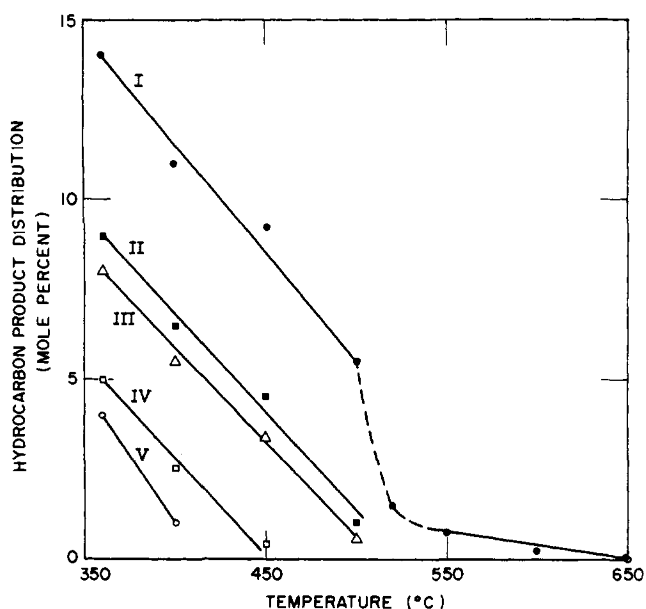

Fig. 1.—Hydrocarbon product distribution *vs.* temperature. Ethane (I), propane (II), ethylene (III), propylene (IV), butane (V), balance methane.

(360°) to 3.3 × 10^{-12} mole per g. sec. (800°). The maximum methane concentration did not exceed 0.01 mole % with no indication of an equilibrium state within this concentration range. The minimum reaction temperature appeared to be between 350 and 360°, where the rate dropped off by two orders of magnitude. The reproducibility of the rates, over a four-month period, indicated a negligible contribution from aliphatic carbon and structural defects. When powdered graphite (100 mesh) replaced the rods, the reaction rates remained the same. This revealed the absence of any mass transport effects.

Probably the most interesting result of this experiment was the increasing proportion of five higher hydrocarbons in the reaction products below 650° (Fig. 1). While the molar ratios of all hydrocarbons to hydrogen remained well below the equilibrium values,[7] the molar ratios of the higher hydrocarbons to methane gradually exceeded the equilibrium ratios as the temperature was lowered. At 360° and 1 atm. of hydrogen, less than 0.1 mole % of these higher hydrocarbons can be expected in the product; yet, they were found to account for some 40 mole % of all hydrocarbons formed.

A mechanism for the hydrogenolysis of graphite has been suggested by Zielke and Gorin.[8] The first step in the proposed reaction is the hydrogenation of exposed edges of the graphite lattice (Fig. 2). It is generally agreed that these are the active sites.[9,10] Six general bond types based on the relative saturation of the attached carbons are indicated. The rate of the first-order reaction above 1000° is probably controlled by the rate of hydrogenation of initially unsaturated "a" bonds, which are the weakest[11] and most exposed. This is supported by the close agreement between the reaction activation energy[2] and the activation energy of hydrogen chemisorption[9] above 1000°. In the transition region, between 1000 and 580°, an increasingly larger fraction of "a" bonds are probably saturated as the reaction order changes from zero to first. The implication of the zero-order reaction below 580° is that the rate-determining step is now the cleavage of saturated "a" bonds; followed by a fast "b" bond cleavage with hydrogenation to form methane.

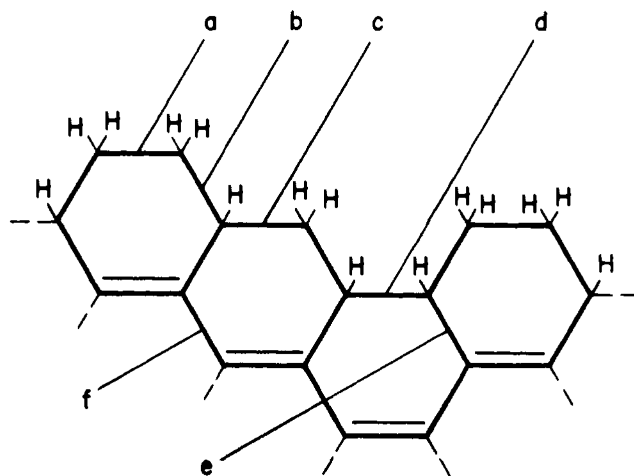

Fig. 2.—Schematic of hydrogenated graphite lattice edge.

The large buildup of ethane and higher hydrocarbons in the reaction products below 515° indicates that "b" and closely related "c" bonds are beginning to cleave at rates comparable to "a" bonds. Besides leading to methane formation, a "c" bond cleavage should promote the breaking of adjacent "a" bonds, which now would be part of a more exposed and saturated hexagon, approaching that of cyclohexane. This effect could account for the lower activation energy of methane formation below 515°.

The stepwise cleavage of alternate saturated "b" bonds with hydrogenation accounts for ethane, while the somewhat less likely simultaneous cleavage of the same bonds without hydrogenation explains the formation of ethylene. Propane formation is governed by the "b–e–d" bond cleavage sequence, while the "b–e–b" sequence accounts for butane. It is significant that no isobutane was detected, even though its equilibrium concentration should be greater than butane.[7] This is reasonable since its formation would involve two unlikely "f" bond cleavages.

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(9) J. P. Redmond and P. L. Walker, Jr., *J. Phys. Chem.*, **64**, 1093 (1960).

(10) R. E. Nightingale, Ed., "Nuclear Graphite," Academic Press, New York, N. Y., 1955, p. 425.

(11) C. T. Mortimer, "Reaction Heats and Bond Strengths," Pergamon Press, New York, N. Y., 1962, p. 62.

On the Role of the Methionine-13 Sulfur in Pancreatic Ribonuclease[1]

Sir:

Hofmann, *et al.*,[2] have shown that S-protein (the subtilisin-produced 104 residue carboxyl terminal part of ribonuclease) can be 70% activated by a synthetic polypeptide containing only the first 13 of the missing amino terminal 20 residues. This synthetic peptide ends in histidine-12-methionine-13, and a peptide lacking these two residues was shown not to activate S-protein. A tridecapeptide very similar to the synthetic one can be readily obtained by cyanogen bromide

(1) This work was supported by a Public Health Service research career development award (GM-K3-7730) and a grant (RG-8197) from the National Institutes of Health, U. S. Public Health Service. Taken from a thesis by M. B. Barancik submitted to the College of Liberal Arts and Sciences in partial fulfillment of the Bachelor of Science Degree in Chemistry.

(2) K. Hofmann, F. Finn, W. Haas, M. J. Smithers, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **85**, 833 (1963).

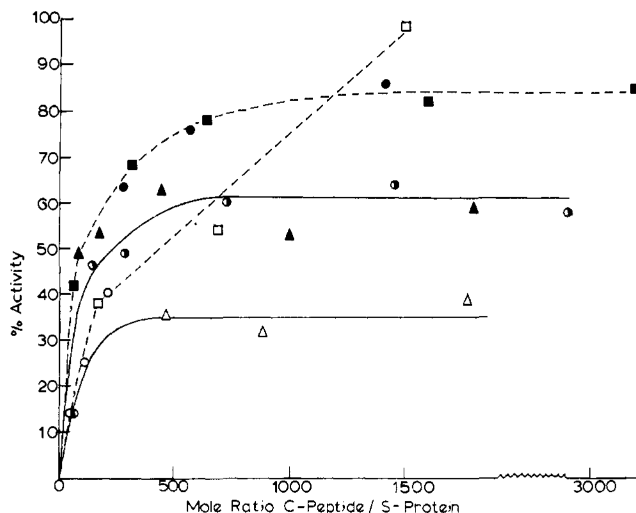

Fig. 1.—Activity toward RNA and cyclic cytidylate induced in S-protein by various C-peptide preparations. The S-protein concentration was $0.030–0.076 \times 10^{-6}$ *M* in the RNA assays and $1.09–1.45 \times 10^{-6}$ *M* in the cyclic cytidylate assays. The S-protein was added to the substrate solution containing the appropriate amount of C-peptide after the C-peptide blank had been run. (As much as 3 mg. of C-peptide gave zero rate in the blank.) The observed rates were compared to the 100% control, in which sufficient S-peptide to saturate the same amount of S-protein was used.

■ Base-treated C-peptide; RNA substrate.
□ Base-treated C-peptide; cyclic cytidylate substrate.
● C-Peptide+ (slow component); RNA substrate.
○ C-Peptide+ (slow component); cyclic cytidylate substrate.
◑ C-Peptide+ (mixture)*; RNA substrate.
◐ C-peptide+ (mixture)*; cyclic cytidylate substrate.
▲ Oxidized C-peptide (mixture)*; RNA substrate.
△ Oxidized C-peptide (mixture)*; cyclic cytidylate substrate.

+, data obtained with both 98.5% and 99.5% pure C-peptide preparations; *, predominantly fast component, but was never obtained completely free of the slow component.

cleavage of RNAse.[3] This peptide, designated C-peptide, is derived from the 13 amino acid amino terminus of RNAse and differs from the synthetic tridecapeptide only in that its carboxyl terminal residue is homoserine lactone instead of methionine. It was of interest to establish whether C-peptide could also activate S-protein.

C-peptide was prepared according to Gross and Witkop[3]; two passes through G-25 Sephadex in 0.2 *M* propionic acid reduced contamination by the larger components to 0.5 and 1.5 mole % in two preparations. The purity was judged by the ratio of tyrosine to phenylalanine absorption in the product and was confirmed by amino acid analysis ($Lys_{2.0}His_{0.94}Arg_{0.96}$-$Asp_{0.08}Thr_{0.85}Ser_{0.12}Glu_{3.0}Gly_{0.05}Ala_{3.00}Meth_{0.02}Ileu_{0.02}$-$Leu_{0.02}Tyr_{0.01}Phe_{1.00}Homoser_{0.78}$ and $Lys_{2.25}His_{1.05}Arg_{1.15}$-$Asp_{0.26}Thr_{1.03}Ser_{0.24}Glu_{3.01}Pro_{0.16}Gly_{0.26}Ala_{3.00}Val_{0.17}Met_{0.05}$ $Ileu_{0.07}Leu_{0.12}Tyr_{0.06}Phe_{1.03}Homoser_{0.76}$, respectively, for the two preparations). The complete absence of unchanged RNAse in the product was demonstrated in both the C-peptide preparations. Electrophoresis on cellulose acetate in 0.04 *M* ammonium bicarbonate at pH 8 showed some preparations to have two components. Both components moved toward the cathode, and the fastest moving component was always the major one in all fresh C-peptide (or oxidized C-peptide) preparations. It was found, however, that treatment with 0.1 *N* NaOH for 1 hr. or less at room temperature quantitatively converted the fast component to the slow component. Exposure to pH 7.1 for 8 hr. at room temperature caused the same transformation, while the fast component remained unaltered after 8 hr. at pH 5.0. Since it was found in control experiments that the same alkali treatments did not affect either the electrophoretic behavior or the activity of unmodified S-peptide, it was concluded that the observed changes in C-peptide cannot be due to hydrolysis of glutamine-11. The most reasonable interpretation of the foregoing observations must, therefore, be that the fast component is C-peptide with an intact homoserine lactone[3] at the carboxyl terminus, and that the slow component is C-peptide in which the lactone has been hydrolyzed to give a carboxyl terminal homoserine.

S-protein was prepared as a by-product of Potts' modification[4] of Richards' method for preparing S-peptide.[5] Its purity was confirmed by amino acid analysis ($Lys_{7.9}His_{3.0}Arg_{2.9}Asp_{14.2}Thr_{7.4}Ser_{9.4}Glu_{9.4}Pro_{3.95}$-$Gly_{2.07}Ala_{7.0}Val_{9.2}Met_{2.6}Ileu_{2.6}Leu_{2.1}Tyr_{5.6}Phe_{2.0}$).

Activity assays using both RNA[6] and cyclic cytidylate as substrates were carried out at 25° on a Cary Model 15 recording spectrophotometer, according to the methods of Kunitz[7] for RNA and of Crook, *et al.*,[8] for cyclic cytidylate. The amounts of S-protein and C-peptide used were calculated assuming that 1 mg./ml. of S-protein has $OD_{278} = 0.71$,[9] and that 5×10^{-3} *M* C-peptide has $OD_{256} = 1.0$, as given for phenylalanine by Bovey and Yanari.[10] The results are shown in Fig. 1.

It is apparent from the figure that C-peptide is indeed capable of activating S-protein, so that methionine-13 sulfur cannot be said to be necessary to the cleavage mechanism. However, in view of the very large amounts of C-peptide required to approach saturation of the S-protein, it was necessary to consider the possibility that the observed activation was due not to C-peptide, but to some methionine-13 containing contaminant in the preparation, which might activate S-protein in a manner similar to the activation of S-protein by oxidized RNAse.[11] In order to eliminate this possibility, a C-peptide preparation was oxidized with performic acid.[12] The oxidized peptide showed a loss of 95% of the small amount (0.05 residues per mole) of contaminating methionine but gave the same activity as the untreated C-peptide. (See the solid RNA curve in Fig. 1.) The fact that there was no activity difference between the 98.5 and 99.5% pure samples of C-peptide is also strong evidence against the possibility that a contaminant is responsible for the observed activity.

Potts, *et al.*, have recently shown that the peptide R^{13}-His-Met-Asp-Ser gave full activity at a ratio of peptide to S-protein of 1:1.[14] Considering then that the peptide R^{13}-His-Met gave about 70% activity at a peptide to S-protein ratio of 10–30:1,[2] and that C-peptide (R^{13}-His-Homoser) as shown in Fig. 1 gave 80–100% activity at a peptide to S-protein ratio of about 600:1, it must be safe to conclude that the sulfur of methionine-13 together with aspartic acid-14 and serine-15 is involved only in the binding of S-peptide to

(3) E. Gross and B. Witkop, *J. Biol. Chem.*, **237**, 1856 (1962).

(4) J. T. Potts, personal communication.

(5) F. M. Richards and P. J. Vithayathil, *J. Biol. Chem.*, **234**, 1459 (1959).

(6) A. M. Crestfield, K. C. Smith, and F. W. Allen, *ibid.*, **216**, 185 (1955).

(7) M. Kunitz, *ibid.*, **164**, 563 (1946).

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(9) J. A. Hermans and H. A. Scheraga, *J. Am. Chem. Soc.*, **83**, 3283 (1961).

(10) F. A. Bovey and S. Yanari, *J. Biol. Chem.*, **236**, 2818 (1960).

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(12) C. H. W. Hirs, *J. Biol. Chem.*, **219**, 611 (1956).

(13) R = Lys-Glu-Thr-Ala-Ala-Lys-Phe-Glu-Arg-GluNH_2-.

(14) J. T. Potts, Jr., D. M. Young, and C. B. Anfinsen, *J. Biol. Chem.*, **238**, 2593 (1963).

S-protein and that apparently no direct function in the catalytic mechanism of RNAse can be ascribed to it. This is in agreement with the previous conclusions of Richards and Vithayathil,[11] based on observations with methionine-13 modified S-peptide, and also with those of Potts, *et al.*,[14] but it appears to disagree with the conclusions of Hofmann, *et al.*, based on their observations with the oxidized synthetic tridecapeptide.[2]

The interesting implications of the effect of alkali on the activity of C-peptide are under investigation.

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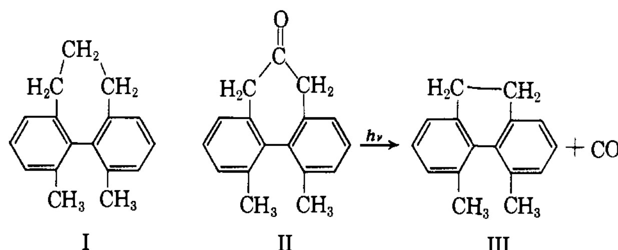
Photoracemization of Biphenyls

Sir:

Among the many photochemical isomerizations which have been studied to date,[1] there appears to be no instance of a transformation whose mechanism does not require bond breaking and bond reformation. We now report the photoracemization of hydrocarbon I as the first example of a light-induced interconversion of conformational stereoisomers.



Hydrocarbon I[2] may be racemized by heating in the dark above 200° (E_{act} 39 kcal./mole). Irradiation (Hanovia high-pressure quartz mercury-vapor lamp, 450 w.) of 0.1–0.2% solutions in ether under nitrogen at 23–25° results in racemization, with initial half-lives varying from 4 to 30 hr.[3] The process appears to be singularly uncomplicated by side reactions. The interposition of a Pyrex shield between the lamp and the solution arrests the racemization; I has λ_{max} 240 mμ (log ε 4.06)[2] and does not absorb significantly above 260 mμ.

Our results may be interpreted most economically by postulating that absorption of energy near 240 mμ (119 kcal./mole) is followed by internal conversion of the initially formed electronically excited molecule to the vibrationally excited "hot" ground state molecule[4] which is directly produced in the classical thermal racemization.[5]

We have also found that ketone II[2] undergoes simultaneous racemization and decarbonylation under the conditions stated. In a typical experiment, irradiation of II ([α]D −635°, benzene) for 6 hr. gave 21% of II ([α]D −582°) and 43% of III ([α]D +194°, benzene), identified by infrared, g.l.c., and rate constant of racemization.[6]

Interposition of a Pyrex shield does not substantially alter these results. In a typical experiment, irradiation of II ([α]D −620°) for 6 hr. gave 34% of II ([α]D −580°) and 32% of III ([α]D +242°). The last finding is not unexpected, since we have previously recognized the spectroscopic interaction of the n → π* and π → π* transitions in II[2] and have looked upon the whole molecule as an extended dissymmetric chromophore.[7] It is surprising, however, that the decarbonylation reaction is 4–5 times as important (in terms of product yield) as racemization. Absorption of radiation at 300 mμ (95 kcal./mole) is more than sufficient to supply the energy required to racemize II (E_{act} 36 kcal./mole), yet degradation of the electronically excited molecule *via* bond cleavage is the preferred pathway.[8]

Compound II is the dibenzo derivative of 3,5-cycloheptadienone, a compound which has never been obtained in optically active form but which has been photochemically decarbonylated[9] to a mixture of isomeric 1,3,5-hexatrienes.[10] We stress that in II and in 3,5-cycloheptadienone, the interaction of the carbonyl group with each of the two benzene rings, respectively double bonds, is identical. Chapman, *et al.*, have attempted to rationalize the absence of light-induced valence tautomerization in 3,5-cycloheptadienones by claiming[9] that "careful examination of the model [of 3,5-cycloheptadienone] shows that the π-orbital of one double bond and the carbonyl π-orbital are well situated for interaction. The remaining double bond is poorly situated for interaction with . . . the carbonyl." This statement is in error, since in actual fact the two double bonds are stereochemically and spectroscopically *completely equivalent;* as we have pointed out some time ago, the carbon–oxygen bond in systems of this type is coincident on a twofold symmetry axis.[11]

We have extended the decarbonylation reaction to other systems (bisnor-II → 9,10-dihydrophenanthrene, doubly bridged diketone[12] → 4,5,9,10-tetrahydropyrene) but the synthetic usefulness is severely limited by the extensive formation of by-products (*inter alia*, the carbinols).

(1) *E.g.*, P. de Mayo and S. T. Reid, *Quart. Rev.* (London), **15**, 393 (1961); "Advances in Organic Chemistry," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1960, p. 367 ff.

(2) K. Mislow, M. A. W. Glass, R. E. O'Brien, P. Rutkin, D. H. Steinberg, J. Weiss, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1455 (1962).

(3) The kinetic results of 15 runs were not quantitatively reproducible. The product of racemization (recovered in nearly quantitative yield) was identical (by infrared and g.l.c.) with starting I. In one experiment the hydrocarbon was racemized to the extent of 98–99%, initial [α]D +244°, final [α]D +4° (benzene).

(4) A similar situation exists in conjugated dienes as has been pointed out by R. Srinivasan, *J. Am. Chem. Soc.*, **84**, 3982 (1962).

(5) Cleavage of bonds to benzyl carbon is not likely to result in racemization, since the resulting diradical is a dissymmetric tetrasubstituted unbridged biphenyl whose racemization energy barrier should greatly exceed that of I.

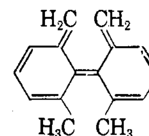
(6) K. Mislow and H. B. Hopps, *J. Am. Chem. Soc.*, **84**, 3018 (1962).

(7) A. Moscowitz, K. Mislow, M. A. W. Glass, and C. Djerassi, *ibid.*, **84**, 1945 (1962).

(8) Whatever the mechanism of the photochemical decarbonylation, we note that the possible intervention of long-lived diradical intermediates will not result in racemization since the blocking substituents ($CH_2CO\cdot/CH_2\cdot$ or $CH_2\cdot/CH_2\cdot$) remain.[5]

(9) O. L. Chapman, D. J. Pasto, G. W. Borden, and A. A. Griswold, *J. Am. Chem. Soc.*, **84**, 1220 (1962).

(10) The corresponding product in our case would have the structure shown. However, we have no evidence for the intervention of such a reactive intermediate.



(11) K. Mislow, *Angew. Chem.*, **70**, 683 (1958).

(12) K. Mislow, E. Simon, and H. B. Hopps, *Tetrahedron Letters*, **No. 22**, 1011 (1962).

(13) National Science Foundation Cooperative Predoctoral Fellow, 1963–1964.

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