XXI.—*Formation of Pyridine Derivatives from Malic Acid.*

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THE nature of the chemical changes which take place in the vegetable organism and lead to the absorption of nitrogen is still unexplained; and although there can be no doubt that the nitrogen enters the plant in the form of ammonia and nitric acid, we are unable to follow the steps by which nitrogenous compounds, especially the alkaloids and albuminoids, are elaborated.

The study of the alkaloids has led, as first suggested by Koenigs (*Studien über die Alkaloide*, München, 1880), to their being regarded as derivatives of pyridine; for although no alkaloid has been artificially prepared hitherto, numerous methods are already known for the synthesis of compounds which must be regarded as pyridine derivatives, and which stand in close relation to the natural alkaloids. Assuming that pyridine compounds are formed in plants, we may endeavour to ascertain what is the nature of the reactions which take place in these natural syntheses.

If, in the formation of alkaloïds, it is taken for granted that nitrogen is supplied in the form of ammonia and amido-compounds, non-nitrogenous carbon compounds must also take part in the reaction. Such processes, however, can only be explained if we know substances which, under the ordinary conditions of pressure and temperature, unite with the above-named nitrogen compounds to form pyridine derivatives. As already remarked by V. Meyer and E. Schulze (*Ber.*, 1884, 1554), no such substances have hitherto been known, but we have now, in *coumalinic acid*, which is very easily formed from malic acid, a body which (in its ethereal salts) fulfils the required conditions.

As one of us has recently shown (*Ber.*, 1884, 936), malic acid when acted on by concentrated sulphuric acid, is split up into formic acid and the half-aldehyde of malonic acid; 2 mols. of the latter in the nascent state then undergo condensation with elimination of the elements of 2 mols. of water, and form coumalinic acid, $C_5H_3O_2.COOH$; this is shown by the equation—

Coumalinic acid at once enters into reaction with ammonia even at the ordinary temperature, forming hydroxynicotinic acid. Thus:—

Primary amines and amido-acids behave similarly with coumalinic acid, reacting with it to form hydroxypyridinecarboxylic acids. The production from malic acid of derivatives of nicotinic acid, which, as is well known, stands in close relation to numerous alkaloïds, is of especial interest, in so far as similar changes probably take place in plants.

The occurrence of chelidonic acid and meconic acid in certain Papaveraceæ is well known, and although the constitution of these compounds is not yet ascertained, important indications of their true nature have been obtained. A comparison of their composition and properties discloses analogies between these vegetable acids and coumalinic acid. They may all be regarded as derivatives of a group of the empirical formula $C_6H_4O_2$, although the constitution of this group does not appear to be invariable. Thus:—

Chelidonic and meconic acids form two series of salts, of which one series is colourless, the other yellow; coumalinic acid also possesses this property. Chelidonic acid and members of the meconic

acid group, as has been specially established in the case of comanic acid, are decomposed when boiled with alkalis or alkaline earths, acetone being one of the products. Under like conditions, coumalinic acid yields a compound having the character of an aldehyde and probably identical with crotonaldehyde. Finally, in their peculiar behaviour with ammonia, these acids exhibit the greatest resemblance, being converted into hydroxypyridinecarboxylic acids. Chelidonic acid, for example, reacts with ammonia to form hydroxypyridinecarboxylic acid—

This reaction corresponds with that of the conversion of coumalinic acid into hydroxypyridinemonocarboxylic acid—

It is to be noted that in the case of coumalinic acid this reaction takes place at the ordinary temperature; whereas, in the case of the other acids, heat or increased pressure is necessary.

Bearing in mind, then, that chelidonic and meconic acids are invariably accompanied in the plants in which they occur, not only by malic acid, citric acid, and similar simple vegetable acids, but also by alkaloids; moreover, that chelidonic and meconic acids are so closely analogous to coumalinic acid; and considering, lastly, the readiness with which coumalinic acid is prepared from malic acid, and may be transformed into pyridine derivatives: it seems probable that the acids in question bear a close genetic relation to malic acid, coumalinic acid, and the pyridine compounds derived from them. Hence the formation of pyridine derivatives from malic acid is of interest from the point of view of vegetable physiology, and affords experimental material which may serve to explain the natural formation of the alkaloids.

The study of the products obtained by the action of ammonia and amido-compounds on coumalinic acid has led to an explanation of their constitution, and affords an insight into the method of their formation. On account of its instability, coumalinic acid is not well adapted for the investigation of this reaction, but the methyl salt described in a previous communication furnishes almost quantitative results.

Ammonia and coumalinic acid, in the form of one of its ethereal salts, as already explained, form a hydroxypyridinecarboxylic acid (hydroxynicotinic acid), which it will be proved has the constitutional formula—

and which is identical with that prepared by Koenigs and Geigy (*Ber.*, 1884, 589) from hydroxyquinolinic acid by elimination of carbonic anhydride. Koenigs and Körner (*Ber.*, 1883, 2152) have proved that when cinchoninic acid is fused with potash, a hydroxyl-group attaches itself to the carbon-atom next to the nitrogen of the pyridine-ring, and from this Koenigs and Geigy have inferred that the hydroxyl-group likewise takes the same position in the hydroxyquinolinic acid prepared by an analogous reaction from quinolinic acid; this conclusion has been confirmed by investigation.

According to Friedländer and Ostermaier (*Ber.*, 1882, 332), in the chloroquinoline prepared by the action of phosphorous pentachloride on carbostyryl, the chlorine is readily displaced by H, C₂H₅O, &c., a behaviour which is characteristic of pyridine-derivatives in which the radicle is represented as contiguous to the nitrogen-atom. Now since hydroxypyridinecarboxylic acid can be converted into a chloropyridinecarboxylic acid of which the chlorine-atom is characterised by the same mobility as that of the chloroquinoline in question, it follows that this atom in the formula of the chlorinated acid, and the corresponding hydroxyl-group in the hydroxypyridinecarboxylic acid, likewise occupy the position contiguous to the nitrogen.

Finally, since the pyridinecarboxylic acid obtained from chloropyridinecarboxylic acid by displacing the chlorine by hydrogen has been proved to be identical with nicotinic acid, and in this it is certain that the carboxyl-group and the nitrogen are relatively in the position N:COOH = 1:3, there remains for hydroxynicotinic acid only the choice between the two following formulæ:—

Of these, the first must be the correct one, as it alone explains the formation of this acid from hydroxyquinolinic acid,

It now remains to explain how coumalinic acid is converted into hydroxynicotinic acid.

Although the correctness of the following formula for coumalinic acid,

proposed by one of us in a former communication, has not yet been experimentally proved, it may be provisionally used, since no observations hitherto made on the acid are irreconcilable with it. The production of such a compound from the half-aldehyde of malic acid, $\text{COH} \cdot \text{CH}_2 \cdot \text{COOH}$, may be supposed to consist in the conversion of the aldehyde—by the alternate addition and loss of water—into a body of the composition $\text{CH}(\text{OH}) \cdot \text{CH} \cdot \text{COOH}$, of which two molecules condense in the following manner:—

The formation of hydroxynicotinic acid from coumalinic acid may be supposed to take place in two stages; in the first place, the elements of ammonia are added to the coumalinic acid, whereby the latter is transformed into an amido-acid, coumalamic acid:—

Although the instability of this compound prevents its isolation, there can be no doubt of its existence, since it is possible to prepare coumalanilidic acid, or rather its acid methyl salt, a perfectly analogous intermediate product formed by the action of aniline:—

This substance has also been analysed.

In the second phase of the reaction, coumalamic acid loses water, forming hydroxynicotinic acid:—

It might be expected that coualmethamic acid, the primary product of the action of methylamine, would resemble pseudoisatin in its behaviour, and would undergo the following change:—

It has been found, however, that the product of this reaction is identical with the methyl salt of the acid obtained by methylating hydroxynicotinic acid,

To explain this fact, it is necessary to assume a movement of the methyl-group from the nitrogen to the oxygen of the neighbouring ketone-group. This intramolecular change acquires additional interest from the analogous observations which Lieben and Haitinger (*Ber.*, 1884, 1507) have made on chelidonic acid and its pyridine-derivatives, showing that the parallelism existing between chelidonic and coumalinic acids may be extended to these characteristic features.

Hydroxynicotinic Acid,

The formation of this acid, and of its ethereal salt, always takes place when ammonia, or an aqueous solution of ammonium carbonate, is brought in contact with coumalinic acid or its ethereal salt. On account of its instability in alkaline solution, coumalinic acid yields but traces of nitrogenous substances; an abundant yield may, however, be obtained from its acid ethereal salts. If, for example, the methyl salt be mixed with dilute ammonia, and the excess of the latter be removed by boiling or allowing the fluid to remain at the ordinary temperature, colourless needles of the methyl salt of hydroxynicotinic acid are obtained. The following is the best process of preparing hydroxynicotinic acid:—1 part of finely powdered coumalinic acid is gradually added to a cold 15 per cent. solution of ammonia, and as soon as it is dissolved 6 parts of caustic soda solution (1 to 5) is added, and the whole boiled for five minutes. On adding concentrated hydrochloric acid to the cool solution, hydroxynicotinic acid separates out in the form of a colourless, heavy, crystalline powder, which may be purified by recrystallising it from 50 per cent. acetic acid. The acid may be obtained in colourless needles by recrystallisation from boiling water to which animal charcoal has been added:—

	Calculated for $\text{C}_6\text{H}_5\text{NO}_3$.	Found.
C	51·8	51·5 per cent.
H	3·6	3·9 "
N	10·1	9·5 "

The acid melts at 303°, becoming discoloured and evolving gas, but it may be sublimed by carefully heating. It is sparingly soluble in boiling water, almost insoluble in alcohol, ether, chloroform, and benzene, but more easily soluble in acid liquids. It has no basic properties,

and can be recrystallised from concentrated hydrochloric acid without undergoing any change. Its aqueous solution gives no precipitates with metallic salts in the cold, but on boiling with copper acetate a light green copper salt separates out. The alkaline salts crystallise well, the lead salt forming white needles containing water of crystallisation. Ferric chloride does not give any characteristic reaction.

On heating it with zinc-dust, pyridine is formed in large quantities. If the acid is heated to a temperature above its melting point, it splits up into carbonic anhydride and hydroxypyridine, $[N:OH = 1:2]$, which is also obtained by the action of hydrochloric acid at 200° , or by distilling the silver salt. The hydroxypyridine gives a reddish-yellow coloration with ferric chloride, and with bromine-water a brominated derivative, crystallising in needles and melting at 206° .

All these facts confirm the identity of the hydroxynicotinic acid with the hydroxypyridinecarboxylic acid of Kœnigs and Geigy.

Chloronicotinic Acid, $C_5H_3NCl.COOH$.

If three molecular proportions of phosphorous pentachloride are gradually added to hydroxynicotinic acid covered with phosphorous oxychloride, and the mixture is heated at $120-130^\circ$, an energetic reaction takes place, and the substance dissolves. On pouring the product of the reaction into ice-cold water, the chlorinated acid separates out as a voluminous crystalline mass, which may be purified by recrystallising from hot water. The yield is theoretical:—

	Calculated for $C_5H_3NClO_2$.	Found.
C	45.7	46.1 per cent.
H	2.6	2.8 ,,
N	8.8	8.7 ,,
Cl	22.5	22.1 ,,

On carefully heating it, the acid sublimes, and melts at 199° with decomposition. It is much more easily soluble in water than the hydroxy-acid, crystallising from it in shining plates; it is also easily soluble in ether, alcohol, and glacial acetic acid, sparingly in chloroform and benzene. It is easily dissolved by concentrated hydrochloric acid, but separates out again on diluting with water. The aqueous solution gives a light green precipitate with copper acetate, especially when hot. By boiling with alcoholic potash, as well as by the action of reducing agents, substances are obtained which are free from chlorine.

Nicotinic Acid, $C_5H_4N.COOH$.

The best way of transforming the chloronicotinic acid into nicotinic acid is by the action of tin and hydrochloric acid. It is heated on the

water-bath until a sample remains clear on addition of water, which, if 5 grams be used, is the case after 15 to 20 minutes. The excess of hydrochloric acid is then got rid of, and the residue is dissolved in water, the tin removed by means of sulphuretted hydrogen, and the solution made slightly alkaline with soda and then boiled with copper acetate. Copper nicotinate is thus obtained as a greenish-blue precipitate, which, after being well washed, is suspended in water, and decomposed with sulphuretted hydrogen. On evaporating the filtrate, slender needles separate out, which melt at 229° , and exhibit all the reactions of nicotinic acid.

Monomethyl Salt of Coumalanilidic Acid,

This well characterised substance may serve as a type of the compounds formed by the action of primary amines on the ethereal salts of coumalinic acid. It is formed as represented by the equation

Bases such as methylamine, ethylamine, and glycocine, form in this way colourless addition-products, whereas the amido-compounds of the aromatic series, as, for example, aniline, toluidine, phenylhydrazine, amidobenzoic acid, and amidoquinoline, give yellow-coloured bodies.

For the preparation of the new compound, the methyl salt of coumalinic acid in alcoholic or dilute acetic acid solution is treated with aniline. If the degree of concentration be rightly gauged, the whole liquid after some moments becomes a solid mass of yellow crystals, a considerable amount of heat being developed. By one recrystallisation from hot alcohol, the substance is obtained in the form of lemon-yellow needles, sufficiently pure for analysis.

	Calculated for $\text{C}_{13}\text{H}_{13}\text{NO}_4$.	Found.	
C	63.16	63.1	62.9 per cent.
H	5.3	5.6	5.8 „
N	5.7	5.75	5.8 „

It melts at 140° with frothing. It is easily soluble in hot alcohol, chloroform, and benzene, sparingly in ether, and insoluble in water. It has the properties of an acid, and dissolves in cold solutions of the alkaline carbonates with a yellow colour, from which solution it is precipitated unchanged by acids. It has no basic properties.

This compound is characterised by the readiness with which it undergoes change. When boiled with alcohol, it is transformed into a colourless, beautifully crystalline body, which is insoluble in alkalis. It also undergoes decomposition by the action of water, acids, or alkalis; on heating it with concentrated ammonium carbonate or sodium acetate, the solution which at first is clear becomes milky, and colourless drops of oil separate. Of these products, only the one obtained by the action of soda has been studied, namely, the acid phenyl salt of hydroxynicotinic acid.

Phenoxynicotinic Acid, $C_6H_5N(OC_6H_5).COOH$.

This acid is formed from the amido-methyl salt just described by boiling it with a moderate amount of soda until the addition of acid to a sample of it causes the formation of a white precipitate after the lapse of a few minutes. The reaction consists in the passage of the phenyl from the nitrogen to the oxygen, of the saponification of the methyl salt, and at the same time of the elimination of the elements of water; this takes place as represented by the equation—

That the acid really has this constitution follows from the analogous and already mentioned mode of formation of methoxynicotinic acid. If the compound which separates out from the alkaline solution when completely decomposed by hydrochloric acid, is recrystallised from boiling water, it forms white needles which melt at 275—280°.

	Calculated for $C_{12}H_9NO_3$.	Found.
C.	67.0	67.0 per cent.
H	4.2	4.6 „
N	6.5	6.4 „

On carefully heating the substance, it sublimes in the form of characteristic voluminous needles, which resemble cotton-wool. It is soluble in hot water, alcohol, and glacial acetic acid, but almost insoluble in ether, chloroform, and benzene; on adding water to its solution in concentrated hydrochloric acid, it is precipitated unchanged. If the aqueous solution is boiled with copper acetate, a sparingly soluble precipitate is formed. On heating it with concentrated hydrochloric acid at 200°, carbonic anhydride is eliminated and a body formed which crystallises beautifully from ether; this

is insoluble in alkalis, and seems to be the phenyl ether derived from hydroxypyridine. This compound would consequently be as stable as the phenyl ether, $(C_6H_5)_2O$.

Methoxynicotinic Acid, $C_5H_3N(OCH_3).COOH$.

This acid, as already stated, can be obtained either by methylating hydroxynicotinic acid or from methylamine and coumalinic acid.

In preparing it from the hydroxy-acid, 3 grams were taken and evaporated to dryness with two molecular proportions of potassium hydroxide, and the finely powdered mass was then heated for three hours at $100-110^\circ$ with methyl alcohol and methyl iodide. After opening the tubes, the methyl alcohol and excess of iodide were expelled by boiling the contents, which were then made slightly acid and the product twice crystallised from boiling water.

To obtain this compound synthetically, the methyl salt of coumalinic acid is added to 4 parts of a 15 per cent. methylamine solution, which is kept cool and then immediately boiled with 5 parts of caustic soda solution (1.5) till completely saponified. When cold, it is carefully acidified and twice crystallised from water. The monomethyl salt of coumalmethamic acid, which may be regarded as an intermediate product, separates out on careful dilution of the solution of the monomethyl salt of coumalinic acid in methylamine in the form of colourless, easily soluble, crystalline needles.

By both methods, methoxynicotinic acid is obtained in very characteristic flattened needles, which contain a molecule of water of crystallisation; they lose the same, however, in a vacuum or at 100° , and become opaque.

	Calculated for $C_7NH_7NO_3 + H_2O$.	Found.
H_2O	10.5	10.5 per cent.

The dried substance gave the following numbers, of which those under I are from the hydroxy-acid, and those under II from material synthetically prepared.

	Calculated for $C_7H_7NO_3$.	Found.	
		I.	II.
C	54.9	54.8	55.3
H	4.6	4.5	4.6
N	9.1	9.6	—

Melting point $237-238^\circ$. The acid is almost insoluble in cold water, but dissolves so readily in boiling water that, as a rule, a hot saturated solution on cooling solidifies to a thick mass of crystals.

It is soluble in alcohol, ether, and glacial acetic acid, insoluble in chloroform and benzene. It has scarcely any basic properties.

The investigation will be continued and extended to other fatty acids.