Campesterol from Methanol Fraction of Brotowali (Tinospora crispa) Stem Bark

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Abstract—***Tinospora crispa* is a plant of the *Menispermaceae* family which is one of the endemic plants in Indonesia, commonly called as Brotowali. T. crispa is traditionally used to treat diabetes, rheumatism, sinusitis and fever. The purpose of this study was to determine the molecular structures of isolated compound of methanol fraction from *T. crispa* stem bark. Extraction using maceration method with *n*-hexane, ethyl acetate and methanol as solvents. The structures of compounds were elucidated with spectroscopic data (^{1}H, ^{13}C, DEPT 135°, HMBC, HMQC, ^{1}H-^{1}H COSY NMR, IR) and MS as well as compared with previously reported spectral data. The structure of isolated compound was finally identified as 24(R)-methylcholesta-5-en-3β-ol (campesterol) by the compared of several physical and spectral data with the literature. In previously study, campesterol have antiangiogenic and anticholesterol activities.**

Keywords—Tinospora crispa, campesterol, Menispermaceae, brotowali

I. INTRODUCTION

The *Tinospora crispa* (Brotowali) of *Menispermaceae* family are used in the form of decoction to treat cholera and hypertension [1]. *T. crispa* is traditionally used for the treatment of gout and reported as analgesic, anti-inflammatory, and antihyperuricemic agents. *T. crispa* of Menispermaceae family is well known as a bitter medicinal plant but it has various efficacy and has been empirically used to treat rheumatism, cholera, diabetes, gout, bruise, hypertension, hypoglycemia, and fever [2][3][4].

Chemical compounds of *T. crispa* were reported as flavonoids, tinocrisposide, steroids, columbine, quaternary alkaloids, saponins, tannins, glycosides, and polyphenols [5][6][7]. Other studies have shown that T. crispa stem extract contains anti-inflammatory [9][7] and analgesic properties [8]. *T. crispa* has the potential compounds to be developed as a raw material of standardized herbal medicine or phytopharmaca.

This plant is very widely used in Indonesia as an herbal medicine, so it is necessary to know the content of secondary metabolites in the *T. crispa* plant. In this research, the methanol fraction was subjected to chromatographic separation to afford steroid compound (24(*R*)-methylcholesta-5-en-3*β*–ol). This compound was first isolated from *T. Crispa* which was obtained from Gorontalo.

II. METHOD

The *Tinospora crispa* was collected at Bubaa village, Boalemo district, Gorontalo, Indonesia in May 2016. It was identified in Herbarium Biology, Faculty of Mathematics and Natural Sciences, Gorontalo State University. The chemicals used in this research are *n*-hexane, ethyl acetate, distilled water, methanol 96%, thin layer chromatography (TLC) silica plate, silica gel G60 (70-320 mesh), TLC, 10% H_2SO_4 in ethanol, and ethanol 70%.

Spectrum measurements were performed using a Infrared spectrometer (Shimadzu FTIR), a ESI-MS spectrometer (UPLC MS/MS TQD type Waters), ^{1}H and ^{13}C-NMR spectrometers were measured using a modern NMR spectrometer (JEOL JNMA-500) which works on frequency 500 MHz..

Extraction and isolation: Dried T. crispa stem bark (700 g) were soaked in 3 L methanol 96% for 3 days. Then the mixture filtered and the filtrate was evaporated under reduce pressure at 45 °C using a Rotary Evaporator to provide 21 g of a gummy concentrate of the crude extract. It was then fractionated with *n*-hexane and followed by ethyl acetate. All extracts were filtered using Whatmann filter paper and corked with cotton, then concentrated by using a vacuum rotary evaporator to provide extracts.

Chromatographic separation: The thin-layer chromatographic (TLC) grade silica gel G60 has already been used in column as the packing material. Then the methanol fraction is entered into column chromatography, and eluted using *n*-hexane followed by mixture of *n*-hexane-ethyl acetate gradient 10%. A total of 11 fractions (01-11) were collected each in 150 mL beakers. The fraction 4 and 5 (0.40 g) was throug to column chromatography over silica gel Kieselgel G60 (mesh 70-230) using a eluent mixture of n-hexane : ethyl acetate (9:1), affording 40 fractions (5.6-01–5.6-40). Fraction 5.6-09 was found to yield crystal on the wall of the beakers. As a final result a needle-shaped crystal is obtained again, and the crystal can be called to be isolated. to determine pure isolates can be done with TLC and ODS using several solvent systems and showed a single spot (> 90% pure).

Test for steroid with Liebermann-Burchard reaction: A crystals isolate was dissolved in chloroform and added a few drops of sulfuric acid and followed by the addition of 2-3 drops of acetic anhydride. After being added, the isolate

turns blue violet and finally forms a green color, which indicates steroids.

III. Results and Discussion

Data from the isolated compound gives molecular formulas $C_{29}H_{48}O$, which was supported by the ^{13}C-NMR and ^{1}H-NMR data spectrum.

Characterization of Isolated Compound

The isolated compound showed characteristic absorption frequencies IR spectrum (KBr) at 3373.6 cm^{-1} (O-H stretching); and 1241cm^{-1} indicated C-O bond vibrations. The C=C vibrations was shown at 1638.8 cm^{-1}; other absorption peaks includes 1454.2 cm^{-1} (CH_2); vibrations at 1383.7 cm^{-1} (O-H), 1045.5 cm^{-1} (cycloalkane), and 883.6 cm^{-1} [10].

The ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) spectra of isolated compound showed δ_H 5.34 (1H, m, H-6), 3.52 (1H, dd, *J* = 7.3, 7.3 Hz, H-3), 2.28 (1H, d, *J* = 7.3 Hz, H-4), 1.01 (3H, s, H-19), 0.92 (1H, d, *J* = 6.6 Hz, H-21), 0.84 (3H, d, *J* = 7.2 Hz, H-27), 0.81 (3H, d, *J* = 7.2 Hz, H-26), 0.80 (3H, d, *J* = 7.0 Hz, H-28), and 0.68 (3H, s, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm) spectra of isolated compound showed δ_C 140.9 (C-5), 121.9 (C-6), 71.9 (C-3), 56.9 (C-14), 56.1 (C-17), 50.1 (C- 9), 42.4 (C-24), 42.3 (C-4), 42.3 (C-13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.1 (C-25), 33.8 (C-22), 31.8 (C-7), 31.8 (C-20), 29.8 (C-8), 28.4 (C-2), 28.4 (C-16), 24.4 (C-28), 21.2 (C-15), 21.2 (C-23), 19.5 (C-18), 21.2 (C-11), 19.5 (C-21), 19.0 (C-27), 21.2 (C-26), and 12.0 (C-19). The data spectrum values ^{1}H and ^{13}C-NMR were assigned on the basis of COSY, HMBC and HMQC correlations and were given i

TABLE I. In addition, the data spectrum ^{1}H and ^{13}C-NMR is supported by comparative literature

Position C	^{13}C-NMR δ_C (ppm)	DEPT 135°	^{1}H -NMR δ_H (Int., mult., *J=Hz*)	HMBC ^{1}H to ^{13}C	COSY ^{1}H - ^{1}H	Comparative Literature [11][12]	
						^{1}H -NMR	^{13}C-NMR
1	37.3	CH_2	0.97 (1H; m); 1.55 (1H; *m*)	C-10,3	H- 3		37.3 [11] [12]
2	28.4	CH_2	1.52 (2H; *m*)	C-4	-		28.2 [12]
3	71.9	CH	3.52 (1H; *dt,*11.0; 3.9)	-	H-1	3.51 (1H) [12]	71.8 [12]
4	42.3	CH_2	1.4 (2H;*m*)	-	-		42.3 [12]
5	140.9	Cq	-	-	-		140.8 [11]
6	121.9	CH	5,3 (1H; *m*)	C-4, 8, 10	-	5,34 (1H; *m*) [12]	121.7 [12]
7	31.8	CH_2	1.33 (2H; *t* , 3.6)	-	-		31.7 [11] [12]
8	29.8	CH	1.73 (1H; *m*)	-	-		
9	50.1	CH	1.53 (1H; *m*)	-	-		50.1 [12]
10	36.5	Cq	-	-	-		36.5 [12]
11	21.2	CH_2	1.13 (2H; *m*)	C-9, 12	H-12		
12	39.8	CH_2	1.21 (2H; *t,* 3.9)	C-9, 18	H-11		40.0 [12]
13	42.3	Cq	-	-	-		42.28 [11]
14	56.9	CH	1.83 (1H; *m*)	-	-		56.9 [12]
15	21.2	CH_2	1.50 (2H; *m*)	-	-		
16	28.4	CH_2	1.53 (1H; *m*); 1.92 (1H;*m*)	-	-		
17	56.1	CH	1.73 (1H; *m*)	-	-		56.1 [11] [12]
18	19.5	CH_3	1.10 (3H;*d, 6.0*)	C-12, 21	H-22		
19	12.0	CH	2.07 (2H; *m*)	-	-		
20	31.8	CH_2	2.17 (H; *m)*	-	-		31.7 [11]
21	19.5	CH	1.30 (H; *m)*	-	-		
22	33.8	CH	1.90 (1H; *m*)	-	H-18		33.9 [12]
23	21.3	CH_2	1.2 (2H; *m*)	C-3, 5, 24	-		
24	42.4	CH_3	1.08 (3H;*t*)	-	-		
25	36.1	CH	1.77 (1H; *m*)	C-5, 9, 10	-		36.1 [12]
26	21.2	CH_3	0.84 (3H; *d, 6.4*)	C-8, 9, 14	-		
27	19.0	CH_3	1.17 (3H; *d, 6.4*)	C-8, 13, 14, 15	-		
28	24.4	CH_3	0.7 (3H; *s*)	-	-		
29	12.4	CH_3	0.98 (3H; *s*)	C-21	-	0.98 (3H; *s*) [11]	

The ^{13}C-NMR (ppm) spectrum showed six methyl (CH_3) groups and one olefenic group at δ_C 140.9 (C-5) and 121.9 (C-6). The deshielded signal at δ_C 71.9 ppm was due to C-3 with a hydroxyl group attached. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublets at δ_C 3.52 ppm. Isolated compound also showed protons at δ_H 5.34 ppm suggesting the presence of a proton corresponding to that of a olefinic bond [11][12]. Thus, the structure of isolated compound was assigned as (24(*R*)-methylcholesta-5-en-3*β*–ol) (campesterol). The physical and spectral data are consistent to the reported literature values of campesterol (Fig. 1).

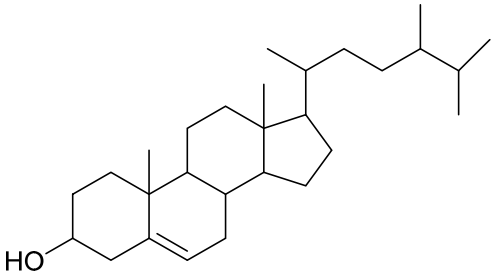

Fig. 1. Isolated compound (campesterol)

IV. Conclusion

The characterization of the isolated compounds as 24(R)-methylcholesta5-en-3 *β* -ol (campesterol) by the comparison of several physical and spectral data with the literature.

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