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STRUCTURE AND STEREOCHEMISTRY OF EUPAFORMONIN, A NOVEL CYTOTOXIC SESQUITERPENE LACTONE FROM *EUPATORIUM FORMOSANUM* HAY.

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The whole plant of *Eupatorium formosanum* HAY. collected in the early spring in Tainan, Taiwan, was previously reported to yield eupatolide[1] (I) (0.4%) as the major cytotoxic agent. A new collection of the plant from a different location[2] has led to the isolation of a new cytotoxic[3] sesquiterpene lactone, eupaformonin (II) (0.002%), in addition to eupatolide (0.001%).

Eupaformonin was isolated as colorless prisms from the ethanolic extract of *E. formosanum* according to an exact literature procedure[1] followed by silica gel column chromatography[5]. Eupaformonin [II, $C_{17}H_{22}O_5$, m.p. 216-218°, m/e 306.1461 (M^+), 264 (M-42) (M-$COCH_3$), 246 (M-60) (M-CH_3COOH), and 228 (M-60-18) (M-CH_3COOH-H_2O); ν_{max} (Nujol) 3409 (OH), 1753 (γ-lactone), 1712 (C=O), 1675 and 1663 cm^{-1} (C=C); δ (pyridine-d_5) 6.41 (1H, d, J = 3.0 Hz, 13-H), 5.72 (1H, d, J = 3.0 Hz, 13H), 2.00 (3H, s, $OCOCH_3$), 2.12 (3H, s) and 1.79 (3H, s) (two vinyl methyl protons)] gave, upon treatment with acetic anhydride in pyridine, an acetate (III) in 50% yield[6]. Compound (III) ($C_{19}H_{24}O_6$, m.p. 178°, colorless needles) showed ν_{max} ($CHCl_3$) 1759 (γ-lactone), 1742 (ester C=O) and 1662 cm^{-1} (C=C); and δ ($CDCl_3$) 2.12 (3H, s), 2.06 (3H, s) (two $OCOCH_3$), 1.92 (3H, s) and 1.79 (3H, s) (two vinyl methyl groups).

The complete molecular structure and relative stereochemistry of eupaformonin were established by a single crystal X-ray analysis. Eupaformonin crystallizes in the orthorhombic system, space group $P2_12_12_1$, a = 14.28(1), b = 11.56(1), c = 9.68(1) Å, Z = 4. The crystal

structure was elucidated by direct phase determining methods using MULTAN[7] and refined by full-matrix least-squares calculations to the present R = 0.106 over 1437 visually estimated reflections from photographic data taken with Cu-K_α (λ = 1.542 Å) radiation.

The X-ray study proves that eupaformonin has constitution (II) and if we assume that the C_7 hydrogen atom is α-oriented as in all known naturally-occurring germacranolides, then (II) also represents the absolute configuration. Eupaformonin is therefore a new member of the $\Delta^{1(10)}$ *trans*, $\Delta^{4(5)}$ *cis* germacradiene class of sesquiterpenes, sub-group 'heliangolides', all previous authentic examples[8] of which have been derivatives of helianginol (IV) and generally have a 3β-hydroxyl function in addition to the 1(10)-epoxide group; typical examples are heliangine[14,15] (V), erioflorin[16] (VI), and erioflorin methacrylate[17] (VII). On the basis of these consistent, but limited, observations it was speculated[17] that the $\Delta^{4(5)}$ *cis* bond may arise from a *trans*-oriented precursor after (or during) the introduction of the oxygen functions. The existence of eupaformonin with its 3α-acetate group and 1(10) *trans* double bond now indicates that the biosynthetic stage at which the *trans-cis*-isomerization occurs may equally well precede the oxidation step.

In (II) the cyclodecadiene ring adopts the boat-chair conformation (VIII) with the C_{14} and C_{15} methyl substituents *anti* with respect to the ring plane. Endocyclic torsion angles around the *cis* and *trans* double bonds are 3° and 169°, respectively. Whereas the former is not significantly different from the ideal unstrained 0° value, the torsion angle at the *trans* double bond departs significantly from 180° and reflects the more strained nature of this bond. The angle found here is similar in magnitude to the corresponding values at the *trans* double bonds in shiromodiol[18] (167°), elephantol[19] (163°), and dihydromikanolide[20] (-163°) but somewhat less than that in the more severely strained melampodin[21] (-155°).

Acknowledgement

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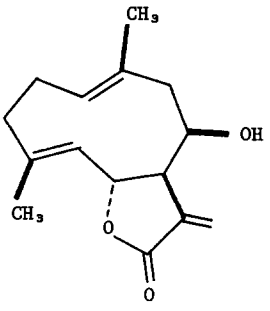
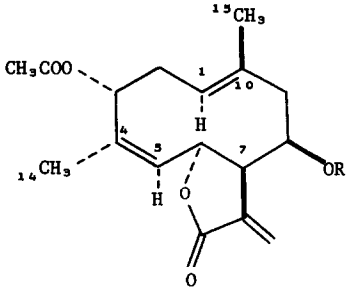
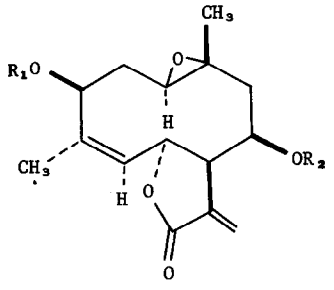


(I)

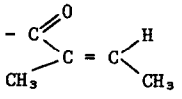


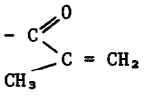
(II) R = H

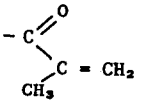
(III) R = $COCH_3$

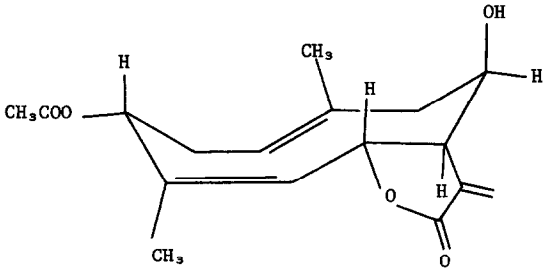


(IV) $R_1 = R_2 = H$

(V) $R_1 = H$, $R_2 =$ 

(VI) $R_1 = H$, $R_2 =$ 

(VII) $R_1 = R_2 =$ 



(VIII)

References and Footnotes

1. K. H. Lee, H. C. Huang, E. S. Huang, and H. Furukawa, J. Pharm. Sci., 61, 629 (1972).

2. Specimens were gathered in early spring, 1973, in Wootai, Pingtong, Taiwan.

3. Eupaformonin showed significant inhibitory activity of the *in vitro* growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H. Ep.-2). Cytotoxicity was assayed by Dr. E. S. Huang, Department of Bacteriology and Immunology School of Medicine, University of North Carolina at Chapel Hill, by literature method[4].

4. E. S. Huang, K. H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, J. Pharm. Sci., 61, 1960 (1972).

5. Mallinckrodt silica gel CC7 was used, and the column was eluted with chloroform-ethyl acetate (9:1).

6. Starting material was recovered in 50% yield. The axial orientation of the C_8 hydroxyl group as shown by the X-ray analysis may well account for the difficulty of this acetylation.

7. G. Germain, P. Main, and M. M. Woolfson, Acta Cryst., A27, 368 (1971).

8. Classification of several other $\Delta^{4(5)}$*cis* germacranolide sesquiterpene lactones into the recently proposed sub-groups[9,10] cannot be made unambiguously for the *cis, trans* nature of the normal reference 1(10) double bond or its equivalent is obscured e.g. eupacunin[11], liatrin[12], or woodhousin[13].

9. S. Neidle and D. Rogers, J.C.S. Chem. Comm., 140 (1972).

10. D. Rogers, G. P. Moss, and S. Neidle, J.C.S. Chem. Comm., 142 (1972).

11. S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, T. Fujita, P. D. Cradwick, A. D. U. Hardy, and G. A. Sim, J. Amer. Chem. Soc., 93, 4914 (1971); S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, and T. Fujita, J. Org. Chem., 38, 2189 (1973).

12. S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Bryan, J. Amer. Chem. Soc., 93, 4916 (1971); S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, R. J. Restivo, and R. F. Bryan, J. Org. Chem., 38, 1853 (1973).

13. W. Herz and S. V. Bhat, J. Org. Chem., 37, 906 (1972).

14. N. Nishikawa, K. Kamiya, A. Takabatake, H. Oshio, Y. Tomiie, and I. Nitta, Tetrahedron, 22, 3601 (1966).

15. It has been pointed out elsewhere[9] that in the original publication[14] the constitutional formula for heliangine was drawn incorrectly as it showed $\Delta^{4(5)}$ *trans* although the crystallographic co-ordinates and molecular diagram indicate a $\Delta^{4(5)}$ *cis* double bond. Unfortunately, while ref. 9 correctly showed the double bond configuration, C_3 and its hydroxyl substituent were omitted. Formula (V) here correctly represents heliangine and erioflorin (VI) which was based upon it.[16]

16. S. J. Torrance, T. A. Geissman, and M. R. Chedekel, Phytochemistry, 8, 2381 (1969).

17. S. Gnecco, J. P. Poyser, M. Silva, P. G. Sammes, and T. W. Tyler, Phytochemistry, 12, 2469 (1973).

18. R. J. McClure, G. A. Sim, P. Coggon, and A. T. McPhail, Chem. Comm., 128 (1970).

19. A. T. McPhail and G. A. Sim, J. Chem. Soc. Perkin II, 1313 (1972).

20. P. J. Cox, G. A. Sim, J. S. Roberts, and W. Herz, J.C.S. Chem. Comm., 428 (1973).

21. S. F. Watkins, N. H. Fischer, and I. Bernal, Proc. Nat. Acad. Sci., 70, 2434 (1973).