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Isolation and characterization of steroids from the stem bark of *Platanus kerrii* Gagnep.

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Abstract *Platanus kerrii* is an evergreen tree, native to Southeast Asia, distributed on the mountains along the borders of countries Vietnam - Laos. From the stem bark of this plant, two substances were isolated in*n*-hexane extract. The structures of these compounds have been identified as stigmast-4-ene-3-one (1) and ergosterol peroxide (2)by modern spectroscopic analysis.

Keywords *Platanus kerrii*, Stem bark, stigmast-4-ene-3-one, ergosterol peroxide.

Introduction

The genus *Platanus* is the unique living member of the Plantaceae family consisting of nine accepted species widespread throughout different regions of the world [1]. This is a small genus of trees known in English as the plane tree. *Platanus* genus is widely planted throughout the cities and the rural areas to make scene and improve the atmosphere [2]. Previous investigations on plantshave shown that this genus has interesting properties. In folk medicine *Platanus orientalis* was used for the treatment of diseases including gastro-intestinal disorders, toothache, skin disease, fever, and body pain [3], kidney stones and itching [4], dermatological, gastrointestinal, rheumatic and inflammatory diseases, blepharitis, conjunctivitis, and hemorrhage [5]. *Platanus acerifolia* was used medicinally for the treatment of different diseases and pathogens, such as dysentery, ophthalmia, toothache and vulnerary [6]. In recent studies, *Platanus orientalis* possesses anti-hepatotoxic, anti-oxidant and cytotoxic activities [7] and elicits anti-inflammatory and antinoceptive effects [8-9]. Previous chemical investigations, triterpenoids, proanthocyanidins and proanthocyanidin glycosides were also isolated from the barks [10-11], tocopherols, esters of phytols with fatty acids and several polyphenols were isolated from the leaves [12-13] and kaempferol derivatives and caffeic acid were isolated from the buds [14].

Platanus kerrii is woody perennial evergreen tree, endemic to Southeast Asia, distributed on the mountains along the borders of countries Vietnam - Laos and Vietnam – Cambodia [15]. The first investigation of our study about chemical composition and biological activity of this plant have been published [16-17]. In this paper, we reported the isolation and characterization of 2 steroids including stigmast-4-ene-3-one (**1**) and ergosterol peroxide (**2**) from the bark of *Platanus kerrii*.

Materials and Methods

Plant Material

The barks of *Platanus kerrii* were collected from the forest in Huong Son district, Ha Tinh province, Vietnam in June 2017. The plant was identified by Dr. Tran The Bach, Institute of Ecology and Biological Resources, VietNam Academy of Science and Technology. A voucher specimen (PK-01) was deposited in Biochemical Laboratory at the

Faculty of Natural Sciences, Hong Duc University. The barks after collection was dried under shade and crushed into powder.

Used equipment and chemicals

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with Ce-Mo stain.

Isolation and characterization

The material powder (2.25 kg) was extracted with *n*-hexane (3 L x 3 times, 24 hours/time) at room temperature. The combined extracts were evaporated *in vacuo* to obtain *n*-hexane residue (3 g). The *n*-hexane residue was chromatographed on silica gel column and eluted using gradient solvents with *n*-hexane-ethyl acetate (100:1 to 0:1, v/v) to afford 12 fractions (H1-H12**)**.The H5 fraction (0.15 g) was subjected to the chromatography column and eluted with dichloromethane- acetone (9:1, v/v)to give 3 sub-fractions H5.1-H5.3.The H5.1 fraction (42 mg) was purified by column chromatography on silica gel with n-hexane-ethyl acetate (9:1, v/v) as eluent to give compound **1** (20 mg).The H12 fraction (0.45 g) was separated by silica gel chromatography column using dichloromethane: methanol = 8: 2 as eluents to give 2 sub-fractions H12.1-H12.2.The H12-2 sub-fraction was purified by silica gel preparative TLC and eluted with dichloromethane: methanol (98: 2) to give compound **2** (4 mg).

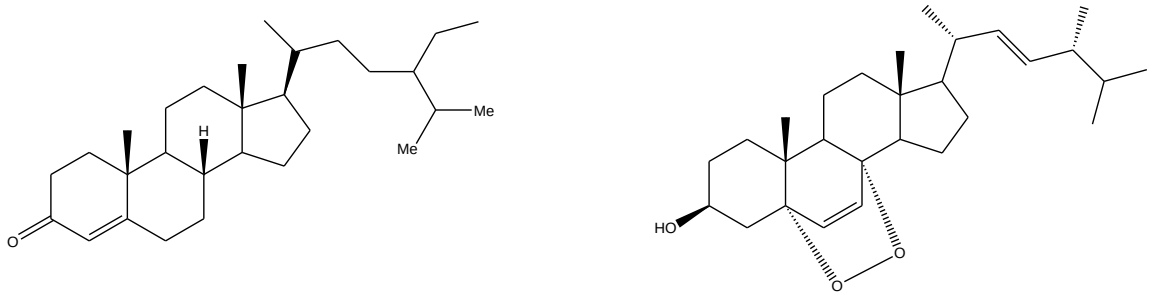
Stigmast-4-ene-3-one (**1**): White solid, mp: 295-298^{o}C.ESI-MS *m/z*413.1 $[M+H]^{+}$.

^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 5.71 (1H, brs, H-4), 1.17 (3H,s, H-19), 0.91 (3H, d, *J* = 6.6 Hz, H-21), 0.84 (3H, t, *J* = 7.5 Hz, H-29), 0.83 (3H,d, *J* = 7.5 Hz, H-26), 0.81 (3H, d, *J* = 7.0 Hz, H-27), 0.70 (3H,s, H-18).^{13}C NMR (125-MHz, $CDCl_3$): 199.6 (C-3),171.7 (C-5), 123.7 (C-4), 56.0 (C-17), 55.9 (C-14), 53.8 (C-9), 45.8 (C-24), 42.3 (C-13), 39.6 (C12), 38.6 (C-10), 36.1 (C-20), 35.7 (C-1), 35.6 (C-8), 34.0 (C-22), 33.9 (C-2), 33.0 (C-6), 32.1 (C-7), 29.1 (C-25), 28.2 (C-16), 26.0 (C-23), 24.2 (C-15), 23.1 (C-28), 21.0 (C-11), 19.8 (C-26), 19.0 (C-27), 18.7 (C-21), 17.4 (C-19), 12.0 (C-18), 11.9 (C-29).

Ergosterol peroxide (**2**): White solid, mp: 173-175 °C. ESI-MS *m/z*429.1 $[M+H]^{+}$

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 6.50 (1H, d, *J* = 8.5 Hz, H-6), 6.24 (1H, d, *J* = 8.5 Hz, H-7), 5.24-5.12 (2H, m, H-22, H-23), 3.99-3.94 (1H, m, H-3), 1.00 (3H, d, *J* = 7.0 Hz, H-21), 0.90 (3H, d, *J* = 6.5 Hz, H-28), 0.88 (3H, s, H-18), 0.83 (3H, d, *J* = 7.0Hz, H-26), 0.82 (3H, s, H-19), 0.81 (3H, d, *J* = 7.0 Hz, H-27). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 135.4 (C-6), 135.2 (C-22), 132.3 (C-23), 130.7 (C-7), 82.1 (C-5), 79.4 (C-8), 66.4 (C-3), 56.2 (C-17), 51.7 (C-14), 51.1 (C-9), 44.5 (C-13), 42.7 (C-24), 39.7 (C-20), 39.3 (C-12), 36.9 (C-4), 36.9 (C-10), 34.7 (C-1), 33.0 (C-25), 30.1 (C-2), 28.6 (C-16), 23.4 (C-15), 20.8 (C-21), 20.6 (C-11), 19.9 (C-26), 19.6 (C-27), 18.1 (C-19), 17.5 (C-28), 12.8 (C-18).

Results and Discussion



Stigmast-4-ene-3-one (**1**) Ergosterol peroxide (**2**)

Figure 1: Chemical structures of isolated steroids compounds from Platanus kerrii

Stigmast-4-ene-3-one (1)

Compound **1** was obtained from *n*-hexane extract as a white solid, mp 295-298^{o}C. The ESI-MS spectrum showed a molecular ion peak *m/z*413.1 $[M+H]^+$, suggested for molecular formula of **1** is $C_{29}H_{48}O_3$ (M= 412). The ^{1}H-NMR spectrum showed typical signals of a sterol compound with 6 methyl groups including 2 singlets at δ_H 1.17 (3H, s, H-19) and 0.70 (3H, s, H-18), 3 doublets at δ_H 0.91 (3H, d, *J* = 6.6 Hz, H-21), 0.83 (3H, d, *J* = 7.5 Hz, H-26), 0.81 (3H, d, *J* = 7.0 Hz, H-27) and a triplet at δ_H 0.84 (3H, t, *J* = 7.5 Hz, H-29). In addition, there is a singlet signal of an olefinic proton at δ_H 5.71. The ^{13}C-NMR and DEPT spectra of **1** showed the presence of 29 carbon signals including 6 CH_3 groups, 11 CH_2 groups and 8 CH groups. The carbonyl signal appeared at δ_C199.6 and olefinic carbon signals were at δ_C171.7 and 123.7.

Analytical NMR and MS data suggest that the structure of **1** is a sterol compound with an *α,β*-unsaturated carbonyl group. Compound **1** is identified as stigmast-4-ene-3-one. The NMR data is in good agreement with values in the reported literature (table 1) [18].

Ergosterol peroxide (5*α*,8*α*-epidioxy-22*E*-ergosta-6,22-dien-3*β*-ol) (2)

The compound **2**wasisolated in white crystalline form, mp = 173-175 °C. The ESI-MS spectrum exhibited protonated peak *m/z* 429.1 [M + H] indicating the molecular weight of **2** is 428, corresponding to the molecular formula $C_{28}H_{44}O_3$.

The ^{1}H-NMR spectrum of **2**also showed characteristic signals ofa sterol compound with 6 methyl groups including 2 singlets at δ_H0.88 (3H, s, H-18), 0.82 (3H, s, H-19) and 4 doublets at δ_H1.00 (3H, d, *J* = 7.0 Hz, H-21), 0.90 (3H, d, J = 6.5 Hz, H-28), 0.83 (3H, d, J = 7.0Hz, H-26), 0.81 (3H, d, J = 7.0 Hz, H-27). There were signals of 2 pairs of germinal olefinic protons at δ_H6.50 (1H, d, *J* = 8.5 Hz, H-6) and 6.24 (1H, d, *J* = 8.5 Hz, H-7), 5.24-5.12 (2H, m, H-22, H-23). In the spectrum, an oxymethine group was found atδ_H3.99-3.94 (1H, m, H-3).

The ^{13}C-NMR spectra and DEPT of compound **2**showed the signals of 28 carbons, including 6 CH_3 groups at δ_C 20.8 (C-21), 19.9 (C-26), 19.6 (C-27), 18.1 (C-19), 17.5 (C-28), 12.8 (C-18), 7 CH_2 groups, 11 CH groups, in which there were signal 2 pairs of olefinic carbons at δ_C 135.4 (C-6), 135.2 (C-22), 132.3 (C-23), 130.7 (C-7), one CH-OH group at δ_C 66.4 (C-3) and 3 quaternary carbons, among that 2 C-O signals were positioned at δ_C82.1 (C-5), 79.4 (C-8).These 2 peaks suggested a epoxide group in the molecule.

On the basis of the above MS, NMR spectral evidences, compound **2** is determined as ergosterol peroxide. The analytical NMR data of **2** are in accordance with those published [19].

Table 1: ^{1}H and ^{13}C-NMR data of compound **1-2** and reference compounds

C	Compound 1				Compound 2			
	$^{a,b}\delta_H$	$^{@}\delta_H$	$^{a,c}\delta_C$	$^{@}\delta_C$	$^{a,b}\delta_H$	$^{\#}\delta_H$	$^{a,c}\delta_C$	$^{\#}\delta_C$
1			35.7	35.7			34.7	34.6
2			33.8	33.9			30.1	30.1
3			199.6	199.6	3.99-3.94 (1H, m)	3.96, m	66.4	66.4
4	5.71 (1H, brs)	5.72 (1H, brs)	123.7	123.7			36.9	36.9
5			171.7	171.7			82.1	82.1
6			32.9	33.0	6.50 (1H, d, 8.5)	6,48 (1H, d, 8.4)	135.4	135.4
7			32.0	32.1	6.24 (1H, d, 8.5)	6.22 (1H, d, 8.4)	130.7	130.7
8			35.6	35.6			79.4	79.4
9			53.8	53.8			51.1	51.1
10			38.6	38.6			36.9	36.9
11			21.0	21.0			20.6	20.6
12			39.6	39.6			39.3	39.3

13			42.4	42.3			44.5	44.6
14			55.8	55.9			51.7	51.7
15			24.1	24.2			23.4	23.4
16			28.1	28.2			28.6	28.6
17			56.0	56.0			56.2	56.2
18	0.70 (3H, s)	0.71 (3H, s)	11.9	12.0	0.88 (3H, s)	0.86, s	12.8	12.9
19	1.17 (3H, s)	1.17 (3H, s)	17.3	17.4	0.82 (3H, s)	0.78, s	18.1	18.2
20			36.1	36.1			39.7	39.7
21	0.91 (3H, d, 6.6 Hz)	0.92 (3H, d, 6.6 Hz)	18.7	18.7	1.00 (3H, d, 7.0)	0.98 (d, 7.0)	20.8	20.9
22			33.9	34.0	5.24-5.12 (2H,	5.19-5.12 (2H,	135.2	135.2
23			26.0	26.0	m)	m)	132.3	132.3
24			45.8	45.8			42.7	42.8
25			29.1	29.1			33.0	33.0
26	0.83 (d, 3H,7.5 Hz)	0.84 (3H, d, 7.5 Hz)	19.8	19.8	0.83 (3H, d, 7.0)	0.82 (d, 7.0)	19.9	19.9
27	0.81 (3H, d, 7.0 Hz)	0.82 (3H, d, 7.0 Hz)	19.0	19.0	0.81 (3H, d, 7.0)	0.79 (d, 7.0)	19.6	19.6
28			23.0	23.1	0.90 (3H, d, 6.5)	0.88 (d, 6.5)	17.5	17.5
29	0.84 (3H, t, 7.5 Hz)	0.85 (3H, t, 7.5 Hz)	11.9	11.9				

[a]125 MHz, [b] $CDCl_3$, [c]500 MHz, @: stigmast-4-ene-3-one [18], # ergosterol peroxide [19].

Conclusion

An investigation of the *n*-hexane extract of the stem bark of *Platanus kerrii* led to the isolation of two steroids compounds including stigmast-4-ene-3-one (1) and ergosterol peroxide or 5α,8α-epidioxy-22E-ergosta-6,22-dien-3β-ol (2). Their chemical structures were elucidated by spectroscopic NMR and MS data and comparison with the reported literatures. These compounds were isolated from *P. kerrii* and *Platanus* genus for the first time.

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