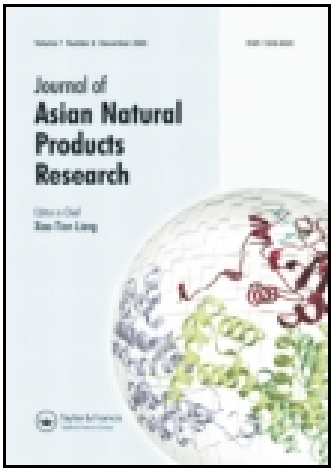

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ORIGINAL ARTICLE

Three new lupane-type triterpenes from *Ceriops tagal*

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Three new lupane-type triterpenes, 3α-*O-trans*-feruloylbetulinic acid (**1**), 3α-*O-trans*-coumaroylbetulinic acid (**2**) and 3β-*O-cis*-feruloylbetulin (**3**), together with 10 known triterpenes (**4**–**13**), were isolated from the aerial parts of the mangrove plant *Ceriops tagal*. The structures of the three new compounds were established by means of spectroscopic data analyses and chemical methods.

Keywords: *Ceriops tagal*; triterpene; 3α-*O-trans*-feruloylbetulinic acid; 3α-*O-trans*-coumaroylbetulinic acid; 3β-*O-cis*-feruloylbetulin

1. Introduction

Ceriops tagal (Perr.) C. B. Rob (Rhizophoraceae) is an important mangrove plant both in ecology and economy. In China, it is distributed mainly on the southern coastal zone [1]. Previous chemical studies on *C. tagal* have resulted in the isolation of several types of triterpenes, diterpenes, and tetraterpenes [2–6]. In our studies on the chemical constituents of *C. tagal*, three new lupane-type triterpenes, 3α-*O-trans*-feruloylbetulinic acid (**1**), 3α-*O-trans*-coumaroylbetulinic acid (**2**), and 3β-*O-cis*-feruloylbetulin (**3**) were isolated together with 10 known triterpenes (**4**–**13**) (Figure 1). The structures of the new compounds were established by means of spectroscopic data analyses, including 1D, 2D NMR, and HR-ESI-MS spectra. The known compounds were identified as 3β-*O-cis*-coumaroylbetulin (**4**) [7,8], 3β-*O-trans*-coumaroylbetulin (**5**) [7,8], 3β-*O-trans*-feruloylbetulin (**6**) [9], 3β-*O-trans*-coumaroylbetulinic acid (**7**) [10], 3β-*O-cis*-coumaroylbetulinic acid (**8**) [10], lupeol (**9**) [11,12], 3-*epi*-betulinic acid (**10**) [12–15], betulin (**11**) [12,15,16], 3-*epi*-betulin (**12**) [17,18], 28-hydroxylup-20(29)-en-3-one (**13**) [16,19] by comparing their spectroscopic data with those reported in the literatures.

2. Results and discussion

Compound **1** was obtained as white powder and deduced to be a triterpene due to a positive Liebermann–Burchard test. Its molecular formula was determined as $C_{40}H_{56}O_6$ by HR-ESI-MS at *m/z* 655.3971 $[M + Na]^+$. The IR spectrum showed characteristic absorptions of OH (3426 cm^{-1}), C = O (1708, 1685 cm^{-1}), and C = C (1635 cm^{-1}). The ^1H and ^{13}C

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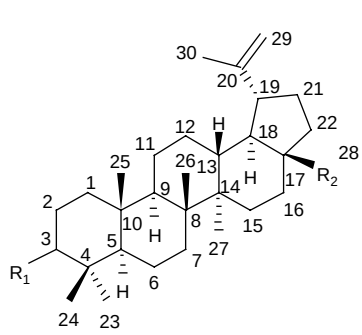

1 R_1 = α-*O*-*trans*-feruloyl, R_2 = COOH
2 R_1 = α-*O*-*trans*-coumaroyl, R_2 = COOH
3 R_1 = β-*O*-*cis*-feruloyl, R_2 = CH_2OH
4 R_1 = β-*O*-*cis*-coumaroyl, R_2 = CH_2OH
5 R_1 = β-*O*-*trans*-coumaroyl, R_2 = CH_2OH
6 R_1 = β-*O*-*trans*-feruloyl, R_2 = CH_2OH
7 R_1 = β-*O*-*trans*-coumaroyl, R_2 = COOH
8 R_1 = β-*O*-*cis*-coumaroyl, R_2 = COOH
9 R_1 = β-*O*H, R_2 = CH_3
10 R_1 = α-*O*H, R_2 = COOH
11 R_1 = β-*O*H, R_2 = CH_2OH
12 R_1 = α-*O*H, R_2 = CH_2OH
13 R_1 = ketone, R_2 = CH_2OH

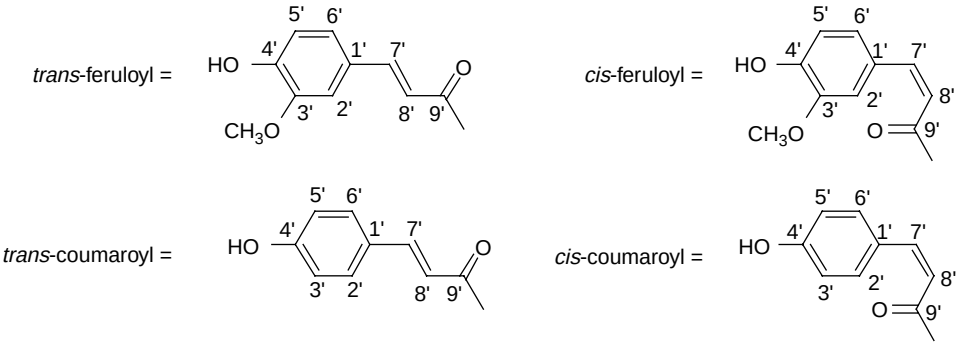

Figure 1. Structures of compounds **1**–**13**.

Table 1. ^{1}H NMR spectral data for compounds **1**–**3** (300 MHz, $CDCl_3$, δ in ppm).

Position	**1**	**2**	**3**
H-3	4.74 (m)	4.75 (m)	4.53 (dd, J = 11.1, 6.0 Hz)
H-19	3.01 (m)	3.03 (m)	2.37 (m)
Me-23	0.88 (s)	0.88 (s)	0.89 (s)
Me-24	0.90 (s)	0.91 (s)	0.87 (s)
Me-25	0.88 (s)	0.88 (s)	0.85 (s)
Me-26	0.96 (s)	0.97 (s)	0.98 (s)
Me-27	1.06 (s)	1.07 (s)	1.05 (s)
H-28a	–	–	3.33 (d, J = 10.5 Hz)
H-28b	–	–	3.79 (d, J = 10.5 Hz)
H-29a	4.74 (br s)	4.75 (br s)	4.68 (s)
H-29b	4.60 (br s)	4.60 (br s)	4.58 (s)
Me-30	1.70 (s)	1.70 (s)	1.68 (s)
H-2′	7.03 (d, J = 1.6 Hz)	7.42 (d, J = 8.1 Hz)	7.62 (d, J = 1.5 Hz)
H-3′	–	6.85 (d, J = 8.1 Hz)	–
H-5′	6.92 (d, J = 8.1 Hz)	6.85 (d, J = 8.1 Hz)	6.87 (d, J = 8.1 Hz)
H-6′	7.10 (dd, J = 1.6, 8.1 Hz)	7.42 (d, J = 8.1 Hz)	7.10 (dd, J = 1.5, 8.1 Hz)
H-7′	7.61 (d, J = 15.6 Hz)	7.62 (d, J = 16.2 Hz)	6.77 (d, J = 12.3 Hz)
H-8′	6.33 (d, J = 15.6 Hz)	6.35 (d, J = 16.2 Hz)	5.81 (d, J = 12.3 Hz)
MeO-3′	3.94 (s)	–	3.81 (s)

Table 2. ^{13}C NMR spectral data for compounds **1**–**3**, **10**, and **11** (100 MHz, $CDCl_3$).

Position	**1**	**2**	**3**	**10**	**11**
1	34.1	34.2	38.6	33.1	38.9
2	22.9	23.2	23.9	25.1	27.5
3	78.3	78.7	80.9	75.8	79.2
4	37.2	37.1	38.1	37.1	39.0
5	49.2	49.5	55.6	48.8	55.5
6	18.1	18.3	18.4	18.1	18.5
7	33.9	34.4	34.3	34.1	34.4
8	40.8	41.1	41.1	40.7	41.1
9	50.4	50.7	50.5	50.1	50.6
10	36.9	37.5	37.3	37.3	37.3
11	20.7	20.9	21.1	20.6	21.0
12	25.5	25.7	25.4	25.4	25.4
13	38.3	38.6	37.5	38.2	37.5
14	42.5	42.7	42.9	42.3	42.9
15	29.7	30.8	27.2	30.4	27.2
16	32.2	32.4	29.4	32.1	29.3
17	56.3	56.6	47.9	56.1	48.0
18	46.9	47.1	48.9	46.9	48.9
19	50.4	50.6	48.1	49.1	48.0
20	150.4	150.6	150.7	150.6	150.6
21	30.5	30.0	29.9	29.5	29.9
22	37.1	37.3	34.2	37.0	34.1
23	27.9	28.2	28.2	28.0	28.1
24	21.7	22.0	16.7	21.9	15.5
25	16.1	16.2	16.4	15.7	16.3
26	16.1	16.3	16.2	15.7	16.1
27	14.9	15.1	14.9	14.4	14.9
28	180.9	182.4	60.7	179.1	60.7
29	109.6	110.0	109.9	109.2	109.8
30	19.4	19.6	19.3	19.0	19.3
1′	127.1	127.4	127.5	–	–
2′	109.5	130.2	113.1	–	–
3′	146.7	116.1	144.6	–	–
4′	147.8	158.1	147.2	–	–
5′	114.7	116.1	114.1	–	–
6′	122.9	130.2	125.8	–	–
7′	144.4	144.5	143.8	–	–
8′	116.3	116.4	117.7	–	–
9′	166.9	167.6	166.6	–	–
MeO-3′	56.1	–	56.2	–	–

NMR spectral data (Tables 1 and 2) showed typical NMR signals of lupane-type triterpene: five methyl singlets at δ_H 0.88 (Me-23), 0.90 (Me-24), 0.88 (Me-25), 0.96 (Me-26), 1.06 (Me-27), one H_β-19 proton signal at δ_H 3.01 (m), and one isopropenyl group with the protons at δ_H 4.74, 4.60 (both br s) assigned to vinylic methylene (H_2-29), the proton at δ_H 1.70 (s) for the attached methyl (Me-30), the carbons at δ_C 150.4 (C-20) and 109.6 (C-29) for the double bond. By comparison of the NMR spectral data with those of 3-*epi*-betulinic acid (**10**) [12–15], which was also isolated from this plant, compound **1** was assigned as a 3-*epi*-betulinic acid derivative with an extra ester moiety. The substituted ester moiety was recognized as *trans*-feruloyl through the ^1H NMR signals at δ_H 7.03 (H-2′, d, $J = 1.6$ Hz), 6.92 (H-5′,

d, $J = 8.1$ Hz), and 7.10 (H-6′, dd, $J = 1.6$, 8.1 Hz), which was characteristic for a 1,3,4-trisubstituted benzene ring, coupled with signals at δ_H 7.61 (H-7′, d, $J = 15.6$ Hz) and 6.33 (H-8′, d, $J = 15.6$ Hz) for the double bond. The attachment of the methoxyl group to C-3′ was evident by the HMBC correlation between MeO-3′ at δ_H 3.94 and C-3′ at δ_C 146.7. A strong HMBC correlation of C-9′ (δ_C 166.9) with H-3 (δ_H 4.74, m) determined the feruloyl residue to be located at C-3. Based on 2D NMR spectral data, compound **1** was characterized as 3α-*O-trans*-feruloylbetulinic acid. The 3α-configuration was deduced from the chemical shift of H-3 at δ_H 4.74 (m), almost identical to that of 3-*epi*-betulinic acid (**10**) [12–15] except for the esterified effect (Table 2). As an additional confirming evidence, compound **1** was subjected to alkaline hydrolysis to yield 3-*epi*-betulinic acid (**10**), according to the same physical and spectroscopic properties as that of the authentic sample.

Compound **2** was obtained as white powder and has a molecular formula of $C_{39}H_{54}O_5$ according to HR-ESI-MS. The NMR spectral data of **2** closely resembled those of **1**, composed of a lupane-type triterpene, 3-*epi*-betulinic acid, and an aromatic ester moiety (Tables 1 and 2). The ester moiety of **2** was established to be *trans*-coumaroyl according to the ^{1}H NMR signals at δ_H 7.42 (2H, d, $J = 8.1$ Hz) and 6.85 (2H, d, $J = 8.1$ Hz), characteristic for a *p*-substituted benzene ring, together with the signals at δ_H 7.62 (H-7′, d, $J = 16.2$ Hz) and 6.35 (H-8′, d, $J = 16.2$ Hz) for the conjugated C = C double bond. The *trans*-coumaroyl moiety placed at C-3 resulted from the similar downfield shifts observed for H-3 and C-3 with those of compound **1** in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). On alkaline hydrolysis, compound **2** also afforded 3-*epi*-betulinic acid (**10**). Therefore, based on the above evidence, the structure of **2** was assigned as 3α-*O-trans*-coumaroylbetulinic acid.

Compound **3** was obtained as white powder and the molecular formula was determined as $C_{40}H_{58}O_5$ from the HR-ESI-MS at *m/z* 641.4186 $[M + Na]^+$. A comparison of the NMR spectral data (Tables 1 and 2) of **3** with those of **1** and **2** revealed the presence of a lupane-type triterpene and an aromatic ester moiety. The triterpene moiety of **3** was identified as betulin through the resembled NMR spectral data (Table 2) with those of betulin and an alkaline hydrolysis of **3** yielding betulin (**11**) [11,14,15]. The ^{1}H NMR spectrum showed two hydroxymethyl protons at δ_H 3.79, 3.33 (each 1H, d, $J = 10.5$ Hz, H_2-28). A *cis*-feruloyl moiety was established from the presence of the *cis*-oriented double bond: δ_H 6.77 (1H, d, $J = 12.3$ Hz, H-7′), 5.81 (1H, d, $J = 12.3$ Hz, H-8′), and the 1,3,4-trisubstituted benzene ring: δ_H 7.62 (H-2′, d, $J = 1.5$ Hz), 6.87 (H-5′, d, $J = 8.1$ Hz), and 7.10 (H-6′, dd, $J = 1.5$, 8.1 Hz). From the above evidence, the structure of **3** was elucidated as 3β-*O-cis*-feruloylbetulin.

By comparison of their spectral data with those reported in the literature, 10 known compounds were identified as 3β-*O-cis*-coumaroylbetulin (**4**), 3β-*O-trans*-coumaroylbetulin (**5**), 3β-*O-trans*-feruloylbetulin (**6**), 3β-*O-trans*-coumaroylbetulinic acid (**7**), 3β-*O-cis*-coumaroylbetulinic acid (**8**), lupeol (**9**), 3-*epi*-betulinic acid (**10**), betulin (**11**), 3-*epi*-betulin (**12**), and 28-hydroxylup-20(29)-en-3-one (**13**).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet FTIR 750 spectrophotometer. ^{1}H NMR, ^{13}C NMR, DEPT, HSQC, and HMBC spectra were recorded with Bruker AMX-300/400 instruments. HR-ESI-MS was carried out

using Micromass Q-Tof Global mass spectrometers. ESI-MS was recorded on a Bruker Esquire 3000 plus spectrometer. All solvents used were of chemical grade and purchased from the Shanghai Chemical Plant (Shanghai, China). MCI Gel CHP20P (75–150 μm) was purchased from Mitsubishi Kasei Chemical Industries (Tokyo, Japan). Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Ltd (Qingdao, China). RP-18 (20–45 μm) was purchased from Fuji Silysia Chemical Ltd (Aichi, Japan). TLC Silica gel GF-254 Plates were purchased from Yantai Huiyou Inc. (Yantai, China).

3.2 Plant material

The aerial parts of *C. tagal* (Perr.) C. B. Rob were collected in August 2008, from Wenchang City, Hainan Province, China. The plant was identified by Pharmacist-incharge Chuan-Shan Fu (Hainan Huatuotianya Pharmaceutical Co. Ltd., Haikou, Hainan, China) and a voucher specimen (No. 20080804) has been deposited at the herbarium of Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.

3.3 Extraction and isolation

The aerial parts of *C. tagal* (10 kg) were powdered and extracted with 95% ethanol at room temperature three times. Evaporation of ethanol left a crude extract (1.2 kg), which was suspended in H_2O, then extracted with EtOAc to afford the EtOAc soluble fraction (272 g). The EtOAc extract was firstly subjected to a column of MCI gel eluted with 30%, 70%, and 100% MeOH/H_2O. Then, 123 g of the MeOH fraction was chromatographed over a silica gel column (petroleum ether–acetone, 25:1–1:10), to yield seven fractions I–VII. Fraction VII (5.3 g) was separated further on an RP-18 column (70–80% MeCN/H_2O) to yield compounds **1** (8.5 mg), **2** (15 mg), **3** (54 mg), **4** (103 mg), **5** (201 mg), **6** (42 mg), **7** (23 mg), and **8** (56 mg). Fraction II (3.6 g) was subjected to a silica gel column eluted with petroleum ether–EtOAc 9:1 to yield compound **9** (602 mg). Fraction III (7.3 g) was subjected to a silica gel column eluted with petroleum ether–EtOAc 8:2 to yield compound **10** (810 mg). Fraction V (5.3 g) was subjected to a silica gel column eluted with petroleum ether–acetone 8:2 to yield compound **11** (81 mg). Fraction IV (6.5 g) was subjected to an RP-18 column eluted with 85% MeCN/H_2O to yield compounds **12** (820 mg) and **13** (930 mg).

3.3.1 3α-O-trans-*Feruloylbetulinic acid* (*1*)

White powder, $[\alpha]_D^{20} = -15$ ($c = 0.02$, $CHCl_3$); IR (KBr) ν_{max}: 3426, 2944, 2871, 1708, 1685, 1635, 1513, 1452, 1261, 1170, 1029 cm^{-1}; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; ESI-MS *m/z*: 655.4 $[M + Na]^+$, 631.9 $[M-H]^-$; HR-ESI-MS (positive) *m/z*: 655.3971 $[M + Na]^+$ (calcd for $C_{40}H_{56}O_6Na$, 655.3975).

3.3.2 3α-O-trans-*Coumaroylbetulinic acid* (*2*)

White powder, $[\alpha]_D^{20} = -12$ ($c = 0.045$, $CHCl_3$); IR (KBr) ν_{max}: 3411, 2946, 2871, 1710, 1685, 1604, 1513, 1272, 1166, 979, 831 cm^{-1}; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; ESI-MS *m/z*: 625.5 $[M + Na]^+$, 601.6 $[M-H]^-$; HR-ESI-MS (positive) *m/z*: 625.3863 $[M + Na]^+$ (calcd for $C_{39}H_{54}O_5Na$, 625.3869).

3.3.3 3β-O-cis-*Feruloylbetulin* (*3*)

White powder, $[\alpha]_D^{20} = +28$ ($c = 0.065$, $CHCl_3$); IR (KBr) ν_{max}: 3440, 2942, 2871, 1702, 1631, 1594, 1515, 1454, 1270, 1172,

1033, 981, 756 cm^{-1}; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; ESI-MS *m/z*: 641.4 $[M + Na]^+$, 617.7 $[M-H]^-$; HR-ESI-MS (positive) *m/z*: 641.4186 $[M + Na]^+$ (calcd for $C_{40}H_{58}O_5Na$, 641.4182).

3.4 Alkaline hydrolysis of 1–3

A sample of **1** (2 mg) was added in 5% KOH–MeOH (5 ml) and the mixture was heated at 65°C for 10 h, the solvent was evaporated under vacuum, then 10 ml water was added and mixed. The mixture was extracted with EtOAc (10 ml ×3) and the EtOAc extract was purified to yield 3-*epi*-betulinic acid (**10**), which was confirmed by ^1H NMR spectrum and TLC comparison with an authentic sample.

Compounds **2** and **3** were treated individually in the same way as described for **1** to yield 3-*epi*-betulinic acid (**10**) and betulin (**11**), respectively.

Acknowledgements

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