CHEMICAL CONSTITUENTS FROM LEAVES OF *SONNERATIA ALBA* J.E. SMITH (SONNERATIACEAE)

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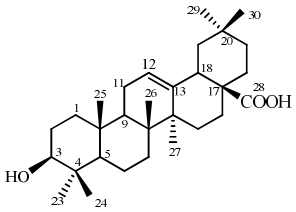
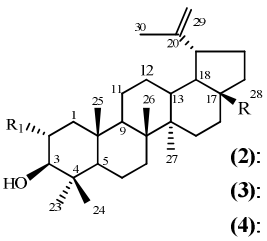
ABSTRACT: *Sonneratia alba J.E. Smith, Sonneratiaceae widely grows in mangrove forests. There were some studies on plants of mangrove forests, and these results showed they contained many interesting bioactive compounds. Nevertheless, Sonneratia alba has not much been studied, especially, has not yet been chemically and biologically studied in Viet Nam. From the petroleum ether extract of the leaves of Sonnertia alba, oleanolic acid (1), betulin (2), betulinic acid (3), alphitolic acid (4), methyl gallate (5) and 5-hydroxymethylfurfural (6) were isolated. Their structures were identified by comparing their NMR data as well as the physical properties with those in literatures. Among them, (1) had high yield (about 0.15% of dried leave) with numerous pharmacological activities including inhibitory activity against HIV-1 protease with IC_{50} of 6.3 μM, anti-inflammatory, anti-cancer. So, Sonneratia alba should be exploited to afford to a valuable source in food and pharmaceutical products. Further studies on this plant are in progress.*

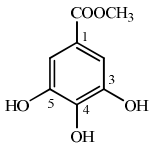
Key words: *Sonneratiaceae, Sonneratia alba, oleanolic acid, betulin, betulinic acid, alphitolic acid, methyl gallate, 5-hydroxymethylfurfural.*



(1)



(2): R = CH_2OH; R_1 = H
(3): R = COOH; R_1 = H
(4): R = COOH; R_1 = OH



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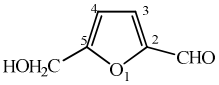

(6)

INTRODUCTION

Sonneratia alba J. E. Smith, Sonneratiaceae (SA, Fig. 1) wildly grows in many mangrove forests in Viet Nam. There were some studies on plants of mangrove forests, and these results showed plants of mangrove forests contained many interesting bioactive compounds such as triterpeneoids, steroids, glycosides, flavonoids, alkaloids, quinonoids… Nevertheless, SA has not much been studied, especially, not yet in Viet Nam. In this paper, the isolation and structural determination of six compounds: oleanolic acid (**1**), betulin (**2**), betulinic acid **(3)**, alphitolic

acid **(4)**, methyl gallate **(5)** and 5-hydroxymethylfurfural **(6)** were reported. Among them, **(1)** was already known in fruits of *Sonneratia ovata* Back. and **(2)**, **(5)** were already isolated from stems and twigs of *Sonneratia caseolaris* (L.) Engl.

Figure 1. *Sonneratia alba* J.E. Smith

MATERIALS AND METHODS

Plant materials

Fresh leaves of the plant were collected in Can Gio mangrove forest in Ho Chi Minh City, Viet Nam in December 2009. The scientific name of the plant was identified by Phan Duc Binh, pharmacist, Associate Editor-in-Chief of the Journal of Drugs and Health. A voucher specimen (No US-B005) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, Vietnam National University - Ho Chi Minh City.

Extraction and isolation

Fresh leaves (28.0 kg) were washed, dried, ground into powder (13 kg) and were extracted by percolation with methanol at room temperature then the methanol extract was evaporated *in vacuo* to give a methanol residue (2 kg). This crude was suspended in water and partitioned against petroleum ether to afford petroleum ether residue (**E**, 700 g). The residue **E** was chromatographed (on a column of si-gel) to afford six compounds **(1)**, **(2)**, **(3)**, **(4)**, **(5)** and **(6)**.

Oleanolic acid (1)

Colourless amorphous powder, mp. 310 °C ($CHCl_3$). The ^{1}H- and ^{13}C-NMR: See Table 1.

Betulin (2)

Colourless amorphous powder, mp. 256-257 °C ($CHCl_3$). The ^{1}H- and ^{13}C-NMR: See Table 1.

Betulinic acid (3)

Colourless amorphous powder, mp. 316-318 °C (CH_3OH). The ^{1}H- and ^{13}C-NMR: See Table 1.

Alphitolic acid (4)

Colourless amorphous powder, mp. 310-314 °C (CH_3OH). The ^{1}H- and ^{13}C-NMR: See Table 1.

Methyl gallate (5)

Colourless amorphous powder, mp. 201-203 °C (CH_3OH). The ^{1}H-NMR, CD_3OD, δppm: 7.01 (2H, s, H-2, H-6), 3.82 (3H, s, CH_3). The ^{13}C-NMR, CD_3OD, δppm: 169.1 (-COO-), 146.5 (C-3, C-5), 139.8 (C-4), 121.6 (C-1), 110.1 (C-2, C-6), 52.3 (CH_3-O-).

5-Hydroxymethylfurfural (6)

Colourless wax. The ^{1}H-NMR, $CDCl_3$, δppm: 6.59 (1H, d, 3.0, H-3), 7.39 (1H, d, 3.0, H-4), 9.60 (1H, s, -CHO), 4.91 (2H, s, -CH_2OH). The ^{13}C-NMR, $CDCl_3$, δppm: 153.4

(C-2), 123.7 (C-3), 110.2 (C-4), 163.0 (C-5), 178.2 (-CHO), 57.5 (-CH_2OH).

RESULTS AND DISCUSSION

Compound **(1)** was a triterpenee with 30 carbons in the ^{13}C-NMR spectrum. Based on two olefinic carbon signals at δ 143.6 and 122.6 and a singlet olefinic proton signal at δ 5.27 as well as seven singlet methyl proton signals at high resonance field showed that **(1)** was a triterpene with olean-12-en skeleton. Resonance at δ 79.0 (O-CH) was oxygenated carbons C-3 as usual. The appearance of the carboxyl signal (-COO-) at δ 183.5 was determined as C-28. Finally, the structure of **(1)** was established as oleanolic acid by comparison with data in the literature [9]. Oleanolic acid was known to have numerous of pharmacological activities including inhibitory activity against HIV-1 protease with IC_{50} of 6.3 μM [10], anti-inflammatory, anti-cancer, and hepato-protective effects, that were tested for their ability to modulate the activities of several cytochrome P450 (CYP) enzymes using human liver microsomes [4], inhibition the growth of ras oncogene-transformed R6 cells without toxicity to the normal cells [8]. Additionally, **(1)** had high yield, about 0.15% of dried leaves, and this material is easily collected in mangrove forest. So *Sonneratia alba* should be exploited to afford a valuable source for food and pharmaceutical products.

Compound **(2)** was a colourless amorphous powder. The ^{13}C-NMR spectral data of **(2)** showed that it was also a triterpene with 30 signals like **(1)**. However, the DEPT spectrum showed that **(2)** had two carbon signals of a disubstituted double bond at δ 109.7 (=CH_2) and 150.5 (=C<), these data supported **(2)** to be a lupane type triterpene. Beside an oxygenated methine group at 79.0 of C-3, **(2)** had another oxygenated methylene carbon signal at 60.6 ppm (C-28), matching with two proton signals [(3.80, *d*, 11.0 Hz, H-28a) and (3.35, *d*, 11.0 Hz, H-28b)]. Comparison spectroscopic data of **(2)** with those in literature [9] suggested that **(2)** was betulin (lup-20(29)-en-3*β*,28-diol).

Table 1. NMR data of compounds **(1)**, **(2)**, **(3)**, **(4)** and related references in CDCl3.

N°	(1)		(1*)	(2)		(2*)	(3)[a]		(3*)	(4)[a]		(4*a)
	δ_H (J=Hz)	δ_C	δ_C	δ_H (J=Hz)	δ_C	δ_C	δ_H (J=Hz)	δ_C	δ_C	δ_H (J=Hz)	δ_C	δ_C
1		38.4	38.5		38.7	38.8		39.3	38.7	-	48.2	48.2
2		27.2	27.4		27.4	27.2		28.3	27.4	4.08 (1H, *td*, 10.0, 4.5)	68.9	68.9
3	3.23 (1H, *d*, 7.0)	79.0	78.7	3.18 (1H, *dd*, 11.0, 5.0)	79.0	78.9	3.45 (1H, *m*)	78.1	78.9	3.39 (1H, *d*, 10.0)	83.8	83.8
4		38.7	38.7		38.9	38.9		39.5	38.8		38.8	38.8
5		55.2	55.2	0.69 (1H, *d*, 10.0)	55.3	55.3		55.9	55.3		56.1	56.1
6		18.3	18.3		18.3	18.3		18.8	18.3		18.8	18.8
7		32.6	32.6		34.3	34.3		34.8	34.3		34.8	34.8
8		39.3	39.3		40.9	40.9		41.1	40.7		41.2	41.2
9		47.6	47.6		50.4	50.4		50.9	50.5		51.0	51.0
10		37.1	37.0		37.2	37.2		37.5	37.2		39.9	39.9
11		22.9	23.1		20.9	20.9		21.2	20.8		21.4	21.4

12		122.6	122.1		25.2	25.3		26.1	25.5		26.1	26.1
13		143.6	143.4		37.4	37.3		38.6	38.4		38.6	38.6
14		41.6	41.6		42.7	42.7		42.8	42.4		42.9	42.9
15		27.7	27.7		27.1	27.0		31.2	30.5		30.2	30.2
16		23.4	23.4		29.2	29.2		32.9	32.1		32.9	32.9
17		46.5	46.6		47.8	47.8		56.6	56.3		56.6	56.7
18	2.83 (1H, *d*, 10.0)	41.0	41.3		48.8	48.8	3.53 (1H, *m*)	47.7	46.8		49.8	49.8
19		45.8	45.8		47.8	47.8		49.8	49.2		47.8	47.8
20		30.7	30.6		150.5	150.6		151.3	150.3		151.3	151.3
21		33.8	33.8		29.8	29.8		30.3	29.7		31.2	31.2
22		32.4	32.3		34.0	34.0		37.6	37.0		37.6	37.6
23	0.90 (3H, *s*)	28.1	28.1	0.97 (3H, *s*)	28.0	28.0	1.22 (3H, *s*)	28.6	27.9	1.05 (3H, *s*)	29.2	29.2
24	0.93 (3H, *s*)	15.5	15.6	0.76 (3H, *s*)	15.4	15.4	0.83 (3H, *s*)	16.3	15.3	0.90 (3H, *s*)	17.7	17.7
25	1.13 (3H, *s*)	15.3	15.3	1.02 (3H, *s*)	16.1	16.1	1.07 (3H, *s*)	16.4	16.0	1.04 (3H, *s*)	17.4	17.4
26	0.92 (3H, *s*)	17.1	16.8	0.83 (3H, *s*)	16.0	16.0	1.00 (3H, *s*)	16.4	16.1	1.04 (3H, *s*)	16.5	16.5
27	0.99 (3H, *s*)	26.0	26.0	0.98 (3H, *s*)	14.8	14.8	1.06 (3H, *s*)	14.9	14.7	1.25 (3H, *s*)	14.9	14.9
28		183.5	181.0	3.79 (1H, *dd*, 11.0, 2.0) 3.34 (1H, *d*, 11.0)	60.6	60.2	-	178.9	180.5		178.9	179.1
29	0.78 (3H, *s*)	33.1	33.1	4.68 (1H, *brs*) 4.58 (1H, *brs*)	109.6	109.6	4.94 (1H, *d*, 2.5) 4.76 (1H, *brs*)	109.9	109.6	4.92 (1H, *d*, 2.0) 4.76 (1H, *d*, 1.5)	109.4	109.9
30	0.76 (3H, *s*)	23.6	23.6	1.68 (3H, *s*)	19.1	19.1	1.79 (3H, *s*)	19.5	19.4	1.77 (3H, *s*)	19.5	19.5

Note: *a: Pyridine-d_5*

**: data in reference.*

Compound **(3)** was a colourless amorphous powder. The ^{13}C and DEPT-NMR spectral data of **(3)** and **(2)** are similar with 30 signals of a triterpene including two olefinic carbon signals at δ 109.9 (=CH_2) and 151.3 (=C<), one oxygenated methine carbon signal at δ 78.1 as usual. It matched with two olefinic proton signals at δ 4.94 and 4.76 and six singlet methyl proton signals at δ 1.8 to 0.8. However, **(3)** had a carboxyl signal at δ 178.9 (C-28) instead of an oxygenated methylene carbon signal. So **(3)** was a triterpenoid acid with a lupane skeleton. Moreover, the NMR spectral data of **(3)** showed a good compatibility to those in reference [9], so **(3)** was proposed to be betulinic acid (3*β*-hydroxylup-20(29)-en-28-oic acid). **(3)** exhibited inhibitory activities against HIV-1 replication in acutely infected H9 lymphocyte cells with EC_{50} values of 1.4 μM and TI values of 9.3 μM [2]. This compound was selectively cytotoxic against several human melanoma cancer cell lines (MEL-1 ED_{50}= 1.1 μg/ml, MEL-2 ED_{50}=2.0 μg/ml, and MEL-4 ED_{50}=4.8 μg/ml). Betulinic acid was then found to be active *in vivo* test using athymic mice carrying human melanomas, with a slight toxicity. Further biological studies indicated that betulinic acid works by induction of apoptosis [7].

Compound **(4)** was a colourless amorphous powder. The ^{13}C and DEPT-NMR spectral data of **(4)** and **(3)** were similar with 30 signals of a lupane skeleton including two olefinic carbon signals at δ 109.9 (=CH_2) and 151.3 (=C<), one

oxygenated methine carbon signal at δ 83.81 (C-3) as usual, one carboxyl signal at δ 178.9 (C-28). It matched with two olefinic proton signals at δ 4.92 and 4.76 and six singlet methyl proton signals at δ 1.8 to 0.8. However, **(4)** had one more oxygenated methine carbon signal at δ 68.9 (C-2) in ^{13}C spectrum, as well as one oxygenated proton signal at δ 4.08 (1H, td, 10.0, 4.5, H-2) in ^{1}H spectrum. The large coupling constant of H-2 supported that the hydroxyl group at C-2 had α-orientation. So **(4)** was alphitolic acid (2α,3β-dihydroxylup-20(29)-en-28-oic acid) *via* comparison with data in literature [4].

Compound **(5)** was a colourless amorphous powder. The ^{13}C and DEPT spectra showed that **(5)** had some carbon signals of one aromatic ring including two oxygenated carbon signals at δ 146.5 (C-3, C-5), 139.8 (C-4), a methine carbon signal at δ 110.1 (C-2, C-6), a quaternary carbon signal at δ 121.6 (C-1). Besides that, it had a carboxyl carbon signal at δ 169.1 and an oxygenated methyl carbon signal at δ 52.3. It corresponded to an aromatic proton signal at δ 7.01 (2H, s) and an oxygenated methyl proton signal at δ 3.82 (3H, *s*) in ^{1}H-NMR spectrum. So **(5)** was methyl gallate through the comparison with data in literature [3].

Compound **(6)** was a colourless amorphous powder. Its ^{1}H-NMR spectrum showed a proton signal at δ 9.60 (1H, *s*) of a formyl group, an oxygenated proton signal at δ 4.91 (2H, *s*), two doublet aromatic proton signals with small coupling contanst of 3.0 Hz at δ 7.39 and 6.59 of an aromatic ring with two *adjacent*-hydrogens. Furthermore, ^{13}C and DEPT spectra of **(6)** showed one signal at δ 178.2 of -**C**H=O, one oxygenated methylene carbon signal at 57.5 ppm and four aromatic carbon signals of a furane ring. Comparison the spectral data of compound (**6)** with those in the literature [6] showed a good compatibility, so the structure of **(6)** was elucidated as 5-hydroxymethylfurfural.

CONCLUSION

From the fresh leaves of *Sonneratia alba* J.E. Smith collected in Viet Nam; oleanolic acid **(1)**, betulin **(2)**, betulinic acid **(3)**, alphitolic acid **(4)**, methyl gallate **(5)** and 5-hydroxymethylfurfural **(6)** were isolated sucessfully. Further studies on this plant are in progress.

THÀNH PHẦN HÓA HỌC CỦA LÁ CÂY BẦN TRẮNG *SONNERATIA ALBA* J.E. SMITH., HỌ BẦN (SONNERATIACEAE)

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TÓM TẮT: *Cây Bần trắng là loài cây đặc hữu của rừng ngập mặn. Mặc dù đã có khá nhiều nghiên cứu trên các cây ngập mặn, tuy nhiên cây Bần trắng chưa được nghiên cứu nhiều trên thế giới. Ở Việt Nam, loài này chưa được tác giả nào khảo sát, nên cây Bần trắng được chọn làm đối tượng nghiên cứu của đề tài này. Từ cao eter dầu hỏa của lá cây Bần trắng, 6 hợp chất đã được cô lập gồm acid oleanolic (1), betulin (2), acid betulinic (3), acid alphitolic (4), methyl gallat (5) và 5-hydroxymethylfurfural (6). Cấu trúc hóa học của các hợp chất này được xác định dựa trên các phương pháp phổ nghiệm kết hợp so sánh với số liệu trong tài liệu tham khảo. Trong số bốn hợp chất trên, acid oleanolic hiện diện với hàm lượng cao, khoảng 0.15% so với bột lá khô. Các kết quả nghiên cứu cho thấy acid oleanolic có hoạt tính mạnh kháng HIV và kháng ung thư. Điều này định hướng cho việc khai thác lá Bần (nếu không thu hái lá cũng rụng bỏ), thu lấy acid oleanolic, chế biến thành các loại thực phẩm và dược phẩm có giá trị. Các nghiên cứu tiếp theo trên cây này vẫn đang được tiếp tục.*

Từ khóa: *Sonneratiaceae, Sonneratia alba, acid oleanolic, betulin, acid betulinic, acid alphitolic, methyl gallat, 5-hydroxymethylfurfural.*

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