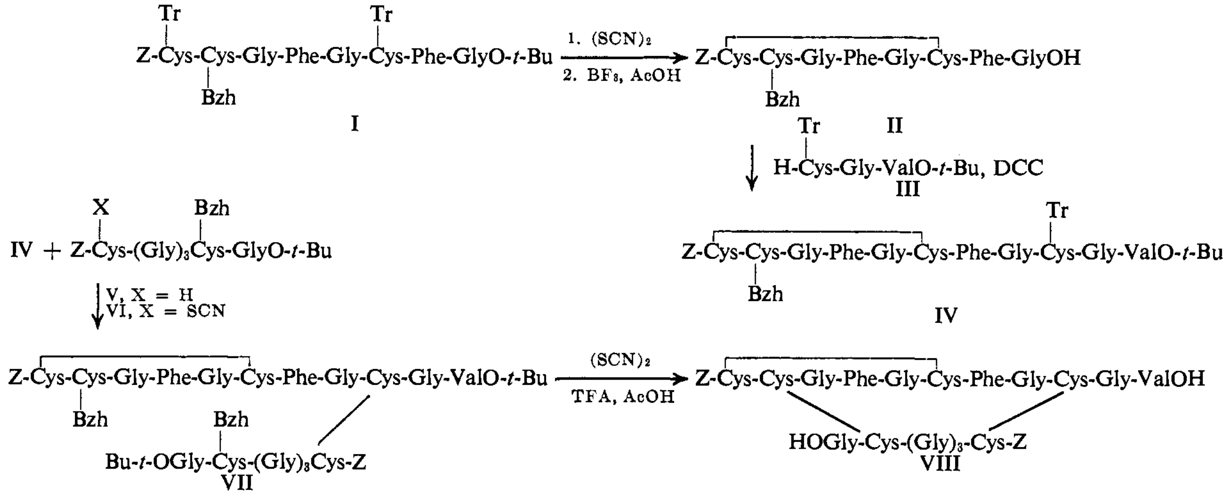
Scheme I



tide III afforded an A chain, IV, containing the A_{1-6} loop and two differentially protected cysteine residues.[2]

The stepwise formation of the two interchain disulfide bridges in VIII was conducted under conditions previously shown to avoid disulfide interchange.[3,4] The sulfenyl thiocyanate VI was generated from the corresponding thiol[5] V and allowed to react with IV at 0° in a mixture of ethyl acetate and acetic acid. The biscystine peptide derivative VII was obtained as a crystalline solid in 76% yield:[6] mp 151–152°; $[\alpha]^{24}\text{D}$ $-28.0°$ (*c* 0.47, DMF); mol wt (calcd) 2214, (found) 2220 (osmometric in *o*-chlorophenol); amino acid analysis after performic acid oxidation and acid hydrolysis: $\text{CySO}_3\text{H}_{6.0}\text{Gly}_{7.7}\text{Phe}_{2.0}\text{Val}_{1.0}$. The symmetrical disulfide resulting from oxidation of the B chain, V, was not observed in the thin layer chromatogram of the reaction mixture.

Selective oxidation of the two remaining S-protected cysteine residues (A_2, B_5) was accomplished by the action of thiocyanogen on VII. The cyclization was conducted in dilute solution (10^{-4} *M*) in a trifluoroacetic acid–acetic acid (1:3 v/v) solvent at 0°. The triscystine peptide derivative, VIII, was obtained in 75% yield; mp 230–236° dec; $[\alpha]^{25}\text{D}$ $-37.5°$ (*c* 0.16, DMF); mol wt (calcd) 1938, (found) 1765 (osmometric in *o*-chlorophenol); amino acid analysis after performic acid oxidation and hydrolysis: $\text{CySO}_3\text{H}_{5.5}\text{Gly}_{7.5}\text{Val}_{1.3}\text{Phe}_{2.2}$.

These data demonstrate that multiple sulfur–sulfur bonds can be selectively introduced into reasonably complicated polypeptides. Degradative experiments, designed to verify by chemical means the apparent location of the sulfur–sulfur bonds in a triscystine derivative related to VIII, are currently in progress.

Acknowledgments. We wish to thank Mrs. Mary W. Pendergraft and Dr. J. D. Morrisett for the amino acid analyses. Metal ion analyses were performed on a AEI-MS-702 spark source mass spectrometer by Dr. R. P. Buck and his associates of the Analytical Laboratory of the Materials Research Center. This research was supported by Research Grant AM-03416 from the Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. Stimulating discussions with Dr. J. A. Maclaren are gratefully acknowledged.

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[3] R. G. Hiskey and D. N. Harpp, *J. Am. Chem. Soc.*, **87**, 3965 (1965).

[4] R. G. Hiskey and M. A. Harpold, *Tetrahedron*, **23**, 3923 (1967).

[5] R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **31**, 2178 (1966).

[6] Satisfactory elemental analyses were obtained for all peptides reported.

Nonunique Ordering of Solute Molecules in Nematic Solvents. Electron Spin Resonance Observation of Coexisting Modes of Solute Alignment

Sir:

We have observed superposition of distinguishable patterns of solute ordering in nematic solvents[1–4] by means of esr spectroscopy of nitroxide radicals[5–9] (Figure 1). This finding supports the hypothesis that the most stable pattern of solute ordering is that of densest packing,[10] but challenges the generality of the uniqueness of this ordering, tacitly assumed in the interpretation of high resolution nmr,[1–3] uv,[10] and ir spectra in nematic solvents.

The nitroxide groups[11–14] are located on roughly planar piperidine-derived rings.[15] The principal axes

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[1] A. Saupe and G. Englert, *Phys. Rev. Lett.*, **11**, 462 (1963).

[2] S. Meiboom and L. C. Snyder, *Science*, **162**, 1337 (1968).

[3] A. Saupe, *Angew. Chem. Intern. Ed. Engl.*, **7**, 97 (1968).

[4] A. D. Buckingham and K. A. McLauchlan, "Progress in High Resolution NMR Spectroscopy," J. W. Emsley, J. Feeney, and L. H. Sutcliffe, Ed., Pergamon Press, Oxford, 1967.

[5] H. R. Falle, G. R. Luckhurst, J. Lemaire, Y. Marechal, A. Rassat, and P. Rey, *Mol. Phys.*, **11**, 49 (1966).

[6] H. Lemaire, *J. Chim. Phys.*, **64**, 559 (1967).

[7] S. H. Glarum and J. H. Marshall, *J. Chem. Phys.*, **47**, 1374 (1967).

[8] H. Lemaire, A. Rassat, P. Rey, and G. R. Luckhurst, *Mol. Phys.*, **14**, 441 (1968).

[9] P. Ferruti, D. Gill, M. A. Harpold, and M. P. Klein, *J. Chem. Phys.*, **50**, 4545 (1969).

[10] G. P. Caesar and H. B. Gray, *J. Amer. Chem. Soc.*, **91**, 191 (1969).

[11] E. Z. Rosantsev and M. B. Neiman, *Tetrahedron*, **20**, 131 (1964).

[12] R. Briere, H. Lemaire, and A. Rassat, *Bull. Soc. Chim. Fr.*, 3273 (1965).

[13] C. L. Hamilton and H. M. McConnell in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman and Co., San Francisco, Calif., 1968, p 115.

[14] A. R. Forrester, J. M. Hay, and R. H. Thomson, "Organic Chemistry of Stable Free Radicals," Academic Press, New York, N. Y., 1968.

[15] The names of radicals I–IV, the synthesis of which will be described in a forthcoming work, are: I = N-4-(2,2,6,6-tetramethyl)-

of the g and nitrogen hyperfine tensors are collinear and are simply related to the molecular geometry.[16] The crucial difference between I and II–IV is in the volume of the group bonded to C_4 of the piperidine ring. In I the single proton does not hinder the chair $\rightleftharpoons$ boat equilibrium.[17,18] Thus the average plane of the ring practically coincides with the x–y plane, and the x axis is *collinear* with the long molecular axis.[5–9] In II–IV, the carboxylic residue on C_4[19] constrains the ring into a boat configuration; as a result the x axis is nearly *perpendicular* to the long molecular axis.

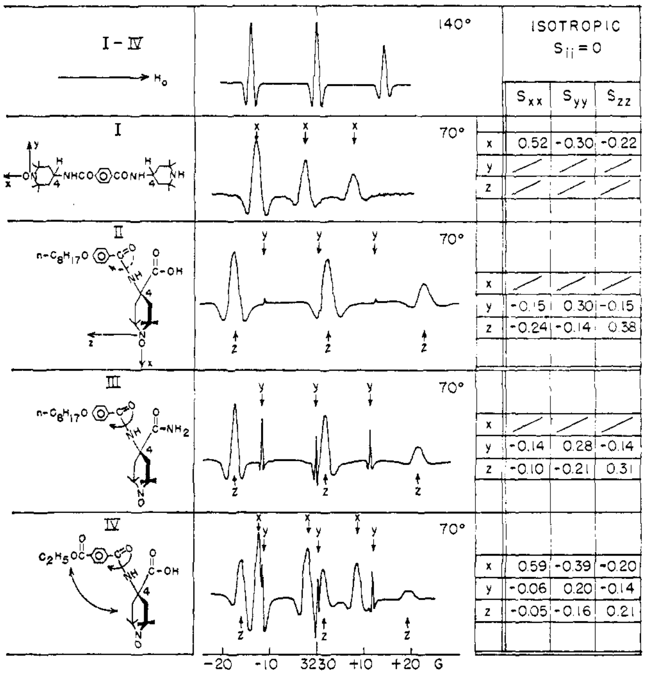
In partially ordered systems the esr lines occur at positions which are linearly interpolated[1–4,9] between the isotropic values and the single crystal[16] values. Spectra of radicals in which one of the principal axes is partially aligned parallel to H_0 are designated x, y, and z, corresponding to the largest positive diagonal element, S_{ii},[1–4,9] of the order parameter matrix (Figure 1, right).

Esr spectra were obtained with a Varian Model E-3 spectrometer equipped with a temperature controller. The solvent, 4-(4-ethoxyphenylazo)-phenyl hexanoate (PEPH, Eastman Organic Chemicals No. 10537), is isotropic above 130°, nematic down to 70°, and supercooled to 65°. Traces of the radicals were mixed with the nematogen and sealed in evacuated tubes after freeze–pump–thaw cycling.

In the isotropic phase (Figure 1, top) the spectra of all the radicals are similar. In the nematic phase, I shows only x alignment.[5–9] II shows predominantly z alignment and, in addition, a very weak y subspectrum which cannot be removed by thermal cycling. III displays simultaneously y and z subspectra. Though it is not quantitatively reproduced, the relative intensity of the y subspectrum is enhanced by lowering the temperature slowly through the nematic range. Molecule IV reveals the coexistence of all x, y, z subspectra.

Our interpretation, based on the densest packing hypothesis,[10] was assisted by reference to space-filling CPK models of the solute and solvent molecules. The long axes of II and III align in a unique pattern parallel to the long axes of the host nematogen. The observed y and z subspectra arise then from two coexistence conformers of II and III.

This interpretation is not adequate for IV, which yields an x subspectrum in addition to the y, z subspectra. Densest packing of L-shaped molecules may be realized in *two stable patterns* in which either one of the arms of the L substitutes for a nematogen molecule. The rate of the 90° jumps between the two roughly isoenergetic states is assumed to equal the 10^6 sec^{-1} flip rate of the long axes of the host molecules.[3,20–23]



	ISOTROPIC $S_{ii} = 0$		
	S_{xx}	S_{yy}	S_{zz}
I x	0.52	-0.30	-0.22
y	/	/	/
z	/	/	/
II x	/	/	/
y	-0.15	0.30	-0.15
z	-0.24	-0.14	0.38
III x	/	/	/
y	-0.14	0.28	-0.14
z	-0.10	-0.21	0.31
IV x	0.59	-0.39	-0.20
y	-0.06	0.20	-0.14
z	-0.05	-0.16	0.21

Figure 1. Second derivative esr spectra of nitroxide radicals I–IV in the isotropic and nematic phases of PEPH. On the left are shown the structural formulas, the prinicpal axes of the spin-Hamiltonian tensors, and amide bonds about which hindered rotations occur. The spectra shown in the center column are grouped into subspectra, denoted by their preferentially aligned principal axes. The y subspectra arise from a 90° twist of the amide bond while the x subspectra result from an alternative mode of solute ordering. On the right are listed the diagonal elements of the order parameter tensors pertaining to the respective subspectra.

In its most plausible configuration, molecule IV is L-shaped and conceivably assumes two patterns of nematic ordering. On the frequency scale set by the ^{14}N hyperfine splitting of the esr spectrum (~50 MHz) the residence time of 10^{-6} sec in each state is very long. If we set the width of the narrowest component line of the observed esr spectrum (~1 MHz) as an upper bound on lifetime broadening, the agreement with the estimated residence time of ~1 μsec is reasonable. This jump rate is, however, very rapid on the frequency scale of nmr in nematic solvents, and one would expect a *unique anisotropic nmr spectrum*[1,2] of diamagnetic solute analogs of molecule IV.

We thus ascribe the x subspectrum of IV to a pattern of ordering in which the x axis is *parallel* to the axis of nematic alignment. The y and z subspectra are assigned to another ordering pattern in which the x axis is *perpendicular* to the axis of nematic alignment.

The L-shape may change into another configuration for which unique ordering is preferred. After 5 hr at 140°, a proton superhyperfine structure appeared in the isotropic phase and the z subspectrum no longer appeared in the nematic phase. The complete dissolution of the heat-treated sample of IV in acetone and its subsequent drying failed to restore the z subspectrum of the initial material.

x alignment is limited neither to radical IV nor to the PEPH solvent, and has been observed in a number of analogous systems.

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piperidyl-N′-4(1-oxyl-2,2,6,6-tetramethyl)piperidyl terephthalamide; II = 1-oxyl-2,2,6,6-tetramethyl-4-(p-octyloxybenzoyl)amino-4-carboxypiperidine; III = 1-oxyl-2,2,6,6-tetramethyl-4-(p-octyloxybenzoyl)-amino-4-carbamidopiperidine; IV = 1-oxyl-2,2,6,6-tetramethyl-4-(p-carbethoxybenzoyl)amino-4-carboxypiperidine.

(16) O. H. Griffith, D. W. Cornell, and H. M. McConnell, *J. Chem. Phys.*, 43, 2909 (1965).

(17) C. Y. Chen and R. J. W. LeFevre, *J. Chem. Soc.*, 3467 (1965).

(18) R. Briere, H. Lemaire, A. Rassat, R. Rey, and A. Rousseau, *Bull. Soc. Chim. Fr.*, 4479 (1967).

(19) A. Rassat and P. Rey, *ibid.*, 816 (1967).

(20) W. Maier and G. Meier, *Z. Naturforsch. A*, **16**, 470, 1200 (1961); *Ber. Bunsenges Physik. Chem.*, **65**, 556 (1961).

(21) G. Meier and A. Saupe, *Mol. Cryst.*, **1**, 515 (1966).

(22) P. Pincus, *Solid State Commun.*, 7, 415 (1969).

(23) M. Weger and B. Cabane, Colloque Sur Les Cristaux Liquides, Montpellier, June 1969.

work was supported, in part, by the U. S. Atomic Energy Commission.

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[25] On leave from the Istituto di Chimica Industriale, Politecnico di Milano, Milan, Italy. Supported by NATO Fellowship.

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Di-π-cyclooctatetraenethorium

Sir:

The relative stability of the recently synthesized di-π-cyclooctatetraeneuranium from cyclooctatetraene (COT) dianion and uranium tetrachloride suggests that significant stabilization is afforded by overlap of the highest occupied (E_2) molecular orbitals of the two rings with vacant f orbitals ($f_{xyz}, f_{z(x^2-y^2)}$) of the central metal.[1] It is clearly important to compare the chemistry of similar complexes with other actinide and with lanthanide rare-earth metals. We report here the preparation and some of the chemistry of the thorium analog.

A suspension of dry thorium tetrachloride in tetrahydrofuran was added to 2 equiv of K_2COT in THF cooled to Dry Ice temperature. The mixture was allowed to warm to room temperature with stirring overnight. The solvent was evaporated from the yellow reaction mixture and the residue was sublimed at 0.01 Torr and 160° to produce the product as fine bright yellow crystals. The mass spectrum showed a parent peak at *m/e* 440 ($Th(COT)_2$) with important fragment peaks at *m/e* 336 (ThCOT), 111 and 109 (unassigned), and 104 (COT). Resublimation gave crystals suitable for X-ray analysis. The compound is isomorphous with di-π-cyclooctatetraeneuranium[2] (uranocene), and the thorium compound therefore also has the D_{8h} sandwich structure.[3]

$Th(COT)_2$ ("thoracene") is decomposed readily by water. It is unstable in air but does not enflame, as does uranocene; crystals of the thorium compound change in color from yellow to brown after a few minutes' exposure to air. The compound decomposes without melting at temperatures above 190° and explodes if heated to red-hot. Thoracene is insoluble in most organic solvents, *e.g.*, $CHCl_3$, CCl_4, THF, benzene, acetone, etc.; it is soluble in DMSO, but the nmr spectrum of this solution shows a complex multiplet at 6.2 ppm rather than the sharp singlet expected for a D_{8h} sandwich structure. On exposure to air for several seconds the nmr spectrum changes to the sharp singlet of COT at 5.75 ppm. We suggest that thoracene forms a complex with DMSO which destroys the symmetry of the rings and changes the structure to that of a diene–transition metal type. This chemistry may be rationalized as follows if bonding interaction with 5f orbitals is important in the di-π-cyclooctatetraeneactinide structures. We have suggested[1] that the two highest energy electrons in uranocene are in a back-bonding $f_{x(x^2-3y^2)}$, $f_{y(3y^2-x^2)}-E_{3u}$ combination. These MO's are vacant in thoracene and could produce a Lewis acid capability that would lead to the observed reactions with Lewis bases such as DMSO and water. The extension of this chemistry to other actinides and to the lanthanide rare earths is in progress.

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[1] A. Streitwieser, Jr., and U. Müller-Westerhoff, *J. Amer. Chem. Soc.*, 90, 7364 (1968).

[2] X-Ray analysis by K. N. Raymond. A complete X-ray structure determination is in progress to determine bond lengths.

[3] A. Zalkin and K. N. Raymond, *J. Amer. Chem. Soc.*, **91**, 5667 (1969).

The Reaction of a Sulfonyl-Chymotrypsin with Hydrogen Peroxide. Generation of a Hydroperoxy Enzyme

Sir:

Generating a reactive free radical at a specific site on a protein and allowing the radical to react with its surroundings would be useful as a chemical probe for three-dimensional structure in solution.[1] The present work was designed to produce a peroxide moiety (which could then be decomposed by photolysis) at the active site of the serine protease chymotrypsin. In this report the preparation of an apparently catalytically active hydroperoxy enzyme is described.

Benzylsulfonyl-chymotrypsin (modified at serine-195) was prepared according to the published procedure.[2] In all experiments it had less than 0.8% catalytic activity. When incubated at pH 7 with H_2O_2 as shown in Figure 1, catalytic activity was fairly rapidly restored until deactivation of enzyme became a significantly competing process (about 12 hr). As previously reported,[2] benzylsulfonyl-chymotrypsin was completely stable under the same conditions without H_2O_2. Native enzyme under the reaction conditions lost activity only very slowly, while the same reaction with the sulfonate and *n*-propyl hydroperoxide resulted in an extremely slow restoration of catalytic activity, probably due to H_2O_2 impurity.[3]

Variation in pH of the reactivation mixture resulted in a regular increase in the rate of reactivation from pH 4 to about pH 7 (the optimum). At much above pH 7 activity was lost at a rate comparable to its restoration.

The product of the H_2O_2 reaction was isolated by exhaustive dialysis (2 to 5 ml *vs.* two changes of 2 l. of 10^{-3} *M* HCl, then four changes of double distilled water) followed by lyophilization. Further experiments were performed on this hydrogen peroxide free material.

From the simple organic system we would expect that the dominant process might be (1) by an S_N2 displace-

$$\begin{aligned} &E\text{–}CH_2OSO_2CH_2C_6H_5 + {}^-OOH \longrightarrow \\ &\qquad\qquad E\text{–}CH_2OOH + C_6H_5CH_2SO_3^- \quad (1) \end{aligned}$$

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[1] A carbene approach to this problem has been taken by Westheimer: J. Shafer, P. Baronowsky, R. Laursen, F. Finn, and F. H. Westheimer, *J. Biol. Chem.*, **241**, 421 (1966); R. Vaughan and F. H. Westheimer, *J. Am. Chem. Soc.*, **91**, 217 (1969).

[2] A. M. Gold and D. Fahrney, *Biochemistry*, **3**, 783 (1964); **5**, 2911 (1966).

[3] *n*-Propyl hydroperoxide was prepared from *n*-propyl methanesulfonate and H_2O_2: H. R. Williams and H. S. Mosher, *J. Am. Chem. Soc.*, **76**, 2984 (1954). After two distillations, analysis still showed *n*-propyl alcohol and H_2O_2 impurities. Work is in progress to clearly define the alkyl hydroperoxide reaction.