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ISOLATION OF CHEMICAL CONSTITUENTS FROM RHIZOMES OF *ETLINGERA SPHAEROCEPHALA* VAR. *GRANDIFLORA*

(Pemencilan Kandungan Kimia Daripada Rizom *Etlingera Sphaerocephala* var. *Grandiflora*)

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Abstract

A phytochemical investigation was conducted on the rhizomes of *Etlingera sphaerocephala* var. *grandiflora* (Zingiberaceae). Steroids of β-sitosterol and stigmasterol, together with a phenolic compound of paeonol were isolated using several chromatographic means. Their structures were established on the basis of extensive spectroscopic (IR, MS, NMR) data analysis and by comparison of the data obtained with those of the literature.

Keywords: Zingiberaceae, *Etlingera sphaerocephala* var. *grandiflora*, β-sitosterol, stigmasterol, paeonol, NMR

Abstrak

Suatu penyelidikan fitokimia dilakukan ke atas rizom *Etlingera sphaerocephala* var. *grandiflora* (Zingiberaceae). Steroid β-sitosterol dan stigmasterol, berserta sebatian fenolik paeonol dipisahkan menggunakan beberapa cara kromatografi. Struktur mereka dibuktikan berasas kepada analisis data spektroskopi (IM, SJ, RMN) yang ekstensif dan secara membandingkan data tersebut dengan data kepustakaan

Kata kunci: Zingiberaceae, *Etlingera sphaerocephala* var. *grandiflora*, β-sitosterol, stigmasterol, paeonol, NMR

Introduction

There are 151 species of Zingiberaceae belonging to 18 genera found in Peninsular Malaysia [1]. The largest genus of Zingiberaceae is *Alpinia* (23 species), followed among others by *Scaphochlamys* (20), *Amomum* (18), *Globba* (15), *Geostachys* (13), *Etlingera* (10) and *Zingiber* (10). The species of *Etlingera* in Peninsular Malaysia has now increased to fifteen [2]. The largest among *Etlingera* species are about 10 m in height. There are more than 100 species of Indo-pacific *Etlingera* growing from sea level to 2500 m [3].

Etlingera sphaerocephala var. *grandiflora* was described by Holttum [4] as a plant with subterranean, sub-globose, sub-cylindric inflorescence where its flowers appearing at soil level; its stature is 2.5 m; its leaves when young are also suffused red or purple below; its labellum of deep crimson lip, white margins, is 6 cm or more in length and 2.7 cm wide; and the base is red in color. *Etlingera sphaerocephala* var. *grandiflora* can be found in many parts of the Peninsular Malaysia and Borneo [5] mainly in lowland forests and at moderate elevation on the mountains. No use has ever been recorded for *E. sphaerocephala* var. *grandiflora* [3]. In this paper, we report the isolation and characterization of β-sitosterol, stigmasterol and paeonol from the rhizomes of *E. sphaerocephala* var. *grandiflora*. These compounds are reported for the first time from *Etlingera*.

Materials and Methods

Plant Material

Rhizomes of the *Etlingera sphaerocephala* var. *grandiflora* were collected in January 2009 from Genting Peras, Hulu Langat, Selangor, Malaysia. A voucher specimen of WYA 386 for the plant has been deposited at the Universiti Kebangsaan Malaysia Herbarium.

Extraction and Isolation

Air-dried rhizomes (315 g) were ground into a fine powder and soaked in *n*-hexane (5.0 L) for six days. The solution was filtered, and the combined filtrates were rotary evaporated under reduced pressure to produce 1.5 g (0.48%) of a brown-gummy rhizomes *n*-hexane extract. The remaining rhizomes powder was soaked in methanol (2.5 L) for another six days. The solution was filtered, and the combined filtrates were concentrated using a rotary evaporator to yield 6.3 g (2.1%) of a brown-oily rhizomes successive methanol extract.

The rhizomes *n*-hexane extract (1.5 g) was subjected to silica gel column chromatography (CC) (Merck 1.09385) by eluting *n*-hexane containing increasing percentages of ethyl acetate and the fractions collected was 100 mL each. Fractions 4 and 5 were combined (96 mg) and then subjected to radial chromatography (RC) (Merck 1.07749) by using increasing polarities of *n*-hexane : ethyl acetate to produce 12.4 mg of β-sitosterol (1). Fractions 6, 7 and 8 were combined (139 mg) and then subjected to silica gel preparative thin layer chromatography (PTLC) (Merck 1.07749) using 100 : 0 to 10 : 90 of *n*-hexane : ethyl acetate to afford 6.6 mg of stigmasterol (2).

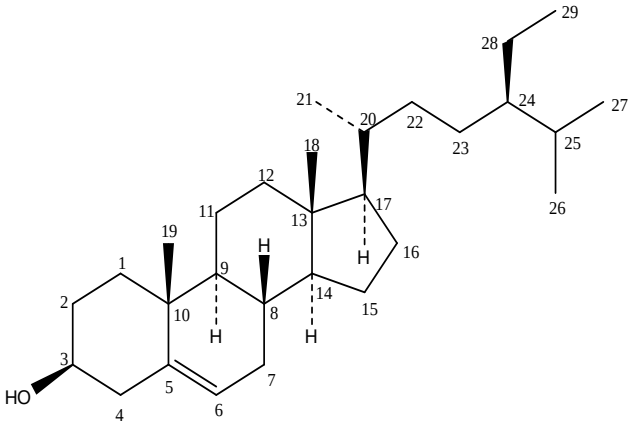
The rhizomes successive methanol extract (6.3 g) underwent silica gel flash CC (Merck 1.07747) using 10% polarity increments from 90 : 10 chloroform : methanol to 100% methanol whereby 100 mL fractions were collected. The flash CC fractions 5, 6 and 7 were combined (170 mg) and subjected to RC to produce five fractions. The combined fractions 1, 2 and 3 were fractionated over silica gel (Merck 1.07749) on PTLC to give 2.4 mg of paeonol (3).

β-Sitosterol (1; si) /Stigmasterol (2; st)

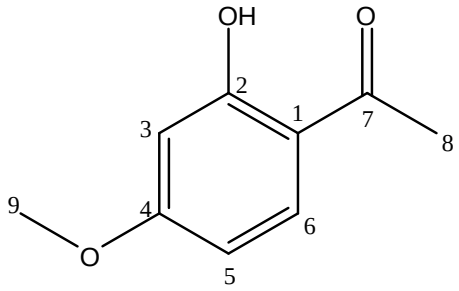
Colorless needles (12.4 mg)/white powder (6.6 mg). IR cm^{-1}: 3393/3434, 2930/2924, 1611/1639, 1457/1457, 1385/1375, 1171/1179. EIMS for β-sitosterol-$C_{29}H_{50}O$ m/z (rel. int.): 414 [M^+] (100%), 396 (30.8%), 381 (14.0%), 329 (13.7%), 303 (22.1%), 255 (11.8%), 213 (12.2%), 145 (18.5%), 95 (21.6%), 81 (21.3%), 55 (25.8%), 43 (45.0%). EIMS for stigmasterol-$C_{29}H_{48}O$ m/z (rel. int.): 412 [M^+] (39.7%), 351 (13.5%), 314 (7.0%), 300 (25.5%), 271 (38.4 %), 229 (8.6%), 213 (10.6%), 55 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): 3.53 (1H, m, H-3), 5.36 (1H, d, J = 5.1/7.3 Hz, H-6), 0.69 (3H, s, H-18), 1.02 (3H, s, H-19), 0.93 (3H, d, J = 6.6/6.2 Hz, H-21), 5.16_{st} (1H, dd, J = 15.6, 8.4 Hz, H-22); 5.02_{st} (1H, dd, J = 15.6, 9.2 Hz, H-23), 0.85 (3H, d, J = 7.0/7.3 Hz, H-26), 0.83/0.81 (3H, d, J = 7.0 Hz, H-27), 0.87/0.83 (3H, t, J = 7.7/7.0 Hz, H-29), 1.05-2.32 (others). ^{13}C NMR ($CDCl_3$, 100 MHz): 141.0 (C-5), 121.9 (C-6), 72.0 (C-3), 57.0/57.1 (C-14), 56.3 (C-17), 50.4/50.3 (C-9), 46.1/51.5 (C-24), 42.6/42.3 (C-4, C-13), 40.0 (C-12), 37.5 (C-1), 36.7 (C-10), 36.4/40.7 (C-20), 34.2/138.5 (C-22), 32.2/32.1 (C-8), 32.1_{si} (C-7), 31.9_{st} (C-7, C-25), 31.9/31.7 (C-2), 29.4_{si} (C-25), 28.5/29.3 (C-16), 26.3/129.5 (C-23), 24.5 (C-15), 23.3/25.6 (C-28), 21.3_{si} (C-11), 21.3_{st} (C-11, C-21), 20.1/19.0 (C-27), 19.6 (C-19), 19.3/21.4 (C-26), 19.0_{si} (C-21), 12.2 (C-29), 12.1 (C-18).

Paeonol (3)

White needles (2.4 mg). IR cm^{-1}: 3432, 2925, 1720, 1383, 1246, 1021. EIMS for $C_9H_{10}O_3$ m/z (rel. int.): 166 [M^+] (31.3%), 151 (100%), 108 (16.5%), 95 (21.0%), 43 (34.6%).^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.56 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.42-6.45 (2H, m, H-3, H-5), 7.63 (1H, d, J=8.8 Hz, H-6), 12.75 (1H, s, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 114.1 (C-1), 165.4 (C-2), 101.0 (C-3), 166.3 (C-4), 107.9 (C-5), 132.5 (C-6), 202.8 (C=O), 55.8 (OCH_3), 26.4 (CH_3).



1 (β-Sitosterol)
2 (*trans*-CH=CH across C22--C23; Stigmasterol)



3 (Paeonol)

Results and Discussion

Purification of the *Etlingera sphaerocephala* var. *grandiflora* rhizomes extracts produced β-sitosterol (1), stigmasterol (2) and paeonol (3). β-Sitosterol (1) was isolated as colorless needles. Column chromatography of the rhizomes *n*-hexane extract gave a 139 mg combined fractions, and its preparative thin layer chromatography yielded 6.6 mg of stigmasterol (2) as white powder. Spectral data of stigmasterol differed minimally from those of β-sitosterol at and around a *trans*-C22-C23 double bond in the former. The EIMS of β-sitosterol/stigmasterol showed the molecular ion m/z 414/412 [M^+] that corresponds to the formula $C_{29}H_{50}O/C_{29}H_{48}O$ and in agreement with other previous spectral data. ^{1}H NMR spectrum of β-sitosterol/stigmasterol indicated the presence of six methyl peaks of H-18, H-27, H-29, H-26, H-21 and H-19 that appeared at respective δ 0.69, 0.83/0.81, 0.87/0.83, 0.85, 0.93 and 1.02. The hydroxymethine proton H-3 of β-sitosterol/stigmasterol came out at δ 3.53 as a multiplet. One proton appeared at δ 5.36 as a doublet with coupling constants of 5.1/7.3 Hz represents the endocyclic double bond proton H-6 of β-sitosterol/*stigmasterol*. Stigmasterol showed two doublet-of-doublets for the other olefinic protons H-23 and H-22 at δ 5.02 (1H, dd, *J* = 9.2, 15.6 Hz) and 5.16 (1H, dd, *J* = 8.4, 15.6 Hz) respectively. The ^{1}H NMR spectral data of stigmasterol are in agreement with those of Hussain et al. [6]. The ^{13}C NMR spectrum of β-sitosterol/stigmasterol showed 28/26 signals for the 29 carbons skeleton with the overlapping of 2/6 carbons. The significant carbon signal for the β-sitosterol/stigmasterol would be the C-3 attached to a hydroxyl group and appeared at δ 72.0. The endocyclic carbon-carbon double bond of β-sitosterol/stigmasterol was represented by two signals at δ 121.9 and 141.0 of C-6 and C-5. Other olefinic carbons of stigmasterol appeared at δ 138.5 and 129.5 for C-22 and C-23. The quaternary C-5 signal is shifted to lower field than those of the tertiary C-6, C-22 and C-23. The similarity of the ^{13}C spectral data of β-sitosterol/stigmasterol with those of the published data in [7]/[8]

confirmed that both two are of the same structure. β-Sitosterol/stigmasterol has never been reported before from any other *Etlingera*. However β-sitosterol was found in many other plant families including Zingiberaceae such as *Zingiber* [9], *Alpinia* [10], *Globba* [11], *Kaempferia* [12], *Costus* [13], *Renealmia* [14], *Aframomum* [15] and *Hedychium* [16], whereas stigmasterol including Zingiberaceae of *Renealmia* [14], *Alpinia* [17, 18] and *Zingiber* [9].

Paeonol (3) was isolated from the rhizomes successive methanol extract as white needles. Mass spectrum of this compound showed a molecular ion peak at m/z 166, which is in agreement with the molecular formula $C_9H_{10}O_3$ of paeonol. ^{1}H NMR spectrum of paeonol showed that there are two singlets at δ 2.56 and 3.84 representing two methyl groups attached to respective quaternary carbonyl, and oxygen. A signal for H-3, H-5 appeared at δ 6.42-6.45 as a multiplet. Proton H-6 showed a doublet which appeared at δ 7.63 with a coupling constant of 8.8 Hz. A hydrogen-bonded, six-membered ring phenolic proton was displayed very down-field at δ 12.75. ^{13}C NMR data of this compound showed that there were nine signals which represents nine different carbons. The signal at δ 202.8 was assigned to the carbonyl group of C-1. Moreover, signals at δ 101.0, 107.9 and 132.5 were assigned to respective aromatic carbons of C-3, C-5 and C-6. Signal at δ 165.4 was assigned to C-2 which is known as an aromatic carbon attached to a hydroxyl group whereas the appearance of the aromatic C-4 with the methoxy substitution was seen at δ 166.3 due to the electron donating feature of the oxygen atoms in those groups. Two up-field signals appeared at δ 26.4 and 55.8 for methyl groups of $COCH_3$ and OCH_3. Comparison of the ^{1}H and ^{13}C NMR data of paeonol with the previously published data in [19] confirmed that both are of the same compound. Paeonol has never been isolated before from any genus of Zingiberaceae. The fact that paeonol was found in the woody plants of *Luculia intermedia* [20], *Rosmarinus officinalis* [21], *Moutan cortex* [22] as well as in the non-woody plants of *Arisaema erubescens* [23], *Exacum affine* [24], *Primula auricula* [25], *Cynanchum paniculatum* [25] supports its existence in our non-woody plant of *Etlingera sphaerocephala* var. *grandiflora*.

Conclusion

The isolation and identification of β-sitosterol (1), stigmasterol (2) and paeonol (3) from the rhizomes of *Etlingera sphaerocephala* var. *grandiflora* was the first ever to be reported from this plant. The work was carried out by utilizing several kinds of chromatographic separation techniques and spectroscopic analyses.

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