We have now demonstrated that IIb is invariably formed from dopamine under conditions of (aut)-oxidation as well as *in vivo*.

A major problem has been the separation of IIb from the isomeric norepinephrine, both compounds having identical $R_f$ values in more than ten different solvent systems. Separation of such mixtures, however, was achieved by treatment with methanolic HCl which methylates IVb quantitatively to IVa[2] but leaves IIb unchanged (Table I).

TABLE I

CHROMATOGRAPHIC RESOLUTION OF MIXTURES OF TRIHYDROXYPHENETHYLAMINE AND NOREPINEPHRINE BY SELECTIVE *O*-METHYLATION

| Compound | PhOH:0.02*N* HCl: KCN 80 g.:20 ml.: trace (saturation of $SO_2$ gas) Anhyd. HCl–$CH_3OH$ Before | After | *sec*-BuOH:HCOOH: $H_2O$ 75:15:10 ($N_2$ atmosphere) Anhyd. HCl–$CH_3OH$ Before | After |
|---|---|---|---|---|
| Trihydroxyphenethylamine (IIb) | 0.29 | 0.30 | 0.25 | 0.25 |
| Norepinephrine (IVb) | 0.29 | 0.58 | 0.25 | 0.51 |
| Mixture of IIb + IVb | 0.30 | 0.30<br>0.58 | 0.25 | 0.25<br>0.51 |
| β-O-Methylnorepinephrine (IVa) | 0.58 | | 0.52 | |

TABLE II

*In Vitro* STUDIES WITH $H^3$ AND $C^{14}$ DOPAMINE

A mixture of dopamine-β,β-$H^3$ and dopamine-α-$C^{14}$ in the ratio of 3.13 was incubated for 3 hours. The "norepinephrine" was isolated using procedures previously described [*cf. Arch. Biochem. Biophys.*, 74, 252 (1958)].

| Incubation with | Counts/min. $C^{14}$ | $H^3$ | $H^3/C^{14}$ | Tritium atoms lost |
|---|---|---|---|---|
| Dog cerebellum homogenate | 248 | 896 | 3.60 | 0 |
| Dog hypothalamus homogenate (boiled) | 1,739 | 5,472 | 3.13 | 0 |
| Ascorbic acid–Fe–Versene[3] | 8,470 | 31,200 | 3.17 | 0 |
| Enzymatically formed norepinephrine[a] | 13,884 | 24,513 | 1.75 | 1 |
| Pure dopamine | ... | ... | 3.13 | ... |

[a] Specific enzymatic side-chain hydroxylation of dopamine occurs in hypothalamus, caudate nucleus, adrenal medulla and similar tissues by the action of an enzyme which may be called "*dopamine β-oxidase*" to differentiate it from any possible *dopamine oxidase* acting on the catechol nucleus.

When a mixture of tritium and carbon labeled dopamines was used for *in vitro* oxidations with various tissues, or with the ascorbic acid–Versene system[3] (Table II), the ratio $H^3/C^{14}$ in the "norepinephrine" fractions was found to be too high except with tissues containing *dopamine β-oxidase*. This suggests that little or no tritium had been lost by hydroxylation of the benzyl position and that norepinephrine was not the product formed. Little or no IVa was detected after treatment with $HCl/CH_3OH$.

Finally, 800 μg. of dopamine-α-$C^{14}$ (4100 c.p.m./μg.) was administered intraperitoneally to rats, and urine was collected in an all glass system. The "norepinephrine" fraction was found to be largely 2,4,5-trihydroxyphenethylamine, representing from 0.5–1% of the administered radioactivity. These are minimal conversion values, since only 1% of injected IIb could be recovered as such in the urine.

Although it remains to be seen whether IIb has any physiological significance, these findings have an important bearing on the validity of biochemical studies with isotopic precursors of norepinephrine.

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## NUCLEAR MAGNETIC RESONANCE SPECTRA. ALLYLMAGNESIUM BROMIDE[1]

*Sir:*

The proton nuclear magnetic resonance (n.m.r.) spectrum of allylmagnesium bromide (I, Fig. 1)

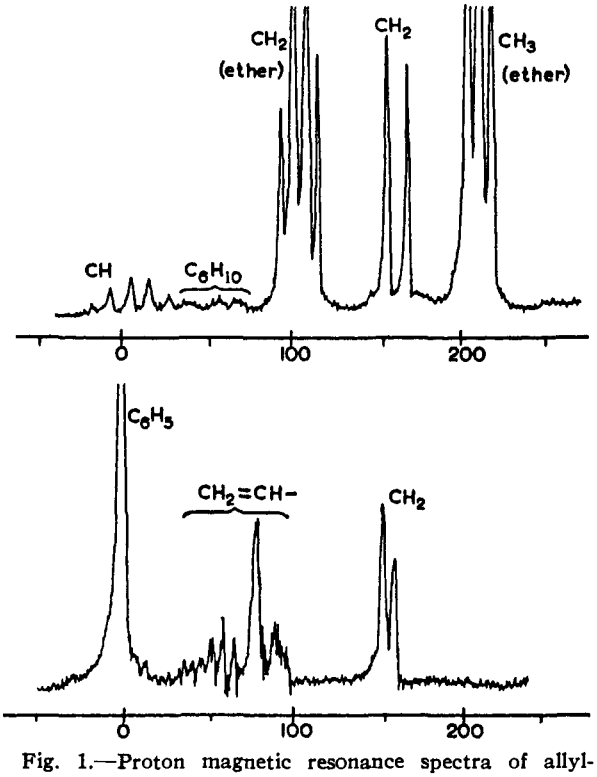



Fig. 1.—Proton magnetic resonance spectra of allylmagnesium bromide in diethyl ether solution (upper) and allylbenzene (lower). The spectra were taken with a Varian Associates High Resolution Spectrometer (V-4300) at 60 Mc. with a 12-inch magnet equipped with a Super-Stabilizer. Chemical shifts are in c.p.s. from benzene (external reference) and were measured by the audio-oscillator sideband superposition method. The signals designated $C_6H_{10}$ in the allylmagnesium bromide spectrum are due to diallyl formed by coupling during preparation of the Grignard reagent, as verified by the spectrum of a sample to which diallyl had been added deliberately.

is extraordinarily revealing with regard to the structure and mobility of this important organometallic compound as ordinarily prepared in diethyl ether solution. In the first place, compared to the

(1) Supported in part by the Office of Naval Research.

(2) B. F. Tullar, THIS JOURNAL, 70, 2068 (1948).

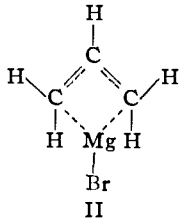
(3) S. Udenfriend, *et al.*, *J. Biol. Chem.*, 208, 731 (1954).

usual allyl compounds (see the spectrum of allylbenzene in Fig. 1), the substance gives an amazingly simple n.m.r. spectrum which, in fact, is of the general pattern expected for $AX_4$, where A and X are nuclei of spin $^1/_2$, having quite different chemical shifts, and all X's are equivalent with respect to A and each other.[2] The mutual interactions of such a system of nuclei result in a spectrum which can be described in terms of a single spin–spin coupling constant $J_{AX}$ which in the present case has the value 12 ± 1 c.p.s. and leads to a symmetrical quintet of resonance lines for A and a doublet for the resonance of X.[3]

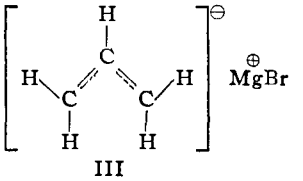
The n.m.r. spectrum of allylmagnesium bromide can be reasonably interpreted only if equilibrium (1) is assumed to be very rapid.[4]

$$BrMg{-}CH_2{-}CH{=}CH_2 \rightleftarrows CH_2{=}CH{-}CH_2{-}MgBr \quad (1)$$

To be sure, the merging of the separate resonances of the 1,3-protons of the allyl radical into an equivalent $X_4$ group could be taken to speak for the rapid equilibrium of eq. (1),[5] (2) a symmetrical bridged structure such as II[6] or the completely ionic structure III.



II



III

However, the fact that the 1,3-protons are in an $X_4$ group with no differentiation into *cis* and *trans* types requires that there be *rapid* rotation (≫30 c.p.s.) around the C–C bonds of the allyl group. This would hardly be possible for II or III because of the considerable double-bond character expected for the C–C bonds of such entities. Thus, the only conclusion is that the two possible covalent forms of the Grignard reagent are in dynamic equilibrium (with $\tau \ll 0.01$ sec.) and the lifetime of each form is long enough to permit rotation around its 1,2 C–C bond.

Further n.m.r. studies of allylmagnesium bromide and other Grignard reagents are in progress.

(2) *Cf.* H. J. Bernstein, J. A. Pople and W. G. Schneider, *Can. J. Chem.*, **35**, 65 (1957).

(3) *Cf.* J. D. Roberts, "Nuclear Magnetic Resonance," McGraw-Hill Book Co., New York, N. Y., 1959, Chap. III.

(4) As far as is known at present, this equilibrium could be established by either inter- or intramolecular processes.

(5) *Cf.* Ref. 3, Chap. IV, for discussion and examples.

(6) W. G. Young and J. D. Roberts, THIS JOURNAL, **68**, 1472 (1946).

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## 2,6-DIAMINO-4-HYDROXYPTERIDINE, A NEW, NATURALLY OCCURRING PTERIDINE

*Sir:*

In hydrogenation experiments on 2-amino-4-hydroxypteridines, using platinum or palladium as catalysts, we noted the persistent occurrence of a greenish-yellow fluorescent compound in reoxidized samples submitted to paper chromatographic analysis. A 20% yield of the new compound was obtained by reduction of 2-amino-4-hydroxypteridine in dilute ammonia solution and reoxidation with manganese dioxide without exposure to air. The bright yellow compound was separated by paper chromatography (1-propanol–1% ammonia) from the other products of the reaction (starting material, and xanthopterin), and was obtained in pure form by eluting the appropriate band from the paper with aqueous ammonia and removing the ammonia. It was very insoluble in water but was readily soluble in dilute acid or alkali. Its elementary analysis indicated an empirical formula $C_6H_6N_6O$ (Calcd. for $C_6H_6N_6O$: C, 40.4; H, 3.37; N, 47.2. Found: C, 40.3; H, 3.12; N, 43.7). Its ultraviolet absorption spectrum had maxima in 0.1 *N* sodium hydroxide at 260 mμ ($E^{1\%}_{1cm.}$, 1092), and 393 mμ ($E^{1\%}_{1cm.}$, 345); maxima in 0.1 *N* hydrochloric acid were at 272 mμ ($E^{1\%}_{1cm.}$, 883) and 377 mμ ($E^{1\%}_{1cm.}$, 310). Some $R_F$ values compared with those for xanthopterin (in brackets) are: 1-propanol, 1% ammonia, (2:1) 0.27 (0.23); 1-butanol, acetic acid, water (4:1:1), 0.26 (0.36); 4% sodium citrate, 0.37 (0.62). We have shown by these reactions that the compound is 2,6-diamino-4-hydroxypteridine: (1) Lumazine under the same conditions of reduction and reoxidation yields a different but very similar compound (presumably 2,4-dihydroxy-6-aminopteridine), indicating that the pyrimidine portion of the ring is unaltered. (2) 2-Amino-4-hydroxypteridine labelled in the 6 and 7 positions with $C^{14}$ loses no radioactivity on conversion to the new compound, indicating that the pyrazine portion is unaffected. (3) The compound if again reduced with platinum in dilute sodium hydroxide solution and allowed to reoxidize in air yields a mixture of 2-amino-4-hydroxypteridine, xanthopterin and starting material. (4) On very gentle treatment with nitrous acid, the compound is almost quantitatively converted to xanthopterin, identified by paper chromatography and by ultraviolet and infrared spectra.

A variety of other pteridines including folic acid and biopterin yield 2,6-diamino-4-hydroxypteridine by the same process, along with xanthopterin and 2-amino-4-hydroxypteridine.

Considerable interest attaches to this compound not only because of the novel method of synthesis but also because we have been able to isolate it by paper chromatography from *Drosophila melanogaster* and from the blue-green algae, *Anacystis nidulans* and *Nostoc muscorum* G and identify it by paper chromatography and ultraviolet and infrared spectra. It is therefore probably widely distributed in nature. The functional significance of the 6-amino group, and the relationship of the compound to xanthopterin, folic acid and other naturally occurring pteridines remain to be elucidated. It is possible, for example, that 2,6-diamino-4-hydroxypteridine is an artifact arising from a very reactive compound (presumably 2-amino-4-hydroxy-5,8-dihydropteridine or 2-amino-4-hydroxy-7,8-dihydropteridine) by addition of ammonia during the isolation procedure.

Additions to the double bonds of the pteridine