THE DETECTION OF ZINC IN CADAVERIC MATERIAL.

THE DECOMPOSITION OF COCAINE IN PRESENCE OF PUTREFYING ORGANIC MATTER.

THE FIXATION OF ALKALOIDS BY FORMALDEHYDE.

BY

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To Professor M. A. Parker, who suggested the problems dealt with herein, and who supervised with the greatest patience all the work carried out in their connection, the thanks and appreciation of the writer are offered.

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INTRODUCTION

INTRODUCTION

In an examination for poisonous substances of a cadaver, Parker (1) obtained the following surprising result.

A sample of the cadaver was treated with hydrochloric acid and potassium chlorate in order to destroy organic matter, and the excess hydrochloric acid removed from the solution by partial evaporation and neutralisation with ammonia. The solution, just acid to litmus, was then saturated with hydrogen sulphide and the precipitate examined as in the ordinary qualitative analysis. Arsenic, and the usual poisonous metals of the second group were soon shown to be absent; but Parker carried the analysis carefully to completion, and obtained a sulphide precipitate in the same position as that of cadmium (which has never been found in connection with a poisoning fatality). This sulphide precipitate was white, however, and was finally shown definitely to be zinc sulphide. The filtrate from the hydrogen sulphide precipitate did not contain any zinc.

In the following pages a study is made of the detection of zinc in cadaveric material, and it is shown that the method for the removal of excess hydrochloric acid used by Parker in the above experiment, is liable to give misleading results, as Parker found. An explanation in theory of the failure of this method is put forth, and an attempt is made to back up the supposition with experimental facts.

THEORETICAL CONSIDERATIONS

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In the analysis of cadaver material for metallic poisons, the organic matter must be destroyed as completely as possible before the regular qualitative tests for metallic ions can be carried through. Perhaps the best known procedure for the destruction of organic matter in this connection is that of R. Fresenius and L. von Babo (2). This is described by Autenrieth (3) as follows:

> " A portion of the original material, previously finely chopped and well mixed with enough water to produce a fluid mass, may --- be used. According to the quantity of material to be destroyed, add 10 - 15 cc. of pure concentrated hydrochloric acid and 1 - 2 grams of potassium chlorate. Set the flask upon a boiling water bath in a good draught, heat and shake frequently so that chlorine comes into intimate contact with material to be destroyed. When the mixture is hot, add 0.3 - 0.5 gram of potassium chlorate every 2 - 3 minutes and shake the flask frequently. Continue in this manner until most of the organic matter is destroyed and the liquid in the flask is clear, or turbid, and has a wine yellow colour. Further addition of potassium chlorate and longer heating should produce no change."

When the organic matter has been destroyed in this way, the mixture is diluted with hot water, a few drops of dilute sulphuric acid added to precipitate possible barium, and the liquid filtered. The filtrate now contains any metals formerly in the original material, along with (in most cases) considerable excess of free hydrochloric acid. Beforethe metals of the hydrogen sulphide group (arsenic, antimony, tin, mercury, lead, copper, bismuth and cadmium) can be precipitated, the excess of free hydrochloric acid must be almost entirely removed.

According to Autenrieth (4) this can be done in either of two ways:

> "--evaporate the solution in a porcelain dish upon the water bath nearly to dryness to remove most of the free hydrochloric acid. This step frequently produces a dark colour which a few crystals of potassium chlorate will discharge.
>
> An alternative procedure is to remove part of the free hydrochloric acid from the filtrate, obtained after treatment with hydrochloric acid and potassium chlorate, by first evaporating to a smaller volume and then adding ammonia until alkaline. Finally add dilute nitric acid until the solution is faintly acid."

The advantage of the second of these two processes is that it can be carried out in a much shorter period of time than the first. The writer has shown, however, first, that when this alternative method is used, Zinc, if present, is precipitated along with the metals of Group II, and may be readily overlooked as it appears in the same position in the analysis scheme as Cadmium, a metal rarely expected in a toxicological investigation. As will be demonstrated in the next section, this type of behaviour indicates the presence of a weak acid. Under the circumstances, the only weak acid to be expected would be a lower (soluble) fatty acid, formed in the oxidation of organic matter by chlorine. The writer has endeavored to prove, therefore, second, that the lower fatty acids are not rapidly decomposed by the treatment described above, and third, that fatty acids are actually present in the filtrate obtained after oxidation with potassium chlorate and hydrochloric acid.

THE DETECTION OF ZINC

Following the usual qualitative analysis scheme, the metals of the copper and arsenic groups (copper, lead, mercury, bismuth and cadmium; arsenic, antimony, and tin) are separated from those of the iron and zinc groups (iron, aluminium, and chromium; cobalt, nickel, manganese, and zinc) by saturating the solution with hydrogen sulphide in presence of a slight excess of hydrochloric or sulphuric acid. This separation depends on the production of a sufficiently high concentration of the hydrogen ion to keep the concentration of the sulphide ion below that necessary for the precipitation of sulphides of iron and zinc and the other metals of this type. If, therefore, the solution contains an acetate, or a salt of any weak acid, e.g. a butyrate or a phosphate, the addition of hydrochloric acid will result, at least at first, in the liberation of the weaker acid and will not produce the excess of hydrogen-ion required for the separation.

Unless provision is made, then, to insure a certain excess of hydrogen-ion, zinc (as well as nickel and cobalt) must be looked for in the hydrogen sulphide precipitate. Stieglitz (5) writes:

> " For the ordinary purposes of analysis, requiring the precipitation of say one milligram of any ion from 100 cc. solution (one-tenth percent, if one gram of substance is used for analysis), a concentration of hydrogen-ion of 0.1 to 0.3 gram-ion per litre forms a good basis for work. The presence of this concentration of hydrogen-ion, irrespective of the possible presence of weak organic or inorganic acids, may be readily insured by a simple test with an appropriate indicator. Methyl-violet is suitable

> "for such a purpose, because it is sensitive only to the rather high concentrations of hydrogen-ion required --- . The blue-green tint, with which one becomes easily familiar, and which can, indeed, always be prepared for matching tints, may be used to recognize speedily, and with sufficient accuracy, a concentration of the hydrogen-ion of the strength desired, irrespective of its source."

Provided this refinement were introduced, there is no doubt that Autenrieth's alternative procedure for the removal of excess hydrochloric acid, i.e. evaporation to more convenient volume and neutralisation with ammonia, might be used with entire satisfaction, even in the presence of zinc.

THE OXIDATION OF LOWER FATTY ACIDS

The fatty acids are well known to be exceedingly stable substances towards oxidising agents; with the exception of formic acid, they are converted into simpler compounds only with difficulty. According to Beilstein (6), acetic acid slowly reduces chromic acid on boiling, with production of an oxy-acetate of chromium; it is scarcely oxidised by dilute alkaline permanganate at 100 to 120 deg. C.; by heating with concentrated alkaline permanganate, oxalic acid is formed; oxidation with potassium persulphate produces a slight amount of succinic acid; and hydrogen peroxide acts on acetic acid giving a small quantity of glyoxylic acid. A few similar references were found to the difficulty of oxidising fatty acids by means of the above reagents, but the action of chlorine in hot aqueous solution does not

appear to have been tried. However, this reagent is a very powerful one, and it would be surprising if fatty acids were not, at least slowly, decomposed under its influence. The writer found difficulty in estimating the fatty acid in a mixture such as is obtained by treatment with potassium chlorate and hydrochloric acid, but the few experiments which were carried out show fairly definitely that acetic and butyric acids are not rapidly decomposed in this way.

EXPERIMENTAL RESULTS

EXPERIMENTAL

THE DETECTION OF ZINC

EXPERIMENT 1. Analysis of another sample of the cadaver examined by Parker.

The organic matter was destroyed by prolonged treatment with hydrochloric acid and potassium chlorate, as described above, and the excess acid removed by the alternative method, i.e., by evaporation to more convenient volume and neutralisation with ammonia. The solution, made just acid to litmus with hydrochloric acid, was heated in a flask on the steam bath, while a stream of hydrogen sulphide was passed through it for about one hour; the treatment with hydrogen sulphide was continued while the flask and its contents cooled. The flask was then loosely stoppered, and allowed to stand over night. In the morning the contents still smelled strongly of hydrogen sulphide, and precipitation was therefore assumed to be complete. The heavy precipitate was filtered off, and washed with saturated hydrogen sulphide solution; the filtrate and washings were combined. The sulphide precipitate was then examined for zinc, as follows.

A warm mixture of 10 cc. of concentrated nitric acid and 20 cc. of water was passed through the filter several times until the precipitate had been entirely dissolved. This solution was made alkaline with strong ammonia, and hydrogen sulphide passed for a short time: a grey precipitate appeared, and was allowed to settle. The liquid was rendered acid with acetic acid, filtered, and washed. After drying,

the filter and precipitate were moistened with a few drops of saturated ammonium nitrate solution and ignited in a porcelain crucible. The contents of the latter, when cold, were extracted with 3 cc. of boiling dilute sulphuric acid, and the resulting solution filtered and divided into two parts. To one part excess sodium hydroxide solution was added, the precipitated ferric phosphate filtered off, and hydrogen sulphide solution added to the filtrate: a flocculent white precipitate appeared on warming, and was shown, by fusion with cobalt nitrate, to contain zinc. The other portion was made strongly alkaline with ammonia, filtered, rendered acid with acetic acid, and saturated with hydrogen sulphide: a white precipitate was again produced, and the presence of zinc confirmed.

The filtrate from the hydrogen sulphide precipitate was evaporated to half volume, made alkaline with ammonia, and excess ammonium sulphide added. A dark precipitate was produced, and allowed to settle. The liquid was rendered faintly acid with acetic acid and allowed to stand over night. After being filtered off and washed, the precipitate was ignited with ammonium nitrate, and the examination for zinc carried through from this point exactly as in the previous case. But here, no indication was obtained of the presence of zinc.

This experiment thus confirms the original observation of Parker, namely, that when the above procedure is followed, zinc appears in the second group of the qualitative analysis scheme, and not in the third, where it belongs. This point is further supported by the results of the following two

artificial experiments.

EXPERIMENT II. Analysis of minced liver containing zinc oxide.

A mixture of about 300 grams of minced beef liver and 0.25 gram of zinc oxide was treated as follows. The material was shaken up with 300 cc. of water and 35 cc. of concentrated hydrochloric acid. 2 grams of potassium chlorate were added and the mixture heated on a boiling water bath; more chlorate was added in small quantities at a time over a period of about 8 hours, the flask being shaken frequently; the temperature of the mass averaged about 85 degrees Centigrade. The liquid was diluted with hot water, filtered, and the filtrate evaporated to a volume of about 200 cc. The latter was made alkaline with ammonium hydroxide, and then just acid to litmus with hydrochloric acid. Precipitation with hydrogen sulphide was carried out exactly as in Experiment 1., and the hydrogen sulphide precipitate shown to contain zinc. The filtrate from the hydrogen sulphide precipitate did not contain zinc.

EXPERIMENT III. Analysis of minced liver containing zinc oxide.

In this experiment, the sample consisted of 440 grams of minced liver and 0.5 gram of zinc oxide. This was mixed with 400 cc. of water and 45 cc. of concentrated hydrochloric acid, and potassium chlorate added at intervals over a period of two and one half hours at a temperature of about 90 degrees C. The filtrate from the oxidised mass was divided into two equal parts.

One part was evaporated to half volume, made alkaline with ammonia, and then just acid with hydrochloric acid (as in Experiments I. and II.). Hydrogen sulphide precipitated all the zinc from this solution, as in the previous experiments.

The other portion was evaporated almost to dryness (Autenrieth's first method for removing the excess hydrochloric acid) and the residue taken up with 150 cc. of water. The analysis of the hydrogen sulphide precipitate obtained from this solution was accidently spoiled, but the presence of zinc in the filtrate was confirmed, so that the loss was of no importance.

This experiment shows that the results obtained in Experiments I. and II. are not due to any peculiar conditions, since in the parallel analyses outlined above, the regular method gave the correct result, while the alternative method gave the anomalous result.

EXPERIMENT IV. Analysis of a mixture of zinc oxide and sodium acetate.

A mixture of 14 grams of sodium acetate and 0.5 gram of zinc oxide was dissolved in 200 cc. of water containing 30 cc. of concentrated hydrochloric acid. This was heated at 90 degrees C. for about two hours while potassium chlorate was being added, and then diluted and divided into two equal parts. In one part the excess hydrochloric acid was removed by the regular method (evaporation almost to dryness) while in the other part it was removed by the alternative method as in Experiments I. and II. above. But in the subsequent analysis

of both portions, the zinc was completely precipitated by hydrogen sulphide, and did not appear in the correct group. This behaviour is readily explained in the case of the part of the solution treated by the alternative method, since the latter could not be expected to remove the acetic acid. In the other case it must be assumed that the quantity of acetate was too large to be completely acted upon by the excess hydrochloric acid, so that acetates as well as chlorides were left after evaporation. And in neither case can the acetic acid have been destroyed to any extent by the treatment with hydrochloric acid and potassium chlorate.

THE OXIDATION OF FATTY ACIDS

The results obtained in the fourth experiment of the preceding section show, decisively enough, that acetic acid is not rapidly destroyed by treatment with chlorine in hot aqueous solution. The experiments described in the next few pages are attempts to estimate quantitatively the extent to which acetic acid and butyric acid are oxidised by heating with hydrochloric acid and potassium chlorate.

EXPERIMENT I. Loss of acetic acid due to evaporation.

Of the variable factors which affect this estimation, the most important is probably that due to loss of acid by evaporation. This will depend on the percentage of fatty acid in the mixture, the temperature, the time of heating, and also, the rate of oxidation of the fatty acid (since this affects its concentration). The following experiment was carried out to determine this factor; the absence of any oxidation makes the value a limiting one, probably too high.

A solution of 4.55 grams of acetic acid in 300 cc. of water was heated in a round flask of the type used in the oxidation experiments for four hours on a boiling water bath. Water was added from time to time to prevent too great reduction in volume. The solution finally occupied 265 cc. Of this, 20 cc. neutralised 5.38 cc. of 0.776 Normal potassium hydroxide. The total acetic acid left, therefore, was

$$\frac{265}{20} \times \frac{5.38}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 3.4 \text{ grams,}$$

and the loss was 1.15 gram from 4.55 grams, or 25.3 per cent.

EXPERIMENT II. Steam distillation of acetic acid.

A common method for removing fatty acids from mixtures containing them consists in setting the acids free (if necessary) by addition of a non-volatile acid, and subsequently distilling the mixture with steam. Before using this method in connection with the present problem, it was necessary to determine the fraction of acetic acid volatile with steam from a solution containing hydrochloric acid and chlorides. The following experiment was therefore carried out.

10.9 grams of acetic acid, 30 grams of potassium chloride, and 10 cc. of concentrated hydrochloric acid were dissolved in 190 cc. of water, and the mixture distilled with steam. The distillate was collected in portions of 250 cc., and each portion titrated against 0.776 Normal potassium hydroxide.

Of the first 250 cc., 24.8 cc. were equivalent to 10 cc. of the alkali solution. The weight of acetic acid in this portion was, therefore,

$$\frac{250}{24.8} \times \frac{10}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 4.7 \text{ grams.}$$

Of the second 250 cc., 38 cc. were neutralised by 5 cc. of the standard potash. The acetic acid present was

$$\frac{250}{38} \times \frac{5}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 1.5 \text{ gram.}$$

39.3 cc. of the third portion exactly neutralised 2 cc. of the standard alkali, so that the quantity of acid distilled

in this case was

$$\frac{250}{39.3} \times \frac{2}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 0.6 \text{ gram.}$$

Tests with silver nitrate showed that very little hydrochloric acid had come over with the distillate. The total acetic acid distilled was 6.8 grams, which is less than 65 per cent of the original. The fact that the quantities obtained in successive portions of the distillate decrease exponentially suggests that the rate of distillation is a logarithm factor of the concentration in the solution being distilled. This means that a small decrease in the concentration of acetic acid in the solution undergoing steam distillation is accompanied by a large decrease in the concentration of acetic acid in the distillate. It follows, also, that if the quantity of acetic acid to be separated is very small, the method of steam distillation may be of little value for this purpose.

EXPERIMENT III. Extent of oxidation of acetic acid.

9.1 grams of acetic acid were dissolved in a mixture of 250 cc. of water and 30 cc. of concentrated hydrochloric acid. The solution was heated on the steam bath and treated with potassium chlorate according to the Fresenius-v.Babo procedure; the total quantity of potassium chlorate added was 10 grams. The mixture was then distilled with steam, until about one litre of liquid had been collected. It was noticed, however, that chlorinous fumes came over with the distillate, rendering it a bright yellow in colour. In order to remove this impurity, hydrogen sulphide was passed into the solution until the

colour had disappeared; the excess hydrogen sulphide was driven off by passing a rapid stream of air through the liquid. The purified distillate was placed in a flask and distilled again with steam until the new distillate measured 530 cc. This was titrated with 0.776 Normal potassium hydroxide, and it was found that 5 cc. of the latter were neutralised by 130 cc. of the distillate. That is, the amount of acetic acid obtained was

$$\frac{530}{130} \times \frac{5}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 0.95 \text{ gram.}$$

Before discussing the full significance of this experiment, the control experiment which was carried out at the same time and under similar conditions should be described.

A solution of 9.1 grams of acetic acid, 30 cc. of concentrated hydrochloric acid, and 5 grams of potassium chloride was made in 250 cc. of water. This was heated on the steam bath for the same length of time as the mixture in the above experiment. The solution was then distilled with steam until 500 cc. of distillate had been collected, and the distillate titrated against standard caustic potash: 5 cc. of 0.776 Normal potassium hydroxidewere exactly neutralised by 32.9 cc. of the acid solution. The total acetic acid, then, was

$$\frac{500}{32.9} \times \frac{5}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 3.5 \text{ grams.}$$

The utilisation of the data provided by these two experiments in deriving the desired result (the percentage of acetic acid destroyed by oxidation) involves a few assumptions and corrections. In the first place, the value given by the control experiment must be corrected for the extra distillation

carried out in the oxidation experiment. Assuming that the result of Experiment II. regarding steam distillation can be applied in this instance, then if the 3.5 grams of acetic acid obtained in the control experiment were subjected to a second distillation, only 65 per cent, or 2.27 grams, would come over with the distillate. For purposes of comparison, therefore, we may say that the control experiment gave only 2.27 grams of acetic acid. This is 1.32 gram more than was obtained in the oxidation experiment. It follows, then, that 1.32 gram, or 14.5 per cent, of the acetic acid has been destroyed by the treatment with chlorine.

We may, on the other hand, argue as follows: the sample of acetic acid taken in the oxidation experiment weighed 9.1 grams; if we accept the result of Experiment I., the loss of acetic acid due to evaporation must have been about 25 per cent, or in this case, 2.3 grams. If there had been no oxidation, then, the quantity of acetic acid subjected to the first distillation would have been 6.8 grams. Now the amount of acid actually present in the second distillate was found to be 0.95 gram; assuming again that each distillation with steam yields only 65 per cent of the acetic acid taken, the quantity brought into the first distillation process must have been

$$\frac{100}{65} \times \frac{100}{65} \times \frac{0.95}{1} = 2.25 \text{ grams.}$$

Subtracting this from the 6.8 grams left (theoretically) after evaporation, we find that 4.55 grams, or 50 per cent, of the acetic acid has been destroyed by oxidation. This value differs enormously from the one obtained above. The second

calculation is, of course, grossly speculative, and this accounts for the deviation between the two answers. But the writer can see no reason why the first value should not be correct, at least in order of magnitude.

EXPERIMENT IV. Estimation of sodium acetate by ignition and titration.

In the absence of carbonates it should be possible to estimate a fatty acid in aqueous solution by neutralisation, evaporation, ignition, and titration of the alkaline residue against standard acid. In order to test this method the following experiment was carried out:

10 grams of sodium acetate and 5 grams of potassium chloride were dissolved in distilled water, the solution rendered just acid with hydrochloric acid, and evaporated completely to dryness. The residue was ignited in a platinum basin until frothing ceased, and the cold mass dissolved in a little distilled water. The solution was filtered to remove undissolved carbon, and titrated against 0.93 Normal sulphuric acid. 120.3 cc. of the acid were required for the whole alkaline solution: the weight of sodium acetate indicated is therefore

$$\frac{120.3}{1000} \times \frac{0.93}{1} \times \frac{82}{1} = 9.2 \text{ grams.}$$

Apparently this method can be used with satisfaction.

EXPERIMENT V. Extent of oxidation of acetic acid.

A mixture of 4.55 grams of acetic acid, 30 cc. of concentrated hydrochloric acid, and 300 cc. of water was heated on

the steam bath and 10 grams of potassium chlorate added gradually over a period of four hours. The liquid was saturated with sulphur dioxide to remove chlorine, and then saturated with hydrogen sulphide to remove the excess sulphur dioxide. Finally a rapid stream of air was passed until the liquid no longer smelled of hydrogen sulphide, the precipitated sulphur filtered off, and the solution rendered alkaline with caustic potash. Sufficient hydrochloric acid was added to give the solution an acid reaction, and it was then evaporated to dryness and ignited in a platinum basin. 46 cc. of 0.93 Normal sulphuric acid were required to neutralise the solution of the residue, so that the quantity of acetic acid left was

$$\frac{46}{1000} \times \frac{0.93}{1} \times \frac{60}{1} = 2.57 \text{ grams.}$$

Assuming that the loss due to evaporation was 25 per cent, or 1.15 gram, the weight of acetic acid destroyed by oxidation must have been 0.83 gram, or 18.2 per cent. This value agrees remarkably well with the one obtained in Experiment III., by the different method.

EXPERIMENT VI. Extent of oxidation of butyric acid.

Only one experiment was carried out in connection with the oxidation of butyric acid. The loss due to evaporation was not studied especially, but it would naturally be less than in the case of acetic acid. The sample weighed 3.88 grams. This was dissolved and oxidised in exactly the same manner as the acetic acid in the previous experiment. The estimation of butyric acid was made by the ignition method, and the quantity found was 0.3 gram. If it is assumed that

15 per cent of the original butyric acid was lost by evaporation, the amount destroyed by oxidation is found to be 77 per cent.

PRODUCTION OF FATTY ACIDS IN THE OXIDATION OF LIVER

Several experiments were performed in an attempt to isolate fatty acids from the product obtained by destroying the organic matter in minced liver according to the Fresenius-v.Babo method. In the case of some of these experiments, technical errors became apparent after the results (all negative) had been obtained. Such experiments, in view of their unimportance, have not been included with the others.

EXPERIMENT I. Production of fatty acids in the destruction of organic matter.

Half a pound of beef liver was finely minced and mixed with 200 cc. of water and 30 cc. of concentrated hydrochloric acid. The fluid mass was heated on the steam bath and treated with potassium chlorate until it was a pale yellow in colour. Altogether 10 grams of chlorate were added over a period of four hours. After diluting with 200 cc. of hot water and filtering, the solution was saturated with hydrogen sulphide to remove any free chlorine. The excess sulphuretted hydrogen was driven off in a rapid stream of air, and the filtered solution distilled with steam until the distillate measured 900 cc. This was made alkaline with sodium hydroxide and evaporated to small volume. Sulphuric acid was added until the solution was strongly acid, and the acid solution, having

a volume of 50 cc. extracted with 150 cc. of ether in three equal portions. The ethereal solution was dried over anhydrous sodium sulphate, decanted, and distilled on a boiling water bath. About 1 cc. of liquid remained, giving an acid reaction, and possessing a sour smell reminiscent of butyric acid. This liquid was heated under a reflux condenser with a little ethyl alcohol and concentrated sulphuric acid for about half an hour. The product had a decidedly ester-like odour, but could not be identified.

EXPERIMENT II. Production of fatty acids in the destruction of organic matter.

Two pounds of beef liver were minced and oxidised in the usual way. The filtrate after oxidation measured 1275 cc. Of this, 100 cc. were neutralised and extracted with ether; the ether layer was discarded. The aqueous portion was then made strongly acid with sulphuric acid and extracted with ether again. The ether was dried and distilled: no acid could be detected in the residue.

600 cc. of the acid filtrate were treated with sulphur dioxide and hydrogen sulphide as in Experiment V. of the preceding section. 50 cc. of concentrated sulphuric acid were added and the solution distilled with steam until 2000 cc. of distillate had been collected. This was made alkaline with caustic potash and evaporated to small volume. 15 cc. of concentrated sulphuric acid were added, bringing the volume to 110 cc., and the solution extracted with 300 cc. of ether in three equal portions. The combined ether extract was shaken out with 30 cc. of dilute sodium carbonate solution

and then with 30 cc. of water; it was dried over calcium chloride, decanted, and allowed to evaporate spontaneously. A very little liquid was left, which gave an acid reaction towards litmus and possessed a sour odour. This was distilled in a very small flask, but the quantity of liquid was not sufficient to give a satisfactory result, and the presence of fatty acid could not be confirmed.

EXPERIMENT III. Production of fatty acids in the oxidation of organic matter.

Two pounds of beef liver were minced and treated according to the Fresenius-v.Babo method. The filtrate from the oxidation mixture was subjected directly to steam distillation, and three litres of distillate collected. The latter was rendered alkaline with potassium hydroxide and evaporated to a volume of 125 cc. All chloride was removed from this solution by treatment with silver sulphate in presence of a little excess sulphuric acid. After filtering off the precipitate of silver chloride, the liquid was distilled with steam until the distillate measured 900 cc. Theoretically, the only acid that could possibly be present in the distillate must be organic acid volatile in steam. The distillate actually had an acid reaction, and on titration, 14.6 cc. of 0.776 Normal caustic potash were required for the entire acid solution. This indicates the presence of an amount of acid equivalent to

$$\frac{14.6}{1000} \times \frac{0.776}{1} \times \frac{60}{1} = 0.68 \text{ gram of acetic acid,}$$

or to

$$\frac{14.6}{1000} \times \frac{0.776}{1} \times \frac{88}{1} = 1.00 \text{ gram of butyric acid.}$$

The neutral solution was evaporated to dryness and the residue tested for fatty acid, but the results were negative.

CONCLUSIONS

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It seems to have been definitely established, that destruction of organic matter containing zinc by the Fresenius-v.Babo procedure, and subsequent removal of the excess hydrochloric acid by the alternative method described by Autenrieth, provides a solution from which the zinc is precipitated by hydrogen sulphide.

This behaviour, as is well known, is a positive indication of the presence of some kind of weak acid; and the only kind of weak acid which could be expected under the circumstances is a weak organic acid. It has been shown conclusively that neither acetic acid nor butyric acid is completely destroyed by subjection to the Fresenius-v.Babo oxidation process for a reasonable length of time. It should be quite possible, therefore, for fatty acids such as these to be found in the product of oxidation of organic matter. Their presence here is made probable by the results obtained in connection with the detection of zinc. The experiments carried out by the writer, however, cannot be said to do more than "hint at" the presence of organic acids in the solution obtained by oxidising meat according to the Fresenius-v.Babo method.

It may be that fatty acids are to be found here only in amounts of the order of the least quantity necessary to disturb the analysis for zinc, which is, of course, very small. If such is the case, the methods used in this work were not sufficiently fine to give a positive result.

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SUMMARY

I. In the detection of zinc in cadaver material, it has been shown that subsequent to the destruction of organic matter by the Fresenius-v.Babo procedure, if the excess hydrochloric acid is removed by partial evaporation and neutralisation with ammonia (an alternative method described by Autenrieth), the zinc will be entirely precipitated by hydrogen sulphide and incorrect results may be obtained.

II. It has been shown that when acetic acid is treated with potassium chlorate and hydrochloric acid according to the Fresenius-v.Babo method, an amount of acetic acid probably between 10 and 20 per cent is destroyed. With butyric acid a larger fraction is destroyed, but the oxidation is far from complete.

III. An attempt has been made to establish the presence of fatty acid in the product obtained by oxidising beef liver according to the Fresenius-v.Babo method. The results indicate that weak organic acids are probably formed, but in such small quantities that their presence could not be confirmed by the methods used.

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SECTION II

THE DECOMPOSITION OF COCAINE IN PRESENCE OF PUTREFYING ORGANIC MATTER

INTRODUCTION

INTRODUCTION

The question as to the stability of cocaine in the living organism and in the cadaver is one which has been answered entirely differently at different times and by different investigators. For instance, Palet (1) states that in cases of chronic cocaine intoxication, examination of the decomposed viscera seven months after death showed the presence of cocaine or its transformation products. Glasenap (2), on the other hand, states that in cases of cocaine poisoning, if death has ensued within two hours, the cocaine will be found unaltered, but if more than four hours have elapsed before death, the cocaine will be found in the urine as ecgonine. He states, further, that cocaine can be detected as such or as ecgonine after thirty-three days exposure to the influence of putrefying flesh or human blood. Wiechowski (3), after administering cocaine to dogs, found 0 to 12 per cent unchanged in the urine. With rabbits, however, neither cocaine nor ecgonine could be found. He concludes that cocaine undergoes a very profound change in the living organism. Autenrieth (4) goes so far as to say:

> "Cocaine probably belongs to those poisons which can very seldom be detected in the cadaver."

It is certainly difficult to draw a definite conclusion from a list of opinions so diverse as the above. Autenrieth's statement, inasmuch as it was published fairly recently, should carry most weight. But it was thought interesting to make a fresh attempt at determining the rate of decomposition of cocaine in putrefying animal material; also to find

out whether preservation by means of low temperature or treatment with formaldehyde prevents the decomposition. No attempt was made to investigate the fate of cocaine introduced into living organisms.

THEORETICAL CONSIDERATIONS

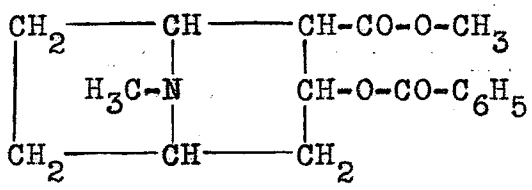
AND

DISCUSSION OF RESULTS

THEORETICAL CONSIDERATIONS

Cocaine, along with several other alkaloids of less importance, is contained in coca-leaves (Erythroxylon coca). It crystallises in colourless prisms, melts at 98 degrees C., and is sparingly soluble in water.

Cocaine possesses the empirical formula $C_{17}H_{21}NO_4$, and forms well-characterised salts, such as the hydrochloride, $C_{17}H_{21}NO_4$,HCl. It is the methyl ester of benzoyltropinecarboxylic acid, being represented structurally as follows:



This substance is readily hydrolised by heating with water, the products being benzoylecgonine and methyl alcohol. If mineral acids, barium hydroxide, or alkalies are used instead of water, the primary product, benzoylecgonine, is further decomposed into ecgonine and benzoic acid. In the living organism the alkaloid is said to be changed rapidly into ecgonine. This hydrolysis is probably brought about by the action of one or more specific enzymes. A cursory examination of the literature of the last decade provided several references to the destruction of alkaloids by some of the common enzymes, although nothing bearing directly on the present phenomenon was found.

It may be assumed, also, that the destruction of cocaine during putrefaction is due to enzyme action. Now the kind and amount of the various enzymes present in decomposing

organic matter may be expected to vary at random from sample to sample, unless, possibly, the growth of organisms in the material were carefully controlled. Provided, therefore, the decomposition is due to enzyme action, it would seem doubtful if a definite general statement could be made regarding the rate of decomposition of cocaine under these circumstances. But it should be possible to find a "probable time limit" after which the presence of cocaine in the material can not be confirmed. As will be seen in the section devoted to experimental work, the results obtained in duplicate experiments frequently do not coincide, thus supporting the idea expressed above regarding the variable nature of the decomposition. Of course, the method used for the estimation of cocaine is sufficiently lacking in accuracy that this variation may be due entirely to the defects of the method, and not at all to the cause cited previously. But the cocaine is undoubtedly destroyed, and the "probable time limit" may be set at 15 days.

The investigations carried out at low temperatures could not be completed, but the results indicate that the rate of decomposition of the cocaine is very much decreased at temperatures below freezing. Assuming that the decomposition is due to enzyme action, then either the specific enzymes are not produced except during putrefaction, or else they cannot bring about destruction of the alkaloid at low temperatures. It is well known that enzymes are altered by heat treatment, and lose their activity.

The corresponding work in which the organic material was

preserved by means of formaldehyde is more complete. Two samples of meat containing cocaine and preserved with formaldehyde were kept for five months and then analysed: the cocaine appeared to be undiminished in quantity. Here again, the formaldehyde may have produced its effect directly by preventing the action of the enzymes, or the behaviour may have been due to the absence of putrefaction.

EXPERIMENTAL RESULTS

EXPERIMENTAL

The animal matter used was in all cases minced beef liver. This was chosen because it could be easily and cheaply obtained, and represented fairly well the kind of material encountered in toxicological examinations.

Experiments were first carried out to determine which of two suggested methods for extracting cocaine was most suited to the work in hand.

EXPERIMENT I. Extraction of cocaine by the Stas-Otto process.

The first method tested was the well known Stas-Otto process, as follows: A solution of 0.25 gram of cocaine in 30 cc. of very dilute tartaric acid was mixed thoroughly with about 200 grams of minced beef liver. The mass was placed in a large flask with 200 cc. of 95 per cent alcohol and 20 drops of 10 per cent tartaric acid. The alcohol had been previously distilled over tartaric acid to remove basic substances. The flask was fitted up with a reflux condenser and heated on the water bath, with frequent shaking, for half an hour. After cooling, the contents of the flask were filtered to remove fat, and the residue washed with alcohol. The filtrate was evaporated to a thin syrup in a glass dish upon the water bath, 100 cc. of cold water added, and the mixture stirred up thoroughly. This was then filtered through a fluted filter paper, and the filtrate evaporated to dryness on the water bath. The residue was taken up carefully with absolute alcohol, the resulting liquid filtered, and the

filtrate again evaporated to dryness. Finally the residue from this evaporation was dissolved in 50 cc. of water, giving an acid solution of the cocaine in the form of the tartrate. This acid solution was extracted directly with 50 cc. of pure ether. After separation, the ether layer was discarded, and the aqueous portion made alkaline by adding saturated sodium carbonate solution. The cocaine was extracted by shaking out six times with pure ether, using 50 cc. each time. The ethereal solution, washed with a little water, was distilled to small volume and evaporated in a weighed dish. The residue weighed 0.045 gram, gave a melting point approaching 92 degrees C., and behaved as cocaine towards the appropriate reagents.

Starting with 0.25 gram of cocaine, then, 0.045 gram, or roughly one fifth of the original weight, was extracted by the Stas-Otto method.

EXPERIMENT II. Extraction of cocaine by the petroleum ether method.

In the second method the animal material is extracted with very dilute aqueous tartaric acid, and the cocaine removed from this by rendering alkaline and extracting with petroleum ether. The following trial was made:

A mixture of 0.25 gram of cocaine and 200 grams of minced liver was made up as in the previous experiment. This was shaken with 200 cc. of very dilute tartaric acid and allowed to stand over night. The mixture was then shaken up again and filtered through fine cloth. (Several hours are required for complete draining.) The filtrate gave an acid reaction, and was of such a deep red colour as to be almost opaque.

It was shaken out with 50 cc. of petroleum ether (previously distilled over tartaric acid to remove basic substances), the acid solution run off, and the ether layer discarded. Saturated sodium carbonate solution was added to the aqueous portion until decidedly alkaline, and then three extractions were made, using for each 100 cc. of petroleum ether. In the first experiment actually carried out, the liquids were roughly shaken up together; but this gave rise to disagreeable emulsions, and in all subsequent experiments each extraction was made by gently rocking the mixture in a large separatory funnel for fully five minutes. The combined ethereal solution was washed with a little water, and after allowing to stand several hours, decanted through a dry filter paper. The filtrate was distilled to small volume using a trap, and evaporated to dryness in a weighed basin. The residue in this case was a waxy crystalline substance weighing 0.07 gram. It melted at close to 95 degrees C., and behaved as fairly pure cocaine.

These experiments indicate that there is little to choose between the two methods with regard to the amount or purity of the cocaine extracted. Consequently, as the second process is much the less tedious, it was chosen for use in all the experiments carried out in connection with this problem.

EXPERIMENTS A. Decomposition of cocaine at ordinary temperatures.

Quantities of minced beef liver containing cocaine were allowed to stand at ordinary temperatures for various periods of time, the cocaine then being estimated as above. In each case, about 225 grams of liver and 0.25 gram of cocaine were used. The cocaine was either dissolved in a small volume of dilute acid and the solution mixed with the liver, or else the solid alkaloid, finely ground, was stirred up thoroughly with the minced meat. Each mixture was kept in a two-litre round flask, the mouth of which was covered with an inverted beaker. The alkaloid was extracted from the mass by treatment with 200 cc. of 0.35 per cent tartaric acid over night, and the rest of the analysis carried out exactly as in Experiment II., above. The cocaine practically always came out as a definitely crystalline, although waxy, solid, and was weighed without being purified.

The results of this set of experiments are given in the following table:

PERIOD OF STANDING	WEIGHT OF COCAINE RECOVERED
0 days	0.012(?) gram
0 "	0.073 "
3 "	0.05 "
8 "	0.045 "
12 "	0.052 "
12 "	0.025 "
13 "	0.04 "
14 "	0.015 "
14 "	0.04 "
15 "	0.025 "
18 "	0.005 "

These figures indicate that twelve days exposure to the decomposing material does not destroy the cocaine to an appreciable extent. The results for the 13, 14, and 15 day periods are indefinite, but by 18 days, the presence of cocaine could certainly not be confirmed. Of course, in these experiments cocaine was known to be the only alkaloid present, and it was consequently easy to establish the existence in the material of even such a small quantity as 0.005 gram. But the positive identification of cocaine is rather difficult, and in an ordinary toxicological examination it is doubtful if any quantity less than 0.02 gram would be sufficient for identification. Of the two 14 day experiments, one gave only 0.015 gram of cocaine, which is hardly sufficient for definite recognition.

In the 15 day experiment, 0.025 gram was obtained, just enough, shall we say, for identification. It thus appears that the presence of cocaine in animal material may not always be readily confirmed after 13 days standing at ordinary temperature. In order to be quite on the safe side, we may put the "probable time limit" at 15 days.

It was noticed that some of the samples became covered with "bread mould" after 8 or 10 days standing, while others failed to show any trace of it even after much longer periods. No attempt was made to control the growth of organisms in the samples, and, as has been suggested, this may be responsible for the variable nature of the results.

EXPERIMENTS B. Decomposition of cocaine at low temperatures.

This set of experiments differed from the above only in that the samples were kept at temperatures below freezing. The results may be tabulated as follows:

PERIOD OF STANDING	WEIGHT OF COCAINE RECOVERED
3 days	0.069 gram
8 "	0.093 "
24 "	0.030 "

It would be necessary to carry out more experiments to complete this study, but these figures are sufficient to show that the destruction of cocaine is considerably slower under these conditions than under those of the first experiments. There was no putrefaction in the above instances, of course.

EXPERIMENTS C. Decomposition of cocaine in presence of formaldehyde.

In these experiments each sample of liver and cocaine was preserved by the addition of 50 cc. of 37 per cent formaldehyde. This treatment rendered the meat pale in colour and leathery in texture, and no decomposition took place with the passage of time. The following data were obtained regarding the quantities of cocaine present after various intervals:

PERIOD OF STANDING	WEIGHT OF COCAINE RECOVERED
0 days	0.065 gram
3 "	0.05 "
8 "	0.05 "
5 months	0.039 "
5 "	0.065 "

It is thus definitely shown that destruction of cocaine does not take place under these conditions during the first five months of standing.

SUMMARY

SUMMARY

I. If beef liver containing cocaine (0.25 gram of cocaine per half-pound of liver) is allowed to putrefy at ordinary temperatures, the presence of cocaine cannot generally be confirmed after 15 days.

II. If the mixture of meat and cocaine is preserved by freezing, the alkaloid is not greatly reduced in quantity after 24 days standing.

III. If the mixture of meat and cocaine is preserved by treatment with formaldehyde, the amount of cocaine is apparently unaltered by 5 months standing at ordinary temperatures.

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SECTION III

THE FIXATION OF ALKALOIDS BY FORMALDEHYDE

INTRODUCTION

INTRODUCTION

Several investigators have pointed out the inadvisability of preserving in any way samples for toxicological examination. The use of formaldehyde in this connection has been criticised especially. And although the preservation of sections for autopsy examination is quite generally considered a bad practise, it is interesting, in view of the recent prevalence of the embalming custom (at least in North America), to find out what effect the presence of formaldehyde in cadaver material has on the detection of poisons therein.

Venturoli and Ciacci (1) carried out experiments to test the influence of formaldehyde, in 10 per cent solution and also in the strength of the commercial product, on a number of poisons which had been mixed with ground animal matter. They found that the detection of codeine, strychnine, quinine, digitoxin, and caffeine is not affected. In the case of morphine they recommend amyl alcohol instead of chloroform for extraction, as a compound is formed with formaldehyde which is more soluble in amyl alcohol than in chloroform. They state that atropine cannot be detected well owing to the formation of gummy substances.

It was thought interesting to start an investigation of this question from a more purely theoretical point of view, and an attempt has been made to determine whether any of the commoner alkaloids react with aqueous formaldehyde in such a way that they cannot be extracted and identified in the usual manner. It was expected that a number of positive results

would be obtained, but of seven alkaloids tested, all gave negative results except one. Chiefly on this account, then, the study was not carried further. An attempt was made to elucidate the chemical mechanism responsible for the single positive result, but without success.

THEORETICAL CONSIDERATIONS

AND

DISCUSSION OF RESULTS

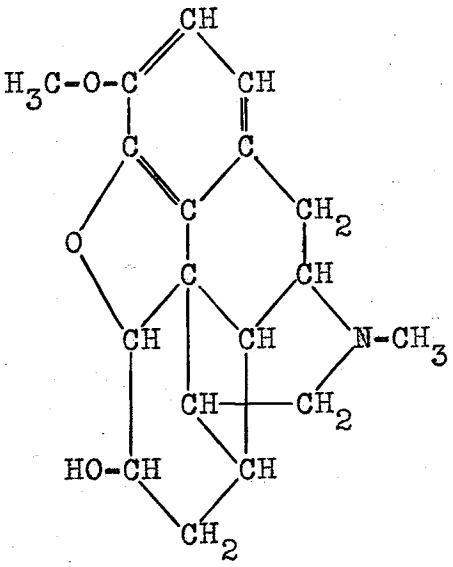
THEORETICAL CONSIDERATIONS

Formaldehyde, as is well known, is a highly reactive substance, and condenses with a great variety of organic compounds, such as aldehydes, ketones, alcohols, phenols, primary and secondary amines, aromatic nitro-compounds, and so on. Some of these condensations may be brought about even in aqueous solution at ordinary temperatures. Under certain conditions, also, formaldehyde unites with more complex substances such as proteins and alkaloids. Some of the formyl derivatives of the alkaloids seem to be well characterised. Thus formyl morphine (2) has a melting point of about 220 degrees C., and at 253 degrees C. decomposes into its progenitors; its salts are crystalline. Formyl codeine is described (2) as a colourless crystalline substance, melting point 180 degrees C., which is insoluble in water, and sparingly so in alcohol and ether. And although these and other formyl alkaloids may be prepared in a variety of ways (generally by heating in sealed tubes, boiling with 100 per cent formic acid, or other drastic means) the condensation of alkaloids with aqueous formaldehyde at ordinary temperatures does not seem to have been studied at all.

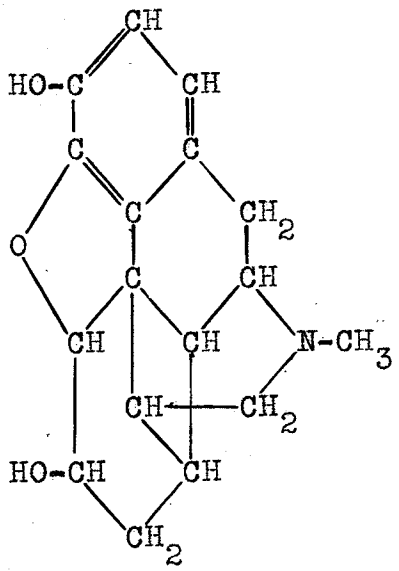
In the present paper the fixation of seven alkaloids by formaldehyde has been studied in the following way: in each case a mixture of the alkaloid and aqueous formaldehyde, allowed to stand at room temperature for several hours, was treated by a method which is known to extract the alkaloid under ordinary conditions, and which was modified only so as to prevent extraction of formaldehyde along with the alkaloid.

The alkaloids tested were novocaine, cocaine, atropine, morphine, codeine, strychnine, and aconitine. Of these, the only one which appeared to be fixed in such a way that it could not be extracted by the ordinary method was morphine. It is most interesting that morphine should behave in this way, while codeine, which bears the same relation to morphine as anisole does to phenol, should not.

The constitutions of morphine and codeine are not known with absolute certainty, but the following structural formulae, put forth by Gulland and Robinson (3), are believed to approach the true ones very closely:



CODEINE

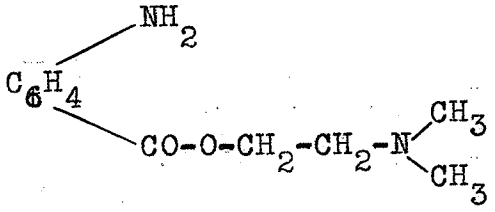


MORPHINE

The result obtained above indicates that the reaction has some connection with the phenolic hydrogen which is present in the morphine molecule, but which is replaced by a methyl group in codeine. But in the formyl derivatives of morphine and codeine mentioned above (2), the alcoholic hydroxyl is the point

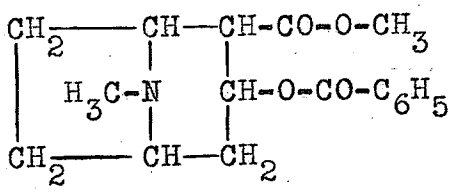
of union.

Primary amines generally condense readily with formaldehyde, and it is consequently surpriding that a negative result should have been obtained in the case of novocaine, which has the formula:

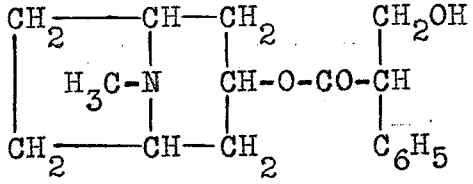


NOVOCAINE

Cocaine, and to a lesser extent atropine, might be expected to be fairly inert towards formaldehyde:

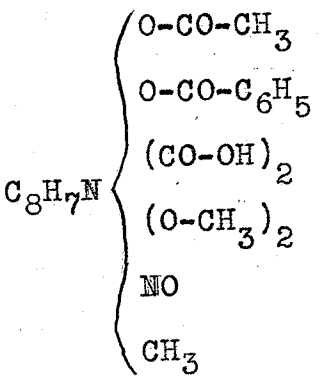


COCAINE



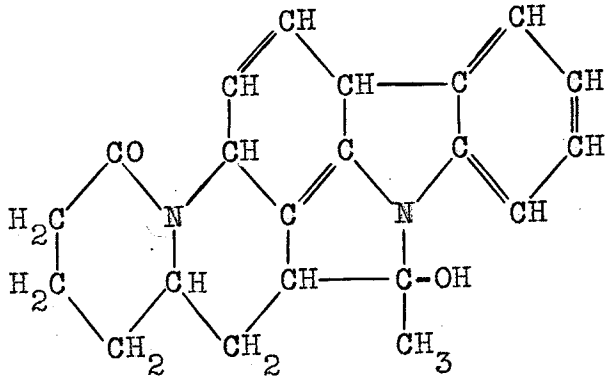
ATROPINE

Very little is known regarding the constitution of aconitine; Brady (4) has put forth the following partial formula:



ACONITINE

Similarly, the problem of the constitution of strychnine has beem only partially solved. Oliveri-Mandala (5) has suggested the following formula, although it does not fulfill all the requirements:



STRYCHNINE

Considerable time was spent in trying to show that a mixture of morphine and aqueous formaldehyde contains some compound of morphine which is not extracted from an ammoniacal solution by hot chloroform or amyl alcohol. The results of this investigation were most unsatisfactory, but in the course of the work it became evident that if the mixture of morphine and formaldehyde is sufficiently concentrated, a part of the morphine may be recovered unchanged. Thus from a solution of 1 gram of morphine sulphate in 27 cc. of 30 per cent formaldehyde, some of the morphine was precipitated by addition of 18 cc. of concentrated ammonium hydroxide, the mixture being cooled in ice.

EXPERIMENTAL RESULTS

EXPERIMENTAL

After a few preliminary trials, the following general method was adopted. Experiments of two types are carried out: first, control experiments to determine what fractional quantity of the alkaloid may be extracted from an aqueous solution in the absence of formaldehyde; and second, experiments involving precisely similar extractions from solutions containing formaldehyde.

A typical control experiment is performed as follows: A known weight of the alkaloid, say 0.05 gram, is dissolved in very dilute acid (generally about 25 cc.) and the solution shaken out with an equal volume of pure ether. The ether layer is discarded and the aqueous solution rendered alkaline by addition of caustic soda, sodium carbonate, or ammonia, depending on the nature of the alkaloid. The alkaline solution is then extracted several times with a suitable solvent (ether or chloroform). The combined extract is washed with a few cubic centimetres of water and allowed to stand over night. It is decanted from any water that may have separated, poured through a dry creased filter paper, and distilled to small volume using a trap. The remaining liquid is washed into a weighed glass basin with a little absolute alcohol and allowed to evaporate. The basin is weighed again, and the amount of alkaloid extracted is at once known. In these experiments it is not necessary to purify the residue.

The formaldehyde experiments follow the same general plan. The weighed quantity of alkaloid is dissolved in fairly strong

formaldehyde solution containing a little acid, and the mixture allowed to stand over night. The extractions are carried out exactly as in the control experiments, but the ether or chloroform extract of the alkaline solution must be washed with ammonia or saturated sodium sulphite to remove formaldehyde, before it is washed with water and evaporated.

Seven of the commoner alkaloids were studied in this way, and the results are listed below. Each experiment is not described in detail unless the procedure differed from that given above. But in every case, the volume and nature of the extracting solvent used is indicated. Caustic soda was the alkali used except for cocaine, atropine, and novocaine, where sodium carbonate was used, and for morphine, in which case ammonia is required. It is to be understood that in each case the residue was subjected to the appropriate tests, and, unless otherwise stated, was shown to consist chiefly of the alkaloid in question.

I. COCAINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.053 gram	Alkaline solution extracted 6 times using 30 cc. of ether each time.	0.046 gram
0.048 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.041 gram
0.051 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.053 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.049 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed twice with strong ammonia (10 cc.)	0.050 gram

II. NOVOCAINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.052 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.051 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.047 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed twice with strong ammonia (10 cc.).	0.056 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed twice with sat. sod. sulphite (25 cc.).	0.050 gram

III. ATROPINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.016 gram
0.050 gram	Alkaline solution extracted 6 times using 30 cc. of chloroform each time.	0.063 gram
0.050 gram	Alkaline solution extracted 6 times using 30 cc. of chloroform each time.	0.037 gram
0.050 gram	Alkaline solution extracted 6 times using 30 cc. of chloroform each time.	0.066 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed twice with sat.sod.sulphite (25 cc.).	0.060 gram
0.050 gram	Alkaline solution extracted 6 times using 30 cc. of chloroform each time. Chloroform extract washed twice with sat. sod. sulphite (75 cc.).	0.048 gram

IV. CODEINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.040 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.051 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed once with sat.sod.sulphite (20 cc.).	0.040 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed once with sat.sod.sulphite (20 cc.).	0.045 gram

V. ACONITINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.040 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time.	0.045 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed once with 20 cc. sat.sod.sulphite.	0.050 gram
0.050 gram	Alkaline solution extracted 10 times using 30 cc. of ether each time. Ether extract washed once with 20 cc. sat.sod.sulphite.	0.046 gram

VI. STRYCHNINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.055 gram	Alkaline solution extracted 3 times using 40 cc. of ether each time.	0.060 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.055 gram	Alkaline solution extracted 3 times using 40 cc. of ether each time. Ether extract washed once with 20 cc. sat.sod.sulphite.	0.040 gram

VII. MORPHINE

Weight of Sample	Details of Extraction	Weight of Residue
	A. CONTROL EXPERIMENTS	
0.050 gram	Ammoniacal solution extracted 3 times using 50 cc. hot chloroform each time.	0.050 gram
0.050 gram	Ammoniacal solution extracted twice using 100 cc. and 25 cc. hot chloroform.	0.047 gram
	B. FORMALDEHYDE EXPERIMENTS	
0.050 gram	Ammoniacal solution allowed to stand over night, then filtered. Several lots of hot chloroform poured thro' paper, and combined chloroform filtrate evaporated.	0.00 gram
0.250 gram	Ammoniacal solution treated as in previous experiment.	0.00 gram
0.050 gram	Ammoniacal solution extracted 3 times using 50 cc. hot chloroform-alcohol (90 vols. to 10 vols.) each time. Chloroform-alcohol extract washed twice using 20 cc. sat.sod.sulphite.	0.017 gram (morphine not detected)

Mention has been made above of the work of Venturoli and Ciacci (1), and of their suggestion that amyl alcohol should be used instead of chloroform for extraction of morphine in presence of formaldehyde. In the following experiment this idea has been tested.

0.250 gram of morphine sulphate was mixed with 10 cc. of 37 per cent formaldehyde and allowed to stand over night. 15 cc. of water and 1 cc. of 10 per cent tartaric acid were added and the solution extracted with 30 cc. of ether. The ether layer was discarded and the aqueous portion placed in a flask with 30 cc. of amyl alcohol and 3 cc. of strong ammonium hydroxide. The mixture was heated for a few minutes on the water bath with occasional swirling, and the amyl alcohol separated and shaken out with 12 cc. of decinormal sulphuric acid. The latter was collected in a separate container, while the amyl alcohol was heated again with the original solution. After separation the amyl alcohol was extracted with another portion of decinormal acid, which was run off into the same container as the first. The whole process was performed a third time, and the combined sulphuric acid solution rendered strongly alkaline with ammonia and allowed to stand over night. After twelve hours no solid appeared to have separated, and the solution was consequently evaporated to very small volume (5 cc.) and again allowed to stand. This time a slight powder separated out and was filtered and washed. The dry residue was extracted with hot alcohol and the solution evaporated in a weighed glass basin. 15 milligrams of solid were left. The colour reactions given by this substance are compared with the corresponding ones given

by morphine in the following table:

REAGENT	COLOUR WITH MORPHINE	COLOUR WITH RESIDUE
Conc. Sulphuric Acid.	pink	olive green
Formalin-Sulphuric Acid.	purple-red, then clear blue	apple green
Conc. Nitric Acid.	orange-red	yellow, with frothing

The conclusion to be drawn from all these experiments is, apparently, that the detection of cocaine, novocaine, atropine, codeine, aconitine, and strychnine in aqueous solutions of these alkaloids is not affected by the presence of formaldehyde, but that morphine becomes fixed in a solution in aqueous formaldehyde (of low concentration), at least towards the ordinary methods of detection.

The simplest explanation of this behaviour on the part of morphine involves the assumption that morphine and formaldehyde react in aqueous solution with production of a compound which is not at all similar to morphine in chemical and physical properties. The experimental work bearing on this question may now be described.

In one experiment it was found that a solution of morphine sulphate in formaldehyde, allowed to stand and then made ammoniacal and filtered, gave, on evaporation, a white amorphous solid. Extraction of this substance with absolute

alcohol yielded a gum, which with sulphuric acid produced the colour reaction given by morphine with formalin-sulphuric acid (purple changing to blue). This indicates that both morphine and formaldehyde nuclei are present.

In another experiment which has already been mentioned, 1 gram of morphine sulphate was dissolved in a mixture of 22 cc. of 37 per cent formaldehyde and 5 cc. of water, and the solution allowed to stand five hours. It was then cooled in ice and 18 cc. of concentrated ammonium hydroxide added slowly. A white crystalline precipitate of morphine was produced, and this was filtered off. Complete evaporation of the filtrate gave a yellow and white powder. The hydrochloride of hexamethylene tetramine was obtained from this by addition of hydrochloric acid. A nitrate was also obtained: lustrous flakes, melting at 163 degrees C.

It was found possible to dissolve morphine (alkaloid) in aqueous formaldehyde, either by stirring mechanically for a few days, or by allowing the mixture to stand for several weeks. Thus 0.54 gram of morphine was dissolved in 50 cc. of 37 per cent formaldehyde by stirring the finely powdered alkaloid with the liquid for three days. One gram of morphine (finely ground) dissolved in 110 cc. of the formalin after standing six weeks. The solutions obtained in this way are bright yellow in colour, neutral towards litmus and congo red, and on evaporation a bulky white solid is gradually thrown down; as the evaporation nears completion, however, the precipitated solid is also yellow. If the evaporation is carried out fractionally, the part of the residue which separates last gives the strongest colour reaction (pink) with sulphuric acid.

Any portion of the residue, on prolonged treatment with cold water, partially dissolves; but subsequently, neither the insoluble fraction nor that obtained by evaporating the water extract gives a pink colour with concentrated sulphuric acid. Instead, the water soluble fractions give a green colour which gradually turns to brown.

In one experiment the solution of morphine in formaldehyde was concentrated to half volume and the precipitated material collected (First Residue). The remaining solution was then evaporated completely to dryness (Second Residue). Each residue was treated with cold water for several hours, the resulting mixture filtered, and the filtrate evaporated. The water-soluble fraction of the second residue was insoluble in dry ether and petroleum ether, slightly soluble in alcohol, chloroform, benzene, and toluene. A quantity was heated with absolute alcohol, and the alcoholic solution filtered and evaporated. The residue gave a green colour with concentrated sulphuric acid, seemed to be partially crystalline, and decomposed with evolution of gas at 95 degrees C. Another portion of the water-soluble fraction of the second residue was extracted with hot chloroform. The dried extract was a white powder which decomposed and frothed up at about 100 degrees C. With concentrated sulphuric acid a red colour changing to purple was produced. The part which did not dissolve in the chloroform gave a green colour changing to brown with sulphuric acid.

This work was not continued, but it seems probable that a thorough and systematic investigation along these lines would produce interesting results.

SUMMARY

I. The detection of cocaine, novocaine, atropine, codeine, quinidine, and strychnine in aqueous solutions of these alkaloids has been shown to be manifested by the presence of formaldehyde.

II. It has been shown that morphine cannot be extracted by any of the ordinary methods from a dilute solution of a morphine salt in aqueous formaldehyde.

III. An unsuccessful attempt had been made to elucidate the chemical reaction which takes place when morphine or one of its salts is dissolved in formalin.

SUMMARY

I. The detection of cocaine, novocaine, atropine, codeine, aconitine, and strychnine in aqueous solutions of these alkaloids has been shown to be unaffected by the presence of formaldehyde.

II. It has been shown that morphine cannot be extracted by any of the ordinary methods from a dilute solution of a morphine salt in aqueous formaldehyde.

III. An unsuccessful attempt has been made to elucidate the chemical reaction which takes place when morphine or one of its salts is dissolved in formalin.

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