Indo. J. Chem., 2010, 10 (2), 222 - 225

6,6'-DIMETHOXY-4,4'-DIHYDROXY-3',2'-FURANO-ISOFLAVANE, A NEW COMPOUND FROM *Melochia umbellata* (Houtt.) Stapf *var*. Degrabrata K. (*Paliasa*)

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Received January 22, 2010; Accepted April 15, 2010

ABSTRACT

Using data from UV, IR, ^{1}H-NMR, ^{13}C-NMR, dan NMR-2D analysis, a compound of 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane (1) was successfully isolated from chloroform fraction of heartwood in Melochia umbellata (Houtt.) Stapf var. Degrabarata K (Paliasa). Hitherto no report of such compound found in literature. The compound however has not shown a significant bioactivity according to either brine shrimp lethality test or evaluation of anti tumor activity against leukemia murine cell (P-388).

***Keywords**: isoflavane, heartwood, chloroform and Melochia umbellata*

INTRODUCTION

Melochia umbellata (Houtt.) Stapf *var*. Degrabrata K (Sterculiaceae) grows well in tropical and subtropical forest [1]. This plant has been known by people in South Sulawesi Province as *paliasa* and used to treat several kinds of disease such as hepatitis, high blood cholesterol concentration, diabetes, and hypertension [2].

Previous studies revealed that methanol extract of heartwood of *M. umbellata* showed high toxicity against *Artemia salina* Linn [3], while β-sitosterol was found in n-hexane fraction [4-5].

This paper reports a new compound, 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane (1) isolated from heartwood of *M. umbellata.*

EXPERIMENTAL SECTION

Materials

The heartwood of *M. umbellata was* collected from Makassar city, South Sulawesi, Indonesia. The plant specimen was identified and deposit at Herbarium Bogoriensis, LIPI Bogor, Indonesia. Flash chromatography with Merck Si gel 60 (230–400 mesh), and TLC analysis on precoated Si gel plates (Merck Kieselgel 60 F 245, 0.25 mm). Hexane, ethyl acetate, acetone, methanol, and chloroform were distillated before using in extraction and chromatographic section.

Instrumentation

Melting point determination was carry out by Fisher Johns apparatus and uncorrected. UV and IR spectra were measured with CARY 100 Conc. EL. 98113099 and PERKIN ELMER Spectrum one L.R. 64912C spectrophotometers, respectively. ^{1}H and ^{13}C-NMR spectra were recorded, with JEOL JNM EX-400 FTNMR spectrometer, operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), using TMS as internal standard. While MS spectrum was measured with MS Mariner Biospectrometry, system ESI (Electrospray Ionization) positive ions mode.

Procedure

Extraction

The amount of 12.7 kg heartwood of *M. umbellata* was ground into powder and extracted exhaustively with methanol for 3 x 24 h at room temperature. The methanol extract was collected, filtered, and concentrated using rotary evaporator to yield 167 g methanol extract. Total extract was further partitioned with n-hexane, chloroform, and ethyl acetate to obtain 9.1, 20, and 10 g respectively.

Isolation and purification

Chloroform extract (20 g) was further fractionated by vacuum chromatography eluting with a gradient of hexane-ethyl acetate to give 35 fractions. The resulting fractions were combined according to their TLC profiles to give ten combined fractions in total; B1 (47.1 mg), B2 (25 mg), B3 (100 mg), B4 (397 mg), B5 (590 mg), B6 (200 mg), B7 (3.476 mg), B8 (1.064 mg), B9 (1.424 mg), B10 (1.149 mg)

Fraction B4 (397 mg) was subjected to flask chromatography eluting with a gradient of 1:9; 2:8, 3:7, 4:6, 0:10 hexane/acetone to obtain ten mayor sub

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fraction; B41 (3.1 mg), B42 (7 mg), B43 (10.9 mg), B44 (14.3 mg), B45 (14 mg), B46 (12 mg), B47 (60 mg), B48 (39 mg), B49 (64 mg), and B4.10 (62 mg).

Fraction B49 (64 mg) was gradually eluted by mixture of hexane/acetone at various proportions yielding three mayor fractions; B491 (13.1 mg), B492 (17 mg), and B493 (13 mg). Fraction B492 was further purified through recrystallization using acetone-chloroform mixture to have 5.8 mg of white powder (compound 1).

Biological assay

Bioactivity of compound was evaluated using brine shrimp *Artemia salina* [6] and P-388 murine leukemia for its toxicity and cytotoxicity [7], respectively.

RESULT AND DISCUSSION

Compound 1 was obtained as white powder (5.8 mg) with an m.p 248-249 °C. The NMR and ESI-MS ($[M - H + 2Na]^{2+}$ at m/z 387) data were consistent with a molecular formula of $C_{19}H_{18}O_6$. The UV spectrum indicates maximum absorption (MeOH) λ_{max}(log ε) at 206 (4.62), 232 (4.27), and 322 (3.92) nm where the last belongs to a group conjugated aromatic which is supported by maximum absorption at IR (KBr) ν_{max} 3452 cm^{-1} corresponding to a hydroxyl group. Infra red spectra also indicated the existence of double bond C-H groups at wave number of 3055 and 3005 cm^{-1} and aromatic C=C stretching 1610 cm^{-1}, aliphatic C-H at 2933, 2906, and 2837 cm^{-1}.

The structure has been established from its ^{1}H- and ^{13}C-NMR (include NMR 2D) spectra. Spectra of its ^{1}H-NMR showed some aromatic signals correlated to a unit ring A coming at δ 7.55 ppm, multiple doublet with coupling constant 2.45 Hz indicated that there was a meta-coupled proton with a proton at δ 7.01 (dd; *J* = 1.80 and 7.95 Hz) ppm, while a chemical shift at 6.91 (d; *J* = 7.95) ppm suggesting that this proton orto-coupled with a proton at δ 7.01 ppm. These protons were located in aromatic ring (ring A) with ABX system. Signal at δ 6.84 (1H; s) is an isolated proton located at pentasubtituted aromatic ring (ring B).

The presence of furan ring (ring C) was indicated by cis-coupled proton signal at δ 7.88 ppm (1H; d; 9.75) and 6.26 ppm (1H; d; 9.75). Proton signals at 3.56 ppm (2H; m), 4.25 ppm (1H; m) and 5.1 ppm (1H; d; 8.6) were specific proton attached to sp^3 carbon at 2, 3 and 4 position of isoflavanol.

^{1}H-NMR spectrum also showed the signals for two methoxy groups at δ 3.83 ppm (3H; s) attached to C-6 and 3.87 ppm (3H; s) which attached to C-6'.

^{13}C-NMR spectra of compound 1 (Table 1) showed all 19 signals represent 19 carbons which were analyzed as five aliphatic carbons (sp^3) and 14 carbon sp^2 (2 C alkenes, and 12 C aromatics). Base on DEPT 135 analysis showed presence of two methyl carbons, one methylene carbon, eight methyne carbons, and eight quarternary carbons.

HMBC spectrum showed a long correlation between proton at δ 7.15 ppm (H-5) and quarternary carbon at δ 148.6 ppm (C-6) and methyne carbon at δ 121.9 ppm (C-7). A long correlation between proton at δ 7.01 ppm (H-7) with methyne carbon at δ 112.3 (C-5) and quarternary carbon at 148.4 ppm (C-9). In addition, long correlation was exhibited by proton with

Table 1. NMR Data of compound 1 in $CDCl_3$

No	δC	δH (number of H; multipl., *J*= Hz)	COSY	HMBC (H⇔C)
2	61.5	3.56 (2H; m)	3	-
3	79.6	4.25 (1H; m)	4	-
4	77.6	5.10 (1H;d; 8.60)	3	5, 1'
5	112.3	7.15 (1H; d; 2.45)	-	6,7, 9
6	148.6	-	-	-
7	121.9	7.01 (1H; dd; 7.95 dan 1.80)	8	8,9
8	115.9	6.91 (1H; d; 7.95)	7	6,10
9	148.4	-	-	-
10	128.5	-	-	-
1'	112.5	-	-	-
2'	160.0	-	-	-
3'	133.3	-	-	-
4'	140.0	-	-	-
5'	101.7	6.84 (1H; s)	-	4', 6'
6'	146.2	-	-	-
7'	145.2	7.88; (1H;d;9.75)	8'	2', 4'
8'	114.4	6.26 (1H;d; 9.75)	7'	-
OMe	56.4	3.83 (3H; s)	-	6'
OMe	56.5	3.87 (3H; s)	-	6

Table 2. Formation of fragments/adducts in ESI-MS spectra of compound 1

No	Fragment/adduct	m/z
1	$[3M]^+$	1026
2	$[2M + 163 (C_{10}H_{11}O_3) + Na]^{2+}$	870
3	$[2M + - H + 97 (C_5H_5O)+Na]^{2+}$	803
4	$[2M+ H +66 (C_4H_2O) + Na]^{2+}$	774
5	$[2M + 66 (C_4H_2O) + Na]^{2+}$	773
6	$[2M - H + 66 (C_4H_2O) + Na]^{2+}$	772
7	$[M -H + 2Na]^{2+}$	387
8	$[M -OH - C_4H_2O]^+$	259
9	$[M + H – 163 (C_9H_7O_3)]^+$	180

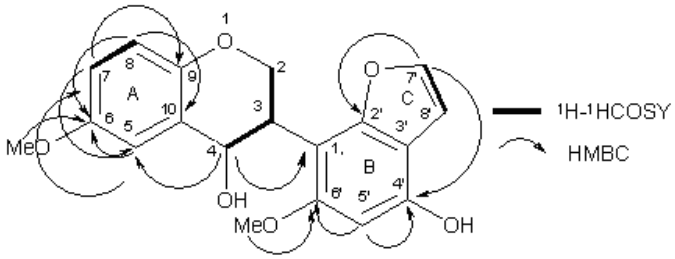

Fig 1. Molecular structure of 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane elaborated by COSY and HMBC correlations

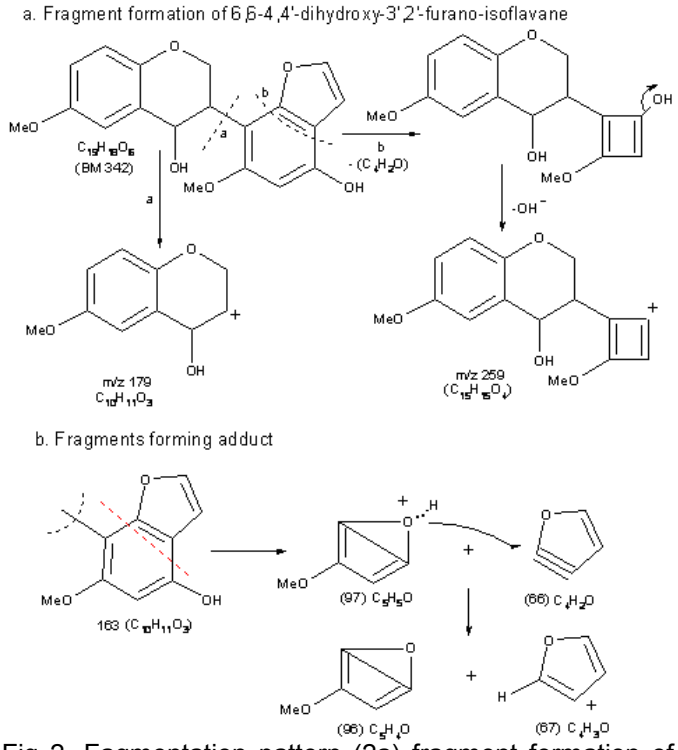

Fig 2. Fagmentation pattern (2a) fragment formation of 6,6'-4,4'-dihydroxy-3',2'-furano-isoflavane and (2b) Fragments forming adduct

δ 6.91 ppm (H-8) with quarternary carbon at δ 148.6 ppm (C- 6) and at δ 128.5 ppm (C-10). It's established the occurrence of A ring.

A long correlation between proton with δ 5.1 (H-4) and methyne carbon at δ 112.3 (C-5) and quarternary carbon at δ 112.5 (C-1') revealed that compound 1 was isoflavane-4-ol, supported by COSY data that there were correlation between H-3 at δ 4.25 ppm with H-4 at δ 5.10 ppm and H-2 at δ 3.56 ppm.

HMBC spectra also showed a long-range correlation between proton at δ 6.84 ppm (H-5') with quaternary carbons at δ140.0 ppm (C-4') and 146.2 ppm (C-6'), and a proton at δ 7.88 (H-7') with methyne carbon at δ 160.0 ppm (C-2') and 140.0 ppm (4') supporting of ring C and D. Ring furan (Fig. 2) was also supported by forming adduct dimmers at spectrum of ESI-MS m/z, 774 $[2M + H + 66 (C_4H_2O) + Na]^{2+}$, 773 $[2M + 66 (C_4H_2O) + Na]^{2+}$, 772 $[2M + (66) C_4H_2O –H + Na]^{2+}$ and m/z 259 $[M-OH - C_4H_2O]^+$ (See also Table 2).

Base on NMR data analysis of compound 1 (Table 1) which was supported by ESI-MS data, it suggesting that compound 1 was 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane as shown in Fig. 1. Literature study revealed that 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane is a new compound isolated from *Melochia umbellata* plant. This compound has not been indicated any significant bioactivity both against *Artemia salina* (LC_{50} >1000 μg/mL) and P-388 murine leukemia cell (IC_{50} >100 μg/mL).

CONCLUSION

A new compound from chloroform extract of the heartwood of *M. umbellata* was discovered, with structure 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavane. With no important bioactivity, it is worth to carry out further investigation on its function in plant.

ACKNOWLEDGEMENT

We thank the Directorate General for Higher Education for financial help and Herbarium Bogoriense, LIPI Bogor, Indonesia, for plant identification.

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