Asian Journal of Chemistry Vol. 13, No.2 (2001), 550–554

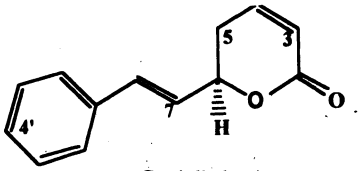
Larvicidal Flavanone and Sesquiterpenes from *Goniothalamus macrophyllus* (Annonaceae)

G.C.L. EE*, K.N. NG, M. RAHMANI and Y.H. TAUFIQ-YAP
Chemistry Department, Faculty of Science and Environmental Studies
University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

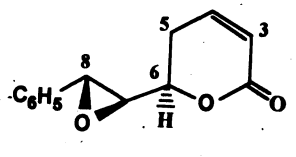
Bioassay-directed fractionation of the stem bark of *Goniothalamus macrophyllus* allowed for the isolation of two styrylpyrones (+)-goniothalamin (**1**) and (+)-goniothalamin epoxide (**2**), one flavanone (–)-pinocembrin (**3**) and a mixture of essential oils which comprises mainly an inseparable mixture of sesquiterpenes (**4**). The structures of these compounds were established by spectral data. (**3**) and (**4**) have not been reported to be present in this plant. Also, larvicidal activities have not been reported for this plant.

INTRODUCTION

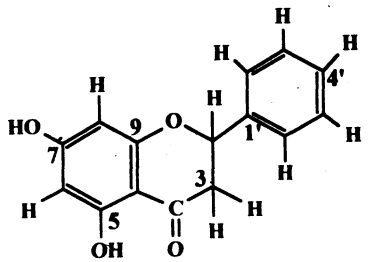
Many plants from the genus *Goniothalamus* have provided bioactive styrylpyrones,[1–4] acetogenins[5, 6] and alkaloids.[7–9] *Goniothalamus macrophyllus* (Bl.) Hook fil. and Thomas is widely distributed in the mixed dipterocarp forests and submontane forests of Sarawak, Malaysia.[10] The roots and stem have a teratogenic effect in mice and this plant is used as an abortifacient in rural areas in North Malaysia.[11] However, a decoction of the roots is used externally for colds and for administering after childbirth.[12]



(+)-Goniothalamin



(+)-Goniothalamin epoxide



Pinocembrin

EXPERIMENTAL

Extraction and isolation of compounds

The dried stem bark of *Goniothalamus macrophyllus* (1.1 kg) was extracted with hexane once for more than 48 h. The same bark was then reextracted with

ethyl acetate and with ethanol twice. This gave 2 g of hexane extract, 21 g of ethyl acetate extract and 73 g of ethanol extract. The hexane extract was partially purified by preparative layer chromatography before being analysed by GC (BP-10 capillary column) for the presence of essential oils and GC-MS (HP5MS capillary column) for identification of sesquiterpenes. The ethyl acetate and ethanol extracts were purified by filtering column chromatography and preparative layer chromatography.

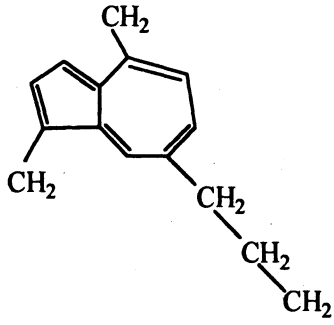
Bioassay

Bioassay tests on *Aedes aegypti* according to the WHO[13] standard procedures were used to investigate the larvicidal activity of samples. A standard stock solution of 5000 ppm (5000 g/mL) was prepared by dissolving 25 mg extract in 5 mL of absolute ethanol. A test solution was made by pipetting a sample of the stock solution (usually 0.5–1.5 mL) into 25 mL of chlorine-free tap water in glass containers. The test solutions were made up to the required concentrations (50–150 ppm). A control was prepared by using 1.5 mL of absolute alcohol in chlorine-free water. The test sample was diluted with chlorine-free water to a volume of 50 mL. Ten late third instar mosquitoes were introduced into each glass and a little larval food was added. Mortality of the mosquito larvae was evaluated after 24 h. A series of at least 5 concentrations in duplicate were needed to obtain LC_{50} values.

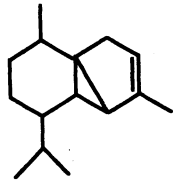
RESULTS AND DISCUSSION

The stem bark extract of *Goniothalamus macrophyllus* showed significant larvicidal activity against larvae of *Aedes aegypti*. It is found that the stem bark contains the very bioactive (+)-goniothalamin **(1)** and (+)-goniothalamin epoxide **(2)** (trace amount) which are styrylpyrones as well as the very bioactive (–)-pinocembrin **(3)**, a flavanone and a mixture of essential oils which comprises an inseparable mixture of sesquiterpenes. Pinocembrin and sesquitepenes have not been reported to be present in this plant. **(1)** and **(3)** were identified through ^{1}H NMR, ^{13}C NMR, 2D NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR) and MS. Sesquiterpene components in **(4)** were identified through GC-MS. We report here spectral data for the major compound **(3)** as well as bioassay data for **(1)**, **(2)**, **(3)** and crude extracts.

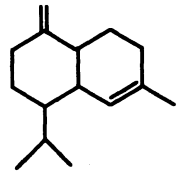
(–)-Pinocembrin was isolated from the ethyl acetate and ethanol extracts as white needles with a melting point of 190–191°C (Lit. 192–193°C)[14] and $[a]_{20}^{D}$ = –44°C (*c*. 1.0, CH_3COCH_3) (Lit = –45.3 (*c*. 0.9, CH_3COCH_3)).[14] EIMS gave an M^+ of 256 which is consistent with the molecular formula $C_{15}H_{12}O_4$. Loss of one water molecule gave the fragment 238 which implied the existence of one hydroxyl group in the molecule. Other fragments were 179 (M^+-77), 152 ($C_7H_4O_4$), 124 ($C_6H_4O_3$), 104 (C_8H_8) and 77 (C_6H_5). The ^{1}H NMR values were assigned using ^{1}H-^{1}H COSY. The ^{1}H NMR spectrum (270 MHz, CD_3COCD_3) gave a multiplet at δ 7.57 of five protons implying a phenyl ring. Other signals were a one-proton sharp singlet at δ 12.25 for hydrogen bonded OH, a two proton sharp singlet at δ 6.09 for two non-equivalent protons at positions C-6 and C-8,



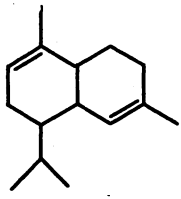
1,4-Dimethyl-7-(propyl)azuline



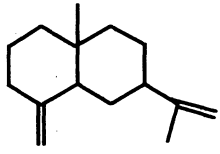
α-Cubebene



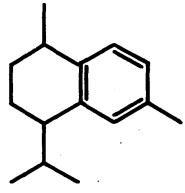
α-Amorphene



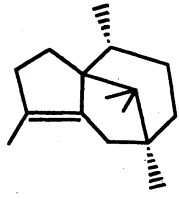
α-Muurolene



β-Selinene



1S, *cis*-calamenene



Cyprene

a dd at δ 5.62 with coupling constant values of 3.3 and 12.8 Hz for the proton at C-2 which is coupled to H-3a and H-3b, respectively. The signal for H-3a appeared at δ 2.87 as a dd with coupling constant values of 3.3 Hz ($J_{3a\text{-}2}$) and 17.2 Hz ($J\ H_{3a\text{-}3b}$) (Table-1). H-3b gave a signal at δ 3.22 with coupling constant values of 12.8 Hz ($J\ H_{3b\text{-}2}$) and 17.2Hz ($J\ H_{3b\text{-}3a}$). The ^{13}C values were assigned from ^{1}H-^{13}C connectivities obtained from ^{1}H-^{13}C HETCOR experiment. The ^{13}C values are given in Table-2.

Compound **(1)**, (+)-goniothalamin was isolated from the hexane and ethyl acetate extracts as colourless crystals with a melting point of 83–84°C. Spectral data match with those from literature.[15] Compound **(2)**, (+)-goniothalamin epoxide was detected as a minor component in the ethanol extract. The mixture of essential oils was found to contain seven sesquiterpenes. They are: α-cubebene, cyprene, α-amorphene, β-selinene, α-muurolene, 1*S*, *cis*-calamenene and 1,4-dimethyl-7-(propyl) azulene. The mass spectra for α-cubebene, cyprene, α-amorphene, β-selinene and α-muurolene indicated molecular masses of 204 whereas those for 1*S*, cis-calamenene and 1,4-dimethyl-7-(propyl) azulene gave molecular masses of 202 and 198 respectively.

TABLE-1
^{1}H NMR (δ) ASSIGNMENTS AND J (Hz) VALUES FOR PINOCEMBRIN (270 MHz, CD_3COCD_3)

Proton	δ	J (Hz)
H-2	5.62 dd	3.3, 12.8
H-3a	2.87 dd	3.3, 17.2
H-3b	3.22 dd	12.8, 17.2
H-6	6.09 s (2H)	—
H-8	6.09 s (2H)	—
Ph	7.57 m	—
5-OH	12.25 s	—

TABLE-2
^{13}C NMR ASSIGNMENTS FOR PINOCEMBRIN (67.8 MHz, CD_3COCD_3)

Carbon	δ
C-2	79.8
C-3	43.5
C-4	196.7
C-5	165.2
C-6	95.9
C-7	167.4
C-8	96.9
C-9	164.0
C-10	103.1
C-1′	138.9
C-2′,6′	127.2
C-3′,5′	129.4
C-4′	129.4

TABLE 3
LARVICIDAL (*Aedes aegypti*) ACTIVITIES OF CRUDE EXTRACTS OF *G. macrophyllus* AND PURE COMPOUNDS

Extracts/pure compounds	LC_{50}/g mL^{-1}
Crude hexane	60.5
Crude ethyl acetate	127.7
Crude ethanol	95.0
(+)-Goniothalamin	15.0
(+)-Goniothalamin epoxide	50–100
(–)-Pinocembrin	33.0

The crude hexane, ethyl acetate and ethanol extracts of *Goniothalamus macrophyllus* as well as pure compounds were bioassayed against the larvae of

Aedes aegypti. The larvae of *Aedes aegypti* were more susceptible to the crude hexane extract (LC_{50} 60.5 $\mu g\ mL^{-1}$) than the ethyl acetate (LC_{50} 127.7 $\mu g\ mL^{-1}$) or ethanol extracts (LC_{50} 95.0 $\mu g\ mL^{-1}$) (Table-3). The hexane extract is most bioactive probably due to the presence of the very bioactive goniothalamin (LC_{50} 15 $\mu g\ mL^{-1}$). LC_{50} values for (+)-goniothalamin , (+)-goniothalamin epoxide and (–)-pinocembrin are given in Table–3.

ACKNOWLEDGEMENTS

The authors wish to thank University Putra Malaysia for financial support.

REFERENCES

1. S.K. Talapatra, D. Basu, T. Deb, S. Goswami and B. Talapatra, *Indian J. Chem.*, **24B**, 24 (1985).
2. A.A.E. El Zayat, N.R. Ferrigni, T.G. McCloud, A.T. McKenzie, S.R. Byrn, J.M. Cassady, C.J. Chong and J.L. McLaughlin, *Tet. Letts.*, **26**, 956 (1985).
3. X.P. Fang, J.E. Anderson, .C-.J Chang and J.L. McLaughlin, *J. Nat. Prod.*, **5**, 1034 (1991).
4. S.H. Goh, G.C.L. Ee, C.H. Chuah and Chen Wei, *Aust. J. Chem.*, **48**, 199 (1995).
5. A. Alkofahi, J.K. Rupprecht, Y.M. Liu, C.J. Chang, D.L. Smith and J.L. McLaughlin, *Experientia*, **46**, 539 (1990).
6. F.Q. Alahi, Y. Zhang, L. Rogers and J.L. McLaughlin, *J. Nat. Prod.*, **60**, 929 (1997).
7. S. Omar, C.L. Chee, F. Ahmad, J.X. Ni, H. Jaber, J. Huang and T. Nakatsu, *Phytochemistry*, **31**, 4395 (1992).
8. S.K. Talapatra, D. Basu, P. Chattopadhyay and B. Talapatra, *Phytochemistry*, **27**, 903 (1988).
9. G.C.L. Ee, *Oriental J. Chem.*, **14**, 41 (1998).
10. J.A.R. Anderson, A Checklist of the Trees of Sarawak, Dewan Bahasa dan Pustaka, Sarawak Branch (1980).
11. T.W. Sam, C.S. Yeu, S. Matsjeh, E.K. Gan, D. Razak and A.L. Mohammad, *Tet. Letts.*, **28**, 2541 (1987).
12. I.H. Burkil, A Dictionary of The Economic Products of the Malay Peninsula, Vol 1, London Crown Agents (1935).
13. World Health Organization: Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides (WHO/VBC/81.807) (1981).
14. J. Buckingham, A DictionaryoOf Natural Products, Vol. 2, p. 1623, Chapman and Hall.
15. J.R. Jewers, J.B. Davis, J. Dougan, A.H. Machanda, G. Blunden, A. Kyi and S. Wetchapinan. *Phytochemistry*, **11**, 2025 (1972).

(*Received*: 19 October 2000; *Accepted*: 1 January 2001) AJC- 2220