The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

BENZOFURAN GLYCOSIDES FROM *STYRAX BENZOIN*

(Glikosida bezofuran daripada *Styrax benzoin*)

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Abstract

Separation of methanol extracts of the fruits and stem bark of *Styrax benzoin* using various chromatography (vacuum liquid chromatography, column chromatography and preparative thin layer chromatography) gave four benzofuran glycosides namely egonol gentiobioside (**1**), egonol gentiotrioside (**2**), egonol glucoside (**3**) and masutakeside (**4**). The compounds were identified by spectroscopic analysis (NMR, mass and infra-red spectral data) and by comparison of the data with that of the literature. Isolation of compounds from this plant has never been reported before.

Keywords: *styrax benzoin*, styracaceae, egonol gentiobioside, egonol gentiotrioside, egonol glucoside, masutakeside

Abstrak

Pemisahan kromatografi ke atas ekstrak metanol buah dan kulit batang *Styrax benzoin* menggunakan pelbagai teknik kromatografi (kromatografi cecair vakum, kromatografi turus dan kromatografi lapisan thin preparatif) memberikan empat benzofuran glikosida yang bernama egonol gentiobiosida (**1**), egonol gentiotriosida (**2**), egonol glukosida (**3**) dan masutakesida (**4**). Sebatian ini telah dikenal pasti dengan analisis spektroskopi (data spektrum RMN, jisim dan infra merah) dan membandingkan data ini dengan data kepustakaan. Pemisahan sebatian daripada tumbuhan ini belum pernah dilaporkan sebelum ini

Kata kunci: *styrax benzoin*, styracaceae, egonol gentiobiosida, egonol gentiotriosida, egonol glukosida, masutakesida

Introduction

Styracaceae is a family consisting of small trees and shrubs, mostly native to tropical and subtropical regions (Asia, Mediterranean, and North to South America) [1]. The genus *Styrax* is different from other genera of this family by the production of resinous material, usually secreted when the barks and trunks are injured by sharp objects [2, 3]. This resin was used by Romans, Egyptians, Phoenicians and Ionians as incense and in therapeutics [1]. Previous studies of this genus led to the isolation of saponins [4 - 7], terpenes [2, 8 -10], lignans [3, 10 - 12], benzofuran derivatives and their glycosides [2 - 4, 8, 9, 11, 13 - 16].

Styrax benzoin (locally known as Pokok Kemenyan) is fairly tall tree and distributed from Malay Peninsula to Sumatra and Western Java [17], where it grows to about 12 metres in maximum height. This species is cultivated as a main source of resin (Sumatra Benzoin or Gum Benjamin) in Indonesia and used as incense and medicine [18]. However, no chemical study on this plant has been reported. We report here the isolation and structural elucidation of four benzofuran glucosides from *S. benzoin*.

Materials and Methods

Plant Material

The fruits and stem bark of *Styrax benzoin* were collected in March 2010 from Teras Jernang, Bangi, Selangor, Malaysia. A voucher specimen (SM2104) is deposited at the Herbarium of Universiti Kebangsaan Malaysia (UKMB) and identified by Mr. Sani Miran.

Extraction and Isolation

The dried powder of fruits of *Styrax benzoin* (0.98 kg) was extracted consecutively at room temperature with EtOAc (3 x 2.5 L), each time for 3 days. After filtration and evaporation of the solvent under vacuum, 13.1 g (1.34 %) of EtOAc extract was obtained. The residue was then extracted with MeOH (3 x 2.5 L) to give 115.5 g (11.79%) of MeOH extract. The dried powder of stem bark (2.75 kg) was extracted similarly with EtOAc (3 x 8.0 L; yield 63.52 g, 2.31 %) and MeOH (3 x 8.0 L, produced 151.37 g, 5.50 %). The MeOH extracts of respective fruits (33.0 g) and stem bark (50.0 g) were fractionated separately by using vaccum liquid chromatography (VLC) eluted with increasing polarity of $CHCl_3$ and MeOH. The eluates that exibited similar profile on silica gel thin layer chromatography (TLC) (Merck 5554) were combined to give 6 fractions (A-F) each for the fruits and stem bark. Purification of fruits, fractions B (6.06 g) and E (5.03 g) by VLC, column chromatography (CC) and preparative TLC using EtOAc and MeOH (8.5:1.5; 7:3) gave compounds **1** (1.2022 g) and **2** (0.1128 g), respectively. Separation of stem bark, fractions D (5.45 g) and E (4.38 g) by VLC, CC and preparative TLC using EtOAc and MeOH (8.5:1.5; 7.5:2.5) afforded compounds **3** (0.0338 g) and **4** (0.0526 g), respectively.

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Egonol gentiobioside 1

(1.2022 g): viscous yellow oil. R_f (6:2:1, ethyl acetate-pyridine-water) = 0.83. ESI-MS (m/z) 673.2071 $[M+Na]^+$, $C_{31}H_{38}O_{15}$. FTIR (ATR) cm^{-1}: 3358, 2934, 1599, 1477, 1227, 1022, 928. ^1H and ^{13}C NMR data: see Table 1

Egonol gentiotrioside 2

(0.1128 g): white amorphous. R_f (6:2:1, ethyl acetate-pyridine-water) = 0.62. ESI-MS (m/z): 835.2620 $[M+Na]^+$, $C_{37}H_{48}O_{20}$. ^1H and ^{13}C NMR data: see Table 1

Egonol glucoside 3

(0.0338 g): viscous yellow oil. R_f (6:2:1, ethyl acetate-pyridine-water) = 0.93. ESI-MS (m/z): 527.1859 $[M+K]^+$, $C_{25}H_{28}O_{10}$. UV (MeOH) λ_{max}: 225, 311 nm. FTIR (ATR) cm^{-1}: 3394, 2941, 1601, 1515, 1485, 1141, 1023, 858. ^1H and ^{13}C NMR data: see Table 1

Masutakeside 4

(0.0526 g): viscous yellow oil. R_f (6:2:1, ethyl acetate-pyridine-water) = 0.81. ESI-MS (m/z): 659.2262 $[M+K]^+$, $C_{30}H_{36}O_{14}$. UV (MeOH) λ_{max}: 224, 311 nm. FTIR (ATR) cm^{-1}: 3546, 3459, 2924, 1595, 1485, 1140, 1036 ^1H and ^{13}C NMR data: see Table 1.

Results and Discussion

Compound **1** was isolated as viscous yellow oil. Its molecular formula was determined as $C_{31}H_{38}O_{15}$ from the molecular ion peak at m/z 673.2071 $[M+Na]^+$ in the ESI-MS. The IR spectrum showed the presence of many hydroxyl groups (3358 cm^{-1}). Comparison of the ^1H and ^{13}C-APT NMR spectra of the compound suggested that it is egonol diglucoside. The ^{13}C-APT NMR spectrum showed the location of twelve out of 31 signals which suggested that the compound is gentiobioside. This was supported by presence of the signals at δ_H 4.13 (*d*, *J* = 7.8 Hz, Glc-H-1') and 4.27 (*d*, *J* = 7.8 Hz, Glc-H-1") in the ^1H NMR spectrum, which could be assigned to two anomeric protons. Thus, the structure of **1** was determined as egonol gentiobioside previously isolated from *Styrax perkinsiae* [2, 11], *S. officinalis* [4] and *S. macranthus* [8].

Compound **2**, $C_{37}H_{48}O_{20}$ appeared as a white amorphous. The mass spectrum showed a molecular ion peak ESI-MS $[M+Na]^+$ at m/z = 835.2620 consistent with a molecular formula of $C_{37}H_{48}O_{20}$. Comparing the ^1H and ^{13}C-APT NMR data of compound **2** with those of compound **1**, compound **2** has three signals at δ_H 4.13 (*d*, *J* = 7.8 Hz, Glc-H-1'), 4.22 (*d*, *J* = 7.8 Hz, Glc-H-1'") and 4.25 (*d*, *J* = 7.8 Hz, Glc-H-1") in the ^1H NMR spectrum, which could be assigned to three anomeric protons. Thus, compound **2** was elucidated as egonol gentiotrioside previously isolated from *Styrax perkinsiae* [2, 11], *S. officinalis* [4] and *S. macranthus* [8].

Compound **3** was obtained as viscous yellow oil. The molecular ion peak at m/z = 527.1859 $[M+K]^+$ in the ESI-MS provided a molecular formula of $C_{25}H_{28}O_{10}$. The UV spectrum absorptions in ethanol at 225 and 311 nm suggested that this compound has the egonol skeleton. The IR (3394 cm^{-1}) of compound **3** showed the presence of many hydroxyl groups. The ^1H NMR spectrum showed only one signal at δ_H 4.12 (*d*, *J* = 7.8 Hz, Glc-H-1') could be assigned to the anomeric proton. Compound **3** was confirmed as egonol glucoside previously isolated from *Styrax perkinsiae* [2, 11], *S. ferrugineus* [3], *S. officinalis* [4] and *S. macranthus* [8] and *S. obassia* [15].

Compound **4** was isolated as viscous yellow oil. The mass spectrum showed a molecular ion peak ESI-MS $[M+K]^+$ at m/z = 659.2262 consistent with a molecular formula of $C_{30}H_{36}O_{14}$. Compound **4** exhibited UV absorptions at 224 and 311 nm which suggested the egonol skeleton. The IR (3546 cm^{-1}) spectrum of the compound suggested the presence of hydroxyl groups. Comparing the ^1H and ^{13}C-APT NMR data of compound **4** with those of compound **1**, compound **4** still has two signals at δ_H 4.12 (*d*, *J* = 7.8 Hz, Glc-H-1) and 4.21 (*d*, *J*_ = 7.8 Hz, Xyl-H-1), but compound **4** has less one signal proton and carbon. Thus spectroscopic data led to the structure of masutakeside previously isolated from *Styrax japonica* [9] and *S. perkinsiae* [11]. The structure of compounds **1**, **2**, **3** and **4** show in Figure 1.

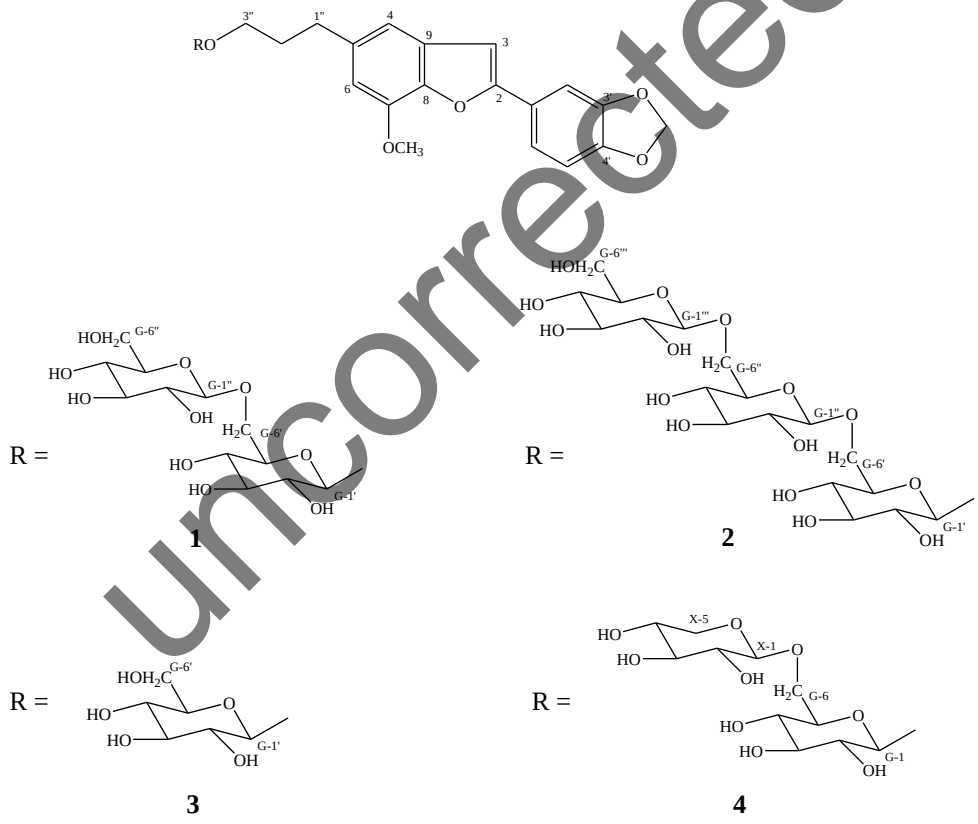

Figure 1. Structure of compounds **1**, **2**, **3** and **4**

Table 1. ^{1}H NMR (600 MHz) spectral data of compounds 1-4 in DMSO-*d*

Position	δ_H (multiplicity, *J* in Hz)			
	Egonol gentiobioside 1	**Egonol gentiotrioside 2**	**Egonol glucoside 3**	**Masutakeside 4**
2	-	-	-	-
3	7.20 (1H, *s*)	7.19 (1H, *s*)	7.21 (1H, *s*)	7.21 (1H, *s*)
4	7.00 (1H, *d*, 0.9)	7.00 (1H, *s*)	7.00 (1H, *s*)	7.01 (1H, *s*)
5	-	-	-	-
6	6.78 (1H, *d*, 0.9)	6.77 (1H, *s*)	6.79 (1H, *d*, 0.6)	6.78 (1H, *d*, 0.6)
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
1'	-	-	-	-
2'	7.42 (1H, *d*, 1.6)	7.40 (1H, *s*)	7.43 (1H, *d*, 1.6)	7.43 (1H, *d*, 1.6)
3'	-	-	-	-
4'	-	-	-	-
5'	7.03 (1H, *d*, 8.2)	7.02 (1H, *d*, 9.0)	7.03 (1H, *d*, 8.0)	7.03 (1H, *d*, 8.2)
6'	7.40 (1H, *dd*, 1.6, 8.2)	7.38 (1H, *dd*, 1.2, 9.0)	7.40 (1H, *dd*, 1.6, 8.0)	7.40 (1H, *dd*, 1.6, 8.2)
1"	2.71 (1H, *t*, 8.6) 2.73 (1H, *t*, 8.6)	2.70 (1H, *t*, 9.6) 2.72 (1H, *t*, 9.6)	2.72 (2H, *t*, 7.8)	2.71 (1H, *t*, 7.8) 2.72 (1H, *t*, 7.8)
2"	1.87 (2H, *q*, 6.9)	1.85 (2H, *q*, 6.9)	1.87 (2H, *q*, 6.8)	1.86 (2H, *q*, 6.9)
3"	3.44 (1H, *m*) 3.80 (1H, *dt*, 6.5, 9.6)	3.44 (1H, *m*) 3.80 (1H, *dt*, 6.3, 9.6)	3.42 (1H, *m*) 3.80 (1H, *dt*, 6.3, 9.3)	3.43 (1H, *m*) 3.79 (1H, *dt*, 5.7, 9.3)
7-OCH_3	3.95 (3H, *s*)	3.94 (3H, *s*)	3.95 (3H, *s*)	3.95 (3H, *s*)
-OCH_2O-	6.09 (2H, *s*)	6.07 (2H, *s*)	6.09 (2H, *s*)	6.09 (2H, *s*)
G-1'	4.13 (1H, *d*, 7.8)	4.13 (1H, *d*, 7.8)	4.12 (1H, *d*, 7.8)	4.12 (1H, *d*, 7.8)
G-2'	2.96 (1H, *br t*, 8.7)	2.98 (1H, *m*)	2.97 (1H, *dt*, 4.4, 8.3)	2.96 (1H, *dt*, 3.4, 8.3)
G-3'	3.13 (1H, *br t*, 9.0)	3.13 (1H, *m*)	3.08 (1H, *dt*, 1.8, 7.8)	3.14 (1H, *br t*, 8.7)
G-4'	3.06 (1H, *br s*)	3.05 (1H, *t*, 3.0)	3.04 (1H, *dt*, 4.4, 9.0)	3.08 (1H, *br t*, 8.7)
G-5'	3.30 (1H, *d*, 1.2)	3.29 (1H, *br t*, 8.1)	3.14 (1H, *dt*, 3.2, 8.2)	3.27 (1H, br t, 7.8)
G-6'	3.58 (1H, *dd*, 6.6, 11.4) 3.97 (1H, *dd*, 1.2, 11.4)	3.97 (1H, *d*, 10.8) -	3.42 (1H, *m*) 3.66 (1H, *dd*, 4.8, 10.8)	3.54 (1H, *dd*, 6.6, 11.4) 3.93 (1H, *dd*, 1.2, 11.4)
G-1"	4.27 (1H, *d*, 7.8)	4.25 (1H, *d*, 7.8)	-	-
G-2"	2.99 (1H, *t*, 8.4)	2.98 (1H, *m*)	-	-
G-3"	3.08 (1H, *br t*, 10.2)	3.13 (1H, *m*)	-	-
G-4"	3.06 (1H, *br s*)	3.13 (1H, *m*)	-	-
G-5"	3.14 (1H, *br t*, 9.3)	3.26 (1H, *br t*, 7.8)	-	-
G-6"	3.44 (1H, *m*) 3.66 (1H, *br d*, 10.8)	-	-	-

(table continues)

Position	**δ_H (multiplicity, *J* in Hz)**			
	Egonol gentiobioside 1	**Egonol gentiotrioside 2**	**Egonol glucoside 3**	**Masutakeside 4**
G-2'''	-	2.98 (1H, *m*)	-	-
G-3'''	-	3.06 (1H, *br s*)	-	-
G-4'''	-	3.13 (1H, *m*)	-	-
G-5'''	-	3.13 (1H, *m*)	-	-
G-6'''	-	3.44 (1H, *m*) 3.65 (1H, *m*)	-	-
X-1	-	-	-	4.21 (1H, *d*, 7.8)
X-2	-	-	-	2.97 (1H, *br t*, 10.6)
X-3	-	-	-	3.08 (1H, *br t*, 8.7)
X-4	-	-	-	3.27 (1H, *br t*, 7.8)
X-5	-	-	-	3.00 (1H, *t*, 10.8) 3.68 (1H, *dd*, 5.4, 11.4)

Table 2. ^{13}C-APT NMR (150MHz) spectral data of compounds 1-4 in DMSO-*d*

Position	**δ_C APT**			
	Egonol gentiobioside 1	**Egonol gentiotrioside 2**	**Egonol glucoside 3**	**Masutakeside 4**
2	155.6	155.6	155.6	155.6
3	101.6	101.6	101.6	101.6
4	112.6	112.6	112.6	112.6
5	138.4	138.4	138.3	138.3
6	108.3	108.3	108.3	108.3
7	144.8	144.7	144.8	144.7
8	142.1	142.0	142.0	142.0
9	130.9	130.9	130.9	130.9
1'	124.5	124.5	124.5	124.5
2'	105.4	105.4	105.4	105.4
3'	148.4	148.4	148.4	148.4
4'	148.3	148.2	148.3	148.3
5'	109.3	109.3	109.3	109.3
6'	119.2	119.2	119.2	119.2
1''	32.3	32.3	32.2	32.3
2''	32.0	32.0	31.9	31.9
3''	68.3	68.4	68.3	68.3
7-OCH_3	56.3	56.2	56.2	56.2
-OCH_2O-	101.9	101.9	101.9	101.9
G-1'	103.3	103.2	103.4	103.3

(table continues)

Position	δ_C APT			
	Egonol gentiobioside 1	**Egonol gentiotrioside 2**	**Egonol glucoside 3**	**Masutakeside 4**
G-2'	73.9	73.9	74.0	73.8
G-3'	77.2	77.0	77.3	77.0/ 77.1
G-4'	70.5	70.4	70.5	70.3
G-5'	76.3	76.0	77.2	76.2
G-6'	68.8	68.8	61.5	68.8
G-1"	103.8	103.6	-	-
G-2"	74.0	73.9	-	-
G-3"	77.4	77.0	-	-
G-4"	70.5	70.3	-	-
G-5"	77.1	76.1	-	-
G-6"	61.5	68.8	-	-
G-1'"	-	103.7	-	-
G-2'"	-	73.9	-	-
G-3'"	-	77.2	-	-
G-4'"	-	70.3	-	-
G-5'"	-	77.1	-	-
G-6'"	-	61.5	-	-
X-1	-	-	-	104.5
X-2	-	-	-	73.8
X-3	-	-	-	77.0/ 77.1
X-4	-	-	-	70.0
X-5	-	-	-	66.1

Conclusion

Four benzofuran glycosides namely egonol gentiobioside **1**, egonol gentiotrioside **2**, egonol glucoside **3** and masutakeside **4** were successfully isolated from the methanol extract of *Styrax benzoin*. This is the first reported phytochemical studies of this plant.

Acknowledgement

This research was financially supported by UKM grant (UKM-GUP-2011-205, UKM-DLP-2012-033, UKM-DLP-2013-18). Special thanks to School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM and Centre for Research and Innovation Management (CRIM) UKM for the technical support on NMR, LC-MSToF, UV and FTIR instrumentation and Mr. Sani Miran for the assistance in identifying plant material.

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