[CONTRIBUTION FROM THE DIVISION OF PHYSICAL CHEMISTRY, UNIVERSITY OF MINN., INSTITUTE OF TECHNOLOGY]

# Preliminary Study of a Metastable Form of Chlorophyll in Fluid Solutions[1]

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The absorption spectra of chlorophyll-*a*, chlorophyll-*b*, pheophytin-*a* and coproporphyrin dimethyl ester in their metastable (triplet?) states have been determined, in the visible and near ultraviolet regions, by an application of the flash photolytic and flash spectrographic techniques to fluid solutions. The half-lives of these metastable molecules, in the solutions studied, fall in the range 100 to 1300 $\mu$seconds. For the case of chlorophyll-*b* in benzene, the decay of the metastable state, G′, appears to obey the following rate equation $-\mathrm{d}[G']/\mathrm{d}t = 5 \times 10^8([G] + [G'])[G']$ within the limits of precision of the experiments.

Chlorophyll molecules in oxygen-free dilute solutions are raised reversibly and almost completely to a metastable state by a single flash of intense light. The absorption spectra of the molecules in their metastable state and the rate of their return to the ground state can be determined by the flash photolytic and flash spectrographic technique which was developed by Dr. G. Porter.[2] The results reported here are based upon an analysis of photographic records obtained at the University of Cambridge with apparatus constructed by Dr. Porter and Mr. Windsor.[3]

## Methods and Procedures

In these experiments the energies of the photolytic flashes were 125 joules or less. Pyrex cuvettes, 10.0 cm. long, were used in most experiments, although a few more concentrated solutions were studied in 5.0-cm. cuvettes. The source and purification of the pigments and solvents has been described elsewhere.[3] The solutions were degassed by boiling off about one quarter of the solvent at ambient or lower temperatures. Ether was added to the solutions in cyclohexanol to facilitate degassing. No special attempt was made to exclude traces of water from the solutions. Ilford Selochrome plates were used in a Hilger E3 spectrograph, and under the conditions of the experiment the useful spectral range was from 340 to 560 m$\mu$. A few spectrograms were taken with a glass prism spectrograph and Ilford HP3 plates, which extended the long wave length limit to about 660 m$\mu$.

The optical densities of each solution were determined at a series of wave lengths, by comparing the blackening of the photographic plate due to the light of a photographic flash which was transmitted by the solution to that of a flash of similar energy which passed through a solvent-filled cell. The average deviation of the densities of photographic plates due to successive, similar flashes appeared to be less than 10%. The coefficient, $\gamma$, of the equation relating the density, $S$, to the exposure, $I$

$$S_{\mathrm{i}} = \gamma \log I_{\mathrm{i}} + a$$

was determined crudely with the aid of a set of oxidized copper gauze screens and a piece of yellow cellophane. A series of exposures of constant flash energy were made separately through the cellophane, and through the several copper screens. The percentage transmission curve of the cellophane was measured with a Unicam spectrophotometer. By determining the wave lengths at which the photographic densities due to an exposure through the cellophane were equal to those due to exposures through the several screens, the percentage transmission of each screen was determined. These transmissivities were used to obtain $\gamma$ as a function of wave length for each developed plate. The plate densities were measured with a *Leeds and Northrup* recording densitometer.[4]

Although no fluorescence was detectable in the wave length range covered, light scattered from the photolytic flash was appreciable in some of the experiments. When necessary, the following relation was used to correct for blackening due to scattered light.

$$-\epsilon cl = \log\ [\text{antilog}\ \frac{Si - S_0}{\gamma} - \text{antilog}\ \frac{Ss - S_0}{\gamma}$$

In this equation $-\epsilon cl$ is the exponent in Beers law, and the subscripts i, o and s refer, respectively, to light transmitted through the solution and through the solvent and to light scattered from the photolytic flash.

Since in the present experiments it was impossible to convert completely the compounds into their metastable forms,[5] it is necessary to correct the observed spectra for the presence of an unknown percentage of the molecules in their ground state. As is well known,[6] it is impossible to obtain a unique solution for either the ratio of the concentrations or for the extinction coefficients of the metastable state. The best that can be done is to determine limits within which these quantities must occur. The spread between these limits depends upon the nature of the spectra, being narrowest if the absorption bands of the two spectra are sharp and well separated and widest if they are broad and overlap. Usually the limits can be greatly narrowed if the probable assumption is made that neither maxima nor minima of one curve correspond (in wave length) with maxima or minima of the other curve.[7] In the present case the uncertainty is increased by departures from the reciprocity law and, for low densities, by unevenness in the background density of the plates. This same uncertainty appears, although to a lesser extent, in the calculated rates of decay of the metastable forms.

## Results and Conclusions

The absorption spectral data for the compounds in their triplet states are presented in the four figures and in the first seven columns of Table I. Number 1, representing chlorophyll-*b* in benzene, is based upon three plates listed in Table I and two additional sets of measurements made with red-sensitive Ilford HP3 plates. The known ratio of the heights of the red and blue peaks in the absorption spectrum of normal chlorophyll-*b* in benzene, was used to reduce the data, taken with different plates and spectrographs, to a common basis. The solid line portion of the triplet curve, T, is reasonably

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(1) This work was made possible by the support of the Office of Naval Research (NR 051,028, Contract N6ori-212, T. O. I) to which the author is indebted. The flash photolytic experiments were performed at the Department of Physical Chemistry, University of Cambridge, while the author was on Sabbatical Furlough from the University of Minnesota and was the recipient of a grant from the Guggenheim Memorial Foundation.

(2) (a) G. Porter, *Proc. Roy. Soc.* (*London*), **200A**, 284 (1950); (b) G. Porter and M. Windsor, *J. Chem. Phys.*, **21**, 2088 (1953).

(3) R. Livingston, G. Porter and M. Windsor, *Nature*, **173**, 485 (1954). The present author is indebted to Dr. Porter for permission to use the apparatus and to Mr. Windsor for valuable assistance in performing the experiments.

(4) These measurements were made at the University of Minnesota and the author is indebted to Mr. V. Subba Rao who made many of them.

(5) The relatively high percentage conversion which was observed is not surprising in view of the facts that the available radiant energy (estimated to be 1% of the total energy of the discharge) corresponds to one hundred or more photons per pigment molecule and that the limiting fluorescent yields are less than 0.25.

(6) E. Q. Adams and L. Rosenstein, THIS JOURNAL, **36**, 1452 (1914).

(7) A. C. Hardy and F. M. Young, *J. Opt. Soc. Am.*, **38**, 854 (1948).

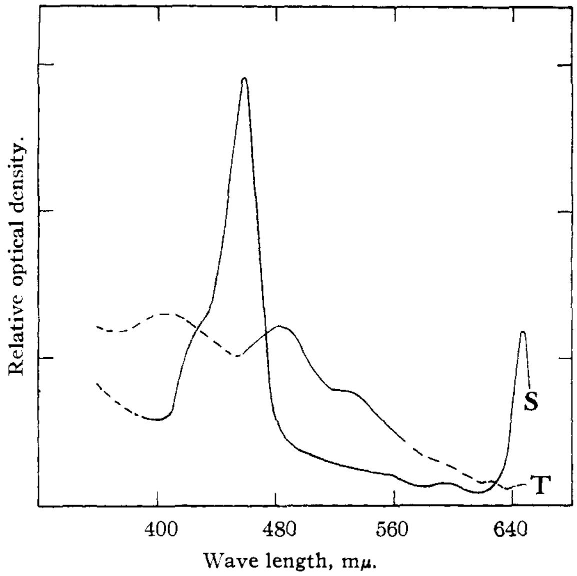

Fig. 1.—Absorption spectra of chlorophyll-*b* in benzene; a composite of several plots: S, ground state; T, lowest triplet state.

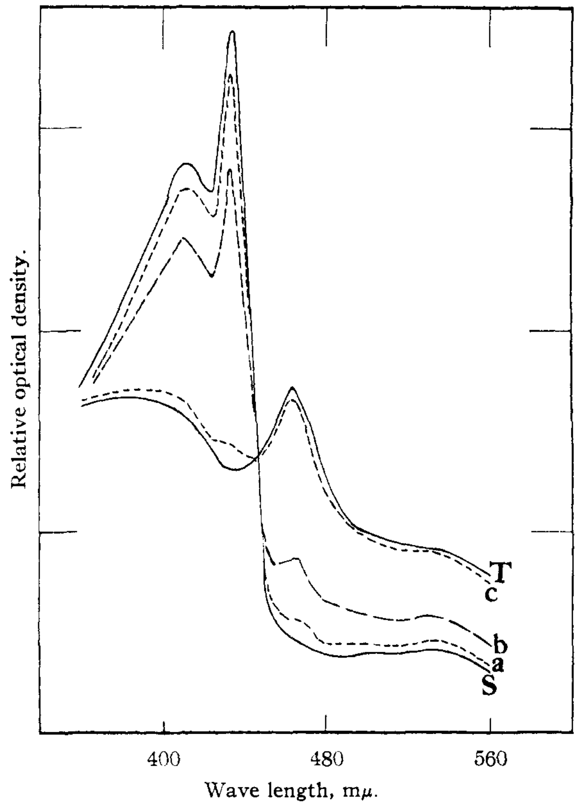

Fig. 2.—Absorption spectra of ($1 \times 10^{-6}$ *m*) chlorophyll-*a* in cyclohexanol: S, ground state; a, 2600 μsec. after flash; b, 900 μsec. after flash; c, "immediately" after flash; T, lowest triplet state.

well determined. While the detailed structure of the dash-line extension of the curve is uncertain, its general form, including its height relative to the singlet curve, is very probably correct.

Each of the remaining three figures is based upon data taken from a single plate, and illustrates the

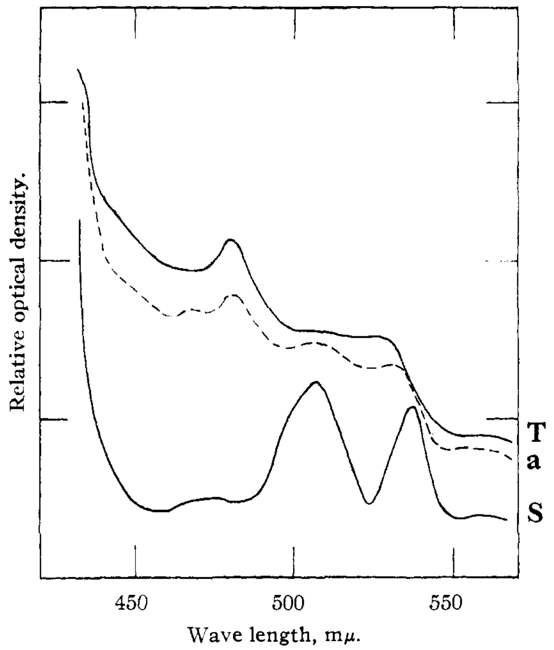

Fig. 3.—Absorption spectra of ($2.5 \times 10^{-6}$ *m*) pheophytin-*a* in benzene: S, ground state; a, "immediately" after flash; T, lowest triplet state.

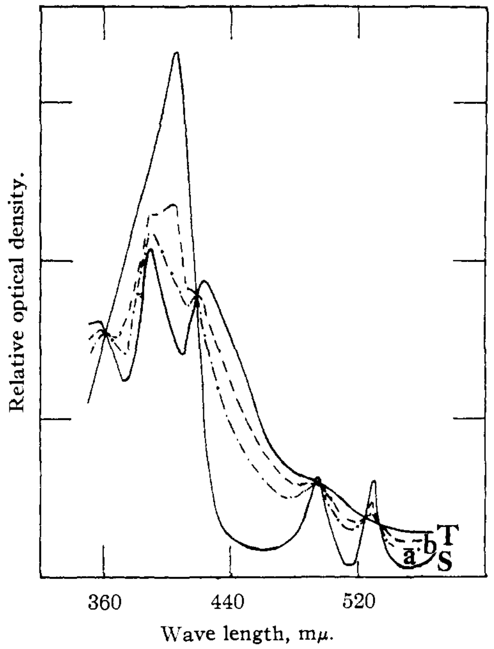

Fig. 4.—Absorption spectra of coproporphyrin dimethyl ester in benzene: S, ground state; a, 120 μsec. after flash; b, "immediately" after flash; T, lowest triplet state.

relation between the absorption curve observed when the spectrographic flash followed the photolytic flash "immediately," and the estimated curve for the triplet state. No attempt was made to include the data obtained with red-sensitive plates for chlorophyll-*a*, since the red absorption band was located beyond the long-wave length limit of the combination. However, these data indicate that the absorption of chlorophyll-*a* in its triplet state decreases more or less continuously from 500 to 660

TABLE I

SUMMARY OF SPECTRAL DATA FOR THE COMPOUNDS IN THEIR METASTABLE STATES

| Compound | Solvent | $m \times 10^6$ (moles/l.) | Maxima, mμ | Δλ, mμ | Max. % of triplet | $\tau_{1/2} \times 10^6$ (sec.) | Order | k(1st order) ($\text{mole}^{-1}$ $\text{sec.}^{-1}$ total pigment concn. |
|---|---|---|---|---|---|---|---|---|
| Chlorophyll-*a* | Benzene | 1.5 | 460 | 28 | 72 | 150 ± 25% | 1st(?) | $3 \times 10^9$ |
| Chlorophyll-*a* | Methanol | 1.1 | 462, 425(?)<br>390(?) | 28 | 90 | 200 ± 25% | ? | $3 \times 10^9$ |
| Chlorophyll-*a* | Cyclohexanol | 1.0 | 465, 360 | 30 | 95 | 600 ± 30% | ? | $1 \times 10^9$ |
| Chlorophyll-*b* | Benzene | 1.0 | 482, 440<br>420(?) | 22 | 90 | 1300 ± 25% | 1st | $5 \times 10^8$ |
| Chlorophyll-*b* | Benzene | 2.2 | 485 | 21 | 90 | 600 ± 30% | 1st | $5 \times 10^8$ |
| Chlorophyll-*b* | Benzene | 9.2 | 485, 430 | 23 | 75 | 200 ± 30% | 1st | $4 \times 10^8$ |
| Chlorophyll-*b* | Methanol | 3.5 | 496, 454<br>420(?), 390(?) | 29 | 80 | 100 ± 40% | ? | $2 \times 10^9$ |
| Pheophytin-*a* | Benzene | 2.5 | 480 | .. | .. | ........ | ... | ..... |
| Porphyrin | Benzene | 5 | 390, 423 | 17 | 80 | 250 ± 50% | ? | $5 \times 10^8$ |

mμ. The spectra for pheophytin-*a*, of Fig. 3, are available for only a limited wave length region; no exposures were made on red-sensitive plates and the solutions used were too concentrated to yield any reliable data in the region of the principal absorption maximum.

For the three compounds for which the data are available, the maximum absorption in the region of the Soret band is much reduced in the triplet state spectra, and it appears possible that this normally sharp band is split into two broad over-lapping components. The separations, Δλ, between the maxima of the longer wave length component and that of the normal Soret bands are given in column five of Table I. The red band is either missing in the triplet spectra or has been shifted to longer wave lengths, beyond the limit of observation. However, the absorption in the region between the major visible absorption peaks is greater in the triplet than in the singlet spectra.

As has been recently emphasized,[8] the absorption spectra of the triplet states of the chlorophylls resemble, in general, the spectra of the Molisch brown phase and of the reversibly oxidized or reduced, labile intermediates of the chlorophylls.

The photochemical formation of the triplet states is a reversible process; any attendant irreversible reactions of the pigments are at most minor side reactions. In the case of chlorophyll-*b* in benzene, the disappearance of the triplet state follows a first-order law, but the specific reaction rate of this process appears to be directly proportional (over a nine-fold range) to the total concentration of the pigment. This is in marked contrast to the behavior of anthracene, where it has been demonstrated unequivocally,[9] not only that the decay of the triplet is first order, but also that the rate constant is independent of the total concentration of anthracene over a one thousand-fold range. Unless the present results have been greatly affected by some undetected systematic error, it follows that the triplet state, G′, of chlorophyll-*b* in benzene disappears principally by a bimolecular reaction between a metastable molecule and another pigment molecule, which may be either in its metastable or normal state. The corresponding mechanism[10] is

$$\begin{aligned}
G + h\nu &\longrightarrow G^* & V_1 &= I_{abs} \\
G^* &\longrightarrow G + h\nu_f & V_2 &= k_2[G^*] = I_f \\
G^* &\longrightarrow G' & V_3 &= k_3[G^*] \\
G + G' &\longrightarrow 2G & V_4 &= k_4[G][G'] \\
2G' &\longrightarrow 2G & V_5 &= k_5[G']^2 \\
G' &\longrightarrow G & V_6 &= k_6[G']
\end{aligned}$$

Even the shortest intervals which are presently used between the photolytic and photographic flashes are sufficient to allow [G*], the concentration of the molecules in their fluorescent state, to fall to zero. Under these conditions

$$-\frac{d[G']}{dt} = (k_4[G] + k_5[G'] + k_6)[G']$$

where $k_4 \cong k_5$ and $k_6 < 2 \times 10^{-7}\, k_4$. It follows that the natural mean life of G′, which would be observed in an infinitely dilute solution, is greater than five milliseconds.

In methanol and in cyclohexanol the rate of decay appears to decrease less rapidly with time than would correspond to a first-order reaction. This behavior is consistent with the measurements of the steady-state reversible bleaching[11] of chlorophyll in alcoholic solutions which indicate that a pair of radicals (?) are formed photochemically, but in rather poor yield, and that they disappear by a bimolecular process to reform normal chlorophyll. While the present results are consistent with such an interpretation they are not reliable enough to confirm it.

The half-lives, $\tau_{1/2}$, column eight of Table I, are empirical values obtained from smooth curves drawn through the experimental points. The first-

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[8] A. Weller, THIS JOURNAL, **76**, 5819 (1954).

[9] G. Porter and M. Windsor, *Discs. Farad. Soc.* (1953).

[10] Compare J. Knight and R. Livingston, *J. Phys. Chem.*, **54**, 703 (1950). These authors concluded, from the analysis of their results on the steady-state, reversible bleaching of chlorophyll, that the chlorophyll molecules were degraded from their metastable to their ground states by a bimolecular reaction with a normal chlorophyll molecule. Since, in their experiments, never more than a few tenths of 1% of the pigment molecules were in their triplet state, it was unnecessary for them to consider the possibility of a bimolecular reaction between two triplet-state molecules.

[11] (a) D. Porret and E. Rabinowitch, *Nature*, **140**, 321 (1937); (b) J. McBrady and R. Livingston, *J. Phys. Chem.*, **45**, 1312 (1941); (c) J. Knight and R. Livingston, *ibid.*, **54**, 703 (1950).

order rate constants, listed in the last column, are based upon the equation

$$k = \frac{\ln 2}{([G] + [G'])\tau_{1/2}}$$

although it must be admitted that the only experimental justification for its use is in the case of chlorophyll-*b* in benzene. It is suggestive that in all cases the calculated values of *k* do not differ by more than a factor of 2.5 from the limiting (maximum) rate constants for a diffusion-controlled process estimated from the approximate equation[12]

$$k\,(\text{diff.}) = \frac{8RT}{3 \times 10^3 \eta}$$

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[12] J. Umberger and V. LaMer, THIS JOURNAL, **67**, 1099 (1945).

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[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NATIONAL RESEARCH COUNCIL]

# Electrolyte Catalysis. I. The Mutarotation of Glucose in Pyridine

BY A. M. EASTHAM, E. L. BLACKALL AND G. A. LATREMOUILLE

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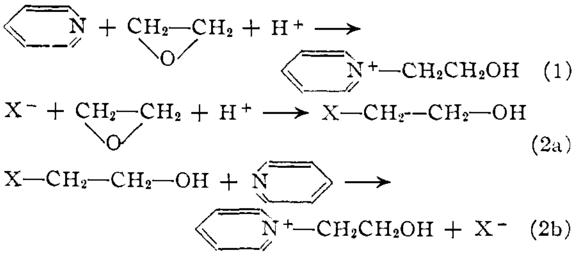
The rates of mutarotation of tetraacetyl- and tetramethylglucoses in anhydrous pyridine and the effect of electrolytes upon the rates have been determined. Lithium iodide and perchlorate are the most effective catalysts; other salts follow in the order $Li^+ > Na^+ > C_5H_5NH^+ > Ag^+$ and $ClO_4^- = I^- > Br^- >$ picrate $> NO_3^- > Cl^-$. The effect of the electrolytes on the two sugars is similar but not identical.

Kinetic studies of the acid-catalyzed reaction of ethylene oxide with anhydrous pyridine show the reaction rate to be markedly dependent upon the acid anion, *i.e.*, $HIO_4 \geqslant HClO_4 > H_2SO_4 > C_6H_5SO_3H > HNO_3$.[1] In a solvent as basic as pyridine, where complete ionization of the acids may be assumed, the proton source in all cases must be the pyridinium ion, but there is no simple explanation for the manner in which the anion influences the proton transfer. The effect is surprising because neutral salts have no apparent effect upon the rate, and because the dependence upon acid concentration is linear between 0.005 and 0.5 *M*.

In catalysis by halogen acids[1] this reaction (1) is complicated by a second mechanism (2)



(1)

(2a)

(2b)

in which step (a) is essentially similar in type to reaction (1). In the case of hydrogen iodide the kinetics of step (2a) appear experimentally as a transition from third order to second order with increasing concentration of iodide ion but can be interpreted as a simple second-order process if one assumes that reaction occurs almost entirely between ethylene oxide and an ion pair of pyridinium iodide and only slightly through the stepwise (third order) addition of hydrogen and iodide ions. A rough estimate of the dissociation constant of pyridinium iodide ion pairs in pyridine can be obtained from the data of Kraus and co-workers[2] and when this value is used to obtain the actual concentration of ion pairs good agreement with the kinetic data is obtained.

If the addition of hydrogen iodide occurs through ion pairs, then in all probability the dependence of reaction (1) upon the nature of the anion is also due to the presence of an ion pair in the active complex. The effect may be particularly marked in this case because the product is itself an ion and its formation may depend upon immediate stabilization. There seems, however, to be no reason to suppose that the more acidic anions would be the most effective stabilizers—ion size should perhaps be a more important factor.

In an effort to obtain more information about these effects a study of the mutarotation of glucose was undertaken. This reaction has been studied extensively in aqueous solution, but has received little attention in aprotic solvents. Lowery and Faulkner[3] found that it took place very slowly in either pyridine or cresol alone but rapidly in a mixture of the two. They suggested that both an acid and a base were necessary for catalysis, a conclusion also reached by Swain and Brown[4] in their recent studies of the mutarotation of tetramethylglucose in benzene solution using pyridine–phenol mixtures as catalyst.

In preliminary experiments glucose proved an unsatisfactory sugar for this work because of its low solubility and rate of solution. Tetra-acetyl-*β*-*d*-glucose and tetra-methyl-*α*-*d*-glucose were therefore selected for further study.

## Experimental

**Materials.**—Pyridine and pyridinium salts were prepared as previously described.[1] Moisture determination by the Karl Fischer method indicated less than 0.005% water in the pyridine. Picrates were prepared from the acid and the corresponding hydroxides and recrystallized several times before use. The remaining salts were thoroughly dried but not otherwise purified. Where necessary the salts were handled in a dry-box.

2,3,4,6-Tetra-acetyl-*β*-*d*-glucose was prepared by the method of Fischer and Hess[5] and recrystallized to a melting point of 135–136° (Pyrex tubes) and a specific rotation $[\alpha]^{25}D$ −2° (ethanol). 2,3,4,6-Tetra-methyl-*α*-*d*-glucose

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[1] A. M. Eastham and B. deB. Darwent, *Can. J. Chem.*, **29**, 585 (1951); A. M. Eastham, *J. Chem. Soc.*, 1936 (1952).

[2] D. S. Burgess and C. A. Kraus, THIS JOURNAL, **70**, 706 (1948); C. J. Carnigan and C. A. Kraus, *ibid.*, **71**, 2983 (1949).

[3] T. M. Lowery and I. J. Faulkner, *J. Chem. Soc.*, **2883** (1925).

[4] C. G. Swain and J. F. Brown, Jr., THIS JOURNAL, **74**, 2534 (1952).

[5] E. Fischer and K. Hess, *Ber.*, **45**, 912 (1912).