photolysis.[5] In addition to extending the number and molecular weight range of the compounds originally reported,[1] these examples include a number of alkanes which contain primary, secondary and tertiary carbon–hydrogen bonds in the same molecule. In summary, the data obtained in the present study provide excellent support for the description of this methylene by Doering *et al.*,[1] as the most indiscriminate reagent yet encountered in organic chemistry.

The difference between methylene reactivity in the gas phase and in solution can be rationalized by consideration of recent spectroscopic evidence. Herzberg[6,7] has obtained spectra of methylene from gas-phase photolysis showing both singlet and triplet states, and demonstrating the singlet state is the shorter lived. Theoretical considerations[7] indicate the excited singlet state is the first formed, the triplet state arising from a later transition.

We suggest that the random reaction of methylene with alkane carbon-hydrogen bonds[8] observed in solution arises from the excited singlet state[9] (paired non-bonding electrons) and, further, that this reaction occurs at a much higher rate than the transition of the singlet to the triplet state (unpaired non-bonding electrons). Thus, there is little or no radical component to the reaction in solution. In the gas phase, we suggest the frequency of collision is sufficiently less that the singlet–triplet transition occurs at a rate of the same order of magnitude as the random singlet insertion. Methylene in the triplet state would be expected to react selectively with carbon–hydrogen bonds as do other radicals. Therefore, methylene reacts with carbon–hydrogen bonds by two paths: a random singlet insertion and a selective triplet abstraction. The abstraction leads to methyl and alkyl radicals which can combine to give apparently selective insertion, attack diazomethane, or react in other ways typical of free radicals. The extent of insertion selectivity and of secondary product formation observed in a particular reaction will thus depend on the ratio of singlet–triplet transition to singlet carbon–hydrogen insertion.

Support for this interpretation is provided by experimental observations. (1) EFFECT OF OXYGEN.—In the photolysis of gaseous diazomethane–alkane mixtures, Frey[10] found secondary products indicating a radical component of the reaction. The presence of oxygen during photolysis prevented formation of the secondary products and altered the ratio of primary products toward that expected for random reaction. Oxygen would be expected to react selectively with methylene in the triplet state and with radicals arising from the reaction of triplet methylene with the alkane. Secondary products, therefore, should be eliminated, and the over-all reaction should exhibit more singlet or random character.

(2) EFFECT OF PRESSURE.—The above rationalization indicates that the gas-phase reaction should be pressure dependent, the triplet character of the reaction increasing as the pressure is lowered. This conclusion is in accord with results from photolysis of diazomethane—*trans*-butene-2-mixtures.[2,11] "Very similar" product compositions were reported for the liquid phase (−70°) and the gas phase (25°) at pressures greater than 1200 mm. As the pressure was lowered the yield of *trans*-1,2-dimethylcyclopropane decreased and the formation of *cis*-pentene-2, 2-methylbutene-1 and *cis*-1,2-dimethylcyclopropane was observed. These C_5 products formed at lower pressure are those expected from the reaction of methylene in the triplet state with the olefin.[12] The observed formation of ethane and other secondary products with decreasing pressure also points to the growing importance of the triplet reaction as the pressure is lowered.

(3) EFFECT OF INERT GAS.—The addition of inert gas should, according to the above interpretation, emphasize the triplet character of the reaction by increasing the ratio of singlet–triplet transition to singlet–hydrocarbon reaction. This requirement is substantiated by the formation of *cis*-1,2-dimethylcyclopropane in the photolysis of diazomethane-*trans*-butene-2 mixtures with argon or nitrogen.[11]

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(5) These data will appear in a forthcoming publication.

(6) G. Herzberg and J. Shoosmith, *Nature*, **183**, 1801 (1959).

(7) G. Herzberg, paper presented at Welch Foundation Conference on Molecular Structure, Houston, Texas, November 16–18, 1959.

(8) Doering and Prinzbach [W. von E. Doering and H. Prinzbach, *Tetrahedron*, **6**, 24 (1959)] have shown this reaction occurs by direct insertion.

(9) Skell and Woodworth [P. S. Skell and R. C. Woodworth, THIS JOURNAL, **78**, 4496, 6247 (1956)] have assigned methylene a singlet structure because of its stereospecific reaction with olefins.

(10) H. M. Frey, *Proc. Chem. Soc.*, 318 (1959).

(11) H. M. Frey, *Proc. Roy. Soc.* (*London*), **A251**, 575 (1959).

(12) R. M. Etter, H. S. Skovronek and P. S. Skell. THIS JOURNAL **81**, 1008 (1959).

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RECEIVED MAY 11, 1960

COSYNTHETIC FACTOR I, A FACTOR INVOLVED IN HYDROGEN-TRANSFER IN *STREPTOMYCES AUREOFACIENS*

Sir:

In two previous communications the accumulation of 7-chloro-5a(11a)-dehydrotetracycline[1] by *Streptomyces aureofaciens* mutant S-1308 and the biological reduction[2] of this compound to 7-chlorotetracycline were described. More recently[3] Cosynthetic Factor I, a stable, water-soluble substance elaborated by many strains of *S. aureofaciens*, was shown to be effective in enabling S-1308 to produce 7-chlorotetracycline instead of the 5a(11a)-dehydro analog. Utilizing this response (Fig. 1) as an analytical method, we have now been able to isolate Cosynthetic Factor I. The isolation and preliminary characterization of this substance are given.

Fermented mash of *S. aureofaciens* mutant W-5 was selected as the source for Cosynthetic Factor I by reason of its relatively high content of the desired product and the fact that it contained no interfering pigments. W-5 was cultivated in the

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(1) J. R. D. McCormick, P. A. Miller, J. A. Growich, N. O. Sjolander and A. P. Doerschuk, THIS JOURNAL, **80**, 5572 (1958).

(2) J. R. D. McCormick, N. O. Sjolander, P. A. Miller, U. Hirsch, N. H. Arnold and A. P. Doerschuk, *ibid.*, **80**, 6460 (1958).

(3) J. R. D. McCormick, U. Hirsch, N. O. Sjolander and A. P. Doerschuk, *ibid.*, **82**, 5006 (1960).

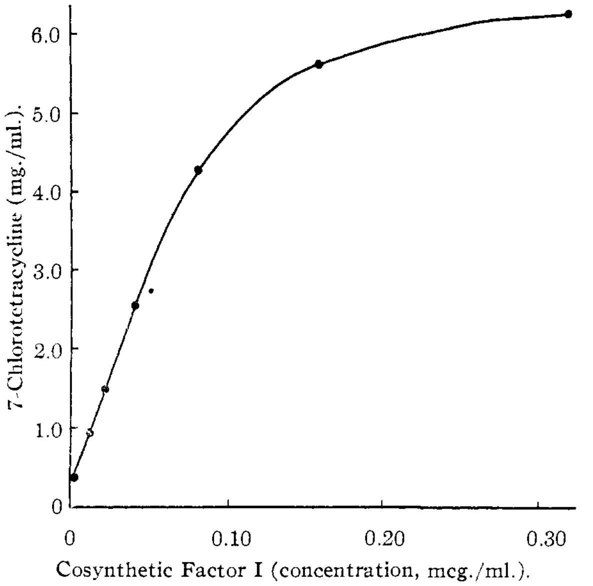

Fig. 1.—Response of S-1308 mash to added Cosynthetic Factor I.

usual corn steep medium.[4] After 120 hours of incubation, the mycelium was separated by filtration and the activity was adsorbed from the filtrate onto activated carbon (Norit A, 35 g./l.). The carbon cake was washed with water and with dimethylformamide, and the activity then was eluted with 0.25 *N* ammonium hydroxide in dimethylformamide. The eluate was concentrated to a sirup *in vacuo;* solid product was precipitated by diluting the sirup with a large volume of 1:25 chloroform–acetone. The solid product was dissolved in water by the addition of ammonium hydroxide to *p*H 10 and fractionated by chromatography on a column of magnesium silicate (Florisil). Colored impurities were washed from the column with water, and a bright yellow-green fluorescent band of Cosynthetic Factor I was then eluted with 1:10 water–methanol. Methanol was removed from the rich fractions by vacuum distillation. Upon adjustment of the aqueous concentrate to *p*H 6.4 with hydrochloric acid, the desired product precipitated as rosettes of yellow needles. Cosynthetic Factor I was recrystallized by dissolving in hot 0.1 *N* hydrochloric acid and cooling. It melted at 287–289° with decomposition. On paper chromatograms it appeared as a single fluorescent spot of R_f 0.30 in butanol–acetic acid–water (3:1:4) and of R_f 0.35 in 0.1 *N* aqueous ammonium hydroxide. Countercurrent distribution in 1:1 phenol–chloroform–0.1 *N* aqueous hydrochloric acid indicated the product to be a single component with a peak location at tube 31 after 50 transfers.

The crystalline product gave the analysis $C_{19}H_{22}N_4O_9$. Found: C, 50.3; H, 5.0; N, 12.2; O (Unterzaucher), 32.6; neut. equiv., 446; pK_a (3:1 dimethylformamide–water), 5.5. The infrared spectrum suggested the presence of OH (2.95 μ) and conjugated carbonyl (5.9 μ). The ultraviolet spectra are presented in Fig. 2.

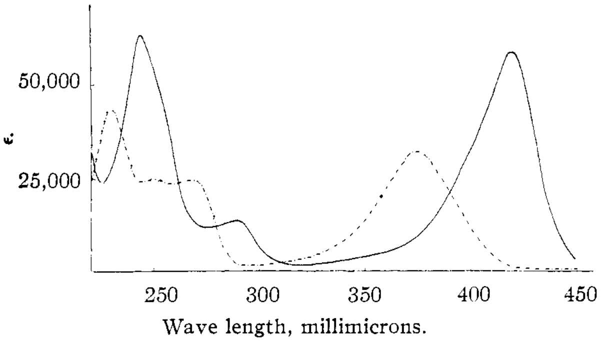

Fig. 2.—Absorption spectra of Cosynthetic Factor I: — · — 0.1 *N* aqueous hydrochloric acid; ——— 0.1 *N* aqueous sodium hydroxide.

Cosynthetic Factor I was found to be dialysable and stable to autoclaving over the *p*H range of 1 to 10. It was reduced readily by aqueous sodium borohydride to a colorless non-fluorescent product which in turn was reoxidized easily by air to the parent compound.

The reversible reduction and oxidation, together with the fluorescence, color, solubility properties, composition and biological origin suggested that Cosynthetic Factor I may be related to the pteridins or flavins. Differentiation from the common members of both these families was possible through the absorption spectra, especially the unusually high extinction coefficient ($\epsilon = \sim 6 \times 10^4$) in alkaline solution (Fig. 2).

The activity of Cosynthetic Factor I is catalytic, as was shown by the fact that one mcg. was sufficient to stimulate the formation of 50,000 mcg. of 7-chlorotetracycline by S-1308 (Fig. 1). The dialysability, stability and high activity per unit weight and the fact that the reaction being promoted is the stereospecific reduction of 7-chloro-5a(11a)-dehydrotetracycline to 7-chlorotetracycline suggest that Cosynthetic Factor I is a cofactor for a hydrogen-transfer enzyme system. Although no other metabolic requirement for this substance by *S. aureofaciens* has been seen, it appears probable that Cosynthetic Factor I may also be involved in more vital hydrogen-transfer processes.

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RECEIVED AUGUST 12, 1960

MECHANISM OF TRANSALKYLATION OF ETHYLBENZENE

Sir:

Ethylbenzene is known to disproportionate to benzene, di- and triethylbenzenes, when treated with strong Lewis acids such as $AlBr_3$ or $GaBr_3$ and HBr. The reaction is known to take place without rearrangement within the ethyl group.[1] In the main, the accepted mechanism due in large part to

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[4] J. J. Goodman, M. Matrishin, R. W. Young and J. R. D. McCormick, *J. Bact.*, **78**, 492 (1959).

[1] R. M. Roberts, G. A. Rapp and O. K. Neville, THIS JOURNAL, **77**, 1764 (1955); R. M. Roberts, S. G. Brandenberger and S. G. Panayides, *ibid.*, **80**, 2507 (1958).