Under the conditions used for the CIDNP studies 4a is converted to 5a in *ca.* 90% yield and the azine 7 (Ar = C_6H_5) is among the products. However, control experiments with authentic samples showed that none

of the polarized lines corresponded to any of the ^{13}C resonances of 7 (Ar = C_6H_5) or of the possible additional products, carbon oxysulfide and the disulfide **8**.

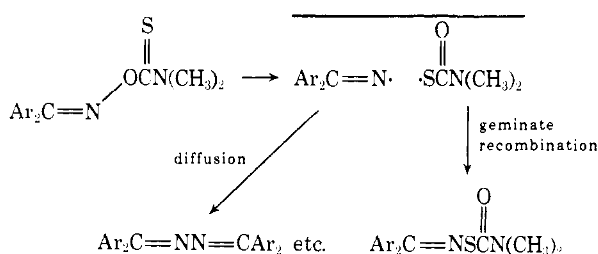
The *g* values for the iminyls[5] **6** are *ca.* 2.003 and for the radical $(CH_3)_2NCOS\cdot$,[11] 2.05. Furthermore the distribution of spin density in iminyls is governed by hyperconjugation,[12] and thus the signs of the hyperfine coupling constants for the C=N and C-1 carbons in **6** will be negative and positive, respectively.

Knowledge of these parameters allows application of Kaptein's rules[2] for net polarization

$$\Gamma_{ne} = \mu\epsilon\Delta g A_i \quad (1)$$

where μ, ϵ, Δg, and A_i have their usual significance. Since the net effects for the C=N and C-1 carbons are respectively E(−) and A(+), it is clear from eq 2 and 3 that ϵ is positive and that polarized **5** therefore arises from cage recombination of the iminyl and $(CH_3)_2$-NCOS· radicals (Scheme I), providing overwhelming support for our proposed pathway.[4]

Scheme I



$$\text{C=N:} \quad \text{sign product} = -+-- \equiv - \text{(E)} \quad (2)$$

$$\text{C-1:} \quad \text{sign product} = -+-+ \equiv + \text{(A)} \quad (3)$$

Azine **7** must arise from iminyls which escape from the cage, and so should show polarization of the C=N and C-1 carbons of the opposite sense to those observed for the rearrangement product **5**. No such polarization could be detected in our spectra. A possible explanation for this lies in the relatively long life[12] of the iminyl radical **6** (in keeping with its ready detection by esr[4,5]). This would allow nuclear relaxation[13] to occur before product formation. We are currently investigating this interesting aspect of the problem.

sult that the C=O signal is masked by that of the C=N. However, these lines are clearly distinguished in the spectrum of authentic product recorded at the reaction temperature.

(11) A. J. Lawson, unpublished work.

(12) J. A. Brivati, K. D. J. Root, M. C. R. Symons, and D. J. A. Tingling, *J. Chem. Soc. A*, 1942 (1969); M. C. R. Symons, *Tetrahedron*, 615 (1973).

(13) This situation was anticipated by Closs and Trifunac as early as 1970: G. L. Closs and A. D. Trifunac, *J. Amer. Chem. Soc.*, **92**, 2186 (1970).

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Possible Sequential Analysis of Small Oligopeptides (Penta to Hepta) with ^1H Nuclear Magnetic Resonance Spectroscopy at 300 MHz

Sir:

In 1966 Sheinblatt[1] in Israel succeeded in determining the amino acid sequences of di- and tripeptides by comparing α-H shift values in their nmr spectra obtained in D_2O at different pH values. The addition of base transforms their zwitterion forms into the corresponding anionic species, whereas acid creates the cationic forms. Therefore, during titration, the H atoms nearest the carboxyl (head side) eventually become either shielded (NaOD added) or deshielded (DCl added) relative to those in the zwitterionic form, and the opposite behavior is found for the α-H atoms nearest the tail. With conventional magnets, the resulting chemical shift change is small; only in the case of the amino acid residues adjacent to the head and the tail positions are these shifts large enough to permit assignment in the nmr spectrum. Since the nmr absorption owing to the penultimate residue in tripeptides are relatively insensitive to pH changes in the surrounding solvent medium, the sequence designation of tripeptides is easily established by this simple nmr procedure.

Sheinblatt's original studies were performed at 100 MHz. With a gain of a factor of three in field strength (300 MHz), we found that the second residue (and sometimes also the penultimate residue) shows demonstrable shift changes in its α-H atom absorption. This suggested to us the possibility of sequence determination by nmr spectroscopy of tetra- and (in favorable cases) of pentapeptides.

We have found now the addition of shift-inducing lanthanide *salts* (Eu^{3+}, Pr^{3+}, Tb^{3+}, Yb^{3+}, Ho^{3+}) to be extremely helpful in increasing the shift variation by an additional factor of *ca.* 10–30. In D_2O, *free* carboxylate anions, but not the undissociated CO_2H groups are good ligands.[2] We have found that free amino functions also show definitive capability to compete favorably with the solvent for ligand formation with the added lanthanide shift reagent (LSR). Shifts of both α-H atoms and β-H atoms can easily be followed, thus facilitating identification of each amino acid residue (Figure 1). Further, the homonuclear indor technique allows the complete identification of that residue together with an assignment of its relative position in the peptide chain.

Figure 2 shows observed shift changes for the α-H atoms in tetraalanine which result upon addition of increasing amounts of the La^{3+}. Pr^{3+} causes downfield shifts whereas Eu^{3+} affords upfield shifts; these results are in the opposite direction from what is found in nonprotic solvents.[2] The effect is presumed[3] to be largest for the head (no. 4) residue (*e.g.*, the residue situated adjacent to the carboxylate group). This

(1) M. Sheinblatt, *J. Amer. Chem. Soc.*, **88**, 2845 (1966).

(2) K. G. Morallee, E. Nieboer, F. J. Rossotti, R. J. Williams, A. Xavier, and R. A. Dwek, *Chem. Commun.*, 1132 (1970); F. A. Hart, G. P. Moss, and M. L. Staniforth, *Tetrahedron Lett.*, 3389 (1971); G. P. Moss, private communication.

(3) The regularity of maximum shift change, *e.g.* for Pr^{3+} being 800–1000 Hz for α(H-4), 60 Hz for α(H-3), and 3–6 Hz for α(H-2), etc., led us to suppose that the peptide chain is almost unfolded and that there is a regular increase in distance of these α-H atoms from the site of coordination. This may not necessarily be true for other (longer) peptides which may contain additional acidic and/or basic functions.

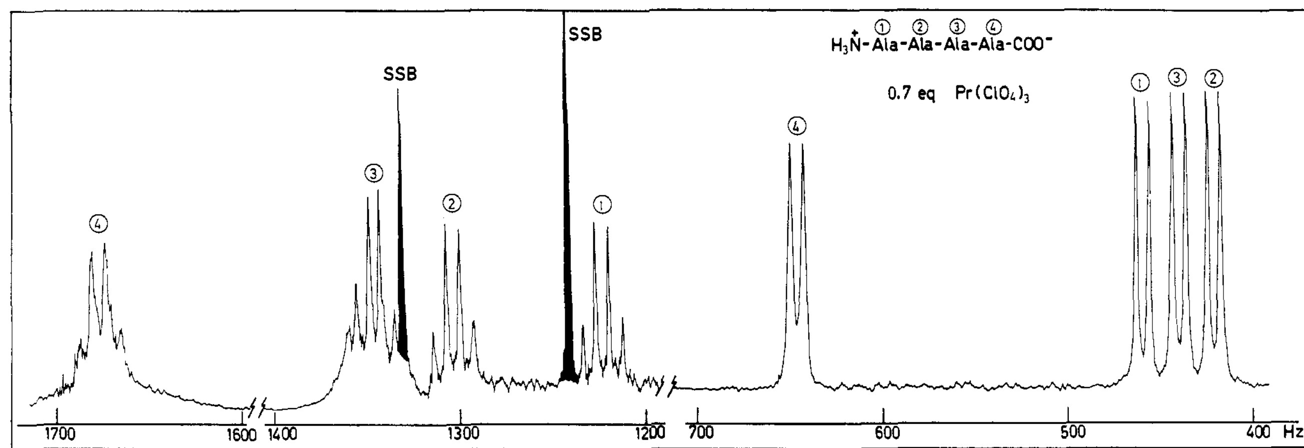

Figure 1. Spectrum of tetraalanine in D_2O after addition of 0.7 equiv of $Pr(ClO_4)_3$. Shift values relative to sodium salt of 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionic acid internal

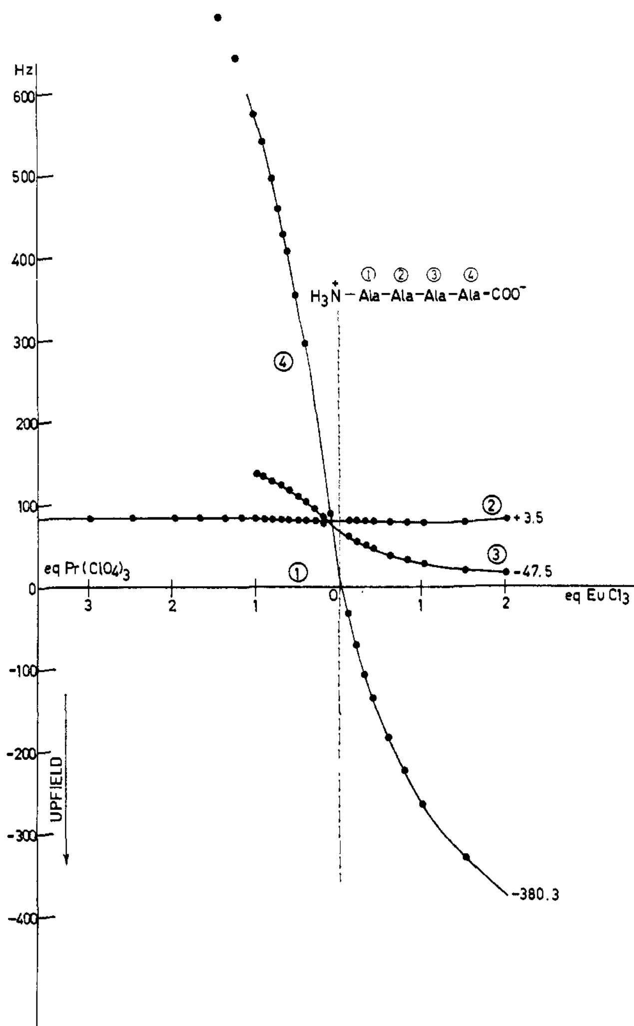

Figure 2. Ligand induced shifts with increasing amounts of respectively Pr^{3+} (deshielding) and Eu^{3+} (shielding) for the α-H atoms of the four residues in tetraalanine. Values in Hz (at 300 MHz) are relative to residue 1, taken as arbitrary reference.

shift becomes decreasingly less important for the residues progressively removed from the CO_2^- group, thus allowing the assignment of the various amino acid residues in the peptide chain starting from the CO_2^- end.[4]

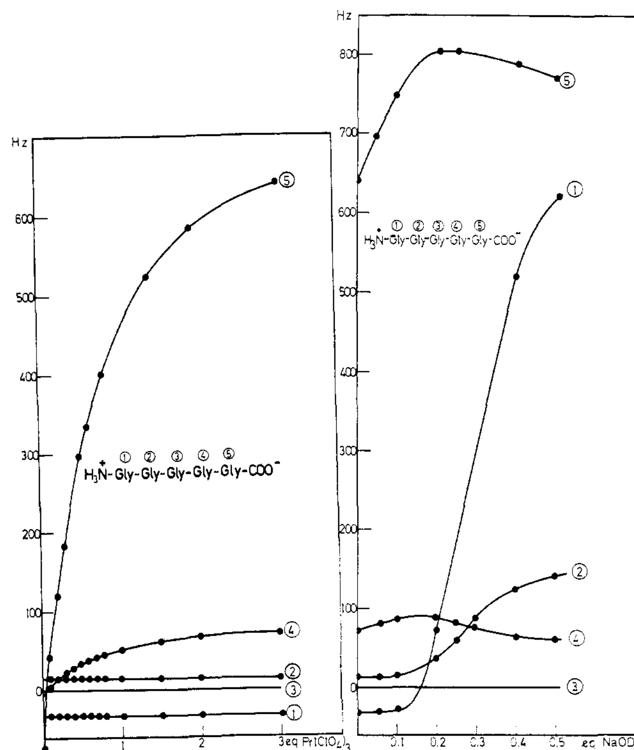

Figure 3. (Left) Effect of Pr^{3+} (deshielding) in pentaglycine and (right) of subsequent addition of base when 3 equiv of Pr^{3+} has been added.

As expected from the foregoing arguments, the effect of the La^{3+} is destroyed by adding increasing amounts of acid (DCl), the original peak locations being almost restored when about 1 equiv of acid has been added. Under these conditions, no suitable functions are present which are capable of complexation with the La^{3+} in D_2O.

The effect of adding base, however, is important (Figure 3). Now the *free* amino function of the tail

(4) The shifts are not expressed in absolute values but instead are relative to an arbitrarily chosen pattern (*e.g.*, α(H-1) in Figure 2). Owing to the great stability of the external field during the entire period of measurement, this field may safely be taken as the absolute reference. In fact, peak locations for all patterns, including the H_2O signals, are greatly altered upon addition of the ligand ion. The H_2O peaks also provide useful references for estimating the relative amount of reagent added. They can also be used as internal references for the spectra.

residue becomes available and the tail-linked residue becomes either highly deshielded (in the presence of Pr^{3+}) or shielded (in the presence of Eu^{3+}). These effects are attenuated for other residues which are successively removed from the tail-linked residue, thus allowing the enumeration of at least two (and in favorable cases, even three) residues from the tail side. These measurements, together with the original lanthanide ion induced shifts observed without added base and the relatively unaffected shift of the "middle" residue, allow the sequential determination of hexa- or even heptapeptides. Additionally, in those cases (*e.g.*, pentaglycine and others) where the sequence could be stated unambiguously by the acid–base titration method,[1] the consecutive addition of lanthanide salts affirmed fully the assignments of the residues as pointed out earlier (see also Figure 3).

Work is in progress to check and optimize the nmr method through systematic investigation of other La^{3+} ions, solvents and mixed polypeptides, and we plan to submit a full account of our findings shortly. Some drawbacks at this stage are already apparent. One difficulty is that the addition of base often causes pronounced broadening of the absorption patterns. Another is that solubility problems are encountered with peptides containing more than five amino acid residues. Thus it will be necessary to consider other potential (binary) solvent (systems) which do not themselves coordinate too strongly with LSR. Further, the occurrence of multifunctional amino acid residues may cause difficulties arising from the presence of multiple potential ligand sites. Thus, at the present stage of our investigation, we are not overly optimistic that the nmr method discussed herein can be universally employed as a nondestructive method for sequential analyses of oligopeptides. The existence of Fourier transform techniques permits the utilization of much smaller quantities of material then used in conventional CW measurements, thus rendering the method interesting in principle for biological purposes.

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Stereochemistry of the Rhodium-Catalyzed "Oxo" Reaction

Sir:

In spite of the great deal of work done on the "oxo" reaction during the last decades,[1] little information is available on the stereochemistry of the addition of the formyl group and hydrogen to the double bond. Although cis addition is generally assumed in the cobalt-catalyzed reaction,[2] conclusive evidence has been presented only in one case[3] and that did not involve an alkene. No information whatsoever is available on the steric course of the rhodium-catalyzed hydroformylation.

We have investigated the hydroformylation of (*E*)-(**1**) and (*Z*)-3-methyl-2-pentene (**2**) in the presence of hydridocarbonyltris(triphenylphosphine)rhodium.[4] Both reactions were carried out as follows: 5 g of olefin and 80 mg of catalyst in 50 ml of benzene were treated at 80° with CO and H_2 (1:1; initial pressure 80 atm). The reactions were stopped when the drop of pressure (25 atm) indicated 50% of conversion. The residual olefins and the products were analyzed by gas chromatography–mass spectrometry (Perkin-Elmer vapor fractometer 990, Hitachi mass spectrometer RMU-6L) against standard samples. The compositions of the reaction mixtures are reported in Table I. The diastereomeric composition of the main products was determined after conversion of the aldehydes into the methyl esters of the corresponding acids;[5] details on the experimental procedure and on the analytical conditions are given elsewhere.[6] No saturated hydrocarbon was detected in the reaction mixture.

Table I. Compositions (%) of Reaction Mixtures

Reaction mixture	Starting olefin: (*E*)-3-Methyl-2-pentene (**1**)	Starting olefin: (*Z*)-3-Methyl-2-pentene (**2**)
Products		
2,3-Dimethylpentanal	86	85
3-Ethylpentanal	3	3
4-Methylhexanal	11	12
Residual olefins		
1	94	8
2	6	92
2,3-Dimethylpentanal		
Erythro	6	92
Threo	94	8

As isomerization accompanying the hydroformylation occurs only to a small extent, the stereochemistry of the reaction is clearly evident from the above results, indicating overwhelming cis addition in both cases.

Although the isomeric composition of the residual olefins suggested that cis–trans isomerization might be responsible for the incomplete diastereomeric purity of the main products, more rigorous evidence was needed to determine the degree of the stereospecificity of the hydroformylation. Therefore, a deuterioformylation of **1** was carried out under the conditions used in the previous hydroformylation,[7] the reaction mixture composition at 50% conversion being in both cases the same. The deuterium content in the residual olefins and in the methyl esters derived from the reaction products[5] was determined by mass spectral analysis: unreacted **1** was more than 90% undeuterated, while the

(1) Extensive reviews are available on the subject; *e.g.* (a) J. Falbe, "Synthesen mit Kohlenmonoxyd," Springer-Verlag, Berlin, 1967; (b) C. W. Bird, "Transition Metal Intermediates in Organic Synthesis," Logos Press, London, Academic Press, New York, N. Y., 1967; (c) A. J. Chalk and R. F. Harrod, *Advan. Organometal. Chem.*, **6**, 119 (1968).

(2) M. Orchin and W. Rupilius, *Catal. Rev.*, **6**, 85 (1972).

(3) A. Rosenthal, *Advan. Carbohyd. Chem.*, **23**, 60 (1968).

(4) C. K. Brown and G. Wilkinson, *J. Chem. Soc. A*, 2753 (1970).

(5) P. Pino, S. Pucci, F. Piacenti, and G. Dell'Amico, *J. Chem. Soc. C*, 1640 (1971).

(6) A. Stefani, *Helv. Chim. Acta*, **56**, 1192 (1973).

(7) An incorporation of one deuterium atom was found by P. Taylor and M. Orchin (*J. Organometal. Chem.*, **26**, 389 (1971)) in the dimethyl maleate–dimethyl fumarate isomerization in the presence of stoichiometric amounts of $DCo(CO)_4$.