

On the other hand, the iodine chloride fundamental vibration is also shifted to  $310\text{ cm.}^{-1}$  by 2,2'-bipyridine in solution.<sup>13</sup> This shows that the donor strengths of PMT and of 2,2'-bipyridine *vis-a-vis* ICl are approximately the same. Yet PMT does not show any appreciable coordinating tendency toward hydrogen ion,<sup>4</sup> while 2,2'-bipyridine is a moderately strong base with a  $pK_b$  of 9.56.<sup>14</sup> One, therefore, should not necessarily relate the donor strength of a compound with its proton affinity, especially if the latter were determined in aqueous solutions.

### Conclusions

In general, it is seen that PMT is a moderately strong donor molecule. Its basic strength *vis-a-vis* iodine halides places it in an intermediate position between weak donors such as aromatic substances and acetonitrile on one hand and the strong donors such as pyridine and trialkylamines on the other. It is interesting to note that most of the convulsant agents, such as strychnine, metathamide, hydra-

zides, etc., likewise have donor properties which seem to be of approximately the same magnitude. In a recent publication Jenney and Pfeiffer report a study on convulsant activity of hydrazides<sup>15</sup> and conclude that there is only a very gross correlation between the  $pK_a$  values of compounds and the convulsant activity. It is possible that electron donor properties and complexing ability of these compounds may show a better correlation with their physiological properties.

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## Some Octet and Bond Refractivities Involving Boron

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The Lorentz-Lorenz molar refractivity  $R$  has been calculated from literature data for  $n$  and  $d$  for a fairly large number of organoboron compounds. A preliminary attempt has been made to evaluate some octet and bond refractivities involving boron. The resulting values show deviations up to 1 cc./mole, and merely approximate average refractivities can be given (in cc./mole for the D-line and  $20^\circ$ ) for the octets  $B:\ddot{O}:C$  (3.1) and  $B:\ddot{Cl}:$  (6.8) as well as for the bond  $B:C_{aliph}$  (1.75). If one assumes in aromatic compounds the Kekulé structure for the  $C_6$  ring, an average apparent value of 3.0 cc./mole results for the bond  $B:C_{arom}$ . A satisfactory additivity of molar refractivity obtains for homologous series of organoboron compounds. For six such series, equations of the type  $R_{obs} = an + b$  have been derived, in which  $n$  is the number of carbon atoms in an alkyl chain.

A search of the literature has disclosed no information regarding the refractivity of any bonds or octet groups in which both elements boron and carbon are involved. An early investigation based on only a few compounds was made<sup>1</sup> in order to evaluate an atomic refraction for boron. However, according to certain investigators,<sup>2</sup> the ordinary atomic refractivities<sup>3</sup> have no simple physical significance, and account must be taken of the electronic structure of the molecules, since optical properties depend on the state of the valence electrons. Values for bond and octet refractivities have been tabulated<sup>4,5</sup> for the generally known Lorentz-Lorenz molar refraction. This molar refraction  $R$  is a measure of the looseness or tightness with which the valence electrons are held by the atomic cores, and is expressed in cc./mole.

(1) A. Ghira, *Atti R. Accad. Lincei*, **2**, 1, 312 (1893); *Z. physik. Chem.*, **12**, 764, 768 (1893).

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The present investigation is concerned with the evaluation of some octet and bond refractivities involving boron. Values for the refractive index  $n$  (for the D-line) and the density  $d$  for a large number of organoboron compounds were selected from a recent comprehensive report on the organic compounds of boron.<sup>6</sup> This information was used to calculate the observed molar refraction,  $R_{obs}$ . Assuming, as a first approximation, exact additivity, the sum of the shares of known<sup>5</sup> bond and octet refractions,  $R_{kn}$ , was subtracted from  $R_{obs}$ , in order to calculate the unknown value,  $R_x$ , involving boron. The difference between  $R_{obs}$  and  $R_{kn}$  has been divided by the number of such bonds or octets present in the compound. For the compounds selected, these octet and bond refractivities were evaluated:  $B:\ddot{O}:C$ ,  $B:\ddot{Cl}:$ ,  $B:C_{aliph}$  and  $B:C_{arom}$ .

The resulting values for the  $B:\ddot{O}:C$  octet range in Table I from 2.92 (compound 17)<sup>7</sup> to 3.51 (compound 9). This variation is hardly due to experi-

(6) M. F. Lappert, *Chem. Revs.*, **56**, [5] 959 (1956).

(7) A still lower value, 2.74, is obtained from compound 19, which contains a phenyl group. See the discussion of Table IV.

TABLE I  
 REFRACTIVITY  $R_x$  OF THE OCTET B: $\ddot{O}$ :C IN CC./MOLE

Compound	$d_{25}^4$	$n_{25}^{20D}$	$M$	$R_{obs}$	$R_{kn}$	$R_x$
1 B(OCH <sub>3</sub> ) <sub>3</sub>	0.9547	1.3610	103.9	24.08	15.30	2.93
2 B(OC <sub>2</sub> H <sub>5</sub> ) <sub>3</sub>	.8635	1.3741	146.0	38.62	29.19	3.14
3 <i>n</i> -B(OC <sub>4</sub> H <sub>9</sub> ) <sub>3</sub>	.8560	1.4085	230.2	66.42	56.97	3.15
4 <i>t</i> -B(OC <sub>4</sub> H <sub>9</sub> ) <sub>3</sub>	.814	1.3883	230.2	66.78	56.70	3.36
5 <i>n</i> -B(OC <sub>5</sub> H <sub>11</sub> ) <sub>3</sub>	.8549	1.4190	272.2	80.41	70.86	3.18
6 <i>i</i> -B(OC <sub>5</sub> H <sub>11</sub> ) <sub>3</sub>	.8514	1.4156	272.2	80.16	70.50	3.22
7 B[OCH(CH <sub>3</sub> )C <sub>3</sub> H <sub>7</sub> ] <sub>3</sub>	.8375	1.4075	272.2	80.09	70.50	3.20
8 <i>t</i> -B[OCH(CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub> ] <sub>3</sub>	.841	1.4151	314.3	93.61	84.30	3.10
9 <i>n</i> -B(OC <sub>7</sub> H <sub>15</sub> ) <sub>3</sub>	.8398	1.4280	356.39	109.18	98.64	3.51
10 <i>n</i> -B(OC <sub>8</sub> H <sub>17</sub> ) <sub>3</sub>	.8548	1.4377	398.47	122.30	112.53	3.26
11 <i>n</i> -B(OC <sub>10</sub> H <sub>21</sub> ) <sub>3</sub>	.8581	1.4440	482.62	149.40	140.31	3.03
12 B[OCH(OCH <sub>3</sub> )CH <sub>3</sub> ] <sub>3</sub>	1.0096	1.4059	236.1	57.43	47.85	3.19
13 B[OCH(Cl)CH <sub>3</sub> ] <sub>3</sub>	1.2780	1.4556	249.3	52.99	43.80	3.06
14 B[OCH <sub>2</sub> COOC <sub>2</sub> H <sub>5</sub> ] <sub>3</sub>	1.160	1.4290	320.1	71.14	61.83	3.10
15 B[OCH(CH <sub>3</sub> )COOC <sub>2</sub> H <sub>5</sub> ] <sub>3</sub>	1.070	1.4215	362.2	85.93	75.54	3.46
16 B(OCH <sub>2</sub> CH <sub>2</sub> COOC <sub>2</sub> H <sub>5</sub> ) <sub>3</sub>	1.108	1.4331	362.2	84.98	75.72	3.09
17 B[OCH(COOC <sub>2</sub> H <sub>5</sub> )CH <sub>2</sub> COOC <sub>2</sub> H <sub>5</sub> ] <sub>3</sub>	1.167	1.4408	578.37	130.82	122.07	2.92
18 B[OCH(CH <sub>2</sub> Cl) <sub>2</sub> ] <sub>3</sub>	1.4028	1.4883	394.8	81.13	72.30	2.94
19 B[OCH(CH <sub>3</sub> )C <sub>6</sub> H <sub>5</sub> ] <sub>3</sub>	1.064	1.5347	374.28	109.46	101.25	2.74
20 <i>n</i> -C <sub>4</sub> H <sub>9</sub> OB(OCH <sub>2</sub> ) <sub>2</sub>	0.9976 <sup>a</sup>	1.4280 <sup>a</sup>	144.0	37.14	26.99	3.38
21 HOCH <sub>2</sub> CH <sub>2</sub> OB(O- <i>n</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	0.9141 <sup>a</sup>	1.4129 <sup>a</sup>	218.1	59.48	50.44	3.01

<sup>a</sup>  $d_{25}^4$ ,  $n_{25}^{20D}$ .

Av. (rounded off) 3.1

 TABLE II  
 REFRACTIVITY  $R_x$  OF THE OCTET B: $\ddot{Cl}$ : IN CC./MOLE

Compound	$d_{25}^4$	$n_{25}^{20D}$	$M$	$R_{obs}$	$R_{kn}$	$R_x$
1 ClB(O- <i>n</i> -C <sub>3</sub> H <sub>7</sub> ) <sub>2</sub>	0.959	1.4028	164.4	41.81	35.00	6.81
2 ClB(O- <i>n</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	.941	1.4132	192.5	51.03	44.26	6.77
3 ClB(O- <i>i</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	.938	1.4055	192.5	50.35	44.08	6.27
4 ClB(O- <i>s</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	.924	1.4017	192.5	50.70	44.08	6.62
5 ClB[OCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> ] <sub>2</sub>	.906	1.4102	220.6	60.35	53.28	7.07
6 ClB[O- <i>t</i> -CH(CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub> ] <sub>2</sub>	.901	1.4165	248.6	69.31	62.48	6.83
7 ClB(O- <i>n</i> -C <sub>5</sub> H <sub>11</sub> ) <sub>2</sub>	.906	1.4380	304.7	88.28	81.30	6.98
8 ClB[O- <i>n</i> -CH(CH <sub>3</sub> )C <sub>6</sub> H <sub>13</sub> ] <sub>2</sub>	.897	1.4277	304.7	87.34	80.88	6.46
9 <i>n</i> -C <sub>3</sub> H <sub>7</sub> OBCl <sub>2</sub>	1.138	1.4094	140.8	30.62	17.50	6.56
10 <i>n</i> -C <sub>4</sub> H <sub>9</sub> OBCl <sub>2</sub>	1.079	1.4162	154.8	36.02	22.13	6.95
11 <i>i</i> -C <sub>4</sub> H <sub>9</sub> OBCl <sub>2</sub>	1.047	1.4088	154.8	36.54	22.04	7.25
12 (CH <sub>3</sub> ) <sub>2</sub> CCH <sub>2</sub> OBCl <sub>2</sub>	1.032	1.4097	168.9	40.52	26.64	6.94
13 <i>n</i> -C <sub>5</sub> H <sub>11</sub> OBCl <sub>2</sub>	1.015	1.4315	211.0	53.87	40.65	6.61
14 Cl(CH <sub>2</sub> ) <sub>4</sub> OBCl <sub>2</sub>	1.254	1.4522	189.3	40.74	26.94	6.90

Av. (rounded off) 6.8

 TABLE III  
 REFRACTIVITY  $R_x$  FOR THE BOND B:C WHEN C IS ALIPHATIC, IN CC./MOLE

Compound	$d_{25}^4$	$n_{25}^{20D}$	$M$	$R_{obs}$	$R_{kn}$	$R_x$
1 B( <i>n</i> -C <sub>3</sub> H <sub>7</sub> ) <sub>3</sub>	0.7225 <sup>b</sup>	1.41352 <sup>b</sup>	140.1	48.41	43.08	1.78
2 B( <i>i</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>3</sub>	.7400 <sup>b</sup>	1.42445 <sup>b</sup>	182.2	62.89	56.70	2.06
3 B( <i>i</i> -C <sub>5</sub> H <sub>11</sub> ) <sub>3</sub>	.7607 <sup>b</sup>	1.43207 <sup>b</sup>	224.2	76.46	70.50	1.99
4 <i>n</i> -C <sub>4</sub> H <sub>9</sub> B(O- <i>n</i> -C <sub>4</sub> H <sub>9</sub> ) <sub>2</sub>	.8300	1.4169	214.2	64.89	63.25	1.64
5 <i>n</i> -C <sub>4</sub> H <sub>9</sub> O( <i>n</i> -C <sub>4</sub> H <sub>9</sub> )BCl	.898	1.4170	176.5	49.43	47.91	1.52
6 ( <i>n</i> -C <sub>5</sub> H <sub>11</sub> ) <sub>2</sub> BOCH <sub>2</sub> CH <sub>2</sub> OB( <i>n</i> -C <sub>5</sub> H <sub>11</sub> ) <sub>2</sub>	.8276 <sup>a</sup>	1.4378 <sup>a</sup>	366.24	116.12	108.76	1.84
7 ( <i>n</i> -C <sub>5</sub> H <sub>11</sub> ) <sub>2</sub> BO- <i>n</i> -C <sub>4</sub> H <sub>9</sub>	.8036	1.4312	310.4	100.02	97.15	1.44

<sup>a</sup>  $d_{25}^4$ ,  $n_{25}^{20D}$ . <sup>b</sup>  $d_{25}^{22.5}$ ,  $n_{25}^{22.6D}$ .

Av. 1.75

mental uncertainties and confirms the conclusion that the multitude of the presently available data cannot be satisfactorily reproduced by a limited number of constant additive increments. Therefore the average value  $3.14 \pm 0.14$  is rounded off at the end of Table I to 3.1 cc./mole.

The mean value, 3.14, for the B: $\ddot{O}$ :C octet refractivity from Table I was used in the calculation

of  $R_{kn}$  for the compounds listed in Table II when needed. The values for the B: $\ddot{Cl}$ : octet refractivity in Table II range between 6.27 and 7.25, and the average  $6.79 \pm 0.21$  has been rounded off to 6.8 cc./mole.

The mean refractivity values for the B: $\ddot{O}$ :C octet, 3.14, and the B: $\ddot{Cl}$ : octet, 6.79, from Tables

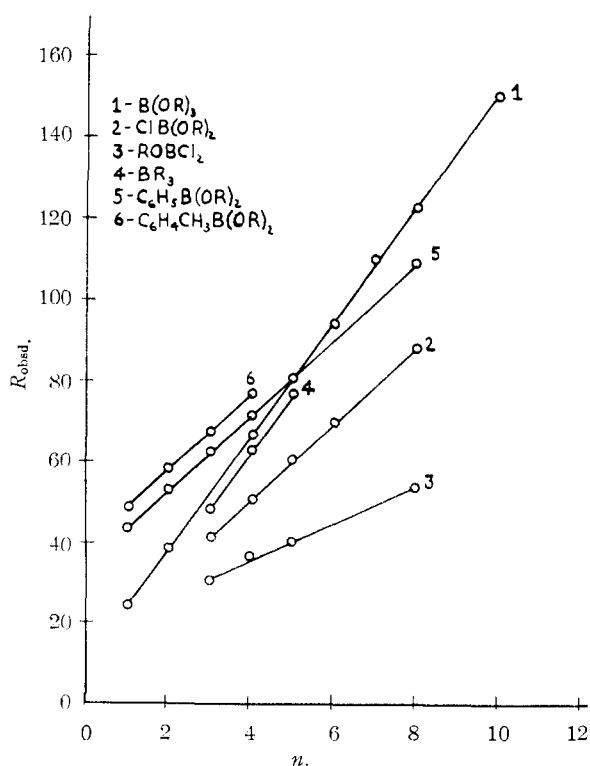
TABLE IV

APPARENT REFRACTIVITY  $R_x$  FOR THE BOND B:C, WHEN THE CARBON IS AROMATIC, IN CC./MOLE

Compound	$d^{25}_D$	$n^{25}_D$	$M$	$R_{obs}$	$R_{kn}$	$R_x$
1 $C_6H_5B(OCH_3)_2$	1.0024 <sup>a</sup>	1.4926 <sup>a</sup>	150.0	43.46	41.03	2.43
2 $C_6H_5B(OC_2H_5)_2$	0.954	1.4785	178.0	52.86	50.29	2.57
3 $C_6H_5B(O-n-C_3H_7)_2$	.9279 <sup>a</sup>	1.4738 <sup>a</sup>	206.1	62.40	59.55	2.85
4 $C_6H_5B(O-n-C_4H_9)_2$	.9245	1.4751	234.1	71.30	68.81	2.49
5 $C_6H_5B(O-i-C_4H_9)_2$	.9163	1.4711	234.1	71.43	68.63	2.80
6 $C_6H_5B(O-s-C_4H_9)_2$	.9073	1.4658	234.1	71.43	68.63	2.80
7 $C_6H_5B(O-s-C_6H_{11})_2$	.8847	1.4666	346.35	108.55	105.43	3.12
8 $o-CH_3C_6H_4B(OCH_3)_2$	.9798 <sup>a</sup>	1.4870 <sup>a</sup>	164.0	48.14	45.63	2.51
9 $o-CH_3C_6H_4B(OC_2H_5)_2$	.9351 <sup>a</sup>	1.4737 <sup>a</sup>	192.1	57.70	54.89	2.81
10 $o-CH_3C_6H_4B(O-n-C_3H_7)_2$	.9206 <sup>a</sup>	1.4721 <sup>a</sup>	220.1	66.96	64.15	2.81
11 $o-CH_3C_6H_4B(O-n-C_4H_9)_2$	.9099 <sup>a</sup>	1.4706 <sup>a</sup>	248.2	76.19	73.41	2.78
12 $m-CH_3C_6H_4B(OCH_3)_2$	.9846 <sup>a</sup>	1.4926 <sup>a</sup>	164.0	48.37	45.63	2.74
13 $m-CH_3C_6H_4B(OC_2H_5)_2$	.9388 <sup>a</sup>	1.4796 <sup>a</sup>	192.1	58.09	54.89	3.20
14 $m-CH_3C_6H_4B(O-n-C_3H_7)_2$	.9226 <sup>a</sup>	1.4757 <sup>a</sup>	220.1	67.25	64.15	3.10
15 $m-CH_3C_6H_4B(O-n-C_4H_9)_2$	.9126 <sup>a</sup>	1.4744 <sup>a</sup>	248.2	76.49	73.41	3.08
16 $p-CH_3C_6H_4B(OCH_3)_2$	.9860 <sup>a</sup>	1.4948 <sup>a</sup>	164.0	48.49	45.63	2.86
17 $p-CH_3C_6H_4B(OC_2H_5)_2$	.9389 <sup>a</sup>	1.4793 <sup>a</sup>	192.1	58.05	54.89	3.16
18 $p-CH_3C_6H_4B(O-n-C_3H_7)_2$	.9225 <sup>a</sup>	1.4770 <sup>a</sup>	220.1	67.41	64.15	3.26
19 $p-CH_3C_6H_4B(O-n-C_4H_9)_2$	.9127 <sup>a</sup>	1.4758 <sup>a</sup>	248.2	76.67	73.41	3.26
20 $CH_3(C_6H_5)BO-n-C_3H_7$	.8985 <sup>a</sup>	1.4880 <sup>a</sup>	162.0	51.95	48.90	3.05
21 $C_2H_5(C_6H_5)BO-n-C_4H_9$	.8940 <sup>a</sup>	1.4864 <sup>a</sup>	190.1	61.09	58.16	2.93
22 $C_6H_5-o-CH_3C_6H_4BO-n-C_3H_7$	.9748 <sup>a</sup>	1.5440 <sup>a</sup>	238.1	77.11	71.20	2.96
23 $o-CH_3C_6H_4-m-CH_3C_6H_4BO-n-C_3H_7$	.9646 <sup>a</sup>	1.5407 <sup>a</sup>	252.2	82.12	75.80	3.16
24 $o-CH_3C_6H_4-p-CH_3C_6H_4BO-n-C_3H_7$	.9648 <sup>a</sup>	1.5420 <sup>a</sup>	252.2	82.27	75.80	3.21
25 $n-C_4H_9O(C_6H_5)BCl$	1.021	1.4996	196.5	56.57	53.47	3.10
26 $n-C_6H_{13}CH(CH_3)O(C_6H_5)BCl$	0.965	1.4865	252.6	75.22	71.78	3.44
27 $C_6H_5BCl_2$	1.194	1.5385	158.8	41.63	38.13	3.50

<sup>a</sup>  $d^{25}_D$ ,  $n^{25}_D$ .

Av. (rounded off) 3.0

Fig. 1.—Dependence of  $R_{obsd}$  (D-line, 20°) for members of homologous series on the number  $n$  of carbon atoms in the alkyl chains, R.I and II, respectively, were used when needed in the calculation of  $R_{kn}$  in Table III. The valuesfor the B:C bond refractivity, when the carbon is aliphatic, range in Table III between 1.44 and 2.06, with an average  $1.75 \pm 0.19$  cc./mole.

In Table I, an especially low value (2.74) was obtained for B: $\ddot{O}$ :C from compound 19, B[OCH-(CH<sub>2</sub>)C<sub>6</sub>H<sub>5</sub>]<sub>2</sub>, which alone contained an aromatic ring, although separated from the oxygen by a methylene group. In calculating this value, the phenyl group was assumed to have the Kekulé structure. This might appear to be justifiable because the refractivity of benzene, 26.18, is close to the sum, 26.30, expected for 6 C:H, 3 C:C and 3 C::C bonds. However, if one uses this assumption for the evaluation of the refractivity of the B:C bond for the compounds of Table IV, in which the carbon is aromatic, a considerable systematic deviation from additivity becomes evident. Data for compounds containing B: $\ddot{O}$ :C octets in which the carbon is aromatic were not available in the report.<sup>6</sup>

The mean values for the octet and bond refractivities from Tables I (3.14), II (6.79) and III (1.75), were used when needed in the calculation of  $R_{kn}$  for the compounds listed in Table IV. The range of the resulting apparent values for B:C<sub>arom</sub> is from 2.43 to 3.50, hence the average value  $2.96 \pm 0.23$  has been rounded off to 3.0 cc./mole, as compared with 1.75 cc./mole for B:C<sub>aliph</sub> in Table III.

Markedly smaller deviations from additivity result when one limits the comparison to series of homologous compounds which differ only with respect to the number of carbon atoms in the alkyl chain. Among the 69 compounds listed in Tables

I-IV, 46 may be classified into one of these six types: (1) B(OR)<sub>3</sub>—compounds 1 to 11, Table I; (2) ClB(OR)<sub>2</sub>—compounds 1 to 8, Table II; (3) ROBCl<sub>2</sub>—compounds 9 to 13, Table II; (4) BR<sub>3</sub>—compounds 1 to 3, Table III; (5) C<sub>6</sub>H<sub>5</sub>B(OR)<sub>2</sub>—compounds 1 to 7, Table IV; (6) C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>B(OR)<sub>2</sub>—compounds 8 to 19, Table IV. If the values for  $R_{obs}$  are plotted against  $n$ , where  $n$  is the number of carbon atoms in a single alkyl group, the following graph is produced.

Since the  $R_{obs}$  values for isomeric compounds show no systematic deviations (the  $n$ -isomers have partly larger, partly smaller values than the  $i$ -isomers), the average value was used in the plot. The fact that almost all of the points fall directly on straight lines may be taken as an indication of the reliability of the values for the density and refractive index as given in the report.<sup>6</sup> From the slopes and intercepts of the straight lines shown, one can derive a set of equations of the type

The resulting increment  $R_{CH_2}$  agrees, except in the series (3), within 0.07 cc. with the value 4.64

	$R = an + b;$	$a$	$R_{CH_2}$
(1) B(OR) <sub>3</sub>	$R_{obsd} = 14.04n + 10.05;$	14.04	$= 3 \times 4.68$
(2) ClB(OR) <sub>2</sub>	$R_{obsd} = 9.18n + 14.27;$	9.18	$= 2 \times 4.59$
(3) ROBCl <sub>2</sub>	$R_{obsd} = 4.92n + 15.73;$	4.92	$= 1 \times 4.92$
(4) BR <sub>3</sub>	$R_{obsd} = 14.14n + 6.04;$	14.14	$= 3 \times 4.71_3$
(5) C <sub>6</sub> H <sub>5</sub> B(OR) <sub>2</sub>	$R_{obsd} = 9.34n + 34.10;$	9.34	$= 2 \times 4.67$
(6) C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> B(OR) <sub>2</sub>	$R_{obsd} = 9.42n + 38.94;$	9.42	$= 2 \times 4.71$

cc. observed for long homologous series. These equations may therefore be used to calculate the molar refractions for any organoboron compounds of the six types mentioned without the need of density and/or refractive index data.

The accuracy of equations 3 and 4 is questionable, since the derivation of the latter is based on only three points (see graph) and for the former the experimental points deviate relatively more from the straight line than for the other equations.

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## The Influence of the Ionization of a Group in the Substrate Molecule on the Kinetic Parameters of Enzymatic Reactions

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Equations have been derived for the single substrate case to include the effects of the ionization of a group in the substrate molecule upon the over-all rate of an enzymatic reaction. These equations have been described in terms of changes in the maximum velocity and Michaelis constant of the reaction. It is shown that there are several distinguishable kinetic cases even when the effects of substrate ionization are complicated by those of groups in the enzymatically active site. These cases may be distinguished by differences in plots of kinetic constants as a function of  $pH$  for the forward and reverse reaction. Provided that enough data are available and that the assumptions made in the derivations are correct, it is possible to tell whether (1) one of both ionic forms of the substrate are utilizable or (2) if only one form is utilizable, whether the other form is a good or poor competitive inhibitor.

The substrates for many enzymes contain groups which ionize in the  $pH$  range most frequently investigated. Since most substrate molecules are of low molecular weight the change in charge associated with the change in degree of ionization of substrate probably will affect the substrate-enzyme interaction in some way and thus will influence the rate of the over-all reaction. The changes in the over-all reaction may in turn be attributed to changes in the kinetic parameters, that is, the maximum velocity and Michaelis constant of the reaction. There has been little attention paid to the effect of substrate ionization on over-all rates of enzymatic reactions. Undoubtedly, much of this lack arises from the already complicated  $pH$ -dependence of the kinetic parameters due to enzymatic ionizations alone.<sup>1-6</sup> However, under suitable conditions, equations describing the over-all reac-

tion rate in the presence of an ionizable substrate may be derived. Provided that there is some difference either in the binding of two different ionized forms of substrate to the enzyme or in the rate of breakdown of the two different enzyme-substrate complexes there will always be some effect of the substrate ionization on the kinetic parameters of the reaction. The derived equations show that changes in enzyme-substrate interaction and breakdown due to changes in the ionization state of the substrate molecule may be detected from a complete study of the kinetic parameters as a function of  $pH$  for the forward and reverse reaction. The ionization constant of the substrate must, of course, be known.

There are two major assumptions made in this paper: first, that the enzyme contains either 0, 1 or 2 ionizable groups which control the  $pH$  dependence of the reaction rate in a "total" way. It will be assumed that there are no more than two such groups. Such an assumption does not seem unlikely in view of the fact that this appears to be the situation for many enzymes as exemplified by fumarase.<sup>6</sup> Secondly, it is assumed that hydrogen ion equilibria are established rapidly.

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