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THE STRUCTURE AND ABSOLUTE CONFIGURATION OF PLENOLIN, A CYTOTOXIC SESQUITERPENE LACTONE

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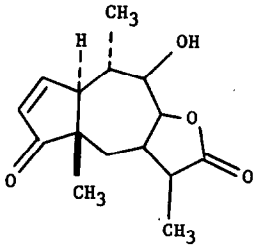
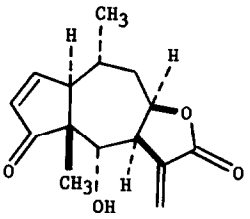
We had previously reported[1] the isolation of a new sesquiterpene lactone, plenolin, from Baileya pleniradiata Harv. and Gray in 0.0003% yield. Too little plenolin was obtained to permit detailed chemical investigation although the structure I was tentatively assigned on the basis of limited spectral data.

While searching for a supply of helenalin (II) for the investigation of the relationship between the sesquiterpene lactone structure and the antitumor/cytotoxic activity,[2] we isolated plenolin as a cytotoxic principle[3,4] from Florida Helenium autumnale L.[5,6] and are reporting our reinvestigation of the structure of plenolin.[7]

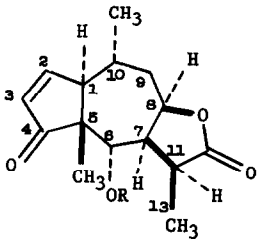
Plenolin was isolated in 0.13% yield from the mother liquor after the removal of helenalin by repeated silica gel column as well as preparative chromatography. Plenolin (III), m.p. 223-226°,[8] has the composition $C_{15}H_{20}O_4$ and shows the presence of a hydroxyl group (3450 cm^{-1}) and a γ-lactone ring (1745 cm^{-1}). The presence of a cyclopentenone ring system in plenolin is indicated by the appearance of an IR band at 1712 cm^{-1} and is substantiated by the presence in the NMR spectrum (in pyridine-d_5) of the characteristic doublets of doublets at δ 7.60 (H-2, J = 2, 6 Hz)[9] and 6.08 (H-3, J = 3, 6 Hz). Plenolin lacks the α-methylene group on the lactone ring, and is the corresponding α-methyl-γ-lactone. Three-proton methyl group signals are seen at δ 1.26 (C-11 methyl, d, J = 7 Hz), 1.08 (C-5 methyl, s) and 1.02 (C-10 methyl, d, J = 7 Hz). The lactonic proton at C-8 is seen as a multiplet at δ 4.87. The one proton doublet[10] at δ 4.73 (J = 4 Hz) is assigned to the proton at the hydroxyl group (at C-6) since it is shifted downfield to δ 5.48 (s) upon acetylation with acetic anhydride in pyridine.



I



II



III, R = H

IV, R = $COCH_3$

V, R = CO-  -I

Phenolin acetate (IV), $C_{17}H_{22}O_5$, has m.p. 174-175° and exhibits NMR ($CDCl_3$) signals at δ 1.05 (3H, s, C-5 methyl), 1.25 (3H, d, J = 7 Hz, C-10 methyl), 1.51 (3H, d, J = 7 Hz, C-11 methyl), 1.94 (3H, s, acetyl), 4.78 (1H, m, H-8), 6.10 (1H, dd, J - 3, 6 Hz, H-3) and 7.75 (1H, dd, J = 2, 3 Hz, H-2).

Considerations from the biogenetic implications observed in the co-occurrence of plenolin (III) and helenalin (II), coupled with the evidence described above, led to the conclusion that the structure of plenolin must be modified to III, i.e. 11,13-dihydrohelenalin. To confirm further the structure of plenolin, the identities of III and IV were established by direct comparison (mixed m.p.; tlc; superimposable IR and NMR spectra) with synthetic samples of dihydrohelenalin and acetyldihydrohelenalin prepared by catalytic hydrogenation of helenalin and acetylation of dihydrohelenalin.

In order to define the stereochemistry at C-11, a single crystal X-ray analysis of the 6-p-iodobenzoate derivative (V) was undertaken. The crystals belong to the orthorhombic system, space group $P2_12_12$, with four molecules in a unit cell of dimensions a = 9.29, b = 26.85, c = 8.45 Å. The structure was solved by the heavy-atom approach and refined by full-matrix least squares calculations incorporating the anomalous-scattering corrections for the iodine atom to R = 0.054 over 1165 reflexions obtained from diffractometer measurements. The analysis shows that V represents the structure and absolute stereochemistry of the p-iodobenzoate derivative from which it follows that III represents dihydrohelenalin, i.e. plenolin.

The potent cytotoxicity exhibited by plenolin is in agreement with our previous hypothesis,[4,11] i.e. the structural requirement for significant cytotoxicity is an O=C-C=CH_2 system as an active center in a lactone, or in a ketone, especially when it is in a cyclopentenone ring system.

ACKNOWLEDGEMENTS

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REFERENCES AND FOOTNOTES

1. T. G. Waddell and T. A. Geissman, Phytochemistry, 8, 2371 (1969).

2. K. H. Lee, J. Pharm. Sci., 62, 1028 (1973), and references cited therein.

3. Plenolin showed significant inhibitory activity of the in vitro growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H.Ep.-2) at 0.814 mcg/ml.[4]

4. K. H. Lee, H. Furukawa, and E. S. Huang, J. Med. Chem., 15, 609 (1972).

5. Specimens were gathered in October, 1971 in Jackson County, Florida. The constituents of Florida H. autumnale was previously examined and reported to contain helenalin in good yield.[6]

6. W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, J. Amer. Chem. Soc., 85, 19 (1963).

7. Plenolin isolated either from H. autumnale or B. pleniradiata was found to be identical on the basis of direct comparison (mixed m.p.; tlc; superimposable IR and NMR spectra).

8. Lit. 1 reported m.p. 216-220°.

9. Partially overlapped with the signal of pyridine.

10. This doublet became a singlet upon addition of D_2O or acetylation indicating the coupling of H-6 with the hydroxyl proton.

11. K. H. Lee, E. S. Huang, C. Piantadosi, J. S. Pagano, and T. A. Geissman, Cancer Res., 31, 1649 (1971).