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STRUCTURE AND STEREOCHEMISTRY OF (−)-ODORINOL, AN ANTILEUKEMIC DIAMIDE FROM *AGLAIA ODORATA**

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Key Word Index—*Aglaia odorata*; Meliaceae; Shu-Lan; (−)-odorinol; NMR; X-ray analysis; antileukemic diamide.

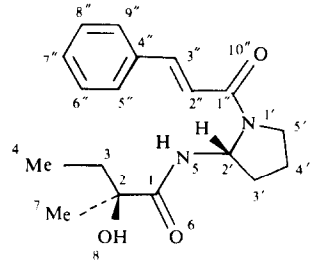
Abstract—Bioassay-directed isolation of an antileukemic extract of *Aglaia odorata* has led to the characterization of (−)-odorinol, a new diamide demonstrating significant *in vivo* antileukemic activity against P-388 lymphocytic leukemia growth in BDF_1 male mice. Its structure and relative stereochemistry were determined from physico-chemical data, spectral evidence and single-crystal X-ray analysis.

INTRODUCTION

Leaves and twigs of *Aglaia odorata*, known as 'Shu-Lan' in Chinese Folklore, are used as a herbal remedy for treatment of human cough, inflammation [1] and traumatic injury [2]. As a result of our continuing searches among Chinese medicinal plants for new naturally occurring potential antitumor agents, the methanolic extract of the leaves and twigs of *A. odorata* was found to show significant inhibitory activity *in vivo* against P-388 lymphocytic leukemia growth in BDF_1 male mice (T/C = 145%) at 50 mg/kg/day, I.P. We report herein on the isolation and structure determination of a new diamide, (−)-odorinol (**1**), which is the major active principle¶ from *A. odorata*.

RESULTS AND DISCUSSION

Compound **1** [$[\alpha]_D^{25}$ − 34.7° ($CHCl_3$, *c* 0.2)] has the molecular formula $C_{18}H_{24}N_2O_3$ as determined by an exact mass measurement of the molecular ion peak in the mass spectrum. Presence of a cinnamoyl moiety was indicated by the appearance of mass peaks at *m/z* 131 ($PhCH{=}CH\overset{+}{C}O$) and 103 (PhCH=CH), by IR bands (KBr) at 1640, 1594, 1525, 1340, and 997 cm^{-1}



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and substantiated by the presence in the ^1H NMR spectrum ($CDCl_3$)** of characteristic lowfield signals at δ 7.57 (1H, *d*, *J* = 15.1 Hz, H-3″), 6.94 (1H, *d*, *J* = 15.1 Hz, H-2″), and 7.20–7.50 (5H, *m*, aromatic protons). Double resonance experiments involving hydrogen atoms bonded to C-3, C-4, C-2′, and C-3′ yielded the following assignments: δ 0.90 (3H, *t*, *J* = 7.5 Hz, H-4), 1.63 (1H, *m*, H-3), 1.37 (1H, *d*, *J* = 16.0 Hz, H-3), 6.10 (1H, *q*, *J* = 9.47 and 6.44 Hz, H-2′), 2.27 (1H, *m*, H-3′), and 1.89–2.20 (3H, *m*, H-3′ and H-4′). A sharp three-proton singlet at δ 1.35 was assigned to the methyl group bonded to the tertiary

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¶ (−)-Odorinol showed significant (T/C ≥ 120%) inhibitory activity against P-388 lymphocytic leukemia growth in BDF_1 male mice (T/C = 136%) at the 5.0 mg/kg level. *In vivo* activity was assayed according to an exact lit. method [3].

**Lit. [4] reported the following different ^1H NMR data for "(+)-odorinol: δ ($CDCl_3$) 0.9 (3H, *t*, *J* = 7 Hz, H-4), 1.4 (3H, *s*, Me-C-2), 1.6 (2H, *q*, *J* = 7 Hz, H-3), 1.5–2.4 (4H, *m*), 3.2–3.9 (2*H*, *br* m, H-5′), 3.5 (1H, *s*, OH), 6.2 (1H, *br* m, H-2′), 6.9 (1H, *d*, *J* = 15 Hz, H-2″), 7.2–7.7 (6H, *m*, aromatic and NH) and 7.6 (1H, *d*, *J* = 15 Hz, H-3″). The differences between these values and those reported here might be due to the use of different instruments.

Table 1. Fractional atomic co-ordinates ($\times 10^4$; $\times 10^3$ for hydrogen atoms) for (−)-odorinol

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
C-1	350	2606	8200	H-3A	27	−171	686
C-2	40	617	7793	H-3B	106	−117	810
C-3	579	−568	7425	H-4A	110	−63	623
C-4	853	337	6627	H-4B	113	170	674
N-5	925	2699	9099	H-4C	28	40	604
O-6	74	4027	7708	H-5	108	146	948
C-7	−160	−536	8616	H-7A	−41	76	894
O-8	−635	895	6985	H-7B	31	−81	911
N-1′	2048	4507	9864	H-7C	−63	−137	825
C-2′	1230	4533	9554	H-8	−59	229	672
C-3′	1106	5035	10 564	H-2′	101	547	902
C-4′	1757	4287	11 397	H-3′A	65	455	1053
C-5′	2397	4536	11 000	H-3′B	114	679	1067
C-1″	2470	4642	9224	H-4′A	164	268	1143
C-2″	2064	4735	8092	H-4′B	185	459	1207
C-3″	2422	4821	7406	H-5′A	273	569	1119
C-4″	2082	5003	6280	H-5′B	275	341	1137
C-5″	1315	5193	5792	H-2″	156	509	785
C-6″	1023	5409	4725	H-3″	294	452	770
C-7″	1473	5450	4127	H-5″	90	498	613
C-8″	2224	5321	4582	H-6″	43	541	445
C-9″	2537	5075	5651	H-7″	126	565	337
O-10″	3151	4677	9587	H-8″	255	544	420
				H-9″	305	535	603

Table 2. Interatomic distances in (−)-odorinol

C-1–C-2	1.542	C-3–H-3A	1.13
C-1–N-5	1.351	C-3–H-3B	1.15
C-1–O-6	1.218	C-4–H-4A	1.07
C-2–C-3	1.527	C-4–H-4B	1.08
C-2–C-7	1.526	C-4–H-4C	1.12
C-2–O-8	1.405	C-5–H-5	1.01
C-3–C-4	1.490	C-7–H-7A	1.18
N-5–C-2′	1.460	C-7–H-7B	0.95
N-1′–C-2′	1.476	C-7–H-7C	1.05
N-1′–C-5′	1.469	C-8–H-8	1.05
N-1′–C-1″	1.368	C-2′–H-2′	0.96
C-2′–C-3′	1.510	C-3′–H-3′A	0.91
C-3′–C-4′	1.475	C-3′–H-3′B	1.24
C-4′–C-5′	1.497	C-4′–H-4′A	1.15
C-1″–C-2″	1.480	C-4′–H-4′B	0.90
C-1″–O-10″	1.229	C-5′–H-5′A	1.01
C-2″–C-3″	1.326	C-5′–H-5′B	1.05
C-3″–C-4″	1.461	C-2″–H-2″	0.94
C-4″–C-5″	1.400	C-3″–H-3″	0.95
C-4″–C-9″	1.404	C-5″–H-5″	1.04
C-5″–C-6″	1.382	C-6″–H-6″	1.06
C-6″–C-7″	1.362	C-7″–H-7″	0.99
C-7″–C-8″	1.364	C-8″–H-8″	0.95
C-8″–C-9″	1.389	C-9″–H-9″	0.97

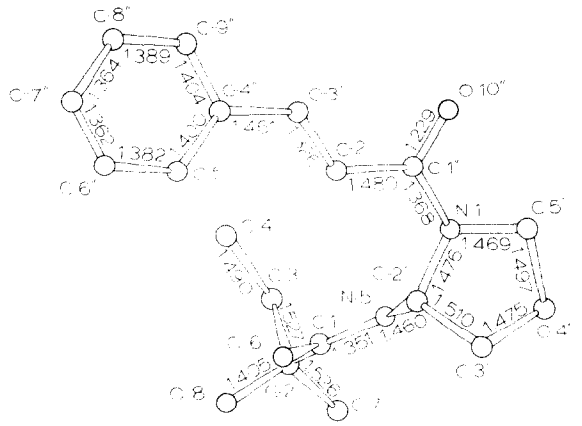

Fig. 1. Structure, relative stereochemistry and interatomic distances (0.004–0.007 Å) for (−)-odorinol; distances not given above are: C-1–C-2 1.542, C-1–O-6 1.218 Å.

center, C-2, bearing a hydroxy group. The presence of a tertiary hydroxy group, which resisted acetylation by acetic anhydride in pyridine at room temperature, was shown by the presence of a strong IR band at 3461 cm^{-1}, a mass peak at *m/z* 298 $[M-H_2O]^+$, and a one-proton singlet in the ^1H NMR spectrum at δ 3.10, which disappeared upon addition of D_2O. The remaining signals in the ^1H NMR spectrum were assigned as follows: δ 3.51 (1H, *m*, H-5′), 3.72 (1H, *m*, H-5′), and 7.46 (1H, *d*, $J = 9.47$ Hz, NHCO). The presence of this amide amino group was also reflected by an IR band at 3265 cm^{-1}. The foregoing evidence led to the conclusion that **1**, with undefined stereochemistry, represented the structure of the active compound, and this was supported by its ^{13}C NMR spectrum ($CDCl_3$): δ 174.86 (*s*, C-1), 165.86 (*s*, C-1″), 142.89 (*d*, C-2″), 134.98 (*s*, C-4″), 129.75 (*d*, C-7″), 128.73 (*d*, C-5″ and C-9″), 128.31 (*d*, C-6″ and C-8″), 118.16 (*d*, C-3″), 62.52 (*d*, C-2′), 46.08 (*t*, C-5′), 76.11 (*s*, C-2), 34.67 (*t*, C-4′), 33.14 (*t*, C-3′), 21.88 (*t*, C-3), 26.11 (*q*, Me-2), and 7.82 (*q*, C-4).

Although the plane structure of **1** is identical with that of odorinol [$[\alpha]_D^{25}+40.5°$ ($CHCl_3$, c 0.01)], isolated previously from the same plant collected in Thailand [4, 5], the opposite signs of their specific rotations suggested that these compounds might be enantiomers.

Unequivocal proof of the structure and relative stereochemistry of **1** was provided by single-crystal X-ray analysis (Tables 1 and 2). Crystals of **1** belong to the monoclinic system, space group $C2$, with a = 19.060(7), b = 6.994(3), c = 13.602(6) Å, β = 109.10(2)° Z = 4. the structure was solved by direct methods by use of MULTAN 76 [6]. Full-matrix least-squares refinement of atomic positional and thermal parameters* (anisotropic C, N, 0; isotropic H) converged to R = 0.050 over 1392 statistically significant [$I > 2.0\sigma(I)$] reflections measured on an Enraf–Nonius CAD-3 automated diffractometer (Ni-filtered Cu-$K\alpha$ radiation, λ = 1.5418 Å; $\theta - 2\theta$ scans) as described elsewhere [7]. A view of the solid state conformation is shown in Fig. 1. The tertiary hydroxy group is intramolecularly hydrogen bonded to the adjacent amide oxygen atom, O(8)...O(6) 2.59 Å.

EXPERIMENTAL

Mps are uncorr. Specific rotations were obtained on an automatic polarimater (l = 0.5 cm). ^1H and ^{13}C NMR spectra were determined (TMS as int. standard) at 250 and 62.89 MHz, respectively. MS were determined at 70 eV using a direct inlet system.

Isolation of (−)-odorinol (**1**). The ground air-dried leaves and twigs (4.55 kg) of *A. odorata*† Lour was exhaustively extracted with $CHCl_3$. Guided by an *in vivo* P-388 lymphocytic leukemia assay in mice [3], the active MeOH extract (255 g) was diluted with H_2O and extracted several times with hexane.

The aq. layer was then concd and extracted several times with $CHCl_3$. The active $CHCl_3$ layers were combined, dried and evaporated *in vacuo* to give 42 g of a residue which was column chromatographed on Si gel (Merck Si gel 60, 230–400 mesh, 1.5 kg, 7.5 × 104 cm) and eluted with $CHCl_3$–MeOH (49 : 1, 19 : 1, 4 : 1 and then 1 : 1) and finally MeOH. Fractions of 300 ml each were collected and examined by TLC. The active component **1** was isolated from fractions 13–17 in 0.01% yield as colorless needles (mp 162–163°) after recrystallization from EtOAc.

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*Atomic co-ordinates have been deposited with the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

†Specimens were gathered in September, 1979 in Pingtung Shen, Taiwan. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.