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Some triterpenoids and steroids from *Bruguiera cylindrica* leaves collected from Can Gio mangrove forest

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Abstract

Bruguiera cylindrica (Rhizophoracea), distributed in the Southeast Asia, has been used as a traditional medicine in the treatment of diarrhea and healing of wounds. Some triterpenoids and steroids were isolated from the dried leaves of *Bruguiera cylindrica*, collected at Can Gio mangrove forest, Ho Chi Minh City, including lupeol (**1**), 3*β*-hydroxyoleana-9(11),12-diene (**2**), a mixture of stigmasterol **(3a)** and *β*-sitosterol (**3b**), cholesta-4-ene-3-one **(4)** and 3*β*-hydroxycholesta-5-ene-7-one (**5**). Their chemical structures were elucidated by spectroscopic analysis as well as comparing their data with the ones in the literature. Although these compounds were known in other plants this is the first time they are reported in this species.

Keywords. *Bruguiera cylindrica*, triterpenoids, steroids.

1. INTRODUCTION

Bruguiera cylindrica Blume (Rhizophoraceae), a mangrove plant, is distributed widely in Southeast Asia [6]. This plant has been used in Vietnamese traditional medicine for the treatment of diarrhea and healing of wounds [6, 7]. Four species of this genus are found in Vietnam including *B. cylindrica*, *B. gymnorhiza*, *B. parviflora* and *B. sexangula* [1]. In the study on Vietnamese mangrove plants, we investigated the chemical constituents of *B. cylindrica* leaves collected in Can Gio mangrove forest, Ho Chi Minh City, Viet Nam.

2. EXPERIMENTAL

Leaves of *Bruguiera cylindrica* were collected at Can Gio mangrove forest in Ho Chi Minh city, Vietnam in September 2015. The scientific name of the plant was authenticated by Dr. Pham Van Ngot, Faculty of Biology, Ho Chi Minh city Pedagogical University. A voucher specimen (**No US-B020**) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University - Ho Chi Minh City.

Column chromatography was carried out on silica gel 60 F_{254} (Merck) and gel Sephadex LH-20.

Extraction of Bruguiera cylindrica

The air-dried leaves *of Bruguiera cylindrica* (6 kg) were dried, ground then extracted with *n*-hexane, ethyl acetate, and methanol (3 x 20 L for each solvent), successively. The *n*-hexane extract (25 g) was subjected to column chromatography, eluted with a gradient of *n*-hexane/acetone to afford nine fractions (A1–A9). Fraction A3 (1.5 g) was purified by silica gel column chromatography to afford compound **1** (10 mg). Compound **3** (5 mg) was isolated from the fraction A5. Fraction A7 (2.49 g) was applied to silica gel column chromatography to give compound **4** (5 mg) and **5** (4 mg)

The ethyl acetate extract (100 g) was subjected to silica gel column chromatography, eluted with a gradient of *n*-hexane/ethyl acetate to afford nine fractions (B1–B9). Fraction B4 was subjected to Sephadex LH-20 column to give five fractions (B4.1–B4.5). Fraction B4.4 was further purified by silica gel column chromatography (chloroform/methanol, 95.5/0.5) to give compound **2** (5 mg).

3. RESULTS AND DISCUSSION

Compound **1** obtained as a white crystal is a triterpene due to the positive sulfuric acid test. The ^{1}H-NMR spectral data of compound **1** gave signals of an isopropenyl group at δ 4.72 (1H, *d*, *J* = 2.0 Hz, H-29a), 4.61 (1H, *dd*, *J* = 2.0, 1.5 Hz, H-29b) and 1.72 (3H, *s*, H-30), a methine proton at δ 3.16 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-3), six singlets belonging to six

methyl groups, including δ 0.79 (3H, *s*, H-24), 0.86 (3H, *s*, H-28), 0.91 (3H, *s*, H-25), 0.99 (3H, *s*, H-23), 1.02 (3H, *s*, H-27) and 1.11 (3H, *s*, H-26). The ^{13}C-NMR spectral data of compound **1** are shown in table 1. All the spectral data of compound **1** were similar to the one of lupeol in the literature [2, 5].

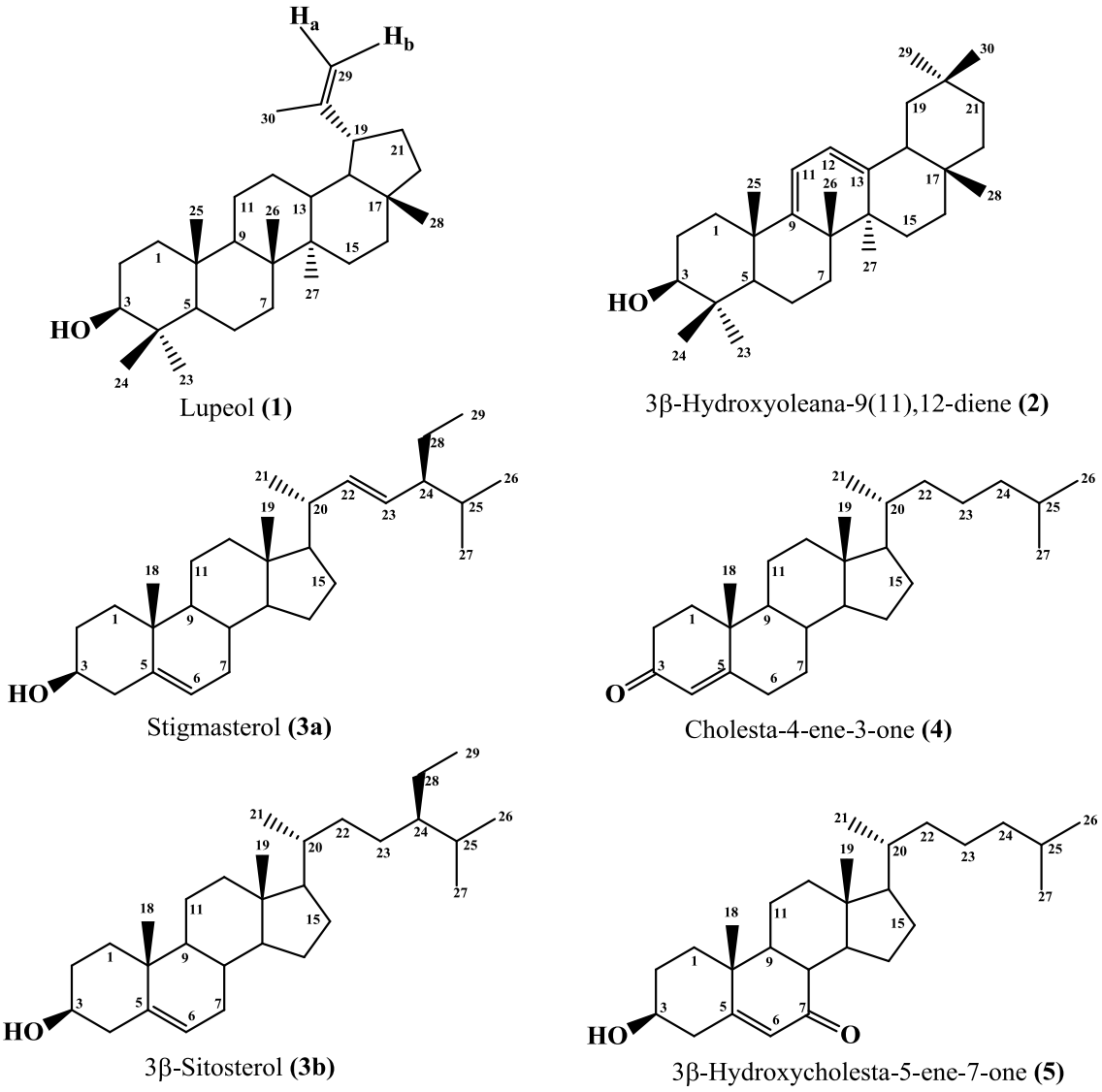

Figure 1: Chemical structure of isolated compounds

Compound **2** obtained as a white crystal. The molecular formula was established as $C_{30}H_{48}O$ from the APCI-MS data at m/z 425.44 $[M+H]^+$ (calc. 425.37). The ^{1}H-NMR spectral data of compound **2** gave one oxymethine doublet of doublet at δ 3.24 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-3), two signals of olefin protons at δ 5.57 (1H, *d*, *J* = 5.5 Hz, H-11) and 5.50 (1H, *d*, *J* = 5.5 Hz, H-12), eight singlets belonging to six methyl groups, including δ 0.81 (3H, *s*, H-24), 0.87 (3H, *s*, H-23), 0.89 (3H, *s*, H-29), 0.90 (3H, *s*, H-30), 0.99 (3H, *s*, H-28), 1.03 (3H, *s*, H-27), 1.14 (3H, *s*, H-25) and 1.19 (3H, *s*, H-26). The ^{13}C-NMR spectral data of compound **2** are shown in table 1. All the spectral data of compound **2** were similar to the ones of 3β-hydroxyoleana-9(11),12-diene in the literature [4, 5].

Compound **3 (3a** and **3b)** was isolated as a white amorphous powder. The APCI-MS gave a molecular ion peaks at *m/z* 397.36 $[M+H-H_2O]^+$ (calc. 397.38) of β-sitosterol and 395.42 $[M+H-H_2O]^+$ (calc. 395.38) of stigmasterol, the molecular ion peak of β-sitosterol is taller. The ^{1}H-NMR spectrum of **3** showed signals of olefin protons at δ 5.35 (3H, *d*, *J* = 5.5 Hz, H-6, H-6'), 5.15 (1H, *dd*, *J* = 15.0, 8.5 Hz, H-22) and 5.01 (1H, *dd*, *J* = 15.0, 8.5 Hz, H-23), along with one oxymethine multiplet at δ 3.52 (3H, *m*, H-3, H-3') and many other signals in the high field from δ 2.5-1.0. The ^{13}C-NMR spectrum showed four olefin carbon signals at δ 140.9 (C-5, C-5'), three olefin methines

at δ 138.5 (C-22), 129.5 (C-23), 121.9 (C-6, C-6') and one oxygenated methine at δ_C 71.9 (C-3, C-3') of a mixture *β*-sitosterol and stigmasterol [3]. All signals showed that **3** was a mixture of *β*-sitosterol and stigmasterol with the ratio of 1:2 based on their protons integrations.

Table 1: NMR data of compounds **1**, **2**, **4** and **5**

Pos.	Compound **1** **(CD_3OD)**		Compound **2** **($CDCl_3$)**		Compound **4** **($CDCl_3$)**		Compound **5** **($CDCl_3$)**	
	δ_C	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)
1	38.8		38.7		35.9		36.5	
2	27.4		27.9		34.1		31.4	
3	79.0	3.16 (1H, *dd*, 11.5, 4.5)	78.7	3.24 (1H, *dd*, 12.0, 5.0)	199.7		70.7	3.67 (1H, *m*)
4	38.7		38.9		123.8	5.72 (1H, *s*)	38.9	
5	55.4		51.2		171.8		165.2	
6	18.3		18.4		33.1		126.3	5.69 (1H, *s*)
7	34.3		32.1		32.2		202.5	
8	40.9		37.1		35.8		45.6	
9	50.5		154.3		54.0		50.1	
10	37.2		40.7		38.8		38.4	
11	21.0		115.7	5.57 (1H, *d*, 5.5)	21.2		21.4	
12	25.2		120.7	5.50 (1H, *d*, 5.5)	39.8		42.9	
13	38.1		147.1		42.6		43.3	
14	42.9		42.8		56.0		50.2	
15	27.5		25.7		24.3		26.3	
16	35.6		27.2		28.3		28.7	
17	43.0		31.9		56.2		54.9	
18	48.4		45.6		12.1	0.71 (3H, *s*)	12.1	0.68 (3H, *s*)
19	48.0	2.44 (1H, *m*)	46.9		17.5	1.17 (3H, *s*)	17.5	1.20 (3H, *s*)
20	151.0		31.1		35.8		35.9	
21	29.9		34.6		18.9	0.91 (3H, *d*, 6.5)	19.1	0.93 (3H, *d*, 6.5)
22	40.0		37.2		36.3		36.2	
23	27.9	0.99 (3H, *s*)	28.2	0.87 (3H, *s*)	23.2		23.2	
24	15.4	0.79 (3H, *s*)	15.4	0.81 (3H, *s*)	39.7		40.0	
25	16.1	0.91 (3H, *s*)	20.1	1.14 (3H, *s*)	28.3		29.3	
26	16.0	1.11 (3H, s)	21.0	1.19 (3H, *s*)	22.8	0.84 (3H, *d*, 7.0)	23.2	0.84 (3H, *d*, 7.0)
27	14.6	1.02 (3H, *s*)	25.3	1.03 (3H, *s*)	22.9	0.81 (3H, *d*, 7.0)	21.6	0.82 (3H, *d*, 7.0)
28	18.0	0.86 (3H, *s*)	28.7	0.99 (3H, *s*)				
29	109.3	4.72 (1H, *d*, 2.0) 4.61 (1H, *dd*, 2.0, 1.5)	23.7	0.89 (3H, *s*)				
30	19.3	1.72 (3H, *s*)	33.2	0.90 (3H, *s*)				

Compound **4** was isolated as a white amorphous powder. Based on the HR-ESI-MS spectrum, the molecular formula of **4** was determined as $C_{27}H_{44}O$ with a pseudomolecular ion peak at *m/z* 385.3448 $[M+H]^+$ (calc. for $C_{27}H_{44}O$+H, 385.3470). The ^{1}H-NMR spectrum of **4** displayed a singlet at *δ* 5.72 (1H, *s*, H-4), three methyl doublets at *δ* 0.91 (3H, *d*, *J* = 6.5 Hz, H-21), 0.84 (3H, *d*, *J* = 7.0 Hz, H-26), 0.81 (3H, *d*, *J* = 7.0 Hz, H-27), and two methyl singlets at *δ* 0.71 (3H, *s*, H-18), and 1.17 (3H, *s*, H-19). The ^{13}C-NMR spectral data of compound **4** were shown in table 1. All signals showed that **4** was cholesta-4-ene-3-one due to the compatibility of its spectroscopic data with the

one in the literature [8].

Compound **5** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of **5** showed one oxymethine multiplet at δ 3.67 (1H, *m*, H-3), an olefin singlet at δ 5.69 (1H, *s*, H-6), three methyl doublets at δ 0.93 (3H, *d*, *J* = 6.5 Hz, H-21,), 0.84 (3H, *d*, *J* = 7.0 Hz, H-26), 0.82 (3H, *d*, *J* = 7.0 Hz, H-27), and two methyl singlets at δ 0.68 (3H, *s*, H-18) and 1.20 (3H, *s*, H-19). The ^{13}C-NMR spectral data of compound **5** were shown in table 1. All signals showed that **5** was 3*β*-hydroxycholesta-5-ene-7-one due to the compatibility of its spectroscopic data with the one in the literature [9].

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