

CHROM. 7815

## COMPLEMENTARY THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF SOME POLYHEDRAL BORANE ANIONS AND THEIR DERIVATIVES

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(Received July 15th, 1974)

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### SUMMARY

Anionic borane derivatives have been separated using ion-exchange and partition thin-layer chromatographic systems. The  $R_F$  order of the borane compounds in the ion-exchange systems was approximately the inverse of that for the partition systems. Derivatives of  $B_{10}H_{10}^{2-}$  and  $B_{12}H_{12}^{2-}$  with similar substituents showed excellent separation, and there is evidence that these systems can resolve some geometrical isomers of disubstituted  $B_{10}H_{10}^{2-}$ .

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### INTRODUCTION

The possible use of boron compounds in neutron-capture cancer therapy has been appreciated for some considerable time<sup>1</sup>. One of the basic requirements for this technique is to have a boron compound which will bind selectively to tumor cells, giving rise to a high tumor-to-blood boron concentration ratio<sup>2</sup> and a high tumor-to-surrounding-normal-tissue boron concentration ratio, while maintaining a high absolute concentration of boron in the tumor. For reasons described elsewhere<sup>1</sup>, the brain appears to be a particularly favorable organ for this technique and in this regard, we have been investigating the derivative chemistry<sup>3</sup> of the polyhedral borane anions  $B_{10}H_{10}^{2-}$  and  $B_{12}H_{12}^{2-}$ .

A major difficulty that we have experienced in working with these ions is in obtaining uncontaminated crystals that can be further used in animal and patient studies. Crystallinity is not necessarily an indication of purity, and seemingly "pure" crystals of these borane anions and their derivatives are often found to be mixtures. For example, we have found that the tetraethylammonium salt of  $B_{12}H_{12}^{2-}$  co-crystallizes with  $B_{11}H_{11}^{2-}$  and that the cesium salts of  $B_{12}H_{11}OH^{2-}$  and  $B_{12}H_{11}SH^{2-}$  co-crystallize. Also, Kendal<sup>4</sup> has shown that crystals of the pentachloro derivative of  $B_{10}H_{10}^{2-}$  contain several isomers. On the other hand, some borane anion derivatives that are only slightly contaminated with impurities may resist all attempts to crystallize them despite a wide choice of cations and solvents.

In order to provide a means of detecting different borane anions in a mixture, we have investigated, and here report, two complementary thin-layer chromatographic (TLC) separation systems for resolving several water- and oxygen-stable borane anions and their derivatives. Preliminary work on the ion-exchange systems was done by Knoth<sup>5</sup>. The other systems involving solvent partitioning on silica gel were developed by Tolpin<sup>6</sup>.

## EXPERIMENTAL

### Compounds

The borane anions and their derivatives were chosen so as to have a range of chemical and physical characteristics. Prepared by literature methods were:  $[(C_2H_5)_3NH]_2B_{10}H_{10}$  (ref. 7);  $Na_2B_{12}H_{12} \cdot 2H_2O$  (ref. 8);  $Cs_2B_{20}H_{18}$  (ref. 9);  $Cs_2B_{12}H_{11}SH \cdot H_2O$  (ref. 10);  $Cs_2B_{12}H_{11}OH$  (ref. 10);  $(CH_3)_4N, 1-B_{10}H_9S(CH_3)_2$  (ref. 11);  $(CH_3)_4N, 1,6-CH_3SB_{10}H_8S(CH_3)_2$  (ref. 11);  $(CH_3)_4N, 1,10-CH_3SB_{10}H_8S(CH_3)_2$  (ref. 11);  $(CH_3)_4N, 1-B_{10}H_{10}NNC_6H_4NHCOCH_3$  (ref. 6);  $(CH_3)_4N, 1-B_{10}H_{10}NNC_6H_4NH_2$  (ref. 6);  $(CH_3)_4N, B_{12}H_{11}S(CH_3)_2$  (ref. 12);  $(CH_3)_4N, 1,12-CH_3SB_{12}H_{10}S(CH_3)_2$  (ref. 13);  $CsB_{12}H_{11}NMP$  (NMP = N-methylpyrrolidone) (ref. 10);  $Cs_2B_{11}H_{13}$  (ref. 14); and  $HCB_9H_{10}C(CH_2)_3NH_3$  (ref. 6). One compound,  $[(CH_3)_4N]_2B_{12}H_{11}SC_6H_3(NO_2)_2$ , has been prepared and analyzed in this laboratory by methods that will be published elsewhere<sup>15</sup>.

### Sorbents

For ion-exchange TLC, Baker-flex DEAE-cellulose and PEI-F-cellulose sheets were cut into  $6.6 \times 2.5$  cm strips. Kodak silica gel sheets 6060 and Baker-flex silica gel 1B sheets were used for partition TLC. These were cut into  $6.6 \times 2.5$  cm strips and the silica gel was removed 1 mm bilaterally to minimize edge effects of the solvent. The silica gel strips were stored in a bell jar containing a saturated solution of sodium nitrite.

### Thin-layer chromatography

Six systems were developed for the TLC of these borane anions. The first three are ion-exchange systems. The sorbents and solvents for these systems are DEAE-cellulose with aqueous 3 M ammonium nitrate (system I), PEI-F-cellulose with aqueous 3 M ammonium hexafluorophosphate (system II), and PEI-F-cellulose with aqueous 6 M nitrate (system III). The second three systems separate borane anions by solvent partitioning on silica gel. For two of these solvent partition systems, a mixed solvent consisting of acetone-heptane-aqueous 0.29 M tetrabutylammonium chloride (12:2:0.5) is used with both Kodak silica gel 6060 (system IV) and Baker-flex silica gel 1B (system V). For the third solvent partitioning system, the mixed solvent acetone-heptane-aqueous 0.072 M tetrabutylammonium chloride (9:3:0.5) is used on Baker-flex silica gel 1B (system VI).

For systems I-V, several milligrams of each compound are dissolved in 0.3 ml of water; if not soluble, 0.2 ml of Bio-Rad AG 50W-X8, 200-400 mesh resin in the hydrogen form, are added to the suspension and the mixture is stirred until most of the compound goes into solution. The solutions are used to spot the strips on a baseline, drawn in pencil, 12 mm from the bottom. After immersing the spotted strip in

solvent to a level of 6 mm, the solvent is allowed to advance to a termination line, also drawn in pencil, 6 mm from the top. The strips are removed and sprayed with a 1% aqueous solution of palladium chloride in hydrochloric acid, purchased from Fisher Scientific Co. (Pittsburgh, U.S.A.). With system VI, each compound is converted to its tetrabutylammonium salt before application to a strip. This is done in the following manner. Several milligrams of the salt are first stirred in 0.3 ml of water and 0.2 ml of the previously mentioned strong acid resin. After filtering the mixture and adding excess tetrabutylammonium chloride solution to the filtrate, the then cloudy filtrate is centrifuged, decanted, and 0.2 ml of acetone are added with stirring to the wet precipitate to produce a solution of the new salt. The solution is used to spot strips which are pencilled, run, and sprayed as described for systems I-V.

## RESULTS AND DISCUSSION

Table I lists  $R_F$  values and standard deviations,  $\sigma = \left\{ \frac{\sum (\bar{R}_F - R_{F_i})^2}{n-1} \right\}^{1/2}$ , for each test compound in ion-exchange systems I-III.  $R_F$  values and standard deviations for the test compounds in partition systems IV-VI are listed in Table II. In the above expression for  $\sigma$ ,  $\bar{R}_F$  is the average value of  $n$  developments where  $n$  is greater than seven and  $R_{F_i}$  is the value in the  $i$ th development. With each test compound, a control spot consisting of  $K_2B_{10}H_{10}$  for systems I-III and of  $Na_2B_{12}H_{12}$  for systems IV-VI was applied. If the  $R_F$  value of the control spot deviated from its mean value by more than  $2\sigma$ , the development data for the test compound were not recorded. The standard deviations for the partition systems IV-VI were generally larger than those for the ion-exchange systems I-III.

TABLE I

### $R_F$ VALUES AND $\sigma$ FOR ION-EXCHANGE SYSTEMS I-III

For  $\sigma < 0.01$ , a value of 0.01 was assigned. System I = DEAE-cellulose with 3 M ammonium nitrate; System II = PEI-F-cellulose with 3 M ammonium hexafluorophosphate; System III = PEI-F-cellulose with 6 M ammonium nitrate.

Compound	System I		System II		System III	
	$R_F$	$\sigma$	$R_F$	$\sigma$	$R_F$	$\sigma$
$K_2B_{10}H_{10}$	0.54	0.02	0.52	0.01	0.47	0.01
$Na_2B_{12}H_{12} \cdot 2 H_2O$	0.35	0.01	0.45	0.01	0.37	0.01
$Cs_2B_{20}H_{18}$	0.00	0.01	0.07	0.01	0.02	0.01
$Cs_2B_{12}H_{11}SH \cdot H_2O$	0.33	0.01	0.40	0.01	0.30	0.01
$Cs_2B_{12}H_{11}OH$	0.61	0.01	0.57	0.03	0.50	0.02
$(Et_3NH)_2B_{12}H_{11}SC_6H_5(NO_2)_2$	0.04	0.01	0.09	0.01	0.03	0.01
$(CH_3)_4N, 1-B_{10}H_9S(CH_3)_2$	0.42	0.03	0.43	0.01	0.37	0.03
$(CH_3)_4N, 1,6-CH_3SB_{10}H_8S(CH_3)_2$	0.44	0.01	0.42	0.01	0.36	0.01
$(CH_3)_4N, 1,10-CH_3SB_{10}H_8S(CH_3)_2$	0.37	0.01	0.35	0.01	0.34	0.01
$(CH_3)_4N, 1-B_{10}H_{10}NNC_6H_4NHCOCH_3$	0.22	0.01	0.32	0.01	0.16	0.01
$(CH_3)_4N, 1-B_{10}H_{10}NNC_6H_4NH_2$	0.15	0.01	0.23	0.01	0.11	0.01
$(CH_3)_4N, B_{12}H_{11}S(CH_3)_2$	0.21	0.01	0.33	0.01	0.24	0.01
$(CH_3)_4N, 1,12-CH_3SB_{12}H_{10}S(CH_3)_2$	0.20	0.01	0.26	0.03	0.25	0.01
$CsB_{12}H_{11}NMP$	0.30	0.02	0.37	0.02	0.32	0.02
$Cs_2B_{11}H_{13}$	0.08	0.01	0.24	0.01	0.04	0.01
$HCB_9H_{10}C(CH_2)_3NH_3$	0.24	0.01	0.49	0.01	0.37	0.01

TABLE II

 $R_F$  VALUES AND  $\sigma$  FOR PARTITION SYSTEMS IV-VI

For  $\sigma < 0.01$ , a value of 0.01 was assigned. System IV = Kodak silical gel 6060 with acetone-heptane-aqueous 0.29 *M*-tetrabutylammonium chloride (12:2:0.5); system V = Baker-flex silica gel 1B with acetone-heptane-aqueous 0.29 *M* tetrabutylammonium chloride (12:2:0.5); system VI = Baker-flex silica gel 1B with acetone-heptane-aqueous 0.072 *M* tetrabutylammonium chloride (9:3:0.5).

Compound	System IV		System V		System VI	
	$R_F$	$\sigma$	$R_F$	$\sigma$	$R_F$	$\sigma$
$K_2B_{10}H_{10}$	0.41	0.01	0.45	0.01	0.24	0.01
$Na_2B_{12}H_{12} \cdot 2 H_2O$	0.56	0.01	0.65	0.04	0.38	0.02
$Cs_2B_{20}H_{18}$	0.74	0.05	0.73	0.02	0.48	0.01
$Cs_2B_{12}H_{11}SH \cdot H_2O$	0.43	0.04	0.56	0.04	0.50	0.01
$Cs_2B_{12}H_{11}OH$	0.33	0.02	0.35	0.01	0.23	0.01
$(Et_3NH)_2B_{12}H_{11}SC_6H_3(NO_2)_2$	0.57	0.02	0.69	0.06	0.50	0.01
$(CH_3)_4N,1-B_{10}H_9S(CH_3)_2$	0.85	0.04	0.74	0.02	0.51	0.01
$(CH_3)_4N,1,6-CH_3SB_{10}H_8S(CH_3)_2$	0.59	0.03	0.74	0.04	0.54	0.01
$(CH_3)_4N,1,10-CH_3SB_{10}H_8S(CH_3)_2$	0.63	0.04	0.77	0.03	0.49	0.01
$(CH_3)_4N,1-B_{10}H_{10}NNC_6H_4NHCOCH_3$	0.79	0.05	0.81	0.02	0.47	0.01
$(CH_3)_4N,1-B_{10}H_{10}NNC_6H_4NH_2$	0.71	0.06	0.82	0.01	0.45	0.01
$(CH_3)_4N,B_{12}H_{11}S(CH_3)_2$	0.71	0.04	0.75	0.05	0.52	0.01
$(CH_3)_4N,1,12-CH_3SB_{12}H_{10}S(CH_3)_2$	0.63	0.03	0.65	0.03	0.53	0.01
$CsB_{12}H_{11}NMP$	0.59	0.03	0.56	0.04	0.45	0.01
$Cs_2B_{11}H_{13}$	0.76	0.02	0.79	0.02	0.62	0.02
$HCB_9H_{10}C(CH_2)_3NH_3$	0.68	0.02	0.66	0.01	0.45	0.02

The  $R_F$  values and standard deviations shown in Tables I and II were computed using thin-layer sheets from the same manufacturer's lot. When different manufacturer's lots were used, the standard deviations and  $R_F$  values for systems I-III remained about the same. Although changing the manufacturer's lots did not change the standard deviations for systems IV-VI, the  $R_F$  values did change appreciably. In particular, with a second lot of Baker-flex silica gel 1B, the  $R_F$  values for systems V and VI varied generally by  $+0.04$  from those given in Table I. Variations in  $R_F$  as much as 0.2 were found in system IV with different lots of Kodak silica gel 6060. Although the  $R_F$  variations from lot to lot of Kodak silica gel 6060 were sometimes large compared with those from lot to lot of Baker-flex silica gel 1B sheets, the system IV, with Kodak silica gel 6060, showed greater resolution of anions than did systems V and VI, which used the Baker-flex silica gel 1B plates. These factors make system IV more useful for complex separations provided reference  $R_F$  values are not needed.

A plot of  $R_F$  values in systems IV versus system I (Fig. 1) shows that generally the  $R_F$  values of compounds in ion-exchange system I are inversely related to their  $R_F$  values in partition system IV. Similar inverse behavior has been seen with analogous halide separation methods in paper chromatography<sup>16</sup>. This inverse behavior also is seen in the tailing downwards of overloaded spots in systems I-III, as opposed to spots tailing upwards in systems IV-VI.

Two general behavioral characteristics of these borane anions in the ion-exchange systems are evident. The first of these is that, for two derivatives of a given skeletal borane cage possessing the same charge, the derivative having the more

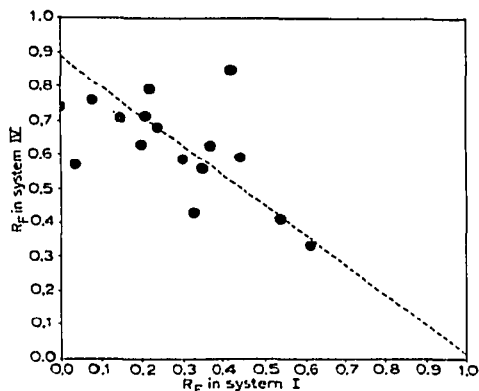


Fig. 1. The  $R_F$  values of the anionic boranes in ion exchange system I (abscissa) are plotted against their values in solvent partitioning system IV (ordinate). The dashed line most strongly weights the control anions  $B_{10}H_{10}^{2-}$  and  $B_{12}H_{12}^{2-}$ .

hydrophilic substituent will give rise to a higher  $R_F$  value. Thus, for example,  $B_{12}H_{11}OH^{2-}$  has a higher  $R_F$  value than  $B_{12}H_{11}SH^{2-}$  because the hydroxyl group is more hydrophilic than the sulfhydryl group.

The second characteristic is that a  $B_{10}H_{10}^{2-}$  cage having a given substituent will possess a higher  $R_F$  value than a  $B_{12}H_{12}^{2-}$  cage having the same substituent. The higher  $R_F$  value of 1- $B_{10}H_9S(CH_3)_2^-$  as compared with  $B_{12}H_{11}S(CH_3)_2^-$  is an example of this behavior.

The ability of these systems to resolve borane isomers is shown by the excellent separation of the 1,6- $CH_3SB_{10}H_8S(CH_3)_2^-$  anion from the 1,10-isomer. Although there is only one example of the separation of the 1,6- and 1,10-isomers of  $B_{10}H_{10}^{2-}$  shown in Tables I and II, one can expect that other 1,6-isomers of this borane anion will show a higher  $R_F$  value than the corresponding 1,10-isomer. The resolution of these two isomers is probably dependent on the magnitude of the interaction between the aqueous phase and the higher-charge-density regions of the skeletal borane cage. Theoretical calculations<sup>17</sup> on the unsubstituted  $B_{10}H_{10}^{2-}$  cage indicate that this borane has a non-uniform charge distribution with the apical boron atoms (Fig. 2:

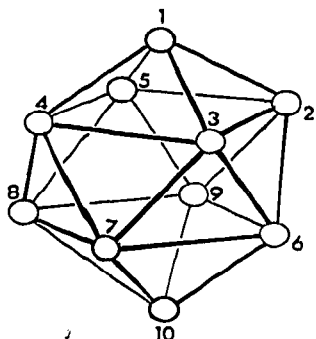


Fig. 2. Structure of  $B_{10}H_{10}^{2-}$ . Each circle represents a boron atom with a terminal hydrogen. Boron atoms 1 and 10 are apical, while 2-9 are equatorial.

atoms 1 and 10) having more negative charge than the equatorial boron atoms (Fig. 2: atoms 2-9). Assuming that the two substituents of 1,6- and 1,10- $\text{CH}_3\text{SB}_{10}\text{H}_8\text{S}(\text{CH}_3)_2^-$  perturb the skeletal  $\text{B}_{10}\text{H}_{10}^{2-}$  charge distribution only slightly, both these isomers have a core charge distribution similar to the  $\text{B}_{10}\text{H}_{10}^{2-}$  anion. However, the aqueous phase can approach more closely the unsubstituted higher-charge-density apical region of the 1,6-isomer than it can either of the substituted apical regions of the 1,10-isomer. For two isomeric derivatives of  $\text{B}_{10}\text{H}_{10}^{2-}$  having the same degree of apical substitution, the isomer having the more non-uniform charge distribution will probably have the higher  $R_F$  value because the aqueous phase can interact more strongly with its electrostatic polar components. In the uniformly charged  $\text{B}_{12}\text{H}_{12}^{2-}$  anion, any substituents on this borane would induce some charge non-uniformity in the cage. Thus, probably the charge non-uniformities and  $R_F$  values of the disubstituted isomers of  $\text{B}_{12}\text{H}_{12}^{2-}$  would be in the order *ortho* > *meta* > *para*.

The dinegative charge of these borane anions suggest that they may reversibly protonate under different solvent conditions. In order to study this possibility, we varied the pH of the ion-exchange systems from 1 to 6.5 with acid resins. This gave rise to insignificant variations in  $R_F$  values. Therefore, the degree of protonation of these ions does not vary over this range of pH values. However, some of the anions, which may be weak bases in a Brønsted sense, may be partially protonated in the partition systems, which involve a low dielectric constant solvent on weakly acidic silica gel. Such a change in protonation with different systems could explain why some anions deviate from the general inverse behavior shown in Fig. 1.

The use of these TLC systems has enabled us to detect over ten components in a single crude preparation of  $\text{Cs}_2\text{B}_{12}\text{H}_{11}\text{SH}$ . Components not separated on the ion-exchange system consequently have been separated on the partition systems and *vice versa*.

#### ACKNOWLEDGEMENTS

E.I.T. wishes to thank S. Hermanek for a useful discussion. This investigation was supported by the Atomic Energy Commission AT(11-1)3334 and by the National Institutes of Health, Cancer Institute, CA-43443 and CA-12246.

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