NON PHENOLIC COMPOUNDS ISOLATED FROM THE LEAVES OF FERN *Chingia sakayensis* (Zeiller) Holtt

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Received 1 March 2006; Accepted 25 March 2006

ABSTRACT

Two non phenolic compounds namely a wax ester hexacosyl hexadecanoic and a steroid β-sitosterol were isolated from the n-hexane extract of the fern Chingia sakayensis (Zeiller) Holtt's leaves. Their structures were elucidated on the basis of the spectrometric evidences (UV, IR, ^{1}H-NMR, ^{13}C-NMR, EIMS and HR-CIMS).

Keywords: *Chingia sakayensis, wax ester, steroid, hexacosyl hexadecanoic, β-sitosterol.*

INTRODUCTION

One of the bioresources of Indonesia was the ferns (Pteridophytes) distributed throughout the Indonesian archipelago. Sastrapradja [1] estimated as many as 1,300 species of the ferns could be discovered in Indonesia. The ferns had been used for a long time as the ornamental plants, vegetables, traditional medicines, protector plants, fertilizer and building material [2].

Chingia sakayensis was one of the ferns in Thelypteridaceae family distributed in Thailand, Malaysia, Serawak, Sumatra and Java. It usually grew in the forest, often near streams, at altitude 150-1200 m. Because of the difference of environment condition, the specimens from Java and Sumatra were much thicker in texture, with very strongly raised veins and sinus membrane on the lower [3].

In the previous paper, we reported the isolation and structure determination of flavonoid kaemferol and matteucinol isolated from the ethyl acetate fraction of the methanol extract and the dichloromethane extract of the *C. sakeyensis*'s leaves, respectively [4,5]. In this paper, we described the isolation and structure determination of wax ester and steroid isolated from the n-hexane extract of the *C. sakayensis*'s leaves.

EXPERIMENTAL SECTION

General Experimental Procedure

Melting point was measured by Fisher John melting point apparatus and was uncorrected. The UV spectra were recorded on Shimadzu LC-9A spectrophotometer. The IR spectrum in KBr film was determined by JASCO FT/IR-5300 spectrophotometer. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100,5 MHz) spectra were measured by JEOL JNM-ECP 400 spectrometer using tetra methyl silane (TMS) as the internal standart. The Mass spectrum (MS) was recorded on JEOL JMS-AM20 spectrometer using ion mode EI^{+} and HR-CIMS. Kieselgel 60 GF-254 (Merck) were used for vacuum liquid chromatography (VLC). Precoated silica gel 60 GF-254 (Merck) was used for thin layer chromatography (TLC) and spots were detected by spraying with the sulphuric acid solution 5% (v/v) in ethanol followed by heating.

Plant Material

The leaves of *C. sakayensis* was collected from Kletak forest, Nongkojajar, Pasuruan, East Java, Indonesia in January 2002. The sample was identified by Mr. Wardaya from the Purwodadi Botanical Garden, Indonesia and a voucher spesimen was deposited at the herbarium of the Purwodadi Botanical Garden, Indonesia.

Isolation

The dried powdered leaves of *C. sakayensis* (1.5 kg) was exhaustively extracted successively with n-hexane (31.5 L), dichloromethane (25.5 L) and methanol (24.0 L) at room temperature. The *n*-hexane extract was evaporated in vacuo to obtain the brown solid (23.4 g). A part of it (8.34 g) was chromatographed by VLC and eluted with solvents of increasing polarity (n-hexane, mixture of *n*-hexane–EtOAc and EtOAc) gave 130 fractions. Futhermore the combined fractions of 7-12 (1.163 g) was chromatographed by VLC and eluted with solvents of increasing polarity (*n*-hexane and mixture of *n*-hexane–EtOAc) yielded 110 fractions. The combined

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fractions of 11-17 (115.3 g) was recrystalized in acetone afforded compound **1** (46.8 mg). It showed a single spot by TLC on silica gel with Rf = 0.71 ($CHCl_3$); Rf = 0.66 (n-hexane : EtOAc = 10 :1) and Rf = 0.06 (n-hexane).

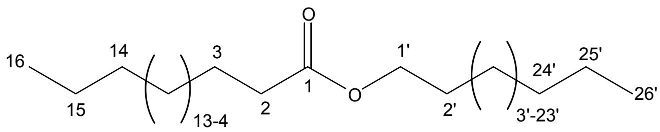
While the combined fractions of 50-57 obtained from VLC of the n-hexane extract, recrystalized in methanol afforded compound 2 (144 mg). It showed a single spot by TLC on silica gel with Rf = 0.08 (n-hexane : $CHCl_3$ = 1 :1), Rf = 0.15 (n-hexane : EtOAc = 9 :1) and Rf = 0.61 (n-hexane : EtOAc = 1:1).

Hexacosyl hexadecanoic **(1)** was obtained as colourless plate ($CHCl_3$ -acetone), mp. 66-68 ^oC, negative on Liebermann-Burchard test. UV (*n*-hexane) λ_{maks} (log ε) = 222 (3.40), 281 (3.16) nm. IR (KBr) ν_{maks} : 2957, 2919, 2849, 1736, 1474, 1464, 1177 cm^{-1} . ^1H-NMR (400 MHz,$CDCl_3$) δ (ppm) : 0.88 (*t*, *J* = 6.78 Hz, H-16, 26'); 1.29 (complex, H-CH_2); 1.60 (*m*, H-2'); 2.29 (*t*, *J* = 7.52 Hz, H-2); 4.05 (*t*, *J* = 6.58 Hz, H-1'). ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ (ppm) : 14.1 (C-16, 26'); 22.7 (C-15,25'); 25.1 (C-3); 26.0 (C-2'); 28.7-29.8 (C -4-13 & C-3'-23'); 32.0 (C-14,24'); 34.5 (C-2); 64.4 (C-1'); 174.0 (C-1). EIMS, m/z (int.rel.) = 620 (M^+) (57), 424 (1), 409 (4), 381 (8), 364 (10), 336 (27), 257 (100), 256 (25), 239 (8), 196 (3), 181 (3), 167 (4), 153 (5), 139 (9), 125 (16), 111 (31), 97 (55), 83 (57), 69 (59), 57 (88); 43 (41). HR-CIMS, m/z : 621, 6549 $[M + H]^+$ (calculated 621.6550 for $C_{42}H_{85}O_2$).

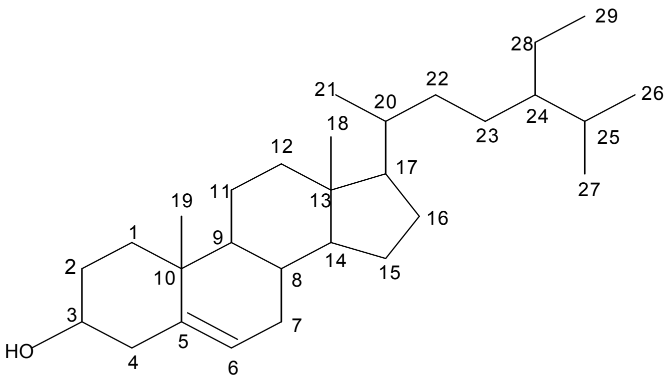
β-sitosterol (2) was obtained as colourless needles (MeOH), mp. 138 ^oC, gave positive test (blue colour) with Liebermann-Burchard test. UV (MeOH) λ_{maks} (log ε) : 211 (3.22) nm. IR (KBr) ν_{maks} : 3416, 2936, 2851, 1647, 1466, 1379, 1053 cm^{-1}. ^1H-NMR (400 MHz,$CDCl_3$) δ (ppm) : 0.68 (*s*, H-18); 0.82 (*d, J* = 7 Hz, H-26,27); 0.85 (*t, J* = 7.7 Hz, H-29); 0.92 (*d, J* = 6.6 Hz, H-21); 1.01 (*s*, H-19); 3.52 (*m*, H-3); 5.35 (*brd, J* = 5.1 Hz, H-6). ^{13}C-NMR (100.5 MHz, $CDCl_3$) δ (ppm) : 11.9 (C-18); 12.0 (C-29); 18.8 (C-21); 19.1 (C-27); 19.4 (C-19); 19.8 (C-26); 21.1 (C-11); 23.1 (C-28); 24.3 (C-15); 26.1 (C-23); 28.3 (C-16); 29.2 (C-25); 29.7 (C-2); 31.7 (C-7); 31.9 (C-8); 34.0 (C-22); 36.2 (C-20); 36.5 (C-10); 37.3 (C-1); 39.8 (C-12); 42.3 (C-13); 42.4 (C-4); 45.9 (C-24); 50.2 (C-9); 56.1 (C-17); 56.8 (C-14); 71.8 (C-3); 121.7 (C-6); 140.8 (C-5). EIMS, m/z (int.rel): 414 (M^+)(7.1), 396 (3.6), 381 (3.6), 329 (10.7), 273 (3.6), 255 (7.1),231 (3.6), 213 (10.7), 43 (100).

RESULT AND DISCUSSION

Compound **1** was isolated from the n-hexane extract of *Chingia sakayensis*'s leaves. The HR-CIMS of **1** showed a quasi molecular ion at m/z 621, 6549 $[M + H]^+$, corresponding to molecular formula $C_{42}H_{84}O_2$. The negative result on Liebermann-Burchard test indicated that **1** was not a triterpenoid and steroid. The IR spectrum of **1** disclosed absorbtion bands for alkyl C-H (2957, 2919, 2849 cm^{-1}), ester carbonyl (1736 cm^{-1}) and ester C-O-C (1177 cm^{-1}). Sharpness of the absorbtion band of alkyl C-H indicated the presence of long chain hydrocarbon. The presence of carbonyl group was confirmed by carbon signal at δ_C 174.0 ppm in ^{13}C-NMR spectrum and the weak absorbtion band at 281 nm in UV spectrum due to electronic transition $n \rightarrow \pi^*$ (forbidden transition) in carbonyl group. The proton signals at δ_H 2.29 (*t*, H-2) and δ_H 4.05 (*t*, H-1') in ^1H-NMR spectrum and the carbon signals at δ_C 174.0 (C-1) ; 34.5 (C-2); 64.4 (C-1') in ^{13}C-NMR spectrum supported the presence ester group. From the above results, **1** was suggested to be a long chain ester (wax ester). Ion fragment at m/z 257 (base peak) was characteristic for the long chain ester of hexadecanoic acid, resulted from fragmentation via double rearrangement of ester group [6]. The presence of hexadecanoyl was also supported by a peak at m/z 256 $[C_{15}H_{31}COOH]^+$, due to fragmentation via McLafferty rearrangement. While fragment ion at m/z 364 corresponded to $[C_{26}H_{52}]^+$ resulted from hexacosyl group $[C_{26}H_{53}]$ which released a hydrogen atom [7]. From the above results, compound **1** was identified as hexacosil hexadecanoic ester.



Compound 2 was isolated from n-hexane extract of the *C. sakayensis*'s leaves. It showed the characteristic colour reaction of a sterol (blue colour) with Liebermann-Burchard test [8]. The EIMS spectrum of 2 showed a molecular ion m/z 414 $[M^+]$, corresponding to molecular formula $C_{29}H_{50}O$. The UV spectrum exhibited maxima at 211 nm, indicating no conjugated double bond. While the IR spectrum disclosed absorbtion bands for alkyl C-H (2936 and 2851 cm^{-1}) and hydroxyl group (3416 cm^{-1}). The presence of OH was also supported by ion fragment at m/z 396 $(M-H_2O)^+$ and the multiplet proton signal at δ_H 3.53 due to the oxyalkyl proton (H-3). Further the proton signal at δ_H 5.35 due to olefinic proton (H-6) and the carbon signals at δ_C 121.7 (C-5) and 140.8 (C-6), supported the presence of a double bond (C=C). The ^{13}C-NMR spectrum of 2 displayed the presence of twenty nine signals, containing a oxyalkyl carbon (δ_C 71.8), two olefinic carbons (δ_C 121.7 and 140.8) and the others were alkyl carbon atom. The ^1H-NMR and ^{13}C-NMR spectral data as well as the mass spectral fragmentation pattern of 2 resembled those of β-sitosterol in literature [9,10]. From the above results, compound 2 was identified as β-sitosterol.



(**2**)

β-sitosterol showed the weak antiinflamatory activity, while its ferulate ester was strong. Moreover β-sitosterol was able to inhibite absorbtion of the bile's cholesterol so it could be used for antilipidemic agent. In addition, β-sitosterol was also able to inhibite 5-alpha-reductase activity so the formation of dihydrotestoterone from testoterone could be inhibited. Thus β-sitosterol could be used to prevent the swelling of the prostat gland [11].

CONCLUSION

A wax ester and steroid namely hexacosyl hexadecanoic and β-sitosterol, respectively, were isolated from the n-hexane extract of *Chingia sakayensis'*s leaves. Hexacosyl hexadecanoic was obtained as colourless plate with mp. 66-68 ^{o}C, while β-sitosterol was obtained as colourless needles, mp.138 ^{o}C. The finding of hexacosyl hexadecanoic was the first time in the fern, while β-sitosterol was found in several species of the fern.

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