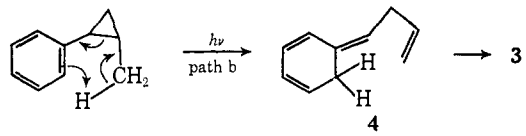
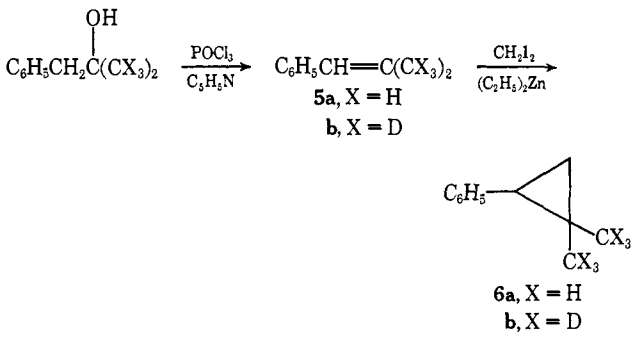
case is of interest since the initial step would represent a novel photochemical 1,5-homocyclic hydrogen migra-



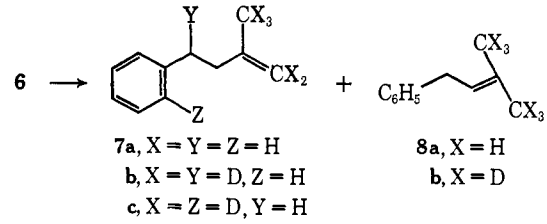
tion[3] whereas the former, if concerted, would be an example of a $\sigma^2 + \sigma^2$ photochemical cycloaddition.[4]

We wish to report the results of a tracer experiment which clearly excludes one of these mechanistic possibilities. Our study is based on the fact that the methylenecyclohexadiene moiety in the hypothetical intermediate **4** has a symmetry plane such that the hydrogen which has migrated from the methyl group and the original aromatic hydrogen become indistinguishable. Subsequent migration of one of the methylene hydrogens to afford **3** should take place regardlesss of the origin of the migrating hydrogen. Since path a must result in the transfer of a methyl hydrogen to the benzylic position, a suitably labeled starting material can provide mechanistic information. The compound chosen for this study was 2,2-dimethylphenylcyclopropane (**6a**) and the synthesis of the required labeled material **6b** is outlined below.



Phosphorus oxychloride–pyridine dehydration of the alcohol obtained from benzylmagnesium chloride and acetone-d_6 afforded a 50% yield of β,β-dimethyl-d_6-styrene (**5b**) which could be easily separated from the accompanying nonconjugated olefin by preparative glpc. Although normal Simmons–Smith conditions are ineffective in this case, treatment of **5b** with diiodomethane in the presence of diethylzinc[5] gave **6b** in 75% yield. The infrared spectrum of **6b** showed pertinent maxima at 2070 and 2205 cm^{-1}. The nmr spectrum consisted solely of a signal ascribed to aromatic protons at τ 2.85 and two multiplets at τ 8.17 and 9.29 due to benzylic and nonbenzylic cyclopropyl protons in the ratio 5:1:2. The methyl resonances at τ 8.80 and 9.22 present in the nmr spectrum of **6a** were absent. The mass spectrum of **6b** showed its parent peak at *m/e* 152.[6]

Irradiation of a cyclohexane solution of **6a** proceeded to give the expected 2-methyl-4-phenyl-1-butene (**7a**)[2] in addition to 2-methyl-4-phenyl-2-butene (**8a**), a previously unreported product.[7,8] In a similar manner irradiation of **6b** afforded the major product **7** whose infrared spectrum showed pertinent maxima at 2300, 2225, 2190, and 2050 cm^{-1}. The mass spectrum had its parent peak at *m/e* 152.[6] The nmr spectrum was most informative



and showed resonances at τ 2.89 (aromatic protons), 7.33 (benzylic proton), and 7.73 (allylic protons) in the ratio 5:1:2. These data are consistent only with compound **7b**. Confirmatory evidence was obtained by alkaline permanganate oxidation of photoproduct **7b**. Mass spectral analysis of the methyl benzoate obtained on diazomethane work-up of the acid indicated that this material was >95% d_0. Since path b would lead to a ratio of **7c**/**7b** of >1 (assuming a primary kinetic isotope effect) whereas path a would result only in the formation of **7b** these experiments clearly establish the course of this reaction as one defined by following path a.[9] A more detailed description of the mechanism of these reactions must await the results of further experiments.

Acknowledgment. We are grateful to the Frederick Gardner Cottrell Fund of the Research Corporation for the partial support of this work.

(3) The thermal analog of this reaction is well known in 2-methylvinylcyclopropanes: H. M. Frey and R. Walsh, *Chem. Rev.*, **69**, 103 (1969).

(4) For examples see: R. Hoffmann, Abstracts of the 21st Organic Chemistry Symposium of the American Chemical Society, Salt Lake City, Utah, June 1969, p 111.

(5) J. Furukawa, N. Kawabata, and J. Nishimura, *Tetrahedron*, **24**, 53 (1968). An adaptation of this method, using diethylzinc generated *in situ*, was employed.

(6) A low-voltage mass spectrum of this material indicated that it was >95% d_6.

(7) The structures of **8a** and **8b** were established by comparison of their nmr and infrared spectra with those of an authentic sample.

(8) The formation of **8b** on irradiation of **6b** indicates that **8** is a primary photoproduct resulting from 1,2-hydrogen migration and that **8** does not result from isomerization of **7**. Since the formation of **8a** and **8b** both take place without the migration of deuterium they may be used as internal standards to calculate a primary kinetic isotope effect for the formation of **7** (*i.e.*, in the initial stages of the reaction k_H/k_D = **7a**/**8a**/**7b**/**8b**). This result has been confirmed by the simultaneous irradiation of 4.9×10^{-3} *M* solutions of **6a** and **6b** in a "merry-go-round" apparatus. Analysis by glpc for **7a** and **7b** at low conversion gives a value of $k_H/k_D = 2.6 \pm 0.3$ for the reaction **6** → **7**.

(9) G. W. Griffin and E. Waldau have come to the same conclusion on the basis of experiments which indicate that *trans,trans*-2,3-dimethylphenylcyclopropane photoisomerizes to terminal olefin faster than *cis,cis*-2,3-dimethylphenylcyclopropane does (G. W. Griffin and E. Waldau, personal communication).

(10) National Science Foundation Undergraduate Research Participant.

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Isolation and Characterization of an Active-Site Peptide from Triose Phosphate Isomerase[1]

Sir:

I have shown that 1-hydroxy-3-iodo-2-propanone phosphate (iodoacetol phosphate, IAP), a reagent structurally similar to the substrate dihydroxyacetone phosphate (DHAP), reacts specifically with a single, essential residue of each catalytic subunit of rabbit-muscle triose phosphate isomerase (TPI); this reaction

(1) This research was sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

results in total loss of enzymic activity.[2] The corresponding chloro (CAP) and bromo (BAP) reagents also react specifically with the active site of TPI and presumably with the same essential residue as does IAP, as judged from autoradiograms of peptide maps.[3] Rose and O'Connell[4] have cited work in press by Coulson and Knowles,[5] who are using BAP to label the active site of TPI. In this communication I report the isolation and amino acid composition of a peptide, containing the incorporated reagent, obtained from a tryptic digest of CAP-modified TPI; and I identify the essential, modified residue as an ester of glutamic acid. Rose and O'Connell[4] reported that glycidol phosphate reacts specifically with an active-site residue of TPI, and they tentatively identified the derivative as an ester on the basis of its lability toward base and hydroxylamine.

The enzyme inactivated with CAP instead of IAP was used in these studies because IAP can oxidize protein sulfhydryl groups, a potentially complicating side reaction.[6]

TPI (150 mg, 5 μmol of catalytic subunit assuming two subunits per molecule with a molecular weight of 60,000[7]) in 10 ml of 0.1 *M* $NaHCO_3$–1 mM EDTA, pH 8.0, was treated with 0.5 ml of 0.04 *M* CAP[8] (20 μmol). After 5 min, inactivation was completed, and the reaction mixture was made 0.01 *M* in β-mercaptoethanol to react with the excess CAP. After cooling the solution to 4°, the carbonyl group of the incorporated reagent was reduced to a hydroxyl group by a 30-min treatment with NaB^3H_4 (4 mg, 0.3 mCi/μmole). In addition to providing a radioactive label, the reduction should stabilize the phosphate moiety and perhaps stabilize the linkage between the protein and reagent. Subsequent to borohydride reduction, the modified TPI was treated with iodoacetic acid in 4 *M* guanidine hydrochloride to carboxymethylate protein SH groups and then dialyzed against 0.1 *M* $(NH_4)_2CO_3$, pH 8.0. The dialyzed solution (34 ml) contained 143 mg of protein having a specific radioactivity of 45.6×10^6 cpm/μmol (30 mg). Native TPI treated with NaB^3H_4 under the same conditions contained only 60×10^3 cpm/μmol.

CAP-modified TPI (the above dialyzed solution) was digested with trypsin (1.5 mg) at 40° for 1.5 hr and then lyophilized to dryness. Purification of the labeled peptide was achieved by a combination of ion-exchange chromatography and gel filtration. A portion of the digest (4.33 μmol, 195×10^6 cpm) was chromatographed on a 2.5×15 cm column of Bio-Rad Aminex AG50W-X2 resin, equilibrated and eluted with 0.25 *M* pyridine–3.8 *M* acetic acid, pH 3.2. The major radioactive fraction (165×10^6 cpm, 84%) eluted at 256–304 ml, and after lyophilization it was passed through a 1.5×230 cm column of Bio-Gel P-4 equilibrated with 0.01 *M* ammonium acetate. Most of the radioactivity (158×10^6 cpm, 3.7 μmol) emerged coincident with a ninhydrin-positive component at 160–200 ml. This peptide appeared homogeneous by paper electrophoresis and chromatography.

The amino acid composition of the purified active-site peptide, deduced from analyses of acid hydrolysates on a Beckman amino acid analyzer, is Trp_2, Lys, Thr, Glu, Pro, Gly_2, Ala_2, Val_2, Ile, Leu, Tyr. (Trp was determined spectrophotometrically.[9]) Agreement in the quantity of peptide analyzed, calculated from the specific radioactivity of CAP-modified TPI before tryptic digestion (0.042 μmol) and from the molar amounts (0.042–0.046) of amino acids in the hydrolysates, substantiates the stated specificity of CAP for a single amino acid of TPI.

The glycerol phosphate moiety was liberated from the isolated peptide by acid, base, and prolonged digestion with trypsin, chymotrypsin, or pronase. Since this lability suggested an ester linkage, the peptide was assayed for ester with hydroxylamine according to Hestrin.[10] A positive test was obtained, and the quantitative results are given in Table I. Electrophoresis showed that the label had been removed. Since glutamine reacted only slightly, hydroxamate was probably formed from a glutamate ester. The low yield (48%) of hydroxamate formation may be due to partial hydrolysis of the ester, caused by the alkaline hydroxylamine used. Neutral hydroxylamine did not liberate the incorporated reagent, and thus necessitated the use of alkaline hydroxylamine.

Table I. Ester Content of Active-Site Peptide as Determined with Hydroxylamine–Ferric Chloride[a]

Sample	Quantity assayed, μmol	Hydroxamate— OD 540 nm	Hydroxamate— μmol
Acetylhydroxamate	0.05	0.31	0.05
	0.10	0.60	0.10
Glycine methyl ester	0.05	0.29	0.048
	0.10	0.58	0.096
Glutamine	0.05	0.015	0.0025
	0.10	0.035	0.0060
Active-site peptide	0.05	0.14	0.023
	0.10	0.28	0.046

[a] The assay was performed as described by Hestrin[10] but was scaled down tenfold.

To verify that the hydroxamate was formed from a glutamate ester, a sample of the hydroxamate derivative of the peptide was subjected to dinitrophenylation followed by base as described by Gallop, *et al.*,[11] to effect Lossen rearrangement. This process converts hydroxamates to amines; 2,4-diaminobutyric acid would therefore result from a glutamate ester. An acid-hydrolyzed sample of the peptide, treated as described, contained only 0.59 mol equiv of glutamic acid and 0.40 mol equiv of a compound which eluted from the short column of the analyzer between lysine and histidine, the same position at which authentic 2,4-diaminobutyric acid eluted. I conclude that CAP esterifies an essential glutamic acid residue at the active site of TPI.

The TPI-catalyzed isomerization of triose phosphates proceeds through a *cis*-enediol intermediate.[12,13] Presumably a basic group at the active site labilizes an α-hydrogen atom of the substrate and promotes intra-

(2) F. C. Hartman, *Biochem. Biophys. Res. Commun.*, **33**, 888 (1968).
(3) F. C. Hartman, *Fed. Proc.*, in press.
(4) I. A. Rose and E. L. O'Connell, *J. Biol. Chem.*, **244**, 6548 (1969).
(5) A. F. W. Coulson and J. R. Knowles, *Chem. Commun.*, in press.
(6) F. C. Hartman, *Biochemistry*, **9**, 1783 (1970).
(7) L. N. Johnson and S. G. Waley, *J. Mol. Biol.*, **29**, 321 (1967).
(8) CAP was synthesized as described by F. C. Hartman, *Biochemistry*, **9**, 1776 (1970).
(9) H. Edelhoch, *ibid.*, **6**, 1948 (1967).
(10) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(11) P. M. Gallop, S. Seifter, M. Lukin, and E. Mielman, *ibid.*, **235**, 2619 (1960).
(12) I. A. Rose, *Brookhaven Symp. Biol.*, **15**, 293 (1962).
(13) I. A. Rose, E. L. O'Connell, and R. P. Mortlock, *Biochim. Biophys. Acta*, **178**, 376 (1969).

molecular proton transfer. Whether a glutamate anion serves this role remains to be determined; however, carboxylate ions can promote enolizations *via* general base catalysis.[14] From the dependence of V_{max} on pH, Rose[12] has calculated that the basic group has a pK of 6.5; and, from chemical studies, Burton and Waley[15] have suggested that the group is histidine.

(14) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969, Chapter 3.
(15) P. M. Burton and S. G. Waley, *Biochem. J.*, 100, 702 (1966).

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1-Phenyl- and 1,3-Diphenyl-2-indanones from the Reaction of α-Halo Ketones and Sodium Methoxide in Methanol

Sir:

The isomeric diphenyl-α-halopropanones $Ph_2CXCOCH_3$ (**1**) and $Ph_2CHCOCH_2X$ (**2**) react with 0.05 *M* sodium methoxide in methanol to give essentially quantitative yields of Favorskii ester $Ph_2CHCH_2CO_2Me$ (**3**). Slow addition of 0.05 *M* NaOMe–MeOH to a methanol solution of **1** gave, however, only 48% **3**; 16% 1-methoxy-1,1-diphenylpropanone (**4**) and 19% methyl *o*-benzoylphenylacetate (**5**) were then formed.

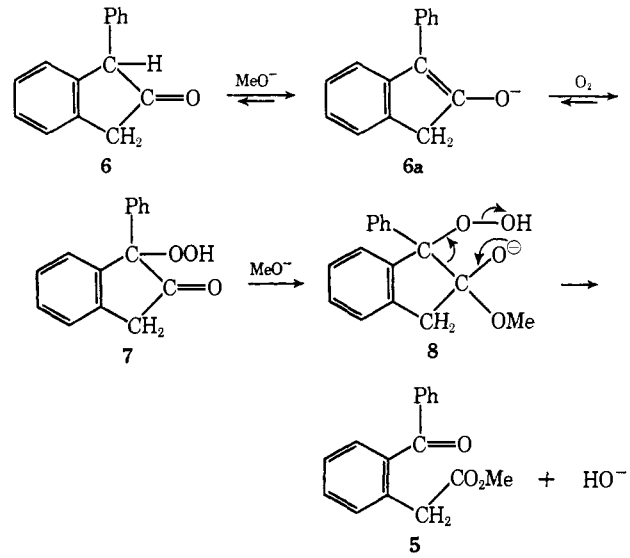
Methanolysis of **1** under these conditions is too slow to account for the formation of more than about one-fourth of the amount of **4** obtained. Methanolysis of an intermediate enol allylic chloride[1] is suggested as the major source of **4**.

When the inverse addition experiment with **1** was carried out under nitrogen, 1-phenyl-2-indanone (**6**) was isolated in place of **5**. Methoxide ion converted **6** ($pK_a \cong 15$ in methanol) almost completely to its enolate ion **6a** (λ_{max} 300 nm) which was found to react at a moderate rate with oxygen to form **5**. The rate of this reaction was followed spectrophotometrically by observing the disappearance of **6a** and also by observing the appearance of **5**. A (pseudo) first-order transformation of **6** to **5** was observed. The mechanistic sequence of Scheme I is suggested, based on this kinetic result and analogy with the mechanistic evidence available from studies of the oxidative cleavage of acyclic ketones.[2]

The novel part of this mechanistic scheme is cleavage of the α C–C bond in the keto hydroperoxide **7** by addition of methoxide ion to the carbonyl group and fragmentation of the resulting adduct (**8**). (The C–C and O–O bonds need not be broken simultaneously in the fragmentation reaction.) This mode of cleavage represents an alternative to that suggested previously for such compounds, namely, the decomposition of a tautomeric form of the α-keto hydroperoxide (a four-membered-ring α-hydroxy peroxide).[3]

(1) F. G. Bordwell, A. C. Knipe, and M. W. Carlson, *J. Amer. Chem. Soc.*, 91, 3949 (1969); F. G. Bordwell and M. W. Carlson, *ibid.*, 91, 3951 (1969).
(2) G. A. Russell, E. G. Janzen, A. G. Bemis, E. J. Geels, A. J. Moye, S. Mak, and E. T. Strom, Advances in Chemistry Series, No. 51, American Chemical Society, Washington, D. C., 1965, pp 112–171.
(3) W. E. Doering and R. M. Haines, *J. Amer. Chem. Soc.*, 76, 482 (1954).

Scheme I



Reaction of **2** (X = Cl) under inverse addition gave **3** and **5**, but no **4**. The ratio of **3** to **5** was identical, within experimental error, with that from **1** (X = Cl). An identical **3**:**5** ratio was also obtained from **2** (X = Br). This is strong evidence for a common intermediate from **1** and **2** which gives rise to both **3** and **5**, since it would be highly unlikely that **3** and **5** would be formed in an identical ratio from all three substrates if they were being formed from each substrate by separate, individual reactions.

Judging from previous work with $PhCHXCOCH_3$ and $PhCH_2COCH_2X$ systems, which has shown that halide ion is lost from a carbanion (enolate ion) intermediate in a rapid step having a high degree of ionic character in the transition state,[4] formation of dipolar ion intermediate **9** from **1** or **2** is reasonable. The common intermediate referred to above could then be an equilibrium mixture of **9** and the corresponding cyclopropanone (**10**).[5–7] Indanone **6** can be visualized as coming from **9** and ester **3** can be visualized as coming from **10**.

Formulation of proton removal as the slow step in the reaction of **1** or **2** with methoxide ion (as shown) is supported by the observation of a relatively small $k_{Br}:k_{Cl}$ leaving group effect in each instance (3.4:1.0 for **1** and 4.2:1.0 for **2**). (Leaving group effects of the order of *ca.* 100:1 are expected when complete preequilibrium is established.[4]) With 0.05 *M* sodium methoxide the conversion of **10** (or its hemiketal) to **3** is the predominant reaction, but at low methoxide ion concentrations this second-order reaction suffers competition from the first-order transformation of **9** to **5**. At high methoxide ion concentrations only **3** is obtained.

Reaction of $Ph_2CXCOCH_2Ph$ or its isomer $Ph_2CHCOCHXPh$ in a nitrogen atmosphere with either

(4) See F. G. Bordwell, R. G. Scamehorn, and W. R. Springer, *ibid.*, 91, 2087 (1969), and references cited therein.
(5) For calculations of the relative energies of cyclopropanones and the corresponding dipolar ions see J. G. Burr, Jr., and M. J. S. Dewar, *J. Chem. Soc.*, 1201 (1954); R. Hoffmann, *J. Amer. Chem. Soc.*, 90, 1475 (1968).
(6) Cycloaddition reactions of cyclopropanones appear to be best explained by assuming reaction *via* a dipolar intermediate [N. J. Turro, S. S. Edelson, J. R. Williams, T. R. Darling, and W. B. Hammond, *ibid.*, 91, 2283 (1969)].
(7) N. J. Turro and W. B. Hammond, *Tetrahedron*, 24, 6017 (1968), have shown that cyclopropanones exist in methanol solution largely in the form of their methyl hemiketals.