Activity-Guided Fractionation of the Leaves of *Ormosia sumatrana* Using a Proteasome Inhibition Assay

Bao-Ning Su,[†,||] Bang Yeon Hwang,[†,▽] Heebyung Chai,[†] Esperanza J. Carcache-Blanco,[†] Leonardus B. S. Kardono,[‡] Johar J. Afriastini,[§] Soedarsono Riswan,[§] Robert Wild,[⊥] Naomi Laing,[⊥,○] Norman R. Farnsworth,[†] Geoffrey A. Cordell,[†] Steven M. Swanson,[†] and A. Douglas Kinghorn*[,†,||]

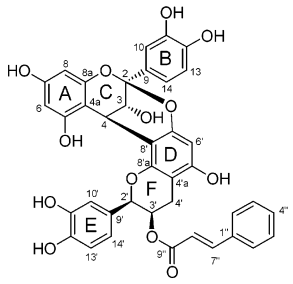
Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, Research and Development Chemistry, Indonesian Institute of Science, Serpong, 15310 Tangerang, Indonesia, Herbarium Bogoriense, Research and Development Center for Biology, Indonesian Institute of Science, 16122 Bogor, Indonesia, and Bristol-Myers Squibb, Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543

Received June 10, 2004

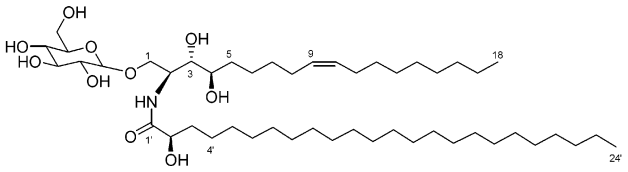
Activity-guided fractionation of a chloroform-soluble extract of the leaves of *Ormosia sumatrana*, using a proteasome inhibition assay, led to the isolation of a new A-type proanthocyanidin derivative, 3′-*O*-cinnamoylprocyanidin A-2 (**1**), and a new cerebroside, sumatranoside (**2**). The structures of these two isolates were determined as epicatechin-(2β→*O*→7′,4β→8′)-epicatechin-3′-*O*-cinnamate (**1**) and 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2*N*-[(2′*R*)-2′-hydroxy-tetracosanoyl]-9*Z*-octadecene-1,3,4-triol (**2**), respectively, by spectroscopic and chemical methods. Sumatranoside (**2**) exhibited proteasome inhibitory activity with an IC_{50} value of 30 μM.

The genus *Ormosia* Jacks. of the subfamily Papilionoideae of the Leguminosae comprises some 130 species, with about 70 species being indigenous to Central America and eastern South America, and the others found in east and southeast Asia and northeast Australia.[1] Previous phytochemical studies on *Ormosia* species have resulted in the isolation of various quinolizidine alkaloids and isoflavonoids.[2−5] No reports have appeared in the literature on the constituents of *O. sumatrana* (Miq.) Prain.

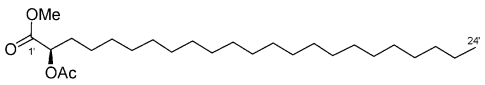
The ubiquitin-proteasome proteolytic system plays an important role in selective protein degradation and regulates cellular events, including cell-cycle progression, apoptosis, and inflammation.[6,7] Since proteasomes interact primarily with endogenous proteins, inhibition of the proteolytic action of the proteasome may block the signaling action of the transcription factor NF-κB and, thus, inhibit the completion of the cell cycle and hence the mitotic proliferation of cancerous cells, leading to cell death by apoptosis, and inhibition of angiogenesis and metastasis.[8] The potential of specific proteasome inhibitors, which may act as anticancer agents, is now of considerable interest in the drug discovery process. As part of a project directed toward the discovery of novel anticancer agents from plants, the chloroform-soluble extract of *O. sumatrana* was found to inhibit the chymotrypsin-like activity of the proteasome in vitro. Bioactivity-guided fractionation of this extract led to the isolation of a new A-type proanthocyanidin derivative, 3′-*O*-cinnamoylprocyanidin A-2 (**1**), and a new cerebroside, sumatranoside (**2**). The isolation, structure elucidation, and proteasome inhibitory activity of compounds **1** and **2** are reported.



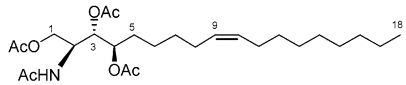
1



2



2a



2b

The molecular formula of **1** was determined as $C_{39}H_{30}O_{13}$ on the basis of HRFABMS (*m/z* 729.1613 $[M + Na]^+$, calcd for $C_{39}H_{30}O_{13}Na$, 729.1584). The IR and UV spectra of **1** revealed the presence of hydroxy (3410 cm^{-1}) and phenolic groups (279 nm, 1691 cm^{-1}). Most of the ^1H and ^{13}C NMR signals of compound **1** were observed in the olefinic and aromatic downfield regions (Table 1). The aromatic signals at δ_H 7.43 (3H, m) and 7.63 (2H, m) and two *trans*-oriented vinyl protons at δ_H 6.45 and 7.43 (each 1H, d, $J = 16.0$ Hz) suggested that compound **1** contains a cinnamoyl moiety. This was confirmed by the HMBC correlations observed from H-7″ to C-1″, C-2″, C-6″, C-8″, and C-9″, and from

* To whom correspondence should be addressed. Tel: +1-614-247-8094. Fax: +1-614-247-8081. E-mail: kinghorn.4@osu.edu.
† University of Illinois at Chicago.
‡ Research and Development Chemistry, Indonesian Institute of Science.
§ Research and Development Center for Biology, Indonesian Institute of Science.
⊥ Bristol-Myers Squibb.
|| Present address: College of Pharmacy, The Ohio State University, Columbus, OH 43210.
▽ Present address: College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea.
○ Present address: Astrazeneca R&D Boston, Waltham, MA 02180.

10.1021/np040134g CCC: $27.50
Published on Web 10/13/2004

Table 1. ^{1}H NMR and ^{13}C NMR Data of Compound **1** in $CDCl_3$[a]

position	δ_C	DEPT	δ_H (J in Hz)
2	100.0	C	
3	67.6	CH	4.22 dd (4.7, 3.2)
4	28.8	CH	4.46 d (3.2)
4a	103.8	C	
5	157.0	C	
6	97.9	CH	6.14 br s
7	158.1	C	
8	96.4	CH	6.14 br s
8a	154.1	C	
9	132.3	C	
10	115.6	CH	7.22 d (2.1)
11	145.1	C	
12	146.2	C	
13	115.3	CH	6.86 d (8.2)
14	119.7	CH	7.09 dd (8.2, 2.1)
2′	80.0	CH	5.27 br s
3′	68.4	CH	5.66 br d (2.9)
4′	27.5	CH_2	3.15 dd (17.6, 4.8), 2.95 d (17.6)
4′a	101.4	C	
5′	156.0	C	
6′	96.7	CH	6.19 br s
7′	152.3	C	
8′	107.1	C	
8′a	151.4	C	
9′	129.9	C	
10′	115.5	CH	7.25 d (1.9)
11′	145.9	C	
12′	146.2	C	
13′	116.0	CH	6.87 d (8.0)
14′	119.8	CH	7.07 dd (7.9, 2.0)
1″	135.2	C	
2″	129.8	CH	7.43 m
3″	129.2	CH	7.63 m
4″	131.3	CH	7.43 m
5″	129.2	CH	7.63 m
6″	129.8	CH	7.43 m
7″	146.0	CH	7.43 d (16.0)
8″	118.8	CH	6.45 d (16.0)
9″	166.2	C	
3-OH			4.39 d (4.7)

[a] Spectra taken at 500 and 125 MHz for proton and carbon, respectively; chemical shift values were assigned on the basis of the observed 2D NMR correlations.

H-8″ to C-1″, C-7″, and C-9″. In addition to the signals assigned to this cinnamoyl unit, the ^{13}C NMR spectrum of compound **1** displayed another 30 resonances, including 24 aromatic carbons and six additional signals at δ_C 100.0 (s, C-2), 80.0 (d, C-2′), 68.4 (d, C-3′), 67.6 (d, C-3), 28.8 (d, C-4), and 27.5 (t, C-4′), comprised of two $C_6-C_3-C_6$ units. The ^{1}H NMR resonances for H-2′, H-3′, H-3, H-4, H-4′a, and H-4′b were assigned at δ_H 5.27 (1H, br s), 5.66 (1H, br d, J = 4.8 Hz), 4.22 (1H, dd, J = 4.7, 3.2 Hz), 4.46 (1H, d, J = 3.2 Hz), 3.15 (1H, dd, J = 17.6, 4.8 Hz), and 2.95 (1H, br d, J = 17.6 Hz), respectively, on the basis of the HMQC data of compound **1**. In the ^{1}H−^{1}H COSY spectrum, correlations from H-3 to H-4, and from H-3′ to H-2′, H-4′a, and H-4′b, were observed. All of the above-mentioned 1D and 2D NMR data suggested that compound **1** is a proanthocyanidin derivative.[9,10]

The chemical shifts of a doubly oxygenated quaternary carbon at δ_C 100.0 (C-2) and a methine at δ_C 28.8 (C-4) suggested that the two flavan units were linked from C-2 to C-7′ through an oxygen ether functionality and directly from C-4 to C-8′ by a C−C bond.[11,12] This was confirmed by the observed HMBC correlations from both H-10 and H-14 to C-2 and from H-4 to C-2, C-7′, C-8′, and C-8′a (Figure 1). The location of the cinnamoyl moiety was assigned to C-3′ on the basis of the observed HMBC correlation from H-3′ to C-9″.

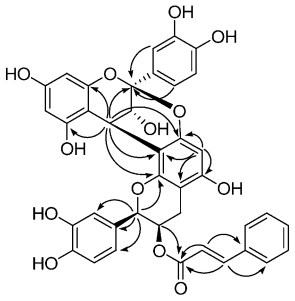


Figure 1. Selected HMBC correlations of compound **1**.

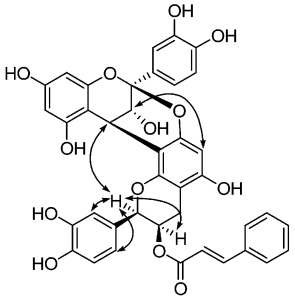


Figure 2. Selected ROESY correlations of compound **1**.

The absolute stereochemistry at C-4 was established by CD measurements. The high-amplitude positive Cotton effect ($[\theta]_{233}$ +1.1 × 10^5) at short wavelength in the CD spectrum of **1** allowed the assignment of the absolute configuration of C-4 as *R*, corresponding to a 2β,4β stereochemistry.[13,14] Moreover, the 3,4-*trans* configuration of the C-ring was further confirmed by the correlation between H-3 and H-6′ in the ROESY spectrum (Figure 2).[15] The appearance of a broad singlet at δ_H 5.27 due to the H-2′ proton indicated a *cis* configuration between H-2′ and H-3′ of the lower flavan unit.[9] The orientation at C-2′ of the lower flavan moiety was assigned as α, as a result of the CD spectral pattern between 270 and 290 nm ($[\theta]_{275}$ −9.70 × 10^4, $[\theta]_{290}$ −1.03 × 10^5), reflecting that the orientation of the B/E-rings in this proanthocyanidin was similar to that of proanthocyanidin A-2, with a 2α-phenyl (C-ring)-2α-phenyl (F-ring) functionality.[16] This configuration was confirmed by a correlation between H-4 and H-2′ in the ROESY spectrum (Figure 2).[17] On the basis of these observations, compound **1** was assigned as epicatechin-(2β→*O*→7′,4β→8′)-epicatechin-3′-*O*-cinnamate (3′-*O*-cinnamoylprocyanidin A-2).

A molecular formula of $C_{48}H_{93}NO_{10}$ was assigned for compound **2** on the basis of the observed sodiated molecular ion peak at *m/z* 866.6738 $[M + Na]^+$ in its HRESIMS. The presence of a β-glucopyranose moiety in **2** was suggested by the chemical shift and coupling constant of the anomeric proton at δ_H 4.95 (1H, d, J = 7.7 Hz, Glc-1), which was supported by the anomeric carbon signal at δ_C 105.6 (d, Glc-1). The ^{1}H and ^{13}C NMR resonances of the glucopyranose unit were assigned on the basis of the observed ^{1}H−^{1}H COSY, HMQC, and HMBC correlations. Other signals displayed in the ^{1}H NMR spectrum of **2** were a downfield doublet at δ_H 8.56 (1H, d, J = 8.2 Hz, NH), eight olefinic, oxygenated, or other heteroatom-attached protons between δ_H 4.48 and 5.56, six methyl protons at δ_H 0.87 (6H, br t, J = 6.3 Hz, CH_3-18 and CH_3-24′), and a strong aliphatic methylene resonance at δ_H 1.26−1.32. The ^{13}C and DEPT NMR spectral data of **2** exhibited a carbonyl group at δ_C 175.7 (C-1′), two olefinic carbons at δ_C 130.4 and 130.2 (C-9 and C-10, d), five oxygenated or other heteroatom-attached carbons at δ_C 75.9 (C-3, d), 72.42 and 72.44 (C-4 and C-2′,

d), 70.5 (C-1, t), and 51.7 (C-2, d), aliphatic methylenes between δ_C 23.0 and 35.6, and two methyls at δ_C 14.4 (CH_3-18 and CH_3-24′, q). A correlation from δ_H 8.56 (NH) to δ_H 5.28 (1H, m, H-2) and correlations from δ_H 8.56 (NH) to δ_C 175.7 (C-1′), 75.9 (C-3), 72.4 (C-2′), 70.5 (C-1, t), and 51.7 (C-2, d) were observed in the $^1H-^1H$ COSY and HMBC spectra of **2**, respectively. These 1D NMR data and 2D NMR correlations indicated the presence of an amide function in the molecule of **2** and suggested that this compound is a cerebroside.[18,19] The location of the sugar moiety was assigned at C-1 on the basis of the clear HMBC correlations observed from both H-1a and H-1b to the anomeric carbon of the β-glucopyranose. The chemical shifts of the allylic methylene carbons at δ_C 27.9 and 27.6 (C-8 and C-11) indicated that the double bond was present in the *Z*-configuration in **2**.[20,21]

Compound degradation studies and acetylation were carried out to determine the lengths of the sphingosine and fatty acid chains, the position of the double bond, and the absolute configuration of cerebroside **2**. Initially, hydrolysis was carried out using LiOH to give a fatty acid methyl ester and a sphingosine glucoside. The acetate of the fatty acid methyl ester **2a** was afforded by acetylation (Ac_2O/pyr) of the fatty acid methyl ester. The sphingosine glucoside was further hydrolyzed using 1 N HCl, and the resulting mixture was then acetylated (Ac_2O/pyr) to give **2b** and pentaacetyl-α-D-glucopyranoside and pentaacetyl-β-D-glucopyranoside. Protonated molecular ion peaks were obtained in the ESIMS at *m/z* 441 and 484 for **2a** and **2b**, respectively. These data enabled the determination of the lengths of the sphingosine and fatty acid units as 18 and 24 carbons, respectively, and that the double bond was located in the sphingosine chain in the molecule of **2**. The location of the double bond at C-9 and C-10 was suggested by the fragmentations obtained in the MS-MS spectrum of **2b**.[22] The optical rotation value of **2a** ($[\alpha]_D$ +16.5°) indicated a 2′*R* absolute stereochemistry in **2**.[23] The absolute configuration of 2*S*, 3*S*, and 4*R* for **2** could be established by comparison of the obtained optical rotation value of **2b** ($[\alpha]_D$ +28.5°) with the literature values of synthetic sphingamines.[23,24] Finally, the absolute configuration of the glucose moiety was determined as D on the basis of the optical rotation value ($[\alpha]_D$ +59.2°) of the mixture of pentaacetyl-α-D-glucopyranoside and pentaacetyl-β-D-glucopyranoside.[25,26] The ^1H NMR spectral data indicated that the pentaacetyl-glucopyranoside obtained in the present study was a mixture of α and β forms in a ratio of about 1:3. Thus, the structure of sumatranoside (**2**) was assigned as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2*N*-[(2′*R*)-2′-hydroxy-tetracosanoyl]-9*Z*-octadecene-1,3,4-triol and is the first isolation of a cerebroside from the genus *Ormosia*.

The new A-type proanthocyanidin derivative, 3′-*O*-cinnamoylprocyanidin A-2 (**1**), was found to be inactive in a proteasome inhibitory assay (<10% inhibition at 28 μM). However, the new cerebroside, sumatranoside (**2**), was demonstrated to be modestly active in the same assay, with an IC_{50} value of 30 μM. Derivatives **2a** and **2b** and the mixture of pentaacetyl-α-D-glucopyranoside and pentaacetyl-β-D-glucopyranoside obtained from compound **2** were inactive in the proteasome inhibitory assay. Both compounds were inactive (ED_{50} > 5 μg/mL) when evaluated in a small tumor cell panel.[27,28] To the best of our knowledge, the proteasome inhibitory activity of ceramides and cerebrosides has not been reported previously. A series of polyhydroxy sterols with approximately the same proteasome inhibition potency as **2** was described recently from a marine sponge (*Acanthodendrilla* sp.).[29]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU-7 spectrometer. IR spectra were recorded on a JASCO FT/IR-410 spectrometer. The CD measurement was performed using a JASCO-710 CD spectropolarimeter. HRFABMS and LRFABMS were recorded on a VG 7070-HF mass spectrometer. ^1H and ^{13}C NMR data (including DEPT, HMQC, HMBC, NOESY, and $^1H-^1H$ COSY spectra) were recorded at room temperature on a Bruker Avance DRX-500 or DPX-360 MHz spectrometer with TMS as internal standard. Column chromatography was conducted on silica gel (70−230 mesh, Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 (Merck, 0.25 mm layer thickness) plates. For visualization of TLC plates, vanillin-sulfuric acid reagent was used.

Plant Material. The leaves of *Ormosia sumatrana* (Miq.) Prain (Leguminosae) were collected at Sub District Riam Durian, District Kota Waringin Lama, Pangkala Bun-Central Kalimantan, Indonesia, in October 1998, and identified by S.R. A voucher specimen (accession No. B-97) has been deposited at the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The milled, air-dried leaves of *O. sumatrana* (1028 g) were extracted with MeOH (4 L × 3) at room temperature, and the solvent was evaporated in vacuo. The dried MeOH extract (68 g) was resuspended in 10% H_2O in MeOH (500 mL) and partitioned with petroleum ether (500 mL × 3) to yield a petroleum ether-soluble residue (16 g). To the aqueous MeOH layer was added H_2O (200 mL), and this was then partitioned with $CHCl_3$ (700 mL × 2) to afford a chloroform-soluble extract (20 g), which was followed by washing with a 1% aqueous solution of NaCl to remove tannins. The chloroform-soluble extract exhibited inhibitory activity in a proteasome assay (IC_{50} 5.3 μg/mL), while both the petroleum ether-soluble and aqueous-soluble extracts were inactive. Hence, the $CHCl_3$-soluble extract (20 g) was subjected to silica gel column chromatography (4.5 × 40 cm) and eluted with a gradient of $CHCl_3$−MeOH (50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 3:1, 0:1, 2000 mL each). Fractions were pooled on the basis of TLC analysis and afforded eight combined fractions (F011−F018). Of these, F015, F016, and F017 showed inhibitory effects in the proteasome assay with IC_{50} values of 3.9, 2.7, and 7.3 μg/mL, respectively. Fractions F015 and F016 (0.4 g) were combined and further chromatographed over silica gel with $CHCl_3$−MeOH (5:1) mixtures and afforded compound **1** (10 mg). Fraction F017 was repeatedly chromatographed over a Sephadex LH-20 column (2.5 × 55 cm), eluted with pure MeOH, affording compound **2** (28 mg).

3′-*O*-Cinnamoylprocyanidin A-2 (1) (epicatechin-(2β→*O*→7′,4β→8′)-epicatechin-3′-*O*-cinnamate): UV (MeOH) λ_{max} (log ε) 218 (4.45), 279 (4.16) nm; IR (dried film) ν_{max} 3410, 2362, 2333, 1691, 1623, 1514, 1405, 1358, 1281, 1178, 1143, 1109 cm^{-1}; CD (MeOH) Δε (nm) +1.24 × 10^5 (217), +1.13 × 10^5 (233), −9.70 × 10^4 (275), −1.03 × 10^5 (290); ^1H and ^{13}C NMR data, see Table 1; LRFABMS *m/z* 729 $[M + Na]^+$; HRFABMS *m/z* 729.1613 $[M + Na]^+$ (calcd for $C_{39}H_{30}O_{13}Na$, 729.1584).

Sumatranoside (2) {1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*)-2*N*-[(2′*R*)-2′-hydroxy-tetracosanoyl]-9*Z*-octadecene-1,3,4-triol}: $[\alpha]^{23}_D$ +9.2° (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.65), 221 (3.18) nm; IR (dried film) ν_{max} 3415, 2982, 1621, 1543, 1430 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 8.56 (1H, d, *J* = 8.2 Hz, NH), 5.43−5.56 (2H, m, H-9 and H-10), 5.28 (1H, m, H-2), 4.95 (1H, d, *J* = 7.7 Hz, Glc-1), 4.71 (1H, dd, *J* = 10.6, 6.8 Hz, H-1a), 4.58 (1H, dd, *J* = 7.7, 3.5 Hz, H-2′), 4.52 (1H, dd, *J* = 10.6, 4.5 Hz, H-1b), 4.48 (1H, dd, *J* = 6.9, 4.8 Hz, H-3), 4.16−4.22 (3H, m, H-4, Glc-3 and Glc-4), 4.00 (1H, t, *J* = 8.0 Hz, Glc-2), 3.86 (1H, m, Glc-5), 2.19−2.26 (4H, m, H-5a, H-8a, H-11a and H-3′a), 2.00−2.09 (4H, m, H-8b, H-11b, H-3′b and H-4′a), 1.92 (1H, m, H-5b), 1.78 (2H, m, H-4′b and H-6a), 1.69 (1H, m, H-6b), 1.26−1.32 (methylene band), 0.87 (6H, t, *J* = 6.3 Hz, CH_3-18 and CH_3-24′); ^{13}C NMR (C_5D_5N, 125 MHz)

δ 175.7 (C-1′, s), 130.4 (C-9 or C-10, d), 130.2 (C-9 or C-10, d), 105.6 (Glc-1, d), 78.6 (Glc-5, d), 78.5 (Glc-3, d), 75.9 (C-3, d), 75.2 (Glc-2, d), 72.42, 72.44 (C-2′ and C-4, d), 71.4 (Glc-4, d), 70.5 (C-1, t), 62.6 (Glc-6, t), 51.7 (C-2, d), 35.6 (C-3′, t), 34.0 (C-5, t), 32.1 (C-16′ and C-22′, t), 30.1−29.5 (other methylenes), 27.9, 27.6 (C-8 and C-11, t), 26.8 (C-4′, t), 25.9 (C-6, t), 23.0 (C-17 and C-23′, t), 14.3 (C-18 and C-24′, q); LRFABMS *m/z* 866 $[M + Na]^+$; HRFABMS *m/z* 866.6738 $[M + Na]^+$ (calcd for $C_{48}H_{93}NO_{10}Na$, 866.6697).

Alkaline Hydrolysis of 2. Sumatranoside (**2**, 8.5 mg) was dissolved in MeOH (2 mL) in a 25 mL round-bottomed flask, and LiOH solution (1 N, 2 mL) was gradually added to the flask. The reaction solution was stirred for 5 h at 50 °C. The resulting mixture was neutralized using 1 N HCl and then extracted with *n*-hexane (3 × 3 mL). The evaporated *n*-hexane-soluble extract was acetylated using pyridine (0.2 mL) and acetic anhydride (0.2 mL) at room temperature overnight to give **2a** (2.1 mg): $[\alpha]^{23}_D$ +16.5° (*c* 0.15, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.98 (1H, t, J = 6.4 Hz, H-2′), 3.74 (3H, s, OMe), 2.14 (3H, s, OAc), 1.77−1.85 (2H, m, H_2-3′), 1.26 (methylene protons), 0.88 (3H, t, J = 6.8 Hz, CH_3-24′); ESIMS *m/z* 441 $[M + H]^+$.

Acid Hydrolysis of the Sphingosine Glucoside. The sphingosine glucoside-containing H_2O layer from the above-described alkaline hydrolysis was sufficiently evaporated at 50 °C under reduced pressure. The residue obtained was further hydrolyzed using 1 N HCl (3 mL), stirring for 7 h at 80 °C. The resulting mixture was neutralized with 10% $NaHCO_3$, dried, and then acetylated using pyridine (0.3 mL) and acetic anhydride (0.2 mL) at room temperature overnight. The acetylation mixture was purified by passage over a Sephadex LH-20 column (1.5 × 10 cm), eluted with MeOH−$CHCl_3$ (1:1), to give **2b** (2.5 mg) and an α and β mixture of pentaacetyl-glucopyranoside (1.8 mg). **2b**: $[\alpha]^{23}_D$ +28.5° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.95 (1H, d, J = 8.9 Hz, NH), 5.30−5.47 (2H, m, H-9 and H-10), 5.08 (1H, dd, J = 7.1, 4.2, H-3), 4.95 (1H, ddd, J = 7.1, 4.2, 3.5 Hz, H-4), 4.47 (1H, m, H-2), 4.29 (1H, dd, J = 11.2, 5.0 Hz, H-1a), 4.01 (1H, dd, J = 11.2, 3.8 Hz, H-1b), 1.96−2.13 (16H, H_2-8, H_2-11, and 4 × OAc), 1.63 (2H, m, H-5), 1.25−1.28 (methylene band), 0.88 (3H, t, J = 7.0 Hz, CH_3-18); ESIMS *m/z* 484 $[M + H]^+$; MS-MS (30 eV) *m/z* 424 (21), 382 (11), 364 (9), 350 (12), 322 (15), 304 (13), 280 (15), 262 (100), 245 (11), 154 (2), 133 (9), 57 (45). Pentaacetyl-α-D-glucopyranoside and pentaacetyl-β-D-glucopyranoside: $[\alpha]^{23}_D$ +59.2° (*c* 0.12, $CHCl_3$); ESIMS *m/z* 391 $[M + H]^+$.

Proteasome Fraction Preparation. A proteasome-enriched fraction from a HL-60 human leukemic cell line was prepared by a modified method reported by Vinitsky et al.[30] HL-60 cells (10^{10}) were harvested by centrifugation at 10 000*g* for 30 min. The pellet was resuspended in PBS, and the cells were pelleted again by centrifugation. The pellet was homogenized in 20 mL of 50 mM Tris-Cl, pH 7.5. The homogenate was centrifuged at 100 000*g* for 30 min. The supernatant was adjusted to 5% poly(ethylene glycol) (w/v, mol wt 8000) and centrifuged at 15 000*g* for 30 min. The resulting supernatant was then adjusted to 12% poly(ethylene glycol) (w/v) and centrifuged at 15 000*g* for 30 min. The supernatant was diluted 2-fold in 10 mM Tris-EDTA, pH 7.5, and clarified by centrifugation at 10 000*g* for 20 min. The resultant fraction was enriched in active proteasome complexes.

Proteasome Inhibition Assay. The assay buffer (155 mM Tris, 10 mM EDTA, pH 7.4) was added to the blank and control wells, respectively, and also dilutions of the positive control were added to the inhibitor wells. Dilutions of the test substances (plant extract, fractions, or pure isolates) were prepared in proteasome assay buffer and added to the appropriate wells. The microtiter plate was allowed to equilibrate to the assay temperature (ambient). The enriched proteasome fraction was diluted to a final assay concentration of 50 μg/mL using assay buffer, and this dilution was then added to each well. Then, the plate was preincubated for 30 min at room temperature to allow inhibitor/enzyme interactions. The enzyme reaction was started by adding Suc-LLVY-AMC substrate to a final concentration of 10 μM and was incubated for 1 h. The chymotrypsin-like proteasome activity was determined by measuring the generation of free AMC using a fluorescent plate reader capable of excitation at a wavelength of 360 nm and detection of emitted light at 460 nm. The Suc-LLVY-AMC substrate was obtained from Calbiochem (San Diego, CA). The boronate proteasome inhibitor PS-341 (bortezomib) was used as positive control (IC_{50} 0.15 μM).

Cytotoxicity Assay. The cytotoxic activity of compounds **1** and **2** was evaluated against a panel of human cancer cell lines according to established protocols.[27,28]

Acknowledgment. This investigation was supported by grant U19-CA52956, funded by the National Cancer Institute, NIH, Bethesda, MD. We thank Dr. R. Kleps, Research Resources Center, University of Illinois at Chicago, for facilitating the running of the 500 MHz NMR spectra. We also thank Drs. J. A. (Art) Anderson and Y. Wang, Research Resources Center, University of Illinois at Chicago, for the mass spectral data.

References and Notes

(1) Polhill, R. M. In *Advances in Legume Systematics. Part I*; Polhill, R. M., Raven, P. H., Eds.; Royal Botanic Gardens: Kew, Richmond, Surrey, U.K., 1981; pp 213−230.
(2) Kinghorn, A. D., Smolenski, S. J. In *Advances in Legume Systematics. Part II*; Polhill, R. M., Raven, P. H., Eds.; Royal Botanic Gardens: Kew, Richmond, Surrey, U.K., 1981; pp 585−598.
(3) Kinghorn, A. D.; Hussain, R. A.; Robbins, E. F.; Balandrin, M. F.; Stirton, C. H.; Evans, S. V. *Phytochemistry* **1988**, *27*, 439−444.
(4) Iinuma, M.; Okawa, Y.; Tanaka, T.; Ho, F.-C.; Kobayashi, Y.; Miyauchi, K.-I. *Phytochemistry* **1994**, *37*, 889−891.
(5) Ricker, M.; Daly, D. C.; Veen, G.; Robbins, E. F.; Sinta, V.; Chota, J.; Czygzan, F.-C.; Kinghorn, A. D. *Brittonia* **1999**, *51*, 34−43.
(6) Hershko, A.; Ciechanover, A. *Annu. Rev. Biochem.* **1998**, *67*, 425−479.
(7) Glickman, M. H.; Maytal, V. *Curr. Top. Microbiol. Immunol.* **2002**, *268*, 43−72.
(8) Almond, J. B.; Cohen, G. M. *Leukemia* **2002**, *16*, 433−443.
(9) Prasad, D.; Joshi, R. K.; Pant, G.; Rawat, M. S. M.; Inoue, K.; Shingu, T.; He, Z. D. *J. Nat. Prod.* **1998**, *61*, 1123−1125.
(10) Calzada, F.; Cerda-Garcia-Rojas, C. M.; Meckes, M.; Cedillo-Rivera, R.; Bye, R.; Mata R. *J. Nat. Prod.* **1999**, *62*, 705−709.
(11) Rawat, M. S. M.; Prasad, D.; Joshi, R. K.; Pant, G. *Phytochemistry* **1999**, *50*, 321−324.
(12) Pant, G.; Nautiyal, A. K.; Rawat, M. S. M.; Sutherland, J. K.; Morris, G. A. *Magn. Reson. Chem.* **1992**, *30*, S 142−S 147.
(13) Lou, H.; Yamazaki, Y.; Sasaki, T.; Uchida, M.; Tanaka, H.; Oka, S. *Phytochemistry* **1999**, *51*, 297−308.
(14) Barrett, M. W.; Klyne, W.; Scopes, P. M.; Fletcher, A. C.; Porter, L. J.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2375−2377.
(15) Cronje, A.; Burger, J. F. W.; Brandt, E. V.; Kolodziej, H.; Ferreira, D. *Tetrahedron Lett.* **1990**, *31*, 3789−3792.
(16) Hatano, T.; Miyatake, H.; Natsume, M.; Osakabe, N.; Takizawa, T.; Ito, H.; Yoshida, T. *Phytochemistry* **2002**, *59*, 749−758.
(17) Barreiros, A. L. B. S.; David, J. P.; de Queiroz, L. P.; David, J. M. *Phytochemistry* **2000**, *55*, 805−808.
(18) Kang, S. S.; Kim, J. S.; Xu, Y. N.; Kim, Y. H. *J. Nat. Prod.* **1999**, *62*, 1059−1060.
(19) Kawatake, S.; Nakamura, K.; Inagaki, M.; Higuchi, R. *Chem. Pharm. Bull.* **2002**, *50*, 1091−1096.
(20) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 2480−2483.
(21) Su, B.-N.; Takaishi, Y. *J. Nat. Prod.* **1999**, *62*, 1325−1327.
(22) Su, B.-N.; Misico, R.; Park, E. J.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **2002**, *58*, 3453−3466.
(23) Murakami, T.; Taguchi, K. *Tetrahedron* **1999**, *55*, 989−1004.
(24) Shimizu, M.; Wakioka, I.; Fujisawa, T. *Tetrahedron Lett.* **1997**, *38*, 6027−6030.
(25) Hashimoto, T.; Tori, M.; Asakawa, Y. *Phytochemistry* **1987**, *26*, 3323−3330.
(26) Hudsen, C. S.; Dale, J. K. *J. Am. Chem. Soc.* **1915**, *37*, 1264−1270.
(27) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.
(28) Seo, E.-K.; Kim, N.-C.; Mi, Q.; Chai, H.; Wall, M. E.; Wani, M. C.; Navarro, H. A.; Burgess, J. P.; Graham, J. G.; Cabieses, F.; Tan, G. T.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 1483−1485.
(29) Tsukamoto, S.; Tatsuno, M.; van Soest, R. W. M.; Yokosawa, H.; Ohta, T. *J. Nat. Prod.* **2003**, *66*, 1181−1185.
(30) Vinitsky, A.; Anton, L. C.; Snyder, H. L.; Orlowski, M.; Bennink, J. R.; Yewdell, J. W. *J. Immunol.* **1997**, *159*, 554−564.

NP040134G