SHORT COMMUNICATION

A STIGMASTEROL GLYCOSIDE FROM THE ROOT WOOD OF *Melochia umbellata* (Houtt) Stapf *var.* degrabrata K.

Ahmad Ridhay[1,2], Alfian Noor[2,*], Nunuk H. Soekamto[2], Tjodi Harlim[2], and Ian van Altena[3]

[1]*Department of Chemistry, Tadulako University, Palu, Indonesia*

[2]*Department of Chemistry, Hasanuddin University, Makassar, Indonesia 90245*

[3]*School of Environmental and Life Science, University of Newcastle, Callaghan, NSW, Australia 2308*

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ABSTRACT

A stigmasterol glycoside (stigmast-5,22-dien-3-O-β-D-glucopyranoside) has been isolated from the chloroform fraction of root wood of Melochia umbellata (Houtt) Stapf var. degrabrata K. The compound structure was determined on spectroscopic evidences including IR, 1D and 2D NMR and compared to previous data. The isolate was also active against Aspergillus niger.

Keywords: *Melochia umbellata; root wood; stigmasterol glycoside; Aspergillus niger*

ABSTRAK

Telah diisolasi stigmasterol glikosida (stigmast-5,22-dien-3-O-β-D-glukopiranosida) dari ftraksi kloroform kayu akar Melochia umbellata (Houtt) Stapf var. degrabrata K. Struktur isolat ditentukan berdasarkan data spektroskopi IR dan NMR (1D dan 2D) dan dibandingkan dengan data sebelumnya. Isolat tersebut juga aktif terhadap jamur Aspergillus niger.

Kata Kunci: *Melochia umbellate; kayu akar; stigmasterol glikosida; Aspergillus niger*

INTRODUCTION

The Sterculiaceae family has approximately 70 genera and 1500 species [1]. Its roots are widely known having contained many bioactive compounds such as flavonoids as an antifungal from *Hildegardia barteri* [2], isoprenylated naphtoquinone from *Firmiana platanifolia* which is active against leukemia cells P-388 [3], the alkaloid quinolinone from *Waltheria douradinha* as antibacterial [4], melochinone and melovinone from *Melochia tomentosa* [5-6], and some alkaloids cyclopeptide from *Melochia chamaedrys* [7].

One of species, *Melochia umbellata* (Houtt) Stapf *var*. degrabrata K. was utilized by local people as traditional medicine for a variety of diseases like hepatitis, liver disease, high cholesterol and hypertension [8]. This ethnobotanical approach can provide an important clue to confirm a scientific evidence for the plant.

In previous paper [9], a compound was found in the heartwood of *Melochia umbellata* (Houtt) Stapf *var*. degrabrata K., i.e. 6.6'-dimetoksi-4,4'-dihydroxy-3',2'-Furano-isoflavan. The isolation and structural determination of stigmasterol glycoside from the chloroform extract of the root wood of *M. umbellata* (Houtt) Stapf *var*. degrabrata K. and its activity as antifungal against *Malazesia furfureus, Candida albicans* and *Aspergillus niger* are reported.

EXPERIMENTAL SECTION

Materials

The root woods of *M. umbellata* were collected in November 2009 from Makassar City, South Sulawesi, Indonesia. Plant identification was conducted in Herbarium Bogoriense Bogor, Indonesia.

Instrumentation

The melting point was determined using a micro melting point measurement (John Fisher). IR determination is done with a Perkin Elmer spectrometer. NMR spectra of ^{1}H, ^{13}C and HMBC were obtained using a Bruker AM 500 spectrometer at 300 MHz (^{1}H) and 125 MHz (^{13}C) with TMS as an internal standard. Separations and identification were conducted with Vacuum liquid chromatograph (VLC) by

* Corresponding author. Tel/Fax : +62-411-585991
Email address : nuklir@indosat.net.id

Table 1. NMR Data of compound **1** in DMSO

No	δ_H (multiplicity, *J* (Hz))	δ_C	HMBC (^1H => ^{13}C)
1	2.36 (m) & 2.13 (m)	38.28	C-2, C-3, C-10, C-5
2	1.30 (m)	33.33	
3	3.42 (m)	76.95	
4	1.80 (br d, 10.2) & 1.16 (br d, 6.6)	36.76	
5	-	140.43	
6	5.32 (br d, 4.5)	121.04	C-7, C-8, C-10
7	1.46 (m)	31.30	
8	1.51 (br s)	31.38	
9	0.99 (br s)	49.57	
10	-	36.15	
11	1.17 (m)	22.59	
12	1.94 (m) & 1.13 (m)	41.69	
13	-	41.80	
14	1.08 (m)	56.20	
15	1.12 (m)	24.72	
16	1.91 (br s) & 1.77 (br s)	29.20	
17	1.01 (m)	56.11	
18	0.65 (s)	11.76	C-12, C-13, C-14, C-17
19	0.99 (s)	18.99	C-1, C-10, C-5, C-9
20	1.34 (m)	35.37	
21	0.91 (d, 6.3)	18.76	C-17, C-20
22	5.18 (dd, 8.4 & 15)	137.85	
23	5.04 (dd, 8.4 & 8.1)	128.79	
24	0.99 (br s)	31.20	C-22
25	1.63 (m)	31.20	
26	0.84 (d, 6.3)	19.28	C-25, C-24, C-27
27	0.80 (d, 6.9)	18.89	C-24, C-25, C-26
28	1.01 (br s)	23.76	
29	0.79 (d, 8.1)	11.58	C-24, C-28
1’	4.23 (d, 7.8)	100.77	C-3, C-5’
2’	2.91 (m)	70.14	
3’	3.15 (m)	76.75	
4’	3.08 (m)	73.43	
5’	3.04 (m)	76.63	
6’	3.66 (m) & 3.50 (m)	61.10	
2’-OH	4.73 (br s)		
3’-OH	4.75 (br s)		
4’-OH	4.71 (br s)		
6’-OH	4.30 (t, 6.0)		

Merck Si gel 60 (230-400 mesh), and thin-layer chromatograph (TLC) on aluminum plates coated with Merck Si gel 60 F254 and thickness of 0.25 mm.

Procedure

Extraction and Isolation

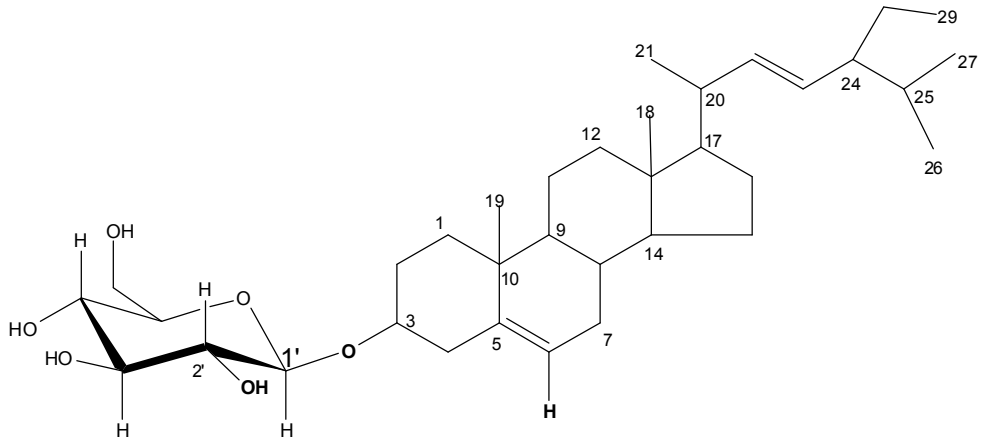
Dry powder of 12 kg of sample was macerated with methanol for 72 h, filtered, and then solvent evaporated until producing a dark brown extract amounting 1.2 kg. The extracts (375 g) were partitioned with n-hexane, chloroform, and ethyl acetate resulting 41 g, 15 g, and 26 g respectively. The chloroform extract was fractionated by VLC and produced nine fractions (1-9). White precipitate found in the fifth fraction was filtered by filtration. Purity test was performed by TLC analysis using three solvent systems and determinated its melting point.

Antifungal Activity Test

Antifungal activity test was done to three species of fungi namely: *Candida albicans*, *Aspergillus niger* and *Malazesia furfureus* using agar diffusion method.

RESULT AND DISCUSSION

The compound obtained, compound 1, is a pale-white powder, mp 278-280 °C, giving an indication of steroid base on Liebermann-Burchard test. Its IR spectrum showed the absorption bands for hydroxyl (3402), aliphatic groups (2940 and 2870), C=C (1635), CH_2 (1458), CH_3 (1370), C-O (1258, 1165, 1072 and



(1)

Fig 1. Stigmast-5,22-dien-3-*O*-*β*-D-glucopyranoside

1026) while strong absorption band at 3402 and 1026 were characterized as a glycoside compound [10].

The ^{1}H NMR spectrum of compound 1 (Table 1) shows two tertiary methyl at *δ* 0.65 (Me-18) and 0.99 (Me-19), three secondary methyl namely at *δ* 0.91 (Me-21), 0.84 (Me-26) and 0.80 (Me-27), one of the primary methyl at *δ* 0.79 (Me-29), one proton with olefinic substitution at *δ* 5.32 (H-6), two protons with substituted olefinic at *δ* 5.18 (H-22) and 5.04 (H-23) and one anomeric proton at *δ* 4.23.

The ^{13}C NMR spectrum of compound 1 (Table 1) showed there are 35 carbon atoms in the molecule. An anomeric carbon signal at *δ* 100.77 indicated the presence of a single monosaccharide moiety. The four methine resonances at *δ* 70.14, 76.75, 73.43, and 76.63 as well as the methylene resonance at *δ* 61.10 were due to C-2', C-3', C-4', C-5' and C-6', respectively of the *β*-*D*-glucopyranoside. The olefinic resonance at *δ* 121.04, 137.85, and 128.79 corresponded to C-6, C-22 and C-23 methine carbons, and a signal at *δ* 140.43 corresponded to the C-5 quarternary carbon of the sterol moiety. The value of *J* = 7.8 on 1' (anomeric proton) reflected that the proton is the axial-axial to C-2' proton which means glucopyranoside moiety binds to the sterol moiety β position [3,11]. Based on the data description above and comparison with physical and spectroscopic data of previously known compound [10], the molecular structure of compound 1 is in Fig. 1 with name of stigmast-5,22-dien-3-*O*-*β*-D-glucopyranoside.

The relationships in the bonding structure was proven through long-range correlation of ^{1}H $\rightarrow$ ^{13}C of HMBC spectrum (Table 1). The existence of long-range correlations of protons at *δ* 4.23 (H-1') with a carbon at *δ* 76.95 (C-3) and 76.63 (C-5') indicates that the group of glucose is bound to C-3 (oxy carbon sp^{3}). Long-range correlations of protons at *δ* 5.32 (H-6) with a methylene carbon at *δ* 31.30 (C-7), methine carbon at 31.38 (C-8), and the quaternary carbon at 36.15 (C-10) strengthen that the C-5 and C-6 are as olefinic bonding in the second ring of sterol skeleton. Long-range correlations of protons at *δ* 0.65 (H-18) with a methylene carbon at *δ* 41.62 (C-12), quaternary carbon at 41.80 (C-13), methine carbon at 56.20 (C-14), and methine carbon at 56.11 (C-17) strengthen that the methyl carbon (C-18) binds to the quaternary carbon (C-13). Then long-range correlations between protons at *δ* 0.99 (H-19) with a methylene carbon at *δ* 38.32 (C-1), quaternary carbon at 36.15 (C-10), quaternary carbon at 140.43 (C-5), and methine carbon at 49.57 (C-9) strengthen that the methyl carbon (C-19) binds to the quaternary carbon (C-10). Distance correlation between proton doublet at *δ* 0.91 (H-21) with carbon methine at *δ* 56.11 (C-17), and carbon methine 35.37 (C-20) strengthen that the methyl carbon (C-21) binds on methine carbon (C-20). Distance correlation between proton doublet at *δ* 0.84 (H-26) with methine carbon at *δ* 31.20 (C-25), methine carbon at 45.14 (C-24), and methyl carbon at 18.89 (C -27) strengthen that the methyl carbon (C-26) binds to the methine carbon (C-25), as well as long-range correlations between proton doublet at *δ* 0.80 (H-27) with carbon (C-24), (C-25), and (C-26) strengthen that the methyl carbon (C-27) binds to the methine carbon (C-25). In addition, long-range correlations between proton doublet at *δ* 0.79 (H-29) with methine carbon at *δ* 45.12 (C-24) and methylene carbon at 23.76 (C-28) strengthen that the methyl carbon (C-29) binds to the methylene carbon (C-28).

Bioassay test of compound 1 on 3 types of fungi namely *Candida albicans*, *Aspergillus niger* and *Malazesia furfureus* showed that compound 1 is active against *Aspergillus niger* only with an area of inhibition zone of 16.3 mm^{2} with the concentration of 200 µg/mL.

CONCLUSION

Compound 1, stigmast-5,22-dien-3-*O*-*β*-D-gluco pyranoside, has been isolated from the root wood of *M. umbellata* (Houtt.) Stapf *var*. degrabrata K. This compound could inhibit the growth of *Aspergillus niger* at a concentration of 200 µg/mL.

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