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A Steroid, 7α-hydroxy-β-sitosterol from the Bark *Chisocheton macrophyllus* (Meliaceae)

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ABSTRACT

The compound 7*α*-hydroxy-β-sitosterol has been isolated from the bark of *Chisocheton macrophyllus*. This compound was obtained by total maceration with methanol and continued partitioning with the solvents *n*-hexane, ethyl acetate, and *n*-butanol. The *n*-hexane extract was separated and purified using various chromatographic techniques to obtain compound **1**. Determination of the structure of compound **1** was identified using various spectroscopic techniques such as MS, IR, 1D NMR, and 2D NMR, as well as comparison of NMR data with reference compounds. Compound **1** was tested for its cytotoxic activity against MCF-7 breast cancer cells and was found to have a weak IC_{50} value of 276.57 µg/mL

Keywords: Chisocheton macrophyllus, steroid, Meliaceae, MCF-7.

INTRODUCTION

The Meliaceae family has approximately 53 genera, 650 species, and almost 25 genera has been explored [1, 2]. Its barks are widely known having contained many bioactive compounds such as ceramicine A from *C. ceramicus* is active against P-388 murine leukemia cells [3], several protolimonoids from *C. paniculatus* showed inhibitory activities on inflammation factor-release (NO and TNF-α) [4,5], the phytosterol oxide from *C. tomentosus* as anticancer [6,7,8], lignan from *C. lasiocarpus* [9], sesquiterpenes from *C. penduliflorus* [10], flavonoids from *C. pentandrus* and *C. celebicus*[11,12], limonoid from *C. cumingianus* [13,14].

Chisocheton macrophyllus has been reported as inhibition of Epstein-BarrI viral antigen promoted tumor [15]. This can be brought into consideration for phytochemical investigation with a view to isolate bioactive novel structure that can be used as drug lead.

In previous investigation [16,17], the compounds were found in the bark of *Chisocheton macrophyllus*, 7-hydroxy-6-methoxy coumarin and *β*-sitosterol-*β*-D-glucose-6-monopalmitate. The isolation and characterized of compound **1** from bark of *Chisocheton macrophyllus* and its activity against the MCF-7 breast cancer cell line have reported.

EXPERIMENTAL SECTION

Materials

The bark of *C. macrophyllus* (Meliceae) was collected from Bogor Botanical Garden, Bogor Indonesia in June 2019. The plant was identified by Mr. Ismail, the staff of the Bogoriense Herbarium, Indonesia Science Institute, Bogor, Indonesia.

Instrumental

IR spectra was measured on One Perkin Elmer infrared-100. NMR data was recorded on Bruker Avance-500 spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR using TMS as an internal standard (Billerica, MA, USA). Chromatograph separations were carried out on silica gel G_{60} (0.063-0.200 mm) (Merck, Darmstadt, Germany), RP18 (0.04-0.063 mm) (Merck, Darmstadt, Germany). TLC plates were precoated with silica gel GF_{254} (Merk, Darmsatadt, Germany, 0.25 mm), and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating

Methods

Extraction and Isolation

The dried powder bark of *C. macrophyllus* (2.5 kg) was extracted gradually with *n*-hexane (5 x 2L), ethyl acetate (5 x 2L), and methanol (5 x 2L) and evaporated to give a crude *n*-hexane extract (10.0 g), ethyl acetate (10,0 g) and methanol (10.0 g) after removal of the solvent. The *n*-hexane extract (10.0 g) was fractionated by vacuum liquid chromatography on Merck GF254 silica gel using a 10% gradient of *n*-hexane-ethyl acetat-methanol solvent to give six fractions (A-F). Fraction D (0.7928 g) was leached with methanol by degrees to remove green extract that stick to the crystals and it gived two subfractions (D1-D2). Subfraction D1 (0.1208 g) was purified by column chromatography on RP-18 silica using 5% gradient MeOH:H_2O to give **1** (1,2 mg).

Anticancer Activity Test

Anticancer activity test was measured by using MTT (Methyl Thiazoldiphenyl-Tetrazoliumbromide) assay against MCF-7 human breast cancer cells.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. Its molecular composition was established to be $C_{30}H_{52}O_2$. The IR spectrum displayed bands at ν_{max} 3400 cm^{-1} (hydroxy), 2959 cm^{-1} and 2851 cm^{-1} (C-H stretching of aliphatic), 1664 cm^{-1} (C=C of olefinic) 1057 cm^{-1} (ether group). The ^{1}H NMR spectrum of compound **1** (Table 1) shows two tertiary methyls at δ_H 0.70 (Me-18) and δ_H 0.99 (Me-19). Three secondary methyl of **1** were seen at at δ_H 0.93 (Me-21), δ_H 0.81 (Me-26) and δ_H 0.83 (Me-27) and one of the primary methyl at δ_H 0.84 (Me-29). One proton with olefinic substitution at δ_H 5.61 (H-6). The oxygenated methane signals were also observed at δ_H 3.58 (H-3) and δ_H 3.85(H-7).

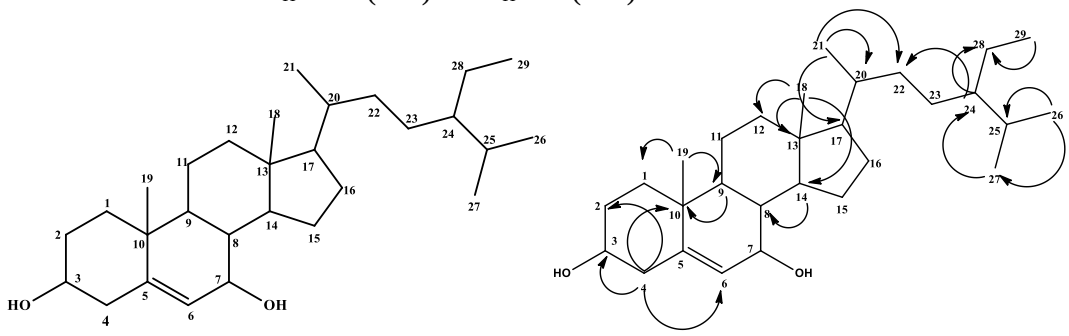

Figure 1**.** Structure of 17α-hydroxy-β-sitosterol and HMBC correlations for **1**.

The ^{13}C NMR spectrum showed 30 carbon resonances, which were classified by their chemical shift and HMQC spectra as 6 methyl groups (2 tertiary, 3 secondary, and 1 primary), 10 methylene carbons, 10

methane carbons (2 oxygenated and 1 olefinic carbons), and 3 quaternary carbons (1 olefinic carbon). The remaining of seventeen degrees of unsaturation consistent with the tetracylic steroidal structure with two hydroxyl and olefinic as additional groups. The significant difference side chain between structure **1** and 7*α*-hydroxy-*β*-sitosterol [6] was the *n*-propyl side chain of C-24. It was indicated by ^{13}C-NMR spectrum of **1** and showed as CH_2 with HMQC.

A comparison of the NMR data of **1** with 7*α*-hydroxy-*β*-sitosterol, *β*-sitosterol, stigmasterol, stigmast-5en-3*α*-acetate compounds indicated that the structure of **1** is very similar to 7*α*-hydroxy-*β*-sitosterol. HMBC correlations from H-4 (δ_H 2.34) with C-3 (δ_c 71.4), C-6 (δ_C 123.6), C-2 (δ_C 31.4), and C-10 (δ_C 37.4) was indicating an olefinic carbon C-5 (δ_C 145.3) similar to 7*α*-hydroxy-*β*-sitosterol and hydroxyl group located in C-3 (δ_C 71.4). The tertiary, secondary, and primary methyl protons has correlation with their neighboring carbon enable the assignment of the two tertiary methyls at C-9, C-10, C-13, and C-17, secondary methyls at C-20 and C-25 (2x), and primary methyl at C-28, respectively. Therefore, the structure of **1** was assigned as 7*α*-hydroxy-β-sitosterol

Compound 7*α*-hydroxy-β-sitosterol was evaluated for its cytotoxicity against the MCF-7 breast cancer cell line using MTT assay and doxorubicin (IC_{50} 19.40 µg/mL) as a positive control and it showed weak activity against MCF-7 with an IC_{50} value of 276.57 µg/mL. This result is in line with previously investigations where a steroid derivative showed weak activity against the breast cancer cell line. IC_{50} values were calculated by the linier regression method using Microsoft Excel software.

Table 1. Data ^{1}H and ^{13}C NMR of compound **1** in $CDCl_3$.

No	Compound **1** δH (∑H, mult, *J*)	δ_C	7*α*-hydroxy-*β*-sitosterol* δ_H (∑H, mult, *J*)	δ_C
1	1,18 (1H, m); 1,12 (1H, m)	37,0	1,80 (1H, m); 1,01 (1H, m)	37,0
2	1,55 (1H, m); 1,87 (1H, m)	31,4	1,80 (1H, m); 1,47 (1H, m)	31,3
3	3,58 (1H, s)	71,4	3,54 (1H, m)	71,3
4	2,34 (2H, m)	42,0	2,29 (2H, d, 5)	42,0
5	-	145,3	-	146,3
6	5,61 (1H, d, 5,15)	123,6	5,55 (1H, d, 5,04)	123,8
7	3,85 (1H, t, 5)	65,3	3,81 (1H, brs)	65,4
8	1,47 (1H, m)	37,5	1,43 (1H, m)	37,5
9	1,21 (1H, m)	42,3	1,15 (1H, m)	42,3
10	-	37,4	-	37.4
11	1,55 (2H, m)	20,7	1,49 (2H, m)	20,7
12	1,98 (1H, m) ; 1,17 (1H, m)	39,2	1,97 (1H, m); 1,12 (1H, m)	39.2
13	-	42,1	-	42,2
14	1,44 (1H, m)	49,5	1,41 (1H, m)	49,4
15	1,70 (1H, m); 1,15 (1H, m)	24,3	1,66 (1H, m);1,08 (1H, m)	24,3
16	1,29 (1H, m); 1,16 (1H, m)	28,3	1,83 (1H, m);1,22(1H,m)	28,3
17	1,19 (1H, m)	55,2	1,14 (1H, m)	55,7
18	0,70 (3H, s)	11,7	0,65 (3H, s)	11,7
19	0,99 (3H, s)	18,4	0,95 (3H, s)	18,4
20	1,35 (1H,m)	36,2	1,33 (1H, m)	36,1
21	0,93 (3H, d)	18,7	0,89 (3H, d, 6,4)	18,3
22	1,31 (2H, m)	33,7	1,24 (2H, m)	33,8
23	1,25 (2H, s)	29,6	1,22 (2H, m)	29,8
24	0,93 (1H, d)	45,7	0,93 (1H, m)	49,4
25	1,66 (1H, s)	29,2	1,64 (1H, m)	29,0
26	0,81 (3H, d)	19,1	0,81 (3H, m)	19,9
27	0,83 (3H, d)	19,8	0,77 (3H, m)	18,9
28	1,25 (2H, s)	23,1	1,22 (2H, m)	23,1
29	0,84 (3H, m)	12,0	0,83 (3H, m)	12,1

*Reference [6].

CONCLUSION

Compound **1**, namely 17*α*-hydroxy-*β*-sitosterol, has been isolated from the bark of *C. macrophyllus* (Meliaceae). This compound was evaluated for its cytotoxicity against the MCF-7 breast cancer cell line using MTT assay and doxorubicin (IC_{50} 19.40 µg/mL) as a positive control and it showed weak activity against MCF-7 with an IC_{50} value of 276.57 µg/mL.

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