

breaking is irreversible⁸ or, in the case of a single transition-state process, bond breaking has not occurred to an extent which may be detected by this means.

In contrast to the extremely rapid reactions of $B_{10}H_{10}^{-2}$, $B_{12}H_{12}^{-2}$ does not react with aryldiazonium ions in acetonitrile.

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(8) L. Melander, "Isotope Effects on Reaction Rates," The Ronald Press Co., New York, N. Y., 1960, p. 103.

(9) Alfred P. Sloan Research Fellow.

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Exchangeable Hydrogen in Chlorophyll and the Path of Hydrogen in Photosynthesis¹

Sir:

Whether labile hydrogen atoms in chlorophyll are involved in photosynthesis is an important question that has attracted much attention but which has not received an unambiguous answer.² The recent demonstration by the method of proton magnetic resonance that chlorophylls *a* and *b* in solution have readily exchangeable hydrogen atoms at the C-10 and δ -methine positions³ has aroused interest in the possibility that an exchange of hydrogen between water and chlorophyll, particularly at the δ position, occurs during photosynthesis. The availability of fully deuterated photosynthetic organisms⁴ now permits a new approach to these questions. Hydrogen incorporated into the chlorophyll of fully deuterated organisms either by chemical exchange or by metabolism can be readily detected by proton magnetic resonance and, because the proton resonances in chlorophyll have been assigned,⁵ the location of the incorporated hydrogen can be determined.

In a typical experiment, fully deuterated *Scenedesmus obliquus* grown in 99.7% D_2O ⁶ with CO_2 as the sole carbon source was harvested by centrifugation and immediately resuspended in fresh H_2O medium.⁴ The resuspended cells were then allowed to continue photosynthesis, receiving 5% CO_2 in nitrogen and a light intensity of 1100 ft.-candles. After 16 hr., the

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) B. Coleman and W. Vishniac, *Natl. Acad. Sci.-Natl. Res. Council, Publ.*, No. 1145, 213 (1963); W. Vishniac and I. A. Rose, *Nature*, **182**, 1089 (1958); W. Vishniac, "Chemical Participation of Chlorophyll in Photosynthesis," in "Comparative Biochemistry of Photoreactive Systems," M. B. Allen, Ed., Academic Press, New York, N. Y., 1960, pp. 377-386; J. W. Weigl and R. Livingston, *J. Am. Chem. Soc.*, **74**, 4211 (1952); T. H. Norris, S. Ruben, and M. B. Allen, *ibid.*, **64**, 3037 (1942).

(3) J. J. Katz, R. C. Dougherty, F. C. Pennington, H. H. Strain, and G. L. Closs, *ibid.*, **85**, 4049 (1963); R. B. Woodward and V. Škarić, *ibid.*, **83**, 4676 (1961); J. H. Mathewson, W. R. Richards, and H. Rapoport, *Biochem. Biophys. Res. Commun.*, **13**, 1 (1963).

(4) H. H. Strain, M. R. Thomas, H. L. Crespi, M. I. Blake, and J. J. Katz, *Ann. N. Y. Acad. Sci.*, **84**, 617 (1960).

(5) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, **85**, 3809 (1963).

(6) H. F. DaBoll, H. L. Crespi, and J. J. Katz, *Biotech. Bioeng.*, **4**, 281 (1962).

algae were again harvested, and the chlorophylls extracted and purified by conventional means.⁴ Each chlorophyll was dissolved in deuterioacetone (0.05 *M*), and the proton magnetic resonance spectrum was recorded.⁷ The p.m.r. spectra showed only a sharp resonance at 6.24 p.p.m. for chlorophyll *a* and 6.22 p.p.m. (relative to TMS as an internal standard) for chlorophyll *b*. These resonances correspond exactly to those assigned to the C-10 proton in ordinary chlorophyll *a* and *b*.⁵ No other proton resonances could be detected, and, in particular, no evidence could be found for the introduction of hydrogen at the δ position. The resonances of the 7 and 8 protons are broad and would be broadened even more by deuterium coupling. Small amounts of hydrogen at these sites are therefore difficult to detect. However, the spectra show no evidence of hydrogen at the 7 and 8 positions. As shown by the n.m.r. observations, chlorophyll synthesis *de novo* in a period of 16 hr. was less than 1%.⁶

Algae allowed to stand 16 hr. in the dark also show hydrogen at the C-10 position. However, the normal extraction and purification procedure is found to introduce hydrogen into this position. Pending the development of other separation procedures, it cannot be decided whether exchange at C-10 is a light or a dark reaction, or whether it is a result of induced exchange during purification. It appears quite certain, however, that none of the other hydrogen atoms in the tetrapyrrole nucleus is involved in a reversible dehydrogenation-hydrogenation cycle with water. Specifically, the present results appear to be inconsistent with recent suggestions that δ -nucleophilicity is involved in photosynthesis.⁸ Furthermore, if chlorophyll undergoes any chemical reaction in photosynthesis, it must do so without affecting the exchangeability of the hydrogen atoms.

When algae are transferred from H_2O to D_2O , severe, if not complete, inhibition of photosynthesis occurs. It is important for the present purpose to demonstrate that a similar inhibition does not occur when the algae are transferred from D_2O to H_2O .

Three-milliliter aliquots of stock cultures of deuterio *S. obliquus* were centrifuged, resuspended in H_2O medium, and allowed to photosynthesize in a Warburg apparatus under the same conditions as used for the exchange experiments. In a typical run the rate of oxygen evolution during the first hour was 4.7 μ moles/min. as compared to 2.1 μ moles/min. for the same culture in D_2O medium. By the 18th and 24th hours the rate of oxygen evolution had dropped to 1.3 μ moles/min., and oxygen production at approximately this rate continued for more than 2 days. Thus, photosynthesis was not significantly slowed or interrupted by transfer of these organisms from D_2O to H_2O , and the number of moles of oxygen produced in 16 hr. per mole of chlorophyll in the sample was roughly 2×10^3 as a lower limit. The closely related green alga *Chlorella vulgaris* suffers severe inhibition of photosynthesis when transferred from D_2O to H_2O , and consequently our experiments were restricted to *S. obliquus*.

Proton magnetic resonance is not a very sensitive procedure, and hydrogen turnover in less than 5% of the chlorophyll would probably not be detected. If hydro-

(7) Measured on a Varian HR-100 n.m.r. spectrometer.

(8) J. H. Mathewson, Abstracts, 146th National Meeting of the American Chemical Society, Denver, Colo., Jan., 1964, p. 16A.

gen exchange occurred at random between any of the deuterium atoms of chlorophyll and water, it must have been confined to less than 5% of the hydrogen atoms at any location. Hydrogen exchange confined to only a few special chlorophyll molecules (constituting less than 5% of the chlorophyll) in a large photosynthetic unit⁹ probably would not be revealed by our procedure.

In these organisms and under the conditions employed in our experiments, then, the bulk of the chlorophyll exists in a form that precludes hydrogen exchange especially at the δ , 7, and 8 positions, either by photosynthesis or by subsequent hydrogen transport.

(9) R. K. Clayton in "Photophysiology," A. C. Giese, Ed., Academic Press New York, N. Y., 1964, p. 159 *et seq.*

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Novel Six-Membered Aluminum-Nitrogen Heterocycles *via* Metalative Cyclization

Sir:

Current interest in novel heterocycles isoelectronic with aromatic hydrocarbons has inspired the synthesis of many unsaturated organoboron ring systems. The physical and chemical properties of borazines¹ and borepins² have been studied with particular attention since π -bonding involving boron might be expected to confer aromatic character upon such heterocyclic nuclei. Dewar's comprehensive studies of boron-nitrogen analogs of aromatic nuclei, such as 10,9-borazarophenanthrene,³ have adduced convincing evidence for such cyclic conjugation. Our recent discovery of a novel metalative cyclization pathway to the hitherto unknown aluminum-nitrogen heterocycles, such as the 10,9-aluminazarophenanthrene system,³ might result from suitably conceived intramolecular metalations. Since the problem of π -bonding between aluminum and adjacent electron-rich centers is still uncertain,⁵ the study of such heterocycles might prove significant in evaluating possible π -electronic interactions.

In light of the foregoing, we are pleased to describe the synthesis and properties of the novel, six-membered, aluminum-nitrogen heterocycle formally isoelectronic (*cf. infra*) with the phenanthrene nucleus. Thus, the heating of an equimolar melt of 2-(methylamino)-biphenyl (I)⁶ and triphenylaluminum at 160° led to the evolution of 1 equiv. of benzene; further heating at 240° produced *ca.* 1 more equiv. of benzene (total benzene (2 equiv.): 93–100%). The resulting pale amber-colored 9-methyl-10-phenyl-10,9-aluminazarophenanthrene (III) did not melt under 500°; however, decomposition commenced at temperatures over 350°.

(1) *Cf., inter alia*, M. J. S. Dewar, *et al.*, *J. Chem. Soc.*, 3073, 3076 (1958); *ibid.*, 2728 (1959); *J. Am. Chem. Soc.*, **84**, 2648 (1962).

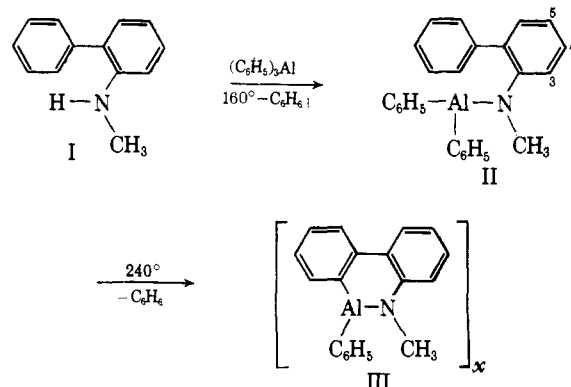
(2) E. E. van Tamelen, G. Brieger, and K. G. Untch, *Tetrahedron Letters*, **No. 8**, 14 (1960).

(3) *Chemical Abstracts* favors the naming of six-membered boron-nitrogen heterocycles as azaborines and this pseudophenanthrene member as 5,6-dihydrodibenz[*c,e*][1,2]azaborine. However, the nomenclature employed in Dewar's papers [*cf.* M. J. S. Dewar and R. Dietz, *J. Chem. Soc.*, 2728 (1959)] conveys a more immediate relationship to pertinent aromatic systems.

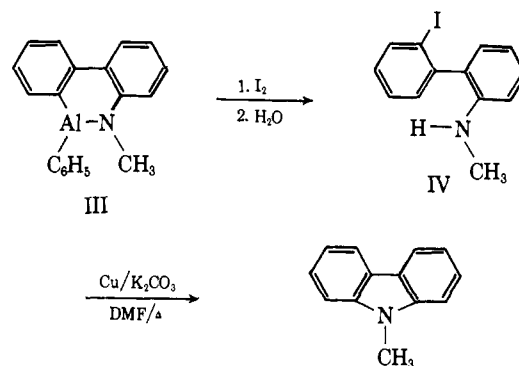
(4) J. J. Eisch and W. C. Kaska, *J. Am. Chem. Soc.*, **84**, 1501 (1962).

(5) A. W. Laubengayer, Special Publication No. 15, The Chemical Society, London, 1961, p. 150.

The product III was insoluble in most aliphatic and aromatic hydrocarbons and only very slightly soluble in anhydrous tetrahydrofuran or N-methylpyrrolidine. Traces of moisture rapidly cleaved III to regenerate I. Ultraviolet spectra of III in the latter two solvents displayed only end absorption in the region >325 m μ .



Although the foregoing reaction product III is clearly a highly associated substance ($x > 1$), it was desirable to demonstrate that benzene evolution had arisen from metalative cyclization leading to the 9-methyl-10-phenyl-10,9-aluminazarophenanthrene monomer (III), and not from metalation *ortho* or *para* to the methylamino group (C-3 or C-5 in II). Therefore, III was treated successively with a benzene solution of iodine and with water to yield, as oils, iodobenzene and *ca.* 80% of an iodo-2-(methylamino)biphenyl (IV)⁶ (picrate,⁶ m.p. 178–180°; *p*-toluenesulfonamide,⁶ m.p. 153–154°). Proof that IV is 2'-iodo-2-(methylamino)-biphenyl was obtained by heating IV with copper bronze in dimethylformamide to produce N-methylcarbazole (m.p. 87–88°; mixture melting point undepressed). Consequently, the structure proof of the molecular unit in III can be summarized in the following manner.



The properties of 9-methyl-10-phenyl-10,9-aluminazarophenanthrene (III) form a sharp contrast to those of the corresponding boron system.⁷ 9-Methyl-10-phenyl-10,9-borazarophenanthrene (V) is reported to be monomeric (m.p. 123°), soluble without decomposition in 95% ethanol, and quite stable to hydrolytic cleavage. Moreover, it exhibits a prominent ultraviolet maximum at 332 m μ , ascribable to boron-nitrogen conjugation. The latter contention is supported by the absence of such absorption in the spec-

(6) All new compounds encountered in this study have been characterized by satisfactory elemental analyses and spectral data consonant with the assigned structures.

(7) M. J. S. Dewar and P. M. Maitlis, *J. Am. Chem. Soc.*, **83**, 187 (1961).