Tetrahedron Letters No. 26, pp 2287 - 2290, 1974. Pergamon Press. Printed in Great Britain.

THE STRUCTURE AND ABSOLUTE CONFIGURATION OF FLORILENALIN, A NEW CYTOTOXIC GUAIANOLIDE FROM FLORIDA HELENIUM AUTUMNALE L.

Kuo-Hsiung Lee*, Toshiro Ibuka, and Mutsuo Kozuka
Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

Andrew T. McPhail* and Kay D. Onan
Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706

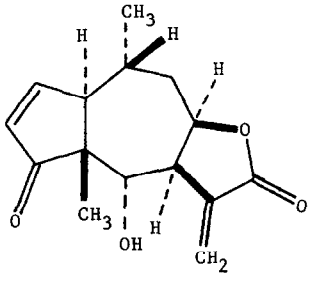
(Received in USA 27 March 1974; received in UK for publication 20 May 1974)

The search for a supply of helenalin (I) for investigations on the relationship between sesquiterpene lactone structure and antitumor or cytotoxic activity[1,2] has led to the isolation, from Florida Helenium autumnale L.[3], of a new sesquiterpene lactone, florilenalin (II), which has significant cytotoxic activity[5].

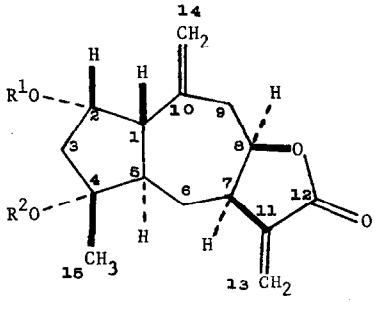
Florilenalin was isolated as an oil from the mother liquor after the removal of helenalin by fractionation involving successive solvent partitions and silica gel chromatography. Florilenalin [(II), $C_{15}H_{20}O_4$[7] (M^+ 264.1360); ν_{max} ($CHCl_3$) 3420 (OH), 1765 (γ-lactone), 1660, 1645, and 1610 cm^{-1} (C=C); δ[8] 4.76 (1H, m, 8-H), 4.37 (1H, m, 2-H)] gave, upon treatment with acetic anhydride in pyridine, a monoacetate [(III), $C_{17}H_{22}O_5$; oil; ν_{max} 3500 (OH), δ 2.08 (3H, s, $OCOCH_3$), 5.34 (1H, m, 2-H)]. Further acetylation of (III) with isopropenyl acetate and p-toluenesulfonic acid afforded a diacetate [(IV), $C_{19}H_{24}O_6$; m.p. 128-129°, m/e 348 (M^+), 288 [M-60(CH_3COOH)], 228 [M-60 (CH_3COOH)-60(CH_3COOH)]. Extensive nmr decoupling experiments (100 MHz) led to the following assignment of protons which fitted into a florilenalin diacetate structure as depicted in (IV); δ 6.33 (1H, d, J = 3.0 Hz, 13-H), 5.74 (1H, d, J = 3.0 Hz, 13-H), 5.12 (1H, br. s, 14-H), 4.94 (1H, br. s, 14-H), 5.32 (1H, m, 2-H), 4.61 (1H, m, 8-H), 3.28 (1H, m, 7-H), 2.02 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$) and 1.43 (3H, s, 15-H). Treatment of (II) with chromium trioxide-pyridine complex resulted in the formation of dehydroflorilenalin (V). Dehydration of (V) with p-toluenesulfonic acid gave rise to a conjugated ketone (VI). The circular dichroism curve of (IV) showed a strong negative Cotton effect at 256 nm; this defined the configuration of the C-7/C-8 lactone grouping as cis-fused[9]. The

configuration of the hydroxyl group at C-2 was established by application of Horeau's method[10,11] and found to be α-oriented. Considerations from the biogenetic implications observed in the co-occurrence of florilenalin (II), a guaianolide, and helenalin (I), a pseudo-guaianolide, led to the conclusion that the stereochemistry of the A/B ring junction and of the C-4 position were as shown, for transformation from the guaianolide (II) to the pseudo-guaianolide was reasonable[12,13] and would be expected to proceed in a stereospecific manner (VII) to lead to these configurations.

Single-crystal X-ray analysis of 4-acetyl-2-p-iodobenzoylflorilenalin (VIII), provided unequivocal proof of the structure, stereochemistry, and absolute configuration of florilenalin. The crystals belong to the orthorhombic system, space group $P2_12_12_1$, a = 14.40, b = 22.62, c = 7.37 Å, Z = 4. The structure was solved by the heavy-atom method from visually estimated photographic data and refined by full-matrix least-squares calculations to R = 0.13 over 1109 independent reflexions. The absolute configuration was assigned by incorporating the anomalous scattering effects of iodine[14] into the structure-factor calculations for which R was significantly smaller[15] for the molecule as represented by VIII than for the mirror image.



(I)

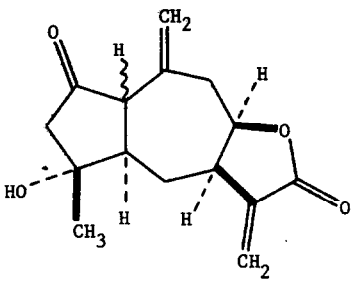


(II) $R^1 = R^2 = H$

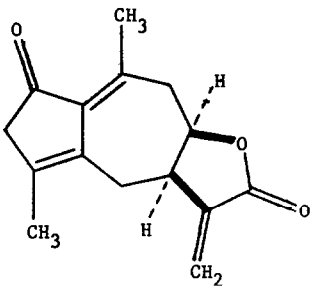
(III) $R^1 = Ac$, $R^2 = H$

(IV) $R^1 = R^2 = Ac$

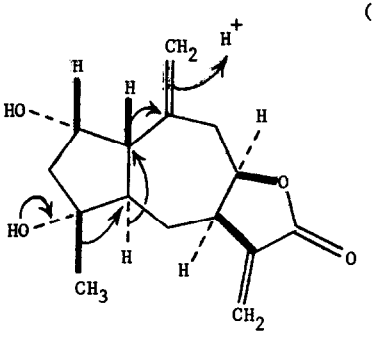
(VIII) $R^1 = p\text{-I-}C_6H_4\text{-CO}$, $R^2 = Ac$



(V)



(VI)



(VII)

ACKNOWLEDGEMENTS

This investigation was supported in part by U.S. Public Health Service Research Grant No. CA 12360 from the National Cancer Institute to K.H.L.

REFERENCES AND FOOTNOTES

1. For previous paper in the series "Antitumor Agents" see: K. H. Lee, T. Ibuka, A. T. McPhail, K. D. Onan, T. A. Geissman and T. G. Waddell, Tetrahedron Letters, in the press.

2. K. H. Lee, J. Pharm. Sci., 62, 1028 (1973).

3. Specimens were gathered in October, 1971 in Jackson County, Florida. The constituents of Florida H. autumnale were previously examined and reported to contain helenalin in good yield.[4]

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

5. Florilenalin showed significant inhibitory activity of the in vitro growth of tissue culture cells originating from human epidermoid carcinoma of larynx (H. Ep.-2) at about 1 μg/ml. 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[6].

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

7. All compounds reported gave satisfactory elemental analysis.

8. NMR spectra were measured in $CDCl_3$ with a 60 MHz instrument using TMS as an internal standard.

9. W. Stöcklin, T. G. Waddell, and T. A. Geissman, Tetrahedron, 26, 2397 (1970).

10. A. Horeau and H. B. Kagan, Tetrahedron, 20, 2431 (1964).

11. T. J. Mabry, W. Renold, H. E. Miller, and H. B. Kagan, J. Org. Chem., 31, 618 (1966).

12. A. Yoshitake and T. A. Geissman, Phytochem., 8, 1753 (1969).

13. T. G. Waddell and T. A. Geissman, Phytochem., 8, 2371 (1969).

14. 'International Tables for X-Ray Crystallography' Vol. III, Kynoch Press, Birmingham, 1962, p. 215.

15. W. C. Hamilton, Acta Cryst., 18, 502 (1965).