# Conformational Aspects of Polypeptide Structure. XXIII. Photoisomerization of Azoaromatic Polypeptides[1,2]

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**Abstract:** Polypeptides with azoaromatic side chains were irradiated at the wavelengths of the absorption maxima for the azoaromatic group (320 m$\mu$ in dioxane) and for its conjugate acid (425 m$\mu$ in trifluoroacetic acid). By comparing the effects with those observed for azobenzene and a model compound N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester, it was possible to assign the transformation to partial *trans–cis* photoisomerizations of azoaromatic side chains. The kinetics of these isomerizations and the dark relaxations of *cis* to *trans* follow first-order kinetics. We employed optical rotatory dispersion to study the structure and isomerizations of the polymers and model compound. The optical rotatory dispersions of the *trans* forms of the conjugate acids of these compounds exhibit large positive Cotton effects at 420 m$\mu$ while the *cis* forms show even larger negative Cotton effects in the same spectral region.

Photoisomerization (*cis–trans*) of azoaromatic compounds has been investigated extensively using absorption spectroscopy.[4–6] We have extended this approach by using optical rotatory dispersion studies to characterize these photoinduced transformations for the copolymers of L-*p*-(phenylazo)phenylalanine. Although most azoaromatic compounds studied to date are not optically active, the azoaromatic group of L-*p*-(phenylazo)phenylalanine exhibits optical activity because of its proximity to an optically active center, the amino acid $\alpha$-carbon. The synthesis, absorption, and ORD spectra of *trans* azo compounds and polymers derived from this amino acid have been described previously.[7]

We irradiated solutions of azobenzene, N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester, and copolypeptides of L-*p*-(phenylazo)phenylalanine with $\gamma$-benzyl-L-glutamate directly in a Cary 60 spectropolarimeter at the $\lambda_{\max}$ of the azoaromatic $\pi$–$\pi^*$ transitions. The *trans* to *cis* isomerization reactions were followed by recording changes in optical rotation with time while the extent of conversion was estimated from the absorption spectra at the $\lambda_{\max}$ of the irradiated and unirradiated solutions. When the irradiated solutions reached a photoequilibrium state, the irradiation was discontinued, and the *cis* to *trans* "relaxation" was followed.

The photoisomerizations were examined in two solvent systems, dioxane and trifluoroacetic acid. In dioxane the azoaromatic groups are unprotonated, and the polypeptide backbone is most probably in an $\alpha$-helical conformation.[7] In trifluoroacetic acid the azoaromatic groups are protonated, and the polypeptide backbone is probably extended.[8] Quantum yields under our irradiation conditions are approximately 0.1 in both solvents (*cf.* Experimental Section).

## Studies in Dioxane

The absorption spectra of azobenzene and N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester in dioxane before irradiation (Figure 1) resemble that found for *trans*-azobenzene in 95% ethanol by Gerson and co-workers.[6] After irradiation at the azo $\pi$–$\pi^*$ absorption maximum (320 m$\mu$), the spectra (Figure 1) are intermediate between those shown for pure *cis*- and pure *trans*-azobenzene in 95% ethanol (Table I). This indicates that complete conversion from *trans* to *cis* is not achieved under our irradiation conditions. Assuming the *cis* spectra of the model compounds in dioxane resemble the spectrum of *cis*-azobenzene reported by Gerson and co-workers,[6] the conversion to *cis* isomer is between 45 and 55% for the model compounds and 40 and 50% for the polypeptides.[9] Thus the equilibrium concentration of *cis* and *trans* isomers after irradiation under identical conditions is similar for the model compounds and the polypeptides.

We showed[7] that the optical rotatory dispersion for the copolymers containing *trans*-azoaromatic residues exhibits a large Cotton effect trough at 233 m$\mu$. After irradiation at 320 m$\mu$, the Cotton effect at 233 m$\mu$ remains essentially unchanged (Figure 2, Table I). These results are consistent with a helical main chain structure for the polypeptides. The reduced intensity of the trough at 233 m$\mu$ ($\sim$11,000° as compared with 13,500–14,000° usually encountered) is attributable to azoaromatic–asymmetric center interactions, as can be seen in Figure 3 where the model compound exhibits a sizeable positive rotation at 233 m$\mu$.

Cotton effects attributable to the azoaromatic side chains decrease upon irradiation at 320 m$\mu$ (Figure 2). However, no new Cotton effects are discernible after irradiation over the spectral range studied. It is clear that the side-chain Cotton effect near 335 m$\mu$ can be ascribed to electronic transitions of the azoaromatic group in the *trans* configuration. Since the *cis* azoaromatic compounds were never isolated, it is still not clear

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[1] For the previous paper in this series, see M. Goodman and E. Peggion, *Biochemistry*, **6**, 1533 (1967).

[2] We gratefully acknowledge support for this research by grants from the National Science Foundation, Grant No. GB-4882, and from the National Institutes of Health, Grant No. GM-08974.

[3] From a thesis submitted by M. L. Falxa to the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the Ph.D. degree.

[4] H. Zollinger, "Diazo and Azo Chemistry," Interscience Publishers, Inc., New York, N. Y., 1961, and references included therein.

[5] H. H. Jaffé and M. Orchin, "Theory and Application of Ultraviolet Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1962, and references included therein.

[6] F. Gerson, E. Heilbronner, A. van Ween, and B. M. Wepster, *Helv. Chim. Acta*, **43**, 1889 (1960).

[7] M. Goodman and A. Kossoy, *J. Am. Chem. Soc.*, **88**, 5010 (1966).

[8] M. Goodman and M. Falxa, submitted for publication.

[9] Copolymers containing more than 79.9 mole % L-*p*-(phenylazo)phenylalanine residues are insoluble in dioxane.

Table I. Photoisomerization of Azoaromatic Compounds

| Sample | Solvent | Irradiation time, hr | λ, mμ | $\epsilon_{320-323}$ | $\epsilon_{420-423}$ | $(R')_{233}$ | $(R')_{315-317}$ | $(R')_{360}$ | $(R')_{425}$ |
|---|---|---|---|---|---|---|---|---|---|
| *a* | Dioxane | None | 320 | 11,500 | | −10,700 | −3,760 | +3,440 | |
| | | 24 | | 7,050 | | −10,600 | −1,880 | +2,380 | |
| *b* | Dioxane | None | 320 | 11,700 | | −11,000 | −6,610 | +6,350 | |
| | | 24 | | 6,170 | | −11,000 | −2,280 | +1,880 | |
| | TFA | None | 425 | | 11,700 | | | | +1,580 |
| | | 24 | | | 8,660 | | | | −4,950 |
| *c* | Dioxane | None | 320 | 17,300 | | −10,600 | −10,700 | +10,600 | |
| | | 24 | | 11,400 | | −11,200 | −2,800 | +2,800 | |
| *d* | Dioxane | Insol | | | | | | | |
| | TFA | None | 425 | | 26,300 | +790[g] | | | +8,300 |
| | | 24 | | | | −240[g] | | | −15,800 |
| *e, h* | 95% EtOH | None | | 22,000 | | | | | |
| *f, h* | 95% EtOH | None | | 4,600 | | | | | |

[a] 37.9 mole % L-*p*-(phenylazo)phenylalanine/62.1 mole % γ-benzyl-L-glutamate copolymer. [b] 49.7 mole % L-*p*-(phenylazo)phenylalanine/50.3 mole % γ-benzyl-L-glutamate copolymer. [c] 79.9 mole % L-*p*-(phenylazo)phenylalanine/20.1 mole % γ-benzyl-L-glutamate copolymer. [d] Poly-L-*p*-(phenylazo)phenylalanine. [e] *trans*-Azobenzene. [f] *cis*-Azobenzene. [g] Measured at 250 mμ. [h] *Cf.* ref 6.

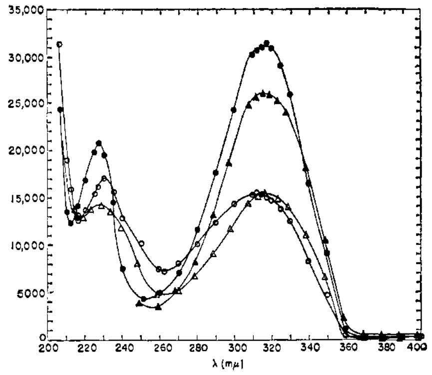

Figure 1. Absorption spectra of azobenzene before (●) and after (○) irradiation at 320 mμ in dioxane and of N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester before (▲) and after (△) irradiation at 320 mμ in dioxane.

Table II. Half-Lives of Azoaromatic Compounds

| Compd[a] | Solvent | $t_{1/2}$, min<br>*trans* to *cis* | <br>*cis* to *trans* |
|---|---|---|---|
| A[b] | Dioxane | 180 | 15 |
| B[c] | Dioxane | 180 | 15 |
| C[c] | Dioxane | 180 | 90 |
| A[b] | TFA | 120 | 15 |
| B[c] | TFA | 120 | 15 |
| C[c,d] | TFA | 120 | 210 |
| D[c] | TFA | 120 | 240 |

[a] A, azobenzene; B, N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester; C, 49.7 mole % L-*p*-(phenylazo)phenylalanine/50.3 mole % benzyl-L-glutamate; D, poly-L-*p*-(phenylazo)phenylalanine; TFA, trifluoroacetic acid. [b] From absorption data. [c] From optical rotatory dispersion and absorption data. [d] There is an induction period before photoisomerization for this compound in TFA which was not observed with the other compounds. We found that the induction period decreases with increasing intensity of irradiation.

whether a weak Cotton effect occurs for these structures or whether a Cotton effect is absent. The amide ester model compound shows similar, but weaker Cotton effects before and after irradiation (Figure 3).[10]

Both the irradiation and relaxation transformations are initially first order in dioxane at 320 mμ for the methyl ester and the copolymers. We plotted the logarithms of the residue rotations as a function of the irradiation and relaxation times and found linear dependencies for the transformations (Table II).

Using the previously described irradiation conditions, the half-life of the *trans* to *cis* photoisomerization reaction is about 180 min for the model compound and the azoaromatic copolymers. The half-life of the *cis* to *trans* relaxation in the dark is approximately 15 min for the model compound[11] and about 90 min for the 50% azo copolymer (Table II).

The over-all appearance of the α-helical polypeptide structure can be visualized as a thin short core composed of the peptide backbone surrounded by a thick sheath composed of the bulky azoaromatic groups. Each *trans* residue is essentially a long, broad electron cloud rigidly oriented about the main chain because of severely restricted rotation about the $C_\alpha$–C bond. Even in the most favorable side-chain orientation about the main chain, there are large interactions between the side chain and main chain which give rise to the side-chain Cotton effects. We demonstrated[7] that the intensity of the peaks and troughs of the azoaromatic Cotton effects varies in a nearly linear fashion with the concentration of azoaromatic residues in the copolymers. On this basis, we believe that side-chain–side-chain interactions are negligible.

Because of the angles required by the *cis*-azo linkage, the electrons of the two aromatic rings do not form the extended delocalized chromophore present in the *trans*

(10) In our previous publication,[7] we presented the ORD curve for the amide ester model compound in dioxane. Since the rotation values are small and the absorption is high through the Cotton effect region (380–240 mμ), it is difficult to obtain reproducible readings. We have repeated the measurement on this compound many times in the present study and Figure 3 represents a much more accurate curve than that reported earlier.

(11) The reported half-lives of *cis* to *trans* conversion in the dark for the model compounds in both dioxane and trifluoroacetic acid are only "order of magnitude" values based upon few experimental points. Only a small number of readings per run could be obtained because the sample is irradiated approximately 1 min each time a reading is taken. We feel that more frequent readings are not possible using our experimental techniques without causing noticeable photoisomerization. A rotation shutter method for following relaxation[12,13] may be preferable instead because it allows much shorter measurement times at regular intervals.

(12) W. R. Brode, J. H. Gould, and G. M. Wyman, *J. Am. Chem. Soc.*, **74**, 4641 (1952).

(13) J. H. Gould and W. R. Brode, *J. Opt. Soc. Am.*, **42**, 380 (1952).

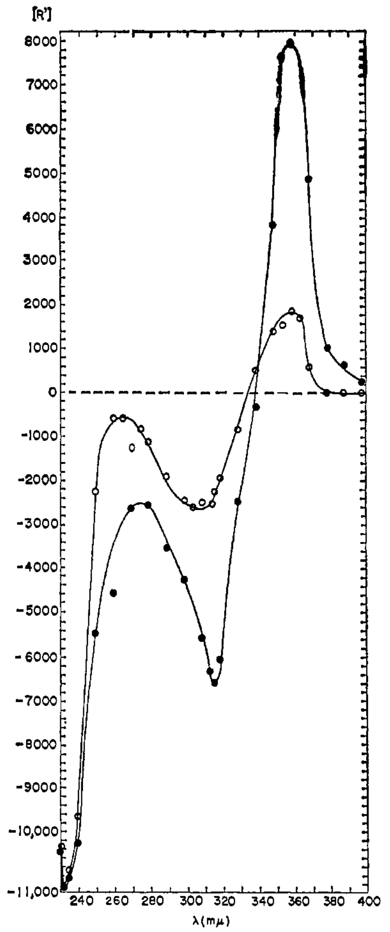

Figure 2. Optical rotatory dispersion of copolymer of L-*p*-(phenylazo)phenylalanine with γ-benzyl-L-glutamate before (●) and after (○) irradiation at 320 mμ in dioxane.

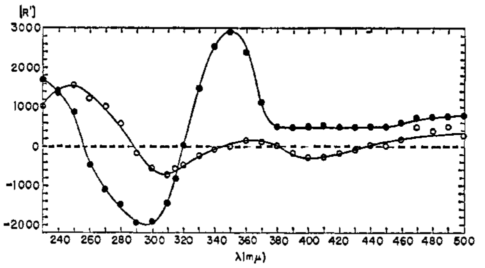

Figure 3. Optical rotatory dispersion of N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester before (●) and after (○) irradiation at 320 mμ in dioxane.

structure. Thus, the properties of poly-L-*p*-(phenylazo)phenylalanine with cisoid side-chain structure resemble poly-L-phenylalanine. In other words, side-chain chromophoric effects are either small or absent completely. There appear to be no *cis–cis* side-chain interactions about the α-helix because of the steric nature of *cis*-azo linkages.

### Studies in Trifluoroacetic Acid[14]

The absorption spectra of the conjugate acids of azobenzene, the monomeric methyl ester, and poly-L-*p*-(phenylazo)phenylalanine in trifluoroacetic acid before irradiation (Figures 4 and 5) resemble those published for the *trans* conjugate acid of azobenzene.[6,14] After irradiation at the azo conjugate acid π–π* absorption maximum (425 mμ), the configurations of the azoaromatic compounds are intermediate between those shown for pure *cis* and pure *trans* azobenzene conjugate acids in ethanol-perchloric acid (1:4).[6] This indicates that complete conversion from *trans* to *cis* is not achieved using our irradiation conditions (Figures 4 and 5). The conversion to *cis* is between 50 and 60% for the model compounds and between 35 and 40% for the polypeptides. The decreased *cis* conversion for the polypeptides may result from steric restrictions between adjacent side groups on the extended polypeptide chain.

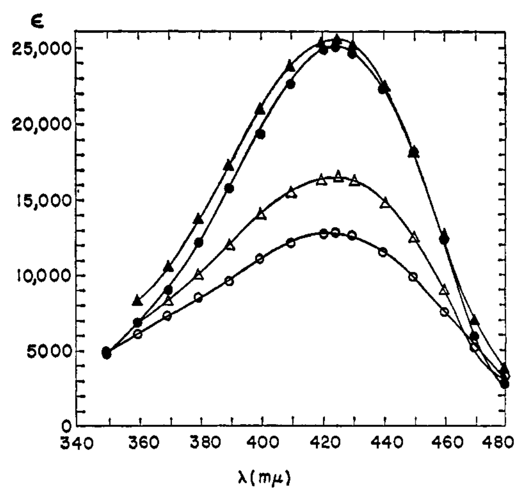

Figure 4. Absorption spectra of azobenzene before (▲) and after (△) irradiation at 425 mμ in trifluoroacetic acid and of N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester before (●) and after (○) irradiation at 425 mμ in trifluoroacetic acid.

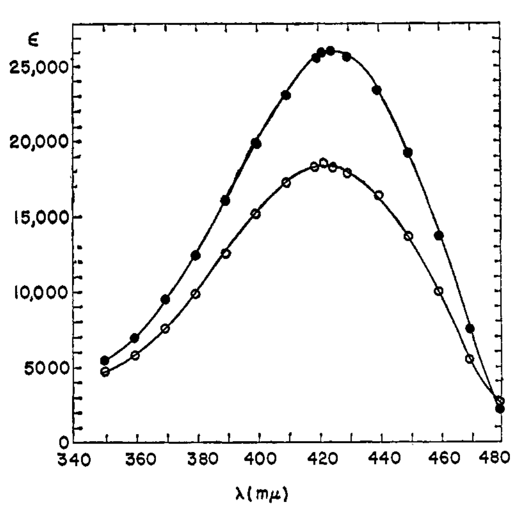

Figure 5. Absorption spectra of poly-L-*p*-(phenylazo)phenylalanine before (●) and after (○) irradiation at 425 mμ in trifluoroacetic acid.

The optical rotatory dispersions of the *trans* and *cis* azoaromatic polypeptides reveal no Cotton effects in the 220–240-mμ region, indicating that the backbone conformation is most probably nonhelical. In a forthcoming paper we will present evidence that the con-

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[14] We were concerned that solutions of the azoaromatic-containing polymers and model compound would degrade in trifluoroacetic acid. We found that the specific viscosities of freshly prepared and 5-day-old solutions of the azoaromatic copolymers in trifluoroacetic acid are identical. This indicates that negligible polymer degradation occurs over this period. No solutions were studied more than 72 hr after preparation.

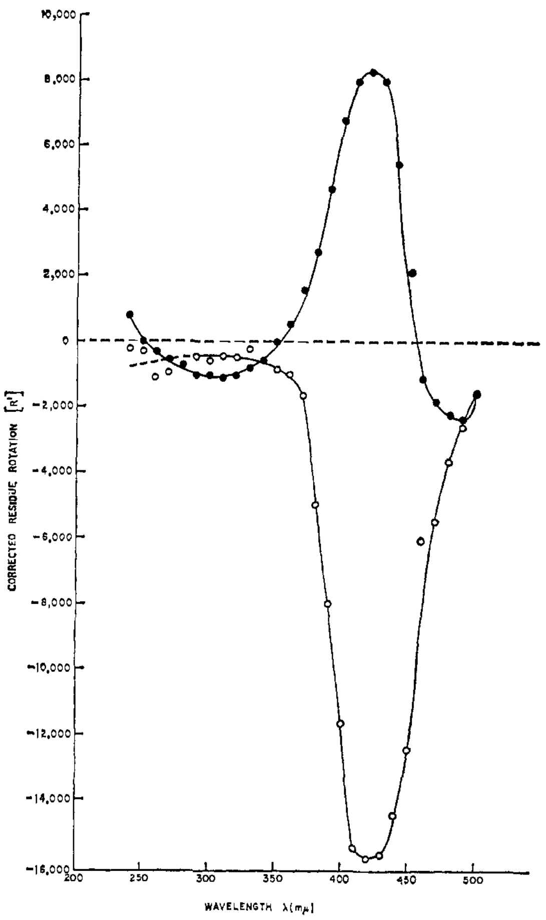

Figure 6. Optical rotatory dispersion of poly-L-*p*-(phenylazo)-phenylalanine before (●) and after (○) irradiation at 425 mμ in trifluoroacetic acid.

formation of the protonated azoaromatic polypeptides is probably extended.[8]

The ORD of *trans* protonated azoaromatic polypeptides exhibit a 425-mμ Cotton effect peak flanked by two shallow troughs near 310 and 490 mμ (Figure 6). This relationship may result from split excitations of the protonated azo structures.[15] After irradiation at 425 mμ, the 425-mμ peak is replaced at somewhat lower wavelengths by a large trough flanked on either side by two shallow peaks (Figure 6). The *cis* isomers of the conjugate azoaromatic acids apparently produce Cotton effects that are of opposite sign and greater magnitudes than those produced by the *trans* isomer. Smaller but similar effects are noted in the monomeric methyl ester.

The observed magnitude of the new rotational trough after irradiation is approximately twice that observed for the 425-mμ peak for the unirradiated azoaromatic compounds (Table I).[16] The side-chain Cotton effect of the protonated *cis*-azoaromatic isomer is probably even greater than that reported here because the observed spectra are derived from incomplete *trans* to *cis* conversion.

Both the irradiation and relaxation transformations are first order for the methyl ester and the copolymers. The half-lives of the irradiated polymers were estimated assuming that the *trans* to *cis* reaction occurred until the residue rotations reached their most negative values[16] rather than until the rotations reached their equilibrium values.

The half-lives for *trans* to *cis* transformation of conjugate azoaromatic acids is about 120 min for the model compounds and for the polypeptides (Table II) using the irradiation conditions described above. The *cis* to *trans* relaxation occurs rapidly for the model compounds, with half-lives of about 15 min. In contrast, the relaxation half-lives of the polypeptides are quite long, 210 min for the 49.7 mole % azoaromatic copolymer and 240 min for the azoaromatic homopolymer (Table II). We believe that steric and electronic side-chain–side-chain interactions about the extended polypeptide main chain tend to hinder the conversion from *cis* to *trans*. In addition, protonation may link pairs of azoaromatic groups and thereby inhibit isomerization.

The *cis* and *trans* Cotton effects of the *p*-(phenylazo)-phenyl groups in trifluoroacetic acid arise primarily from induced optical activity derived from neighboring asymmetric centers of the amino acid α-carbon. We suggest this origin since both the acetyl amino acid methyl ester and the polypeptides exhibit the same side-chain Cotton effects. Molecular models show that there is appreciable interaction between the bulky azoaromatic side chains in both the *cis* and *trans* configurations. These interactions are undoubtedly responsible for the larger magnitudes of the side-chain Cotton effects for the polypeptides when compared with the model compound.

In Figures 4 and 5 we present the ultraviolet spectra of the conjugate acids of *trans* and *cis* azoaromatic compounds. It is not possible to assign absorption bands for the *cis* structure. The situation is quite different for the optically active azoaromatic compounds where we observe a strong positive optical rotatory dispersion band in the 420-mμ region for the *trans* structures. After irradiation these peaks are converted into troughs of larger magnitude (Figure 6) which must arise from *cis* azoaromatic contributions. We are preparing a series of optically active azoaromatic compounds in order to investigate the general relationship between the electronic spectra and the *cis* and *trans* configurations about the azoaromatic group.

## Experimental Section

**Materials.** Dioxane (Fisher) was refluxed over and distilled from sodium.[17] Dioxane used for rotatory dispersion, circular dichroism, and ultraviolet spectroscopy ("spectro quality," Matheson Coleman and Bell) was passed through a column of neutral alumina just prior to use in order to remove water and peroxides.[17]

Trifluoroethanol (Halocarbon Corp.), hexafluoroacetone trihydrate (Hynes Chemical Corp. and Allied Chemical), dichloroacetic acid (Fisher, purified), and trifluoroacetic acid (Distillation Products Industries) were used without further purification. Hexafluoroacetone trihydrate and trifluoroacetic acid were stored under refrigeration.

Samples of azobenzene, N-acetyl-L-*p*-(phenylazo)phenylalanine methyl ester, L-*p*-(phenylazo)phenylalanine–γ-benzyl-L-glutamate random copolymers containing 37.9, 49.7, and 79.9 mole % azopeptide, and poly-L-*p*-(phenylazo)phenylalanine were kindly supplied by Dr. Aaron Kossoy.[7] The weight-average molecular weights of the copolymers were between 20,000 and 30,000.

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(15) I. Tinoco, Jr., *J. Am. Chem. Soc.*, **86**, 297 (1964).

(16) There is an "overshooting" of the rotational values (of several hundred degrees) before equilibrium is reached. Apparently, more *cis* isomer is present for 6 hr of irradiation than at equilibrium.

(17) L. F. Fieser "Experiments in Organic Chemistry," 3rd revised ed, D. C. Heath and Co., Boston, Mass., 1955, pp 284, 264.

**Apparatus.** The optical rotatory dispersion measurments were recorded on the Cary ORD 60 automatic recording polarimeter equipped with a 450-w Osram xenon lamp. The instrument was continuously flushed with nitrogen to keep moisture and oxygen from entering. The nitrogen flow was 12 ft$^3$/hr during operation and for 1 hr before and after use. The nitrogen flow was about 2 ft$^3$/hr during inoperative periods. Gaseous nitrogen was supplied from a Linde LC-3 liquid nitrogen tank with a built-in vaporizer. The nitrogen passed through about 20 ft of copper tubing before entering the spectropolarimeter to allow the nitrogen to equilibrate with room temperature. A special water-cooled cell holder was used to ensure constant temperature for our measurements as well as to allow controlled changes of temperature.[18] The polarimeter cooling water (27 ± 0.1°) was normally passed through the cell holder.

All ultraviolet and visible spectra were recorded on a Perkin-Elmer Model 350 spectrophotometer using 0.01-cm matched Suprasil (quartz) cells. The settings used were resolution, 3; speed, 2; pen response, 3. Tungsten (visible) or deuterium (ultraviolet) light sources were used.

Viscosity measurements were carried out using a Cannon-Ubbelohde 150 viscometer. The viscometer bath temperature was 25 ± 0.1°.

**Measurements. 1. Optical Rotatory Dispersion and Absorption Spectra.** All absorption and optical rotatory dispersion measurements of azo compounds were carried out in 0.100-mm fused quartz cells. Extreme care was used in cleaning the cell and in the placement of the cells in the cell holder to avoid shifts in the base line. The short-path-length cells were also used for the photoisomerization studies.

Azo solutions were kept in total darkness at least 24 hr before scanning to ensure complete *trans* configuration at the start of measurement.

When azo sample runs were made, the cells were quickly filled and inserted into the spectrophotometer. The visible (for TFA solutions) or ultraviolet (for dioxane) spectra were rapidly measured in less than 3 min. The cells were then immediately inserted into the spectropolarimeter and the dispersion spectra obtained at a scan speed of 15 m$\mu$/min. No observable photoisomerization occurred during scanning of the polymer solutions at this speed. A scan from 500 to 230 m$\mu$ can be accomplished in less than 20 min. This seems to be the maximum speed for recording rotations without an intolerable signal-to-noise ratio while operating at high sensitivities. However, noticeable photoisomerization takes place during ORD scans recorded at speeds less than 10 m$\mu$/min.

Checks of unwanted isomerization were made by scanning through the side-chain Cotton effect region (370–300 or 450–400 m$\mu$), and the observed rotations agreed with those taken on the longer scans within experimental error. The ORD of the monomeric model compound was measured in 75-m$\mu$ segments to minimize photoisomerization. After measurements were made, the solutions were returned to the dark. Samples were reirradiated on successive days to check reproducibility.

Solvent base lines were always run *before* and *after* each ORD scan to ensure that the instrument base line was flat and that the cell was clean.

Since azobenzene is optically inactive, the photoirradiation of this compound was followed by observing the changes in photomultiplier dynode voltage on the Cary ORD 60. The photoisomerization was assumed to be complete when no changes in dynode voltages were noted over a period of at least 30 min. As before, the absorption spectra were measured before and after irradiation.

**2. Photoisomerization.** After the rotatory dispersion scan was completed, the wavelength was set at the *trans*-azo $\lambda_{\max}$, and the sample was irradiated.[19] The sample rotation was then followed at constant wavelength as a function of time. The protonated azo solutions were irradiated at 425 m$\mu$, and the unprotonated azo solutions were irradiated at 320 m$\mu$. Although we irradiated at the $\lambda_{\max}$ of the $\pi$–$\pi^*$ transition, higher quantum yields have been reported irradiating at 436 m$\mu$, the $\lambda_{\max}$ of the n–$\pi^*$ transition in nonpolar solvents.[20]

Samples were initially irradiated using the programmed slit widths (constant energy program). The programmed slit width was 0.5 mm at 425 m$\mu$ and 1.5 mm at 320 m$\mu$. When the rotation of the irradiated sample became constant, the automatic slit control was closed and the slits were fully opened (2.4 mm). Irradiation was continued until no further rotational changes were observed, if any. After the photoisomerization was completed, the ORD and ultraviolet or visible ranges were rapidly measured.

During irradiation, the chart scan drive was set at "synchronous." The chart gears were chosen to produce chart speeds of 250, 500, or 1000 sec per chart division. This design feature of the Cary ORD 60 enabled accurate recording of the rotational changes with time.

**3. "Dark Relaxation."** The following experimental scheme was devised to compare the *cis* to *trans* reaction which occurs in the dark with the light-sensitive reverse reaction. After the sample had been irradiated and the full spectrum ORD and ultraviolet (or visible) scans obtained, the sample was replaced in the spectropolarimeter and the wavelength reset at the wavelength of irradiation, $\lambda_{\max}$. The rotation was recorded and compared with the rotation observed at the completion of the irradiation to ascertain if isomerization occurred during scanning. No changes were observed except for the model compounds. The model compounds were reirradiated at $\lambda_{\max}$ until their rotation (or absorption) reached the equilibrium values again.

To initiate the "relaxation" cycle the compensator, pen, and slit controls were switched off, and the slit was closed manually. The chart drive remained on synchronous, producing a time axis upon which the "dark relaxation" measurements were recorded. Every 15 or 30 min for the polymers and every 5 or 15 min for the model compounds a rotation reading was recorded. The slit drive was first switched on, followed by the compensator. When the dynode voltage was stabilized, and the movement of the compensator gears could not be heard, the pen was turned on. The pen was kept off the paper until the pen movement stabilized, and then the rotational trace was recorded for 30 sec. The above cycle was repeated until enough points had been recorded to establish the reaction half-life and the order of the reaction.

This method gives accurate readings and avoids removing the sample from the instrument during the "relaxation period." The disadvantage is that the sample is irradiated approximately 1 min each time a rotation reading is taken so that frequent readings are not possible without causing noticeable photoisomerization. This probably makes little practical difference for measurements of polymer relaxation because the irradiation time is short compared to the "dark time." Relaxation measurements of model compound solutions were less accurate. Here the *cis* to *trans* reversion is very rapid (see Results); thus only two or three readings per run could be taken. More frequent readings resulted in appreciable photoisomerization.

**4. Quantum Yield.** The radiation intensity was measured using an Eppley spectrum-type bismuth-silver thermopile[21] with a 1-mm quartz window. The thermopile was placed 9.0 cm from the left side of the sample chamber. The thermopile window was 1.90 mm high and 2.76 mm long with a calibrated sensitivity of 0.392 $\mu$v/$\mu$w/cm$^2$. The light source had been operated for 70 hr before these studies were employed. This provided the maximum beam intensity and stability and minimum noise. No measurable change in lamp intensity occurred during the quantum yield experiments (about 50 hr). Because of the lamp design and the large off-axis elliptical source mirror, there is negligible arc wandering after the initial 40–60 hr of lamp operation.

The azoaromatic samples were irradiated in dioxane solutions at 320 m$\mu$ employing a 1.5-mm slit width. The radiation intensities ($I_a$) absorbed by the sample solutions were approximately $2 \times 10^{12}$ quanta/sec with *trans:cis* quantum yields about 0.1 under these conditions.

The protonated azoaromatic samples in trifluoroacetic acid solutions were irradiated at 425 m$\mu$ employing a 0.5-mm slit width. The radiation intensities ($I_a$) absorbed by the sample solutions were approximately $1.5 \times 10^{12}$ quanta/sec for a slit width of 0.5 mm. The *trans:cis* quantum yields under these conditions are also about 0.1.

**Acknowledgment.** We wish to thank Dr. Aaron Kossoy of our laboratory for the preparation of the azo model compounds and polymers.

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[18] This thermostated brass holder was built by M. Schwartz and R. Parla based on a design developed by Dr. E. Dratz of the Laboratory of Chemical Biodynamics, University of California, Berkeley, Calif.

[19] The solutions were irradiated at the $\lambda_{\max}$ of the *trans*-azo form because at this wavelength there is the greatest difference in absorbance between the *cis* and *trans* forms. This yields the greatest driving force for the maximum conversion of *trans* to *cis*.

[20] D. R. Kearns, *J. Phys. Chem.*, **69**, 1062 (1965).

[21] J. G. Calvert and J. N. Pitts, "Photochemistry," John Wiley and Sons, Inc., New York, N. Y., 1966.