of phosphonites[2b] with polyhalomethanes and with mercaptans,[3] disulfides[4] and alkylsulfenyl halides[5] (RSCl), we wish to describe some of our observations.[6]

Triphenylphosphine did not react appreciably with purified **bromoform** at room temperature. Reaction was observed under the following conditions: (1) near the boiling point of bromoform, when an 85% yield of **triphenyl-(dibromomethyl)-phosphonium bromide (II)** was obtained in a few seconds; (2) at room temperature under irradiation with a Hanovia ultraviolet lamp (80% yield of II[7]); (3) at *ca.* 80° in benzene solution containing traces of benzoyl peroxide (over 80% of II[7] in 30 minutes); in the absence of peroxide, under comparable conditions, no reaction was observed.

This reaction clearly involves free radicals[8] and probably constitutes a chain process. These steps are proposed

$$(C_6H_5)_3P + \cdot CHBr_2 \longrightarrow \underset{\text{I}}{(C_6H_5)_3\dot{P}\text{-}CHBr_2} \quad (1)$$

$$\underset{\text{I}}{(C_6H_5)_3\dot{P}\text{-}CHBr_2} + BrCHBr_2 \longrightarrow \underset{\text{II}}{[(C_6H_5)_3\overset{(+)}{P}\text{-}CHBr_2]\overset{(-)}{Br}} + \cdot CHBr_2 \quad (2)$$

An intermediate with pentacovalent phosphorus may be involved in step (2). The fate of the postulated neutral *phosphoranyl radical*, I, with an expanded phosphorus valence shell, could depend on the nature of the groups attached to the phosphorus atom[9]; for instance, the process $R_3\dot{P}\text{-}X \rightarrow R\cdot + R_3P\text{-}X$ is conceivable. Propagation steps with R· radicals may then be involved. Haszeldine[1] recently has suggested an initial nucleophilic attack by phosphorus on halogen in order to explain the formation of dimethyl-(trifluoromethyl)-phosphine, $(CH_3)_2P\text{-}CF_3$, and tetramethylphosphonium iodide $(CH_3)_4P^+I^-$, in the reaction of trimethylphosphine with iodotrifluoromethane.

The reaction of triphenylphosphine with polyhalomethanes seems to be a case in which a salt is formed in a free radical process.

Triphenyl-(dibromomethyl)-phosphonium bromide (II) had m.p. 235° (from methanol–ethyl acetate), was moderately soluble in water, had ionic bromine and exhibited a band at 3.6μ characteristic[10] of structure $R_3P^+\text{-}C\text{-}H$. Calcd. for $C_{19}H_{16}Br_3P$: C, 44.3; H, 3.1. Found: C, 43.9; H, 2.8. The phosphonium bromide II is of interest in connection with our studies on stable phosphine-methylenes[10,11] $R_3P^+\text{-}C^-XY$.

(3) F. W. Hoffmann, R. H. Ess, T. C. Simmons and R. S. Hanzel, THIS JOURNAL, **78**, 6414 (1956).

(4) H. I. Jacobson, R. G. Harvey and E. V. Jensen, *ibid.*, **77**, 6064 (1955).

(5) D. C. Morrison, *ibid.*, **77**, 181 (1955).

(6) For earlier work on the reaction of tertiary phosphines with polyhalomethanes: (a) H. Hantzsch and H. Hibbert, *Ber.*, **40**, 1508 (1907); (b) A. W. Hofmann, *Proc. Roy. Soc.*, **11**, 291 (1859). For the action on disulfides: A. Schöenberg, *Ber.*, **68**, 163 (1935).

(7) A second substance (m.p. 314°) was also produced in small yield.

(8) A chain-reaction mechanism has been proposed for the photochemical or peroxide-initiated addition of polyhalomethanes to olefins (M. S. Kharasch, E. V. Jensen and W. H. Urry, THIS JOURNAL, **69**, 1100 (1947); M. S. Kharasch, O. Reinmuth and W. H. Urry, *ibid.*, **69**, 1105 (1947)).

(9) In the related case of the trialkyl phosphites[2a] (and of the phosphonites[2b]) one of the possibilities would be: $(RO)_3\dot{P}\text{-}X \rightarrow R\cdot + (RO)_3P(O)X$ (*cf.* G. Kamai and Z. Kbarrasova, *Zhur. Obshchei. Khim.*, **27**, 953 (1957)). In a reinvestigation of the reaction of trialkyl phosphites with mercaptans, C. Walling and R. Rabinowitz have reached similar conclusions THIS JOURNAL, **79**, 5326 (1957).

(10) F. Ramirez and S. Dershowitz, *J. Org. Chem.*, **22**, 41 (1957).

(11) (a) F. Ramirez and S. Levy, THIS JOURNAL, **79**, 67 (1957); (b) F. Ramirez and S. Dershowitz, *ibid.*, **78**, 5614 (1956).

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IDENTIFICATION OF SELENOMETHIONINE IN THE PROTEINS OF *Escherichia coli* EMPLOYING THE CHROMATOGRAPHIC "FINGERPRINT" METHOD

Sir:

The wide distribution of organic selenium compounds throughout the plant and animal kingdoms has been recognized for many years. It has been frequently suggested that selenium may replace sulfur in the methionine and cystine of proteins, although no selenium containing organic compound obtained from natural sources has been unequivocally identified.[1]

Cowie and Cohen have observed that selenomethionine could completely replace methionine for the normal exponential growth of a methionine-requiring mutant of *Escherichia coli*, and that selenium from radioactive selenite was incorporated into proteins.[2] Recent experiments by Schwarz and Foltz lead to the conclusion that selenium is an essential trace element.[3]

We wish to report the identification of selenomethionine from a hydrolysate of the proteins of *E. coli* grown in the presence of radioactive selenium, supplied as selenite, using the chromatographic "fingerprint" method.[4]

Wild-type *E. coli*, strain B, was grown in a sulfur-deficient glucose-salts medium under conditions which permitted rapid incorporation of selenium into proteins.[5] The cells were harvested and fractionated by the method of Roberts, *et al.*[6] One milligram of the highly radioactive protein fraction was successively hydrolyzed enzymatically with pepsin, pancreatin, and erepsin. In order to purify the hydrolysate and to eliminate peptides and inorganic selenium salts, the hydrolysate was loaded on an eight per cent. cross-linked Dowex 50 cation exchange column in the hydrogen form. The column was then washed with water to remove traces of selenite and selenate. Elution of the column with 1.5 normal hydrochloric acid produced a highly radioactive eluate which, after drying in a vacuum desiccator, was loaded on a 3-ml. Dowex 2 anion exchange column in the hydroxide form. No radioactivity could be removed by washing with water. Most of the activity could be eluted

(1) For a review of the literature, see S. F. Trelease and O. A. Beath, "Selenium," Publ. by the Authors, New York, N. Y., 1949.

(2) D. B. Cowie and G. N. Cohen, *Biochem. Biophys. Acta*, in press.

(3) K. Schwarz and C. M. Foltz, THIS JOURNAL, **79**, 3292 (1957).

(4) R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton and R. J. Britten, "Studies of Biosynthesis in *Escherichia coli*," Carnegie Institution of Washington Publ. 607, Washington, D. C., 1955, pp. 190–192.

(5) T. W. Tuve and H. H. Williams, presented before the 132nd Meeting of the American Chemical Society, New York, N. Y., Sept. 8–13, 1957; see Abstracts, p. 10-C.

(6) R. B. Roberts, *et al.*, ref. 4, pp. 13–14.

from the Dowex 2 with 1.5 normal hydrochloric acid in the second column volume, along with the neutral amino acids. The amphoteric material thus obtained was chromatographed in two dimensions, using *sec*-butyl alcohol/formic acid/water (70/10/20) as the first dimension and phenol/water/ammonia (80/20/0.3) as the second. A radioautograph was made of the chromatogram to locate the radioactive material, and the material was "fingerprinted" as follows: The radioactive material occurring in the region which corresponded in location to selenomethionine,[7] and which contained approximately one μg. of selenium, was eluted from the first chromatogram and divided into two equal fractions. One fraction was treated with ninhydrin and gave no reaction. The second fraction was mixed with 150 μg. of carrier selenomethionine and chromatographed. A radioautograph was taken of this chromatogram, and the chromatogram was then sprayed with ninhydrin. The location, shape, size, and form of the spot on the radioautograph corresponded precisely with that spot produced by the ninhydrin test for carrier selenomethionine.

Thus, a chromatographic "fingerprint" identified the amphoteric radioactive material obtained after column fractionation and chromatography of the protein hydrolysate as selenomethionine. To further substantiate the identification, mild oxidation, followed by chromatography, of a mixture of the unknown radioactive material and carrier selenomethionine produced exact "fingerprints" which chromatographically corresponded to the selenoanalogs of a sulfoxide and a sulfone.

Our data indicate that selenocystine may also be produced by *E. coli* grown in the presence of selenite, and studies concerning the identification of this compound will be the subject of a forthcoming paper.

(7) The authors are indebted to Mr. D. B. Cowie, Department of Terrestrial Magnetism, Carnegie Institution of Washington, for a generous sample of selenomethionine, which was originally synthesized by Dr. Alex Shrift, of the University of Pennsylvania, and which was used in the experiments described in reference (2).

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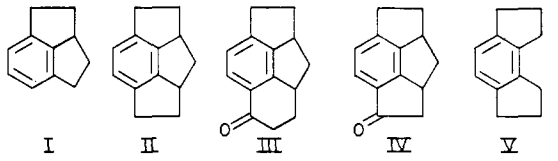
2,2a,3,3a,4,5-HEXAHYDRO-1H-CYCLOPENT[jkl]-AS-INDACENE

Sir:

Recently the synthesis of 2,2a,3,4-tetrahydro-1H-cyclopent[cd]indene (I) was reported.[1] Although previous statements had predicted a very high degree of strain for this system, possibly to the point of excluding its existence, this was found not to be the case. Very little evidence of strain was manifest, the only departure from normal being the extreme ease of hydrogenation.

Continuing the search for a compound in which the strain imparted by a fused ring system would be sufficient to bend the benzene ring and hence decrease significantly its resonance stabilization, we have prepared 2,2a,3,3a,4,5-hexahydro-1H-cyclopent[jkl]-as-indacene(II). Examination of its properties clearly demonstrates a departure from the usual aromaticity of benzenoid compounds and the appearance of olefinic-type characteristics.

The synthesis of III was achieved by starting with 1-oxo-2,2a,3,4-tetrahydro-1H-cyclopent[cd]-indene,[1] which was reduced with sodium borohydride to the alcohol. Hydrogen chloride gave the 1-chloro compound which was converted to the acetic acid with malonic ester, and thence to the propionic acid [m.p. 95.7–96.1°. *Anal.* Calcd. for $C_{14}H_{16}O_2$: C, 77.8; H, 7.5; equiv. wt., 216. Found: C, 77.7; H, 7.4; equiv. wt., 214] by Arndt–Eistert homologation. The propionic acid was cyclized quantitatively with hydrogen fluoride to the ketone (III), which was ring-opened to the carboxyacetic acid by oximination, rearrangement with benzenesulfonyl chloride, and hydrolysis. Pyrolysis of the dibasic acid lead salt gave the ketone IV [m.p. 89–91°. *Anal.* Calcd. for $C_{13}H_{12}O$: 84.8: H, 6.6; mol. wt., 184. Found: C, 85.0; H, 6.5; mol. wt. (Rast), 190]. Wolff re-



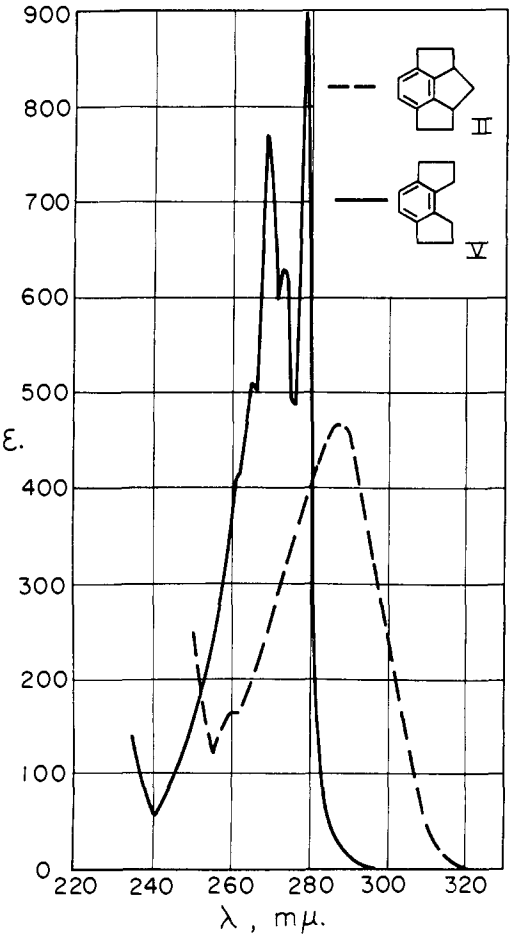


Fig. 1.—Ultraviolet absorption spectra in hexane.

(1) H. Rapoport and J. Z. Pasky, THIS JOURNAL, 78, 3788 (1956).