caused by the presence of a small amount of β-phenoxylactic acid which could not be removed by fractional crystallization.

The assistance given by Professor S. M. McElvain during the course of this work is gratefully acknowledged.

Summary

The monophenyl ethers of glyceric acid, β-phenoxylactic acid and α-phenoxyhydracrylic acid, and a number of their derivatives have been prepared and characterized.

The possibility that both of these isomers may be formed simultaneously in the reaction between β-chlorolactic acid and sodium phenoxide has been pointed out.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 626]

THE INFLUENCE OF CERTAIN NEUTRAL SALTS UPON THE ACTIVITY OF MALT AMYLASE

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Recent work[1] has shown that pancreatic amylase not only is dependent for its activity upon the presence of electrolytes but that it differs markedly in its response in activity to the presence of different salts. In view of this fact and of the recent findings of several investigators[2] that the influence of electrolytes upon enzymic activity is dependent on several interrelated factors, it seemed of special interest to continue our studies of typical starch-splitting enzymes of plant and animal origin by ascertaining whether quantitative differences might also be obtained in the action of malt amylase by the presence or absence of certain salts when the other factors were very fully controlled in the light of all present knowledge. One phase of this work, that dealing with the influence of acetate and phosphate upon the activity of malt amylase, has already been described.[2] The results of the quantitative study of the influence of other salts upon the activity of malt amylase are reported briefly here.

Experimental

The general procedure employed in this investigation consisted in allowing the enzyme to act at 40° (±0.01°) for thirty minutes upon 2% starch solutions which differed among themselves with respect to either the salt content or the hydrogen-ion activity or both as explained below. At the

[1] (a) Sherman, Caldwell and Dale, THIS JOURNAL, **49**, 2596 (1927); (b) Sherman, Caldwell and Adams, *ibid.*, **50**, 2529, 2535, 2538 (1928).

[2] Sherman, Caldwell and Boynton, *ibid.*, **52**, 1669 (1930), and references therein contained.

expiration of this half-hour period, the extent to which the starch had been converted to reducing sugars, mainly maltose,[3] was established according to the gravimetric method previously described[4] and this was taken as the measure of the relative activity of the enzyme.

The hydrogen-ion activities of the substrates were adjusted by mixtures of sodium acetate and acetic acid, present in a total concentration of 0.01 *M* acetate. Such mixtures had been shown[2] to be satisfactory for work with this enzyme. By changing the proportions of equimolar sodium acetate and acetic acid, it was possible to adjust the solutions to different desired hydrogen-ion activities without changing the total concentration of acetate. The total concentration of 0.01 *M* acetate was chosen for several reasons. It had been found to afford the desired buffer effect and at the same time was low enough not to interfere with the study of the influence of other salts. Previous work had also shown[2] that acetate as well as phosphate exerts a very slight favorable influence upon malt amylase. This influence, in the case of acetate, is practically constant through a wide zone of concentrations including 0.01 *M*, which is therefore a suitable concentration to use. The hydrogen-ion activities of all solutions were measured electrometrically. In all of the experiments here reported, the conditions of time, temperature and the concentrations of starch, enzyme and total acetate were constant.

As regards the factors which were varied, the plan of the work was as follows: (1) in order to determine whether or not sodium chloride has any influence upon the activity of malt amylase, direct comparisons were made of the enzymic activity (a) at graded hydrogen-ion activities in the presence and absence of the salt at fixed concentrations, and (b) in the presence of graded concentrations of the salt at fixed hydrogen-ion activities. (2) The other neutral salts studied, potassium chloride, sodium nitrate and sodium sulfate, were compared in a similar manner as to their effect upon the hydrolysis of starch with malt amylase. The enzymic activity in the presence of these salts at a single concentration and hydrogen-ion activity was compared with that exerted in the absence of salt both at the same hydrogen-ion activity and at the optimal hydrogen-ion activity for the enzyme and conditions here employed. (3) Finally, the effect of different concentrations of each salt upon the activity of malt amylase was established at each of three hydrogen-ion activities: (a) at the optimum, (b) on the alkaline side of the optimum, (c) on the acid side of the optimum.

A dry preparation of malt amylase, purified from dialyzed malt extract according to a modification of the method described by Sherman and Schlesinger,[5] was used.

[3] Sherman and Punnett, THIS JOURNAL, **38**, 1877 (1916).

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

[5] Sherman and Schlesinger, *ibid.*, **37**, 643 (1915).

All reagents were carefully purified. The salts were of the highest purity obtainable and were recrystallized three times in all cases with the exception of sodium chloride, which was prepared and purified by the method of Richards and Wells.[6] Soluble starch which constituted the substrate was washed repeatedly and air dried. Redistilled water was used for all solutions.

Results with Sodium Chloride.—Parallel measurements of the activity of the enzyme in the presence of 0.01 *M* sodium chloride and in its absence, each at closely graded hydrogen-ion activities from P_H 4.0 to P_H 6.2, showed that the enzymic activity was greater in the presence of the sodium chloride in the more acid solutions, P_H 4.3 to P_H 4.0, and in the more alkaline solutions, P_H 5.1 to P_H 6.2. In solutions of intermediate reaction from approximately P_H 4.3 to P_H 5.1, however, the presence of sodium chloride had no appreciable influence upon the activity of the enzyme. These solutions correspond approximately to the optimal hydrogen-ion activity previously established[2] for this enzyme in the presence of this concentration of acetate. Similar results were obtained in a series of experiments with 0.02 *M* sodium chloride. Thus sodium chloride in concentrations of 0.01 and 0.02 *M* did not increase the activity of the enzyme at the optimal, but rendered it more active in solutions of less favorable, hydrogen-ion activity.

This was also found to be true when, in another series of experiments, the influence of several concentrations of sodium chloride (0.01, 0.02, 0.05, 0.07 and 0.10 *M*) was carefully and repeatedly measured: at the optimal hydrogen-ion activity (P_H 4.5), in solutions more acid (P_H 4.0) and in solutions less acid (P_H 5.5).

At its optimal hydrogen-ion activity, the enzyme was as active without as with the sodium chloride; but the presence of this salt helped to overcome the retarding influence of an unfavorable hydrogen-ion activity upon the action of the enzyme.

This was demonstrated for all of the five concentrations of sodium chloride tested (0.01 to 0.10 *M*), the lower concentrations of the salt appearing more favorable on the acid, and the higher concentrations on the more alkaline side.

If the activity of the enzyme at its optimal hydrogen-ion activity (P_H 4.5) be taken as 100%, its activity at P_H 4.0 was 89% without added salt and 99% with 0.01 *M* sodium chloride; while at P_H 5.5 the activity of the enzyme was 77% without neutral salt and 97% with 0.10 *M* sodium chloride.

Thus malt amylase showed a practically optimal action through a wider range of hydrogen-ion activity when sodium chloride was present in suitable concentration than it did in the absence of the salt.

Results with Potassium Chloride.—The influence of potassium chloride

[6] Richards and Wells, THIS JOURNAL, 27, 459 (1905).

was studied in the same way as that of sodium chloride and with nearly the same results. The differences between the data obtained with these two salts were no greater than might reasonably be allowed for experimental error, but the average activity of the enzyme was in most cases slightly higher in the presence of the potassium, than of the sodium, salt.

Results with Sodium Sulfate.—At the optimal hydrogen-ion activity, P_H 4.5, the average enzymic activity was slightly higher in the presence of 0.01 *M* to 0.10 *M* sodium sulfate than in its absence, but here again, as in the case of potassium chloride, the differences were so small as to be of doubtful significance. At hydrogen-ion activities on either side of the optimum, (*viz.*, P_H 4.0 and P_H 5.5, respectively) the activity of the enzyme was increased by the presence of the sulfate (0.01 to 0.10 *M*), but this favorable influence was less than that exerted by the same concentrations of sodium or potassium chloride under the same conditions and decreased with increasing concentrations of the salt in solutions on the acid side of the optimum.

Results with Sodium Nitrate.—Sodium nitrate was found to have a less favorable influence upon the activity of malt amylase than the same concentrations of sodium or potassium chloride when tested under the same conditions.

Summary and Conclusions

The influence of sodium chloride, potassium chloride, sodium sulfate and sodium nitrate upon the saccharogenic activity of malt amylase has been investigated and special consideration has been given to the influence of changes in hydrogen-ion activity and salt concentration.

Under the experimental conditions here described, neutral salts are not essential to the activity of malt amylase nor do they increase the saccharifying action of the enzyme above that shown in their absence at the optimal hydrogen-ion activity.

The activity of malt amylase was increased at unfavorable hydrogen-ion activities by the presence of these salts, but not to the same extent by different salts. Those studied here may be arranged according to the decreasing magnitude of their effect upon the activity of the enzyme at unfavorable hydrogen-ion activities as follows: sodium and potassium chlorides, sodium sulfate, sodium nitrate.

In solutions of unfavorable hydrogen-ion activity, the addition of sodium chloride in the concentrations most favorable to the action of the amylase (0.01 *M* at P_H 4.0 or 0.10 *M* at P_H 5.5) restored the enzyme to an activity practically equal to that obtaining in the absence of salt at the optimal hydrogen-ion activity, P_H 4.5. Thus with the addition of the proper amount of sodium chloride, malt amylase exerts its highest activity in solutions differing more widely in hydrogen-ion activity than in its absence.

The findings here reported explain the contradictory evidence presented by various workers from time to time regarding the influence of salts upon the activity of this enzyme and emphasize anew the importance of controlling the hydrogen-ion activities of the solutions in which the enzyme action occurs.

The influence of salts upon the activity of malt amylase appears to be specific.

These findings as a whole establish quite definitely a difference, in dependence of optimal activity upon neutral salts, between pancreatic and malt amylases, which are being studied in detail as representative and analogous enzymes of animal and plant origin, respectively.

NEW YORK CITY

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES, ELI LILLY AND COMPANY]

DIALKYL BARBITURIC ACIDS

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Nearly a hundred different 5,5-disubstituted barbituric acid derivatives have been prepared in the quarter of a century since Fischer and Dilthey[1] and Fischer and von Mering[2] found that certain of these derivatives could be used therapeutically as sedatives and hypnotics. These investigators were aware that certain of the compounds were more active as hypnotics than others and that the effect could be markedly changed by varying the chemical nature of the substituent radical.

Subsequent investigations[3] have shown that those barbituric acids in which the sum of the C atoms in the two substituent groups is 6, 7 or 8 are the most effective. When evaluated on rats, cats, rabbits or mice, by the intravenous or subcutaneous injection of their sodium salts, this group shows, in most instances, a wider margin of safety between the anesthetic or down dose and the toxic dose than does, for example, diethylbarbituric acid.

The investigation was started in 1927 for the purpose of studying the various isomeric amyl-ethyl and amyl-allyl derivatives of the barbituric acids because of the commercial availability of certain of the amyl alcohols, and to extend the study of the secondary alkyl-ethylbarbituric acid series.[4]

[1] Fischer and Dilthey, *Ann.*, **335**, 334 (1904).

[2] Fischer and von Mering, *Therap. d. Gegenwart.*, **44**, 97 (1903); **45**, 145 (1904).

[3] (a) Carnot and Tiffeneau, *Compt. rend.*, **175**, 242 (1922); (b) Shonle and Moment, THIS JOURNAL, **45**, 243 (1923); (c) Volwiler, *ibid.*, **47**, 2236 (1925); (d) Nielson, Higgens and Spruth, *J. Pharmacol.*, **26**, 371 (1926); (e) Swanson and Page, *ibid.*, **31**, 1 (1927); (f) Dox and Hjort, *ibid.*, **31**, 455 (1927).

[4] (a) During the course of this investigation Dox and Jones, THIS JOURNAL, **50**, 2033 (1928), have reported on 5-*n*-amyl-5-ethylbarbituric acid. (b) Volwiler and Tabern described a number of amyl-ethyl and amyl-allyl derivatives at the Minneapolis Meet-