gether with studies by Davis[6] and Kost,[7] cast doubt on our earlier conclusions and suggest that a different explanation be sought for the remarkable dependence[3] of sulfenamide torsional barriers on the electronegativity of the substituent at sulfenyl sulfur.[8]

References and Notes

(1) (a) For part 31 of this series, see G. Yamamoto and M. Raban, *J. Org. Chem.*, **41**, 3788 (1976). (b) This work was supported by the National Science Foundation.
(2) Reviews: (a) C. Brown and B. T. Grayson, *Mech. React. Sulfur Compd.*, **5**, 93 (1970); (b) F. A. Davis, *Int. J. Sulfur Chem.*, **8**, 71 (1973).
(3) (a) D. Kost and M. Raban, *J. Am. Chem. Soc.*, **98**, 8333 (1976); (b) M. Raban, D. A. Noyd, and L. Bermann, *J. Org. Chem.*, **40**, 752 (1975); (c) M. Raban, E. H. Carlson, S. K. Lauderback, J. M. Moldowan, and F. B. Jones, Jr., *J. Am. Chem. Soc.*, **94**, 2738 (1972); (d) M. Raban and F. B. Jones, Jr., *ibid.*, **93**, 2692 (1971); (e) M. Raban, G. W. J. Kenney, Jr., and F. B. Jones, Jr., *ibid.*, **92**, 6677 (1969).
(4) The three compounds other than **1a** and **2a** and their barriers are: *N*-(1-phenylethyl)-*N*-benzenesulfonylbenzenesulfenamide,[3c] 13.0 kcal/mol; *N*-(1-phenylethyl)-*N*-*p*-toluenesulfonyl-2-nitrobenzenesulfenamide,[3c] 18.4 kcal/mol; *N*-(1-phenylethyl)-*N*-*p*-toluenesulfonyl-4-nitrobenzenesulfenamide,[5] 14.7 kcal/mol. The first of these differs from the others in having a benzenesulfonyl residue rather than a *p*-toluenesulfonyl residue at nitrogen. Previous experiments[3d] have shown, however, that this change does not affect the barriers of the closely related *N*-isopropyl-*N*-arenesulfonylbenzenesulfenamides.
(5) S. K. Lauderback, Ph.D. Dissertation, Wayne State University, 1974.
(6) F. A. Davis and E. W. Kluger, *J. Am. Chem. Soc.*, **98**, 302 (1976).
(7) D. Kost and A. Zeichner, *Tetrahedron Lett.*, 3239 (1975). See also: D. Kost and M. S. Sprecher, *ibid.*, 1089 (1977).
(8) (a) All new compounds reported here received satisfactory elemental analyses and had NMR spectra in accord with the assigned structures. The trinitrobenzenesulfenamides were prepared by reaction of trinitrobenzenesulfenyl chloride[8b] with the appropriate amine or the silver salt of the appropriate *N*-alkylsulfonamide. (b) G. Yamamoto and R. Raban, *J. Org. Chem*, **42**, 597 (1977).
(9) (a) A. P. Sloan Fellow, 1972–1976. (b) "Faculty of Science, University of Tokyo".

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A Spin Labeling Study of a Polysaccharide Support Matrix for Affinity Chromatography

Sir:

Although affinity chromatography is widely used for the separation and purification of a variety of biological molecules,[1] little information is available at the molecular level about the interactions upon which the technique depends, the microscopic structures of support materials, or the empirical observation that a "spacer arm" is sometimes required between the matrix and the immobilized ligand for maximum efficiency of separation.[2] The spacer, however, has been observed to confer upon the matrix a further binding selectivity independent of the immobilized ligand,[3] and hydrophobic chromatography[4] relies on this type of interaction.

We describe herein the use of the spin labeling technique[5] to investigate agarose,[6] a commonly used affinity chromatographic support matrix. Cyanogen bromide-activated agarose[6,7] was coupled to ω-aminocarboxylic acids, $H_2N(CH_2)_nCO_2H$, where n = 1, 3, 5, and 10, in 0.1 M bicarbonate buffer, pH 8.3, and each of these spacer–agarose conjugates was linked, after being washed free of unreacted amino acid, in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride[8] (EDC), at pH 5, to each of the stable nitroxide radicals 4-amino-2,2,6,6-tetramethylpiperidino-1-oxy (**1**) and 3-amino-2,2,5,5-tetramethylpyrrolidino-1-oxy[9] (**2**). In addition the two nitroxides were coupled directly to the activated polysaccharide. Each labeled product was exhaustively washed on a sintered glass filter using bicarbonate (pH 8.3) and acetate (pH 4.0) buffers to remove noncovalently bound, adsorbed, spin label.[10]

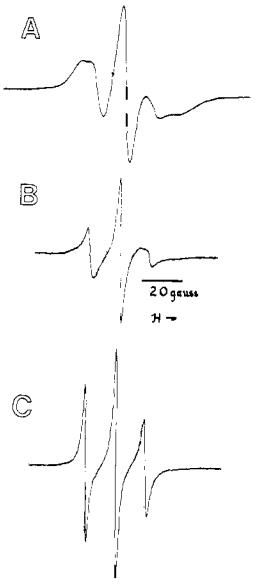
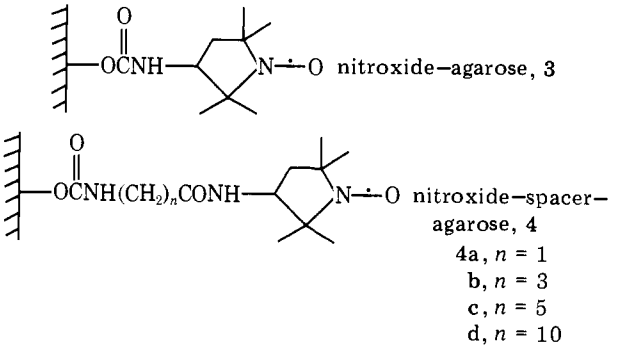


Figure 1. EPR spectra of: (A) compound **3**, (b) compound **4a** ($\tau = 1.4 \times 10^{-9}$ s), and (c) compound **4c** ($\tau = 4 \times 10^{-10}$ s), in aqueous suspension recorded at 28 °C on a Varian E-3 spectrometer.



The EPR spectra of the products[11] were found to become sharper with increasing n (Figure 1), and the rotational correlation times τ[12] of the nitroxide decrease, approaching a limiting value of 3×10^{-10} s at large n.[11] For $1 \leq n \leq 10$, i.e., in those cases where a spacer arm is present, the spectrum is characteristic of a "weakly immobilized" nitroxide moiety, while for directly bound label a "moderately immobile" spectrum is obtained, and two partially resolved spectral components are present. Both nitroxides gave similar results.

The increased freedom of rotational motion experienced by the nitroxide (ligand) in the presence of a spacer arm reflects its greater "availability" to the surrounding solution. Steric hindrance of the ligand by the support matrix has been suggested[13] as a factor tending to decrease binding efficiency of substrate in affinity chromatography in cases where the spacer is insufficiently long, and the quantitative (increase in τ) and qualitative (appearance of a second spectral component) changes in the spectra upon decreasing the length of the spacer and finally removing it completely corroborate this idea. It has also been found that the increase of the anchoring arm beyond a certain limiting length, typically corresponding to that of about an eight-atom chain, causes no further improvement in separation efficiency;[14] this is paralleled in our results by the approach of the correlation time of the nitroxide to a minimum value at about the same chain length.

The directly attached label appears to be present in two distinct environments characteristic of the support material.

We suggest that these two types of reaction site, which we shall call "open" (lower τ) and "masked" (higher τ), are in fact present in all the labeled matrices; where, however, we have interposed a spacer between the label and its point of attachment to the solid phase, the two components of the EPR spectrum are not resolved; the rotational freedom of the label is not sensitive to the site of attachment of the spacer to the matrix.

Evidence for this contention was obtained by subjecting the ten labeled agaroses to a cycle of solvent changes: water to ethanol, from this to 1,4-dioxane and finally to *n*-heptane.[15] A progressively less polar environment is thus experienced by a label exposed to free solution. The agarose was observed to contract increasingly during the cycle, and two components were now visible in the EPR spectrum of each product in ethanol.[11] All these changes were completely reversible; that is, by addition of water at any point in the cycle one could return to spectra such as those shown in Figure 1.

These observations are in accord with a model for the structure of precipitated agarose in which separate polysaccharide strands associate,[16] probably by hydrogen bonding, giving rise to some form of tertiary structure. We propose that "open" ligand attachment sites are located on regions of single stranded, unassociated chain, and that "masked" sites are created by chain associations.

However, line shapes resembling that in Figure 1A can also arise as a result of very anisotropic rotational reorientation of a spatially isotropic distribution of spin labels.[17] Thus rapid rotation could occur about the single bonds joining a label to the polysaccharide matrix, motion perpendicular to this being more restricted.

Further investigations aimed at the elucidation and extension of this model are in progress.

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(2) See, e.g., (a) B. R. Baker and H. U. Siebeneick, *J. Med. Chem.*, **14**, 799 (1974); (b) E. Steers, Jr., P. Cuatrecasas, and H. B. Pollard, *J. Biol. Chem.*, **246**, 196 (1971).
(3) See, e.g., B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, **50**, 751 (1973).
(4) S. Shaltiel and Z. Er-el, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 778 (1973).
(5) L. J. Berliner, "Spin Labelling, Theory and Applications", Academic Press, New York, N.Y., 1976.
(6) Agarose was obtained as gifts of Sepharose 4B and CNBr-activated Sepharose 4B from Pharmacia, and as a gift from Marine Colloids, Ltd. (SKA-Me 11335).
(7) In cases where the activation was performed in the laboratory, the method of S. C. March, I. Parikh, and P. Cuatrecasas, *Anal. Biochem.*, **60**, 149 (1974), was used.
(8) Obtained from Sigma.
(9) Obtained from Eastman.
(10) In the absence of a spacer, and for small *n*, the EPR spectrum of the products was sufficiently broad that the presence of noncovalently bound label, characterized by a sharp triplet, was obvious. This enabled us to design a thorough washing procedure; six washes with 10 mL of buffer to 0.1 g of product, using each buffer alternately, were generally enough.
(11) Detailed results to be published in the full paper.
(12) Calculated according to the method of T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 1010 (1965), which assumes Lorentzian line shape and isotropic tumbling of the label. Because these conditions are not fulfilled, and because there is, for example, an estimated 20% error in the calculation for Figure 1B, and no estimate of τ has been quoted for Figure 1A, we attach only order-of-magnitude significance to the figures.
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(14) P. Cuatrecasas in "Biochemical Aspects of Reactions on Solid Supports", G. R. Stark, Ed., Academic Press, New York, N.Y., 1971.
(15) The solvent change was accomplished via two intermediary, two component mixtures in each case; thus for example between water and ethanol the sample was exposed to 7:3 and 3:7 water:ethanol mixtures, being sucked free of supernatant between each change.
(16) D. A. Rees, *Adv. Carbohydr. Chem. Biochem.*, **24**, 267 (1969), has proposed a double helix.
(17) E. L. Wee and W. G. Miller, *J. Phys. Chem.*, **77**, 182 (1973). In the treatment of Mason et al. (*ibid.*, **78**, 1324 (1974)) which is based on the rigorous theory of Freed (*ibid.*, **75**, 3385 (1971)), axially symmetric rotation is analyzed in terms of components along two perpendicular axes.

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Gas-Phase Acidities: Alkanethiols

Sir:

The order of acidity of the simple aliphatic alcohols in the gas phase is known to be $H_2O < MeOH < EtOH < i\text{-}PrOH < t\text{-}BuOH$.[1-3] This order is reversed, however, upon solvation in a variety of solvents.[4-6] Calorimetry has revealed that in dimethyl sulfoxide (Me_2SO) the reversal results primarily from the effect of alkyl substituents upon the heat of solvation of the anions.[7] It appears that increasing bulk of the alkyl substituent strongly hinders solvation of the anion and leads to destabilization of the anion relative to the neutral acid, but the extensive ion-pairing of alkoxides in Me_2SO[8] makes interpretation of the calorimetric results somewhat uncertain. Ion-pairing does not occur in water, but unfortunately only the free energies of ionization, $\Delta G°_i{}^{H_2O}$, are known there. The low acidity of the alcohols seems to have prevented determination of the entropies, $\Delta S°_i{}^{H_2O}$, and the enthalpies, $\Delta H°_i{}^{H_2O}$, of ionization.

The entropies and enthalpies of ionization in water are known for the alkanethiols, the next higher group 6A acids.[9] We have, therefore, measured the acidities of several thiols in the gas phase to determine the intrinsic effect of alkyl groups on acidity and to compare gas-phase acidities with aqueous acidities where ion-pairing is absent.

Pulsed ion cyclotron resonance (ICR) mass spectrometry was used to measure equilibrium constants for proton-transfer reactions between the thiols, RSH,

$$A^- + RSH = RS^- + HA \qquad (1)$$

and various reference acids, HA, using established techniques.[3,10-13] Methyl nitrite was used as the source of negative ions. Double resonance between A^- and RS^- was observed for all acid pairs measured, indicating that the reactions are fast compared to the time scale of the experiment. Multiple overlaps with different reference acids provided results which agreed to within ±0.2 kcal/mol for all acid pairs measured.

The data in Table I show the effects of alkyl groups on the relative acidity of alkanethiols and alcohols in the gas phase and in aqueous solution. The thiols are quite similar to the alcohols in substituent and solvation effects on acidity: increasing the size of the alkyl group results in increased gas-phase acidity and decreased aqueous acidity. In the gas phase the incremental change in going from Me to Et is larger for O than for S acids, but Et to *i*-Pr and *i*-Pr to *t*-Bu produces the same acidity increases for both series. In aqueous solution there is a complete inversion in the order of acidities for both the alcohol series and the alkanethiol series. This trend is analyzed for the thiols in Table II in terms of the effects of alkyl substituents on the relative enthalpy and entropy of ionization in the gas phase and in aqueous solution. In the gas phase the entropy change is very small for proton-transfer reactions such as in Table II. The numbers shown were calculated using literature values for the absolute entropies of the alkanethiol