the consecutive first-order reaction sequence

$$\mathrm{I} \xrightarrow{k_1} \left[\mathrm{V} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{VI}\right] \xrightarrow{k_3} \mathrm{II} + \mathrm{III}$$

where $k_2 > k_{-2} > k_3 > k_1$ and $k_3/k_1 = 52$ (by extrapolation to 141.8° from Table I), calculations[8] give $[\mathrm{VI}]_{max}/[\mathrm{I}]_0 = 1.77\%$ and $t_{max} = 24$ min. The observed concentration profile of VI demands that the principal energy surface connecting I and cyclopentenes II and III passes through the equilibrium system V $\rightleftarrows$ VI.[9]

The observed $\Delta H^{\ddagger}$ values (Table I) are consistent with transition states resembling intermediates $A^{\ddagger}$, $B^{\ddagger}$, or $C^{\ddagger}$ in which the migrating carbon (starred) p orbital is orthogonal to a nearly planar pentadienyl or allyl species[10] and thus reflect contributions from (1) the summed delocalization energies of each orthogonal component and (2) nonbonding interactions arising from s-cis *vs.* s-trans conformations of either a migrating allyl group of the initial pentadienyl chain. It is this second contribution, clearly destabilizing $C^{\ddagger}$ relative to $B^{\ddagger}$, and precluding direct cyclopentene formation from $A^{\ddagger}$ by approximately 2.3 kcal/mol,[11] which in effect retards direct closure of either $A^{\ddagger}$ or $B^{\ddagger}$ to a five-membered ring.

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(8) G. Pannetier and P. Souchay, "Chemical Kinetics," Elsevier, New York, N. Y., 1967, pp 186–188.

(9) Thermodynamic considerations require that $C^{\ddagger}$ be nearly equally accessible from V and VI.

(10) The probable role of orthogonal trimethylenemethanes in the thermolysis of methylenecyclopropanes has been discussed by W. E. Doering and H. Roth, *Tetrahedron*, **26**, 2825 (1970), and by J. J. Gajewski, *J. Amer. Chem. Soc.*, **93**, 4450 (1971); possibly orthogonal species such as IV have been recently invoked by W. R. Roth and H. Schmidt, *Tetrahedron Lett.*, 3639 (1971), to explain stereoconvergence from thermolysis of 1,2-cis and 1,2-trans ring-disubstituted allylidenecyclopropane derivatives.

(11) *Cf. s-cis- vs. s-trans*-butadiene, $\Delta F_{eq} \simeq 2.3$ kcal/mol: J. G. Aston, G. Szasz, H. W. Wooley, and F. G. Brickwedde, *J. Chem. Phys.*, **14**, 67 (1946).

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Proton Magnetic Resonance Studies in Trifluoroethanol. Solvent Mixtures as a Means of Delineating Peptide Protons

Sir:

One of the most important steps in determining the secondary structure of small polypeptides by pmr spectroscopy is the separation of peptide protons into groups according to whether they are exposed to the solvent or shielded from the solvent either sterically or through hydrogen bonds. The two most common methods of accomplishing this delineation are through the temperature dependence of the peptide proton chemical shifts[1,2] and by deuterium proton exchange rates. The peptide protons exposed to the solvent will have the higher temperature dependence and the higher deuterium–proton exchange rates. Here we would like to suggest another method of differentiating between these protons in cyclic polypeptides of relatively fixed conformation by using mixtures of TFE (2,2,2-trifluoroethanol) with other solvents.

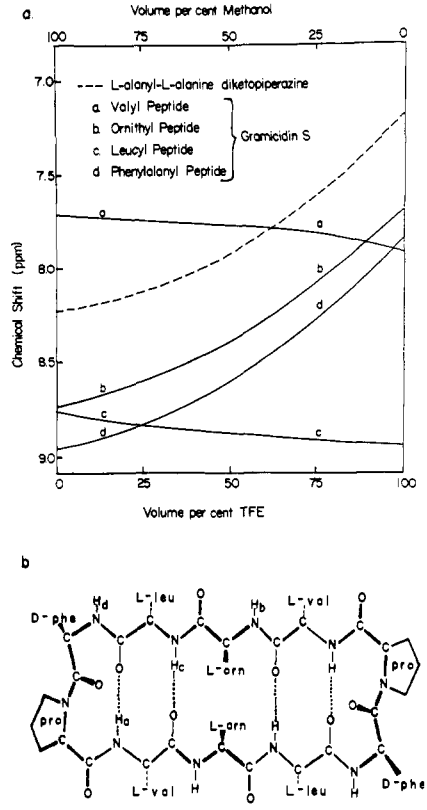

Figure 1. (a) Chemical shifts of the peptide protons of gramicidin S and L-alanyl-L-alanine diketopiperazine as functions of volume per cent of methanol and trifluoroethanol. Spectra were recorded at 220 MHz. (b) Secondary structure of gramicidin S.

As an example we take gramicidin S. Pmr studies on gramicidin S have been conducted by Conti,[3] Stern, *et al.*,[4] and Ohnishi and Urry.[2] A summary of the steps leading to the elucidation of the secondary structure of gramicidin S has been presented by Urry and Ohnishi.[5] The peptide region of the pmr spectrum of gramicidin S in methanol consists of four doublets, each corresponding to two amino acid residues. As the volume per cent of TFE is increased in a methanol–TFE mixture, the phenylalanyl and ornithyl peptide resonances undergo a dramatic upfield shift, the valyl and leucyl, a slight downfield shift (Figure 1). The peptide proton–α-proton coupling constants are independent of the TFE–methanol solvent ratios and the α protons show very little change in splitting patterns. This suggests there is no change in backbone conformation and very little if any change in side chain conformations. Similar behavior is observed in TFE–dimethyl sulfoxide mixtures. The solution conformation of gramicidin S

(1) K. D. Kopple, M. Ohnishi, and A. Go, *J. Amer. Chem. Soc.*, **91**, 4264 (1969).

(2) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969).

(3) F. Conti, *Nature (London)*, **221**, 777 (1969).

(4) A. Stern, W. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci. U. S.*, **61**, 734 (1968).

(5) D. W. Urry, "Spectroscopic Approaches to Biomolecular Conformation," American Medical Association, Chicago, Ill., 1970, pp 227–281.

as presented by Stern, *et al.*,[4] has the valyl and leucyl peptide protons intramolecularly hydrogen bonded and the phenylalanyl and ornithyl peptide protons exposed to the solvent. It is, therefore, the resonances of the peptide protons exposed to the solvent which undergo the large upfield shift as the per cent of TFE is increased. The pmr spectra of gramicidin S in both deuteriomethanol and deuterio-TFE are very sharp suggesting no aggregation. As a reference compound, the position of the peptide proton resonance for DKP (L-alanyl-L-alanine diketopiperazine) was observed in various TFE–methanol mixtures. The result, as shown in Figure 1, is consistent with an upfield shift for solvent exposed peptide protons and very closely parallels the result for the nonintramolecularly hydrogen bonded protons of gramicidin S.

The exact nature of the solvent–peptide proton interaction giving rise to this phenomenon is not entirely clear at present. However, a decrease in the extent of hydrogen bonding with the solvent is consistent with an upfield shift for the corresponding resonance.[6] Goodman and Rosen[7] found for a series of L-glutamate oligomers that TFE induced secondary structure. Circular dichroism results by Urry, *et al.*,[8] indicate that TFE mimics the natural environment of membrane proteins insofar as the membrane proteins in 80% TFE give ellipticities very similar to those calculated for membrane proteins in their natural environment. We would like to raise the possibility that TFE does not hydrogen bond as well with the peptide protons as does methanol. This would in turn favor intramolecular hydrogen bonding, thereby increasing the probability of a fixed solution conformation.

There are other lines of experimental evidence supporting this idea. In deuteriomethanol the phenylalanyl and ornithyl peptide protons exchange very rapidly; whereas in deuterio-TFE exchange is slow. Also temperature dependence studies in TFE show the temperature coefficients for the ornithyl and phenylalanyl peptide protons to be very similar to the valyl and leucyl, in sharp contrast to methanol studies where the differentiation between internally and noninternally hydrogen bonded protons is very clearcut.[2] This means that in TFE, exposure of peptide protons to solvent has little effect on the temperature dependence.

The similar but opposing dipoles for the CF_3 and CH_2OH moieties may, in part, be responsible for the unusual solvent properties of TFE. This and other possible mechanisms are under consideration. The essential point of this communication, however, is the presentation of a third method for delineating peptide protons in small, relatively rigid cyclic polypeptides and thereby elucidating their secondary structure. Investigations are presently underway concerning the general applicability of this method.

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Reactions of Methylene in Solution. Formation of Olefins

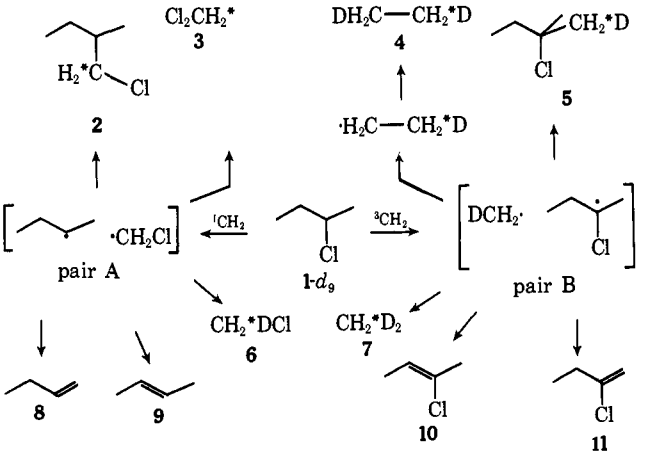
Sir:

Recently we described the application of chemically induced dynamic nuclear polarization (CIDNP) as a mechanistic tool in the study of carbene reactions.[1] In our continuing investigations we have uncovered a novel reaction of methylene, namely, the dehydrogenation or dehydrochlorination of suitable substrates to form olefins and, respectively, methane or chloromethane.

It had been observed that the insertion of methylene into the carbon–chlorine bond of optically active 2-chlorobutane (**1**) proceeded with a high degree of racemization.[2] We reinvestigated this reaction employing direct photolysis and photosensitized decomposition of diazomethane for generating $^1CH_2$ and $^3CH_2$, respectively. In order to simplify the nmr spectra of prospective polarized products a perdeuterated substrate was used (**1**-d_9). The resulting spectra are shown in Figure 1a, c. The signal directions of the polarized products were analyzed in the framework of the radical-pair theory.[3] We assumed that all radicals involved have π character ($a_\alpha < 0$; $a_\beta > 0$) and that radicals bearing a chlorine have larger g values than their alkyl counter radicals.

Similar to the reactions with $CDCl_3$[1b] the two spin states of methylene were selective in their abstraction reactions with 2-chlorobutane (Scheme I). Thus,

Scheme I[a]



[a] An asterisk denotes polarization.

the polarized products observed during direct irradiation could be explained by attack at the chlorine atom ($^1CH_2$), whereas the polarized products observed in

(1) H. D. Roth, *J. Amer. Chem. Soc.*, **93**, 1527, 4935 (1971).
(2) W. von E. Doering, private communication.
(3) R. Kaptein, *Chem. Commun.*, 932, (1971), has suggested simple qualitative rules for the evaluation of CIDNP spectra. References to the radical-pair theory of CIDNP may be found therein.

(6) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N.Y., 1959, Chapter 15.
(7) M. Goodman and I. G. Rosen, *Biopolymers*, 2, 537 (1964).
(8) D. W. Urry, L. Masotti, and J. R. Krivacic, *Biochim. Biophys. Acta*, 241, 600 (1971).