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Ouregidione from *Goniothalamus velutinus* (Annonaccae)

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Abstract. The structures of ouregidione, aristolactam B(11), goniothalenol, goniothalamin and annonacin, chemicals that were isolated from the stem-bark of *Goniothalamus velutinus*, were established by NMR spectroscopy. The five compounds were evaluated for their larvicidal activity against *Aedes aegypti.*

Abstrak. Struktur ouregidione, aristolactam B(11), goniothalenol, goniothalwnin dan annonacin, bahan kimia yang ditapis dari kulit tumbuhan *Goniothalamus velutinus*, ditentukan secara pektroskopi RMN. Aktiviti larvisiid bahan tersebut dikaji terhadap *Aedes aegypti.*

Introduction

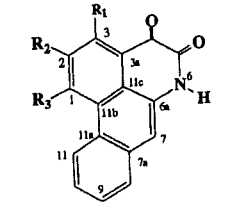
Our earlier structural studies [l-3] on styrylpyrones (from *Goniothalamus dolichocarpus*) and acetogenins (from *Disepalum anomalum*) have established the larvidical activity of these types of compounds against the *Aedes aegypti* mosquito. The present study reports the isolation of compounds from the stem-bark and leaves of *G. velutinus*, a North Bornean plant that is used indigenously as an insect repellent in Sarawak.

Experimental

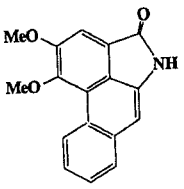
The stem-bark of *G. velutinus* was collected from a highland forest in Sarawak, East Malaysia. The sample was identified at the Herbarium, Forest Depattment, Kuching, Sarawak, at which a voucher specimen (S 34824) has been deposited. The dried bark (1 kg) of *G. velutinus* was ground to a coarse powder and extracted by *n*-hexane for 48 hours. The extraction gave 22 g of crude material, which was chromatographed by preparative layer chromatography to give goniothalamin 5 (0.1 g) and a mixture of sesquiterpenes. The powdered bark was again extracted by ethanol to provide, upon removal of the solvent, 55 g of a semi-solid material. This was partitioned between chloroform and water. The chloroform fraction was further partitioned between 90% aqueous methanol and hexane. The methanol fraction was evaporated to dryness to give 48 g of material, which was chromatographed on an SiO_2 column (Merck 9385, Kieselgel 60) by using chloroform followed by gradient elution with methanol-chloroform to afford ouregidione **1** (2 g), aristolactam B(11) **2** (0.2 g), goniothalenol **3** (0.2 g) and annonacin **4** (0.1 g). Proton and carbon NMR spectra were recorded on a JEOL JNM-GSX 270 spectrometer and $CDC1_3$ or C_5D_5N was used as solvent (Tables 1 and 2). Mass spectra were recorded on a VG Prospec instrument.

Needle-shaped orange **1** (m.p. 274-276°C) shows no optical rotation arising from the absence of a chiral carbon center. The EIMS spectrum shows a molecular peak at m/z 337.10 that corresponds to $C_{19}H_{15}O_5N$. Proton and carbon NMR of **1** are tabulated in Table 1 and Table 2. The HNMR spectrum showed three methoxy singlets at **8** 4.10, 4.17 and 4.21; the signals were assigned on the basis of comparison with compounds **6 7** and **8**. The broad singlet at 6 11.75 implicated the N-H proton. The H-11 proton of the D-ring was assigned by comparison with values reported [5] for compounds **6**, **7** and **8**.

The linkages between the methoxy protons at 8 4.10, 4.17, 4.21 to the aromatic signals at δ 147.5, 159.0, 160.5 were shown by HMQC. The HMQC spectrum also demonstrated the connectivities between the two overlapping protons at δ 7.66 to the two carbon signals at 8 127.3 and 127.5, and also between that at 6 7.90 to the carbon at 5 128.3. The UMBC spectrum gave the ^{3}J coupling of both amino and the H-7 (δ 7.70) protons to the quaternary carbon (δ 120.3), and also between a singlet at 8 9.51 (H-1 1) to the carbon signal at δ 121.3 (C-1lb). A ^{2}J coupling of the proton at δ 9.51 (H- 11) to the carbon signal at δ 127.6 (C-11a) was also distinct. Finally, the through- space interaction of the NH proton to H-7 proton and OMe-1 to H-11 proton from the NOE-difference experiment confirmed the assignment of ouregidione.



1 $R_1 = R_2 = R_3 = OMe$
6 $R_1 = H$, $R_2 = R_3 = OMe$
7 $R_1 = R_3 = H$, $R_2 = OH$
8 $R_1 = H$, $R_2 = OH$, $R_3 = OMe$



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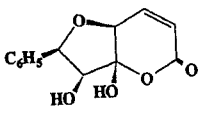

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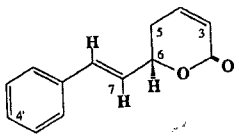

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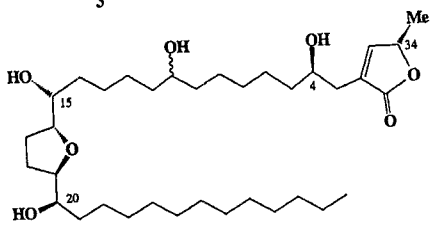

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Bioassays of the pure compounds were performed on the larvae of *Aedes aegypti* mosquito in 1% aqueous ethanol. Larvicidal tests were performed according to the protocols of the World Health Organisation [4]. A known quantity of the compound was dissolved in absolute ethanol to provide a stock solution; serial dilutions of the stock were prepared in 250 mL drinking glasses containing 25 ml of water. Ten 3rd instar mosquito larvae were introduced into each solution and the volume made up to 50 mL. A little liver powder was added for the larvae to consume. The number of larvae still living after 24 hours was then noted (Table 3).

Results and Discussion

Our previous study [1] on *G. dolichocarpus* demonstrated that annonacin **4** constituted the principal compound; other studies [6-9] on other *Goniothalamus* species reported only oxoaporphines and styrylpyrones. Tetrahydrofuranoid acetogenins are sometimes found in certain genera of the Annonaceae family, and the insecticidal and cytotoxic effects of these plants have been attributed to these compounds. The present study on *G. velutinus* has yielded ouregidione, together with aristolactam B(11), goniothalenol, annonacin and (+)-goniothalamin. The occurrence of the dioxoaporphine alkaloid in this genus has not been previously reported.

The compunds when bioassayed on *Aedes aegypti* mosquitoes showed high larvicidal activity (Table 3). The dioxoaporphine ouregidione showed relatively higher larvicidal activity in comparision with the oxoaporphine aristolactam B(11), probably because of its dioxo-nature of the compound. Goniothalenol showed lower larvicidal activity in comparison with (+)-goniothalamin probably because of the absence of the styrenyl double bond after forming the diol and cyclization.

Acknowledgements

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Table 1. Comparison HNMR values of **1** (in $ClCl_3$) with **1**, **6**, **7** and **8** (in C_5D_5N) [5]

Proton	**1** (in $ClCl_3$)	**1**	**6**	**7**	**8**
OMe-1	4.10 s	4.03 s	3.94 s		4.20 s
OMe-2	4.17 s		4.16 s	4.12 s	
OMe-3	4.21 s	4.20 s			
N-H	11.75s				
H-1				8.96 d (2.5 Hz)	
H-3			8.42 s	8.55 d (2.5 Hz)	8.63 s
H-7	7.70 s	7.89 s	7.78 s	7.68 s	7.73 s
H-8	7.90 dd (9, 2Hz)	8.03 dd (7, 2 Hz)	7.99 m	7.96 dd (8, 2 Hz)	7.95 m
H-9	7.66 td (9, 2Hz)	7.72 ddd (7, 7, 2 Hz)	7.74 m	7.65 ddd (8, 1.5 Hz)	7.63 m
H-10	7.66 td (9, 2Hz)	7.71 ddd(7, 7, 2 Hz)	7.67-9.77 m	7.62 ddd (8, 2 Hz)	7.71-9.79 m
H-11	9.51 dd (9, 2Hz)	9.7 br d (7 Hz)	7.67-9.77 m	8.79 br d (8 Hz)	7.71-9.79 m

Table 2: CNMR values for **1** in $CDCl_3$ and **6**, **7**, **8** in C_5D_5N [5]

Carbon	**1**	**6**	**7**	**8**
C-1	147.5	154.9	117.1[a]	154.1
C-2	159.0	153.4	159.3	152.8
C-3	160.5	113.2	117.0[a]	118.4
C-3a	128.5	124.8[a]	131.2[b]	126.4
C-4	175.4	178.0	179.0	178.3
C-5	157.6	156.9	157.2	157.0
C-6a	131.7	131.5	132.1[b]	131.8
C-7	116.0	113.2	110.6	112.3
C-7a	131.7	133.5	133.5[b]	133.6
C-8	128.3	129.0	128.7	128.9
C-9	127.3	128.3[b]	128.4	128.2[a]
C-10	127.5	127.2[b]	126.3	126.9
C-11	127.3	128.3[b]	123.0	128.1[a]
C-11a	127.6	127.2	127.2	127.2
C-11b	121.3	125.8[a]	133.2[b]	125.3
C-11c	120.3	119.5	116.4	118.4

OMe-1	61.8	60.3	-	59.9
OMe-2	61.2	56.3	-	-
OMe-3	62.1	-	-	-

[a,b]Assignments with the same superscript may be interchanged.

Table 3. Larvicidal activity of compounds isolated from *G. velutinus*

Compound	**1**	**2**	**3**	**4**	**5**
LC_{50} (μg mg^{-1})	5-20	50-100	100-150	9-10	15-16