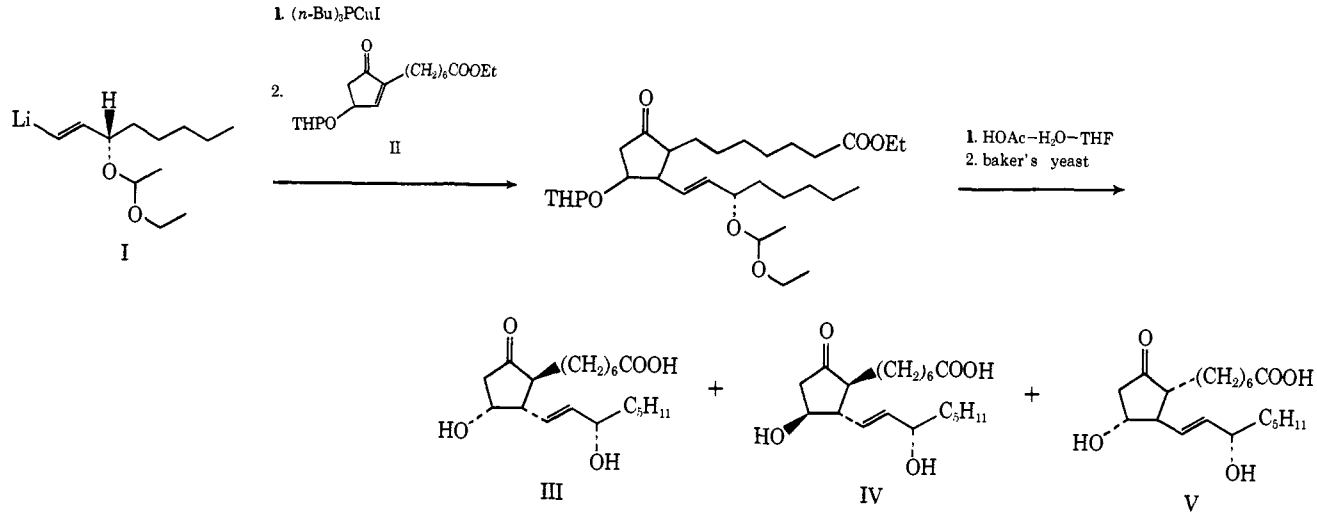
Scheme I



$[\alpha]^{23}D$ −22.3° (*c* 10 in ether) (reported values: $[\alpha]D$ −19.8°[4] and $[\alpha]D$ −21°[5]), into 3(*S*)-hydroxy-1-iodo-1-*trans*-octene, which was in turn transformed into 3(*S*)-(α-ethoxy)ethoxy-1-iodo-1-*trans*-octene by reaction with ethyl vinyl ether in the presence of an acid catalyst. Treatment of the latter compound with lithium metal gave I in an overall yield of about 25%. The procedure for the preparation of 2-(6′-carboethoxyhexyl)-2-cyclopentene-4-tetrahydropyranoxy-1-one (II) was previously described.[2]

The prostanoic acid skeleton was constructed by condensing II (500 mg) with 2 molar equiv of I, in presence of 1 molar equiv of tri-*n*-butylphosphine–copper(I) iodide complex[6] at 0° in ether. After cleaving the protecting groups by acid treatment[7] and the ester grouping with baker's yeast,[8] the mixture was chromatographed over a silicic acid–Celite column. Two major diastereomeric products[9] were obtained by elution of the column with a gradient system comprised of benzene–ethyl acetate. The first product (113 mg) was obtained as an oil, whose physical constants[10] were in good agreement with 15-epi-*ent*-PGE_1 (IV). Its circular dichroism (CD) spectrum exhibited a positive Cotton effect ($[\theta] \times 10^{-3} = +12.05°$ at λ 296 nm), whereas its acid dehydration product, 15-epi-*ent*-PGA_1, λ_{max}^{alc} 217 nm (ε 11,000), afforded a negative Cotton effect ($[\theta] \times 10^{-3} = -50.4°$ at 231 nm), in contradistinction to the CD curves[11] of natural PGE_1 and PGA_1, respectively. The second product (107 mg), mp 115–116° $[\alpha]^{20}D$ −54.3° (*c* 1.0, THF),[12] $[\alpha]^{20}D$ −65.1° (*c* 0.43, ethanol), was found to be identical (infrared, nuclear magnetic resonance, and mass spectra) with an authentic specimen of natural PGE_1, prepared by biosynthesis.[13] Aside from these major products, a small quantity of 11,15-epi-*ent*-PGE_1 (III)[14] (26 mg) was also formed in the reaction (Scheme I). However, no apparent 8-iso-PGE_1 and 11-epi-PGE_1 were detectable.[15] This product profile reveals that this method possesses considerable stereoselectivity in that the 1,4 addition by the vinyl copper reagent appears to proceed almost exclusively from the least hindered side of II and that the relative stereochemistry of the substituents at C-8, C-12, and C-11 is all trans.[16]

Acknowledgment. The authors are indebted to Professor Josef Fried for a sample of the (−)-α-phenylethylamine salt of 3(*S*)-octynol phthalic ester and to Dr. John Pike for samples of 11-epi-, 15-epi-, and 8-isoprostaglandins.

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(8) The prostanoic acid esters were exposed to 15 g of dried yeast (Red Star) in 500 ml of 0.1 *M* phosphate buffer, pH 7.0, for 16 hr at 25° on a rotary shaker.

(9) The concept of utilizing the optically active hydroxylated 8-carbon side chain for the resolution was first employed by J. Fried, *et al.*, *J. Amer. Chem. Soc.*, **93**, 5594 (1971).

(10) The chromatographic behavior and infrared, nuclear magnetic resonance, and mass spectra of this substance were identical with those of an authentic sample of 15-epi-PGE_1, provided to us by John Pike.

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(12) Under the same conditions, our sample of natural PGE_1 (mp 115–116°) afforded values of $[\alpha]^{20}D$ −55.2° (*c* 1.6, THF) and −66.1° (*c*, 0.57, ethanol). This differs somewhat from the reported value, $[\alpha]^{25}D$ −58.3° (*c* 0.47, THF): E. J. Corey and R. K. Varma, *J. Amer. Chem. Soc.*, **93**, 7319 (1971).

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(14) The infrared, nuclear magnetic resonance, and mass spectra of this substance and its chromatographic behavior were identical with those of an authentic sample of 11,15-epi-PGE_1.

(15) PGE_1, 8-iso-PGE_1, and 11-epi-PGE_1 can be separated on thin layer plates by two developments in the solvent system, $CHCl_3$–EtOH–HoAC (90:4:6).

(16) This investigation was supported in part by research grants from the Wisconsin Alumni Research Foundation and the National Institutes of Health (AM-4874 and AM-9688).

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Proton Magnetic Resonance Line Broadening Produced by Association with a Nitroxide Radical in Studies of Amide and Peptide Conformation[1]

Sir:

Morishima, Endo, and Yonezawa[2] have reported contact shifts produced by hydrogen bonding between di-*tert*-butyl nitroxide and several simple organic mole-

(1) This work was supported by a grant from the U. S. Public Health Service, GM 14069, and by a Public Health Service Research Career Development Award, GM 47357, both from the Institute of General Medical Sciences.

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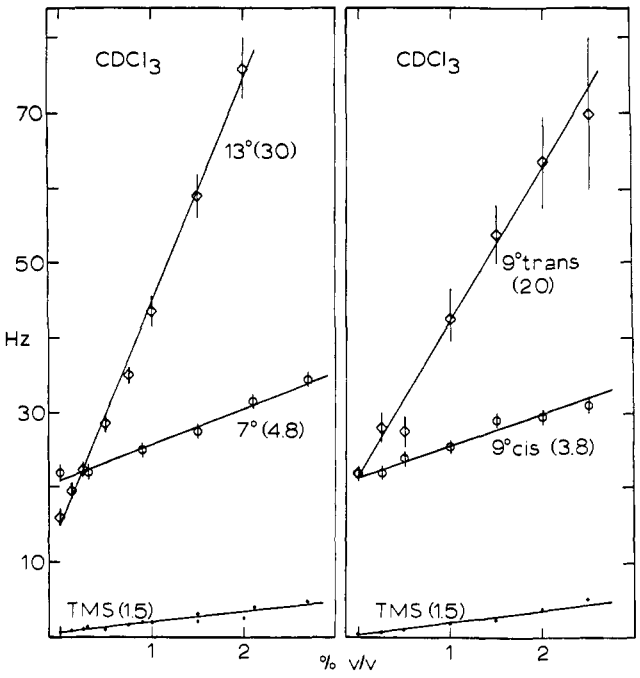

Figure 1. Line-broadening effect of radical I on amide proton resonances of cis and trans lactams in chloroform, 30°. At left are shown widths at half-height *vs.* volume per cent radical in the solvent for the trans lactam 2-azacyclotridecanone (13°) and the cis lactam 2-azacycloheptanone (7°). At right are data for the resonances of the coexisting cis and trans forms of 2-azacyclononanone. The slopes of the least-squares lines (Hz/vol %) are given in parentheses. Data for tetramethylsilane are also shown. Measurements were made at 60 MHz, using solutions of about 15 wt % lactam.

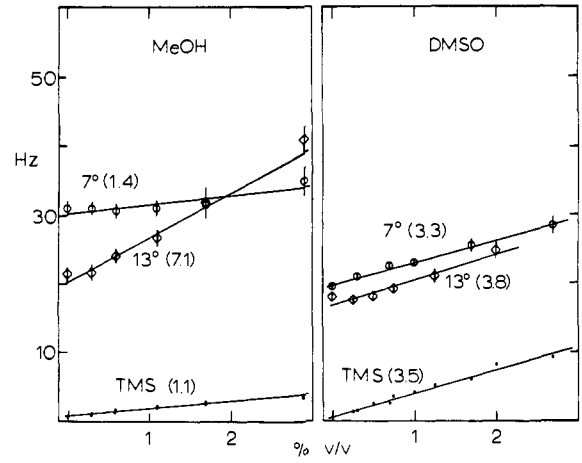

Figure 2. Line-broadening effect of radical I on amide proton resonances of the cis and trans lactams 2-azacycloheptanone (7°) and 2-azacyclotridecanone (13°) in methanol (left) and in dimethyl sulfoxide (right). Slopes of the least-squares lines (Hz/vol %) are given in parentheses. Data were obtained at 30°, 60 MHz, using *ca.* 15 wt % solutions in methanol and 5–10 wt % solutions in dimethyl sulfoxide.

cules in carbon tetrachloride solution. The effects on peptide proton (amide N–H) resonances produced by hydrogen bonding with a stable free radical might prove useful in conformational studies of peptides. For this it is necessary to find a radical–solvent combination in which peptides are soluble and in which radical–peptide association competes sufficiently with radical–solvent and/or peptide–solvent hydrogen bonding. We have found that this condition is met, and differential line-broadening effects are produced, by 1–3% solutions of 3-oxyl-2,2,4,4-tetramethyloxazolidine (I) in chloroform or methanol.

The radical I was prepared by chloroperbenzoic acid oxidation of the corresponding oxazolidine.[3] Our preparation is an orange liquid, fp 4–5°, containing, according to gas chromatography, about 5% pentane (used in the work-up) but no oxazolidine or other impurities. Its mass spectrum showed the parent peak at *m/e* 144.

The broadening effect of radical I on the amide proton resonances of cis and trans lactams is shown in Figures 1 and 2. 2-Azacycloheptanone (caprolactam) and 2-azacyclotridecanone have respectively cis and trans amide links.[4,5] Dielectric studies of benzene solutions have indicated that cis lactams are associated as dimers and trans lactams are in linear chains.[6] 2-Azacyclononanone is a mixture of cis and trans forms in solution;[6] the amide proton resonances of the two forms are readily distinguished at 60 MHz. In chloroform solutions (Figure 1) the N–H lines of the trans amides are more affected by a given concentration of radical than are those of the cis forms. In either case, the N–H lines are much more strongly broadened than the tetramethylsilane resonance, indicating that the radical associates specifically with the lactam. The distinction between cis and trans amides is probably a demonstration that the cis dimer is more stable than the trans polymer in the equilibrium

$$(\mathrm{RCONH})_n + \mathrm{O{-}N}\!\!<^{\mathrm{R}} \rightleftharpoons \mathrm{RCONH\cdots O{-}N}\!\!<^{\mathrm{R}} + (\mathrm{RCONH})_{n-1}$$

and is not an indication that the N–H of the trans lactams is hindered from solvation.

Figure 2 shows the effect of radical on the 9- and 13-membered lactams in two solvents common for peptide nmr work. In methanol, only the trans lactam appears to associate with the radical in competition with radical–solvent, amide–solvent, and (since high lactam concentrations were used) amide–amide hydrogen bonding. The observed line broadening in the trans case is the result of association with radical and not of base-catalyzed proton exchange with solvent. Pyridine at comparable concentrations does not affect the line width of either lactam in methanol. In dimethyl sulfoxide neither N–H line width is affected more than that of tetramethylsilane, suggesting that the amide protons are completely solvated by dimethyl sulfoxide, distinguishing it from methanol. No association between radical and dimethyl sulfoxide seems likely; rather, the TMS line is more strongly broadened by radical in dimethyl sulfoxide than in methanol or chloroform, which suggests solvophobic association of radical and reference.

Not shown in Figures 1 and 2 are measurements on aqueous solutions of *N*-methylacetamide. In water, a given concentration of radical causes the same line width increment (*ca.* 4 Hz/vol % at 30°) for both the amide proton resonance and the methyl proton reso-

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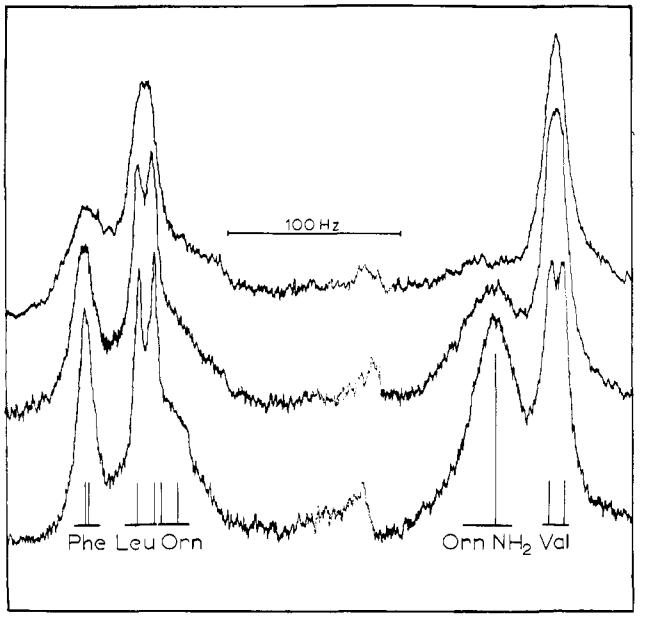

Figure 3. 220-MHz spectra of the N–H resonances of gramicidin S in methanol (10 wt %, 20°) showing the effect of additions of 0, 1, and 2% radical. The assignments shown are from the literature.[7,9] The dotted absorption appears to be an impurity in the sample used. We thank Dr. J. J. Katz, Argonne National Laboratory, for the use of the 220-MHz spectrometer.

nance of reference 2,2-dimethyl-2-silapentane-5-sulfonate ion.

We have examined the effect of radical on peptide spectra using the cyclic decapeptide gramicidin S, cyclo(L-Pro-L-Val-L-Orn-L-Leu-D-Phe)$_2$. Nmr and deuterium exchange studies of this peptide[7-10] agree in supporting a solution conformation of C_2 symmetry in which the N–H proton of each valine and leucine residue is directed inward, in the approximate plane of the peptide backbone, and transannularly hydrogen bonded to the carbonyl residue of an opposing leucine or valine residue. The N–H protons of these residues are thus shielded from interaction with other species. The effect of added radical on the N–H region of the spectrum of gramicidin S in methanol is shown in Figure 3. Although quantitative evaluation is difficult because of the overlap and multiplet structure of the resonances, it is clear from Figure 3 that only the protons assigned[7,9] to the valine and leucine residues escape extensive broadening. Measurements in dimethyl sulfoxide solutions also distinguish among the observed N–H proton resonances. The exposed ornithine (*ca.* 20 Hz/%) and phenylalanine (*ca.* 11 Hz/%) protons have greater line width increments than the shielded leucine proton (*ca.* 7 Hz/%). The valine proton is obscured by the aromatic proton absorption.

We think that this technique may be a useful adjunct to other methods, such as exchange rate and solvent or temperature dependence of chemical shift, in determining the environment of amide protons in studies of peptide conformation.

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On the Detection of the Helix–Coil Transition of Polypeptides by Ultrasonic Absorption Measurements in the Megahertz Range. Case of Poly-L-glutamic Acid

Sir:

The finding of ultrasonic absorption maxima on the plots of absorption *vs.* pH for aqueous solutions of poly-L-glutamic acid[1] (PLGA) and poly-L-ornithine[2] (PLO) has been recently reported. Moreover, Parker, *et al.*,[3] have shown that the excess ultrasonic absorption of poly-L-lysine (PLL) solutions increases with pH up to pH 10.2 where precipitation occurs. These three studies were performed at ultrasonic frequencies above 1 MHz and the results were interpreted as indicating a sensitivity of the ultrasonic absorption in the megahertz range to the equilibria between the helical and coiled conformations of the above polypeptides.

Following these reports similar absorption maxima on the plots of absorption *vs.* pH in protein solutions[4] have been found and have been interpreted as being due to proton transfer processes. Furthermore, the "site binding" of counterions by polyions in polyelectrolyte solutions has been shown to give rise to an excess ultrasonic absorption.[5,6] This effect, in conjunction with the variation of absorption associated with the conformational transition from coil to helix, has been interpreted[7] to be responsible for the ultrasonic absorption maximum appearing on the plots of absorption *vs.* concentration of added salt relative to PLL solutions.[7] The question remains, therefore, as to whether proton transfer and/or counterion site binding occur in solutions of synthetic polypeptides and, if so, as to their contribution to the observed absorption maxima in the cases of PLGA[1] and PLO[2] and to the excess absorption of PLL.[3] The purpose of this paper is to present methods by which a distinction can be made between the contributions of the three processes occurring (proton transfer, counterion site binding, helix–coil transition) and to show how these methods can be applied to ultrasonic studies of PLGA. Results from studies on PLL and PLO are reported elsewhere.[8]

Before discussing the experimental results it must be first pointed out that all of the studies of the effect of pH on the ultrasonic absorption of PLGA[1,9,10] have actually been performed on the sodium salt of PLGA

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