taining concentrations of reactants comparable to those used to estimate the yield of the reaction spectrophotometrically, were prepared and allowed to proceed to completion. The product was isolated by removing the acetonitrile, dissolving the residue in chloroform, and extracting with an aqueous zinc chloride solution. After enough EDTA was added to the aqueous phase to chelate all the zinc ion present, the product was re-extracted into chloroform. The chloroform solution was then dried over sodium sulfate and evaporated to dryness. The residue yielded ultraviolet and infrared spectra that were very similar to those of an authentic sample of 1,10-phenanthroline-2-carbinol. When part of the residue was dissolved in an ethanolic solution of dimedone and chromatographed on Whatman No. 3M paper using isopropyl alcohol–water–trichloroacetic acid–ammonia (75 ml:25 ml:5 g:0.2 ml) as the solvent system, a spot indistinguishable from an authentic sample of 1,10-phenanthroline-2-carbinol was obtained.

The mass spectra of the residue and that of independently synthesized 1,10-phenanthroline-2-carbinol (II) were very similar and consistent with the calculated composition $C_{13}H_{10}N_2O$ for II. Each spectrum possessed the intense and characteristic molecular ion peak at m/e 210 as well as an isotope peak at m/e 211 that was 14.5% as intense as the molecular ion peak.[19]

As further proof that II was the product and in order to investigate if the reaction proceeded by direct hydrogen transfer, 1-propyl-4,4-dideuterionicotinamide (86% isotopically pure by nuclear magnetic resonance) was used as the reductant in place of the corresponding dihydronicotinamide. The product was then isolated as indicated above. An intense molecular ion peak at m/e 211, corresponding to monodeuterated 1,10-phenanthroline-2-carbinol ($C_{13}H_9DN_2O$) and accounting for 70% of the product, was observed when the mass spectrum of the reaction product was obtained at the approximate appearance potential of the P − 1 (m/e 209) peak of undeuterated 1,10-phenanthroline-2-carbinol. These results provide further evidence that II is the primary product of the reduction. They also demonstrate that II is formed by direct hydrogen transfer from III to the carbonyl carbon of Zn^{2+}–I. Even though direct hydrogen transfer is very likely in the absence of any readily exchangeable hydrogens in anhydrous acetonitrile, its demonstration is essential if this this system is to be considered an appropriate model for the alcohol dehydrogenase reaction.[20,21]

In summary, the zinc ion catalysis of the reaction between 1,10-phenanthroline-2-carboxaldehyde and N-propyl-1,4-dihydronicotinamide strongly suggests that either coordination or, at the least, proximity to a metal ion is a feasible and efficient method for activation of a carbonyl group for reduction. The catalytic efficiency of the zinc ion must be significant since (a) no reaction could be detected in the absence of the metal ion and (b) 1,10-phenanthroline-2-carboxaldehyde is the first aldehyde to be reduced by a dihydronicotinamide in a nonenzymic system. Although the present experiments cannot be considered proof that zinc ion serves a similar

catalytic function in alcohol dehydrogenase, they certainly strengthen the view that coordination or proximity of the carbonyl to the zinc ion could be a very important feature of the enzymic catalysis.

Donald J. Creighton,[22] David S. Sigman*
*Department of Biological Chemistry*
University of California at Los Angeles, School of Medicine
Los Angeles, California 90024

Intermediacy of a 1,2-Benzotropilidene in the Photochemical Rearrangement of a Benzonorcaradiene to a Benzobicyclo[3.2.0]hepta-2,6-diene

Sir:

The photochemical rearrangement of the benzonorcaradiene moiety 1 to the benzobicyclo[3.2.0]hepta-2,6-diene skeleton 2 has been noted by several groups.[1]
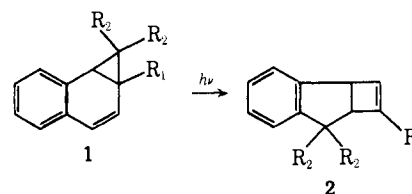
a, $R_1 = R_2 = H$
b, $R_1 = CO_2CH_3; R_2 = H$
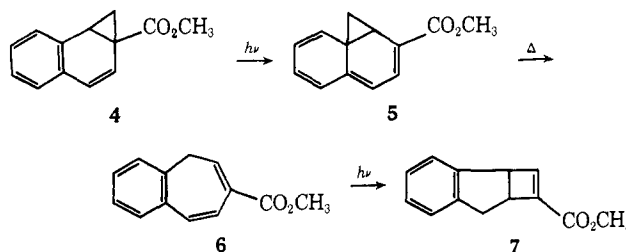
One mechanism for this conversion involves the intermediacy of 3 which undergoes thermal rearrangement to 2 (Scheme I). A second possible mechanism for

Scheme I. The One-Photon Route for the Benzonorcaradiene to Benzobicyclo[3.2.0]hepta-2,6-diene Conversion
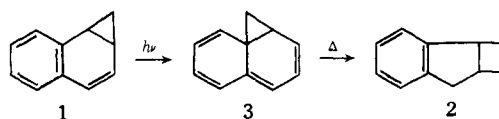
this process is a two-photon sequence wherein diene 6 is a direct precursor of 7 (Scheme II).[1d] While

Scheme II. The Two-Photon Route for the Benzonorcaradiene to Benzobicyclo[3.2.0]hepta-2,6-diene Conversion

(19) R. M. Silverstein and G. Clayton Bassler, "Spectrophotometric Identification of Organic Compounds," Wiley, New York, N. Y., 1968.
(20) F. H. Westheimer, H. F. Fisher, E. E. Conn, and B. Vennesland, *J. Amer. Chem. Soc.*, 73, 2403 (1951).
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published work without quantum efficiency data supported the one-photon process for the unsubstituted compound,[1c] we felt that this might not be the most efficient or common reaction scheme for the general case. We report here the wavelength dependence of the photolysis of 4 and suggest that these results support the two-photon route and the intermediacy of 6 in the 4 → 7 conversion.

Our first objective was to study quantitatively the photochemistry of 4 and 6. Irradiation of 4 at 300 nm[2] led solely to the formation of cyclobutene 7 in 90% yield. Quantum yields[3] for the disappearance of 4 and appearance of 7 are recorded in Table I.[4] While the

Table I. Quantum Yields for Photolysis of 4 at 300 nm

Concn of 4, M	% conversion	Quantum yields[a] 7	4
10^{-3}	4.8	0.17	0.15
10^{-3}	5.3	0.17	0.16
10^{-3}	8.9	0.18	

[a] Reliability of these values is judged to be ±10%.

quantum yields for the disappearance of 4 and appearance of 7 were of moderate efficiency, they do not exclude the possibility that these results arise from two efficient photochemical steps.[5] Since a low quantum efficiency for the closure of diene 6 to cyclobutene 7 could immediately exclude the two-photon route, the photochemistry of 6 and its quantum efficiency were studied. Preparative photolysis of 6 afforded 7 (87%) and 4
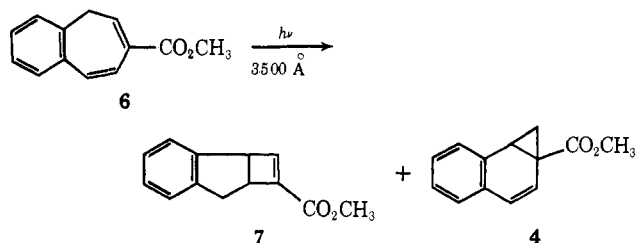
(13%) in nearly quantitative yield.[6] The quantum yields for disappearance of 6 and appearance of 4 and 7 at 330 nm are recorded in Table II.

Since the high efficiency (0.61) of the electrocyclic ring closure of 6 to 7 did not exclude the two-photon route on the basis of an inefficient second step, we sought to establish conditions under which 6 might be detected in the 4 photolysis. The high efficiency of photocyclization coupled with the stronger absorption of diene 6 relative to 4 in the 300–330-nm region suggested that

(2) Preparative photolyses were performed in cyclohexane over purified nitrogen with a Rayonet PRP 3000- or 3500-Å source.

(3) (a) Quantum yields were measured using a Bausch and Lomb high-intensity monochrometer and potassium ferrioxalate actinometry. (b) For a description of the apparatus, see J. S. Swenton, J. A. Hyatt, T. J. Walker, and A. L. Crumrine, *J. Amer. Chem. Soc.*, 93, 4808 (1971).

(4) In the quantum yield determinations, small amounts of a material identical in vpc retention time with the diene 6 were observed.

(5) The maximum overall quantum efficiency for a two-photon process is 0.5 (both steps having unit efficiency).

(6) (a) These results are in marked contrast with those noted in the parent 1,2-benzotropilidene, which yields 1a as the major product with only small amounts (<1%) of the cyclobutene 2a.[1c] The different amounts of hydrogen-shift vs. electrocyclic product in the parent 1,2-benzotropilidene relative to the carbomethoxy derivative 6 may be due to a substituent effect.[6b] (b) For a study of the influence of substituents on 1-substituted cycloheptatriene photochemistry, see A. P. ter Borg, E. Razenberg, and H. Kloosterziel, *Chem. Commun.*, 1210 (1967).

Table II. Quantum Yields of 6 Photolysis at 330 nm

Concn of 6, M	% conversion	Quantum yields 6[a]	7[b]	4[b]
1.8×10^{-2}	10.0		0.62	0.090
1.8×10^{-2}	14.0	0.60	0.57	0.083
1.8×10^{-2}	14.0	0.62		0.093
1.8×10^{-2}	17.0	0.65	0.64	0.085
	Avg.	0.62	0.61	0.088

[a] At these low conversions, the calculations of the quantum yields for disappearance involve a difference between two large numbers. Thus we believe these numbers to be the least accurate. [b] Reliability of these values is judged to be ±10%.

further attempts to unambiguously identify 6 as the intermediate in the 4 photolysis in this irradiation region would be quite difficult. Thus, we sought an irradiation wavelength where the absorption of 4 was maximized with respect to 6. From comparison of the uv spectrum of 4 with that of 6, this condition was met at 270 nm.[7] Interestingly, photolysis of 4 at 270 nm produced only traces of cyclobutene 7 at low conversion, the major product being the diene 6. Furthermore, the quantum yield for disappearance of 4 increased from its value of 0.17 at 300 nm to 0.47 at 270 nm[8,9] (see Table III).

Table III. Quantum Yields for Photolysis of 2 at 2675 Å

Concn of 4, M	% conversion	Quantum yields 4	6	7
1.7×10^{-2}	8	0.45	0.35	~0.03
1.2×10^{-2}	10	0.47	0.37	~0.03
1.2×10^{-2}	13	0.47	0.33	~0.03

These results strongly suggest that the major pathway from 4 to 7 in our system is the two-photon route involving the intermediacy of 6.[9] The inability to observe significant quantities of 6 from irradiation of 4 in the 300-nm region is due to the strong relative uv absorption and high efficiency of cyclization of 6.[10] The different mechanisms for the photolyses of 4 and of the unsubstituted benzonorcaradiene, 1a, are of some interest. An important factor in this anomaly appears to be the effect of the carbomethoxy substituent, not on the benzonorcaradiene photochemistry, but on the photolysis of the 1,2-benzotropilidene system. For the unsubstituted 1,2-benzotropilidene, the major photochemical process is hydrogen shift to yield benzonorcaradiene, 1a.[11] This would serve as a means of converting any 1,2-benzotropilidene formed in the photolysis of 1a, back to 1a. In contrast, the major photochemical process of 5-carbomethoxy-1,2-benzotropilidene, 6, is ring closure to the

(7) The ratio of extinction coefficients of 4:6 at 300 nm is 0.16, while at 270 nm this ratio has its maximum value of 1.5.

(8) The poorer agreement between the quantum yields for disappearance of 4 and appearance of 6 at the shorter wavelength is unexplained.

(9) Using the efficiencies of the 6 → 7 and the 4 → 7 transformations (0.61 and 0.17), and assuming that the two-photon route is the exclusive reaction path, the calculated quantum efficiency of the 4 → 6 process would be 0.55.

(10) A dramatic wavelength effect on the basic photochemistry of 4 could also account for our results; i.e., the one-photon route is followed at 300 nm, the two-photon route at 270 nm. However, we feel that with a difference of only 30 nm in excitation wavelength this is an unlikely possibility.

(11) M. Pomerantz and G. W. Gruber, *J. Amer. Chem. Soc.*, 89, 6799 (1967).

cyclobutene **4**. We are presently exploring the nature and generality of substituent effects in these systems.

Denise M. Madigan, John S. Swenton[*12]

Department of Chemistry, The Ohio State University
Columbus, Ohio 43210

1-Hydroxybenzotriazole as a Racemization-Suppressing Reagent for the Incorporation of *im*-Benzyl-L-histidine into Peptides

Sir:

Racemization during the synthesis of polypeptides can be a serious problem because a high degree of steric homogeneity is usually necessary for biological or physical studies. The detection of small amounts of diastereoisomeric contaminants and their removal from synthetic polypeptide preparations is often difficult, so synthetic procedures which minimize the risk of racemization are commonly used. The most widely used procedure, particularly for the synthesis of relatively short polypeptide chains, involves the stepwise synthesis from the carboxyl end using urethane-protected amino acids.[1] The popularity of this method rests largely on the belief that racemization does not occur. Recently, however, it was shown that considerable racemization of *tert*-butyloxycarbonyl-*im*-benzyl-L-histidine [Boc-His-(Bzl)] had occurred under the somewhat more demanding conditions of solid-phase peptide synthesis.[2,3] In this case, removal of the contaminating isomers was not possible. Subsequent studies utilizing a model system based upon the separation and quantitation of diastereoisomers of *im*-benzylhistidylglutamic acid [His(Bzl)-Glu] on an amino acid analyzer demonstrated that Boc-His(Bzl) racemizes under a variety of conditions, including standard solution reactions.[4] It was found that the presence of 1 equiv of N-hydroxysuccinimide virtually eliminated racemization in the dicyclohexylcarbodiimide-mediated coupling of Boc-His(Bzl) with Glu-(OBzl)-O-polymer. However, in agreement with the findings of Kaiser, *et al.*,[5] the yield in this reaction was unacceptably low (70%). The presence of 30% β-alanine in the peptide hydrolysates suggests that the low yield was due to competitive acylation of the amino function by succinimidoxycarbonyl-β-alanine-N-hydroxysuccinimide ester, a known product of the reaction between dicyclohexylcarbodiimide and N-hydroxysuccinimide.[6,7]

Recently, König and Geiger[8] showed that 1-hydroxybenzotriazole was as effective as N-hydroxysuccinimide in suppressing racemization in dicyclohexylcarbodi-

imide-mediated couplings where oxazolone formation is possible. Since a side reaction comparable to the one between dicyclohexylcarbodiimide and N-hydroxysuccinimide seemed unlikely for 1-hydroxybenzotriazole, it seemed worthwhile to investigate the utility of this reagent in the synthesis of His(Bzl) peptides. Boc-His-(Bzl), 34.5 mg (0.1 mmol), Glu(OBzl)-OBzl·Tos, 50 mg (0.1 mmol), Bu_3N, 0.024 ml (0.1 mmol), and 1-hydroxybenzotriazole, 13.5 mg (0.1 mmol), were dissolved in 1 ml of CH_2Cl_2.[9] To this solution was added dicyclohexylcarbodiimide, 20.5 mg (0.1 mmol); the solution was kept at 23° overnight. The precipitated dicyclohexylurea was removed by filtration and the filtrate evaporated at 23° under vacuum. The residue was dissolved in 5 ml of CF_3COOH, and HBr was bubbled through the solution for 2 hr. The solution was then evaporated under vacuum at 23° and the residue was dissolved in 30 ml of 0.2 N sodium citrate, pH 2.2. A 0.5-ml aliquot was chromatographed on a 0.9 × 18 cm column of Beckman PA-35 resin using 0.35 N sodium citrate, pH 5.26, for elution at 70 ml/hr. Under these conditions D-His(Bzl)-L-Glu elutes in 44 min and L-His(Bzl)-L-Glu in 55 min. Less than 0.1%[10] D-His-(Bzl)-L-Glu was found in this case, compared with 1.8% when 1-hydroxybenzotriazole was omitted.

For the solid-phase test, 330 mg (0.04 mmol) of Glu(OBzl)-O-polymer was agitated gently with a solution of 55 mg (0.16 mmol) of Boc-His(Bzl) and 22 mg (0.16 mmol) of 1-hydroxybenzotriazole in 1.5 ml of CH_2Cl_2. After 10 min, 33 mg (0.16 mmol) of dicyclohexylcarbodiimide was added, and agitation continued overnight. The polymer was collected on a sintered filter, washed with CH_2Cl_2 (three 3-ml portions), 50% CF_3COOH in CH_2Cl_2 (three 3-ml portions), and CF_3COOH (3 ml), then suspended in 5 ml of CF_3COOH, and HBr was bubbled through the suspension for 2 hr. After filtration and evaporation, the crude reaction product was submitted to chromatographic analysis as before. Less than 0.3% D-His(Bzl)-L-Glu was found, compared with 11% in the absence of 1-hydroxybenzotriazole.

In order to evaluate the yield achievable during solid-phase synthesis, Pro-Phe-O-polymer was acylated with Boc-His(Bzl), 1-hydroxybenzotriazole, and dicyclohexylcarbodiimide as described above. A portion of the crude product obtained by HBr cleavage was hydrolyzed in constant-boiling HCl at 110° under N_2 for 24 hr. An aliquot of the hydrolysate subjected to amino acid analysis had the expected amino acids in equimolar amounts: His(Bzl), 1.03;[11] Pro, 0.98; Phe, 1.00. An L-amino acid oxidase digest[2] of the hydrolysate had His(Bzl), 0.00; Pro, 1.00; Phe, 0.00 (after correcting for the amount of racemization occurring during acid hydrolysis), providing independent proof that racemization had been negligible. The equality of Pro and Phe in the acid hydrolysate shows that no side reactions had occurred which could have resulted in irreversible modification of Pro, thus validating the use of amino acid ratios to evaluate cou-

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(8) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

(9) Neither compound is soluble in CH_2Cl_2 at this concentration; however, the solubility of each is enhanced by the other, and complete solubilization is achieved.

(10) The sensitivity limit of the test.

(11) His(Bzl) was determined as described in J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969, p 54.