It should be emphasized that "hardening" the potential functions to account for the observed isotope effect in acetyl chloride and *t*-butyl chloride inevitably leads to grossly overestimated effects in systems where hyperconjugation is impossible.

In summary, we conclude: (1) in ordinary systems with hyperconjugation possible, less than 10%—probably 2–5%—of the observed isotope effect is due to nonbonded interactions; reasonable estimates of the nonbonded isotope effect might be obtained by using Bartell's procedure with the Scott and Scheraga functions.

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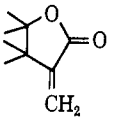
The Isolation and Structural Elucidation of Euparotin Acetate, a Novel Guaianolide Tumor Inhibitor from *Eupatorium rotundifolium*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of *Eupatorium rotundifolium* L. (Compositae)[3] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[4] We report herein the isolation and structural elucidation of euparotin acetate, a novel tumor-inhibitory sesquiterpene of the guaianolide type from *E. rotundifolium*.

Fractionation of the ethanol extract, guided by assay against KB, revealed that the active principles were concentrated, successively, in the chloroform layer of a chloroform–water partition and in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition. Further fractionation involving silicic acid chromatography yielded euparotin acetate (III),[5] $C_{22}H_{26}O_8$: mol wt (mass spectroscopy),[6] 418; mp 156–157° (vac); $[\alpha]^{30}D$ −191° (*c* 0.54, EtOH); λ_{max}^{EtOH} end absorption at 210 mμ (ε 18,400); λ_{max}^{KBr} 2.91, 5.67, 5.74, 5.85, 6.03, 6.04, 6.06, 7.49, 7.95, 8.90, 9.75, 10.20 μ; and nmr signals (in $CDCl_3$) at τ 3.62 and 4.33 (2 H, doublets, J = 3.5 cps, I), 3.93 (1 H, q, J = 7 cps, vinyl H), 4.28 (3 H, multiplets, vinyl H and 2 >CH–O), 5.18 (1 H, d, J = 8 cps, >CH–O), 5.78 (1 H, m), 7.12 (1 H, br s, OH), 7.30 (2 H, s, II), 7.97 (3 H, s, –O–$COCH_3$), 8.02 and 8.18 (9 H, multiplets, vinyl methyls).



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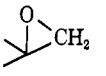


II

Further chromatography yielded euparotin (IV), $C_{20}H_{24}O_7$: mol wt (mass spectroscopy),[6] 376; mp 199–200° (vac); $[\alpha]^{32}D$ −124° (*c* 1.25, EtOH); λ_{max}^{EtOH} end absorption at 213 mμ (ε 17,800); λ_{max}^{KBr} 2.90, 5.68, 5.86, 6.05, 6.08, 6.17, 7.98, 8.73, 9.88 μ; and nmr signals (in $CDCl_3$) at τ 3.61 and 4.36 (2 H, doublets, J = 3.5 cps, I), 3.96 (1 H, q, J = 7 cps, vinyl H), 4.26 (1 H, m, vinyl H), 5.17 (1 H, d, J = 8 cps, >CH–O), 5.17 and 5.87 (2 H, multiplets), 7.28 (2 H, s, II), 8.04 and 8.20 (9 H, multiplets, vinyl methyls). Acetylation of IV gave III.

Acylation of euparotin with bromoacetic anhydride gave the bromoacetate V. Euparotin bromoacetate crystallized from benzene–petroleum ether as a benzene solvate, $C_{22}H_{25}BrO_8 \cdot 0.5C_6H_6$, mp 156–157°, $[\alpha]^{32}D$ −142° (*c* 0.38, EtOH), the crystals of which belong to the monoclinic system, space group C2, with four units of $C_{22}H_{25}BrO_8 \cdot 0.5C_6H_6$ in a cell of dimensions a = 34.85, b = 7.04, c = 10.90 A, β = 106° 35′; 1947 independent $|F_0|$ values were derived from the three-dimensional X-ray intensity data which were recorded on equiinclination Weissenberg photographs and visually estimated.

The initial position of the bromine atom was obtained from the three-dimensional Patterson synthesis. The carbon and oxygen atoms were located in three-dimensional electron-density distributions for which the Fourier coefficients were weighted according to the method proposed by Sim.[7] The atomic coordinates were subsequently refined by the least-squares method and the present value of R is 13.1%. The bromine atom was assigned anisotropic temperature factors, but the carbon and oxygen atoms were assigned only isotropic values.

The results of the X-ray analysis establish that the bromoacetate has structure V and it follows, therefore, that euparotin has structure IV, and euparotin acetate, structure III. The absolute configuration was deduced by Bijvoet's anomalous dispersion method.[8]

Euparotin appears to be the most highly oxygenated guaianolide reported to date (*i.e.*, six oxygen functions in the basic C_{15} nucleus) and the first recognized to contain a spiroepoxide.[9] It is noteworthy that euparotin

(1) Tumor Inhibitors. XX. Part XIX: S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *J. Pharm. Sci.*, in press.

(2) Supported by grants from the National Cancer Institute (CA-04500), the American Cancer Society (T-275), the National Science Foundation (GB-2878), and a contract with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health (PH 43-64-551).

(3) Leaves, stems, flowers, and roots were gathered in Florida, Sept 1960. The authors acknowledge with thanks 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 the Cancer Chemotherapy National Service Center.

(4) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

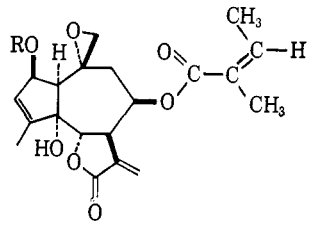
(5) Euparotin and euparotin acetate showed significant cytotoxicity (ED_{50}) against KB (human carcinoma of the nasopharynx) cell culture at 0.21 μg/ml. Euparotin acetate showed significant inhibitory activity against Walker carcinosarcoma 256 in rats at 75 mg/kg.[4]

(6) The authors thank Professor A. L. Burlingame and Dr. H. K. Schnoes, University of California, Berkeley, for the mass spectral data.

(7) G. A. Sim, *Acta Cryst.*, **12**, 813 (1959); **13**, 511 (1960); "Computing Methods and the Phase Problem in X-ray Crystal Analysis," R. Pepinsky, J. M. Robertson, and J. C. Speakman, Ed., Pergamon Press, Oxford, 1961, p 227.

(8) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature*, **169**, 271 (1951).

(9) For a comprehensive review, see F. Sorm and L. Dolejš, "Guaianolides and Germacranolides", Editions Scientifiques Hermann, Paris, 1966.



III, R = $COCH_3$
IV, R = H
V, R = $COCH_2Br$

acetate, like the previously reported tumor inhibitors withaferin A,[10,11] elephantin,[12] and elephantopin,[12] possesses epoxide and α,β-unsaturated lactone functions. Investigations are in progress which are aimed at evaluation of the significance of the latter functions, and of other structural features, in relation to the tumor-inhibitory activity of the respective compounds.

(10) S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. Saenz Renauld, *J. Am. Chem. Soc.*, 87, 5805 (1965).

(11) Withaferin A has recently been found to show significant inhibitory activity against Walker carcinosarcoma 256 in rats at 40 mg/kg.[4]

(12) S. M. Kupchan, Y. Aynehchi, J. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame, *J. Am. Chem. Soc.*, 88, 3674 (1966).

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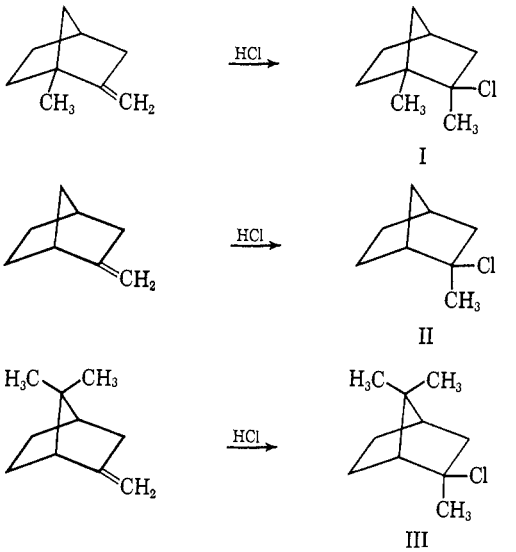
The Hydrochlorination of 2-Methylenenorbornane and Related Derivatives. Evidence for the Absence of a Symmetrical Nonclassical Intermediate in the Hydrochlorination of 1-Methyl-d_3-2-methylenenorbornane

Sir:

We wish to report that the hydrochlorination of 1-methyl-d_3-2-methylenenorbornane can be controlled to yield predominantly unscrambled 1-methyl-d_3-2-methyl-*exo*-norbornyl chloride. Consequently, the formation of a symmetrical intermediate, the 1,2-dimethylnorbornyl nonclassical cation, cannot be significant in this electrophilic addition reaction.

The automatic hydrochlorination technique developed recently[1] provides a simple method for achieving the hydrochlorination of an olefin with a minimum exposure of the product to the further action of hydrogen chloride. This encouraged us to undertake a study of the hydrochlorination of norbornane derivatives in an attempt to see whether the results would throw light on the question of bridged nonclassical intermediates in the carbonium ion reactions of these compounds.

Application of the Wittig reaction to 1-methyl-2-norbornanone produced 1-methyl-2-methylenenorbornane, bp 135–137°, $n^{20}D$ 1.4680, in 70% yield. *Anal.* Calcd for C_9H_{14}: C, 88.45; H, 11.55. Found: C, 88.50; H, 11.55. Treatment of 1-methyl-2-methylenenorbornane with hydrogen chloride at 0° yielded 99% pure 1,2-dimethyl-*exo*-norbornyl chloride (I) in a total reaction time of 2 min. Similarly, 2-methylenenorbornane yielded 99% pure 2-methyl-*exo*-norbornyl chloride (II) in 2 min, and α-fenchene yielded over 90% pure 2,7,7-trimethyl-*exo*-norbornyl chloride (III), together with the Wagner–Meerwein rearranged secondary product,[2] in 8 min.



I

II

III

The structure of 2,7,7-trimethyl-*exo*-norbornyl chloride, mp 19–20°, was confirmed as the *exo* derivative, without significant contamination by the *endo* isomer, by the nmr results described below and by the constancy of its rate of ethanolysis over more than 80% reaction, $k_1^{25} = 8.5 \times 10^{-4}$ sec.$^{-1}$. That no skeletal rearrangement had occurred is confirmed by the results of the earlier borohydride-trapping experiments.[3] The nmr spectrum exhibited methyl proton absorption at δ 1.05, 1.41, and 1.64. The latter peak is assigned to the 2-methyl group, and the large difference in the chemical shift of the first two methyl peaks (attributed to the 7-methyls) is similar to that observed in apoisobornyl chloride (δ 1.03 and 1.33) and isobornyl chloride (δ 0.88 and 1.10), with *exo* chloride substituents, rather than to bornyl chloride (δ 0.88 and 0.92), with its *endo* substituent.

Polar hydrohalogenation of olefinic compounds has long been postulated to proceed *via* intermediates with carbonium ion character.[4] The intermediate leading to the product-determining step might be either a classical carbonium ion pair[5] or a π complex.[6,7] It has also been suggested that the reaction might proceed in some cases *via* a concerted *trans* attack by both the electrophile and the nucleophile on the double bond.[7] The last two possibilities cannot be important in the present cases because the products are almost exclusively tertiary *exo* chlorides. The result with α-fenchene is of particular interest since it has been argued that

(1) H. C. Brown and M.-H. Rei, *J. Org. Chem.*, 31, 1090 (1966). We utilized a commercial unit from Delmar Scientific Laboratories, Maywood, Ill. 60154.

(2) W. Hückel and D. Volkmann, *Ann.*, 664, 31 (1963).

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(4) P. B. D. de la Mare and R. Bolton, "Electrophilic Additions to Unsaturated Systems," Elsevier Publishing Co., New York, N. Y., 1966.

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(6) M. J. S. Dewar and A. P. Marchand, *Ann. Rev. Phys. Chem.*, 16, 321 (1965).

(7) G. S. Hammond and T. D. Nevitt, *J. Am. Chem. Soc.*, 76, 4121 (1954); G. S. Hammond and C. H. Collins, *ibid.*, 82, 4323 (1960).