Summary

Phenylboric acid was prepared in a pure state, and the three isomeric nitrophenylboric acids were obtained from this acid by nitration under appropriate conditions. *m*-Aminophenylboric acid, its acetyl and benzoyl derivatives, and the benzoyl derivative of *o*-aminophenylboric acid were prepared. The physical characteristics of these substances are reported.

Bacteriological tests showed that phenylboric acid and the three nitrophenylboric acids exert a bacteriostatic effect upon *Staphylococcus aureus;* *m*-aminophenylboric acid and its derivatives do not produce a bacteriostatic effect.

The nitrophenylboric acids are more highly bacteriostatic than phenylboric acid, which in turn is much more effective than boric acid. Although no direct comparison was made, it appears that *m*-nitrophenylboric acid (1:200) approaches or slightly surpasses phenol (1:70) in its bactericidal action toward *B. typhosus*, but phenylboric acid (1:200) is much weaker than phenol (1:70).

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THE EFFECT OF ETHYLENE UPON THE HYDROLYSIS OF SALICIN BY EMULSIN

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The nature of the transformations brought about by the use of ethylene in the preparation of fruits and vegetables for marketing and the mechanism of the action continue to attract attention. It is generally believed that the changes produced are due to acceleration of enzymic activity. In an earlier paper[1] a brief consideration of the subject from this point of view, along with the results of experiments as to the effect of ethylene upon diastase and invertase with pure substrate material, was presented. No significant difference between the ethylene-treated and untreated materials was observed. The enzymes investigated were selected as representative of those concerned with normal ripening changes of carbohydrates and the original plan of experimentation anticipated studies upon other enzymes more definitely associated with color changes. It has been pointed out by Harvey[2] that an overdose of ethylene causes bananas to become brown. This coloration may be similar to leaf darkening and result from an increased hydrolysis of some glucoside followed by oxidase action upon the non-sugar component. It also seems probable that other coloration phenomena might have, as an initial stage, the change of a

[1] Englis and Zannis, THIS JOURNAL, 52, 797 (1930).

[2] Harvey, Bulletin 247, U. of Minn., Agr. Exptl. Sta., October, 1928.

glucoside into its simpler constituents. Since emulsin, which is associated with hydrolysis of β-glucosides, has apparently not received attention in connection with ethylene treatments, a study of this enzyme was undertaken. Several factors influenced the selection of salicin as the substrate material. It can be obtained readily in a high state of purity. Its hydrolysis by the enzyme has been thoroughly studied by Hudson and Paine[3] and others[4] and the rate can be followed readily by both physical and chemical methods.

Experimental

Materials.—The emulsion was prepared from sweet almonds according to the directions of Morrow.[5] One portion of the emulsion was preserved in the dry state. Another portion was made into a 1% solution and preserved with toluene.

The salicin was obtained from the Pfanstiehl Chemical Company. Its melting point was 195–197° and the ash content 0.05%.

The ethylene was a commercial product supplied by the U. S. Industrial Alcohol Company.

Method.—The general procedure was the same as that employed in the earlier study of diastase. The substrate of salicin was buffered with an acetic acid–sodium acetate buffer at a P_H of 4.66 before adding the enzyme. The progress of the hydrolysis was followed by polarimetric observations and by determination of the reducing sugar by the Munson and Walker method.

Experiment I

Procedure.—6.250 grams of salicin was dissolved in 200 cc. of buffer solution and the solution divided into two parts. One part was saturated with ethylene. Both portions were then warmed to 40° and 25 cc. of the fresh emulsin solution added to each. At various time intervals portions were withdrawn and sodium carbonate added to stop the action of the enzyme and hasten the establishment of the equilibrium value following mutarotation. The portions were then filtered and polarized in a 2-dm. tube.

TABLE I

EFFECT OF ETHYLENE UPON THE HYDROLYSIS OF SALICIN BY EMULSIN. PROGRESS OF REACTION FOLLOWED BY POLARIMETRIC METHOD

Salicin 2.5 g. per 100 cc.; temperature, 40°; P_H 4.66

Experiment	Emulsin	Time, minutes	Rotation, 2-dm. tube Control, °V.	Ethylene treated, °V.
		0	−9.0	−9.0
		15	−7.1	−6.9
I	Fresh 0.2 g. per 100 cc.	30	−5.3	−5.0
		80	+0.4	−0.5
		1500	+4.0	+4.0
	Dry (after 30 days)	0	−8.9	−8.9
		15	−8.1	−8.2
II	0.2 g. per 100 cc.	30	−7.2	−7.1
		200	−1.4	−1.0

[3] Hudson and Paine, THIS JOURNAL, 31, 1242 (1909).

[4] Kuhn and Sobotka, *Z. physik. Chem.*, 109, 65 (1924).

[5] Morrow, "Biochemical Laboratory Methods," John Wiley and Sons, New York 1927, p. 286.

Experiment II

In a second experiment carried out thirty days later a 1% solution of the dry emulsin was prepared and the same procedure followed as in the first experiment. The results of the experiments are given in Table I.

Experiments III and IV were analogous to I and II except that the reaction was followed by determination of the reducing sugar produced. The quantities of materials used and results obtained are given in Table II.

TABLE II

EFFECT OF ETHYLENE UPON THE HYDROLYSIS OF SALICIN BY EMULSIN

Salicin, 1.25 g. per 100 cc.; temperature, 40°; P_H 4.66

Experiment	Emulsin	Time, minutes	Cu_2O Control, g.	Cu_2O C_2H_4-treated, g.	Glucose Control, g.	Glucose C_2H_4-treated, g.	Hydrolysis Control, %	Hydrolysis C_2H_4-treated, %
		0	0.0065	0.0065	0.0027	0.0027	..	..
III	Fresh 0.2 g. per	40	.2783	.2555	.1266	.1155	63	57
	100 cc.	80	.3795	.3664	.1777	.1709	89	86
		0	.0065	.0065	.0027	.0027	..	..
IV	Dry after 30	15	.0361	.0300	.0152	.0126	6	5
	days 0.2 g.	30	.0754	.0757	.0324	.0324	16	16
	per 100 cc.							

Discussion

An examination of the tables shows practically no difference in the rate of hydrolysis in the salicin in the ethylene-treated samples and the controls in any of the experiments. The slight differences observed in favor of the controls are probably not significant. The emulsin on drying lost a great deal of its activity but the results with regard to ethylene were the same as with the fresh material.

In connection with the findings of this paper and the one preceding it[1] the results of a number of other studies of similar nature may be mentioned. Hirschfelder and Ceder[6] have carried out some very interesting experiments with ethylene attempting to extend the work upon fruits and vegetables to animals. Rats were given ethylene-treated drinking water and grown in atmospheres of varying concentrations of ethylene. The growth of the rats was not favorably affected. In supplementary work with enzymes they found that pepsin, trypsin and liver lipase were not activated by ethylene. However, they report that pancreatic amylase was activated. Johnson and Wormall[7] also report that potassium thiocyanate, a material which has been used to break the dormant period of potatoes and may be considered to fall in the same group of chemicals as ethylene, accelerated the first stages of hydrolysis of starch by saliva, malt and potato diastase but did not markedly increase the rate of formation of

[6] Hirschfelder and Ceder, *Am. J. Physiol.*, 91, 624 (1930).

[7] Johnson and Wormall, *Proc. Leeds Lit. Soc. Sci.*, Sec. 1, 318-324 (1928).

reducing sugars. Denny and associates,[8] who have been perhaps the most consistent contributors in this general field, have observed that under the conditions of their experiments sodium thiocyanate, thiourea and ethylene chlorohydrin all break the dormancy of potatoes. However, the increases in enzyme activity which were found from the treatment of the potatoes with chemicals were not direct effects of the chemicals on the enzymes but were indirect and brought about by the action of the chemical upon the tubers, since the treated juice of the potato failed to show significant differences from the control. This study relative to the treatment of the juice corresponds in principle to the method followed in this paper and the results obtained here are in agreement with its findings. Perhaps the apparent contradiction in the results obtained by Hirschfeld and Ceder[6] can be attributed to a lack of buffering or some other difference in experimental detail.

The results of these experiments suggest that a very complex system of reactions is taking place. The question might be raised as to whether or not studies as to the effect of ethylene or similar substances upon purified enzymes with pure substrate material should be abandoned. Perhaps mixtures of enzymes may be studied and some "key" reaction in the chain found. If not, one must come to the tentative conclusion of Denny that the chemicals seem to induce the living matter to produce larger amounts of (or more active) enzymes and their effect is not a direct one upon the enzymes.

Summary

The fact that ethylene hastens coloration of many fruits and vegetables gave rise to the belief that its effect might be associated with the activation of the enzyme responsible for the hydrolysis of many glucosides. For this reason a study was made of its influence upon the rate of hydrolysis of salicin by emulsin.

The rate of hydrolysis was followed by polarimetric observations and determinations of reducing sugar.

No acceleration of the reaction was observed in favor of the treated samples over the controls.

The results are in accord with the idea that ethylene does not directly affect the activity of enzymes, but acts indirectly through its effect upon the living matter.

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[8] Denny, Miller and Guthrie, *Am. J. Bot.*, 17, 483 (1930).