Alkaloids from the Roots of *Goniothalamus griffithii*

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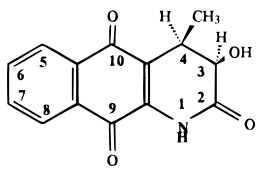
Three new alkaloids—griffithazanone A (**1**), griffithdione (**2**), and griffithinam (**3**)—were isolated from the roots of *Goniothalamus griffithii*, along with six known compounds, 4-methyl-2,9,10-(2H)-1-azaanthracenetrione (**4**), velutinam, aristololactam BI, aristololactam BII, aristololactam AII, and norcepharanone B. Their structures were elucidated on the basis of spectral and chemical methods. The absolute configuration of griffithazanone A (**1**) was determined by the preparation of Mosher's esters.

Alkaloids, including phenanthrene lactams, occur in several genera of the family Annonaceae.[1−4] Lactams with conjugated carbonyl groups are assumed to be biogenetic intermediates of phenanthrene lactam.[5] Azaanthraquinone-type alkaloids are also found in a few members of the Annonaceae.[6−7] *Goniothalamus griffithii* Hook. f. et Thoms (Annonaceae) is a tropical plant distributed in southern mainland China, India, and Thailand.[8] In a screen for antitumor agents from Annonaceous plants, an ethanolic extract of the roots of *G. griffithii* was found to be significantly cytotoxic against a number of human cancer cell lines. Purification of this extract yielded three new alkaloids, griffithazanone A (**1**), griffithdione (**2**), and griffithinam (**3**), along with six known alkaloids, 4-methyl-2,9,10-(2H)-1-azaanthracenetrione (**4**),[9] velutinam,[4] aristololactam BI,[12] aristololactam BII,[11] aristololactam AII,[11] and norcepharanone B.[10,15]



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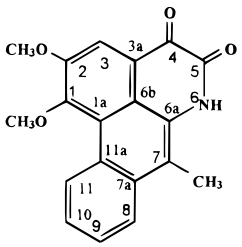

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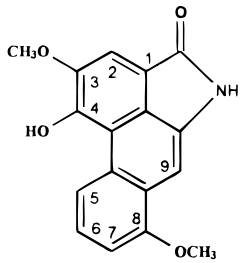

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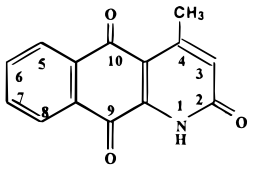

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Griffithazanone A (**1**) was obtained as yellow needles with mp 208−210 °C. Its HREIMS gave a molecular ion at *m*/*z* 257.0692 (calcd 257.0688), compatible with a molecular formula of $C_{14}H_{11}NO_4$. The ^{1}H NMR spectrum showed signals of four coupled aromatic protons, indicating an *ortho*-disubstituted benzene moiety. The two aromatic signals at low field (*δ* 8.10 and 8.15) indicated that the respective protons were affected by a *peri*-carbonyl as shown in structure **1**. Four methine carbon signals at *δ* 126.8, 126.5, 133.5, and 135.0 in the ^{13}C NMR spectrum supported this partial structure. Three other coupled signals in the ^{1}H NMR spectrum of **1** (*δ* 1.13, 3H, d, *J* = 7.0 Hz; *δ* 3.71, 1H, dq, *J* = 7.0 Hz; *δ* 4.48, 1H, d, *J* = 7.0 Hz) suggested the presence of a −CH(CH$_3$)−CH(OH)− moiety. The NMR spectral data of **1** were similar to those of cleistopholine[6] and scorazanone.[7] Based on a HMBC correlation, the methyl group was allocated to C-4. The methine proton at *δ* 3.71 correlated with the carbonyls at *δ* 181.8 and 171.2, the oxymethine carbon at *δ* 69.2, the methyl group at *δ* 11.2, and two quaternary carbons at *δ* 125.5 and 137.0. Other HMBC correlations provided the assignments of all carbon and proton signals in **1** (Table 1). In an NOE difference experiment, irradiation of the methyl signal at *δ* 1.13 only caused enhancement of the methine signal at *δ* 3.71 (H-4); irradiation of the methine signal at *δ* 3.71 (H-3) caused enhancements of the methine signal at *δ* 4.48 and the methyl signal at *δ* 1.13, while irradiation of the methine signal at *δ* 4.48 only caused enhancement of the methine signal at *δ* 3.71. This suggested a *cis*-configuration for H-3 and H-4 (3*R*/4*R* or 3*S*/4*S*). In addition, **1** was converted to **4** when treated with $SOCl_2$ in pyridine at room temperature overnight, confirming the elucidation. The absolute configuration of **1** was established using Mosher ester methodology based on the differences between the ^{1}H NMR chemical shifts of its (*R*)- and (*S*)-methoxytrifluoromethylphenylacetic acid ester (MTPA) (Mosher ester). The Δ*δ*s−*δ*r of NH, H-4, and CH$_3$-4 of the Mosher ester of **1** were −0.02, +0.13, and +0.15, respectively (Table 2). According to Mosher's assumption,[14] only the *R* configuration of C-3 could have greater shielding of NH and less shielding of both H-4 and CH$_3$-4 in the (*S*)-MTPA derivatives of **1**. Thus, the structure of **1** has an absolute configuration of 3*R* and 4*R*. The structure of **1** was assigned as (3*R*)-hydroxyl-(4*R*)-methyl-3,4-dihydro-2,9,10-(2H)-1-azaanthracenetrione.

Griffithdione (**2**) was obtained as orange needles with mp 216−218 °C. Its HREIMS indicated a molecular ion at *m*/*z* 321.0990 (calcd 321.1001), corresponding to a molecular formula of $C_{19}H_{15}NO_4$. The UV spectrum, when run in methanol, showed a characteristic phenanthrene chromophore[11−13] with absorption maxima at 244 and 295 nm. The ^{1}H NMR spectrum of **2** revealed the presence of one methyl group (*δ* 2.73), two methoxyl groups (*δ* 4.09 and 4.13), an aromatic proton (*δ* 8.29, s), four coupled aromatic protons (*δ* 7.68, t; 7.73, t; 8.12, d; 9.64, d; each *J* = 7.0 Hz), and an imine proton (*δ* 11.22 in DMSO-d_6 and 9.08 in $CDCl_3$).

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Table 1. NMR Spectral Data of Compounds **1** and **4** ($CDCl_3$, δ)

carbon no.	**1**		**4**	
	δ_H	δ_C	δ_H	δ_C
2		171.2		160.4
3	4.48 (d, J = 7.0 Hz)	69.2	6.69 (d, J = 1.0 Hz)	127.7
4	3.71 (dq, J = 7.0 Hz)	30.6		152.3
CH_3-4	1.13 (d, J = 7.0 Hz)	11.2	2.71 (d, J = 1.0 Hz)	22.8
4a		125.5		116.1
5	8.15 (dd, J = 7.6 1.2 Hz)	126.8	8.23 (dd, J = 7.7, 1.2 Hz)	127.5
6	7.74 (dt, J = 7.6 1.2 Hz)	133.5	7.78 (dt, J = 7.7, 1.2 Hz)	133.7
7	7.80 (dt, J = 7.6 1.2 Hz)	135.0	7.87 (dt, J = 7.7, 1.2 Hz)	135.6
8	8.10 (dd, J = 7.6 1.2 Hz)	126.5	8.19 (dd, J = 7.7, 1.2 Hz)	126.7
8a		132.2		133.2
9		178.7		177.9
9a		137.0		139.8
10		181.8		181.4
10a		130.2		129.9
NH	8.07 (br s)		9.78 (br s)	

Table 2. $\Delta\delta_H$ ($\delta_s-\delta_r$) Data of (*S*)- and (*R*)-MTPA Derivatives of **1** ($CDCl_3$, δ)

proton	**1*S***	**1*R***	$\Delta\delta_H$ (δs−δr)
H-3	5.83 (d, J = 7.0 Hz)	5.84 (d, J = 7.0 Hz)	
H-4	3.76 (dq, J = 7.0 Hz)	3.63 (dq, J = 7.0 Hz)	+0.13
CH_3-4	1.23 (d, J = 7.0 Hz)	1.08 (d, J = 7.0 Hz)	+0.15
NH	8.06 (s)	8.08 (s)	−0.02
H-5	8.16 (d, J = 7.6 Hz)	8.15 (d, J = 7.6 Hz)	
H-6	7.75 (t, J = 7.6 Hz)	7.75 (t, J = 7.6 Hz)	
H-7	7.81 (t, J = 7.6 Hz)	7.81 (t, J = 7.6 Hz)	
H-8	8.12 (d, J = 7.6 Hz)	8.12 (d, J = 7.6 Hz)	

The IR spectrum of **2** showed that there were two carbonyl groups in the molecule (1681 and 1658 cm^{-1}), and the signals at δ_c 197.3 and 176.6 in ^{13}C NMR spectrum supported this suggestion. The M^+ at *m*/*z* 321 was the base peak, and *m*/*z* 293 was obtained by the loss of CO from *m*/*z* 321. It was apparent that **2** had the same structural skeleton as cepharadione.[5] However, only one aromatic proton singlet was observed in the ^1H NMR spectrum of **2**. The locations of the methyl and two methoxyl groups were determined by NOE difference experiments. Irradiation of the methoxyl signals at δ 4.13 and 4.09 and the methyl singlet at δ 2.73 caused enhancements of the singlet at δ 8.29 and of two doublets at δ 9.64 and 8.12, respectively. These suggested that the two methoxyls and the methyl group should be located at C-3, C-4, and C-9, respectively, while the aromatic proton singlet at δ 8.29 should be assigned to H-2. Thus, the structure of griffithdione (**2**) was assigned as 1,2-dimethoxyl-4,5-dioxo-7-methyl-6a,7-dehydroaporphine.

Griffithinam (**3**) was obtained as pale yellow needle crystals, mp 262−264 °C. Its molecular formula, $C_{17}H_{13}NO_4$, was deduced from the molecular ion at *m*/*z* 295 in the EIMS and by elemental analysis. The UV spectrum showed a characteristic phenanthrene chromophore,[11−13] and the bathochromic shift of the maxima produced by the addition of alkali suggested the presence of a phenolic hydroxyl group in the molecule. The appearance of bands at 3406, 3174, 1705, 1660, and 1654 cm^{-1} in the IR spectrum revealed the presence of hydroxyl, imine, and lactam carbonyl groups, respectively. The ^1H NMR spectrum (DMSO-d_6) of **3** confirmed the presence of an imine, a hydroxyl (δ 10.60, δ 10.61, each 1H, s), and two methoxyl groups (δ 3.98 and 4.03, 3H each). The aromatic proton region of the ^1H NMR spectrum closely resembled that of the C-2, C-5, C-6, C-7, C-9 unsubstituted aristololactams.[12] Two aromatic singlets at δ 7.78 (1H) and 7.42 (1H) could be ascribed to H-2 and H-9, respectively. The positions of both methoxyl groups in **3** were determined by an NOE difference experiment. Thus, on irradiation of the methoxyl at δ 3.98, the signals at δ 7.16 and 7.42 had a 7−8% intensity enhancement, indicating the methoxyl group was at C-8, and the signals at δ 7.16 and 7.42 were assigned to H-7 and H-9, respectively. On the other hand, irradiation of the methoxyl at δ 4.03 resulted in a 7−8% intensity enhancement of the signal at δ 7.78, suggesting this methoxyl should be allocated to C-3. Therefore, the hydroxyl group was located at C-4. The ^{13}C NMR spectrum in DMSO-d_6 revealed two methoxyl peaks at δ 57.2 and 55.8, a carbonyl signal at δ 168.7, and 14 aromatic carbon signals, including three oxygenated aromatic carbon signals at δ 155.1, 149.5 and 148.2. Thus, griffithinam was deduced to be 10-amino-4-hydroxy-3,8-dimethoxyphenanthene-1-carboxylic acid lactam (**3**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert Nr 229 micromelting point apparatus and were uncorrected. The optical rotation was obtained on a Perkin−Elmer 241 polarimeter. UV spectra were run on a Shimazu UV-240 spectrometer. IR spectra (KBr) were measured on a Perkin−Elmer 683 infrared spectrometer. ^1H and ^{13}C NMR spectra, along with NOE and HMBC experiments, were obtained on a Bruker AM 500 spectrometer in $CDCl_3$ or DMSO-d_6 with TMSi as internal standard. EIMS and HREIMS data were recorded on a ZAB-2F mass spectrometer. Elemental analyses were determined on a MOD 1106 elemental analyzer.

Plant Material. The plant material (roots) was collected from Jinghong County, Yunnan Province, People's Republic of China, in July 1996, and identified as *Goniothalamus griffithii* Hook. f. et. Thoms by Professor Shaorong Guo, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, where a voucher specimen (96021) is deposited.

Extraction and Isolation. The dried roots (9.1 kg) of *G. griffithii* were extracted exhaustively with 95% EtOH and evaporated under a vacuum to yield extract F_1 (1 kg). This was partitioned between H_2O and $CHCl_3$ (1:1), giving a H_2O-soluble fraction F_2 (300 g) and a $CHCl_3$-soluble fraction F_3 (373 g), as well as an insoluble fraction F_6 (320 g). F_3 was first dissolved in 90% MeOH and defatted with petroleum ether to give the 90% MeOH-soluble fraction F_4 (268 g), which was subjected to Si gel (160−200 mesh) column chromatography and eluted with gradient mixture of petroleum ether and Me_2O. Fractions of similar composition (as indicated by TLC) were combined. The residue (19.2 g) obtained by elution with petroleum ether−Me_2CO (8:2) was subjected to Si gel chromatography and eluted with petroleum ether−EtOAc (7:3). Fifty fractions of 100 mL each were collected. From fractions 21−35 and fractions 39−42, griffithdione (**2**, 16 mg) and 4-methyl-2,9,10-(2H)-1-azaanthracenetrione (**4**, 58 mg) were obtained, respectively. The residue (1.2 g) obtained by elution with petroleum ether−Me_2CO (9:1) was subjected to Si gel

chromatography, eluting with petroleum ether−EtOAc (8:2). Thirty-five fractions of 100 mL each were collected. Fractions 3−6 furnished griffithazanone A (**1**, 7 mg). The residue obtained by elution with petroleum ether−Me_2CO (3:2) on further chromatography over Si gel followed by crystallization from MeOH furnished griffithinam (**3**, 345 mg). Additional column chromatography resulted in the isolation of other five compounds, velutinam (38 mg), aristololactam BI (14 mg), aristololactam BII (12 mg), aristololactam AII (58 mg), and norcepharanone B (6 mg).

Griffithazanone A (1): yellow needles (Me_2CO); mp 208−210 °C; $[\alpha]^{25}_D$ +146° (*c* 0.06, $CHCl_3$); UV ($CHCl_3$) λ_{max} 203 (4.07), 218 (4.00), 255 (4.29), 286 (3.99), 335 (3.36) nm; IR (KBr) ν_{max} 3427, 3284, 1730, 1699, 1670, 1637, 1591, 1454, 1301, 1217, 1103, 933, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 257 $[M]^+$ (100), 242 (13), 228 (87), 214 (25), 200 (61), 183, 105, 77; HREIMS *m*/*z* 257.0692 (calcd for $C_{14}H_{11}NO_4$, 257.0688).

Griffithdione (2): orange needles ($CHCl_3$); mp 216−218 °C; UV ($CHCl_3$) λ_{max} 244 (4.49), 295 (4.02), 307 (4.14), 330 (4.13), 460 (3.98) nm; IR (KBr) ν_{max} 3564, 1681, 1658, 1581, 1367, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.29 (1H, s, H-3), 9.64 (1H, d, J = 7.0 Hz, H-11), 7.68 (1H, t, J = 7.0 Hz, H-10, 7.73 (1H, t, J = 7.0 Hz, H-9), 8.12 (1H, d, J = 7.0 Hz, H-8), 2.73 (3H, s, CH_3-7), 4.13 (3H, s, OCH_3-2), 4.09 (3H, s, OCH_3-1), 11.22 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.1 (C-1), 118.9 (C-1a), 152.8 (C-2), 123.5 (C-3), 118.2 (C-3a), 197.3 (C-4), 176.6 (C-5), 132.2 (C-6a), 123.5 (C-6b), 112.9 (C-7), 126.3 (C-7a), 128.2 (C-8), 128.1 (C-9), 124.6 (C-10), 127.2 (C-11), 124.0 (C-11a), 12.7 (CH_3-9), 60.6 (OCH_3-1), 56.6 (OCH_3-2); EIMS *m*/*z* 321 $[M^+]$ (100), 293 $[M\text{-}CO]^+$ (30), 278 (9), 263 (10), 250 (2), 235 (20), 207 (8), 179 (11); HREIMS *m*/*z* 321.0990 (calcd for $C_{19}H_{15}NO_4$, 321.1001).

Griffithinam (3): pale yellow needles (MeOH); mp 262−264 °C, UV (MeOH) λ_{max} 239 (4.43), 257 (4.51), 289 (4.25), 325 (4.05), 340 (3.94), 385 (3.92) nm, (MeOH + NaOH) 235 (4.55), 322 (4.33), 335 (4.02), 395 (4.04) nm; IR (KBr) ν 3406 (OH, NH), 3174, 1705 (C═O), 1660, 1654, 1540, 1469, 1381, 1043 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 7.78 (H-2), 8.89 (1H, d, J = 8.0 Hz), 7.47 (1H, t, J = 8.0 Hz), 7.16 (1H, d, J = 8.0 Hz), 7.42 (1H, s), 4.03 (3H, s, OCH_3-3), 3.98 (3H, s, OCH_3-8), 10.61 and 10.60 (1H each, br s, NH and OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.7, 155.1, 149.5, 148.2, 134.6, 127.4, 125.1, 125.0, 124.1, 129.0, 115.9, 114.4, 108.9, 107.4, 97.7, 57.2, 55.8; EIMS *m*/*z*: 295 $[M^+]$ (100), 280 (80), 252 (12); *anal*. C 69.12%, H 4.45%, N 4.64%, calcd for $C_{17}H_{13}NO_4$, C 69.13%, H 4.44%, N 4.74%.

Griffithazanone A MTPA Derivatives (1*R* and 1*S*): Griffithazanone A (6 mg) was divided into two parts and treated with (*R*)- and (*S*)-MTPA, respectively, in the presence of *N*,*N*′-cyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) at room temperature overnight. The (*R*)- and (*S*)-MTPA derivatives of **1** were purified by preparative TLC on Si gel with petroleum ether−EtOAc (3:2) as the developing solvent. ^1H NMR ($CDCl_3$, 500 MHz) are shown in Table 1.

Dehydration of Griffithazanone A (1): Griffithazanone A (1 mg) was dissolved in pyridine and treated with $SOCl_2$ at room temperature overnight, 4-methyl-2,9,10-(2H)-1-azaanthracenetrione (**4**) was detected on a TLC plate.

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