Diterpenes from *Leucas aspera* Inhibiting Prostaglandin-Induced Contractions

Samir Kumar Sadhu,[†] Emi Okuyama,*[,‡] Haruhiro Fujimoto,[§] and Masami Ishibashi[⊥]

Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh, Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan, Faculty of Pharmaceutical Sciences, Teikyo Heisei University, 2289-23 Uruido, Ichihara, Chiba 290-0193, Japan, and Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Received November 4, 2005

Investigation of the inhibitory fraction of *Leucas aspera* on prostaglandin-induced contraction in guinea pig ileum provided four new diterpenes, leucasperones A (**1**) and B (**2**) and leucasperols A (**3**) and B (**4**), and three new isopimarane glycosides, leucasperosides A, B, and C (**5**−**7**), together with the known compounds asperphenamate, maslinic acid, (−)-isololiolide, and linifolioside. The structures of the compounds were determined by detailed spectroscopic analysis. The configurations of **1** and **2** and the acetylated derivatives of **3** and **4** were determined by differential NOE analysis and CD data. Leucasperone A (**1**), leucasperosides A (**5**) and B (**6**), and linifolioside showed inhibition of prostaglandin-induced contractions.

In our continuing study on traditional medicines, the bioactive components responsible for their medicinal uses have been investigated. *Leucas aspera* L. (Labiatae) is a medicinal plant of Bangladesh, used for its analgesic-antipyretic and anti-inflammatory properties.[1] Previously, 12 compounds were obtained from this herb by activity-directed isolation using inhibition against prostaglandin (PG)-induced contractions in the guinea pig ileum and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activities.[2] This paper deals with the structures and PG inhibitory activities of a series of new diterpenoids (**1**−**7**).

Results and Discussion

In our previous study, fraction 1B was obtained by Sephadex LH-20 column chromatography of the *n*-BuOH layer from the extract of *L. aspera*.[2] The fraction showed inhibition against both PGE_1- and E_2-induced contractions at 9×10^{-5} g/mL in guinea pig ileum by the Magnus method, while no clear DPPH-radical scavenging activity was observed. Fraction 1B was further separated by repeated column chromatography to yield new compounds **1**−**7**, along with the known compounds asperphenamate[3−5] (**8**), maslinic acid[6] (**9**), linifolioside[7] (**10**), linoleic[8] and oleic acids,[9] and β-sitosterol and stigmasterol 3-*O*-β-glucosides.[10,11] The ^{1}H and ^{13}C NMR spectral data of **11** were identical with those of isololiolide.[12−14] However, the specific rotation ($[\alpha]^{25}_D$ −40.3, $CHCl_3$) and CD ($\Delta\epsilon_{214}$ −4.6, MeOH) were the reverse of and smaller than the published data of (+)-isololiolide ($[\alpha]^{15}_D$ +80.6, $CHCl_3$; CD $\Delta\epsilon_{212}$ +10.39, MeOH).[15,16] The compound was, therefore, determined as a mixture of enantiomers, in which the (−)-form was predominant, although chiral-HPLC analysis did not separate them under the conditions applied (column, Shiseido CD-Ph 4.6 × 250; mobile phase, EtOH−*n*-hexane, 4:6; flow rate, 0.5 mL/min; UV detection, 220 nm).

Compounds **1** and **2** were isolated as new diterpenes and named leucasperones A and B, respectively. Compound **1**, a colorless amorphous solid, $[\alpha]^{23}_D$ +116 (MeOH), was assigned the molecular formula $C_{26}H_{38}O_8$, by HRFABMS [*m/z* 517.2180 $(M + K)^+$]. The ^{13}C NMR and DEPT spectra showed 26 carbon signals for seven methyls, six methylenes, four methines, and nine quaternary carbons. The chemical shifts of the quaternary carbons were one carbonyl at δ 212.3, three ester carbonyls at δ 170.3, 170.5, and 170.9, two olefinic carbons at δ 130.3 and 146.8, and an oxygenated carbon at δ 72.1. Additionally, three oxygenated carbons were assigned as one methylene at δ 68.5 and two methines at δ 67.0 and 72.2. In the ^{1}H NMR spectrum, one OH resonance was observed at δ 2.05 (1H, br s) and three acetoxy methyl signals appeared at δ 1.91, 1.95, and 2.00, corresponding to the signals at δ 21.4, 20.8, and 21.5 in the ^{13}C NMR spectrum. Three mutually coupled protons at δ 4.94 (1H, dd, J = 10.7, 0.9 Hz, H-17*cis*), 5.12 (1H, dd, J = 17.1, 0.9 Hz, H-17*trans*), and 5.80 (1H, dd, J = 17.1, 10.7 Hz, H-16) indicated a second double bond, which was assigned to the olefinic carbon signals for one methylene at δ 111.1 (C-17) and one methine at δ 144.5 (C-16) in the ^{13}C NMR spectrum. Two oxymethines and one oxymethylene signal in the ^{1}H NMR spectrum were observed at δ 5.36 (H-9) and 5.44 (H-13), and 4.04 (H-11a) and 4.22 (H-11b), respectively. After analysis of the COSY and HMQC spectra, the final structure of **1** was determined by an HMBC experiment, as follows: Me-12 showed cross-peaks with C-1, C-2, C-10, and C-11. Both of the coupled methylenes, H_2-3 and H_2-4, shared correlations with the carbonyl at C-2 and the olefin carbon at C-5, and the H_2-4 gave an additional cross-peak with the olefinic C-10. These correlations suggested a hexenone ring (A ring), of which both olefinic carbons were correlated with H-9. Since a partial structure of C(9)H−C(8)H_2−C(7)H−C(19)H_3 was determined from the COSY spectrum, the correlations of one of the methylene protons of C-8 at δ 1.64 with C-9 and C-10 and of Me-18 with C-5, C-6, C-7, and C-13 facilitated formulation of the B ring. The chain substituent at C-6 was determined by the ^{1}H NMR chemical shifts and the coupling constants together with the HMBC correlations of Me-20 with C-14, C-15, and C-16.

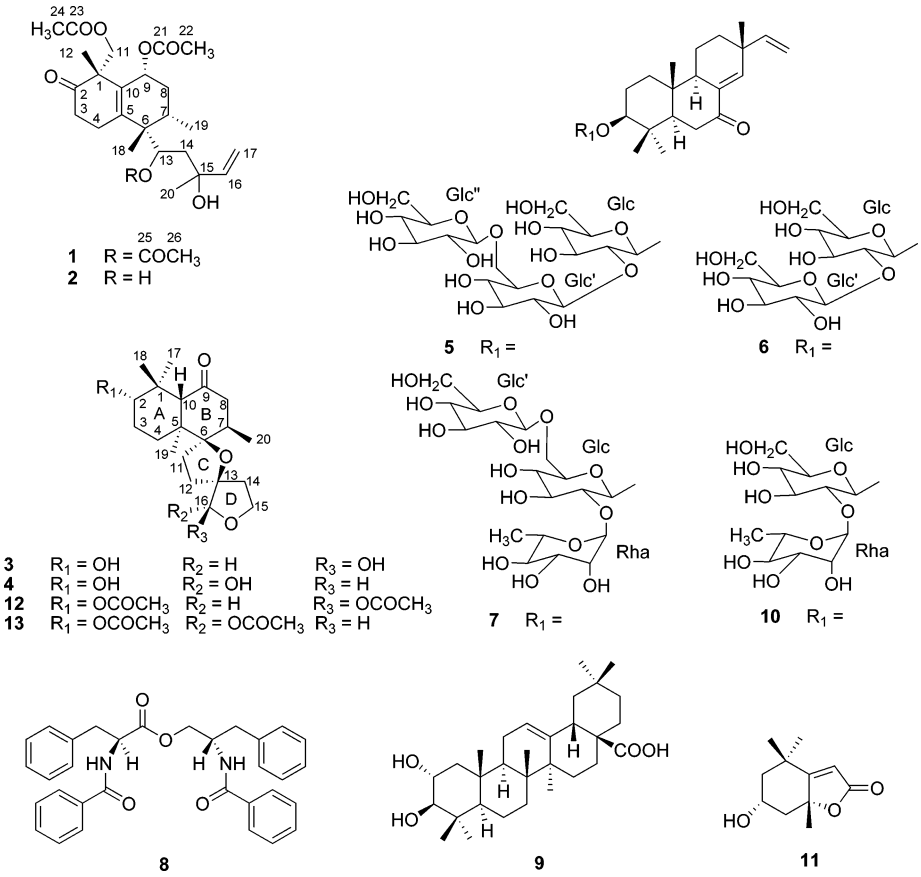
The positions of the three acetoxy groups were determined by the observed correlations of H-9, H_2-11, and H-13 with the carbonyl carbons at C-21, C-23, and C-25, respectively, which were also correlated with the methyl protons Me-22, Me-24, and Me-26, respectively. The ^{1}H and ^{13}C NMR data are summarized in Table 1.

For determination of the relative configuration of **1**, a differential NOE experiment was carried out as shown in Figure 1. Irradiation of Me-12 and Me-19 showed 11% and 4% NOE enhancement with H-9 and H_2-14, respectively. Considering the coupling constants between (H-9)−(H_2-8)−(H-7), the relative configuration was evident. The CD spectrum exhibited a single positive Cotton effect at 291 nm. Therefore, the octant rule seems to be applicable for determination of the absolute configuration of **1** except for the chain substituent at C-6, if a stable conformer is obtained. We carried out density functional theory (DFT) computational studies to obtain

* To whom correspondence should be addressed. Tel: +81-475-53-4606. Fax: +81-475-53-4556. E-mail: emioku@jiu.ac.jp.
[†] Khulna University.
[‡] Josai International University.
[§] Teikyo Heisei University.
[⊥] Chiba University.

10.1021/np058118m CCC: $33.50
Published on Web 06/29/2006

Chart 1



the optimized stable structure for one isomer (13*S*, 15*S*). Calculations were executed using Gaussian 98[17] at the B3LYP level of theory[18,19] with the 6-31G(d) basis set.[20] All atoms were allowed to move freely during the optimization for each molecule. Application of the octant rule for the obtained stable conformer of the (13*S*,-15*S*)-isomer resulted in the absolute configuration of **1** shown in Figure 2, except for the C-13 and C-15 positions in the C-6 side chain.

Leucasperone B (**2**), a colorless amorphous solid, was assigned the molecular formula $C_{24}H_{36}O_7$, as determined by HRFABMS [*m/z* 475.2100 $(M + K)^+$]. The ^1H and ^{13}C NMR data (Table 1) resembled those of **1**, except for the C-13 methine proton, which was shielded at δ 4.15 in **2** compared to that of **1** (δ 5.44), and an additional OH (δ 3.41), consistent with the presence of the C-13 hydroxyl group. The absolute configuration was determined as being the same as that of **1**, because **2** also showed a similar Cotton effect in the CD spectrum.

Compounds **3** and **4** were isolated as a 5:2 mixture of diastereomers. After acetylation of the sample, two diacetylated components, **12** and **13**, were separated.

Compound **12**, a white solid, $C_{24}H_{36}O_7$, showed 24 carbon signals in the ^{13}C NMR spectrum, which included six methyls, seven methylenes, four methines, and seven quaternary carbons. The chemical shifts of the quaternary carbons comprised one carbonyl at δ 210.1, two ester carbonyls at δ 170.5 and 170.9, and two oxygenated carbons at δ 90.0 and 92.3. Additionally, three oxygenated carbon atoms were assigned at δ 65.9 (CH_2), 79.6 (CH), and 97.0 (CH), which were correlated to the ^1H NMR signals at δ 3.84 (H-15a) and 4.11 (H-15b), 4.29−4.32 (H-2), and 5.77 (H-16), respectively, in the HMQC spectrum. The C-17 and C-18 methyl protons gave HMBC cross-peaks with C-1, C-2, and C-10, and the C-19 methyl protons correlated with C-4, C-5, C-6, and C-10, suggesting a cyclohexane ring A, including a C(2)H−C(3)-H_2−C(4)H_2 partial structure as determined from the COSY spectrum. The H-10 methine proton at δ 2.75 in the A ring showed cross-peaks with the C-9 carbonyl and C-6, and Me-20 correlated with C-6, C-7, and C-8. One of the C-8 methylene protons at δ 2.04 showed a correlation with C-10. Therefore, the B ring fused to the A ring as a decalin unit could be proposed. The presence of a substituent at C-6 was suggested by the HMBC correlations of H_2-11 with C-5, C-6, and C-7. The H-16 methine proton at δ 5.77 showed cross-peaks with C-13, C-14, and C-15 and additionally with C-12, which indicated a tetrahydrofuran D ring connected to an ethyl group at C-12. Considering the molecular formula of the compound and the downfield chemical shifts of C-6 and C-13 in the ^{13}C NMR spectrum, the ether linkage between these positions formed a tetrahydrofuran C ring. The positions of the two acetoxy groups were determined by the observed correlations of H-2 and H-16 with the carbonyl carbons at δ 170.5 and 170.9, respectively, which were also correlated with the methyl protons at δ 2.01 [C(2)−$OCOCH_3$] and 2.23 [C(16)−$OCOCH_3$]. The ^1H and ^{13}C NMR data of **12** are summarized in Table 2.

Compound **13** has the same molecular formula as that of **12** by HRFABMS. The ^1H and ^{13}C NMR spectra of **13** were similar to those of **12**. Some differences, however, were observed in the ^1H NMR chemical shifts assigned to the C- and D-ring protons. The H-16 methine proton was shifted downfield to δ 6.27 (5.77 in **12**). From these data, **13**, with same molecular mass as of **12**, was estimated to be the isomer of **12** at the C-16 acetal position. The ^1H and ^{13}C NMR data of **13** are given in Table 2.

For determination of the relative configuration of **12** and **13**, differential NOE experiments were carried out. In **12**, irradiation of H-10 showed NOEs with H-2, H-4β, H-8β, and Me-18. Irradiation of Me-19 and Me-20 led to NOEs with H-7, H-11α, and Me-17, and with H-8β and H-14α, respectively. The configuration of the C-16 position was determined by the observed NOEs with H-4β and H-12β by irradiation of H-16. Compound **13** showed an NOE pattern similar to **12**, except for the H-16 position.

Table 1. NMR Data for Leucasperones A (**1**) and B (**2**) ($CDCl_3$, δ ppm, J in Hz)

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1		51.1		51.2
2		212.3		212.9
3	2.41−2.54 m	36.9	2.38 ddd (15.9, 10.4, 6.1) 2.52 dt (15.9, 5.2)	37.2
4	2.53−2.68 m	24.2	2.62 ddd (17.4, 10.4, 5.2) 2.95 dtl (17.4, 5.2)	24.4
5		146.8		147.9
6		45.4		45.3
7	1.92 br s	34.3	1.86−1.91 m	34.1
8	1.64 br dd (11.7, 1.9) 1.93 br d (11.7)	34.7	1.64 dt (14.3, 2.7) 1.82 ddd (14.3, 4.3, 1.2)	34.8
9	5.36 br s	67.0	5.36 br s	67.5
10		130.3		129.4
11	4.04 d (10.7) 4.22 d (10.7)	68.5	4.04 d (10.7) 4.27 d (10.7)	68.6
12	1.14 s	19.3	1.15 s	19.1
13	5.44 t (6.1)	72.2	4.15 dd (9.5, 2.7)	71.3
13-OH			3.41 br s	
14	1.76 d (6.1)	44.9	1.52 dd (13.4, 9.5) 1.55 overlapped	44.9
15		72.1		73.9
15-OH	2.05 br s		3.41 br s	
16	5.80 dd (17.1, 10.7)	144.5	5.91 dd (17.4, 10.7	145.9
17	4.94 dd (10.7, 0.9) 5.12 dd (17.1, 0.9)	111.1	5.03 dd (10.7, 0.9) 5.19 dd (17.4, 0.9)	111.8
18	1.11 s	23.0	1.24 s	23.0
19	1.15 d (6.7)	17.4	1.04 d (7.0)	17.0
20	1.24 s	29.8	1.36 s	26.4
21		170.5		170.6
22	2.00 s	21.5	2.01 s	21.6
23		170.3		170.4
24	1.95 s	20.8	1.97 s	20.8
25		170.9		
26	1.91 s	21.4		

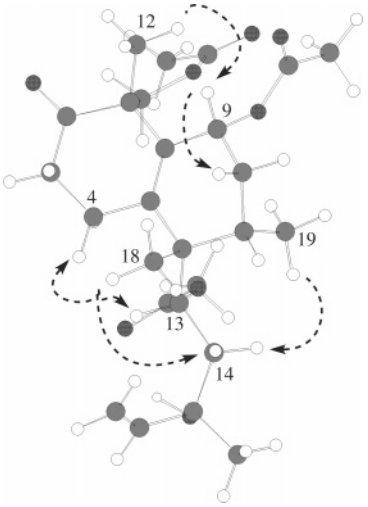


Figure 1. Key NOEs (dashed arrows) observed for **1** [carbons (gray), hydrogens (blank), and oxygens (black) circles].

Irradiation of H-16 showed an NOE with H_2-4β but not with H_2-12. Measurement of the atomic distance for their stable conformers obtained from DFT analysis also supported both structures. The differential NOE results of **12** and **13** are presented in Figure 3.

Both **12** and **13** showed a single positive Cotton effect at 297 nm in their CD spectrum. The octant rule was considered to be applicable for the determination of their absolute configurations using their stable conformers. Since the acetyl groups were mobile to project on both + and − sectors of the octant rule, they were excluded from the consideration. Therefore, the absolute stereostructures of **12** and **13** were determined as shown in Figure 4.

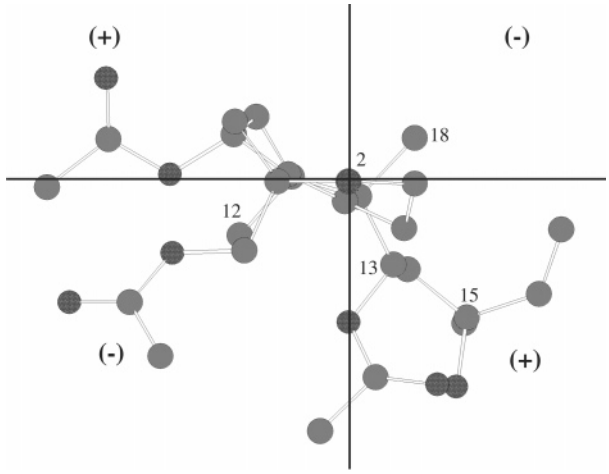


Figure 2. Stable conformer of the (13*S*,15*S*)-isomer of **1** after DFT analysis [carbons (gray) and oxygens (black) circles].

Table 2. NMR Data for Compounds **12** and **13** ($CDCl_3$, δ ppm, J in Hz)

	12		**13**	
position	δ_H	δ_C	δ_H	δ_C
1		36.5		36.7
2	4.29−4.32 m	79.6	4.38 dd (12.3, 4.0)	79.7
3α	1.58−1.64 m	23.3	1.63 qd (12.3, 4.0)	23.4
3β			1.75 dq (12.3, 4.0)	
4α	1.41−1.44 m	29.8	1.51 dt (12.3, 4.0)	30.66[a]
4β	1.58−1.63 m		1.83 td (12.3, 4.0)	
5		47.1		47.5
6		92.3		92.9
7	2.10−2.17 m	38.7	2.10−2.17 m	38.3
8α	2.04 dd (13.2, 4.3)	48.7	2.02 dd (13.1, 4.3)	48.7
8β	2.44 td (13.2, 1.0)		2.41 t (13.1)	
9		210.1		209.8
10	2.75 s	58.3	2.67 (s)	58.3
11α	1.76−1.82 m	28.8	1.77−1.82 m	29.0
11β	2.13 dd (9.1, 3.4)		1.98−2.03 m	
12α	1.99 dd (9.5, 3.4)	36.9	1.98−2.06 m	30.70[a]
12β	2.10 dd (9.5, 2.5)		2.09−2.14 m	
13		90.0		92.7
14α	1.93 ddd (11.6, 7.3, 2.1)	35.0	2.25 ddd (13.1, 5.2, 4.6)	36.4
14β	2.53 td (10.1, 11.6)		2.03−2.09 m	
15	3.84 ddd (10.1, 8.8, 7.3) 4.11 ddd (10.1, 8.8, 2.1)	65.9	4.08 ddl (9.1, 5.2)	67.5
16	5.77 s	97.0	6.27 s	100.5
17	1.22 s	16.0	1.22 s	16.4
18	0.96 s	27.6	0.97 s	27.6
19	0.88 s	19.8	0.90 s	20.1
20	0.96 d (6.4)	17.0	0.98 d (6.7)	17.8
$OCOCH_3$-2	2.01 s	21.1	2.02 s	21.2[b]
$OCOCH_3$-16	2.23 s	21.2	2.05 s	21.3[b]
$OCOCH_3$-2	$OCOCH_3$-2	170.5		170.0
$OCOCH_3$-16	$OCOCH_3$-16	170.9		170.5

[a,b] Interchangeable.

The major (**3**) and minor (**4**) components in the original mixture were estimated from the ^1H and ^{13}C NMR spectra of the acetylated derivatives. The positions of the acetylation were clearly determined by the observation of the upfield-shifted signals for H-2 {δ 3.11 (**3**) and 3.07 (**4**)} and H-16 {δ 4.81 (**3**) and 5.33 (**4**)}, compared to those of the acetylated derivatives. From these data, **12** and **13** seemed to be derived from **3** and **4**, named leucasperols A and B, respectively.

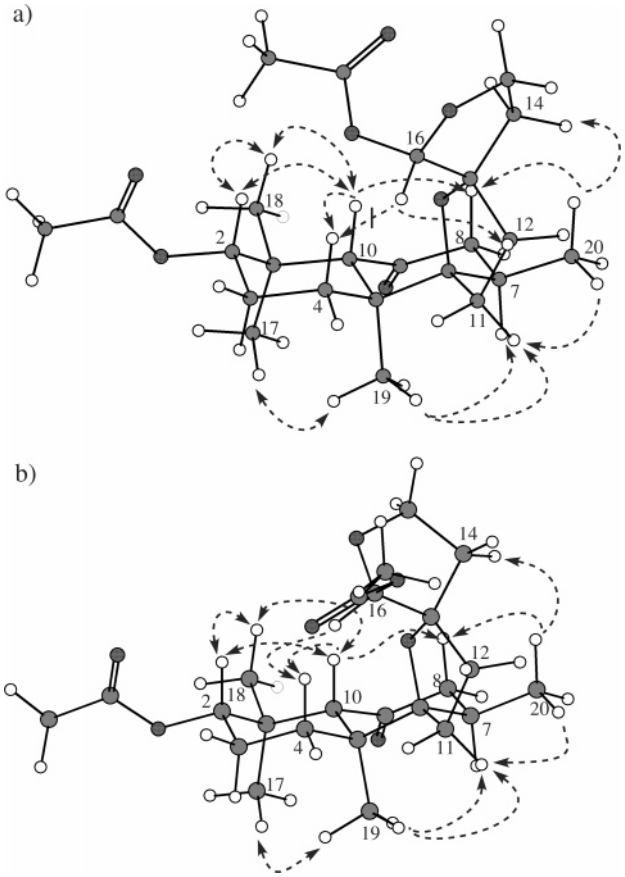


Figure 3. Key NOEs (dashed arrows) observed for (a) **12** and (b) **13** [carbons (gray), hydrogens (blank), and oxygens (black) circles].

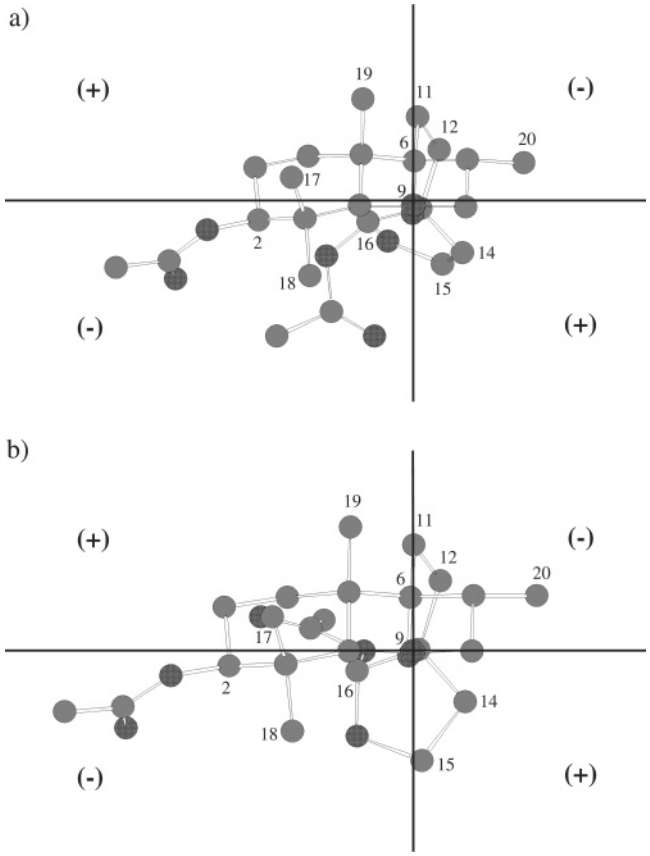


Figure 4. Stable conformers of (a) **12** and (b) **13** after DFT analysis [carbons (gray) and oxygens (black) circles].

Compounds **5**−**7** and **10** were determined by ^{1}H and ^{13}C NMR to be isopimarane glycosides based on the same aglycone. The new components **5**−**7** were named leucasperosides A, B, and C, respectively.

The molecular formula, $C_{38}H_{60}O_{16}$, for **7** was established by HRFABMS [*m*/*z* 795.3800 $(M + Na)^+$], and the ^{1}H and ^{13}C NMR suggested an additional glucose moiety compared with **10**. The position of glucosylation was determined by the HMBC correlation between glc′ H-1 at δ 4.26 (d, $J = 7.6$ Hz) and glc C-6 at δ 68.6. The coupling constant of glc′ H-1 indicated a β-anomer. After hydrolysis of **7**, D-glucose and L-rhamnose were determined by HPLC analysis with a chiral detector. The structure of **7** was therefore deduced as isopimara-8(14),15-diene-7-keto-3-*O*-β-D-glucosyl(1→6)-[α-L-rhamnosyl(1→2)]-β- D-glucoside.

Compound **6** showed an $(M + H)^+$ peak at *m*/*z* 627.3361 in the HRFABMS, corresponding to the molecular formula $C_{32}H_{50}O_{12}$. Analysis of the ^{1}H and ^{13}C NMR spectra indicated that a glucose unit replaced the rhamnose moiety in **10**. The connectivity of the two glucose units was determined by the HMBC cross-peak between glc′ H-1 at δ 4.43 (d, $J = 7.9$ Hz) and glc C-2 at δ 81.3. Considering the coupling constant of each anomeric proton of **6**, a 3-*O*-β-D-glucosyl(1→2)-β-D-glucoside structure was obtained.

The molecular formula of **5**, $C_{38}H_{60}O_{17}$, was assigned from *m*/*z* 827.3505 for $(M + K)^+$ in the HRFABMS. Accordingly, the structure suggested the presence of three hexose units. The ^{1}H and ^{13}C NMR spectra indicated that an additional glucose unit was bonded to the sugar portion of **6**. The position was determined by the correlation of glc″ H-1 at δ 4.42 (d, $J = 7.6$ Hz) with glc′ C-6 at δ 70.0 in the HMBC experiment, and the coupling constant of glc″ H-1 indicated a β-anomer. Consequently, **5** was determined as isopimara-8(14),15-diene-7-keto-3-*O*-β-D-glucosyl(1→6)-β-D-glucosyl(1→2)-β-D-glucoside.

The absolute configurations of **5**−**7** were supported by CD analysis. The CD data of **5**−**7** and **10** were similar to that of linifoliol ($\Delta\epsilon_{246}$ −5.1, MeOH), the aglycone of linifolioside,[7] but opposite that of oryzalexin A (−CE_{210}, +CE_{250}, +CE_{335}), isolated from *Oryza sativa*.[21]

Compounds **1**, **5**−**7**, **9**, and **10**, and the mixture of **3** and **4**, were tested for inhibitory activity against PG-induced contractions in the guinea pig ileum. Among the compounds tested, **6** exhibited the most potent effect at concentrations of 1×10^{-5} g/mL (16 μmol) and 3×10^{-5} g/mL (48 μmol) against PGE_1- and PGE_2-induced contractions, respectively. Compounds **1** and **5** inhibited both types of contractions at 6×10^{-5} g/mL (126 and 76 μmol, respectively), while **10** showed a less potent inhibition against only PGE_1-induced contraction at 6×10^{-5} g/mL (98 μmol). Compounds **7** and **9** at 3×10^{-5} g/mL (39 and 64 μmol, respectively) and a 5:2 mixture of **3** and **4** at 6×10^{-5} g/mL (170 μmol) had no inhibitory activity. In the case of the isopimarane glycosides, variation in both the type and number of sugar units contributed to PG inhibitory activity. None of the isolated compounds showed DPPH-radical scavenging activity. Compound **9** and stigmasterol glucoside have been reported for the anti-inflammatory activity evaluated by carrageenan-induced edema inhibition in mice.[22] Some highly oxygenated isopimarane diterpenes also have been reported for the nitric oxide inhibitory effect.[23,24] Accordingly, **1**, **5**, **6**, and **10** together with other compounds in our previous paper[2] may contribute to the anti-inflammatory activity of *L. aspera*.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. CD spectra were obtained in a JASCO J-720WI spectropolarimeter. EIMS was measured on a JEOL GC-Mate, FABMS on a JEOL JMS-AX500, and HRMS by a JEOL HX-110A spectrometer. NMR spectra were recorded on a JEOL A 500 spectrometer with a deuterated solvent, the chemical shift of which

Table 3. ^{1}H NMR Data for Compounds **5**−**7** and **10** (δ ppm, J in Hz)

position	**5**[a]	**6**[b]	**7**[b]	**10**[a]
1	1.36 td (13.4, 3.7)	1.19 td (13.5, 3.6)	1.30−1.34 m	1.33 td (13.4, 3.4)
	1.82 br d (13.4)	1.68 br d (13.5)	1.64 br d (13.4)	1.80 dt (13.4, 3.3)
2	1.72−1.75 m	1.54−1.57 m	1.54 br s	1.70−1.74 m
	2.03 dd (13.4, 3.7)	1.96 dd (13.4, 3.6)	1.92 dd (12.8, 4.3)	2.04−2.08 m
3	3.30 overlapped	3.11−3.13 m	3.16−3.21 m	3.26−3.29 m
5	1.58 dd (13.5, 5.2)	1.51 dd (13.4, 5.2)	1.52 dd (13.1, 5.5)	1.59 dd (13.4, 5.2)
6	2.35 dd (18.9, 13.5)	2.25 dd (18.6, 13.4)	2.26 dd (18.6, 13.1)	2.35 dd (18.9, 13.4)
	2.50 dd (18.9, 5.2)	2.36 dd (18.6, 5.2)	2.35 dd (18.6, 5.5)	2.49 dd (18.9, 5.2)
9	2.02−2.09 m	1.99−2.05 m	2.00−2.04 m	2.04−2.08 m
11	1.45 dd (13.1, 2.8)	1.36 td (13.1, 2.5)	1.33−1.39 m	1.50−1.54 m
	1.75−1.78 m	1.63−1.67 m	1.65−1.70 m	
12	1.51 td (13.4, 2.8)	1.43 td (13.4, 2.5)	1.46 td (13.4, 2.2)	1.46−1.50 m
	1.64 dd (13.4, 1.5)	1.57−1.60 m	1.57 br s	1.63−1.67 m
14	6.65 dd (2.4, 1.5)	6.48 dd (2.4, 1.5)	6.47 br s	6.65 dd (2.4, 1.5)
15	5.82 dd (17.7, 10.7)	5.82 dd (17.7, 10.4)	5.83 dd (17.7, 10.3)	5.83 dd (17.0, 10.6)
16	4.97 dd (10.7, 0.9)	4.95−4.99 m	4.96−4.99 m	4.97 dd (10.6, 1.0)
	5.00 dd (17.7, 0.9)			5.00 dd (17.0, 1.0)
17	1.10 s	1.05 s	1.05 s	1.10 s
18	1.07 s	0.96 s	0.92 s	1.04 s
19	0.94 s	0.82 s	0.81 s	0.95 s
20	0.86 s	0.75 s	0.74 s	0.85 s
Glc				
1	4.47 d (7.3)	4.29 d, 7.4	4.29 d, 7.3	4.42 d (7.6)
2	3.56 dd (8.6, 7.3)	3.30−3.34 m	3.24−3.30 m	3.46 t (7.6)
3	3.36 tl (8.6)	3.34−3.38 m	3.30 overlapped	3.43 t (7.6)
4	3.22−3.24 m	3.09−3.11 m	3.03 t (9.1)	3.28−3.30 m
5	3.27 m	3.06−3.09 m	3.34−3.38 m	3.23 ddd (9.8, 5.5, 2.1)
6	3.67 dd (11.9, 5.5)	3.42 dd (11.0, 5.1)	3.56 dd (11.9, 7.1)	3.66 dd (11.9, 5.5)
	3.87 dd (11.9, 2.1)	3.65 br d (11.0)	3.94 br d (11.9)	3.84 dd (11.9, 2.1)
Glc′				
1	4.66 d (7.6)	4.43 d (7.9)	4.26 d (7.6)	
2	3.23 m	2.98 t (8.9)	2.96 t (8.4)	
3	3.37 tl (8.5)	3.14 t (8.9)	3.04 t (9.5)	
4	3.34 m	3.01−3.04 m	3.05 m	
5	3.48 ddd (9.9, 6.1, 2.1)	3.04−3.07 m	3.16 m	
6	3.79 dd (11.9, 6.1)	3.47 dd (11.3, 4.6)	3.43 dd (11.9, 4.9)	
	4.12 dd (11.9, 2.1)	3.62 dd (11.3, 1.9)	3.68 br d (11.9)	
Glc″				
1	4.42 d (7.6)			
2	3.22 m			
3	3.20 m			
4	3.26 m			
5	3.57 m			
6	3.62 dd (11.9, 5.5)			
	3.82 dd (11.9, 2.1)			
Rha				
1			5.24 br s	5.37 d (1.8)
2			3.69 overlapped	3.93−3.95 m
3			3.47 dd (9.8, 3.3)	3.73 dd (9.5, 3.3)
4			3.14−3.20 m	3.38 t (9.5)
5			3.81 dq (9.5, 6.1)	3.95−3.99 m
6			1.06 d (6.1)	1.21 d (6.1)

[a] Measured in CD_3OD. [b] Measured in DMSO-d_6.

was used as an internal standard. Preparative HPLC was performed on a Waters 600E multisolvent delivery system connected with a Waters 486 tunable absorbance detector using a Senshu Pak ODS-5251-S column (20 × 250 mm i.d.). PGE_1 and PGE_2 (Cayman Chemical, Ann Arbor, MI) and SC-51089 (BIOMOL Research Laboratories Inc., Plymouth Meeting, PA) were dissolved in DMSO (Nacalai Tesque, Inc., Kyoto, Japan) and were further diluted with Dulbecco's phosphate-buffered saline (−) (Nacalai Tesque, Inc., Kyoto, Japan). L-Rhamnose monohydrate (98%) purchased from Aldrich Chemical Co. (Milwaukee, WI) was used. All other chemicals used in the experiment were of analytical grade.

Plant Material. The whole plants of *L. aspera* were collected from Khulna, Bangladesh, in March 2000. After shade drying for 15 days, most of the leaves were separated. The remaining plant parts were cut into pieces and dried in an oven at 40 °C for 3 h before grinding. A voucher specimen (No. LNP 20010-01) has been deposited in the Laboratory of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan.

Extraction and Isolation. The extraction and isolation procedure of *L. aspera* (1.55 kg) was carried out as described previously.[2] The remaining major fraction, 1B (4.84 g), exhibited inhibition against both PGE_1- and E_2-induced contractions at 9×10^{-5} g/mL, but did not show clear DPPH-radical scavenging activity. Fraction 1B was separated by passage over a silica gel column eluted with *n*-hexane−acetone to obtain fractions 12A−12G, which were further separated independently. Fraction 12C (179 mg) was chromatographed by an ODS flash column with MeOH−H_2O (1:1) followed by Sephadex LH-20 with MeOH to afford **1** (19 mg) and **2** (2 mg). Fraction 12D (169 mg) was separated by silica gel flash chromatography (*n*-hexane−acetone, 4:1) and then by ODS flash column chromatography. Compound **8** (3 mg) was obtained from the MeOH−H_2O (2:1) eluate after further purification, and **9** (7 mg) from the MeOH−H_2O (3:1−4:1) eluate. Fraction 12E (212 mg) afforded **11** (3 mg) by ODS flash column chromatography with MeOH−H_2O (1:2−2:3) and then by ODS HPLC using MeOH−H_2O (2:1) and CH_3CN−H_2O (3:7). Fraction 12F (267 mg) was fractioned by ODS flash column chromatography eluted with MeOH−H_2O (1:1), and the obtained fraction (43 mg) was purified by Sephadex LH-20 column chromatography to afford a mixture of **3** and **4** (25 mg). Fraction 12G (1960 mg) was applied to an ODS flash column eluted with MeOH−H_2O (1:2−1:0). The MeOH−H_2O (2:1) eluates, fractions

13B and 13C, were further separated. Fraction 13C (120 mg) was chromatographed by silica gel flash column chromatography eluted with $CHCl_3$−MeOH (9:1)−acetone to give a fraction (26 mg), which was further purified by ODS HPLC (MeOH−H_2O, 7:3) and by passage over Sephadex LH-20 (MeOH) to give **10** (7 mg). Fractions 14B (50 mg) and 14C (92 mg), which were separated from fraction 13B by silica gel ($CHCl_3$−MeOH, 3:1 and 1:1, respectively), were further purified by ODS HPLC. Compound **6** (22 mg) was obtained from fraction 14B with MeOH−H_2O (7:3), and **5** (32 mg) and **7** (10 mg) were obtained from fraction 14C with MeOH−H_2O (3:2) after further purification.

Leucasperone A (1): colorless, amorphous solid; $[\alpha]^{23}_D$ +116 (*c* 1.2, MeOH); CD (*c* 0.0005 mol, MeOH, 22 °C) $\Delta\epsilon$ (nm) +2.1 (291, max.); IR (KBr) ν_{max} 3489, 2979, 1727, 1373, 1234, 1024 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 460 (1), 400 (19), 358 (6), 340 (22), 273 (14), 267 (32), 262 (33), 201 (100), 189 (47), 173 (53), 159 (91); HRFABMS (NBA/PEG) *m*/*z* 517.2180 (calcd for $C_{26}H_{38}O_8K$, 517.2204).

Leucasperone B (2): colorless, amorphous solid; CD (*c* 0.001 mol, MeOH, 22 °C) $\Delta\epsilon$ (nm) +2.1 (291, max); IR (neat) ν_{max} 3446, 2968, 1732, 1716, 1373, 1240, 1023 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS (NBA/PEG) *m*/*z* 475.2100 (calcd for $C_{24}H_{36}O_7K$, 475.2098).

Leucasperol A (3) and Leucasperol B (4) (5:2 mixture): white powder; ^1H NMR of **3** ($CDCl_3$, δ ppm, *J* in Hz) 4.81 (1H, s, H-16), 4.11 (1H, ddd, 9.8, 8.7, 2.7, H-15b), 3.79 (1H, td, 8.7, 7.3, H-15a), 3.11 (1H, dd, 11.6, 4.3, H-2), 2.63 (1H, s, H-10), 2.43 (1H, dt, 11.9, 9.8, H-14b), 2.41 (1H, td, 13.1, 0.9, H-8b), 2.14 (1H, dd, 9.4, 3.6, H-11b), 2.10−2.19 (1H, m, H-7), 2.06 (1H, dd, 13.1, 4.3, H-8a), 2.02 (1H, dd, 9.1, 3.6, H-12b), 1.98 (1H, dd, 9.1, 4.3, H-12a), 1.92 (1H, ddd, 11.9, 7.3, 2.7, H-14), 1.86 (1H, m, H-4b), 1.83 (1H, m, H-11a), 1.64−1.68 (1H, m, H-3b), 1.59 (1H, dd, 11.6, 3.7, H-3a), 1.44 (1H, dtl, 12.5, 3.7, H-4a), 1.16 (3H, s, H-17), 1.08 (3H, s, H-18), 0.95 (3H, d, 6.4, H-20), 0.88 (3H, s, H-19); ^{13}C NMR of **3** ($CDCl_3$, δ ppm) 210.3 (C-9), 99.0 (C-16), 92.7 (C-6), 91.3 (C-13), 78.2 (C-2), 64.8 (C-15), 58.5 (C-10), 48.7 (C-8), 47.3 (C-5), 38.6 (C-7), 37.4 (C-1), 36.6 (C-12), 35.5 (C-14), 29.9 (C-4), 29.0 (C-11), 27.7 (C-18), 26.5 (C-3), 20.0 (C-19), 16.9 (C-20), 14.9 (C-17); ^1H NMR of **4** ($CDCl_3$, δ ppm, *J* in Hz) 5.33 (1H, s, H-16), 4.04 (1H, td, 8.5, 3.9, H-15b), 3.97 (1H, td, 8.5, 7.0, H-15a), 3.07 (1H, dd, 11.3, 4.3, H-2), 2.64 (1H, s, H-10), 2.36 (1H, td, 13.1, 1.0, H-8b), 2.01 (1H, overlapped, H-8a), 1.15 (3H, s, H-17), 1.07 (3H, s, H-18), 0.96 (3H, d, 6.4, H-20), 0.87 (3H, s, H-19); ^{13}C NMR of **4** ($CDCl_3$, δ ppm) 210.6 (C-9), 101.6 (C-16), 93.0 (C-6), 92.5 (C-13), 78.4 (C-2), 66.3 (C-15), 58.1 (C-10), 48.9 (C-8), 47.5 (C-5), 37.8 (C-7), 37.4 (C-1), 37.2 (C-12), 31.1 (C-14), 28.7 (C-4), 31.2 (C-11), 27.6 (C-18), 26.5 (C-3), 20.2 (C-19), 17.6 (C-20), 15.0 (C-17); EIMS *m*/*z* 352 $[M]^+$ (20), 334 (85), 316 (9), 251 (6), 219 (10), 203 (8), 191 (13), 183 (100), 170 (28), 151 (21), 137 (21); HRFABMS (NBA/PEG) *m*/*z* 353.2338 (calcd for $C_{20}H_{33}O_5$, 353.2328). The sample (5.8 mg) was acetylated using Ac_2O (100 μL) and pyridine (200 μL) for 48 h at room temperature. After normal workup, the mixture (6.8 mg) was separated by silica gel column chromatography with *n*-hexane−acetone (7:1) to give **12** (2.2 mg) and **13** (2.0 mg).

Compound 12: white solid; CD (*c* 0.0013 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +1.0 (297, max); IR (KBr) ν_{max} 2958, 1735, 1706, 1375, 1240, 1097 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS (NBA/PEG) *m*/*z* 436.2435 (calcd for $C_{24}H_{36}O_7$, 436.2461).

Compound 13: white powder; CD (*c* 0.0012 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +1.1 (297, max.); IR (KBr) ν_{max} 2981, 1739, 1706, 1367, 1254, 1122, 1088 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HREIMS *m*/*z* 436.2451 (calcd for $C_{24}H_{36}O_7$, 436.2461).

Leucasperoside A (5): white powder; CD (*c* 0.0007 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +4.9 (204, max), −3.0 (254, min), −0.5 (345, min), 0 (372); ^1H and ^{13}C NMR, see Tables 3 and 4, respectively; HRFABMS (NBA/PEG) *m*/*z* 827.3505 (calcd for $C_{38}H_{60}O_{17}K$, 827.3468).

Leucasperoside B (6): white powder; CD (*c* 0.0009 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +4.1 (205, max.), −2.7 (252, min.), −0.4 (332, min.), 0 (372); ^1H and ^{13}C NMR, see Tables 3 and 4, respectively; HRFABMS (NBA/PEG) *m*/*z* 627.3361 (calcd for $C_{32}H_{51}O_{12}$, 627.3381).

Leucasperoside C (7): white powder; CD (*c* 0.0006 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +4.9 (205, max.), −3.0 (251, min.), −0.6 min (332, min.), 0 (369); ^1H and ^{13}C NMR, see Tables 3 and 4, respectively; HRFABMS (NBA/PEG) *m*/*z* 795.3800 (calcd for $C_{38}H_{60}O_{16}Na$, 795.3779).

Table 4. ^{13}C NMR Data for Compounds **5**−**7** and **10** (δ in ppm)

position	**5**[a]	**6**[b]	**7**[b]	**10**[a]
1	37.9	36.1	36.3	38.0
2	27.4	25.7	26.0	27.4
3	90.3	87.7	86.8	89.4
4	40.4	38.5	38.5	40.2
5	50.9	48.8	49.1	51.3
6	37.7	36.4	36.4	37.7
7	202.9	199.0	199.0	202.8
8	136.6	135.1	135.2	136.6
9	51.8	49.7	49.6	52.0
10	36.7	35.0	35.0	36.7
11	20.1	18.5	18.3	20.2
12	35.2	33.2	33.2	35.2
13	39.7	38.1	38.1	39.8
14	145.6	142.4	142.4	145.6
15	147.8	146.5	146.5	147.8
16	112.2	111.7	111.7	112.2
17	26.2	25.6	25.6	26.2
18	27.7	26.7	26.6	27.7
19	15.9	15.0	15.2	16.2
20	14.2	13.4	13.5	14.2
Glc				
1	105.2	103.5	103.4	105.6
2	81.3	81.3	76.3	79.5
3	78.25[c]	76.5	77.9	78.9
4	71.9[d]	69.9	70.3[d]	72.2[c]
5	77.8[c]	76.4	75.7	77.7
6	62.8	60.9	68.6	62.8
Glc′				
1	104.6	103.9	103.3	
2	76.3	75.2	73.6	
3	78.0[c]	76.1	76.77[c]	
4	71.7[d]	69.9	70.4[d]	
5	77.0	76.8	76.80[c]	
6	70.0	61.0	61.1	
Glc″				
1	104.9			
2	78.1[c]			
3	75.2			
4	71.5[b]			
5	78.3[c]			
6	63.1			
Rha				
1			99.8	101.9
2			70.1[d]	72.07[c]
3			70.7[d]	72.11[c]
4			72.0	74.0
5			68.0	70.0
6			17.8	18.0

[a] Measured in CD_3OD. [b] Measured in DMSO-d_6. [c,d] Interchangeable in the same column.

Asperphenamate (8): white solid; CD (*c* 0.002 mol, MeOH, 22 °C) $\Delta\epsilon$ (nm) −5.5 (229, min) {$[\theta]$ (nm) −18 000 (229, min.)}, {lit.,[5] CD (MeOH) $[\theta]$ (nm) −23 530 (227)}; FABMS (NBA) *m*/*z* 507 $[M + H]^+$.

Linifolioside (10): white powder; $[\alpha]^{20}_D$ −60 (*c* 0.14, MeOH), {lit.,[7] $[\alpha]_D$ −70.5 (MeOH)}; CD (*c* 0.0009 mol, MeOH, 23 °C) $\Delta\epsilon$ (nm) +4.2 (207, max.), −3.0 (252, min.), −0.4 (338, min.), 0 (375); ^1H and ^{13}C NMR, see Tables 3 and 4, respectively; FABMS (NBA) *m*/*z* 633 $[M + Na]^+$.

[(−)-Isololiolide (3*R*,5*R*)] (11): white powder; $[\alpha]^{25}_D$ −40.3 (*c* 0.14, $CHCl_3$), {lit.[15] for (+)-isololiolide, $[\alpha]^{15}_D$ +80.6 ($CHCl_3$)}; CD (*c* 0.0009 mol, MeOH, 22 °C) $\Delta\epsilon$ (nm) −4.6 (214, min), {lit.[16] CD for isololiolide, (MeOH) $\Delta\epsilon$ (nm) +10.39 (212)}; the assignment of C-6 and C-8 should be reversed in the ^{13}C NMR data in ref 14.

Acid Hydrolysis of Compound 7. A solution of **7** (2.5 mg) in 1,4-dioxane (0.5 mL) and 5% aqueous H_2SO_4 (0.5 mL) was stirred at 100 °C for 2 h. After cooling to room temperature, H_2O was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated to afford an aglycone, which was identified as linifoliol[7] by ^1H NMR and CD spectroscopy. The aqueous layer was neutralized by passage through Amberlite IRA96SB eluted with H_2O,

which was evaporated in vacuo to give a sugar fraction. The identification of sugars was performed by HPLC analysis with a chiral detector. The sugar fraction gave the corresponding peaks of D-glucose (t_R, 33 min) and L-rhamnose (t_R 18 min). HPLC conditions: column, Shodex RSpak DC-613 (6.0 × 150 mm i.d.); solvent, CH_3CN-H_2O, 3:1 (v/v); flow rate, 0.5 mL/min; temperature, 40 °C; RI detection, Shodex RI-72; and chiral detection, JASCO OR-1590.

Prostaglandin Inhibition Assay. Prostaglandin inhibition was evaluated by the Magnus assay modified as previously reported[2] using Hartley male guinea pig ileum (350−550 g, 4−6 weeks, Japan SLC). The animals were conditioned at least one week in a 12 h light/dark cycle room with controlled temperature and humidity in accordance with the experimental animal welfare guidelines of Chiba University. After the animals were sacrificed, the ileum was maintained at room temperature in Kreb's solution (118 mmol of NaCl, 4.7 mmol of KCl, 2.5 mmol of $CaCl_2$, 1.2 mmol of KH_2PO_4, 25 mmol of $NaHCO_3$, 1.2 mmol of $MgSO_4$, 10.0 mmol of glucose) bubbled with a gas mixture of 95% O_2 and 5% CO_2. A resting tension of 0.5 g was applied to each ca. 1 cm ileum preparation, which was then equilibrated in 5 mL of organ bath solution bubbled with the gas mixture at 28 °C. PGE_1 or E_2 was added at concentrations of 3×10^{-7} mol/1×10^{-6} mol and 1×10^{-7} mol/3×10^{-7} mol, respectively. When the muscle contraction became stable, each sample dissolved or suspended in 5% DMSO−H_2O solution was used in the experiments. A force-displacement transducer (TB-611T, Nihon Kohden, Japan) coupled to an amplifier (AP-601G, Nihon Kohden, Japan) was used for the measurement of isometric contractions, which were recorded on a chart recorder (TI-102, Tokai Irika, Japan). Activity was evaluated on the basis of the inhibitory effect after two applications of each sample. SC-51089, a PGE_2 (EP_1 receptor) antagonist, was used as a positive control, at a concentration of 3 μmol.

Antioxidant Assay. An antioxidant activity, based on the DPPH-radical scavenging effect, was carried out as described in a previous paper.[2]

Acknowledgment. We thank Dr. T. Hoshino, Dr. M. Hata, and Mr. H. Fuji, for access to the DFT computational facilities. The computations were carried out by the DRIA system at the Graduate School of Pharmaceutical Sciences, Chiba University. We also thank Dr. M. Kitajima and Dr. K. Katakawa for the use of HPLC with a chiral detector, and the Chemical Analysis Center of Chiba University for the measurement of HRMS.

Supporting Information Available: Chart showing the isolation scheme of compounds **1**−**11** and table of the HMBC correlations of leucasperone A (**1**) and compound **12**. These are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Ghani, A. *Medicinal Plants of Bangladesh, Chemical Constituents and Uses*; Asiatic Society of Bangladesh: Dhaka, 1998; pp 215−216.
(2) Sadhu, S. K.; Okuyama, E.; Fujimoto, H.; Ishibashi, M. *Chem. Pharm. Bull.* **2003**, *51*, 595−598.
(3) Jakupovic, J.; Chen, Z.-L.; Bohlman, F. *Phytochemistry* **1987**, *26*, 2777−2779.
(4) McCorkindale, N. J.; Baxter, R. L.; Roy, T. P.; Shields, H. S.; Stewart, R. M.; Hutchinson, S. A.; *Tetrahedron* **1978**, *34*, 2791−2795.
(5) Clark, A. M.; Hufford, C. D. *Lloydia* **1977**, *40*, 146−151.
(6) Yamagashi, T.; Zhang, D. C.; Chang, J. J.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. *Phytochemistry* **1988**, *27*, 3213−3216.
(7) Mahato, S. B.; Pal, B. C. *Phytochemistry* **1986**, *25*, 909−912.
(8) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st ed.; Aldrich: Milwauke, WI; Vol. I, 1993; p 782.
(9) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st ed.; Aldrich: Milwauke, WI, Vol. I, 1993; p 785.
(10) Faizi, S.; Ali, M.; Saleem, R.; Irfanullah; Bibi, S. *Magn. Reson. Chem.* **2001**, *39*, 399−405.
(11) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st ed.; Aldrich: Milwauke, WI, Vol. III, 1993; p 569.
(12) Isoe, S.; Katsumura, S.; Heyon, S. B.; Sakan, T. *Tetrahedron Lett.* **1971**, *16*, 1089−1092.
(13) Behr, D.; Wahlberg, I.; Nishida, T.; Enzell, C. R. *Acta Chem. Scand.* **1979**, *B 33*, 701−704.
(14) Mori, K.; Khlebnikov, V. *Liebigs Ann. Chem.* **1993**, *1*, 77−82.
(15) Isoe, S.; Heyon, S. B.; Katsumura, S.; Sakan, T. *Tetrahedron Lett.* **1972**, *25*, 2517−2520.
(16) Machida, K.; Kikuchi, M. *Phytochemistry* **1996**, *41*, 1333−1336.
(17) Frisch, M. J.; Trucks, G. W.; Robb, H. M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratman, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudi, K. N.; Strain, C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Pis, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.
(18) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(19) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(20) Hehre, W. J.; Random, L.; Schleyer, P. V. R.; Pople, J. A. *Ab initio Molecular Orbital Theory*; Wiley: New York, 1986.
(21) Kono, Y.; Takeuchi, S.; Kodama, O.; Akatsuka, T. *Agric. Biol. Chem.* **1984**, *48*, 253−255.
(22) Ortega, C. A.; Rotelli, A. E.; Gianello, J. C. *Planta Med.* **1998**, *64*, 778.
(23) Awale, S.; Tezuka, Y.; Banskota, A. H.; Kadota, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 31−35.
(24) Awale, S.; Tezuka, Y.; Banskota, A. H.; Adnyana, I. K. Kadota, S. *J. Nat. Prod.* **2003**, *66*, 255−258.

NP058118M