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THE CRYSTAL STRUCTURE AND CYTOTOXICITY OF GONIODIOL-7-MONOACETATE FROM *GONIOTHALAMUS AMUYON*

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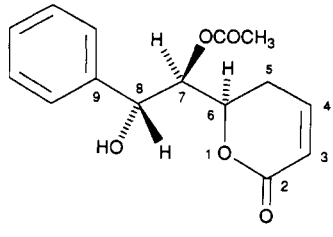
ABSTRACT.—The styrylpyrone, goniodiol-7-monoacetate [**1**] [6*R*-(7*R*,8*R*-dihydro-7-acetoxy-8-hydroxystyryl)-5,6-dihydro-2-pyrone], has been isolated from *Goniothalamus amuyon*, and its detailed molecular structure has been determined by X-ray crystallographic anaylsis. Goniodiol-7-monoacetate showed potent (ED_{50} values <0.1 μg/ml) cytotoxicities against KB, P-388, RPMI, and TE671 tumor cells.

Goniothalamus amuyon (Blanco) Merr. (Annonaceae) is a small tree or shrub indigenous to southern Taiwan near the coastal regions (1). Extracts of the seeds of *G. amuyon* have been used for the treatment of edema and rheumatism (2). In a previous phytochemical study, the stems of this plant were found to contain five isoquinoline alkaloids (3). Further investigations on structurally novel bioactive compounds from the leaves of *G. amuyon* have now resulted in the isolation of a cytotoxic styrylpyrone which from spectral data has been identified as the known goniodiol-7-monoacetate [**1**]. Compound **1** was first isolated from the leaves and twigs of *Goniothalamus sesquipedalis* and the bark of *Goniothalamus grifithii* by Talapatra *et al.* (4), who elucidated its constitution and proposed a 6*S*,7*S*,8*S* absolute stereochemistry from ^{1}H-nmr spectral data and steric arguments in conjunction with biogenetic considerations based on the 6*S* configuration assigned to goniothalamin (5). Gesson *et al.* (6), however, have subsequently suggested that, since several total syntheses of (+)-goniothalamin have shown it to possess a 6*R* configuration, the overall stereochemistry of **1** should be revised to 6*R*,7*R*,8*R*. An X-ray crystallographic analysis has now established the latter relative stereochemistry beyond doubt and provided details of the molecular geometry. The cytotoxicity of **1** is demonstrated for the first time.



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RESULTS AND DISCUSSION

The crystal structure of **1** was solved by direct methods. Fractional atomic coordinates are listed in Table 1[1]. The asymmetric unit consists of a pair of hydrogen-bonded [O-20 . . . O-15′ = 2.800(4) Å, O-20′ . . . O-15 = 2.782(4) Å] crystallographically independent molecules. Corresponding pairs of bond lengths and bond angles in each of these molecules agree well (max $\Delta = 0.029$ Å, mean $\Delta = 0.011$ Å for lengths; max $\Delta = 1.4°$, mean $\Delta = 0.66°$ for angles) and all are in accord with expectations (7). The conformations adopted by the molecules in the solid state, illustrated in Figure 1, are very similar except for the disposition of their phenyl rings, where the obviously different orientations with respect to the rest of the molecule may be ascribed to packing forces.

TABLE 1. Fractional Atomic Coordinates ($\times 10^4$, $\times 10^3$ for H[a]) for the Two Crystallographically Independent Molecules of Goniodiol-7-monoacetate [**1**], with Estimated Standard Deviations in Parentheses.

Atom	x	y	z	Atom	x	y	z
O-1	0(–)[b]	0(–)[b]	0(–)[b]	O-1′	4349(3)	−2334(2)	−2391(4)
C-2	−1273(4)	−570(2)	−969(7)	C-2′	3388(4)	−1778(2)	−1792(7)
C-3	−2502(4)	−644(3)	351(8)	C-3′	1820(4)	−1755(3)	−3549(8)
C-4	−2527(4)	−59(3)	2185(8)	C-4′	1281(4)	−2343(3)	−5404(10)
C-5	−1190(4)	638(2)	3136(7)	C-5′	2279(4)	−3032(3)	−5988(9)
C-6	281(4)	324(2)	2634(6)	C-6′	3997(3)	−2691(2)	−4998(6)
C-7	1654(4)	1005(2)	3160(5)	C-7′	5118(3)	−3384(2)	−5085(5)
C-8	3133(4)	692(2)	2529(6)	C-8′	6853(3)	−3031(2)	−4268(6)
C-9	4501(4)	1384(2)	3359(6)	C-9′	7920(3)	−3762(2)	−4271(5)
C-10	5540(5)	1390(3)	5730(7)	C-10′	7764(4)	−4295(3)	−6439(7)
C-11	6777(5)	2043(3)	6510(8)	C-11′	8741(5)	−4970(3)	−6406(8)
C-12	6969(5)	2702(3)	4974(9)	C-12′	9884(4)	−5103(2)	−4253(7)
C-13	5947(5)	2703(3)	2599(9)	C-13′	10053(5)	−4578(3)	−2102(8)
C-14	4732(5)	2057(3)	1782(7)	C-14′	9071(4)	−3907(2)	−2116(7)
O-15	−1345(3)	−962(2)	−2996(5)	O-15′	3842(3)	−1352(2)	222(5)
O-16	1215(3)	1744(1)	1615(4)	O-16′	4918(2)	−4071(1)	−3429(4)
C-17	1265(4)	2534(2)	2777(6)	C-17′	4271(4)	−4868(2)	−4511(6)
O-18	1556(4)	2662(2)	5034(5)	O-18′	3664(4)	−4995(2)	−6744(5)
C-19	900(5)	3217(2)	866(8)	C-19′	4426(6)	−5533(3)	−2546(8)
O-20	3472(3)	−88(2)	3802(5)	O-20′	7066(3)	−2409(2)	−6069(5)
H-3	−331(5)	−109(3)	−6(7)	H-3′	124(6)	−134(3)	−311(9)
H-4	−348(6)	−2(4)	302(9)	H-4′	34(6)	−235(4)	−635(10)
H-5A	−84(6)	75(3)	499(10)	H-5′A	198(5)	−313(3)	−784(8)
H-5B	−150(6)	118(4)	247(10)	H-5′B	203(5)	−351(3)	−467(8)
H-6	53(3)	−13(2)	368(5)	H-6′	416(4)	−221(2)	−612(6)
H-7	192(4)	128(2)	519(6)	H-7′	485(3)	−362(2)	−683(5)
H-8	286(4)	59(2)	70(6)	H-8′	720(4)	−276(2)	−238(6)
H-10	539(5)	91(3)	673(7)	H-10′	698(5)	−420(3)	−797(7)
H-11	754(6)	198(3)	839(9)	H-11′	856(6)	−541(3)	−795(9)
H-12	781(5)	324(3)	559(7)	H-12′	1060(6)	−559(4)	−410(10)
H-13	607(6)	308(3)	158(9)	H-13′	1096(6)	−466(4)	−41(10)
H-14	424(5)	205(3)	32(7)	H-14′	919(5)	−353(3)	−46(8)
H-19A	78(6)	303(4)	−92(9)	H-19′A	383(6)	−606(4)	−336(9)
H-19B	−16(7)	339(4)	104(10)	H-19′B	542(6)	−560(4)	−210(10)
H-19C	161(5)	377(3)	153(8)	H-19′C	419(6)	−531(4)	−78(10)
H-20	335(5)	−54(3)	262(8)	H-20′	760(8)	−189(4)	−485(11)

[a]Hydrogen atoms bear the same labels at the atoms to which they are bonded.
[b]The coordinates of O(1) were held constant throughout to define the space group origin.

[1]Atomic coordinates for goniodiol-7-monoacetate [**1**] have been deposited at the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2, 1EW, UK.

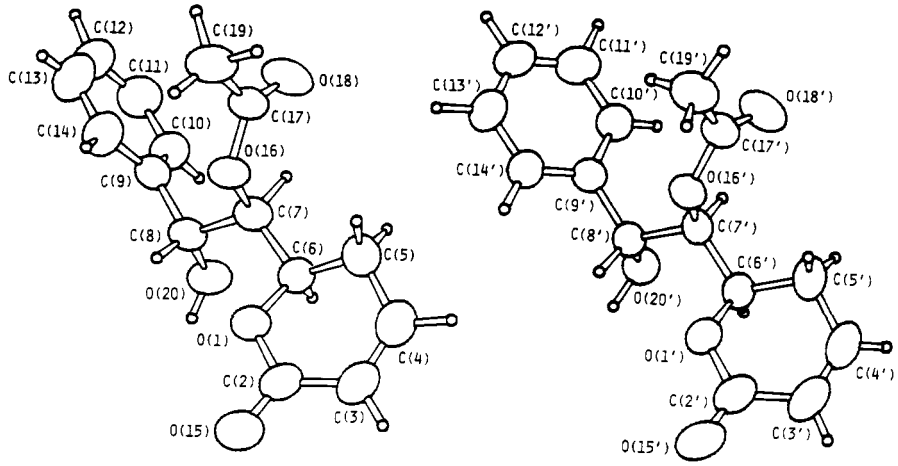

FIGURE 1. Atom numbering scheme and solid-state conformation of each of the two molecules of goniodiol-7-monoacetate [**1**] in the asymmetric crystal unit. Small circles represent hydrogen atoms.

Endocyclic torsion angles in the 5,6-dihydro-2-pyrone ring are related by an approximate mirror plane of symmetry passing through C-3 and C-6 and, with those around the adjacent C-2–C-3 and C-3–C-4 bonds being fairly small, the ring conformation is best described as a distorted half-boat (or envelope or sofa); a like conformation is adopted by the corresponding ring in goniotriol (8). Torsion angles (ω_{ij}, ± 0.4–$0.7°$) about the ring bonds in the unprimed molecule of **1**, with those for the primed molecule in parentheses, follow: $\omega_{1,2}$ 16.8(17.3), $\omega_{2,3}$ 12.4(10.7), $\omega_{3,4}$ −6.7(−5.2), $\omega_{4,5}$ −24.8 (−24.5), $\omega_{5,6}$ 50.4(48.8), $\omega_{6,1}$ −48.7(−47.7)°. Corresponding values, calculated by us from the coordinates provided in Alkofani *et al.* (8), which in fact are for the enantiomer of the structure shown and are of considerably lower precision (±2–3°) than those derived in the present study, follow: 21, 8, −6, −22, 46, −48°. The H-7–C-7–C-8–H-8 torsion angles at −168(3)° and −179(3)° in the unprimed and primed molecules, respectively, indicate that the solid-state conformation is consistent with that deduced earlier by Talapatra *et al.* (4) from ^{1}H-nmr spectral data as the preferred solution conformer.

Compound **1** demonstrated potent cytotoxicity against human epidermoid carcinoma of the nasopharynx (KB), murine lymphocytic leukemia (P-388), human melanoma (RPMI), and CNS carcinoma (TE671) with ED_{50} values of <0.1 μg/ml in all screens, but it lacked cytotoxic activity against human lung carcinoma A-549 and colon HCT-8 tumor cells when screened at 4.0 μg/ml. However, it is interesting to note that goniodiol, isolated from *Goniothalamus giganteus*, was found to show significant and selective cytotoxicity against A-549 human lung tumor cells ($ED_{50} = 0.12$ μg/ml) (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp was taken on a Yanagimoto micro-melting point apparatus and is uncorrected. The uv spectrum was obtained using a Hitachi 200-20 Spectrophotometer. The ir spectrum was measured on a Hitachi 260-30 Spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded at 200 and 50 MHz, respectively, in $CDCl_3$ using TMS as an internal standard with a Varian Gemini NMR Spectrometer. Eims spectrum was determined on a JEOL JMS-HX 110 mass spectrometer at 70 eV with a direct inlet system. Si gel 60 (Merck, 230–400 mesh) was used for cc, pre-coated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were employed for analytical tlc, and pre-coated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were utilized for preparative tlc.

PLANT MATERIAL.—The leaves of *G. amuyon* used in this investigation were collected in Hengchun, Pingtung Hsien, Taiwan in August 1989. A voucher specimen was deposited in the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

EXTRACTION AND ISOLATION OF GONIODIOL-7-MONOACETATE [**1**].—The fresh leaves (3.5 kg) of *G. amuyon* were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and further partitioned to yield *n*-hexane, $CHCl_3$, and aqueous extracts. Cytotoxicity bioassays in KB and P-388 cells detected the activity ($ED_{50} \leq 10$ μg/ml in each cell line) in the $CHCl_3$ extract. Chromatography of the $CHCl_3$ extract (48 g) on Si gel (1.8 kg, 43 × 6 cm) using $CHCl_3$/EtOAc/MeOH mixtures of increasing polarity as an eluting solvent yielded 50 fractions (200 ml each). The fractions (7.80 g) eluted with $CHCl_3$-EtOAc (10:1) were found to show significant cytotoxicity and were further purified by Si gel cc, eluted with a gradient of *n*-hexane and EtOAc. Goniodiol-7-monoacetate [**1**] was crystallized from fractions eluting with *n*-hexane–EtOAc (1:1). Recrystallization with a mixture of $CHCl_3$ and EtOAc afforded **1** (1.58 g) as white prisms: mp 148–149°; $[\alpha]^{25}D + 144.4°$ ($c = 0.1$, $CHCl_3$); ir (KBr) (cm^{-1}) ν max 3480 (OH), 1740, 1700, and 1240 (2-pyrone and acetyl), 760 and 750 (unsubstituted phenyl); uv λ max (MeOH) 215 nm (log ε 3.84). The eims exhibited peaks at *m/z* 170 (51%), 110 (100%), 107, and 105, suggesting a styrylpyrone skeleton (4,6). The ^{1}H nmr (200 MHz, $CDCl_3$) showed signals at δ 1.82 (3H, s, 7-OAc), 2.34 (2H, m, H-5), 3.12 (1H, br s, 8-OH), 5.08 (1H, ddd, $J = 9.6$, 4.8, 1.0 Hz, H-6), 5.12 (1H, d, $J = 8.0$ Hz, H-8), 5.16 (1H, dd, $J = 8.0$, 4.8, H-7), 6.02 (1H, ddd, $J = 9.6$, 2.2, 1.0 Hz, H-3), 6.90 (1H, ddd, $J = 9.6$, 4.8, 2.2 Hz, H-4), and 7.37 (5H, m, Ar-H). The ^{13}C-nmr (50.3 MHz, $CDCl_3$) spectrum revealed signals at δ 170.48 (s, C-2), 121.32 (d, C-3), 146.50 (d, C-4), 26.40 (t, C-5), 70.64 (d, C-6), 75.62 (d, C-7), 165.07 (s, $OCOCH_3$), 20.55 (q, $OCOCH_3$), 75.49 (d, C-8), 141.44 (s, C-9), 128.70 (d, C-10 and C-14), 127.54 (d, C-11 and C-13), and 128.70 (d, C-12).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF GONIODIOL-7-MONOACETATE [**1**].—Crystal data: $C_{15}H_{16}O_5$, MW = 276.29, triclinic, space group *P1* (No. 1), $a = 8.831(1)$ Å, $b = 15.321(1)$ Å, $c = 5.451(1)$ Å, $\alpha = 91.93(1)°$, $\beta = 105.08(1)°$, $\gamma = 94.40(1)°$ (from 25 orientation reflections, $42° < \theta < 47°$), $V = 709.5(3)$ Å^3, $Z = 2$, $D_c = 1.293$ g·cm^{-3}, μ(CuKα radiation, $\lambda = 1.5418$ Å) 7.7 cm^{-1}; crystal dimensions 0.04 × 0.10 × 0.50 mm.

Preliminary unit cell dimensions and space group information were derived from oscillation, precession, and Weissenberg photographs. One hemisphere of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, graphite monochromator; ω–2θ scans; scanwidth (1.00 + 0.14 tan θ)°; θ max = 75°]. The intensities of four reference reflections, remeasured every 2 h during data collection, showed no significant variation (<1%). From a total of 2912 non-equivalent measurements, those 2241 reflections with $I > 3.0\sigma(I)$ were retained for the analysis following corrections for the usual Lorentz and polarization effects.

The crystal structure was solved by direct methods (MULTAN-11/82). Initial carbon and oxygen atom coordinates were obtained from an *E* map. Hydrogen atoms were all located in a series of difference Fourier syntheses evaluated following several rounds of full-matrix least-squares adjustment of non-hydrogen atom positional and thermal parameters (at first isotropic, then anisotropic). With the inclusion of hydrogen atom positional and isotropic thermal parameters as well as an extinction correction (g) as variables in the subsequent least-squares iterations, the refinement converged (max shift, ESD = 0.01) at $R = \Sigma||F_o|-|F_c||/\Sigma|F_o| = 0.037$, $R_w = [\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.052$, $g = 1.9(1) \times 10^{-5}$, $GOF = [\Sigma w(|F_o|-|F_c|)^2/(N_{observations} - N_{parameters})]^{1/2}$. A final difference Fourier synthesis contained no unusual features (max. $\Delta\rho = 0.13$ e/Å^3).

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from the literature (10). During the least-squares iterations, $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o|-|F_c|)]$ was minimized.

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