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.

RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

RICHARD M. LEMMON
FRANCO MAZZETTI[8]
FREDERICK L. REYNOLDS
MELVIN CALVIN

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

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.

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.

(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).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
BETHESDA 14, MARYLAND

HARVEY A. ITANO
ELIZABETH ROBINSON

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

| Order of reflection | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Substituted p.a. | $I_c$ | 275 | 0 | 15 | 12 | 2 | 2 | 7 | 0 | 2 | 4 |
| 4-Keto-p.a. | $I_0$ | st | ... | wk$^+$ | wk$^+$ | wk$^-$ | vwk | wk$^+$ | vwk | vwk | wk |
| 4-Hydroxy-p.a. | $I_0$ | st | ... | wk$^+$ | m | wk$^-$ | wk | m | vwk | wk | wk$^+$ |
| 11-Substituted p.a. | $I_c$ | 94 | 7 | 87 | 2 | 2 | 0 | 3 | 13 | 1 | 3 |
| 11-Keto-p.a. | $I_0$ | m$^-$ | vwk | m$^+$ | ... | vwk | ... | vwk | wk$^+$ | ... | vwk |
| 11-Hydroxy-p.a. | $I_0$ | m$^-$ | wk | st | wk | wk | vwk | wk$^-$ | m$^+$ | wk | wk$^+$ |
| 12-Substituted p.a. | $I_c$ | 80 | 2 | 87 | 8 | 10 | 0 | 0 | 2 | 2 | 13 |
| 12-Keto-p.a. | $I_0$ | m$^+$ | ... | st | m$^-$ | m | ... | ... | wk | wk | m |
| 12-Hydroxy-p.a. | $I_0$ | wk$^+$ | vwk | m | wk | wk | ... | ... | wk$^-$ | wk$^-$ | wk |

p.a. = palmitic acid, st = strong, m = medium, wk = weak, v = very, $I_c$ = calculated intensity, $I_0$ = observed intensity.

being produced by the "guest" molecules behaving as a one-dimensional crystal along the channel direction of the "host" structure, and the molecular length is readily calculated from the line positions.[1]

We have now examined the intensity sequence at the centers (corresponding to $(00l)$-reflections) of such lines, produced by a series of compounds where the chain length is held constant and the position of a substituent on the chain is varied. In general, a unique pattern of intensities was found for each position isomer, suggesting the possibility of determining the position of substituents on a hydrocarbon chain. For all of the substituted palmitic acids examined (twelve keto-acids and six hydroxy-acids) very good agreement was obtained between the calculated and the observed intensities of the lines, three examples of each series of which are listed. The same calculated values apply to methyl substituted acids.

In such a one-dimensional problem the calculation of relative intensities $I_c$ $(00l)$ can be done by the formula

$$I_c\ (00l) = C_\theta \cdot |F_{(00l)}|^2$$

where

$$C_\theta = (1 + \cos^2 2\theta)/\sin 2\theta$$

and

$$F_{(00l)} = \sum_{n=1}^{N} f_n\ (\cos 2\pi l z_n + i \sin 2\pi l z_n)$$

or

$$F_{(00l)} = 2\sum_{n=1}^{N/2} f_n \cos 2\pi l z_n$$

for the general and the centrasymmetrical cases, respectively. The function $C_\theta$ of the diffraction angle $\theta$ corrects for the Lorentz factor and the polarization factor. In the formula for the structure factor $F_{(00l)}$, $f_n$ is the atomic scattering factor and $z_n$ the coordinate in the chain direction of the $n^{th}$ atom, taking the period length of the adducted compound (repeat distance) as unity, whereas $l$ is the Laue index of the reflection order. The summation is taken over all N atoms of a repeat unit, or if the origin is put on a center of symmetry, over all $N/2$ centrosymmetrical pairs of atoms.

For the palmitic acid dimer, $c$ = 45.1 Å. Since the hydrocarbon chains are nearly stretched out in the urea channels, the coördinates $z_n$ were computed assuming 1.26 Å. and 3.74 Å. for the $c$-projection of the C–C single bond length and of the van der Waals distance between two neighboring methyl end groups, respectively. For simplicity, a uniform atomic scattering factor, $f_n$, was introduced for all carbon and oxygen atoms; this is justified, since carbon on the average is connected to two hydrogen atoms thus having the same number of electrons as oxygen has. The good agreement between calculated and observed intensities shows that calculations based upon such a simplified model are adequate for identification purposes.

More details and applications will appear in *Zeitschrift für Kristallographie.*

The compounds were kindly supplied by Dr. Robert Meyer, Dept. of Pharmacy, University of Wisconsin, Madison, Wisconsin, and we thank Dr. K. C. Peng for taking most of the X-ray photographs.

(1) N. Nicolaides and F. Laves, THIS JOURNAL, **76**, 2596 (1954); other references found there.

(2) Department of Medicine, The University of Chicago, Chicago 37, Illinois. Guggenheim Fellow for the year 1955–56.

MINERALOGISCHES INSTITUT
EIDG. TECHN. HOCHSCHULE
ZÜRICH, SWITZERLAND

N. NICOLAIDES[2]
F. LAVES
A. NIGGLI

RECEIVED NOVEMBER 2, 1956

## FORMATION OF AN ORGANO BORON PEROXIDE BY REACTION OF OXYGEN AND TRIMETHYLBORANE[1]

*Sir:*

The reaction of trimethylborane with oxygen at pressures below the explosion limit produces a 1:1 reaction product as previously reported by Bamford and Newitt[2] and observed by Coleman.[3] However, we have found that this product is not

TABLE I

| Molar flow ratio $O_2:B(CH_3)_3$ | % $B(CH_3)_3$ reacted | Molar product[a] $O_2:B(CH_3)_3$ | Milliequivalents[b] oxidizing power per g. |
|---|---|---|---|
| 0.8:1 | 63 | 0.91:1 | .... |
| 1.0:1 | 73 | 0.95:1 | .... |
| 1.2:1 | 92 | 0.91:1 | .... |
| 1.4:1 | 95 | 1.03:1 | .... |
| 1.5:1 | 96 | 0.98:1 | .... |
| 2.0:1 | 99 | 0.96:1[c] | 11.02 ± 0.05 |
| 2.3:1 | 99 | 0.93:1[c] | 11.15 ± .05 |
| 2.0:1 | 99 | ... | 11.00 ± .02 |

[a] Calculated from the weight of product and the amount of $B(CH_3)_3$ consumed in the reaction. [b] One gram $(CH_3)_2BOOCH_3$ is equivalent to 11.38 millimoles. Reported values are the means of six determinations in each case. Neither $B(CH_3)_3$ nor $CH_3B(OCH_3)_2$ liberate iodine by the procedure employed. [c] Molecular weight determinations on product from these preparations gave $M$ = 88.5 and 87.4.

(1) This work was supported in part by the Ohio State University Research Foundation.

(2) C. H. Bamford and D. M. Newitt, *J. Chem. Soc.*, 695 (1946).

(3) J. E. Coleman, J. A. Lovinger, R. C. Petry and F. H. Verhoek, Final Report OSU Research Foundation Project 116-C, June 16, 1955.