Ligulaverin A, a structurally novel sesquiterpenoid-derived metabolite possibly formed *via* a biological Diels–Alder reaction

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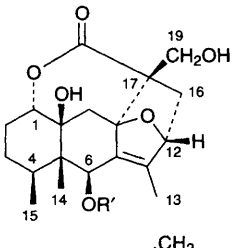
The crystal structure of Ligulaverin A 1, isolated from *Ligularia veitchiana* (Hemsl.) Greenm., reveals a novel carbocyclic skeleton which is likely to be derived from a hydroxymethacrylate ester of an eremophilane sesquiterpenoid *via* an intramolecular Diels–Alder reaction.

Pericyclic reactions are rare but not unknown in biological systems. However, considerable controversy attends the contention that the Diels–Alder reaction is amongst Nature's repertoire of enzyme-catalysed processes.[1] Indeed, tentative evidence that a true enzyme-catalysed Diels–Alder reaction might actually occur *in vivo* has only recently emerged from cell-free studies on the biosynthesis of the solanapyrones.[2] Here we report the structure of a new biologically active terpenoid, the skeleton of which appears to be biogenetically derived *via* an intramolecular Diels–Alder process.

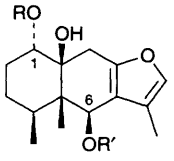
As part of a wider study of medicinally active constituents of the Chinese flora, we investigated the biologically active principles extracted from *Ligularia veitchiana* (Hemsl.) Greenm. (Compositae), tinctures of which have traditional use in Chinese medicine[3,4] as a treatment for influenza, coughs, ulcers and tuberculosis. Fractionation of a ethyl acetate extract of *L. veitchiana* whole plant tissue by partition and adsorption chromatography afforded ligulaverin A **1**, as a major bioactive metabolite. Spectroscopic studies of **1** (^{1}H and ^{13}C NMR, IR, MS *etc.*) showed it to be an eremophilane derivative with 19 skeletal carbon atoms, but the limited quantities of the active compound available precluded a complete spectroscopic structural assignment. Eventually we were able to obtain suitable crystals for an X-ray crystallographic study (using Cu radiation) and were able to assign the structure **1** to the metabolite,‡ and then to complete assignment of the spectroscopic data.§

The asymmetric unit in the crystal structure of **1** contains four molecules, which have essentially identical structures, but differ in the conformations of the substituent hydroxy, hydroxymethyl and hydroxymethacrylate ester groups (Fig. 1). The molecules are linked by a complex network of hydrogen bonds involving 11 of the 12 hydroxy group hydrogen atoms. The carbocyclic structure of **1** is unprecedented, but can be formally regarded as being related to the known eremophilane sesquiterpenoid, euryopsol **2**, which has previously been isolated from *Euryops* spp. (Compositae).[5] A plausible biosynthetic origin for the skeleton of **1** emerges from consideration that it is derived from the 1-(hydroxy)methacrylate ester **2a** [or the 1,6-di(hydroxymethacrylate) ester **2b**] of **2** *via* an intramolecular Diels–Alder reaction in which the polarised double bond of the hydroxymethacrylate ester acts as a dienophile. Modelling studies (AM1 calculations) support this contention, showing that the side-chain double bond can achieve overlap with the furan to yield a classical [4 + 2] product, although the energy demands of attaining the strained conformation of the transition state are only partially compensated by the energy released in the addition reaction. It therefore seems probable that the biosynthetic route to the carbocyclic skeleton of **1** involves an internal Diels–Alder reaction of **2a** or **2b**.

There remain three possible hypotheses for the origin of this skeleton. The possibility that the compound **1** is an artifact produced during the isolation can be ruled out because the isolation conditions did not involve the use of elevated temperature, nor did heating of the extracts increase the yields of the metabolite. Two other plausible rationalisations remain:



1 R′ = CO–C(=CH$_2$)–CH$_2$OH



2 R = R′ = H

2a R′ = H; R = CO–C(=CH$_2$)–CH$_2$OH

2a R = R′ = CO–C(=CH$_2$)–CH$_2$OH

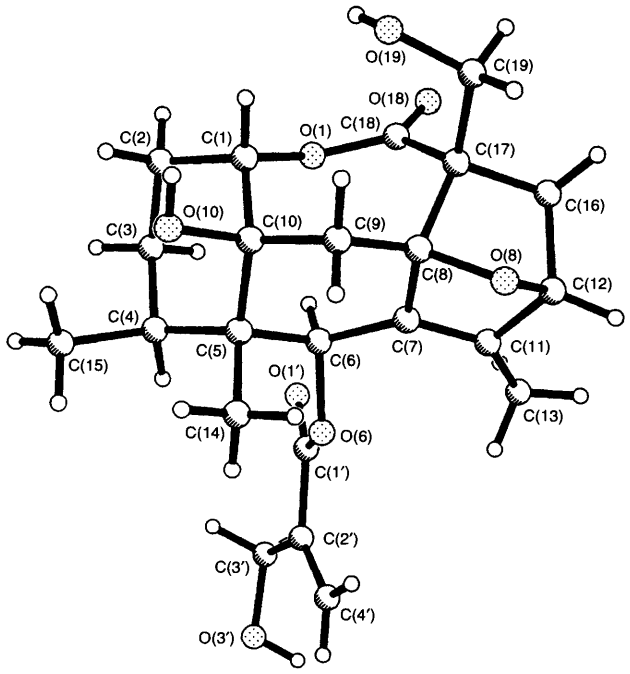


Fig. 1 The structure of **1**: there are four such molecules in the asymmetric unit, differing only in the orientations of the substituent groups

(*a*) that **1** (or its 6-hydroxy precursor **1a**) is produced by a non-enzymatic Diels–Alder reaction by the effect of solar heating of **2b** or **2a**, respectively, in the plant tissue, or that (*b*) the metabolite is the product of a true enzymatic Diels–Alder'ase process. As the AM1 calculations indicate that compound **1** is about 15 kJ mol^{-1} less stable than its precursor **2b**, which is not among the products isolated from the plant, there is strong evidence tht the third hypothesis is indeed the correct one. These latter questions are the subject of current studies in our laboratories.

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Footnotes

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‡ *Crystal data* for **1**: $C_{23}H_{30}O_8$, M = 434.47, monoclinic, $P2_1$, a = 12.179(4), b = 19.598(7), c = 18.769(5) Å, β = 108.16(2)°, V = 4257(2) Å^3 [from 2θ values for 31 reflections measured at ±ω (40 < 2θ < 44°), λ = 1.54184 Å], Z = 8, D_{calc} = 1.356 g cm^{-3}, $F(000)$ = 1856, T = 150 K, μ(Cu-Kα) = 0.849 mm^{-1}. Colourless plate developed in (001), 0.62 × 0.51 × 0.02 mm^3. Data were collected in the range 5 < 2θ < 120° ($-14 \leq h \leq 14$; $-2 \leq k \leq 22$, $0 \leq l \leq 21$) using Cu-Kα radiation and ω–2θ scans on a Stoe Stadi-4 four-circle diffractometer equipped with an Oxford Cryosystems low-temperature device operating at 150 K (J. Cosier and A. M. Glazer, *J. Appl. Crystallogr.*, 1986, **19**, 105). Following application of an absorption correction based on psi-scan measurements (T_{min} = 0.449; T_{max} = 1.00) the structure was solved by direct methods (SIR92, A. Altomare, G. Cascarano, C. Giacovazzo and A. Guagliardi, *J. Appl. Crystallogr.*, 1993, **26**, 343). Refinement by full-matrix least-squares versus F^2 (SHELXTL-96, G. M. Sheldrick, University of Göttingen, Germany, 1996) was performed with hydrogen atoms placed in calculated positions and allowed to ride on the atoms to which they are attached. Hydroxy group hydrogens were placed so as to form the 'best' hydrogen bonds while being staggered with respect to the bonds made by the neighbouring carbon atoms. There is two-fold rotational disorder in the CH_2OH substituent on C-17 in one of the four molecules comprising the asymmetric unit, and this was modelled using two alternative positions for O-19 in the ratio 70:30; the two fragments were constrained to have similar geometries in subsequent cycles of refinement. Anisotropic displacement parameters were refined for all non-hydrogen atoms except for the minor component of the disordered CH_2OH group; global similarity and rigid bond restraints were placed on the thermal parameters. At convergence $R1$ = 7.09% [based on F and 4792 unique data with $F > 4\sigma(F)$], w$R2$ = 19.42% (based on F^2 and all 6715 unique data used for refinement) for 1210 parameters; ΔF max. and min. were 0.37 and −0.43 eÅ^{-3}. The Flack absolute structure parameter refined to −0.2(3). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See information for Authors, Issue No. 1. Any request to the CCDSC for this material should quote the full literature citation and the reference number 182/249.

§ *Selected spectroscopic data* for **1**: ^{13}C NMR (100 MHz, CD_3OD) δ 10.4 (C-13), 16.8 (C-14), 17.4 (C-15), 24.1 (C-3), 24.5 (C-2), 34.4 (C-4), 35.5 (C-16), 41.7 (C-9), 46.1 (C-5), 61.4 (C-17), 61.6 (C-19), 67.2 (C-3′), 71.7 (C-1), 74.9 (C-10), 83.4 (C-12), 84.9 (C-6), 87.2 (C-8), 125.7 (C-4′), 134.9 (C-2′), 141.6 (C-11), 146.8 (C-7), 167.1 (C-1′) and 176.9 (C-18); ν_{max}(KBr)/cm^{-1} include 3469, 1751 and 1730; MS *m/z* (rel. int.): 434 [M^+, 3], 416 [$M - H_2O^+$, 17], 332 (29), 248 (40), 230 (100), 213 (40), 188 (30), 170 (32), 162 (27), 124 (51) and 85 (62).

References

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