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β-Sistosterol and β-sitostenone from *Eucalyptus deglupta*

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Research on natural products has been rapidly increasing recently, as isolation and synthetic techniques have progressed, and a wide variety of biological properties have been found. This work is planned to analyze the phytochemical constituents of the stem of *Eucalyptus deglupta*. Two known compounds, β-sitosterol (**K-1**) and β-sitostenone (**K-2**), were isolated from the hexane extract of *Eucalyptus deglupta*. Two compounds have been identified by extensive techniques of spectroscopy (IR, NMR, and high-resolution MS) and by comparison with the literature data. The two compounds are known and isolated from other plants, but they are recorded for the first time from this plant. In addition, β-sitostenone (**K-2**) was isolated for the first time in genus *Eucalyptus*.

Keywords: *Eucalyptus deglupta*, Myrtaceae, spectroscopic techniques, β-sitosterol, β-sitostenone.

Introduction

Eucalyptus deglupta blume belongs to the family Myrtaceae and it is found mainly in tropical areas, including Indonesia. This genus is rich in essential oil. Pharmacological studies have shown that essential oil from *Eucalyptus* spp. have anti-bacterial, anti-inflammatory, anti-fungal, anti-viral, and cytotoxic activities. Moreover, a significant number of species were used to treat flue, cold, fever, and bronchial infections in traditional Brazilian medicine. In previous research on the leaves of these species, β-diketone derivatives such as 4-hydroxytritriacontane-16,18-dione and 16-hydroxy-18-tritria-contanone, flavonoids such as cypellogins A, B, C, eucalyptine, and terpenoids such as 3β-acetate-12,20(29)-lupadien-28-oic acid, ursolic acid, β-sitosterol, amirinic acid, ursolic acid lactone, betulinic acid, oleanolic acid, 2α,3β,7β-trihydroxy-11α-methoxyurs-12-en-28-oic acid, 2α,3β,7β-trihydroxyurs-11-en-28,13β-olide, nerolidol, and squalene were isolated, but there was no information on the β-sitosterol components of *Eucalyptus deglupta* Blume, and β-sitostenone components of *Eucalyptus* spp.[1–12]. In the current study, we express the isolation and structure elucidation of β-sitosterol and β-sitostenone from the hexane extract of *Eucalyptus deglupta* Blume.

Experimental

General:

To collect physical data, the following equipments were used: melting point, Fisher Johns; IR spectra, FTIR-8400S (Shimadzu) spectrophotometer; HR-MS spectra, JEOL JMS-700 mass spectrometer; ^{1}H NMR spectra, AVANCE NEO (500 MHz); and ^{13}C NMR AVANCE NEO (125 MHz) spectrometer with a residual solvent as an internal standard. For chromatography, the following techniques have been used: silica gel column chromatography, Merck Kieselgel gel 60 (0.063–

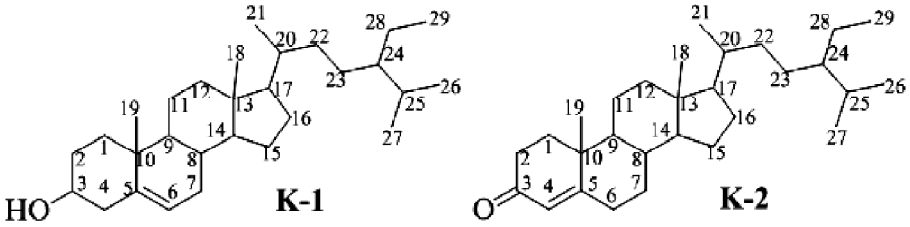

Fig. 1. Chemical structures of compound **K-1**, and **K-2** from the stem of *Eucalyptus deglupta.*

0.200 mm); preparative TLC, precoated silica gel 60 F_{254} plates, Merck, Darmstadt, Germany; and detected under UV lamp before dipping in anisaldehyde solution followed by heating. All solvents used for chromatographic separation were obtained from commercial chemicals and solvent available in Surabaya, Indonesia, and distilled before use.

Plant materials:

In July 2019, stem of *Eucalyptus deglupta* Blume was collected in Primary Forest ("Hutan Primer"), Palu city, North Sulawesi, Indonesia, and determined by the Biology Department, Faculty of Science and Technology, Universitas Airlangga. A sample voucher was saved in the Biology Department.

Extraction and isolation:

The *Eucalyptus deglupta* dried powder stem (4600 g) was extracted at room temperature with methanol over 72 h. The solutions were then filtrated and evaporated by using the rotary vacuum evaporator to concentrate under heat. 1.2 kg of crude extract was obtained. The extract was subsequently partitioned with numerous organic solvents to provide the following fractions, hexane, EtOAc, and MeOH fractions. 1.2 g of hexane fraction was chromatographed with silica gel column, eluted with gradient solvent mixtures of increasing polarity: hexane-EtOAc, 9:1–7:3, and then 100% EtOAc. A total of 6 combined fractions (ED-1 to ED-6) were obtained. Fraction ED-3 (212 mg) was then purified by preparative TLC (silica gel, n-hexane/$CHCl_3$, 6:4) to obtain white solids of **K-1** (30 mg) and oily form of **K-2** (5.7 mg).

Results and discussion

K-1 was obtained as white solids. HR-MS, 1D NMR and 2D NMR data identified the molecular formula $C_{29}H_{50}O$ (*m/z* 414.3862). The proton NMR spectrum revealed six methyl signals, four doublets (δ_H 0.90, 0.81, 0.79), and 0.83, and two singlet (δ_H 0.66 and 0.99) signals. These signals were allocated to the carbon atom positions H_3-18, H_3-19, H_3-21, H_3-26, and H-27, respectively. Besides this, there were an olefinic multiplet signal (δ_H 5.33), and the multiplet methine proton (δ_H 3.50) signal that connected to the carbon which attached to the OH group. These two signals were allocated to H-6 and H-3 protons. According to the molecular formula, in the ^{13}C NMR and DEPT experimental classification (Table 1), 29 carbon resonances comprising 9 methine (1 sp^2 and 8 sp^3) 11 methylene, 3 quaternary (1 sp^2 and 2 sp^3), and 6 methyl carbons were resolved. In HMBC experiment, the following correlations were observed (Fig. 2); the methyl signal at δ_H 0.66 (H_3-18) connected with four sp^3 carbons, including two methine, one methylene, and one quaternary carbon, at δ_C 56.8, 56.1, 39.8, and 42.3; the methyl signal at δ_H 0.99 (H_3-19) connected with four carbons, including two quaternary carbons (1 sp^3 and 1 sp^2), one sp^3 methine carbon, and one sp^3 methylene carbon, at δ_C 140.8, 36.5, 50.2, and 37.3; the methyl signal at δ_H 0.90 (H_3-21) connected with three sp^3 carbons at 56.1, 36.2, and 33.9; the methyl signal at δ_H 0.81 (H_3-26) correlated with three sp^3 carbons, comprising two quaternary carbons, and one methylene carbon, at δ_C 45.9, 29.2, and 19.1; the methyl signal at δ_H 0.79 (H_3-27) connected with two sp^3 carbons, comprising of two methine carbons, and one methyl carbon, at δ_C 45.9, 29.2,

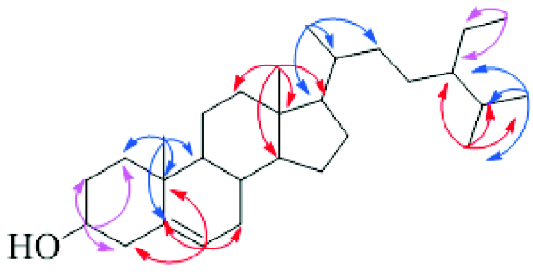

Fig. 2. HMBC key correlations in compound **K-1**.

Table 1. ^{1}H, and ^{13}C NMR data of **K-1**, and compared with those obtained from literature M. D. Greca *et al*., 1990

Carbon atom	M. D. Greca *et al*., 1990, *J. Nat. Prod.* ^{13}C NMR	**K-1** ^{13}C NMR	M. D. Greca *et al*., 1990, *J. Nat. Prod.* ^{1}H NMR	**K-1** ^{1}H NMR
1	37.3	37.3	–	–
2	31.6	31.7	–	–
3	71.7	71.8	3.52, m	3.50, m
4	42.2	42.3	–	–
5	140.7	140.8	–	–
6	121.6	121.7	5.35, m	5.33, m
7	31.9	31.9	–	–
8	31.8	31.9	–	–
9	51.1	50.2	–	–
10	36.4	36.5	–	–
11	21.1	21.1	–	–
12	39.8	39.8	–	–
13	42.4	42.3	–	–
14	56.8	56.8	–	–
15	24.2	24.3	–	–
16	28.3	28.3	–	–
17	56.0	56.1	–	–
18	11.8	11.9	0.69, s	0.66, s
19	19.5	19.4	1.01, s	0.99, s
20	36.1	36.2	–	–
21	18.7	18.8	0.92, d, 6.4 Hz	0.90, d, 6.4 Hz
22	33.9	33.9	–	–
23	26.1	26.1	–	–
24	45.8	45.9	–	–
25	29.2	29.2	–	–
26	19.8	19.8	0.83, d, 6.8 Hz	0.81, d, 6.8 Hz
27	19.2	19.1	0.81, d, 6.9 Hz	0.79, d, 6.9 Hz
28	23.1	23.1	–	–
29	11.0	11.9	0.85, t, 7.8 Hz	0.83, d, 7.8 Hz

and 19.8; the methyl signal at δ_H 0.83 (H_3-29) connected with two sp^3 carbons, comprising one methylene, and one methine, at δ_C 23.1, and 45.9; the olefinic signal at δ_H 5.33 (H-6) connected with four carbons (1 sp^2 and 3 sp^3), including two quaternary, and two methylene, at δ_C 140.8, 36.5, 42.3, and 31.9; the methine signal at δ_H 3.51 (H-3) connected with three sp^3 carbons, including three methylene, at δ_C 37.3, 31.7, and 42.3, respectively. Therefore, ^{1}H NMR and ^{13}C NMR data of **K-1** (Table 1) showed a high similarity to the data reported earlier for β-sitosterol[13–15].

K-2 was obtained as oily crystal form with molecular formula $C_{29}H_{48}O$ (*m/z* 412.3705) as identified by HR-MS spectral data, along with 1D and 2D spectral data. The integration of the proton NMR spectrum of **K-2** indicated the presence of 48 protons: 11 methine protons, 6 methyl protons, and 8 methine protons. One double bond comprising an olefinic proton may easily be deduced from the ^{1}H NMR spectrum. This olefinic proton was allocated as H-4. In addition to this, six methyl signals, including two singlet signals, and four doublet signals were also allocated as, δ_H 0.69 (H_3-18),

Table 2. ^{1}H and ^{13}C NMR data of **K-2**, and compared with those obtained from literature M. D. Greca *et al*., 1990

Carbon atom	M. D. Greca *et al*., 1990, *J. Nat. Prod.* ^{13}C NMR	**K-2** ^{13}C NMR	M. D. Greca *et al*., 1990, *J. Nat. Prod.* ^{1}H NMR	**K-2** ^{1}H NMR
1	35.7	35.7	„	„
2	33.9	33.9	„	„
3	198.9	199.6	5.74, d, 2.2 Hz	5.70, s
4	123.6	123.7	„	„
5	171.0	171.7	„	„
6	32.9	32.9	„	2.25, 2.37, m
7	32.1	32.1	„	„
8	35.7	35.7	„	„
9	53.8	53.8	„	„
10	38.6	38.6	„	„
11	21.0	21.1	„	„
12	39.5	39.6	„	„
13	42.4	42.4	„	„
14	55.9	55.9	„	„
15	24.1	24.2	„	„
16	28.1	28.2	„	„
17	56.1	56.0	„	„
18	11.9	11.9	0.73,s	0.69, s
19	17.4	17.4	1.19,s	1.16, s
20	36.1	36.1	„	„
21	18.7	18.71	0.93,d, 6.6 Hz	0.90, d, 6.6 Hz
22	34.0	34.0	„	„
23	25.9	26.1	„	„
24	45.8	45.9	„	„
25	29.1	29.2	„	„
26	19.8	19.8	0.84, d, 6.1 Hz	0.81, d, 6.1 Hz
27	19.2	19.0	0.82, d, 6.8 Hz	0.79, d, 6.8 Hz
28	23.1	23.1	–	–
29	11.1	11.9	0.85, d, 7.2 Hz	0.83, d, 7.2 Hz

1.16 (H_3-19), 0.90 (H_3-21), 0.81 (H_3-26), 0.79 (H_3-27), and 0.83 (H_3-29). The presence of 29 carbons resonance was confirmed by the ^{13}C NMR spectrum. The ^{1}H NMR and ^{13}C NMR data of **K-2** (Table 2) showed a high similarity to the data reported earlier for β-sitostenone[14,15]. This was further established by the long range ^{1}H-^{13}C HMBC connections (Fig. 3): one methine proton at δ_H 5.79 (H-4) connected with sp^3 methylene carbon at δ_C 33.9, and sp^3 quaternary carbon at δ_C 38.6; one methyl proton at δ_H 0.69 (H_3-18) connected with one sp^3 methylene carbon at δ_C 39.6, two sp^3 methine carbons at δ_C 55.9 and 56.0; and sp^3 quaternary carbon at δ_C 42.4; one methyl proton at δ_H 1.16 (H_3-19) connected with two quaternary carbons (1 sp^3 and 1 sp^2) at δ_C 171.7 and 38.6, one sp^3 methylene carbon at δ_C 35.7,

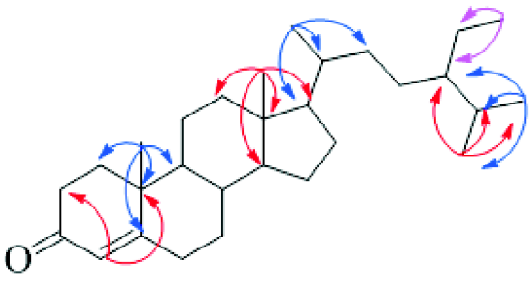

Fig. 3. HMBC key correlations in compound **K-2**.

and one methine carbon at δ_C 53.9; the methyl doublet proton at δ_H 0.90 (H_3-21) connected with two sp^3 methine carbons at δ_C 56.0 and 36.1, and one sp^3 methylene carbon at δ_C 34.0; the methyl doublet proton at δ_H 0.81 (H_3-26) connected with two sp^3 methine carbons at δ_C 45.9 and 29.2, and one sp^3 methyl carbon at δ_C 19.0; the methyl doublet proton at δ_H 0.79 (H_3-27) connected with one sp^3 methyl carbon at δ_C 19.8, and two sp^3 methine carbons at δ_C 45.9 and 29.2; the methyl doublet signal at δ_H 0.83 (H_3-29) connected with one sp^3 methylene carbon at δ_C 23.1, and sp^3 methine carbon at δ_C 45.9, respectively.

Conclusions

In summary, β-sitosterol (**K-1**) and β-sitostenone (**K-2**) were obtained from the hexane extract of *Eucalyptus deglupta* Blume. These two structures were identified based on the advance spectroscopic analyses, such as FTIR, NMR, and HR-MS. This is the first study that recognizes β-sitosterol (**K-1**) and β-sitostenone (**K-2**) present in *Eucalyptus deglupta.* In addition, there was no information on the β-sitostenone component in *Eucalyptus* spp.

Acknowledgements

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Supporting Information

All NMR spectra are available on the online version.

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