SUPPLEMENTARY MATERIAL

Two rare antioxidative prenylated terpenoids from loop-root Asiatic mangrove *Rhizophora mucronata* (Family Rhizophoraceae) and their activity against pro-inflammatory cyclooxygenases and lipoxidase

Vamshi Krishna Raola¹ and Kajal Chakraborty^{*,1}

Affiliation

Central Marine Fisheries Research Institute, Ernakulam North P.O., P.B. No. 1603, Cochin 682018, India

* Corresponding author. Address: Marine Bioprospecting Section of Marine Biotechnology Division, Central Marine Fisheries Research Institute, Ernakulam North, P.B. No. 1603, Cochin, India. Tel.: +91 484 2394867; fax: +91 484 2394909.

E-mail address: kajal_cmfri@yahoo.com (K. Chakraborty).

¹ Equal contribution

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ABSTRACT

Two new biogenic prenylated terpenoids were isolated from the methanol extract of *Rhizophora mucronata*. The extended C₂₀ sesquiterpenoid with prenylated guaiane framework was characterized as (4*E*, 8*Z*)-3, 3a, 6, 7-tetrahydro-3, 9-dimethyl-5-(6-methylheptan-2-yl) cycloocta[b]furan-2-(9*aH*)-one (**1**). (35*E*)-1,2,3,5,6,6-icosahydro-4,4,8b,10,14,17,20,20-octamethylpicen-3-yl-34,35-dimethyloct-31-enoate (**2**) represents the first example of naturally occurring C₄₀ prenylated oleanane type triterpenoid, whereas one 4,5-dimethyloct-5-enoate side chain remains attached at C-3 position of the oleanane framework formed by the E-ring closure of C₃₀ baccharide moiety. The structures of the compounds were elucidated using NMR and mass spectrometric analysis. Compound **1** was found to have significantly greater antioxidant activities (IC₅₀ ~ 0.75 mg/mL) compared to **2** (IC₅₀ > 0.80 mg/mL). No significant differences in anti-cyclooxygenase-2 of these compounds was discernable (IC₅₀ 0.8 – 0.9 mg/mL), whilst compound **1** showed greater anti-5-lipoxidase activities (IC₅₀ ~ 0.8 mg/mL) than that of **2** (IC₅₀ 0.96 mg/mL). Bioactivities of the prenylated terpenoids were inversely proportional to lipophilic and bulk descriptors.

Keywords: *Rhizophora mucronata*; prenylated terpenoids; anti-inflammatory; antioxidant; structure-activity relationship

Supplemental file legend

Table S1. NMR spectroscopic data of **1** and **2** in CDCl₃^a

Figure S1. ¹³C-NMR spectrum of **1**(125 MHz, CDCl₃)

Figure S2. (a) ¹H-¹H COSY, (b) HSQC, (c) HMBC and (d) NOESY spectra of **1**. The key ¹H-¹H COSY couplings have been represented by the bold face bonds. The key HMBC couplings have been indicated as double barbed arrow. The NOESY spectrum has been indicated as two sided dotted arrows.

Figure S3. GC-MS spectrum of **1**

Figure S4. ¹³C-NMR spectrum of **2**(125 MHz, CDCl₃)

Figure S5. (a) ¹H-¹H COSY, (b) HSQC, (c) HMBC and (d) NOESY spectra of **2**. The key ¹H-¹H COSY couplings have been represented by the bold face bonds. The key HMBC couplings have been indicated as double barbed arrow. The NOESY spectrum has been indicated as two sided dotted arrows.

Figure S6. GC-MS spectrum of **2**

Figure S7. UV spectrum of **1**

Figure S8. UV spectrum of **2**

1 **2**

C. No.	¹³ C NMR (δ, ppm)	¹ H NMR (int., mult., J in Hz) ^b	¹ H- ¹ H COSY	HMBC (¹ H- ¹³ C)	¹³ C NMR (δ, ppm)	¹ H NMR (int., mult., J in Hz) ^b	¹ H- ¹ H COSY	HMBC (¹ H- ¹³ C)
1	173.67		20-H,3-H		38.62	1.73(t,1H ^a) 1.04(t,1H ^β)	H-2	-
2	34.87	2.29(m,1H)	10-H,4-H	C-1,3	27.53	1.61(m,1H ^a) 2.03(m,1H ^β)	H-3	C-1,3
3	25.69	1.64(t,1H)	3-H	C-4	80.59	4.50(dd,J=5.41H)	-	C-2,4,31
4	124.27	5.11(d,1H)	-	C-5,11	39.82	-	-	-
5	131.22	-	7-H	-	55.59	0.84(t,1H)	-	C-6
6	32.21	2.01(t,2H)	8-H	C-5	18.16	1.53(m,1H ^a) 1.36(m,1H ^β)	H-5	C-7
7	31.93	2.06(q,2H)	-	C-8,9	34.54	1.34(t,1H ^a) 1.49(t,1H ^β)	H-6	C-8
8	125.02	5.15(t, J=6.7,1H)	-	C-9,7	41.73	-	-	-
9	135.19	-	3-H	-	51.14	1.29(t,1H)	-	C-8
10	80.57	4.50(d,1H)	12-H,18-H	C-9	37.38	-	-	-
11	39.73	1.97(m,1H)	13-H	C-5,6	21.1	1.55(m,1H)	-	C-12
12	27.98	1.52(m,1H)	-	-	26.19	1.77(m,1H ^a) 1.50(m,1H ^β)	H-11	-
13	16.56	0.98(m,2H)	15-H	-	39.1	2.00(dd,1H)	-	C-14,18
14	29.36	1.37(m,3H)	16-H,17-H	C-13	43.34	-	-	-
15	22.69	1.29(m,1H)	15-H	C-14,17	28.07	2.01(t,1H ^a) 1.79(m,1H ^β)	H-16	C-16
16	14.11	0.87(d,3H)	-	C-15	37.76	1.44(m,1H ^a) 1.23(m,1H ^β)	-	C-17
17	16.01	1.60(dd,3H)	2-H	C-2,3	33.35	-	-	-
18	26.4	1.69(s,3H)	-	C-12	142.67	-	-	-
19	29.7	1.26(d,3H)	11-H	C-9,8	129.77	4.87(s,1H)	-	C-20
20	17.68	0.90(d,3H)	-	-	32.61	-	-	-
21	-	-	-	-	33.56	1.48(m,1H ^a) 1.10(m,1H ^β)	H-22	C-20
22	-	-	-	-	37.87	1.76(t,1H ^a) 1.72(t,1H ^β)	-	C-17
23	-	-	-	-	28.07	0.87(s,3H)	-	-
24	-	-	-	-	15.56	0.85(s,3H)	-	C-4
25	-	-	-	-	16.1	1.08(s,3H)	-	C-9
26	-	-	-	-	16.78	0.97(s,3H)	-	C-9
27	-	-	-	-	14.56	0.73(s,3H)	-	C-14
28	-	-	-	-	25.19	1.02(s,3H)	-	C-17
29	-	-	-	-	31.34	0.95(s,3H)	-	-
30	-	-	-	-	29.37	0.93(s,3H)	-	C-20
31	-	-	-	-	173.68	-	-	-
32	-	-	-	-	34.91	2.31(t,1H ^a) 2.28(t,1H ^β)	H-33	C-31,33
33	-	-	-	-	38.32	2.27(m,1H ^a) 1.64(m,1H ^β)	-	C-31
34	-	-	-	-	47.56	1.94(t,1H)	-	C-40
35	-	-	-	-	145.2	-	-	-
36	-	-	-	-	121.65	5.18(t,1H)	-	C-34
37	-	-	-	-	23.46	1.88(m,1H ^a) 1.63(m,1H ^β)	H-36	C-36
38	-	-	-	-	14.11	0.89(d,3H)	H-37	C-37
39	-	-	-	-	26.04	1.14(s,3H)	-	C-35
40	-	-	-	-	33.27	0.88(d,3H)	H-34	-

^a NMR spectra recorded using Bruker AVANCE III 500 MHz (AV 500) spectrometer in CDCl₃ as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm). ^bValues in ppm, multiplicity and coupling constants (J= Hz) are indicated in parentheses. The assignments were made with the aid of the ¹H-¹H COSY, HSQC, HMBC and NOESY experiments.

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Figure S1

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Figure S2

Figure S3

Figure S4

Figure S5

Figure S6

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Figure S7

Figure S8

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