Protein and Hydrogen Ion Control of Photochromism in Aminoazobenzene Compounds[1]

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Abstract: Several ordinary *N,N*-dialkylaminoazobenzenes (*n*-alkyl groups, *n*-methyl to *n*-butyl) were found to be photochromic in water, with dark cis → trans isomerizations in the $t_{1/2}$ relaxation time range from a few to several hundred seconds. The studies were carried out with free dyes in water, and with aqueous protein bound dyes, principally serum albumin. The heretofore lack of photochromism of such compounds in water now appears due to H^+, which is an effective catalyst for the dark reaction even at neutral pH. Typically H^+ has a second-order rate contribution, k_{H^+}, of the order 10^9–10^{12} sec^{-1} M^{-1}. At sufficiently alkaline pH, 8–12, photochromism in water of such compounds is readily studied. Without the *n*-alkyl N-substituents, photochromism is still not accessible on the time scale $t_{1/2} > 3$ sec. The studies were largely performed at 4°, with, however, observable rates at 25°. It is considered that H^+ catalyst probably acts at the *n* electrons on the azo linkage of the cis isomer. β-Lactoglobulin behaves similarly to serum albumin. At large protein concentrations, where the dyes are continuously bound, the effect of proteins is to decrease k_{H^+}, relative to that for free azobenzene compounds.

Cis–trans isomerizable azobenzene dyes may become interesting optical probes for proteins for several reasons. They engage in major but reversible geometry switching reactions and hence are subject to steric constraint imposed by proteins. The dyes are easily obtained in wide variety, and large absorption spectra changes accompany the geometry switching. The dyes are potentially light energy storage systems, and they can in turn control certain macromolecular properties. For instance, Deal, Erlanger, and Nachmansohn[2] used azobenzene derivative photochromism to obtain photoregulation in membranes, and polymer viscosity has been controlled[3,4] by means of light energy, using azobenzene photochromism. Azobenzene photochromism has recently been reviewed by Ross and Blanc.[5]

"Optical probes" for proteins means that hopefully the chromophores reflect something of the nature of the macromolecule region in which they are bound. In free solution azobenzene photochromism depends on the nature of the solvent, and sometimes is sensitive to the presence of catalytic species in the solvent. In turn, response to such catalysts is much determined by substituent groups on the azobenzene rings. As an example, azobenzene itself and 4-aminoazobenzene have similar photochromic properties in hydrocarbon solvents and in very dry alcohols. But if much moisture is present, they differ a great deal in their rates of cis → trans conversion in the dark.

So far, it appears from the literature that a compound like methyl orange is either not photochromic in water, or at best is not observably so using ordinary reaction timing methods in the seconds to minutes range. It might have been expected to be discernibly photochromic with fast reaction techniques, but out of reach if cis → trans apparent first-order rate constants are greater than a sec^{-1} or so. However, we have now found that N-substituted aminoazobenzenes are slowly photochromic in water in the sense just described, and discussed before,[6] if they are simply observed at alkaline pH avalues. Probably the reason why hydroxylic solvents have so far seemed to so markedly destabilize the cis form of aminoazobenzenes is because of the presence of protons, and not because of the nature of the solvent molecules alone.

General acid catalysis probably is not a major contributor to cis → trans reaction velocities described below, but specific catalysis by protons is a major contributor. The apparent first-order rate constants, k_a, are expressed by

$$k_a = k_0 + k_{H^+}(H^+) \quad (1)$$

in which k_0 is the water contribution to the rate constant, and k_{H^+} is the specific constant for H^+. In general, adsorption binding of the azobenzene compound to protein considerably lowers k_{H^+}, but does not decrease it to zero. The extent of amino group alkylation also bears on the dye's behavior. With N-alkylation, that is with *n*-alkyl substituents, there arises some stability of the cis, or high energy, form of the dye, and decreasing tendency for protons to catalyze the cis → trans conversion, compared to unsubstituted aminoazobenzenes.

This paper may serve to sum up three points concerning the cis → trans reaction of substituted azobenzenes. (i) Proceeding through a series of ring substituents such as alkoxy, amide, semicarbazide, hydroxy, and unsubstituted amino groups, there is generally an increasing tendency for the reaction to come under increasing influence of various catalytic species other than solvent molecules. (ii) There has been considerable discussion in the literature concerning rather widely varying photochromic behavior as affected by solvents. Particularly contrasting are hydroxylic and hydrocarbon solvents. Generally, hydroxylic solvents (alcohol, water, and their mixtures) seemed to

(1) Work supported by U. S. Army DA-31-124-ARO(D)-409, and National Institutes of Health Grant GM 18807-02.

(2) W. J. Deal, B. F. Erlanger, and D. Nachmansohn, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1230 (1969).

(3) R. Lovrien, *Proc. Nat. Acad. Sci. U. S.*, **57**, 236 (1967).

(4) G. Van Der Veen, "Photoregulation of Polymer Conformation," Thesis, University of Groningen, Netherlands, 1969.

(5) D. L. Ross and J. Blanc in "Photochromism. Techniques of Chemistry," Vol. III, G. H. Brown, Ed., Wiley-Interscience, New York, N. Y., 1971, Chapter 5.

(6) R. Lovrien and J. Waddington, *J. Amer. Chem. Soc.*, **86**, 2315 (1964).

Table I. Azobenzene Dye Photochromism in Water and in Aqueous Serum Albumin, 0.02 Ionic Strength, 4°[a]

	Abbreviation	Free dye	With protein[b]
N,N-Dialkylaminoazobenzenes			
4-Dimethylaminoazobenzene (butter yellow)	DMAA	$k_0 = 1 \times 10^{-3}$ $k_{H^+} = 2.4 \times 10^9$	$k_0' = 1 \times 10^{-3}$ $k_{H^+}' = 1.4 \times 10^8$
4-Dimethylamino-4'-carboxylazobenzene (para methyl red)	PMR	$k_0 = 4.2 \times 10^{-3}$ $k_{H^+} = 1.3 \times 10^{11}$	$k_0' = 9.6 \times 10^{-3}$ $k_{H^+}' = 4.1 \times 10^7$
4-Dimethylamino-2'-carboxylazobenzene (ortho methyl red)	OMR	$k_0 = 2.8 \times 10^{-3}$ $k_{H^+} = 1.4 \times 10^{11}$	$k_0' = 4.2 \times 10^{-2}$ $k_{H^+}' = 8.7 \times 10^9$
Methyl orange	MO	$k_0 = 4.7 \times 10^{-2}$ $k_{H^+} = 1.6 \times 10^{11}$	$k_0^* = 1.7 \times 10^{-2}$ $k_{H^+}^* = 2.5 \times 10^8$
Butyl orange	BO	$k_0 = 8.2 \times 10^{-2}$ $k_{H^+} = 4.8 \times 10^{11}$	$k_0^* = 6.0 \times 10^{-3}$ $k_{H^+}^* = 1.0 \times 10^8$
Nonsubstituted aminoazobenzenes			
4-Aminoazobenzene	AAB	} Not photochromic	} Not photochromic
4-Amino-4'-dimethylaminoazobenzene	ADAB		
4-Phenylazo-1-naphthylamine	PAN		

[a] Rate constants according to eq 1, in sec^{-1}, and $sec^{-1}\ M^{-1}$. [b] Primed quantities, as in k_0', from the case in which k_0 values dependent on [P] were extrapolated to lim $1/[P] \rightarrow 0$. Asterisked quantities, as k_0^*, are from the case in which $[P] = 6.8 \times 10^{-4}\ M$ protein.

destabilize the cis isomer. Often photochromism failed to be observed. We have now found values of k_{H^+} of the order of 10^8–$10^{12}\ M^{-1}\ sec^{-1}$ for N-alkylated aminoazobenzenes. For amide-substituted azobenzenes, we[6] previously found a value of the order of 10^0; Wettermark, *et al.*,[7] found *ca.* 10^7 for hydroxyazobenzenes. With $k_{H^+} \sim 10^{11}$, therefore, it appears that part of the reason for instability of the cis isomers may have been due to the presence of small amounts of H^+, simply from solvent self-ionization, even in binary mixtures. In plain, or neat water, photochromism becomes observable if simply the pH is controlled, *i.e.*, if the solution is made alkaline. There are temperature effects also, but it is adequate to descend to the 0–5° region to readily observe photochromism. Even at room temperature, however, photochromism of a dye such as aqueous methyl orange is observable, at high pH. (iii) Once pH controls are imposed, and k_{H^+} is accounted for in the case of free dyes, protein binding (mostly serum albumin in the present study) exerts its own influence. In effect, binding lowers k_{H^+}, relative to the free dye. Then aqueous photochromism of *N*-alkylaminoazobenzenes can be seen, using simple equipment, down into the mild alkaline region, pH ~9.

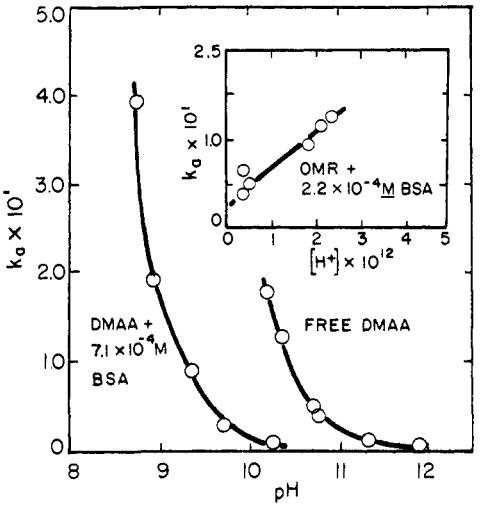


Figure 1. Experimental rate constants for dye DMAA (Table I) cis → trans dark reaction, after irradiation with light to produce cis isomer. With the high concentration of bovine serum albumin, the dye is bound most of the time. Insert: Plot of such data for dye OMR, according to eq 1.

Results

The azobenzene dyes which were examined are listed in Table I. Cis → trans rate behavior is reported for various dyes in the order given in the table.

For azobenzene itself, in benzene solvent, Halpern, *et al.*,[8] give a rate constant $k = 3.6 \times 10^{-6}\ sec^{-1}$ at 25°. From Hartley's[9] data, $k = 1.6 \times 10^{-6}\ sec^{-1}$. Aqueous isoionic serum albumin according to our data binds two azobenzene molecules from the crystal. These adsorption bound molecules have an apparent first-order cis → trans value, k_a, of $8.0 \pm 2 \times 10^{-6}\ sec^{-1}$ (25°). Thus it appears so far as if aqueous serum albumin bound azobenzene behaves in a way similar to that of azobenzene in benzene solvent. Alkaline serum albumin also binds azobenzene, but up to pH 11, the rate constants change but little from those of the neutral (isoionic) pH case. Because of the water insolubility of azobenzene, examination of nonbound azobenzene was not attempted.

Ordinary aminoazobenzenes, with no N-alkylation, mostly failed to be photochromic in water, even in very high alkali, at temperatures down to 4°. Some of these are listed in Table I. It is likely that with fast reaction equipment the cis form could be discerned. Fleeting photochromism may be seen. But the cis → trans conversion is too fast for our present apparatus, and the photostationary state greatly favors the trans form, leaving an inadequate absorption spectral range.

In contrast, the N-alkylated azobenzenes are readily studied in alkaline pH. Figure 1 illustrates the pH dependency of the apparent first-order rate constant, k_a, as a function of pH, for one of these compounds in the free or nonbound case, and in the presence of $7 \times 10^{-4}\ M$ bovine serum albumin. We cannot follow reactions in which $k_a > 5 \times 10^{-1}\ sec^{-1}$. Nevertheless, there is adequate range for discerning that: (i) as H^+ concentration decreases, the N-alkylated dyes are indeed photochromic in water; (ii) the protein markedly decreases k_{H^+} at each pH. The protein stabilizes the

(7) G. Wettermark, M. Langmuir, and D. Anderson, *J. Amer. Chem. Soc.*, 87, 478 (1965).
(8) J. Halpern, G. Brady, and C. Winkler, *Can. J. Res., Sect. B*, 28, 140 (1950).
(9) G. S. Hartley, *J. Chem. Soc.*, 633 (1938).

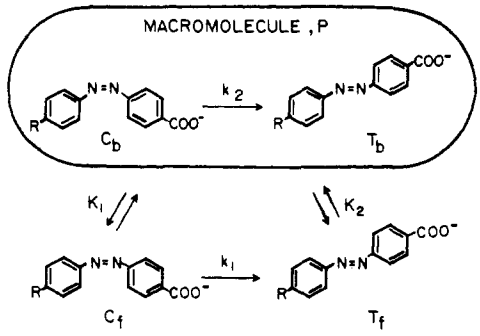


Figure 2. Pathways for cis → trans relaxation, in free and bound dyes. C_b and C_f are bound and free cis isomers, T_b and T_f corresponding trans isomers.

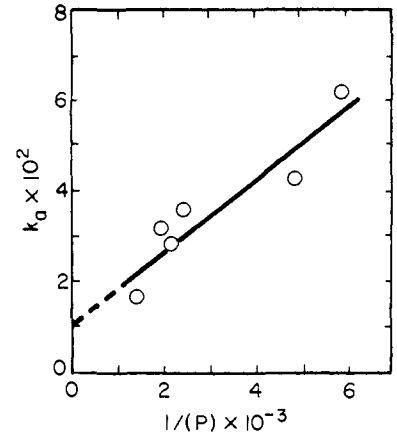


Figure 3. Application of eq 2 to the system DMAA dye and serum albumin. Intercept: k_2.

cis isomer. The insert of Figure 1 shows the application of eq 1 to some pH-dependent data for a dye, obtaining k_0 and k_{H^+}.

The influence of the protein on the dark reaction depends on two factors: (i) the nature of the cis dye interaction with the protein, and (ii) the pathways *via* which the cis form returns to the trans form. Namely, an isomerization while the dye remains continuously bound, or a pathway in which the cis isomer unbinds from the protein, returns to the trans form in free solution, and then reequilibrates with the protein as the trans isomer. Let C_f and C_b represent free and bound cis isomer obtained by irradiation, and T_f and T_b the corresponding trans isomers. Figure 2 illustrates the simplest complete set of pathways for relaxation to the dark steady state. In Figure 2, K represents the equilibrium binding constant, and k values are rate constants. The assumptions are made that: (i) the binding reactions to the macromolecule are much faster than the geometry switching reactions, so that equilibrium binding holds throughout. (Binding relaxation times for other azobenzene dyes, with serum albumin in the acid region, have been listed by Alberty[10] in the millisecond ranges.) (ii) As the protein concentration, [P], is increased, binding of the cis isomer becomes independent of competition for binding sites by the trans isomer. In the limit of $1/[P] \rightarrow 0$, the equilibria of T_f with P does not affect the other reactions. In that case, protein binding of the trans form of the dye, characterized by K_2, does not affect the rates of the other three reactions in Figure 2. Short of the limit, but still at protein concentrations, [P], of the order of a hundred times greater than total dye concentration, this assumption is likely to be valid for serum albumin. It has many sites for binding organic ions, and as shown below, its molar binding constant for such dyes is of the order of 10^4 in the alkaline pH range.

Using the three remaining reactions which are in principal control over the rate process

$$k_a = (k_1/K_1[P]) + k_2 \qquad (2)$$

giving k_2 when k_a is extrapolated to the lim $1/[P] \rightarrow 0$. Rate parameters for free or nonbound dyes, from eq 1, are given in Table I. These may be used for evaluating k_1, in eq 2, at various pH values.

In the presence of protein, the rate behavior depends, at a minimum, on protein concentration, and on the nature of how H^+ acts as a catalyst when the dye is bound to the protein. Accordingly, there are two general ways by means of which the data were analyzed. (i) The pH dependency of photochromism with varying protein concentrations was determined. At each protein concentration, a value of k_0 and k_{H^+} was obtained, as in eq 1. These values were extrapolated as a function of 1/[P], to the limit. They are given in Table I as primed quantities, k_0' and k_{H^+}', for three dyes, butter yellow, or DMAA, and the two methyl red dyes, PMR and CMR. This amounts to an empirical way of treating the data, but gives a ready comparison of free and bound dye, in terms of eq 1. (ii) The second means of analyzing the data involved determining k_a for a series of protein concentrations, all at the same pH. These were plotted according to eq 2, as illustrated in Figure 3. The value of k_1 was calculated according to eq 1 from the parameters for the free dye at that pH. Thence, K_1, as defined in Figure 2, was obtained. The value of k_2, for the isomerization of the continuously bound dye, gives a fair qualitative idea of the photochromic lifetimes of the isomerization, from $t_{1/2} = 0.69/k_2$. Representative values for K_1, and $t_{1/2}$, for some of the dyes are listed in Table II.

From serum albumin's tendency to bind hydrocarbons and organic anions even at high pH, it was thought that increasing the size of the flexible hydrocarbon groups on the alkylaminoazobenzenes might enhance stabilization of the cis form of the alkylaminoazobenzenes, compared to the case for dimethylamino groups. Accordingly, corresponding diethylamino and dipropylamino analogs to methyl orange were synthesized and examined. Butyl orange is commercially available.

Longer chain hydrocarbons do not confer unusual photochromic rate properties. Representative values are given in Table I, for methyl orange, M.O., and butyl orange, B.O. Although the sulfonated azobenzenes are also photochromic in sufficiently alkaline systems, and although serum albumin has a large effect in the same way it has for the carboxylic dyes, M.O. and B.O. behave in a way similar to the rest of the alkylamino dye family. So do the diethyl and the dipropyl analogs. A complete study with protein concentration variation was not made for M.O. and B.O., but they were studied

(10) R. A. Alberty, Abstracts, 140th National Meeting of the American Chemical Society, Chicago, Ill., 1961, 10T.

Table II. Application of Eq 1 and 2. Values of Binding Constants, K_1, and Half Lifetimes, $t_{1/2}$, for Bound Cis Isomers. Bovine Serum Albumin, 0.02 *M* KCl, 4°

DMAA, butter yellow			OMR, ortho methyl red			PMR, para methyl red		
pH	$K_1 \times 10^{-4}$, M^{-1}	$t_{1/2}$, sec	pH	$K_1 \times 10^{-4}$, M^{-1}	$t_{1/2}$, sec	pH	$K_1 \times 10^{-4}$, M^{-1}	$t_{1/2}$, sec
9	3.6	5	10.5	1.2	2	9	2.5	14
9.5	3.5	16	11	1.2	7	9.5	2.3	31
10	3.1	58	11.5	1.3	9	10	2.0	50
10.5	2.2	310	12	1.8	11	10.5	1.3	63
11	1.2	660				11	0.6	63

at a fairly large protein concentration, 6.8×10^{-4} *M*, about 5% by weight. The corresponding parameters in Table I are asterisked, *e.g.*, k_{H^+}*, to distinguish the values from the case where the values were taken to the lim $1/[P] \rightarrow 0$ for the family with primed constants.

With no alkyl protection on the amino group, photochromism of the three dyes, AAB, ADAB, and PAN, in Table I was not observed. Although increasing the length of the alkyl chains has little effect, complete absence of them renders the amino dyes nonphotochromic on our time scale. In comparing sizes and positions of ring substituents, the major difference seems to lie in the methyl red pair, OMR and PMR. Here, the ortho and para carboxylate groups yield a difference of 800 between their k_{H^+}' values, whereas k_0', k_0, and k_{H^+} values are quite comparable in magnitude.

There are three other results of interest. (i) The protein dependent phenomena are by no means confined to serum albumin. β-Lactoglobulin and other proteins control azobenzenoid photochromic behavior. (ii) The phenomena can easily be observed up to 25–30°. Hence activation thermodynamics parameters can be determined. (iii) The charged dyes exhibit expected Coulombic effects, in comparing for example PMR with the neutral dye DMAA. These matters probably will be pursued further. As usual with azobenzene chromophores, the reaction is quite reversible and can be recycled many times. There is no evidence in these aqueous systems, that any of the various species react chemically to form new compounds. The dyes maintain their integrity.

Discussion

It is not obvious how H^+ catalyst is likely to enter the cis → trans isomerization process, partly because the mechanism(s) of this transformation in the noncatalyzed case is quite unclear. We tend to the view that hydrogen ion probably interacts with *n* electrons of the azo link, when the molecules are in the cis form. The p*K*'s of *N*,*N*-dialkylammonium groups in aminoazobenzenes are almost all between 2 and 5, in hydroxylic solvents, rather far from any apparent p*K* region we discern from our data. Admittedly, this p*K* pertains to the ground state of the trans form of the compound, but from consideration of the absorption spectra, and p*K* values for the corresponding monoaromatic amines, it is not likely that cis isomer dialkylamino p*K* values should be much higher than those for the trans forms.

In azobenzene itself, Lewis[11] has said that *cis*-azobenzene is clearly a stronger base than is the case for *trans*-azobenzene. Rau[12] in a recent review notes that the n → π* absorption intensity is greater for *cis*-azobenzene than for the trans isomer, and that the cis isomer has a high n → π* oscillator strength. These factors tend to assign H^+ interaction at the cis form's *n* electrons.

It is still unknown whether the main mechanism underlying geometry switching involves rotation around a nitrogen–nitrogen bond which has temporary single bond character, or a mechanism involving nitrogen rehybridization and a linear transition state. Some authors[13,14] consider that there is an intermediate state between the cis and trans form, which confers thermal sensitivity on the isomerization, at least in azobenzene. It is possible that H^+ has its effect on this state, perhaps tending to stabilize it.

The protein molecule imposes its own pH dependent features on the apparent pH dependent behavior of the dye isomerization. There may be two opposing forces in the dye–protein interaction, in the case of the charged dyes. The mild alkaline pH range of serum albumin is one in which there is a substantial and sometimes an increasing binding energy for nonpolar compounds,[15] usually with an association standard free energy from −3 to −6 kcal/mol. In the high alkaline range, pH >11, there occurs massive unfolding which often is reversed to some extent by nonpolar compound binding. As pH increases, the protein becomes increasingly negatively charged, which would tend to repel anionic dyes. In fact, we observe a minimum in our rate data, centered at pH about 11, with carboxylated dyes. As the pH increases beyond 11, cis → trans return rates increase, despite decreasing $[H^+]$, probably because of Coulombic interactions leading to decreased binding. This behavior is considerably less the case with the neutral dye, DMAA.

The reason for the fairly marked difference in K_1, upon simply changing the position of the carboxyl groups on the methyl red dyes, is quite unknown. There is an enormous number of ring positional isomer pairs of azobenzenes which may be obtained, and it will be useful to see if this is a general pattern, or whether the case here is rather seldom encountered. From the behavior of the azobenzene itself, and the k_0 and k_0' comparisons made above, it appears that the cis → trans geometry switching process for these monoazo dyes is roughly independent of the nature of the protein–dye interaction stereochemistry. Contrast between free and bound dyes appears mainly in the accessibility to H^+ acting as a catalyst. It was found

(11) G. E. Lewis, *J. Org. Chem.*, **25**, 2194 (1960).
(12) H. Rau, *Angew. Chem., Int. Ed. Engl.*, 12, 224 (1973).
(13) H. Stegemeyer, *J. Phys. Chem.*, **66**, 2555 (1962).
(14) G. Zimmerman, L. Y. Chow, and U. J. Paik, *J. Amer. Chem. Soc.*, **80**, 3528 (1958).
(15) J. Steinhardt and J. Reynolds, "Multiple Equilibria in Proteins," Academic Press, New York, N. Y., 1969, Chapter 4.

before[16] that monoazo dye molecules seem to be under rather little control by serum albumin, even when they are bound to it. It is as if the protein cannot really grip them strongly; but with larger substrates, long chain aliphatics and disazobenzene dyes, the interaction is strong enough to affect a number of events beyond the simple binding step.

Experimental Section

The full names of the dyes (abbreviated in Table I) and their source are as follows: *N,N*-dimethyl-*p*-phenylazoaniline (DMAA), *p*-(*p*-dimethylaminophenylazo)benzoic acid (PMR), *o*-(*p*-dimethylaminophenylazo)benzoic acid (OMR), *p*-(*p*-dimethylaminophenylazo)benzenesulfonate (MO), *p*-(*p*-dibutylaminophenylazo)benzenesulfonate (BO), 4-phenylazoaniline (AAB), *N,N*-dimethyl-4,4′-azodianiline (ADAB), and 4-phenylazo-1-naphthylamine (PAN) were all purchased from Eastman Co. as the sodium salts, and recrystallized from ethanol–water. The dyes ethyl orange and propyl orange {*p*-(*p*-diethylaminophenylazo)benzenesulfonate and *p*-(*p*-dipropylaminophenylazo)benzenesulfonate} were synthesized by coupling sulfanilic acid with the appropriate *N,N*-dialkylaniline, and chromatographing on silica gel to isolate the pure dyes.

The source and general techniques for handling the proteins were much the same as before,[16] in which we started by deionizing the bovine serum albumin (recrystallized fraction V). The photochromism investigations also used previous methods,[6] except that a Cary 14 was used, and the data were computer fitted. The lower (4°) temperatures were achieved by water jacketing all surfaces with which the absorption cells were in contact both during irradiation and spectrophotometry, plus impinging cold nitrogen across the cell faces in both stages. Temperatures were measured by immersing an Anschutz thermometer directly into the cell contents at the end of each run. The 4° pH measurements were calibrated by standard 0.05 *M* inorganic phosphate buffer (pH 6.98) and 0.05 *M* carbonate buffer (pH 10.22).

(16) R. Lovrien and T. Linn, *Biochemistry*, **6**, 2281 (1967).

Communications to the Editor

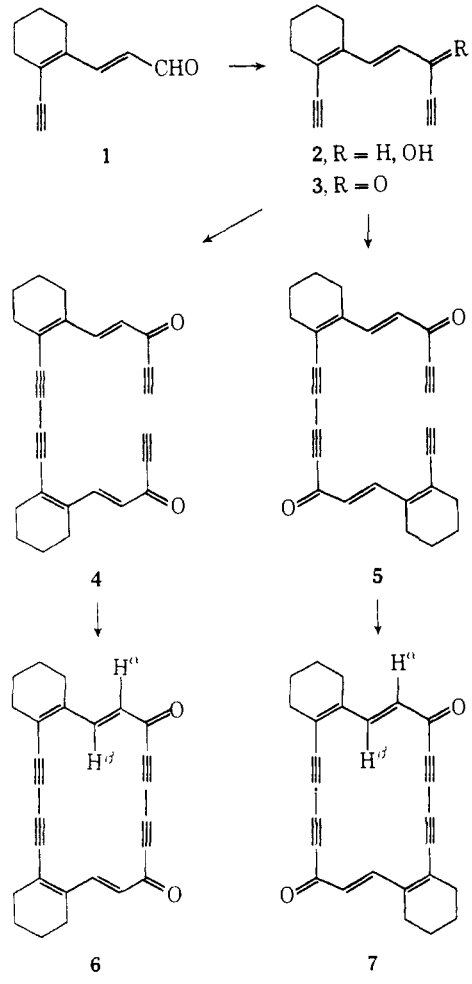
Synthesis of Alkylated Cyclooctadecatetraenetetrayne-1,6-diones and 1,10-diones (Tetradehydro[18]annulenediones)[1]

Sir:

We have recently described[2] a synthesis of the tetraalkylated tetradehydro[18]annulene-1,6-dione (**6**), but no determination was made as to whether it possessed quinonoid character. We have now synthesized the related tetraalkylated tetradehydro[18]annulene-1,10-dione (**7**), as well as the dialkylated tetradehydro[18]-annulene-1,6-dione (**11**) and -1,10-dione (**12**). The electrochemistry of these diketones has been investigated by Breslow, *et al.*,[3] as described in the following communication, and evidence for the quinonoid nature of **6**, **11**, and **12** has been obtained.

The aldehyde **1** has been prepared[4] in 45% yield by homologation of 2-ethynyl-1-cyclohexenecarboxaldehyde, and the yield has now been increased to 60–65% by careful attention to reaction conditions.[5] Treatment of **1** with 1.4 mol equiv of ethynylmagnesium bromide[6] in tetrahydrofuran for 16 hr at room temperature gave 85–90% of **2** (mp 62–63°),[7] which was oxidized to **3** (mp 59–60°)[7] in 80–85% yield with manganese dioxide[8] in ether for 30 min at room temperature.

Oxidative coupling of **3** with oxygen, cuprous chloride, ammonium chloride, and concentrated hydrochloric acid in aqueous ethanol and benzene ("Glaser conditions")[9] for 2 hr at 60–65°, followed by chromatography on silica gel (Woelm, activity III), led mainly to the diketones **4** (30% yield) and **5** (30% yield, yellow



(1) Unsaturated Macrocyclic Compounds. 102. For part 101, see P. J. Beeby and F. Sondheimer, *Angew. Chem.*, **85**, 406 (1973); *Angew. Chem., Int. Ed. Engl.*, 12, 411 (1973).

(2) K. Yamamoto and F. Sondheimer, *Angew. Chem.*, **85**, 41 (1973); *Angew. Chem., Int. Ed. Engl.*, **12**, 68 (1973).

(3) R. Breslow, D. Murayama, R. Drury, and F. Sondheimer, *J. Amer. Chem. Soc.*, **96**, 249 (1974).

(4) R. H. McGirk and F. Sondheimer, *Angew. Chem.*, **84**, 897 (1972); *Angew. Chem., Int. Ed. Engl.*, **11**, 834 (1972).

(5) R. H. McGirk, private communication.

(6) E. R. H. Jones, L. Skatteböl, and M. C. Whiting, *J. Chem. Soc.*, 4765 (1956); *Org. Syn.*, **39**, 56 (1959).

(7) Uv, ir, and ^{1}H nmr spectra compatible with the assigned structures were obtained for all new compounds.

(8) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

(9) See G. Eglinton and W. McCrae, *Advan. Org. Chem.*, **4**, 225 (1963).