Structure and Stereochemistry of Microlenin, a Novel Antitumour Dimeric Sesquiterpene Lactone from *Helenium microcephalum*; *X*-Ray Crystal Structure

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Summary The structure and stereochemistry of microlenin, a novel antitumour sesquiterpene lactone isolated from *Helenium microcephalum*, have been established from spectral and single-crystal *X*-ray analyses.

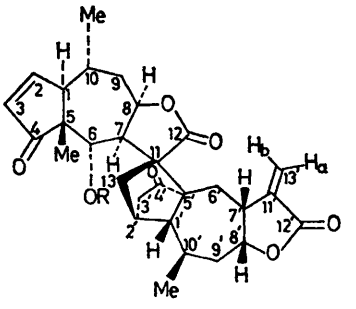
WE reported recently the structure determination of a new antitumour pseudoguaianolide, microhelenalin-A,† which was isolated in addition to helenalin from *Helenium microcephalum*.‡ Further examination of *H. microcephalum* has now resulted in the isolation of a novel dimeric sesquiterpene lactone, microlenin (I), which also has significant antitumour activity.§ Microlenin appears to arise from a Diels–Alder type condensation involving the 11,13-double bond of helenalin and the enol form of the cyclopentenone ring of a norpseudoguaianolide.

† K. H. Lee, Y. Imakura, and D. Sims, *J. Pharm. Sci.*, submitted. For the previous paper in the series 'Antitumor Agents,' see K. H. Lee, T. Kimura, M. Okamoto, C. M. Cowherd, A. T. McPhail, and K. D. Onan, *Tetrahedron Letters*, in the press.

‡ Specimens were gathered in June, 1972, in Texas. We thank Professor John J. Sperry, Texas A & M University, for collecting and identifying the plant material. The constituents of *H. microcephalum* were examined previously and reported to contain helenalin in good yield (R. Adams and W. Herz, *J. Amer. Chem. Soc.*, 1949, **71**, 2546).

§ Microlenin showed significant (T/C $\geqslant$ 125%) inhibitory activity against Walker 256 carcinosarcoma in rats (T/C 173%) at the 2·5 mg/kg level. *In vivo* activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, by a literature method [R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.* (*Part* 3), 1972, **3**, 1].

Microlenin (I), $C_{29}H_{34}O_7$, m.p. 280 °C (decomp.), $[\alpha]_D^{22}$ + 10·0 (*c.* 1·00, pyridine), shows i.r. bands (KBr) at 3521 (OH), 1763 (γ-lactone), 1756, 1664 (α-methylene-γ-lactone), 1744 (cyclopentanone), and 1707 cm^{-1} (cyclopentenone). Acetylation of (I) with acetic anhydride in pyridine gave a monoacetate (II), $C_{31}H_{36}O_8$, m.p. 278 °C (decomp.), *m/e* 536·2407



(I) R = H
(II) R = COMe

(M^+). The ^1H n.m.r. spectrum ($CDCl_3$) of (II) indicated the presence of three methyl groups [δ 0·94 (3H, s, 5-Me), 1·23 (3H, d, *J* 7·0 Hz, 10-Me), and 1·18 (3H, d, *J* 7·0 Hz, 10′-Me)], an acetyl group [δ 1·97 (3H, s, 6-OAc)], a cyclopentenone ring system [δ 7·67 (1H, dd, *J* 2·0 and 6·0 Hz, 2-H) and 6·06 (1H, dd, *J*. 3·0 and 6·0 Hz, 3-H)], and an α-methylene-γ-lactone unit bearing a proton at the β-position (7′-H) [δ 6·28 (1H, d, *J* 3·0 Hz, 13′-H_a) and 5·52 (1H, d, *J* 3·0 Hz, 13′-H_b)]. Extensive double-resonance experiments identified other proton signals at δ 5·62 (1H, s, 6-H), 2·48 (1H, d, *J* 8·0 Hz, 7-H),¶ 4·95 (1H, m, 8-H), 3·29 (1H, m, 7′-H), and 4·71 (1H, m, 8′-H).

The co-occurrence of (I) with helenalin coupled with the foregoing evidence suggested that it had one of two possible dimeric sesquiterpene lactone structures resulting from a Diels–Alder type condensation which involved either the 11,13-double bond of helenalin and the enol form of the cyclopentenone ring of a nor-pseudoguaianolide as shown in (I), or conversely, the 11′–13′-double bond of a nor-pseudoguaianolide and the cyclopentenone ring of helenalin. Of these, the former was considered the more probable as the high-resolution mass spectrum of (II) revealed diagnostically important peaks at *m/e* 95 (C_7H_{11}), 123 ($C_8H_{11}O$ and $C_7H_7O_2$), and 124 ($C_8H_{12}O$ only), which are indicative of the presence of a cyclopentenone-bearing helenalin-type pseudoguaianolide with a 4-, 6-, and 8-oxygenation pattern and in which the exocyclic double bond at C(11) is saturated.[1]

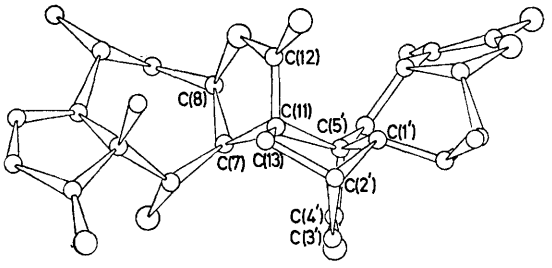


FIGURE. Conformation of (I).

The structure and stereochemistry of (I) were established unequivocally by single-crystal *X*-ray analysis. Crystals of (I) are monoclinic, space group $P2_1$, with a = 14·23(1), b = 12·28(1), c = 7·26(1) Å, β = 106·3(1)°, Z = 2. The crystal structure was solved by direct non-centrosymmetric phase-determining procedures using MULTAN.[2] Positional and anisotropic thermal parameters of the non-hydrogen atoms were refined by full-matrix least-squares calculations to R 0·050 over 1606 statistically significant [$I > 2{\cdot}0\,\sigma(I)$] reflections measured on an Enraf-Nonius CAD-3 automated diffractometer (θ–2θ scans; Ni-filtered Cu-K_α radiation, λ = 1·5418 Å). The solid-state conformation is shown in the Figure.

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¶ The upfield shift of this proton is due to an anisotropic effect caused by the proximity of the carbonyl group at C(4′) as shown by the solid-state geometry and reproduced in Dreiding molecular models; it is also indicative of the absence of a double bond at C(11).

[1] L. Tsai, R. J. Highet, and W. Herz, *J. Org. Chem.*, 1969, **34**, 945.
[2] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.