	88.5	89.1
4	170.6	173.3	173.2	170.3	173.1
5	101.1	102.9	103.0	101.8	103.4
6	163.9	165.0	164.8	160.4	161.6
7	46.0	46.5	46.4	55.1	55.1
8	43.2	44.0	44.1	38.9	39.7
9	128.8	133.1	133.0	127.0	131.1
10	129.3	129.7	129.8	129.3	130.0
11	116.0	117.7	117.7	116.0	117.7
12	157.8	158.1	158.1	158.4	158.7
13	116.0	117.7	117.7	116.0	117.7
14	129.3	129.7	133.0	129.3	130.0
2′	163.6	167.0*	167.0	164.4	167.3
3′	87.8	88.2	88.3	92.4	92.3
4′	170.6	173.3	173.2	170.3	172.7
5′	101.1	102.9	103.0	46.0	46.9
6′	163.9	165.0	164.8	79.5	80.8
7′	46.0	46.5	46.4	122.6	124.5
8′	43.2	44.2	44.1	131.0	131.8
9′	128.8	129.8	133.0	127.7	131.7
10′	129.3	129.8	129.8	128.6	129.1
11′	116.0	116.2	117.7	116.3	117.9
12′	157.8	157.5	158.1	159.1	159.2
13′	116.0	116.2	117.7	116.3	117.9
14′	129.3	129.8	129.8	128.6	129.1
4-OMe	55.4	56.8	56.9	55.5*	57.0
4′-OMe	55.4	56.8	56.9	55.3*	56.4
1″		102.3	102.2		102.1[a]
2″		74.9	74.9		74.9
3″		78.1	78.1		78.2
4″		71.3	71.3		71.4
5″		78.0	78.0		78.0
6″		62.5	62.5		62.5
1‴			102.2		102.2[a]
2‴			74.9		74.9
3‴			78.1		78.2
4‴			71.3		71.4
5‴			78.0		78.0
6‴			62.5		62.5

[a] Exchangeable signals.

of **1** supported the half-structure ($C_{14}H_{12}O_4$). The positive HRFABMS of compound **1** gave a quasi-molecular ion peak at *m/z* 489.1570 $[M + H]^+$, which suggested the molecular formula $C_{28}H_{24}O_8$ for **1**. From these data, compound **1** was thought to be a dimer of half-structure ($C_{14}H_{12}O_4$). In the HMBC spectrum, the proton signals of H-7 and H-8 were correlated with the carbon signals of C-7 and C-8. The NOE correlation of H-7 and H-8 was also observed. The $[\alpha]_D$ value of compound **1** was almost 0°. From these results, the structure of compound **1** was concluded to be the point symmetry dimer as shown.

Achyrodimer B (**2**), obtained as an amorophous powder, showed hydroxy and carbonyl bands at 3319 and 1690 cm^{-1} in the IR spectrum. The ^1H NMR spectrum showed two methoxyl protons, two disubstituted benzene rings, two γ-pyrone rings [δ_H 5.32, 5.33, 5.96, and 5.98 (each 1H, d, J = 2.0 Hz)], four methine protons [δ_H 4.25 (2H, dd, J = 8.1, 10.2 Hz), 4.34 and 4.38 (each 1H, dd, J = 8.1, 10.2 Hz)], and a sugar moiety. The ^{13}C NMR spectrum showed 34 signals including two methoxyl carbons, two disubstituted benzene rings, two γ-pyrone rings, four methine carbons, and one sugar moiety (glucose: δ_C 62.5, 71.3, 74.9, 78.0, 78.1, and 102.3). The ^{13}C NMR data of compounds **1** and **2** were very similar except for the glucose moiety in compound **2**. The positive HRFABMS of compound **2** gave the quasi-molecular ion peak at *m/z* 651.2054 $[M + H]^+$, suggesting the molecular formula $C_{34}H_{34}O_{13}$. From these results, compound **2** was estimated to be a glycoside of compound **1**. In the HMBC spectrum, the glucose anomeric proton signal at δ_H 4.85 (1H, d, J = 7.3 Hz) was correlated with the carbon signal at δ_C 158.1 (C-12), indicating that the glucose was on C-12 and had a β-configuration. The relative stereochemistry of the cyclobutane ring was determined to be the same as that of compound **1** from the coupling constants of the methine protons (J = 8.1 and 10.2 Hz). Thus, the structure of achyrodimer B (**2**) was assigned as shown.

Achyrodimer C (**3**) has the molecular formula $C_{40}H_{44}O_{18}$ based on the HRFABMS (*m/z* 813.2619 $[M + H]^+$). The ^{13}C NMR (Table 1) and ^1H NMR data of **3** were compared with those of the closely related **2**. These data suggested that the only difference was that compound **3** has two glucose units but compound **2** has only one glucose moiety. The ^{13}C NMR spectrum of **3** showed the same chemical shift values as those of compound **2**. In the HMBC spectrum, the glucose anomeric protons at δ_H 4.90 (d, J = 7.3 Hz) were correlated with the carbon signals at δ_C 158.1 (C-12 and C-12′), indicating that the glucose moieties are on C-12 and C-12′, and are β. Thus, the structure of anhyrodimer C (**3**) was assigned as shown.

Achyrodimer D (**4**), obtained as an amorphous powder, showed hydroxy and carbonyl bands at 3341 and 1690 cm^{-1} in the IR spectrum, and the UV spectrum indicated the presence of an aromatic moiety (268 nm). The ^1H NMR spectrum of **4** showed signals due to two disubstituted benzene rings [δ_H 7.10, 7.19, 7.34, and 7.53 (each 2H, d, J = 8.4 Hz)], two methoxyl protons, one *trans*-double bond [δ_H 6.87 and 7.26 (each 1H, d, J = 15.9 Hz)], one γ-pyrone ring [δ_H 5.44 and 6.11 (each 1H, d, J = 2.0 Hz)], the same as compounds **1**–**3**, and four methine protons [δ_H 3.75 (1H, d, J = 9.6 Hz), 4.48 (1H, dd, J = 9.6, 11.5 Hz), 4.61 (1H, d, J = 11.5 Hz), and 5.65 (1H, s)]. The ^{13}C NMR spectrum of **4** showed 28 signals, including two disubstituted benzene rings, one γ-pyrone ring, one *trans*-double bond, and two methoxyl carbons. The positive HRFABMS of compound **4** gave the quasi-molecular ion peak at *m/z* 489.1524 $[M + H]^+$, suggesting the molecular formula $C_{28}H_{24}O_8$. In the HMBC spectrum of **4**, the proton signal at δ_H 4.61 (H-7) was correlated with the carbon signals at δ_C 101.8 (C-5), 160.4 (C-6), and 79.5 (C-6′), the proton signal at δ_H 4.48 (H-8) with the carbon signal at δ_C 129.3 (C-10 and C-14), the proton signal at δ_H 3.75 (H-5′) with the carbon signals at δ_C 79.5 (C-6′) and 92.4 (C-3′), the proton signal at δ_H 6.87 (H-7′) with the carbon signals at δ_C 79.5 (C-6′) and 127.7 (C-9′), and the proton signal at δ_H 7.26 (H-8′) with the carbon signals at δ_C 79.5 (C-6′) and 128.6 (C-10′ and 14′). From these results and the methine proton coupling pattern, the structure of **4** was estimated to be another dimer of *p*-hydroxy-5,6-dehydrokawain[7] similar to compound **1**. This type of dimer was reported from *Alpinia speciosa* and named AS-2 (= aniba dimer A).[8] Compound **4** has two more hydroxy groups in comparison with AS-2. The positions of the hydroxy groups were determined, as indicated from the HMBC results, to be on C-12 and C-12′. The relative stereochemistry of **4** was determined to be the same as that of AS-2 (aniba dimer A) from the coupling constants of H-7, H-8, and H-5′. Thus, the structure of anhyrodimer D (**4**) was assigned as shown.

Anhyrodimer E (**5**) had the molecular formula $C_{40}H_{44}O_{18}$ based on HRFABMS (*m/z* 813.2549). The ^1H NMR and ^{13}C NMR data of **5** were very similar to those of compound **4** except for the two additional glucose moieties in **5**. In the HMBC spectrum of **5**, the anomeric proton signals at δ_H 4.95 (H-1″) and 4.96 (H-1‴) were correlated with the carbon signals at δ_C 158.7 (C-12) and 159.2 (C-12′), respectively. From this correlation and the anomeric proton coupling

Table 2. Inhibitory Effects for Cytokine Release of Compounds **2**, **3**, **5**, **6**, and **11**[a]

compound	cytokine production ratio		
	IL-10	IL-12	TNF-α
2	0.08	3.33	1.63
3	0.01	5.74	1.48
5	1.67	2.28	1.45
6	0.03	0.05	0.40
11	0.03	9.68	1.65
prednisolone	0.14	0.24	0.44

[a] PBMCs were treated with lipopolysaccharide (LPS) in the presence of compounds **2**, **3**, **5**, **6**, and **11** (each 10 μg/mL). Prednisolone (0.3 μg/mL) was used as a reference sample. Data were expressed as ratios to cytokine production induced by LPS.

constants (J = 7.4 and 7.2 Hz), the positions and configurations of glucose were determined to be on C-12 and C-12′, and β. Thus, the structure of anhyrodimer E (**5**) was determined as shown.

The known compounds 5-hydroxy-7,8-dimethoxyflavone,[9] 3,5-dihydroxy-7,8-dimethoxyflavone,[9] 3,5-dihydroxy-6,7,8-trimethoxyflavone,[9] pinoresinol,[10] syringaresinol,[11] *p*-hydroxy-5,6-dehydrokawanin,[7] scoporone,[12] scopoletin,[12] 10,-11-dihydro-10-hydroxytremeton,[13] ursolic acid, and 3-*O*-(β-D-glucopyranosil)sitosterol[14] were also isolated and were identified from spectroscopic data comparison with literature values.

Several of the isolated compounds, **2**, **3**, **5**, 5-hydroxy-7,8-dimethoxyflavone, and *p*-hydroxy-5,6-dehydrokawanin, were assayed for cytokine-inducing activity on human peripheral blood mononuclear cells (PBMCs) to investigate potential antitumor effects. No cytokine production was observed by the stimulation of human PBMCs by any of the test samples. We next examined the effect of the test samples in LPS-induced cytokine production. Data were expressed as ratios to cytokine production induced by LPS (Table 2). 5-Hydroxy-7,8-dimethoxyflavone markedly inhibited the LPS-induced production of IL-12, TNF-α, and IL-10. Interestingly, compounds **2**, **3**, and *p*-hydroxy-5,6-dehydrokawanin augmented the LPS-induced production of IL-12 and TNF-α, whereas compounds **2**, **3**, and *p*-hydroxy-5,6-dehydrokawanin markedly inhibited the LPS-induced IL-10 production. Helper T (Th) cells that are major cytokine-producing cells are devided into two subpopulations, Th1 and Th2. Th1 cells are induced by IL-12 and by IL-18 and produce IFN-γ, IL-2, TNF-α, and TNF-β. Th2 cells, induced by IL-4, produce IL-4, IL-5, IL-6, and IL-10.[15] It is reported that Th1-type and Th2-type T-cell responses can promote different immunopathological reactions. Th1-type responses appear to be involved in organ specific autoimmunity. In contrast, Th2-type responses are induced against common environmental allergens and are responsible for triggering allergic atopic disorders.[16] Thus, it is suggested that Th1 inducers as well as Th2 inhibitors may be effective for the treatment of allergic disorders and that Th2 inducers may be potential drugs for autoimmune diseases. Furthermore, it is also reported that Th1-type cytokines are effective in eliminating cancer cells in patients with malignancies.[17]

The data from the current study suggested that 5-hydroxy-7,8-dimethoxyflavone may be a strong inhibitor for whole cytokines and that the other samples, except for compound **5**, may be potent inducers of Th1-type T-cell response and inhibitors of the Th2-type response. These compounds may be potential therapeutic agents for several types of diseases as regulators in Th1/Th2 cytokine balance.

Experimental Section

General Experimental Procedures. NMR spectra (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both using TMS as internal standard) were measured on a Bruker ARX-400 instrument and a Bruker AVANCE-400 instrument. MS were obtained on a JEOL JMSD-300 instrument. CC: Silica gel 60 (Merck), Toyopearl HW-40 (Tosoh), and Sephadex LH-20 (Pharmacia); HPLC: GPC (gel-permeation chromatography: Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC (Hibar RT 250-25, LiChrosorb Si 60, $CHCl_3$−MeOH system). UV spectra were measured on a Beckman DU-650 spectrophotometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material. The aerial parts of *Achyrocline bogotensis* were collected from Guatavita, Colombia, in August 2001 and identified by Dr. Cristina Garzon. A voucher specimen (01JC010) is deposited in the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogota, Colombia.

Extraction and Isolation. The dried aerial parts (1.8 kg) of *A. bogotensis* were extracted with MeOH. The MeOH extracts were concentrated in vacuo to give a residue (77 g), which was suspended in H_2O and partitioned sequentially with EtOAc and *n*-BuOH. The EtOAc layer was concentrated to give a residue (26 g), which was subjected to silica gel column chromatography (1.2 kg, 11 × 100 cm) and eluted with solvents of increasing polarity (*n*-hexane−EtOAc, EtOAc, EtOAc−MeOH, MeOH) to give 16 major fractions (1−16). Fraction 10 (0.3 g) was subjected to Sephadex LH-20 (MeOH) to give eight fractions (10.1−10.8). Fractions 10.3 and 10.4 were chromatographed on a silica gel column and eluted with solvents of increasing polarity ($CHCl_3$, $CHCl_3$−MeOH, MeOH) to give **4** (17.5 mg). Fractions 12 (1.8 g) and 13 (1.0 g) were chromatographed on silica gel and eluted with solvents of increasing polarity ($CHCl_3$−MeOH, MeOH) to give nine fractions (12.1−12.9). Fraction 12.4 was subjected to HPLC (silica gel, $CHCl_3$) and preparative TLC ($CHCl_3$−MeOH) to give **1** (9.1 mg). Fractions 14−16 (4.4 g) were chromatographed on a silica gel column and eluted with solvents of increasing polarity ($CHCl_3$−MeOH, MeOH) to give 14 fractions (14.1−14.14). Fraction 14.9 was separated by GPC (MeOH) to give **3** (25.9 mg) and **5** (16.4 mg). Fraction 14.10 was subjected to chromatography on Sephadex LH-20 ($CHCl_3$−MeOH, 1:2) and GPC (MeOH) to give **2** (23.6 mg).

Known compounds were also isolated: 5-hydroxy-7,8-dimethoxyflavone (28.0 mg), 3,5-dihydroxy-7,8-dimethoxyflavone (3.7 mg), 3,5-dihydroxy-6,7,8-trimethoxyflavone (3.5 mg), pinoresinol (11.3 mg), syringaresinol (4.0 mg), *p*-hydroxy-5,6-dehydrokawanin (10.4 mg), scoporone (7.6 mg), scopoletin (7.6 mg), 10,11-dihydro-10-hydroxytremeton (14.5 mg), ursolic acid (5.3 mg), 3-*O*-(β-D-glucopyranosil)sitosterol (217.0 mg).

Compound 1 (achyrodimer A; 8,8′-di(12-hydroxyphenyl)-7,7′-di[6-(4-methoxy-2-pyronyl)]cyclobutane): colorless amorphous powder; $[\alpha]_D$ 0° (*c* 0.9, $CHCl_3$−MeOH); IR (KBr) ν_{max} 3394, 2921, 1689, 1637, 1561, 1516, 1257 cm^{-1}; UV ($CHCl_3$−MeOH) λ_{max} nm (log ϵ) 285 (4.23); ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 7.47 (4H, d, J = 8.4 Hz, H-10,10′,14,14′), 7.16 (4H, d, J = 8.4 Hz, H-11,11′,13,13′), 6.28 (2H, d, J = 2.0 Hz, H-4,4′), 5.38 (2H, d, J = 2.0 Hz, H-2,2′), 4.63 (2H, dd, J = 7.3, 9.5 Hz, H-8,8′), 4.43 (2H, dd, J = 7.3, 9.5 Hz, H-7,7′), 3.39 (6H, s, 4,4′-OMe); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRFABMS *m/z* 489.1570 $[M + H]^+$ (calcd for $C_{28}H_{25}O_8$, 489.1549).

Compound 2 (achyrodimer B; 8-(12-β-D-glucopyranosylphenyl)-8′-(12′-hydroxyphenyl)-7,7′-di[6-(4-methoxy-2-pyronyl)]cyclobutane): colorless amorphous powder; $[\alpha]_D$ −25.0° (*c* 0.9, MeOH); IR (KBr) ν_{max} 3319, 2881, 1710, 1690, 1679, 1565, 1513, 1412, 1250 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 284 (4.05); ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.24 (2H, d, J = 8.6 Hz, H-10,14), 7.14 (2H, d, J = 8.5 Hz, H-10′,14′), 7.01 (2H, d, J = 8.6 Hz, H-11,13), 6.69 (2H, d, J = 8.5 Hz, H-11′,13′), 5.98 (1H, d, J = 2.0 Hz, H-4′), 5.96 (1H, d, J = 2.0 Hz, H-4), 5.33 (1H, d, J = 2.0 Hz, H-2′), 5.32 (1H, d, J = 2.0 Hz, H-2), 4.85 (1H, d, J = 7.3 Hz, H-1″), 4.38 (1H, dd, J = 8.1, 10.2 Hz,

H-8), 4.34 (1H, dd, J = 8.1, 10.2 Hz, H-8′), 4.25 (2H, dd, J = 8.1, 10.2 Hz, H-7,7′), 3.87(1H, d, J = 11.3 Hz, H-6″), 3.72 (6H, s, 4,4′-OMe), 3.68 (1H, m, H-6″), 3.38−3.45 (4H, m, H-2″,3″,4″, 5″); ^{13}C NMR (CD_5OD, 100 MHz), see Table 1; HRFABMS *m/z* 651.2054 $[M + H]^+$ (calcd for $C_{34}H_{35}O_{13}$, 651.2078).

Compound 3 (achyrodimer C; 8,8′-(12-β-D-glucopyranosylphenyl)-7,7′-di[6-(4-methoxy-2-pyronyl)]cyclobutane): colorless amorphous powder; $[\alpha]_D$ −32.2° (*c* 1.7, MeOH) IR (KBr) ν_{max} 3265, 2882, 1711, 1690, 1679, 1565, 1512, 1412, 1254 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 282 (4.38); ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.31 (4H, d, J = 8.5 Hz, H-10,10′,14,-14′), 7.07 (4H, d, J = 8.5 Hz, H-11,11′,13,13′), 6.04 (2H, d, J = 2.2 Hz, H-4,4′), 5.38 (2H, d, J = 2.2 Hz, H-2,2′), 4.90 (1H, d, J = 7.3 Hz, H-1″,1‴), 4.45 (2H, dd, J = 8.3, 8.9 Hz, H-8,8′), 4.39 (2H, m, H-7,7′), 3.92 (1H, d, J = 11.8 Hz, H-6″,6‴), 3.77 (6H, s, 4,4′-OMe), 3.72 (2H, m, H-6″,6‴), 3.42−3.50 (8H, m, H-2″,3″,4″,5″,2‴,3‴,4‴,5‴); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HRFABMS *m/z* 813.2619 $[M + H]^+$ (calcd for $C_{40}H_{45}O_{18}$, 813.2606).

Compound 4 (achyrodimer D, 4′-methoxy-8-(12-hydroxyphenyl)-7-[6-(4-methoxy-2-pyronyl)]-6-(12′-hydroxy-trans-styryl)-1′-oxabicyclo[4,2,0]octa-4′-en-2′-one): colorless amorphous powder; $[\alpha]_D$ +27.0° (*c* 1.5, $CHCl_3$−MeOH); IR (KBr) ν_{max} 3341, 2923, 1710, 1690, 1679, 1564, 1514, 1254 cm^{-1}; UV ($CHCl_3$−MeOH) λ_{max} nm (log ϵ) 268 (4.66); ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 7.53 (2H, d, J = 8.4 Hz, H-10′,14′), 7.34 (2H, d, J = 8.4 Hz, H-10,14), 7.26 (1H, d, J = 15.9 Hz, H-8′), 7.19 (2H, d, J = 8.4 Hz, H-11,13), 7.10 (2H, d, J = 8.4 Hz, H-11′,13′), 6.87 (1H, d, J = 15.9 Hz, H-7′), 6.11 (1H, d, J = 2.0 Hz, H-5), 5.65 (1H, s, H-3′), 5.44 (1H, d, J = 2.0 Hz, H-3), 4.61 (1H, d, J = 11.5 Hz, H-7), 4.48 (1H, dd, J = 9.6, 11.5 Hz, H-8), 3.75 (1H, d, J = 9.6 Hz, H-5′), 3.43 (3H, s, 4′-OMe), 3.29 (3H, s, 4-OMe); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRFABMS *m/z* 489.1524 $[M + H]^+$ (calcd for $C_{28}H_{25}O_8$, 489.1549).

Compound 5 (achyrodimer E, 4′-methoxy-8-(12-β-D-glucopyranosylphenyl)-7-[6-(4-methoxy-2-pyronyl)]-6-(12′-β-D-glucopyranosyl-*trans*-styryl)-1′-oxabicyclo[4,2,0]-octa-4′-en-2′-one): colorless amorphous powder; $[\alpha]_D$ +58.1° (*c* 0.7, MeOH); IR (KBr) ν_{max} 3319, 2884, 1710, 1690, 1678, 1565, 1512, 1412, 1243 cm^{-1}; UV (MeOH) λ_{max} nm (log ϵ) 260 (4.51); ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.41 (2H, d, J = 8.7 Hz, H-10′,14′), 7.32 (2H, d, J = 8.4 Hz, H-10,14), 7.12 (2H, d, J = 8.7 Hz, H-11,13), 7.09 (2H, d, J = 8.7 Hz, H-11′,13′), 6.92 (1H, d, J = 15.9 Hz, H-8′), 6.53 (1H, d, J = 15.9 Hz, H-7′), 6.11 (1H, d, J = 2.0 Hz, H-5), 5.50 (1H, d, J = 2.0 Hz, H-3), 5.42 (1H, s, H-3′), 4.96 (1H, d, J = 7.2 Hz, H-1‴), 4.95 (1H, d, J = 7.4 Hz, H-1″), 4.43 (1H, d, J = 11.4 Hz, H-7), 4.29 (1H, dd, J = 9.6, 11.4 Hz, H-8), 3.93 (2H, d, J = 10.0 Hz, H-6″,6‴), 3.78 (3H, s, 4′-OMe), 3.74 (2H, m, H-6″,6‴)3.72 (2H, d, J = 9.6 Hz, H-5′), 3.44−3.52 (8H, m, H-2″,3″,4″,5″,2‴,3‴,4‴,5‴), 3.41 (3H, s, 4-OMe); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HRFABMS *m/z* 813.2549 $[M + H]^+$ (calcd for $C_{40}H_{45}O_{18}$, 813.2606).

Treatment of Human PBMCs and Cytokine-Inducing Assay. Human peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll-Hypaque gradient density centrifugation according to standard procedures.[15] PBMCs (1×10^6/mL) were cultured in RPMI 1640 medium containing 10% fetal calf serum in the presence or absence of the compounds (1 or 10 μg/mL) for 48 h at 37 °C, then cytokines in the supernatants of these cultures were analyzed by commercial ELISA kits. OK-432 (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), a *Streptococcus pyogenes*-derived immunopotentiator that is commonly used for immunotherapy in malignancies, was used as a positive control. ELISA kits for human IFN-γ, TNF-α, IL-4, IL-6, IL-10, and IL-12 were purchased from BioSource International, Inc. (Camarillo, CA).

References and Notes

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