Vernolepin, a Novel Elemanolide Dilactone Tumor Inhibitor from *Vernonia hymenolepis*[1,2]

Sir:

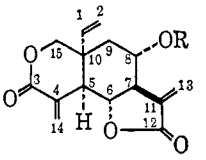
In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of *Vernonia hymenolepis* A. Rich (Compositae)[3] showed significant activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[4] We report herein the isolation and structural elucidation of two novel elemanolide dilactones, vernolepin (I) and vernomenin (IV). Vernolepin showed significant inhibitory activity against the Walker intramuscular carcinosarcoma 256 in rats at 12 mg/kg.[4]

Fractionation of the ethanol extract, guided by assay against KB, revealed that the cytotoxic activity was concentrated, successively, in the methanol layer of a 10% aqueous methanol–petroleum ether (bp 60–68°) partition and in the ethyl acetate layer of an ethyl acetate–water partition. Further fractionation involving silicic acid chromatography yielded vernomenin (IV) followed by vernolepin[5] (I).

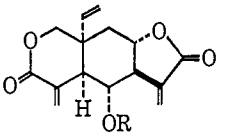
Vernolepin (I, $C_{15}H_{16}O_5$, M^+ *m/e* 276[6]) showed mp 181–182°; $[\alpha]^{28}D$ +72° (*c* 1.04, acetone); λ_{max}^{MeOH} 208 mμ (end absorption, ε 20,300); $\lambda_{max}^{CHCl_3}$ 2.90, 3.32, 3.44, 5.64, 5.80, and 6.18 μ; nmr signals (in pyridine-d_5) at τ 3.25 and 4.13 (2 H, d, J = 1.5 cps, exocyclic $=CH_2$), 3.25 (1 H, br s, –OH), 3.74 (2 H, d, J = 3 cps, exocyclic $=CH_2$), 4.58 (3 H, complex multiplet, vinyl H), 5.25 and 5.74 (2 H, d, J = 12 cps, and dd, J = 12 and 1.5 cps, respectively, $-CH_2O$), 5.75 (1 H, t, J = 10 cps, >CHO), 5.75 (1 H, m, >CHO), 6.87 (1 H, m, –CH), 7.12 (1 H, tt, J = 10 and 3 cps, C-7 H), and 7.80 and 8.11 (2 H, m_{ABX}, J_{AB} = 14, J_{AX} = 4.5, J_{BX} = 10 cps, C-9). The equivalent weight (by titration) was found to be 143, indicative of a dilactone structure.

Vernolepin was converted into several crystalline derivatives: the acetate II (by treatment with acetic anhydride and pyridine), $C_{17}H_{18}O_6$, mp 146–147°, $[\alpha]^{25}D$ +134° (*c* 0.89, $CHCl_3$); the tetrahydro derivative (by hydrogenation with 10% palladium on charcoal), $C_{15}H_{20}O_5$, mp 145–146°, $[\alpha]^{28}D$ +55° (*c* 1.00, acetone); the methanol adduct VI (by transesterification with 1% methanolic hydrochloric acid), $C_{16}H_{20}O_6$, M^+ *m/e* 308, mp 172–173°; $[\alpha]^{28}D$ +47° (*c* 1.09, acetone), $\lambda_{max}^{CHCl_3}$ 5.83, 5.90, and 6.13 μ. Upon treatment with acetic anhydride and pyridine, the methanol adduct VI yielded a diacetate, VII, $C_{20}H_{24}O_8$, M^+ *m/e* 392, mp 148–149°, $[\alpha]^{27}D$ +8° (*c* 1.17, acetone).

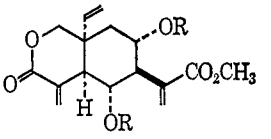
Treatment of vernolepin with *p*-bromobenzenesulfonyl chloride in a mixture of pyridine and benzene gave vernolepin *p*-bromobenzenesulfonate (III, mp 178–179°, $[\alpha]^{25}D$ −16° (*c* 0.69, acetone)), which was crystallized from methanol in the monoclinic system, of space group $P2_1$, with two molecules of $C_{21}H_{19}BrO_7S$ in a cell of dimensions a = 9.80, b = 10.10, c = 11.04 Å; β = 90° 12′. Equiinclination Weissenberg photographs of the *h0l* through *h7l* layers yielded 1146 measurable intensities. The carbon and oxygen atoms were located in three-dimensional electron-density distributions, the elucidation of the structure being hindered by pseudosymmetry and the very large anisotropic thermal vibration of the bromine atom. The present value of R is 20%, and refinement of the atomic parameters is being continued by least-squares calculations. The results of the analysis establish that the derivative has the constitution and relative stereochemistry III, and it follows, therefore, that vernolepin has structure I.



I, R = H
II, R = $COCH_3$
III, R = $SO_2C_6H_4Br$



IV, R = H
V, R = $COCH_3$



VI, R = H
VII, R = $COCH_3$

Vernomenin (IV, $C_{15}H_{16}O_5$, M^+ *m/e* 276) was isolated as an amorphous solid, $[\alpha]^{27}D$ −62° (*c* 1.44, acetone); λ_{max}^{MeOH} 210 mμ (end absorption, ε 20,000); $\lambda_{max}^{CHCl_3}$ 2.78 (sharp), 2.91 (broad), 3.28, 3.41, 5.63, 5.79, 5.97, and 6.15 μ. The nmr signals of vernomenin (in pyridine-d_5) were similar to those of vernolepin (I). Upon acetylation with acetic anhydride–pyridine mixture at room temperature, vernomenin gave a crystalline monoacetate (V), $C_{17}H_{18}O_6$ (M^+ *m/e* 318 and base peak *m/e* 276). The acetate V did not melt below 300° but softened at 212–214°; $[\alpha]^{26}D$ −135° (*c* 2.00, $CHCl_3$). When treated with methanolic hydrochloric acid, vernomenin gave the methanol adduct VI previously obtained from vernolepin, indicative that both differ only in the attachment of the γ-lactone function. This structural difference was revealed by the nmr spectra of their respective acetates. In vernomenin acetate (V), the triplet centered at τ 4.78 (J = 9 cps) could be assigned to the proton at acetate-bearing C-6, while the multiplet centered at τ 5.90 corresponded to the proton (spin coupled to three protons) at lactone-bearing C-8. In contrast, the spectrum of vernolepin acetate (II) showed a multiplet at τ 4.95, assigned to the proton at acetate-bearing C-8, while the lactone proton signal appeared as a triplet centered at τ 5.96 (J = 9 cps), indicative of attachment to C-6. From the nmr spectra and the structure of vernolepin, it follows that vernomenin has structure IV.

A germacranolide monolactone, vernolide, has been isolated from *Vernonia colorata*.[7] Several elemane monolactones, *e.g.*, saussurea lactone[8] and isolindera-

(1) Tumor Inhibitors. XXXI. Part XXX: S. M. Kupchan, J. E. Kelsey, M. Maruyama, and J. M. Cassady, *Tetrahedron Letters*, in press.

(2) Supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with the Cancer Chemotherapy National Service Center (C.C.N.S.C.), National Cancer Institute, National Institutes of Health (PH 43-64-551).

(3) Leaves were collected in Ethiopia in Jan 1965. The authors acknowledge with thanks the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by C.C.N.S.C.

(4) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the C.C.N.S.C. by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(5) Vernolepin and vernomenin showed cytotoxicity (ED_{50}) against KB cell culture at 2 and 20 μg/ml, respectively.[4]

(6) We thank Dr. G. Van Lear and Dr. F. W. McLafferty of the Purdue Mass Spectrometry Center, supported under U. S. Public Health Service Grant FR-00354, for the mass spectral data.

(7) R. Toubiana and A. Gaudemer, *Tetrahedron Letters*, 1333 (1968).

lactone,[9] have been reported; in each case, the isolation procedure included exposure to high temperatures. In view of the demonstrated transformation of germacranolide precursors to elemanolides by heating, the question has been raised as to whether some of the elemanolides may be artifacts.[8,9] In contrast, when an isolation procedure was devised which involved cold aqueous extraction of *V. hymenolepis*, vernolepin was isolated in a yield comparable to that obtained by hot ethanol extraction. This fact supports the view that vernolepin is, indeed, a naturally occurring compound.

Vernolepin and vernomenin appear to be the first recognized elemanolide dilactones.

(8) A. S. Rao, A. Paul, Sadgopal, and S. C. Bhattacharyya, *Tetrahedron*, **13**, 319 (1961).
(9) K. Takeda, H. Minato, and M. Ishikawa, *J. Chem. Soc.*, 4578 (1964).

S. Morris Kupchan, Richard J. Hemingway
Dieter Werner, A. Karim
Department of Pharmaceutical Chemistry
University of Wisconsin, Madison, Wisconsin 53706

A. T. McPhail, G. A. Sim
Chemical Laboratory
University of Sussex, Brighton, England
Received May 2, 1968

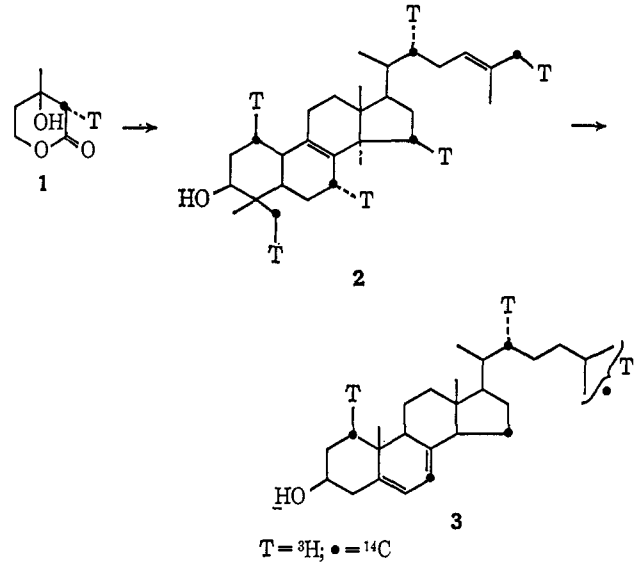
The Fate of the 15β Hydrogen of Lanosterol in Cholesterol Biosynthesis

Sir:

In cholesterol biosynthesis the three methyl groups, 4α, 4β, and 14α, of lanosterol are removed by oxidation to carbon dioxide.[1] The 14α-methyl group is believed to be transformed into a carboxyl group and eliminated by decarboxylation, which is facilitated by the 8,9 double bond.[2,3]

If the 8,9 double bond is not the only promoter of the elimination of the 14α-carboxyl group, this reaction could be facilitated by one of the following mechanisms: (a) oxidation of 14α-carboxylic acid with formation of a carboxyl radical and its elimination with the involvement of one of the hydrogen atoms in position 15, (b) oxidation in position 15 and concerted elimination of the carboxyl group, (c) previous formation of double bond in position 15,16 facilitating the elimination of carbon in 14. All these possibilities imply the removal of hydrogen atoms in position 15. The fate of these hydrogen atoms during cholesterol biosynthesis has been followed by determining the position of labeled hydrogens in lanosterol, 5α-cholest-7-en-3β-ol, and cholesta-5,7-dien-3β-ol biosynthesized from labeled mevalonic acids. Actual $^3H:^{14}C$ ratios and atom equivalents of all the significant compounds are shown in Table I.

Accumulation of labeled cholesta-5,7-dien-3β-ol (**3**) has been obtained by incubating liver homogenates[4] of rats pretreated with AY 9944,[5] in the presence of the same inhibitor[6] and of 3(±)-(2*S*)-[2-^{14}C-2-^{3}H]mevalonic acid lactone (**1**) (10 μCi of ^{14}C, $^3H:^{14}C$ 10.00).[7] Radioactive carbon atoms in cholesta-5,7-dien-3β-ol correspond to the positions shown in formula **3**.[8] Since the radioactive precursor is asymmetrically labeled, tritium should be localized at positions 1α, 15β, 22*R*, and 26 or 27, the 7 position being excluded on the basis of our previous results.[9] The $^3H:^{14}C$ ratio should be 4:5. If the 15β hydrogen is exchanged with the medium, this ratio should be 3:5.



The unsaponifiable residue from homogenates was acetylated, carrier cholesta-5,7-dien-3β-ol acetate was added, and the mixture was separated by column chromatography on silver nitrate–kieselgel G–Celite.[10] The obtained cholesta-5,7-dien-3β-ol acetate[11] was diluted again with nonradioactive material and hydrogenated in presence of tris(triphenylphosphine)rhodium chloride[6] to yield 5α-cholest-7-en-3β-ol acetate.[11] After column chromatography on silver nitrate–kieselgel G–Celite,[10] this compound (0.565 μCi of ^{14}C/mmole) showed a $^3H:^{14}C$ ratio of 6.07, corresponding to 3.02 labeled hydrogens out of 5 radioactive carbon atoms. The ^{14}C radioactivity and the $^3H:^{14}C$ ratio were constant after several crystallizations. Furthermore oxidation with osmium tetroxide of some of the radioactive 5α-cholest-7-en-3β-ol acetate produced the mixture of the epimeric *cis*-5α-cholestane-3β,7,8-triol 3β-acetates[6,11] (0.563 μCi of ^{14}C/mmole) which showed an unchanged $^3H:^{14}C$ ratio with respect to 5α-cholest-7-en-3β-ol acetate. The same ratio was found in the mixture of the epimeric *cis*-5α-cholestane-3β,7,8-triol 3β-acetates[6,10] (0.189 μCi of ^{14}C/mmole) obtained from radioactive 5α-cholest-7-en-3β-ol acetate which could be isolated in small amounts[6] from liver homogenates. The expected constant $^3H:^{14}C$ ratio was also found

(1) J. A. Olson Jr., M. Lindberg, and K. Bloch, *J. Biol. Chem.*, **226**, 941 (1957).
(2) F. Gautschi and K. Bloch, *J. Am. Chem. Soc.*, **79**, 684 (1957).
(3) M. Lindberg, F. Gautschi, and K. Bloch, *J. Biol. Chem.*, **238**, 1661 (1963).
(4) N. L. R. Bucher and K. McGarrahan, *ibid.*, **222**, 1 (1956).
(5) *trans*-1,4-Bis(2-chlorobenzylaminomethyl)cyclohexane hydrochloride, a specific inhibitor of cholesta-5,7-dien-3β-ol-Δ⁷-reductase, according to D. Dvornik, M. Kraml, J. Dubuc, M. Givner, and R. Gaudry, *J. Am. Chem. Soc.*, **85**, 3309 (1963).
(6) L. Canonica, A. Fiecchi, M. G. Kienle, A. Scala, G. Galli, E. G. Paoletti, and R. Paoletti, *Steroids*, **11**, 282 (1968).
(7) Incubation experiments were performed at least in duplicate; reproducibility of results was excellent.
(8) O. Isler, R. Ruegg, J. Würsch, K. F. Gey, and A. Pletscher, *Helv. Chim. Acta*, 40, 2369 (1957).
(9) L. Canonica, A. Fiecchi, M. G. Kienle, A. Scala, G. Galli, E. G. Paoletti, and R. Paoletti, *Steroids*, in press.
(10) G. Galli and E. G. Paoletti, *Lipids*, 2, 72 (1967); 2, 84 (1967).
(11) The chemical purity of all compounds was established by comparing melting points, optical rotation values, mass spectra, and glpc retention times on a 1% phenylsilicone glass column with those of authentic samples.