and regained by reversibly denaturing the protein in 8 *M* urea at pH 3.0.

The reversible heat and urea denaturation of ChT–NAP shows the new absorption band is dependent on the conformation of the protein. Since charge-transfer interactions require the close proximity of the donor and acceptor moieties, a charge-transfer interaction in a protein should be destroyed when gross conformational changes of the protein occur (*e.g.*, such as those induced with heat and urea).

The above lines of evidence are therefore consistent with the identification of the new absorption bands in both ChT–NAP and ChT–DNAP as due to charge-transfer interactions. It is then pertinent to inquire as to the nature of the donor moiety of the enzymic charge-transfer complex.

To aid in identifying which aromatic amino acid residue (phenylalanine, tyrosine, or tryptophan) was serving as the donor in the enzymic charge-transfer interaction, reversible, noncovalent complexes composed of toluene, *p*-cresol, and indole[9] were prepared with both α-bromo-4-nitroacetophenone and α-bromo-2,4-dinitroacetophenone in acetonitrile as solvent. The charge-transfer complexes of these compounds with the nitroacetophenones showed new absorption bands in the region 300–400 mμ. For example, the absorption maxima of the charge-transfer complexes that toluene, cresol, and indole form with α-bromo-2,4-dinitroacetophenone are 312, 315, and 345 mμ, respectively. Since the absorption maximum of the charge-transfer complex with indole most closely corresponds to that of ChT–DNAP (λ_{max} 365 mμ),[10] these results suggest that the donor in the enzymic charge-transfer complex is a tryptophan residue.

Another fact which is consistent with (but not proof of) the identification of tryptophan as the donor in the enzymic charge-transfer complex is that the characteristic tryptophan fluorescence of chymotrypsin is 60% quenched in ChT–NAP and ChT–DNAP. The quenching of the fluorescence of indole residues involved in charge-transfer complexes has been observed in other systems.[11]

In summary, α-bromo-4-nitroacetophenone alkylates methionine-192 of chymotrypsin and forms a conformationally dependent charge-transfer complex with a vicinal tryptophan residue. Because BrNAP performs two functions, it can be termed a chemical–optical bifunctional reagent. Further, it can be noted that this reagent (and other similar reagents) provides a method for determining the maximum distance between the site of alkylation and the donor of the charge-transfer complex. In chymotrypsin, for example, we conclude that the methionine-192 sulfur can be no more than 8 A from the center of the indole moiety of a tryptophan residue.

(9) Toluene, *p*-cresol, and indole serve as models for the aromatic nuclei of phenylalanine, tyrosine, and tryptophan, respectively.

(10) The charge-transfer complex indole forms with α-bromo-4-nitroacetophenone has λ_{max} 330 mμ (*cf.* ChT–NAP, λ_{max} 350 mμ). The absorption maxima of the charge-transfer transitions of ChT–NAP and ChT–DNAP are each shifted 20 mμ to the red of the maxima of the corresponding model charge-transfer complexes containing indole. This red shift may be due to a solvent effect or to the presence of the positive charge on methionine-192 which results from sulfonium salt formation as a consequence of alkylation.

(11) S. Shifrin, *Biochim. Biophys. Acta*, **81**, 205 (1964).

Acknowledgments. We are pleased to acknowledge the support of this work by U. S. Public Health Service Grant No. AM-07300. Important initial experiments were ably performed by Mr. P. John Flory, Jr. Professor William White of the University of Vermont had kindly informed us of the inactivation of chymotrypsin by α-bromoacetophenone prior to the start of this work.

(12) National Institutes of Health Postdoctoral Fellow, 1965–1966.

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Deoxygenation by Carbon Atoms

Sir:

The large value for the heat of combination of a carbon atom with an atom of oxygen (256.7 kcal/mole)[1] suggests that the deoxygenation of oxygen-containing compounds by carbon atoms should be a thermodynamically favored process. We are able to report several examples of this process in the condensed phase.

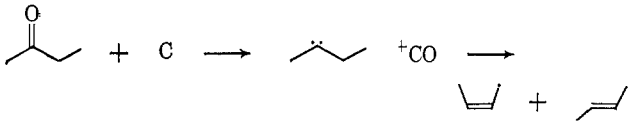
Reactions of carbon atoms, produced by nuclear transformation, with carbon dioxide, oxygen, and ethylene oxide have been reported to cause deoxygenation with production of radioactive carbon monoxide.[2,3]

The reaction system employed in this study has been reported previously.[4] Simultaneous deposition of carbon vapor with acetone at −196° results in the formation of carbon monoxide and propylene. This observation may be understood on the basis of an oxygen abstraction by the atomic carbon to form carbon monoxide and a dialkylcarbene. The resultant carbenes are known to rearrange to isomeric olefins.[5,6]

$$CH_3\overset{\overset{O}{\|}}{C}CH_3 + C \longrightarrow CH_3\ddot{C}CH_3 + CO$$

$$CH_3\ddot{C}CH_3 \longrightarrow CH_3CH{=}CH_2$$

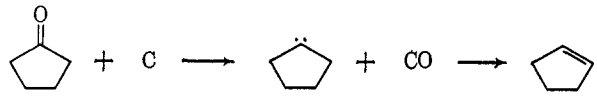
Other ketones show similar behavior; the reaction with 2-butanone gave *cis*-2-butene and *trans*-2-butene in yields[7] of 67 and 16%, respectively. Small amounts of 1-butene and 1,3-butadiene are also observed in the 2-butanone reaction. A determination of the amount of carbon monoxide formed showed it to be roughly equimolar with the 2-butenes.



The reaction with cyclopentanone gave cyclopentene in 42% yield and a small amount of cyclopentadiene.

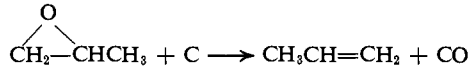
(1) A. E. Douglas, *J. Phys. Chem.*, **59**, 109 (1955).
(2) C. MacKay and R. Wolfgang, *Radiochim. Acta*, **1**, 42 (1962).
(3) C. MacKay and R. Wolfgang, *Science*, **148**, 899 (1965).
(4) P. S. Skell, L. Wescott, Jr., J. P. Goldstein, and R. R. Engel, *J. Am. Chem. Soc.*, **87**, 2829 (1965).
(5) L. Friedman and H. Schechter, *ibid.*, **81**, 5512 (1959).
(6) W. Kirmse and B. V. Bülow, *Chem. Ber.*, **96**, 3316 (1963).
(7) Yields are calculated in the following manner: (mole of product formed/mole of C_1 vaporized) × 100. The amount of C_1 in these vaporizations has been estimated at 30% of the weight of vaporized carbon (P. S. Skell and R. F. Harris, unpublished results).

At present, we have no satisfactory explanation for the formation of doubly unsaturated products.

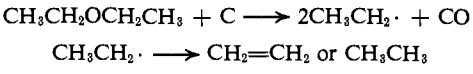


A side reaction to be considered in the deoxygenation of ketones by carbon vapor is photolysis by radiation from the arc. The extent of this side reaction has been estimated in the case of 2-butanone by measuring the relative amounts of methane and carbon monoxide: CH_4:CO = 1:25. Since methane and carbon monoxide are formed in similar amounts during photolysis,[8] the photolytic pathway does not appear significant in the 2-butanone reaction. The olefins observed in the deoxygenation reactions are not produced by photolysis.

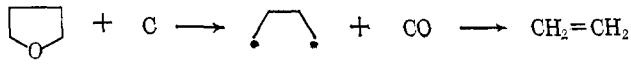
The reaction of propylene oxide with carbon atoms leads to propylene in 160% yield. The yield value greater than 100% indicates that the C_2 and/or C_3 present in the carbon vapor is also participating in oxygen abstraction. This result is consistent with a deoxygenation by a carbon atom to form carbon monoxide with the simultaneous formation of propylene.



The reaction of ethyl ether with carbon atoms gives ethylene and ethane in 40 and 35% yields, respectively (yields calculated on the basis of 1 mole of ethylene and 1 mole of ethane/mole of C_1). This result may also be explained by a deoxygenation process to produce two ethyl radicals and a molecule of carbon monoxide. A small amount of *n*-butane, the radical coupling product, was also observed.

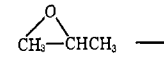
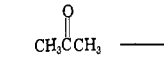
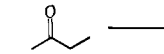
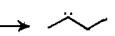
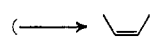
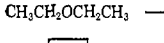
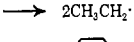
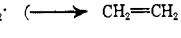
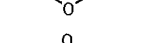
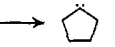
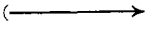


By analogy to the ethyl ether reaction, the reaction with tetrahydrofuran (THF) was expected to form a 1,4-diradical. The same 1,4-diradical has been examined in the vapor phase[9] and gives ethylene and cyclobutane as the major products. In the carbon atom reaction with THF, ethylene was formed in 27% yield (yield calculated on the basis of 2 moles of ethylene/mole of C_1) with a trace of cyclobutane. Also recovered from the THF reaction were 1-butene and traces of *n*-butane and *cis*-2-butene.[10] The failure to observe cyclobutane as a major product is surprising and may be an indication that a "hot" 1,4-diradical is formed in the highly exothermic deoxygenation process.



Deoxygenation is not observed with simple alcohols and water, even though it is thermodynamically favored, and other reactions dominate.[11] Table I summarizes the results to date with the calculated ΔH's required for the removal of the oxygen atom from each substrate.[12] In calculating the ΔH values, it was assumed C_1 abstracted the oxygen. While C_1 must be the major abstracting species, C_2 and C_3 might also function as deoxygenating agents.

Table I

Substrate	Intermediate	Products	ΔH(calcd) for O-abstrn step, kcal/mole
			135–145
			85–100
			85–100
			85–100
			85–100
			85–100

Although it seems probable that the metastable singlet states (^1S and ^1D) of carbon are the major reactants, experiments are in progress which will indicate the reactive species.

Acknowledgment. We acknowledge the financial support of the Air Force Office of Scientific Research.

(8) C. H. Bamford and R. G. W. Norrish, *J. Chem. Soc.*, 1504 (1935).

(9) S. W. Benson and G. B. Kistiakowsky, *J. Am. Chem. Soc.*, **64**, 80 (1942).

(10) The formation of C_4 olefins from 1,4-dibromobutane and alkali metal in solution has been reported. See W. S. Smith, *J. Org. Chem.*, **23**, 509 (1958).

(11) P. S. Skell and R. F. Harris, unpublished results.

(12) All required bond energies taken from R. L. Cottrell, "The Strengths of Chemical Bonds," 2nd ed, Butterworth & Co. (Publishers) Ltd., London, 1958.

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Received February 3, 1967

The Instability of Muscle Aldolase Subunits in Dilute Alkali[1]

Sir:

Previous reports from this laboratory showed that, upon exposure to mildly alkaline conditions, the molecular weights of native aldolase ($\bar{M}_w^0$ = 142,000) and succinyl aldolase subunits ($\bar{M}_w^0$ = 54,500) decreased to values of approximately 2.24 and 2.70 × 10^4, respectively.[2] This led to the conjecture that aldolase might be comprised of six fundamental subunits rather than three, as postulated by previous investigators.[3]

Further confusion regarding the quaternary structure of muscle aldolase was generated by the recent communication of Kawahara and Tanford which indicated

(1) This investigation was supported by U. S. Public Health Service Grant AM08130 from the National Institutes of Health.

(2) L. F. Hass and M. S. Lewis, *Biochemistry*, **2**, 1368 (1963); L. F. Hass, *ibid.*, **3**, 535 (1964).

(3) A. Kowalsky and P. D. Boyer, *J. Biol. Chem.*, **235**, 604 (1960); E. Stellwagen and H. K. Schachman, *Biochemistry*, **1**, 1056, (1962); W. C. Deal, W. J. Rutter, and K. E. Van Holde, *ibid.*, **2**, 246 (1963).