Structure and Stereochemistry of Amorphispironone, a Novel Cytotoxic Spironone Type Rotenoid from *Amorpha fruticosa*

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A novel cytotoxic spironone type rotenoid, amorphispironone **1** has been isolated from the leaves of *Amorpha fruticosa* and its structure and stereochemistry have been established from spectral data in conjunction with single-crystal X-ray analysis.

Crude extracts of *Amorpha fruticosa* have been shown to exhibit feeding deterrence along with insecticidal, antiparasitic, antimicrobial and hypotensive activities.[1] As a result of our continuing searches for novel cytotoxic antitumour compounds from plants, amorphispironone **1**, a novel rotenoid, has been isolated from the leaves of *Amorpha fruticosa* as an active principle.† We report herein, the isolation and characterization of **1**.

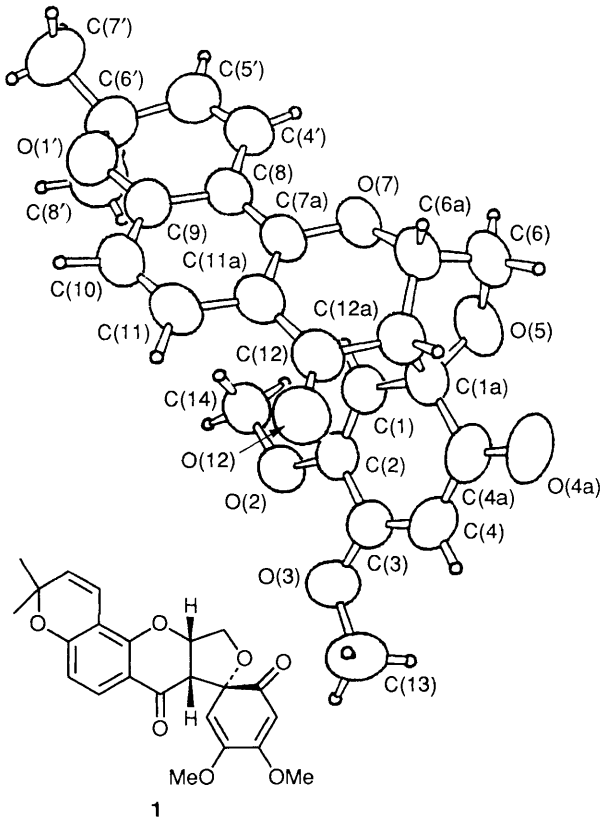

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Fig. 1 Structure and solid-state conformation of amorphispironone **1**; small circles represent hydrogen atoms

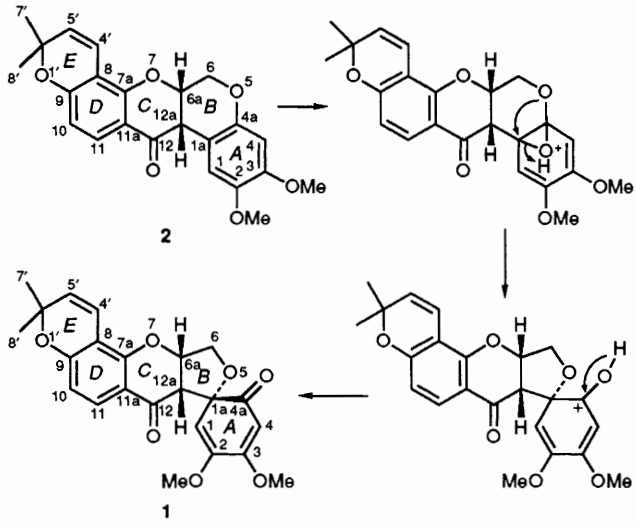
† Amorphispironone (1) showed significant (ED_{50} ≤4.0 μg ml^{-1}) selective cytotoxicity in KB (ED_{50} = 0.58 μg ml^{-1}) and RPMI (malignant melonoma) (ED_{50} = 0.61 μg ml^{-1}) cells. Compound **1** was inactive against A-549 (lung), HCT-8 (colon) and TE671 (human medulloblastoma) tumour cells at 10 μg ml^{-1}. The cytotoxicity assay was carried out according to literature methods.[6,7]

Amorphispironone **1**‡ {$C_{23}H_{22}O_7$, colourless crystals from 80% aqueous MeOH, m.p. 152–152.5 °C, $[\alpha]_D$ −61.6° (*c* 0.162 in MeOH), λ_{max} nm (MeOH) (log ε) 269 (4.51) and 316 (4.05)} was isolated from the crude chloroform extract of *Amorpha fruticosa* by silica gel chromatography. Many rotenoids have been isolated from the fruit[2,3] and the root bark[4] of this same plant. The general features of the NMR spectra of **1** suggested that it also was a rotenoid. Comparison of the ^{1}H NMR spectral data for **1** with those of the known rotenoid deguelin **2**[5] revealed that the *C–D–E* ring protons were similar but the *A–B* ring protons were different. These data suggested that **1** was a rotenoid, like **2** but differing in the *A/B* ring region. The complete structure and stereochemistry of amorphispironone were established unequivocally by single-crystal X-ray analysis.§¶ A view of the solid-state conformation is provided in Fig. 1. In general, bond lengths

‡ EI mass *m/z*: 410.1364 (M^+). $C_{23}H_{22}O_7$ requires *m/z* 410.137. IR (KBr): ν_{max}/cm^{-1} 3010, 2970, 1665, 1640, 1625, 1575, 1393, 1270, 1110, 1070 and 820. NMR spectral assignments were made on the basis of ^{1}H–^{13}C COSY and long range ^{1}H–^{13}C COSY spectra. ^{1}H NMR ($CDCl_3$, 300 MHz, *J* in Hz) δ 5.02 (1H, s, 1-H), 5.52 (1H, s, 4-H), 4.50 (1H, dd, *J* 3 and 10, 6-H_a), 4.64 (1H, d, *J* 10, 6-H_b), 5.27 (1H, dd, *J* 3 and 4.5, 6a-H), 6.50 (1H, d, *J* 9, 10-H), 7.67 (1H, d, *J* 9, 11-H), 3.44 (1H, d, *J* 4.5, 12a-H), 3.25 (3H, s, 2-OMe), 3.84 (3H, s, 3-OMe), 6.67 (1H, d, *J* 10, 4'-H), 5.64 (1H, d, *J* 10, 5'-H) and 1.44 (3H, s, 7'-H or 8'-H), 1.47 (3H, s, 7'-H or 8'-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.8 (d, C-1), 84.1 (s, C-1a), 148.3 (s, C-2), 166.2 (s, C-3), 99.8 (d, C-4), 198.8 (s, C-4a), 76.0 (t, C-6), 82.7 (d, C-6a), 160.1 (s, C-7a or C-9), 109.4 (s, C-8), 156.3 (s, C-7a or C-9), 111.7 (d, C-10), 127.6 (d, C-11), 114.5 (s, C-11a), 186.0 (s, C-12), 60.1 (d, C-12a), 55.1 (q, C-2-OMe), 55.7 (q, C-3-O*Me*), 115.6 (d, C-4'), 129.2 (d, C-5'), 77.7 (s, C-6'), 28.0 (q, C-7' or C-8') and 28.2 (q, C-7' or C-8').

§ *Crystal data* for **1**: $C_{23}H_{22}O_7$, *M* = 410.43, monoclinic, space group $P2_1$, *a* = 12.596(2), *b* = 8.635(1), *c* = 9.763(1) Å, β = 102.03(1)°, *U* = 1038.6(4) Å^3, *Z* = 2, D_c = 1.312 g cm^{-3}, μ(Cu-Kα radiation, λ = 1.5418 Å) = 7.7 cm^{-1}. Intensity data [±*h*, + *k*, + *l*; θ_{max} = 75°, scan width (0.75 + 0.14tanθ)°; 2279 non-equivalent reflections] were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, graphite monochromator). The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement of atomic parameters (anisotropic C, O; isotropic H) converged at *R* = 0.032 (R_w = 0.047) over 1975 reflections with $I > 3.0\sigma(I)$. Crystallographic calculations were performed by use of the Enraf-Nonius SDP package. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

¶ The absolute stereochemistry represented by structure **1** could not be established from the X-ray data. It follows, however, by assuming identical configurations at common asymmetric centres in **1** and another rotenoid derivative with which it co-occurs and for which the absolute stereochemistry has been established in our laboratory by an X-ray crystallographic analysis.



Scheme 1

agree well with expected values. Bond strain, however, is evident in the elongated C(1a)–C(12a) [1.572(3) Å] and C(1a)–C(4a) [1.548(4) Å] bonds. Ring *A* is fairly flat but puckered slightly towards a shallow envelope form with C(1a) as the out-of-plane atom,‖ rings *B* and *C* have envelope conformations with C(6a) as the out-of-plane atom in each case, ring *D* is planar, while ring *E* approximates to a 1,3-diplanar form.

The unusual spiro *A*–*B* ring system in **1** is probably biogenetically derived from **2** in the manner indicated in Scheme 1.

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‖ Endocyclic torsion angles (ω_{ij}, σ ±0.2–0.4°) about the bonds between atoms *i* and *j* follow: $\omega_{1a,1}$ 12.8, $\omega_{1,2}$ −3.3, $\omega_{2,3}$ −6.4, $\omega_{3,4}$ 4.9, $\omega_{4,4a}$ 5.6, $\omega_{4a,1a}$ − 13.8° in ring *A*; $\omega_{1a,5}$ 0.1, $\omega_{5,6}$ −24.1, $\omega_{6,6a}$ 38.4, $\omega_{6a,12a}$ −37.2, $\omega_{12a,1a}$ 23.5° in ring *B*; $\omega_{6a,7}$ 45.4, $\omega_{7,7a}$ −19.0, $\omega_{7a,11a}$ −6.3, $\omega_{11a,12}$ 2.8, $\omega_{12,12a}$ 24.2, $\omega_{12a,6a}$ −47.5° in ring *C*; $\omega_{7a,8}$ 3.6, $\omega_{8,9}$ −0.6, $\omega_{9,10}$ −2.0, $\omega_{10,11}$ 1.6, $\omega_{11,11a}$ 1.3, $\omega_{11a,7a}$ −3.9° in ring *D*; $\omega_{8,9}$ −1.1, $\omega_{9,1'}$ 26.8, $\omega_{1',6'}$ −37.9, $\omega_{6',5'}$ 26.9, $\omega_{5',4'}$ −4.2, $\omega_{4',8}$ −10.3° in ring *E*.

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