the mixture was connected to a water pump through two soda lime U-tubes and was evacuated with stirring for 2 hr to remove ammonia and methanol. The evaporation was completed with a vacuum pump for 3 additional hr.[20] The cleaved material was extracted with DMF (three 10-ml portions) and methanol (two 10-ml portions) and the resin was removed by filtration. The solvents were evaporated at 30° on a rotary evaporator and the residue was dried *in vacuo* over $P_2O_5$ to remove residual solvents. Upon trituration with 95% ethanol followed by washing with 95% ethanol and diethyl ether and further drying *in vacuo* over $P_2O_5$, the protected nonapeptide amide Z-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-$NH_2$ was obtained as a white amorphous powder, weight 355 mg, mp 246–248°, $[\alpha]^{22}D$ −50.5° (*c* 1, dimethylformamide). *Anal.* Calcd for $C_{72}H_{92}N_{12}O_{14}S_2$: C, 61.1; H, 6.53; N, 11.9. Found: C, 61.2; H, 6.82; N, 11.8.

The yield of the protected nonapeptide amide from the cleavage was 73% of the amount expected based on the increase in weight of the resin. The over-all yield based on the amount of glycine originally esterified to the resin was 59%. Amino acid analysis[21] gave: Asp, 1.00; Glu, 1.12; Pro, 0.90; Gly, 1.07; Ile, 1.02; Leu, 1.14; Tyr, 0.83; Bzl-Cys, 1.95; Cys, 0.07; $NH_3$, 3.1. The total time required for the synthesis and cleavage was 6 days. The protected nonapeptide (100 mg) was treated with sodium in liquid ammonia as described by du Vigneaud, *et al.*,[2] and the resulting free thiol groups were oxidized by treatment with an aqueous solution of potassium ferricyanide[22] to give a solution possessing a total of 15,900 IU of oxytocic activity.[23] Pure oxytocin (32.5 mg, 46%) was obtained from a lyophilizate of this solution by gel filtration on Sephadex G-15,[24] $[\alpha]^{22.5}D$ −24.0° (*c* 0.5, 1 *N* acetic acid). *Anal.* Calcd for $C_{43}H_{66}N_{12}S_2 \cdot CH_3COOH \cdot H_2O$: C, 49.80; H, 6.69; N, 15.49. Found: C, 49.93; H, 6.85; N, 15.37. Amino acid analysis gave: Asp, 1.00; Glu, 1.00; Pro, 1.10; Gly, 1.0; Cys, 1.80; Ile, 0.89; Leu, 1.00; Tyr, 0.85; $NH_3$, 3.0. When examined by thin layer and paper chromatography the product was shown to be homogeneous and to give the same $R_f$ values as those reported for oxytocin.[25] The material exhibited an oxytocic activity of ~430 IU/mg. This value may be increased by ~12% if the water and acetic acid content of the lyophilized product are taken into account. The over-all yield of biologically active oxytocin was 27%. The total time required for the synthesis starting with Boc-amino acids and ending with chromatographically pure oxytocin was 10 days.

(20) During the evacuation at the vacuum pump the methanolic ammonia was trapped by interposing between the flask containing the suspension and the vacuum pump a glass freeze-drying apparatus (Scientific Glass Apparatus Co., Inc., Bloomfield, N. J., catalog no. JD-9379) having a Dry Ice–acetone mixture in the central compartment and the central flask, containing 100 ml of a 1:1 mixture of 12 *N* HCl and glacial acetic acid, immersed in a Dry Ice–acetone bath at −80°.

(21) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).

(22) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).

(23) P. Holton, *Brit. J. Pharmacol.*, 3, 328 (1948); R. D. Munsick, *Endocrinology*, **66**, 860 (1960).

(24) M. Manning and T. C. Wuu, unpublished data.

(25) M. Manning and V. du Vigneaud, *J. Am. Chem. Soc.*, **87**, 3978 (1965).

The advantages of rapidity and efficiency which characterize the Merrifield method have been fully demonstrated in the extension of the method for use in the synthesis of the key intermediate required for the synthesis of oxytocin. Further application is being made toward the synthesis of protected nonapeptides required for the synthesis of lysine-vasopressin[26] and of analogs of oxytocin and lysine-vasopressin. It is also worthy of note that Beyerman[27] has carried out the solid-phase synthesis of the partially protected nonapeptide leading to 9-deamido-oxytocin[28] and Takashima, du Vigneaud, and Merrifield[29] have utilized the solid-phase technique for the synthesis of deamino-oxytocin.[22]

**Acknowledgment.** I wish to thank Dr. Murray Saffran for extensive use of laboratory facilities and Dr. Tim Wuu and Mr. Levon Guluzian for performing the amino acid analyses and the bioassays.

(26) During the preparation of this report the approach outlined herein has been used to synthesize in 60% yield (based on the amount of glycine originally esterified to the resin) an analytically pure protected nonapeptide amide with the amino acid sequence of lysine-vasopressin: Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-Gly-$NH_2$, mp 228–231°, $[\alpha]^{22.5}D$ −42° (*c* 1, dimethylformamide). An aliquot (100 mg) of this material upon reduction with sodium in liquid ammonia[2] and subsequent oxidation with potassium ferricyanide[22] gave a solution possessing a total of 15,000 units of pressor activity: "The Pharmacopeia of the United States of America," 16th revision, Mack Publishing Co., Easton, Pa., 1960, p 793.

(27) H. C. Beyerman, C. A. M. Doers-Boonekamp, W. J. Van Zoest, and D. Van Den Berg, "Peptides," H. C. Beyerman, A. Van de Linde, and W. M. Van Den Brink, Ed., North Holland Publishing Co., Amsterdam, 1967, p 117.

(28) B. M. Ferrier and V. du Vigneaud, *J. Med. Chem.*, **9**, 55 (1966).

(29) Personal communication from Drs. Herbert Takashima, Vincent du Vigneaud, and Bruce Merrified.

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## The Stereochemistry and Inversion of Trivalent Oxygen

*Sir:*

The most succinct example of a unimolecular thermal reorganization is the pyramidal inversion of molecules that possess lone pairs. The product is either enantiomeric, diastereomeric, or equivalent to the starting material. Nearly all previous studies have focused on the inversion properties of nitrogen,[1] although other atoms have received some attention.[2,3] We have initiated a general program to broaden the scope of knowledge in this field to include other atoms. In the present communication and the next,[4] we wish to report the first observation of the thermal stereomutation of oxygen and of arsenic.

Oxygen must necessarily be trivalent for inversion studies. Although the planar pyrilium salts are clearly

(1) (a) For a very complete review, see G. Binsch, "Topics in Stereochemistry," Vol. III, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, Inc., New York, N. Y., in press; (b) for calculations of inversion barriers, see G. W. Koeppl, D. S. Sagatys, G. S. Krishnamurthy, and S. I. Miller, *J. Am. Chem. Soc.*, **89**, 3396 (1967).

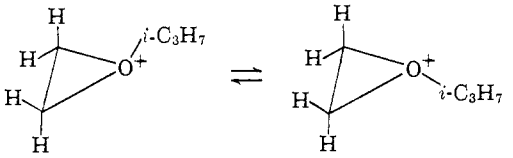
(2) Sulfur: (a) D. R. Rayner, E. G. Miller, P. Bickart, A. J. Gordon, and K. Mislow, *ibid.*, 88, 3138 (1966); (b) P. Haake and P. C. Turley, *ibid.*, **89**, 4611, 4617 (1967); (c) E. W. Abel, R. P. Bush, F. J. Hopton, and C. R. Jenkins, *Chem. Commun.*, 58 (1966); (d) D. Darwish and G. Tourigny, *J. Am. Chem. Soc.*, 88, 4303 (1966).

(3) Phosphorus: (a) J. B. Lambert and D. C. Mueller, *ibid.*, 88, 3669 (1966); (b) L. Horner and H. Winkler, *Tetrahedron Letters*, 461 (1964).

(4) J. B. Lambert and G. F. Jackson, III, *J. Am. Chem. Soc.*, **90**, 1350 (1968).

unsuitable, Meerwein's trialkyloxonium salts, isoelectronic to amines,[5] offer an acceptable possibility. The stereochemistry of Meerwein's salts and of protonated alcohols and ethers[6] is presumed but not proved to be tetrahedral, by analogy with the hydronium ion.[7] The configurational stability of trivalent oxygen may be tested by examination of the nmr spectrum of a methylene group adjacent to trivalent oxygen and contained in a rigid cyclic system of appropriate symmetry. Configurational stability would be indicated by an AB spectrum (complicated by coupling to adjacent protons); rapid inversion or fortuitous degeneracy would give an $A_2$ spectrum.

We have examined the methyl, ethyl, and isopropyloxonium salts of oxirane, oxetane, tetrahydrofuran, and tetrahydropyran[8] in liquid $SO_2$. At 40° the spectrum of the isopropyloxonium salt of ethylene oxide (oxirane) contains a very sharp singlet 149 Hz below internal cyclopentane due to the ring protons, a doublet centered at 13 Hz below cyclopentane due to the methyl protons,[9] and a septet at 182 Hz below cyclopentane due to the methinyl proton. As the temperature is lowered, the ring proton resonance broadens to coalescence at −50° ($T_c$) and sharpens to a closely coupled ($\nu_{AB}$ ~3 Hz) AA'BB' spectrum at −70°. The half-height method,[10] the coalescence temperature, and a complete line-shape analysis above $T_c$ all indicate an activation energy ($E_a$) of 10 ± 2 kcal/mole. Analogously reversible effects were observed for the methyl and the ethyl salts. The physical basis for these changes must be a slowing of the rate of oxygen inversion that results in magnetically nonequivalent ring protons at the lowest temperatures. The stereochemistry of these trialkyloxonium salts must therefore be tetrahedral, rather than planar.



The spectra of the oxonium salts of the six-membered tetrahydropyran are temperature independent.[11,12] The rates of oxygen inversion in these salts must therefore be too rapid to cause spectral alterations even at −70°. The situation is similar to that of nitrogen, the inversion of which was first examined in aziridines.[13]

The spectral data described thus far could also be explained in terms of a bimolecular mechanism in which inversion is effected by rapid intermolecular exchange of the alkyl groups between two oxonium ions or between one oxonium ion and a free ether molecule. This mechanism has been excluded by two lines of reasoning. The rate of a bimolecular reaction should be concentration dependent. We have examined the methyloxonium salt of ethylene oxide over the concentration range 0.2–2.0 mmole/ml and found the observed rate process to be independent of concentration. Various competition experiments were also carried out to test the exchange properties of the alkyl substituents. (1) Two equivalents of tetrahydropyran was allowed to react with 1 equiv each of $CH_3I$ and $AgBF_4$. The nmr spectrum showed equal amounts of complexed ($\alpha$ protons at 198 Hz below cyclopentane) and uncomplexed ($\alpha$ protons at 130 Hz) tetrahydropyran. Rapid exchange would have required only one averaged resonance for the $\alpha$ protons. (2) The O-methyl, O-ethyl, and O-isopropyl derivatives of ethylene oxide were prepared in the usual manner;[8] after several days, 1 equiv of tetrahydropyran was added to each. The nmr spectra of the three-membered-ring oxonium complexes remained unchanged, and the only resonances from the six-membered ring were of uncomplexed tetrahydropyran. Thus, none of the O-alkyl substituent was transferred from the three-membered ring to the six-membered ring. (3) To show that tetrahydropyran is not at a thermodynamic disadvantage in obtaining an O-alkyl substituent in the presence of ethylene oxide, 1 equiv of each was allowed to compete for 1 equiv of alkylating agent. Under these conditions both complexes formed. It is thus clear from both the concentration and the competition experiments that the alkyl groups do not undergo an intermolecular interchange on the nmr time scale of observation. The physical process causing the spectral changes must therefore be the unimolecular thermal inversion of oxygen.

(5) H. Meerwein in Houben-Weyl's "Methoden der Organischen Chemie," Vol. 6, Part 3, Georg Thieme Verlag, Stuttgart, 1965, p 325.

(6) G. A. Olah and D. H. O'Brien, *J. Am. Chem. Soc.*, **89**, 1725 (1967).

(7) L. W. Schroeder and J. A. Ibers, *ibid.*, 88, 2601 (1966), and references therein. These studies may, however, be exceptional because the experiments were on the solid state and the materials were extensively hydrogen bonded.

(8) All oxonium salts were prepared *in situ* by reaction of an alkyl halide with silver tetrafluoroborate and an ether. About 1 mmole

$$RX + AgBF_4 + R_2'O \longrightarrow R_2'RO^+BF_4^- + AgX\downarrow$$

each of $AgBF_4$ and the ether were placed in an nmr tube under anhydrous conditions. The tube was cooled to −78° and the volume was brought to 1 ml with $SO_2$. One equivalent of the halide was added, together with the standard (cyclopentane), and the tube was sealed and shaken for a short time. After about 1 day the silver halide was centrifuged to one end of the tube. All spectra were recorded on a Varian A-60.

(9) The position of the methyl doublet serves as a convenient indicator for the charge on the methinyl carbon. The methyls of 2-bromopropane in $SO_2$ center at 11 Hz below cyclopentane. When $AgBF_4$ is added to form the dimethylcarbonium ion (no ether present), the methyls respond to the adjacent positive charge by shifting downfield to 48 Hz below cyclopentane. When the ether is then added to form the oxonium salt, the methyls move back upfield to 13 Hz below cyclopentane, indicating that the charge has passed from the adjacent carbon to the more distant oxygen.

(10) J. B. Lambert, *Tetrahedron Letters*, 1901 (1963).

(11) The spectra of the four- and five-membered rings were too complicated for a straightforward analysis. The isopropyl derivative of tetrahydropyran did not form,[9] presumably for the same steric reason that Meerwein failed to isolate triisopropyloxonium tetrafluoroborate; *cf.* H. Meerwein, E. Battenberg, H. Gold, E. Pfeil, and G. Willfang, *J. Prakt. Chem.*, **154**, 96 (1939).

(12) The nmr spectrum of such an ion was reported by F. Klages, J. E. Gordon, and H. A. Jung, *Ber.*, **98**, 3748 (1965).

(13) A. T. Bottini and J. D. Roberts, *J. Am. Chem. Soc.*, 78, 5126 (1956).

(14) This work was supported by the National Science Foundation (Grant GP-6611).

(15) National Institutes of Health Predoctoral Fellow, 1967–1968.

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## The Stereomutation of Arsenic

*Sir:*

Because of its configurational stability, trivalent arsenic is capable of supporting optical activity.[1,2]

(1) V. I. Sokolov and O. A. Reutov, *Russ. Chem. Rev.*, 34, 1 (1965).

(2) K. Mislow, "Introduction to Stereochemistry," W. A. Benjamin, Inc., New York, N. Y., 1966, pp 87–88.