New Sesquiterpenoid and Triterpenoids from the Fruits of *Rhizophora mucronata*

Surat LAPHOOKHIEO, Chatchanok KARALAI,* and Chanita PONGLIMANONT

Department of Chemistry, Faculty of Science, Prince of Songkla University; Hat-Yai, Songkhla 90112, Thailand.
Received March 1, 2004; accepted April 22, 2004

A new sesquiterpene (1) and two new pentacyclic triterpenoid esters (2, 3) together with three known compounds (4—6) were isolated from the fruits of *Rhizophora mucronata*. Their structures were elucidated by analysis of their spectroscopic data. The new compounds were characterized as 3-hydroxy-3,7,11-trimethyl-9-oxododeca-1,10-diene (mucronatone, 1), 3β-*E*-caffeoyltaraxerol (2) and 3β-*Z*-caffeoyltaraxerol (3).

Key words *Rhizophora mucronata*; mucronatone; taraxerol; caffeoyl

Rhizophora mucronata (Rhizophoraceae), is a mangrove tree. The bark of this plant has been used by the local Thai people in a folk medicine for treatment of nausea, vomiting, diarrhea and stop bleeding in fresh wounds.[1)] This plant has been investigated by many groups and diterpenoids,[2)] triterpenoids,[3)] and steroids[4)] were elucidated. As part of our chemical investigation on Thai medicinal mangrove plants,[5—7)] we reported herein the isolation and structure elucidation of two new pentacyclic triterpenoid esters and a new sesquiterpene along with three known compounds, 3β-*E*-*p*-coumaroyltaraxerol,[8)] 3β-*Z*-*p*-coumaroyltaraxerol[8)] and β-taraxerol[9)] from hexane and methylene chloride extracts. The structures of these compounds were elucidated through spectral studies including 1D and 2D NMR analysis.

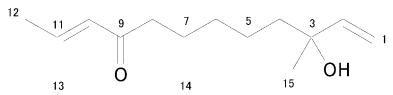
Compound **1** was obtained as a pale yellow oil. The ESI-TOF MS gave a molecular ion peak at *m*/*z* 261.1823 $[M+Na]^+$ (Calcd 261.1830) consistent with the molecular formula $C_{15}H_{26}O_2$, which implied three degrees of unsaturation. The UV spectrum showed maximum at 223 nm indicating the presence of enone chromophore. The IR spectrum exhibited absorption bands at 3461 cm^{-1} (hydroxyl), 1679 cm^{-1} (conjugated carbonyl), 1616 and 916 cm^{-1} (double bond). The ^{13}C-NMR spectral data (Table 1) showed 15 carbons, attributable to three methine carbons (δ 145.2, 124.1, 29.6); five methylene carbons (δ 111.4, 51.7, 42.3, 37.3, 21.2); four methyl carbons (δ 27.5, 27.5, 20.6, 19.8) and three quaternary carbons (δ 201.1, 154.7, 73.0), as determined by DEPT experiment. Furthermore, four methyl signals in the ^{1}H-NMR spectral data of **1** (Table 1) at δ 2.13 (d, J=1.2 Hz), 1.87 (d, J=1.2 Hz), 1.26 (s) and 0.87 (d, J=6.6 Hz) were in agreement with a linear sesquiterpene, thus suggesting compound **1** to have a farnesane framework.[10)]

The location of two double bonds and a carbonyl group were established as follows. The olefinic proton signals at δ 5.02 (1H, dd, J=1.5, 10.8 Hz), 5.20 (1H, dd, J=1.5, 17.4 Hz) and 5.90 (1H, dd, J=10.8, 17.4 Hz) were assigned to a monosubstituted double bond that must be located at C-1 (δ 111.4) and C-2 (δ 145.2). Furthermore, H-2 (δ 5.90) showed correlation in the HMBC spectrum (Table 1) with the carbinol carbon at C-3 (δ 73.0) and Me-15 (δ 27.5). A signal at δ 6.06 (1H, m) exhibited cross-peak in the COSY spectrum with the vinylic methyl signal at δ 2.13 (d, J=1.2 Hz, Me-13) and 1.87 (d, J=1.2 Hz, Me-12), which was additionally correlated with a signal of carbonyl carbon at δ 201.1 in the HMBC spectrum. These correlations were consistent with the location at C-10 (δ 124.1) and C-11 (δ 154.7) of the remaining trisubstituted double bond and the carbonyl group was located at C-9 (δ 201.1). Finally, the downfield methylene protons at C-8 (δ 2.38, 1H, dd, J=6, 15 Hz and 2.18, 1H, dd, J=7.8, 15 Hz) showed cross-peak in the COSY spectrum with H-7 at δ 2.00 (1H, m), and the methine H-7 was also coupled with the Me-14 signal at δ 0.87 (3H, d, J=6.6 Hz)

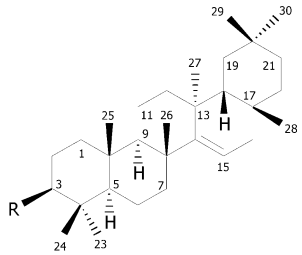
Table 1. ^{1}H-, ^{13}C-, DEPT, COSY and HMBC Spectral Data of Compound **1** (300 MHz and 75 MHz in $CDCl_3$)

No.	^{1}H	^{13}C	DEPT	COSY (^{1}H→^{1}H)	HMBC (^{1}H→^{13}C)
1a	5.02, dd, 1.5, 10.8 Hz;	111.4	CH_2	2, 1b	2, 3
1b	5.20, dd, 1.5, 17.4 Hz			2, 1a	
2	5.90, dd, 10.8, 17.4 Hz	145.2	CH	1	3, 4, 15
3	—	73.0	C	—	—
4	1.30, m; 1.50, m	42.3	CH_2	—	2, 3, 5, 6, 15
5	1.30, m	21.2	CH_2	—	6, 7
6	1.15, m; 1.35, m	37.3	CH_2	—	—
7	2.00, m	29.6	CH	14	5, 6, 8, 9
8a	2.38, dd, 6, 15 Hz;	51.7	CH_2	7, 8b	6, 7, 9, 10
8b	2.18, dd, 7.8, 15 Hz			7, 8a	
9	—	201.1	C	—	—
10	6.06, m	124.1	CH	12, 13	9, 11, 12, 13
11	—	154.7	C	—	—
12	1.87, d, 1.2 Hz	27.5	CH_3	10	9, 10, 11
13	2.13, d, 1.2 Hz	20.6	CH_3	10	9, 10, 11
14	0.87, d, 6.6 Hz	19.8	CH_3	7	6, 7, 8
15	1.26, s	27.5	CH_3	—	2, 3, 4

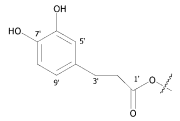
* To whom correspondence should be addressed. e-mail: kchatcha@ratree.psu.ac.th



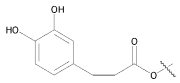
1



2 R =



3 R =



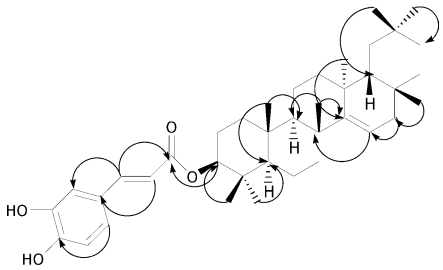


Fig. 1. Selective HMBC Correlation of **2**

and 2H-6 (δ 1.15, 1H, m and δ 1.35, 1H, m). Therefore, the structure of **1** was elucidated as 3-hydroxy-3,7,11- trimethyl-9-oxododeca-1,10-diene and was given the trivial name as mucronatone.

Compound **2** was obtained as a white solid with a molecular formula of $C_{39}H_{56}O_4$ based on the $[M-H]^-$ ion at *m/z* 587.4151 in the ESI-TOF MS experiment (Calcd 587.4101). The presence of a triterpenoid skeleton was suggested by the violet vanillin sulfuric acid test. The IR spectrum exhibited absorption bands at 3409 cm^{-1} (hydroxy), 1705 cm^{-1} (carbonyl) and the UV spectrum showed maxima at 237, 294 and 327 nm suggesting the presence of considerable conjugation in the molecule. The ^{1}H-NMR signals of eight methyl singlets at δ 0.82 (Me-28), 0.89 (Me-27), 0.91 (Me-23), 0.91 (Me-30), 0.96 (Me-24), 0.96 (Me-29), 0.98 (Me-25) and 1.10 (Me-26), one oxymethine proton at δ 4.57 (t, *J*=9 Hz, H-3) and one olefinic proton at δ 5.54 (dd, *J*=3, 8.1 Hz, H-15) suggested a typical of pentacyclic triterpene which was identified as β-taraxerol by the combination of 1D (Table 2) and 2D NMR spectral data and comparison with data reported previously for taraxerol.[9,11] Moreover, clear separations of the proton signals identified as an *E*-caffeoyl moiety were observed at δ 7.52 (1H, d, *J*=15.9 Hz, H-3′), 7.05 (1H, d, *J*=1.8 Hz, H-5′), 6.94 (1H, dd, *J*=1.8, 8.1 Hz, H-9′), 6.82 (1H, d, *J*=8.1 Hz, H-8′) and 6.23 (1H, d, *J*=15.9 Hz, H-2′).[12,13] This substituent group was attached to an oxygen atom of β-taraxerol at C-3 (δ 81.1) as a result of the downfield effect observed on H-3 (δ 4.57) and additionally confirmed by the correlation between H-3 (δ 4.57) and C-1′ (δ 167.9) in the HMBC spectrum (Fig. 1). The NOE correlation between CH_3-23 (δ 0.91) and H-3 (δ 4.57) was observed in the NOESY spectrum of **2**. This result supported the relative stereochemistry at C-3. Therefore, compound **2** was assigned as 3β-*E*-caffeoyltaraxerol.

Compound **3** was obtained as a yellowish solid. The ESI-TOF MS gave a molecular ion peak at *m/z* 587.4091 $[M-H]^-$ (Calcd 587.4101), consistent with the molecular formula $C_{39}H_{56}O_4$. The UV and IR spectrum closely resembled to those of **2**. The ^{1}H- and ^{13}C-NMR spectral data (Table 2) were very similar to those of **2** except for the appearance of signals of *Z*-caffeoyl moiety (δ 5.77, d, *J*=12.6 Hz, H-2′; 6.76, d, *J*=12.6 Hz, H-3′) instead of an *E*-caffeoyl moiety (δ 6.23, d, *J*=15.9 Hz, H-2′; 7.52, d, *J*=15.9 Hz, H-3′). Thus, compound **3** was assigned as 3β-*Z*-caffeoyl taraxerol.

Compounds **4**—**6** were characterized as 3β-*E*-*p*-coumaroyltaraxerol,[8] 3β-*Z*-*p*-coumaroyltaraxerol[8] and β-taraxerol,[9] respectively, based on comparison of ^{1}H- and ^{13}C-NMR spectral data with the literature reports.

Experimental

General Experimental Procedures Melting points were determined on the Electrothermal melting point apparatus. UV spectra were measured with a SPECORD S100 spectrophotometer (Analytikjena). The optical rotation values were determined with a Polarimeter ADP 220 (Bellingham+Stanley Ltd.). The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. The ^{1}H-NMR were recorded using 500 MHz Varian UNITY INOVA and Bruker Avance 300 MHz spectrometer in $CDCl_3$ and CD_3OD. The ESI-TOF mass spectra were obtained from a Micromass LCT mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100, respectively. Precoated plates of silica gel 60 GF_{254} were used for analytical purposes.

Plant Material The fruits of *Rhizophora mucronata* were collected in August, 2003 at Sigao district, Trang Province, Thailand. The plant was identified by Dr. Kitichate Sridith and a voucher specimen has been deposited at Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand (Collection No. P. Seni 1 (PSU)).

Extraction and Isolation Air-dried fruits of *Rhizophora mucronata* (17 kg) were extracted with hexane and methylene chloride successively. The hexane extract (32 g) was subjected to QCC over silica gel and eluted with a gradient of hexane–acetone to afford 11 fractions (D1—D11). Fraction D2 (444.3 mg) was crystallized from acetone–hexane to give compound **6** (50 mg). Fraction D3 (7.88 g) was subjected to CC using 70% CH_2Cl_2–hexane as eluent to give compound **1** (132.2 mg), **4** (80.6 mg) and **5** (202.2 mg). Fraction D4 (1.20 g) upon washing with hexane gave a white solid (700 mg) which was further subjected to CC using 5% diethyl ether–CH_2Cl_2 as eluent to give compound **4** (69.4 mg) and **5** (100.2 mg). Fraction D7 (2.10 g) was further subjected to CC using 10% diethyl ether–CH_2Cl_2 as eluent to give compound **2** (80.5 mg), **4** (49.4 mg) and **5** (30.2 mg).

The methylene chloride extract (23 g) was subjected to QCC over silica gel and eluted with a gradient of $CHCl_3$–methanol to afford 8 fractions (E1—E8). Fraction E2—E4 (5.02 g) was crystallized from $CHCl_3$ to give a mixture of compounds **4** and **5** (4.34 g). Fraction E5 (1.10 g) was further subjected to CC using 5% methanol–$CHCl_3$ as eluent to give compound **2** (56.7 mg), **3** (25.1 mg), **4** (46.4 mg) and **5** (35.4 mg). Fraction E6—E7 (4.50 g) was crystallized from $CHCl_3$ and methanol to give compound **2** (2.3 g).

Mucronatone (**1**): Colorless viscous oil, $[\alpha]_D^{27}$ −16.66° (*c*=0.04, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 223 (4.16). IR (Neat) cm^{-1}: 3461, 1679, 1616, 916. ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz), see Table 1. ESI-TOF MS *m/z*: 261.1823 (Calcd for $[C_{15}H_{26}O_2+Na]^+$: 261.1830).

3β-*E*-Caffeoyltaraxerol (**2**): White solid, mp 246—248 °C. $[\alpha]_D^{27}$ +28.84° (*c*=0.052, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 237 (3.33), 294 (3.29), 327

Table 2. ^{1}H-, ^{13}C-NMR and DEPT Spectral Data of Compounds **2** and **3** (300 MHz and 75 MHz in $CDCl_3$+CD_3OD)

No.	**2**		**3**		DEPT
	^{13}C	^{1}H	^{13}C	^{1}H	
1	37.3	0.95, m; 1.28, m	37.5	0.96, m; 1.32, m	CH_2
2	23.5	1.65 , m	23.5	1.68, m	CH_2
3	81.1	4.57, t, 9 Hz	81.3	4.48, t, 8.4 Hz	CH
4	37.5	—	37.6	—	C
5	55.6	0.90, m	55.9	0.89, m	CH
6	18.6	1.45, m	18.8	1.40, m; 1.55, m	CH_2
7	36.6	1.65, m	36.8	1.65, m	CH_2
8	38.9	—	38.8	—	C
9	48.6	0.92, m	49.3	0.95, m	CH
10	37.8	—	37.8	—	C
11	17.4	1.45, m	17.6	1.45, m	CH_2
12	35.0	1.30, m	35.2	1.35, m	CH_2
13	37.8	—	37.8	—	C
14	157.9	—	157.6	—	C
15	116.8	5.54, dd, 3, 8.1 Hz	117.3	5.54, dd, 3, 8.1 Hz	CH
16	37.6	1.65, m; 1.94, dd, 2.7, 14.7 Hz	37.8	1.65, m; 1.92, dd, 2.7, 14.7 Hz	CH_2
17	35.7	—	35.9	—	C
18	49.1	1.45, m	48.9	1.46, m	CH
19	41.1	1.35, m; 2.05, m	41.3	1.35, m; 2.04, m	CH_2
20	28.7	—	28.9	—	C
21	33.6	1.30, m	33.2	1.34, m	CH_2
22	33.0	1.62, m	33.8	1.55, m	CH_2
23	27.9	0.91, s	28.1	0.84, s	CH_3
24	16.6	0.96, s	16.6	0.95, s	CH_3
25	15.4	0.98, s	15.5	0.86, s	CH_3
26	25.8	1.10, s	26.0	1.09, s	CH_3
27	29.8	0.89, s	30.0	0.91, s	CH_3
28	29.7	0.82, s	29.9	0.82, s	CH_3
29	33.2	0.96, s	33.4	0.95, s	CH_3
30	21.2	0.91, s	21.4	0.91, s	CH_3
1′	167.9	—	166.5	—	C
2′	115.2	6.23, d, 15.9 Hz	117.1	5.77, d, 12.6 Hz	CH
3′	145.0	7.52, d, 15.9 Hz	143.9	6.76, d, 12.6 Hz	CH
4′	126.8	—	127.0	—	C
5′	114.0	7.05, d, 1.8 Hz	116.9	7.41, d, 1.8 Hz	CH
6′	144.8	—	144.5	—	C
7′	147.3	—	147.3	—	C
8′	115.2	6.82, d, 8.1 Hz	117.1	6.79, d, 8.1 Hz	CH
9′	121.8	6.94, dd, 1.8, 8.1 Hz	124.3	7.05, dd, 1.8, 8.1 Hz	CH

(3.33). IR (KBr) cm^{-1}: 3409, 1705. ^{1}H-NMR ($CDCl_3$+CD_3OD, 300 MHz) and ^{13}C-NMR ($CDCl_3$+CD_3OD, 75 MHz), see Table 2. ESI-TOF MS (negative mode) *m/z*: 587.4151 (Calcd for $C_{39}H_{55}O_4$ $[M-H]^-$: 587.4101).

3β-*Z*-Caffeoyltaraxerol (**3**): White solid, mp 246 °C (dec.). $[\alpha]_D^{27}$ −100° (c=0.04, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 223 (2.91), 296 (2.65), 316 (2.64). IR (KBr) cm^{-1}: 3416, 1701. ^{1}H-NMR ($CDCl_3$+CD_3OD, 300 MHz) and ^{13}C-NMR ($CDCl_3$+CD_3OD, 75 MHz), see Table 2. ESI-TOF MS (negative mode) *m/z*: 587.4091 (Calcd for $C_{39}H_{55}O_4$ $[M-H]^-$: 587.4101).

Transesterification of 2 A solution of **2** (10 mg in $CHCl_3$: MeOH (3 ml, 1 : 1) and 0.5 ml conc. HCl) was refluxed for 24 h. Then the reaction mixture was extracted with $CHCl_3$ and washed with $NaHCO_3$. The product was purified by preparative TLC to afford β-taraxerol[9] (1.8 mg), methyl caffeoate (1.9 mg) and a starting material (3 mg).

Acknowledgments We are grateful to the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0007/2546), the Higher Education Development Project: Postgraduate Education and Research Program in Chemistry and Prince of Songkla University through Natural Products from Mangrove Plants and Synthetic Materials Research Unit for financial support.

References

1) Boonyapraphat N., Chockchaicharaenphorn C., "Thai Medicinal Plants," Vol. II, Prachachon Ltd., Bangkok, 1998, pp. 311—312.
2) Anjaneyulu A. S. R., Anjaneyulu V., Rao V. L., *J. Asian Nat. Prod. Res.*, **4**, 53—61 (2002).
3) Ahmed S. S., Hiader S. I., Rabbani M. M., *Fitoterapia*, **59**, 79—81 (1988).
4) Ghosh A., Misra S., Dutta A. K., Choudhury A., *Phytochemistry*, **24**, 1725—1727 (1985).
5) Chumkaew P., Karalai C., Ponglimanont C., Chantrapromma K., *J. Nat. Prod.*, **66**, 540—543 (2003).
6) Laphookhieo S., Cheenpracha S., Karalai C., Chantrapromma S., Rat-a-pa Y., Ponglimanont C., Chantrapromma K., *Phytochemistry*, **65**, 507—510 (2004).
7) Chantrapromma S., Usman A., Fun H. K., Laphookhieo S., Karalai C., Rat-a-pa Y., Chantrapromma K., *Acta Crystallogr.*, **C59**, o68—o70 (2003).
8) Kokpol U., Chavasiri W., Chitrawong V., Miles D. H., *J. Nat. Prod.*, **53**, 953—955 (1990).
9) Corbett R. E., Cumming S. D., *J. Chem. Soc. Perkin Trans. I*, **1972**, 2827—2829 (1972).
10) Rueda A., Zubia E., Ortega M. J., Salva J., *J. Nat. Prod.*, **64**, 401—405 (2001).
11) Mahato S. B., Kundu A. P., *Phytochemistry*, **37**, 1517—1575 (1994).
12) Alvarenga N., Ferro E. A., *Fitoterapia*, **71**, 719—721 (2000).
13) Tommasi N. D., Simone F. D., Pizza C., Mahmood N., Moore P. S., Conti C., Orsi N., Stein M., *J. Nat. Prod.*, **55**, 1067—1073 (1992).