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GONIOTHALAMIN AND ITS DISTRIBUTION IN FOUR *GONIOTHALAMUS* SPECIES

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Abstract—Goniothalamin, a biologically active styryldihydropyrone isolated from the bark of *Goniothalamus andersonii* Sinclair, has been shown to be the (+)-(5*S*)-δ-lactone of 5-hydroxy-7-phenylhepta-2,6-dienoic acid previously obtained from *Cryptocarya caloneura* (Scheff.) Kostermans. The distribution of goniothalamin in *G. andersonii*, *G. macrophyllus* Miq., *G. malayanus* Hook. f. et Thoms., and *G. velutinus* Airy-Shaw has been studied. The lactone was detected in all parts of *G. andersonii* and *G. macrophyllus*, and in some samples of *G. malayanus*, but was not present in the samples of *G. velutinus* examined. Young stems and leaves were found to have the lowest goniothalamin content, and the fruit of *G. andersonii* was the richest source of the lactone (6·1%).

INTRODUCTION

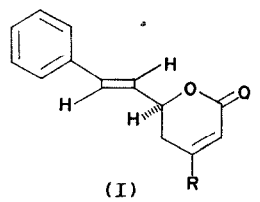
THE GENUS *Goniothalamus* (Annonaceae) comprises some 115 species of shrubs and trees which grow in Asia.[1] A number of the plants have been used for timber,[2–4] as fibre sources,[2,3] and for ornamental[5] and medicinal purposes,[3,6] but no phytochemical studies of the genus have been reported. As part of a study of the peat-swamp species of Sarawak[7] as sources of useful biologically active compounds, extract of *Goniothalamus andersonii* and *G. malayanus* were tested for biological activity, and the light petroleum extracts of both plants were found to have antifungal and CNS activity.[8] This paper reports on the isolation of an active principle from both plants and presents evidence which leads to the constitution of the compound and its distribution in four species of *Goniothalamus*.

RESULTS AND DISCUSSION

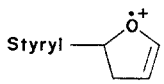
A biologically active compound, to which we have assigned the trivial name goniothalamin, crystallized from the light petroleum extract of *Goniothalamus andersonii* bark, when concentrated, in a yield of 1·2%. Goniothalamin, $C_{13}H_{12}O_2$ (M^+, *m/e* 200·0804), m.p. 85°, $[\alpha]_D$ +170·3°, (c, 1·38; $CHCl_3$), showed strong absorption in its UV spectrum at

[1] H. K. AIRY-SHAW, in *A Dictionary of the Flowering Plants and Ferns* (edited by WILLIS) (7th edition), p. 489, University Press, Cambridge (1966).
[2] *The Wealth of India*, Vol. 4, p. 169, Council of Scientific and Industrial Research, New Delhi (1956).
[3] I. H. BURKILL, *A Dictionary of the Economic Products of the Malay Peninsula*, Vol. 1, p. 1097, Crown Agents, London (1935).
[4] G. WATT, *A Dictionary of the Economic Products of India*, Vol. 3, p. 533, Allen, London (1890).
[5] E. J. H. CORNER, *Wayside Trees of Malaya*, Vol. 1, p. 134, Government Printing Office, Singapore (1940).
[6] E. QUISUMBING, *Medicinal Plants of the Philippines*, p. 314, Bureau of Printing, Manila (1951).
[7] J. A. R. ANDERSON, *The Gardener's Bull. Singapore* **20**, 131 (1964).
[8] A. BARRANCO, R. T. BONE, A. PINEGAR and H. M. ROSE, unpublished data.

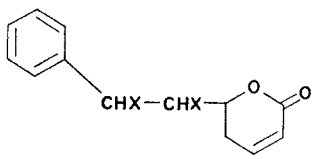
207, 253 and 284 nm (log ε 4·49, 4·29 and 3·21), indicative of a styryl residue, which underwent a bathochromic shift on addition of base.[9] Strong IR absorption at 1720, 1660, 1250 and 760 cm^{-1} was assigned to an α,β-unsaturated δ-lactone moiety, and 1575, 1492, and 970 cm^{-1} to a styryl group. The latter deduction was supported by aromatic proton (δ 7·3, m, 5H) and olefinic proton absorptions (δ 6·68, dd, 1H, *J* 15·7 and 1 Hz; δ 6·21, dd, 1H, *J* 15·7 and 6 Hz) in the NMR spectrum, which also showed that the olefinic protons had a *trans* configuration.[10] The NMR spectrum was also instructive with regard to the α,β-unsaturated δ-lactone moiety. Two olefinic protons were observed at δ 6·05 (1H, dt, *J* 9·6 and 1·7 Hz) and δ 6·85 (1H, dt, *J* 9·6 and 4·2 Hz), which were assigned to H_3 and H_4 respectively; an allylic methylene at δ 2·45 (2H, m); and a proton on a carbon bearing the oxygen of the lactone group at δ 5·03 (1H, m). Double irradiation experiments showed that the proton on the carbon bearing the oxygen of the lactone group was coupled both to the olefinic protons of the styryl group and the allylic methylene protons.



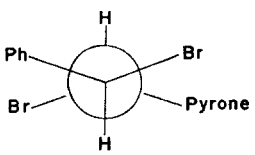
(I)
Goniothalamin (a) R = H
Kawain (b) R = OMe



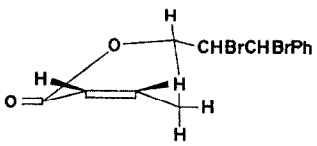
(II)



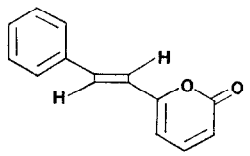
(III)
(a) X = Br *(erythro)*
(b) X = Br *(threo)*



(IV)



(V)



6-Styryl-2-pyrone (VI)

The above spectroscopic evidence indicated that goniothalamin was identical with the (+)-(5*S*)-δ-lactone of 5-hydroxy-7-phenylhepta-2,6-dienoic acid (Ia) previously isolated from the dried bark of *Cryptocarya caloneura* (Scheff.) Kostermans (Lauraceae) in a yield of 0·16%.[11] Confirmatory evidence for this assignment was obtained in the following way. The base peak of the high resolution mass spectrum was observed at *m/e* = 68. This ion, which corresponds to ionized furan,[12] is derived from the molecular ion by a two step process involving the ion (II) *m/e* = 172. Strong ions in the spectrum at *m/e* = 77, 91, 104 and 115 correspond to the phenyl, tropylium, styrene and indenyl ions, and the ion at *m/e* = 131 is probably the cinnamyl ion.[13] Similar fragments have been observed in the mass spectrum of kawain (Ib).[14]

[9] A. I. SCOTT, *Interpretation of Ultraviolet Spectra of Natural Products*, p. 98, Pergamon Press, London (1964).
[10] P. BEAK and H. ABELSON, *J. Org. Chem.* **27**, 3715 (1962).
[11] J. R. HLUBECEK and A. V. ROBERTSON, *Austral. J. Chem.* **20**, 2199 (1967).
[12] W. H. PIRKLE, *J. Am. Chem. Soc.* **87**, 3022 (1965).
[13] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, p. 201, Holden-Day, San Francisco (1967).
[14] M. PAILER, G. SCHADEN and R. HÄNSEL, *Monatsch. Chem.* **96**, 1842 (1965).

Treatment of goniothalamin with NBS gave three products which could be partially separated by trituration with ether. The components of the ether insoluble fraction were separated by TLC. The major product, $C_{13}H_{12}O_2Br_2$ (M^+, *m/e* 358, 360, 362), m.p. 175–177° (dec.), $[\alpha]_D$ −6° (c, 0·63; $CHCl_3$) was also obtained by the treatment of goniothalamin with bromine in carbon tetrachloride. Strong absorption in its IR spectrum at 1730 cm^{-1} indicated that the molecule still contained an α,β-unsaturated lactone moiety. The mass spectrum was similar to that of goniothalamin; it differed mainly in the region above *m/e* = 200 where ions corresponding to the sequential loss of two bromine atoms from the molecular ion was observed. The NMR spectrum showed one proton resonances at δ 5·50 (1H, d, *J* 11·4 Hz) and δ 4·55 (1H, dd, *J* 11·4, 1·4 Hz), and the absence of styryl olefinic protons. This indicated that bromine addition had occurred at the styryl double bond, and that the conformation of the side chain is (IV) with H_7 and H_8 trans-diaxial and the dihedral angle between H_6 and H_7 close to 90°. The two olefinic protons of the dihydropyrone moiety and H_6 were observed as doublets of quartets at δ 6·07 (1H, dq, *J* 9·8, 2·7 and 0·8 Hz), δ 6·97 (1H, dq, *J* = 9·8, 6·0 and 2 Hz), and δ 5·31 (1H, dq, *J* 11·5, 4·5 and 1·4 Hz) respectively, indicating that the conformation of the lactone ring had changed as a result of bromination. The spectroscopic data showed that the lactone ring had adopted the conformation (V) with one of the allylic methylene protons approximately coplanar with the two olefinic protons and the other trans-diaxial with H_6. Examination of Dreiding models showed that such a change would be expected in *erythro*-7,8-dibromogoniothalamin (IIIa), but not the *threo*-isomer, in order that non-bonded interaction between the bromine atom on C_7 and the allylic methylene protons would be minimized. The minor component of the ether insoluble fraction was shown to be *threo*-7,8-dibromogoniothalamin (IIIb), $C_{13}H_{12}O_2Br_2$(M^+, *m/e* = 358, 360 and 362), m.p. 168° (dec.), $[\alpha]_D$ +162° (c, 0·74; $CHCl_3$). Its IR spectrum showed strong absorbtion at 1720 cm^{-1}, indicative of a dihydropyrone functionality, and differed from the spectrum of the *erythro*-isomer in the fingerprint region. The MS of the two compounds were similar showing that bromine addition had occured in both compounds at the styryl double bond. The NMR spectrum (d_6-acetone) indicated the absence of styryl olefinic proton resonances and their replacement by resonances at δ 5·08 (1H, dd, *J* 11 and 1 Hz) and δ 5·50 (1H, dd, *J* 11 and 3 Hz), and showed the olefinic protons of the lactone moiety at δ 5·97 (1H, dt, *J* 9·8 and 1·8 Hz) and δ 7·12 (1H, dt, *J* 9·8 and 4 Hz). The spectroscopic data showed that the conformation of the lactone ring of the *threo*-isomer was similar to that of goniothalamin with the plane bearing the olefinic protons bisecting the angle between the allylic methylene protons.

Chromatographic separation of the ether soluble portion of the reaction products derived from treating goniothalamin with NBS afforded 6-styryl-2-pyrone (VI), $C_{13}H_{10}O_2$ (M^+, *m/e* = 198), m.p. 116°. The IR spectrum showed strong absorption at 1720 cm^{-1} indicating a 2-pyrone functionality.[15–17] The NMR spectrum showed that the lactone ring had been dehydrogenated. No resonances were observed in the δ 0–6 ppm region of the spectrum; one of the styryl olefinic protons was observed at δ 6·58 (1H, d, *J* 16·2 Hz); and H_3 and H_5 were observed at δ 6·19 (1H, dd, *J* 9·6 and 0·9 Hz) and δ 6·12 (1H, dq, *J* 6·7, 0·9 and 0·4 Hz) respectively. The mass spectrum showed strong ions at *m/e* = 170(M^+–CO), 142(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), which were consistent with a 6-styryl-2-pyrone form-

[15] W. B. Mors, M. T. Magalhães and O. R. Gottlieb, *Fortsch. Chem. Org. Nat.* **20**, 132 (1962).
[16] D. Herbst, W. B. Mors, O. R. Gottlieb and C. Djerassi, *J. Am. Chem. Soc.* **81**, 2427 (1959).
[17] J. D. Bu'Lock and H. G. Smith, *J. Chem. Soc.* **502** (1960).

ulation.[18] This compound was subsequently reported as a constituent of *Aniba parviflora* (Meissn.) Mez.[19] Styryl-2-pyrones have previously been reported as constituents of the Lauraceae,[11,19,20] Piperaceae[21,22] and Basidiomycetes,[23] but have not been found before in the Annonaceae. In view of the pharmacological activity displayed by goniothalamin and

TABLE 1. GONIOTHALAMIN CONTENTS OF DIFFERENT MORPHOLOGICAL PARTS OF *Goniothalamus* SPECIES

Species	Part	Goniothalamin yield (% air-dried wt) Sample A	B	C	D
G. andersonii	Stem bark	2·10	0·63	0·49	—
	Stem wood		1·36	0·53	
	Young stems	0·25			
	Leaves	+	+	+	
	Root bark			0·75	
	Whole root	3·00			
	Fruit	6·10			
G. macrophyllus	Stem bark	0·95			
	Stem wood	1·52			
	Leaf	+			
	Root bark	0·68			
	Root wood	0·83			
G. malayanus	Stem bark		—	0·22	—
	Stem wood		—		
	Young stems	0·05			
	Leaves		—		
	Root bark		+		
	Root wood		—		
	Whole root	1·40			
G. velutinus	Stem bark	—	—		
	Stem wood	—	—		
	Leaves	—	—		

+ Positive for goniothalamin, but very low yield.
— Goniothalamin not detected.

its possible taxonomic significance for the genus *Goniothalamus*, we decided to investigate its distribution in four species of *Goniothalamus* growing in the peat swamps of Sarawak.

A linear relationship has been found[24] to exist between the logarithm of the weight of a substance applied to a thin-layer chromatogram and the square root of the area of the spot

[18] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, p. 208, Holden-Day, San Francisco (1967).
[19] A. M. BITTENCOURT, O. R. GOTTLIEB, W. B. MORS, M. T. MAGALHÃES, S. MAGESWARAN, W. D. OLLIS and I. O. SUTHERLAND, *Tetrahedron* **27**, 1043 (1971).
[20] O. R. GOTTLIEB, *Bôtanica* **4**, 113 (1967).
[21] H. ACHENBACH and G. WHITTMAN, *Tetrahedron Letters* 3259 (1970).
[22] R. HÄNSEL, *Pacific Sci.* **21**, 293 (1968).
[23] G. M. HATFIELD and L. R. BRADY, *Abstr. Internat. Meeting Med. Plant Res.* p. 9, Vienna (1970).
[24] S. J. PURDY and E. V. TRUTER, *Analyst* **87**, 802 (1962).

produced, and has been employed for the assay of steroidal sapogenins.[25,26] This relationship has been found to apply to goniothalamin, from at least 20–80 μg with an error of ±5–6%, thus showing that densitometric TLC provides a suitable method for the assay of goniothalamin. Different organs of *Goniothalamus andersonii*, *G. macrophyllus*, *G. malayanus* and *G. velutinus* were assayed for goniothalamin (Table 1). In all cases, the TLC method well separated goniothalamin from interfering compounds. Goniothalamin was detected in all parts tested of *G. andersonii* and *G. macrophyllus* and in some samples of *G. malayanus*, but was not detected in the leaves, stem bark or stem wood of two samples of *G. velutinus*. In the goniothalamin-containing species, the lowest yield was always found in the leaves. In the root and stem samples tested, the goniothalamin yield was usually higher in the wood than the bark. The yield of goniothalamin was very low in the young stem of *G. andersonii* and *G. malayanus* and it would seem that the yield increases with the age of the plant part. This age factor could be the reason for the inconsistent results obtained from *G. malayanus*, as stem bark sample D, in which goniothalamin was not detected, was from young stems. Age may also be a factor in sample B of *G. malayanus*, in which goniothalamin was not detected in the bark, stem wood, leaves or root wood, but was detected in very low quantity in the root bark. All samples of *G. malayanus*, when examined microscopically, showed similar characters, and could be differentiated from the other *Goniothalamus* species tested in this study. These results will be reported elsewhere.

EXPERIMENTAL

M.ps were determined on a Kofler Block and are uncorrected. IR spectra were measured as KBr discs, and NMR spectra in $CDCl_3$ unless otherwise specified. MS were recorded on Hitachi–Perkin–Elmer RMU 6 single focussing and A.E.I. 902 double focussing instruments at ionising potentials of 70 eV. Specific rotations were determined on a ETL-NPL Automatic Polarimeter Type 143A. TLC was carried out on Silica Gel Stahl (Merck).

Isolation of goniothalamin from G. andersonii *bark*. Dried *Goniothalamus andersonii* bark (1800 g) was exhaustively extracted with light petroleum in a Soxhlet. On concentration of the light petroleum extract to one-third of its original volume, goniothalamin (Ia; 21·4 g) crystallized as rods, m.p. 85°, $[\alpha]_D$ +170·3° (c, 1·38; $CHCl_3$).

Treatment of goniothalamin with NBS. Goniothalamin (0·387 g) and NBS (0·344 g) were refluxed in dry CCl_4 (75 ml) for 5 hr. The reaction mixture was cooled in ice-water and the unreacted NBS and succinimide filtered off. Evaporation of the filtrate gave an oil (0·536 g), which furnished on ether insoluble solid (0·155 g) and an ether-soluble oil (0·33 g) on trituration with ether. TLC on thick layers of SiO_2 of a sample of the solid (0·142 g) using CH_2Cl_2 as solvent afforded *threo*-7,8-*dibromogoniothalamin* (IIIb; 6 mg), m.p. 168° (dec) $[\alpha]_D$ +162° (c, 0·74; $CHCl_3$) as the faster running component, and *erythro*-7,8-*dibromogoniothalamin* (IIIa; 79 mg), m.p. 175–177° (dec), $[\alpha]_D$ −6° (c, 0·63; $CHCl_3$) as the slower running component. Chromatography of the ether soluble oil on silica gel (Merck 0·05–0·2 mm; 10 g) gave on elution with light petroleum–Et_2O (19:1 and 9:1)6-styryl-2-pyrone (VII; 170 mg) m.p. 116°, and unreacted goniothalamin (112 mg) on elution with light petroleum–Et_2O (9:1).

Quantitative TLC procedure for the estimation of goniothalamin. Goniothalamin, in $CHCl_3$, was applied in weights of 20, 40 and 60 μg to air-dried layers of silica gel G, wet thickness 250 μ, and developed with ether. Following development, goniothalamin was detected by spraying the plates uniformly with $SbCl_3$ in conc. HCl (3:1, w/v) and heating in a circulating air stream at 100° until the spots were a deep purple colour, this normally taking about 5 min. The plates were allowed to cool before the absorbances of the spots were estimated by a Vitatron densitometer using a slit width of 20 × 2 mm and a tungsten lamp source. The values so obtained were calculated by an automatic integrator. Spots were scanned in the direction opposite to that of solvent flow and erratic readings from extraneous light were avoided by the use of a light-tight box. The percentage error in the method was calculated by applying 20, 40 and 60 μg of goniothalamin to thin-layer plates and estimating the variations observed in spot intensities. Ten chromatograms, each of four

[25] G. Blunden, R. Hardman and J. C. Morrison, *J. Pharm. Sci.* **56**, 948 (1967).
[26] G. Blunden and R. Hardman, *J. Chromatogr.* **34**, 507 (1968).

spots, were prepared for each weight of gonothalamin. The results obtained by this method for gonothalamin were as follows:

Wt. applied (μg)	Goniothalamin recovered μg	%
20	20·5 ± 1·1	102·5 ± 5·5
40	39·8 ± 2·1	99·4 ± 5·2
60	59 6 ± 3·5	99·3 ± 5·8

Method of assay for goniothalamin in plant material. Goniothalamin was extracted from a known weight of plant material with light petroleum (40–60°) for 16 hr, the extract evaporated to dryness, re-dissolved in $CHCl_3$ and made up to a known volume. The extract was applied to a TLC plate along with a standard goniothalamin solution. The applied spots of the plant and standard solutions, of approximately equal goniothalamin concentration, were alternated on the plate to minimise the effects of variation in thickness and opacity of the adsorbent layer. The goniothalamin concentration was calculated relative to the standard solution, and the resultant value obtained from the mean of 6 plates, each containing 2 spots of the plant extract.

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Key Word Index—Goniothalamus andersonii; Annonaceae; goniothalamin; 5-hydroxy-7-phenylhepta-2,6-dienoic acid δ-lactone; 6-styryl-2-pyrone.