SUPPLEMENTARY MATERIAL

Self Gelating Isoracemosol A, New Racemosaceramide A, and Racemosol E from *Barringtonia racemosa*

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Abstract:

Phytochemical investigation of the CHCl₃ extract of the fruits of *Barringtonia racemosa* resulted in the isolation of two novel metabolites along with isoracemosol A and betulinic acid as known metabolites. The new compounds were characterized as phytosphingosine-type ceramide [(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-hexacos-8(*E*)-ene-1,3,4-triol, **1**] and racemosol E [21 -acetoxy-22 -(2-methylbutyroxyl)-olean-12-ene-3,16,28-triol, **2**] on the basis of extensive spectroscopic data analysis and chemical modifications. In addition, the self gelating property of isoracemosol A (**3**) was investigated for the first time, which leads to the unexpected agglomerated porous like morphology.

Keywords: *Barringtonia racemosa*, racemosaceramide A, racemosol E, isoracemosol A, morphology and gelating property.

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1. General Experimental Procedures

Optical rotations were measured on a Horiba SEPA-300 high sensitive Polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer with K Br pellets. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded with Bruker Avance II spectrometer in $\text{C}_5\text{D}_5\text{N}$ with TMS as an internal standard. Coupling constants (J) were given in Hz. The ESIMS data were recorded on an Agilent 1100 MSD with an ESI SL trap. The HR-ESIMS data were acquired on an Agilent 6510 Q-TOF and ESI probe. Column chromatography was performed with silica gel (100-200, 230-400 mesh, Sigma). Silica gel F254 (0.25 mm, Merck) was used for analytical TLC. Field Emission Scanning electron microscopy (FESEM) experiments were carried out using Zeiss Ultra Plus scanning electron microscope.

2.Plant material

Fruits of *B. racemosa* were purchased from a local market in Trivandrum in March 2011 from Kerala, India, and authenticated by Prof. B. Kondala Rao, Department of Marine Living Sources, Andhra University, Visakhapatnam. A voucher specimen (#IIC-MG-116) has been deposited at the Herbarium of Natural Product Chemistry, IICT, Hyderabad, India.

The fruits of (2.0 kg) of *B. racemosa* were ground to a fine powder and extracted with CHCl_3 (10 l) in a Soxhlet apparatus for 18 h. After filtration, the filtrate was concentrated under reduced pressure to yield a pale yellow residue (26.7g). A part of this residue (25 g) was subjected to VLC over silica gel (230-400 mesh, Merck)

using solvents of increasing polarity from *n*-hexane through acetone resulted in 12 main fractions of 800 mL each. Fraction-6 was subjected to Si gel CC afforded racemosaceramide A (**1**, 10 mg). Fraction-8 was submitted to silica gel CC eluted with *n*-hexane-acetone (8: 2) resulted in the isolation of racemosol E (**2**, 9 mg), isoracemosol A (3, 300 mg). Fraction 12 was repeatedly purified by silica gel CC using *n*-hexane-acetone (6: 4) to obtain pure betulinic acid, 4 (20 mg).

3.Acetylation of compound 2

Compound **2** (1 mg) was dissolved in a mixture of C₅H₅N (0.2 mL) and Ac₂O (0.2 mL) and the solution was left overnight at ambient temperature. After usual work-up, the product was purified by recrystallization from *n*-hexane to afford **2a** (1 mg).

4.Alkaline hydrolysis of 2

Mixture (2 mg) of compounds **2** was refluxed for 3 h in a solution of 3% KOH (2 mL). The reaction mixture was extracted with EtOAc (5 mL), and the organic phase was washed with H₂O and evaporated to yield barringtogenol C (2 mg).

5.Methanolysis and GC-MS analysis of 1

Compound **1** (1 mg) was dissolved in 5% HCl/MeOH (1 mL) and the solution was refluxed at 80 °C for 18 h. The reaction mixture was then extracted with *n*-hexane and concentrated in vacuo. The residue was subjected to Si gel column chromatography in *n*-hexane/EtOAc (7:3) to yield a FAME. The FAME from **1** was subjected to GC-MS [column: ZB5MS 30MX 0.32 m, i.d. 0.25 mm, column temp 150 – 300 °C (rate of temp increase = 10 °C/min), injection volume 1 μ L, split less]. FAME from **1** (methyl-2-hydroxyhexadecanoate): t_R [min] = 13.0, m/z 286 [M^+], 227 [$M - COOCH_3$] $^+$.

6.DMDS Derivative of **1**

Compound **1** (0.5 mg) was dissolved in CS₂ (0.2 mL) with the addition of dimethyl disulfide (DMDS) (0.2 mL) and 20 μ L of iodine solution (60 mg of I₂ in 1 mL of diethyl ether). After stirring for 48 h at 60 °C in a 10 mL tube sealed with a Teflon-lined cap, the reaction mixture was diluted with 0.2 mL of Na₂S₂O₃ solution (5% in distilled water). The organic phase was refluxed and the aqueous phase was extracted twice with 0.2 mL of *n*-hexane. The organic extracts were concentrated, and the DMDS derivative was analysed by EIMS, which showed

molecular	ion	peak	at	m/z	775.
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HR-ESIMS Spectrum of racemosaceramide (1)

m/z	Ion	Formula	Abundance
704.6174	(M+Na)+	C ₄₂ H ₈₃ NNaO ₅	4493.9

Best	Formula (M)	Ion Formula	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm)	DBE
☒	C ₄₂ H ₈₃ NO ₅	C ₄₂ H ₈₃ NNaO ₅	100		681.62818	681.62712	-1.55	1.55	2

Isotope	Abund%	Calc Abund%	m/z	Calc m/z	Difference (ppm)
1	100	100	704.6174	704.6163	-1.5
2	38.19	46.94	705.6211	705.6197	-1.94
3	19.23	11.8	706.6267	706.6229	-5.4
4	6.49	2.09	707.629	707.6259	-4.41

FAB MS Spectrum of racemosaceramide (1)

¹H NMR Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

^{13}C NMR Spectrum of racemosaceramide (1) (600MHz, $\text{C}_5\text{D}_5\text{N}$)

DEPT Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

HSQC Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

HMBC Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

HMBC Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

HMBC Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

COSY Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

COSY Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

COSY Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

NOESY Spectrum of racemosaceramide (1) (600MHz, C₅D₅N)

^{13}C NMR Spectrum of fatty acid methyl ester of racemosaceramide (1) (600MHz, CDCl_3)

HR-ESIMS of racemosol E (2)

m/z	Ion	Formula	Abundance
639.4259	(M+Na)+	C37 H60 Na O7	43879.5

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Match	Mass Match	m/z	DGE
✓	C37 H60 O7	C37 H60 Na O7	639.4231	81.87		616.4367	616.4339	-4.55	4.56	93.4	76.44	77.67	639.4259	8

Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)	Abund Sum%
1	100	100	65.69	639.4259	639.4231	-4.41	67.23
2	37.1	40.97	26.91	640.4291	640.4265	-4.04	24.95
3	9.67	9.62	6.32	641.4267	641.4296	4.47	6.5
4	1.97	1.65	1.08	642.4491	642.4324	-25.91	1.33

^1H NMR Spectrum of racemosol E (600MHz, $\text{C}_5\text{D}_5\text{N}$)

^1H NMR Spectrum of racemosol E (600MHz, $\text{C}_5\text{D}_5\text{N}$)

^1H NMR Spectrum of racemosol E (600MHz, $\text{C}_5\text{D}_5\text{N}$)

^{13}C NMR Spectrum of racemosol E (500 MHz, $\text{C}_5\text{D}_5\text{N}$)

^{13}C NMR Spectrum of racemosol E (600 MHz, $\text{C}_5\text{D}_5\text{N}$)

^{13}C NMR Spectrum of racemosol E (600 MHz, $\text{C}_5\text{D}_5\text{N}$)

DEPT Spectrum of racemosol E (600MHz, C₅D₅N)

DEPT Spectrum of racemosol E (600MHz, C₅D₅N)

HSQC Spectrum of racemosol E (600MHz, C₅D₅N)

HSQC Spectrum of racemosol E (600MHz, C₅D₅N)

HSQC Spectrum of racemosol E (600MHz, C₅D₅N)

HMBC Spectrum of racemosol E (600MHz, C₅D₅N)

HMBC Spectrum of racemosol E (600MHz, C₅D₅N)

COSY Spectrum of racemosol E (600MHz, C₅D₅N)

NOESY Spectrum of racemosol E (600MHz, C₅D₅N)

NOESY Spectrum of racemosol E (600MHz, C₅D₅N)

Gelation test results in pure solvents before and after heating

S=Soluble I=Insoluble FN=fine needles PS=Partially Soluble PG=Partial Gel

Entry	Solvent	State(initial)	State(final)
1	<i>n</i> -Hexane	I	FN
2	Acetone	S	S
3	Ethyl Acetate	S	S
4	Methanol	S	S
5	Benzyl alcohol	S	S
6	Ethylene glycol	I	PS
7	Chloroform	S	S
8	Carbon tetra chloride	I	PG
9	DMSO	S	S
10	DMF	S	S
11	Water	I	I
12	Benzene	I	PS
13	Toluene	I	I
14	Glycerol	I	I
15	Ethyl alcohol	S	S
16	<i>n</i> -Butyl alcohol	PS	S

Images of prepared Gel samples in different proportions

Scanning Electron Microscopy Images of (3) before gel

Scanning Electron Microscopy Images of (3) Gel

Plot of T_{gel} vs. V/V % of Acetone and Hexane

Gelation test results of Isoracemosol A with different proportions of *n*-hexane and acetone combination at constant concentration.

Entry	Solvent (1 ml)	Concentration (W/V %)	State	T _{Gel} (in °C)
1	98:02 <i>n</i> -Hexane/Acetone	0.2	PG	-
2	96:04 <i>n</i> -Hexane/Acetone	0.2	PG	-
3	94:06 <i>n</i> -Hexane/Acetone	0.2	PG	-
4	92:08 <i>n</i> -Hexane/Acetone	0.2	PG	-
5	90:10 <i>n</i> -Hexane/Acetone	0.2	G	48
6	88:12 <i>n</i> -Hexane/Acetone	0.2	G	51
7	86:14 <i>n</i> -Hexane/Acetone	0.2	G	49
8	84:16 <i>n</i> -Hexane/Acetone	0.2	PG	-
9	82:18 <i>n</i> -Hexane/Acetone	0.2	PG	-
10	Carbon tetrachloride	0.2	PG	-
G=Gel PG= Partial Gel				

Table S1. ^1H and ^{13}C NMR Chemical Shifts of Compounds **1**, **2**, **2a** ($\text{C}_5\text{D}_5\text{N}$)

^1H NMR (in 600 MHz)					^{13}C NMR (in 150 MHz)				
position	1	position	2	2a	position	1	position	2	2a
N-H	8.57 (1H, d, $J = 8.8$ Hz)	1	1.58 (m)		1'	174.9	1	39.1	39.2
Aliphatic chain	1.28	1b	1.03 (m)		2'	72.2	2	28.1	28.2
1	4.49 (1H, dd, $J = 4.9, 3.8$ Hz)	2	1.87 (m)		3'	35.4	3	78.0	78.1
1	4.40 (1H, dd, $J = 4.9, 10.8$ Hz)	2 b			4'	25.6	4	40.1	39.4
2	5.10 (1H, m)	3	3.47 (dd, $J = 5.1, 11.0$)		1	61.7	5	55.8	55.9
3	4.32 (1H, m)	4		3.48 (1H, d, $J = 9.0, 5.5$ Hz)	2	52.7	6	18.8	18.8
4	4.27 (1H, m)	5	0.88 (d, $J = 7.0$)		3	76.5	7	33.2	33.3
8/9	5.50 (2H, m)	6	1.57 (m)		4	72.6	8	39.4	40.1
2'	4.61 (1H, dd, $J = 3.6, 7.5$ Hz)	6 b	1.39 (m)		5	33.6	9	47.1	47.2
5b, 8b, 4', 9 b, 3' b	1.98 (5H, m)	7	1.62 (m)		6	26.5	10	37.2	37.3
H-18, H-24'	0.88 (6H, $J = 7.9$)	7 b	1.33 (m)		7	33.1	11	23.9	23.9
		8			8	130.6	12	123.0	123.0
		9	1.78 (m)		9	130.4	13	142.9	144.0
		10			10	32.7	14	41.7	42.1
		11	1.93 (m)				15	34.8	34.3
		11 b	1.85 (m)				16	68.3	67.9
		12	5.44 (t, $J = 3.3$)	5.45 (1H, m)			17	48.2	47.4
		13					18	40.1	41.3
		14					19	47.2	48.3
		15	2.02 (m)				20	36.3	36.4
		15 b	1.64 (m)				21	79.3	78.7
		16	4.48 (r s)	5.03 (1H, m)			22	73.5	77.3
		17					23	28.7	28.8
		18	3.08 (m)				24	16.9	16.6
		19	3.08 (m)				25	16.6	15.9
		19 b	1.41 (m)				26	16.9	17.1
		20					27	27.4	27.4
		21	6.54 (d, $J = 10.1$)	4.62 (1H, $J = 10.0$ Hz)			28	63.6	68.4
		22	6.28 (d, $J = 10.1$)	4.79 (1H, $J = 10.0$ Hz)			29	29.4	30.6
		23	1.25 (s)				30	20.1	
		24	0.92 (s)				21-COCH ₃	170.8	
		25	0.91 (s)				21-COCH ₃	21.2	
		26	1.05 (s)				1'	176.7	
		27	1.81 (s)				2'	41.7	
		28a	3.68 (d, $J = 10.5$)	3.73 (1H, $J = 10.0$ Hz)			3'	26.9	
		28	3.42 (d, $J = 10.5$)	4.03 (1H, d, $J = 10.0$ Hz)			4'	11.9	
		29	1.06 (s)				5'	15.8	
		30	1.28 (s)						
		21-COCH ₃	2.10 (s)						
		21-COCH ₃							
		1'							
		2'	2.27 (sext, $J = 7.0$ Hz)						
		3'a	1.72 (m)						
		3'	1.38 (m)						
		4'	0.79 (t, $J = 7.4$)						
		5'	1.12 (d, $J = 7.0$)						

Table S2 . Key HMBC, ^1H - ^1H COSY, and ^1H - ^1H NOESY correlations of Compounds (1-2)

1				2			
position	HMBC	COSY	NOESY	position	HMBC	COSY	NOESY
N-H	C-1, C-2, C-3			1	C-2	1b-H	
H-1	C-2, C-3			1b		1 -H,	
H-2	C-1', C-1,	1-Ha,	5-H	2		1-H	
	C-3, C-4	1-H , 3-H					
H-3	C-1, C-2, C-4,	4-H		2 b			
	C-5						
H-4	C-2, C-3, C-6	5-H		3	C-23, C-24		
H-5	C-6, C-7		2-H	4			
H-6	C-5, C-6			5	C-6, C-10, C-25		23-H
H-7/10	C-5, C-6			6		5-H	
H-2'	C-1', C-3', C-4'	3'-H		6 b			
H-3'	C-2', C-4'			7			
H-4'	C-2'			7 b			
				8			
				9			
				10			
				11			
				11 b			
				12	C-9, C-11, C-14, C-18		
				13			
				14			
				15	C-27		
				15 b			
				16	C-14, C-18		28 -H
				17			
				18	C-12, C-13, C-17, C-19, C-20	19 b-H	
				19	C-18, C-29, C-30	19 b-H	
				19 b		19 -H	
				20			
				21	COCH ₃ , C-20, C-22, C-29, C-30		29-H
				22	C-1', C-16, C-17, C-20, C-21, C-28		30-H
				23	C-3, C-5, C-24		5-H
				24	C-5		
				25			
				26			
				27	C-8, C-13, C-14, C-15		
				28a	C-17, C-18, C-22		
				28			
				29	C-19, C-20, C-21, C-30		
				30	C-19, C-20, C-21, C-29		
				21-COCH ₃			
				21-COCH ₃			
				1'			
				2'	C-1', C-3', C-4', C-5'		
				3'			
				4'			
				5'	C-1', C-2', C-3'		

Figure S1. Key COSY and HMBC correlations of compounds (1) and (2)

Figure S2. Key NOESY correlations of compound (2)

Figure S3. FAB Mass fragments of compound (1)

Figure S4. Field Emission Scanning Electron Microscopy (FESEM) Images of isoracemosol A (3)

a) Before gelation

) After gelation