Myo-inositol-Derived Glycolipids with Anti-inflammatory Activity from *Solanum lanceolatum*†

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Lanceolitols A1−A7 (**1**−**7**) and B1−B7 (**9**−**15**), two series of new *myo*-inositol-derived glycolipid analogues, in which a sugar moiety is replaced by a fatty acid esterified *myo*-inositol moiety, were isolated from the leaves of *Solanum lanceolatum*. Their structures were elucidated on the basis of spectroscopic analysis (^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and HRFABMS), as well as chemical analysis. All the compounds showed in vivo anti-inflammatory activity against ear edema in mice produced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In vitro enzyme inhibition studies showed that the mixture of lanceolitols A1−A7 inhibited by 58.56% phospholipase A_2 from bee venom, while the mixture of lanceolitols B1−B7 was cyclooxygenase-2 (COX-2) inhibitors (IC_{50} = 237 μM).

A previous field ethnobotanical study carried out in 200 small rural communities of the highlands of Chiapas, México, indicated that there are a number of *Solanum* species known as "sosa" including *S. chrysotrichum*, *S. hispidum*, and *S. lanceolatum* employed in treating several skin mycosis conditions, principally athlete's foot, as well as for curing ulcers, wounds, and burns.[1−3] Chemical and pharmacological investigations of *S. chrysotrichum*[4,5] and *S. hispidum*[6] demonstrated that the steroidal saponins are the antimycotic components. In our ongoing research on the active principles of Mexican *Solanum* species, it was found that the methanolic extract of the leaves of *S. lanceolatum* Cav. exhibited in vivo anti-inflammatory activity against TPA-induced mouse ear edema. Purification of this extract by conventional methods afforded two series of seven new pseudoglycolipid homologues named lanceolitols A1−A7 (**1**−**7**) and lanceolitols B1−B7 (**9**−**15**) as the anti-inflammatory principles of the title plant, along with four known compounds. Herein, we report the structural elucidation of these *myo*-inositol-derived glycolipids and their anti-inflammatory activities.

Results and Discussion

Leaves of *S. lanceolatum* were extracted with MeOH at room temperature. This extract showed inhibition on TPA-induced mouse ear edema (15.06%, at a dose of 0.1 mg/ear). The extract was then partitioned between CH_2Cl_2 and H_2O, and the CH_2Cl_2 phase was subjected to repeated silica gel column chromatography to yield 154.6 mg of the mixture of lanceolitols A1−A7 (**1**−**7**) and 40 mg of the mixture of lanceolitols B1−B7 (**9**−**15**), apparently "pure" by TLC and HPTLC.

The presence of hydroxyl and ester groups in the mixture of **1**−**7** was indicated by intense bands in the IR spectrum at 3423 and 1751 cm^{-1}, respectively. Its ^{13}C and DEPT NMR spectra identified 11 oxygen-bearing carbon resonances, of which, one (δ 61.75) is a methylene and 10 are methines, indicating the presence of a glycoconjugate. The presence of long aliphatic acyl chains was indicated by an intense band at δ 1.26 in the ^{1}H NMR spectrum, as well as by a cluster of methylene signals around δ 29.7 and various carbonyl carbon signals between δ 172.8 and 172.0 in the ^{13}C NMR spectrum.

The FABMS spectrum of the peracetate derivatives of lanceolitols A1−A7 (**1a**−**7a**) showed a prominent molecular ion peak at *m/z* 901 and less intense peaks at *m/z* 872 and 844, in agreement with the molecular formula $C_{45}H_{72}O_{18}$, $C_{43}H_{68}O_{18}$, and $C_{41}H_{64}O_{18}$, respectively, suggesting the presence of a mixture of homologues, which where not further separated.

In the ^{1}H NMR spectrum, only one anomeric proton in the β orientation was observed at δ 4.61 (1H, d, J = 6 Hz), and HSQC showed its attachment to the anomeric carbon at δ 99.00. This served as the starting point for tracing connectivity within the sugar spin system by COSY and NOESY experiments. Once the ^{1}H signals of the xylopyranose residue have been assigned (Table 1), the six remaining ^{1}H resonances, all between δ 3.94 and 5.61, were assigned to the *myo*-inositol ring, and connectivity was delineated from ^{1}H−^{1}H COSY and TOCSY experiments with the characteristic signal of H-2 at δ 5.61 (1H, t, J = 2.8 Hz) as the starting point.[7] The carbon to which each hydrogen was attached was defined from HSQC. The most downfield of these carbons (δ 74.29) in the cyclitol ring was the point of linkage to xylose. Coupling constants ($J_{1,2}$ = 2.8 Hz; $J_{1,6}$ = 10.4 Hz) observed for its hydrogen (δ 3.94, H-1) and those observed for the hydrogen assigned to H-2 at δ 5.61 ($J_{1,2} = J_{2,3}$ = 2.8 Hz) and H-3 at δ 4.94 ($J_{2,3}$ = 2.8 Hz; $J_{3,4}$ = 10.8 Hz) confirmed assignment of the δ 3.94 resonance to an axial hydrogen with adjacent axial and equatorial hydrogens. The remaining assignments for the *myo*-inositol ring were straightforward. Full assignments of the proton and carbon resonances were secured from the TOCSY, COSY, NOESY, HSQC, and HMBC data and were in full agreement with those described for *myo*-inositol.[8]

Based on the HMBC correlations, the ester moiety was linked to C-2 of the cyclitol ring on the basis of long-range correlation between H-2 of the *myo*-inositol and the carboxyl carbon at δ 172.81. Similar analysis established that the xylose residue was β-linked to O-1 of *myo*-inositol because of the correlation between H-1 of xylose at δ 4.61 and C-1 of the cyclitol at δ 74.29. Due to symmetry of the

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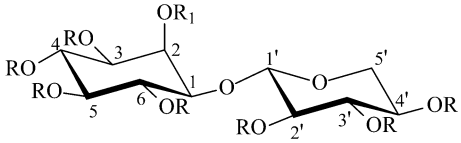

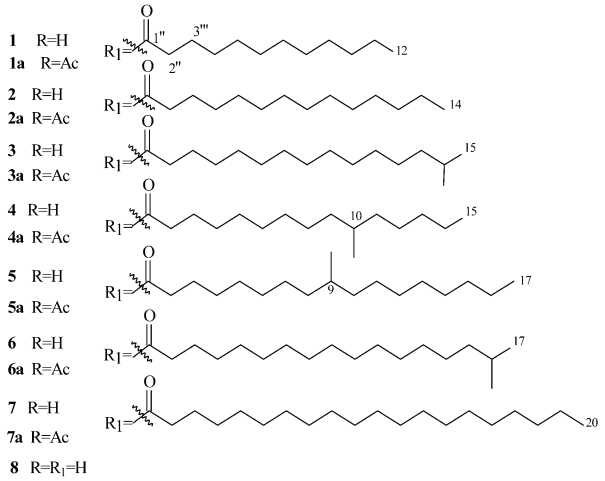

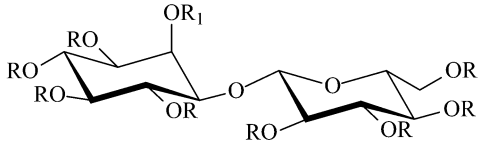

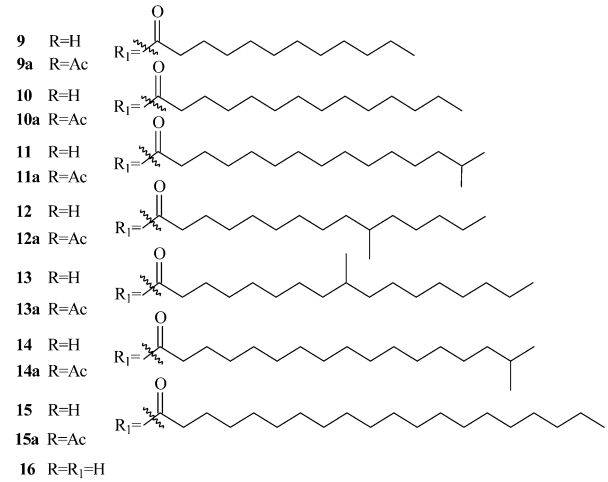

myo-inositol ring, substitution at C-1 or C-3 results in equivalent structures, and the smaller number is assigned.

Analysis of the high-field region of the ^{1}H NMR spectrum showed one methyl triplet at δ 0.89 and two methyl doublets at δ 0.89 and δ 0.88, indicating the presence of linear, *iso*, and *anteiso* acyl chains. To identify the acyl chains, the mixture of **1**−**7** was subjected to methanolysis with 2 equiv of LiOH−MeOH for 3 h. TLC of the crude reaction mixture afforded the alcohol **8** as a single pure substance, as judged by TLC, and a mixture of fatty acid methyl esters. This mixture was subjected to GC-MS quantitative analysis, and seven different compounds were identified as indicated in Table 3. Three unbranched fatty acid methyl esters were identified on the basis of comparison of their retention times and mass spectra with those of authentic samples as methyl dodecanoate, methyl tetradecanoate, and methyl eicosanoate. Two *iso*-branched and two *anteiso*-branched fatty acid methyl esters were identified as methyl 14-methylpentadecanoate, methyl 16-methylheptadecanoate, methyl 10-methylpentadecanoate, and methyl 9-methylheptadecanoate. The position of branching of methyl 14-methylpentadecanoate and methyl 16-methylheptadecanoate was evidenced by the presence of peaks at *m/z* 227 and 255, respectively, originated by cleavage of the isopropyl part ($M^+ - 43$), as well as the diagnostic peaks at *m/z* 195 (227 − MeOH), 177 (227 − MeOH − H_2O), 223 (255 − MeOH), and 205 (255 − MeOH − H_2O). The position of branching at C-9 of methyl 9-methylheptadecanoate was indicated by the presence of two relatively intense peaks separated by 28 amu at *m/z* 185 ($C_{11}H_{21}O_2$) and 157 ($C_9H_{17}O_2$), originated from α-cleavage with respect to the tertiary carbon atom carrying the methyl branch. Furthermore, diagnostic peaks at *m/z* 153 ($C_{11}H_{21}O_2$ − MeOH) and 135 ($C_{11}H_{21}O_2$ − MeOH − H_2O) were observed. Analogously, the methyl branching of methyl 10-methylpentadecanoate was indicated by peaks at *m/z* 199 ($C_{12}H_{23}O_2$), 171 ($C_{10}H_{19}O_2$), 167 ($C_{12}H_{23}O_2$ − MeOH), and 149 ($C_{12}H_{23}O_2$ − MeOH − H_2O) and the absence of the peak at *m/z* 185 ($C_{11}H_{21}O_2$).[9] Therefore, lanceolitols A1−A7 (**1**−**7**) can be formulated as D-*myo*-inositol-2-*O*-dodecanoyl-1-*O*-*β*-D-xylopyranoside (**1**), D-*myo*-inositol-2-*O*-tetradecanoyl-1-*O*-*β*-D-xylopyranoside (**2**), D-*myo*-inositol-2-*O*-14-methylpentadecanoyl-1-*O*-*β*-D-xylopyranoside (**3**), D-*myo*-inositol-2-*O*-10-methylpentadecanoyl-1-*O*-*β*-D-xylopyranoside (**4**), D-*myo*-inositol-2-*O*-9-methylheptadecanoyl-1-*O*-*β*-D-xylopyranoside (**5**), D-*myo*-inositol-2-*O*-16-methylheptadecanoyl-1-*O*-*β*-D-xylopyranoside (**6**), and D-*myo*-inositol-2-*O*-eicosanoyl-1-*O*-*β*-D-xylopyranoside (**7**). The absolute configuration of the esterified *myo*-inositol ring and the sugar moiety was not determined due to scarcity of the compound.

The FABMS spectrum of the peracetate derivative of the mixture of lanceolitols B1−B7 (**9a**−**15a**), like the mixture of **1a**−**7a**, showed molecular ion peaks at *m/z* 972, 944, and 917, in agreement with the molecular formulas $C_{48}H_{76}O_{20}$, $C_{46}H_{72}O_{20}$, and $C_{44}H_{68}O_{20}$, respectively, indicative of a mixture of homologues. Analyses of the NMR data of lanceolitols B1−B7 (**9a**−**15a**) and comparison with those of the mixture of lanceolitos A1−A7 (**1a**−**7a**) showed that **9a**−**15a** differ from **1a**−**7a** only in the absence of the xylopyranosyl unit linked at C-1 of the *myo*-inositol ring. Instead, it is attached to a glucopyranosyl unit (Tables 1 and 2). The ^{13}C NMR spectrum showed one more methine-bearing-oxygen carbon than **1a**−**7a** (Table 2). The presence of the glucopyranosyl unit was deduced from the ^{1}H NMR anomeric proton signal at δ 4.60 (d, J = 7.6 Hz), which was compatible with the anomeric carbon signal at δ 99.50. Also, in this case, the proton-coupling network within the pseudo-disaccharide residue was established using a combination of ^{1}H−^{1}H COSY, NOESY, TOCSY, HSQC, and HMBC experiments (Tables 1 and 2). Once again, direct evidence of the sugar sequence and the linkage sites was derived from the HMBC experiments. These results established a *β*-D-glucopyranosyl unit linked at C-1 of a *myo*-inositol unit and the acyl chains attached to C-2 of the cyclitol ring. Quantitative GC-MS analysis of the mixture of fatty acid methyl esters obtained by basic methanolysis permitted the identification of methyl dodecanoate, methyl tetradecanoate, methyl 14-methylpentadecanoate, methyl 16-methylheptadecanoate, methyl 10-methylpentadecanoate, methyl 9-methylheptadecanoate, and methyl eicosanoate, which is similar to that obtained from lanceolitols A1−A7 (**1**−**7**), but in different relative percentage as reported in Table 3. Therefore, lanceolitols B1−B7 (**9**−**15**) can be formulated as D-*myo*-inositol-2-*O*-dodecanoyl-1-*O*-*β*-D-glucopyranoside (**9**), D-*myo*-inositol-2-*O*-tetradecanoyl-1-*O*-*β*-D-glucopyranoside (**10**), D-*myo*-inositol-2-*O*-14-methylpen-

Table 1. ^{1}H NMR Data of Compounds **1**−**7** and **9**−**15** and Their Peracetate Derivatives (**1a**−**7a** and **9a**−**15a**) (400 MHz, $CDCl_3$)

position	**1**−**7**[a]	**1a**−**7a**	**9**−**15**[a,b]	**9a**−**15a**
H-1	3.76 dd (10, 2.8)	3.94 dd (10.4, 2.8)	3.92 m	3.98 dd (10.4, 2.8)
H-2	5.56 t (2.8)	5.61 t (2.8)	5.58 m	5.61 t (2.8)
H-3	4.95 dd (10.8, 2.8)	4.94 dd (10.8, 2.8)	5.06 t (9.6)	4.94 dd (10.4, 2.8)
H-4	5.39 t (10)	5.48 t (10.4)	5.54 m	5.46 t (9.6)
H-5	5.05 t (9.6)	5.16 t (10)	5.06 m	5.19 t (10)
H-6	3.82 t (9.6)	5.40 t (10)	3.84 m	5.38 t (9.6)
H-1′	4.32 d (7.2)	4.61 d (6)	4.37 d (8)	4.60 d (7.6)
H-2′	3.21 m	4.75 dd (8, 5.6)	3.30 m	4.85 dd (7.6, 9.2)
H-3′	3.41 t (8.4)	5.08 t (8)	3.39 m	5.13 t (9.2)
H-4′	3.54 m	4.85 dt (7.2, 4.8)	3.30 m	5.05 t (9.2,10)
H-5β′	3.91 dd (11.6, 4.8)	4.07 dd (12.2, 4.8)	3.45 m	3.72 dddd (2.4, 2, 4.4, 9.8)
H-5α′	3.21 m	3.43 dd (12.2, 7.2)		
H-6a′			3.57 m	4.36 dd (4.4, 12.4)
H-6b′			3.79 m	4.06 dd (2, 12.4)
H-2″	2.21 m	2.41 m	2.35 m	2.41 m
H-3″	1.56 m	1.63m	1.58 m	1.63 m
$-(CH_2)_n-$	1.19 sa	1.26 sa	1.22 sa	1.26 m
ω-methyl groups	0.87 t (6.4)	0.89 t (6.8)	0.89 d (7)	0.911 d (6.8)
isopropyl methyl groups	0.82 d (6.4)	0.89 d (6.8)	0.86 d (6.2)	0.906 d (6.8)
branch methyl groups	0.80 d (6.8)	0.88 d (6.8)	0.87 t (6.8)	0.86 t (6.6)
acetyl CH_3		2.11, 2.10, 2.09, 2.06, 2.01, 2.01 (each, 3H, s)		2.00, 2.01, 2.02, 2.03, 2.05, 2.06, 2.08, 2.09 (each, 3H, s)

[a] Dissolved in $CDCl_3$−DMSO. [b] Taken at 200 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**−**7** and **9**−**15** and Their Peracetate Derivatives (**1a**−**7a** and **9a**−**15a**) (100 MHz, $CDCl_3$)

position	**1**−**7**[a]	**1a**−**7a**	**9**−**15**[a,b]	**9a**−**15a**
C-1	75.29	74.29	75.18	75.03
C-2	65.50	66.94	65.20	67.37
C-3	69.75	69.10	69.10	69.15
C-4	72.29	69.38	71.12	69.33
C-5	69.16	70.55	69.52	70.23
C-6	72.00	70.13	71.51	69.88
C-1′	103.15	98.90	101.12	99.50
C-2′	69.35	70.31	71.24	71.35
C-3′	78.64	70.71	76.40	73.10
C-4′	69.35	68.75	71.69	67.85
C-5′	68.72	61.75	72.49	71.91
C-6′			63.56	61.73
C-1″	173.49−169.25	172.8−172.07	173.8−169.64	172.72−170.25
C-2″	43.32, 43.05	43.20, 43.10	43.30, 43.02	43.14, 43.04
C-3″	34.41, 32.00	34.16, 32.10	34.32, 32.04	34.12, 32.03
$-(CH_2)_n-$ acyl chain	29.75−25.21,	29.88−25.65	29.85−25.68	29.79−25.10
isopropyl methyl groups	22.81	22.87	22.80	22.80
branch methyl groups	22.34	21.03	22.36	22.24
ω-methyl groups	14.32	14.32	14.33	14.25
CH_3*C*OO−		170.16−169.36		169.67−169.34
CH_3COO−		20.99, 20.79		20.77, 20.68

[a] Dissolved in $CDCl_3$−DMSO. [b]Taken at 200 MHz.

tadecanoyl-1-*O*-β-D-glucopyranoside (**11**), D-*myo*-inositol-2-*O*-10-methylpentadecanoyl-1-*O*-β-D-glucopyranoside (**12**), D-*myo*-inositol-2-*O*-9-methylheptadecanoyl-1-*O*-β-D-glucopyranoside (**13**), D-*myo*-inositol-2-*O*-16-methylheptadecanoyl-1-*O*-β-D-glucopyranoside (**14**), and D-*myo*-inositol-2-*O*-eicosanoyl-1-*O*-β-D-glucopyranoside (**15**). The absolute configuration of the esterified *myo*-inositol ring and the sugar moiety was not determined due to scarcity of the compound.

The mixtures of lanceolitols A1−A7 (**1**−**7**) and B1−B7 (**9**−**15**) were evaluated with respect to their anti-inflammatory activity against TPA-induced inflammation in mice, and the inhibitory activities were compared with that of indomethacin, a commercially available anti-inflammatory drug. At a dose of 0.02 mg/ear, the mixture of lanceolitols A1−A7 (**1**−**7**) displayed 57.84% inhibition of ear edema; this activity was weaker than or almost equivalent with that of indomethacin (69.64% at a dose of 0.02 mg/ear),

Table 3. Fatty Acid Methyl Esters Obtained from Lanceolitols **1**−**7** and **9**−**15**

fatty acid methyl ester	t_R (min)	**1**−**7** (%)	**9**−**15** (%)
dodecanoate	3.48	9.90	2.50
tetradecanoate	5.61	56.50	7.62
14-methylpentadecanoate	7.36	6.85	14.12
10-methylpentadecanoate	7.39	3.48	2.44
9-methylheptadecanoate	9.07	1.09	8.06
16-methylheptadecanoate	9.03	1.49	5.48
eicosanoate	10.72	7.92	48.20

while the mixture of lanceolitols B1−B7 (**9**−**15**) inhibited markedly the TPA-induced inflammation by 78.65% at the same dose. When these compounds were tested as inhibitors of *c*PLA_2 enzyme from bee venom using an enzymatic method[10] and betulinic acid, a natural product with anti-cPLA$_2$ activity,[11] as the positive control, the mixture of lanceolitols A1−A7 (**1**−**7**) showed the best inhibitory potency with 58.56% inhibition, at a concentration of 100 μg/mL, 2.5% higher than that of betulinic acid (15.93%) at the same concentration, while the mixture of lanceolitos B1−B7 (**9**−**15**) did not show inhibition.

The in vitro ability (IC_{50} values) of both series of lanceolitols to inhibit the isozyme COX-2 was determined by an enzymatic inhibitory assay.[12] In this assay the mixture of lanceolitols B1−B7 (**9**−**15**) showed the best inhibitory potency ($IC_{50} = 237 \pm 2.0$ μM, relative to **15**), while lanceolitols A1−A7 (**1**−**7**) were less potent COX-2 inhibitors ($IC_{50} = 763 \pm 0.09$ μM, relative to **2**), relative to the reference drug indomethacin ($IC_{50} = 0.75$ μM).

Lanceolitols A1−A7 and B1−B7 are two series of *myo*-inositol-derived pseudoglycolipid homologues, in which a sugar moiety is replaced by a *myo*-inositol, attached to a fatty acid through an ester linkage. These or similar compounds have not been previously reported from a natural source, even though some other complex cyclitol glycosides, such as fagopyritols A and B,[13−15] the trisaccharide MGI,[16] and disaccharides,[17,18] have been reported from terrestrial plants, as well as the glycolipids crasserides and isocrasserides isolated from marine sponges.[9,19−21]

Our research on this plant indicated that *S. lanceolatum* is a source of inhibitors of phospholipase A_2 (PLA_2) and COX-2 enzymes. The in vivo anti-inflammatory activity displayed by these compounds indicated this plant as a possible candidate for the development of new drugs to treat symptoms associated with inflammatory diseases, such as osteoarthritis and arteriosclerosis. Further studies on the assessment of the COX-1/COX-2 selectivity index and inhibitory potency are in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher Johns melting point apparatus. The IR spectra were measured on a Bruker Vector 22 spectrometer. HRFABMS spectra in a matrix of glycerol were recorded on a JEOL JMX-AX 505 HA mass spectrometer. GC-MS spectra were acquired using an Agilent 6890 series GC system and Agilent 5973 mass selective detector, employing a fused-silica column, 30 m × 0.32 mm HP-5MS (cross-linked 5% Ph Me silicone, 0.25 μm film thickness). The temperature of the column was varied, after a delay of 2 min from the injection, from 40 to 250 °C with a slope of 10 °C/min and a stay of 5 min at this temperature. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. All NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 MHz for ^{1}H NMR, ^{1}H−^{1}H COSY, HMBC, HSQC, and ^{1}H−^{1}H TOCSY and 100 MHz for ^{13}C NMR and ^{13}C DEPT using DMSO-d_6 and $CDCl_3$ as solvents. Chemical shifts are reported in ppm (δ) relative to TMS signal. Column chromatography and TLC were carried out on silica gel 60 (0.063−0.2 mm, Merck).

Plant Material. The leaves of *S. lanceolatum* were collected near the village of Santiago el Pinar, municipality of San Andrés Larrainzar, Chiapas, province of México, on January 20, 1997, and were identified by Biol. Abigaíl Aguilar from the IMSS-México Herbarium (IMSSM), where voucher specimens have been deposited.

Extraction and Isolation. The leaves (800 g) were extracted by maceration at room temperature three times with MeOH (5 L) and concentrated to dryness under reduced pressure. The MeOH extract (102 g) was partitioned between CH_2Cl_2 and H_2O to yield a CH_2Cl_2-soluble fraction and an aqueous-soluble fraction. The CH_2Cl_2 extract (86 g, 10.75%) was adsorbed on silica gel (86 g) and chromatographed on CC over silica gel 60 (860 g), using *n*-hexane−EtOAc−CH_2Cl_2−MeOH mixtures of increasing polarity as eluent to yield six fractions: F-1 (8.7 g, 100:0:0:0), F-2 (2.87 g, 4:1:0:0), F-3 (9.56 g, 3:2:0:0), F-4 (12.1 g, 0:0:95:05), F-5 (5.8 g, 0:0:9:1), and F-6 (32.72 g, 0:0:3:2). F-2 was subjected to silica gel column chromatography eluted with *n*-hexane−EtOAc (4:1) to yield four fractions; fraction 1 (1.11 g) was applied to a silica gel column using *n*-hexane−CH_2Cl_2 (1:1) as the isocratic eluent system to yielded 43.4 mg of betulaprenol 6[22] (0.0054%) and 5 mg of lupeol[23] (0.000625%, mp 211−213 °C); fraction 4 was ground with acetone to yield 60 mg of β-sitosterol (0.0075%, mp 135−139 °C). Fraction F-4 of the main chromatography was applied to a silica gel column using mixtures of CH_2Cl_2−acetone; fractions 38−42 eluted with 93:7 afforded 154.6 mg of lanceolitols A1−A7 (**1**−**7**, 0.019%). F-6 was subjected to silica gel column chromatography using a gradient system of *n*-hexane−EtOAc−MeOH; fractions 71−125 eluted with 5:5:0.5 yielded 44 mg of the inseparable mixture of β-sitosteryl and stigmasteryl glycosides (0.0055%, mp 284−285 °C), and fractions 131−152 eluted with 5:5:1 afforded 40 mg of lanceolitols B1−B7 (**9**−**15**, 0.005%). Betulaprenol 6, lupeol, β-sitosterol, and the mixture of glycosides of β-sitosterol and stigmasterol were identified by direct comparison (IR, TLC) with authentic samples, while the mixtures of lanceolitols A1−A7 (**1**−**7**) and B1−B7 (**9**−**15**) were characterized by spectroscopic measurements of their peracetate derivatives, as well as by chemical degradation.

General Acetylation Procedure. Mixtures of compounds **1**−**7** (50 mg) and **9**−**15** (15 mg) were each treated with Ac_2O (1 mL) and pyridine (0.5 mL) for 6 h. The reaction was quenched with ice (5 mL) and extracted with EtOAc (3 × 10 mL), and the residue was purified on a silica gel column with CH_2Cl_2−acetone mixtures to give the peracetate derivatives **1a**−**7a** (52 mg) and **9a**−**15a** (16 mg).

Lanceolitols A1−A7 (1−7): white amorphous powder; mp 130−140 °C; $[\alpha]_D^{25}$ − 6.6 (*c* 0.61, MeOH); IR (KBr) ν_{max} 3423 (OH), 2926, 2856 (CH), 1751 (C=O), 1100 (C−O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$−DMSO-d_6), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$−DMSO-d_6), see Table 2; FABMS (assignment, relative intensities) *m/z* 634 [**5**, **6**, M + K + H]$^+$ (17), 622 [**7**, M]$^+$ (5), 518 [**2**, M + K − AcO + H]$^+$ (7), 515 [**1**, M + K − AcO]$^+$ (10), 472 [**7**, M − $C_5H_9O_5$]$^+$ (15).

Lanceolitols A1−A7 peracetate (1a−7a): white powder; mp 80−90 °C; $[\alpha]_D^{25}$ − 9.07 (*c* 0.65, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2925, 1759 (ester), 1432, 1374, 1041 (C−O−C), 728 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS (assignment, relative intensities) *m/z* 940 [**7a**, M + K + H]$^+$ (10), 924 [**7a**, M + Na]$^+$ (17), 901 [**7a**, M + H]$^+$ (5), 896 [**5a**, **6a**, M + Na + H]$^+$ (9), 883 [**3a**, **4a** M + K]$^+$ (1.5), 872 [**5a**, **6a** M]$^+$ (2), 844 [**3a**, **4a**, M]$^+$ (2), 841 [**7a**, M − AcO]$^+$ (1.8), 813 [**5a**, **6a**, M − AcO]$^+$ (1), 626 [**7a**, M − $C_{11}H_{15}O_8$]$^+$ (30), 331 [**5a**, **6a**, $C_{29}H_{50}O_9$; **7a**, M − $C_{31}H_{54}O_9$]$^+$ (20).

Lanceolitols B1−B7 (9−15): white amorphous powder; mp 140−160 °C; $[\alpha]^{25}{}_D$ − 11 (*c* 0.66, MeOH); IR (KBr) ν_{max} 3415 (OH), 2927, 2857 (CH), 1747 (C=O), 1099 (C−O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$−DMSO-d_6), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$−DMSO-d_6), see Table 2; FABMS (assignment, relative intensities) *m*/z 690 [**15**, M + K + H]$^+$ (35), 689 [**15**,

M + K]$^+$ (11), 662 [**13**, **14**, M]$^+$ (7), 651 [**15**, M + H]$^+$ (7), 622 [**13**, **14**, M]$^+$ (7), 605 [**10**, M]$^+$ (7), 594 [**11**, **12**, M]$^+$ (5), 538 [**9**, M]$^+$ (7).

Lanceolitols B1−B7 peracetate (9a−15a): viscous oil; $[\alpha]^{25}_D$ −7.7 (*c* 1.4, CH_2Cl_2); IR (CH_2Cl_2) ν_{max} 2961, 1758 (ester), 1430, 1375, 1044 (C−O−C), 730 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS (assignment, relative intensities) *m/z* 1012 [**15a**, M + K + H]$^+$ (20), 996 [**15a**, M + Na + H]$^+$ (18), 984 [**13a**, **14a**, M + K + H]$^+$ (10), 983 [**13a**, **14a**, M + K]$^+$ (7), 973 [**15a**, M + H]$^+$ (7), 972 [**15a**, M]$^+$ (10), 944 [**13a**, **14a**, M]$^+$ (5), 928 [**10a**, M + K + H]$^+$ (7), 917 [**11a**, **12a**, M + H]$^+$ (7), 916 [**11a**, **12a**, M]$^+$ (10), 914 [**15a**, M − AcO + H]$^+$ (25), 829 [**10a**, M − AcO]$^+$ (7), 626 [**15a**, M − $C_{14}H_{19}O_{10}$]$^+$ (55), 331 [**10a**, M − $C_{28}H_{46}O_{11}$; **15a**, M − $C_{34}H_{58}O_{11}$]$^+$ (100).

Methanolysis of Lanceolitols 1−7 and 9−15. Mixtures of compounds **1**−**7** (80 mg) and **9**−**15** (10 mg) were separately stirred at room temperature for 3 h with 2 equiv of LiOH in MeOH (3 mL). Ice was added, and the reaction mixture was extracted with EtOAc (3 × 5 mL). The EtOAc fraction was evaporated to dryness in a vacuum to yield a mixture of fatty acid methyl esters (15 mg from **1**−**7** and 3 mg from **9**−**15**), which were analyzed by GC-MS.

Analysis of the Methyl Ester Mixture. The identification of the fatty acid methyl esters obtained from methanolysis of lanceolitols **1**−**7** and **9**−**15** was based upon their GC-MS retention times and GC-MS spectra. Methyl dodecanoate, methyl tetradecanoate, and methyl eicosanoate were identified on the basis of comparison of their retention times and mass spectra with those of authentic samples, while methyl 14-methylpentadecanoate, methyl 16-methylheptadecanoate, methyl 10-methylpentadecanoate, and methyl 9-methylheptadecanoate were identified on the basis of the analysis of their fragmentation pattern in their MS spectra. Percentages were based on the peak areas of the GC spectra.

Chemicals and Enzymes. TPA (12-*O*-tetradecanoylphorbol 13-acetate), sedalphorte, reference drug indomethacin, DMSO, EtOH, and MeOH were purchased from Sigma Chemical Co., and *c*PLA_2 assay buffer, DTNB (5,5′-dithiobis(2-nitrobenzoic acid), arachidonoyl thio-PC, *c*PLA_2 from bee venom, prostaglandin screening EIA (enzyme inmunoassay) antiserum, prostaglandin screening AChE (acetylcholinesterasa) tracer, prostaglandin screening EIA standard, EIA buffer (10 ×), wash buffer (400 ×), tween 20, mouse anti-rabbit IgG coated plate, Ellman's reagent, reaction buffer (10 ×), COX-2 (human recombinant), heme, arachidonic acid (substrate), potassium hydroxide, hydrochloric acid, and stannous chloride were purchased from Cayman Chemical Co.

Experimental Animals. Adult male Wistar CD-1 mice with a body weight ranging from 20 to 25 g were used. All animals had free access to food and water and were kept on a 12/12 h light−dark cycle.

TPA-Induced Mouse Ear Edema. Mouse ear edema was evaluated following the protocol previously described,[24] using groups of three male CD-1 mice. Edema was induced by topical application of 2.5 μg per ear of TPA dissolved in EtOH. Solutions of the mixtures of lanceolitols A1−A7 (**1**−**7**) and B1−B7 (**9**−**15**) (0.02 mg/ear) and the standard drug indomethacin (0.02 mg/ear) as reference, dissolved in DMSO and acetone, respectively, were applied to both sides of the right ear (10 μL each side) simultaneously with TPA. The ear swelling was measured before TPA application and 4 h after, and the edema was expressed as the increase in thickness.

***c*PLA_2 Assay System.** Activity of *c*PLA_2 was evaluated by the test described in the *c*PLA_2 kit No. 76502, purchased from Cayman Chemical Co.[10] Briefly, to a supplied ThioPC/Triton X-100 substrate solution (2 mM ThioPC, 4 mM Triton X-100, and 30% glycerol in 80 mM Hepes, pH 7.4, 150 mM NaCl, 10 mM $CaCl_2$, and 1 mg/mL BSA), distributed in a 96-well plate (200 μL) was added a solution of 100 μg/mL in DMSO, and then 500 ng of *c*PLA_2 dissolved in 5 μL of assay buffer was added to the wells. The plate was shaken for 20 s on high to mix and then incubated for 60 min at 37 °C. For blanks, buffer was added to some wells. After 60 min, 10 μL of a 25 mM DTNB/475 mM EGTA mixture was added to all substrate-containing wells to quench the reaction and initiate color development. The absorbance was measured at 405 nm. The average absorbance of the blanks was subtracted from that of the enzyme-containing wells to correct for the absorbance due to the substrate, DTNB, and EGTA. The difference in absorbance was used to calculate enzyme activity.

Cyclooxygenase Inhibition Studies. All compounds described herein were tested for their ability to inhibit COX-2 using a COX-(human recombinant)-inhibitor screening kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2, and $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μL, 0.1 M Tri-HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with COX-2 (10 μL) enzyme in the presence of heme (10 μL) was added 10 μL of various concentrations of test drug solutions (10, 100, and 500 μM in a final volume of 1.15 mL). These solutions were incubated for a period of 2 min at 37 °C, after which 10 μL of AA (100 μM) was added, and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay. This assay is based on the competition between PGs and a PG−acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody−PG complex binds to a mouse antirabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents, and then Ellman's reagent, which contains the substrate to acetylcholinesterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 414 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation. Percent inhibition was calculated by comparison of compound-treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration−inhibition response curve (duplicate determinations).

Statistical Analysis. Data are expressed as a mean SEM. Data were analyzed by using Dunnett's test for the *c*PLA_2 assay, Anova test for the TPA-induced ear edema test, and Bonferroni test for the COX-2 assay. $p < 0.05$ was considered to be significant. Analysis of the regression line was used to calculate IC_{50} values.

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