

such as acquisition time and disk storage space. In most cases, the assignment of a cross-peak in a 2-D NOE spectrum can be made from prior knowledge of likely interactions, but ambiguities sometimes result. For instance, the interaction between Phe NH and Pro-5 H α can be seen in the 2-D NOE spectrum, but the 2-D NOE spectrum does not allow the cross-peak to be assigned to Pro-5 H α as opposed to Phe H α . In general, 1-D and 2-D NOE spectroscopy provide consistent and complementary information.

Comparison of Solution and Crystal Interproton Distances.

Distances between protons calculated from X-ray derived coordinates for crystalline cyclo(D-Phe-Pro-Gly-D-Ala-Pro) are given in Table IV along with the 1-D and 2-D NOE derived distances. Analysis of these interproton distances serves as an indicator of the consistency of the two structural approaches, and more importantly, it allows a direct comparison of the solution and crystal conformations of this model peptide. There is a remarkable correspondence of the distances from NOE and from X-ray; nearly all are the same within experimental error. Deviations between X-ray and NOE results in two distances involving Pro-2 H β 's are outside the range of experimental error, and they may reasonably be correlated with a site of conformational change between crystal and solution. In a previous detailed analysis of the proline ring geometries of this cyclic pentapeptide using coupling constants,¹⁹ Pro-2 was found to adopt a different conformational distribution in solution from that in the crystal while Pro-5 was fixed in the same ring geometry in both states. In this previous study, an equilibrium between two oppositely puckered ring conformations was proposed in solution for Pro-2. The present data are consistent with the previous study in indicating a difference between crystal and solution conformations around Pro-2, but they offer no information on the dynamic aspect of the conformational description, except that the Overhauser effects are transmitted to and from Pro-2 despite any conformational averaging.

Conclusions

Quantitative interproton distances in a cyclic pentapeptide were obtained from both 1-D and 2-D NOE buildup rates, and there is excellent agreement between the distances obtained by the two methods. A single correlation time for all protons in this rigid peptide was assumed in all distance calculations, and this as-

sumption is justified by T_1 and NOE results. A two-spin approximation was also used in all distance calculations. This is a good approximation for all interactions studied except between Gly NH and both Gly H α and Ala NH, and between Pro-2 H α and the Phe aromatic ring protons. Consequently, these three interproton distances are only estimates of the actual distances since the two-spin approximation is not strictly valid for these interactions. Nonetheless, striking agreement was observed between interproton distances from NOE and those for the crystal structure of this peptide as solved by X-ray diffraction. Not only does this correspondence indicate that the methods employed in the NOE analysis seem sound, but it also affords a direct comparison of the crystal and solution conformations of a cyclic model peptide. Only in the region of the Pro-2 pyrrolidine ring does there appear to be a significant conformational change in the peptide between the two environments. In both states, the peptide adopts a conformation with two transannular hydrogen bonds, one in a β turn around D-Phe-Pro-2-Gly-D-Ala and the other in a γ turn around D-Ala-Pro-5-Gly. The proximity of Gly NH to Pro-2 H α , which gives rise to a strong NOE, is a hallmark of a type II β turn when accompanied by evidence for a 4-1 intramolecular hydrogen bond as previously found for this peptide.¹⁸ The proximity of Phe NH to Pro-5 H α is definitive evidence for a trans proline, and it is consistent with the presence of a γ turn; additional data previously reported¹⁸ indicated the existence of the 3-1 intramolecular hydrogen bond. The turn is further supported by the (weak) NOE between the Gly NH and the Ala NH. Other conformationally informative NOE's placed the Gly NH with respect to the Gly H α 's and allowed unequivocal assignment of these two methylenes. Furthermore, NOE data supported the preferred rotamer of the Phe ring as over the Pro-2 ring, hence accounting for the widely separated Pro H β protons.

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Relative ^{36}S - ^{34}S Kinetic Isotope Effects

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Abstract: Two sulfur kinetic isotope effects, k_{32}/k_{34} and k_{32}/k_{36} , were measured for the thermal isomerization of bis(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanyl) sulfide (1) to *P*-oxo-*P'*-thionobis(5,5-dimethyl-1,3,2-dioxaphosphorinanyl) oxide (2). The r value is equal to 1.92 ± 0.11 for the relative ^{36}S - ^{34}S kinetic isotope effect and is independent of isotopic composition and solvent used. This value is in the range predicted earlier for carbon and heavier atoms.

It has been 3 decades since Bigeleisen,¹ on the basis of the simple models, predicted that the ^{14}C kinetic isotope effect ($k_{12}/k_{14} - 1$) should be about 2 times larger than the corresponding ^{13}C isotope effects ($k_{12}/k_{13} - 1$). Relative ^{14}C - ^{13}C kinetic isotope effects were analyzed in detail by Stern and Vogel.² The calculated values were compared with experimental results, and a range 1.8-2.0 for the ratio r (eq 1) was obtained. Exceptions

$$r = \frac{\ln(k_{12}/k_{14})}{\ln(k_{12}/k_{13})} \quad (1)$$

to this generalization are usually considered to result from experimental errors,³ but other explanations such as anomalous temperature dependence² or reaction complexity⁴ can be encountered.

Although some other elements such as oxygen and sulfur have suitable stable isotopes, to the best of our knowledge, there are no data on r values for kinetic isotope effects of any heavy atoms except for carbon. An r value has been used qualitatively for the comparison of two measured sulfur isotope effects: k_{32}/k_{35} and k_{32}/k_{34} .⁵ In order to verify whether the range of r values estimated

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Table I. Experimental Values of k_{32}/k_{34} , k_{32}/k_{36} , and r for Isomerization of **1** to **2**

solvent	$^{34}\text{S}_0 = ^{34}\text{S}/^{32}\text{S}$	k_{32}/k_{34}^a	$^{36}\text{S}_0 = ^{36}\text{S}/^{32}\text{S}$	k_{32}/k_{36}^a	$r = (\ln(k_{32}/k_{36}) / \ln(k_{32}/k_{34}))$
propylene carbonate	0.3955	1.0108 ± 0.0008	1.5798	1.0210 ± 0.0011	1.93 ± 0.18 ^b
benzotrile	0.3954	1.0123 ± 0.0011	1.5814	1.0255 ± 0.0017	2.06 ± 0.23
1-methylnaphthalene	0.3956	1.0133 ± 0.0005	1.5821	1.0240 ± 0.0006	1.80 ± 0.08
1-methylnaphthalene	0.2486	1.0130 ± 0.0008	0.9946	1.0250 ± 0.0011	1.91 ± 0.14
1-methylnaphthalene	0.1632	1.0125 ± 0.0028	0.6522	1.0240 ± 0.0010	1.91 ± 0.43
					1.92 ± 0.11 ^c mean

^a Averaged over six individual measurements; statistical errors of means are given on the 0.95 confidence level. ^b Statistical error calculated from equation $\Delta r = r(\sum(\Delta\alpha_i/\alpha_i \ln \alpha_i)^2)^{1/2}$, $i = 1$ or 2 , where $\alpha_1 = k_{32}/k_{34}$, $\alpha_2 = k_{32}/k_{36}$. ^c Statistical error of the mean value was calculated as $(\sum \Delta r_i^2)^{1/2}/5$.

for carbon could be applied to heavier elements, we have measured ^{34}S and ^{36}S kinetic isotope effects on the same reaction. This is the first time the kinetic isotope effect of sulfur-36 has been measured.

Theory

The relation between different carbon isotope effects was roughly estimated by Melander and Saunders³ for the diatomic model

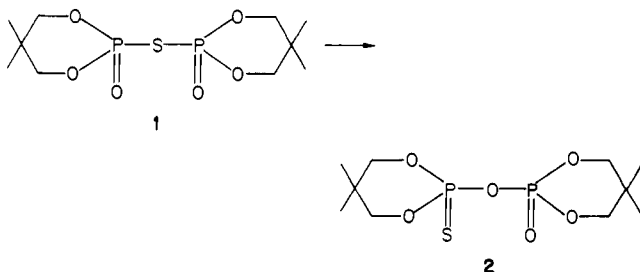
$$\frac{\ln(k_L/k_{H2})}{\ln(k_L/k_{H1})} = \frac{1 - \left[\frac{1/12 + 1/m}{1/(12 + n_2) + 1/m} \right]^{1/2}}{1 - \left[\frac{1/12 + 1/m}{1/(12 + n_1) + 1/m} \right]^{1/2}} \quad (2)$$

where the isotope carbon atoms have the mass $12 + n$ ($n = 0, 1$, or 2) and m is the mass of the atom attached to carbon. We adopted this equation to sulfur isotope effects by changing 12 into 32 and $n = 0, 2$, or 4 . For $m = 1, 12, 31$ (phosphorus), and ∞ , we obtained r values equal to 1.89, 1.90, 1.90, and 1.92, respectively. These calculations show that r values close to 1.90, which are nearly the same as the value for carbon isotope effects, should be expected for sulfur isotopes.

Experimental Section

Spontaneous rearrangement of organic monothiopyrophosphates, first observed by Michalski,⁶ can serve as a convenient model reaction for experimental measurement of relative ^{36}S - ^{34}S kinetic isotope effects. The mechanism of this type of reaction has been studied in our laboratory.⁷ We chose isomerization of bis(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinanyl) sulfide (**1**) to *P*-oxo-*P*-thionobis(5,5-dimethyl-1,3,2-dioxaphosphorinanyl) oxide (**2**) for this purpose.

The reaction mechanism is complex. In dilute solution, however, the reaction is first order, and the rate-determining step is the first, monomolecular step.⁸ We synthesized⁹ compound **1** containing 80 atom %



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^{36}S and 20 atom % ^{34}S from elemental sulfur of the same isotopic constitution. This isotopically modified compound was diluted with material having the natural isotopic abundance (Table I). Compound **2** labeled with ^{36}S and ^{34}S was obtained by quantitative isomerization of compound **1**. Kinetic isotope effects were measured at 130.8 ± 0.2 °C. The initial concentration of **1** was about 0.01 M, and three solvents of different polarity were used. Usually six samples of the reaction mixture were heated in ampules sealed under nitrogen. The reaction was allowed to proceed for different time periods, giving f (the extent of reaction) in the range 0.040–0.635. After the reaction was quenched by cooling, the solvent was evaporated under the vacuum and the reagents were isolated by thin-layer chromatography on silica gel using benzene–chloroform–acetone (4:2:1 v/v) as the developing mixture. It was demonstrated in separate experiments that the isolation and purification does not cause isotope fractionation. Reaction progress was followed radiochemically in parallel experiments by means of a trace amount of ^{35}S -labeled substrate which was added to the same reaction mixture. Ampules containing the radioactive material were removed from the thermostat at the same time as those with stable isotopes only. Twenty microliters of the solution was chromatographed on TLC plate. Gel with adsorbed compound **1** or **2** was placed in vials and measured by liquid scintillation by using an Intertechnique SL 30 counter.

Isotope ratios $^{34}\text{S}/^{32}\text{S}$ and $^{36}\text{S}/^{32}\text{S}$ for substrate (S_0) and for product isolated after fraction of reaction f (R) were measured by a modified Kwart procedure¹⁰ by using Finnigan 4000 mass spectrometer. Ion currents of molecular peaks M (m/e 300), $M + 2$ (332), and $M + 4$ (334) were monitored with the aid of a MID unit, and a correction for natural abundance of other isotopes was made. We found that for the enriched material that we used, 300–500 individual scans were enough to obtain the required precision for kinetic isotope effects (see Table I). Usually each sample was introduced 4 times to the mass spectrometer, and a mean value of these four measurements was used in calculations of kinetic isotope effect from the equation

$$k_L/k_H = \frac{\ln(1-f)}{\ln(1-fR/S_0)} \quad (3)$$

Values obtained for different extents of reaction f were averaged. The mean and its statistical error at the 0.95 confidence level were used for calculation of r values. Isotopic fractionation during fragmentation in the spectrometer is considered to be the main source of error in the above procedure. The extend of fragmentation may change dramatically with the change of the energy of the bombarding electrons. For both compounds under investigation, there is no dependence of isotopic ratios on the energy of bombarding electrons in the range 15–70 eV.

Results and Discussion

The obtained values of kinetic isotope effects and the corresponding r values are presented in Table I. As could be expected, the relative kinetic isotope effects are independent of isotopic composition and the solvent used. The mean r value obtained lies in the middle of the range 1.8–2.0 predicted for relative carbon kinetic isotope effects and is practically equal to the value predicted for relative sulfur kinetic isotope effects on the basis of diatomic model. Thus, these results are consistent with the theoretical range of r values postulated earlier.¹

Registry No. **1**, 16956-55-1; **2**, 15762-04-6; ^{34}S , 13965-97-4; ^{36}S , 14682-80-5.

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