LII.—*The Catalytic Racemisation of Amygdalin.*

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In a communication to the Royal Society of Canada in May, 1902, it was shown by the author and another that, under certain conditions, amygdalin yields, on hydrolysis, inactive mandelic acid instead of the lævo-variety. The latter is obtained, along with ammonium chloride and dextrose, when amygdalin is heated with concentrated hydrochloric acid. This result furnishes, as is well known, one of the arguments for ascribing to the glucoside the constitutional formula CHPh(M)·CN, where M represents a disaccharide radicle, most probably maltose (E. Fischer, *Ber.*, 1895, **28**, 1510). The change consists in a reaction between amygdalin and water leading to the formation

of *l*-mandelic acid, $CHPh(OH)\cdot CO_2H$, the essential product from the point of view of this research. When amygdalin is boiled with a dilute aqueous solution of an alkali, it is converted into a salt of amygdalinic acid, $CHPh(M)\cdot CO_2H$ (Liebig and Wöhler, *Annalen*, 1837, **22**, 11). From analogy, it might be expected that aqueous hydrochloric acid would hydrolyse amygdalinic acid into dextrose and *l*-mandelic acid. And since even dilute hydrochloric acid could be employed without fear of benzaldehyde being produced—as happens when amygdalin and dilute hydrochloric acid are heated together—it was anticipated that by this method the yield of *l*-mandelic acid would be better than that obtained by the direct hydrolysis of amygdalin with strong hydrochloric acid. Instead of yielding the active acid, however, amygdalinic acid gave invariably inactive mandelic acid, both when caustic potash and baryta were used in its preparation and also when it was hydrolysed in daylight or in darkness with hydrochloric acid varying in strength from seminormal to concentrated. The phenomenon seemed of the same nature as several which have been recorded where racemisation takes place when an atom or group directly attached to an asymmetric carbon atom is replaced by another radicle. Besides the supposed analogy to similar cases, however, this one possesses a special interest because, if the racemisation of amygdalinic acid takes place when the maltose radicle is replaced by hydroxyl, some as yet unknown influence must be at work to prevent racemisation when the same substitution is being effected by the same agent under the same conditions in the molecule of amygdalin. The study of the reaction has therefore been continued, and the results show that most probably racemisation does not take place either during the course of substitution of the maltose radicle, or when the nitrile is being hydrolysed to carboxyl. On the contrary, it is effected, without any apparent chemical action, whenever amygdalin is dissolved in dilute alkaline solutions. Whether the immediate product of this change is racemic amygdalin or not is not yet absolutely certain, since the substance is non-crystalline and its aqueous solution evaporates to a gum not easily purified for analysis, but the experimental results are readily explained on this assumption; moreover, the amygdalinic acid of Liebig and Wöhler (*loc. cit.*) is racemoid as regards its mandelic asymmetric carbon atom, whilst its lævo-isomeride, possessed of a much higher degree of optical activity, is to be obtained by the partial hydrolysis of amygdalin with strong hydrochloric acid. The isolation and further study of these substances is in progress.

Experimental.

The method adopted was to examine polarimetrically the rate of progress of the various reactions involved. The specific rotation of the amygdalin was determined in aqueous solution at 20°:

$l = 1$ dm., $c = 10$, $\alpha_D = -3{\cdot}54°$, hence $[\alpha]_D^{20°} = -35{\cdot}4°$.
„ 2 „ „ 4, „ $-2{\cdot}915°$, „ „ $-36{\cdot}45°$.

The rotatory power therefore changes but slightly with the concentration. The transformation of amygdalin into amygdalinic acid by treatment with aqueous baryta is a reaction which proceeds very readily. The baryta solution contained 2·338 grams of $Ba(OH)_2$ in 100 c.c., and 25 grams of amygdalin were dissolved in 250 c.c. of solution. After filtration, the reading in a 1 dcm. tube was $-4{\cdot}3°$. A portion was heated on a boiling water-bath for 10 minutes under a reflux condenser, cooled, and again examined. The observed angle was now $-5{\cdot}75°$, and it showed no further change after heating for 30 minutes longer. After remaining at 20° for 6 days, the rotation of the solution was found to have changed to $-5{\cdot}75°$; hence, in this case also, the reaction was complete. When this solution, concentrated by evaporation, was hydrolysed with strong hydrochloric acid for 2 hours on the water-bath, it yielded, of course, inactive mandelic acid. The substitution of hydroxyl for the maltose radicle, on the other hand, although it proceeds quite rapidly at 100°, takes, at the ordinary temperature, a much longer time than the hydrolysis of the nitrile.

Amygdalin (50 grams) was boiled in an open flask with 500 c.c. of the baryta solution until all the ammonia was expelled, the theoretical quantity of oxalic acid was then added to precipitate all the barium, and the filtered solution evaporated to a syrup. When cold, this was dissolved in hydrochloric acid of sp. gr. 1·115, the solution being made up to 250 c.c. with the same solvent. This solution, when examined at intervals in the polarimeter, gave the following results ($l = 2$ dm., $t = 20°$ approximately):

Table I.

Time.	α_D.	Time.	α_D.
0	$-22{\cdot}0°$	4 weeks	$-5{\cdot}0°$
12 hours	$-21{\cdot}7$	8 „	$+2{\cdot}0$
3 days	$-20{\cdot}5$	15 „	$+17{\cdot}0$
2 weeks	$-12{\cdot}0$		

The final reading is not accurate to less than one degree, as the solution had become dark in colour, but it corresponds within the limit of accuracy with the value to be expected from the amount

of dextrose (+15·8°). The mandelic acid produced from amygdalinic acid, even at the ordinary temperature, is therefore also inactive.

The difference in the rates at which amygdalinic acid is produced by dilute baryta solution, and hydrolysed by comparatively strong hydrochloric acid, was very striking, and suggested an examination of the velocity of hydrolysis of amygdalin by hydrochloric acid alone. The following data show clearly the separation of the reaction into two parts, one of which proceeds with a velocity comparable with that due to baryta and is complete after six days. Most probably the first consists also in the transformation of the nitrile radicle into carboxyl, whilst the second—the replacement of the maltose by hydroxyl—goes on very slowly, as indicated in Table I. Ten grams of amygdalin were dissolved in hydrochloric acid (sp. gr. 1·1), the solution being made up to 50 c.c. with the same solvent and filtered rapidly. The observed readings were as follows ($l = 2$ dm., $t = 20°$ approximately):

TABLE II.

Time.	α_D.	Change of α_D.	Time.	α_D.	Change of α_D.
0	−13·5°	—	71 hours	−40·0°	26·5°
1 hour	−14·43	0·93°	95 ,,	−41·3	27·8
3 hours	−16·12	2·62	119 ,,	−42·44	28·94°
6·5 ,,	−18·8	5·30	143 ,,	−42·78	29·28°
22 ,,	−27·5	14·00	220 ,,	−40·7	
24·5 ,,	−28·35	14·85	346 ,,	−35·8	
47 ,,	−35·68	22·18			

The change has not yet been followed further, but the final rotation which the solution should show when all the amygdalin has been converted into dextrose and *l*-mandelic acid is easily calculated from the known specific rotations of these two substances. The concentration of the mandelic acid will be 6·28 per cent., its specific rotation, from my own determinations, is about −163°, and the corresponding values for dextrose are 14·89 per cent. and +53°. In a 1 dcm. tube, the resulting rotation will therefore be −10·24° + 7·89° = −2·33°. Practically the final rotation was easily reached by warming a portion of the solution for 7 minutes on the water-bath. The liquid became somewhat red in colour, and therefore the observation was not very exact, but it was found to be about −2°, the value remaining constant after warming in the same way for half an hour longer. After 2 hours' heating, the solution became so dark in colour that polarimetric observations could not be taken, but on treatment with ether it gave a very active ethereal extract, which, on evaporation, left a crystalline solid, easily identified by its melting point as *l*-mandelic acid. A comparison of the values in the above table shows that the first reaction is almost complete in 120 hours, while

the second has proceeded to a very slight extent. One may therefore, with only a small error, take 30° as the total optical change produced in passing from the nitrile to the carboxylic acid, since the rotation of the small amount of mandelic acid formed almost exactly neutralises that of the corresponding amount of dextrose. An almost constant value of K is obtained on applying to the above values the equation for a unimolecular reaction, $K=\frac{1}{t}\log_e\frac{A}{A-x}$, where t = time in hours, $A=30°$, and $x=0{\cdot}93°$.

TABLE III.

$t=1$	$K=0{\cdot}01368$	$t=47$	$K=0{\cdot}01242$
3	0·01323	71	0·01314
6·5	0·01299	95	0·01194
22	0·01241	119	0·01220
24·5	0·01211		

The results in Table I, which indicate the velocity of the second reaction (the hydrolysis of the maltose radicle), when treated in the same way, also give an approximately constant value 0·0003, so that this change proceeds about 40 times more slowly than the first.

The results indicated in Tables I and II were obtained with solutions of the same concentration with respect to amygdalin. The first table shows that Liebig and Wöhler's amygdalinic acid produced by baryta gives in 20 per cent. solution in the 2 dcm. tube an angle of rotation of $-22°$, whilst the second shows that the amygdalinic acid produced by hydrochloric acid has a rotation of about $-42°$ in the same tube, and at the same concentration. Liebig and Wöhler's amygdalinic acid is, therefore, already racemoid with respect to its mandelic asymmetric carbon atom. The results thus far, therefore, pointed to racemisation taking place in the preparation of the amygdalinic acid, that is, during the hydrolysis of the nitrile, since the second stage of the reaction, namely, the substitution of the maltose radicle, is the same in both cases. A reaction of this nature would be sufficiently remarkable to warrant further investigation, because, although instances of racemisation taking place when one of the atoms or groups directly attached to an asymmetric carbon atom is replaced by another atom or group are fairly common when a halogen is the atom undergoing replacement, yet there are but few recorded cases of its occurrence when the atom replaced is not so situated (propoxyphenylacetic acid, McKenzie, Trans., 1899, **75**, 764). Another consideration, however, led to the conclusion that racemisation might take place even earlier than this. The specific rotation of amygdalin freshly dissolved in hydrochloric acid solution was

found in the last experiment to be $-33{\cdot}75°$, a value differing but little from that in pure water, whilst in a baryta solution newly prepared and having no odour of ammonia it was found in the first experiment to be about $-43°$. The discrepancy between this and the value $-35{\cdot}4°$, found in aqueous solution, might, however, be due to the action of the baryta on one of the hydroxyl groups of the maltose radicle. To settle this point, 10 grams of amygdalin were dissolved in 50 c.c. of the baryta solution. After 10 minutes, and before any odour of ammonia could be detected, 50 c.c. of a dilute sulphuric acid solution, containing the theoretical quantity of H_2SO_4 to precipitate all the barium, were added. After settling and filtering, the rotation of the solution at 20° was found to be $\alpha_D = -4{\cdot}32°$, $l = 1$ dcm., hence $[\alpha]_D^{20°} = -43{\cdot}2°$. The increased rotation of the amygdalin is therefore not due to the presence of, or to combination with, the baryta, since the specific rotation remains unaltered after the barium has been precipitated. When evaporated down, either on the water-bath or spontaneously, this solution did not crystallise, even after some time, but left a viscous gum. It appears, therefore, that solution in dilute aqueous baryta produces in a very short time, without any appreciable hydrolysis of the nitrile radicle having taken place, a very marked change, not only in the rotatory power, but also in the solubility of amygdalin. In order to determine whether racemisation had already taken place, a similar solution to the last one was prepared and, after 10 minutes, was almost neutralised with hydrochloric acid; it was then evaporated to a syrup which, when cold, was dissolved in hydrochloric acid (sp. gr. 1·1), the volume being made up to 50 c.c. with the same solvent. The rotation of this solution in the 1 dcm. tube was $-10°$. A portion of the solution, when heated for 10 minutes to 67° only, to avoid darkening in colour, gave a rotation of $-8{\cdot}55°$, and after 10 minutes' additional heating at the same temperature $+7{\cdot}25°$. The rotation due to the dextrose alone if all were hydrolysed would be $+7{\cdot}9°$. The solution was further heated on a boiling water-bath for 10 minutes and, as it had become too dark for examination in the polarimeter, was extracted with ether. The ethereal extract showed only very little activity, and left on evaporation, a crystalline solid, easily identified as *i*-mandelic acid by a determination of its melting point. Racemisation is therefore effected by simple solution in dilute aqueous baryta. A second portion of the solution in aqueous hydrochloric acid was examined in the 2 dcm. tube at the room-temperature (about 20°) at various intervals:

TABLE IV.

Time.	α_D.	Change of α_D.	Time.	α_D.	Change of α_D.
0	−20·0°	—	67 hours	−20·95°	−0·95°
4 hours	−20·1	−0·1°	76 ,,	−20·91	−0·91
19 ,,	−20·85	−0·85	250 ,,	−14·2	+5·80
43 ,,	−21·0	−1·0	296 ,,	−13·32	+6·68
			∞ ,,	+15·8	

In this case also, as with *l*-amygdalin (Table II), it is evident that the hydrolysis takes place in two stages, and a very strong indication that the new substance is racemoid amygdalin is seen in the general correspondence of the velocities of the two reactions as indicated by the change in rotation. The total change from the most lævo- to the most dextro-reading is about 37° in both cases, and after 300 hours both have become altered to approximately the same extent, the amount of transformation being 7/37ths of the entire change represented by the second stage. In the case of the racemoid substance, however, the first stage is accompanied by only a very small change in optical power, whilst with *l*-amygdalin the corresponding stage involves a very large change of rotation.

If the racemisation of amygdalin is, as the foregoing observations seem to indicate, a catalytic action induced by the presence of alkalis, a very small quantity of the latter ought to effect the change, although not so rapidly. It might also be brought about in a longer time by the alkaline carbonates. The following experiments confirm this supposition. Amygdalin (10 grams) was placed in a 50 c.c. flask and some water was added. Then 10 c.c. of the baryta solution were run in and the flask was filled to the mark with water. It took 15 minutes' shaking to effect solution, whereas 50 c.c. of the same baryta solution dissolve 10 grams of amygdalin almost instantly. The rotation observed in the 1 dcm. tube was −10·15°, and this remained unchanged after 16 hours. Twenty-five c.c. of the solution were then saturated with carbonic acid and filtered. The optical power was almost unchanged, namely, −9·88°. The solution was evaporated to a syrup, the residue being dissolved in hydrochloric acid (sp. gr. 1·1), the final volume of which is made up to 25 c.c. The rotation was now −9·57°, $l = 1$ dcm., and this remained almost constant for two days, as was found previously for the racemoid substance (see Table IV). A portion of the hydrochloric acid solution was warmed on the water-bath at a temperature of nearly 100° for 10 minutes, when its rotation changed to −4·58°, and after 10 minutes' additional heating to +5°. The mandelic acid

produced was therefore inactive, this final dextro-rotation being due to the dextrose produced. A similar experiment was performed in which only 5 c.c. of the baryta solution were employed for the same weight of amygdalin, and, although the glucoside took a slightly longer time to dissolve, yet the rotation of the resulting solution was the same. In this last experiment, the proportion is only 0·03 mol. of barium hydroxide to 1 mol. of amygdalin, and the effect is the more striking as it causes 10 grams of amygdalin to dissolve in 50 c.c., whereas 120 c.c. of pure water are required for the same weight of the glucoside at the ordinary temperature. A very small quantity of potassium carbonate was also found to induce racemisation. Ten grams of amygdalin were placed in a 50 c.c. flask, which was filled to the mark with a solution containing 0·1886 gram K_2CO_3 in 100 c.c. of water, this being equivalent to using 10 c.c. of the baryta solution. It took two hours' constant shaking to effect solution, and the rotatory power in the dcm. tube was almost the same as in the last two experiments, namely, $\alpha_D = -9·7°$. The racemisation of amygdalin is apparently, therefore, a catalytic action, depending most probably on the presence of hydroxyl ions in the solution.

That daylight has no effect in inducing the action in the presence of baryta was shown by an experiment similar to one already described, where 10 grams of amygdalin, 50 c.c. of baryta solution, and 50 c.c. of an equivalent sulphuric acid solution were employed. The whole operation was performed in the dark, but in this case also the product was racemoid.

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