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Chemistry of Arylboric Acids VIII. The Relationship between Physico-chemical Properties and Activity in Plants

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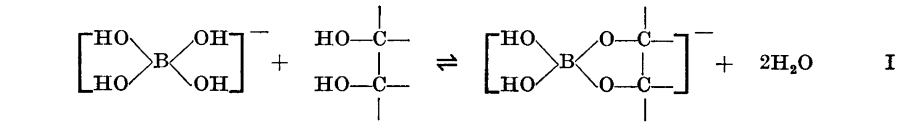
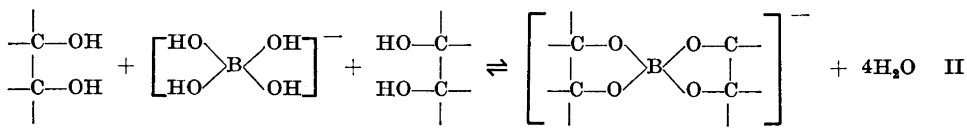
Ionization constants for a number of arylboric acids and their complexes with sugars have been measured. Further, the effect of arylboric acids on retrogradation of starch and viscosity of borax-galactomannan solutions have been investigated. Results of these physico-chemical investigations have been compared with the root cell elongation effect of the compounds in order to validate a hypothesis on the mode of action of arylboric acids in plants. Evidence is presented for a correlation between root growth promoting effect and ability of the compounds to form complexes with polyols.

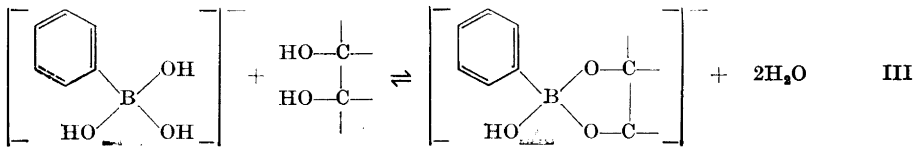
It has been known for a long time that polyhydroxy compounds enhance the acidity of boric acid through complex formation [1,2]. The following equilibria are involved:





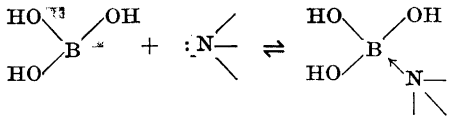
It is obvious that if compound I is a stronger acid than boric acid, the acidity must increase when the polyalcohol is added to a boric acid solution. For steric reasons compound II must be completely dissociated.

Phenylboric acid behaves in the same manner but can bind only one polyalcohol [3].



III

Boric acid can also interact with other functional groups, *i.a.* an amino group:



but in comparison to the B—O bond the B—N bond is much weaker [4].

From a chemical point of view it seems, therefore, reasonable to associate the plant physiological effects of boric acid and arylboric acids with an effect on hydroxyl-rich compounds. Thus we can expect that boric acid or arylboric acids may act upon: a) an enzyme system where either the coenzyme or the substrate contains a polyhydroxyl compound; b) the polysaccharide chains in the cell wall; Schmucker [5], Torssell [6], Odnoff [7] and O'Kelley [8*]; c) translocation of sugars; Gauch and Dugger [9].

a. *Effect of boric acid and phenylboric acid on enzymes.* The effect of boric acid on various enzymes has been tested by several workers (Ref.[10], reviews). As a general rule it may be said that boric acid has no or very little effect at low concentrations but inhibits enzyme activity at higher concentrations *in vitro*.

The action of phenylboric acid on the enzymes tested shows the same general picture but the inhibiting effect seems to be stronger than that of boric acid [11]. It was found that the activity of invertase, α-amylase and phosphorylase *in vitro* and the fermentation activity of baker's and brewer's yeast were not affected by phenylboric acid at concentrations less than 5×10^{-3} M. In the present paper three other enzymes have been tested: pectinase, pectin esterase and the "Zwischenferment" with its coenzyme TPN. Pectins presumably play a prominent role as cell wall constituents and as the boric acids seem to regulate the cell stretching it was of interest to test whether or not phenylboric acid has an influence on enzymes controlling the breakdown and de-esterification of pectins. In the first two enzyme systems phenylboric acid can couple with the substrate and in the third with both the coenzyme and the substrate, glucose-6-phosphate.

b. *Effect of boric acid and arylboric acids on the cell wall.* Schmucker [5] found that boron is essential for the germination of pollen and growth of the pollen tube and attributed this to an effect of the boric acid — polyalcohol complexes on cell wall formation. Boron deficiency symptoms appear first in meristematic tissues, root tips and buds, *i.e.* parts of the plant characterized

* *Added in proof.* Recently A. R. Spurr, *Am. J. Bot.* **44** (1957) 637, also hypothesized that boron affects the carbohydrate deposition into the cell wall.

by rapid growth rate. By discontinuous addition of boric acid to boron deficient bean plants, Odnoff [7] has shown the role of boron in the stretching process of the roots. On the basis of experiments with arylboric acids on wheat roots, Torssell [6] proposed the hypothesis that arylboric acids and boric acid control the deposition and orientation of polysaccharides in the cell wall through complex formation and thereby also regulate the stretching of the cell. More evidence for such a view is presented in the present work.

It has already been pointed out that the effects of arylboric acids on plants probably are not associated with an auxin effect [6]. The activity of synthetic auxin or antiauxin compounds strongly depends upon what kind of nuclear substituents they have and on the general structure of the molecule whereas this is of less importance in the arylboric acid series.

It is more likely that the effects of arylboric acids are associated with the properties of the dioxyboron group than with an overall effect of the molecule. The paper chromatographic experiments show, however, that an adsorption of arylboric acids on polysaccharides also may play a role. In order to explain the action of arylboric acids on amylose retrogradation, we have to take into consideration both complex formation and adsorption.

If now the arylboric acid-polyalcohol complexes play the proposed role in cell wall formation and in a wider sense in the cell stretching, we would expect to find a correlation between the plant physiological activity of different arylboric acids and their ability of form sugar complexes. The delaying effect of arylboric acids on amylose retrogradation [6] that served as a model experiment for the aging process of the polysaccharide chains in the cell wall, should further parallel their activity in plants.

In proposing this working hypothesis, however, we should not neglect two other factors that can greatly affect the activity of the compounds in plant. The uptake and translocation may be different with compounds that have the same intrinsic effect at the site of action. Secondly, the toxicity of some derivatives may be so high that side reactions completely counteract the cell elongation effect.

We have been looking for other model experiments more closely related to the proposed stabilization mechanism and have investigated the effect of arylboric acids on borax-galactomannan gels [12]. Galactomannan is a polysaccharide, consisting of a chain of 1,4′ linked D-mannopyranose units with single side units of D-galactopyranose linked 1,6′ to every other D-mannose unit [13]. The addition of borax to dilute solutions of this polysaccharide gives rise to an increased viscosity.

c. *Effect of boric acid and arylboric acid on sugar translocation.* Gauch *et al.*[9,14] have postulated that boric acid plays a role in the translocation of sugars in plants. There is evidence in favor of this hypothesis but other evidence is not in accord with it. McIlrath and Palser [15] found that in roots of boron deficient cotton plants, where no phloem necrosis was observed, the total sugar content was equal to that of normal plants and in the leaves it was lower. Spraying of buds with sucrose solution did not inhibit the boron deficiency symptoms. Odnoff [7] reported that the sugar level in the roots of boron deficient young bean plants was higher than in normal control plants.

Provided that the mechanism of action of arylboric acids and boric acid is the same, it is not possible to explain their cell stretching effect only on basis of an increased sugar translocation [14]. We probably have to account for several modes of action of boron in plants, of which the translocation effect may be one.

EXPERIMENTAL

Determination of enzyme activities in the presence of phenylboric acid

1. *Pectinase.* The effect of phenylboric acid on pectinase was tested viscosimetrically and by measurement of reducing groups produced.

a. *Viscosimetrically.* The following solutions were prepared: (I) sodium pectate, 1 %, (Sunkist Growers, Inc., Ontario, Calif., U.S.A.), buffered to pH 4.0 with acetate, 0.05 M. (II) Pectinase, 0.01 % (Nutritional Biochemicals Corp., Cleveland, Ohio). The crude pectinase was first purified by ultra filtration according to McClendon and Somers [16]. (III) Phenylboric acid 7.0×10^{-2}, 7.0×10^{-3} and 7.0×10^{-4} M. To 20 ml (I) + 2 ml (III) (or 2 ml water in the control run) equilibrated at 30° in a water bath were added 2 ml enzyme solution (II). The mixture was shaken rapidly. The time of efflux was measured at intervals with an Ostwald viscosimeter. In one experiment the enzyme was first incubated with 3.5×10^{-2} M phenylboric acid for 1.5 h and then added to the pectin solution. Fig. 1 shows the efflux time (extent of degradation) as a function of time.

b. *Measurement of reducing groups.* To 50 ml of sodium pectate (I) and 20 ml phenylboric acid (III) equilibrated at 30° 2 ml of 0.1 % enzyme solution was added. The time was noted and 10 ml aliquots were removed at various times and analyzed for reducing groups according to Willstätter and Schudel [17]. Fig. 2 shows the I_2 consumption (liberated reducing groups) as a function of time.

2. *Pectinesterase.* (Pectin from Nutritional Biochemicals Corp., Cleveland, Ohio and Pectinesterase from Worthington Biochemical Sales Co., Freehold, New Jersey.) The enzyme activity was tested according to the procedure of Kertesz [18]. Fifty ml of 1 % pectin solution containing 0.1 M NaCl and a suitable amount of phenylboric acid was equilibrated at 30° and adjusted to pH 6. Two ml of 0.01 % enzyme solution was added; followed by thorough mixing. Sodium hydroxide (0.01 M) was added at a rate to keep the pH constant at 6. In Fig. 3 the alkali consumption is plotted against time.

3. *Glucose-6-phosphate dehydrogenase (Zwischenferment).* The assay method, described by Kornberg and Horecker [19] was employed. The reduction of the coenzyme in presence of various phenylboric acid and boric acid concentrations was followed (Fig. 4) by measuring at various times the intensity of the absorption band (at 340 mμ) of the reduced form. (Zwischenferment from Sigma Chemical Co., St. Louis, Mo.; TPN, Nutritional Biochemicals Corp., Cleveland, Ohio). Veronal buffer pH 7.5 was used instead of glycylglycin.

Complex constant measurements

Complex constants of arylboric acids with fructose, maltose, starch (Lintner, potato) and galactomannan were measured. The method, described in an earlier paper [3], was employed with the difference that a saturated calomel electrode was used and the measurements were carried out in pure water instead of 0.1 M KCl. The constants in Table 1 are mean values from two or more runs. The arylboric acids tested were synthesized according to Torssell [20].

Effect of arylboric acids on amylose retrogradation

Because identically prepared amylose solutions may retrograde with different velocity, all substances were tested [6] at the same time with the same amylose solution in order to get comparable values of their inhibiting effect. Into 5 ml of 4×10^{-2} M arylboric acid

were pipetted 15 ml 0.5 % maize amylose containing 0.02 M buffer (pH 5.0 acetate; pH 6.5 and 6.9 phosphate; and pH 7.9 veronal). The final arylboric acid concentration was 10^{-2} M. Because of insolubility of some compounds (*p*-nitro-phenylboric acid, 3-nitro-4-carbomethoxy-phenylboric acid) they were tested at a concentration of 5×10^{-3} M. Intensity of the scattered light was measured at various times with a *Leifo*-photometer. The experiments were duplicated at various pH's.

Effect of arylboric acids on the viscosity of borax-galactomannan solutions

Galactomannan from guar seed (Stein Hall, New York, U.S.A.; trade name Jaguar A-20-D) was reprecipitated once [21] and 1.6 g of the dry ground product was dispersed in 400 ml vigorously stirred hot water (*ca.* 80°). After standing overnight the solution was heated to boiling for 10 min, filtered hot with suction through porous filter paper and was buffered to pH 8.9 with glycine buffer (0.02 M).

Four series of solutions were prepared: a) 10 ml galactomannan (I) + 10 ml borax of various concentrations ($10^{-3}-10^{-2}$ M); b) 10 ml (I) + 10 ml phenylboric acid (half neutralized in order to minimize pH changes) in various concentrations ($10^{-3}-10^{-2}$ M); c) 10 ml (I) + 10 ml of a mixture in various proportions of borax (10^{-2} M) + phenylboric acid (10^{-2} M); d) 10 ml (I) + 5 ml borax (3.0×10^{-3} M, final concn.) + 5 ml of various arylboric acids (half neutralized, 1.2×10^{-3} M, final concn.). The mixtures were shaken vigorously and equilibrated for 2 h at 25°. Their viscosity was measured with an Ostwald viscometer. Efflux times are shown for series a–c in Table 5. The decrease in efflux time (viscosity) caused by addition of various arylboric acids to the borax-galactomannan solution, series d, has been calculated as percent of the viscosity increase caused by borax alone, when added to the galactomannan solution, Table 1.

Paper chromatography

A technique, elaborated by Rockland and Dunn [22] was employed. R_F-values refer to pure water as solvent. The arylboric acids (0.3 % water-methanol solution) were placed upon the paper, W 52, by means of a drawn out melting point capillary. Colors were developed with 0.4 % alcoholic curcuma solution, which gives brown-red spots with boron on a yellow background when the paper strips were left to dry for 5 min in an oven at 90° [27].

RESULTS AND DISCUSSION

Effects on enzymes. Presence of phenylboric acid does not affect the time course of pectic acid degradation by pectinase when followed viscosimetrically (Fig. 1) but a slight inhibition (*ca.* 10 % after 20 h, 10^{-3} M) could be detected by measuring reducing groups, (Fig. 2). Rather high concentrations of phenylboric acid (10^{-2} M) must be used before the inhibiting effect on pectinesterase is significant (Fig. 3). Glucose-6-phosphate dehydrogenase is also left unaffected by phenylboric acid in concentrations $<10^{-3}$ M. Boric acid is less active (Fig. 4). Thus when summing up results from the enzymatic work carried out in previous [11] and present papers we find, with one exception — cholinesterase [11] (which, however, does not occur in plant tissues) — that phenylboric acid at concentrations $<10^{-3} - 5 \times 10^{-3}$ M has no effect on the tested enzymes and at higher concentrations acts as an inhibitor.

According to Eliasson [23] phenylboric acid has no effect on the respiration of excised wheat roots.

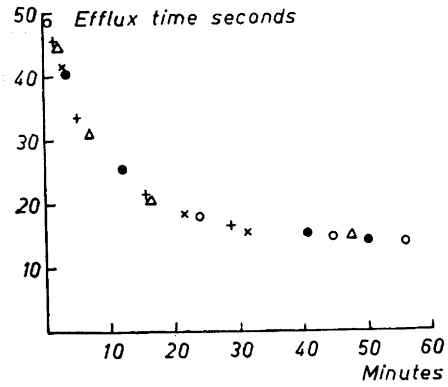


Fig. 1. Effect of phenylboric acid on the activity of pectinase. O Control, + 0.58 × 10^{-4} M phenylboric acid, × 0.58 × 10^{-3} M PhBA, ● 0.58 × 10^{-2} M PhBA, △ Enzyme incubated for 1.5 h with 0.58 × 10^{-2} M PhBA. The degradation of pectin is measured viscosimetrically.

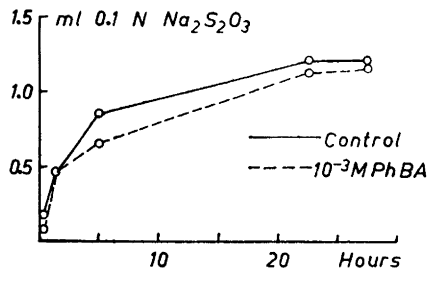


Fig. 2. Effect of phenylboric acid on the activity of pectinase. Degradation is followed by measurements of reducing groups, (equivalent to ml $Na_2S_2O_3$).

The effects of phenylboric acid on enzymes are in no cases striking and it seems to be difficult to draw any conclusions about relationships between the action of phenylboric acid on enzyme systems and its cell stretching effect in plants. However, the possibility may not be excluded that boric acid might be a constituent of an enzyme or act in some way *via* an enzyme.

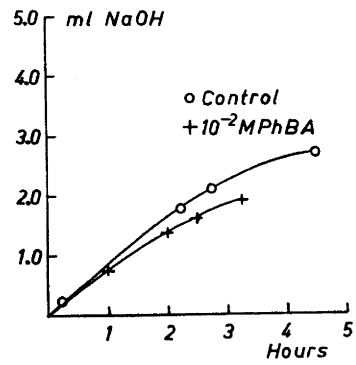


Fig. 3. Effect of phenylboric acid on the activity of pectinmethylesterase.

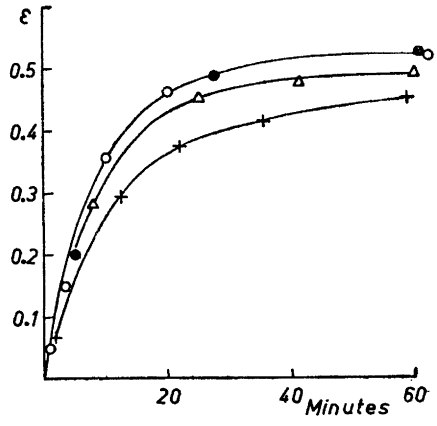


Fig. 4. Effect of phenylboric acid and boric acid on glucose-6-phosphate dehydrogenase. O Control, ● 2.3 × 10^{-3} M H_3BO_3, △ 2.5 × 10^{-3} M phenylboric acid, + 0.92 × 10^{-2} M phenylboric acid.

Table 1. Ionization constants for arylboric acids and their complexes with fructose; root growth promoting effect of arylboric acids and their effect on the viscosity of galactomannan-borax solutions. PhBA = Phenylboric acid.

	Compound	Ionization constant k_s $RBH \rightleftharpoons RB^- + H^+$ at 25°; $\times 10^9$	Complex constant k_1 $D + RB^- \rightleftharpoons RBD^-$ at 25°; $\times 10^{-3}$	$k_s \times k_1 \times 10^6$ $D + RBH \rightleftharpoons RBD^- + H^+$	Reduction of viscosity; per cent	Activity in plants
1	Boric acid	0.653 a)	0.69 ($k_2 = 0.83 \times 10^5$)	0.45	—	— b)
2	Phenylboric acid	1.37 c)	5.18 d)	7.10	55	+++
3	4-NO_2-PhBA	70.7	2.49	176	86	+++
4	3-NO_2-PhBA	50.0	2.50	125	73	++
5	2-NO_2-PhBA	~3	~1.5	~4.5	—	++
6	3-CHO-PhBA	16.0	3.00	48.0	57	+(+)
7	2-CHO-PhBA	48.6	0.837	40.7	62	+
8	2-CH_2OH-PhBA	41.5	0.832	34.5	76	0
9	4-OCH_3-PhBA	0.482	5.76	2.78	26	+++
10	2-NO_2-5-NH_2-PhBA	0.101	1.54	0.156	0	0
11	2-NO_2-4-NH_2-PhBA	0.230	2.73	0.63	32	0
12	2-NO_2-4-$COOCH_3$-PhBA	4.35	1.67	7.26	46	0(+)
13	4-NHAc-PhBA	1.87	5.35	10.0	54	+(+)
14	2-CH_3-3,5-diNO_2-PhBA	125	3.70	462	89	++
15	4-CH_3-PhBA	—	—	—	32	++
16	4-NO_2-phenol	—	—	—	24	0

a) Torssell, K. *Arkiv Kemi* 3 (1952) 571. b) Ref.[6] c) Ref.[24] d) Ref.[3]

Relationship between complex constants, $k_s \times k_1$ and growth promoting effect. Ionization constants, k_s:

$$k_s = \frac{[RBH]}{[H^+]\,[RB^-]}$$

and complex constants [3] k_1 (arylboric acid — fructose):

$$k_1 = \frac{[RBD^-]}{[RB^-]\,[D]} = \frac{([H^+] + [Na^+]) \cdot [H^+] - k_s\,[RBH]}{k_s\,[RBH]\,[D]}$$

Table 2. Complex constants for some arylboric acids with different polyalcohols. The complex constants for fructose, mannose and starch have been calculated on the basis that only one complex can be formed per molecule and for galactomannan that one complex can be formed per sugar unit. Temp. 25° C.

Compound	Fructose $k_1 \times 10^{-3}$	Maltose $k_1 \times 10^{-3}$	Starch (Lintner) $k_1 \times 10^{-3}$	Galactomannan $k_1 \times 10^{-3}$
Phenylboric acid	5.18	0.0410	0.072	0.0082
3-NO_2-PhBA	2.50	0.0259	—	—
2-CH_2OH—PhBA	0.832	0.0165	—	—

(RBH = Phenylboric acid; RB^- = ionized acid; D = polyalcohol) have been measured for a number of arylboric acids which show various degrees of cell stretching effect (Table 1). When comparing the complex formation ability of arylboric acids ($k_s \times k_1$) with their action in plants, the assumption has to be made that the relative magnitude of complex constants for one sugar, in this case fructose, is about the same for another polyalcohol. This has been proved to be true for maltose, which forms much weaker complexes than fructose (Table 2). The systems, Lintner starch-phenylboric acid and galactomannan-phenylboric acid have also been investigated. Calculation of complex constants can be only very approximate as the units within the chain differ in complex forming ability. Galactose residues do not form as strong complexes as the mannose units of galactomannan. Also at end groups or at groups where branching occurs, the conditions for complex formation are different. As a first approach toward an investigation of the nature of these complexes, an end group determination (reducing groups) was carried out according to Willstätter and Schudel. This gives some idea concerning the average molecular weight (lower limit). It was found with starch that the concentration of the complex always was less than the reducing group concentration and calculation of the complex constant on the basis of reducing group concentration gave a more constant value than a calculation on a basis that every glucose unit takes part in complex formation to the same degree. The fact that methyl glucoside [1] gives very weak complexes with boric acid in comparison with glucose favors the idea that in starch the end groups preferentially combine with phenylboric acid. The complex constant for the starch-phenylboric acid system when only end groups are taken into consideration is of the same magnitude as that of the maltose-phenylboric acid system, in which the formation of a complex containing two phenylboric acid per maltose seems to be negligible, Table 2. It may therefore be assumed that complex formation between starch and phenylboric acid preferably takes place at the end groups and that to some extent weaker complexes are formed with the other units of the chain. Willstätter-Schudel end-group determination of starch usually gives too high values. According to the analysis, the starch molecule contains on an average 15—20 units, which seems to be too low for this kind of starch (Lintner potato starch). A lower end group concentration gives a higher complex constant k_1 but on the other hand a formation of weak complexes between phenylboric acid and some of the units in the chain has the opposite effect on k_1.

In the galactomannan-phenylboric acid system it was found that the complex formation is higher than the amount of reducing groups, *i.e.* the units in the chain are as important as the end groups for complex formation. This is to be expected from steric reasons; mannose and galactose have adjacent *cis*-hydroxyl groups at carbon atoms 2,3 and 3,4 respectively, which favors complex formation while the hydroxyl groups of the glucose unit in starch are all *trans* and only the end group has adjacent *cis*-hydroxyls (in 1,2 position). Table 3 shows some of the original measurements and in column 7 the concentration of the complex is calculated. A Willstätter-Schudel end group determination gave a polymerization degree of about 30, a value that for this kind of polysaccharide is too low. From the last row of column 7,

Table 3. 40.00 ml of a galactomannan-phenylboric acid solution is titrated with 0.1668 N NaOH. (D_{tot}) is the concentration of sugar residues = molecular concentration x degree of polymerization. Temp. 25° C.

ml NaOH	$[Na^+]$ $\times 10^2$	$[RBH_{tot}]$ $\times 10^2$	$[RBH]$ $\times 10^2$	$[D_{tot}]$ $\times 10^2$	$[D]$ $\times 10^2$	$[RBD^-]$ $\times 10^2$	pH	$[H^+]$ $\times 10^8$	k_1
—	—	2.18	2.18	1.50	1.50	—	5.75	178	—
1.20	0.486	2.12	1.63	1.46	1.42	0.041	8.30	0.501	6.5
2.20	0.869	2.07	1.20	1.42	1.34	0.084	8.68	0.209	8.0
3.47	1.332	2.01	0.68	1.38	1.25	0.130	9.11	0.0775	8.7

however, we find that the concentration of the complex is 0.130×10^{-2} M which is about one tenth of the total sugar concentration in the solution. That means that, as an average, every tenth sugar unit has formed a complex with phenylboric acid at this pH and we must therefore have more than one complex per molecule. The complex constant for galactomannan, Table 2, has been calculated on the assumption that every chain unit gives equally strong complexes and that already formed complexes do not affect the formation of adjacent ones. The complex constants for starch and galactomannan are not comparable as they are calculated on basis of different assumptions. In order to get an idea about their relative magnitude the galactomannan constant has to be multiplied by the polymerization number of galactomannan.

In the last column of Table 1 the activities of the compounds are tabulated according to an arbitrary scale: +++ means a root elongating effect of 175 % or more, ++ = 150—175 %, + = 125—150 % and 0 = 100 % (none) — 125 %. The values are taken from a previous paper [6] and by the gradation the position of the maximum (concentration at which maximum occurs) has been considered.

The active acids 3, 4, 6 and 14 (see Table 1 for identification) all have high ionization constants, k_s, and also high complex formation ability, $k_s \times k_1$, in comparison with the inactive acids 10, 11 and 12. The $k_s \times k_1$ values of the highly active 2, 5 and 9 are higher than that of the inactive 10 and 11 but of the same magnitude as 12. Numbers 7 and 8 also are strongly dissociated and have high $k_s \times k_1$ values and we would therefore expect that they should be active in plants which, however, is not the case. A closer examination of their activity curves shows that they are more toxic to the roots, than the other compounds. Number 8, for example, inhibits the root growth completely at a concentration of 10^{-5} M whereas 10, 11 and 12 at 10^{-4} M do not show any inhibiting effect. *o*-Hydroxymethyl-phenylboric acid, 8, shows in comparison with other tested arylboric acids a strong bacteriostatic effect [11]. Therefore, it is probable that they actually are active, but their toxicity reverses the cell elongating effect by disturbing other essential reactions of importance for root growth. It has already been pointed out in the introduction that uptake and translocation may influence the activity of the compounds. When these factors are of the same magnitude for different compounds the complex constant alone ought to determine the activity in plants. Isomeric compounds as the *o*-, *m*- and *p*-nitrophenylboric acids are probably affected

equally by the mentioned factors and we find that complex constants $k_s \times k_1$ and root growth promoting activity (Ref.[6], Fig. 1) increases in the same order:

$$o\text{-}NO_2 < m\text{-}NO_2 < p\text{-}NO_2\text{-phenylboric acid}$$

Thus, it may be concluded that a correlation exists between plant physiological action of arylboric acids and their ability to form complexes with polyalcohols.

Relation between retrogradation effect, degelation effect and growth promoting effect. The inhibiting effect of arylboric acids on amylose retrogradation decreases in the following order:

$$3\text{-}NO_2 > 4\text{-}OCH_3 > 2\text{-}CH_2OH > \text{-}H \geq 2\text{-}NO_2\text{-}5\text{-}NH_2 > 2\text{-}NO_2 \geq 2\text{-}CHO > H_3BO_3$$

Relative order for some compounds may slightly change from experiment to experiment; the main order, however, is the same. The sequence above represents an average obtained from several runs at various pH's. Some compounds were tested in 0.005 M solution (4-NO_2- and 2-NO_2-4-$COOCH_3$-phenylboric acid) as they precipitated at higher concentrations. Their activity decreased in the following order:

$$4\text{-}NO_2 > 2\text{-}NO_2\text{-}5\text{-}NH_2 > 2\text{-}NO_2\text{-}4\text{-}COOCH_3$$

In Fig. 5 the effect of phenylboric acid at various concentrations is demonstrated.

It is obvious that complex formation alone cannot be responsible for the retrogradation inhibiting effect, because 4-methoxy-phenylboric acid and phenylboric acid show stronger inhibitions than 2-formyl-phenylboric acid that has a higher complex constant $k_s \times k_1$. Adsorption of arylboric acids on the growing amylose aggregates probably plays a role. Therefore a similarly built aromatic compound, 3-nitrophenol, (inactive in plants) was tested and found to have the same activity as phenylboric acid; aniline, 0.01 M, has no activity.

The measurements of starch complexes show further that units in the chain form only very weak complexes and that phenylboric acid is mainly linked to the end groups, which explains the fact that correlation between complex constants and the effect on retrogradation is not good. Even if the compounds most active in root growth are found in the beginning of the sequences above, the value of the retrogradation experiment as a true model for deposition of cell wall material is decreased, because of the fact that other compounds inactive in plants, *e.g.* nitrophenol, also hamper the retrogradation.

The chromatographic behavior of arylboric acids demonstrates clearly the importance of adsorption effects. It is possible to separate arylboric acids on paper with pure water as eluent. R_F values of different derivatives are shown in Table 4.

Table 4. R_F values of arylboric acids. Water as solvent. Whatman 52. The numbers refer to compounds in Table 1.

Compound	1	2	3	4	5	7	8	9	10	11	12	15	16
R_F	~1	0.74	0.56	0.58	0.57	0.87	0.66	0.62	0.64	0.70	0.79	0.67	0.70

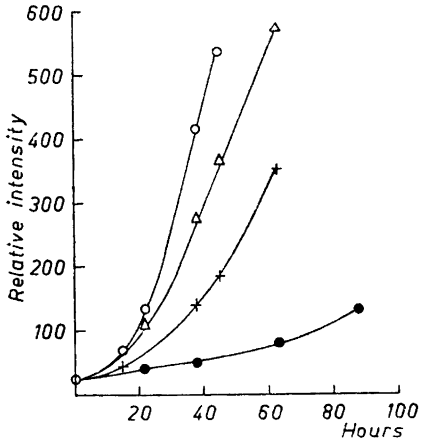


Fig. 5. Effect of various phenylboric acid concentrations on the retrogradation rate of maize amylose at pH 6.90—6.96. O Control, △ 2×10^{-3} M PhBA, + 10^{-2} M PhBA, ● 4×10^{-2} M PhBA.

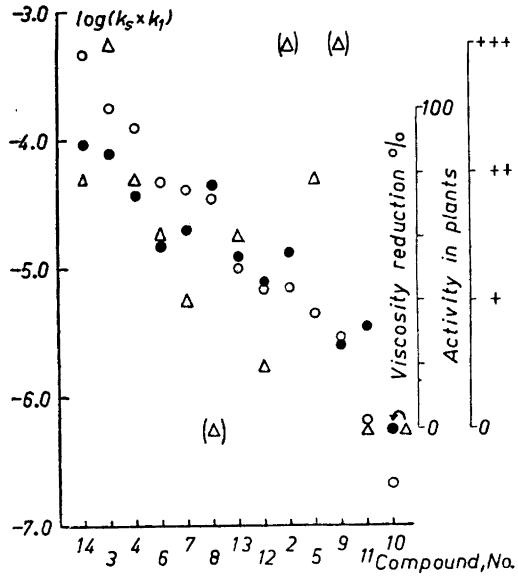


Fig. 6. Comparison of complex constants for arylboric acids, O, with their effect in plants, △, and on the viscosity of galactomannan-borax solutions, ●.

Borax forms gels with galactomannans; in dilute solution a viscosity increase takes place. Repeated cross linkages between galactomannan and and boric acid causes increased chain length and molecular weight, which results in an increased viscosity or, finally, in a gel formation [12]. Arylboric acids reduce the viscosity; probably in the following way. When an arylboric acid and boric acid are present in the polysaccharide solution at the same time, they will compete for the favorably oriented hydroxyl groups of the chain. As arylboric acids are not able to combine with more than one sugar residue, the formation of such complexes will decrease the chain length. The negatively charged arylboric acid-polysaccharide complexes have furthermore a repelling effect on other charged chains, which make it more difficult for boric acid to link them together.

Table 5. Effect of boric and phenylboric acids on the viscosity of galactomannan solutions (~ 0.15 %) pH 8.95.

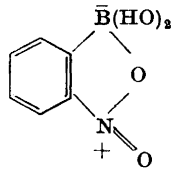
Boric acid concn. M	—	2×10^{-3}	5×10^{-3}	10^{-2}	—	2×10^{-3}	5×10^{-3}
Phenylboric acid conc. M	—	—	—	—	10^{-2}	2×10^{-3}	2×10^{-3}
Efflux time, sec	37.2	43.2	248	∞	38.0	41.5	120

The galactomannan-boric acid-phenylboric acid system illustrates the proposed role of boric acid as a regulator of deposition and orientation of cell wall material and the role of arylboric acids as an inhibition in this process. Tables 1 and 5 show the effect of arylboric acids in decreasing the viscosity of a borax-galactomannan solution. Derivatives with high complex constant reduce the viscosity strongly and compounds low in complex forming ability have less effect (see also Fig. 6). Thus the degelation effect parallels complex formation and consequently the root growth promoting effect. Nitrophenol was also tested but showed here little effect in comparison with active compounds.

In order to clarify the relationship between complex constants of arylboric acids, their action on the viscosity of borax-galactomannan solutions and their activity in plants, the compounds have been arranged along the x-axis of Fig. 6 in the order of their ability to form complexes. log ($k_s \times k_1$) is plotted along the y-axis, open rings. Also along the y-axis are plotted: 1) The reduction in viscosity due to arylboric acids in the borax-galactomannan mixtures (as percent of the viscosity increase caused by borax alone, filled rings); 2) The cell elongating effect of the compounds (triangles). Results from the viscosity measurements parallel fairly well the complex constants. With three exceptions (triangles in parenthesis) the points for the cell elongating effect follow the pathway or the other two sets of data. The deviation of number 8 has already been discussed. 2 and 9, phenylboric acid and *p*-methoxy-phenylboric acid have in comparison with their complex constants, a very high action on roots. But here, as is pointed out earlier, uptake, transport and toxicity can play a role. The activity curves of these two compounds have a very broad maximum in comparison to the other tested compounds [6].

Note on the ionization constants of some arylboric acids

Ionization constants of a number of arylboric acids have been measured earlier and the results discussed on the basis of negativities and resonances of the groups involved [24-26]. Additional data for some new derivatives are presented here. From negativity and resonance interaction of the formyl group with the benzene ring it is to be expected that the *o*-compound should be a stronger acid than the *m*-compound and both of them stronger than phenylboric acid; this is the case (Table 1). The anomalous weakness of *o*-nitrophenylboric acid is ascribed to formation of a ring structure imposing a negative charge on the boron atom [25], a phenomenon which appears also with the three



other nitroderivatives 10, 11 and 12. As expected, the 3,5-dinitro compound is a strong acid in comparison with phenylboric acid — about 100 times stronger.

The k_s for *o*-amino-phenylboric acid has not been measured but it ought to be considerably weaker than phenylboric acid because of resonance interaction between the nitrogen and boron atoms. Its acetyl derivative, however, has a k_s value very slightly greater than that of phenylboric acid, which means that the free electrons at the nitrogen atom are completely neutralized by the carbonyl group. We can find the same general trend in the benzoic acid series.

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