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(10) At least two more minor isomers of II were present, but these and the 11,13-di-cis isomer could result from degradation of the 11-cis isomer in a dark process. Isomers of I are thermally more stable.
(11) Altex ½ in. × 1 ft 5-μ Lichrosorb column, 10% ether in hexane for I and 5% ether in hexane for II.
(12) Assignments confirmed by computer simulated spectra. We thank Dr. T. Bopp for his instruction and the use of his program.
(13) A solvent mixture of 5% dioxane-d_8 in CCl_4 (by volume) was used for all ^1H NMR samples. The signal of isotopic impurity of dioxane (3.59 ppm) does not interfere with those of the retinal. Other NMR data, e.g., the four-bond H, F coupling constants, will be discussed in a future full paper.
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Interproton Distances for the β-Turn Residues of the Peptide Gramicidin S Determined from Nuclear Overhauser Effect Ratios

Sir:

We wish to report the determination of the distances between both Pro $C^\delta H$ protons and the Phe $C^\alpha H$ proton of the D-Phe-Pro sequence in gramicidin S, cyclo(D-Phe-Pro-Val-Orn-Leu)$_2$. They quantitatively confirm our earlier nuclear Overhauser effect (NOE) observations[1] and the conclusion that this sequence possesses a type II′ β-turn conformation. This measurement represents an accurate method of determining the angle ψ in peptides and specifically ψ (Phe) in gramicidin S. The calculated interproton distances approximate the sum of the Van der Waals radii of the two protons.

Both relaxation time and NOE measurements can in principle be used to determine interproton distances and dihedral angles in peptides.[1-7] Since the NOE's arise principally from dipolar coupling,[2,8,9] their determination by difference double resonance[1] or INDOR[1] promises significant advances in conformational analysis of complex peptides in solution.

The proton magnetic resonance spectra and difference double resonance spectrum of gramicidin S obtained by irradiating the Pro $C^{\delta 2}H$ are shown in Figure 1. The value of all six NOE's shown in Figure 2 were obtained by comparing the areas of the observed NOE's in double resonance difference spectra with the area of the $C^\alpha H$ region of the normal spectrum. Cancellation of nondipolar coupled resonances are better than 0.5% in all cases.

Even though the extreme narrowing condition is not met ($\tau_c \sim 10^{-9}$ s here[5]), the ratios of NOE's and the interproton distances are still related[8] as shown in eq 1-3 where, for example, $NOE_{\delta 1}(\delta 2)$ is the fractional intensity change in the resonance

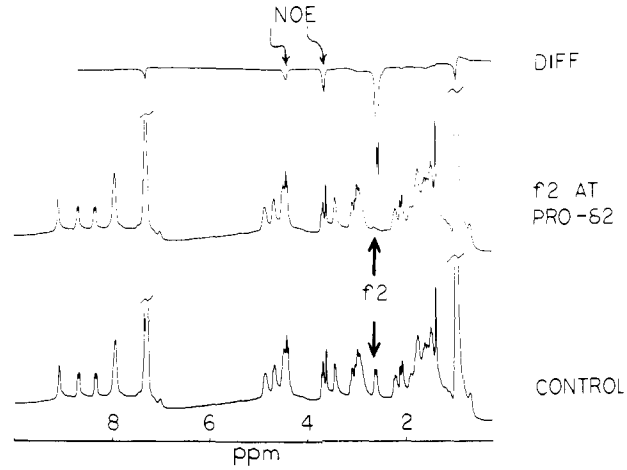

Figure 1. Three ^1H NMR spectra are shown: a "control" spectrum with the decoupling frequency set off resonance, a spectrum obtained with the decoupler frequency set to irradiate the Pro $C^{\delta 2}H$, and the difference obtained by subtracting the former from the latter spectrum. The two NOE's discussed in the text are indicated by arrows.

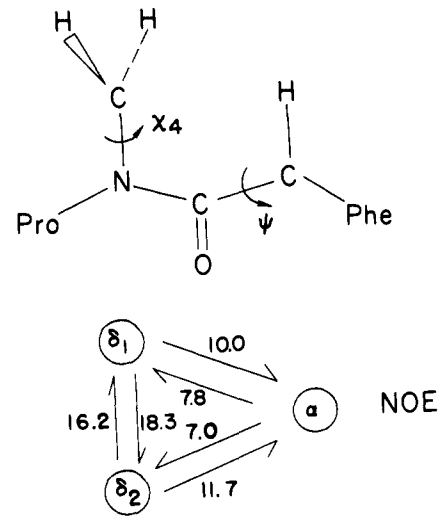

Figure 2. The fragment of gramicidin S containing the Phe $^4C^\alpha H$ and the two Pro $C^\delta H$'s is shown. Also shown are the values of the six NOE's (the arrows point from the irradiated proton to the proton at which the stated percent decrease in intensity is observed).

from the Pro $C^{\delta 1}H$ when the Pro $C^{\delta 2}H$ is irradiated and $r_{\delta 1-\alpha}$ is the interproton distance between the Pro $C^{\delta 1}H$ and the Phe $C^\alpha H$.

$$\frac{r_{\delta 1-\alpha}}{r_{\delta 1-\delta 2}} = \left[\frac{NOE_{\delta 1}(\delta 2) + NOE_{\delta 1}(\alpha)NOE_{\alpha}(\delta 2)}{NOE_{\delta 1}(\alpha) + NOE_{\delta 1}(\delta 2)NOE_{\delta 2}(\alpha)}\right]^{1/6} = 1.15 \quad (1)$$

$$\frac{r_{\delta 2-\alpha}}{r_{\delta 2-\delta 1}} = \left[\frac{NOE_{\delta 2}(\delta 1) + NOE_{\delta 2}(\alpha)NOE_{\alpha}(\delta 1)}{NOE_{\delta 2}(\alpha) + NOE_{\delta 2}(\delta 1)NOE_{\delta 1}(\alpha)}\right]^{1/6} = 1.21 \quad (2)$$

$$\frac{r_{\alpha-\delta 1}}{r_{\alpha-\delta 2}} = \left[\frac{NOE_{\alpha}(\delta 2) + NOE_{\alpha}(\delta 1)NOE_{\delta 1}(\delta 2)}{NOE_{\alpha}(\delta 1) + NOE_{\alpha}(\delta 2)NOE_{\delta 2}(\delta 1)}\right]^{1/6} = 1.04 \quad (3)$$

The internuclear distance between the two Pro $C^\delta H$'s can easily be obtained by using the C-H bond length of 1.1 Å and a H--C--H angle of 107°.[10] This value of 1.77 Å plus the ratios above $r_{\delta 1-\alpha} = 2.03$ and $r_{\delta 2-\alpha} = 2.14$ for the distances between each Pro $C^\delta H$ and the Phe $C^\alpha H$. This use of NOE ratios for determining interproton distances is well established[8] and ^{13}C relaxation studies[11] have demonstrated that all carbon atoms

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of the backbone of gramicidin S have correlation times equal to the molecular reorientation time. Despite this, extensive reevaluation of all the assumptions behind this method are required; until then the best accuracy that can be claimed for the interproton distances is 2.0 ± 0.2 Å, even though the precision (~0.04) of the measurements is much higher.

Model building (3 cm/Å) assuming $\psi = -120°$ for a classical type II′ β turn yields 2.23 and 2.57 Å for these distances while Dygert et al.[12] using semiempirical calculations determined these distances as 2.24 and 2.21 Å and the ψ angle as −137°. The interproton distances of 2.0 ± 0.2 Å determined from the NOE measurements plus the independent determination of the ratio of these distances (eq 3) agree best with the latter and indicate a ψ (Phe) of $-130° \pm -15°$. Thus the protons of the Pro $C^{\delta}H$--Pro $C^{\delta}H$--Phe $C^{\alpha}H$ moiety approach within their Van der Waals contact distance and appear to be a tightly packed system. This suggests that any χ_4 motions of the Pro ring system will be coordinated with χ rotation of the Phe residue.

This report confirms quantitatively what was previously suggested and qualitatively shown:[1] NOE's can be used to determine dihedral angles in peptides, can specifically give the previously unmeasured angle ψ to reasonable accuracy, and can be used quantitatively as criteria for common conformational features of peptides such as β turns, extended structures, and helices. This approach offers considerable promise for the future of peptide (and protein) conformational analysis since now a combination of NMR techniques exists which can produce a number of measured parameters equal to or slightly greater than the number of unknown dihedral angles to be determined.

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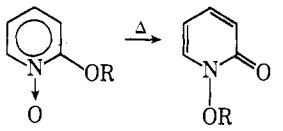
Effect of Pressure on Concerted and Stepwise Sigmatropic Shifts[1]

Sir:

Innumerable data are now available[2] supporting the conjecture[3] that concerted cycloadditions should have smaller transition states than the diradicaloid stepwise analogues, the simple reason being that the former have two new bonds in the process of formation and the latter one. While this criterion is subject to confusion by special features such as a highly dipolar starting component[4] or intermediate,[5] when these considerations have been taken into account, there is little room for doubt about the mechanism.

Similar arguments should be possible about other types of pericyclic reactions. Thus, concerted sigmatropic shifts should involve primarily the formation of a new bond and hence a volume contraction, whereas stepwise shifts characterized by initial bond cleavage to give radicals should be characterized by a volume expansion. The concerted reactions should therefore be accelerated, and the stepwise ones should be retarded. To date few of these reactions have been studied. Claisen[6,7] and Cope[7,8] rearrangements have negative activation volumes and hence they fit their description as concerted in this respect; stepwise shifts via diradicals are indicated by pressure-inhibited rates in the racemization of benzyl phenyl sulfoxide[9] and in the rearrangement of *N*-(1-cyanocyclohexyl)pentamethyleneketenimine to bi(1-cyanocyclohexyl).[10] No examples have been reported, however, of chemically very similar sigmatropic shifts of contrasting mechanism, such as was done by Stewart,[11] for example, for cycloadditions.

The thermal 1,4 shift of 2-alkoxypyridine *N*-oxide to *N*-alkoxy-2-pyridone[12] offers such a contrast: with R as benzyl (I), the rearrangement is concerted, but with benzhydryl (II)



it proceeds via diradicals. Both reactions have rates virtually independent of solvent, but only the latter exhibits strong CIDNP signals as it progresses.

The pressure effects have now been measured at 100 °C in diglyme solution. The benzyl reaction was followed by NMR analysis and the benzhydryl by means of UV; the pressure

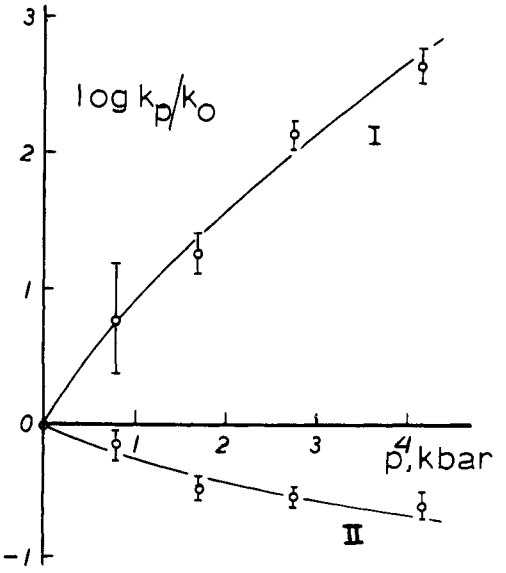


Figure 1. Effect of pressure on the rearrangement rates of substrates I and II.

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