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THE STRUCTURE AND STEREOCHEMISTRY OF EUPAHYSSOPIN, A NEW ANTITUMOR GERMACRANOLIDE FROM *EUPATORIUM HYSSOPIFOLIUM*[1]

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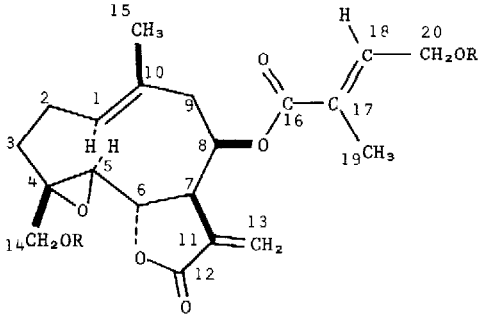
As a result of the continuing search for new and novel naturally occurring potential antitumor agents[1], the chloroform extract of the whole plant of *Eupatorium hyssopifolium* L.[2] was found to show significant inhibitory activity against Walker 256 carcinosarcoma as well as P-388 lymphocytic leukemia. We report herein the isolation and structure determination of eupahyssopin (I), the major active principle[3,4] from *E. hyssopifolium*.

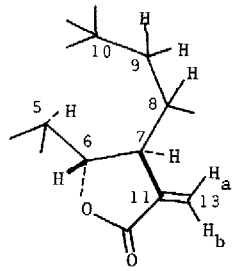
Eupahyssopin, a new germacranolide, was isolated in 0.434% yield as colorless prisms from the chloroform extract of *E. hyssopifolium* according to an exact literature procedure[5] followed by successive silica gel column chromatography. Eupahyssopin [(I), m.p. 125°, $[\alpha]_D^{25}$ -138.9° (c = 1.45, $CHCl_3$), m/e 378.1684 (M^+)] has molecular formula[6] $C_{20}H_{26}O_7$. The presence of an α-methylene-γ-lactone moiety bearing a proton at the β-position (H-7) in (I) is indicated by the appearance of ir bands (KBr) at 1775 and 1665 $cm.^{-1}$ and is substantiated by the presence in the nmr spectrum[7] of a characteristic pair of low field doublets at δ 5.76 (1H, J = 3.0 Hz, H_a-13) and 6.36 (1H, J = 3.0 Hz, H_b-13). Double resonance experiments involving H_a and H_b established the location of the H-7 multiplet at δ 3.22. The *trans*-diaxial relationships between the protons at C-5, C-6, and C-7, with

H-5α, H-6β, H-7α[8] is seen in the signal for H-6 which occurs as a well-defined one-proton triplet at δ 4.91 (J = 9.0 Hz), a feature common to this class of compounds. Irradiation at the frequency of H-6 converted the doublet at δ 3.04 (J = 9.0 Hz, H-5) into a singlet and also affected the multiplet due to H-7. Irradiation at the frequency of H-8 (δ 5.76, overlapped m) sharpened the multiplet at δ 3.22 (H-7) and collapsed two well-separated doublets of doublets at δ 2.79 (J = 5, 14 Hz) and 2.20-2.50 (partially overlapped) into two doublets (J = 14 Hz), demonstrating that H-8 was adjacent to two protons at C-9 which in turn was adjacent to a fully substituted C-10. The foregoing observations establish the relationship between the protons in the C-5 — C-9 region to be as in partial structure (III).



(I) R = H

(II) R = $COCH_3$



(III)

The presence of two primary hydroxyl groups in (I) was shown by the presence of a strong ir band at 3420 $cm.^{-1}$, a pair of AB-type doublets at δ 4.02 (1H, J = 12 Hz, H-14) and 3.84 (1H, J = 12 Hz, H-14) as well as a two-proton doublet of doublets at δ 4.33 (J = 1, 6 Hz, H-20) in the nmr spectrum. These signals shifted downfield to δ 4.78, 3.80, and 4.76, respectively, in the diacetate [(II), m.p. 112.5°, $C_{24}H_{30}O_9$, m/e 462.1894 (M^+), δ 2.10 (3H, s), 2.15(3H, s) (2 OAc)], obtained from acetylation of (I) with acetic anhydride in pyridine.

The ^{13}C nmr spectrum of (I) displayed two carbonyl carbon signals at δ 165.96 and 168.77. One of these was due to the lactonic carbonyl; the other was from an ester which was also indicated by an ir band at 1710 $cm.^{-1}$ Spin decoupling led to the following assignment of

protons consistent with the 2-methyl-4-hydroxy-2-butenoate structure for the ester group as depicted in (I): δ 1.80 (1H, d, J = 1.6 Hz, H-19), 6.81 (1H, dt, J = 1.6, 6 Hz, H-18), and 4.33 (2H, dd, J = 1, 6 Hz, H-20). Further confirmation for these assignments was obtained by diagnostically important mass peaks at m/e 262 and 304 which were due to loss of HOOC-$C(CH_3)$=CH-CH_2OH and HOOC-$C(CH_3)$=CH-CH_2OCOCH_3 in (I) and (II), respectively.

The nature of the protons and the methyl group at C-2, C-1, and C-10 in (I) were deduced as follows. The nmr spectrum of (I) exhibited a three-proton singlet at δ 1.73, slightly split by allylic coupling (J 0.5 Hz), thereby indicating the presence of a vinyl methyl group at C-10. The olefinic proton at C-1 appeared as a multiplet at δ 5.37 which upon irradiation collapsed the C-10 methyl doublet to a singlet and affected the two-proton multiplet due to H-2 (δ 2.43). Conversely, irradiation at the frequency of the C-10 methyl doublet converted the multiplet at δ 5.37 (H-1, X part of an ABX system) [H-1 now only coupled to two protons at C-2 (A and B components of an ABX system)] to a double doublet (J = 4, 11 Hz).

The remaining oxygen atom of (I) was assigned as a 4,5-epoxide by off-resonance proton decoupling in the ^{13}C nmr spectrum which showed a doublet at δ 66.82 for the -CH(O)- linkage at the 5-position and a singlet at δ 64.28 for the >C(O)-C moiety at the 4-position.

Unequivocal proof of the structure and stereochemistry of (I) was provided by single-crystal *X*-ray analysis of the diacetate (II) which crystallizes in the orthorhombic system, space group $P2_12_12_1$, a = 11.04(1), b = 24.81(1), c = 8.79(1) Å, Z = 4. The crystal structure was solved by direct non-centrosymmetric phase determining procedures using MULTAN.[9] Atomic positional and anisotropic thermal parameters for the non-hydrogen atoms have been refined to R 0.131 over 1087 statistically significant [$I > 2.0\sigma(I)$] reflections measured on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered Cu-K_α radiation, λ = 1.5418 Å) operating in the θ-2θ scanning mode. The 4,5-epoxide is *trans*; the C-4-CH_2OH and C-10 methyl groups are *syn* oriented on the β face of the molecule. The ten-membered ring conformation defined by the endocyclic torsion angles, $\omega_{1,2}$ -101, $\omega_{2,3}$ 55, $\omega_{3,4}$ -82, $\omega_{4,5}$ 146, $\omega_{5,6}$ -131, $\omega_{6,7}$ 101, $\omega_{7,8}$ -80, $\omega_{8,9}$ 55, $\omega_{9,10}$ -111, $\omega_{1,10}$ 176°, closely resembles that of eupatolide[10] for which the corresponding values are -98, 50, -86, 155,-136, 90, -76, 67, -115, and 167°.

Acknowledgements

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References and Footnotes

1. Antitumor Agents. 18. Part 17: K. H. Lee, Y. Imakura, and D. Sims, submitted for publication.

2. Specimens were gathered in 1971, in Virginia. We thank Dr. M. E. Wall and Mr. H. L. Taylor, Research Triangle Institute, N. C., for providing the plant material (Voucher No. PR 21479).

3. Eupahyssopin showed significant (T/C $\geq$ 125%) inhibitory activity against Walker 256 carcinosarcoma in rats (T/C 330%) 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[4].

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5. K. H. Lee, R. F. Simpson, and T. A. Geissman, Phytochemistry, 8, 1515 (1969).

6. All compounds reported gave satisfactory elemental analysis.

7. ^{1}H and ^{13}C nmr spectra were measured in $CDCl_3$ (TMS) with a Varian XL-100-FT instrument.

8. Assuming that the C-7 hydrogen is oriented α as in all known naturally-occurring germacranolides from higher plants.

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