TWO ALKYLATED FLAVONOID ISOLATED FROM THE STEM OF THE FERN Chingia sakayensis (Zeiller) Holtt

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ABSTRACT

Two known alkylated flavonoid namely matteucinol dan matteucinol-7-O-β-D-glucoside was isolated for the first time from the fern Chingia sakayensis (Zeiller) Holtt's stem. Their structures were elucidated on the basis of spectroscopic evidence and by comparation with those reported data in literature.

Keywords: *Fern, Chingia sakayensis, stem, alkylated flavonoid, matteucinol, matteucinol-7-O-β-D-glucoside*

INTRODUCTION

Chingia sakayensis was one of the ferns belong the 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 ticker in texture, with very strongly raised veins and sinus membrane on the lower [1]. The young fronds of the plant can be eaten cooked or raw, an extract of mature fronds in water some times sprinkled on fever, and a decoction was used as tonic after childbirth [2].

Previous work on the chemical constituents of the fern *C. sakayensis*'s leaves had led to identification three known compounds namely hexacosyl hexadecanoic, β-sitosterol, and kaempferol [3,4]. In continuation of our studies, we examined the phenolic constituents of the *C. sakayensis*'s stem and separated two known alkylated flavonoid namely matteucinol (**1**) and matteucinol-7-O-β-D-glucoside (**2**). It was isolated for the first time from this species and of the genus *Chingia*. The structure of the isolated compound was assigned on the basis of their spectroscopic (UV, IR, ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC) and mass spectrometric data, as well as by comparison with those reported in the literature. In this paper, we reported the isolation and structure determination of matteucinol and matteucinol-7-O-β-D-glucoside isolated from *C. sakayensis*'s stem.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting point was measured by Fisher John melting point apparatus and was uncorrected. The UV spectra were recorded on Shimadzu Pharmaspec UV-1700 spectrophotometer. The IR spectrum in KBr film was determined by JASCO FT/IR-5300 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were measured by JEOL JNM-AL 400 spectrometer [operating at 400 MHz (^{1}H) and 100.4 MHz (^{13}C)] using tetra methyl silane (TMS) as the internal standard. The Mass spectrum (MS) was recorded on JEOL LX 1000 spectrometer using ion mode FAB^{+} [3-nitro benzyl alcohol (m-NBA) as matrix]. Kieselgel 60 GF-254 (Merck) and silica gel G 60 63-200 μm (Merck) were used for vacuum liquid chromatography (VLC) and flash chromatography (FC), respectively. Precoated silica gel 60 F-254 (Merck) 0.25 mm, 20 x 20 cm 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 stem of *C. sakayensis* was collected from Kletak forest, Nongkojajar, Pasuruan, East Java, Indonesia in January 2002. A voucher specimen was deposited at the herbarium of the Purwodadi Botanical Garden, Indonesia.

Isolation

The dried powdered stem of *C. sakayensis* (677 g) was exhaustively extracted successively with n-hexane (4 L x 3), dichloromethane (4 L x 3), and methanol (4 L x 3) at room temperature. The methanol extract was evaporated *in vacuo* to afford the concentrate methanol extract (65 g). Furthermore it was extracted with ethyl acetate-water mixture (1 : 1) (400 mL x 3). Removal of the solvent under reduced pressure of the ethyl acetate soluble fraction yielded a brown residue (10 g). A portion of it (5 g) was chromatographed by VLC and eluted with solvents of increasing polarity (n-hexane, n-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$-MeOH) yielded 225 fractions (15 mL each). The

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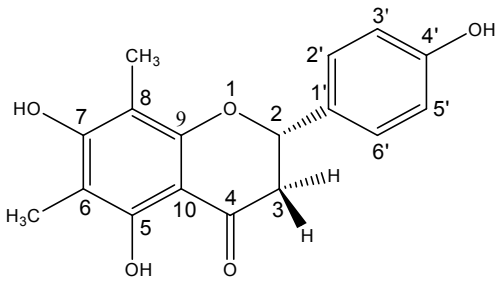
combined fractions of 34-50 (216 mg) were purified by FC with n-hexane-EtOAc (3:2) as eluen yielded compound **1** (30 mg). Recrystalization of the combined fractions of 128-135 (360 mg) in $CHCl_3$-MeOH afforded compound **2** (58 mg).

Compound 1 was obtained as pale yellow crystal (benzene), mp. 167-168°C, $[\alpha]_D^{20}$ = -26° (MeOH, *c*.0.1), gave positive test with $FeCl_3$ (green) and shinoda test (Mg-HCl)(red). It showed a single spot by TLC on silica gel with Rf = 0.27 (*n*-hexane-EtOAc = 4: 1), 0.44 ($CHCl_3$), and 0.73 ($CHCl_3$ -EtOAc = 1 :1). UV (MeOH) λ_{maks} (log ε) : 294 (3.47), 350 (sh) (2.77) nm; (MeOH + NaOH): 339 (3.72)nm; (MeOH+$AlCl_3$): 295 (3.45), 360 (sh)(2.69) nm; (MeOH+$AlCl_3$+HCl): 297 (3.42), 358 (2.69) nm; (MeOH+NaOAc): 340 (3.51) nm; (MeOH+NaOAc+H_3BO_3): 296 (3.44), 345 (sh)(2.99) nm. IR (KBr) ν_{maks} : 3453 (OH), 3005 (aromatic C-H), 2922, 2840 (alkyl C-H), 1630 (chelated C=O), 1520 (aromatic C=C), 1454, 1397 cm^{-1}. ^{1}H-NMR (400 MHz,$CDCl_3$) δ (ppm) : 2.03(3H, *s*, 8-CH_3), 2.05 (3H, *s*, 6-CH_3), 2.78 (1H, *dd*, *J* = 17 Hz, 3 Hz, H-3β), 3.03 (1H, *dd*, *J* = 17 Hz, 13 Hz, H-3α), 3.83 (3H, *s*, 4'-OCH_3), 5.32 (1H, *dd*, *J* = 13 Hz, 3 Hz, H-2), 6.95 (2H, *d*, *J* = 9 Hz, H-3',5'), 7.39 (2H, *d*, *J* = 9 Hz, H-2',6'), 12.29 (1H, *s*, chelated 5-OH). ^{13}C-NMR (100.4 MHz,$CDCl_3$) δ (ppm) : 6.9 (8-CH_3), 7.6 (6-CH_3), 43.1 (3α,3β), 55.2 (OCH_3), 78.2 (C-1), 102.3 (C-8), 102.7 (C-10), 103.5 (C-6), 114.0 (C-3',5'), 127.4 (C-2',6'), 131.0 (C-1'), 157.7 (C-9), 158.8 (C-5), 159.6 (C-4'), 162.1 (C-7), 196.5 (C-4). EIMS, m/z (rel.int.): 314 (M^+)(100), 207 (M-C_7H_7O)$^+$(9), 206 (M-C_7H_7O-H)$^+$(6), 180 (M-$C_9H_{10}O$)$^+$(89), 152 (M-$C_9H_{10}O$-CO)$^+$(70), 134 (M-$C_9H_8O_4$)$^+$(36), 121 (24), 91 (10), 77 (5), 69 (5), 55 (4).

Compound 2 was obtained as pale yellow crystal (MeOH-$CHCl_3$), mp. 135-136 °C, $[\alpha]_D^{20}$ = +7° (MeOH, *c*.0.1), gave positive test with $FeCl_3$ (green) and shinoda test (Mg-HCl)(pale red). It showed a single spot by TLC on silica gel with Rf = 0.14 ($CHCl_3$ - EtOAc = 1 : 4), 0.28 ($CHCl_3$ -MeOH = 9 :1) and 0.38 ($CHCl_3$-MeOH = 5:1). UV (MeOH) λ_{maks} (log ε) : 282 (3.41), 361 (sh) (2.77) nm; (MeOH + NaOH): 284 (3.33), 372 (sh) (2.88)nm; (MeOH+$AlCl_3$): 281 (3.37), 362 (sh)(2.73) nm; (MeOH+$AlCl_3$+HCl): 283 (3.36), 363 (sh) (2.76) nm; (MeOH+NaOAc): 282 (3.41), 362 (sh) (2.77) nm; (MeOH+NaOAc+H_3BO_3): 282 (3.41), 363 (sh) (2.74) nm. IR (KBr) ν_{maks} : 3432 (OH), 2928 (alkyl C-H), 1636 (chelated C=O), 1516 (aromatic C=C), 1456, 1356, 1125, 1069, 835 cm^{-1}. ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm) : 2.05(3H, *s*, 8-CH_3), 2.07 (3H, *s*, 6-CH_3), 2.84 (1H, *dd*, *J* = 17.2 Hz, 2.8 Hz, H-3β), 3.04 (*m*, H-5"), 3.12 (1H, *m*, H-4"), 3.21 (1H, *m*, H-3"), 3.28 (1H, *m*, H-2"), 3.32 (1H, *brd*, *J* = 4.8 Hz, H-3α), 3.60 (1H, *dd*, J = 10.4 Hz, 4.8 Hz, H-6"), 3.40 (1H, *dd*, J = 11,6 Hz, 5.6 Hz, H-6"), 3.76 (3H, *s*, 4'-OCH_3), 4.58 (1H, *d, J* = 7.2 Hz, H-1"), 5.54 (1H, *m*, H-2), 6.98 (2H, *d*, *J* = 8.4 Hz, H-3',5'), 7.45 (2H, *d*, *J* = 8.8 Hz, H-2',6'), 12.10 (1H, *s*, chelated 5-OH). ^{13}C-NMR (100.4 MHz, DMSO-d_6) δ (ppm) : 8.70 (6-CH_3), 9.27 (8-CH_3), 42.19 (3α,3β), 55.16 (OCH_3), 61.05 (C-6"), 69.86 (C-4"), 74.06 (C-2"), 76.34 (C-3"), 77.03 (C-5"), 77.84 (C-2), 104.19 (C-1"), 109.96 (C-10), 110.13 (C-8), 111.21 (C-6), 113.94 (C-3',5'), 127.99 (C-2',6'), 130.82 (C-1'), 157.27 (C-9), 157.85 (C-5), 159.36 (C-4'), 161.40 (C-7), 198.46 (C-4). FABMS, m/z (rel.int.): 515 (M+K^+)(2), 477 (M+H^+) (1), 345 (2 m-NBA+K^+)(22), 315 (aglycone+H^+), 314 (aglycone) (3), 307 (2 m-NBA+H^+)(14), 192 (m-NBA+H^+)(100), 136 (m-NBA-OH) (86).

RESULT AND DISCUSSION

Compound 1 was isolated from ethyl acetate soluble fraction of methanol extract of the *C. sakayensis*'s stem as a pale yellow needles (benzene), mp.167-167 °C, $[\alpha]_D^{20}$ = -26° (MeOH, *c*.0.1), gave positive test with $FeCl_3$ (green) and shinoda test (Mg-HCl) (red). The EIMS spectrum of **1** showed a molecular ion peak at m/z 314, corresponds a molecular formula $C_{18}H_{18}O_5$. The UV spectrum of **1** indicated absorbtion characteristic of flavanone-type compounds at 297 nm (band II) and 343 nm (sh) (band I) [5]. The absorbtion bands of alkyl C-H (2922, 2840 cm^{-1}), chelated carbonyl group (1630 cm^{-1}), and aromatic C=C (1520 cm^{-1}) in the IR spectrum, together with the existence of the ABX-type proton signals at δ_H 2.78 (*dd*, H-3β), 3.05 (*dd*, H-3α), and 5.35 (*dd*, H-2) in the ^{1}H-NMR spectrum (Table 2) also supported that **1** was a flavanone [6]. The batochromic shift of band II (42 nm) on adding NaOH and NaOAc reagent showed the presence of a hydroxyl group at C-7 [5]. The presence of OH group at C-5 was supported by the batochromic shift of band II (11 nm) on adding $AlCl_3$ + HCl reagent. No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that **1** didn't have ortho-dihydroxyl group at the A-ring. The chelated proton signal at δ_H 12.29 (*s*) indicated the presence of a hydroxyl group at C-5. Futher the ^{1}H-NMR spectrum of 1 showed the existence of two aromatic methyl groups [δ_H 2.03 (*s*), 2.05 (*s*)] and a methoxyphenyl group [δ_H 3.85 (*s*)] in the flavanone skeleton (Table 1). In the HMBC spectrum of **1**, the proton signal of the first aromatic methyl group (δ_H 2.03) showed correlation with carbon signals of C-5 (δ_C 158.8), C-6 (δ_C 103.5), C-7 (δ_C 162.1), while the proton signal of the second aromatic methyl group (δ_H 2.05) correlated with carbon signals of C-7 (δ_C 162.1), C-8 (δ_C 102.3), C-9 (δ_C 157.7) (Table 1). These results indicated that the first and the second aromatic methyl groups should be located at C-6 and C-8, respectively. The correlation between proton signal of methoxyphenyl group (δ_H 3.85) with carbon signal of C-4' (δ_C 159.6) in the HMBC spectrum, together with the appearance of two aromatic proton signals at δ_H 6.95 (*d, J* = 9.0 Hz, H-3',5') and 7.39 (*d, J* = 8.7 Hz, H-2',6') due to two pairs of ortho-coupled



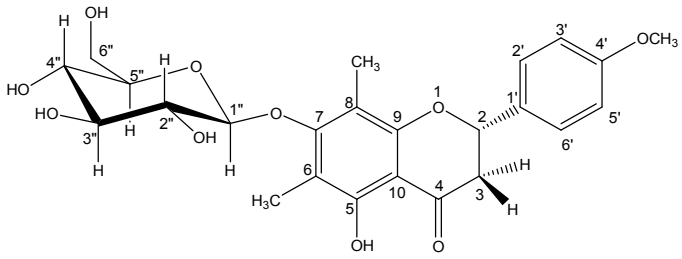
(1)

Table 1. ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of **1** in $CDCl_3$

Position of Atom C	^{1}H-NMR (δ ppm, mult.,*J*)	^{13}C-NMR (δ ppm)	^{1}H-^{13}C HMBC
1	-	-	-
2	5.32 (*dd*,*J*=13 Hz,3 Hz)	78.2	C-1',C-2',C-6'
3α	3.03 (*dd*,*J*=17 Hz,13 Hz)	43.1	C-4, C-10
3β	2.78 (*dd*,*J* = 17 Hz,3 Hz)	43.1	C-2, C-4, C-1'
4		196.5	-
5	-	158.8	-
6	-	103.5	-
7	-	162.1	-
8	-	102.3	-
9	-	157.7	-
10	-	102.7	-
1'	-	131.0	-
2'	7.39 (*d*,*J*=9 Hz)	127.4	C-2, C-3', C-4', C-6'
3'	6.95 (*d*,*J*= 9 Hz)	114.0	C-1',C-4', C-5'
4'	-	159.6	-
5'	6.95 (*d*,*J*= 9 Hz)	114.0	C-1',C-4', C-5'
6'	7.39 (*d*,*J*=9 Hz)	127.4	C-2, C-2', C-4', C-5'
6-CH_3	2.03 (*s*)	6.9	C-5, C-6, C-7
8-CH_3	2.05 (*s*)	7.6	C-7, C-9
4'-OCH_3	3.83 (*s*)	55.2	C-4'
5-OH	12.29 (s)	-	-

aromatic protons in the B-ring indicated the presence of a methoxy group at C-4'. The other significant correlations of **1** can be seen in Table 1. Further supporting evidence of structure 1 for matteucinol came from comparison of the ^{1}H-NMR, ^{13}C-NMR, and EIMS spectral data with those of reported data in literature [6,7]. From the above results, compound **1** was identified as matteucinol (5,7-dihydroxy-4'-methoxy-6,8-dimethyl flavanone).

Compound 2 was isolated from the ethyl acetate soluble fraction of methanol extract of the *C. sakayensis*'s stem as a pale yellow powder ($CHCl_3$-MeOH), mp. 135-136 ^{o}C, gave positive test with $FeCl_3$ test (green) and Shinoda-test (Mg-HCl) (pale red). The



(2)

Table 2. ^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR data of **2** in DMSO-d_6

Position of Atom C	δ_H (mult.,*J* in Hz)	δ_C	^{1}H-^{13}C HMBC
1	-	-	-
2	5.54 (*m*)	77.84	C-1',C-2', C-6', C-4
3α	3.32 (*brd*,4.8)	42.19	C-4
3β	2.84 (*dd*,17.2,2.8)	42.19	C-4
4		198.46	-
5	-	157.85	-
6	-	111.21	-
7	-	161.40	-
8	-	110.13	-
9	-	157.27	-
10	-	109.96	-
1'	-	130.82	-
2'	7.45 (*d*,8.8)	127.99	C-2, C-3', C-4', C-6'
3'	6.98 (*d*,8.4)	113.94	C-1',C-2', C-4', C-5'
4'	-	159.36	-
5'	6.98 (*d*,8.4)	113.94	C-1',C-3', C-4', C-6'
6'	7.45 (*d*,8.8)	127.99	C-2, C-2', C-4', C-5'
6-CH_3	2.05 (*s*)	8.70	C-5, C-6, C-7
8-CH_3	2.07 (*s*)	9.27	C-7, C-8,C-9
4'-OCH_3	3.76 (*s*)	55.16	C-4'
5-OH	12.10 (*s*)	-	C-5,C-6,C-8
1"	4.58 (*d*, 7.2)	104.19	C-7
2"	3.28 (*m*)	74.06	
3"	3.21 (*m*)	76.34	
4"	3.12(*m*)	69.86	
5"	3.04 (*m*)	77.03	
6"	3.60 (*dd*,10.4, 4.8), 3.40 (*dd*, 11.6, 5.6)	61.05	

FABMS spectrum of **2** a quasi molecular ion peak at m/z 477 $[M+H^{+}]$, suggesting a molecular formula $C_{24}H_{28}O_{10}$. The absorption maxima at 282 nm (band II) and 361 nm (sh) (band I) in the UV spectrum supported that compound supported that compound **2** was a flavanone [5]. The presence of absorbtion bands for alkyl C-H (2928 cm^{-1}), chelated carbonyl group (1636 cm^{-1}), and aromatic C=C (1516 cm^{-1}) in the IR spectrum, together with the existence of the ABX-type

signals at δ_H 2.84 (*dd*, H-3β), 3.32 (*brd*, H-3α), and 5.54 (*m*, H-2) in the ^{1}H-NMR spectrum (Table 2) also supported that compound **2** had the flavanone skeleton. No batochromic shift of band II on adding NaOH and NaOAc reagent showed that **2** didn't have a free hydroxyl group at C-7. No batochromic shift on adding NaOAc + H_3BO_3 reagent supported that **2** didn't have ortho-dihydroxy group at A-ring. The chelated proton signal at δ_H 12.10 (s) indicated the existence of a hydroxyl group at C-5. The ^{1}H-NMR spectrum of 2 exhibited proton signals due to a 4'-methoxyphenyl group at δ_H 3.76 (3H, s, 4'-OCH_3), 6.98 (2H, d, J=8.4 Hz), and 7.45 (2H, d, J=8.8 Hz), two aromatic methyl group at δ_H 2.05 (3H, s) and 2.07 (3H, s) as well as a glycosyl group at δ_H 4.58 (1H, d, J = 7.2 Hz, H-1") and δ_H 3.04-3.60 (6-H glycosyl)(Table 2). The glycosyl group of **2** could be identified as a glucosyl group because its carbon signals resembled those of reported data in literature [5]. In the HMBC spectrum of **2**, proton signal of methoxyphenyl group (δ_H 3.76) showed correlation with carbon signal of C-4' (δ_C 159.36), proton signal of the first aromatic methyl group (δ_H 2.05) correlated with carbon signals of C-5 (δ_C 157.85), C-6 (δ_C 111,21), C-7 (δ_C 161.40), and the proton signal of the second aromatic methyl group (δ_H 2.07) correlated with carbon signals of C-7 (δ_C 161.40), C-8 (δ_C 110.13), C-9 (δ_C 157.27) (Table 2). These results suggested the presence of methoxyphenyl group at C-4' and aromatic methyl group at C-6 and C-8, respectively. The correlation between proton signals of anomeric proton of glucosyl group (δ_H 4.58) with carbon signal of C-7 (δ_C 161.40) in the HMBC spectrum of **2** showed the presence of glucosyl group at C-7. Meanwhile the coupling constant value of the anomeric proton was 7.2 Hz, indicated the presence of a β-glycosydic linkage to a aglycone [8]. Further supporting evidence of structure **2** for matteucinol-7-O-β-D-glucoside came from comparison of the ^{1}H-NMR and ^{13}C-NMR spectral data with those of reported data in literature [6]. From above results compound **2** was suggested to be a matteucinol-7-*O*-β-D-glucoside.

CONCLUSION

Two alkylated flavanone, matteucinol and matteucinol-7-*O*-β-D-glucoside had been isolated from the ethyl acetate soluble fraction of the methanol extract of the fern *C. sakayensis*'s stem. This is the first report of the chemical constituents from this species and the genus *Chingia* of the fern.

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