labile, or of order less than unity, or both. Also, since tetraborane furnishes three $BH_3$ groups per molecule, for reaction with trimethylamine, it would appear that the experimental facts concerning its ammonia chemistry[4] require an even more thorough reinterpretation than would be obvious from the intervening work on ammoniated diborane.[5]

### Experimental Part

**Preparation of Tetraborane.**—Pure tetraborane was prepared by the action of hydrogen upon the unstable pentaborane $B_5H_{11}$, during ten minutes at 100°,[6] followed by careful fractionation and a check of the vapor tension of the product at 0°.[7] The $B_5H_{11}$ was easily obtained by flow-heating of diborane at 120°,[6] using the apparatus indicated by Fig. 1. This demountable attachment to the vacuum system operated much in the manner of the original fixed apparatus, but was far more convenient.

**Reaction of Tetraborane with Trimethylamine.**—A 41.1-cc. (gas at S.C.) sample of $B_4H_{10}$ was added in five equal portions over a period of 5 hours, to 185 cc. (gas at S.C.) of $(CH_3)_3N$ dissolved in 1.7572 g. of tetrahydrofuran. The mixture was magnetically stirred between additions of $B_4H_{10}$, and subsequently for a period of 72 hours. During the first 48 hours, the temperature was −78°; thereafter it was raised gradually to −30°, with evolution of 1.26 cc. of hydrogen. The solvent was then removed and separated by fractional condensation (through −45° and trapped at −78°; weight 1.7616 g.), and the other components were brought together again in the reaction tube; during 15 hours at 0°, the recoverable $(CH_3)_3N$ fell from 48.7 to 46.8 cc.

The volatile solid was identified as $(CH_3)_3NBH_3$ by its melting point (94.5–95°) and vapor tensions (77.2 mm. at 96.9° or 48.3 mm. at 86.8°—1% above literature values)[8] as well as by a hydrolytic analysis (31.6 cc. gas at S.C. yielded 90.8 cc. of $H_2$ and 32.9 cc. of B calcd. as gas atoms). Its weight was equivalent to 122.0 cc. of standard gas, so that the ratio $B_4H_{10}$:$(CH_3)_3N$ (used):$(CH_3)_3NBH_3$ was 1:3.37:2.98. Hence the residual solid had the composition $BH \cdot 0.37(CH_3)_3N$.

Preliminary experiments had shown that this solid was not entirely hydrolyzed by aqueous HCl alone; hence in this case it was first heated with ethanol in a sealed tube (110°), yielding 51.4 cc. of hydrogen, and then during 15 hours with dilute HCl at 110° it gave a further 3.16 cc. of hydrogen. Since the hydrogen was expected to amount to 82.2 cc., and since the boric acid titration gave only 32.3 cc. of B-gas instead of 41.1, it was evident that the hydrolysis is very difficult to complete. After the indicated treatment, the odor of B–H solids persisted.

In a second experiment very like the one just described, the ratio $B_4H_{10}$:$(CH_3)_3N$ (used):$(CH_3)_3NBH_3$ was found to be 1:3.54:2.98.

In two experiments in which no solvent was used, such ratios were 1:2.98:2.49 and 1:2.86:2.54, respectively. The non-volatile white or yellowish solids failed to react with either trimethylamine or diborane, the latter having been used in an attempt to regain tetraborane from any possible amine-complex of a B–B bonded material.

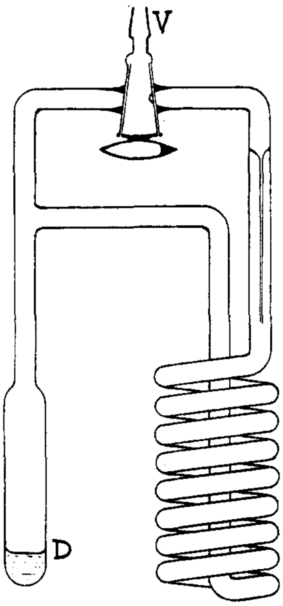

Fig. 1.—Diborane from D at −78° goes through the coil at 120° and is trapped at −196° in the vacuum system beyond the joint V. For further runs, the stopcock (Apiezon L grease) is turned the other way and the diborane is distilled back to D, leaving the higher boranes in the main system.

**Reaction of Tetraborane with Ethanol.**—The action of a deficient proportion of ethanol upon tetraborane at −78° was undertaken in the hope of obtaining a volatile B–B linked compound. No such substance could be obtained in any of a considerable number of experiments; instead, about 13% of the boron from the unrecovered $B_4H_{10}$ appeared in a colorless polymer, evidently containing ethoxy groups, while the rest was recovered as di- and triethoxyborines and a trace of diborane. In a typical experiment, 97.0 cc. of ethanol and 22.4 cc. of $B_4H_{10}$ (gases at S.C.) were mixed at −78°, yielding 92.1 cc. of $H_2$ during 60 hours at that temperature. Fractional condensation then gave 0.55 cc. of $B_2H_6$ and 11.8 cc. of $B_4H_{10}$ (confirmed by hydrolysis to yield boric acid and hydrogen in amounts corresponding to the stated gas volumes); a further 0.4 cc. of $B_4H_{10}$ was demonstrated as excess $B(OH)_3$ and $H_2$ from the hydrolysis of the $(C_2H_5O)_2BH$ fraction; it had been shown by work with a known mixture, that a trace of $B_4H_{10}$ is very difficult to remove from $(C_2H_5O)_2BH$ by distillation methods alone. The $(C_2H_5O)_2BH$ amounted to 13.6 cc., and the $(C_2H_5O)_3B$ to 19.8 cc. (gases at S.C.). A fraction of $(C_2H_5O)_2BH$, which was purified after recovering from the $(C_2H_5O)_3B$ fraction, showed a vapor tension of 36.5 mm. at 0° (literature 37.5).[9] The glassy solid, remaining in the original reaction tube, was hydrolyzed to form 5.5 cc. of $H_2$ and $B(OH)_3$ equivalent to 5.4 cc. of B-gas. Thus it appeared that 10.2 cc. of $B_4H_{10}$ had reacted to form 92.1 cc. of $H_2$, 0.55 cc. of $B_2H_6$, 13.6 cc. of $(RO)_2BH$, 19.8 cc. of $(RO)_3B$ and 5.4 cc. of BH material. This result would account for 98% of the B and 102% of the $H_2$ which would correspond to the hydrolysis of 10.2 cc. of $B_4H_{10}$.

**Acknowledgment.**—The generous support of this work by the Office of Naval Research is gratefully acknowledged.

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[5] H. I. Schlesinger and A. B. Burg, This Journal, **60**, 290 (1938).

[6] A. B. Burg and H. I. Schlesinger, *ibid.*, **55**, 4009 (1933).

[7] A. Stock and E. Kusz, *Ber.*, **56B**, 795 (1923).

[8] A. B. Burg and H. I. Schlesinger, This Journal, **59**, 785 (1937).

[9] H. C. Brown, H. I. Schlesinger and A. B. Burg, *ibid.*, **61**, 676 (1939).

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## Interactions of the Aminobenzoate Ions and Serum Albumin[1]

By R. K. Burkhard

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It has been shown that the anions derived from methyl red and two of its isomers exhibit differences in the extents of their interactions with bovine and human serum albumins. The anion derived from "*meta*-methyl red" (4′-dimethylaminoazobenzene-3-carboxylic acid) was found to react with these two proteins to a greater extent than the anions derived from either of its isomers, methyl red or "*para*-methyl red" (4′-dimethylaminoazobenzene-2-carboxylic acid and 4′-dimethylaminoazobenzene-4-carboxylic acid, respectively).[2] Preliminary

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[1] Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] I. M. Klotz, R. K. Burkhard and J. M. Urquhart, *J. Phys. Chem.*, **56**, 77 (1952).

investigations into these phenomena revealed that of these three isomeric dyes the *m*-isomer was the strongest acid.[3] Thus it appeared that there might be a relationship between protein binding ability and acid strength. Accordingly, this study was undertaken to determine whether the correlation between protein binding ability and acid strength as observed in the case of the anions of the methyl reds could be carried over to the aminobenzoate ions.

### Experimental

The systems chosen for study involved bovine serum albumin (BSA) at 0.2% concentration, methyl orange (MO) and the aminobenzoate ions. Considerable difficulty was encountered in attempts to obtain data by equilibrium dialysis of the aminobenzoate ions themselves with the protein, and accordingly the spectral displacement technique and competitive equilibrium dialysis technique as developed by Klotz and his co-workers were used.[4,5] The spectral studies were performed at room temperature and *p*H 7.3. The competitive equilibrium dialysis studies were performed at 0° and *p*H 6.8. Phosphate buffers were used to maintain the *p*H values. In all cases the extent of interaction was measured by studying the effect that the aminobenzoate ions had on the interaction between methyl orange and bovine serum albumin. The molar ratios of aminobenzoate to methyl orange ranged from 62/1 to 925/1. All spectral studies and analyses were performed on the Beckman model DU spectrophotometer. The protein samples were obtained from the Armour Company.

### Results and Discussion

A study of protein interactions involving the anions of anthranilic acid and *p*-aminobenzoic acid has been made by Klotz.[4] It was shown at that time that the anthranilate ion was more effective than the *p*-aminobenzoate ion in displacing methyl orange from protein surfaces. The results obtained in this study which pertain to the *o*- and *p*-isomers agree entirely with this earlier work. The *m*-isomer which was not studied at that time yielded very interesting results showing that the *m*-isomer had no greater effect on the displacement of methyl orange than did the *p*-isomer.

TABLE I

CHANGES IN THE MOLAR EXTINCTION COEFFICIENT OF METHYL ORANGE WHEN IN THE PRESENCE OF VARIOUS ADDITIVES (*p*H 7.3, ROOM TEMP.)

Concentration of methyl orange (MO) $1.58 \times 10^{-5}$ mole/l.; concentration of bovine serum albumin (BSA) and the aminobenzoic acids 0.2%; ratio of aminobenzoic acids to MO 925/1.

| System | $\Delta\epsilon$ at 4700 Å. |
|---|---|
| MO | 0 |
| MO, BSA | −4200 |
| MO, BSA, anthranilic acid | −1000 |
| MO, BSA, *m*-aminobenzoic acid | −1800 |
| MO, BSA, *p*-aminobenzoic acid | −1800 |

TABLE II

BINDING OF METHYL ORANGE AND BOVINE SERUM ALBUMIN IN THE PRESENCE OF THE AMINOBENZOIC ACIDS (*p*H 6.8, 0°)

Concentration of methyl orange (MO) $0.942 \times 10^{-5}$ mole/l.; concentration of bovine serum albumin (BSA) 0.2%; concentration of the aminobenzoic acids $5.8 \times 10^{-4}$ mole/l.; ratio of aminobenzoic acids to MO 62/1.

| System | Mole of MO bound *per mole protein* |
|---|---|
| MO, BSA, anthranilic acid | 0.16 |
| MO, BSA, *m*-aminobenzoic acid | .33 |
| MO, BSA, *p*-aminobenzoic acid | .33 |

It has been pointed out by Klotz that the presence of groups capable of hydrogen bonding such as are found in the anthranilic acid molecule enhance protein binding.[4] The ions derived from *m*- and *p*-aminobenzoic acids, however, cannot engage in such bonding and hence acid strength and molecular configuration should be the paramount considerations in their interactions with proteins. The fact that these two isomers show no difference in their ability to displace methyl orange from protein surfaces strongly suggests that the interactions of these two isomeric ions are similar. If this be true then one would suspect that the results observed with the methyl reds might be due to factors other than acid strength. Biochemical implications of these findings are now being investigated.

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[3] R. K. Burkhard, Ph.D. Thesis, Northwestern University, 1950.

[4] I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

[5] I. M. Klotz, F. M. Walker and R. B. Pivan, *ibid.*, **68**, 1486 (1946).

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## Simultaneous Vicinal Dichlorination, a Correction[1]

BY E. CAMPAIGNE AND JOSEPH R. LEAL[2]

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We have been unable to confirm the phenomenon of "simultaneous vicinal dichlorination" previously reported from this Laboratory.[3] Using conditions which were as nearly as possible a duplication of those used in the earlier experiments, complex mixtures of halogenated products were obtained which could not be completely separated. It is possible that small amounts of vicinal chloro derivatives may have escaped detection in these mixtures. The situation is apparently further complicated by the existence of molecular compounds of two or more of the halogenated hydrocarbons which have relatively sharp melting points near those reported for pure compounds.

A study of the use of iodine trichloride as an aromatic halogenating agent and its reaction with benzene under various conditions has been made. When a carbon tetrachloride solution of acetanilide, for example, was refluxed with iodine trichloride, a 17% yield of *p*-chloroacetanilide[4] was found in the aqueous washings of the reaction mixture. The crystalline material remaining after the removal of the solvent, however, was not completely resolved into its components, even after repeated fractional crystallizations from various solvents.

Benzene, on the other hand, gave reaction mixtures from which moderate yields of chlorobenzene

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[1] Contribution No. 568 from the Chemical Laboratories of Indiana University.

[2] Taken from a portion of the thesis submitted by Joseph R. Leal in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[3] E. Campaigne and W. Thompson, THIS JOURNAL, **72**, 629 (1950).

[4] E. Crepaz, *Atti Reale ist. Veneto sci.*, **94**, 555 (1934-1935) [*C. A.*, **31**, 6209 (1937)] obtained N-dichloroiodoacetanilide by treatment of acetanilide in cold chloroform with potassium chloroiodate (III), ($KICl_4$).