*Anal.* Calcd. for $C_6H_{11}N_5O_2$: C, 38.91; H, 5.99; N, 37.80; $CH_3O$, 33.53. Found: C, 38.92; H, 5.93; N, 37.72; $CH_3O$, 33.78.

An attempt to carry out this reaction in water solution was unsuccessful. Refluxing began around 80°, the solution turned progressively darker, and the temperature dropped to 60°. Apparently the methoxyl groups of the nitrile are easily hydrolyzed by aqueous alkali, but not by alcoholic alkali, before reaction with dicyandiamide takes place.

**Diethoxyacetoguanamine.**—A mixture of the diethoxyacetonitrile (25.8 g., 0.2 mole), dicyandiamide (18.6 g., 0.22 mole), potassium hydroxide (0.66 g., 0.01 mole) and 2-methoxyethanol (30 ml.) was heated as described above and worked up in the same manner, giving 40 g. (94%) of white crystalline product, m.p. 195–197°. It was recrystallized from 60% aqueous ethanol; m.p. 194–194.5°. It is thermally stable at least to 260°.

*Anal.* Calcd. for $C_8H_{15}N_5O_2$: C, 45.04; H, 7.09; N, 32.83; $C_2H_5O$, 42.25. Found: C, 45.26; H, 7.23; N, 32.72; $C_2H_5O$, 42.17.

When the quantities of reagents were tripled in this reaction, the exothermic reaction became so violent that half of the contents of the flask were expelled. Attempts to moderate the violence of this reaction were only partially successful. Portionwise addition of the dicyandiamide to the other reagents gave a 48% yield of the guanamine. Addition of the nitrile to the hot mixture of dicyandiamide and alkali gave an 84% yield on a 0.1-mole scale but only 38% on a 0.35-mole scale. When 2-methoxyethanol was replaced by 1-butanol, the reaction rate was decreased (probably because of lower boiling point and lower solubility of dicyandiamide) without impairing the yield; foaming, however, was troublesome. Weaker bases such as piperidine and potassium carbonate gave only dark, tarry products. Use of water as solvent caused hydrolysis of the nitrile.

**Di-*n*-butoxyacetoguanamine.**—A mixture of dibutoxyacetonitrile (13.0 g., 0.07 mole), dicyandiamide (6.7 g., 0.08 mole), sodium hydroxide (0.3 g., 0.007 mole) and 2-methoxyethanol (35 ml.) was heated to 110°. A mildly exothermic reaction caused the temperature to rise to 130°. The mixture was refluxed for 15 minutes, cooled and poured into water. The filtered and dried product was a white, crystalline material, 16.4 g. (87.2%), m.p. 165–168°. It was recrystallized from methanol; m.p. 166.5–167.5°.

*Anal.* Calcd. for $C_{12}H_{23}N_5O_2$: C, 53.50; H, 8.61; N, 25.97. Found: C, 53.62; H, 8.35; N, 25.91.

The reaction proceeded similarly when larger amounts of material were used.

**Bis-(2-ethyl-1-hexyloxy)-acetoguanamine.**—A mixture of bis-(2-ethyl-1-hexyloxy)-acetonitrile (38.2 g., 0.13 mole), dicyandiamide (16.0 g., 0.19 mole), potassium hydroxide (1.0 g., 0.015 mole) and 2-methoxyethanol (50 ml.) was heated as described above for the butyl analog. Ammonia was evolved and insoluble material (melamine[7] and similar materials) formed. The insoluble material was removed by filtration and the filtrate was poured into several volumes of water. The guanamine precipitated as an oil which rapidly hardened to a white, waxy solid. This was washed with water in a Waring blendor and dried. The weight of the crude product was 47.5 g. (96%), m.p. *ca.* 100°. It was recrystallized from hexane; m.p. 115–116°.

*Anal.* Calcd. for $C_{20}H_{39}N_5O_2$: C, 62.97; H, 10.31; N, 18.34. Found: C, 62.91; H, 10.26; N, 18.43.

**Attempted Experiments.**—α,α-Dimethoxypropionitrile did not give a guanamine under the conditions used in this study. It did not react exothermically with dicyandiamide and, after refluxing, a very dark solution was obtained which apparently contained none of the desired guanamine.

Heating dialkoxyacetoguanamines in the presence of an acidic condensing agent, such as zinc chloride, transformed them into brittle resins with the loss of the etherifying alcohol.

(7) Dicyandiamide, when heated under alkaline conditions, loses ammonia to form melamine and more complex triazine condensation products. This is especially true in the guanamine synthesis when relatively unreactive nitriles are used. See, for example, D. W. Kaiser, U. S. Patent 2,606,904 (Aug. 12,1952).

AMERICAN CYANAMID CO.
STAMFORD, CONNECTICUT

# COMMUNICATIONS TO THE EDITOR

## A POLAROGRAPHIC INVESTIGATION OF THE MECHANISM OF MUTAROTATION OF *d*-GLUCOSE

*Sir:*

It has been shown previously[1] that the polarographic reduction of equilibrium *d*-glucose is a completely rate-controlled process in a $10^{-2}$ molar solution of LiOH and in a phosphate buffer.

In the present case a solution which was 0.655 molar in *d*-glucose, 0.0183 molar in $NaH_2PO_4$, 0.0458 molar in $Na_2HPO_4$ and 0.0916 molar in LiCl gave a wave height of the limiting current of glucose of 57 mm. These current–voltage curves were recorded with a Sargent–Heyrovsky Model XII polarograph at 25°, some 40 minutes after the α-glucose was dissolved, and at $1/5$ of the maximum galvanometer sensitivity (0.0052 μa./mm.). Within the limits of the accuracy obtained this wave height was independent of the height of the mercury level. Immediately after dissolving the α-glucose a current–voltage wave was obtained with a limiting current considerably higher than 57 mm. This limiting current decreased with time, approaching the equilibrium value of 57 mm. The change in wave height was recorded as a function of time, yielding a continuous current–time curve of glucose in the phosphate buffer at 25°.

If the mechanism of mutarotation is considered to be essentially

$$\alpha \underset{k_1'}{\overset{k_1}{\rightleftarrows}} \mu \underset{k_2}{\overset{k_2'}{\rightleftarrows}} \beta \qquad (1)$$

where μ presumably is the aldehyde form, then the kinetic current due to α- and β-glucose separately is given by (*cf.*[2,3])

$$i_k(\alpha) = nF \times 10^{-3} \times 3/5 \times 0.85(mt)^{2/3} \sqrt{D}(k_1/\sqrt{k_1' + k_2'})C_\alpha \qquad (2)$$

and

$$i_k(\beta) = nF \times 10^{-3} \times 3/5 \times 0.85(mt)^{2/3} \sqrt{D}(k_2/\sqrt{k_1' + k_2'})C_\beta \qquad (3)$$

where all symbols have their conventional meaning ($mt$ = 0.00475 g., $D = 6.16 \times 10^{-6}$ cm.²/sec.)[4].

(1) K. Wiesner, *Collection Czechoslov. Chem. Commun.*, **12**, 64 (1947).

(2) K. Wiesner, *Chem. Listy*, **41**, 6 (1947).

(3) J. Koutecky and R. Brdicka, *Collection Czechoslov. Chem. Commun.*, **12**, 337 (1947).

(4) L. Friedman and P. G. Carpenter, THIS JOURNAL, **61**, 1745 (1939).

The concentration of the $\beta$ form present in the solution at the time $t$ being given by $x = x_\infty(1 - e^{-kt})$, it follows that

$$\ln(i_k - i_{k,\infty}) = \ln 0.00690x_\infty(k_1 - k_2)/\sqrt{k_1' + k_2'} - kt \quad (4)$$

where

$i_k$ = total kinetic limiting current at time $t$
$i_{k,\infty}$ = total kinetic limiting current at time $\infty$
$= 0.00690\,[k_1a + (k_2 - k_1)x_\infty]/\sqrt{k_1' + k_2'}$ (5)
$= 1.43\,\mu$A.
$a$ = total concentration of the glucose = 0.655 mole/liter
$k$ = conventional first order rate constant of mutarotation[5]
$= 1/2[k_1 + k_1' + k_2 + k_2' - \sqrt{(k_1 + k_1' - k_2 - k_2')^2 + 4k_1'k_2'}]$[6]

or, if $k_1', k_2' \gg k_1, k_2$

$$k = (k_1k_2' + k_1'k_2)/(k_1' + k_2') \quad (6)$$

The over-all equilibrium constant is

$$x_\infty/(a - x)_\infty = k_1k_2'/k_1'k_2 = 1.740 \quad (7)[6]$$

A plot of ln $(i_k - i_{k,\infty})$ *vs.* $t$, derived from the current–time curve, could be fitted quite well by a straight line, as required by equation (4). The slope of this line is

$$k = 3.17 \times 10^{-3}\ \text{sec.}^{-1} \quad (8)$$

and the intercept is

$$\ln 0.00690x_\infty(k_1 - k_2)/\sqrt{k_1' + k_2'} = 0.115 \quad (9)$$

From the equations (5), (6), (7), (8) and (9), values for the constants in equation (1) are found: $k_1 = 5.80 \times 10^{-3}$ sec.$^{-1}$, $k_2 = 1.77 \times 10^{-3}$ sec.$^{-1}$, $k_1' = 69$ sec.$^{-1}$, $k_2' = 37$ sec.$^{-1}$, from which values the concentration of the free aldehyde form may be calculated. It was found to be $2.0 \times 10^{-5}$ mole/liter, which is 0.0030% of the total glucose concentration.

A more complete report will be published later, together with work now in progress pertaining to a more detailed elucidation of the mechanism as given by equation (1).

(5) T. M. Lowry and W. T. John, *J. Chem. Soc.*, **97**, 2634 (1910).
(6) J. C. Kendrew and E. A. Moelwyn-Hughes, *Proc. Roy. Soc. (London)*, **A176**, 352 (1940).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NEW BRUNSWICK
FREDERICTON, N. B., CANADA
J. M. LOS
K. WIESNER

RECEIVED NOVEMBER 9, 1953

## A NEW SOLUBLE CYTOCHROME

*Sir:*

*Rhodospirillum rubrum*, a photosynthetic heterotrophic bacterium, contains large amounts of a haemprotein which may be obtained by treatment with warm trichloroacetic acid, by extraction with phosphate buffer from cell residues after acetone treatment and by sonic disruption of cell suspensions. The protein so obtained can be purified in good yield by a modified Keilin–Hartree procedure.[1] The same protein is also obtained with similar yields from another photoheterotrophe, *Rhodopseudomonas spheroides*. Although this haem protein (I) possesses many properties usually attributed to mammalian cytochrome-c (II) it is, in fact, a new cytochrome. Thus, although I, like II, can be reduced with DPNH via DPNH-cytochrome-c reductase (prepared either from *R. rubrum* or pig heart), is not auto-oxidized, can be reversibly reduced or oxidized by reagents such as the ferro-ferricyanide couple, hydrosulfite and ascorbate, and exhibits an absorption spectrum in the visible identical with that of reduced II, it differs from II in the following important particulars: (a) I is not oxidized in air in the presence of the cytochrome oxidase system, whether the enzyme is prepared from pig heart, pig kidney or rat kidney. Preparations of I, purified electrophoretically (see below), do not inhibit the oxidase, as evidenced by unabated activity of the enzyme in catalyzing oxidation of II in the presence of I. (b) I is not absorbed on the $NH_4^+$ form of Amberlite IRC-50 ion exchange resin, prepared according to the directions of Margoliash.[2] (c) I

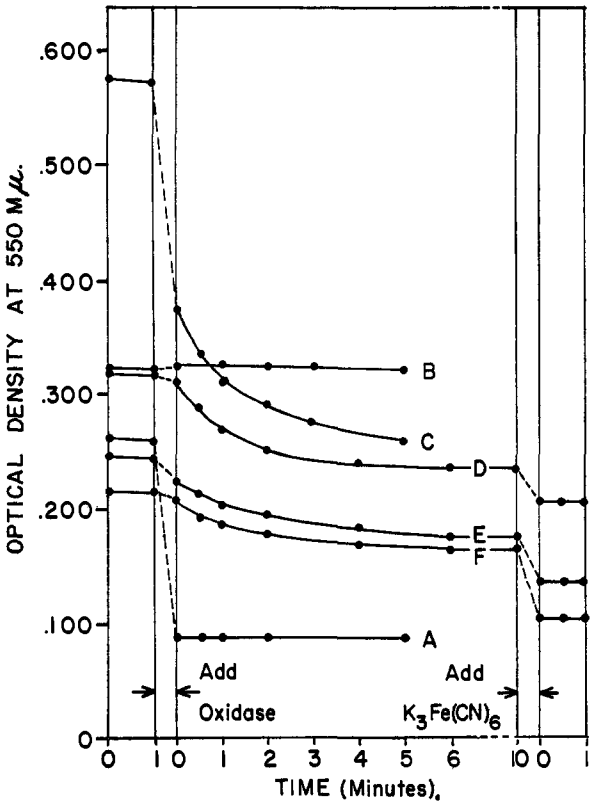



Fig. 1.—Action of cytochrome oxidase on bacterial cytochrome: tests were performed with a Beckman DU spectrophotometer, using absorption cells in which were placed 1.0-ml. volumes of solutions containing 50 $\mu$moles of phosphate buffer *p*H 7.4, 0.4 $\mu$mole of $AlCl_3$, 1 mg. of protein of a pig heart cytochrome oxidase preparation and cytochrome preparations as indicated. Reduced cytochromes were prepared with stoichiometric amounts of ascorbic acid. Oxidase was added to the test systems after an initial one minute period of observation as well as in equal amounts to the water blank. Mixing time was about 15 seconds and all values for optical density prior to mixing were corrected for the dilution: A, 0.01 $\mu$mole of reduced cytochrome c; B, 0.012 $\mu$mole of reduced bacterial cytochrome; C, 0.01 $\mu$mole of reduced cytochrome c plus 0.012 $\mu$mole of reduced bacterial cytochrome. D, E and F all contain 0.008 $\mu$mole of reduced bacterial cytochrome with 0.01, 0.004 and 0.002 $\mu$mole of oxidized cytochrome c, respectively.

(1) L. P. Vernon, *Arch. Biochem. Biophys.*, **43**, 492 (1953).
(2) E. Margoliash, *Nature*, **170**, 1014 (1950).

can be only partially oxidized by a ferro–ferricyanide oxidation–reduction buffer (20:1) under conditions which permit complete oxidation of II. (d) The cathodic mobility of I on ionophoresis in 1% ammonium acetate on Whatman no. 5 paper is less than that of II by at least one order of magnitude.

Preparations of I, as obtained by the Keilin–Hartree procedure, usually exhibit a purity of 10–50%, based on spectral absorption properties and the assumption that the extinction coefficients at the characteristic absorption maxima are closely similar to those for II. Electrophoretic treatment of such preparations results in a 2- or 3-fold purification because of removal of colorless impurities which migrate rapidly toward the cathode.

The conclusion that I is not identical with II is further strengthened by the observation that I can be oxidized via mammalian cytochrome oxidase, if some II is added to the reaction mixture (see Fig. 1). Complete oxidation in this manner cannot be attained, indicating in agreement with (c) above, that the oxidative–reduction potential of I is slightly positive with respect to II.

It is tempting, but premature, to discuss the many intriguing possibilities raised by the presence of this new cytochrome in photosynthetic organisms. We may note, however, that the existence of the bacterial cytochrome may provide the basis for a mechanism of photochemical H-transport and an explanation for the absence of a light-stimulated respiration in the photoheterotrophes. It is suggested that I be called tentatively "cytochrome-c2".[3] Further researches on the properties of this and other haem-proteins in the photosynthetic bacteria are proceeding.

The investigations at Washington University have been made possible by grants from the C. F. Kettering Foundation and the U. S. Public Health Service. We are indebted to Dr. S. Velick, Dept. of Biochemistry, Washington University Medical School, for aid in the experiments on electrophoretic purification of the bacterial cytochrome.

(3) R. Hill and D. Keilin, private communication.

A.R.C. UNIT OF MICROBIOLOGY, THE UNIVERSITY
SHEFFIELD, ENGLAND S. R. ELSDEN
THE EDW. MALLINCKRODT INSTITUTE
OF RADIOLOGY, WASHINGTON
UNIVERSITY MEDICAL SCHOOL M. D. KAMEN
SAINT LOUIS, MISSOURI L. P. VERNON

RECEIVED DECEMBER 3, 1953

## BEHAVIOR OF AN ION-EXCHANGE RESIN IN LIQUID AMMONIA

*Sir:*

In the course of a study of ion species present in liquid ammonia solution, use has been made of the cation exchange resin Dowex-50.[1] The ammonium form of the resin, dried 16 hours *in vacuo*, was washed copiously at −33° by repeatedly condensing fresh anhydrous ammonia upon it and then filtering. The resin was exposed, in a column operation, to a solution of liquid ammonia which contained the products of the reaction of potassium with monoammino boron trifluoride[2,3] (0.1901 g. K, 0.4380 g. $BF_3 \cdot NH_3$).

The total product from the 0.6281 g. of reactants dissolved in about 35 ml. of ammonia, was passed through a resin column, one cm.$^2$ × 12 cm. at a rate that varied between 1 and 2 ml. per minute. The resin, which was largely in the ammonium form, quantitatively removed potassium from the solution. Boron and fluorine passed through the resin bed. The eluate solution was evaporated to dryness and the solid residue, which weighed 0.4457 g. after evacuation, was shown to be mainly $BF_3 \cdot NH_2$ by X-ray diffraction analysis.[4]

After the resin column had been washed until the washings gave negative tests for boron, a solution of ammonium chloride was passed through the column. The 11.7 g. of resin had a calculated capacity of 58.5 meq. cation, 10.9 meq. of potassium being on the resin after two exchange experiments. The passage of a total of 134 meq. ammonium ion (in approx. 1.5 *M* $NH_4Cl$ solution) through the column resulted in the elution of only 0.4 meq. potassium.

The behavior of Dowex-50 in liquid ammonia parallels its behavior in water. A solution of ammonium chloride in ammonia behaves like a solution of hydrogen chloride in water: neither are efficient in stripping the resin of potassium ions. Potassium ion efficiently displaces ammonium ion from the resin in ammonia just as it displaces hydronium ion from the resin in water solution.

It appears that ion-exchange techniques of separations, purifications, and syntheses can be used successfully in the liquid ammonia medium.

(1) These studies were inspired by a paper which described the use of a cation exchange column in the study of $KBF_3OH$: see C. A. Wamser, THIS JOURNAL, **73**, 419 (1951).

(2) In a forthcoming publication it will be shown that $BF_3 \cdot NH_3$ reacts with *only one* equivalent of potassium in dilute ammonia solutions. When the resulting solution is evaporated to dryness and analyzed by X-ray diffraction the pattern of KF is found. Quantitative recovery of the total solid products and analysis of the gaseous and solid products support the following interpretation:

$$K + BF_3 \cdot NH_3 \longrightarrow KF + BF_2NH_2 + 1/2\ H_2$$

This equation accounts for all our observations, but it is emphasized that we have not as yet isolated the boron compound.

(3) For a description of the reaction of sodium with $BF_3 \cdot NH_3$ in liquid in ammonia see C. A. Kraus and E. H. Brown, *ibid.*, **51**, 2690 (1929).

(4) The X-ray powder diffraction of $BF_3 \cdot NH_3$ as obtained in this laboratory does not check with that reported by A. W. Laubengayer and G. F. Condike, *ibid.*, **70**, 2274 (1948). As prepared here in two ways, or as recrystallized from either water or liquid ammonia, the compound yields the following lines:

4.87 (m) 4.01 (s) 3.65 (s) 3.37 (s) 2.87 (m) 2.72 (s)
2.45 (m) 2.34 (m) 2.24 (s) 1.97 (w) 1.81 (m) 1.65 (w)

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TENNESSEE C. W. KEENAN
KNOXVILLE, TENNESSEE W. J. MCDOWELL

RECEIVED NOVEMBER 20, 1953

## THE STRUCTURE OF PITHECOLOBINE

*Sir:*

Some time ago we described an alkaloid from the bark of *Pithecolobium saman* (Benth.)[1] $C_{22}H_{46}N_4O_2$ (one amide group. one hydroxy group, one ring, no double bond) which on reduction with $LiAlH_4$ gave desoxypithecolobine $C_{22}H_{48}N_4$ (one ring, no double bond). Analytical difficulties with even highly

(1) K. Wiesner, D. M. MacDonald, Z. Valenta and R. Armstrong, *Can. J. Chem.*, **30**, 761–772 (1952).

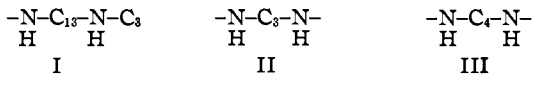
purified samples make it impossible to distinguish these formulas from those containing one more $CH_2$. We have now identified all the carbons as recognizable fragments and established thereby the $C_{23}$ formula. Desoxypithecolobine contains four secondary nitrogens, as by acetylation it gave a glassy *neutral* tetraacetyl derivative which by Li-$AlH_4$ reduction gave tetraethyldesoxypithecolobine, characterized by a homogeneous countercurrent distribution peak and distillation of the peak fractions (b.p. 211–212°, collar flask 0.05 mm.; found: C, 75.05; H, 13.22; N, 11.60). In this latter compound all nitrogens are tertiary as there is no N–H peak in the infrared and the compound is recovered unchanged from acetylation (identity of infrared spectrum).

Hofmann degradation of methylated desoxypithecolobine gave[1] in the first stage tetramethyl-1,4-*tetra*methylenediamine and a mixture of bases. The second stage performed on these bases gave a diene $C_{13}H_{24}$ (b.p. 107–112°, collar flask 12 mm., U.V. $\lambda_{max.}$ 227 m$\mu$, log $\epsilon$ = 4.38; found: C, 86.55; H, 13.26; uptake of hydrogen, 2 moles). Measurements of boiling point and molecular weight performed on the tetrahydro product distinctly favor the $C_{13}$ formula. There was further isolated[1] from the mixture of bases a mixture of $N_1$ bases analyzing quite well for $C_{17}H_{33}N$. These took up 2 moles of hydrogen on hydrogenation and on subsequent Hofmann degradation gave a hydrocarbon $C_{13}H_{26}$ and dimethyl-*n*-propylamine identified by mixed melting point and infrared spectrum of the picrate and analysis. The $C_{13}H_{26}$ hydrocarbon took up one mole of hydrogen and gave a hydrocarbon $C_{13}H_{28}$ (found: C, 84.77; H, 15.09; b.p. 129–132°, collar flask 40 mm.). This leaves only 3 carbons to be identified. These in view of the low N-$CH_3$ values obtained on desoxypithecolobine (found: (N)$CH_3$, 3.11) must form one fragment.

Hofmann degradation of methylated pithecolobine gave in the first stage a large neutral fraction which was hydrogenated, after which the individual compounds were separated by chromatography. In addition, tetramethyl-1,3-*tri*methylenediamine was identified in good yield by mixed melting point, analysis, and infrared spectrum of the picrate. From the hydrogenated neutral substances a compound $C_{13}H_{27}ON$ (m.p. 88–90°, found: C, 73.14; H, 12.72; N, 6.66) has been isolated which has all the characteristic infrared bands of a saturated primary amide. Further, a compound $C_{16}H_{33}ON$ (m.p. 49–50°, found: C, 75.14; H, 13.00; N, 5.52) with all the characteristic infrared bands of a saturated secondary amide was obtained. This on hydrolysis gave *n*-propylamine identified by infrared spectrum of the hydrochloride. As two $C_3$ fragments have now been obtained which must have a different origin, 23 carbons have thus been accounted for.

Chromatography of the neutral substances before hydrogenation gave a small yield of a monounsaturated primary amide $C_{13}H_{25}ON$ (m.p. 67–69°, found: C, 73.88; 12.02; N, 6.81). An oily secondary amide was obtained which on acid hydrolysis slowly liberated propionic aldehyde identified by paper chromatography and ultraviolet absorption spectrum of the 2,4-dinitrophenylhydrazone. This may be explained by an isomerization and hydrolysis of an allylamide.

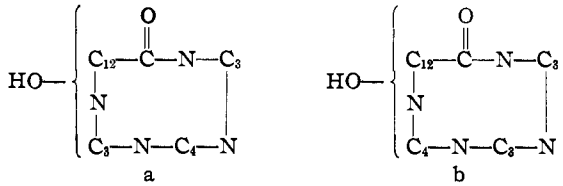
Keeping in mind the secondary nature of all nitrogens in desoxypithecolobine the presence of



I II III

has been demonstrated.

The evidence thus points to structure (a) or (b)



a b

for pithecolobine and the corresponding structures, with the amide and hydroxy group reduced, for desoxypithecolobine. On the basis of indirect suggestive evidence to be published later, we favor (a) for the arrangement of fragments.

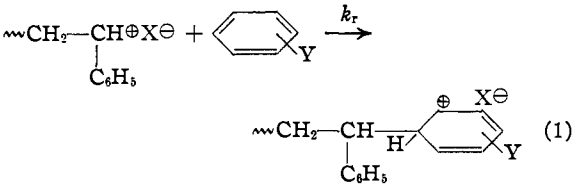
CHEMISTRY DEPARTMENT K. WIESNER
UNIVERSITY OF NEW BRUNSWICK D. M. MACDONALD
FREDERICTON, N. B., CANADA C. BANKIEWICZ

RECEIVED NOVEMBER 10, 1953

## IONIC POLYMERIZATION. A METHOD FOR MEASURING THE RELATIVE RATES OF ATTACK OF A CARBONIUM ION PAIR ON AROMATIC COMPOUNDS IN HOMOGENEOUS SOLUTION. NUCLEOPHILICITY FACTORS

*Sir:*

We have been able to utilize the useful chain transfer equation developed for free radical polymerization[1] as a sensitive method for measuring quantitatively the relative rates of attack of a carbonium ion pair on aromatic compounds in homogeneous solution. *With this treatment it is possible to assign a relative numerical factor, here called a nucleophilicity factor, for attack of such an ion pair on an aromatic nucleus*



(1)

It is of primary interest and importance that the Mayo equation (2) is applicable to substances which retard the rate of cationic catalyzed polymerization. This is not the case for free radical polymerization due to the occurrence of termination involving two radicals, which leads to dependence of the degree of polymerization on the radical concentration. Thus a typical $1/\overline{P}_n$ *vs.* [S]/[M] plot will not be linear for a retarder in a radical system. In a cationic chain process, however, it is reasonable to assume that termination involving two ion pairs does not occur, and the degree of polymerization should be independent of the concentration of ion pairs.

(1) F. R. Mayo, THIS JOURNAL, **65**, 2324 (1943).

Retardation and chain transfer were studied in the polymerization of styrene by stannic chloride catalyst in carbon tetrachloride–nitrobenzene solvent mixture. The data were correlated by means of the aforementioned equation which may be written as

$$\frac{1}{\bar{P}_n} - \frac{1}{\bar{P}_{no}} = \frac{k_r}{k_p}\frac{[R]}{[M]} \qquad (2)$$

[R]/[M] represents the mole ratio of retarder or chain transfer agent (R) to monomer, $k_r/k_p$ the ratio of the rate constant for reaction of ion pair with R to the propagation rate constant, and the terms $\bar{P}_n$ and $\bar{P}_{no}$ the number average degree of polymerization in the presence and absence of R, respectively.

For each compound reported in Table I polymerizations were carried out at about six different concentrations of R, and at least two control polymerizations containing no R were carried out at the same time. The concentrations of monomer, catalyst and nitrobenzene were the same in all runs, and the total volume per cent. of carbon tetrachloride plus R was also maintained constant.[2]

Polymerizations were ordinarily stopped at 10–15% conversion by precipitation of the polymer in methanol. The degrees of polymerization were determined from measurements of intrinsic viscosities in benzene solution at 30°, using the intrinsic viscosity–molecular weight relation of Pepper[3] for unfractionated polystyrene. $\bar{P}_{no}$ is about 200 under the conditions of our experiments, and the concentrations of R were chosen over a range so that at the highest concentration $\bar{P}_n$ was about one-third to one-half of $\bar{P}_{no}$.

In each case a plot of $1/\bar{P}_n - 1/\bar{P}_{no}$ *vs.* [R]/[M] is linear and passes through the origin. The values of $k_r/k_p$ obtained from the slopes of these plots are listed in Table I, together with relative values of $k_r$. These values are consistent with the mechanism proposed (1), based on qualitative knowledge of the Friedel–Crafts reaction.

TABLE I

POLYMERIZATION DATA[a]

| Compound (R) | $k_r/k_p$ | $k_r$ (relative) neucleophilicity factor[b] |
|---|---|---|
| *p*-Cymene | 0.0044 | 0.42 |
| *p*-*t*-Butyltoluene | 0.0062 | 0.69 |
| *p*-Chloroanisole | 0.0082 | 0.78 |
| *p*-Xylene | 0.0105 | 1 |
| Thiophene | 0.98 | 93 |
| Anisole | 1.62 | 154 |

[a] Initial monomer concentration = 1.95 *M*; stannic chloride concentration = 0.023 *M*; temperature 0°. [b] The value of $k_p$ is assumed to be identical in all cases; a value of $k_r = 1$ is arbitrarily assigned to *p*-xylene.

Relative rates of polymerization were measured in the absence and presence of additional compounds, using a precipitation technique. Over the measured range, about 10–80% conversion, under the conditions employed here the reaction is pseudo first order with respect to monomer.

The hydrocarbons have little or no effect on the rate, indicating that only chain transfer is occurring with these compounds. However, thiophene retards the rate strongly and anisole relatively weakly.

This method provides a sensitive means of studying the effect of different alkyl groups in mono- and higher substituted alkyl benzenes on the reactivity toward an ion pair of the general type involved in the Friedel–Crafts reaction. Where possible, such results could be compared with published data concerning complex formation with electrophilic species[4,5,6] and relative rates of halogenation.[7,8]

Other related problems are under investigation using this general experimental procedure.

We gratefully acknowledge the generous support of the Office of Naval Research.

(2) This was done in order to keep the dielectric of the medium approximately constant, since the aromatic hydrocarbons in the table all have dielectric constants close to that of carbon tetrachloride. In the case of *p*-chloroanisole, this procedure may have permitted a significant variation in solvent dielectric; for anisole and thiophene the concentrations employed were small (<0.025 *M*) and the dielectric was probably not appreciably affected.

(3) D. C. Pepper, *J. Polymer Sci.*, **7**, 347 (1951).

(4) L. J. Andrews and R. M. Keefer, THIS JOURNAL, **71**, 3644 (1949).

(5) D. A. McCaulay and A. P. Lien, *ibid.*, **73**, 2013 (1951); D. A. McCaulay, B. H. Shoemaker and A. P. Lien, *Ind. Eng. Chem.*, **42**, 2103 (1950).

(6) H. C. Brown and J. D. Brady, THIS JOURNAL, **74**, 3570 (1952).

(7) P. B. D. De la Mare and P. W. Robertson, *J. Chem. Soc.*, 279 (1943).

(8) E. Berliner and F. Berliner, THIS JOURNAL, **71**, 1195 (1949).

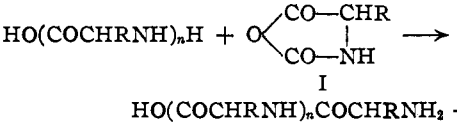
DEPARTMENT OF CHEMISTRY
INSTITUTE OF POLYMER RESEARCH C. G. OVERBERGER
POLYTECHNIC INSTITUTE OF BROOKLYN GERARD F. ENDRES
BROOKLYN 1, NEW YORK

RECEIVED SEPTEMBER 23, 1953

## THE MECHANISM OF POLYMERIZATION OF N-CARBOXY-α-AMINO ACID ANHYDRIDES

*Sir:*

The polymerization of N-carboxy-α-amino acid anhydrides (I) is believed to proceed according to reaction (1).[1,2] It was assumed, by analogy with



$$\text{HO(COCHRNH)}_n\text{H} + \underset{\text{I}}{\text{O}\!\!<\!\!\begin{matrix}\text{CO—CHR}\\ \text{CO—NH}\end{matrix}} \longrightarrow \text{HO(COCHRNH)}_n\text{COCHRNH}_2 + \text{CO}_2 \qquad (1)$$

the polymerization of ethylene oxide, that no termination reaction occurs, and that propagation proceeds by the addition of I to the terminal free amino group present in each polypeptide chain. Water-initiated polymerizations should therefore lead to polypeptides with equal numbers of amino and carboxyl terminal groups, while amine-initiated polymers should contain amino, but no carboxyl terminal groups. Furthermore, it should be possible to prepare poly-α-amino acids of very high molecular weight.

The fact that the N-carbonyl group of I reacts to a certain extent with sodium methoxide,[3] suggested to us that reaction (2), which constitutes a *termination* reaction, should also occur. Termina-

(1) S. G. Waley and J. Watson, *Proc. Roy. Soc.* (*London*), **A199**, 499 (1949).

(2) L. Gold, *J. Chem. Phys.*, **21**, 1190 (1953).

(3) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1154 (1953).

$$HO(COCHRNH)_nH + I \longrightarrow HO(COCHRNH)_nCONHCHRCOOH \quad (2)$$
II

tion of polymerization takes place when the amino group of a growing peptide chain reacts with carbon 2 of I, leading to an urea derivative (II), with the formation of a free carboxyl group. The presence of II (R = $CH_2C_6H_5$) in poly-DL-phenylalanine (prepared by bulk polymerization of I, R = $CH_2C_6H_5$, *cf.* substance 1 in the table) was demonstrated as follows: The polymer was hydrolyzed in acetic acid–hydrochloric acid and 5-benzylhydantoin-3-β-phenylpropionic acid[4] (III, R = $CH_2C_6H_5$) was separated from DL-phenylalanine by ether extraction. Both the racemic and *meso* forms of III were isolated (30 mg. from 1.5 g. of polymer) and identified by mixed melting points with authentic samples.

$$II \xrightarrow{H^+} (n-1)H_2NCHRCOOH + \text{III} \quad (3)$$

Since the polypeptides contain both terminated and unterminated chains, an excess of carboxyl groups is to be expected. The number average degree of polymerization should therefore be calculated from the total number of end groups (*i.e.*, both carboxyl and amino groups), present in the polymer.[5] We have found that the terminal amino and carboxyl groups of poly-α-amino acids can be determined by titration in anhydrous dimethylformamide with perchloric acid and sodium methoxide respectively, using thymol blue as an indicator. Results are summarized in the table.

TABLE I

| Poly-α-amino[a] acid | Number of terminal groups per amino acid residue: COOH (titr.) (A) | $NH_2$ (titr.) (B) | $NH_2$ (Van Slyke) (C) | Calcd. degree of polymerization: 1/C | 2/(A+B) |
|---|---|---|---|---|---|
| 1[b] | 0.033 | 0.011 | 0.010 | 100 | 45 |
| 2[c] | .048 | .017 | .014 | 72 | 31 |
| 3[d] | .118 | .059 | .059 | 17 | 11 |
| 4[d] | .091 | .020 | .019 | 53 | 18 |
| 5[d] | .072 | .016 | .016 | 63 | 23 |
| 6[d] | .056 | .016 | .012 | 83 | 28 |
| 7[e] | .143 | .012 | .010 | 100 | 7[f] |

[a] Methods of preparation *cf.* E. Katchalski, *Advances in Protein Chemistry*, 6, 123 (1951.) [b] Poly-DL-phenylalanine. [c] Poly-δ,N-carbobenzoxy-DL-ornithine. [d] Poly-ε,Nc-carbobenzoxy-L-lysine (different samples). [e] Poly-β,N-carbobenzoxy-DL-α,β-diaminopropionic acid (polymerization in anhydrous dioxane initiated by diethylamine). [f] Calculated from 1/A + B.

There is fairly good agreement between the amino group titration and the Van Slyke analysis. The table clearly illustrates the considerable excess of carboxyl groups over amino groups in the polypeptides investigated. This fact, as well as the presence of carboxyl groups in amine-initiated polymers,[6] and the relatively low molecular weight of the polymerization products, are readily explained by the termination reaction.

(4) F. Wessely and M. John, *Z. physiol. Chem.*, **170**, 98, 167 (1927); F. Wessely, K. Schlögl and G. Korger, *Monatsh.*, **83**, 1156 (1952).

(5) The corrected degrees of polymerization appear in column 6 of the Table. Hitherto the values appearing in column 5 of the table were considered to represent the average degree of polymerization.

(6) J. H. Fessler and A. G. Ogston, *Trans. Faraday Soc.*, **47**, 667 (1951).

DEPARTMENT OF BIOPHYSICS
WEIZMANN INSTITUTE OF SCIENCE
REHOVOTH, ISRAEL
MICHAEL SELA
ARIEH BERGER

RECEIVED OCTOBER 30, 1953

## DIRECT INTERACTION BETWEEN METAL ATOMS IN THE CRYSTALS OF BIS-(DIMETHYLGLYOXIME)-NICKEL(II) AND -PLATINUM(II)

*Sir:*

Previously the present writers have studied the dichroism of the crystals of Magnus green salt,[1,2] its related compounds,[2] and salts of tetracyanoplatinate(II),[3] and arrived at the conclusion that in the above crystals there exists a direct interaction between central platinum atoms of the planar complexes. Recently Godycki and Rundle[4] reported on the crystal structure of bis-(dimethylglyoxime)-nickel(II), suggesting the existence of a similar, though weak, interaction between nickel atoms. They have mentioned of the pleochroism of the nickel compound, but have not given any value for absorption coefficients. Moreover, their description on the pleochroism contradicts our results, though the conclusion reached agrees with ours. In this letter we wish to report on the results of the quantitative dichroism measurement, demonstrating the non-existence of the metal–metal interaction in the crystal of bis-(dimethylglyoxime)-copper and the possible existence of the metal–metal interaction in the crystals of bis-(dimethylglyoxime)-nickel(II) and -platinum(II).

Quantitative dichroism measurement by Tsuchida–Kobayashi's microscopic method[5] was performed with a microcrystal in the region from 2400 to 7000 Å. First, measurement was made with black prismatic crystals of bis-(dimethylglyoxime)-copper(II) (Fig. 1).[6] For the absorption band at the longest wave length region (Fig. 1), which is considered as due to transitions related to the metal–ligand linkages, a marked dichroism was observed. The following data were obtained: for ∥ absorption,[7] $\nu = 54 \times 10^{13}$/sec. and log $\alpha = 1.91$[8]; for ⊥ absorption, $\nu = 57.6 \times 10^{13}$/sec. and log $\alpha = 1.65$. The relation on the dichroism with this compound agrees with that induced for planar complexes of an ordinary type,[5b] indicating that there exists

(1) S. Yamada and R. Tsuchida, *J. Chem. Soc. Japan*, **70**, 44 (1949).

(2) S. Yamada, THIS JOURNAL, **73**, 1579 (1951).

(3) S. Yamada, *Bull. Chem. Soc. Japan*, **24**, 125 (1951).

(4) L. E. Godycki and R. E. Rundle, *Acta Cryst.*, **6**, 478 (1953).

(5) (a) R. Tsuchida and M. Kobayashi, "The Colours and the Structures of Metallic Compounds," Zoshindo, Osaka, Japan, 1944, p. 180. (b) See, for example, S. Yamada, THIS JOURNAL, **73**, 1182 (1951).

(6) About the crystal structure, see S. Bezzi, E. Bua and G. Schiavinato, *Gazz. chim. ital.*, **81**, 856 (1951).

(7) ∥ and ⊥ refer to results with polarized lights having their electric vectors parallel and perpendicular to the planes of the complexes, respectively.

(8) α denotes absorption coefficient per mm. of the crystal.

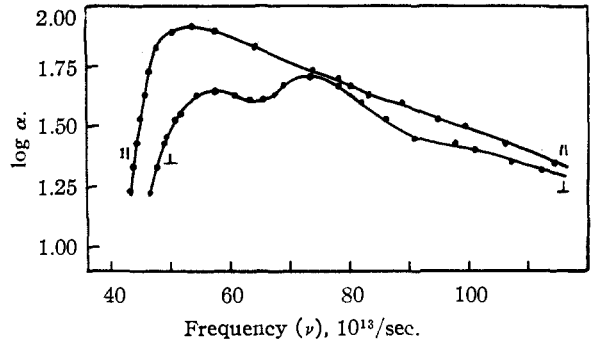



Fig. 1.—Absorption spectra of bis-(dimethyloxime)-copper.

no metal–metal interaction. Similar measurement (Fig. 2) revealed that the corresponding nickel compound exhibits a remarkable dichroism for the corresponding absorption band, maximum absorption being observed with electric vector along the *c*-axis.[9] The following data were

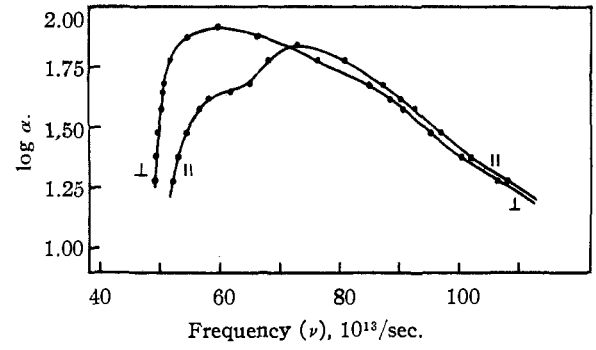



Fig. 2.—Absorption spectra of bis-(dimethylglyoxime)-nickel.

obtained: for ∥ absorption, $\nu = 60 \times 10^{13}$/sec. and log $\alpha = 1.62$; for ⊥ absorption, $\nu = 59.2 \times 10^{13}$/sec. and log $\alpha = 1.91$. These data indicate that the relation on the dichroism with this crystal is reverse to that in the ordinary case. A direct interaction between nickel atoms may be considered as responsible for the reversal of the effect. Similar dichroism measurement was made with chocolate-colored acicular crystals of the corresponding platinum complex. Although the crystal structure has not been determined, judging from the data of analogous compounds, planar complexes are supposed to be arranged parallel to each other to a greater or lesser extent, with their planes nearly perpendicular to the needle-axis. On the basis of the assumed structure, it is established that ⊥ absorption is bathochromic and hyperchromic to ∥ absorption. This relation is reverse to that for planar complexes of an ordinary type.[5b] The metal–metal interaction, as in the crystal of the corresponding nickel compound, may be expected in the platinum compound.

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE — SHOICHIRO YAMADA
OSAKA UNIVERSITY — RYUTARO TSUCHIDA
NAKANOSHIMA, OSAKA, JAPAN

RECEIVED OCTOBER 28, 1953

(9) Godycki, *et al.*, reported (ref. 4) that maximum absorption was observed with the electric vector perpendicular to the *c*-axis.

## BIOLOGICAL ACTIVITY OF A METABOLITE OF *p*-AMINOBENZOIC ACID (PABA) IN A HYDROXYLATING SYSTEM

*Sir:*

Sloane, Crane and Mayer[1] reported that resting cells of *Mycobacterium smegmatis* (101) hydroxylate aniline to *p*-aminophenol. Further investigations[2,3] indicated that the hydroxylation is an energy-coupled reaction. It was determined that chlortetracycline (Aureomycin[4]) and oxytetracycline (Terramycin[5]) compounds which uncouple oxidative phosphorylation[6,7] inhibit the hydroxylation reaction without affecting the oxygen uptake of mycobacterial cells[8] at concentrations ranging from 1.3 to 5.4 $\times 10^{-6}$ *M*.[9] Penicillin, chloramphenicol, dihydrostreptomycin, viomycin and neomycin do not inhibit the hydroxylation reaction by the mycobacteria at these concentrations.[9]

Isochlortetracycline, the biologically inactive rearrangement product of chlortetracycline[10] does not inhibit the hydroxylation, thus the antibiotic activity of chlortetracycline parallels its ability to inhibit the hydroxylation reaction.

It is the purpose of this communication to describe some biological and chemical properties of a metabolite of PABA, which non-competitively reverses the activity of chlortetracycline and oxytetracycline in this system. The data are shown in Table I. The metabolite appears to function as a

TABLE I

THE CHLORTETRACYCLINE–OXYTETRACYCLINE REVERSING ACTIVITY OF PABA-METABOLITE IN THE HYDROXYLATING SYSTEM

| | *M. Tuberculosis* (#607 ≃ 600 mg. dried cells per 25 ml. of buffer–citrate–metals solution per 250-ml. flask (vigorous aeration (1)) | μM. *p*-aminophenol, 16 hr. |
|---|---|---|
| 1 | Cells + buffer–citrate–metals + aniline (107 μM.) | 4.62 |
| 2 | 1 + 0.136 μM. chlortetracycline | 0 |
| 3 | 2 + 9.12 μM. PABA—metabolite[a] | 1.54 |
| 4 | 2 + 4.56 μM. PABA—metabolite[a] | 1.54 |
| 5 | 2 + 2.28 μM. PABA—metabolite[a] | 0.93 |
| 6 | 2 + 1.14 μM. PABA—metabolite[a] | 0.49 |
| 7 | 2 + 0.57 μM. PABA—metabolite[a] | 0.13 |
| 8 | 4 without aniline | 0 |
| 9 | 4 without aniline and chlortetracycline | 0 |
| 10 | Buffer–citrate–metals + 4.56 μM. PABA metabolite + 107 μM. aniline | 0 |

[a] Maximum solubility of metabolite is 4.5 μM. per 25 ml.

cofactor or cosubstrate in the hydroxylation reaction. Amino acids, purines, pyrimidines and vita-

(1) N. H. Sloane, C. Crane and R. L. Mayer, *J. Biol. Chem.*, **193**, 453 (1951).

(2) N. H. Sloane, M. Samuels, C. Ritter, C. Crane and R. L. Mayer, *Federation Proc.*, **11**, 288 (1952).

(3) N. H. Sloane, M. Samuels and R. L. Mayer, *J. Biol. Chem.*, in process of publication.

(4) Aureomycin is the registered trade name of Lederle Laboratories Division, American Cyanamid Co.

(5) Terramycin is the registered trade name of Chas. Pfizer and Co.

(6) W. F. Loomis, *Science*, **111**, 474 (1950).

(7) Y. Miura, Y. Nakamura, H. Matsudaira and T. Komeiji, *Antibiotics and Chemotherapy*, **2**, 152 (1952).

(8) Non-pathogenic mycobacteria perform this hydroxylation.

(9) N. H. Sloane, unpublished data.

(10) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).

mins did not reverse the activity of chlortetracycline at varying concentrations. The metabolite does not affect the antibiotic activity of these compounds, as determined by the standard antibiotic assay technique.

It is interesting to note that while 2,4-dinitrophenol ($5 \times 10^{-4}$ *M*) also inhibits the reaction[2,3] the action of this uncoupling agent[11] is not reversed by the PABA metabolite. These data suggest that different inhibitors of oxidative phosphorylation do not necessarily effect the same locus in the enzyme complex. Witter, Newcomb and Stotz[12] have previously discussed this concept.

The compound designated PABA-metabolite was obtained as a crystalline free base from culture filtrates of *M. smegmatis* which was grown in the presence of 0.1% PABA. The compound was purified by partition between water and ethyl acetate (*p*H 10.0) after removal of the silver precipitable material from the culture filtrate at *p*H 7. The metabolite (ethyl acetate soluble at *p*H 10) was crystallized from water (*p*H 6.8) and then from ethyl acetate, chloroform–petroleum ether mixtures or hot methanol to constant analyses. The yield of recrystallized metabolite is in the order of 500 μg. per liter. The compound has the empirical formula $C_{14}H_{14}N_2 \cdot 1/2H_2O$[13] and melts[14] at 198–199° uncor., with darkening.

*Anal.* Calcd. for $C_{14}H_{14}N_2 \cdot 1/2H_2O$: C, 76.69; H, 6.90; N, 12.78; mol. wt., 219. Found:[15] C, 76.96; H, 7.23; N, 12.81; mol. wt., 248 (Signer, $10^{-3}$ *M* in acetone).

The ultraviolet absorption spectrum of the metabolite (free base) in ethanol shows maxima at 258 mμ (ε 23,400) and at 295 mμ (ε 3900). The ultraviolet absorption spectrum of the hydrochloride in ethanol shows a marked change, the absorption peaks at 258 and 295 mμ are replaced by an inflection at 255 mμ (ε 3600). The metabolite shows one aromatic amine group by Bratton–Marshall test[16] per mole (slow development of the color).

The major infrared bands are at 2.9, 6.14, 6.54, 7.07, 7.57, 7.96, 8.45, 8.91, 9.27, 10.03 (broad), 10.67, 11.45, 12.25 and 13.25 microns. Work is now in progress for the elucidation of the structure of this compound.

(11) W. Loomis and F. Lipmann, *J. Biol. Chem.*, **173**, 827 (1948).

(12) R. F. Witter, E. H. Newcomb and E. Stotz, *ibid.*, **202**, 291 (1953).

(13) Microanalyses were performed by Mr. Louis Brancone and coworkers.

(14) The compound decomposes if heated slowly; a sharp melting point is obtained if compound is placed in preheated bath in vacuum capillary.

(15) Analyses of five preparations agreed.

(16) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY NATHAN H. SLOANE
PEARL RIVER, NEW YORK

RECEIVED OCTOBER 28, 1953

# BOOK REVIEWS

**Physical Chemistry of Metals.** By LAWRENCE S. DARKEN, Ph.D., Research Laboratory, United States Steel Corporation; and ROBERT W. GURRY, Ph.D., Research Laboratory, United States Steel Corporation; with a collection of problems by MICHAEL B. BEVER, Sc.D., Department of Metallurgy, Massachusetts Institute of Technology. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York 36, N. Y. 1953. ix + 535 pp. 16.5 × 23.5 cm. Price, $8.50.

This book will appeal to both the chemist and the metallurgist although it was written expressly for the latter. As a text for students who have completed the usual one-year introductory course in physical chemistry, it should find its place in many metallurgy curricula. It will be a valuable guide to the research worker in this field of applied physical chemistry.

After a brief introductory chapter the next four, approximately one-fourth of the book, are devoted to gases, solids and liquids. The discussion of atomic structure is excessively brief but contains a complete table of arrangement of orbital electrons. Bonding and resonance phenomena are treated briefly but competently. In addition the chapter on solids discusses plastic deformation, Hume-Rothery's classification, atomic radii and crystallography of the elements and imperfections in crystals. It contains a rather full exposition of Pauling's theory of valence and atomic radius in metals.

The chapter on solid solutions and intermetallic compounds includes quantitative discussions of the effect of size factor and electronegativity on extent of solid solubility. Long and short range order and intermediate phases are discussed.

Chapter 5 contains an excellent summary of the structure of liquids as deduced from X-ray diffraction data. The authors make a strong case for ordering in certain liquid metallic solutions. A discussion of the "hole" theory of liquid structure contains the only mention of viscosity.

Chapters 6–10 present the classical approach to thermodynamics with applications to metallic solutions and other systems of especial metallurgical interest. Statistical mechanics is considered beyond the scope of the book but its conclusions are used freely, especially in connection with the third law. This procedure is likely to prove baffling to the student who has not been told about the relation between entropy and randomness.

The treatment of solutions will be especially helpful to research metallurgists. The authors employ the function $\alpha_1 = \ln \gamma_1/(1 - N_1)^2$ which is useful in interpolating activity data and in graphical integration of the Gibbs–Duhem equation. This chapter and a later one on free energy–composition diagrams are especially recommended to the physicist or metallurgist who is unfamiliar with the elegant methods developed by chemists for the thermodynamic treatment of solutions.

Two chapters on the phase rule and heterogeneous equilibria contain the basic principles applicable to one- and two-component systems. Systems of three or more components are not discussed.

Chapter 14 contains summaries of many useful metallurgical data on the free energy of formation of oxides, sulfides, carbides, nitrides and chlorides. In it also is found a brief summary of the authors' own fine work on the system iron–oxygen. The two following chapters are treatises on the two important systems Fe–N and Fe–C. They include