Table I. Parameters for the Trypsin- and AT$_{6.7}$-Catalyzed Hydrolysis of TAME and BAEE

Enzyme	Substrate	$k_L \times 10^{-2}$, sec^{-1}	$K_L \times 10^5$, M	$k_{LL} \times 10^{-2}$, sec^{-1}	$K_L' \times 10^2$, M
Trypsin	TAME	1.51 ± 0.22	1.52 ± 0.28	7.85 ± 0.60	5.86 ± 0.44
AT$_{6.7}$	TAME	1.79 ± 0.31	0.74 ± 0.14	26.6 ± 1.4	2.80 ± 0.17
Trypsin	BAEE	0.32 ± 0.05	0.71 ± 0.07	. . .	. . .
AT$_{6.7}$	BAEE	0.38 ± 0.02	0.57 ± 0.03	0.52 ± 0.05	2.01 ± 1.11

mediate and the rate-determining pseudo-first-order solvolysis of this intermediate.[5] The phenomenon, which has been observed previously for the trypsin-catalyzed hydrolysis of TAME,[6–8] has been attributed to the formation of ternary as well as binary complexes, the velocity of product formation from the ternary complex being greater than that from the binary complex in which the substrate is bound only to the single active site.

Figure 2 illustrates the effect of BAEE concentration on the rate of hydrolysis of this substrate by trypsin and AT$_{6.7}$ under the same experimental conditions. As previously reported[6] the enhancement of k_{obsd} at high substrate concentrations in the case of the trypsin-catalyzed hydrolysis is minimal. With acetylation of the enzyme the effect becomes clearly demonstrable even though the magnitude is much smaller than one observes using TAME as substrate.

The theoretical lines in Figures 1 and 2 were calculated by regression analysis[9] using the treatment and symbols employed by Trowbridge, *et al.*[6]

The results are listed in Table I. The parameters for the trypsin-catalyzed hydrolysis of TAME are consistent with those determined by Trowbridge, *et al.*,[6] and the binary parameters for BAEE agree with the K_m reported by Inagami[10] and the k_3 of Bernhard and Gutfreund[5] when allowances are made for differences in pH, ionic composition, and enzyme purity. Small decreases in the magnitude of K_L and K_L' with acetylation are observed with both substrates. These are consistent with enhanced affinity for these positively charged compounds due to the change in net charge on the protein.

The primary change responsible for the selective increase in molecular activity for hydrolysis of TAME upon acetylation of the enzyme appears in the parameter k_{LL}. While uncertainty exists concerning the nature of the elementary steps of trypsin-catalyzed hydrolysis, particularly under the conditions of apparent substrate activation, this change does not appear to be explainable on the basis of enhanced substrate binding. It is also in this parameter, which has the form of the catalytic rate constant for formation of product from the ternary complex, that the dramatic difference between the rates of hydrolysis of TAME and BAEE at high substrate concentrations is observed. Thus the difference in effectiveness of these two substrates as activators does not appear to reflect differences in binding. Whether the activator serves as an allosteric effector, as suggested by Bechet and Yon,[7] or interacts directly with the substrate molecule bound to the active site, the nature of the acylamido substituent group appears to be an important determinant of the activation process.

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(5) S. A. Bernhard and H. Gutfreund, *Proc. Natl. Acad. Sci. U. S.*, **53**, 1238 (1965).

(6) C. G. Trowbridge, A. Krehbiel, and M. Laskowski, Jr., *Biochemistry*, **2**, 843 (1963).

(7) J. Bechet and J. Yon, *Biochim. Biophys. Acta*, **89**, 117 (1964).

(8) N. J. Baines, J. B. Baird, and D. T. Elmore, *Biochem. J.*, **90**, 470 (1964).

(9) A Control Data Corporation 1604 computer was employed for computation.

(10) T. Inagami, *J. Biol. Chem.*, **239**, 787 (1964).

Evidence for the Species BH_2^+ and $BH(OH)_2$ in Aqueous Solutions. The Reaction of Diborane with Hydroxide

Sir:

We wish to report stoichiometric evidence for the formation of intermediates in the hydrolyses of diborane and hydroborate in aqueous solutions at temperatures around −70°. The data for strongly acidic solutions are consistent with the formation of the aquated BH_2^+ ion. The data for unacidified solutions of ethanol and water correspond to the formation of $BH(OH)_2$ (or its hydrogen-bridged dimer), probably mixed with the various possible ethoxy derivatives. Studies with potassium hydroxide, in solution and in the solid state, suggest the formation of the $BH(OH)_3^-$ ion.

Evidence for BH_2^+. Potassium hydroborate reacts with 8 M HCl at −70° to form 2 moles of hydrogen/mole of hydroborate and a solution which is capable of reducing iodine and which yields another 2 moles of hydrogen when warmed above −20°.[1] Boron-11 nmr spectra of cold solutions as concentrated as 2 N in the reducing species (determined both by iodine titration and hydrogen evolution) showed only an extremely broad peak[2] centered 29 ppm upfield from the signal of BCl_3. After allowing such solutions to decompose at 0°, the broad peak disappeared, and a precipitate of boric acid formed.

Diborane reacts slowly[6] with 8 M HCl at −75° to form always 2 moles of hydrogen/mole of diborane; 4 more moles of hydrogen is evolved upon warming the solution above −20°. The properties of the cold solu-

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(1) Such data were obtained from experiments carried out in closed reaction vessels. However, when potassium hydroborate was added to 8 M HCl at −75° while rapidly pumping the evolved gas through a trap at −78° and a trap at −196°, a small amount (corresponding to a 2% yield) of diborane was collected in the −196° trap.

(2) McAchran and Shore[3] similarly were unable to observe a well-defined spectrum of the $BH_2(OSMe_2)_2^+$ ion, and Schaeffer, *et al.*,[4] only observed a very broad $BH_2(dioxane)_2^+$ signal. Apparently ^{11}B quadrupole relaxation occurs in ions of the type $BH_2(base)_2^+$.[5]

(3) G. E. McAchran and S. G. Shore, *Inorg. Chem.*, **4**, 125(1965).

(4) R. Schaeffer, F. Tebbe, and C. Phillips, *ibid.*, **3**, 1475 (1964).

(5) S. G. Shore, C. W. Hickam, Jr., and D. Cowles, *J. Am. Chem. Soc.*, **87**, 2755 (1965).

(6) Only about one-half of a 0.6-mmole sample of diborane reacted with 10 ml of stirred 8 M HCl at −75° in a period of 3 hr.

tion are identical with those of the solutions prepared by the addition of hydroborate to cold 8 *M* HCl. To determine whether chloride ion has a specific influence on the course of the reaction, the reaction of diborane with 5.4 *M* $HClO_4$ at −55° was studied. In two such experiments, 2.70 and 2.15 moles of hydrogen were slowly evolved per mole of diborane. We believe the principle reaction in these experiments was the same as that occuring in 8 *M* HCl at −75°, but that some extra hydrolysis occurred because the experiments were necessarily carried out at a considerably higher temperature and with a lower concentration of hydrogen ion.

We believe that the reactions of hydroborate and diborane with cold aqueous acid may be expressed by the equations

$$BH_4^- + 2H^+ \longrightarrow 2H_2 + BH_2^+$$

$$B_2H_6 + 2H^+ \longrightarrow 2H_2 + 2BH_2^+$$

and that the decomposition of the aquated BH_2^+ species above −20° may be written as follows

$$BH_2^+ + 3H_2O \longrightarrow 2H_2 + B(OH)_3 + H^+$$

Evidence for $BH(OH)_2$. When diborane reacts with ethanol–water solutions at temperatures around −75°, always 4 moles of hydrogen is evolved per mole of diborane. The resulting solution yields another 2 moles of hydrogen when warmed above −20°. These data suggest the formation and subsequent decomposition of $BH(OH)_2$, $BH(OH)(OEt)$, and $BH(OEt)_2$.[7] Thus we might write, for the formation of $BH(OH)_2$

$$B_2H_6 + 4H_2O \longrightarrow 4H_2 + 2BH(OH)_2$$

and for the decomposition of $BH(OH)_2$

$$BH(OH)_2 + H_2O \longrightarrow H_2 + B(OH)_3$$

Of course, it is conceivable that hydrogen-bridged species such as $(HO)_2BH_2B(OH)_2$, $(HO)_2BH_2B(OH)(OEt)$, etc., are formed, or that a cyclic compound such as boroxine ($B_3O_3H_3$) is formed, but in the absence of further data we prefer the above simple formulation.

When a solution prepared by the reaction of diborane with an ethanol–water solution at −75° is treated with an equal volume of 8 *M* HCl at the same temperature, no appreciable hydrogen is evolved. The resulting solution decomposes at approximately the same rate as solutions to which no acid has been added (at corresponding temperatures above −20°). These results suggest that the decomposition is not acid catalyzed.

When a solution prepared by the reaction of diborane with an ethanol–water solution at −75° is treated with an ethanol–water solution of potassium hydroxide at the same temperature, only a small amount of hydrogen is evolved. When the resulting solution is warmed to room temperature, approximately 1.3 moles of hydrogen/mole of diborane is rapidly evolved. A final 0.7 mole of hydrogen is very slowly evolved during a period of several days at room temperature, bringing the total evolved hydrogen to 6 moles/mole of diborane. We tentatively suggest that the addition of hydroxide to the cold solution causes the formation of a series of species such as $BH(OH)_3^-$, $BH(OH)_2(OEt)^-$, etc., and that these species undergo hydrolysis upon warming the solution.[10] Other experiments which may be interpreted as indicating the formation of $BH(OH)_3^-$ are described below.

Reaction of B_2H_6 with KOH. When diborane reacts at −30° with an excess of coarsely pulverized reagent grade KOH which has been allowed to absorb about 7% extra water, approximately 1.13 moles of hydrogen is evolved per mole of consumed diborane.[14] When the resulting solid is dissolved in cold water, a small amount of hydrogen (about 0.15 mole/mole of consumed diborane) is rapidly evolved, and the resulting solution slowly evolves hydrogen for several days at room temperature.[16] The latter observation is very significant in that it shows that, after the initial rapid evolution of hydrogen, the solution contains more reducing power than corresponds to the formation of equimolar amounts of borate and hydroborate. The stoichiometry may be explained by assuming that the principal over-all reaction of the diborane with potassium hydroxide is

$$H_2O + 2KOH + B_2H_6 \longrightarrow H_2 + KBH_4 + KBH(OH)_3$$

with the following side reaction occurring to some extent.

$$2H_2O + 2KOH + B_2H_6 \longrightarrow 2H_2 + KBH_4 + KB(OH)_4$$

The latter reaction can be made essentially quantitative by allowing diborane to react with a concentrated KOH solution at −30°.[14,17] All our attempts to obtain the boron-11 nmr spectrum of the $BH(OH)_3^-$ ion were unsuccessful; solutions of the B_2H_6–solid KOH reaction product only yielded resonances attributable to $B(OH)_4^-$ and BH_4^-. Possibly ^{11}B nuclear quadrupole relaxation effects are responsible for our inability to see a signal. In this respect it is interesting to note that we were similarly unable to observe a proton nmr signal for the hydridic hydrogen of the aqueous trimethoxyhydroborate ion. Solutions prepared from $NaBH(OMe)_3$ showed, however, a signal corresponding to the hydrobo-

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[7] Weiss and Shapiro[8] found that 4 moles of hydrogen was evolved/mole of diborane consumed in the reaction with ice at −80°. They proposed the formation of –O–BH–O– groups attached to the surface of the ice. Lehmann, *et al.*,[9] showed that $BH(OEt)_2$ is a relatively stable material formed by the reaction of ethanol with excess diborane.

(8) H. G. Weiss and I. Shapiro, *J. Am. Chem. Soc.*, **75**, 1221 (1953).

(9) W. J. Lehmann, H. G. Weiss, and I. Shapiro, *J. Chem. Phys.*, **30**, 1222 (1959).

(10) Apparently only on the basis of the evolved hydrogen, Mikheeva and Surs[11] postulated the formation of $BH(OH)_3^-$ as one of several intermediates in the reaction of B_2H_6 with KOH solutions. Mochalov and Gil'manshin[12] presented polarographic evidence for the same species, but Gardiner and Collat[13] disputed their interpretation.

(11) V. I. Mikheeva and V. Y. Surs, *Dokl. Akad. Nauk SSSR*, **93**, 67 (1953); *Chem. Abstr.*, **48**, 7470*c* (1954).

(12) K. N. Mochalov and G. G. Gil'manshin, *Dokl. Akad. Nauk SSSR*, **132**, 134 (1960); *Zh. Fiz. Khim.*, **36**, 1089 (1962).

(13) J. A. Gardiner and J. W. Collat, *Inorg. Chem.*, **4**, 1208 (1965).

(14) These observations are in close agreement with those of Stock and Kuss,[15] who observed that 1.1 moles of hydrogen/mole of diborane was evolved in the reaction of diborane with their driest sample of KOH. With increasing moisture content, the hydrogen evolution increased; 30% KOH solution yielded 2 moles of hydrogen/mole of diborane. Stock and Kuss explained their data in terms of the reaction $B_2H_6 + 2KOH \rightarrow 2KOBH_3 + H_2$.

(15) A. Stock and E. Kuss, *Chem. Ber.*, **47**, 810 (1914).

(16) We cannot now account for the rapid initial evolution of hydrogen. Possibly this is attributable to a relatively unstable $BH_2(OH)_2^-$ ion. The final rate of hydrogen evolution appears to be greater than that observed for KBH_4 in a solution of comparable alkalinity.

(17) Davis and Gottbrath[18] obtained a 10–17% yield of hydroborate by bubbling diborane through NaOH solutions at 0°. Winternitz has alluded to the reaction, and has stated that he was unable to verify Stock's findings with regard to the reaction of diborane with potassium hydroxide.[19]

(18) R. E. Davis and J. A. Gottbrath, *Chem. Ind.* (London), 1961 (1961).

(19) P. F. Winternitz, "Borax to Boranes," Advances in Chemistry Series, No. 32, American Chemical Society, Washington, D. C., 1961, p 174.

rate ion. The latter species may be attributable to impurity in the $NaBH(OMe)_3$ sample or to a disproportionation upon dissolution.[20]

Acknowledgment. We wish to thank Messrs. Malcolm Judkins and Robert Marianelli for obtaining the ^{11}B nmr spectra. This research was supported by the U. S. Atomic Energy Commission.

(20) H. C. Brown, E. J. Mead, and P. A. Tierney, *J. Am. Chem. Soc.*, **79**, 5400 (1957).

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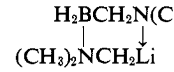
A Lithium-Substituted Borane Derivative and New Classes of Boranes

Sir:

We wish to report the isolation of a novel lithium-substituted borane derivative, lithiomethyldimethylamino(dimethylaminomethyl)dihydroborane (I). A

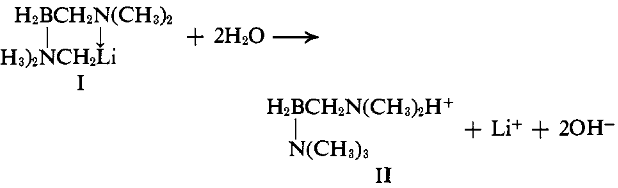


$$\begin{array}{l} H_2BCH_2N(CH_3)_2 \\ \quad\;\, | \qquad\quad \downarrow \\ (CH_3)_2NCH_2Li \end{array}$$

I

cyclic structure is probable in view of its constitution and volatility. It is a white, highly crystalline solid, melting at 112–114°, subliming at 80° under high vacuum. It is soluble in benzene, hexane, and ether. On exposure to air it reacts vigorously, nearly inflaming after a short induction period.

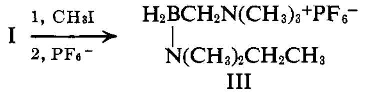
The composition of I was determined by a number of chemical methods.[1] A neutralization equivalent of 67 ± 1 agrees favorably with the value of 68.0 calculated from the equation



$$\underset{\text{I}}{\begin{array}{l} H_2BCH_2N(CH_3)_2 \\ \quad | \qquad\quad \downarrow \\ (CH_3)_2NCH_2Li \end{array}} + 2H_2O \longrightarrow \underset{\text{II}}{\begin{array}{l} H_2BCH_2N(CH_3)_2H^+ \\ \quad | \\ N(CH_3)_3 \end{array}} + Li^+ + 2OH^-$$

The titration curve that would be expected for the simultaneous titration of a strong base and a moderately strong base was obtained. The hydrolysis of the carbon–lithium bond and the $-CH_2N(CH_3)_2$ group account for the formation of 2 moles of hydroxide/mole of I. Concentration of the titrant solution and addition of ammonium hexafluorophosphate solution led to the isolation of the hexafluorophosphate salt of the new borane cation II. Its structure was identified by independent synthesis as discussed later.

Reaction of I with methyl iodide gave a white solid iodide salt which was converted to the hexafluorophosphate salt by metathesis. *Anal.* Calcd for $H_2B[N(CH_3)_2C_2H_5]CH_2N(CH_3)_3PF_6$: C, 31.6; H, 7.95; N, 9.2; P, 10.2. Found: C, 31.9; H, 7.98; N, 9.2; P, 10.3.



$$\text{I} \xrightarrow[2,\ PF_6^-]{1,\ CH_3I} \underset{\text{III}}{\begin{array}{l} H_2BCH_2N(CH_3)_3{}^+PF_6^- \\ \quad | \\ N(CH_3)_2CH_2CH_3 \end{array}}$$

The presence of the $H_2B<$ moiety in III, and consequently in I, was confirmed by hydrolysis of III in the presence of platinum.[2] The hydrolytic hydrogen value of $6.7 \pm 0.2 \times 10^{-3}$ mmole/mg agreed well with that calculated for III, 6.6×10^{-3} mmole/mg. Recovery of 85% of the ethyldimethylamine (identified by infrared analysis) liberated in the hydrolysis substantiated the $-H_2BN(CH_3)_2C_2H_5$ group in III. Isolation of this amine clearly confirms the position of the lithium–carbon bond in I.

Synthesis of I was effected by stirring 2 moles of butyllithium with 1 mole of $H_2B[N(CH_3)_3]_2{}^+Cl^-$ for several hours. The product was then isolated by volatilization from the residue after evaporation of hexane solvent. The reaction temperature and stoichiometry are critical. If about 1.5 moles of butyllithium are used, different products result, one of which is the new borane-substituted amine, trimethylamine (dimethylaminomethyl)borane (IV). It was found that the best yields of IV

$$\underset{\text{IV}}{(CH_3)_3NBH_2CH_2\ddot{N}(CH_3)_2}$$

were obtained by rapid addition of the butyllithium while keeping the temperature near 20°.

Because of the fact that a boron–nitrogen bond between tertiary boron and nitrogen is analogous to a carbon–carbon bond, IV is structurally similar to N,N-3,3-tetramethylbutylamine. It is a colorless liquid of low volatility (vapor pressure less than 1 mm at room temperature) which freezes to a crystalline solid melting at −25.5 to −23°. It decomposes slowly, about 75% per day, at room temperature according to

$$2\underset{\text{IV}}{(CH_3)_3NBH_2CH_2\ddot{N}(CH_3)_2} \longrightarrow 2(CH_3)_3N\colon + \underset{\text{V}}{[BH_2CH_2N(CH_3)_2]_2}$$

This decomposition reaction was useful in characterizing IV. A 55.0-mg sample of IV was heated several hours at 120° in a closed vessel. The volatile product at −40° was distilled and found to be 0.404 mmole (96% of theory) of trimethylamine. The residue remaining was 0.208 mmole (98.5% of theory) of (dimethylaminomethyl)borane cyclic dimer (V),[3] identified by its infrared spectrum.

Trimethylamine (dimethylaminomethyl)borane (IV) dissolves readily in water to give a basic solution from which the moderately soluble hexafluorophosphate salt of cation II can be precipitated. The infrared spectrum of this salt shows a sharp single NH absorption at 3260 cm⁻¹ and a multiplet BH absorption at 2350 cm⁻¹.[4] The somewhat low value of the BH stretching frequencies has also been observed for amine alkyboranes.[5,6] Hydrolysis gave a hydrolytic hydrogen value of 0.0072 ± 0.0001 mmole/mg, identical with that calculated for the formation of 2 moles of hydrogen/formula weight of $(CH_3)_3NBH_2CH_2N(CH_3)_2H^+PF_6^-$.

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(1) Because of its reactivity I has not given satisfactory elemental analyses.

(2) A modification of the general technique suggested by H. C. Miller (personal communication) was used. See N. E. Miller, J. A. Forstner, and E. L. Muetterties, *Inorg. Chem.*, **3**, 1690 (1964).

(3) N. E. Miller and E. L. Muetterties, *ibid.*, **3**, 1196 (1964).

(4) The splitting of the BH stretching absorptions was similar to that observed in solid-state spectra of $H_2B(NR_3)_2{}^+$ salts.[5]

(5) N. E. Miller and E. L. Muetterties, *J. Am. Chem. Soc.*, **86**, 1033 (1964).

(6) M. F. Hawthorne, *ibid.*, **83**, 831 (1961).