CHAPTER IV

RESULTS AND DISCUSSION

The dried stem bark of *Goniothalamus tenuifolius* (2 kg) was macerated in petroleum ether (8 L, 1 day) and then filtered. The marc was extracted with 95 % ethanol (8 L, 5 days, each). The ethanol extract was partitioned between chloroform and water. The dried chloroform extract was then separated by repetitive chromatography to afford six compounds. The structures of the isolated pure compounds were determined based on their UV, IR, NMR and MS data, and subsequently confirmed by comparison of these values with those reported in the literature. The antimalarial activity of each pure compound was evaluated by radioisotope microdilution technique.

1. Structure Determination of Isolated Compounds

1.1 Identification of Compound GT-A [I]

Compound GT-A was obtained as a pale brown solid from fractions G3 and G4 of the chloroform extract by repetitive chromatographic technique.

The EIMS of compound GT-A (Figure 2) revealed its molecular ion peak at *m/z* 196, suggesting the molecular formula $C_{10}H_{12}O_4$. The UV absorption spectrum (Figure 3) showed maximal absorptions at λ_{max} 213, 240, 262, 285 and 297 nm.

Compound GT-A could be assigned as the known compound 2,4-dihydroxy-6-methylbenzoic acid ethyl ester by analysis of its ^1H and ^{13}C NMR spectral properties.

From the ^1H NMR spectrum (Figures 4a-4b), the structure of compound GT-A contained two methyl groups at δ 1.41 (3H, s) and δ 2.51 (3H, s). One methylene group and two aromatic methine protons were found at δ 4.39 (2H, ddd, J = 7.02, 7.02, 7.02 Hz), 6.23 (1H, d, J = 2.14 Hz) and 6.28 (1H, d, 2.14 Hz),

respectively. The most downfield signal at δ 11.80 (1H, s) was assigned to 2-OH group because of its intramolecular hydrogen bond to the C-7 carbonyl oxygen.

The assignments of these aromatic protons were based on the NOE difference experiments (Figures 5a-5c). An NOE enhancement was observed for the 6-methyl protons at δ 2.51 when H-5 at δ 6.23 was irradiated, and vice versa (Figures 5a-5b). On the other hand, no NOE enhancement was detected when H-3 at δ 6.28 was irradiated (Figure 5c). According to the coupling constants of the two methine aromatic protons (J = 2.14 Hz), they could be classified as meta coupling.

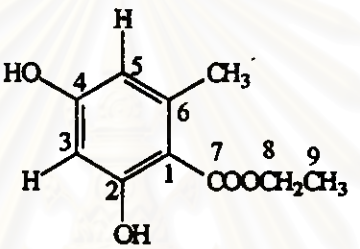
The ^{13}C NMR spectrum (Figure 6) showed ten carbons which could be classified as two methyl carbons, one methylene carbon, two methine carbons and four quaternary carbons. The most downfield carbon signal was assigned to a carbonyl group. Two of the quarternary carbons were downfield because of the deshielding effect from hydroxy group substitution.

The HMQC spectrum (Figure 7) revealed correlations between the directly coulpled ^{1}H and ^{13}C nuclei. According to the HMQC spectrum, all protonated carbons of compound GT-A could be assigned. The directly coupled ^{1}H and ^{13}C are summarized in Table 4.

Table 4 Carbon-proton correlations of compound GT-A observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-3	101.27	6.28
C-5	111.25	6.23
C-8	61.26	4.39
C-9	14.23	1.41
6-CH_3	24.36	2.51

The HMBC spectrum (Figures 8a-8b) showed correlations of the long-range coupling between ^{1}H and ^{13}C nuclei, providing information for assignment of the quaternary carbons. The 6-CH_3 protons showed correlations with C-5, C-6 and C-1, suggesting the position of methyl substitution at C-6. This was comfirmed by the correlation between H-5 and C-6. The 2-OH proton showed correlations with C-2, C-3 and C-1, suggesting the right position of hydroxy group at C-2. The H-3 proton, showed correlations with C-1, C-5, C-4 and C-2 as expected. Therefore, the complete proton and carbon assignments of compound GT-A (Table 5) were obtained through analysis of the HMQC and HMBC spectra.



[I]

Table 5 ^{1}H and ^{13}C NMR spectral data of compound GT-A (in chloroform-*d*)

Position	Compound GT-A	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
1	105.76	-
2	165.38	-
3	101.27	6.28 (d, *J* = 2.14)
4	160.14	-
5	111.25	6.23 (d, *J* = 2.14)
6	144.00	-
7	171.67	-
8	61.26	4.39 (ddd, *J* = 7.02, 7.02, 7.02)
9	14.23	1.41 (dd, *J* = 7.02, 7.02)
6-CH_3	24.36	2.51 (s)
2-OH	-	11.80 (s)

1.2 Identification of Compound GT-B [II]

Compound GT-B, recrystallized from methanol as yellow needles, was obtained from fractions G5 and G6 by repetitive chromatography and gel filtration chromatography.

The empirical formula of compound GT-B was determined by EIMS (Figure 9) to be $C_{17}H_{13}NO_3$. The mass spectrum showed a molecular ion peak at m/z 279 and other peaks at 264 $[M-Me]^+$ and 236 $[M-CO-Me]^+$. The UV spectrum in methanol showed characteristics of a phenanthrene chromophore (Figure 10) with maximal absorptions at λ_{max} (log ε) 231 (2.85), 262 (2.75), 275 (2.79), 286 (2.78), 315 (2.23) and 382 (2.15) nm. The IR spectrum (KBr) revealed the presence of NH (3316 cm^{-1}) and C=O (1715 cm^{-1}) functionalities in the structure (Figure 11). Compound GT-B was identified as aristolactam BII (cepharanone B) by analysis of its NMR spectral data and comparison of these data with those previously published.

According to the ^1H NMR spectrum (Figures 12a-12b), two singlet signals at δ 4.03 (3H, s) and 4.04 (3H, s) were attributed to two methoxy groups which were placed at C-3 and C-4, respectively. The most downfield signal at δ 10.84 (1H, s) was assigned to NH. Six aromatic protons appearing at δ 7.13 (1H, s), 7.55 (1H, dd, J = 7.81, 7.81 Hz), 7.58 (1H, dd, J = 7.81, 7.81 Hz), 7.85 (1H, s), 7.94 (1H, d, J = 7.81 Hz) and 9.11 (1H, d, J = 7.81 Hz) were assigned to H-9, H-6, H-7, H-2, H-8 and H-5, respectively. The equal coupling constant (J = 7.81 Hz) revealing that H-5, H-6, H-7 and H-8 protons are in the same ring (ring D). The assignment of all protons and the two methoxy groups were confirmed by a series of NOE difference experiments (Figures 13a-13e).

In an NOE difference experiment, the H-8 (δ 7.94) signal showed correlation with H-7 (δ 7.58) and H-9 (δ 7.13) signals (Figure 13a). When H-9 was irradiated, the signal of H-8 was enhanced (Figure 13b). When H-5 (δ 9.11) was irradiated, the signals of H-6 (δ 7.55) and 4-OCH_3 were enhanced (Figure 13c). The

H-2 (δ 7.85) proton also displayed an NOE with 3-OCH_3 (δ 4.04) (Figure 13d). When both 4-OCH_3 (δ 4.03) and 3-OCH_3 (δ 4.04) protons were irradiated, the signals of H-2 (δ 7.85) and H-5 (δ 9.11) were enhanced (Figure 13e).

The ^{13}C NMR spectrum (Figure 14) showed 17 carbons which could be classified by examination of the DEPT 135 spectrum (Figure 15). These spectral data suggested the presence of one carbonyl group, six methine carbons, two methoxy groups and eight quaternary carbons. According to the HMQC spectrum of GT-B (Figures 16a-16c), all protonated carbon could be assigned as shown in Table 6.

Table 6 Carbon-proton correlations of compound GT-B observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	109.91	7.85
C-5	126.84	9.11
C-6	125.47	7.55
C-7	127.46	7.58
C-8	129.03	7.94
C-9	104.60	7.13

The long-range C-H correlations of compound GT-B could be observed from the HMBC spectrum (Figures 17a-17d). The H-2 proton showed correlations with C-4, C-10a, C=O, C-3 and C-1, providing assignments for these quaternary carbons. The correlations between 3-OCH_3 protons and C-3, and between 4-OCH_3 protons and C-4 confirmed the assignments of C-3 and C-4. The assignments of C-4b and C-10 were made, based on the correlations between H-9 and C-4b, and between H-9 and C-10. The H-5 proton showed correlations with C-4a, C-8a and C-7. The results from the HMBC experiment confirmed the structure of compound GT-B and all of the quarternary carbon resonances were assigned completely. The proton and

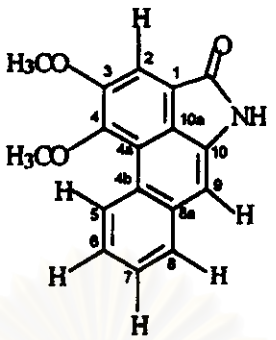
compared with those of aristolactam BII (Omar *et al.*, 1992), as summarized in Table 7.

Table 7 ^{1}H and ^{13}C NMR spectral data of compound GT-B (in DMSO-d_6) and aristolactam BII

Position	Compound GT-B		Aristolactam BII	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C* (ppm)	δ_H** (ppm) (multiplicity), J (Hz)
1	121.55	-	125.2	-
2	109.91	7.85 (s)	110.2	7.85
3	154.24	-	155.0	-
4	150.40	-	151.8	-
4a	119.91	-	122.8	-
4b	125.92	-	127.5	-
5	126.84	9.11 (d, J = 7.81)	127.9	9.12 (m)
6	125.47	7.55 (dd, J = 7.81, 7.81)	126.0	7.55 (m)
7	127.46	7.58 (dd, J = 7.81, 7.81)	128.1	7.55 (m)
8	129.03	7.94 (d, J =7.81)	129.4	7.93 (m)
8a	134.82	-	131.4	-
9	104.61	7.13 (s)	105.5	7.13 (m)
10	135.12	-	136.4	-
10a	123.33	-	121.5	-
C=O	168.39	-	170.3	-
3-OCH_3	56.93	4.04 (s)	57.3	4.06
4-OCH_3	59.91	4.03 (s)	60.6	4.06
NH	-	10.84 (s)	-	10.78

* From (Omar *et al.*, 1992) (in pyridine-d_5)

** From (Priestap, 1985) (in DMSO-d_6)



[II]

Aristolactam BII was first isolated from *Aristolochia argentina* (Aristolochiaceae) in 1974 (Crohare *et al.*, 1974). It also occurred in *Stephania cepharantha* (Menispermaceae) (Akasu, Itokawa and Fujita, 1974), *Schefferomitra subaequalis* (Annonaceae) (Dyke and Gellert, 1978), *Piper attenuatum, P. boehimerifolium* and *P. longum* (Piperaceae) (Desai *et al.*, 1989), *Saururus cernuus* (Saururaceae) (Rao and Reddy, 1990), *Houttuynia cordata* (Saururaceae) (Proble and Bauer, 1992) and *Goniothalamus velutinus* (Omar *et al.*, 1992). Therefore, this is the second report of aristolactam BII from the genus *Goniothalamus*.

1.3 Identification of Compound GT-C [III]

Compound GT-C was obtained as a yellowish-green solid from fractions G5 and G6 by repetitive chromatogragphic technique including gel filtration chromatography.

The EIMS of compound GT-C (Figure 18) exhibited a molecular ion peak at *m/z* 309, consistent with a molecular formula of $C_{18}H_{16}NO_4$, and also showed other major peaks at 294 $[M\text{-}Me]^+$ and 251 $[M\text{-}CO\text{-}2Me]^+$. The UV spectrum in methanol (Figure 19) demonstrated absorption maxima in methanol at λ_{max} (log ε) 210 (3.37), 242 (2.85), 254 (2.85), 247 (2.47), 400 (2.17)nm which were characteristics of a phenanthrene chromophore. The IR spectrum (Figure 20) showed absorption bands at

3700-3500 (NH stretching), 3400-3000 (CH streching of aromatic compound) and 1652 (C=O stretching) cm^{-1}, suggesting a lactam group.

Regarding the ^1H NMR spectrum (Figures 21a-21b), the presence of the following features was indicated: three methoxy groups at δ 3.98, δ 4.01 and δ 4.04; five aromatic protons at δ 7.21 (1H, br d, J = 7.81 Hz), 7.42 (1H, s), 7.51 (1 H, br dd, J = 7.81, 7.81 Hz), 7.87 (1H, s), 8.75 (H, br d, J = 7.81 Hz). The most downfield signal at δ 10.97 was assigned to NH. From the ^1H NMR spectrum, it could be inferred that GT-C bore a close resemblance to GT-B, but compound GT-C was substituted with three methoxy groups whereas compound GT-B was substituted with two methoxy groups .

The ^{13}C NMR spectrum (Figure 23) showed 18 carbons. These spectral data suggested the presence of one carbonyl group, three methoxy groups, five methine carbons and nine quaternary carbons. Among the nine quaternary carbons, three were deshieled by the inductive effect of the attached methoxy groups.

To determine the positions of the methoxy groups, a NOESY experiment was carried out (Figures 22a-22e). The H-7 (δ 7.21) and H-9 (δ 7.42) signals showed NOE interactions with the resonance of 8-OCH_3 at δ 3.98. The H-2 (δ 7.83) and H-5 (δ 8.75) protons showed NOE interaction with the resonances of 3-OCH_3 (δ 4.04) and 4-OCH_3 (δ 4.01), respectively. This fact confirmed the positions of the three methoxy groups at C-3, C-4 and C-8.

By comparing the above spectral information with previously reported data (Priestap, 1985), compound GT-C was identified as aristolactam BI or taliscanine. Taliscanine is also a component of *Aristolochia taliscana* (Aristolochiaceae) (Maldonado, Herran and Romo, 1966 cited in Priestap, 1985), *Goniothalamus sesquipedalis* (Annonaceae) (Talapatra *et al.*, 1988). Talapatra *et al.*, 1988 and

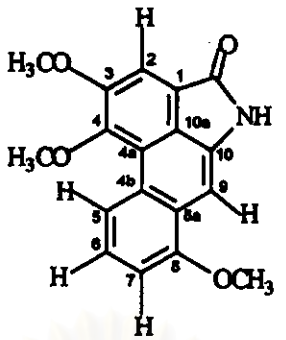
Priestap, 1985 have also described the proton assignments of taliscanine; however, there has been no report of the carbon assignments of taliscanine.

The HMQC spectrum (Figures 24a-24c) revealed correlations between the directly coupled ^{1}H and ^{13}C nuclei. According to the HMQC spectrum, all protonated carbons of compound GT-C could be assigned. The directly coupled ^{1}H and ^{13}C are summarized in Table 8.

Table 8 Carbon-proton correlations of compound GT-C observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	110.31	7.87
C-5	119.25	8.75
C-6	125.69	7.51
C-7	108.25	7.21
C-9	97.89	7.42

The HMBC spectrum (Figures 25a-25e) showed correlations of the long range coupled ^{1}H and ^{13}C nuclei. The correlations of the 3-OCH_3 protons with C-3, the 4-OCH_3 protons with C-4 and the 8-OCH_3 protons with C-8, confirming the three positions of methoxy group. The correlation of H-2 proton with C-1, C-10a and C=O, provided assignments for these quaternary carbons. After all correlations were examined by the same procedure as described in the compound GT-B, the assignments of all quaternary carbons could be assigned. In this work, all carbons and protons were assigned completely through analysis of the HMQC and HMBC spectra, as shown in Table 9.



[III]

Table 9 ^{1}H and ^{13}C NMR spectral data of compound GT-C (in DMSO-d_6) and taliscanine (in DMSO-d_6) (Priestap, 1985)

Position	Compound GT-C		Taliscanine	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	121.58	-	-	-
2	110.31	7.87 (s)	-	7.85
3	154.28	-	-	-
4	150.60	-	-	-
4a	119.92	-	-	-
4b	126.70	-	-	-
5	119.25	8.75 (br d, J = 7.81)	-	8.72 (dd)
6	125.69	7.51 (br dd, J = 7.81, 7.81)	-	7.50 (t)
7	108.25	7.21 (br d, J = 7.81)	-	7.18 (dd)
8	155.29	-	-	-
8a	124.80	-	-	-
9	97.89	7.42 (s)	-	7.43 (s)
10	134.60	-	-	-
10a	123.07	-	-	-
C=O	168.29	-	-	-
3-OCH_3	56.98	4.04 (s)	-	4.04 (s)

Table 9 (Continued)

Position	Compound GT-C		Taliscanine	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
4-OCH_3	59.94	4.01 (s)	-	4.04 (s)
8-OCH_3	55.89	3.98 (s)	-	4.04 (s)
NH	-	10.79 (s)	-	10.73 (s)

1.4 Identification of Compound GT-D [IV]

Compound GT-D was obtained as a yellow solid from fraction G10 by repetitive chromatographic technique.

The EIMS of GT-D (Figure 26) revealed a molecular ion at *m/z* 295, suggesting a molecular formula of $C_{17}H_{13}NO_4$. Important peaks at *m/z* 280 $[M\text{-}Me]^+$, 252 $[M\text{-}CO\text{-}Me]^+$ were observed. The UV spectrum (Figure 27) showed charactertics of a phenanthrene chromophore at λ_{max} (log ε) 245 (2.48), 293 (2.03), 406 (1.82) nm. The IR spectrum (Figure 28) revealed maximal absorptions at 3531-3000 (OH and NH strecthing), 1672 (C=O streching), 1283 (C-O stretching) cm^{-1}. These features suggested the presence of lactam and hydroxyl functionalities.

The ^1H NMR spectrum (Figures 29a-29b) indicated the presence of a hydroxy group at δ 10.11 and two methoxy groups at δ 3.99 and δ 4.03. The other proton NMR signals were quite similar to those of GT-C, suggesting that the structure of GT-D was close to that of GT-C except for the replacement of one of the methoxy groups in GT-C with a hydroxyl group in GT-D.

Several NOE difference experiments were performed to determine the substituted positions (Figures 30a-30f). The H-9 (δ 7.40) signal showed NOE

interaction with NH (δ 10.78) and a hydroxy group at δ 10.11 (Figure 30f) whereas H-7 (δ 7.06) showed NOE interaction with H-6 (δ 7.36) and the hydroxyl group at δ 10.11 (Figure 30a), suggesting the placement of the hydroxy group at C-8. When the methoxy groups at δ 3.99 and δ 4.03 were irradiated, the resonances of H-5 (δ 8.61) and H-2 (δ 7.84) were enhanced respectively (Figures 30b-30c), confirming the positions of the two methoxy groups at C-3 and C-4. Similarly, when H-2 (δ 7.84) was irradiated, the signal of 3-OCH_3 was enhanced (Figure 30d).

The ^{13}C NMR spectum (Figure 31) and DEPT 135 spectrum (Figure 32) provided signals for one carbonyl, two methoxy groups, five methine carbons and nine quaternary carbons.

Based on the information obtained from the HMQC spectrum (Figures 33a-33c), all protonated carbons of compound GT-C were assigned, as shown in Table 10.

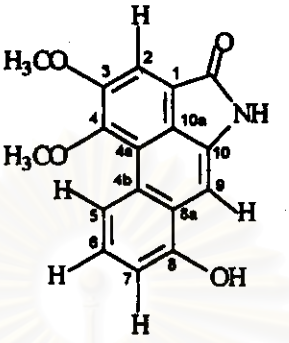
Table 10 Carbon-proton correlations of compound GT-C observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	109.92	7.84
C-5	117.97	8.61
C-6	125.78	7.36
C-7	112.13	7.06
C-9	98.71	7.40

The assignments of quaternary carbons of compound GT-D and the positions of the methoxy groups were confirmed by examination of the HMBC spectrum (Figures 34a-34c).

Comparison of its ^1H and ^{13}C NMR spectra with reported data (Omar *et al.*, 1992), suggested that compound GT-D was identical with velutinam, an aristolactam

previously obtained from the stem bark of *Goniothalamus velutinus*. The complete proton and carbon assignments of compound GT-D and those of velutinam are shown in Table 11.



[IV]

Table 11 ^{1}H and ^{13}C NMR spectral data of compound GT-D (in DMSO-d_6) and velutinam (in pyridine-d_5) (Omar *et al.*, 1985)

Position	Compound GT-D		Velutinam	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	121.61	-	125.2	-
2	109.92	7.84 (s)	110.5	8.06 (s)
3	154.21	-	154.9	-
4	150.54	-	151.9	-
4a	120.19	-	123.1	-
4b	127.08	-	128.7	-
5	117.97	8.61 (d, J = 8.30)	119.3	9.14 (dd, J = 7.8, 1.1)
6	125.78	7.36 (dd, J = 8.30, 8.30)	126.2	7.55 (dd, J = 7.8, 7.8)
7	112.13	7.06 (d, J = 8.30)	112.9	7.42 (dd, J = 7.8, 1.1)
8	153.74	10.11 (s, 8-OH)	155.4	-
8a	123.96	-	125.9	-
9	98.71	7.40 (s)	99.9	8.23
10	133.89	-	135.2	-
10a	123.31	-	121.7	-

Table 11 (Continued)

Position	Compound GT-D		Velutinam	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
C=O	168.35	-	170.0	-
3-OCH_3	56.94	4.03	57.0	3.91
4-OCH_3	59.87	3.99	60.2	4.13
8-OH	-	10.11		
NH	-	10.78	-	-

1.5 Identification of Compound GT-E [V]

Compound GT-E, an orange solid, was obtained from fraction G10 by repetitive gel filtration chromatography.

The EIMS of compound GT-E (Figure 35) displayed a molecular ion peak at *m/z* 307, corresponding to $C_{18}H_{13}NO_4$. Its mass spectrum showed a [M-28] peak at m/z 279 indicated a facile loss of carbonyl group. Other fragment ions at *m/z* 264 [M-CO-Me]$^+$ and 236 [M-CO-Me-CO]$^+$ were also observed. This particular fragmentation pattern is characteristic of 4,5-dioxoaporphine alkaloids (Desai *et al.*, 1988). This compound showed typical phenanthrene UV absorptions (Figure 36) at λ_{max} (log ε) 209 (3.61), 235 (3.54), 301 (3.16), 315 (3.19), 440 (3.06) nm. The IR spectrum exhibited NH and C=O groups at 3611-3300 and 1700 cm^{-1}, respectively.

The ^1H NMR spectrum of compound GT-E (Figures 38a-38b) disclosed the presence of two methoxy groups, six aromatic protons and an NH group. This spectrum is quite similar to that of compound GT-B . The substitution pattern was also similar to that of GT-B, as determined by a series of NOE experiments (Figures 39a-39d). Comparison of the mass spectrum of compound GT-B with that of compound GT-E suggested that the latter contained an additional carbonyl group.

This was further evidenced from its ^{13}C NMR spectrum (Figure 40) which displayed two carbonyl groups at δ 170.86 and 155.53. In addition, two methoxy groups, six methine carbons and eight quaternary carbons were also observed.

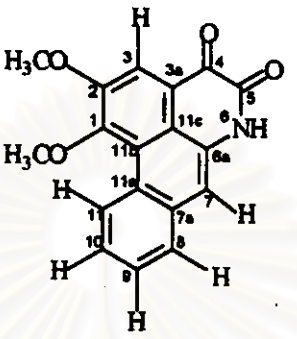
Compound GT-E was identified as norcepharadione B by analysis of NMR spectrum, including the HSQC and HMBC spectra. Its NMR properties are in good agreement with the literature values (Achenbach, Frey and Waibel, 1991 and Desai *et al.*, 1988). Norcepharadione B was isolated from the callus tissue of *Stephania cepharantha* (Menispermaceae) (Akasu, Itokawa and Fujita, 1975). This compound was also found in *Guatteris ouregou* (Annonaceae) (Cortes *et al.*, 1986), *Piper attenuatum, P. boehimerifolium, P. hamiltonii* and *P. longum* (Piperaceae) (Desai *et al.*, 1988 and Desai *et al.*, 1989), *Monoclanthus vignei* (Annonaceae) (Achenbach, Fray and Waibel, 1991) and *Houttuynia cordata* (Saururaceae) (Probstel and Bauer, 1992). However, this is the first time that 4,5-dioxoaporphine was found in the genus *Goniothalamus.*

From the HSQC experiment (Figures 41a-41c), six protonated carbons could be assigned, as summarized in Table 12.

Table 12 Carbon-proton correlations of compound GT-E observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-3	112.62	8.16
C-7	112.79	7.52
C-8	128.50	7.93
C-9	126.02	7.60-7.68
C-10	128.02	7.60-7.68
C-11	127.23	9.43

The assignments of quaternary carbons were obtained from the HMBC correlations (Figures 42a-42e). The proton and carbon assignments of compound GT-E and norcepharadione B are summarized in Table 13.



[V]

Table 13 ^{1}H and ^{13}C NMR spectral data of compound GT-E (in DMSO-d_6) and norcephadione B.

Position	Compound GT-E		Norcepharadione B	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C* (ppm)	δ_H** (ppm) (multiplicity), J (Hz)
1	153.95	-	154.9	-
2	152.33	-	153.4	-
3	112.62	8.16 (s)	113.2	8.22
3a	124.66	-	124.8	-
4	170.86	-	178.0	-
5	155.53	-	156.9	-
6a	130.18	-	131.5	-
7	112.79	7.52 (s)	113.2	7.58
7a	132.41	-	133.5	-
8	128.50	7.93 (d, J = 7.57)	129.0	7.96 (m)
9	126.02	7.60-7.68 (m)	128.3	7.69 (m)
10	128.02	7.60-7.68 (m)	127.2	7.69 (m)
11	127.23	9.42	128.3	9.46 (m)

Table 13 (Continued)

Position	Compound GT-E		Norcepharadione B	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C* (ppm)	δ_H** (ppm) (multiplicity), *J* (Hz)
11a	125.98	-	127.2	-
11b	123.61	-	125.8	-
11c	118.23	-	119.5	-
1-OCH_3	60.14	4.05 (s)	60.3	4.10
2-OCH_3	56.49	4.09 (s)	56.3	4.14
6-NH	-	12.05 (s)	-	12.1

* From (Achenbach, Frey and Waibel, 1991) (in Pyridine-d_5)

** From (Desai *et al.*, 1988) (in DMSO-d_6)

1.6 Identification of Compound GT-F [VI]

Compound GT-F was obtained as a pale brown solid from fractions G7, G8 and G9 by a combination of several techniques, including gel filtration chromatography.

Compound GT-F gave a molecular ion $[M]^+$ at *m/z* 265 in the EIMS (Figure 43), suggesting a tentative molecular formula of $C_{16}H_{11}NO_3$. The IR spectrum (Figure 45) indicated the presence of OH and NH (3600-3048 cm^{-1}) and conjugates C=O (1706 cm^{-1}) functional groups. The UV spectrum displayed UV absorptions at λ_{max} (log ε) 233 (2.90), 275 (3.86), 285 (3.86), 389 (2.26) nm (Figure 44), characteristic of a phenanthrene chromophore.

The ^1H NMR spectrum of compound GT-F (Figures 46a-46b) which displayed signals at δ 4.01 (3H, s), 7.08 (1H, s), 7.54 (1H, ddd, *J* = 7.81, 7.81, 1.47 Hz), 7.56 (1H, ddd, *J* = 7.81, 7.81, 1.47 Hz), 7.61 (1H, s), 7.93 (1H, dd, *J* = 7.81, 1.47 Hz), 9.09 (1H, dd, *J* = 7.81, 1.47 Hz,), 10.78 (1H, s) was strikingly similar to that of compound GT-B. The only exceptions were that GT-F had one less methoxy

group and that the H-2 proton of compound GT-F appeared rather upfield at δ 7.61 in comparison to a similar proton in compound GT-B at δ 7.85. This could be due to the shielding effect of a hydroxy group at C-3. This was confirmed by NOE difference experiments (Figures 47a-47e). Only the H-5 proton showed NOE with the signal of the methoxyl group. This proved that the methoxy group was attached to C-4. As expected, no effect could be observed between H-2 and any other protons. The structure of compound GT-F was determined to be aristolactam AII by comparing these spectral data with the literature values (Priestap, 1985)

Examination of the ^{13}C NMR spectrum (Figure 48) revealed the presence of one methoxy group, six methine carbons, eight quarternary carbons and one carbonyl carbon.

Based on the information obtained from the HMQC spectrum (Figures 49a-49b), all protonated carbons of compound GT-F were assigned, as shown in Table 14.

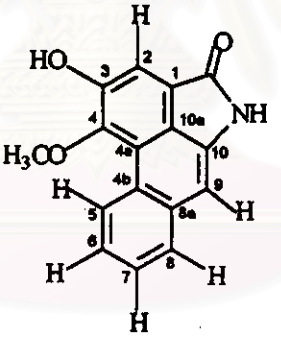
Table 14 Carbon-proton correlations of compound GT-F observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	113.40	7.61
C-5	126.78	9.10
C-6	125.28	7.36
C-7	127.28	7.56
C-8	128.97	7.93
C-9	103.88	7.08

From the HMBC spectrum (Figures 50a-50c), the signals at δ 121.55, 154.24, 150.40, 119.99, 125.92, 134.82, 135.12 and 123.33 ppm were assigned to C-1, C-3, C-4, C-4a, C-4b, C-8a, C-10 and C-10a, respectively

Regarding the assignments of ^{1}H and ^{13}C NMR resonances of Aristolactam AII, only aromatic oxygenated carbons and methoxyl carbons have been reported. All of the protons and carbons of this compound were completely assigned in this study, as shown in Table 15.

Aristolactam AII was first isolated from the root extract of *Aristolochia argentina* (Aristolochiaceae) in 1974 (Crohare *et al.*, 1974). Aristolactam AII could also be found in *Aristolochia indica* (Achari *et al.*, 1982), *Pararistolochia flos-avis* (Aristolochiaceae) (Sun *et al.*, 1987), *Goniothalamus sesquipedalis* (Annonaceae) (Talapatra *et al.*, 1988), *Piper attenuatum, P. boehimerifolium, P. hamiltonii* and *P. longum.*(Piperaceae) (Priestap, 1985 and Desai *et al.*, 1989), *Monocyclanthus vignei* (Annonaceae) (Achenbach, Frey and Waibel, 1991) and *Houttuynia cordata* (Saururaceae) (Probstel and Bauer, 1992).



[VI]

Table 15 ^{1}H and ^{13}C NMR spectral data of compound GT-F (in DMSO-d_6) and aristolactam AII (in DMSO-d_6)

Position	Compound GT-F		Aristolactam AII*	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
1	121.81	-	-	-
2	113.40	7.61 (s)	-	7.62
3	152.27	-	152.2	-

Table 15 (Continued)

Position	Compound GT-F		Aristolactam AII*	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
4	149.83	-	148.9	-
4a	120.35	-	-	-
4b	125.35	-	-	-
5	126.78	9.09(dd, *J* = 7.81, 1.47)	-	9.13 (m)
6	125.28	7.54 (ddd, *J* = 7.81, 7.81, 1.47)	-	7.54 (m)
7	127.28	7.56 (ddd, *J* = 7.81, 7.81, 1.47)	-	7.54 (m)
8	128.97	7.93 (dd, *J* = 7.81, 1.47)	-	7.93 (m)
8a	134.85	-	-	-
9	103.88	7.08 (7.08 (s)	-	7.08
10	135.32	-	-	-
10a	122.28	-	-	-
3-OH	-	-	-	-
4-OCH_3	59.45	4.01 (s)	59.5	4.03
C=O	168.49	-	-	-
NH	-	10.78 (s)	-	10.77

* From (Priestap, 1985)

The co-occurrence of aristolactam and 4,5-dioxoaporphine in a previous report (Achari *et al.*, 1982) and in this study is of considerable biogenetic significance. The isolation of 4-hydroxy, 5-oxo and 4,5-dioxoaporphines from *Stephania cepharantha* (Menispermaceae), *Piper sanctum* (Piperaceae), *Fusea longifolia* (Papaveraceae) and *Glaucium flavum* (Papaveraceae) has led to the idea that 4,5-dioxoaporphines might arise by oxidation of aporphines and also function as intermediates in the biosynthesis of aristolactams (Priestap, 1985). This is consistent with the biogenetic pathway of aristolactams proposed earlier in 1976. (Castedo, Suau and Mouriño, 1976).

2. Antimalarial Activity of Pure Compounds

Evaluation of the antimalarial activity of the isolated pure compounds was carried out using a radioisotope microdilution technique (Desjardins *et al.*, 1979). The results were compared with two currently used drugs chloroquine and pyrimethamine, as shown in Table 16.

Table 16 Antimalarial activity of isolated pure compounds from *Goniothalamus tenuifolius* and current drugs

Compound	EC_{50} (μg/ml)
GT-A	33
GT-B	11
GT-C	10.5
GT-D	7.5
GT-E	7.5
GT-F	9.5
Pyrimethamine	2.8
Chloroquine	0.03

Compound GT-A, a benzoic acid derivative, appeared to have no activity against the parasites. The alkaloids GT-B, GT-C, GT-D, GT-E and GT-F exhibited inhibitory effects on the growth of *Plasmodium falciparum*. Although the intensity of the response was not equivalent to the known antimalarial agents investigated, appreciable activity was demonstrated with these compounds. Several biological activities such as cytotoxicity (Sun *et al.*, 1987) and antiinflammatory activity (Probstel and Bauer, 1992), have been reported for phenanthrene lactams and 4,5-dioxoaporphine alkaloids, but their antimalarial activity has never been described. This investigation is the first report of the antimalarial activity of these groups of natural products.