staying together of electron and sodium $t_{Na} \geq 3 \times 10^{-7}$ second.

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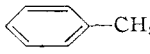
EFFECT OF 1-PHENYL-2-HYDRAZINOPROPANE, A POTENT MONOAMINE OXIDASE (MAO) INHIBITOR, ON BRAIN LEVELS OF NOREPINEPHRINE AND SEROTONIN

Sir:

We have synthesized certain hydrazine analogs,[1] in the hope of obtaining sympathomimetic substances with greater affinity for the cell receptor sites and increased stability toward metabolic degradation. One such compound, α-methylphen-



—$CH_2CH(CH_3)$—NH—NH_2·HCl (JB-516)

ethylhydrazine hydrochloride exhibited amphetamine-like activity. In addition, it was demonstrated by Horita[2] to be an MAO inhibitor, many times more potent than iproniazid.

The therapeutic significance of the MAO inhibitor iproniazid in the treatment of depressed mental conditions[3] prompted us to report on the preparation, preliminary pharmacological and biochemical effects of this new hydrazine derivative. Phenyl-2-propanone was treated with methanolic hydrazine hydrate to form phenyl-2-propanone hydrazone in 80% yield, b.p. 101–103° (0.30 mm); found: N, 18.46; $n^{20}D$ 1.5613. The hydrazone was reduced to α-methylphenethylhydrazine with platinum oxide in ethanol–acetic acid solution, b.p. 82–86° (0.50 mm.); yield 55–60% (found: N, 18.71; $n^{20}D$ 1.5401). The monohydrochloride melted at 122–124° (found: Cl, 18.97; C, 57.95; H, 8.10; N, 15.01.)

Five mg./kg. of JB-516 produced marked central stimulatory effects in rabbits similar to those of amphetamine. Lower doses (1 mg./kg.) exerted no obvious effects. However, this dose appeared to inhibit brain monoamine oxidase activity,[4,5] since rabbits pretreated with 1 mg./kg. of JB-516 and then given 5 mg./kg. reserpine exhibited excitation instead of depression.

Monoamine oxidase has been shown to have a major role in the physiologic inactivation of both serotonin and norepinephrine in brain.[6]

Since these substances may be involved in the regulation of certain brain functions,[7,8] it was of interest to investigate the effect of JB-516 in low dosage on serotonin and norepinephrine levels in brain. JB-516 (1 mg./kg.) and iproniazid (10 mg./kg.) were administered daily (s.c.) to rabbits for five days. The levels of the amines, in brain stem, measured by methods previously described[9,10] rose slowly and reached two to three times the normal value within five days. By the third or fourth day central stimulation was evident. The administration of 5 mg./kg. of iproniazid and 50 mg./kg. of isoniazid daily for 5 days elicited neither excitation nor an increase in the levels of the brain amines. The effects of the various drugs on brain levels of norepinephrine and serotonin are summarized in Table I. Each value represents the average of three animals.

Mg./kg.				
JB-516	Iproniazid		Isoniazid	
1.0	10	5	50	Controls
Norepinephrine levels (γ/g. tissue)				
0.95	1.1	0.43	0.40	0.40
Serotonin levels (γ/g. of tissue)				
1.6	0.92	0.60	0.58	0.58

These results suggest that JB-516 is at least ten times as active as iproniazid in eliciting central excitation and a rise in brain levels of nor-epinephrine and serotonin. The increase in nor-epinephrine may be related to central action of JB-516; in this regard it is noteworthy that large doses of 3,4-dihydroxyphenylalanine, a norepinephrine precursor, cause central excitation which is enhanced by pretreatment with iproniazid.[11]

In summary, the replacement of an amino group by a hydrazino moiety in amphetamine has yielded a compound which embodies the effects of amphetamine but is in addition a very potent MAO inhibitor. The compound is now undergoing extensive clinical investigation for treatment of depressed mental conditions.

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INTERCONVERSION OF VOLATILE BORANES BY BASIC REAGENTS

Sir:

All of the known effective methods of converting one volatile boron hydride to another have been adjustments of physical conditions governing decomposition-type reactions—as in the conversion of diborane mostly to pentaborane-9 by fast-flow methods at elevated temperatures,[1] or mostly to pentaborane-11 by flow at higher pressures and lower temperatures,[2,3] or to tetraborane by a partial reversal of the process.[2] We now report that some borane interconversions can be done efficiently by the action of appropriately chosen chemical reagents, well below room temperature and

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under conditions adaptable to large-scale production.

One example is the quantitative process $B_5H_{11} + 3H_2O \rightarrow 2H_2 + B(OH)_3 + B_4H_{10}$. This represents an efficient synthesis of B_4H_{10} because B_5H_{11} is efficiently made from B_2H_6.[2] Another example is the reaction of $[(CH_3)_2N]_2BH$ with B_5H_{11}, converting nearly half of the latter to B_5H_9 and 3.6% to B_6H_{10}. This represents the best known way to obtain the hitherto very rare hexaborane, the yield of which probably can be improved further. The conversion of B_5H_{11} to B_5H_9 was not necessarily direct, for B_4H_{10} also was formed, and we have found that $[(CH_3)_2N]_2BH$ converts as much as 25% of a B_4H_{10} sample to B_5H_9. These reactions suggest interesting new ideas about the mechanisms of borane interconversions by the classical gas-phase processes.

In the experiments described all volatile products were identified by their known physical properties and hydrolytic analyses. Numbers preceding formulas represent millimole quantities.

Tetraborane from Pentaborane-11.—Nearly pure B_5H_{11} (0.509 mmole) was hydrolyzed during one minute at 0°, yielding 0.491 B_4H_{10} and 0.506 $B(OH)_3$; and 0.017 B_5H_9 impurity was recovered. Again, the hydrolysis of 0.392 B_5H_{11} gave 0.360 B_4H_{10}, 0.767 H_2, and 0.366 $B(OH)_3$, with recovery of 0.023 B_5H_9. Allowing for the B_5H_9 impurity, the yields of B_4H_{10} were 99.8 and 97.6%. The process also shows how to analyze B_5H_{11} for B_5H_9, an impurity formerly difficult to estimate.

Pentaborane-9 and Hexaborane from Pentaborane-11.—A reaction between 1.187 B_5H_{11} and 0.696 $[(CH_3)_2N]_2BH$ was noticed at −78°. During slow warming to 0°, volatile products formed at increasing rates; final stoichiometry: 0.192 B_2H_6, 0.165 B_4H_{10}, 0.583 B_5H_9, 0.009 B_5H_{11}, 0.035 B_6H_{10}, 0.074 $(CH_3)_2NB_2H_5$, and non-volatile material. The last was heated with dimethylamine, forming 0.626 $[(CH_3)_2N]_2BH$ and 1.02 $(CH_3)_2NBH_2$; final recovery of boron as volatile compounds, 91%. The 3.6% yield of B_6H_{10} was not an impurity in the B_5H_{11}, for partial hydrolysis of parallel samples would have disclosed any B_6H_{10} (easily separable from B_4H_{10} and B_5H_9).

Pentaborane-9 from Tetraborane.—An adduct empirically formulated as $[(CH_3)_2N]_2BH \cdot B_4H_{10}$ was formed at −78°. On warming to −15° during 5 hr., 0.692 mmole evolved 0.031 B_2H_6, 0.120 B_4H_{10}, 0.037 $[(CH_3)_2N]_2BH$, 0.092 $(CH_3)_2NBH_2$, and 0.116 B_5H_9. The pentaborane represented 25% of the boron in the unrecovered tetraborane.

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THE SYNTHESIS OF HEPARIN IN MOUSE MAST CELL TUMOR SLICES

Sir:

Heparin appears to be entirely localized in the mast cells which occur generally throughout all connective tissue. This had led to the general assumption that these cells are the site of synthesis of heparin although the possibility has never been excluded that they serve only a storage or excretory function. The availability of mouse mast cell tumors,[1] 2 to 2.5 g. subcutaneous masses of essentially homogeneous tissue, made possible a study of the metabolism of heparin in mast cells, *in vitro*.

Slices of tumor were incubated with either C^{14}-glucose or S^{35}-sulfate and heparin of relatively high purity isolated.

TABLE I

Approximately 5 g. of tissue slices of mouse mast cell tumor were incubated in Krebs–Ringer phosphate buffer with either C^{14}-glucose (5.5 μM./ml., 0.36 μC/μM.) or S^{35}-sulfate (1.5 μM./ml., 3 μC/μM.) at 37° in an atmosphere of O_2. The tissue was then homogenized, boiled, cooled, adjusted to *p*H 8.5 and incubated with 25 mg. of pancreatin overnight. The samples were then dialyzed for 18 hours against running tap water, the insoluble material was removed by centrifugation and sufficient NaCl added to the supernatant solutions to make them 1 *M*. Heparin was precipitated by the addition of an excess of cetyltrimethylammonium bromide; the complex was then redissolved in 4 *M* NaCl and re-precipitated by dilution to 1 *M*. This was repeated three times. Finally, the heparin complex was washed well with water, dissolved in 2 *M* NaCl and the quaternary amine removed as the insoluble thiocyanate salt. The solution of heparin was thoroughly dialyzed against distilled water before counting. Heparin was determined by metachromatic and carbazole assays using commercial heparin (Upjohn, 120 units/mg.) as a standard. The assays were in good agreement and approximately 70% of the weight was accounted for by heparin.

Expt.	Radioactive precursor	Incubation time, hours	Heparin Mg.	Heparin C.p.m./mg.
I	Glucose	0	2.1	14
	Glucose	1	2.0	4,040
	Glucose	2	2.0	7,560
II	Sulfate	0	2.0	20
	Sulfate	2	2.0	34,000

The following evidence supports the view that, in both experiments, the radioactive compound isolated was heparin. (1) A crude chondroitin sulfate fraction, which was also isolated, was found to have less than 20% the radioactivity and less than 15% the specific activity of the heparin. (2) When a ten-fold excess of unlabeled heparin and chondroitin sulfate were added to radioactive samples over 90% of the radioactivity was re-isolated with the heparin. (3) Shaking an aqueous solution of radioactive material with chloroform:octanol[2] (9:1) did not remove any detectable protein or radioactivity. (4) Upon electrophoresis in 0.02 *M* citrate buffer, *p*H 4.5 for 4 hours at 500 volts, single metachromatic and radioactive spots, which exactly corresponded, were observed. (5) After digestion with microbial heparinase[3] no radioactivity was recoverable upon the addition of carrier heparin. (6) After hydrolysis of the C^{14}-labeled heparin for 20 minutes at 100° in 7.5% H_2SO_4 70% of the radioactivity remained non-dialyzable. Both chondroitin sulfate and hyaluronic acid are completely degraded under these

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