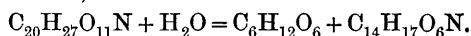


# CXXI.—*The Hydrolysis of Amygdalin by Emulsin.* *Part II.*

By S. J. MANSON AULD, Ph.D.

By the action of an extract of yeast (Hefenenzym), amygdalin is hydrolysed with the separation of only one molecule of glucose and the formation of a true glucoside, mandelonitrile glucoside (Fischer, *Ber.*, 1899, **28**, 1509):



Mandelonitrile glucoside is hydrolysed by emulsin into benzaldehyde, hydrocyanic acid, and glucose.

From the above decomposition of amygdalin by yeast extract, the active enzyme of which was supposed to be maltase\* (the ferment which decomposes maltose into two molecules of glucose), Fischer assumed that amygdalin is a derivative of maltose or of an exactly similarly constructed sugar. Latterly it has been generally the custom to regard amygdalin as the maltoside of benzaldehyde-cyanohydrin, and as such it is described in most books of reference. Thanks chiefly to the work of Emil Fischer, it is now practically certain that whilst emulsin only decomposes  $\beta$ -glucosides, maltase will only hydrolyse those glucosides which are derived from  $\alpha$ -dextrose, hence there exists a distinct stereochemical relationship between these sugars and their specific enzymic hydrolysts.

These facts seemed to render it probable, or indeed certain, that the two molecules of glucose in the biose residue of amygdalin are not of similar constitution owing to their different behaviour towards the complementary enzymes, emulsin and maltase. It was first pointed out by the author (*Proc.*, 1907, **23**, 72) that amygdalin is probably the derivative of an unknown  $\alpha\beta$ -disaccharide capable of hydrolysis both by emulsin ( $\beta$ -enzyme) and maltase or amygdalase ( $\alpha$ -enzyme). This view has since been confirmed by Rosenthaler (*Arch. Pharm.*, 1907, **245**, 684). It was also pointed out that in the hydrolysis of amygdalin by emulsin the decomposition could take place in three ways, depending on the mode of attachment of the enzyme to the glucoside molecule. Either mandelonitrile glucoside and dextrose are first produced with subsequent hydrolysis of the former, or benzaldehydecyanohydrin and the disaccharide are formed and the latter

\* It has now been shown by Caldwell and Courtauld (*Proc. Roy. Soc.*, 1907, **79**, B, 350) that the yeast enzyme which hydrolyses amygdalin is not identical with maltase. This, however, has no effect on the above arguments as "amygdalase" is also an  $\alpha$ -enzyme.

then resolves into two molecules of dextrose, or, lastly, amygdalin may undergo fission into the three components by direct abstraction of the dextrose residue attached to the benzaldehydecyanohydrin molecule.

Thanks to the admirable work of E. F. Armstrong on the correlation of the stereoisomeric  $\alpha$ - and  $\beta$ -glucosides with the corresponding glucoses (Trans., 1903, **83**, 1305), the first part of the problem could be attacked in the same manner as was adopted for the determination of the structure of the  $\alpha$ -glucoside phaseolunatin (Dunstan, Henry, and Auld, *Proc. Roy. Soc.*, 1907, **79**, B, 315). This method depends on the formation of sugar from the glucoside by hydrolysis and observation of its change of rotation on assuming the ordinary form of glucose which has been shown by Lowry (Trans., 1903, **83**, 1314) to be an equilibrium mixture of two stereoisomerides. The production of equilibrium is hastened by the addition of a drop of alkali.

*Rotation of Amygdalin.*—With regard to the optical rotation of amygdalin, two different numbers are recorded. Bouchardat (*Compt. rend.*, 1844, **19**, 1175) found for amygdalin, dried over lime at  $45^{\circ}$ ,  $[\alpha]_D -35.5^{\circ}$ , and this value is still quoted in many books of reference. Schiff (*Ber.*, 1899, **32**, 2701), repeating the experiments, obtained the value  $-40.26^{\circ}$ , and declared Bouchardat's figures to refer to  $[\alpha]_R$  and not to  $[\alpha]_D$ . Owing to these different results, the rotation of amygdalin was carefully measured, with the result that Schiff's rotations were also found to be not quite correct, the average rotation found being  $[\alpha]_D -41^{\circ}36'$ . For 3.883 per cent. Schiff found  $[\alpha]_D -41.1^{\circ}$ .

Amygdalin, per cent.	<i>l.</i>	$\alpha_D^{18}$ .	$[\alpha]_D^{18}$ .	(Schiff.) $[\alpha]_D^{17}$ .	$[\alpha]_D$ calculated from Bouchardat's results by Schiff.
1.636	200	$1^{\circ}22'$	$41^{\circ}16'$	—	—
3.272	200	2 45	42 1	$40^{\circ}18'$	$41^{\circ}57'$
5.418	200	4 30	41 31	—	—

Mean =  $41^{\circ}36'$ .

*Characterisation of the Dextrose Residues in Amygdalin.*—Owing to the comparative slowness with which the yeast enzyme attacks amygdalin and the consequent spontaneous production of equilibrium in the dextrose which is formed together with the one molecule of mandelonitrile glucoside, it was found impossible to identify the sugar by the method proposed by E. F. Armstrong. To determine the nature of the second dextrose molecule, namely, that attached to the benzaldehydecyanohydrin nucleus, the decomposition of mandelonitrile glucoside by emulsin was employed. The mandelonitrile glucoside was prepared from amygdalin by the action of yeast extract according to the directions given by Fischer (*Ber.*, 1895, **28**, 1509). After recrystallisation from chloroform it melted at  $148^{\circ}$ ,

and a proof of its purity was obtained by analysis. 0.5 Gram of emulsin was added to 5 grams of mandelonitrile glucoside dissolved in 50 c.c. of water, thoroughly mixed by shaking and kept in the thermostat in a stoppered bottle at 40°. For each observation, a few c.c. of the liquid were withdrawn, mixed with a small definite volume of alumina cream, and filtered through asbestos. The rotation of the clear liquid was then observed, before and after adding a drop of ammonia solution to establish equilibrium. Great care was taken to avoid the presence of any traces of alkali which might induce premature equilibrium, as in some cases it was found that the presence of a small proportion of tap-water was sufficient immediately to cause the dynamic change :

Time of action, hours.	Initial rotation.	Rotation after adding alkali.	Change of rotation.
—	- 1°48'	- 1°48'	0°0'
0.5	- 1 18	- 1 4	+ 0 14
1.0	- 0 45	- 0 20	+ 0 25
1.5	- 0 22	+ 0 2	+ 0 24
24.0	+ 0 32	+ 0 35	+ 0 3

Taking into account the following rotations :

Mandelonitrile glucoside .....	- 26.1°
$\alpha$ -Glucose.....	+ 105.0
$\beta$ -Glucose .....	+ 22.0
Equilibrium mixture of $\alpha$ - + $\beta$ -glucose .....	+ 52.5

it is obvious from the results set forth above that in the decomposition of mandelonitrile glucoside there is primarily liberated the low rotating  $\beta$ -glucose, which, on the addition of ammonia, is at once transformed into the equilibrium mixture of higher rotation, thus proving, what has been generally assumed, that Fischer's glucoside is a  $\beta$ -glucoside. As a result of this, it is certain also that the dextrose residue of the amygdalin biose which is attached to the benzaldehydecyanohydrin nucleus has also a  $\beta$ -configuration.

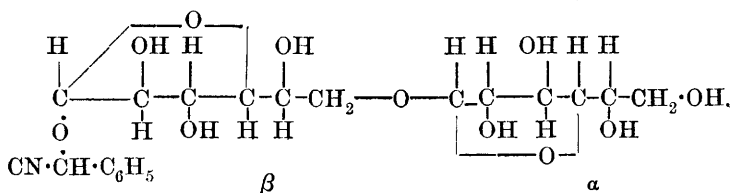
Although, as mentioned previously, it is impossible similarly to obtain direct evidence of the remaining dextrose molecule, the result can be attained by utilising the decomposition of amygdalin itself in a manner similar to that used for mandelonitrile glucoside. If both molecules of the biose are of  $\beta$ -configuration the positive change in rotation should be greatly increased, whereas, if the second molecule is derived from  $\alpha$ -glucose, the optical rotation of the sugars produced on liberation with emulsin will to a certain extent neutralise each other, and on production of the equilibrium there will be no change, or only a very slight decrease in the rotation. This was actually found to be the case, as by using strong solutions of amygdalin a slight decrease in the rotation could be detected :

Temperature = 40.5°.

Weight of amygdalin in 50 c.c. of H <sub>2</sub> O, grams.	Weight of emulsin added, gram.	Time of action, hours.	Initial rotation.	Rotation after adding alkali.	Change of rotation.
4.45	0.3	—	-6°48'	-6°48'	0° 0'
4.45	0.3	0.5	-3 54	-4 4	-0 10
4.45	0.3	1.0	-1 30	-1 45	-0 15
4.45	0.3	2.0	+2 51	+2 43	-0 8
4.45	0.3	24.0	+6 26	+6 26	-0 0
3.8	0.25	—	-4 48	-4 48	-0 0
3.8	0.25	0.66	-3 9	-3 18	-0 9
3.8	0.25	1.33	-0 23	-0 33	-0 10
3.8	0.25	20.0	+2 14	+2 14	0 0

Rotations measured in 2-dcm. tube.

From these results it would therefore seem to have been definitely proved that the biose from which amygdalin is derived is not maltose, but an  $\alpha\beta$ -diglucose, and that amygdalin should be formulated as follows :



Of extreme interest in this connexion is the fact, made apparent by the following experiments, that maltose, unlike glucose, has practically no inhibiting effect on the hydrolysis of amygdalin by emulsin.

*Effect of Maltose on the Velocity of Hydrolysis.*—For each experiment, 15 c.c. of 2 per cent. amygdalin solution were mixed with 3 c.c. of emulsin solution :

Temperature = 41.5°.

Time of action, mins.	Maltose added, gram.	HCN formed, gram.	Amygdalin decomposed, per cent.
30	—	0.00287	17.46
30	0.05	0.00284	17.27
30	0.10	0.00281	17.10
30	0.20	0.00283	17.23
30	0.30	0.00280	17.04

*Mode of Procedure of the Hydrolysis.*—Taking into account the three possible methods of decomposing amygdalin by emulsin, it will be observed that each involves the production of different quantities of the end-products. If mandelonitrile glucoside is formed as an intermediate product of the reaction and is hydrolysed more slowly than amygdalin (which seems to be the case), then estimation of the

hydrocyanic acid and the benzaldehyde formed should give (relatively) lower results than those obtained from the dextrose. The same holds good in the equilibrium stage of the hydrolysis, as practically no enzyme action is complete and consequently a certain amount of the intermediate product may be expected to remain undecomposed. Similarly, should the emulsin after attaching itself to the  $\beta$ -glucoside molecule, as it apparently must do in its rôle of  $\beta$ -enzyme, cause the intermediate formation of the unknown  $\alpha\beta$ -disaccharide, then the dextrose estimations should fall below those of the hydrocyanic acid and benzaldehyde; only in the event of the enzyme causing the disruption of the amygdalin molecule by simultaneous cleavage of the "biose" and "cyanhydrin" linkings would hydrocyanic acid, benzaldehyde, and dextrose be formed in quantities corresponding with the ordinary equation of amygdalin hydrolysis. It will be observed that the first method leads to the apparent anomaly of amygdalin being decomposed in the same manner by both yeast enzyme (Caldwell and Courtauld's amygdalase) and emulsin, which enzymes are of course really complementary to each other in their action. From one or two preliminary experiments carried out it was originally thought that the biose was formed as an intermediate product. This view, however, had to be considerably modified after careful and exhaustive series of experiments had been carried out. It was eventually found, in fact, that estimations of hydrocyanic acid and benzaldehyde constantly gave lower results than those of dextrose.

Care had to be taken to obtain the maximum hydrolysis in as short a time as possible so as to avoid any error due to partial decomposition of hydrocyanic acid on standing. A correction had also to be applied for the reducing power of the enzyme added, which was found to be considerable.

To avoid repetition and to save space, only a certain number of the results obtained will be quoted, and all these are included in the following table:

Time of action, hours.	Amygdalin used, grams.	Emulsin used, gram.	HCN formed, gram.	Cu <sub>2</sub> O formed (corrected), grams.	Decomposition from HCN, per cent.	Decomposition from C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> , per cent.*	Decomposition from C <sub>6</sub> H <sub>5</sub> ·CHO, per cent.†
0·5	0·4	0·03	0·00556	0·6090	23·5	27·8	—
1·0	0·4	0·03	0·01000	1·0520	42·5	48·0	—
1·25	0·4	0·03	0·01187	1·2800	50·2	58·4	49·9
2·25	0·4	0·03	0·01772	1·8610	75·0	85·1	75·8
16·0	1·2	0·10	0·06501	—	91·7	—	91·0
20·0	1·5	0·20	0·07665	2·0800	93·4	94·9	94·0
4·0	0·3	0·06	0·01430	1·9900	87·0	90·8	—
1·75	0·3	0·06	0·01330	1·9300	81·5	88·1	—
0·25	0·3	0·04	0·00469	—	28·4	—	28·6
24·0	1·5	0·30	0·07969	2·1358	97·0	97·5	96·5

\* Estimated gravimetrically.

† Estimated by Ripper's method.

These results make it appear certain that the reaction proceeds (at any rate mostly) in two stages, and that the biose linking in amygdalin is less resistant towards emulsin than that of the benzaldehydecyanohydrin. In this way mandelonitrile glucoside is formed preferentially and should appear among the reaction products. By stopping the reaction at a suitable stage, Fischer's glucoside can actually be separated. 0.5 Gram of emulsin was allowed to act on 20 grams of amygdalin and the reaction stopped when 75 per cent. of the amygdalin had been decomposed as calculated from the hydrocyanic acid liberated. The liquid was boiled to coagulate the protein matter, filtered and evaporated to dryness with animal charcoal, the charcoal being subsequently extracted with dry ethyl acetate. After repeating this process, the concentrated syrup was allowed to stand, when the mandelonitrile glucoside separated in small needles, melting, after recrystallisation, at 148°.

Considering this remarkable intermediate formation of mandelonitrile glucoside in the hydrolysis of amygdalin by emulsin, which is thus identical with that of acids (Caldwell and Courtauld, *Trans.*, 1907, **91**, 666), it is curious that Fischer's glucoside has not so far been discovered co-existent with amygdalin in Nature.

CHEMICAL DEPARTMENT,  
EAST LONDON COLLEGE.

---