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Chemical constituents and bioactive compounds of *Goniothalamus tortilipetalus* Hend (Annonaceae)

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ABSTRACT A Malaysian plant, *Goniothalamus tortilipetalus* Hend (Annonaceae) was studied for its chemical constituents and biological activities. The stem bark of *Goniothalamus tortilipetalus*, produced two isoquinolines namely discretamine (**1**) and liriodenine (**2**). The leaves yielded asimilobine (**3**), liriodenine (**2**) and lanuginosine (**4**). In addition, non-alkaloidal aromatic compounds were also purified from the petroleum ether extract. They were 6-styryl-2-pyrone (**5**) and goniothalamin (**6**). The pharmacological study revealed that 6-styryl-2-pyrone possesses a vasorelaxant effect on rat aorta and it is cytotoxic to KB cells (ED_{50} = 48.5 μg/ml).

ABSTRAK Satu tumbuhan Malaysia, *Goniothalamus tortilipetalus* Hend., (Annonaceae) telah dikaji kandungan kimia dan aktiviti biologinya. Kulit batang *Goniothalamus tortilipetalus*, menghasilkan dua alkaloid isokuinolina; diskretamina (**1**) dan liriodenina (**2**). Manakala asimilobina (**3**), liriodenina (**2**) dan lanuginosin (**4**) telah dipisahkan daripada daun *G. tortilipetalus*. Sebagai tambahan, sebatian bukan alkaloid juga telah ditulinkan daripada ekstrak petroleum eter; 6-stiril-2-piron (**5**) dan goniothalamin (**6**). Kajian farmakologikal juga mendapati 6-stiril-2-piron memberikan kesan positif terhadap pengenduran otot licin aorta tikus dan ianya sitotoksik terhadap sel KB (ED_{50} = 48.5 μg/ml).

(Alkaloid, Annonaceae, Cytotoxicity, *Goniothalamus tortilipetalus*, Vasorelaxant effect).

INTRODUCTION

Chemical constituents of *Goniothalamus tortilipetalus*, were investigated which yielded four alkaloids; diskretamine (**1**), liriodenine (**2**), asimilobine (**3**) and lanuginosine (**4**) and two non-alkaloidal compounds; 6-styryl-2-pyrone (**5**) and goniothalamin (**6**). Structural elucidation was performed with the aid of spectroscopic methods; $^{1}H/^{13}C$ - NMR, IR, UV, MS.

EXPERIMENTAL

Goniothalamus tortilipetalus Hend., was collected at Grik, Perak. 1kg of the dried and milled stembark of *Goniothalamus tortilipetalus* Hend., were moistened with 15 % NH_4OH, soaked in CH_2Cl_2 for 3 days (cold extraction) or extracted with soxhlet apparatus (17 hours). The CH_2Cl_2 extract were reduced to 500 ml followed by basic extraction using 5 % HCl until Mayer's test is negative. The aqueous solution obtained was basified to pH ≈ 10 and reextracted with CH_2Cl_2, followed by washing with distilled H_2O and dried over anhydrous sodium sulphate. Finally, the extract was concentrated to give crude alkaloids of 3.0 g in weight. The same procedure was followed for the dried leaves of *G. tortilipetalus* Hend., except for the stembark of *Goniothalamus tortilipetalus* Hend., which was previously defatted with petroleum ether. The petroleum extract afforded two non-alkaloidal aromatic compounds.

Discretamine (**1**): UV λ_{max} (log ε) nm: 207 (4.20), 230 (4.10), 282 (3.19); IR υ_{max} cm^{-1}: 3450, 1350, 950, 1025; Mass spectrum m/e (%): 327 (M^+), 312, 176, 162, 150, 135; ^{1}H NMR ($CDCl_3$) ppm: 3.82 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.18 (1H, d, J = 16 z, H - 8eq), 6.68 (1H, s, H -1 or H - 4), 670 (1H, s, H -1 or H - 4), 6.83 (1H, d, J =

6 Hz, H - 11, or H - 12), 6.81 (1H, J = 6 Hz, H - 11, or H - 12).

Liriodenine (**2**): mp 278 - 280°C (chloroform dec.) lit [1]. 275 - 276°C); UV λ_{max} (log ε) nm: 248 (3.91), 275 (4.1), 310 (3.18) sh, 415 (3.10); IR υ_{max} cm^{-1}: 1660, 960, 860; Mass spectrum m/e (%): 275 (100), 247, 219, 189, 188, 162; ^{1}H NMR ($CDCl_3$) ppm: 6.34 (2H, s, -OCH_2O-), 7.17 (1H, s, H - 3), 7.62 - 7.80 (2H, m, H - 9, 10), 8.63 (1H, dd, J = 8 Hz, J' = 1 Hz, H - 8), 8.71 (1H, dd, J" = 8 Hz, J''' = Hz, H - 11), 7.77 (1H, d, J = 5.4 Hz, H - 4), 8.96 (1H, d, J = 5.4 Hz, H - 5).

Asimilobine (**3**): UV λ_{max} (log ε) nm: 273 (4.21), 308(3.51); IR υ_{max} cm^{-1}: 3255, 3550, 1035.; Mass spectrum m/e (%): 267 (70), 266 (100), 238, 223 252, 236, 194, 177.;^{1}H NMR (270 MHz) ppm: 3.59 (3H, s, 1-OCH_3), 6.70 (1H, s, H-3), 8.3 (1H, m, H-11), 2.8 3.2 (3H, m, H - 8, H - 9, H - 10); ^{13}C NMR ($CDCl_3$): 48.3 (C-1), 142 - 9 (C -2), 125 (C - 1a), 132 (C - 1b), 114.6 (C - 3), 127 (C - 3a), 28.9 (C - 4), 43.2 (C - 5), 53.6 (C - 6a), 36.1 (C - 7), 136.1 (C - 7a), 129.8 (C - 8), 128.0 (C - 9), 128.3 (C -10), 127.2 (C - 11), 131 (C - 11a), 60.4 (OCH_3 - C - 1).

Lanuginosine (**4**): mp 318-320°C (decomp) (lit [2]. 315-317°C); UV λ_{max} (EtOH) nm: 211, 247, 273, 432 (log ε: 421, 4.30, 420 and 3.68); λ_{max} (EtOH - HCl) nm: 258, 283, 396 (log ε 4.31, 4.23, 3.66); IR υ_{max} cm^{-1}: 1655 (conjungated C = O) and 945); Mass spectrum m/e (%): 305 (M^+ 100), 290 (12), 277 (4.3), 276 (10), 275 (11), 262 (8), 247 (5); ^{1}H NMR ($CDCl_3$) ppm: 4.0 (3H, s, 9 - OCH_3), 6.35 (2H, s, OCH_2O), 7.15 (1H, s, H - 3), 7.33 (1H, dd, J_m = 3 Hz J_o = 9 Hz, H - 10), 7.78 (1H, d, J = SH_2, H - 4), 8.03 (1H, d, J = 3 Hz, H - 8), 8.57 (1H, d, J = 9 Hz, H - 11), 8.89 (1H, d, J = 5 Hz, H - 5).

6-Styryl-2-pyrone (dehydrogoniothalamin) (**5**):, mp 115 - 116°C (lit [3]. 116°C); UV λ_{max} (log ε) nm: 363, 269 nm; IR υ_{max} cm^{-1}: 1726.5, 965; Mass spectrum m/e (%):198 (100), 170, 141, 131, 103, 95, 77, 39, 28; ^{1}H NMR ($CDCl_3$) ppm: 6.58 (1H, d, J = 16.2 Hz), 6.19 (1H, dd, J = 9.6 Hz and 0.9 Hz, H - 3), 6.12 (1H, dq, J = 6.7, 0.9 and 0.4 Hz, H - 5).

Goniothalamin (**6**): UV λ_{max} (log ε) nm: 207, 255 and 284; IR υ_{max} cm^{-1}: 1720, 1577, 1663, 1492,970, 760; Mass spectrum m/e (%): 200, 172, 131, 115, 104, 91, 77, 68 (100); ^{1}H NMR ($CDCl_3$) ppm: 2.45 (2H, m, CH_2 -5), 5.03 (1H, m, H - 6), 6.05 H, dt, J = 9.7, J' = 1.8 Hz, H - 4), 6.85 (1H, dt, J = 9.7 Hz, J" = 4.2 Hz, H - 3), 6.21 (1H, dd, J = 15.7 Hz, J" = 1 Hz, H - 8), 6.68 (1H, dd, J = 15.7 Hz, J" + 1 Hz, H - 8), 7.35 (5H, m, 5 H aromatic).

RESULTS AND DISCUSSION

Alkaloids

The crude alkaloid (0.3 % of bark) and (0.35 % of leaves) were subjected to chromatography over silice gel, followed by subsequent purification of the fractions collected.

Two alkaloids were isolated from the bark of *Goniothalamus tortilipetalus*; discretamine (**1**) liriodenine (**2**) while the leaves produced three alkaloids; liriodenine (**2**) asimilobine (**3**) and lanuginosine (**4**). The petroleum ether extract yielded two non-alkaloidal aromatics; 6-styryl-2-pyrone (**5**) and goniothalamin (**6**).

A tetrahydroprotoberberine alkaloid, discretamine (**1**) was isolated from the bark as brownish amorphus solid. The UV spectrum showed a maximum at 284, 262 and 208 nm. The IR spectrum showed absorption at 3543cm^{-1} typical of an intramolecular hydrogen bonded hydroxyl group. Another peak was also observed at 1332 cm^{-1} which is due to the in plane O-H bend.

The mass spectrum revealed a molecular ion peak at m/e 327 which corresponded to a molecular formula of $C_{19}H_{21}NO_4$.Two important peaks were also observed in the mass spectrum at m/e 149 (48%) and m/e 178 (97%). The former peak was indicative of a tetrahydroprotoberberine bearing a hydroxyl group in ring D, while the latter was formed by an expulsion of a proton which is typical for the 9-methoxy, 10-hydroxy, substitution. Other peaks observed were m/e 326, 312, 176, 162, 150 and 135.

Interestingly, the ^{1}H NMR revealed the downfield resonances in the region of 6.60 - 7.00 ppm corresponding to a tetrahydroprotoberberine skeleton with substitutions at C-2, C-3, C-9 and C-10. An AB doublet of doublet (dd) centred at 6.82 ppm with a coupling constant of 6 Hz was observed which is attributable to H-11 and H-12. Furthermore a singlet corresponding to two aromatic protons, which may be ascribed to H-1 and H-4 was observed at 6.68 ppm. A half quartet was observed at 4.18 ppm (J = 16 Hz) due to the

resonance of the C-8 equatorial proton. Moreover, ^{1}H NMR spectrum also showed two sharp singlets, belonging to two methoxyl groups appeared at 3.82 ppm and 3.92 ppm.

Liriodenine (**2**) was obtained as fine yellow needles from chloroform with m.p. 278 - 280°C. An oxoaporphines nature was deduced for the major alkaloid, based on its intense yellow colour, strongly fluorescent chloroform solution and the deep red colouration it produced in acid medium [5]. This was supported by data obtained from UV-Vis and IR spectroscopy. The former showed absorption bands at 262, 248, 310, and 415 nm. The latter showed a very significant peak at 1658 cm^{-1}. This peak was due to the stretching of a highly conjugated carbonyl group [6]. In addition an absorption characteristic of a methylenedioxy was also observed at 969 cm^{-1}. Finally a peak at 865 cm^{-1} was present as a result of the C-H out of plane deformation of a single isolated aromatic proton which is attached to C-3 [4]. All these data signified the presence of a highly conjugated chromophore with a ketone group enwrapped within the system. Its mass spectrum gave a very significant molecular ion peak at m/e 275, giving the possibility of the molecular formula to be $C_{17}H_9NO_3$.

Furthermore, the ^{1}H NMR spectrum revealed the characteristic AB quartet typical of H-4 and H-5 at 7.5 ppm and 8.87 ppm, respectively with a coupling constant of 5.3 Hz. A one proton singlet was observed which is attributable to H-3 at 7.17 ppm. In addition a two proton singlet at 6.36 ppm indicative of a methylenedioxy group was also present. More over two sets of multiplets at 7.56 - 7.80 ppm and 8.55 - 8.63 ppm corresponding to four protons suggested that ring D is not substituted.

Asimilobine (**3**), was isolated and crystallized from acetone to give a colourless prism, with m.p 177 – 179 °C. It was also characterized by the development, on exposure to iodine vapour, of an orange-coloured spot that gradually darkened to a green colour when the plate was allowed to stand exposed to the atmosphere [7].

The mass spectrum showed a molecular ion peak at m/e 267, which corresponded to a molecular formula of $C_{17}H_{17}O_2N$. In the UV region, it absorbed at 273 and 308 nm and in alkaline medium it experienced a bathochromic shift, hence suggested that hydroxyl substituent may be present. Moreover, the IR spectrum showed a strong absorption at 3255^{-1} and 3550 cm^{-1} due to the stretching of OH and N-H. An absorption by the methoxyl was observed at 1035 cm^{-1}. The mass spectrum revealed a peak at m/e 238 $[M-29]^+$ due to the loss of methylene imine and a base peak at m/e 266 $[M-1]^+$ was also present.

The ^{1}H NMR spectrum exhibited four aromatic proton signals as a series of multiplets. The multiplets centred at 8.30 ppm and those between 7.20 - 6.80 ppm, was integrated for one proton and for three protons, respectively. The former was attributed to H-11 since in aporphine this proton would always be found more down field than the other aromatic protons due to the deshielding effect caused by the facing ring A. One methoxyl singlet was observed at 3.59 ppm which is rather shielded compared to the normal aromatic methoxyls since the protons of the methoxyl were forced to place themselves on top of ring D where the electron density is high. A singlet at 6.70 ppm was also observed which is attributable to H-3.

The last alkaloid, lanuginosine (**4**) was isolated as an orange red needles with m.p 318 - 320°C. Rf: 0.67 [$CHCl_3$: MeOH, 9:1]. It formed strong red solution in mineral acids and showed a strong green fluorescence in $CHCl_3$ solution [8]. The UV spectrum showed maxima at 211 nm (4.21), 247 (4.30), 273 (4.20) and 432nm (3.68). Its IR spectrum revealed absorption bonds at 1655 cm^{-1} assignable to conjugated ketone.

Furthermore, the mass spectrum showed a base peak at m/e 305 corresponded to a molecular formula of $C_{18}H_{11}NO_4$. Other significant peaks were observed at m/e 290 and m/e 275 due to the $[M\text{-}CH_3]^+$ and $[M^+\text{-}CH_2O]^+$ fragment, respectively.

The ^{1}H NMR spectrum showed a distinct methoxyl peak at 4.00ppm and a strong methylenedioxy peak of oxoaporphine at 6.35 ppm. The chemical shifts of methoxyl and methylenedioxy protons vary with their location [8]. The spectrum showed a singlet at 7.15 ppm which can be ascribed to H-3. The characteristic A-B doublet of doublet of the ring B protons (H-4, H-5) centered at 7.78 ppm and 8.89 ppm with a coupling constant 5 Hz was also present Furthermore the spectrum revealed a doublet belonged to H-8 at 8.03 ppm ($J_{8,10}$ = 3 Hz) and a doublet of doublet at 7.33 ppm of H-10. The highly deshielded proton, H-11 resonated as a doublet doublet at 8.55 ppm ($J_{10,11}$ = 9 Hz, $J_{11,8}$ = 3

Hz), which proved that C-9 was substituted by methoxyl group.

Non Alkaloidal Aromatic Compounds

The aromatic derivatives of monocyclic 2-pyrones are phytochemically associated with *goniothalamus* species [9,10] and *aniba* species [11]. 6-Styryl-2-pyrones (**5**) have previously been reported as constituents of the Lauraceae [12] Piperanceae and Basidiomycetes [13]. In this study, the author isolated two major compounds from *Goniothalamus tortilipetalus* Hend labelled as compound (**5**) and (**6**).

6-Styryl-2-pyrones (**5**) was crystalized from chloroform to form yellow hexagonal crystal with m.p. 115 - 116°C. The UV spectrum showed maxima at 363 and 269 nm. Its IR spectrum showed strong absorption at 1726 cm^{-1} indicating a 2-pyrone functionality [14,15]. The peak at 965 cm^{-1} was reminiscent of a trans-disubstituted ethylene associated with a styrenoid residue [16].

The mass spectrum showed a strong peak at m/e 198 thus suggesting the molecular formula of $C_{13}H_{10}O_2$. It also showed strong ion peaks at m/e 170 $[M - 2CO]^+$, 141 $[M - CO - CHO]^+$, 131 $[C_8H_7CO]^+$, 103 $[C_8H_7]^+$, 95 $[M - C_8H_7]^+$, 77 $[C_6H_5]^+$, 39 $[C_3H_3]^+$ and 28[CO].

Such a structure was also evident from the ^1H NMR data. The ^1H NMR spectrum showed that the lactone ring had been dehydrogenated. No resonance was observed in the 0-6 ppm region of the spectrum. One of the styryl olefinic protons was observed at 6.58 ppm (1H, d, J = 16.2 Hz) while H-3 and H-5 were observed at 6.19 ppm (1H, dd, J = 9.6 and 0.9 Hz) and 6.12 (1H, dq, J = 6.7, 0.9 and 0.4 Hz), respectively.

The ^{13}C NMR data indicate that all the 13 carbons resonated in the aromatic region. The high field signal of C-2 was observed at 161.8 ppm and the low field signal of C-8 was observed at 104.9 ppm. A peak at 135.2 ppm was assigned to the quartenary carbons C-6 and C-9.

The last non-alkaloidal compound, goniothalamine (**6**) was isolated in white crystals from chloroform with mp 83°C. It showed strong bands in IR spectrum at 1722, 1249, 752 cm^{-1} corresponding to the resonance of the α, β-unsaturated γ-lactone moiety. Additional bands were detected at 1494 and 965 cm^{-1} which belong to the styryl group.

In addition its mass spectrum showed a molecular ion peak at m/e 200 giving possibility to a molecular formula of $C_{13}H_{12}O_2$. The base peak was observed at m/e 68 corresponding to the ionized furan [17].

The ^1H-NMR spectrum revealed aromatic protons at 7.3 ppm as multiplet, and olefinic proton peaks at 6.68 (dd, 1H, J = 15.7 and 1Hz) and 6.21 ppm (dd, 1H, J = 15.7 and 6Hz), respectively with a trans configuration. ^1H-NMR were also indicative of an α,β-unsaturated δ-lactone moiety. Two olefinic protons were observed at 6.05 (1H, dt, J = 9.6 and 1.7 Hz) and 6.85 ppm (1H, dt, J = 9.6 and 4.2 Hz) which were assigned to H-3 and H-4, respectively. An allylic methylene was observed as a multiplet at 2.45 ppm and a proton on a carbon bearing the oxygen of the lactone group appeared as a multiplet at 5.03 ppm.

Bioactivity

1. Vasorelaxant activity on rat aorta

In anaesthetized rats, intravenous administration of 6-styryl-2-pyrone (10mg/kg) caused a transient drop in systolic (-50mm of Hg) and diastolic (-30 mm of Hg) blood pressure. A slight drop in heart rate was also observed but with variable results. In the isolated rat thoracic aorta [18] 6-styryl-2-pyrone caused a dose-dependent inhibition of the contractile responses to the phenylephrine (0.1μM) and high K (80 μM). The vasorelaxant effect of 6-styryl-2-pyrone is probably responsible for the hypotensive effect observed in anaesthesized rats. The mechanism of the vasorelaxant effect of the 6-styryl-2-pyrone may be due to the inhibition of calcium influx through both receptor and voltage operated calcium channels which were activated by phenylephrine and high potassium concentration, respectively.

2. Cytotoxic activity on KB cells

Two of the compounds isolated from *Goniothalamus tortilipetalus* Hend., were tested for their activity on KB cells [19] and positive results were obtained. They are liriodenine and 6-styryl-2-pyrone. Figure 1 illustrates the mortality curve of liriodenine and 6-styryl-2-pyrone. The ED_{50} of liriodenine is below 13 μg/ml and the ED_{50} for 6-styryl-2-pyrone is 48.5 μg/ml.

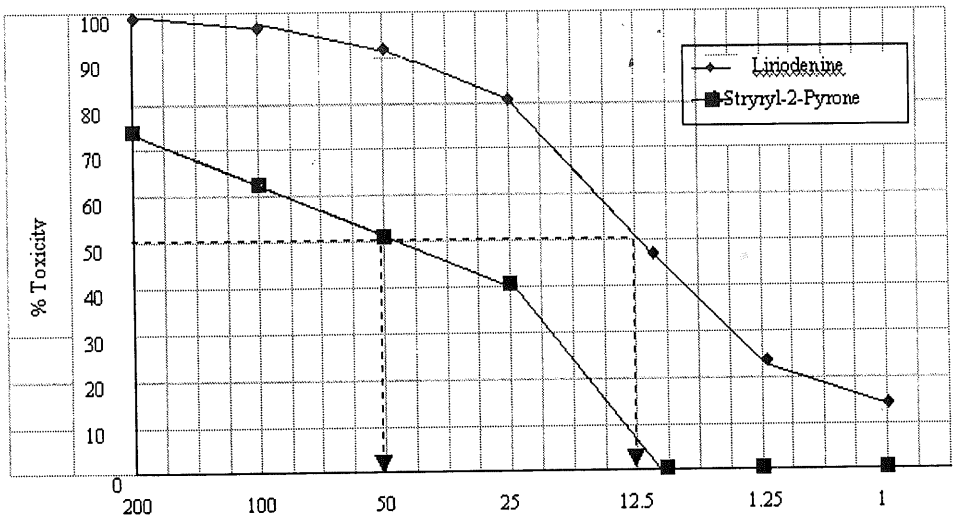
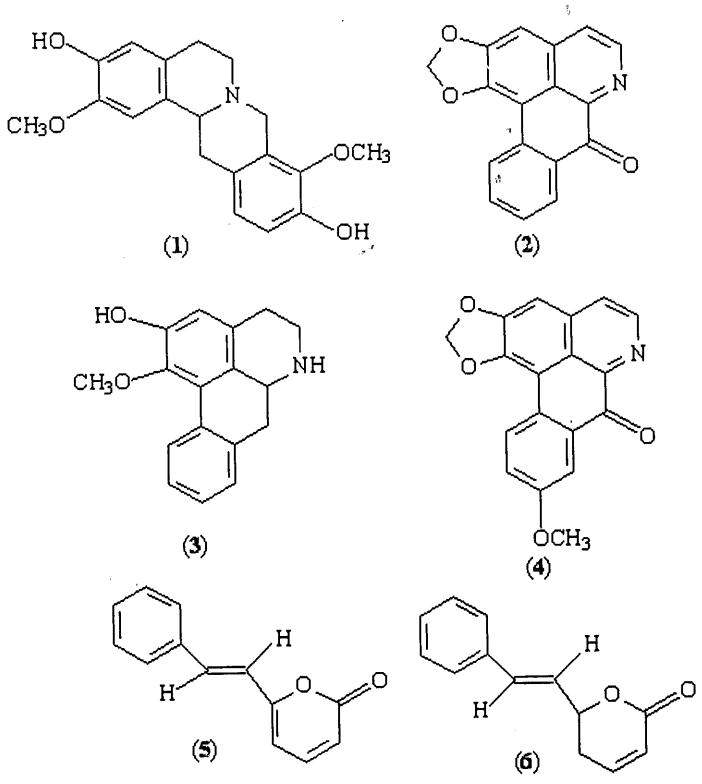


Figure 1: % Toxicity versus concentrations of liriodenine and 6-Styryl-2-pyrone



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