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Inversion at Pyramidal Oxygen and Sulfur[1]

Sir:

Inversion about pyramidal nitrogen is a well-established phenomenon.[2] One way in which the rate of this inversion has been measured in solution is by proton magnetic resonance spectroscopy of tertiary benzyl amines.[3,4] Pyramidal geometry about tricoordinate oxygen has been established for $H_3O^+Cl^-$ in the solid,[5] and probably for $(CH_3)_2OBF_3$ in the gas phase.[6] Pyramidal geometry about oxygen in the liquid phase and the barrier to inversion have been determined for the oxonium salts of oxirane,[7] but not for a neutral species. Pyramidal configuration about trigonal sulfur was established many years ago by isolation of optically active trialkyl sulfonium salts.[8] The barrier to inversion in these salts is so high that racemization occurs by dissociation and recombination of the sulfonium ion.[9,10]

When a CH_2 group is bonded to a carbon atom bearing three different substituents the two methylene protons will be magnetically nonequivalent regardless of the rate of rotation about the carbon-carbon bond between the asymmetric center and the methylene group.[11] A similar situation prevails if the asymmetric center has only three groups but is held in a pyramidal configuration.[12] Upon inversion about the pyramidal center the nonequivalence of the methylene protons will be lost.

The BF_3 complex of benzyl ethyl ether was prepared by condensing equimolar quantities of the two reactants, separated by the sulfur dioxide solvent, into an NMR sample tube at liquid nitrogen temperature on a vacuum line. Upon thawing and mixing the complex is formed. The proton resonance spectrum of such a sample at 100 MHz and −65 °C is interpreted as having nonequivalent methylene protons. These results indicate slow inversion about pyramidal oxygen in the complex.

The ether alone, in sulfur dioxide as solvent, has sharp single lines from the aromatic protons at 7.26 ppm and the benzylic protons at 4.42 ppm, a quartet at 3.51 ppm from the methylene protons, and a triplet at 1.16 ppm from the methyl protons at both 25 and −65 °C. The para proton is shifted to 6.4 ppm and the ortho and meta protons to 7.0 ppm upon reaction with 1 equiv of BF_3. The three envelopes remain broad from 25 to −65 °C. The methyl triplet is shifted downfield to 1.46 ppm and the methylene quartet to 4.30 ppm at 25 °C. By −65 °C a complex but sharp pattern of lines is observed for the ethyl group and may be analyzed for the following parameters.

$$\delta CH_3 = 1.45 \text{ ppm} \qquad J_{CH_3\text{-}A} = 7.3 \text{ Hz}$$
$$\delta CH_A = 4.24 \text{ ppm} \qquad J_{CH_3\text{-}B} = 7.3 \text{ Hz}$$
$$\delta CH_B = 4.36 \text{ ppm} \qquad J_{A\text{-}B} = 14.4 \text{ Hz}$$

The broadening of the phenyl and benzyl proton signals in the complex may be explained by chemical shifts which would give a multiplicity of unresolved lines in this complicated spin system.

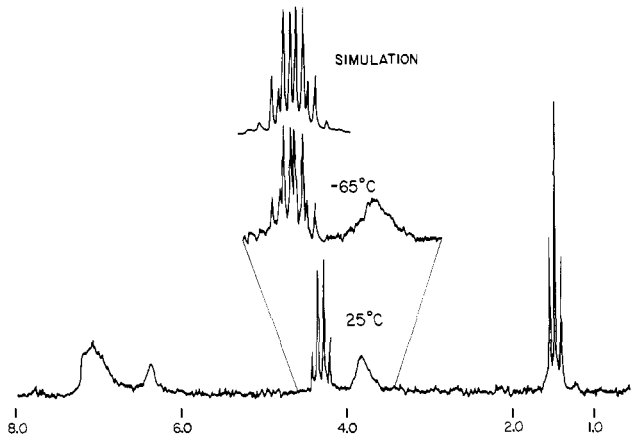

Figure 1. Experimental and theoretical proton resonance spectra of the benzyl ethyl ether boron trifluoride complex.

Experimental spectra at 25 and −65 °C are shown in Figure 1, along with a simulated spectrum of the methylene region in the slow exchange limit. The effects of temperature upon the spectrum are completely reversible. From a comparison of experimental spectra as a function of temperature and theoretical spectra as a function of exchange rate,[13] $\Delta H^{\ddagger}$ is calculated to be 4.1 ± 0.3 kcal/mol.

An unambiguous explanation of the spectral results in terms of hindered inversion about pyramidal oxygen requires refutation of the possible existence of two BF_3 complexes (with oxygen and the aromatic π electrons) and exchange between the two complexes. If there were two complexes one would expect different chemical shifts for the methyl as well as methylene protons, but the methyl lines remain narrow over the entire temperature range. The observed spectra were simulated by the spin system ABC_3. They cannot be simulated by exchange between two systems of type A_2B_3 even if the two groups of methyl protons are given the same chemical shift.

Nonequivalence of the methylene protons of benzyl ethyl ether complexes with tantalum pentafluoride has also been observed. However, complexes with niobium pentafluoride, boron trichloride, phosphorus pentafluoride, aluminum trichloride, and germanium tetrafluoride do not give nonequivalent methylene protons at low temperature. Phenetole, 2-ethoxynapthalene, diethyl ether, and diallyl ether complexes also gave no evidence of nonequivalent methylene protons.

Nonequivalence of the methylene protons in a complex of diethyl sulfide with BH_3 has been observed and attributed to hindered inversion about sulfur.[12] Hindered inversion has also been observed in platinum chloride complexes of dibenzyl sulfide and the barrier to inversion was found to be 18 kcal/mol.[14] In the present study nonequivalent methylene protons were observed in the complex between diethyl sulfide and boron trichloride, but not in complexes with boron trifluoride, tantalum pentafluoride, niobium pentafluoride, germanium tetrafluoride, titanium tetrafluoride, aluminum trichloride, and tungsten hexafluoride. Additional Lewis acids and other ethers and sulfides are being investigated to determine the factors which allow nonequivalent methylene protons to be ovserved in complexes of ethers and sulfides.

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Synthesis of a Protein with the Properties of the Apolipoprotein C-I (ApoLP-Ser)

Sir:

We wish to report the synthesis of apolipoprotein C-I (apoLP-Ser), a constituent of very low density lipoproteins (VLDL) of human plasma.[1] Apolipoprotein C-I was shown to be an activator of lecithin:cholesterol acyltransferase[2] and thus may play an important role in cholesterol metabolism.[3] The amino acid sequence of C-I was recently determined[4] and was characterized by a high lysine content (16%) and the absence of cysteine, tyrosine, and histidine in the 57 residue sequence.

Heterogeneity of synthetic products is recognized as a serious problem in the solid phase synthesis[5] of peptides larger than ten residues.[6,7] The formation of incorrect sequences has often been attributed to incomplete coupling reactions[8-11] and premature removal of side chain protection.[12] Several variations were introduced into the automated synthetic procedure used to prepare apoC-I in an attempt to overcome some of these problems. A 1% cross-linked polystyrene resin support was used with a very low Boc(*O*-benzyl)serine substitution in an attempt to reduce deletion sequences.[8,13] The use of shrink and swell washes[8] and a mixed solvent system for coupling reactions[9] were used in an attempt to expose all of the reactive sites during the synthesis. The premature deprotection of the ϵ-amino group of lysine[14] was minimized by use of the *o*-chlorobenzyloxycarbonyl (*o*-Cl-Z)[15] derivative. The single Trp residue was protected by the N^{im}-formyl group.[16] Symmetrical anhydrides of Boc amino acids[17] were used in all coupling reactions, as it had been reported that this coupling method improves yields in longer syntheses.[18]

The synthesis was based on Boc(*O*-benzyl)serine esterified to the polystyrene resin[19] (0.086 mmol/g). The amino acid resin (4 g) was then treated to the automated stepwise addition[20] of a three- to fourfold excess of suitably protected amino acids. The Boc group was used for α-amino protection, and the side chains were protected as follows: Asp (β-OBzl), Glu (γ-OBzl), Ser (Bzl), Thr (Bzl), Lys (*o*-Cl-Z), Arg (NO_2), Trp (CHO). The coupling steps, including the addition of Asn and Gln, were carried out with a twofold excess of the symmetrical anhydride[17] (generated in situ by addition of half the required amount of DCC at the commencement of a coupling and the remainder after 1 h). With Asn and Gln the coupling reaction was carried out in the presence of an equimolar amount of 1-hydroxybenztriazole. An identical second coupling reaction was performed, except that a solvent system of dichloromethane and dimethylformamide (1:1, v/v) was used. Boc groups were removed by two treatments of the resin with trifluoroacetic acid in dichloromethane (50%, 25%, v/v) for 5 and 30 min, respectively. The peptide was cleaved from the resin (1 g) with liquid HF (20 ml) at 0° for 60 min in the presence of anisole (1 ml).[21] The formyl group was removed under mildly basic conditions (4°, pH 7.6, 3 days) and examination of the uv absorption at 280 and 300 nm[16] showed good removal of the protecting group. The crude protein was then purified by gel filtration (see Figure 1) and ion exchange chromatography (see Figure 2).

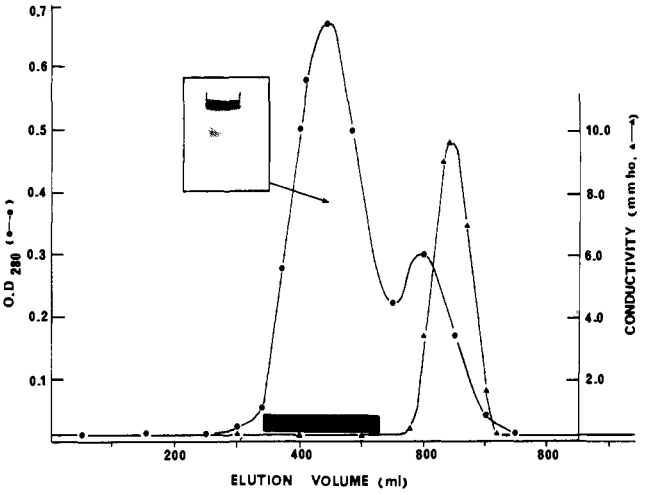

Figure 1. Gel filtration of the synthetic apoC-I after HF cleavage. The product was dissolved in 0.02 M potassium phosphate, pH 7.0 (50 ml), and applied to a column of Sephadex G50 (90 × 2.5 cm). The column was run with the same buffer as eluent, at a flow rate 100 ml/h and 10 ml/fraction. Each fraction was monitored by OD_{280} measurements (●—●) and conductivity (▲—▲). The solid bar shows the fractions which contained significant activity as an activator of LCAT, and the inset shows the result of polyacrylamide gel electrophoresis (7.5% cross-linked gel, pH 8.9, 8 M urea).

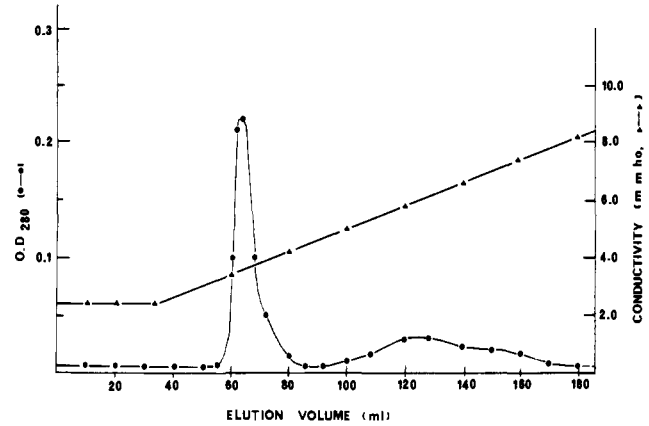

Figure 2. Purification of synthetic apoC-I by ion exchange chromatography on Sephadex SP-25. Synthetic apoC-I was applied to a column of SP-Sephadex (9 × 1.5 cm) equilibrated in potassium phosphate buffer (pH 4.0, 0.05 M) and the product was eluted with a linear gradient of potassium phosphate buffers (0.02 M, pH 4.0, 200 ml to 0.2 M, pH 7, 200 ml), at a flow rate of 200 ml/h. Fractions (7 ml) were monitored by OD_{280} (●—●) and conductivity (▲—▲). The recovery of purified synthetic apoC-I (first peak pH 4.2, conductivity 3.5 $m\Omega^{-1}$) was variable (20-50%), and was probably caused by the tendency of apoC-I to aggregate in the absence of denaturants.[28]

Examination of the crude product after HF cleavage indicated that the modifications to the synthetic procedure had indeed led to a more homogeneous product, as shown by a good yield of cleaved peptide,[22] by amino acid analyses at strategic points during the synthesis, and by electrophoretic and amino acid analysis of the crude product.[23]

The product after purification behaved as a homogeneous protein in that it eluted as a single narrow peak on gel filtration and ion exchange chromatography. Synthetic apoC-I migrated as a single band in a position identical with native apoC-I on