O,O-diethyl S-(*p*-bromophenyl)-phosphate, b.p. 115–117° (40 μ), $n^{25}\text{D}$ 1.5445 (calcd. for $C_{10}H_{14}O_3PSBr$: 36.93; H, 4.34; S, 9.62; Br, 24.39); 96% of triethyl phosphate, b.p. 33° (0.15 mm.), $n^{25}\text{D}$ 1.4036; and 81% of ethyl chloride.

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RECEIVED OCTOBER 24, 1956

THE DESULFURIZATION OF MERCAPTANS WITH TRIALKYL PHOSPHITES

Sir:

The attempted extension of the transesterification of triethyl phosphite with aliphatic alcohols[1] to mercaptans resulted in the formation of triethyl thionophosphate with simultaneous conversion of the alkanethiol to the corresponding alkane according to the equation $(C_2H_5O)_3P + RSH \rightarrow (C_2H_5O)_3PS + RH$. This unusual desulfurization reaction proceeds practically to completion at reflux temperature during several hours, or at a slower rate at room temperature, and is effectively catalyzed by ultraviolet irradiation.

Thus, a mixture of 83 g. (0.5 mole) of triethyl phosphite (previously distilled from sodium) and 73 g. (0.5 mole) of *n*-octyl mercaptan in a Pyrex flask fitted with a 24-in. fractionating column was irradiated with a General Electric 100-watt S-4 bulb at a distance of 5 inches from the flask. After 6.25 hours of irradiation, the mixture was distilled to give 50.3 g. (88%) of octane, b.p. 122.0–124.5°, $n^{25}\text{D}$ 1.3951–1.3959 (reported,[2] b.p. 125.59°, $n^{25}\text{D}$ 1.3953) and 90.9 g. (0.459 mole) of triethyl thionophosphate, b.p. 45° (0.50 mm.), $n^{20}\text{D}$ 1.4461 (reported,[3] b.p. 105–106° (20 mm.), $n^{20}\text{D}$ 1.4480, calcd. $n^{25}\text{D}$ 1.4460).

Likewise, 83 g. (0.5 mole) of triethyl phosphite and 62 g. (0.5 mole) of benzyl mercaptan were treated in an identical manner to give 43.1 g. (93.6%) of crude toluene, b.p. 90–110°, $n^{25}\text{D}$ 1.4945 (reported[4] 110.56°, $n^{20}\text{D}$ 1.4963, calcd. $n^{25}\text{D}$ 1.4942) and 94 g. (95%) of crude triethyl thionophosphate, b.p. 55° (0.9 mm.), $n^{25}\text{D}$ 1.4461–1.4500, contaminated with some benzyl mercaptan, b.p. 194°, $n^{25}\text{D}$ 1.5715.

Preliminary results show that a wide variety of thiols undergoes the reported desulfurization reaction with triethyl and other trialkyl phosphites. The results of a current investigation of the scope and mechanism of the reaction will be reported later.

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(1) F. W. Hoffmann, R. P. Usinger, Jr., and R. J. Ess, THIS JOURNAL, **78**, 5817 (1956).

(2) A. F. Shepard, A. L. Henne and T. Midgley, Jr., *ibid.*, **53**, 1948 (1931).

(3) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 258.

(4) W. T. Richard and J. H. Wallace, Jr., THIS JOURNAL, **54**, 2705 (1932).

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RECEIVED NOVEMBER 12, 1956

LABELING OF BENZENE WITH A CARBON-14 ION BEAM[1]

Sir:

A recent communication[2] described the production of labeled organic material with accelerated tritium ions, and the suggestion was made that C^{14} ions might be used for the same purpose. The labeling of organic compounds by C^{14} atoms derived from the $N^{14}(n,p)C^{14}$ reaction has been reported by several workers.[3] However, organic compounds thus labeled (using a nuclear reactor) are subjected to high neutron and gamma ray fluxes, and, in addition, the energy of the C^{14} atom (about 45,000 e.v.) is fixed. Both of these factors cause radiation decomposition to become a serious problem. During the past several months we have been observing the effects produced on irradiating solid benzene with a C^{14} ion beam in a mass spectrometer. Work in this direction has already been reported by Croatto and Giacomello.[4] Since there is great theoretical and practical interest in this type of experiment, we wish to present the results we have obtained in determining the efficiency of a C^{14} ion beam in labeling solid benzene. Benzene was chosen for this work because it is resistant to radiation decomposition and because its purification may be carried out with high efficiency by means of vapor-phase chromatography.

The carbon-14 ion beam was obtained using a 60°-sector, 15-cm. radius mass spectrometer. Labeled carbon dioxide (23% C^{14}) was introduced into the ion source and the singly charged, mass-14 beam thus produced (energy = 2 kv.) amounted to between 10^{-9} and 10^{-10} amp. During the irradiations, benzene vapor was directed slowly and continuously onto the surface of a cold trap at the target end of the spectrometer. This trap was maintained at $-155 \pm 5°$; a lower temperature would have led to condensation of carbon dioxide from the ion source.

The irradiated benzene (about 500 mg.) was purified by repeated passages through vapor-phase chromatographic columns[5] (elution-partition type); two different substrate liquids, silicone and Carbowax, were used.[6] Since labeled toluene is also a possible product in these irradiations,[7] toluene was added to the benzene after an irradiation and the toluene fraction was purified on the chromatographic columns along with the benzene. The specific radioactivity of the benzene and toluene fractions was determined by liquid-scintillation counting.

Two passages through Carbowax columns are

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(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) R. Wolfgang, T. Pratt and F. S. Rowland, THIS JOURNAL, **78**, 5132 (1956).

(3) *E.g.*, P. E. Yankwich, *et al.*, *J. Chem. Phys.*, **14**, 131 (1946); G. Giacomello, *Ricerca sci.*, **21**, 1211 (1951); A. P. Wolf and R. C. Anderson, THIS JOURNAL, **77**, 1608 (1955); A. G. Schrodt and W. F. Libby, *ibid.*, **78**, 1267 (1956).

(4) Abstracts of the Naples, 1954, Meeting of the Italian Society for Scientific Progress (S.I.P.S.). See also *Ricerca sci.*, **26**, 529 (1956).

(5) We wish to thank Drs. K. P. Dimick and J. Corse, Department of Agriculture, Western Utilization Research Branch, Albany, California, for the use of their vapor-phase chromatography apparatus.

(6) K. P. Dimick and J. Corse, *J. Food Tech.*, **10**, 360 (1956).

(7) A. G. Schrodt and W. F. Libby, THIS JOURNAL, **78**, 1267 (1956).

enough to bring both the benzene and the toluene to constant specific activity. Further passages through either carbowax or silicone lead to no diminution in the specific activity, and the front and back parts of a given peak, or fraction, of benzene have the same specific activities. Within a factor of two (the insecurity in our knowledge of the C^{14} ion beam intensity) we have found that approximately 2% of the C^{14} ions striking the benzene replace a C^{12} atom and form a C^{14}-labeled benzene molecule. About half this figure, or 1%, of the ions are found as C^{14}-labeled toluene. At the present time no other products of irradiation have been identified.

Our experiments indicate that organic compounds may be labeled to a comparatively high specific activity by this technique. A methyl homolog, such as toluene, may be 100% labeled. In the case of the benzene, we find that a 10^{-9} amp. beam running for 10 hours gives about 1,000 dis./min. in the chromatographically purified product. If one were to use a microamp. beam one should be able to get approximately one microcurie of C^{14} into a few milligrams of benzene in one day's irradiation.

[8] U. S. Foreign Operations Administration Fellow, 1954–56.

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RECEIVED NOVEMBER 19, 1956

DEMONSTRATION OF INTERMEDIATE FORMS OF CARBONMONOXY- AND FERRIHEMOGLOBIN BY MOVING BOUNDARY ELECTROPHORESIS

Sir:

Oxidation of carbonmonoxyhemoglobin to ferrihemoglobin by ferricyanide results in the acquisition of a positive charge at acid *p*H and the release of carbon monoxide by each heme (Hb) oxidized. Since each hemoglobin molecule contains four hemes, the reaction occurs in four successive steps: $(HbCO)_4 \rightarrow (HbCO)_3Hb^{+1} \rightarrow (HbCO)_2Hb_2^{+2} \rightarrow (HbCO)Hb_3^{+3} \rightarrow Hb_4^{+4}$.

Although attempts have been made in the past to obtain physical evidence for intermediates in hemoglobin reactions,[1] the present experiments, in which $(HbCO)_3Hb^{+1}$, $(HbCO)_2Hb_2^{+2}$, and $(HbCO)Hb_3^{+3}$ were separated by moving boundary electrophoresis, provide the first conclusive demonstration of their existence.

Aliquots of normal human adult carbonmonoxyhemoglobin solution were diluted to about 1 g./100 ml. with potassium phosphate buffer of ionic strength 0.01 and *p*H 6.85 (measured at 25°), and 0.01 *M* $K_3Fe(CN)_6$ was added in volumes equivalent to about 25% to a three-fold excess of hemes present. The reaction mixtures were further diluted with buffer to a hemoglobin concentration of 0.5 g./100 ml. and allowed to stand overnight under carbon monoxide at 4° after which they were dialyzed for at least 40 hours with two or three changes of buffer to remove ferricyanide and ferrocyanide ions. The dialyzed solutions were analyzed in the same buffer in a Spinco Model H electrophoresis instrument at 0.5°. The percentage of each sample oxidized to ferrihemoglobin was determined in a Cary model 14 recording spectrophotometer. Spectrophotometric measurements in a series of phosphate buffers of ionic strength 0.01 indicated that ferrihemoglobin is almost entirely in the acidic form at *p*H 6.85.[2] When mixtures prepared from carbonmonoxyhemoglobin ($(HbCO)_4$) and ferrihemoglobin (Hb_4^{+4}) were analyzed electrophoretically, neither the alteration of proportions nor the appearance of components of intermediate mobility was discernible; thus, no detectable intermolecular reaction occurs during electrophoresis. On the other hand, each of the samples prepared by partial oxidation of $(HbCO)_4$ had components with intermediate mobilities. Since boundary anomalies may interfere with the interpretation of electrophoretic patterns obtained in dilute buffers,[3] control experiments with known mixtures were performed. It was found that proportions obtained from ascending limb patterns agreed well with the true proportions present. Therefore, it was possible to identify the components in each sample by direct comparison of electrophoretic and spectrophotometric analyses. For example, Fig. 1 shows the pattern obtained from a preparation that was 22% oxidized according to its absorption spectrum. The only interpretation consistent with both analyses is that, of the three major components, the slow, intermediate, and fast components are $(HbCO)_4$, $(HbCO)_3Hb^{+1}$, and $(HbCO)_2Hb_2^{+2}$, respectively. The small component migrating ahead of the others is probably $(HbCO)Hb_3^{+3}$. As the percentage oxidized was increased, components identified as $(HbCO)Hb_3^{+3}$ and Hb_4^{+4} appeared in increasingly higher proportions. More detailed descriptions and analyses of these experiments will be published.

Fig. 1.—Ascending limb pattern of 22% oxidized carbonmonoxyhemoglobin after electrophoresis for 22,440 sec. at 9.08 volts/cm. Total hemoglobin concentration 0.5 g./100 ml. in potassium phosphate buffer of *p*H 6.85, ionic strength 0.01; δ-boundary at right.

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[1] For discussions, see J. B. Conant, *Harvey Lectures*, Ser. 28, 159 (1932–1933); R. Lemberg and J. W. Legge, "Hematin Compounds and Bile Pigments," Interscience Publishers, Inc., New York, N. Y., 1949, p. 271.

[2] For *pK* determinations of ferrihemoglobin at low ionic strength, see P. George and G. Hanania, *Biochem. J.* (*London*), **55**, 236 (1953).

[3] L. G. Longsworth, *J. Phys. Colloid Chem.*, **51**, 171 (1947).

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RECEIVED NOVEMBER 7, 1956

A CONVENIENT METHOD OF LOCATING SUBSTITUENTS ON THE HYDROCARBON CHAIN OF MOLECULES ADDUCTING WITH UREA

Sir:

Attention is called to the series of continuous layer lines which form when a Laue X-ray diffraction pattern is taken of a urea (or thiourea) adduct single crystal. Those lines may be interpreted as