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LASIODIPLODIN, A POTENT ANTILEUKEMIC MACROLIDE FROM *EUPHORBIA SPLENDENS**

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Key Word Index—*Euphorbia splendens*; Euphorbiaceae; lasiodiplodin; NMR; X-ray; antileukemic macrolide.

Abstract—*In vivo* P-388 assay-directed fractionation of an active extract from *Euphorbia splendens* has led to the isolation of lasiodiplodin, a potent antileukemic macrolide, the structure of which was established from spectral data and a single-crystal X-ray analysis.

The stem, root and latex of *Euphorbia splendens* Bojer¶ (*Euphorbia milii* Ch. Des Moulins) [1, 2] are known as 'Chi-Lin-Hua' or 'Tie-Hai-Tang' in Chinese folklore and as herbal remedies for human hepatitis and abdominal edema [2]. As a result of the continuing search among plants for novel naturally occurring potential antitumor agents, the chloroform extract of the stems and leaves of *E. splendens* was found to show significant *in vivo* inhibitory activity against growth of the P-388 lymphocytic leukemia in the BDF_1 male mouse [3]. In vivo activity was assayed according to ref. [3]. Compound **1** (lasiodiplodin) demonstrated significant ($T/C \geq 120\%$) antileukemic activity in P-388 lymphocytic leukemia ($T/C > 140\%$) at 0.2 mg/kg/day I.P. Bioassay-directed fractionation of the active extract has now led to the isolation and characterization of **1** as the potent antileukemic principle. This appears to be the first instance in which a resorcylate macrolide has been demonstrated to have such activity.

Compound **1**, $C_{17}H_{24}O_4$, *m/z* 292.1676 $[M]^+$, mp 178–180° (from acetone–petrol) (ref. [4] reported mp 183–184° for lasiodiplodin), $[\alpha]_D^{20} + 4.8°$ ($CHCl_3$; *c* 1.2)[the $[\alpha]_D^{20}$ ($CHCl_3$; *c* 1.0) measured for an authentic sample of lasiodiplodin was +5.9°], was isolated in 0.00018% yield by repeated Si gel CC, prep. TLC, and HPLC of an active chloroform extract of *E. splendens*. The IR spectrum (KBr) of **1** contained absorption bands at 3382(OH), 1690(conjugated CO), and 1604 cm^{-1} (aromatic ring). A pair of narrowly split doublets at δ 6.24 (1H, *d*, $J = 1.5$ Hz, H-3) and 6.22 (1H, *d*, $J = 1.5$ Hz, H-5) in the ^1H NMR spectrum of **1** (250 MHz, $CDCl_3$)** indicated the presence of two *meta*-disposed aromatic protons. In addition, this spectrum contained one methoxy group signal as a sharp three-proton singlet at δ 3.71 (OMe-2), and one secondary methyl group signal as a doublet at δ 1.38 (3H, $J = 5.6$ Hz, Me-9′). A low-field one-proton multiplet at δ 5.29 was assigned to proton (H-8′) bonded to the carbon bearing a secondary methyl group as, upon irradiation of this multiplet, the Me-9′ doublet collapsed to a singlet; two one-proton multiplets at δ 1.92 and 1.67 were also simplified by this procedure. Conversely, irradiation at δ 1.38 (Me-9′) simplified both multiplet signals at δ 5.29 (H-8′) and 1.92, whereas irradiation at δ 1.67 simplified only the signal at δ 5.29. Consequently, the signals at δ 1.92 and 1.67 were assigned to two methylene protons (H_a-7′ and H_b-7′) which were adjacent to two protons at C-6′ as well as to H-8′, with the carbon (C-8′) bearing this latter proton in addition to Me-9′ being bonded either to an oxygen or to a fully substituted carbon atom. The two multiplets at δ 2.64 (1H) and 2.46 (1H) were assigned to the benzylic methylene protons H_c-1′ and H_d-1′. Upon irradiation of these two signals, the multiplets at δ 1.56–1.74 were simplified, indicating that H_c-1′ and H_d-1′ were adjacent to a methylene group (δ 1.56–1.74, 2H) at C-2′. Signals for the remaining methylene protons at C-3′–C-6′ occurred as overlapped multiplets in the δ 1.26–1.47 region.

The ^{13}C NMR (62.89 MHz, $CDCl_3$) spectrum confirmed the presence of one aromatic methoxy group (δ 55.87, *q*), a tetrasubstituted aromatic ring in which C-2 and C-4 were *O*-substituted, and C-1 and C-6 were *C*-substituted [δ 117.40, *s* (C-1); 158.07, *s* (C-2); 97.17, *d* (C-3); 157.87, *s* (C-4); 108.49, *d* (C-5) and 143.07, *s* (C-6)]††, one proton bonded to a carbon

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¶Specimens were gathered in 1979 in Taipei, Taiwan. A voucher specimen is available for inspection at the Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan.

**Ref. [4] reported the ^1H NMR (100 MHz, $CDCl_3$, TMS) assignments as follows: δ 6.12 (ArH), 5.22 (CHOR), 3.58 (OMe), 2.50 ($ArCH_2$) and 1.28 (Me).

††These assignments are in good agreement with those expected [5].

Table 1. Non-hydrogen atom fractional co-ordinates ($\times 10^4$) for **1**, with standard deviations in parentheses

Atom	x	y	z
C-1	−862(7)	4177(5)	1320(13)
C-2	−1176(7)	4516(6)	2122(12)
C-3	−1672(7)	5335(6)	2965(15)
C-4	−806(9)	5803(6)	3008(16)
C-5	−23(8)	5497(7)	2265(15)
C-6	−33(8)	4658(6)	1446(14)
C-7	−935(7)	3293(6)	429(13)
O-8	−1150(5)	3364(4)	−1201(9)
O-9	−743(6)	2585(4)	1140(11)
O-10	−2423(4)	3985(4)	1971(10)
C-11	−3291(8)	4308(7)	2734(17)
O-12	−861(6)	6616(5)	3870(13)
C-1′	863(9)	4306(8)	604(20)
C-2′	1083(12)	4805(10)	−1087(24)
C-3′	1746(19)	4409(15)	−2186(36)
C-4′	1451(20)	3445(17)	−2909(25)
C-5′	782(21)	3189(21)	−4394(23)
C-6′	7(21)	3337(16)	−4197(25)
C-7′	−373(20)	2470(13)	−3190(36)
C-8′	−1173(9)	2576(7)	−2297(19)
C-9′	−2072(17)	2592(13)	−3148(36)

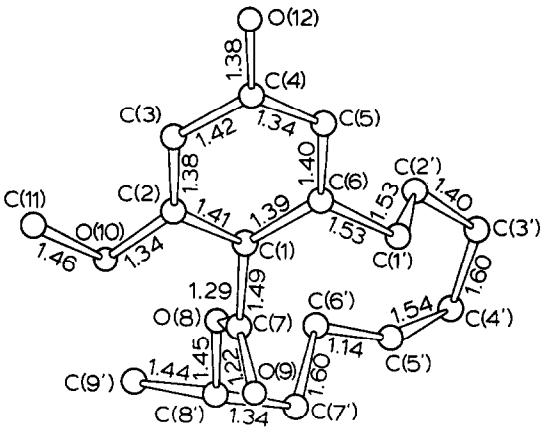
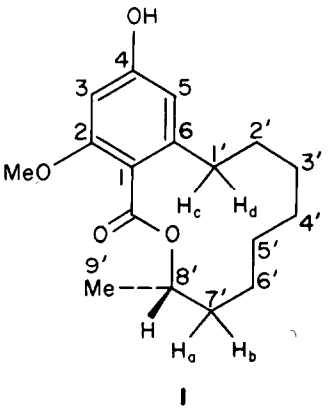


Fig. 1. Structure and solid-state conformation and inter-atomic distances of **1**.



1

atom bearing an oxygen function [δ 72.49, *d* (C-8′)] and seven methylene triplets [δ 32.46 (C-1′); 30.46, 30.08, 26.47, 25.55, 24.26 and 21.47 (C-2′–C-7′)],† indicating the presence of a polymethylene system in **1**.

The foregoing evidence suggested that **1** was a macrolide in which the lactone carbonyl group was bonded directly to an aromatic ring. A survey of the literature indicated the similarity of **1** and the resorcyclic acid lactone lasiodiplodin which is a fungal metabolite of *Lasiodiplodia theobromae* [4] and for which the structure had been determined on the basis of oxidative and spectral studies [5].

Unequivocal proof of the structure of **1** was provided by a single-crystal X-ray analysis. Crystals of **1** belong to the orthorhombic system, space group $P2_12_12_1$, with $a = 14.313(5)$ Å; $b = 14.920(5)$ Å; $c = 7.667(4)$ Å; $U = 1637$ Å^3; $Z = 4$; $D_{calc.} = 1.186$ g/cm^3. Intensity data, recorded on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered Cu-K_α radiation, $\lambda = 1.5418$ Å; θ–2θ scans), yielded 705 statistically significant [$I > 2.0\sigma(I)$] (see ref. [6]) reflections. The structure was solved by use of MULTAN [7]. Full-matrix least-squares refinement of atomic positional and thermal (anisotropic C, O; fixed H contributions) parameters‡ converged to $R = 0.081$. A view of the structure showing the solid-state conformation and inter-atomic distances (± 0.01–0.04 Å‖) is shown in Fig. 1. Molecules of **1** are associated in the crystal by an intermolecular O . H . . . O hydrogen bond between the aromatic hydroxy and lactone carbonyl groups (O . . . O 2.71 Å).

Confirmation that **1** and lasiodiplodin were identical was obtained by direct comparison (mmp, co-TLC, IR, NMR and specific rotation) with an authentic sample.

†These signal assignments may be reversed.

‡A list of co-ordinates for the non-hydrogen atoms in **1** has been deposited at the Cambridge Crystallographic Data Centre.

‖Conformational flexibility of the C-2′–C-7′ polymethylene moiety of the 12-membered lactone ring is reflected in the very anisotropic thermal parameters (U_{ii} range 0.07–0.56 Å^2) for these atoms as well as C-9′. Consequently, the least-squares derived s.d.s are grossly under-estimated in the polymethylene moiety, and the apparent extremely short C-5′–C-6′ single bond distance arises from the especially ill-defined positions of this pair of atoms which are furthermost from the more rigid aromatic ring system and its immediately bonded substituents.

EXPERIMENTAL

Extraction of Euphorbia splendens. The ground air-dried stems (9.5 kg) of *E. splendens* were exhaustively extracted with $CHCl_3$. Guided by an *in vivo* P-388 lymphocytic leukemia assay in mice, the $CHCl_3$ extract (120 g) was added

with H_2O and extracted several times with hexane. The aq. layers were concd and then extracted with $CHCl_3$. The active $CHCl_3$ layers were dried and evaporated *in vacuo* to give 47 g residue.

Isolation of lasidiplodin (1). CC was performed on the above residue (47 g) using Si gel (Merck Si gel 60, 70–230 mesh, 8 × 120 cm) with $CHCl_3$, $CHCl_3$–MeOH (95:5, 90:10, 80:20 and then 50:50) and MeOH as the eluting solvents. Fractions of 300 ml each were collected and examined by TLC. Further CC was performed on fractions 6–7 (7 g), which concd the activity, using Si gel [Merck Si gel 60, 300 g, 3.5 × 104 cm; $CHCl_3$, $CHCl_3$–MeOH (95:5) and MeOH were used as the eluting solvents, and fractions of 200 ml each were collected] to yield fractions (Nos. 4 and 5) (1.3 g) which showed potent antileukemic activity. Prep. TLC of these two fractions led to the isolation of the active principle, 1 (17 mg). Further purification of 1 was achieved by HPLC (Si, Partisil M9, 50 × 9.4 mm, $CHCl_3$, 4 ml/min flow rate).

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