Although sodium chloride was used in relatively stronger concentration, its effect was less than that of any of the other chemicals tried. There is a progressive inhibition proportionate to the concentration. The most marked difference between the effect of sodium chloride and the other chemicals tried is that it shows as large an effect, if not larger, on the low-grade as on the straight flour.

The different behavior of these two flours toward these various chemicals indicates a very profitable line of investigation. The chemical study of flour has been confined too much to the constituents which occur in relatively large quantities. If measured quantitatively the amount of amylolytic enzymes present in the flour would be very small as compared with other constituents, yet their power is so great that digestion with water at a suitable temperature results in transforming more than two-fifths of the weight of the flour into soluble substances calculated as maltose. Yet the sensitiveness of these enzymes is such that a very small amount of acid and still smaller amount of alkali inhibits action.

Summary.

As the line of investigation presented in this paper is comparatively new with respect to flour, the methods used have been fully described. We have shown that the optimum temperature for the production of the maximum amount of reducing sugars is very near 65°; that the best proportion of water and flour lies between 1 : 4 and 1 : 10, and that there is little difference between these two limits. It has also been shown that the largest transformation takes place during the first hour; approximately 88% of the total change occurs during the first hour. The inhibiting effect of various chemicals has been shown. The inhibiting action is greater toward straight flour than toward low-grade.

ENZYMES. ASYMMETRIC SYNTHESES THROUGH THE ACTION OF OXYNITRILASES. PART I.

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In a previous communication to This Journal,[1] it was pointed out that certain samples of emulsin when acting on amygdalin leave a residue of *l*-mandelonitrile. As the active nitrile in amygdalin is the dextro form, the nitrile found must be a synthetical product. The same sample of emulsin, however, when allowed to act on benzaldehyde and hydrocyanic acid, gave *d*-mandelonitrile, a result which had been previously noted by Rosenthaler. Rosenthaler,[2] however, found that his emulsin left *d*-mandelonitrile as a residue when acting on amygdalin. A plausible explanation for these divergent results is that there are varying quantities of two

[1] Krieble, This Journal, **34**, 716 (1912).

[2] Rosenthaler, *Arch. Pharm.*, **246**, 365 (1908).

oxynitrilases in emulsin, one correlated to the *d*-nitrile and the other to the *l*-nitrile.

If there are two oxynitrilases, one would expect to find them separately in the plants which contain either prunasin or amygdalin, and sambunigrin, because in the one case there is a glucoside containing a *d*-nitrile and in the other an *l*-nitrile. In our search for these two enzymes separately, our efforts have been only partly successful. We were able to find an enzyme activating the formation of the *d*-nitrile from benzaldehyde and hydrocyanic acid in the leaves and bark of the black wild cherry tree, Prunus Serotina, and also in the leaves of the peach tree. We were very much surprised not to find the enzyme activating the formation of the *l*-mandelonitrile in the leaves of the common elder, as Bourquelot and Danjou[1] have shown that they contain sambunigrin. The leaf extract from the elder acting on benzaldehyde and hydrocyanic acid did not show any optical activity at all. Whether there is any nitrile formed has not been definitly determined. There is no doubt that the extract hastens the decomposition of the nitrile, as ½ cc. of nitrile in 20 cc. of the cherry-leaf extract gives a very strong odor of hydrocyanic acid if kept at 40° for a short time, while in a solution without the enzyme the hydrocyanic acid can scarcely be detected. It is possible that the enzymes hastening the formation of the *d*- and *l*-nitrile are present in equal quantities. This point still remains to be determined.

Another point of interest was the fact that ordinary emulsin hastens the formation of both *d*- and *l*-mandelonitrile—the dextro slightly more than the levo. This is not surprising, as emulsin hydrolyzes both the *l*- and *d*-amygdalin, which contain the *d*- and the *l*-mandelonitrile, respectively. The enzmyes from the peach or wild cherry leaf seems to hasten the formation of the *d*-nitrile only. Of course the nitrile formed under the influence of the enzyme of the leaf is not the pure dextro compound, but the small amount of the racemic form may easily be due to the spontaneous combination of benzaldehyde and hydrocyanic acid. The amount of the racemic nitrile formed in the presence of emulsin can hardly be explained in this way and, as we have already said, it seems plausible to suppose that there are two enzymes at work. We will have more to say on this subject in our paper on emulsins, which is not yet completed for publication.

Experimental.

The leaves of the black wild cherry were collected in Pennsylvania in August, 1912, at the time the cherries were ripe. They were dried in the sun and kept in a dry place until examined in May. The enzyme was extracted by soaking the leaves in water for 36 hours, adding a few drops of toluene to keep the solution steril. Usually 5 grams were added to 50

[1] Bourquelot and Danjou, *Compt. rend.*, 141, 59, 598 (1905).

cc. of water, but in one case 15 grams were used, but results show that the nitrile obtained was not any more active. The synthetical experiments were carried out in the following way: The leaves were filtered off and a certain number of cc. of the filtrate or extract were treated with an equal quantity of a 4% solution of hydrocyanic acid and 1 cc. of benzaldehyde. The mixture was kept at the room temperature for 2½ hours with an occasional shaking. It was then thoroughly extracted with ether, the ether boiled off in a water bath and the nitrile hydrolyzed with strong hydrochloric acid. The resulting mandelic acid was made up to a definit volume and examined in a polariscope.

TABLE I.

No. of grams of leaves or bark.	No. of cc. of extract used.		Vol. of mandelic acid solution.	Activity at room temp. in 2 dm. tube.
(1) 15 (W. cherry).....	25		25	— 8.00°
(2) 5 (W. cherry).....	25	(boiled 10 min.)	20	— 0.00°
(3) 5 (W. cherry).....		(Extract saturated with $MgSO_4$. Used 22 cc.)	20	— 0.5°
(4) 5 (W. cherry, collected in June, 1913).....	20		20	— 3.50°
(5) 5 (Peach leaves, collected in May, 1913).	25		20	—14.75°
(6) 5 (W. cherry bark)....	20		20	— 4.40°

Experiment number 2 shows that the asymmetric synthesis is due to an enzyme and not to an optically active compound present in the leaf. Experiment number 3 indicates that the enzyme is not completely salted out by magnesium sulfate. The solution was supersaturated by warming it with excess of magnesium sulfate at 40°. When cold it was filtered twice through a folded filter, but the results show that there was a small amount of enzyme left. From the fourth experiment one would gather that the amount of enzyme varies materially at different times of the year. We hope to investigate this point more thoroughly. The enzyme from the peach leaves seems surprisingly active. From the results in Table III one sees that more than 80% of the benzaldehyde was converted into mandelonitrile and 71% of this amount was dextro active.

The following experiments were carried out to see when the maximum quantity of active mandelonitrile was present. Five grams of the wild cherry leaves were added to 100 cc. of water. They were allowed to stand 36 hours, then the leaves were filtered out and the extract used. 20 cc. of the extract were added to an equal amount of 4% hydrocyanic acid and 1 cc. of benzaldehyde. The mixtures were allowed to stand for the number of hours indicated with an occasional shaking. The nitrile was

extracted, hydrolyzed, and the resulting mandelic acid was made up to 20 cc. It was examined in a 2 dm. tube at the room temperature.

TABLE II.

Time in hours.	Rotation in degrees.
2	—11.0
5	—11.0
10	—10.0
24	— 7.0

From these results it is evident that the reaction is a fairly rapid one.

The enzyme in the wild cherry leaf is free from what Armstrong calls amygdalinase.[1] This was proved in the following way: Five grams of leaves were treated with 50 cc. of water for 36 hours. To 25 cc. of this extract 35 cc. of 99% alcohol were added. The slightly turbid solution was filtered. After the filter paper was dry, it was treated with 20 cc. of water and the solution shaken frequently during one-half hour. It was filtered and 1 gram of amygdalin added to the filtrate. After adding a few drops of toluene the solution was kept at 40° for 48 hours. It was then made up to 25 cc. and examined in the polariscope in a 2 dm. tube; $\alpha = 3.37°$, or $[\alpha]_D = -42.1°$ at the room temperature. The solution was also tested with Fehling's solution but it did not show the slightest reduction. In another experiment, 1 gram of salicin was used instead of amygdalin and at the end of 48 hours it showed the following results in a 2 dm. tube: $\alpha = -4.95°$, or $[\alpha]_D = -61.9°$, the correct rotation for pure salicin. This solution also did not reduce Fehling's solution. In a second experiment with salicin, the solution was allowed to stand in the thermostat for 72 hours, but the specific rotation was about the same, namely, —62.25°. It did not reduce Fehling's solution. The oxynitrilase is therefore free of the β glucosidase necessary to hydrolyze salicin. Whether it is free of prunase[1] or whether the formation of the nitrile is due to prunase could not be determined, as we did not have the corresponding glucoside prunasin. Until it is shown that the enzyme which hastens the formation of the nitrile is the same as the enzyme which hydrolyzes prunasin we hope to call the enzyme which hastens the former reaction oxynitrilase. The enzyme which hastens the formation of the *d*-nitrile we will call dextro oxynitrilase and the one which hastens the formation of the *l*-nitrile levo oxynitrilase.

The last series of experiments were carried out to determin the relative proportions of the active and the racemic mandelonitriles from the same concentrations of benzaldehyde and hydrocyanic acid under the influence of different enzymes. We tried Kahlbaum's emulsin, the enzyme from the peach leaves, and the enzyme precipitated from the extract of the wild cherry leaves. The peach-leaf extract was prepared by soaking 5 grams of leaves in 50 cc. of water for 36 hours. We used 25 cc. The wild cherry leaves were treated the same way, but instead of using the extract directly

[1] H. E. and E. F. Armstrong, *Proc. Roy. Soc.*, (*B*) **85**, 359 (1912).

the enzyme was precipitated and redissolved. 25 cc. of the extract were added to 35 cc. of alcohol, the solution was filtered and the precipitate was redissolved in 20 cc. of water, which was afterwards made up to 25 cc. In the case of the emulsin 25 cc. of water were added to 0.5 gram of the emulsin. To each of the 25 cc. of enzyme solution an equal quantity of 4% hydrocyanic acid solution was added and 1 cc. of benzaldehyde. The resulting solutions were occasionally shaken and kept at the room temperature for 2½ hours. The nitriles were extracted immediately. In the peach and wild cherry experiment this could be done directly, but in the emulsin experiment the emulsin was first precipitated with a drop of acetic acid and filtered off. Both the precipitate and filtrate were extracted with ether. The nitriles were hydrolyzed and the mandelic acid extracted and dried to constant weight. It was dissolved in water and made up to 50 cc., after which it was examined in a polariscope and an aliquot portion (10 cc.) titrated with a 0.22 *N* barium hydroxide. The following were the results obtained:

TABLE III.

	Rotation in a 2 dm. tube.	Cc. of 0.22 *N* $Ba(OH)_2$ used.
Wild cherry	—3.85	5.65
Peach	—5.5	7.6
Emulsin	—0.8	6.5

Grams of mandelic acid.	Grams of racemic acid.	Grams of active mandelic acid.	Per cent. of active acid.
0.9447	0.3115	0.6332	67.0
1.2707	0.2661	0.9046	71.2
1.0868	0.9553	0.1315	12.0

These results are interesting in two respects. In the first place one would hardly expect so much of the benzaldehyde and hydrocyanic acid to combine; and as we have already pointed out in the introduction, it is a surprise to find such a large variation in the proportion of the active and racemic mandelic acid, a result which can only be explained by assuming that there are two oxynitrilases present in emulsin. In one of the experiments with the enzyme from the wild cherry leaf, fully 85% of the resulting mandelic acid was active, which shows that there can be very little, if any, *l*-oxynitrilase present in this leaf.

We are continuing this investigation.

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NOTE.

Water of Crystallization of the Calcium Salt of Lauronolic Acid.—In a previous article[1] we stated that the calcium salt of Lauronolic (Laurolenic) acid crystallized with three molecules of water of crystallization, thus

[1] THIS JOURNAL, 34, 178.