an order of magnitude faster than the ultrafast reaction of $NH_4^+:NH_3$ hydrogen exchange (II).[7] We infer that the chemical mobility of the electrons in these solutions matches their physical mobility (*e.g.*, electrical conductivity), and that this may be correlated with the physical models being proposed[8] to describe metal–ammonia solutions. In these solutions, the electron attack on an electron-attracting entity appears to be *via* a mechanism quite different from that of ordinary ionic transport.

(7) Because the reactant ratio of $Li:NH_4^+$ is high, there is the possibility that only the ammonia associated with one equivalent of lithium should be considered as exchanging with the ammonia associated with ammonium ion. This seems improbable, since it would involve the mixing of all the NH_4Br solution with only a part of the Li solution. It is more straight-forward to assume that the mixing involves approximately equal amounts of both solutions at all times, that both solutions mix fairly uniformly in order for all the NH_4^+ to react, that both reactions I and II are diffusion controlled, and that the rate of attack of NH_4^+ ions by electrons competes effectively with the rate of attack (exchange) of NH_4^+ ions by NH_3 molecules.

(8) G. Lepoutre and J. F. Dewald, THIS JOURNAL, **78**, 2953 (1956); E. Becker, R. H. Lindquist and B. J. Alder, *J. Chem. Phys.*, **25**, 971 (1956); M. C. R. Symons, *ibid.*, **30**, 1628 (1959).

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RECEIVED FEBRUARY 26, 1960

THE SYNTHESIS OF 2-DEOXY-D-RIBOFURANOSE 1-PHOSPHATE

Sir:

Cleavage of a ribonucleoside with a nucleoside phosphorylase was reported by Kalckar[1] to give a ribose 1-phosphate. Subsequently, this product was shown to be α-D-ribofuranose 1-phosphate on the basis of certain chemical tests, as well as by chemical synthesis of both anomers.[2] In a presumably similar fashion, the action of the appropriate nucleoside phosphorylases on deoxyribonucleosides leads to the formation of an extremely acid-labile 2-deoxy-D-ribofuranose 1-phosphate,[3-5] which has been isolated as a crystalline di-(cyclohexylammonium) salt.[4,5] The optical rotation of this material, $[\alpha]_D$ +38.8°,[5] and the manner in which it is enzymatically synthesized, suggest[5] that, like its D-ribose analog, it possesses the α-configuration. This conclusion is supported by the present work, in which we describe the chemical synthesis of a 2-deoxy-D-ribofuranose 1-phosphate of high biological activity.

Crystalline 3,5-di-*O*-*p*-toluoyl-2-deoxy-D-ribofuranosyl chloride[6] was condensed in benzene solution with disilver phosphate[7] (previous workers who have used this material for the synthesis of phosphates[8] have termed it "monosilver phosphate"). The product of the condensation was saponified in aqueous alcohol with lithium hydroxide, the lithium replaced by cyclohexylamine using an ion exchange resin and the crude dry salts extracted with *n*-propyl alcohol to remove cyclohexylammonium *p*-toluoate. Inorganic phosphate then was removed as magnesium ammonium phosphate and the resulting material crystallized as the di-(cyclohexylammonium) salt from methanol–ether or from aqueous acetone. The product, which was obtained in a yield of *ca.* 30%, based on the chloride, showed $[\alpha]^{20}D$ of about +22° in water. *Anal.* Calcd. for $C_{17}H_{37}N_2O_7P$ (412.47): C, 49.49; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.33; H, 9.32; N, 6.63; P, 7.53.

In *n*-propyl alcohol–ammonia–water and in isopropyl alcohol–ammonia–water the material is chromatographically homogeneous, and possesses the same R_f as authentic, enzymatically prepared 2-deoxy-D-ribofuranose 1-phosphate. Fractional crystallization of the synthetic salt has not been entirely successful; however, using methanol–ether, fractions were obtained with $[\alpha]^{20}D$ as high as +30° (C, 49.44; H, 8.76; N, 6.87; P, 7.48). One of these fractions, when assayed enzymatically using fish nucleoside phosphorylase,[5] had about 80% of the activity of natural 2-deoxy-D-ribofuranose 1-phosphate.

Since crystalline acylated 2-deoxy-D-ribofuranosyl halides have been shown[6] to give rise to anomeric mixtures of 2-deoxy-D-ribofuranosides, it seems probable that the phosphate obtained here is a mixture of anomers. A comparison of our product and the natural material on the basis of optical rotation and biological activity supports the assumption[5] that the natural substance of $[\alpha]_D$ +38.8° is the α-anomer and, furthermore, one may calculate that the β-anomer would have an $[\alpha]_D$ of approximately −10°.

The authors are grateful to Dr. H. L. A. Tarr for carrying out the enzymatic assays and for providing a sample of enzymatically prepared 2-deoxy-D-ribofuranose 1-phosphate. Also, we wish to thank Dr. W. E. Scott of Hoffmann–La Roche, Inc., for certain as yet unpublished experimental details.

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RECEIVED FEBRUARY 15, 1960

THE PHOTOREDUCTION OF PORPHYRINS AND THE OXIDATION OF AMINES BY PHOTO-EXCITED DYES

Sir:

Two distinct series of hydro-porphyrins are known. The hydrogens may be located on the peripheral "pyrrole" ring carbons (chlorophyll and bacteriochlorophyll), or they may be on the methine carbons (porphomethenes,[1] I and II, and porphyrinogens). The photoreduction of uroporphyrin produces, in addition to a transitory compound absorbing at 440 and 735 mμ, a more stable substance absorbing at 500 mμ which is related to the second series of hydroporphyrins, the porphomethenes.

Porphyrins may be photoreduced by a wide variety of compounds, *e.g.*, ascorbic acid, glutathione and tertiary amines. The reduction of uroporphyrin with ethylenediaminetetraacetic acid

(1) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(2) R. S. Wright and H. G. Khorana, THIS JOURNAL, **78**, 811 (1956); G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **79**, 441 (1957).

(3) M. Friedkin and H. M. Kalckar, *J. Biol. Chem.*, **184**, 437 (1950).

(4) M. Friedkin, *ibid.*, **184**, 449 (1950); M. Friedkin and D. Roberts, *ibid.*, **207**, 257 (1954).

(5) H. L. A. Tarr, *Can. J. Biochem. and Physiol.*, **36**, 517 (1958).

(6) M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, THIS JOURNAL, **81**, 4112 (1959).

(7) R. Flatt and G. Brunisholz, *Helv. Chim. Acta*, **34**, 692 (1951).

(8) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944).

(1) D. Mauzerall and S. Granick, *J. Biol. Chem.*, **232**, 1141 (1958).

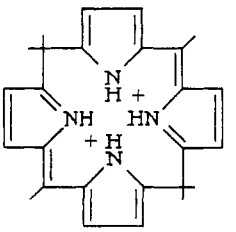
proceeds readily in deoxygenated, neutral, aqueous solution when the porphyrin is excited with light of 400, 500 or 605 mμ wave length. These wave lengths were isolated with interference filters together with suitable blocking filters from the light of a 500 watt projection lamp. Both isomer I and III uroporphyrin react similarly. The first readily detectable product of the photoreduction ($t >$ 10 seconds) is a transitory compound having intense absorption bands at 440 ($E_m \cong 10^5$) and 735 mμ ($E_m \cong 5 \times 10^4$). In the absence of light and oxygen, it is stable for at least thirty hours at neutral *p*H and room temperature. In the presence of air it has a half life of about five minutes. This autoxidation is sensitive to light. The substance also reacts rapidly with iodine. Krasnovsky and co-workers[2] have noted an absorption band at 740 mμ on illuminating hematoporphyrin in pyridine containing ascorbic acid. They favor the possibility of a radical-ion-pair, but a variety of dihydroporphyrins exclusive of structure I are also possible.

On continued illumination, an intense band at 500 mμ ($E_m = 5\text{–}10 \times 10^4$) arises as the 440–735 mμ and the uroporphyrin absorptions fall. Over 95% of the porphyrin can be reduced, and further reduction beyond the 500 mμ stage appears to be very slow. Titration of this product with iodine reforms the porphyrin in 95 ± 5% yield. About 5 moles of iodine was required per mole of reduced porphyrin. This high value may be due to side reactions of the iodine. The reaction of iodine with tertiary amines is highly sensitive to light. The recovered porphyrin was identified by the position and by the relative ratios (6 independent values) of the intensities of its absorption bands in neutral (4 bands) and acid (3 bands) solution. Molar absorption coefficients of the reduced species were calculated from changes in the porphyrin absorption assuming only two reduced products to be present. No evidence for the presence of any colorless reduction product (*e.g.*, uroporphyrinogen) was obtained.

The photoreduced porphyrin absorbing at 500 mμ has spectral reactions characteristic of the dipyrrylmethene structure[1] of porphomethenes: (1) The absorption of porphomethenes is also at 500 mμ (with $E_m \cong 10^5$); (2) the absorption band decreases in intensity and shifts to shorter wave lengths in alkaline solution with a *pK* of about 9.5; and (3) the absorption vanishes in the presence of sulfite ion at *p*H 7.

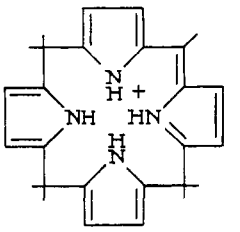
Seely and Calvin claim that the photoreduction of the zinc chelate of α,β,γ,δ-tetraphenylporphyrin by benzoin[3] forms reduced porphyrins of the chlorin and bacteriochlorin series. Under our conditions described above, the zinc chelate of uroporphyrin shows little if any reaction, and the copper chelate is not visibly affected.

Many secondary and tertiary amines will act as photoreducing agents toward a variety of dyes, *e.g.*, thiazines and flavines.[4] Ethylenediaminetetraacetic acid[5] is especially reactive. The claim is often made that the water molecule is split during the reaction[5,6] presumably with concomitant formation of the amine oxide. However, oxidation of the amine at the α carbon is more reasonable. In general the reactivity of the amine is related to its ease of oxidation at the α-carbon: *e.g.*, sparteine is very reactive, primary amines are far less reactive, and ammonium ions are unreactive. The direct isolation of the oxidized amine product is complicated by the facile autoxidation of the reduced dye and by the possible decomposition of the amine oxide if formed. In an attempt to circumvent these difficulties, the fact that small ring bridgehead amines are very resistant to oxidation at the α-carbon, yet readily form N-oxides, was used. No reasonable intermediate or transition state can be stabilized due to ring strain (Bredt's rule). In fact, the rate of photoreduction of thionine by 1,4-diazabicyclo[2,2,2]octane was found to be less than $^1/_{5000}$th that with N,N′-dimethylpiperazine. This bicyclodiamine is also vastly less susceptible to photoöxidation by iodine.



dihydroporphyrin
porphodimethene

I



tetrahydroporphyrin
porphomethene

II

Among the more interesting non-nitrogeneous photo-reducing agents we have found for these dyes are ethyl acetoacetate and 2-carbethoxycyclopentanone. Chlorophyll has a somewhat similar active "methylene" group at position 10 and possibly this molecule can act both[7] as a photo-oxidant and photo-reductant.

Acknowledgment.—I am grateful to Dr. S. Granick for continual encouragement, to Dr. G. Oster for valuable discussions and wish to thank the Houdry Process Corporation for a generous gift of the bicyclodiamine (DABCO).

(2) A. A. Krasnovsky, *J. Chim. Phys.*, **55**, 968 (1958).

(3) G. R. Seely and M. Calvin, *J. Chem. Phys.*, **23**, 1068 (1955).

(4) G. Oster and N. Witherspoon, THIS JOURNAL, **79**, 4836 (1957); F. Millich and G. Oster, *ibid.*, **81**, 1357 (1959).

(5) J. R. Merkel and W. J. Nickerson, *Biochem. Biophys. Acta*, **14**, 303 (1954).

(6) M. Koizumi and H. Obata, *Bull. Chem. Soc. Japan*, **30**, 136 (1957).

(7) E. I. Rabinowitch, "Photosynthesis," Vol. II, Pt. 2, Interscience Publishers, Inc., New York, N. Y., 1956, p. 1487.

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RECEIVED JANUARY 13, 1960

MEMBRANE DIFFUSION STUDIES WITH PROTEINS AND NUCLEIC ACIDS

Sir:

Previous studies[1,2] dealing with separation by differential diffusion through cellophane mem-

(1) L. C. Craig, T. P. King and A. Stracher, THIS JOURNAL, **79**, 3729 (1957).

(2) L. C. Craig, Wm. Konigsberg, A. Stracher and T. P. King, "Symposium on Protein Structure," Methuen & Co. Ltd., London, 1958, p. 104.