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Isolation and Characterization of Styryllactone of *Goniothalamus ridleyi*

(Pemencilan dan Pencirian Stirillakton *Goniothalamus ridleyi)*

SAMSIAH JUSOH, ZURIATI ZAKARIA, FASIHUDDIN B. AHMAD & LAILY B. DIN*

ABSTRACT

Phytochemical studies were conducted on the stem bark, stem, root and fruit of Goniothalamus ridleyi *(Annonaceae) collected at Post Brooke, Gua Musang, Kelantan, Malaysia. Extraction using organic solvent followed by extensive purification using standard procedure afforded an epoxystyryllactone, 5-acetoxyisogoniothalamin oxide (1) from the stem bark and fruit; a styryllactone, 5-acetoxygoniothalamin (2) and a styrylpyrone, dehydrogoniothalamin (3) from the stem and root; a styryllactone, 5-hydroxygoniothalamin (4) from the root and styrylpyrone as well as goniothalamin (5) from the fruit. These compounds were characterized using spectroscopic techniques.*

Keywords: Annonaceae; goniothalamin derivatives; Goniothalamus; *NMR*

ABSTRAK

Kajian fitokimia telah dijalankan ke atas kulit batang, batang, akar dan buah Goniothalamus ridleyi *(Annonaceae) yang diperoleh dari Post Brooke, Gua Musang, Kelantan, Malaysia. Pengekstrakan menggunakan pelarut organik diikuti dengan pemisahan ekstensif menggunakan prosedur piawai menghasilkan epoksistirillakton, 5-asetoksiisogoniotalamin oksida (1) daripada kulit batang dan buah; stirillakton, 5-asetoksigoniotalamin (2) dan stirilpiron, dehidrogoniotalamin (3) daripada batang dan akar; stirillakton, 5-hidroksigoniotalamin (4) daripada akar dan stirilpiron manakala goniotalamin (5) daripada buah. Sebatian-sebatian ini dikenal pasti menggunakan teknik spektroskopi.*

Kata kunci: Annonaceae; Goniothalamus*; NMR; terbitan goniothalamin*

INTRODUCTION

The genus *Goniothalamus* (Annonaceae) is an archaic shrub or treelets which grow in shady primary rainforest of tropical Asia (Wiart 2007). *Goniothalamus ridleyi* is a sub-canopy tree which can grow up to 7-15 m tall and 8-20 cm diameter. Stipules are absent and the leaves are alternate, simple and penni-veined. The size of the flower petals are approximately 5-10 cm long, reddish-brown and are placed in apocarps containing several seeds. This plant could be found in undisturbed forests up to 500 m in altitude, usually on hillsides and ridges. In the secondary forests, it is usually present as a pre-disturbance remnant. The bark decoction is used to treat stomach-ache (Mat-Salleh & Latiff 2002). Phytochemical studies on *Goniothalamus* spp. showed that the presence of interesting secondary metabolites particularly styryllactones and annonaceous acetogenins and alkaloids. All the secondary metabolites isolated from this genera especially styryllactone and acetogenin showed significant cytotoxicity against several human cell lines. In the previous study on *G. ridleyi*, goniothalamin, goniothalamin oxide and isoaltholacton have been isolated (Ee et al. 1999). The objectives of this research was to isolate and characterize secondary metabolites from different parts of *G. ridleyi* and to investigate the correlation of these compounds in the plant.

MATERIALS AND METHODS

PLANT

G. ridleyi was collected from Post Brooke, Gua Musang, Kelantan, Malaysia and separated into stem bark, stem, root and fruit. The samples were oven dried at 50°C and ground to powder form prior to extraction. This species has been identified by a botanist, Dr. Shamsul Khamis (UPM). The voucher specimen of *G. ridleyi* (SK1739/10) was deposited at Herbarium Universiti Putra Malaysia (UPM), Serdang.

EXTRACTION AND ISOLATION

The extraction of samples were carried out using three different solvent i.e. hexane, chloroform and methanol, which afforded three crude extracts. Each crude extract was separated using vacuum liquid chromatography (VLC, if the extract was more than 3 g), column chromatography (CC) and preparative thin layer chromatography (PTLC). The purification of the crude extracts afforded 5-acetoxyisogoniothalamin oxide (1), 5-acetoxygoniothalamin (2), dehydrogoniothalamin (3), 5-hydroxygoniothalamin (4) and goniothalamin (5).

The chloroform crude extract of the stem bark of *G. ridleyi* (5 g) was separated using VLC using mixture of chloroform, ethyl acetate and as mobile phase.

Fractions 4-11 which showed similar profile based on thin layer chromatography (TLC) were combined and further separated using CC with hexane, chloroform and methanol as mobile phase. Fractions 115-118 were combined and further separated using PTLC with a solvent mixture of hexane:chloroform (3:7). The purified compound was collected and crystallised using hexane and chloroform to give white needles, GRCB 1 (27.7 mg) identified as 5-acetoxyisogoniothalamin oxide (1) an epoxystyryl-lactone which has been previously found in *G. sesquipedalis* (Hasan et al. 1994).

The chloroform crude extract of the stem (2.34 g) was separated on CC using a mixture of hexane:ethyl acetate (6.5:3.5). Fractions 61-63 was combined and further separated using PTLC with similar elution system as for the CC and afforded GRDB 5 (white needles). This compound gave R_f value 0.36 in similar solvent system as for the PTLC. Spectroscopic data showed that GRDB 5 is similar to 5-acetoxygoniothalamin (2) that has been reported from *Goniothalamus uvaroides* (Fasihuddin et al. 1991).

The methanol crude extract of the stem of *G. ridleyi* (2.26 g) was separated using column chromatography. A mixture solvent of ethyl acetate and methanol at different ratio was used as eluent and afforded 144 fractions. Fractions 4-9 were combined and further purified using PTLC and a mixture of hexane:ethyl acetate (8:2) as eluting solvent. PTLC separation afforded a pure orange coloured compounds, GRDC 2. Based on the spectroscopic data, GRDC 2 has been identified as dehydrogoniothalamin (3). Compound 3 was previously reported from *G. umbrosus* by Fasihuddin and Din 2002.

The ethyl acetate crude extract of the root (9.57 g) was separated using vacuum liquid chromatography (VLC) with ethyl acetate and methanol as eluent to give a total of 12 fractions. Fraction 1-3 which showed similar TLC was further separated using column chromatography with mobile phase consisted of hexane and ethyl acetate which afforded 178 fractions. Fractions 157-165 was combined and further purified using PTLC and hexane:ethyl acetate (4.5:5.5) as mobile phase. PTLC purification afforded a pure compounds GRAB 6 (61.7 mg, yellowish oily amorphous) with R_f 0.4616 in hexane:ethyl acetate (1:1). Spectroscopic data indicated that GRAB 6 is identical with 5-hydroxygoniothalamin (4) reported by Goh et al. 1995.

The ethyl acetate crude extract of *G. ridleyi* fruit (1.35 g) was separated using CC and a mixture of hexane, ethyl acetate and methanol as mobile phase which afforded 119 fractions. Fractions 33-40 was combined and further purified using PTLC with solvent system hexane:ethyl acetate (7:3) and afforded GRFB 8 (77.1 mg) as white crystal. GRFB 8 gave R_f 0.30 in hexane:ethyl acetate (7:3) as mobile phase. Based on the spectroscopic data, GRFB 8 was identified as goniothalamin (5). The presence of **5** was reported in various *Goniothalamus* spp. by Jewers et al. 1972.

COMPOUND IDENTIFICATION

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ using BRUKER spectrometer (600 MHz for proton and 125 MHz for ^{13}C). Mass spectrometer (MS) spectra were recorded on Shimadzu GCMS QP5050A. Column chromatography (CC) and vacuum liquid chromatography (VLC) was carried out using Merck Silica gel 60 (230-400 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were performed on Merck TLC Silica gel 60 F_{254} 0.25 mm and detected by UV light (254 and 365 nm).

Results and Discussion

The ^1H NMR spectrum of GRCB 1 showed disubstituted epoxide protons H-7 and H-8 at δ 3.36 (dd, J = 1.8, 6.0 Hz, H-7) and 4.04 (d, J = 1.2 Hz, H-8); a methine proton next to an acetate (δ 5.43 (q, J = 3.0 Hz, H-5) and a disubstituted double bond at δ 6.27 (d, J = 9.6 Hz, H-3); 7.11 (dd, J = 5.4, 9.6 Hz, H-4) in conjunction with a lactone with a secondary terminus at δ 4.45 (q, J = 3.0 Hz, H-6). The other phenyl ring for H-10, H-11, H-12, H-13 and H-14 were shown at δ 7.39-7.28 (m). The methyl group for acetyl compound was observed at δ 2.09 (s). Based on ^{13}C data, two carbonyl carbons were detected i.e. at δ 169.8 and 161.33 for acetyl and phenyl ring, respectively. Substituted phenyl ring were observed at δ 125.73 (C-10, C-14), 128.78 (C-12) and 128.63 (C-11, C-13). The spectrum showed a disubstituted epoxide carbon at δ 57.44 (C-7) and 58.19 (C-8), C-5 at 62.08 and disubstituted double bond at 124.7 (C-3) and 140.33 (C-4). Mass spectrum showed *m/z* 274 ($[M]^+$, 2), 258 ($[M - O]^+$, 2), 216 ([*m/z* 258 - $C_2H_2O]^+$, 2), 198 ([*m/z* 258 - $C_2H_4O_2]^+$, 2), 139 (100), 107 (100), 91 (100), 65 (40) and 43 ($[C_2H_3O]^+$, 100). Based on the spectroscopic data and published information (Hasan et al. 1994), GRCB 1 was identified as 5-acetoxyisogoniothalamin oxide (1). ^1H NMR spectrum of GRDB 5 gave strong absorption of acetoxy peak at δ 2.05 which appeared as singlet. The spectrum also showed that the olefinic protons at δ 6.25 (d, J=9.6 Hz, H-3), 6.99 (dd, J=5.4, 6.0, 9.9 Hz, H-4), 6.22 (dd, J=6.0, 6.6, 15.9 Hz, H-7), 6.82 (d, J=16.2 Hz, H-8) and aromatic phenyl ring at 7.35 (d, J=4.2 Hz, H-10, H-14), 7.34 (t, J=5.4, 7.8 Hz, H-11, H-13) and 7.29 (d, J=7.8 Hz, H-12). The presence of H-5 and H-6 were observed at δ 5.19 (dddd, J=1.2, 3.0, 6.6 Hz) and 5.37 (dd, J=3.0, 5.4 Hz). The ^1H NMR spectrum was supported by ^{13}C NMR signal at δ 162.36 (CO), 121.18 (C-3), 140.69 (C-4), 63.90 (C-5), 79.09 (C-6), 134.90 (C-7), 124.81 (C-8), 135.71 (C-9), 126.80 (C-10, C-14), 128.73 (C-11, C-13), 128.53 (CO) and 20.53 (CH_3). Mass spectrum of GRDB 5 showed M^+ at *m/z* 258 (10). Other peaks were observed at *m/z* 216 ([M - $C_2H_2O]^+$, 5), 198 ([M - $C_2H_4O_2]^+$, 15), 175 ($[C_{11}H_{11}O_2]^+$, 20), 133 (100), 125 ($[C_6H_6O_3]^+$, 74), 115 ($[C_9H_7]^+$, 26), 84 (100) and 43 ($[C_2H_3O]^+$, 78). Based on MS and NMR (proton, ^{13}C, HMBC, HMQC and COSY) data and comparison with published information data (Fasihuddin et al. 1991), GRDB 5 was identified as 5-acetoxygoniothalamin (2). The

TABLE 1. The comparison data of ^{1}H of compounds with published data

Position of H	5-Acetoxyisogoniothalamin oxide		5-Acetoxygoniothalamin		Dehydrogoniothalamin		5-Hydroxygoniothalamin		Goniothalamin	
	H of GRCB 1	H of reference [a]	H of GRDB 5	H of reference [b]	H of GRDC 2	H of reference [c]	H of GRAB 6	H of reference [d]	H of GRFB 8	H of reference [e]
3	6.27 (d, *J*=9.6 Hz)	6.23 (dd, *J*=9.9, 0.7 Hz)	6.25 (d, *J*=9.6 Hz)	6.20 (dd, *J*=10.0, 2.0 Hz)	6.22 (d, *J*=4.8 Hz)	6.16 (d, *J*=5.3 Hz)	6.12 (d, *J*=9.6 Hz)	6.12 (d)	6.09 (tt, *J*=10.8, 1.2 Hz)	6.05 (dt, *J*=9.6, 1.7 Hz)
4	7.10 (dd, *J*=9.6, 5.4Hz)	6.88 (dd, *J*=9.9, 5.0 Hz)	6.99 (dd, *J*=9.9, 6.0 Hz)	6.90 (dd, *J*=10.0, 5.0 Hz)	7.33 (dd, *J*=11.4, 4.8 Hz)	7.48 (dd, *J*=11.3, 5.3 Hz)	7.00 (dd, *J*=9.9, 6.0 Hz)	6.97 (dd)	6.93 (dddd, *J*=4.8, 3.6 Hz)	6.85 (dt, *J*=9.6, 4.2 Hz)
5	5.43 (q, *J*=3.0 Hz)	5.64 (ddd, *J*=5.0, 3.9, 0.7 Hz)	5.19 (dddd, *J*=6.6, 3.0 Hz)	5.26 (dd, *J*=6.0, 3.0 Hz)	6.03 (d, *J*=11.4 Hz)	6.00 (d, *J*=11.3 Hz)	4.27 (dd, *J*=4.2, 3.0 Hz)	4.25 (m)	2.53 (m)	2.45 (m)
6	4.45 (q, *J*=3.0 Hz)	4.60 (t, *J*=4.0 Hz)	5.37 (dd, *J*=5.4, 3.0 Hz)	5.15 (dd, *J*=5.0, 3.0 Hz)			5.01 (dddd, *J*=3.0, 1.2 Hz)	5.03 (ddd)	5.09 (m)	5.03 (m)
7	3.36 (dd, *J*=6.0, 1.8 Hz)	3.33 (dd, *J* =4.1, 2.1 Hz)	6.22 (dd, *J*=15.9, 6.0 Hz)	6.16 (dd, *J*=15.0, 6.0 Hz)	7.33 (d, *J*=15.6 Hz)	7.27 (d, *J*=15.8 Hz)	6.39 (dd, *J*=16.2, 6.6 Hz)	6.31 (dd)	6.28 (dd, *J*=15.9, 6.6 Hz)	6.21 (dd, *J*=15.7, 6.0 Hz)
8	4.04 (d, *J*=1.2 Hz)	3.93 (d, *J*=2.1 Hz)	6.82 (d, *J*=16.2 Hz)	6.80 (dd, *J*=15, 1.0 Hz)	6.85 (d, *J*=15.6 Hz)	6.80 (d, *J*=15.9 Hz)	6.82 (d, *J*=16.2 Hz)	6.81 (d)	6.73 (d, *J*=15.6 Hz)	6.68 dd, *J*=15.7, 1.0 Hz)
10, 14	7.39-7.28 (m)	7.25 (m)	7.35 (d, *J*=4.2 Hz)	7.26 (m)	7.52 (d, *J*=7.2 Hz)	7.30-7.39 (m)	7.41 (dd, *J*=7.5, 1.8 Hz)	7.30 (m)	7.40 (d, *J*=7.8 Hz)	
	7.3 (m)									
11, 13		7.35 (m)	7.34 (t, *J*=7.8Hz)							
		7.38 (dd, *J*=18.0, 7.2Hz)		7.30 (tt, *J*=6.6, 3.0 Hz)		7.34 (t, *J*=7.8 Hz)				
12			7.29 (d, *J*=7.8 Hz)		7.44 (d, *J*=5.4 Hz)		7.27 (dd, *J*=3.0, 5.7 Hz)		7.26 (t, *J*=7.8 Hz)	
5-OH							3.04 (br s)	2.10 (br s)		
OCO**CH**$_3$	2.09 (s)	2.04 (s)	2.05 (s)	2.02 (s)						

[a] = Hasan et al. 1994, [b] = Fasihuddin et al. 1991, [c] = Fasihuddin & Din 2001, [d] = Goh et al. 1995, [e] = Jewers et al. 1972

TABLE 2. The comparison data of ^{13}C of compounds with published data

Position of C	5-Acetoxyisogoniothalamin oxide		5-Acetoxygoniothalamin		Dehydrogoniothalamin		5-Hydroxygoniothalamin		Goniothalamin	
	C of GRCB 1	C of reference [a]	C of GRDB 5	C of reference [b]	C of GRDC 2	C of reference [c]	C of GRAB 6	C of reference [d]	C of GRFB 8	C of reference [e]
2	161.33	161.30	162.36	162.30 (d)	169.57	169.80	163.62	163.00	163.99	163.90
3	125.04	124.70	121.18	124.70 (d)	121.57	121.60	122.62	123.00	121.61	121.60
4	140.33	139.80	140.69	140.70 (d)	142.89	143.10	144.98	144.40	144.83	144.80
5	62.08	62.90	63.90	63.90 (d)	115.10	115.20	63.06	63.10	29.89	29.90
6	77.96	77.00	79.09	79.10 (d)	149.04	149.20	81.36	80.90	78.01	77.90
7	57.44	54.00	134.90	121.10 (d)	118.74	118.70	121.83	121.50	125.67	125.80
8	58.19	59.50	124.81	134.80 (d)	138.42	138.50	135.18	135.30	133.13	133.10
9	135.53	135.50 (s)	135.71	135.60 (d)	136.38	136.40	135.70	135.60	135.77	135.80
10, 14	125.73	125.70 (2d)	126.80	126.70 (d)	127.21	127.30	126.86	126.80	126.73	126.80
11, 13	128.78	128.60 (2d)	128.73	128.70 (d)	128.87	128.90	128.68	128.70	128.73	128.70
12	128.63	128.70 (d)	128.53	128.50 (d)	129.04	129.10	128.48	128.60	128.40	128.20
O**C**OCH$_3$	169.80	169.90	169.99	169.90						
OCO**CH$_3$**	20.53	20.50	20.53	20.50						

[a] = Hasan et al. 1994, [b] = Fasihuddin et al. 1991, [c] = Fasihuddin & Din 2001, [d] = Goh et al. 1995, [e] = Jewers et al. 1972

data and comparison of proton and carbon with published data was recorded in Tables 1 and 2, respectively. The different structure of these five styryllactones was recorded in Figure 1.

GRDC 2 was isolated as an orange compound and showed aromatic proton of phenyl ring at δ 7.52 (d, *J*=7.2 Hz, H-10, H-14), 7.38 (dd, *J*=7.2, 4.2, 18.0 Hz, H-11, H-13) and 7.44 (d, *J*=5.4 Hz, H-12) in ^{1}H NMR spectrum. Two olefinic proton were observed at δ 7.33 (d, *J*=15.6 Hz, H-7) and 6.85 (d, *J*=15.6 Hz, H-8). The configuration of these two protons were trans based on their *J* value i.e. 15.6 Hz. The other protons were observed at δ 6.22 (d, *J*=4.8 Hz, H-3), 7.33 (dd, *J*=11.4, 4.8 Hz, H-4) and 6.03 (d, *J*=11.4 Hz, H-5). The ^{13}C NMR spectrum showed 13 peaks which represent 13 carbon in GRDC 2. The carbonyl ester in ring appeared at δ 169.57. The other carbon were observed at δ 121.57 (C-3), 142.89 (C-4) and 115.10 (C-5), while aromatic carbon were detected at 136.38 (C-9), 127.21 (C-10, C-14), 128.87 (C-11, C13) and 129.04 (C-12). The olefinic carbon, C-7 and C-8 appeared at δ 118.74 and 138.42, respectively. GRDC 2 showed M^+ and base peak at *m/z* 198. The other peaks were observed *m/z* 170 ($[M\text{-}CO]^+$, 33), 141 ($[C_{11}H_9]^+$, 46), 115 ($[C_9H_7]^+$, 81), 89 (10) and 63 (10). Based on the spectroscopic data and published information (Fasihuddin & Din 2002). GRDC 2 was identified as dehydrogoniothalamin (3).

NMR spectrum of GRAB 6 showed the presence of 11 protons and 12 carbons. Chemical shifts at δ 7.41 (2H, dd, *J*=1.8, 7.5 Hz, H-10, H-14), 7.30 (2H, tt, *J*=3.0, 6.6 Hz, H-11, H-13) and 7.27 (1H, dd, *J*=3.0, 5.7 Hz, H-12) showed the presence of phenyl ring. This is supported by the ^{13}C NMR data which gave signals at δ 126.86 (C-10, C-14), 128.68 (C-11, C-13) and 128.48 (C-12). The hydroxyl group attached to C-5 (63.06) appeared at δ 3.04 (br s). The other signal of four olefinic protons were observed at δ 6.12 (1H, d, *J*=9.6 Hz, H-3), 7.00 (1H, dd, *J*=5.4, 6.0, 9.9 Hz, H-4), 6.40 (1H, dd, *J*=6.6, 16.2 Hz, H-7) and 6.82 (1H, d, *J*=16.2 Hz, H-8). Signal of H-5 and H-6 appeared at δ 4.27 (1H, dd, *J*=3.0, 4.2 Hz) and 5.01 (1H, dddd, *J*=1.2, 3.0Hz) respectively. The other carbon showed at δ 163.62 (C-2), 122.62 (C-3), 144.98 (C-4), 81.36 (C-6), 121.83 (C-7), 135.18 (C-8) and 135.70 (C-9). Based on NMR data and comparison with published data (Goh et al. 1995), GRAB 6 was identified as 5-hydroxygoniothalamin (4).

GRFB 8 as yellowish white needles showed 12 protons and 13 carbons. The NMR ^{1}H spectrum showed CH_2 at δ 2.53 (2H, m, H-5) while CH could be detected at 6.07 (1H, dt, *J*=1.8, 9.9 Hz), H-3), 6.92 (1H, dddd, *J*=3.6, 4.8 Hz, H-4), 5.09 (1H, m, H-6), 6.27 (1H, dd, *J*=6.6, 15.9 Hz, H-7), 6.72 (1H, d, *J*=16.2 Hz, H-8), 7.39 (2H, d, *J*=5.6 Hz, H-10, H-14), 7.17-7.35 (2H, m, H-11, H-13) and 7.26-7.30 (1H, m, H-12). The NMR ^{13}C spectrum showed CO at δ 164.08 (C-2), CH_2 at 29.86 (C-5) and CH at 121.56 (C-3), 144.91 (C-4), 78.03 (C-6), 125.66 (C-7), 133.16 (C-8), 135.77 (C-9), 126.73 (C-10, C-14), 128.72 (C-11, C-13) and 128.39 (C-12). MS data of this GRFB 8 observed $[M]^+$ at *m/z* 200 and base peak at *m/z* 68 (cyclopentenyl ion). The other peaks were *m/z* 172 ($[M\text{-}CO]^+$), 131 (cinnamyl ion), 122, 115 (indenyl ion), 104 (styrenyl ion), 91 (tropilium ion), 77 (phenyl ion), 51 and 39. The NMR and MS data was compared to Jewers et al. 1972 and it is identical with goniothalamin (5).

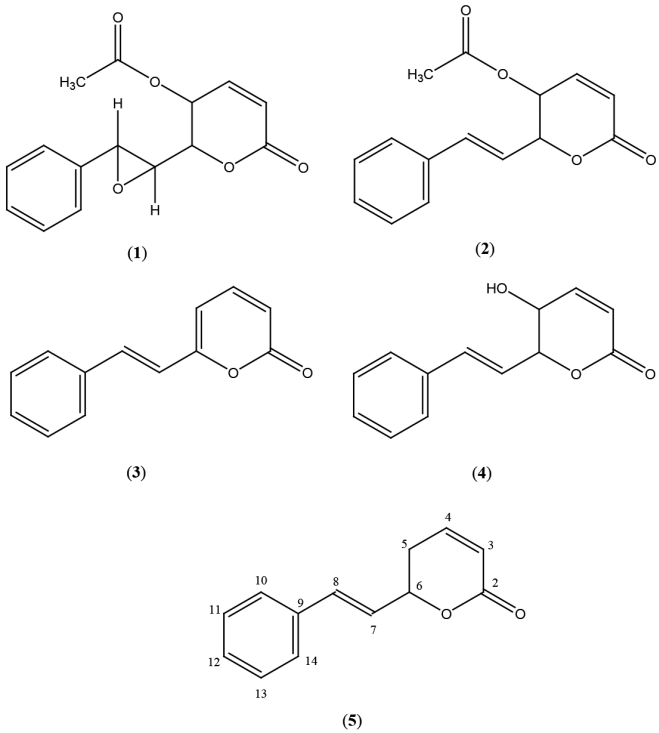

FIGURE 1. Styryllactones in *G. ridleyi*

Conclusion

Phytochemical studies on *G. ridleyi* resulted in the isolation of five styryllactone identified as 5-acetoxyisogoniothalamin oxide (1), 5-acetoxygoniothalamin (2), dehydrogoniothalamin (3), 5-hydroxygoniothalamin (4) and goniothalamin (5). Compound 1 was isolated from the chloroform crude extract of the fruit and stem bark; compound 2 from the chloroform crude extract of the stem and the hexane, chloroform and methanol crude extract of the root; compound 3 from the methanol crude extract of the root and stem; compound 4 from the chloroform crude extract of the root and compound 5 from the chloroform crude extract of the fruit. Compound 1-5 were isolated through extensive chromatography especially column chromatography and preparative thin layer chromatography. This is the first report on the isolation of compound 1-4 in *G. ridleyi*. Hence, this is part of our continuous phytochemical studies on *Goniothalamus* found.

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Samsiah Jusoh
Rice and Industrial Crop Research Centre
Malaysian Agricultural Research and Development Institute (MARDI)
P.O. Box 12301, 50774 Kuala Lumpur
Malaysia

Zuriati Zakaria
Malaysia Japan Institute of Technology
Universiti Teknologi Malaysia
54100 Kuala Lumpur
Malaysia

Fasihuddin B. Ahmad
Department of Chemistry
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
94300 Kota Samarahan, Sarawak
Malaysia

Samsiah Jusoh & Laily B. Din*
School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: lbdin@ukm.edu.my

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