A Flavanoid Compound Isolated From The Dichloromethane Extract Of The Silver Fern (Pityrogramma Calomelanos)

by Suyatno Sutoyo

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Abstract. A flavanoid compound, namely 2',6'-dihydroxy-4'-methoxydihydrochalcone had been separated from the dichloromethane extract of the silver fern (*Pityrogramma calomelanos).* It was obtained as pale yellow needles crystal with m.p. of 167 – 168 ºC. Its structure was identified based on the spectroscopic evidence and by comparison with reported literature data.

1. Introduction

Pityrogramma calomelanos is one of the ferns belonging the Polypodiaceae family widely distributed in tropical Asia, especially Indonesia. The origin of silver fern is from tropical America, but it is now widespread in the tropical Asia region. It usually grew in open region, near streams, slope of mountain, and old wall [1]. This fern was used as the ornamental plant and phytoremediation land polluted mercury, lead, arsenic, and zink [2]. The silver fern also can be used as the ground cover plant because it can cover the garden with good. Several flavonoid compounds in dihydrochalcone type had been separated from the fern species in *Pityrogramma* genus. Flavonoid 2',6'-dihydroxy-4,3'-dimethoxy-4',5'-metilendioxy dihydrochalcone was isolated from *Pityrogramma ebenea* [3]. While from Pityrogramma triangularis had been separated dihydrochalcone namely 2',6',4-trihydroxy-3'-methyl-4'-methoxy dihydrochalcone and 2',6',4-trihydroxy-3',5'-dimethyl-4'-methoxy dihydrochalcone [4]. Beside it was also found chalcone, namely ceroptene (2'-hydroxy-3',3'-dimethyl-4'-methoxy-6-oxo chalcone) and triangularine (2',6',4-trihydroxy-3'-methyl-4'-methoxy chalcone) Therefore, the chemical constituents of the dichoromethane extract of *P. calomelanos* had not been reported. In the course of our studies, a flavonoid in dihydrochalcone type namely 2',6'-dihydroxy-4'-methoxydihydrochalcone had been isolated from the aerial part of *P. calomelanos*'s dichloromethane extract. In this paper, we reported the isolation and structure determination of this isolate.

2. Data and methods

2.1. General experimental procedures

Melting point was measured by Fisher John melting point apparatus and was uncorrected. UV spectra were recorded on Shimadzu Pharmaspec UV-1700 spectrophotometer. IR spectrum in KBr film was determined by Shimadzu FTIR-8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured by JEOL JNM ECA-500 spectrometer [operating at 500 MHz (^{1}H) and 125.7 MHz (^{13}C)]. Mass spectrum (MS) was recorded on Shimadzu QP-5000 spectrometer using electron impact (EI) ion mode. Kieselgel 60 GF-254 (Merck) were used for vacuum liquid chromatography (VLC). 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.

2.2. Plant materials

The aerial part of *P. calomelanos* was collected from Kletak forest, Nongkojajar district, Pasuruan, East Java, Indonesia April 2017. A voucher spesimen was identified and deposited at the herbarium of the Purwodadi Botanical Garden, East Java, Indonesia.

2.3. Extraction and isolation

The dried powdered of *P. calomelanos*'s aerial part (5 k was exhaustively extracted successively with *n*-hexane (8 L x 3) and dichlorometane (8 L x 3) at room temperature. The dichloromethane extract was evaporated by reduced pressure, revealed the blackish green solid (98 g).

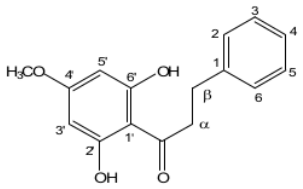
A portion of dichloromethane extract (5.0 g) was separated by VLC and eluted with solvents of increasing polarity (*n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate) yielded 120 fractions (15 mL each). Removal of the solvent under reduced pressure of the combined fractions of 68-78 gave the dark green solid (525 mg). It was recrystalized in chloroform-n-hexane afforded a flavonoid 2',6'-dihydroxy-4'-methoxydihydrochalcone (**1**) (134 mg).

2',6'-dihydroxy-4'-methoxydihydrochalcone (**1**) was obtained as pale yellow crystal (chloroform-n-hexane), mp. 167-168 °C, which gave positive test with Shinoda test (Mg-HCl) (yellow) and $FeCl_3$ (greenish yellow). It showed one spot TLC using three eluents system with Rf of 0.86 (chloroform-ethyl acetate = 9 : 1), 0.57 (*n*-hexane-ethyl acetate = 4 : 1), and 0.41 (*n*-hexane-ethyl aceate = 9 : 1). UV (MeOH) λ_{max} (log ε) : 285 (3.72), 330 (sh) (2.89) nm; (MeOH + NaOH): 295 (3.68), 364 (sh) (3.21) nm; (MeOH+$AlCl_3$): 305 (3.76), 374 (sh) (2.83) nm; (MeOH+$AlCl_3$+HCl): 288 (3.68), 375 (sh) (2.50) nm; (MeOH+NaOAc): 285 (3.75) nm; (MeOH+NaOAc+H_3BO_3): 285 (3.76) nm. IR (KBr) ν_{max} : 3253 (OH), 3014 (aromatic C-H), 2969, 2862 (alkyl C-H), 1646 (chelated C=O), 1593, 1527 (aromatic C=C), 1435, 1384, 1216, 1074 cm^{-1}. ^{1}H-NMR (500 MHz,$CDCl_3$) δ (ppm) : 3.02 (2H, *t*, *J* = 7.95 Hz, H-β), 3.40 (2H, *t*, *J* = 7.3 Hz, H-α), 3.79 (3H, *s*, 4'-OCH_3), 5.93 (2H, *s*, H-3' and H-5'), 7.25 (5H, *m*, H-2,3,4,5,6). ^{13}C-NMR (125.8 MHz,$CDCl_3$) δ (ppm) : 30.7 (C-β), 45.8 (C-α), 55.7 (4'-OCH_3), 94.6 (C-3',5'), 104.9 (C-1'), 126.1 (C-4), 128.6 (C-2,6), 128.7 (C-3,5), 141.8 (C-1), 165.7 (C-2',4',6'), 204.7 (C=O). EIMS, m/z (rel. int., %): 272 (25), 255 (6), 177 (3), 167 (100, base peak), 140 (38), 136 (3), 124 (3), 111 (6), 104 (6), 91 (22), 77 (6), 69 (6), 51 (6), 39 (6).

3. Results and analysis

Compound **1** showed the positive results on phenolic test and flavonoid test using the $FeCl_3$ reagent (yellowish green) and Shinoda test (Mg + HC1) (yellow), respectively. It showed that isolate was a flavonoid compound [5,6]. The absorption bands of IR spectrum at 3253 (OH), 3014 (aromatic C-H), 2969, 2862 (alkyl C-H), 1646 (chelated C=O), 1593, 1527 (aromatic C=C) supported that isolate was a flavonoid. The UV spectrum of **1** showed absorbtion characteristic of dihydrochalcone-type compounds at 285 nm (band II) and 330 nm (sh) (band I) [6,7]. No significant bathochromic shift of band II on adding of NaOH and NaOAc reagents indicated that the isolate did not have a free hydroxyl group at C-4'. The bathochromic shift of band II on adding of $AlCl_3$ + HCl reagent supports the existence of an free hydroxyl group at C-2'. While the addition of NaOAc + H_3BO_3 did not cause the bathochromic shift of band II. This showed that flavonoid isolate did not have the ortho-dihydroxy group at A ring. Two triplet proton signal at δ_H 3.02 and 3.40 ppm due to H-α and H-β, respectively, supported that **1** had a basic skeleton of dihydrochalcone. While the presence of singlet proton signal at δ_H 3.79 ppm indicated the presence of methoxy group at C-4'. Multiplet proton signal at δ_H 7.25 ppm indicated that structure of **1** was similar with the B ring of pinocembrine [7,8,9] that was not substituted. The ^{13}C-NMR spectrum of **1** exhibited 11 carbon signals represented 16 carbon signals, consisted of alkyl carbon [δ_c 30.7 (C-β), 45.8 (C-α)], methoxy carbon [δ_c 55.7 (4'-OCH_3)], aryl carbon [δ_c 94.6 (C-3',5'), 104.9 (C-1'), 126.1 (C-4), 128.6 (C-2,6), 128.7 (C-3,5), 141.8 (C-1)],

oxyaryl carbon [δ_c 165.7 (C-2',4',6')] and carbonyl carbon [δ_c 204.7 (C=O)]. The EIMS spectrum of **1** showed a molecular ion peak at m/z 272, corresponding a molecular formula $C_{16}H_{16}O_4$. A peak at m/z 167 was caused by benzoil group from the A ring, while a peak at m/z 104 indicated the presence of 2-phenyl ethyl group from the B ring. A peak at m/z 16 was supported that the B ring did not have subtituents. From the above results, compound **1** was identified as 2',6'-dihydroxy-4'-methoxy-dihydrochalcone (**1**). This flavonoid is the first time reported from the dichloromethane extract of *P. calomelanos*'s aerial part. However its existence was ever reported from the *Notholaena sulphurea* and *Populus spp* [10].



(**1**)

4. Conclusions

A flavonoid compounds namely 2',6'-dihydroxy-4'-methoxy-dihydrochalcone was separated from the dichloromethane extract of *P. calomelanos*'s aerial part. It was obtained as pale yellow needles crystal with m.p. of 167 – 168 ^oC. This flavonoid is the first reported from the dichloromethane extract of *P. calomelanos*'s aerial part.

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References

[1] Steenish V, Holttum RE 1982 *Flora Malesiana* London: Junk Publisher.
[2] Visoottiviseth P, Francesconi K, Sridokchan W 2002 *Environ. Pollut.* **118 (3)** 453-61.
[3] Miraglia MDC, Padua APD, Mesquita, AAL, Gottlieb OR. 1985 *Phytochemistry* **24** (**5**) 1120
[4] Wollenweber E, Dietz VH, Schilling G, Favre-Bonvin J, Smith DM.1985 *Phytochemistry* **24** (**5**) 965-971.
[5] Robinson T 1991 *The Organic Constituents of Higher Plants*. 6th ed North Amherst, MA : Cordus Press.
[6] Markham KR 1982 *Techniques of Flavonoid Identification* London: Academic Press.
[7] Sutoyo S, Indrayanto G, Zaini NC 2007 *Natural Product Communications* **2 (5)** 579-580.
[8] Markham KR , Geiger H 1994 ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In *The Flavonoids. Advances in research since 1986*. Harborne JB (Ed) London: Chapman & Hall.
[9] Bick IRC, Brown RB, Hillis WE 1972 *Aust. J. Chem.* 25, 449-451.
[10] Bohm BA 1994 The minor flavonoids. In *The Flavonoids. Advances in research since 1986*. Harborne JB (Ed). London: Chapman & Hall.

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