Cerebrosides and a Monoacylmonogalactosylglycerol from *Clinacanthus nutans*

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A mixture of nine cerebrosides and a monoacylmonogalactosylglycerol were seperated from the leaves of *Clinacanthus nutans*. The structures of the cerebrosides were characterized as 1-*O*-β-D-glucosides of phytosphingosines, which comprised a common long-chain base, (2*S*,3*S*,4*R*,8*Z*)-2-amino-8(*Z*)-octadecene-1,3,4-triol with nine 2-hydroxy fatty acids of varying chain lengths (C_{16}, C_{18}, C_{20-26}) linked to the amino group. The glycosylglyceride was characterized as (2*S*)-1-*O*-linolenoyl-3-*O*-β-D-galactopyranosylglycerol. The structures were established on the basis of the spectroscopic data and chemical reactions.

Key words *Clinacanthus nutans*; Acanthaceae; leave; cerebroside; glycosphingolipid; monoacylmonogalactosylglycerol

The genus *Clinacanthus consists* of two species, *C. nutans* LINDAU and *C. siamensis* BREM. and belongs to the family Acanthaceae.[1] Both species are small shrubs occurring throughout South East Asia. *C. nutans* (Thai name: phaya yo or phaya plongtong) is often confused with *C. siamensis* (Thai name: lin nguu hao). *C. nutans* has long been used in Thailand as a traditional medicine for the treatment of insect- and snake-bite and skin rashes, including herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions. The anti-inflammatory activity of a *n*-BuOH-soluble fraction from the leaves has been reported.[2] In a series of *in vitro* models a crude extract of the leaves showed significant inhibitory activity on VZV.[3] Likewise, ethanol extracts of *C. nutans* were found to be virucidal against HSV-2 *in vitro*.[4] However, negative results have also been reported.[5] Nonetheless, clinical trials have reported the successful use of a *C. nutans* preparation (cream) for treatment of genital herpes and varicella-zoster lesions in patients.[6—9] Previous chemical studies on *C. nutans* have revealed the presence of lupeol, β-sitosterol, stigmasterol and myricyl alcohol.[10,11] Six known *C*-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin 7-*O*-β-glucopyranoside, orientin and isoorientin have been isolated from the *n*-BuOH- and water-soluble portion of the methanolic extract of the stems and leaves of *C. nutans* collected in Thailand.[12] Five sulfur-containing glucosides were isolated from the *n*-BuOH-soluble portion of a methanolic extract of the stems and leaves of plant material said to be *C. nutans*.[13]

As part of our work on bioactive substances from natural sources, a mixture of cerebrosides (**1**) and a monoacylmonogalactosylglycerol (**4**) were isolated from the EtOAc-soluble fraction of the ethanolic extract of the fresh leaves of *C. nutans*. We are now reporting the isolation and structure elucidation of **1** and **4** by spectroscopic methods and chemical reactions.

The fresh leaves of *C. nutans* were extracted with EtOH at room temperature. After evaporation of the extract, the residue obtained was dissolved in H_2O and partitioned with EtOAc and then *n*-BuOH. The EtOAc-soluble fraction was subjected to flash column chromatography to give 13 fractions, among which fraction 9 possessed anti-HSV-1 activity with IC_{50} value of 7.86 μg/ml. Further chromatographic purification of this fraction gave **1** as a colorless solid and **4** as a pale yellow wax.

The IR spectrum of **1** showed bands at 3315, 1635, 1075 and 1036 cm^{-1} indicating the presence of hydroxyl, amide and C–O functional groups. The ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1) indicated the presence of a sugar moiety (δ_H 4.30, 1H, d, J=8.4 Hz, anomeric proton; δ_C 102.7), an amide function (δ_H 7.62, 1H, d, J=9.0 Hz, NH; δ_C 174.0), and long chain aliphatic and olefinic functions (δ_H 0.87, t, J=6.6 Hz, CH_3; δ_H 1.25, br s, CH_2, δ_H 5.33 and 5.34, 1H each, both dt, J=9.6, 4.8 Hz; δ_C 128.9, 129.2). The data were suggestive of a glycosphingolipid structure.

The ^{13}C-NMR spectrum of **1** (Table 1) was assigned by a combination of distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation

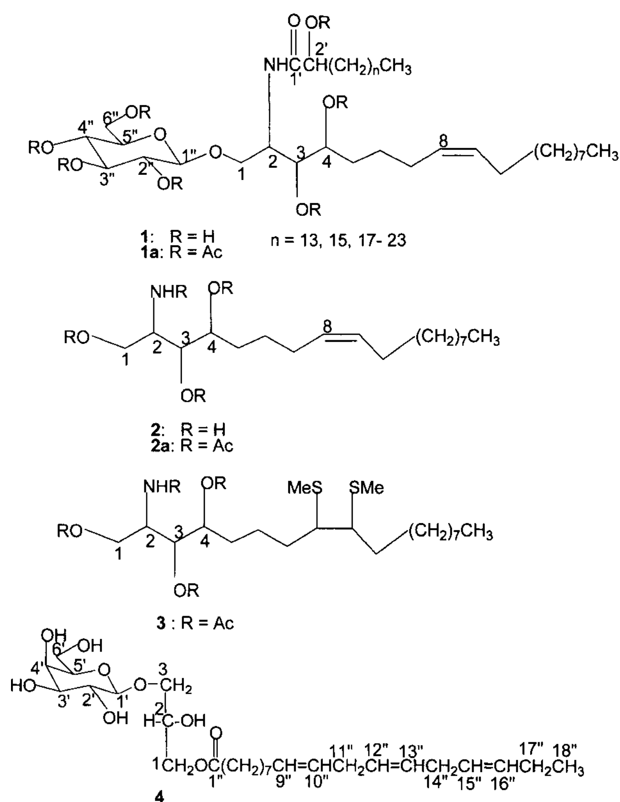

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1** ($CDCl_3$–DMSO-d_6, 3 : 2) and **1a** ($CDCl_3$)

Position	**1** (δ_H)	**1** (δ_C)	**1a** (δ_H)	**1a** (δ_C)
1a	3.58 (dd, 11.0, 3.3)	68.1	3.68 (dd, 11.0, 3.3)	66.7
1b	4.02 (dd, 11.0, 5.9)	—	3.86 (dd, 11.0, 3.3)	—
2	4.26 (m)	49.6	4.27 (tt, 9.0, 3.3)	48.2
3	3.52 (m)	73.7	5.13 (dd, 9.0, 3.3)	74.0
4	3.53 (m)	70.8	4.88 (dt, 9.0, 3.3)	73.1
5	1.55 (m)	33.8	1.60 (m)	27.9
7 and 10	2.05 (m)	26.3, 26.4	1.97 (m)	26.3, 27.2
8 or 9	5.33 (dt, 9.6, 4.8)	128.9	5.31 (dt, 9.6, 6.4)	128.7
9 or 8	5.34 (dt, 9.6, 4.8)	129.2	5.36 (dt, 9.6, 6.4)	130.6
NH	7.62 (d, 9.0)	—	7.73 (d, 9.0)	—
1′	—	174.0	—	169.9
2′	3.95 (m)	70.9	5.15 (t, 5.0)	71.6
3′	1.55 (m)	33.8	1.83 (m)	31.7, 31.9
1″	4.30 (d, 8.4)	102.7	4.48 (d, 8.0)	100.4
2″	3.22 (t, 8.4)	72.6	4.89 (dd, 9.5, 8.0)	71.2
3″	3.41 (t, 8.4)	75.9	5.18 (t, 9.5)	72.7
4″	3.35 (t, 8.4)	69.5	5.06 (t, 9.5)	68.1
5″	3.28 (m)	75.6	3.69 (ddd, 9.5, 4.0, 2.5)	71.9
6a″	3.68 (dd, 12.1, 4.8)	61.0	4.13 (dd, 12.5, 2.5)	61.7
6b″	3.82 (dd, 12.1, 2.2)	—	4.25 (dd, 12.5, 4.0)	—
CH_3	0.87 (t, 6.6) (2×)	13.2, 13.3	0.88 (t, 7.2) (2×)	14.1
$(CH_2)_n$	1.25 (br s)	21.7, 24.4, 25.2 28.2, 28.6, 30.9 30.9, 31.6	1.25 (br s)	22.7, 24.9, 25.9 29.2, 29.3, 29.5, 29.7
CO$\underline{CH_3}$	—	—	1.99, 2.02, 2.05 (2×), 2.07, 2.09, 2.24, all s	20.5 (3×), 20.6, 20.7, 20.9, 21.0
$\underline{CO}CH_3$	—	—	—	169.27, 169.34, 169.7, 170.16, 170.20, 170.6, 171.0

(HMBC) experiments. Important long-range correlations were observed between C-1″ and Hab-1; C-1 and H-1″, H-2, H-3; C-2 and NH and C-1′ and NH and H-2′ (Fig. 1). These results again supported the glycosphingolipid structure.

The ^{1}H-NMR spectrum of the heptaacetate derivative (**1a**) of **1** (Table 1) was much clearer, with well-resolved signals. The signal of the anomeric proton of a β-D-glucopyranose appeared at δ 4.48 as a doublet ($J_{1,2}$=8.0 Hz, diaxial) and other glucose protons were assigned (Table 1) from ^{1}H–^{1}H-COSY spectrum. Signals from two olefinic protons at δ 5.31 and 5.36 (each dt, J=9.6, 6.4 Hz), two methyl groups at δ 0.88 (t, J=7.2 Hz) and the long-chain methylene protons at δ 1.25 (br s) suggested the presence of two long aliphatic chains, one of which possessed a *cis* double bond. A doublet at δ 7.73 (J=9.0 Hz) was assigned to the NH of the amide moiety. The spectrum of **1a** also showed signals for two oxygenated methylene protons as two doublets of doublets at δ 3.68 (J=11.0, 3.3 Hz, Ha-1) and 3.86 (J=11.0, 3.3 Hz, Hb-1), and four methine protons as a triplet of triplets at δ 4.27 (J=9.0, 3.3 Hz, H-2), a doublet of doublets at δ 5.13 (J=9.0, 3.3 Hz, H-3), a doublet of triplets at δ 4.88 (J=9.0, 3.3 Hz, H-4) and a triplet at δ 5.15 (J=5.0 Hz, H-2′). These data, together with the other ^{1}H–^{1}H COSY correlations of **1a** (Fig. 1), supported the structure as the 1-β-D-glucopyranoside of a 3,4-dihydroxysphingosine-type ceramide possessing a 2-hydroxy fatty acid acyl group.

The ^{13}C-NMR of **1a** (Table 1) was assigned by a combination of DEPT, HMQC and HMBC experiments. In particular, the long-range correlations which were observed in the HMBC spectrum (Fig. 1) also supported the substitution pattern in **1a**.

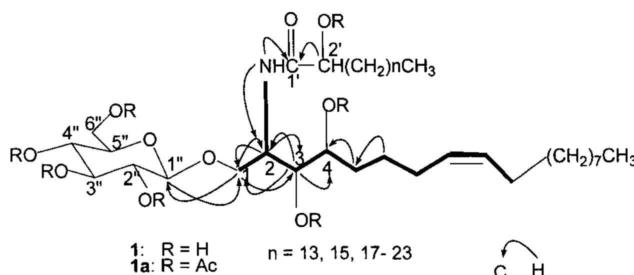

Fig. 1. Selected ^{1}H–^{1}H COSY (Bold Lines) Correlations of **1a** and Selected HMBC (Full-Line Arrows) Correlations of **1** and **1a**

Methanolysis[14] of **1** yielded methyl glucoside, a mixture of fatty acid methyl esters and a trihydroxy long-chain base (**2**). Therefore, **1** must be a mixture of cerebrosides. The fatty acid methyl esters were identified by GC/MS as methyl 2-hydroxypalmitate (4.6%), methyl 2-hydroxystearate (6.9%), methyl 2-hydroxyeicosanoate (2.7%), methyl 2-hydroxyheneicosanoate (1.1%), methyl 2-hydroxydocosanoate (29.3%), methyl 2-hydroxytricosanoate (6.8%), methyl 2-hydroxytetracosanoate (29.0%), methyl 2-hydroxypentacosanoate (4.5%) and methyl 2-hydroxyhexacosanoate (12.4%). The MS spectrum of **1** showed a series of molecular ion $[M+H]^+$ peaks at m/z 872, 858, 844, 830, 816, 802, 788, 760 and 732 and fragment ions at m/z 710 $[872-glc]^+$, 696 $[858-glc]^+$, 682 $[844-glc]^+$, 668 $[830-glc]^+$, 654 $[816-glc]^+$, 640 $[802-glc]^+$, 626 $[788-glc]^+$, 598 $[760-glc]^+$ and 570 $[732-glc]^+$. Identification of the nine fatty acids mentioned above indicated that **1** was comprised of a common long-chain base (**2**) acylated by 2-hydroxy fatty acids of varying

chain lengths. The absolute configuration at C-2 of the 2-hydroxy fatty acid was presumed to be *R* from the specific rotation of the mixture of fatty acid methyl esters ($[\alpha]_D$ −11.0°).[15—17]

The long-chain base tetraacetate (**2a**) showed a $[M]^+$ peak at *m/z* 483. The ^{1}H-NMR spectrum of **2a** (Experimental) (well-resolved signals) contained a doublet at δ 5.92 (J=9.0 Hz) of the NH of the amide function, two doublets of triplets at δ 5.30 (J=10.8, 7.2 Hz) and 5.38 (J=10.8, 6.8 Hz) of the two *cis* olefinic protons and a triplet of a methyl group at δ 0.88 (J=6.6 Hz) and a broad singlet at δ 1.28 of the methylene protons. The ^{1}H-NMR spectrum also had signals of two doublets of doublets at δ 4.00 (J=11.7, 3.2 Hz, Ha-1) and 4.29 (J=11.7, 4.5 Hz, Hb-1), a multiplet centered at δ 4.47 (H-2), a doublet of doublets at δ 5.10 (J=8.1, 3.2 Hz, H-3), a doublet of triplets at δ 4.94 (J=9.9, 3.2 Hz, H-4) and four acetoxy methyl groups apparent as three singlets at δ 2.03, 2.05 (2×) and 2.15. The assignments were supported by the ^{1}H–^{1}H COSY spectrum (Fig. 2). The ^{13}C-NMR spectral data of **2a** (Experimental) was assigned by combination of DEPT, HMQC and HMBC experiments. The ^{1}H- and ^{13}C-NMR spectra of **2a** were shown to be almost identical to (2*S*,3*S*,4*R*,9*Z*)-2-acetamino-1,3,4-triacetoxy-9-docosene,[15] and (2*S*,3*S*,4*R*,13*Z*)-2-acetamino-1,3,4-triacetoxy-13-docosene.[18] The optical rotations of **2a**, (2*S*,3*S*,4*R*,9*Z*)-2-acetamino-1,3,4-triacetoxy-9-docosene,[15] and (2*S*,3*S*,4*R*,13*Z*)-2-acetamino-1,3,4-triacetoxy-13-docosene[18] (+25.4°, +17.6°, +26.5°, respectively) suggests that **2a** also has 2*S*, 3*S*, 4*R* configuration.

In order to establish the position of the double bond, the tetraacetate (**2a**) was treated with dimethyl disulfide (DMDS) and I_2 and the product subjected to electron impact (EI)-MS analysis. The EI-MS spectrum of the DMDS derivative showed a molecular ion at *m/z* 577 and significant fragment ions at *m/z* 530, 482, 422, 390, 330 and 187 arising from selective fragmentation at the C-8–C-9 position of C18 chain, thus confirming the position of the double bond at C-8–C-9 in the long-chain base. Similar results have been reported for another cerebroside containing **2** as the long-chain base.[19] The Δ^8 double bond in **2a** was determined to be *cis* (*Z*) by the upfield shifted carbon chemical shifts of C-7 (δ 26.8) and C-10 (δ 27.3)[17,20] and the relative small coupling constant of H-8 at δ 5.30 (dt, J=10.8, 7.2 Hz) and H-9 at δ 5.38 (dt, J=10.8, 6.8 Hz).

The above evidence led to the assignment of **2a** as (2*S*,3*S*,4*R*,8*Z*)-2-acetamino-1,3,4-triacetoxy-8(*Z*)-octadecene and therefore **2** as the long-chain base present in **1**. This base is also present in a cerebroside isolated from *Euphorbia biglandulosa*[19] and in cerebrosides from Phytolaccae Radix.[17] The (*E*)-isomer is present in aralia cerebroside from *Aralia elata*.[21]

Therefore **1** was characterized as a mixture of (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxypalmitoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxysteroyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxyeicosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxyheneicosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxydocosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxytricosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxytetracosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxypentacosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol and (2*S*,3*S*,4*R*,8*Z*)-1-*O*-(β-D-glucopyranosyl)-2*N*[(2′*R*)-2′-hydroxyhexacosanoyl]-2-amino-8(*Z*)-octadecene-1,3,4-triol.

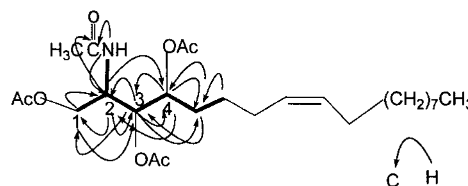

Fig. 2. Selected ^{1}H–^{1}H COSY (Bold Lines) and HMBC (Full-Line Arrows) Correlations of **2a**

The spectral data of **4** indicated the presence of a sugar and a long-chain unsaturated aliphatic system strongly suggesting a glycolipid. The ^{1}H- and ^{13}C-NMR spectra of **4** (Experimental), together with ^{1}H–^{1}H COSY, HMQC and HMBC spectra, led to the assignments of all the ^{1}H- and ^{13}C-NMR signals for the sugar and glycerol moieties. The signals at δ 103.4, 71.1, 73.1, 68.6, 74.7 and 61.1 in the ^{13}C-NMR spectrum and peaks at δ 4.25 (d, $J_{1',2'}$=7.6 Hz, diaxial, H-1′) and 3.51 (dd, $J_{3',4'}$=3.0 Hz, axial-equatorial, 9.0 Hz, H-3′) in the ^{1}H-NMR spectrum suggested that the sugar in **4** was a β-D-galactopyranose. Signals at δ 64.9, 68.2 and 70.9 and a doublet at δ 4.14 (J=6.8 Hz), a multiplet at δ 4.00 and two doublets of doublets at δ 3.71 (J=10.5, 3.5 Hz) and 3.89 (J=10.5, 6.3 Hz) in the ^{13}C- and ^{1}H-NMR spectra of **4**, respectively, were indicative of a glycerol moiety. A carbonyl carbon signal was observed at δ 174.1. A broad triplet of four protons at δ 2.81 (J=5.8 Hz), a quartet of two protons at δ 2.06 (J=8.0 Hz), a quintet of two protons at δ 2.09 (J=8.0 Hz) and a triplet of two protons at δ 2.35 (J=8.0 Hz) were due to the methylene hydrogens lying between two double bonds, a double bond and a methylene group, a double bond and a methyl group and next to a carbonyl moiety, respectively. Six olefinic carbons at δ 126.8, 127.4, 127.9, 128.0, 129.9 and 131.6 were observed in the ^{13}C-NMR spectrum of **4**, indicating the presence of three double bonds in the structure. This was in good agreement with the presence of a multiplet (triplet-like) signal of six protons of the three double bonds at δ 5.36 in the ^{1}H-NMR spectrum of **4**. The narrow width of the olefinic protons in the ^{1}H-NMR spectrum at 5.36 (triplet-like) and the absence of IR absorption at 965—975 cm^{-1} in **4** indicates that the three double bonds are *cis*.[23,24] These spectral data suggested the long chain fatty acid in **4** was linolenic acid. Treatment of **4** with NaOMe in MeOH gave methyl ester of linolenic acid which was identified by GC/MS and (2*R*)-1-*O*-β-D-galactopyranosylglycerol ($[\alpha]_D$ −7.1°)[22,24,25] (Experimental).

Important long-range correlations were observed between C-1′ and Hab-3, C-1″ and H-1 and H-2″ and C-3 and H-1′ in the HMBC spectrum of **4** (Fig. 3). These results suggested that linolenic acid and β-D-galactopyranose were connected to C-1 and C-3 of glycerol, respectively. Compound **4** was thus characterized as (2*S*)-1-*O*-linolenoyl-3-*O*-β-D-galac-

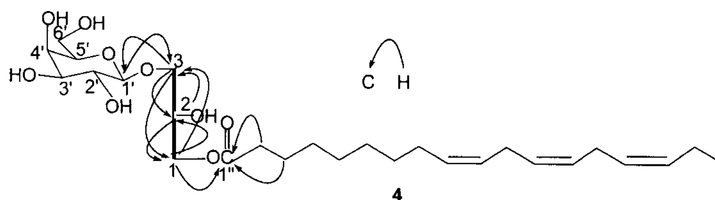

Fig. 3. Selected ^{1}H–^{1}H COSY (Bold Lines) and HMBC (Full-Line Arrows) Correlations of **4**

topyranosylglycerol. Unfortunately, the mixture of cerebrosides (**1**) did not show antiviral (HSV-1) and antiinflammatory (COX-1 and COX-2) activities. The monoacylmonogalactosylglycerol (**4**) was not tested because the sample had decomposed.

Experimental

Melting point: uncorrected. IR spectra: Jasco A-302 Spectrophotometer. ^{1}H-NMR: $CDCl_3$, $CDCl_3$/DMSO-d_6, $CDCl_3/CD_3OD$ Bruker Avance 400 (400 MHz), TMS as internal standard. ^{13}C-NMR: $CDCl_3$, $CDCl_3$/DMSO-d_6, $CDCl_3/CD_3OD$, 100 MHz, TMS as internal standard. MS: VG 7070 mass spectrometer operating at 70 eV for EI-MS and a VG Quattro triple quadrupole instrument for the electrospray MS (LSI). GC/MS was performed with a Hewlett Packard 5890 Series II gas chromatograph attached to a Hewlett Packard 5989B MS employing the EI mode (70 eV). Conditions: HP-5 capillary column (30 m×0.25 mm×0.25 μm); column temperature: 150—270 °C (3 °C min^{-1}), then held at 270 °C; carrier gas: He; injector and detector temperatures: 250 °C and 280 °C, respectively. Optical rotation: MeOH or $CHCl_3$. TLC: precoated PLC_{254} plate (Merck); spots were detected by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. CC: silica gel 70—230 mesh (Merck), silica gel 230—400 mesh (Merck), Lichroprep RP-18 25—40 μm. A voucher specimen (BRU. 350) was deposited at the National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand.

Extraction and Isolation The fresh leaves of *Clinacanthus nutans* (2.45 kg) were extracted with 95% EtOH at room temperature. After filtration, the filtrate were evaporated to give a dark green thick oil which was partitioned between water (300 ml) and EtOAc (3×300 ml) and the water layer then extracted with *n*-BuOH (3×250 ml). Removal of the solvent of each fraction gave the EtOAc fraction as a dark green thick oil (20.9 g), the *n*-BuOH fraction as a brown thick oil (15.8 g) and the water fraction as a brown thick oil (111.4 g). The EtOAc fraction (20.0 g) was separated by flash column chromatography using silica gel (230—400 mesh, diameter 13.0 cm×height 5.5 cm) and the column was eluted with (500 ml each) hexane, hexane/EtOAc (4 : 1, 3 : 2, 2 : 3, 1 : 4), EtOAc, EtOAc/%MeOH (5, 10, 20, 40, 60, 80) and MeOH to give 13 fractions. Fraction 9 (2.1 g) which possessed strong anti-HSV-1 activity (IC_{50}=7.86 μg/ml) was purified by flash column chromatography using silica gel (230—400 mesh, diameter 7.0 cm×height 3.0 cm) and the column was eluted with (3×50 ml each) EtOAc and EtOAc/%MeOH (2, 4, 6, 8, 10, 20). The fractions were combined on the basis of their behaviour on TLC and evaporated to give 7 fractions. Fraction 4 (771 mg) was further chromatographed on a column of silica gel (70—230 mesh, 75 g) using CH_2Cl_2/MeOH/H_2O (lower layer) (40 : 3 : 1, 30 : 3 : 1, 20 : 3 : 1, 15 : 3 : 1) as the eluent to give a mixture of cerebroside **1** and monoacylmonogalactosylglycerol **4** as a colorless solid (332 mg). The mixture (152 mg) was separated on PLC (silica gel, layer thickness 1.0 mm) using CH_2Cl_2/MeOH/H_2O (lower layer) (15 : 3 : 1, 4 runs and 10 : 3 : 1, 2 runs) to give cerebroside **1** as a colorless solid (83 mg). The mixture (123 mg) was separated by C-18 reverse-phase gravity column chromatography eluting with MeOH/H_2O (5 : 1), MeOH/H_2O (10 : 1) and MeOH to give **4** as a pale yellow wax (50 mg) and **1** as a colorless solid (41 mg).

Cerebroside 1 A colorless solid, IR ν_{max}^{Nujol} cm^{-1}: 3315 (broad), 2921, 2851, 1635, 1588, 1075, 1036. Liquid secondary ion (LSI)-MS *m/z* (rel. int. %): 872 $[M+H]^+$ (13%), 858 $[M+H]^+$ (6), 844 $[M+H]^+$ (37), 830 $[M+H]^+$ (15), 816 $[M+H]^+$ (71), 802 $[M+H]^+$ (5), 788 $[M+H]^+$ (11), 760 $[M+H]^+$ (25), 732 $[M+H]^+$ (5), 710 $[872-glc]^+$ (20), 696 $[858-glc]^+$ (5), 682 $[844-glc]^+$ (54), 668 $[830-glc]^+$ (24), 654 $[816-glc]^+$ (100), 640 $[802-glc]^+$ (5), 626 $[788-glc]^+$ (12), 598 $[760-glc]^+$ (27), 570 $[732-glc]^+$ (4). High resolution (HR)-LSI-MS *m/z*: 872.7165 $[M+H]^+$, $C_{50}H_{98}NO_{10}$ requires 872.7185; 844.6854 $[M+H]^+$, $C_{48}H_{94}NO_{10}$ requires 844.6873; 816.6567 $[M+H]^+$, $C_{46}H_{90}NO_{10}$ requires 816.6560; 760.5914 $[M+H]^+$, $C_{42}H_{82}NO_{10}$ requires 760.5934. Other molecular ion peaks were too weak to allow the measurement of high resolution data. ^{1}H- and ^{13}C-NMR data see Table 1.

Acetylation of 1 A mixture of **1** (19 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was heated at 85 °C for 2 h. After the usual work up, the acetate (**1a**) was obtained as a light yellow wax (23 mg) which was purified by column chromatography using silica gel (3 g) and hexane/EtOAc as the eluent to give **1a** as a colorless gum (17 mg). IR $\nu_{max}^{thin\ film}$ cm^{-1}: 2924, 2853, 1752, 1686, 1522, 1467, 1435, 1370, 1226, 1041. ^{1}H- and ^{13}C-NMR data see Table 1.

Methanolysis of 1 Compound **1** (43 mg) was refluxed with 0.9 M HCl in 82% aq. MeOH (10 ml) for 18 h. The mixture was extracted with hexane and the combined organic phase was washed with water and dried over Na_2SO_4. Removal of the solvent gave a colorless wax (22 mg) which was chromatographed on silica gel [hexane/EtOAc (20 : 1, 5 : 1)] to yield a mixture of fatty acid methyl esters as a colorless wax (15 mg). $[\alpha]_D^{25}$ −11.0° (c=0.80, $CHCl_3$). The mixture of the esters was analyzed by GC/MS. Peak 1 (t_R 8.50 min, 2-hydroxypalmitic acid methyl ester), EI-MS *m/z*: 286 $[M]^+$, 254 $[M-CH_3OH]^+$, 227 $[M-CH_3COO]^+$, 208, 182, 159, 145, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 2 (t_R 9.822 min, 2-hydroxystearic acid methyl ester), EI-MS *m/z*: 314 $[M]^+$, 282 $[M-CH_3OH]^+$, 255 $[M-CH_3COO]^+$, 236, 210, 159, 146, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 3 (t_R 11.01 min, 2-hydroxyeicosanoic acid methyl ester), EI-MS *m/z*: 342 $[M]^+$, 310 $[M-CH_3OH]^+$, 283 $[M-CH_3COO]^+$, 238, 207, 159, 146, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 4 (t_R 11.57 min, 2-hydroxyheneicosanoic acid methyl ester), EI-MS *m/z*: 356 $[M]^+$, 297 $[M-CH_3COO]^+$, 278, 236, 159, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 5 (t_R 12.13 min, 2-hydroxydocosanoic acid methyl ester), EI-MS *m/z*: 370 $[M]^+$, 338 $[M-CH_3OH]^+$, 311 $[M-CH_3COO]^+$, 266, 160, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 6 (t_R 12.63 min, 2-hydroxytricosanoic acid methyl ester), EI-MS *m/z*: 384 $[M]^+$, 352 $[M-CH_3OH]^+$, 325 $[M-CH_3COO]^+$, 280, 207, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 7 (t_R 13.15 min, 2-hydroxytetracosanoic acid methyl ester), EI-MS *m/z*: 398 $[M]^+$, 366 $[M-CH_3OH]^+$, 339 $[M-CH_3COO]^+$, 294, 159, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 8 (t_R 13.62 min, 2-hydroxypentacosanoic acid methyl ester), EI-MS *m/z*: 412 $[M]^+$, 353 $[M-CH_3COO]^+$, 281, 207, 145, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57. Peak 9 (t_R 14.54 min, 2-hydroxyhexacosanoic acid methyl ester), EI-MS *m/z*: 426 $[M]^+$, 394 $[M-CH_3OH]^+$, 367 $[M-CH_3COO]^+$, 322, 281, 207, 159, 127 $[C_9H_{19}]^+$, 111 $[C_8H_{15}]^+$, 97 $[C_7H_{13}]^+$, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69 $[C_5H_9]^+$, 57.

The aq. MeOH layer was neutralized with NH_4OH and extracted with EtOAc. The combined EtOAc extract was washed with H_2O, dried over Na_2SO_4 and evaporated to give the long-chain base (**2**) as a slightly yellow wax (12 mg). The aq. MeOH layer was then evaporated to dryness and chromatographed on silica gel [CH_2Cl_2/MeOH/H_2O (lower layer) (20 : 3 : 1, 10 : 3 : 1, 7 : 3 : 1)] to give methyl glucopyranoside (mixture of α- and β-anomer) as a colorless solid (3 mg). TLC [silica gel, CH_2Cl_2/MeOH/H_2O (lower layer) (10 : 3 : 1)] of the resulting methyl glucopyranoside (α- and β-anomer) was identical to that of the standard methyl α-D-glucopyranoside and methyl β-D-glucopyranoside.

Acetylation of 2 A mixture of **2** (10 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was stirred at room temperature overnight. After the usual work up, the tetraacetate **2a** was obtained as a light yellow oil (14 mg) which was chromatographed on silica gel [hexane/EtOAc (10 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, 2 : 1)] to give **2a** as a colorless wax (4 mg). $[\alpha]_D^{25}$ +25.4° (c=0.35, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 0.88 (3H, t, J=6.6 Hz, CH_3), 1.28

(14H, br s, 7×CH_2), 1.40 (2H, m, H-6), 1.65 (2H, m, H-5), 2.01 (4H, m, H-7, H-10), 2.03, 2.05 (2×), 2.15 (12H, all s, 4×OAc), 4.00 (1H, dd, J=11.7, 3.2 Hz, Ha-1), 4.29 (1H, dd, J=11.7, 4.5 Hz, Hb-1), 4.47 (1H, m, H-2), 4.94 (1H, dt, J=9.0, 3.2 Hz, H-4), 5.10 (1H, dd, J=8.1, 3.2 Hz, H-3), 5.30 (1H, dt, J=10.8, 7.2 Hz, H-8 or H-9), 5.38 (1H, dt, J=10.8, 6.8 Hz, H-9 or H-8), 5.92 (1H, d, J=9.0 Hz, NH). ^{13}C-NMR ($CDCl_3$): δ 14.1 (CH_3), 20.7 (2×), 21.0, 23.3 (4×OCO$\underline{CH_3}$) 22.7, 29.3 (2×), 29.6 (2×), 29.7, 31.9 (7×CH_2), 25.6 (C-6), 26.8, 27.3 (C-7, C-10), 27.9 (C-5), 47.7 (C-2), 62.8 (C-1), 72.0 (C-3), 72.9 (C-4), 128.8 (C-8 or C-9), 130.7 (C-9 or C-8), 169.8, 170.1, 170.8, 171.1 (4×O$\underline{CO}$$CH_3$). EI-MS m/z (rel. int. %): 483 $[M]^+$ (0.5%), 423 $[M-CH_3COOH]^+$ (14), 364 $[423-CH_3COO]^+$ (12), 304 $[364-CH_3COOH]^+$ (13), 262 (12), 244 (13), 184 (44), 144 (20), 102 (70), 84 (100), 67 (24).

Dimethyl Disulfide Derivative (3) of 2a Tetraacetate **2a** (6 mg) was dissolved in carbon disulfide (0.5 ml) and dimethyl disulfide (0.5 ml) and iodine (10 mg) added. The reaction mixture was then kept at 60 °C for 48 h in a small sealed vial. The reaction was quenched with 5% aq. $Na_2S_2O_3$ and the mixture was extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4, filtered and concentrated to give the dimethyl disulfide derivative (**3**) as a light yellow solid (6 mg). $[\alpha]_D^{25}$ +14.6° (c=0.32, $CHCl_3$). EI-MS m/z (rel. int. %): 577 $[M]^+$ (5%), 530 $[M-CH_3S]^+$ (5), 482 $[M-CH_3S-CH_3S]^+$ (6), 422 $[M-95-60]^+$ (8), 390 $[M-187]^+$ (33), 330 $[M-187-60]^+$ (100), 187 $[C_{11}H_{23}S]^+$ (25).

Monoacylmonogalactosylglycerol (**4**): A pale yellow wax, $[\alpha]_D^{25}$ −3.3° (c=0.70, MeOH). IR ν_{max}^{neat} cm^{-1}: 3369, 3010, 1738, 1069. ^{1}H-NMR ($CDCl_3$–CD_3OD): δ 0.98 (3H, t, J=8.0 Hz, CH_3), 1.32 (6H, br s, H-5″, H-6″, H-7″), 1.34 (2H, m, H-4″), 1.62 (2H, quintet, J=8.0 Hz, H-3″), 2.06 (2H, br q, J=8.0 Hz, H-8″), 2.09 (2H, br quintet, J=8.0 Hz, H-17″), 2.35 (2H, t, J=8.0 Hz, H-2″), 2.81 (4H, br t, J=5.8 Hz, H-11″, H-14″), 3.51 (1H, dd, J=9.0, 3.0 Hz, H-3′), 3.52 (1H, m, overlapped signal, H-5′), 3.58 (1H, dd, J=9.0, 7.6 Hz, H-2′), 3.71 (1H, dd, J=10.5, 3.5 Hz, Ha-3), 3.76 (1H, dd, J=11.2, 5.3 Hz, Ha-6′), 3.82 (1H, dd, J=11.2, 6.0 Hz, Hb-6′), 3.89 (1H, dd, J=3.0, 1.2 Hz, H-4′), 3.89 (1H, dd, J=10.5, 6.3 Hz, Hb-3), 4.00 (1H, m, H-2), 4.14 (2H, d, J=6.8 Hz, H-1), 4.25 (1H, d, J=7.6 Hz, H-1′), 5.36 (6H, m, triplet-like signal, H-9″, H-10″, H-12″, H-13″, H-15″, H-16″). ^{13}C-NMR ($CDCl_3$–CD_3OD, 6 : 1): δ 13.8 (C-18″), 20.2 (C-17″), 24.5 (C-3″), 25.2, 25.3 (C-11″, C-14″), 26.9 (C-8″), 28.8 (2×), 28.9 (C-5″, C-6″, C-7″), 29.3 (C-4″), 33.8 (C-2″), 61.1 (C-6′), 64.9 (C-1), 68.2 (C-2), 68.6 (C-4′), 70.9 (C-3), 71.1 (C-2′), 73.1 (C-3′), 74.7 (C-5′), 103.4 (C-1′), 126.8, 127.4, 127.9, 128.0, 129.9, 131.6 (C-9″, C-10″, C-12″, C-13″, C-15″, C-16″), 174.1 (C-1″). MS m/z (rel. int. %): 515 $[M+H]^+$ (20%), 353 (100), 309 (37), 291 (50), 270 (75), 235 (50), 219 (70). HR-LSI-MS m/z: 515.3205 $[M+H]^+$, $C_{27}H_{47}O_9$ requires 515.3217. Found: 515.3205.

Alkaline Hydrolysis of 4 A solution of **4** (50 mg) in 5% NaOMe/MeOH (5.0 ml) was kept at 40 °C for 2 h. The mixture was extracted with hexane (3×10 ml). The hexane layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to give methyl linolenoate as a colorless wax (19 mg). Further purification of the ester by a column of silica gel with hexane/EtOAc (50 : 1) gave the methyl ester as a colorless wax (16 mg) which showed a single peak on GC/MS (t_R=19.00 min), EI-MS m/z: 292 $[M]^+$ identical with methyl linolenoate. The MeOH-soluble fraction was neutralized with 2 N HCl and evaporated to give a solid residue which was chromatographed on a column of silica gel (6 g) eluting with CH_2Cl_2/MeOH/H_2O (lower layer) (20 : 3 : 1, 10 : 3 : 1) to give (2R)-1-O-β-D-galactopyranosylglycerol (12 mg) as a pale yellow powder, $[\alpha]_D^{25}$ −7.1° (c=0.88, MeOH) (lit.[22] −9.0 °C, lit.[24] −7.0 °C, lit.[25] −9.5 °C). The optical rotation of (2S)-1-O-β-D-galactopyranosylglycerol was reported to be +6.2 °C.[26]

Antiviral Activity Assay The colorimetric method previously described by Skehan and coworkers[27] was employed for antiviral assay. Herpes simplex virus type 1 (HSV-1) was maintained in the Vero cell line (kidney fibroblast of an African monkey), which was culture in the Eagle's minimum essential medium (MEM) with the addition of heat-inactivated fetal bovine serum (FBS) (10%) and antibiotics. The test samples were put into wells of a microtiter plate at the final concentrations ranging from 20 to 50 μg/ml. The viral HSV-1 (30 PFU) was added into 96 well plate, followed by plating of Vero cells (1×10^5 cells/ml); the final volume was 200 μl. After incubration at 37 °C for 72 h, under 5% of CO_2 atmosphere, cells were fixed and stained, and optical density was measured at 510 nm. Under the screening conditions, the reference compound, Acyclovir, typically exhibited the antiviral HSV-1 with the IC_{50} of 2—5 μg/ml.

Antiinflammatory Activity Assay Immortalized COX-1$^{-/-}$ and Cox-2$^{-/-}$ mouse lung fibroblast cells were plated at 1×10^5 cells/ml in complete Dulbelcco's Modified Eagle Medium (DMEM) containing 0.1 mM non-essential amino acids, 292 mg/ml L-glutamine, 50 mg/ml ascorbic acid and 10% fetal vovine serum, in 96-well flat-bottomed tissue culture plates at 83 μl/well. Cells were incubated at 37 °C for 72 h in a humidified incubator with 5% CO_2. Subsequently, cells were washed with phosphate buffer saline solution and incubated for 30 min in 83 μl serum-free DMEM medium containing test compounds. DMEM media containing drug vehicle, DMSO (0.1%), and aspirin were used as a control for 100% COX activities and a positive control, respectively. The medium was then replaced with serum-free DMEM containing the same amount of drugs or DMSO and 2 mM of calcium ionophore A23187, and cells were incubated for 30 min. Culture supernatants were collected at the end of incubation time and assayed for prostaglandin E_2 (PGE_2) concentrations by the radioimmunoassay method previously described by Kirtikara and coworkers.[28] The inhibition of COX activity was determined from the percent reduction of PGE_2 produced by drug treated cells relative to PGE_2 produced by cells treated with DMSO alone. IC_{50} values of COX-1 and COX-2 were determined using SOFTmax software (Molecular Devices, Sunnyvale, CA, U.S.A.). Aspirin was used as a positive control and almost equally effective against COX-1 and COX-2. IC_{50} values of aspirin for COX-1 and COX-2 are 2.06 μg/ml and 3.57 μg/ml, respectively.

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