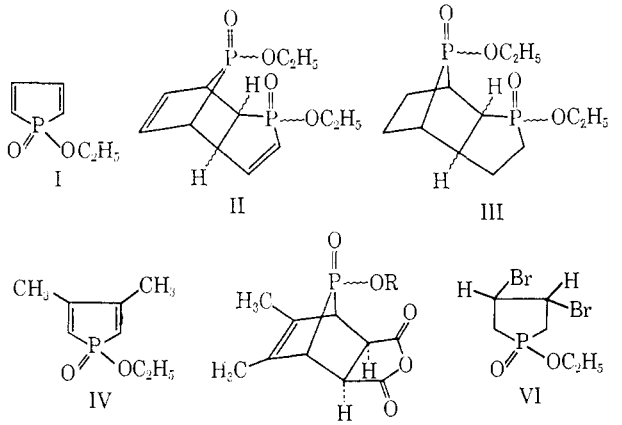
with other dienes[5] to be a better diene and poorer dienophile (and consequently more stable) than the unsubstituted ethoxyphosphole oxide. A crude reaction mixture has been obtained that reacts with maleic anhydride, presumably to form Va, which on hydrolysis forms Vb. The latter has been identified by analysis, analytical mass spectrum, and nmr spectrum. The nmr spectrum in D_2O shows a singlet at δ 3.71 (2 H), a doublet at 2.89 (J = 11 cps, 2 H), and a singlet at 1.80 (6 H). The simplicity of the spectrum confirms the assigned structure. Apparently the signal from the bridgehead hydrogen atoms is split by the ^{31}P; the lack of interaction between these hydrogen atoms and those adjacent to the carbonyl groups suggests that the compound is the *exo* isomer.[6]

II, III, and V constitute a new class of phosphinates with highly compressed C–P–C bond angles. Their mass spectra show, in addition to the parent ion, a strong peak corresponding to the loss of the bridge, with an accompanying appropriate metastable peak. Presumably $O{=}POC_2H_5$ or $O{=}POH$ is formed as the uncharged cleavage product. These are monomeric metaphosphites, previously postulated[7] as intermediates in the hydrolysis of phosphites.



I II III IV VI

Va, R = C_2H_5
b, R = H

II was best prepared from VI, obtained by the bromination of 1-ethoxy-3-phospholene 1-oxide[8] in chloroform at 0°, needles from ethyl acetate–hexane, mp 47–48°; nmr triplet at δ 1.37 (3 H), multiplets from 1.9 to 3.2 (4 H), and overlapping sextet and quintet 3.82 to 5.20 (4 H); principal infrared bands at 3.31, 7.17, 7.75, 8.00, 8.22, 9.67, 10.40, 11.43, and 12.05 μ. *Anal.* Calcd for $C_6H_{11}Br_2O_2P$: C, 23.56; H, 3.59; P, 10.12; Br, 52.26. Found: C, 23.49, 23.47; H, 3.75, 3.70; P, 10.27, Br, 52.02.

VI (25 g) in 125 ml of carbon tetrachloride was allowed to react with 25 g of dry triethylamine at 0° for 10 hr to yield triethylammonium bromide (removed by filtration) and a solution of II. The concentrate from the carbon tetrachloride solution was chromatographed over Florisil by elution with 3:1 carbon tetrachloride–ethyl acetate. The fractions were evaporated, and those containing product were dissolved in ethyl acetate and crystallized by the addition of *n*-hexane; rhombic crystals, mp 125–126°; exact mass, calcd 288.0680, found 288.0688; principal infrared bands (KBr) at 3.34, 6.39, 7.20, 7.54, 8.19, 8.33, 9.19, 9.75, 10.54, 11.83, 12.62, 13.06, 13.71, and 14.47 μ; the nmr spectrum is compatible with that expected for II. *Anal.* Calcd for $C_{12}H_{18}O_4P_2$; C, 50.00, H, 6.30, P, 21.50. Found: C, 49.36, H, 6.25, P, 21.57.

Crystalline II was hydrogenated in absolute ethanol with 10% platinum on charcoal at 3 atm for 24 hr. After removing solvent, the product was crystallized from ethyl acetate–hexane; needles, mp 80–81°; exact mass: calcd 292.0993, found 292.1009; principal infrared bands (CCl_4) at 3.30, 7.88, 8.04, 8.13, 8.21, 9.19, 9.65, 10.50, and 11.49 μ; the nmr spectrum is compatible with that expected for III. *Anal.* Calcd for $C_{12}H_{22}O_4P_2$: C, 49.30, H, 7.59, P, 21.21. Found: C, 49.42; H, 7.50; P, 21.20.

The crude reaction mixture from 1-ethoxy-3,4-dimethyl-2-phospholene 1-oxide[8] and N-bromosuccinimide was treated with 1 equiv of sodium ethoxide in ethanol; a compound (presumably 1-ethoxy-3,4-dimethylphosphole 1-oxide), λ_{max} 298 mμ, was generated. After 5 min a slight excess of acetic acid was added; the solution of the phosphole was stable at room temperature for several days, as evidenced by ultraviolet spectroscopy. The solution was evaporated at room temperature, and the carbon tetrachloride soluble portion of the residue was treated with maleic anhydride. Vb formation could be prevented by the use of superdry solvents or accelerated by the addition of a small amount of water or acetic acid. After crystallization from tetrahydrofuran, the compound melted at 261–263°; principal infrared bands (KBr) at 3.30, 5.29, 5.58, 6.88, 7.72, 8.11, 8.50, 9.22, 10.22, 10.70, 10.85, and 14.67 μ. *Anal.* Calcd for $C_{10}H_{11}O_5P$: C, 49.60, H, 4.58; P, 12.79. Found, C, 49.4; H, 4.54; P, 12.62.

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(7) E. T. Kaiser, M. Panar, and F. H. Westheimer, *ibid.*, **85**, 602 (1963).
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Biological Activities of Some Terminally Modified Squalene and Squalene 2,3-Oxide Analogs

Sir:

Recent findings in our own[1,2] and another[3] laboratory have shown that squalene 2,3-oxide (I) and not squalene (II) is the substrate which undergoes enzymic cyclization to lanosterol in preparations of rat liver. The cyclase system occurs in the microsomal fraction and requires neither oxygen nor NADPH.[2] The molecular asymmetry of squalene 2,3-oxide permits new approaches to the study of the mechanism of enzymic cyclization, and it is now possible to synthesize selected analogs of squalene oxide and to test them as substrates for the cyclase system in the expectation that the structures of their products will shed new light on the mode of interaction between this enzyme system and its substrate.

As a first approach to a systematic study of this type

(1) E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *J. Am. Chem. Soc.*, **88**, 4752 (1966).
(2) E. E. van Tamelen, J. D. Willett, and R. B. Clayton, *ibid.*, **89**, 3371 (1967).
(3) E. J. Corey and W. E. Russey, *ibid.*, **88**, 4750 (1966).

we have prepared radiolabeled 2,3-dihydrosqualene (III), 2,3-dihydrosqualene 22,23-oxide (IV), 1,1′,2-trisnorsqualene (V), and 1,1′,2-trisnorsqualene 22,23-oxide (VI) and have examined their metabolism in preparations of rat liver *in vitro*. We wish to report results which demonstrate the anaerobic enzymic cyclization of IV and VI and the oxidation and cyclization of III and V to sterols. ^{3}H-Labeled III and IV were prepared from I[4] as follows (all tlc separations were carried out with silica gel with 10% $CaSO_4$). Hydrolysis of I in 3% $HClO_4$ in aqueous glyme gave squalene 2,3-glycol (VII) which was cleaved to 1,1′,2-trisnorsqualene-3-aldehyde (VIII) by sodium metaperiodate. Reaction of VIII with isopropylmagnesium bromide gave 2,3-dihydro-3-hydroxysqualene (IX), which was converted to its *p*-tosylate (X) by treatment with *p*-tosyl chloride and pyridine. Lithium aluminum tritide reduction of X yielded (after continuous tlc with hexane) 75% III (124,000 dpm of ^{3}H/μg) and 25% II which was unlabeled and cleanly separated from III. Hydrocarbon III, radiochemically homogeneous by glpc on DEGS (retention time relative to II, 1.44 at 195°, N_2 flow 120 cc/min), was converted to ^{3}H-labeled IV *via* the 22,23-bromohydrin by the established procedure.[4] Correct elemental analyses and infrared, nmr, and mass spectral data consistent with the proposed structures were obtained for IV, VII, VIII, and IX, which behaved as radiochemically pure products on tlc and, with the exception of IV,[5] on glpc.

Reduction of VIII with sodium borohydride to the corresponding alcohol, followed by conversion to the tosylate and reduction with lithium aluminum tritide, gave ^{3}H-labeled V (118,000 dpm/μg, R_f on tlc with 1% EtOAc–hexane, 0.61), which was radiochemically homogeneous by glpc on DEGS (retention time relative to II, 2.51 at 198°, N_2 flow, 120 cc/min) and gave infrared, nmr, and mass spectral data consistent with the assigned structure.

The 22,23-oxide VI was prepared in the usual way *via* the corresponding 22,23-bromohydrin of V. Tlc of VI in 5% EtOAc–hexane provided a product (R_f 0.33) which gave nmr, infrared, and mass spectra in accord with the assigned structure.[6]

Incubations with Rat Liver Preparations. All incubations were carried out for 2 hr at 37° and were either aerobic, with a whole homogenate[7] containing an NADPH-generating system, or anaerobic, with a microsomal suspension.[8,9] Products were recovered by saponification, extraction of nonsaponifiable material, and separation by tlc.

From an anaerobic incubation of IV (4.5×10^6 dpm, 1.26 mg) material containing 3.6×10^5 dpm, moving as dihydrolanosterol (XI) (R_f 0.4), was isolated by tlc in 25% EtOAc–hexane. Glpc of the trimethylsilyl ether (TMSE) of this material on DEGS gave the major radioactive peak with a retention time relative to III of 3.88, corresponding to the TMSE of XI. The mass spectrum of this material was identical with that of the authentic TMSE of XI.

Anaerobic incubation of VI (3×10^6 dpm, 26.5 μg) gave a product (4.2×10^5 dpm) moving with 25,26,27-trisnorlanosterol (XII) (R_f 0.60 on tlc with 35% EtOAc–hexane). A portion of this material (203,000 dpm) was cocrystallized with 52 mg of authentic XII (mp 170–175°, prepared from lanosterol 24,25-oxide by a sequence analogous to that used in the preparation of V and fully characterized by infrared, nmr, and mass spectral analysis). Two crystallizations of the free sterol followed by two crystallizations after acetylation gave the acetate of XII (mp 147–148.5°, correct analysis, infrared) of constant specific activity 1680 dpm/mg. Hence, 48% of the activity of this fraction was retained in XII. On glpc of further portions (as the TMSE) *ca.* 50% of the effluent radioactivity behaved as the TMSE of XII on both Carbowax (retention time relative to V, 3.65) and DEGS (retention time relative to V, 3.75). The remaining activity (unidentified) was distributed at shorter retention times. From a duplicate anaerobic incubation of VI, further material (4.0×10^5 dpm), behaving chromatographically as XII, was similarly isolated and extensively purified by repeated tlc as the free sterol and as the acetate. Authentic XII (200 μg) was added to this material (2.06×10^5 dpm) and a part was shown by glpc, as TMSE, both on Carbowax and DEGS, to contain 70% ^{3}H-labeling corresponding to XII, the relative specific activity of which was 126.2 dpm/cm^2 peak area under specified glpc conditions. The remaining material (1.65×10^5 dpm) was converted by Moffat oxidation to 25,26,27-trisnorlanostenone (XIII), then to 25,26,27-trisnorlanostene (XIV) by Wolff–Kishner reduction. Glpc of XIV on DEGS under the conditions used for analyzing the TMSE of the starting material showed 70% effluent activity coincident with authentic XIV (correct analysis, infrared, nmr), with no significant change in specific activity. Finally, identity of enzymically produced XII was confirmed by its mass spectral comparison with an authentic specimen.

The foregoing results demonstrate the enzymic cyclization of IV and VI to the expected lanosterol analogs, XI and XII, respectively. Since these results were obtained with the microsomal fraction which is known to contain the enzyme system that cyclizes the natural product squalene 2,3-oxide to lanosterol, it seems likely that this enzyme system is responsible for the conversions described here, though this requires confirmation by studies with the purified enzyme.

To obtain information concerning the metabolic potential of the hydrocarbons III and V, aerobic incubations were performed as follows. Aerobic incubation of III (6×10^5 dpm, 4.8 μg) and the usual isolation procedures showed *ca.* 6% conversion to cholesterol and 2% conversion to dihydrolanosterol (R_f 0.42 and 0.54, respectively, on tlc in 25% EtOAc–hexane). Material with the R_f of cholesterol (27,400 dpm) was combined with 31.7 mg of pure cholesterol, acetylated, recrystallized, and purified *via* the dibromide with only 4.3% loss of specific activity.

From aerobic incubation of V (7.5×10^5 dpm, 6.3

(4) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, **3**, 121 (1962).

(5) IV, in common with I and VI, has proved unstable under glpc conditions.

(6) The structure of VI was further confirmed by acid hydrolysis to the 22,23-glycol and cleavage with metaperiodate to 4,8,13,17-tetramethyleicosa-4,8,12,16-tetraenal, for which correct infrared, nmr, and mass spectral data were also obtained.

(7) N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.*, **222**, 1 (1956).

(8) T. T. Tchen and K. Bloch, *ibid.*, **226**, 921 (1957).

(9) Detailed procedures will be described elsewhere: J. D. Willett, K. B. Sharpless, K. Lord, E. E. van Tamelen, and R. B. Clayton, *ibid.*, in press.

μg) similar procedures indicated *ca.* 1%[10] conversion to material behaving like cholesterol [or 25,26,27-trisnorcholesterol (XV)] on tlc. Cocrystallization of a portion of this material (4536 dpm) with authentic XV, 23.7 mg (mp 132–133.5°, synthesized by standard methods from 3β-hydroxy-Δ⁵-cholenic acid, correct infrared, nmr, and elemental analysis), gave a loss of 30% of the activity. Acetylation and further recrystallization gave no further loss of activity, but purification (as for cholesterol) *via* the dibromide resulted in retention of only 10% of the original radioactivity.

The results suggest that both 2,3-dihydrosqualene and 1,1′,2-trisnorsqualene can act as substrates for the squalene oxidase that normally converts squalene to the 2,3-oxide, and that trisnorlanosterol may be converted to other sterols of the trisnorcholestane series (but with poor efficiency) by the enzymes that normally oxidize lanosterol to cholesterol. Conversion of dihydrolanosterol and other 24,25-dihydrosterols to cholesterol is already well established.[11]

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(10) It should be noted that the present studies do not permit conclusions concerning the relative efficiencies of utilization of the oxides IV and VI, or of the hydrocarbons III and V, since different concentrations of substrates and different liver enzyme preparations were used in the various experiments. Further work directed toward a quantitative comparison of these substrates is in progress.

(11) R. B. Clayton, *Quart. Rev.* (London), **19**, 168 (1965).

(12) National Institutes of Health Predoctoral Fellow.

(13) National Institutes of Health Postdoctoral Fellow.

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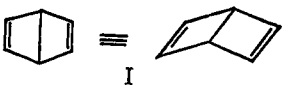
Nonaromatization Reactions of Bicyclo[2.2.0]hexa-2,5-diene

Sir:

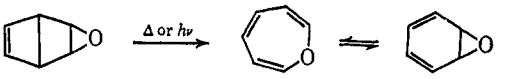
Despite the passage of some years since the first bicyclo[2.2.0]hexa-2,5-dienes (nonplanar "Dewar benzenes") (I) were reported,[1] the subsequent identification of additional cases in other laboratories,[2a,b] and the commercial availability of one of these,[3] very little is known about the chemical behavior of this high-



I

energy strained system. We have studied the interaction of parent "Dewar benzene" with various electrophilic species, finding that the reactions usually do not involve aromatization but characteristically provide nonbenzenoid transformation products.

Oxidation of bicyclo[2.2.0]hexa-2,5-diene[4] with 0.46 *M* *m*-chloroperbenzoic acid in diethyl ether at room temperature afforded the 2,3-oxide II, a colorless oil at room temperature (75% yield). The nmr spectrum of the oxide exhibited signals at τ 3.61 (H-5, -6; broad singlet), 5.97 (H-2, -3; doublet) and 6.68 (H-1, -4; broad singlet), all in the integrated ratio 1:1:1. In a mass spectral determination, the molecular ion (*m/e* 94) appeared as a major peak, accompanied by more intense peaks at *m/e* 78 and 44. After being heated neat at 115° ($t_{1/2} \cong 16$ min) or being irradiated with a low-



II III

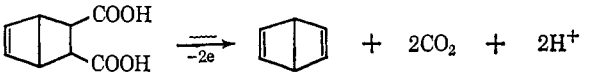
pressure mercury lamp at room temperature in *n*-pentane solution for *ca.* 1 hr, epoxide II isomerized to oxepin–benzene oxide (III), which was identified (vpc, nmr, ultraviolet) by comparison with authentic material.[5] Although no phenol was detected after epoxidation of I, it was produced during the thermolysis and photolysis reactions of II.

On being treated with 0.5 mmole of bromine in alkane solution at 0° for 10 min, "Dewar benzene" (*ca.* 1 mmole) was converted in 97% yield to an oily mixture of dibromides (IV).[6] No bromobenzene was detected. After rapid chromatography of IV, a pure component, the 2,3-*trans*-dibromide IVa, could be isolated [nmr spectrum: τ 3.52 (broad singlet), 3.67 (broad singlet), 5.32 (multiplet), 5.80 (doublet; J = 4 cps), 6.25 (multiplet); integrated ratio 1:1:1:1:2, respectively]. Ozonolysis of IVa, including an oxidative work-up with

IVa IVb

peracetic acid, provided 3,4-*trans*-dibromocyclobutane-*cis*-1,2-dicarboxylic acid (V), which as the diester was found to be identical (infrared, nmr) with an authentic specimen.[7] Catalytic hydrogenolysis [5% Pd–C, Et_3N (fourfold excess)] of V dimethyl ester led to dimethyl cyclobutane-*cis*-1,2-dicarboxylate ester (VI), direct comparison with the known substance being carried out.[8]

(4) The hydrocarbon used herein was prepared by the electrolytic decarboxylation of acid i, a reaction first performed by Mr. T. Whitesides (unpublished results secured in this laboratory).



i

(5) E. Vogel, W. A. Boll, and H. Günther, *Tetrahedron Letters*, 609 (1965).

(6) With larger proportions of bromine, "Dewar benzene" is converted to a crystalline tetrabromide, mp 120–120.5°.

(7) E. Vogel, *Ann.*, **615**, 14 (1958).

(8) N. L. Allinger, *J. Org. Chem.*, **30**, 1945 (1965).

(1) (a) E. E. van Tamelen and S. P. Pappas, *J. Am. Chem. Soc.*, **84**, 3789 (1962); (b) E. E. van Tamelen and S. P. Pappas, *ibid.*, **85**, 3297 (1963).

(2) For a partial list, see: (a) E. E. van Tamelen, *Angew. Chem.*, 77, 759 (1965); *Angew. Chem. Intern. Ed. Engl.*, **4**, 738 (1965); (b) H. G. Viehe, *Angew. Chem.*, 77, 768 (1965); *Angew. Chem. Intern. Ed. Engl.*, **4**, 746 (1965).

(3) W. Schäfer, *Angew. Chem.*, **78**, 716 (1966).