Novel Diterpene Lactones from *Suregada multiflora*

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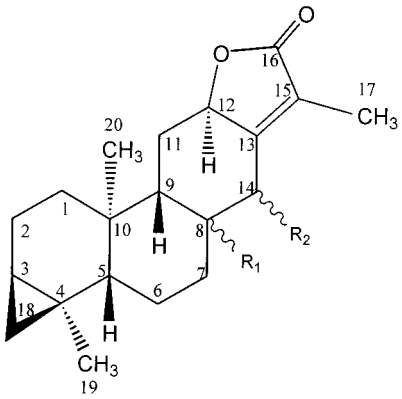
Two new diterpene lactones, suregadolides A (**1**) and B (**2**), were isolated from a dichloromethane extract of *Suregada multiflora* bark. These compounds possess a novel skeleton, which contains a cyclopropane ring bridging C-3 and C-4 of the abietane skeleton. The structures were established on the basis of one- and two-dimensional NMR and other spectroscopic studies. Compound **1** showed moderate inhibitory activity in a mutant yeast strain bioassay.

Suregada multiflora A. Juss. (syn. *Gelonium multiforum*) (Euphorbiaceae) is reported to be used medicinally for hepatic disorders and for the treatment of gum disease.[1] Several diterpene lactones,[2,3] kaurane-type diterpenes,[4] and flavonoids[4−6] have been reported from the leaves, roots, and seeds of this plant. A number of triterpenoids[7,8] have also been isolated from the bark of the same plant. GAP31, a protein which inhibits HIV-1[9,10] and is effective in a human breast cancer model,[11] has also been reported from *S. multiflora*.

In the current study, a dichloromethane−methanol (1:1) extract of *S. multiflora* was found to possess significant cytotoxicity for non-small cell lung (NCI-H322M), colon (SW-620), CNS (U251), and breast (MDA-MB-435) human tumor cell lines in the National Cancer Institute 60-cell line tumor panel.[12] The extract also showed inhibitory activity toward a DNA-repair-deficient yeast mutant in a mechanism-based yeast bioassay.[13] The mutant *Saccharomyces cereviseae* yeast assay utilized in the present study is based on the differential response on DNA-repair-deficient and repair-proficient yeast strains toward the test sample. A genetically engineered yeast strain that lacks the specific recombination pathway that is associated with the repair of double strand breaks and meiotic recombination, known as RAD 52, was used for activity-guided isolation of **1** and **2** from the CH_2Cl_2−MeOH extract of *S. multiflora*.

Compound **1** was isolated as a white amorphous solid. The molecular formula of **1** was deduced as $C_{20}H_{28}O_4$ from an exact mass measurement (*m/z* 332.4334). On the basis of mass, 1D NMR, and IR spectral evidences, compound **1** could be concluded to be a diterpene with an α,*β*-unsaturated *γ*-lactone chromophore (1760 cm^{-1}), a vinylic methyl (δ_H 1.78, δ_C 7.8), one secondary hydroxyl-bearing (δ_H 4.43, δ_C 72.2) carbon, and one tertiary hydroxyl-bearing (δ_C 74.2) carbon. Besides these functionalities, the presence of a cyclopropyl ring (δ_H −0.01 and 0.40; δ_C 21.8) and two additional methyl groups (δ_H 0.93, δ_C 23.5; δ_H 1.11, δ_C 14.6) was also inferred. The complete structure was then deduced with the help of 2D NMR studies and from comparative studies with reported diterpene lactones[3,14] and the metasequic acids, as described below.[15]

From the $^1H-^1H$ (COSY-45°) and HMQC spectra of **1** (Table 1), it was inferred that H-12 (δ_H 5.15; δ_C 77.2) of the α,*β*-unsaturated lactone chromophore was coupled with two nonequivalent geminal methylene protons (H-11a, δ_H 1.50 m; H-11b, δ_H 2.42 dd, $J_{11a,11,b}$ = 13.3 Hz, $J_{11b,12}$ = 6.4 Hz; δ_C 29.1) along with the vinylic C-17 methyl protons (δ_H 1.78, d, $J_{17,12}$ = 1.8 Hz; δ_C 7.80) (homoallylic coupling). Coupling of the H-11a methylene proton with the C-9 methine proton (δ_H 1.39, d, $J_{9,11a}$ = 7.6 Hz; δ_C 52.7) indicated the presence of a CH−CH_2−CH−O− moiety in the molecule. Since H-9 could not be correlated with any other proton except the C-11a, a methylene proton, it was concluded that this must have two adjacent quaternary carbons.



1 $R_1 = R_2 =$ β-OH
2 $R_1 = R_2 =$ α-O

The ^1H NMR spectrum of **1** clearly indicated the presence of a trisubstituted cyclopropane ring in the molecule. Two upfield signals of the C-18 cyclopropane methylene protons [H-18 (exo), δ_H −0.01, dd, $J_{18exo,3}$ = 5.7 Hz, $J_{18exo,18endo}$ = 4.2 Hz and H-18 (endo), δ_H 0.40 (dd, $J_{18endo,3}$ = 9.2 Hz, $J_{18\ exo,18endo}$ = 4.0 Hz); δ_C 21.8], the H-3 signal at δ_H 0.54 (ddd, $J_{3,2}$ = 9.0 Hz, $J_{3,18endo}$ = 8.5, $J_{3,18exo}$ = 6.0 Hz; δ_C 19.1), and one quaternary carbon signal (C-4, δ_C 16.1) confirmed unambiguously the presence of a trisubstituted cyclopropane ring. These values are also consistent with the values reported for the metasequic acids,[15] which contain a trisubstituted cyclopropyl substituent in ring A of a labdane skeleton. COSY and HMBC interactions further confirmed the position of the cyclopropyl group in **1**.

The HMBC spectrum of **1** (Figure 1) showed a correlation of the C-20 methyl protons (δ_H 1.11, δ_C 14.6) with C-9 (δ_C 52.7), whereas the C-19 and the C-20 methyl protons (δ_H

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Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$ + CD_3OD) Data of Suregadolides A (**1**) and B (**2**)

	1		**2**	
position	δ_H	δ_C	δ_H	$\delta_{^{13}C}$[a]
1a	0.55 (m)	35.8 (t)	0.66 (m)	33.2 (t)
1b	1.55 (m)		1.40 (m)	
2a	1.64 (m)	23.8 (t)	1.64 (m)	23.8 (t)
2b	1.84 (m)		1.79 (m)	
3	0.54 (ddd, 9.0, 8.5, 6.0)	19.1 (d)	0.50 (ddd, 9.2, 9.3, 5.9)	16.4 (d)
4		16.1 (s)		14.2 (s)
5	1.05 (dd,12.8, 3.2)	51.2 (d)	1.09 (dd, 13.6, 3.0)	50.9 (d)
6a	1.60 (m)	19.5 (t)	1.19 (m)	20.8 (t)
6b	1.82 (dd, 12.0, 5.5)		1.60 (dd, 14.3, 4.5)	
7a	1.52 (m)	41.2 (t)	1.17 (m)	34.4 (t)
7b	2.25 (m)		1.19 (m)	
8		74.2 (s)		61.5 (s)
9	1.39 (d, 7.6)	52.7 (d)	1.67 (d, 6.8)	45.3 (d)
10		37.4 (s)		35.5 (s)
11a	1.50 (m)	29.1 (t)	1.20 (m)	29.3 (t)
11b	2.42 (dd, 13.3, 6.4)		2.1 (dd, 13.1, 5.7)	
12	5.15 (ddd, 12.0, 6.4, 1.6)	77.2 (d)	4.18 (ddd, 10.3, 5.5, 2.3)	77.7 (d)
13		162.7 (s)		157.5 (s)
14	4.43 (s)	72.2 (d)	3.64 (s)	55.5 (d)
15		122.5 (s)		128.0 (s)
16		175.7 (s)		175.0 (s)
17	1.78 (d, 1.8)	7.8 (q)	1.75 (d, 2.0)	8.0 (q)
18a	−0.01 (dd, 5.7, 4.2), exo	21.8 (t)	−0.09 (dd, 5.5. 4.6), exo	18.4 (t)
18b	0.40 (dd, 9.2, 4.0), endo		0.30 (dd, 9.3, 4.2), endo	
19	0.93 (s)	23.5 (q)	1.1 (s)	23.8 (q)
20	1.11 (s)	14.6 (q)	0.8 (s)	16.4 (q)

[a] Determined from the DEPT NMR spectrum.

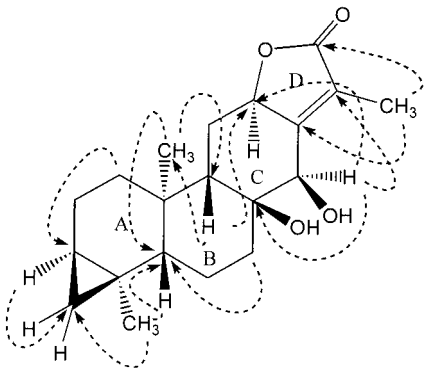

Figure 1. Important HMBC interactions of compound **1**.

0.93 and 1.11) and H-7b (δ_H 2.25) were correlated with the C-5 (δ_C 51.2). The C-19 methyl protons were also found to be correlated with C-4 (δ_C 16.1) and C-18 (δ_C 21.8), while H-3 (δ_H 0.54) exhibited a correlation with C-18.

In ^{1}H and ^{13}C NMR spectra ($CDCl_3$ + CD_3OD) of **1**, the downfield signals of H-14 (δ_H 4.43 and δ_C 72.2), and the quaternary C-8 (δ_C 74.2) indicated the presence of one secondary hydroxyl group and one tertiary hydroxyl group in the molecule. The ^{1}H NMR spectrum of **1**, when recorded in C_5D_5N, showed signals for the two OH protons (δ_H 7.65 and 5.79), which disappeared when the ^{1}HNMR spectrum was recorded in C_5D_5N with a few drops of D_2O. The H-14 signal was found to be correlated with C-8 (δ_C 74.2), C-13 (δ_C 162.7), C-15 (δ_C 122.5), and C-12 (δ_C 77.2). On the other hand H-9 (δ_H 1.39) was correlated with C-8 and C-14.

The stereochemistry of the A/B ring junction of **1** was inferred as being *trans* on biogenetic grounds.[3] The ster-

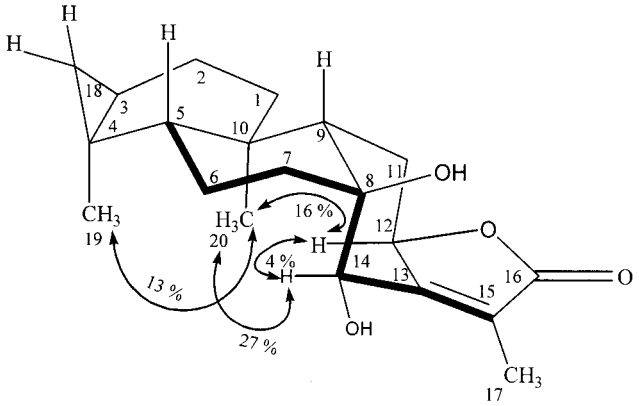

Figure 2. Importane NOE interactions of compound **1**.

eochemistry of H-12 was determined as α (axial) and H-9 as β (pseudoaxial) from the coupling constants and multiplicities of the H-9, H-11, and H-12 signals. The H-12 (δ_H 5.15, ddd, $J_{12,11a}$ = 12.0 Hz, $J_{12,11b}$ = 6.4 Hz, $J_{12,17}$ = 1.6 Hz) resonance appeared as a doublet of doublet of doublets, while H-11b (δ_H 2.42, $J_{11a,11b}$ = 13.3 Hz, $J_{12,11b}$ = 6.4 Hz) was a doublet of doublets and H-9 (δ_H, 1.39, $J_{9,11a}$ = 7.6 Hz) appeared as a doublet. These values were consistent with the values reported for gelomulide F, which contained H-12α (axial) and H-9β functionalities.[3]

The NOE effect on H_3-20 and H-12 by irradiation of H-14 indicated their spatial proximity in **1**. Since the H-12 methine has been found to be α-oriented in all other abietane diterpene lactones reported from the same plant, it was therefore presumed that H-14 is α-oriented and thus OH-14 β-oriented. The NOE effect on H_3-20 by irradiation of the H-12 signal and also the effect on H_3-19 by irradiation of H_3-20 supported the α-orientation of the C-19 methyl and C-20 methyl groups and thus the β-orientation of the cyclopropane ring in compound **1**. From the Drieding model of compound **1**, it was observed that these interactions were possible only when the C-8 hydroxyl group is β-oriented (Figure 2). The structure of compound **1** was therefore elucidated as 3,4,18-cyclopropa-8β,14β-dihydroxy-13,15-abieten-16,12-olide and was named suregadolide A.

Compound **2**, $C_{20}H_{26}O_3$ (*m*/*z* 314.4180), was isolated as a white amorphous solid. The complete structure of **2** was deduced by comparing its spectral data with those of **1** and gelomulide A.[3] A comparative study of the NMR chemical shifts of C-8, C-14, and H-14 with gelomulide A indicated the presence of a trisubstituted oxirane group at C-8 and C-14 of **2**. The stereochemistry of the A/B ring junction was inferred as being *trans* on biogenetic grounds.[3] The same stereochemistry was deduced for H-12 (α) and H-9 (β) as for compound **1**. The strong NOE interactions between H-12 and H_3-20 confirmed the orientation of H_3-20 as α. No NOE interactions were observed between H-12 and H-14 or between H_3-20 and H-14. On the other hand, H-14 showed a NOE interaction with H-9, which indicated its β-orientation. However, H-5 showed no NOE interaction with H_3-20 but exhibited a strong NOE interaction with H-18, which confirmed the β-orientation of the cyclopropane ring. The structure of compound **2** was therefore elucidated as 3,4,18-cyclopropa-8,14α-epoxy-13,15-abieten-16,12-olide and was named suregadolide B.

The crude extracts, fractions, and pure compounds were screened using this mechanism-based yeast bioassay.[16] Compound **1** exhibited IC_{12} values of 35 and 70 μg/mL in the RAD 52 and RAD^+ mutant yeast assays, respectively. A positive control, streptonigrin, exhibited comparative IC_{12}

values of 0.4 and >1 μg/mL, respectively. Compound **2** was not tested in these bioassays.

Experimental Section

General Experimental Procedures. The melting points were recorded on a Buchi 535 melting point apparatus and are uncorrected. Optical rotations were measured on a Polartronic D polarimeter. The UV spectra were measured on a Hitachi UV-3200 spectrophotometer. The IR spectra were recorded on a JASCO A-302 spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker AM-400 and AMX 500 NMR spectrometers using a UNIX data system at 400 and 500 MHz, respectively, while the ^{13}C NMR spectra were recorded on the same instruments at 100 and 125 MHz, respectively. The chemical shift (δ) values are reported in ppm, and coupling constants (J) are in Hz. The LREIMS and HREIMS were recorded on a JEOL HX 110 mass spectrometer with a DA 500 data system. Precoated TLC plates were used to check the purity of compounds. Flash silica gel G_{254}, 240−300 mesh (E. Merck), was used for column chromatography.

Plant Material. The plant material (1.65 kg) was collected near Cox's Bazaar, Chunati Game Reserve, Harbang Beat, Bangladesh, by Mr. Fazle Rabbi and Mr. Ali Hussain in April 1999. The plant was identified by Prof. M. Salar Khan of the Bangladesh National Herbarium (BNH), Dhaka, Bangladesh. A herbarium specimen of this plant was deposited at BNH (voucher No. DACB, accession No. 28004).

Extraction and Isolation. Air-dried bark (1.5 kg) of *Suregada multiflora* was extracted by 10 L of dichloromethane and methanol (1:1) to obtain a crude extract (115.8 g). This crude extract was tested at the National Cancer Institute, Bethesda, MD, and was found to possess cytotoxicity against several cancer cell lines (see text). The crude extract was partitioned with dichloromethane and water (1:1, v/v), and a CH_2Cl_2 extract (38.2 g) and aqueous soluble parts (73.5 g) were thus obtained. The aqueous extract was again partitioned with BuOH (1:1, v/v), and a BuOH extract (25.1 g) was obtained. The crude extracts were tested in a mechanism-based yeast assay.[13] The dichloromethane-soluble part showed inhibitory activity in the yeast assay, while the other extracts were not active. Further fractionation of the CH_2Cl_2 extract was then carried out by VLC (vacuum-liquid chromatography) on silica gel using gradients of hexane−CH_2Cl_2−MeOH, which yielded 12 fractions. Out of these fractions, three fractions, SM-3, SM-8, and SM-10, were found to be active, with IC_{12} values in the range 100−200 μg/mL.

Fraction SM-8 (6.5 g) was then fractionated into eight further fractions by silica gel column chromatography using hexane, EtOAc, and MeOH as eluents. The active fraction, SM-8-5 (300 mg, IC_{12} 60 μg/mL), was subjected to further separation by repeated column chromatography to afford several subfractions. Subfraction SM-8-5-3-3 was subjected to column chromatography by elution with acetone and hexane (1:4) to afford compound **1** (6 mg). Subfraction SM-8-5-3-2 was eluted with acetone and hexane (0.9:4.1) to yield compound **2** (10 mg). The percentage w/w yields were 4×10^{-4} (**1**) and 6.7×10^{-4} (**2**). The R_f values of **1** and **2** (hexane−acetone, 4:1) were 0.11 and 0.30, respectively.

Suregadolide A (**1**): white amorphous powder (6 mg), mp 233−234 °C; $[\alpha]_D^{25}$ −41.7° (*c* 0.08, $CHCl_3$); UV λ_{max} MeOH (log ϵ) 218 nm (2.23); IR ν_{max} ($CDCl_3$) 3340, 3300 (OH), 2940 (C−H), 1760 (α, β-unsaturated γ-lactone C=O), 1450, 1410, 1110, 1060, and 1010 cm^{-1}, ^{1}H and ^{13}C NMR ($CDCl_3$ + CD_3OD), Table 1; EIMS *m*/*z* 332 $[M]^+$ (30), 314 (12), 192 (63), 177 (89), 135 (68), and 55 (100); HREIMS *m*/*z* 332.4334 (calcd for $C_{20}H_{28}O_4$, 332.4339).

Suregadolide B (**2**): white amorphous powder (10 mg); $[\alpha]_D^{25}$ −2.40° (*c* 0.04, MeOH); UV λ_{max} MeOH (log ϵ) 224 nm (1.82); IR ν_{max} ($CDCl_3$) 2860 (C−H), 1740 (α,β-unsaturated γ-lactone C=O), 1440, 1380, 1025, 880 cm^{-1}, ^{1}H and ^{13}C NMR ($CDCl_3$ + CD_3OD), Table 1; EIMS *m*/*z* 314 $[M]^+$ (24), 272 (17), 203 (12), 161 (38), 93 (80), and 55 (100); HREIMS *m*/*z*, 314.4180 (calcd for $C_{20}H_{26}O_3$, 314.4186).

Bioassay. The assay was performed according to the method reported by Gunatilaka et al.[13] Strains of genetically engineered yeast (*Saccharomyces cervisiae*) were provided by Mr. Leo Faucette of SmithKline Beecham Pharmaceuticals. The strains RAD 52 (RS322YK) and wild-type RAD^+ were plated on YPD agar (7 mm layer) in 170 mm × 170 mm plates. Wells (6 mm diameter) were made in the plates (9 wells in 170 mm × 170 mm plates) with a sterile cork borer, test samples were dissolved in DMSO−MeOH (1:1), and a 100 μL sample concentration was placed in each well. Streptonigrin was used as a positive control for RAD 52. Plates were read in terms of zone of inhibition after incubation for 48 h at 30 °C. IC_{12} values were determined as the dose that gives an inhibition zone of 12 mm using a 6 mm diameter well.

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