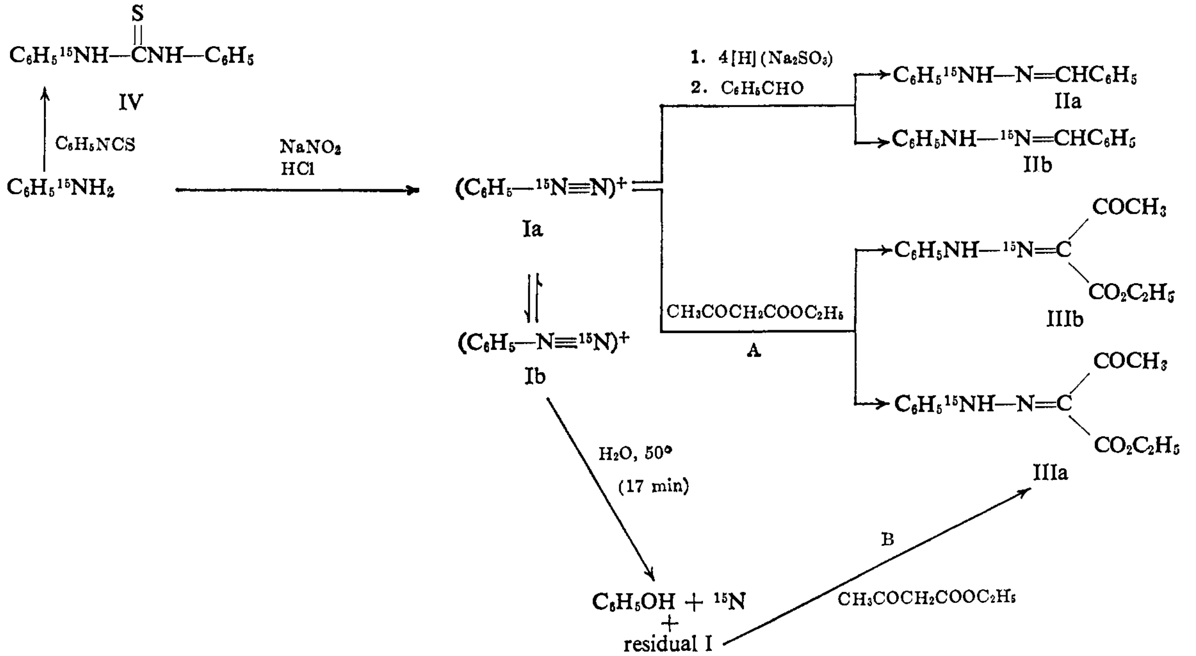
Scheme I

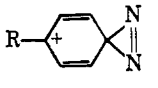


with about 80-mg samples in 0.25 ml of solvent were studied. In order to increase (where necessary) the signal to noise ratio and thus enhance the accuracy of measurements we have used a time-averaging computer (Varian C-1024 CAT) for recording pmr spectra.

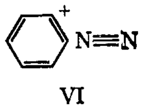
A comparison of the area under the doublet corresponding to ^{15}N-*H* signal and the singlet due to ^{14}N-*H* in III (and II) indicated that 96.5 ± 0.3% of the nitrogen adjacent to the phenyl ring and directly bonded to a proton corresponded to ^{15}N. The ^{15}N content of the starting material was determined to be 96.6 ± 0.3% from the pmr spectrum of ^{15}N-diphenylthiourea (IV) prepared from ^{15}N-aniline.[2]

If the isotope rearrangement Ia → Ib had taken place, the species IIb and IIIb would have been generated along with IIa and IIIa. The fact that the ^{15}N content of the nitrogen adjacent to the phenyl ring in hydrozones II and III was the same as that of the starting material indicated that no noticeable isotope randomization had taken place.

Recently Lewis and Insole[3] reported that when ^{15}N-diazonium fluoroborate is subjected to hydrolysis at 50° until about 80% of the diazo compound is converted to phenol, the residual diazo compound is found to have undergone about 2.3% randomization. In order to explain the apparent rearrangement Ia → Ib accompanying the hydrolysis of diazonium salts, Lewis and Insole suggest the presence of the intermediate V along with the caged pair VI.



V



VI

We have duplicated the experimental conditions of Lewis and Insole. ^{15}N-Labeled phenyldiazonium fluoroborate was subjected to decomposition in water for 17 min at 50°. The residual diazo compound was converted to the hydrazone III and its pmr spectrum (obtained by time averaging over 80 runs) was studied. From the measurement of areas, the ^{15}N content of the N-H group was found to be 96.5 ± 0.2% in the samples of III obtained by route A as well as route B. The same ^{15}N content for both of these samples was also found from their mass spectra.

To verify the accuracy of the pmr method for isotope level determination we diluted the above sample of hydrazone III with unlabeled III so that the resulting ^{15}N content was 95.5%. Using the "CAT" with a sample size of 15 mg, the isotope level at the α-nitrogen in this sample was determined to be 95.7 ± 0.4%. This indicates that had the isotope rearrangement taken place to the extent of 2–3% as suggested by Lewis and Insole, our spectral measurements would have detected it.

Apparently the observations of Lewis and Insole were affected by large limits of error inherent in their method of analysis. Our findings are that the diazonium ion Ia does not undergo rearrangement to Ib during reduction, coupling, or hydrolysis.

The formation of the intermediate V during hydrolysis of phenyldiazonium ion is improbable in the light of our observations. Our conclusions are consistent with recently reported kinetic investigations of Brown and Drury.[4]

Acknowledgment. We wish to thank Dr. P. T. Funke and Dr. E. R. Malinowski for useful discussions. This research was supported in part by a grant (GM-12122) from the U. S. Public Health Service.

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[2] ^{15}N-Aniline was purchased from Merck Sharp and Dohme of Canada. The isotopic purity was reported to be 97%.

[3] J. M. Insole and E. S. Lewis, *J. Am. Chem. Soc.*, **85**, 122 (1963); **86**, 32, 34 (1964).

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The Optical Rotatory Properties of Poly-L-proline

Sir:

Despite considerable study by several groups of investigators,[1–6] the optical rotatory properties of poly-L-proline do not appear to have been satisfactorily described. It is well established that it exists in a strongly levorotatory form, designated as II, in water and organic acids; Blout, *et al.*,[4] report a strong negative Cotton band centered at 203 mμ ($[m']_{216}$ *ca.* $-32{,}000°$) for this form in water. It is also known that, as normally prepared, poly-L-proline exists in a different form, designated as I, which is weakly dextrorotatory at 589 mμ and is stable in aliphatic alcohols[3,6] but unstable in water and organic acids. Upon dissolving in these latter solvents, it mutarotates over a period of several hours to give form II.[1,2,3] The structures of solid state forms corresponding to I and II have been established; I[7] is a right-handed helix with a residue translation of 1.85 A and with the peptide bonds in the *cis* configuration, whereas II[8,9] is left-handed with a residue translation of 3.12 A and with the peptide bonds *trans*.[10] The mutarotation in solution very probably arises principally from *cis–trans* isomerization about the peptide bonds.

Form I has been stated by Blout, *et al.*,[4] to exhibit a negative Cotton band in the far ultraviolet even stronger than that of II. We have investigated this question further by observing the mutarotation of poly-L-proline at ambient temperature (*ca.* 30°) in trifluoroethanol solution, observing both ORD (at $1\text{-}2 \times 10^{-2}\ M$ in residues) and absorption over the range 280 to 185 mμ.[11] Some results of a typical experiment are shown in Figure 1. It is observed that initially the poly-L-proline (degree of polymerization 140) exhibits a strong *positive* Cotton band, apparently centered at about 217 mμ.[12] This gives place (half-life about 1.5 hr at *ca.* 30°) to a weaker negative Cotton band, centered at 202 mμ, corresponding to that reported by Blout, *et al.*[4]

We find the absorption spectrum (in trifluoroethanol) of the trifluoroethanol-stable form (presumably form II) to have a maximum at 202 mμ (ϵ 7100) while that of the initial form (assumed to be I) is at 210 mμ (ϵ 8900), a wavelength difference even greater than that reported by Gratzer, *et al.*,[5] for aqueous solutions. The absorption maximum for II corresponds to the inflection point of the ORD curve, but that for I is markedly shifted to shorter wavelength. The positive lobe of the form I ORD curve is considerably weaker ($[m]_{223} = +47{,}500 \pm 500°$) than the negative lobe ($[m]_{208} = -90{,}000 \pm 500°$),[13] both values extrapolated to zero time; they are more nearly equal (Figure 1 and ref 4) for form II.

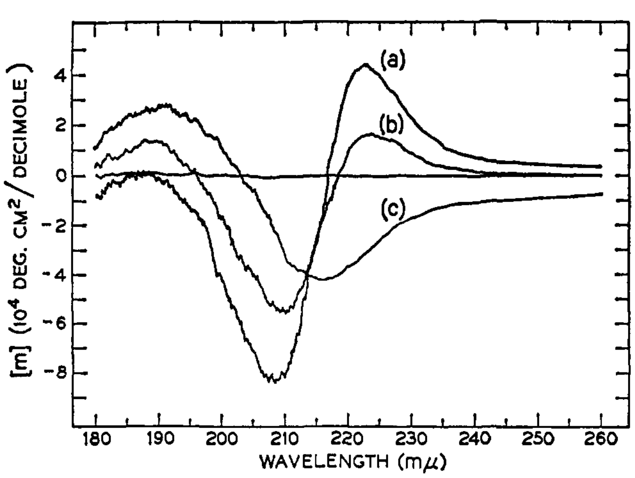

Figure 1. ORD spectra of poly-L-proline, $2.05 \times 10^{-2}\ M$ in trifluoroethanol, *ca.* 30°. Curves a, b, and c are run at successive times. Because the rates of scanning and of mutarotation are comparable, curves a and b show some kinetic distortion. For these, the three intervals given below represent the times (minutes after initial dissolution of polymer) at which the first maximum, first minimum, and second maximum were observed: (a) 8, 11, 22 min; (b) 64, 68, 80 min; (c) 20 hr.

The assignment of the form I Cotton band is not yet certain. It is probable that it is a π–π^* band, but in view of the marked n-π^* Cotton band observed in the closely related model compound cyclic di-L-proline,[14] an n–π^* contribution cannot be excluded.

Acknowledgment. The authors wish to thank Dr. Yoh-Han Pao, Dr. James Longworth, and Professor Albert Moscowitz for valuable advice and Professor Murray Goodman for kindly confirming certain of our observations on another instrument.

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(10) For a review and illustrations of these structures see W. F. Harrington and P. H. von Hippel, *Advan. Protein Chem.*, **16**, 1 (1961).

(11) Absorption and ORD spectra were obtained using a Durrum-Jasco Model ORD/UV-5 spectrophotometer and ORD recorder. Quartz cells (Opticell Cell Co., Inc., Brentwood, Md.) of 0.1- and 1-mm path length were employed. Poly-L-proline was obtained from Yeda (Rohovoth, Israel) in two lots having degrees of polymerization of 15 and 140.

(12) Since the completion of this work, Carver and Blout (private communication) have reported a similar band in polyproline I. E. R. Blout and E. Schechter (*Biopolymers*, **1**, 565 (1963)) have reported similar bands for poly-L-proline films grown from a catalytic surface.

(13) Very similar positive Cotton bands are observed in both 90:10 (v/v) 1-butanol–trifluoroethanol and 90:10 acetonitrile–trifluoroethanol, and these show no change after 30 days at room temperature. We interpret this to mean that the high molecular weight polymer is substantially entirely in form I in these solvents (and at zero time in trifluoroethanol) as it would not be expected that one would coincidentally choose three solvent systems in all of which the same equilibrium mixture of I and II happened to prevail. On the other hand, the low molecular weight polymer, while showing generally similar behavior, exhibits at the start an ORD curve indicating the presence of form II; it also shows somewhat weaker rotations for both forms.

(14) F. A. Bovey, F. P. Hood, and J. Longworth, unpublished observations.

The Biosynthesis *in Vitro* of Methylenebisphloroglucinol Derivatives

Sir:

We have recently shown that the methyl groups as well as the central methylene unit of *Dryopteris* fern constituents such as *p*-aspidin (IV) are derived from methionine *in vivo*.[1] In addition, methylaspidinol (I) has been shown[1] to be incorporated in IV, presumably *via* oxidation to the quinone methide species (II), followed by Michael addition of the anion of butyryl-

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