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Sterols and Sterol Glycosides from the Leaves of *Gynura procumbens*

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Abstract – A mixture of sterols containing β-sitosterol and stigmasterol (**1**), and sterol glycosides containing 3-O-β-D-glucopyranosyl β-sitosterol and 3-O-β-D-glucopyranosyl stigmasterol (**2**) were isolated from the leaves of *Gynura procumbens*. After acetylation of **2** with pyridine-acetic anhydride, 3-O-β-D-tetra-O-acetylglucopyranosyl β-sitosterol (**3**) was isolated.

Key words – *Gynura procumbens*, β-sitosterol, stigmasterol, 3-O-β-D-glucopyranosyl β-sitosterol, 3-O-β-D-tetra-O-acetylglucopyranosyl β-sitosterol.

Introduction

Gynura procumbens (Lour.) Merr., known in Indonesia as 'sambong nyawa', is a herb of the Compositae family. The plant is believed to be useful as a febrifuge in a few eruptive fevers, and a remedy for kidney trouble, prevention of rheumatism, elimination of lethargy especially in old people, and treatment for rashes (Perry, 1980). The fresh leaves of the plant frequently eaten raw together with rice as an appetizer.

To our knowledge, the presence of steroid compounds in this plant has not yet been reported. In the present paper we report the isolation and characterization of sterols and their corresponding glycosides.

Experimental

Instrumentation – The melting points (uncorrected) were determined on Electrothermal 9100 capillary melting point apparatus. GLC analysis of sterols were recorded on Shimadzu GC 14A using 5% phenylmethyl silicone capillary column.

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^{1}H (300 MHz) and ^{13}C-NMR (75 MHz) were recorded in $CDCl_3$ and pyridine-d_5 using TMS as internal standard on a BRUKER BZH 300 spectrometer. The IR spectra (KBr) were recorded on a FTIR BOMEM spectrometer.

Plant Material – Dried sample leaves of *G. procumbens* were obtained from Jakarta, Indonesia and milled into powder. A voucher specimen is deposited at the School of Pharmaceutical Sciences, Universiti Sains Malaysia.

Extraction and Isolation – Dried powdered leaves (400 g) were first extracted with petroleum ether (60-80 °C, 3 liters), followed by extraction of the marc with chloroform (3 liters), and finally with methanol (3 liters) in a soxhlet extractor for 16 hr each.

The petroleum ether extract (14.0 g) was subjected to flash column chromatography on silica gel using petroleum ether-chloroform as eluent. From the fractions eluted with pet. ether-chloroform (1:1), further purification with preparative TLC (pet. ether-ethyl acetate 4:1) afforded compound **1** (100.0 mg).

The chlorofom extract (9.5 g) was also subjected to silica gel column chromatography using chloroform-methanol as eluent affor-

ded compound 2 (200.0 mg).

β-sitosterol and stigmasterol (1) – Recryst. (EtOH) gave white powder, m.p. 144-146 °C; IR (KBr) 3400 (OH), 2900 (CH), 1640 (C=C) cm^{-1}; ^1H- NMR (300 MHz, $CDCl_3$, δ) 5.35 (1H, *brs*, H-6 of β-sitosterol and stigmasterol), 5.15 (1H, *dd*, J=15.2, 8.4 Hz, H-22 of stigmasterol), 5.03 (1H, *dd*, J=15.1, 8.2Hz, H-23 of stigmasterol), 3.52 (1H, *m*, H-3 of β-sitosterol and stigmasterol), 1.02 (3H, *d*, J=6.6 Hz, Me-21 of stigmasterol), 1.0 (3H, *s* , Me-19 of β-sitosterol and stigmasterol), 0.92 (3H, *d*, J=6.6Hz, Me-21 of β-sitosterol), 0.86 (3H, *t* , J=7.1 Hz, Me-29 of β-sitosterol), 0.84 (3H, *d*, J=6.4 Hz, Me-26 of stigmasterol), 0.83 (3H, *d*, J=6.6Hz, Me-26 of β-sitosterol), 0.82 (3H, *t*, J=7.2Hz, Me-29 of stigmasterol), 0.81 (3H, *d*, J=6.6Hz, Me-27 of β-sitosterol), 0.79 (3H, *d*, J= 6.6Hz, Me-27 of stigmasterol), 0.70 (3H, *s*, Me-18 of stigmasterol), 0.68 (3H, s, Me-18 of β-sitosterol); ^{13}C- NMR (75 MHz, $CDCl_3$, δ)-see Table 1.

3-O-β-D-glucopyranosyl β-sitosterol and 3-O-β-D-glucopyranosyl stigmasterol (2) – Recryst. (EtOH) gave white solid, m.p. 270-272 °C; IR (KBr) 3400 (OH), 2900 (CH), 1640 (C=C) cm^{-1}; ^1H-NMR (300 MHz, pyridine-d_5, δ) 5.33 (1H, *brs*, H-6 of β-sitosterol and stigmasterol), 5.15 (1H, *dd*, J=15.2, 8.4Hz, H-22 of stigmasterol), 5.09 (1H, *dd*, J=15.1, 8.2 Hz, H-23 of stigmasterol), 5.03(1H, *d*, J=7.6 Hz, H-1'), 4.54 (1H, *dd*, J=12.

Table 1. ^{13}C NMR spectral data for compounds 1 ($CDCl_3$), 2(pyridine-d_5) and 3 (pyridine-d_5) [(ppm) relative to TMS].

C	1		2		3
	β-sitosterol	stigmasterol	β-sitosterol glucoside	stigmasterol glucoside	
1	37.25	37.25	39.65	40.27	37.40
2	28.20	28.20	28.60	29.27	28.50
3	71.78	71.78	78.75	78.75	79.30
4	39.75	39.75	40.29	40.29	39.60
5	140.74	140.74	142.34	141.34	140.60
6	121.69	121.69	121.69	121.69	122.30
7	31.90	31.90	32.40	32.40	32.20
8	31.60	31.60	32.28	32.28	32.10
9	50.12	51.23	50.76	51.60	50.37
10	36.50	36.50	37.20	37.20	36.90
11	21.06	21.06	21.50	21.50	21.30
12	37.78	37.78	40.29	40.29	39.30
13	42.30	42.30	42.80	42.60	42.50
14	56.76	56.76	57.18	57.18	56.90
15	24.30	24.30	24.70	25.20	24.53
16	26.08	26.08	27.07	27.07	26.40
17	56.05	56.05	56.69	56.06	56.30
18	11.84	12.04	12.18	12.34	11.99
19	19.02	19.39	19.49	19.49	19.39
20	36.10	36.10	36.50	36.50	36.40
21	18.80	19.00	19.24	19.37	19.23
22	33.90	138.30	34.60	138.80	34.25
23	29.43	129.30	30.50	129.90	30.02
24	45.83	50.10	46.49	46.49	47.07
25	29.10	28.90	29.98	29.98	29.50
26	19.80	21.20	20.07	20.07	19.98
27	19.40	21.10	19.58	19.58	19.03
28	23.10	23.10	23.77	23.77	23.41
29	11.97	12.20	12.34	12.51	12.17

Table 2. ^{13}C NMR spectral data for sugar moiety in 2 and its acetyl derivatives in 3.

C	β-sitosterol glucoside 2	stigmasterol glucoside 3
C-1'	102.87	99.83
C-2'	75.48	72.27
C-3'	78.30	72.17
C-4'	72.20	69.24
C-5'	78.60	73.60
C-6'	63.29	62.50
	-	170.5
OAc	-	170.3
	-	169.8
OAc	-	169.6
	-	20.64
OAc	-	20.64
	-	20.43
OAc	-	20.42

1, 2.4 Hz, H-6'a), 4.39 (1H, *dd*, J=12.1, 5.3 Hz, H-6'b), 4.26 (2H, *m*, H-3', H-4'), 4.03 (1H, *dd*, J=8.2, 7.6Hz, H-2'), 3.95 (2H, *m*, H-5' and H-3'), 1.02 (3H, *d*, J=6.6Hz, Me-21 of stigmasterol), 0.98 (3H, *d*, J=6.6Hz, Me-21 of β-sitosterol), 0.95 (3H, *s*, Me-19 of β-sitosterol and stigmasterol), 0.89 (3H, *t*, J=7.2Hz, Me-29 of β-sitosterol and stigmasterol), 0.85 (3H, *d*, J=6.6Hz, Me-26 of β-sitosterol and stigmasterol), 0.83 (3H, *d*, J=6.6Hz, Me-27 of β-sitosterol and stigmasterol), 0.67 (3H, *s*, Me-18 of stigmasterol), 0.66 (3H, *s*, Me-18 of β-sitosterol); ^{13}C-NMR (75 MHz, pyridine-d_5, δ)-see Table 1 and Table 2.

Hydrolysis of 2– Compound 2 (15 mg) was treated with 2M HCl at 100 °C for 6 hr gave an aglycone and a sugar. The aglycone was identified as a mixture of β-sitosterol and stigmasterol (Rf. 0.83), and the sugar was identified as D-glucose (Rf 0.12) by direct comparison with respective authentic samples on TLC [$CHCl_3$-CH_3OH-H_2O (8:4:1)].

Acetylation of 2– Compound 2 (50 mg) was treated with dry pyridine (7 ml) and acetic anhydride (4 ml). The mixture was stirred at room temperature for 12 hr. After evaporation of the solvent, the residue was subjected to silica gel column chromatography using petroleum ether-ethyl acetate as eluent afforded compound **3** (30 mg), m.p. 158-160 °C (from hexane-ethyl acetate). IR (KBr) 2920 (CH), 1761 (C=O) 1640 (C=C) cm^{-1}; MS m/z $[M+Li]^+$ 751; ^1H-NMR (300 MHz, pyridine-d_5, δ) 5.76 (1H, *dd*, J=9.7, 9.4 Hz, H-3'), 5.51 (1H, *dd*, J=9.7 , 9.4 Hz, H-4'), 5.47 (1H, *dd*, J=9.7, 7.9 Hz, H-2'), 5.42 (1H, *brs*, H-6), 5.10 (1H, *d*, J=7.9 Hz, H-1'), 4.66 (1H, *dd*, J=12.3, 2.4 Hz, H-6' a), 4.40 (1H, *dd*, J=12.3, 4.7 Hz, H-6' b), 4.16 (1H, *m*, H-5'), 3.75 (1H, *m*, H-3), 2.54-2.40 (2H, *m*, H-4), 2.11 (3H, *s*, AcO), 2.02 (3H, *s*, AcO), 2.01 (3H, *s*, AcO), 2.00 (3H, *s*, AcO), 0.98 (3H, *d*, J=6.5 Hz, Me-21), 0.94 (3H, *s*, Me-19), 0.91 (3H, *t*, J=7.3 Hz, Me-29), 0.88 (3H, *d*, J=6.6 Hz, Me-26), 0.85 (3H, *d*, J=6.6 Hz, Me-27), 0.67 (3H, *s*, Me-18); ^{13}C- NMR (75 MHz, pyridine-d_5, δ)-see Table 1 and Table 2.

Results and Discussion

Compound **1** on TLC gave positive test on steroid which showed one dark pink spot with Rf 0.37 (pet. ether-ethyl acetate, 4:1) after spraying with 10% H_2SO_4. The spot has the same Rf compared to standard β-sitosterol and stigmasterol in the same solvent system; both β-sitosterol and stigmasterol were reported (Xu, 1988) to have the same Rf. In the ^1H-NMR spectrum, both H-6 proton for β-sitosterol and stigmasterol resonated at δ 5.35 (*brs*). The presence of stigmasterol component in **1** was clearly shown by resonating peaks at δ 5.15 (*dd*, J=15.2, 8.4 Hz, H-22), δ 5.03 (*dd*, J=15.1, 8.2 Hz, H-23), and δ 0.70 (*s*, Me-18); the resonating peak for Me-18, β-sitosterol was shown at δ 0.68. The chemical shifts for C-5, C-6, C-22, and C-23 in the ^{13}C-NMR were shown at δ 140.74, 121.69, 138.30, and 129.30, respectively.

GLC analysis of **1** (Fig. 1) using capillary column 5% phenylmethyl silicone at 250 °C further confirmed that **1** was a mixture of β-sitosterol (Rt=37.46 min) and stigmasterol (Rt=32.52 min) in 3:1 ratio. The Rt values of β-sitosterol and stigmasterol were det-

ermined by direct comparison with the respective authentic samples.

On the basis of the above observations, **1** was identified as a mixture of β-sitosterol and stigmasterol.

Compound **2** on TLC showed one pink spot with Rf 0.33 ($CHCl_3$-MeOH, 8.5:1.5) after spraying with 10% H_2SO_4, and responded positively to Molisch test (Trease, 1972) for carbohydrate. It showed that **2** was a sterol glycoside. The presence of aglycone β-sitosterol and stigmasterol were shown by similarity in chemical shifts to **1** in the ^{1}H-NMR spectrum. In addition to the resonating peaks belong to β-sitosterol and stigmasterol, the ^{1}H-NMR also showed additional 7 protons in the region from δ 5.05 to δ 3.8, which indicated the presence of sugar D-glucose moiety (Campos, 1991). Anomeric proton H-1' appeared as a dublet at δ 5.03 (J=7.6Hz), and two H-6' protons were appeared as a dublet-dublet at δ 4.54 (J=12.1, 2.4 Hz) and δ 4.39 (J=12.1, 5.3 Hz), respectively. H-3' and H-4' protons were resonated as multiplet at δ 4.26, H-2' as a dublet-dublet at δ 4.03, (J=8.2, 7.6 Hz) and H-5' as a multiplet at δ 3.95. The ^{13}C-NMR data of **2** (Table 1 and Table 2) also demonstrated the presence of six additional carbons when compared to ^{13}C-NMR data of **1** (Table 1 and Table 2). The chemical shifts at δ 102.87 (C-1'), 78.6 (C-5'), 78.3 (C-3'), 75.5 (C-2'), 72.2 (C-4') and 63.3 (C-6') were assigned to six carbons in D-glucopyranosyl moiety of the glycoside.

Acid hydrolysis of **2** with 2M HCl in MeOH-H_2O under reflux gave a mixture of β-sitosterol and stigmasterol as the aglycone which was identified by TLC, ^{1}H-NMR, and GLC analysis (Fig. 1) in comparison with **1** and standard β-sitosterol and stigmasterol. The sugar component was confirmed as D-glucose by direct comparison with authentic sample on TLC. The ratio of β-sitosterol glucoside to stigmasterol glucoside in **2** was about 3:1 as was shown by GLC analysis of the aglycone (Fig. 1).

Acetylation of **2** with pyridine-acetic anhydride and purification by silica gel column chromatography gave the acetate **3** which on TLC showed a pink spot Rf 0.95 ($CHCl_3$-

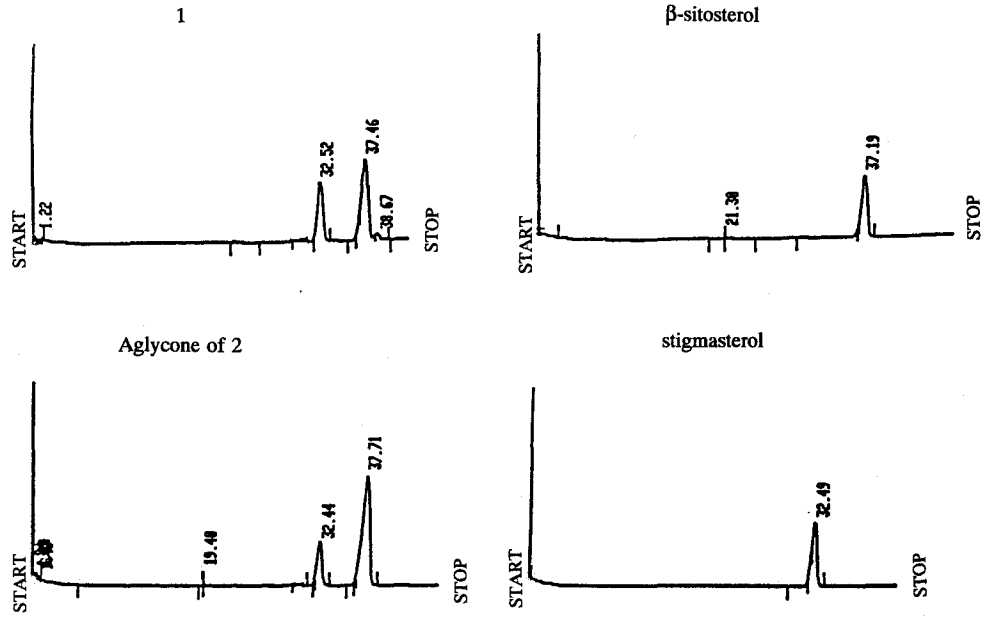

Fig. 1. GC Analysis of 1, β-sitosterol, stigmasterol and the aglycone of 2 on 5% phenylmethyl silicone capillary column under isothermal condition at 250 °C.

MeOH, 8.5:1.5) after spraying with 10% H_2SO_4. Only aglycone β-sitosterol was present in **3** which was shown by the absence of chemical shifts at δ 5.15 (*dd*, J=15.2, 8.4 Hz, H-22 stigmasterol), δ 5.09 (*dd*, J=15.1, 8.2 Hz, H-23 stigmasterol) in the ^{1}H-NMR spectrum and δ 138.80 (C-22 stigmasterol) and δ 129. 90 (C-23 stigmasterol) in the ^{13}C-NMR, respectively. The presence of four AcO groups in **3** were shown in the ^{1}H-NMR spectrum as four singlets at δ 2.11, 2.02, 2.01, and 2.00. The carbonyl chemical shifts of four AcO groups in the ^{13}C-NMR clearly were demonstrated at δ 170.5, 170.3 169.8, and 169.6.

Compound **3** in FAB mass spectrometry with the addition of LiI showed m/z peak at 751 $[M+Li]^+$, which gave the 744 $[M^+]$ and was in agreement with 3-O-β-D-tetra-O-acetyl-glucopyranosyl β-sitosterol, molecular formula $C_{43}H_{68}O_{10}$.

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