Like precipitates were readily obtained from bromanil and tetrachloro-*o*-quinone.

Estimation of Iodine Set Free.—For the purpose of determining the amount of free iodine formed in the reaction of iodides with bromanil, 10 grams of the powdered crystals, melting at 292–293°, were boiled two hours with 10 grams of potassium iodide in alcohol. The solution was then filtered, the precipitate washed with alcohol till no more iodine color was visible in the alcohol used for washing, and the alcohol filtrate and washings made up to 250 cc. This solution was then titrated for iodine with a thiosulfate solution, 1 cc. of which was equivalent to 0.01 gram of iodine. Much difficulty was found in estimating the end-point, as the reduction products of the quinones present darkened the liquid considerably, and obscured the blue color, so it seemed best to use but 10–15 cc. and titrate rapidly. In nine titrations, the following values were found for 1 cc. of the thiosulfate solution in terms of the iodine solution: 2.20, 2.23, 2.19, 1.19, 1.88, 2.17, 2.29, 2.34, 2.36. The average of these is 2.16, which corresponds to 1.1 grams of free iodine in solution as reaction product. The insoluble quinones from the reaction weighed 9.6 grams and melted at 253–258°. Calculated as dibromodiiodoquinone, it is equivalent to 7.9 grams bromanil, but as a matter of fact it is equivalent to less bromanil, as it was not all dibromodiiodoquinone. This corresponds roughly, then, to the disappearance as various reduction products of 2.1 grams bromanil. The 1.1 grams of iodine formed would be set free by 2.0 grams of bromanil, showing that the iodine formed, as was to be expected, really does come from reduction of the bromanil which does not appear as iodo derivative.

Potassium Iodide in Cold Alcohol on Bromanil.—Ten grams of pure bromanil, 10 grams potassium iodide and 100 cc. of alcohol were placed in an Erlenmeyer flask, closed with a rubber stopper, and allowed to stand with no heating, and with only occasional shaking, for four months. No green salt was visible at any time. The final substance in the flask was red brown; isolated as usual, it melted at 242–245°, and on crystallization from ethyl acetate, showed that the effect had been in no wise different from that produced when heated.

THE AMYGDALINS AND THEIR INTER-REACTIONS WITH EMULSIN.

By VERNON K. KRIEBLE.

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In a previous communication[1] from this laboratory, it was proved that the rotation of a racemized amygdalin solution is independent of the nature and of the concentration of the alkali and that the equilibrium point is independent of the temperature and of the concentration of the

[1] Walker and Krieble, *J. Chem. Soc.*, **95**, 1437.

amygdalin. It was pointed out that racemic amygdalin could be partially resolved into its optical isomers. *l*-Amygdalin or ordinary amygdalin was obtained pure from the racemic by recrystallization from aqueous alcohol and afterwards from water. A part of the more soluble portion containing the dextro form was obtained with alcohol of crystallization at low temperature in absolute alcohol. The actual rotation of these anhydrous crystals did not agree with the calculated rotation of a solution made up of *d*- and *l*-amygdalin in proportion to the *d*- and *l*-mandelic acid obtained when the crystals were hydrolyzed with acid. It had also been noted that the amounts of glucose liberated by emulsin from *l*- and *r*-amygdalin was 4 : 3, which suggested that emulsin did not split off the second molecule of glucose from the dextro compound and should, therefore, have formed *d*-mandelonitrile glucoside or sambunigrin, though this substance could not be extracted. It was not sambunigrin, however, as it was not broken up by emulsin into benzaldehyde, glucose and hydrocyanic acid. It was also noted that a racemic solution dried on a water-bath for some hours had an increase in the specific rotation and that such solutions could not be hydrolyzed by emulsin to the same extent as the unheated racemic modification. These last two facts indicated that a more complicated transformation than the simple racemization was taking place and it was suggested that probably the change was from an α- to a β-glucoside.

In the present communication it is shown that the minutest trace of hydroxyl ion is capable of racemizing amygdalin and that the cyanide radicle is necessary to effect this change. The composition of the racemic amygdalin is definitely proved and shown to be made up of 56.25% of dextro and 43.75% of the levo form. The cause of the increase in rotation when racemic solutions are dried on the water bath is found to be due to a very slight amount of hydroxyl ion coming from the hydrolysis of the barium salt from an unknown acid always associated with amygdalin in minute quantities. The nature of the change giving rise to the increased rotation is conclusively proved to be a transformation of the CN radicle. When the cause was known it could easily be eliminated, after which it was possible to isolate dextro amygdalin in a pure form. Its properties are very similar to that of the levo compound, as was expected.

A very interesting fact was brought to light in connection with the hydrolysis of the amygdalin with emulsin. It had been pointed out by other investigators that emulsin not only hydrolyzes but also synthesizes active benzaldehydecyanhydrin from hydrocyanic acid and benzaldehyde. The curious part is, however, that Feist, Rosenthaler and Auld always found *d*-benzaldehydecyanhydrin in their hydrolytic solutions while we invariably obtained the levo modification in the case of certain samples of emulsin. With benzaldehyde and hydrocyanic acid

the dextro antipode is produced, which agrees with the results of the above investigators.

Experimental.

The experimental results will be taken up under the following headings:

1. The effect of the strength of alkalies upon the equilibrium between the levo and dextro amygdalin. 2. Composition of the racemic amygdalin. 3. Cyanide radicle necessary for racemization. 4. Cause of the increase in rotation of the racemic amygdalin when it is dried on a water-bath. 5. Nature of change described above as due to hydroxyl ion. 6. Resolution of racemic amygdalin: (*a*) Isolation of the levo form; (*b*) isolation of the dextro form. 7. Hydrolysis of the *d*-amygdalin by strong sulfuric acid. 8. Hydrolysis of the *d*-amygdalin by strong hydrochloric acid. 9. Action of emulsin on the amygdalin. 10. Synthesis by emulsin of *d*-benzaldehydecyanhydrin from hydrocyanic acid and benzaldehyde.

1. The Effect of the Strength of Alkalies upon the Equilibrium between Levo and the Dextro Amygdalin.

Walker[1] showed that when ordinary amygdalin is treated with dilute alkali it is rapidly changed into a substance which is much more soluble and which yields a slight preponderance of *d*-mandelic acid when hydrolyzed. During the course of the investigation it was found that this change is brought about by extremely minute quantities of hydroxyl ions, as the following experiments will show.

Amygdalin is never entirely neutral, even though it has been recrystallized five or six times. In this particular lot, 10 grams required more than 1 drop 0.25 *N* alkali, but less than 2 drops to give a pink color with phenolphthalein. When 1 drop is diluted with 25 cc. of water, it can be added to 10 grams of amygdalin, also dissolved in water to which a drop of phenolphthalein has been added, without bringing out the pink color. When this solution is boiled down a thick syrup is left instead of crystals; if it is redissolved in water and made up to 100 cc. it has the following rotation: $c = 6.944$, $t = 24°$, $l = 2$ dcm., $\alpha = -9.57°$, hence $[\alpha]_D^{24}$ $-53.5°$. Now 1 cc. of 0.25 *N* alkali solution contains 0.00425 gram of OH ion and, assuming that there are 20 drops in 1 cc., we would have used 0.000225 gram to racemize 10 grams, or 2 in 100,000, provided the barium salt of the acid present is completely hydrolyzed. In another experiment, 10 cc. of a 10% solution was made up to 25 cc. with a solution of barium carbonate in carbonic acid. After standing for 2 hours it still had a specific rotation of $-38.4°$ at 23°. The solution was then immersed in boiling water for upwards of twenty minutes. Its specific rotation had changed to $-48.9°$, which showed that it was partly racemized. When this solution was boiled to dryness and then hydrolyzed with hydrochloric acid, it gave mandelic acid with a specific rotation of $+5.5°$ (racemic gives $+18°$). Here, too, the barium salt

[1] *J. Chem. Soc.*, **83**, 478 (1903).

of the acid present is formed and no doubt this is hydrolyzed and gives rise to hydroxyl ions.

It is hard, however, to explain the two following experiments in this way. One cc. 0.25 *N* barium hydroxide was just neutralized with sulfuric acid, then boiled to dryness. Ten cc. of a 10% solution were added to the barium sulfate and boiled to dryness again. The syrup left was made up to 25 cc. and examined in the polariscope, $\alpha = -3.18°$, $c = 3.5776$, $t = 23°$, $l = 2$ dcm., hence $[\alpha]_D^{23}$ —44.4°. In another experiment the same quantity of barium hydroxide was neutralized with sulfuric acid until the pink color of phenolphthalein disappeared, then 10 cc. of *l*-amygdalin added and the solution boiled to dryness. When it was made up to 25 cc. and examined in the polariscope, $\alpha = -3.90°$, $t = 24°$, $l = 2$ dcm., hence $[\alpha]_D^{24}$ —54.5°. So barium sulfate freshly precipitated seems to racemize faster than when it has once become perfectly dry. This difference might be due to occluded barium hydroxide in the barium sulfate crystals, which later diffused and affected the change in rotation or to the fact that the first solution is saturated with barium sulfate and the second is not.

2. Composition of the Racemic Amygdalin.

Walker, who first discovered the racemic amygdalin,[1] pointed out that when it was completely hydrolyzed with hydrochloric acid, the ethereal extract always showed an excess of *d*-mandelic acid, indicating the production of excess of *d*-amygdalin. This has been confirmed by Dakin[2] and by a number of our own observations. Since the equilibrium could not be shifted and since the amygdalinic acid gives inactive acid, it was held that the racemic was an equal mixture of *l*- and *d*-amygdalin.[3] The reason given for the production of excess of *d*-mandelic acid was the inequality in the rates of hydrolysis of the two varieties by acid, accompanied by a slow racemization. This is not borne out by experiment. Five grams of *l*-amygdalin (3 H_2O) were hydrolyzed with hydrochloric (D 1.118) at 60° to 70°, for 5 hours, extracted with ether 4 times and the residue from the ether made up to 50 cc. and examined in the polariscope, $\alpha = -8.44°$, $l = 2$ dcm., $t = 25°$, and 20 cc. needed 29.2 cc. of 0.125 *N* alkali, or $c = 2.775$; hence $[\alpha]_D^{25}$ —152°. So there is no racemization during the hydrolysis of the levo form, and the same is true of the dextro form from results which appear later on. As proof that the continued boiling did not racemize the mandelic acid, 1 gram was dissolved in hydrochloric acid (D 1.118) and made up to 50 cc. This rotated polarized light —3.30° in a 1 dcm. tube. The solution was heated for 10 hours, then cooled and examined

[1] *J. Chem. Soc.*, 83, 472 (1903).

[2] *Ibid.*, 85, 1512 (1904).

[3] Walker and Krieble, *Ibid.*, 95, 1437 (1909).

again; it now had an angle of —3.28° at the same temperature. So the excess of *d*-mandelic acid must represent a corresponding excess of *d*-amygdalin in the racemic. Several grams were racemized and then hydrolyzed with hydrochloric acid (D. 1.118) for 6 hours at 60°–70° and the acid solution extracted with ether. The ether residue was made up to 50 cc. and examined in the polariscope. $\alpha = +0.85°$, $t = 25°$, $l = 2$ dcm., 20 cc. needed 25.45 cc. 0.125 *N* alkali to neutralize it, but 1.65 cc. was hydrochloric acid, so $c = 2.2610$; hence $[\alpha]_D^{25} + 18.8°$. At this temperature mandelic acid has a specific rotation of 150°, so the % excess of dextro in the above solution is $18.8/150 \times 100$, or 12.75. The racemic is therefore composed of 56.25% *d*-amygdalin and 43.75 *l*-amygdalin. Near the close of this investigation, the *d*-amygdalin was isolated and found to have a specific rotation of —61.2° at 19° for a 5% solution. A *l*-amygdalin solution under the same conditions has a rotation of —39.2°. If we calculate what the specific rotation of a solution ought to be if it was made up of 56.25 parts of dextro and 43.75 of levo from the above rotations we find it to be —51.6°, while the observed rotation is 52.2° for the same temperature. This is conclusive proof that the racemic is not made up of equal parts of levo and dextro amygdalin.

3. The Cyanide Radicle Necessary for Racemization.

No one has as yet suggested a theory to explain the racemization but it can be demonstrated by the following experiment that it is not possible to racemize the asymmetric carbon atom in the mandelic acid radicle—the one racemized when amygdalin is treated with alkali—unless the cyanide group is attached to it; by treating a metallic salt of active amygdalinic acid with alkali. To do this, it was necessary to prepare active amygdalinic acid. Fifty grams of *l*-amygdalin (3 H_2O) were dissolved in 450 cc. of 0.25 *N* barium hydroxide and the solution boiled to expel all the ammonia. The barium was precipitated with sulfuric acid and the solution allowed to settle. The clear liquid was siphoned off into a 1 liter measuring flask, the barium sulfate carefully washed and the washings also run into the measuring flask. It was filled up to the mark and a rotation taken: $\alpha = -5.44°$, $t = 21.5°$, $l = 2$ dcm.; hence $[\alpha]_D^{21.5}$ —60.8° for anhydrous amygdalinic acid. To 500 cc., 8.2 grams of strychnine were added, which is the theoretical quantity necessary to produce the acid salt. This dissolved readily on boiling. The strychnine salt did not crystallize on cooling, however, so the solution was concentrated in a number of stages and then allowed to stand for some time, but there was no sign of crystallization. So another lot of 8.2 grams of strychnine was added and dissolved by boiling. When this solution was concentrated to about 100 cc. and allowed to stand for one week, very thin, long, transparent, needle-shaped crystals came down. When filtered from the mother liquor and exposed to the air they became opaque and finally

crumbled to powder, showing that they contained water of crystallization. When dried to constant weight, they weighed 18 grams. A 1% solution had a specific rotation of $[\alpha]_D$ —26.5°. One and two-tenths grams dissolved in water required 11 cc. of 0.125 *N* alkali to neutralize the acid. Amygdalinic acid ($C_{19}H_{27}O_{11}COOH$) has a molecular weight of 476; therefore, 11 cc. of 0.125 *N* solution would represent 0.6545 gram. Strychnine ($C_{21}H_{22}N_2O_2$) has a molecular weight of 334, so for every 476 parts of amygdalinic acid there would be 334 of strychnine or 0.6545 gram of amygdalinic acid would be combined with 0.4592 gram of strychnine. It is evident, therefore, that the 1.2 grams of the strychnine salt contained 0.0863 gram of water, which corresponds to $3^1/_2$ molecules of water of crystallization. This was verified by drying a portion over phosphorus pentoxide in a tube exhausted to 50–70 mm. and heated to 90°. 0.83 gram of the salt lost 0.0612 gram H_2O, corresponding to $3^1/_2$ molecules.

To show that optically active amygdalinic acid cannot be racemized, 5 grams of crystallin strychnine amygdalinate were dissolved in water and 1 cc. more 0.25 *N* barium hydroxide added than was necessary to precipitate the strychnine. After one hour the alkali was neutralized and the solution made up to 50 cc. The specific rotation of the barium salt was $c = 4.605$, $\alpha = -3°$, $t = 21.5°$, $l = 2$ dcm., hence $[\alpha]_D^{21.5}$ —32.5°. The solution was filtered and concentrated to a thick syrup, then hydrolyzed with hydrochloric acid. The extracted mandelic acid was made up to 50 cc. and examined in the polariscope. It had an angle of +2.75° in a 2 dcm. tube at 22°; its concentration was 1.7765 grams; hence $[\alpha]_D^{22}$ +77.4°. The excess of dextro in the crystals is, therefore, 77.4/149 × 100, or 52%, which shows that the amygdalin radicle cannot be racemized if the cyanide group is absent.

As active mandelonitrile was obtained near the end of this investigation it was found that the asymmetric carbon atom present could be racemized if the nitrile was dissolved in ether and a few drops of dilute alkali added.

4. Cause of the Increase in Rotation of the Racemic Amygdalin.

As already pointed out in the introduction, we were considerably baffled by the behavior of the racemic amygdalin. We always had a large portion which could not be obtained crystallized. Emulsin did not seem to hydrolyze the racemic forms completely and the end-point of the hydrolysis seemed to be influenced by the length of time the racemic compound was dried on the water bath. It took a long time, however, to find out what caused this change. At first it was thought to be due to the presence of barium carbonate, which slightly dissolves in the racemic solution when saturated with carbon dioxide. So oxalic and sulfuric acids were used to neutralize the barium hydroxide but in almost every case the rotation increased, as the following table shows.

A 10% stock solution of *l*-amygdalin (3 H_2O) was made up. Ten cc. were used in each experiment, to which was added 1 cc. of 0.25 *N* barium hydroxide and then treated as follows:

(1) Passed in carbon dioxide until neutral to phenolphthalein, filtered and boiled for five hours on water-bath. Made up to 25 cc. and examined in the polariscope.

$\alpha = -4.60°$, $t = 24°$, $l = 2$ dcm., hence $[\alpha]_D^{24}$ —64.3°.

(2) Passed in carbon dioxide until saturated, then boiled ten minutes before filtering. Filtrate was boiled for 5 hours.

$\alpha = -4.70°$, $t = 24°$, $l = 2$ dcm., hence $[\alpha]_D^{24}$ —65.7°.

(3) Added oxalic acid until neutral to phenolphthalein. Evaporated and heated 5 hours.

$\alpha = -4.60°$, $t = 25°$, $l = 2$ dcm., hence $[\alpha]_D^{25}$ —64.3°.

(4) Added sulfuric acid until neutral to phenolphthalein, then evaporated and boiled 5 hours.

$\alpha = -4.56°$, $t = 23°$, $l = 2$ dcm., hence $[\alpha]_D^{23}$ —63.7°.

(5) (12.5 cc. used instead of 10 cc.) Neutralized with theoretical quantity of oxalic acid, evaporated and heated for $5^1/_2$ hours then made up to 25 cc.

$\alpha = -4.70°$, $t = 20°$, $l = 2$ dcm., hence $[\alpha]_D^{20}$ —52.5°.

These are only a few selected at random from the notebook. There were very few where the rotation did not increase. Finally, it was noticed that all the solutions which had an increased rotation gave a faint precipitate with sulfuric acid, while solutions heated like number 5 did not give a precipitate. This gave a clue to the situation, as it suggested that the amygdalin solutions were faintly acid and that when the barium hydroxide was added this acid was changed to the barium salt. When the excess of barium hydroxide was neutralized, this barium would not be precipitated, as there was just enough added to make the solution neutral to phenolphthalein. To show that this was actually the case, 1 drop of barium hydroxide was added to 10 grams of *l*-amygdalin (3 H_2O) in solution, but as I have already said, this did not change the phenolphthalein red. When the solution was evaporated to a syrup and then made up to 100 cc., it had a specific rotation of $c = 8.944$, $\alpha = -9.57°$, $l = 2$ dcm., $t = 24°$; hence $[\alpha]_D^{24}$ —53.5°. Ten cc. of this solution were evaporated to dryness and heated for 5 hours, then made up to 25 cc. It now had a rotation of $c = 3.5776$, $\alpha = -4.70°$, $l = 2$ dcm., $t = 24°$; hence $[\alpha]_D^{24}$ —65.7°. This was repeated with the same result.

To prove that this change must be due to the hydroxyl ions caused by the hydrolysis of the barium salt, 1 drop of *N* sulfuric acid was added to 10 cc. of the above 100 cc. and heated for 5 hours and then made up to 25 cc. again. It had a rotation of $c = 3.5776$, $\alpha = -3.75°$, $t = 24°$, $l = 2$ dcm.; hence $[\alpha]_D^{24}$ —52.4°. This was repeated with a 10-gram lot to which 5 cc. excess oxalic acid (0.33 *N*) were added when the barium hydroxide was neutralized, with the same result. A very small amount of hydroxyl ions, therefore, does not only racemize, but also appears

to change the amygdalin in some way, when present during the evaporation of the solution.

5. Nature of the Change Caused by Hydroxyl Ions in Racemic Solutions when Evaporated to Dryness and Baked on Water Bath.

The most natural thing to expect when the specific rotation went up was that there was more of the dextro form produced. Six grams were, therefore, dissolved in water and added to a solution of 100 cc. containing 2 cc. of barium hydroxide, which had been precipitated and redissolved by passing in carbon dioxide. The solution was taken down to a very thick syrup. This was afterwards made up to 50 cc. and a rotation taken: $\alpha = -12.44°$, $l = 2$ dcm., $t = 23°$, $c = 10.7328$; hence $[\alpha]_D^{23}$ $-58°$. The solution was evaporated to a thick syrup and hydrolyzed with hydrochloric acid (D 1.118) for 4 hours at 60°–70°, the mandelic acid extracted and made up to 50 cc. It had a dextro rotation of 0.20° in a 1 dcm. tube at 22°, 20 cc. used up 38.1 cc. 0.125 N alkali or $c = 3.6195$; hence $[\alpha]_D^{22}$ $+5.5°$. This shows that the equilibrium has not been shifted in favor of the dextro form.

Since barium could always be precipitated from solutions which had this high rotation and racemic solutions whose rotation did not go up did not contain barium, it was conceivable that the barium was in some way united with the glucose part of the molecule which caused this increase in rotation. One gram of l-amygdalin (3 H_2O) was, therefore, dissolved in water, $^1/_2$ cc. of barium hydroxide added, and then oxalic acid added until the color of the phenolphthalein had just disappeared. The solution was evaporated to dryness and baked on the water-bath for 5 hours. It was then made up to 50 cc. and had the following rotation: $\alpha = -4.55°$, $t = 25°$, $l = 2$ dcm., $c = 3.5776$; hence $[\alpha]_D^{25}$ $-63.9°$. Twenty-five cc. of this solution were treated with dilute sulfuric acid until it no longer gave a precipitate, and then evaporated to 25 cc. and another rotation taken. It now rotated polarized light $-4.40°$ at 23° in a 2 dcm. tube, or $[\alpha]_D^{23}$ $-63°$, which shows that the combination of barium with the glucose radicle is not the cause of the increase in rotation.

If the cause of this high rotation exists in the glucose part of the molecule, then the corresponding ammonium amygdalinate ought also to have an increased rotation. So 2.5 grams l-amygdalin (3 H_2O) were boiled with excess of barium hydroxide until all the ammonia was expelled, then ammonia and carbon dioxide were passed in to precipitate all the barium. The solution was filtered, the precipitate carefully washed and the clear filtrate evaporated to 50 cc. It had a sepcific rotation of $\alpha = -6.47°$, $l = 2$ dcm., $t = 19°$, $c = 4.472$; hence $[\alpha]_D^{19}$ $-72.3°$. This was repeated some time later when $[\alpha]_D^{24}$ was found to be $-71.8°$. When the ammonium amygdalinate was prepared from the racemic form which had the increased rotation, it was found, however, to be the same. Two

and five-tenths grams of *l*-amygdalin (3 H_2O) were dissolved in water, 2 cc. barium hydroxide added and after 10 minutes carbon dioxide passed in. The solution was evaporated to dryness and baked for 7 hours, then made up to 50 cc. The specific rotation was $c = 4.472$, $\alpha = -5.43°$, $l = 2$ dcm., $t = 24.5°$; hence $[\alpha]_D^{24.5}$ —60.7°. Twenty-five cc. of the solution were changed to ammonium amygdalinate by the method described above. When it was made up to 25 cc., it had a specific rotation of $[\alpha]_D^{23}$ —71.6°. This shows that the change of rotation in the racemic form when heated is not due to any change in the glucose part of the molecule or else the corresponding ammonium amygdalinate would not have the same rotation. As further proof, ammonium amygdalinate was heated for 7 hours in the presence of a small quantity of freshly precipitated barium carbonate, but when redissolved it was found to have the same rotation.

From these results one would suspect that the cyanide group was changed or hydrolyzed in some way. This could easily be proved as it was pointed out[1] that strong sulfuric acid when allowed to act on *l*-amygdalin for several hours at a high temperature, produced about 85% of the theoretical *d*-mandelonitrile, so if the cyanide has been hydrolyzed during the change that takes place when the rotation of the racemic form goes up, it ought not to give any nitrile when hydrolyzed with strong sulfuric acid. Twenty grams of the racemic amygdalin were, therefore, heated with a small quantity of barium carbonate for 6 hours when the specific rotation went up to —67.4°. The solution was again evaporated and the thick syrup dissolved in 60 cc. of water and 40 cc. of concentrated sulfuric acid. This solution was heated to 90° and kept at this temperature for $1^1/_2$ hours, after which it was cooled and extracted with 100 cc. of benzene. The benzene solution did not have any activity. It was evaporated to a small volume, then poured into a small beaker and when free of the solvent and moisture it weighed 0.287 gram, which is only an eighth of the nitrile obtained from *l*-amygdalin. The aqueous hydrolytic solution was extracted with ether and the mandelic acid obtained was dissolved and made up to 100 cc. with water. It had a slight dextro activity. Ten cc. required 26.6 cc. of 0.125 *N* alkali or the concentration of mandelic acid was 5.12 grams. This demonstrates that the nitrile was hydrolyzed before the sulfuric acid acted, because when *l*-amygdalin is treated for the same length of time with the same strength of acid it yields about 85% of nitrile while in this case 86% of mandelic acid was obtained.

It is difficult to say to what the nitrile radicle is transformed when the racemic amygdalin is heated, as it is impossible to get the new substance pure. One might expect it to be ammonium amygdalinate from its rotation. There are no ordinary tests, however, which can be applied

[1] Walker and Krieble, *J. Chem. Soc.*, **95**, 1369 (1909).

in this case, so the electrical resistance of this substance was compared with that of ammonium amygdalinate. The cell used had a constant of 0.1037 and a 5% *l*-amygdalin (3 H_2O) solution had a conductivity of 0.424×10^{-4}. Ammonium amygdalinate was prepared according to methods already described.

The following are some of the conductivities observed, measurements being made at 25°.

Per cent of NH_4 amygdalinate.	Specific rotation.	Specific conductivity.
5	—71.8°	6.74×10^{-3}
4	..	5.18×10^{-3}
$3\frac{1}{8}$	..	4.45×10^{-3}
2	..	3.45×10^{-3}
3% of NH_4 amygdalinate + 1% of *l*-amygdalin	—63°	3.99×10^{-3}

The following are the solutions which had been heated:

4	—65.4°	5.94×10^{-4}
4	—58.2°	3.61×10^{-4}
4	—67.5°	5.98×10^{-4}
4	—64.6°	5.08×10^{-4}

From these results it is quite evident that this new substance is not ammonium amygdalinate nor even a mixture of amygdalin and ammonium amygdalinate. It might be the amide or some other nitrogen derivative of amygdalinic acid, but it cannot be the acid itself as it is very nearly neutral in reaction, nor can it be the barium salt, as there is only the faintest trace of barium in it. At this point our attention was turned to the isolation of the *d*-amygdalin, as we had found out how to evaporate the racemic form without bringing about any internal change.

6. Resolution of Racemic Amygdalin.

Seventy-five grams were dissolved in several hundred cc. of luke-warm water and 6 cc. of barium hydroxide added. After one-half hour, 1.4 cc. of 0.33 *N* oxalic acid were added in excess of the quantity needed to make the solution neutral. This was evaporated to a moderately thick syrup and 250 cc. of 95% alcohol added, which caused 30 grams of crystals to separate. It was impossible to get any more crystals by concentrating the mother liquor, so the alcohol was distilled off *in vacuo* and the syrup drawn out to a froth. The flask was then broken and the residue dried in a vacuum desiccator, after which it was dissolved in 500 to 600 cc. of boiling absolute alcohol. When the solution cooled to the room temperature, a small quantity (5 grams) of a heavy syrup separated. This is due to the fact of not using enough alcohol as it was not obtained in subsequent experiments. So the clear solvent was decanted and cooled to —5° for an hour, which caused 7.6 grams of a fine crystallin precipitate to separate. This was rapidly filtered and washed with cooled ab-

solute alcohol. When the temperature went up a few degrees it liquefied completely giving off its alcohol of crystallization. The mother liquor was concentrated to $^1/_3$ of its original volume and allowed to stand at the room temperature for several days. During this time 11.8 grams of crystals separated, which, so far as could be ascertained, did not contain alcohol of crystallization. The solution was again concentrated, but no more crystals separated, so it was cooled with salt and ice, which caused 4 more grams of crystals with alcohol of crystallization to separate. It was concentrated a third time, which caused another crop of 2.6 grams to come out, at room temperature. When the mother liquor was concentrated a fourth time and cooled with a freezing mixture, 3.2 grams separated. The solvent was then completely evaporated which left a residue of 3.34 grams. It is possible, therefore, to resolve the racemic amygdalin practically completely into three different mixtures all of which, however, are crystallin. The final residue of 3.34 grams was rather sticky and when tested was found to be slightly acidic in nature, which showed that prolonged boiling hydrolyzes a very small fraction.

(a) Isolation of the Levo Amygdalin.

The first crop of crystals (30 grams) contains a large preponderance of *l*-amygdalin. A 5% solution in a 2 dcm. tube at 20° rotated polarized light —4.11°. When 1.366 grams were dried over phosphorus pentoxide *in vacuo* at reduced pressure it lost 0.0928 gram or 6.8% of water of crystallization; hence $[\alpha]_D^{20}$ —44°. Five grams were hydrolyzed in the usual way and the mandelic acid extracted, dissolved and made up to 50 cc. It had a specific rotation of α = —6.40°, l = 2 dcm., t = 24.5° and 20 cc. required 31.4 cc. of 0.125 N alkali, or c = 2.983; hence $[\alpha]_D^{24.5}$ —107.3°. At this temperature mandelic acid has a rotation of —150.5°, therefore, this fraction contains about 85.6% of the levo isomer and 14.4 of the dextro. If one calculates what the rotation of such a mixture should be at 20°, it comes to —42.5°, which is not very far from the value found when one takes into consideration the number of experimental facts that the theoretical depends on. About 20 grams of the above 30 were dissolved in a small quantity of hot water. When the solution cooled, the levo compound crystallized out in rosets. These were twice recrystallized and then air-dried. A 5% solution (3 H_2O) showed a rotation of —3.43° in a 2 dcm. tube at 25°, or $[\alpha]_D^{25}$ —38.3°, which agrees very well with the *l*-amygdalin we started with, namely $[\alpha]_D^{25}$ —38.6° for the same concentration. Five grams of it were hydrolyzed and the mandelic acid obtained without purification, had a specific rotation of α = —65.9°, t = 26.5°, 20 cc. required 23.7 cc. 0.125 N alkali, or c = 2.2344; hence $[\alpha]_D$ —152.6° at 17°, which compares favorably with —154°, the value found for carefully purified mandelic acid. Whether this crop of crystals which has a specific rotation of —44° is a definit compound,

made up of 3 parts of levo to 1 of dextro, saturated with the mother liquor which contains a big excess of dextro; or whether it is *l*-amygdalin saturated with the mother liquor is hard to determin. The crystals themselves come out of the alcoholic solution in the form of rosets and have all the characteristics of *l*-amygdalin. Then their rotation is not constant, for if they are washed with a small quantity of alcohol it comes down as low as —42.5°. If it is a definit compound it is very loosely bound together, as there is no difficulty whatever in isolating the pure levo form from it in good quantities.

(b) Isolation of the Dextro Amygdalin.

After the greater part of the *l*-amygdalin is separated from the racemic, the *d*-amygdalin is obtained in two crystallin modifications as already stated. The second fraction (11.8 grams) has a specific rotation of $c = 4.82$, $t = 20.5°$, $\alpha = -5.66°$; hence $[\alpha]_D^{20.5}$ —59°. Three and five-tenths grams were hydrolyzed in the usual way with hydrochloric acid. The mandelic acid was extracted with ether, dissolved and made up to 50 cc. with water. It showed a rotation of +6.65° in a 2 dcm. tube at 18.5°, 20 cc. required 24.6 cc. 0.125 *N* alkali; *c*, therefore, = 2.337; hence $[\alpha]_D^{18.5}$ +142°. So this fraction is about 95% pure *d*-amygdalin. After recrystallizing this fraction twice from absolute alcohol, it had a specific rotation of $c = 6$ (anhyd.), $\alpha = -7.27°$, $l = 2$ dcm., $t = 27°$; hence $[\alpha]_D^{27}$ —60.6°. When hydrolyzed, as we shall see later, it yields pure *d*-mandelic acid. It melts sharply at 212°, is easily soluble in 95% alcohol, fairly insoluble in absolute alcohol and crystallizes out slowly from a supersaturated solution in very fine fluffy crystals. It dissolves in less than its own weight of water, but assumes a crystallin form when evaporated to a thick syrup and allowed to stand for a day or two. It has a bitter taste and is hydrolyzed to glucose, hydrocyanic acid and benzaldehyde by emulsin.

The following table shows that the specific rotation of the *d*-amygdalin varies with temperature and concentration, as was conjectured from the rotation of the racemic:

C.	α.	T.	l.	Specific rotation.
8.5	—10.62°	14	2 dcm.	—62.5°
8.5	—10.43°	24.5	2	—61.4°
8.5	—10.19°	36.5	2	—60°
4.25	—5.13°	27	2	—60.4°
2.125	—2.51°	26.5	2	—59.2°

The other modification of the dextro form, namely, the one which crystallizes with alcohol of crystallization, has a specific rotation of $c = 4.96$, $l = 2$ dcm., $t = 26°$, $\alpha = -5.57°$; hence $[\alpha]_D^{26}$ —56.1°. About 3 grams were hydrolyzed and the mandelic acid extracted. It was made up with water to 50 cc. and a rotation taken: $l = 2$ dcm., $t = 27°$, $\alpha = +3.23°$,

20 cc. required 20.16 cc. of 0.125 *N* alkali; hence $[\alpha]_D^{27}$ +84.3°, which corresponds to 78.3% of dextro and 21.7% of levo amygdalin. When one calculates what the specific rotation ought to be, it comes to —56.4° at 19° which is practically the same as the one observed. This modification seems to be a definit compound composed of 3 parts dextro and 1 of levo, mixed with a small quantity of dextro. It crystallizes with alcohol of crystallization, which the dextro compound alone does not appear to do. It is much more readily soluble in absolute alcohol than the dextro form. When such a solution is cooled, a part of the dextro form crystallizes out; if it is not filtered out, however, but allowed to stand for some hours, it reunites with the portion still in the mother liquor, forming a thick syrup which can be redissolved by heating the solvent and the process repeated. At a temperature slightly higher than the room temperature this modification, therefore, seems to be unstable, allowing the dextro form to separate.

7. Hydrolysis of Dextro Amygdalin with Strong Sulfuric Acid.

As the *l*-amygdalin acted so peculiarly towards acids, it seemed worth while to hydrolyze the *d*-amygdalin and see whether it acted in the same way. Five grams of *d*-amygdalin were, therefore, hydrolyzed in a solution containing 10 grams of sulfuric acid and 15 grams of water for 1 hour and 15 minutes at 90°. Fifteen minutes after the heating was begun, the solution turned milky and very soon beads of oil began to appear. At the end of the time specified, the solution was cooled and extracted with 50 cc. of benzene. It rotated polarized light —3° in a 1 dcm. tube. The benzene solution was collected and evaporated to dryness which left a dark colored oil free from the odor of benzaldehyde. It weighed 1.235 grams or about 85% of the theoretical nitrile in 5 grams. The aqueous hydrolytic solution was extracted with ether and the mandelic acid obtained was dissolved and made up to 25 cc. It had an angle of +2.95° in a 2 dcm. tube at 27°, 20 cc. required 11.8 cc. of 0.125 *N* alkali, or the 25 cc. contained 0.279 gram of mandelic acid. This demonstrates that the dextro compound loses its glucose before the cyanide radicle is attacked in a strong sulfuric acid solution. This is identical with the hydrolysis of the levo form.[1]

8. Hydrolysis of Dextro Amygdalin with Hydrochloric Acid (D. 1.18) at the Room Temperature.

Five grams of *d*-amygdalin were dissolved in hydrochloric acid (D. 1.118), the solution being made up to 25 cc. The hydrolysis was carried on at the room temperature and followed by taking readings in a 1 dcm. polariscope tube.

[1] *J. Chem. Soc.*, **95**, 1374 (1909).

Time.	Rotation.	Time.	Rotation.
5	—11.85°	119	+10.75°
7	—7.80°	143	+12°
23	—1.20°	167	+13°
30	+0.73°	215	+15°
47	+3.95°		

I tried to take another reading 48 hours later, but the mandelic acid had already started to crystallize out. What was left of the hydrolytic solution was extracted with ether and the mandelic acid obtained was dissolved in water and the solution made up to 50 cc. It had an angle of $+5.75°$, $t = 27°$, $l = 2$ dcm., 20 cc. required 20.7 cc. 0.125 N alkali, or $c = 1.966$; hence $[\alpha]_D^{27} +147°$. Since carefully purified mandelic acid has a specific rotation of $+149°$ at this temperature, our *d*-amygdalin must have been pure. By comparing the polariscope readings with those taken in the hydrolysis of the *l*-amygdalin, we notice that there is a very sharp drop in the rotation of the former and a corresponding rise in the latter. This strengthens the view expressed by Walker[1] that the first product formed is principally amygdalinic acid, which is then hydrolyzed to glucose and mandelic acid.

9. Action of Emulsins on the Amygdalin.

Liebig and Wohler[2] first pointed out that amygdalin was decomposed into glucose, benzaldehyde and hydrocyanic acid by emulsin. Since then several papers have appeared on this subject by Tammann,[3] Caldwell and Courtlaud,[4] and Auld.[5]

The following are the main conclusions arrived at: First, that for small concentration of the enzyme the velocity is proportional to its concentration. As the concentration is increased this relationship ceases and finally a further increase does not produce a corresponding increase in velocity. Second, that a constant quantity and not a constant fraction of amygdalin is hydrolyzed in a unit time, at least when a large excess of amygdalin is present. Third, that amygdalin is almost completely hydrolyzed into glucose, benzaldehyde and hydrocyanic acid by emulsin. Liebig believed that the hydrolysis went on as long as the benzaldehyde dissolved in water. Tammann noted a decomposition of 60% at 40°. Caldwell and Courtlaud, repeating Tammann's experiments, find that at the end of 67 and 90 hours, 98.2 and 98.5%, respectively, is decomposed. Fourth, that mandelonitrile glucoside is first formed, which in turn breaks down to benzaldehyde and hydrocyanic acid. The first part of the hydrolysis, therefore, takes place at the biose linking just as it does with

[1] *J. Chem. Soc.*, 83, 476 (1903).
[2] *Ann.*, 22, 1.
[3] *Z. physik. Chem.*, 16, 271 (1892).
[4] *J. Chem. Soc.*, 91, 670 (1907).
[5] *Ibid.*, 93, 1251 (1908).

maltose,[1] but the hydrolysis of maltose stops here, while emulsin goes one step further.

The action of emulsin on the racemic amygdalin has not been the subject of much investigation. Dakin[2] noted that if a solution of emulsin is added to a racemized solution of amygdalin and kept at 40° for some time, benzaldehyde and hydrocyanic acid were found present in the solution. This only shows, however, that there is still *l*-amygdalin in the solution, but proves nothing concerning the action of emulsin on the *d*-amygdalin. So a series of comparative experiments were carried out to determin this action on the various amygdalins. The emulsin used was Kahlbaum's preparation. The solution employed was made up by adding 1 gram of the emulsin to 100 cc. of water. This was stirred at intervals during several hours, after which it was filtered into a bottle and several drops of toluene added. The extent of the hydrolysis in most cases was estimated by determining the amount of hydrocyanic acid produced. At first, Auld's method[3] was used, which consists of adding an excess of sodium acid carbonate and then titrating the hydrocyanic acid with dilute standard iodine solution. It was not very satisfactory, as the end was by no means sharp. A second method that was tried, was to make the hydrolytic solution alkalin with magnesium hydroxide and add several drops of potassium chromate, then titrate with silver nitrate until silver chromate is precipitated. Still another method employed was to add excess of silver nitrate to the emulsin solution then titrate the excess with ammonium thiocyanate, using ferric alum as an indicator. These methods were tested by estimating standard potassium cyanide in an emulsin solution containing benzaldehyde, but the amount of cyanide determined was always too low. Correct values could be obtained when the emulsin was left out, which seemed to indicate that the silver nitrate in some way united with the emulsin.

A new method was, therefore, devised which gave better results than any of those mentioned above. It consisted in sucking a slow current of pure air through the Erlenmeyer flask in which the hydrolysis was carried on and then through a U-tube which held a caustic soda solution. In this way most of the hydrocyanic acid was fixed as it was liberated, which prevented it from being oxidized to formic acid. The last traces of the acid were removed by boiling the hydrolytic solution at the end of the reaction. This was not boiled into the caustic soda solution, however, but into a U-tube which contained a given amount of standard silver nitrate. This was afterwards transferred to a 100 cc. measuring flask, 2 cc. of concentrated nitric acid added, and then the caustic soda

[1] Fischer, *Ber.*, **28**, 1508.
[2] *J. Chem. Soc.*, **85**, 1517 (1904).
[3] *Ibid.*, **93**, 1264 (1908).

solution containing the cyanide, and filled up to the mark. The silver cyanide was filtered out and the filtrate titrated with ammonium thiocyanide. The reason that the air was passed through caustic soda at first and not through silver nitrate was because the silver cyanide which precipitated clogged up the small glass tubes with which the U-tube was filled. This would stop the flow of air and consequently the result was of no use. This method was tested and found satisfactory by putting standard potassium cyanide solution into the Erlenmeyer flask and then admitting dilute sulfuric acid, the hydrocyanic acid being carried over and estimated as specified above. The Erlenmeyer flasks in which the hydrolysis was carried on were kept in a thermostat at 41° to 43° for the length of time specified.

Amount of *l*-amygdalin used. Cc.	Length of time hydrolyzed. Hours.	Number of emulsin used. Cc.	Amount of HCN produced. Gram.	Per cent decomposition.
10 of 3% sol. (3 H_2O)	24	10	0.01499	94.9
10% (3 H_2O)	24	10	0.01510	95.6
10	9	10	0.01240	78.5
10 of 3% (anhyd.)	4	10	0.00879	49.6
	Racemic Amygdalin.			
10 of 3% (3 H_2O)	9	10	0.01085	68.9
10	24	10	0.01223	77.5
10	24	10	0.01206	76.3
10	42	10	0.01292	81.8
10	42	10	0.01269	80.3
	Dextro Amygdalin.			
10 of 3% (anhydrous)	24	10	0.01607	90.7
10	24	10	0.01558	88.0
10	4	10	0.00803	45.4

It is obvious from the above results that the dextro amygdalin as well as the levo amygdalin is hydrolyzed into benzaldehyde, glucose and hydrocyanic acid, though at a slower rate. It seems hard to explain, however, why it is that the racemic form is decomposed more slowly than both the levo and the dextro separately. There were a great many more experiments carried out than the ones quoted here, but none of them showed greater decomposition. The glucose was also estimated, both at the end of 9 hours and at 24 hours. At the end of 9 hours, 1.92 grams were liberated from 3 grams of racemic amygdalin (3 H_2O), or 91%, and at the end of 24 hours, 2 grams, or 94.7%. This showed that the most of the undecomposed substance was the mandelonitrile, which was verified by the following experiment:

Fifteen grams of racemic amygdalin were made up approximately to 250 cc. with water, 1 gram of emulsin added and the flask thoroughly shaken. It was kept in the thermostat at 41° for 15 hours and then extracted twice with 150 cc. of ether each time. When the ether was

evaporated to a volume of 50 cc. it was examined in a polariscope and found to be quite inactive. The rest of the ether evaporated spontaneously leaving an oily residue which had a very strong odor of benzaldehyde. This residue was hydrolyzed with hydrochloric acid and evaporated to dryness, then extracted 3 times with ether. It left a solid, 0.776 gram, which was filtered off and dried. This was dissolved and the solution made up to 100 cc., 20 cc. were added to 35 cc. 0.1 *N* silver nitrate. It required 28.35 cc. of *N*/47 ammonium thiocyanate to titrate the excess of silver. From this titration the 100 cc. would contain 0.7768 gram of ammonium chloride, which would be the amount yielded from 1.929 grams of nitrile, or 49.4% of the total quantity in 15 grams of amygdalin.

10. Synthesis of Active Benzaldehydecyanhydrin from Benzaldehyde and Hydrocyanic Acid with Emulsin.

During the time the above experiments were being carried out there appeared a paper by Feist[1] in which it was pointed out that if emulsin was allowed to act on a solution containing amygdalin for several days and then extracted with ether, the ether was always found to be dextro rotatory. This activity was shown to be due to *d*-mandelonitrile, which Feist supposed was one of the primary decomposition products. Several months later, Rosenthaler[2] showed that benzaldehyde and hydrocyanic acid in the presence of emulsin formed *d*-mandelonitrile, which put Feist's supposition in doubt, because the chances are even that the active nitrile which he isolated was a synthetic product and not a decomposition product. This was followed by a paper by Auld,[3] who showed that the nitrile was produced much faster from benzaldehyde and hydrocyanic acid than from an equivalent concentration of *l*-amygdalin, using the same quantity of emulsin. Auld argued, therefore, that it was exceedingly probable that the nitrile always found in emulsin hydrolytic solutions was a secondary product formed from the benzaldehyde and hydrocyanic acid and not a primary decomposition product as supposed by Feist. Feist repeated his original experiment with a new sample of emulsin and found that the activity was much less than in his first experiment. This demonstrated that not all emulsins were alike, which made us curious to see what our emulsin did, especially since the ethereal extract from the racemic amygdalin showed no activity.

Five grams of *l*-amygdalin were dissolved in 75 cc. of water and 0.5 gram emulsin added. The flask was thoroughly shaken and kept in a thermostat at 41° for $3^1/_2$ hours. The emulsin was precipitated, the solution filtered and extracted with ether. The solvent was distilled to 15 cc. and examined in a polariscope tube, but it was found to be inactive.

[1] *Arch. Pharm.*, 246, 206–9 (1908).
[2] *Ibid.*, 246, 365–6 (1908).
[3] *J. Chem. Soc.*, 95, 927 (1909).

Since this was inactive, Rosenthaler's experiment was tried. Four-tenths gram of emulsin was dissolved in 25 cc. of water to which was added 20 cc. of 1.62% hydrocyanic acid and 2 cc. of benzaldehyde. This solution was kept at 25° for $3^1/_2$ hours, then filtered through a filter covered with freshly precipitated alumina and the filtrate extracted with 20 cc. of ether. In the first two experiments there was no activity noticed in this ethereal solution, but the third had a dextro rotation of 0.15° in a 2 dcm. tube. This is very small, however, as Rosenthaler, with the same conditions, got an activity of over a degree. Consequently this sample of emulsin contains very little of the synthetic enzyme. As there was another unopened sample of Kahlbaum's emulsin in the laboratory, this was also tried. In an experiment carried out identically to the one above the ether extract when examined in a 2 dcm. tube had a dextro rotation of 0.95°. This was repeated and the second time the activity in a 2 dcm. tube was 0.98°. This time the ether was completely evaporated and the nitrile which was left was hydrolyzed with hydrochloric acid. The mandelic acid was not extracted, but the hydrolytic solution itself (50 cc.) was examined in the polariscope. In a 2 dcm. tube its activity was —1.80°. This confirmed Feist's results that emulsin does not always contain the same quantity of the synthetic enzyme.

l-Amygdalin was also hydrolyzed with this emulsin to see if more active nitrile could be obtained than with the old emulsin. Five grams of *l*-amygdalin were dissolved in 75 cc. of water and 1 gram of the new emulsin added. It was kept at 40° for $3^1/_2$ hours, then extracted with 20 cc. of ether. This had a levo activity of 0.17° in a 2 dcm. tube. In the second experiment only $^1/_2$ gram of emulsin was used. The activity this time was —0.15°. The third time the hydrolysis was allowed to go on for 24 hours instead of $3^1/_2$ and this time the activity was only —0.12°. The ether solutions from the three experiments were poured together, the solvent evaporated and the residue hydrolyzed with hydrochloric acid. The mandelic acid was extracted and dissolved in enough water to make 25 cc. This solution had an angle of +0.6° in a 2 dcm. tube. This is a surprising result as Feist, Rosenthaler, and Auld always got a dextro nitrile yielding levo mandelic acid upon hydrolysis. To make perfectly sure that our nitrile was actually levo, the experiment was repeated using 10 grams of *l*-amygdalin and 1 gram of emulsin. But the ether solution again had an activity of —0.10°, and when the nitrile was hydrolyzed and the mandelic acid made up to 25 cc., it had a dextro rotation of 0.35°. Here then we have an example where emulsin produces a dextro mandelonitrile from commercial benzaldehyde and hydrocyanic acid and a levo nitrile when these two chemical compounds are obtained from *l*-amygdalin, because we cannot argue that the levo nitrile obtained in the second case is a primary decomposition product since the *l*-amyg-

dalin contains dextro mandelonitrile. This looks as though there might be two different benzaldehydes but this assumption could not be proved definitely. As the first sample of emulsin produced very little active nitrile, it was used to hydrolyze *l*-amygdalin and the benzaldehyde obtained was used instead of the commercial in a synthetic experiment. The experiment was carried out in the following manner: Ten grams of *l*-amygdalin and 1 gram emulsin (old) were dissolved in 150 cc. water and kept at 40° for $3^1/_2$ hours. The enzyme was precipitated with a drop of acetic acid and filtered out. The filtrate was extracted with ether and the solvent evaporated. To 2 cc. of this residue $^1/_2$ gram of emulsin, dissolved in 20 cc. water, was added and 25 cc. of the hydrocyanic solution (1.62%). This solution was kept at 25° for $3^1/_2$ hours, when the emulsin was again precipitated and the clear filtrate extracted with ether. The residue after the solvent was evaporated was hydrolyzed with hydrochloric acid. The hydrolytic solution (25 cc.) had an angle of —0.90° in a 2 dcm. tube, showing that dextro nitrile was produced. If the formation of the two different nitriles is due to two different varieties of benzaldehyde, the benzaldehyde in the last case must have been transformed to the commercial variety during extraction because it yielded the same nitrile when treated with emulsin.

Since the *l*-amygdalin gave *l*-nitrile when treated with new emulsin, it seemed interesting to see what nitrile the *d*-amygdalin produced. Five grams of *d*-amygdalin and $^1/_2$ gram of new emulsin were dissolved in 75 cc. of water and kept at 40° for $3^1/_2$ hours. After the emulsin was precipitated and filtered out the solution was extracted with ether. When the solvent was evaporated to 20 cc. it was found to have an activity of —0.12° in a 2 dcm. tube. The nitrile was hydrolyzed with hydrochloric acid and the hydrolytic solution (15 cc.) had an angle of +0.85° in a 2 dcm. tube. Whether all the *l*-mandelonitrile in this case is a primary product of decomposition or a secondary product is hard to say, but it is exceedingly likely that a large part is a secondary product because the same nitrile is formed when *l*-amygdalin is used, where it must be secondary.

There seems to be only one other apparent explanation for the production of these two mandelonitriles, if one does not assume that there are two benzaldehydes. There might be something in the solution which added on in some way to the emulsin and this addition product might then synthesize the other optically active nitrile. Now the only substance present in both the hydrolysis of the *l*- and the *d*-amygdalin and not when the nitrile is synthesized from benzaldehyde and hydrocyanic acid is glucose. The following experiment was carried out to exclude this possibility:

Five grams emulsin and 2 grams glucose were dissolved in 25 cc. water

to which 25 cc. of hydrocyanic solution and 2 cc. benzaldehyde were added. After $3^1/_2$ hours, the usual extraction was effected and the nitrile hydrolyzed with hydrochloric acid. The hydrochloric acid solution (50 cc.) showed a rotation of —1.45° in a 2 dcm. tube. The presence of the glucose, therefore, did not change the activity from dextro to levo.

Another experiment was carried out similar to the one above only substituting for glucose *l*-amygdalin, but the resulting mandelic acid was levo active.

Investigations will be continued on this subject to discover if possible the cause for the production of these two nitriles. The barks and leaves of wild cherry and elder berry will also be extracted to see if there is any difference in the emulsin produced.

In conclusion, I wish to thank Dr. Walker for proposing this investigation and for his suggestions and interest during its progress; also Dr. McIntosh for many practical suggestions.

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STUDIES ON ENZYME ACTION. I. SOME EXPERIMENTS WITH THE CASTOR BEAN LIPASE.

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This investigation was undertaken with the object of studying the action of a fat or ester splitting ferment (lipase) more particularly from the point of view of the ferment itself and its behavior under varying conditions. The lipase present in castor beans was chosen as most suitable for the present work.

The lipase preparation used in the experiments was prepared as follows: The castor beans[1] were ground roughly, extracted a number of times with carbon tetrachloride or chloroform,[2] then ground to a fine powder and passed through a 100-mesh sifter. In this way a large portion of the shells were separated. The fine powder was then extracted exhaustively with ether (100 times or more) in a Soxhlet extractor, practically all of the fat being removed in this way.[3] The following experiment shows the activity of the preparation at the different stages: 2 cc. olive oil and 50 cc. water were treated with the substances stated for 17 hours at 38° and the acid present then titrated with a 0.1 *N* sodium hydroxide solution, using phenolphthalein as indicator. No castor bean present = 0.6 cc. alkali required; 0.2 gram castor beans before sifting = 1.0 cc.; 0.2 gram castor beans after sifting (shells removed) = 1.9 cc.; 0.2 gram

[1] The castor beans were supplied by the Baker Castor Oil Company of New York.

[2] This extraction was carried out by Mr. C. W. Otto.

[3] Cf. A. E. Taylor, *J. Biol. Chem.*, **2**, 87 (1906), for the preparation of the lipase material from castor beans and a careful study of its properties.