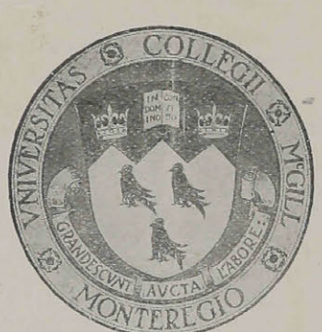
AMYGDALINS & THEIR INTERACTIONS WITH EMULSIN



In a previous communication from this laboratory (Walker and Krieble, J. Chem. Soc.95,1457) it was proved that the rotation of a racemised 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 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 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 hydrolized 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 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 hydrolized by emulsin to the same extent as the unheated racemic modification. These last two facts indicated that a more complicated transformation than the simple racemication was taking place and it was suggested that probably the change was from an α to a B glucoside.

In the present communication it is shown that the minutest trace of hydroxyl ion is capable of racemising amygdalin and that the Cn radicle is necessary to effect this change. The composition of the racemic is definitely proved and shown to be made up of 56.25% of dextro and 43.75% of laevo. 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 Ba salt from an unknown acid always associated with amygdalin in minute quantities. The nature of the change giving rise to the increased rotation was 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 laevo 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 hydrolyses but also synthesis active benzaldehyde cyanhydrin 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 laevo 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 alkalis upon the equilibrium between the laevo and dextro amygdalin.

2- Composition of the racemic amygdalin.

3- Cn Radicle necessary for racemication.

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 laevo.
   (b) Isolation of the dextro.

7- Hydrolysis of the dextro amygdalin by strong sulphuric acid.

8- Hydrolysis of the dextro 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 ALKALIS UPON THE EQUILIBRIUM BETWEEN THE LAEVO AND THE DEXTRO AMYGDALIN.

Walker (J. Chem. Soc. 1903, 83, 478) 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 dextro mandelic acid when hydrolized. 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 N/4 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=8.944, t=24°, l=2 dcm. $\alpha = -3.57°$; hence $[\alpha]_D^{24} = -55.5°$. Now 1 cc. of a N/4 alkali solution contains .00425 gram of O H ion and, assuming that there are 20 drops in 1cc., we would have used .000225 gram to racemise 10 grams or 2 in 100,000, provided the barium salt of the acid present is completely hydrolized. In another experiment, 10 cc.

of a 10 per cent 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 C. 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 racemised. When this solution was boiled to dryness and then hydrolized with hydrochloric acid, it gave mandelic acid with a specific rotation of +5.5°, -(racemic gives +18°.) Here too the barium salt of the acid present is formed and no doubt this is hydrolized and gives rise to hydroxyl ions.

It is hard, however, to explain the two following experiments in this way. 1 cc. N/4 barium hydrate was just neutralized with sulphuric acid, then boiled to dryness. 10 cc. of a 10 per cent solution was added to the barium sulphate 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 hydrate was neutralized with sulphuric 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 sulphate freshly precipitated seems to racemise faster than when it has once become perfectly dry. This difference might be due to occluded barium hydroxide in the barium sulphate crystals which

later diffused and affected the change in rotation due to the fact that the first solution is saturated with barium sulphate and the second is not.

2. COMPOSITION OF THE RACEMIC.

Walker, who first discovered the racemic amygdalin,(J.Chem. Soc. 1903, 83, 472) pointed out that when it was completely hydrolized with hydrochloric acid, the ethereal extract always showed an excess of dextro-mandelic acid, indicating the production of excess of dextro amygdalin. This has been confirmed by Dakin (J. Chem. Soc. 1904, 85, 1512) and by a number of ~~my~~ 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 laevo and dextro amygdalin, (Walker and Krieble, J. Chem. Soc. 1909, 95, 1457.) The reason given for the production of excess of dextro-mandelic acid was the inequality in the rates of hydrolysis of the two varieties by acid, accompanied by a slow racemisation. This is not borne out by experiment. 5 grams of l-amygdalin ($3\ H_2O$) were hydrolized with hydrochloric (D 1.118) at 60° to 70° C. for 5 hours, extracted with ether 4 times and the residue from the ether made up to 50 cc. and examined in the polariscope, $\alpha = -8.41°$, l=2 dcm., t=25° and 20 cc. needed 29.2 cc. of N/8 alkali or c=2.775; hence $[\alpha]_D^{25}$ -152°. So there is no racemisation during the hydrolysis of the laevo and the same is true of the dextro from results which appear later on. As proof that the continued boiling did not

racemise the mandelic acid, 1 gram was dissolved in hydrochloric acid (D 1.118) and made up to 50 cc. This showed a rotation of -3.30° in a 1 dcm. tube. The solution was heated for 10 hours, then cooled and examined again; it now had an angle of -3.28° at the same temperature. So the excess of dextro mandelic acid must represent a corresponding excess of dextro amygdalin in the racemic. Several grams were racemised and then hydrolized with hydrochloric acid (D 1.118) for 6 hours at 60°-70° C. and the acid solution extracted with ether. The ether residue was made up to 50 cc. and examined in the polariscope. $\alpha = +.85°$, $t = 25°$, $l = 2$ dcm., 20 cc. needed 25.45 N/8 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 per cent excess of dextro in the above solution is 18.8/150 X 100 or 12.75. The racemic is therefore composed of 56.25 per cent dextro amygdalin and 43.75 laevo amygdalin. Near the close of this investigation, the dextro amygdalin was isolated and found to have a specific rotation of -61.2° at 19° C. for a 5 per cent solution. A laevo 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 laevo 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 laevo and dextro amygdalin.

3. THE CN RADICLE NECESSARY FOR RACEMISATION.

No one has as yet suggested a theory to explain the racemisation but it can be demonstrated by the following experiment that it is not possible to racemise the asymmetric carbon atom in the mandelic acid radicle,- the one racemised when amygdalin is treated with alkali - unless the Cn 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. 50 grams of l-amygdalin (3 H_2O) were dissolved in 450 cc. of N/4 barium hydroxide and the solution boiled to expell all the ammonia. The barium was precipitated with sulphuric acid and the solution allowed to settle. The clear liquid was siphoned off into a 1 liter measuring flask, the barium sulphate carefully washed and the washings also run into the measuring flask. It was filled up to the mark and a rotation taken: $\alpha = -5.44^\circ$, $t = 21.5^\circ$, $l = 2$ dcm.; hence $[\alpha]_D^{21.5} -60.8^\circ$ 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 per cent solution had a specific rotation of $[\alpha]_D$ -33.5°. 1.2 grams dissolved in water required 11 cc. of N/8 alkali to neutralize the acid. Amygdalinic acid ($C_{19}H_{27}O_{11}COOH$) has a molecular weight of 476; therefore 11 cc. of N/8 solution would represent .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 .6545 grams of amygdalinic acid would be combined with .4592 gram of strychnine. It is evident therefore that the 1.2 grams of the strychnine salt contained .0863 gram of water which corresponds to 3 1/2 molecules of water of crystallization. This was verified by drying a portion over P_2O_5 in a tube exhausted to 50-70 mm. and heated to 90° C.

23.086	weight of boat and strichnine salt	23.086
22.25	" " " After drying	23.0248
.836 grams,	weight of salt	.0614 water
.0614	water	
.7746	anhydrous salt	

.9614/.7746 x 810 = 64 parts or 3 1/2 molecules of water.

To show that optically active amygdalinic acid cannot be racemized, 5 grams of crystalline Strychnine Amygdalinate were dissolved in water and 1 cc. N/4 more Baryta 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, $\alpha = -3^\circ$, t=21.5°, l = 2 dcm., hence $[\alpha]_D^{21.5} -32.5^\circ$. The solution was filtered and concentrated to a thick syrup, then hydrolized 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 32°; its concentration was 1.7765 grams; hence $[\alpha]_D^{22} +77.4^\circ$. The ~~per cent~~ excess of dextro in the crystals is therefore 77.4/149x100 or 52 per cent which shows that the amygdalin radicle cannot be racemised if the Cn 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 racemised 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 behaviour of the racemic. We always had a large portion which could not be obtained crystallized. Emulsin did not seem to hydrolize the racemic completely and the end point of the hydrolysis seemed to be influenced by the length of time the racemic 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 sulphuric acids were used to neutralize the baryta, but in almost every case the rotation increased, as the following table shows.

A 10 per cent stock solution of laevo amygdalin($3\ H_2O$) was made up. 10 cc. was used in each experiment to which was added 1 cc. of N/1 baryta 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 sulphuric 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 $[\alpha]^{20} -52.5°$.

These are only a few selected at random from the notebook . There were very few where the rotation did not go up. Finally, it was noticed that all the solutions which had an increased rotation gave a faint precipitate with sulphuric 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 baryta was added this acid was changed to the barium salt. When the excess baryta 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 baryta 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=8.044, α=-9.57°, l= 2 dcm. t=24°; hence $[\alpha]_D^{24}$ -53.5°. 10 cc of this solution was evaporated to dryness and heated for 5 hours, then made up to 25 cc. It now had a rotation of c 3.5776, α -4.70°, l= 2 dcm., t=24°; hence $[\alpha]_D^{24}$ -65.7°. This was repeated with the same result.

To prove that it mest be due to the hydroxyl ions caused by the hydrolysis of the barium salt, 1 drop of N sulphuric 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, α=-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 (N/3) was added when the baryta was neutralized, with the same result. A very

small amount of hydroxyl ions, therefore, does not only racemise but also appears to change the amygdalin in some other 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 dextro produced. 6 grams were, therefore, dissolved in water and added to a solution of 100 cc. containing 2 cc. of baryta 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: $\alpha = -12.44^\circ$, $l = 2$ dcm., $t = 23^\circ$, c=10.7328; hence $[\alpha]_D^{23} -58^\circ$. The solution was evaporated to a thick syrup and hydrolized with hydrochloric acid (D. 1.118) for 4 hours at 60-70 c., the mandelic acid extracted and made up to 50 cc. It had a dextro rotation of .20 in a 1 dcm. tube at 22 C., 20 cc. used up 38.1 N/8 alkali or c=3.6195; hence $[\alpha]_D^{22} +5.5^\circ$. This shows that the equilibrium has not been shifted in favor of the dextro.

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. 1 gram of l. amygdalin (3 H_2O) was, therefore, dissolved in water, 1/2 cc. baryta 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 $[\alpha]_D^{25} -63.9°$. 25 cc. of this solution was treated with dilute sulphuric acid until it no longer gave a precipitate, and then evaporated to 25 cc. and another rotation taken. It now showed a rotation of -4.40° at 23° C. 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 ($3H_2O$) were boiled with excess of baryta 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 specific 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 which had the increased rotation it was found, however, to be the same. 2.5 grams of l. amygdalin ($3 H_2O$) were dissolved in water, 2 cc. baryta 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, $\alpha = -5.45°$, $l = 2$ dcm., $t = 24.5°$; hence $[\alpha]_D^{24.5} = -60.7°$. 25 cc. of the solution was 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 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 furthur 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 Cn group was changed or hydrolized in some way. This could easily be proved as it was pointed out (Walker and Krieble, J. Chem. Soc. 1909, 95, 1369) that strong sulphuric acid when allowed to act on l.amygdalin for several hours at a high temperature, produced about 85 per cent of the theoretical d.mandelonitrile, so ~~now~~ if the Cn has been hydrolized during the change that takes place when the rotation of the racemic goes up, it ought not to give any nitrile when hydrolized with strong sulphuric acid. 20 grams of the racemic 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. concentrated sulphuric 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 small volume, then poured into a small beaker and when free of the solvent and moisture it weighed .287 grams which is only an eighth of the nitrile obtained from laevo 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. 10 cc. required 26.6 cc. of N/8 alkali or the concentration of mandelic acid was 5.12 grams. This demonstrates that the nitrile was hydrolized before the sulphuric acid acted because when l.amygdalin is treated for the same length of time with the same strength of acid it yields about 85 per cent of nitrile while in this case 86 per cent of mandelic acid was obtained.

It is difficult to say to what the nitrile radical is transformed when the racemic 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 in this case, so the electrical resistance of this substance was compared with that of ammonium amygdalinate. The cell used had a constant of .1037 and a 5 per cent l.amygdalin ($\frac{1}{2}H_2O$) solution had a conductivity of .424 x 10^{-4}. Ammonium amygdalinate was prepared according to methods already described.

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

Per cent of NH_4 amygdalinate	Specific Rotation	Specific conductivity.
5	-71.8°	6.74 x 10^{-3}
4		5.18 x 10^{-3}
3 1/3		4.45 x 10^{-3}
2		3.45 x 10^{-3}
3 per cent of Am. Amygdalinate		
1 " " " l. Amygdalin	-65°	3.99 x 10^{-3}
The following are the solutions which had been heated.		
4	-65.4°	5.94 x 10^{-4}
4	-58.2°	3.61 x 10^{-4}
4	-67.5°	5.98 x 10^{-4}
4	-64.6°	5.08 x 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 derivitive 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 dextro amygdalin as we had found out how to evaporate the racemic without bringing about any internal change.

6. RESOLUTION OF RACEMIC AMYGDALIN.

75 grams were dissolved in several hundred cc. of luke-warm water and 6 cc. of baryta added. After one half hour, 1.4 cc. of N/3 oxalic acid were added in excess to the quantity needed to make the solution neutral. This was evaporated to a moderately thick syrup and 250 cc. of 95 per cent 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° C. for an hour, which caused 7.6 grams of a fine crystalline precipitate to separate. This was rapidly filtered and washed with cooled absolute alcohol. When the temperature went up a few degrees ~~they~~ it liquefied completely giving off ~~their~~ 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 crystalline. 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 hydrolizes a very small fraction.

(a) ISOLATION OF THE LAEVO AMYGDALIN.

The first crop of crystals (30 grams) contains a large preponderance of laevo amygdalin. A 5 per cent solution in a 2 dcm. tube at 20° C., ~~had an angle of~~ rotated polarized light -4.11°, ~~when~~ When 1.366 grams were dried over P_2O_5 in vacuo at reduced pressure it lost .0928 gram or 6.8 per cent of water of crystallization; hence, $[\alpha]_D^{20} -44°$. 5 grams were hydrolized in the usual way and the mandelic acid extracted dissolved and made up to 50 cc. It had a specific rotation of $\alpha = -6.40°$, $l = 2$ dcm., $t = 24.5°$ and 20 cc. required 31.4 cc. of N/8 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 per cent of the laevo isomer and 14.4 of the dextro. - If one calculates what the rotation of such a mixture should be at 20° C, 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 laevo crystallized out in rosettes. These were twice recrystallized and then air-dried. A 5 per cent solution (3 H_2O) showed a rotation of ~~had an angle of~~ -3.43° in a 2 dcm. tube at 25 C. or $[\alpha]_D^{25}$ -38.3°, which agrees very well with the laevo amygdalin we started with, namely $[\alpha]_D^{25}$ -38.6° for the same concentration. 5 grams of it were hydrolized and the mandelic acid obtained without purification had a specific rotation of α = -65.9°, t=26.5, c=20 cc. 23.7 N/8 alkali or $\frac{c}{\wedge}$ 2.2344; hence $[\alpha]_D^{26.5}$ -152.6 at 17° C. 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 definite compound, made up of 3 parts of laevo to 1 of dextro, saturated with the mother liquor which contains a big excess of dextro; or whether it is laevo amygdalin saturated with the mother liquor is hard to determine. The crystals themselves come out of the alcoholic solution in the form of rosettes and have all the characteristics of laevo amygdalin. Then their rotation is not constant, for if they are washed with a small quantity of alcohol it comes down as low as -43.5°. If it is a definite compound it is very loosely bound together as there is no difficulty whatever ~~to~~ in isolating pure laevo from it in good quantities.

(b) ISOLATION OF THE DEXTRO AMYGDALIN.

After the greater part of the laevo is separated from the racemic, the dextro is obtained in two crystalline modifications as already stated. The second fraction (11.8 grams) has a specific rotation of c=4.82, t=20.5, α = -5.66 ; hence $[\alpha]_D^{20.5}$ -59°. 3.5 grams were hydrolized in the usual way with hydrochloric acid. The mandelic acid was extracted with ether, dissolved and made up to 50 cc. with water. It ~~had an angle of~~ rotated polarized light + 6.05° in a 2 dcm. tube at 18.5° C. 20 cc. required 24.6 cc. N/8 alkali; c, therefore, = 2.537; hence $[\alpha]_D^{18.5}$ +142°. So this fraction is about 95 per cent pure dextro. By recrystallizing this fraction twice from absolute alcohol, it had a specific rotation of c=6 (anhyd), α -7.27°, l =2 dcm., t=27°; hence $[\alpha]_D^{27}$ -60.6°. When hydrolized, as we ~~will~~ shall see later, it yields pure dextro mandelic acid. It melts sharply at 212° C., is easily soluble in 95 per cent 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 crystalline form when evaporated to a thick syrup and allowed to stand for a day or two. It has a bitter taste and is hydrolized to dextrose, hydrocyanic acid and benzaldehyde by emulsin.

The following table shows that the specific rotation of the dextro varies with temperature and concentration as was conjectured from the rotation of the racemic:

C.	α	T.	L.	
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, namely, the one which crystallizes with alcohol of crystallization, has a specific rotation of c=4.96, l=2 dcm., t=26°, α=-5.57°; hence $[\alpha]_D^{26}$=-56.1°. About 3 grams were hydrolized and the mandelic acid extracted. It was made up with water to 50 cc. and a rotation taken; l=2 dcm., t=27°, α=+3.33°, 20 cc. required 20.16 cc. of N/8 alkali, hence $[\alpha]_D^{17}$+84.3° which corresponds to 78.3 per cent of dextro and 21.7 per cent of laevo amygdalin. When one calculates what the specific rotation ought to be, it comes to -56.4° at 19°C which is practically the same as the one observed. This modification seems to be a definite compound composed of 3 parts dextro and 1 of laevo, mixed with a small quantity of dextro. It crystallizes with alcohol of crystallization which the dextro alone does not appear to do. It is much more readily soluble in absolute alcohol than the dextro. When such a solution is cooled, a part of the dextro 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 to separate.

(7) HYDROLYSIS OF DEXTRO AMYGDALIN WITH STRONG SULPHURIC ACID.

As the laevo amygdalin acted so peculiarly towards acids, it seemed worth while to hydrolize the dextro and see whether it acted in the same way. 5 grams of dextro amygdalin were, therefore, hydrolized in a solution containing 10 grams sulphuric acid and 15 grams water for 1 hour and 15 minutes at 90°C. 15 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 had an angle of -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 per cent 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. dcm. tube at 27°C. 20 cc. required 11.8 cc. of N/3 alkali or the 25 cc. contained .279 gram of mandelic acid. This demonstrates that the dextro loses its glucose before the Cn radicle is attacked in a strong sulphuric acid solution. This is identical with the hydrolysis of the laevo.

(J. Chem. Soc. 1909, 95, 1374.)

(8) HYDROLYSIS OF DEXTRO AMYGDALIN WITH HYDROCHLORIC ACID (D. 1.118) AT THE ROOM TEMPERATURE.

5 grams of dextro 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.

Time.	
5	-11.85°
7	- 7.80°
23	-- 1.30°
30	+ .75°
47	+ 3.95°
119	+ 10.75°
143	+ 12.°
167	+ 13.°
215	+ 15.°

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. N/8 alkali or c=1.906, hence $[\alpha]_D^{27}$ +147°. Since carefully purified mandelic acid has a specific rotation of +149° at this temperature, our dextro amygdalin must have been pure. By comparing the polariscope readings with those taken in the hydrolysis of the laevo

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 (J. Chem. Soc. 1903, 83,476) that the first product formed is principally amygdalinic acid which is then hydrolized to glucose and mandelic acid.

(9) ACTION OF EMULSINS ON THE AMYGDALIN.

Liebig and Wohler (A., 22,1) first pointed out that amygdalin was decomposed into glucose, benzaldehyde and hydrocyanic acid by emulsin. Since then several papers have appeared on this subject, Tammann, Zeitsch. Physikal Chem., 1892, 16, 271; Caldwell and Courtlaud, J. Chem. Soc. 1907, 91, 670; and Auld, J. Chem. Soc. 1908, 1251.

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 furthur increase does not produce a corresponding increase in velocity. Second, that a constant quantity and not a constant fraction of amygdalin is hydrolized im a unit time, at least when a large excess of amygdalin is present. Third, that amygdalin is almost completely hydrolized 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 per cent at 40°C Caldwell and Courtlaud, repeating Tammann's experiments, find that at the end of 67 and 90 hours, 98.2 and 98.5 per cent

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 maltase, (Fischer, Ber. 28, 1508) but the hydrolysis of maltase stops here, while emulsin goes one step furthur.

The action of emulsin on the racemic has not been the subject of much investigation. Dakin (J. Chem. Soc. 85, 1904,1517) noted that if a solution of emulsin is added to a racemised solution of amygdalin and kept at 40° C 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 dextro amygdalin. So a series of comparative experiments were carried out to determine 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 the amount of hydrocyanic acid produced. At first Auld's method (J. Chem. Soc. 1908, 1264) 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 alkaline with magnesium hydrate 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 sulphocyanide, 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 the ones 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 oxidised 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 solution

containing the cyanide, and filled up to mark. The silver cyanide was filtered out and the filtrate titrated with ammonium sulphocyanide. 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 erlemeyer flask and then admitting dilute sulphuric 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° C. for the length of time specified.

Amount of l-amygdalin used	Length of time hydrolized	Number of cc. of emulsin used.	Amount of HCN produced	Per cent decomposition
10 cc. of 3% Sol. ($3H_2O$)	24 hrs.	10 cc.	.01499 gr.	94.9
10 cc "	24 hrs.	10 cc.	.01510 gr.	95.6
10 cc.	9 hrs.	10 cc.	.01240 gr.	78.5
10 cc. 3%(Anhyd)	4 hrs.	10 cc.	.00879 gr.	49.6
	Racemic Amygdalin			
10 cc. 3%($3 H_2O$)	9 hrs.	10 cc.	.01085 gr.	68.9
10 cc. "	24 hrs.	10 cc.	.01223 gr.	77.5
10 cc. "	24 hrs.	10 cc.	.01206 gr.	76.3
10 cc. "	42 hrs.	10 cc.	.01292 gr.	81.8
10 cc. "	42 hrs.	10 cc.	.01269 gr.	80.3

Dextro Amygdalin.

10 cc. of 3% Anhydrous	24 hrs.	10 cc.	.01607 gr.	90.7
10 cc "	24 hrs.	10 cc.	.01558 gr.	88.
10 cc. "	4 hrs.	10 cc.	.00803 gr.	45.4

It is obvious from the above results that the dextro amygdalin as well as the laevo amygdalin is hydrolized into benzaldehyde, glucose and hydrocyanic acid though at a slower rate. It seems hard to explain, however, why it is that the racemic is decomposed more slowly than both the laevo 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 per cent and at the end of 24 hours .2 grams or 94.7 per cent. This showed that the most of the undecomposed substance was the mandelonitrile which was verified by the following experiment.

15 grams racemic 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° C. for 15 hours and then extracted twice with 150 cc. 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 hydrolized with hydrochloric acid and evaporated to

dryness, then extracted 3 times with ether. It left a solid 0.776 grams which was filtered off and dried. This was dissolved and the solution made up to 100 cc., 20 cc. was added to 35 cc. N/10 silver nitrate. It required 28.35 cc. of N/47 ammonium sulphocyanide to titrate the excess of silver. From this titration the 100 cc. would contain .7768 grams of ammonium chloride which would be the amount yielded from 1.929 grams of nitrile or 40.4 per cent of the total quantity in 15 grams of amygdalin.

10. SYNTHESIS OF ACTIVE BENZALDEHYDECYANHYDERIN FROM BENZALDEHYDE AND HYDROCYANIC ACID WITH EMULSIN.

During the time the above experiments were being carried out there appeared a paper by Feist (Ard. Pharm. 246, 206-209 1908) 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 rotatary. This activity was shown to be due to dextro mandelonitrile which Feist supposed was one of the primary decomposition products. Several months later Rosenthaler (Arch. Pharm. 246, 365-366, 1908) showed that benzaldehyde and hydrocyanic acid in the presence of emulsin formed dextro 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 (J. Chem. Soc. 95, 1909, 927) 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 showed no activity.

5 grams of l.amygdalin were dissolved in 75 cc. of water and .5 grams 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. Since this was inactive, Rosenthaler's experiment was tried. .4 grams of emulsin were dissolved in 25 cc. water to which was added 20 cc. of 1.62 per cent of hydrocyanic acid and 2 cc. of benzaldehyde . This solution was kept at 25° C. 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 .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 angle of .95°. This was repeated and the second time the activity in a 2 dcm. tube was .98°. This time the ether was completely evaporated and the nitrile which was left was hydrolized 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 hydrolized with this emulsin to see if more active nitrile would be obtained than with the old emulsin. 5 grams of laevo amygdalin were dissolved in 75 cc. of water and 1 gram of the new emulsin added. It was kept at 40° C. for 3 1/2 hours, then extracted with 20 cc. of ether. This had a laevo activity of .17° in a 2 dcm. tube . In the second experiment only 1/2 gram of emulsin was used. The activity this time was -.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 -.12°. The ether solutions from the three experiments were poured together, the solvent evaporated and the residue hydrolized with hydrochloric acid.

The mandelic acid was extracted and dissolved in enough water to make 25 cc. This solution had an angle of +.6° in a 2 dcm. tube. This is a surprising result as Feist, Rosenthaler, and Auld always got a dextro nitrile yielding laevo mandelic acid upon hydrolysis. To make perfectly sure that our nitrile was actually laevo, the experiment was repeated using 10 grams of l.amygdalin and 1 gram of emulsin. But the ether solution again had an activity of -.10° and when the nitrile was hydrolized and the mandelic acid made up to 25 cc. it had a dextro angle of .35°. Here then we have an example where emulsin produces a dextro mandelonitrile from commercial benzaldehyde and hydrocyanic acid and a laevo nitrile when these two chemical compounds are obtained from l.amygdalin, because we cannot argue that the laevo nitrile obtained in the second case is a primary decomposition product since the l.amygdalin 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 hydrolize 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. 10 grams of l.amygdalin and 1 gram emulsin (old) were dissolved in 150 cc. water and kept at 40° C. for 3 1/2 hours. The The emzyme 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° C. 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 -.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 dextro amygdalin produced. 5 grams of dextro amygdalin and 1/2 gram of new emulsin were dissolved in 75 cc. water and kept at 40° C. 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 -.12° in a 2 dcm. tube. The nitrile was hydrolized with hydrochloric acid and the hydrolytic solution (15 cc.) had an angle of +.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 synthesise the other optically active nitrile. Now the only substance present in both the hydrolysis of the laevo and the dextro amygdalin and not when the nitrile is synthesised from benzaldehyde acid and hydrocyanic acid is dextrose. The following experiment was carried out to exclude this possibility:

5 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 hydrolized 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 laevo.

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

Investigations will be continued on this subject to discover if possible the cause for the production off 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.

McGill University,

Montreal, Can.

COMMITTEE
ON
GRADUATE STUDIES

April 19th, 12

Dear Dr. Ruttan:

I enclose a thesis submitted by Mr. Victor Kriebel for the degree of Ph.D. Will you please forward it to Dr. MacInnes after you have examined it.

I am yours truly

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