are stable substances at room temperature and pressure, whereas with the higher homologs the complex polymer is perhaps only stable at high pressures. The effect of pressure on the aliphatic aldehydes is in many ways the most interesting result of our work, since it is the one case which have discovered where a reaction can be brought about only by the application of high pressures. We seem to be concerned here not only with increasing the rate of a process but with an actual change of the equilibrium conditions.

Summary

1. The rate of polymerization of isoprene under high pressures has been studied. The reaction is subject to positive catalysis by peroxides and negative catalysis by hydroquinone. Although the reaction is of a high order, the rate is approximately in accord with a first order reaction presumably because of an autocatalytic effect. The temperature and pressure coefficients of the rate have been estimated. The solubility and elasticity of the product depend on the extent to which the isoprene has been polymerized; when the polymerization is practically complete at room temperature at 12,000 atm. the product is very insoluble

2. The action of high pressures on *n*-butyraldehyde produces a solid only slightly soluble in organic solvents. It reverts to *n*-butyraldehyde rapidly. It is suggested that this polymer is similar to the well-known polymers of formaldehyde but that the energy relationships are such that the polymer is stable only at high pressures.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY, No. 622]

A QUANTITATIVE STUDY OF THE INFLUENCE OF ACETATE AND OF PHOSPHATE ON THE ACTIVITY OF MALT AMYLASE

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In previous work upon the enzymic activity of malt amylase by different investigators, acetate and phosphate have been used somewhat interchangeably as electrolytes helpful to the activity and conservation of the enzyme. The use of phosphate mixtures, which are not effective buffers at the hydrogen-ion activities employed in work with malt amylase (P_H of about 4.5), has probably come about largely through the thought that the phosphate ion is a constant accompaniment of this enzyme when acting in its natural environment and that it may have some specific activating effect. Recent work[1] has shown that the optimal hydrogen-ion activity

[1] (a) Hahn and Michalik, *Z. Biol.*, **73**, 10 (1921); (b) Hahn and Meyer, *ibid.*, **76**, 227 (1922); (c) Myrback, *Z. physiol. Chem.*, **149**, 1 (1926); (d) Luers and Nichimura, *Wochschr. für Brauerei*, **43**, (No. 38) 415 (1926); (e) Sherman, Caldwell and

for the action of a given enzyme may differ with different environmental conditions, including the kinds and concentrations of electrolytes present, and that, therefore, the apparent influence of an electrolyte may be due to favorable or unfavorable changes in the environment of the enzyme rather than to any specific influence upon the enzyme itself, or that a combination of both these effects may be influencing the results obtained. The experiments here described were, therefore, undertaken with the aim of separating as far as possible the effects of these factors and establishing more definitely the relations of acetate and phosphate to the enzymic activity of malt amylase and to the hydrogen-ion activities which induce its optimal enzymic action.

Experimental

All reagents were carefully purified. A preparation of malt amylase obtained according to a modification of the method of Sherman and Schlesinger[2] was used throughout the work. The method previously described[3] for the determination of the saccharogenic or sugar-forming activity of the enzyme was followed with slight modifications. The enzyme acted in the presence of 2% starch containing known total concentrations of acetate or phosphate. The hydrogen-ion activities of all solutions were measured electrometrically. The general plan of the work involved three steps: first, determinations were made to establish the optimal hydrogen-ion activities for the enzyme when acting in the presence of each of several different concentrations of acetate or of phosphate; second, direct comparisons were made of the activity of the enzyme in the presence of the different concentrations of each salt, when the solutions were in each case adjusted to the optimal hydrogen-ion activity as previously determined; and third, the activity of the enzyme was compared in the absence of any added salt and in the presence of the selected concentration of phosphate or of acetate, each solution being adjusted to the most favorable hydrogen-ion activity.

Results

Relation of Hydrogen-Ion Activity and Concentration of Acetate to the Activity of Malt Amylase.—Direct comparisons were made of the enzymic activity at eight different hydrogen-ion activities between P_H 4.0 and 6.0 in the presence of each of the following total concentrations of acetate: 0.01 *M*, 0.03 *M*, 0.06 *M* and 0.1 *M*. The total acetate concentration was kept constant in each case, but the proportions of equimolar acetic acid and sodium acetate used depended on previous electrometric titrations and were changed in order to obtain the desired hydrogen-ion activities. These were verified electrometrically in all cases. The most favorable hydrogen-ion activities were also established in a similar manner for total acetate concentrations of 0.00005 and 0.0001 *M*.

The averages of several determinations at each concentration of acetate show that the hydrogen-ion activity for the optimal enzymic activity

Dale, THIS JOURNAL, **49**, 2596 (1927); (f) Sherman, Caldwell and Adams, *ibid.*, **49**, 2000 (1927); **50**, 2529, 2535, 2538 (1928).

[2] Sherman and Schlesinger, *ibid.*, **35**, 1617 (1913).

[3] Sherman and Walker, *ibid.*, **43**, 2461 (1921).

changes with the concentration of acetate, from P_H 4.5 to 4.8 at 0.00005 M to P_H 5.0 to 5.4 at 0.1 M.

The results of the direct comparisons of the enzymic activity in the presence of the different concentrations of acetate at these optimal hydrogen-ion activities showed that the activity of the enzyme was slightly increased by the lower concentrations of acetate, while the activities at 0.01 to 0.10 M showed no significant further increase.

Relation of Hydrogen-Ion Activity and Concentration of Phosphate to the Activity of Malt Amylase.—Similar experiments were made with phosphate mixtures. Again, in each direct comparison, the total phosphate concentration was kept constant, the proportions of phosphoric acid and of acid and alkaline sodium phosphates being changed in order to obtain the desired hydrogen-ion activities. These were verified electrometrically for each solution.

The results are similar to those obtained in the presence of acetate, that is, the optimal hydrogen-ion activity for the enzymic activity changes with the concentration of phosphate from P_H 4.5 for 0.00005 M to P_H 4.9 for 0.10 M phosphate.

The average results of several direct comparisons of the enzymic activity in the presence of the different concentrations of phosphate at these optimal hydrogen-ion activities show a slight increase in the activity of the enzyme with increasing concentrations of phosphate, but as the phosphate in concentrations higher than 0.01 M seemed to interfere with the accuracy of the determinations of reducing sugar formed, it was not feasible to establish the precise optimum.

Comparison of the Activity of Malt Amylase in the Presence of Acetate and Phosphate.—In order to obtain a quantitative comparison of the activity of the enzyme in the presence of the two salts studied, parallel determinations were carried out, using starch dispersions containing the same concentration, 0.01 M, of acetate and of phosphate but adjusted to the optimal hydrogen-ion activity for each case.

The enzyme showed the same activity in the presence of 0.01 M acetate or 0.01 M phosphate provided the systems had been adjusted to the optimal hydrogen-ion activity in each case. Inasmuch as acetate and phosphate in appropriate concentration are equally favorable to the action of malt amylase and the acetate is a much more efficient buffer at the hydrogen-ion activities to be used with this enzyme, the acetate is plainly preferable to the phosphate here.

Summary

The optimal hydrogen-ion activities for malt amylase in the presence of different concentrations of acetate and of phosphate have been quantitatively established and found to depend on the concentration of the salt.

Even in very low concentrations, both acetate and phosphate increase the activity of malt amylase slightly.

Acetate, well known to be much the more efficient buffer in the range of hydrogen-ion activities suitable for work with this enzyme, is here found to be as effective in activating the enzyme as is phosphate, and to be experimentally applicable over a wider range of concentration.

Acetate is, therefore, preferable to phosphate as a buffer salt for use with malt amylase.

In an acetate concentration of 0.01 *M* the optimal activity was found at P_H 4.5 to 4.8, and in a concentration of 0.1 *M*, at P_H 5.0 to 5.4.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF BRISTOL]

THE CONSTITUTION OF CATECHIN. X

BY M. NIERENSTEIN

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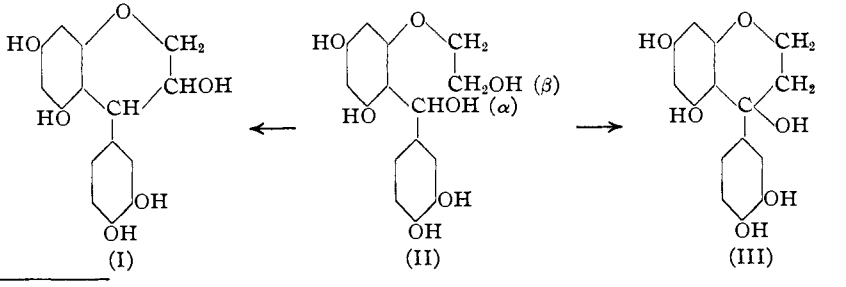
Only the heart-wood of the cutch-producing acacias,[1] *Acacia Catechu*, Willd., *A. Catechuoides*, Benth. and *A. Sundra*, D. C. is used in the manufacture of cutch, since it is generally believed that the other parts of the plant contain no catechin.[2] An examination of the sap-wood, bark and young twigs of these three acacias, carried out in this Laboratory, supports this generally accepted view. Furthermore, it was found that the catechin content of the heart-wood increases as the medulla is approached, which would seem to indicate that catechin is a final product of metabolism in these acacias,[3] and must therefore be derived from some other product formed in the living plant. This hypothesis is confirmed by the presence of *l*-leucomaclurin-glycol ether (II) in the young twigs of *Acacia Catechu*, Willd. The production of acacatechin (I) and iso-acacatechin (III) from *l*-leucomaclurin-glycol ether (II) would then follow as a matter of course.



(I) (II) (III)

[1] Prain, *J. Asiatic Soc. Bengal*, **66**, 508 (1897).

[2] Wiesner, "Die Rohstoffe des Pflanzenreiches," 3d ed., Leipzig und Berlin, **1914**, Vol. I, p. 605.

[3] These considerations obviously do not apply to gambier-catechin present in the leaves of *Umcaria Gambier* and allied species.