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STEROIDS FROM THE SUPER RED DRAGON FRUIT (*Hylocereus costaricensis*)

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ABSTRAK

Dua senyawa steroid, 7α-Hydoxy β-sitosterol (**1**) dan β-sitosterol (**2**), telah diisolasi dari ekstrak etil asetat Buah Naga Merah Super (*Hylocereus costaricensis*). Struktur kimia senyawa **1** dan **2** diidentifikasi berdasarkan data-data spektroskopi meliputi, UV, IR, NMR-1D, NMR-2D dan massa serta perbandingan data spektra dari penelitian sebelumnya. Senyawa **1** dan **2** pertama kali dilaporkan pada buah naga merah (*Hylocereus costaricensis*).

Kata kunci: 7α-Hydoxy β-sitosterol, β-sitosterol, *Hylocereus costaricensis*, steroids.

ABSTRACT

Two steroids compounds, 7α-Hydoxy β-sitosterol (**1**) and β-sitosterol (**2**), have been isolated from ethyl acetate extract of the fresh Super Red Dragon Fruit (*Hylocereus costaricensis*).The chemical structure of compounds **1** and **2** were identified by spectroscopic data including UV, IR, NMR-1D, NMR-2D and mass as well as by comparing with previously reported spectral data. Compounds **1** and **2** were reported for the first time from dragon fruit (*Hylocereus costaricensis*).

Keywords: 7α-Hydoxy β-sitosterol, β-sitosterol, *Hylocereus costaricensis*, steroids.

INTRODUCTION

Steroids is an important class of secondary metabolites, widely widespread in plants, animals, marines as well as fungi and have similarity to cholesterol in structure (Saeidnia et al., 2014), including β-sitosterol, campesterol, stigmasterol and cycloartenol (Ostlund, 2002). Sterols, especially β-sitosterol was reported to have interesting activity including anti-inflammatory (Prieto et al., 2006), inducing apoptosis (Chai et al., 2008; Park et al., 2007; Ju et al., 2004), chemoprotective or chemopreventive effects (Ovesna et al., 2004), hypocholesterolemic (Zak et al., 1990), angiongenic effect (Moon et al., 1999), anti-diabetic (Gupta et al., 2011; Jamaluddin et al., 1994; Radika et al., 2013), and anti-oxidant (Baskar et al., 2012; Vivancos and Moreno, 2005).

Super Red Dragon fruit (*Hylocereus costaricensis*), known in Indonesia as "Naga Merah Super" is a promising tropical fruit which can be cultivated in different tropical and subtropical parts of the world such as Southeast Asia, and Central and South America. The demand for Super Red Dragon Fruit extensively increases and the fruit today can be found on almost all exotic fruit markets around the world (Salakpetch, 2000; Mohd, 2010).

Previous phytochemical studies on the species of *H. costaricensis* have revealed the presence mostly of polyphenolic compounds

with interesting biological activities, including (Strack et al., 2003; Karamaae et al., 2006; Wong and Siow, 2015; Wybraniec et al., 2001).

Although polyphenolic compounds of this species have been investigated previously, the steroid composition of *H. costaricensis* is yet to be reported. The isolation and structure identification of these isolated compounds are described herein.

MATERIAL AND METHODS

General Experimental Procedure

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Synapt G2 mass spectrometer instrument. NMR data were recorded on a Jeol ECZ-500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, and TMS as internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating.

Plant material

The fresh fruit of *H. costaricensis* were collected in Plantation Plant at Cicalengka District, West Java Province, Indonesia in April 2017. The plant was identified by the Mr. Joko Kusmoro, staff of the Laboratory of Plant Taxonomy, Department of Biology, Universitas Padjadjaran and a voucher specimen was deposited at the herbarium.

Extraction and isolation

The fresh fruit (25 kg) was extracted with methanol (30 L) at room temperature for 3 days. After removal of the solvent under vacuum, the concentrated of MeOH extract (120.5 g) was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-BuOH, successively. Evaporation resulted in the crude extracts of *n*-hexane (10.3 g), EtOAc (30.4 g), and *n*-BuOH (21.6 g), respectively. The *n*-hexane soluble fraction (10.3 g) was fractionated by vacuum liquid chromatography on silica gel 60 using a gradient *n*-hexane and EtOAc to give nine fractions (A–I). Fraction A (6 g) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane–CH_2Cl_2 (10:0–1:1) to give ten subfractions (A01–A10). Subfraction A03 was chromatographed on a column of silica gel, eluted with *n*-hexane:$CHCl_3$ (9:1) to give **1** (12.4 mg). Fraction B (12.4 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane and EtOAc to give eight fractions (J–Q). Fraction K (927.6 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane–EtOAc (10:0–0:10) to give **2** (10.2 mg).

RESULT AND DISCUSSION

The phytochemical test by using Lieberman-Buchard reagents for the *n*-hexane extract showing the presence of steroids. By using phytochemical test to follow separations, the *n*-hexane fraction was separated by column chromatography over silica gel by gradient elution. The fractions were repeatedly subjected to normal-phase column chromatography on silica gel to produce two steroids **1** and **2** (Figure 1).

7*α*-Hydroxy-*β*-sitosterol (1). White needle-like crystals, m.p. 138-140 ^{o}C; IR (KBr) ν_{max} 3450, 2940, 2860, 1469, 1365, 1045 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm): 1.70 (1H, m, H-1a), 1.72 (1H, m, H-1b), 1.48 (1H, m, H-2a), 1.58 (1H, m, H-2b), 3.50 (1H, m, H-3), 2.20 (1H, m, H-4a), 2.41 (1H, m, H-4b), 5.43 (1H, t, *J*=5.2, H-6), 4.21 (1H, m, H-7), 0.89 (1H, m, H-8), 1.45 (1H, m, H-9), 1.40 (1H, m, H-11a), 1.45 (1H, m, H-11b), 1.18 (1H, m, H-12a), 1.30 (1H, m, H-12b), 0.90 (1H, m, H-14), 1.54 (1H, m, H-15a), 1.06 (1H, m, H-15b), 1.45 (1H, m, H-16a), 1.25 (1H, m, H-16b), 1.28 (1H, m, H-17), 1.15 (3H, s, Me-18), 0.71 (3H, s, Me-19), 1.84 (1H, m, H-20), 0.90 (3H, d, *J*=6.2, Me-21), 2.14 (1H, m, H-22a), 2.23(1H, m, H-22b), 2.17 (1H, m, H-23a), 2.33 (1H, m, H-23b), 1.60 (1H, m, H-24), 1.82 (1H, m, H-25), 0.86 (3H, d, *J*=6.5 Hz, Me-26), 0.85 (3H, d, *J*=5.2 Hz, Me-27), 1.30 (1H, m, H-28a), 1.48 (1H, m, H-28b), 1.81 (3H, t, *J*=5.2 Hz, Me-29); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; TOFMS (negative ion mode) *m/z* 413.3811 $[M-H]^-$, (calcd. $C_{29}H_{49}O^-$, *m/z* 413.3844).

***β*-sitosterol (1).** White needle-like crystals, m.p. 143-145 ^{o}C; IR (KBr) ν_{max} 3440, 2935,

2869, 1452, 1368, 1045 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm): 1.75 (1H, m, H-1a), 1.82 (1H, m, H-1b), 1.65 (1H, m, H-2a), 1.72 (1H, m, H-2b), 3.65 (1H, m, H-3), 2.34 (1H, m, H-4a), 2.38 (1H, m, H-4b), 5.21 (1H, t, *J*=5.0, H-6), 2.56 (1H, m, H-7a), 2.72 (1H, m, H-7b), 0.90 (1H, m, H-8), 1.48 (1H, m, H-9), 1.50 (1H, m, H-11a), 1.55 (1H, m, H-11b), 1.25 (1H, m, H-12a), 1.35 (1H, m, H-12b), 0.96 (1H, m, H-14), 1.64 (1H, m, H-15a), 1.16 (1H, m, H-15b), 1.60 (1H, m, H-16a), 1.41 (1H, m, H-16b), 1.32 (1H, m, H-17), 1.35 (3H, s, Me-18), 0.81 (3H, s, Me-19), 1.84 (1H, m, H-20), 0.96 (3H, d, *J*=6.2, Me-21), 2.35 (1H, m, H-22a), 2.50 (1H, m, H-22b), 2.26 (1H, m, H-23a), 2.53 (1H, m, H-23b), 1.90 (1H, m, H-24), 1.92 (1H, m, H-25), 0.96 (3H, d, *J*=6.5 Hz, Me-26), 0.88 (3H, d, *J*=5.2 Hz, Me-27), 1.46 (1H, m, H-28a), 1.57 (1H, m, H-28b), 1.91 (3H, t, *J*=5.2 Hz, Me-29); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; TOFMS (negative ion mode) *m/z* 413.3862 $[M-H]^-$, (calcd. $C_{29}H_{49}O^-$, *m/z* 413.3789).

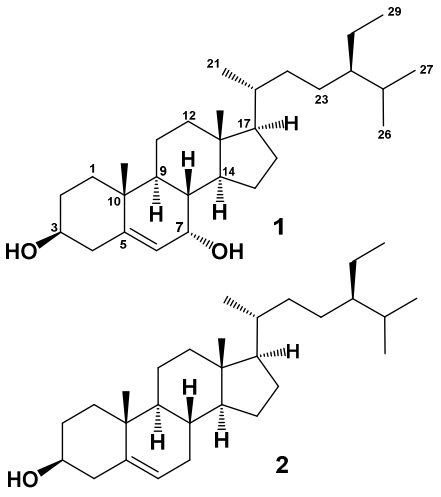

Figure 1. Chemical Structure of **1** and **2**

Compound **1** was obtained as a white needle-like crystal. The TOFMS spectrum showed $[M-H]^+$ *m/z* 413.3811 (calcd. *m/z* 413.3844), which coresponded to the molecular formula $C_{29}H_{50}O$ and thus required requiring hydrogen deficiency index of five, originating from one pairs of C sp^2 and the remaining tetracyclic stigmastane-type steroid. The IR spectra showed absorption peaks at 3450 cm^{-1} (OH), 2940 and 2860 cm^{-1} (aliphatic), 1469 cm^{-1} (C=C), 1365 and 1240 cm^{-1} (*gem*-dimethyl groups), and 1045 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 500 MHz) spectrum showed the presence of six methyl groups, two tertiary methyl groups resonating at δ_H 1.15 (Me-18), 0.71 (Me-19), three secondary methyl groups resonating at δ_H 0.90 (3H, d, *J* = 6.2 Hz, Me-21), 0.86 (3H, d, *J* = 6.5 Hz, Me-26), and 0.85 (d, *J* = 5.2 Hz, Me-27), one primary methyl group resonating at δ_H 1.81 (t, *J* = 5.2 Hz, Me-29), which was indicate the presence of stigmastane-type steroid skeleton (Cayme and Ragasa, 2004, Farabi et al., 2017). One olefinic methine group, resonating at δ_H 5.43 (1H, t, *J*=5.2, H-6), and oxymethine group resonating at δ_H 3.50 (1H, m, H-3) also observed at ^{1}H NMR spectra. The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Figure 2). ^{1}H-^{1}H COSY cross peak observed at H-2/H-3/H-4 indicated that position of hydroxy group at C-3. The cross peak also observed at H-6/H-7/H-8 that indicated the position of double bond at C-5/C-6. The ^{13}C-NMR ($CDCl_3$ 125 MHz) and HMQC and DEPT 135° spectra showed the presence of six methyl groups, one olefinic methine, one olefinic quartenary carbon, and a oxygenated methine group, resonating at δ_C 71.5 (C-3) and 74.5 (C-7), indicated the characteristic of stigmastane-type steroid (Cayme and Ragasa, 2004; Farabi et al., 2017). These functionalities accounted for one of total five degree of unsaturations. The remaining four degrees of unsaturation were consistent with the stigmastane-type steroid. Correlation of H-2 and H-4 to an oxygenated carbon at δ_C 71.5, indicated that hydroxyl group attached at C-3. Another hydroxyl group was located at C-7 based on the correlation of H-6 and H-8 to oxygenated carbon at C-7 (δ_C 74.5). A comparison of the NMR data of **1** with the data for 7α-Hydroxy-β-sitosterol (Chaturvedula and Prakash, 2012; Farabi et al., 2017), revealed that the structure of the two compounds were very similar, consequently compound **1** was identified as a 7α-Hydroxy-β-sitosterol, which shown in this plant for the first time.

Compound **2** was obtained as a white needle-like crystal. The IR spectra showed absorption peaks at 3480 cm^{-1} (OH), 2960 and 2865 cm^{-1} (aliphatic), 1472 cm^{-1} (C=C), 1382 and 1238 cm^{-1} (*gem*-dimethyl groups), and 1040 cm^{-1} (C-O). The NMR spectra of **2** very similar with **1**, the main difference was that compound **2** the absence of hydroxyl group at [δ_H 4.21 (1H, m, H-7), δ_C 74.5] and the presence of the methylene signal at [δ_H 2.56 (1H, m, H-7a),

2.72 (1H, m, H-7b), δ_C 32.4], suggested that **2** is 7-dehydroxy derivative of **1**. In comparison of **2** with literature data of a stigmast-5-en-3β-ol (β-sitosterol) (Chaturvedula and Prakash, 2012; Harneti et al., 2014; Farabi et al., 2017), showed good agreement, therefore compound **2** was identified as a stigmast-5-en-3β-ol (β-sitosterol), which shown in this plant for the first time.

Table 1. NMR data for compounds **1** and **2***

Position of Carbon	**1** δ_C (mult.)	**2** δ_C (mult.)
1	36.6 (t)	37.1 (t)
2	31.4 (t)	32.6 (t)
3	71.5 (d)	72.2 (d)
4	42.8 (t)	42.3(t)
5	145.2 (s)	146.1 (s)
6	122.5 (d)	123.6 (d)
7	74.5 (d)	34.6 (t)
8	32.4 (d)	31.4 (d)
9	50.6 (d)	51.6 (d)
10	36.6 (s)	37.6 (s)
11	21.5 (t)	22.5 (t)
12	40.2 (t)	41.2 (t)
13	42.6 (s)	41.6 (s)
14	57.4 (d)	58.4 (d)
15	26.7 (t)	27.7 (t)
16	28.8 (t)	27.9 (t)
17	56.4 (d)	56.9 (d)
18	12.6 (q)	12.2 (q)
19	19.1 (q)	18.5 (q)
20	36.2 (d)	35.2 (d)
21	19.0 (q)	18.5 (q)
22	34.4 (t)	34.2 (t)
23	26.0 (t)	26.5 (t)
24	45.4 (d)	44.8 (d)
25	29.6 (d)	29.4 (d)
26	19.0 (q)	18.5 (q)
27	20.4 (q)	20.2 (q)
28	23.0 (t)	22.5 (t)
29	12.5 (q)	12.4 (q)

*measured in $CDCl_3$ (500 MHZ for ^1H and 125 MHz for ^{13}C)

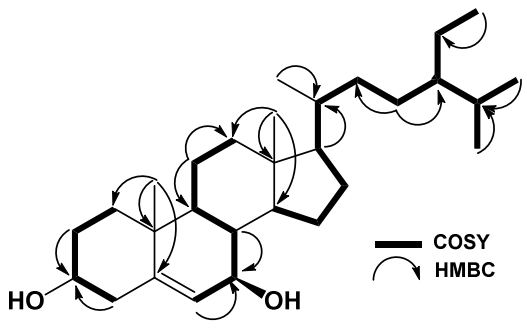

Figure 2. Selected ^1H-^1H COSY and HMBC correlation for compound **1**

CONCLUSIONS

Two steroids have been isolated from the fresh fruit of *H. costaricensis* and identified by spectroscopic methods as 7*α*-Hydroxy-*β*-sitosterol and β-sitosterol (**2**), The investigation of these steroids were shown in this species for the first time.

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