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Cytotoxic Steroids from the Stem Barks of *Pandanus tectorius*

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ABSTRACT

n-Hexane extract of the stem barks of *Pandanus tectorius* Parkins. ex J. P. du Roi collected in Thuathien-Hue province, Vietnam has shown a good cytotoxicity activity against KB (a human epidermal carcinoma) cell line with the IC_{50} value of 45.33 μg mL^{-1}. Investigation on the chemical constituents of this extract has resulted in the isolation of four compounds: (a) Stigmast-4-en-3-one, (b) Stigmasta-4,22-dien-3-one, (c) Cycloeucalenol and (d) Stigmast-22-en-3β-ol. Their structures were determined by various spectroscopic methods. In addition, compounds (a-c) were evaluated their cytotoxic activity against KB cell line, compound b has moderate activity (IC_{50} value of 88.96 μg mL^{-1}).

Key words: *Pandanus tectorius*, stem barks, steroid, cytotoxicity

INTRODUCTION

The genus *Pandanus* (Pandanaceae) currently comprises approximately 600 species that are widely distributed in tropical and subtropical regions. Vietnam has four *Pandanus* species, including *P. amaryllifolius, P. tectorius, P. humilis* and *P. tonkinensis*. They are widely growing from the Northern to the Southern of Vietnam. In addition, *P. tectorius* is the most popular one (Vo, 1999). Several *Pandanus* species are used as a remedy for toothache and rheumatism and as diuretic, cardiotonic, etc. (Vo, 1999). Chemical investigation on the arial parts of these plants revealed that they contain many interesting secondary metabolites, for instance, monoterpenes and sesquiterpenes from *P. latifolius* (Macleod *et al*., 1982), alkaloids from *P. amaryllifolius* (Nonato *et al*., 1993), fatty acids from the fruits of *P. conoideus* (Southwell and Harris, 1992). n-Hexane extract of the stem barks of *Pandanus tectorius* has shown a good cytotoxicity activity against KB cell line with the IC_{50} value of 45.33 μg mL^{-1} that led us carry out the phytochemical study on this sample. This paper describes the isolation and cytotoxicity of four compounds from stem barks of the Vietnamese *Pandanus tectorius*.

MATERIALS AND METHODS

Materials: Stem barks of *Pandanus tectorius* Parkins. ex J. P. du Roi were collected Thuathien-Hue province in November 2012 and identified by MSc. Nguyen The Anh (Institute of Chemistry, VAST, Vietnam). Voucher specimens are deposited at Faculty of Chemistry, Hanoi University of Education (HOA01-2012).

METHODS

General: TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck). Preparative HPLC was performed on a Jasco PU-2087 instrument with a UV-2070 and RI-2031 detectors using a waters

5 SL-II column (10.0×250 mm), flow rate of 1.0 mL min^{-1}. NMR spectra were recorded on Varian Bruker Avance 500 MHz, using $CDCl_3$ as solvent. Chemical shifts are referenced to internal TMS (0 ppm, ^1H) and $CDCl_3$ (77.0 ppm, ^{13}C), respectively. The positive ion high-resolution ESI-MS were recorded on a Bruker Apex III Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer, equipped with a 7 Tesla superconducting magnet.

Extraction and isolation: Dried *P. tectorius* stem barks powders (5,000 g) were extracted with methanol (10 L×3). The methanolic extract was concentrated to give a residue (140 g) which was further partitioned into n-hexane, EtOAc, BuOH and water. The *n*-hexane crude extract (13.5 g) was chromatographed by Sephadex LH-20 column, eluting by $CHCl_3$/MeOH (1/1) solvent system to give two fractions. Fr.1 (10.5 g) was purified by silica gel column chromatography, using hexane/EtOAc gradient to give 10 fractions. Sub-Fr. 5 (97 mg) was purified by preparative HPLC, using hexane/EtOAc (15/1) to yield compound (a) (2.3 mg), compound (b) (2.6 mg). Compound (c) (9 mg) was obtained from sub-fr. 6 (185 mg) by prep. HPLC, hexane/EtOAc (9/1). Compound (d) (54 mg) was obtained from Fr. 2 (3 g) by silica gel column chromatography, eluting by hexane/EtOAc (6/1) as a solvent system.

Compound a: ESI-FTICR-MS: m/z $[M+H]^+$ calcd for $C_{29}H_{49}O$: 413.3783; found 413.3778. ^1H NMR (500 MHz, $CDCl_3$): δ 5.72 (1H, s, H-4), 1.18 (3H, s, H-19), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.85 (3H, m, H-29), 0.84 (3H, d, J = 6.8 Hz, H-26), 0.82 (3H, d, J= 6.8 Hz, H-27), 0.71 (3H, s, H-18). ^{13}C NMR: (Table 1).

Compound b: ESI-FTICR-MS: m/z $[M+H]^+$ calcd for $C_{29}H_{47}O$: 411.3627; found 411.3621. ^1H NMR (500 MHz, $CDCl_3$): δ 5.72 (1H, s, H-4), 5.15 (1H, dd, J = 9.0, 15.5 H-22), 5.03 (1H, dd, J= 8.5, 15.5 Hz, H-23), 1.18 (3H, s, H-19), 1.02 (3H, d, J = 7.5 Hz, H-21), 0.85 (3H, d, J = 6.0 Hz, H-27), 0.81 (3H, m, H-29), 0.80 (3H, d, J = 6.0 Hz, H-26), 0.73 (3H, s, H-18). ^{13}C NMR: (Table 1).

Compound c: ESI-FTICR-MS: m/z $[M+H]^+$ calcd for $C_{30}H_{51}O$: 427.3940; found 427.3934. ^1H NMR (500 MHz, $CDCl_3$): δ 4.72 (1H, s, H-30), 4.67 (1H, s, H-30), 1.03 (3H, d, J = 7.0 Hz, H-26), 1.02 (3H, d, J = 7.0 Hz, H-27), 0.98 (3H, d, J = 7.5 H-29), 0.97 (3H, s, H-18), 0.91 (3H, d, J = 4.5 Hz, H-21), 0.87 (3H, s, H-28), 0.39 (1H, d, J = 4.0 Hz, H-19), 0.14 (1H, d, J = 4.0 Hz, H-19). ^{13}C NMR: (Table 1).

Compound d: EI-MS: m/z 412 $[M]^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 5.35 ppm (1H, brd m, H-6), 5.14 (1H, m, H-23), 5.03 (1H, m, H-22), 3.49 ppm (1H, m, H-3), 1.00 (3H, s, H-19), 0.92 (3H, d, J = 5.5 Hz, H-21), 0.85 (3H, m, H-29), 0.82 (3H, d, J = 6.5 Hz, H-26), 0.81 (3H, d, J = 6.5 Hz, H-27), 0.68 (3H, s, H-18). ^{13}C NMR: (Table 1).

Cytotoxicity assay: The crude extract and compounds (a-c) were tested on cytotoxicity against KB cell line from American type culture collection according to the method described by Scudiero *et al.* (1988).

RESULTS AND DISCUSSION

The hexane extract of *P. tectorius* was subjected to silica gel column chromatography and followed by prep. HPLC to give four steroids (a-d).

Table 1: ^{13}C NMR spectral data for compounds a-d (ppm, in $CDCl_3$)

	Compound			
No.	a	b	c	d
1	35.70	35.7	30.8	37.3
2	34.00	34.0	34.8	31.7
3	199.70	199.6	76.6	71.8
4	123.80	123.8	44.6	42.3
5	171.70	171.7	43.4	140.8
6	33.90	33.0	24.7	121.7
7	33.00	32.1	28.1	31.9
8	35.70	35.7	46.9	31.9
9	53.90	55.9	23.6	50.2
10	38.60	38.6	29.7	36.5
11	21.10	21.0	25.2	21.1
12	39.70	39.6	32.9	39.8
13	42.40	42.3	45.4	42.3
14	55.90	53.9	48.9	56.8
15	24.20	24.3	35.0	24.3
16	28.20	28.9	27.2	28.3
17	56.10	56.0	52.2	56.1
18	12.00	12.2	17.8	11.9
19	17.40	17.4	27.0	19.4
20	36.10	40.5	36.1	40.5
21	18.70	21.2	18.4	18.8
22	32.10	138.1	35.4	138.3
23	26.10	129.5	31.3	129.3
24	45.90	51.3	156.9	51.3
25	29.20	31.9	33.8	29.2
26	19.80	19.0	22.0	19.8
27	19.10	21.1	21.9	19.1
28	23.10	25.4	19.1	23.1
29	12.00	12.2	14.4	12.0
30			105.9	

The molecular formula of compound (a) was found to be $C_{29}H_{48}O$ by FT-ICR-MS (m/z 413.3778, $[M+H]^+$). The ^1H NMR spectrum shows the presence of six methyl groups at 1.18, 0.92, 0.85, 0.84, 0.82, 0.71 ppm and one olefinic proton at 5.72 ppm. The ^{13}C NMR spectrum of compound (a) shows 29 carbon signals, including two olefinic carbons at 123.8 and 171.7 ppm, one conjugated ketone at 199.7 ppm (Table 1). Interpretation of its 2D NMR (HSQC and HMBC) suggests that compound (a) is a sterol which has identical NMR spectral data with those of stigmast-4-en-3-one (Su *et al*., 2009). Therefore, compound a is found to be stigmast-4-en-3-one as shown in Fig. 1.

Compound (b) was also obtained from hexane extract of *P. tectorius*. Its molecular formula was identified as $C_{29}H_{46}O$ by FT-ICR-MS. Its ^1H NMR spectrum has signals of three olefinic protons at 5.72, 5.15, 5.03 ppm and six methyl groups. In addition, its ^{13}C NMR spectrum show the presence of 29 carbons. Compound (b) has very similar spectral data with those of compound (a) except for presence of two olefinic protons instead of two methylenes. Two olefinic protons are determined to be H-22 and H-23 based on its HMBC spectrum. Consequently, compound (b) is found to be stigmasta-4,22-dien-3-one (Balde *et al*., 2000) as shown in Fig. 1.

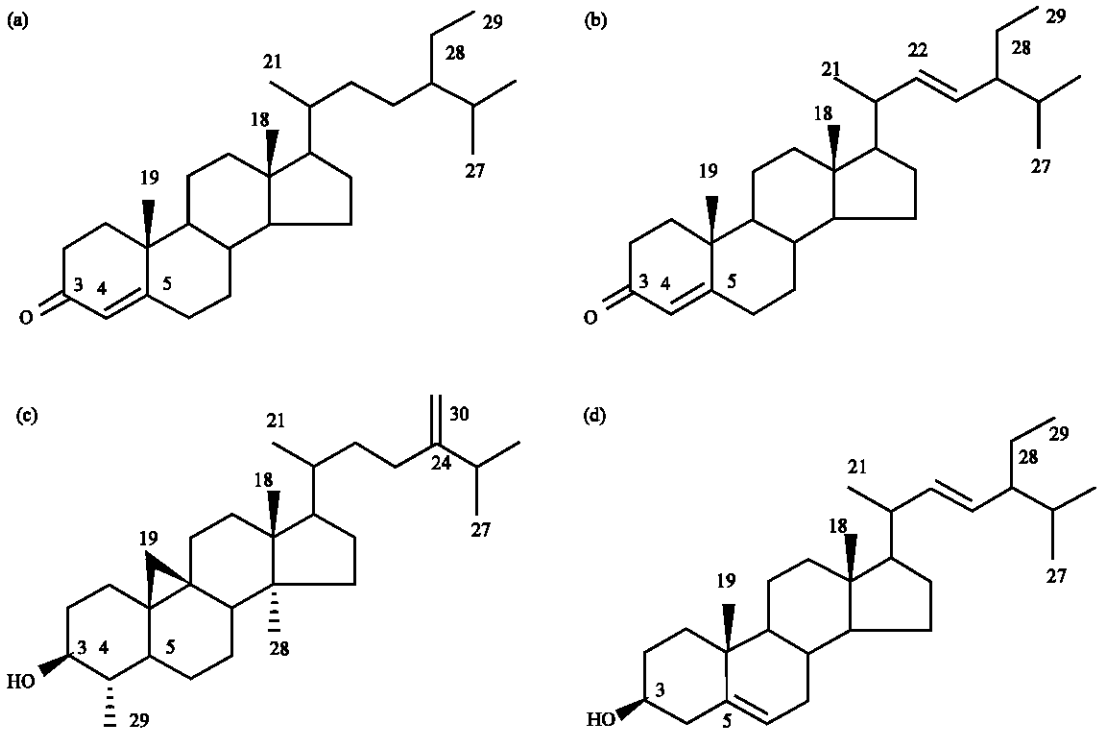

Fig. 1(a-d): Structures of (a) Stigmast-4en-3-one, (b) Stigmasta-4, 22-dien-3-one, (c) Cycloeucalenol and (d) Stigmast-22-en-3β-ol

The molecular formula of compound (c) was found to be $C_{30}H_{50}O$ by FT-ICR-MS. Analysis of its ^1H NMR spectra revealed that it has an *exo*-methylene (δ_H = 4.72 and 4.67 ppm), six methyl groups and two signals at very high filed (δ_H = 0.39 and 0.14 ppm) suggesting the presence of a cyclopropane ring. The ^{13}C NMR spectrum has resonances of 30 carbons. Compound (c) has very similar spectral data with those of cycloeucalenol (Song *et al.*, 2007). Therefore, compound (c) was determined as cycloeucalenol.

From the EI-MS data of (d) was afforded m/z 412 $[M]^+$, corresponding to a molecular formula of $C_{29}H_{48}O$. The ^1H NMR signals indicates the presence of three olefinic protons at 5.35, 5.03, 5.14 ppm and six methyl signals. The ^{13}C NMR spectrum (Table 1) revealed 29 carbon signals. The NMR spectral data suggest that it is a sterol. The mass, ^1H and ^{13}C NMR spectral data identified (d) as stigmast-5,22-dien-3β-ol (Good and Akisha, 1997).

Cytotoxic assay of compounds (a-c) was carried out to evaluate their anticancer property against KB cell line. The result shows that compound (b) has moderate activity (IC_{50} value of 88.96 μg mL^{-1}).

CONCLUSION

Chemical composition of n-hexane extract of the stem barks of *Pandanus tectorius* collected in Thuathien-Hue province, Vietnam has been investigated. Four compounds, stigmast-4-en-3-one (a), stigmasta-4,22-dien-3-one (b) cycloeucalenol (c) and stigmast-22-en-3β-ol (d) were isolated and structural elucidated by HREIMS and 1D, 2D NMR spectroscopies. Compound (b) has moderate cytotoxicity activity towards KB cell line (IC_{50} value of 88.96 μg mL^{-1}).

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