A Study of the Glucoside Solanine.

The Detection of Cocaine in Cadaveric Matter.

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by

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1932.

A Thesis Presented

to the

Graduate School of Manitoba University,

in Candidacy for the

Degree of Master of Science.

1933.

The writer wishes to acknowledge his indebtedness to Professor M. A. Parker for the suggestion of the problems set forth, and to express his appreciation of the helpful suggestions and advice given during the progress of the investigations.

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Section I.

A Study of the Glucoside Solanine.

INTRODUCTION.

INTRODUCTION

The alkaloid solanine, which is at the same time a glucoside (gluco-alkaloid) occurs in the potato planet (Solanum tuberosum), and in other Solanaceae such as Solanum niger (deadly nightshade), Solanum dulcamara and Solanum lycopersicum (tomato). It has also been found in the family Scopoliaceae, such as Scopolia orientalis and Scopolia atropoides.

Solanine is not uniformly distributed in all parts of the potato plant, being most abundant in the berry-like fruit, which is rare; also, in the chlorophyll-free sprouts which appear when the tubers germinate.

In the preparation of solanine, from any of the above mentioned plants, the use of mineral acids should be avoided, on account of the ease with which the substance is hydrolyzed.

Many methods for the extraction of solanine from potatoes are mentioned in the literature. A few of the better known methods are given below.

Firbas[1] macerates the fresh potato shoots with water containing 2% of acetic acid, renders the

liquid alkaline with ammonia and extracts the precipitate (after drying) with boiling alcohol, from which the solanine crystallizes on cooling.

Cazeneuve and Breteau[2] grind the shoots (which should be shorter than four inches) with half their weight of lime water, dry the pulp at room temperature and extract with 93% alcohol. On evaporation of the alcohol in vacuo a syrup remains, which crystallizes on standing. The crystals are washed with ligroin and ether and are recrystallised three times from boiling 95% alcohol. Yield 1 gram per kilo of shoots.

Another two methods in use, those of von Morgenstern[3], and O. Schmiedberg and G. Meyer, are described in the experimental work.

Solanine is deposited from hot alcohol in fine, colorless silky needles, which are generally stated to melt (decompose) at about 240°. Cazeneuve and Breteau give the melting point 280°.

Very considerable doubt exists as to the formula of this substance; it seems possible that in various species different alkaloids occur; thus the solanine in Solanum sodomoeum of Oddo and Colombano[4] appears to be

different from that in the potato.

Among the various formulae assigned to solanine two may be mentioned: $C_{52}H_{93}O_{18}N$, proposed by Firbas (loc. cit.) and more recently confirmed by Whittmann[5], and the simpler formula $C_{28}H_{47}O_{10}N$, due to Cazeneuve and Breteau (loc. cit.). The chief objection to the former formula is that according to Firbas, the action of acids on solanine results in the liberation of water:

$$C_{52}H_{93}O_{18}N \quad C_{40}H_{61}O_2N \quad 2C_6H_{12}O_6 \quad 4H_2O.$$

On the other hand, Firbas' formula of the fission product solanidine has been confirmed by the analyses and molecular weight determinations of Whittmann. The above equation for the decomposition of solanine cannot be quite accurate, for according to the investigations of Votoček and Vondraček[6], and of Zeisel and Whittmann[7], both d-galactose and rhamnose are formed from solanine; a third sugar, which is perhaps a disaccharide, is also present.

Solanine is odorless when dry, but when moist exhales an odor recalling that of potatoes while cooking. The taste of solanine is somewhat bitter and pun-

gent.It leaves on the pharynx a persistently acrid sensation. Solanine is poisonous, producing in dogs and cats violent vomiting, followed by somnolence, and sometimes accompanied by paralysis of the lumbar muscles. One grain killed a rabbit in six hours, and ½ grain is strongly nauseating to a man.

Solanine is nearly insoluble in water, and only very slightly soluble in cold alcohol, but dissolves readily in hot alcohol. It is insoluble in ether, chloroform, benzene, or petroleum-spirit, but is soluble in amyl alcohol, which may be employed to extract solanine from its alkaline solutions. Hot, saturated solutions of solanine in both amyl and ethyl alcohols gelatinize upon cooling.

Solanine is said to have a faintly alkaline reaction. It is a very feeble base, the salts being mostly decomposed by excess of water. The acid sulphate, however, is said to be very stable and not decomposed by water, even on heating, in contradistinction to the neutral salt. It is amorphous and very bitter. The hydrochloride is precipitated as a jelly on adding ether to a solution of solanine in alcohol acidified with hydrochloric acid.

Solanine is not affected by alkalies, even when boiling. It does not reduce Fehling's solution, but reduces silver ammonio-nitrate on heating.

Concentrated nitric acid dissolved solanine in the cold to a liquid which is at first colorless, but rapidly acquires a magnificent purple colour, which soon disappears. With strong hydrochloric acid, solanine gives a yellow coloration. Concentrated Sulphuric acid dissolves solanine with orange colour, changing to deep violet and brown. In contradistinction to this, the solanine of Cazeneuve and Breteau was hardly colored yellow by sulphuric acid; with fuming nitric acid a pink colour was only developed on long standing. Hydrochloric acid did not colour it.

When warmed with a mixture of equal volumes of alcohol and strong sulphuric acid, solanine dissolves with rose-red coloration. This reaction is stated to be characteristic.

As a micro-chemical test for solanine in plants, Schaarschmidt lays the section to be examined in moderately concentrated sulphuric or nitric acid, when in a few seconds the presence of the alkaloid is

indicated by a beautiful rose colour. In this manner, Schaarschmidt recognized the presence of solanine in the tuber and stalk of Solanum tuberosum; and also in S. nigrum, S. dulcamara, Capsicum annuum, Lycopersicum esculentum, and Mandragora officinalis.

The potato being such a common article of food, Professor Parker[8] suggested the desirability of determining the amount of solanine present in various parts of the potato. In pursuance of this suggestion, the experimental work demanded by this problem was undertaken. The work was divided into two parts. The first was the actual determination of the amount of solanine present; the second was an attempt to determine the empirical formula of solanine.

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS.

It was thought desirable to try the two methods outlined in Autenreith[9] for the extraction of solanine from potatoes, and to find which would yield the best results. The first method employed was that of O. Schmiedberg and G. Meyer, which is as follows.

500 grams of finely minced potatoes were mixed with distilled water, and the liquid pressed out as completely as possible by means of a potato-masher. The liquid was decanted from the starch which settled out, the starch was mixed with water, and the latter decanted when the starch had settled. The entire liquid was now carefully neutralized with ammonia and evaporated to a syrupy consistency on the steam bath. In the meantime, the press-cake was mixed with several times its volume of boiling alcohol - previously distilled over tartaric acid to remove basic impurities - and the alcohol pressed out after standing for several hours. Two such extractions were made. The combined alcoholic extracts were then filtered, and the residue, consisting of starch, was washed with hot alcohol. Any solanine present in the residue from the aqueous extract was isolated by extraction with the alcoholic filtrate , the residue being

washed with hot alcohol. The liquid was filtered from the precipitate which formed after standing one hour, and was evaporated to a syrupy consistency on the steam bath. The residue was dissolved in water containing sulphuric acid, filtered, and the residue washed with water. The clear liquid was very gently warmed, saturated with ammonia, and set aside for one day. The precipitate which had collected at the end of this time was filtered through a previously weighed filter; washed first with water, then with ether, and finally dried at 100°.

The weight of the residue - presumably all solanine - was .002 grams.

METHOD OF VON MORGANSTERN.

As much liquid as possible was expressed from 500 grams of finely-grated potatoes, using a potato-masher. Two extractions of the press cake were made with water, the liquid being expressed each time. Protein substances were precipitated from the combined liquids by adding 1 cc. of acetic acid and warming for about an hour on the steam bath. After being filtered, the filtrate was evaporated to a syrupy consistency, and hot 95% alcohol was added until the cloudiness which

formed had vanished. The solution was decanted after an interval of 12 hours, and the residue twice extracted with hot alcohol. The combined alcoholic extracts were evaporated on the steam bath, the residue warmed with water containing acetic acid, and then filtered. The filtrate was heated to boiling, and ammonia was added to precipitate the solanine. After standing for about ten minutes on the steam bath, the precipitate was filtered off, washed with water containing ammonia, and then dissolved in boiling alcohol. The resulting solution was evaporated to near-dryness on the steam bath, the residue dissolved in water containing acetic acid, and then filtered. The filtrate was heated to boiling, and the solanine was precipitated with ammonia. The precipitate was collected on a filter - previously dried and weighed - washed with 2% ammonia, and dried at 90°.

The residue - presumably entirely solanine - weighed .0019 grams.

Each of the above methods is open to criticism. The former procedure is faulty in that it employs sulphuric acid to dissolve the solanine, which has the effect of hydrolyzing the solanine. The second, while

free from this objection, does not remove the starch which settles out from the expressed liquid: the starch, on being heated on the water bath as described in the method, breaks up to form a heavy gelatinous mass which is very difficult - almost impossible - to filter. Also, the press-cake is disregarded: this contains some solanine, hence the method, although not rigorously quantitative, is made less so, by omitting the press-cake.

In the subsequent experiments, in which solanine was extracted from the potatoes, a combination of both the foregoing methods was employed. An outline of the method used in the following trials is described below.

The potatoes were ground up, and the liquid expressed by means of a potato-masher. Water was added to the press-cake, and the liquid again expressed. This was done twice. The combined liquids were then decanted from the deposit of starch, and filtered. Acetic acid was added to the filtrate to precipitate protein substances, the entire liquid warmed on the steam bath for about one hour, and then filtered. The filtrate was then evaporated to the consistency of a syrup on the steam bath. In the meantime, the press-cake was mixed with twice its volume of 95% alcohol, and refluxed on the steam bath for one hour. At

the end of this time, the alcohol was expressed by means of a potato-masher, filtered, and the residue washed with hot alcohol. The solanine in the residue from the aqueous extract was obtained by extraction with the alcoholic filtrate. The liquid was filtered after two hours, and the alcohol evaporated on the steam bath. Water containing acetic acid was added to the residue, filtered, and the residue washed with water containing acetic acid. The filtrate was heated to boiling, and solanine precipitated by the addition of ammonia. After standing on the steam bath for ten minutes, the solanine was filtered, and washed with 2% ammonia. The residue was dissolved in boiling alcohol, filtered, and evaporated to near-dryness on the steam bath. The residue was dissolved in water containing acetic acid, filtered, the filtrate heated to boiling, and the solanine precipitated by adding ammonia. After standing on the steam bath for ten minutes, the solanine was collected on a previously weighed filter, and dried at 100°.

Nov. 10/32. Extraction of Solanine from Entire Potato.

From the potatoes used in the experiments, 1000 grams were taken, and the remainder placed in

a warm, dry room. The 1000 gram sample yielded, on treatment, 0.0048 grams solanine.

Feb. 25/33.

A further 1000 gram sample, analyzed on this date, yielded 0.045 grams solanine.

It is apparent from the above results that the solanine content of potatoes increases with age.

On Nov. 29/32, a quantity of solanine was obtained from a sample of the 1931 potato crop, using the sprouts. A determination was also made of the amount of solanine present in the entire potato, and the amount present in the very small potatoes of the same crop. These potatoes were very small indeed, the largest ones not exceeding $\frac{3}{4}$ inch in diameter, and the smallest ones being about $\frac{1}{2}$ inch in diameter.

The normal sized potatoes, on treatment, yielded 0.061 grams solanine per kilogram of potatoes, while the small potatoes yielded 0.163 grams solanine from 230 grams of sample, which would be 0.708 grams per kilogram.

The results obtained above, clearly indicate that small potatoes are much richer in solanine than

large potatoes of the same variety.

The solanine obtained in each of the above extractions was combined and recrystallized from alcohol. The melting point was found to be 270°, decomposition taking place at 265°. The solanine was again crystallized from alcohol, and the melting point this time was 240° C, decomposition taking place at the same time. Two more crystallizations were made, the melting point in each case being 240°, with decomposition. The solanine, if such it was, was as pure as could be obtained.

To establish the identity of the substance as solanine, two colour tests were made, one with conc. sulphuric acid, and the other with ethyl alcohol + sulphuric acid. In the first test, the substance dissolved to give an orange colored solution which became brownish-red on warming. The second test employed an alcoholic solution of solanine, added to conc. sulphuric acid as an upper layer, to produce a red zone at the junction of the two liquids, and below this, a yellowish zone.

The amount of solanine obtained after the repeated crystallizations was not very large, but

enough had been obtained to run a few combustions. The purpose of the combustions was a further confirmation of the identity of the substance. If the results obtained for carbon and hydrogen were near the theoretical answers, obtained from the various formulae, it was fairly safe to assume that the substance obtained was solanine.

The combustions were made using an electric furnace.

The results obtained, from these combustions, are given below.

First Trial	Second Trial	Third Trial
C 70.2%	C 62.1%	C 62.1%
H 15.09%	H 11.6%	H 10.4%

The percentages of carbon, calculated from the various formulae proposed for it, vary from 55% to 67%. The results obtained, taking 62.1% as the result, is within this range. This indicates that the substance obtained is solanine.

Determination of Moisture and Nitrogen Content of Potatoes.

Determinations were made, during the week of Nov. 20/32, of the moisture and nitrogen content of the potatoes on hand. The results obtained were then compared with

the results from similar determinations made later. The reason for these experiments was to find if, as the solanine content of the potatoes increased, the nitrogen content would also increase.

The first set of determinations were made on Nov. 20/32, and the results obtained were -

	Moisture Content.	Nitrogen Content.
First Trial	73.76 %	.797%
Second Trial	73.74 %	.796%

The next set of determinations were made on March 5/33, nearly four months later. The results obtained at this time are -

	Moisture Content.	Nitrogen Content.
First Trial	71.64 %	.83 %
Second Trial	71.63 %	.825 %
Third Trial		.831 %

The nitrogen content of potatoes sprouts was also determined, and found to be 1.19%.

The results obtained above are just what are to be expected. The moisture content had decreased after four months, while the nitrogen content had increased slightly. The loss in moisture can be explained by the fact that the potatoes were stored in a dry room.

The potato, as a rule, is very efficient in keeping its moisture within it, but in a dry atmosphere, the loss of a little bit of water is to be expected. The increase in nitrogen would be due, in part, to the loss in weight of the potatoes, and in part to the fact that some of the carbon present in the potatoes had been used up in respiration during germination.

The potatoes, at this time, possessed quite large-sized sprouts, and the solanine was extracted from them.

The method used in extracting the solanine from the sprouts is due to Firbas, and, in brief, is to extract the fresh young shoots with water containing acetic acid, rendering the filtered liquid alkaline with ammonia, and extracting the precipitate, after drying, with boiling alcohol, from which the solanine crystallizes upon cooling.

The extraction was carried out as follows. 750 grams of the fresh young sprouts - not exceeding four inches in length - were ground in a machine, and the dark colored liquid which had been expressed from the shoots, by means of a potato-masher, was made acidic with acetic acid, and then filtered. The

press-cake was macerated with water containing acetic acid, and filtered. This was done twice. The acid filtrates were combined, made strongly alkaline with ammonia, and set aside for one day. The solanine, which had precipitated as a dark brown mass, was filtered, washed with water containing ammonia, and dried over conc. sulphuric acid in a vacuum dessicator. The solanine present in the dark, dried mass was extracted by heating with alcohol, and filtering the hot, alcoholic extract from which the solanine crystallizes on cooling. The yield of solanine was 1.6 grams. The melting point of the crystals obtained was 238°- 240° (decomp.)

The mother liquor remaining, after the solanine had been separated, was thought to contain a considerable amount of solanine, as on standing, the entire solution jelled.

An attempt was made to obtain a further yield of solanine from this jell. The jell was dissolved in water containing acetic acid, and ammonia was added to the clear solution, which caused the solanine to precipitate. The solanine, after it had been been filtered, washed and dried, was dissolved in

the minimum amount of hot alcohol, and then cooled under the cold water tap, but no solanine crystallized out. On standing, the solution again jelled. the procedureoutlined above, was employed three times, but no solanine could be crystallized.

The solanine was finally obtained as follows.

To the alcoholic solution of solanine, acetone was added until the solanine had all been precipitated. The precipitate was filtered, dried, and dissolved in the minimum amount of hot alcohol. This time, on cooling the hot solution, the solanine crystallized out.

Yield 0.3 grams. M.P. 238°- 240° (decomp.)

Combustions were carried out for carbon and hydrogen on the solanine which was now on hand. This time a gas furnace was employed.

The results obtained are given below.

	Carbon.	Hydrogen.
First Trial	61.31 %	9.21 %
Second Trial	61.30 %	9.23 %
Third Trial	61.28 %	9.18 %

Fourth Trial	61.25 %	9.17 %
Fifth Trial	61.26 %	9.17 %
Sixth Trial	61.27 %	9.15 %
Mean	61.27 %	9.18 %

The percentage of carbon obtained with the new sample of solanine is very close to that obtained with the previous solanine. This would indicate that the two samples of solanine obtained were identical.

The percentage of nitrogen in the solanine was also determined, using the Kjeldahl method. The results were -

Trial I	Trial II	Trial III	Trial IV	Trial V
1.38 %	1.385 %	1.371 %	1.36 %	1.39 %

Mean 1.37 %

The results obtained with the later solanine were presumed to be more accurate, since the number of combustions done was greater than in the previous case, and also, the gas furnace could be handled much better.

Using the figures for carbon, hydrogen, and nitrogen obtained, the empirical formula for the substance was worked out as follows.

C = 61.27 ÷ 12 = 5.1 ÷ .097 = 52

H = 9.18 ÷ 1 = 9.18 ÷ .097 = 94

N = 1.37 ÷ 14 = .097 ÷ .097 = 1

O (by difference) = 28.18 ÷ 16 = 1.76 ÷ .097 = 18.

Empirical formula is then - $C_{52}H_{94}NO_{18}$.

This formula agrees very closely with the one given by Firbas as $C_{52}H_{93}NO_{18}$. This would seem to indicate that the substance obtained from the potato sprouts is solanine.

SUMMARY

SUMMARY

1. The solanine content of potatoes increases as the potatoes age.

2. The sprouts of germinating potatoes are much richer in solanine than the potatoes themselves.

3. Small potatoes contain more solanine than large potatoes of the same variety.

4. The nitrogen content of the sprouts is greater than that of the potatoes themselves. This would indicate that the solanine passes from the potato into the sprouts as the potatoes germinate.

5. The empirical formula of solanine was determined to be $C_{52}H_{94}NO_{18}$.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Firbas, Monatsh, 1889, 10, 541.
2. Cazeneuve and Breteau, Compt. Rend., 1889, 128, 887.
3. von Morganstern, Landw. Vers. Stat., 1907, 65, 300.
4. Oddo and Colombano, Gazzetta, 1908, (1), 38, 27.
5. Whittmann, Monatsh.,1905, 26, 445.
6. Votoček and Vondraček, Ber., 1903, 36, 4372.
7. Zeisel and Whittmann, Ibid, 1903, 36, 3554.
8. Professor Parker, Private Communications.
9. Autenreith, Laboratory Manual for the Detection of Poisons and Powerful Drugs.

Section II.

The Detection of Cocaine in Cadaveric Matter.

INTRODUCTION.

INTRODUCTION.

Many different and contradictory statements appear in the literature concerning the fate of cocaine in the living organism.

Glasenap[1] states that in cases of cocaine poisoning, where death has ensued within two hours, cocaine can be detected as such, but if more than four hours have elapsed, the cocaine can be found as ecgonine in the urine, showing that the breakdown of cocaine into ecgonine occurs more rapidly in the living, than in the dead organism. He also states that cocaine can be detected after thirty-three days as such, or as the decomposition product, ecgonine.

The two above statements of Glasenap, concerning the detection of unchanged cocaine in the organism are contradictory, and therefore are not to be relied upon.

Palet[2] says that seven months after death, in a case of chronic cocaine intoxication, the presence of cocaine , or its products of decomposition, could be confirmed.

Sonnie-Morett[3] maintain that small amounts of cocaine are quickly decomposed in the organism,

and cannot be detected by chemical means. The presence of cocaine, they go on to say, can only be confirmed when the examination is proceeded with immediately after death. According to Wiechowski[4], after cocaine had been administered to dogs, 10 - 12% of it had been eliminated, unchanged, by way of the kidneys. With rabbits, however, neither cocaine nor ecgonine could be found. They conclude that the behaviour of cocaine in the animal body is analogous to atropine, in that both undergo profound decomposition.

Madelaine Magnette[5] says: " The alkaloids which yield ecgonine on hydrolysis, as benzoylecgonine, and cocaine, and ecgonine itself, cannot be detected in putrefying cadavers. The identification of the products of hydrolysis of cocaine does not serve as conclusive evidence of the presence of the latter - these products either occur occasionally, as methyl alcohol, or else they exist normally in cadavers, as benzoic acid."

This statement by Madelaine Magnette is open to criticism. It is quite possible that methyl alcohol and benzoic acid may occasionally occur in

cadavers, but ecgonine certainly does not. Therefore, the presence of ecgonine would establish without a doubt, the existence of an alkaloid capable of producing ecgonine on hydrolysis.

As the result of an examination for the purpose of determining whether a solution of cocaine instead of novocaine had been injected into two female patients who had died with symptoms of cocaine poisoning, Popp[6] was unable to establish the presence of either cocaine or novocaine in the organs received five days after death. In connection with this investigation, Popp, together with Mannich and Ellinger conducted experiments upon rabbits, injecting into them varying quantities of cocaine and novocaine. The animals were killed at different intervals after injection, and the extracts from their organs tested for cocaine by their anaesthizing action, and by the potassium permanganate and iodic acid tests. Only in a single instance was cocaine detected at the place of injection three hours after death.

To prove the capability of alkaloids to resist putrefaction, Proelss[7] mixed 500 grams of

meat and blood with 0.5 grams of cocaine hydrochloride. After standing for 14 days, 100 grams of the mixture - corresponding to 0.1 grams of cocaine - were examined. After repeated evaporations and redissolving, the residue was obtained in crystalline form. The residue was proven to be cocaine by means of the following tests; its bitter taste, the anaesthesia produced on the tongue, its alkaline reaction, its ability to reduce calomel, and the green colour it imparted to a solution of potassium dichromate in sulphuric acid. He thus proved, beyond doubt, that cocaine could be detected, unchanged, after fourteen days. Neither cocaine nor ecgonine could be detected after 173 days, or after 268 days.

From the number of conflicting statements quoted above it is rather difficult to arrive at a definite conclusion. It was thought interesting, therefore, to make a fresh attempt at determining the rate of decomposition of cocaine in putrefying animal matter, and also to find out what effect freezing had on the rate of decomposition.

THEORETICAL CONSIDERATIONS

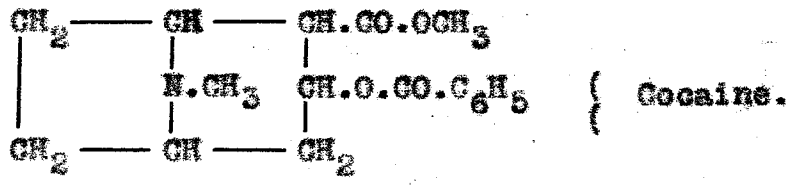
THEORETICAL CONSIDERATIONS.

Cocaine, $C_{17}H_{21}NO_4$, occurs to the extent of 0.2 - 0.8 percent in coco leaves, the leaves of the South American shrub, Erythroxylon coco.

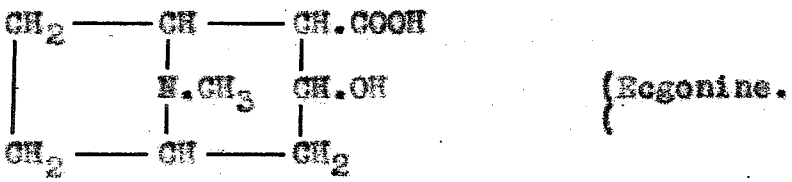
It crystallizes best from hot alcohol or petroleum ether, and is obtained in large, colorless monoclinic prisms melting at 98°C. Its solutions are laevo-rotatory and have a strong alkaline reaction. It has a faintly bitter taste, producing upon the tongue temporary anaesthesia. Cocaine is difficultly soluble in water, but dissolves easily in alcohol, ether, benzene, chloroform and acetic ether. Dilute acids dissolve cocaine, and usually form well-crystallized salts. The fixed alkalies, such as ammonia and alkaline carbonates precipitate the free base from solutions of its salts.

Cocaine is a monacid base, forming salts with one equivalent of acid. The most important cocaine salt is the hydrochloride, used in medicine, and having the composition $C_{17}H_{21}NO_4.HCl$. The tertiary nature of this alkaloid is shown by

the fact that at 100°C it combines with methyl iodide, forming the well crystallized cocaine iodomethylate, $C_{17}H_{21}NO_4CH_3I$. It contains a methyl group attached to nitrogen, that is, the group $N.CH_3$, because methylamine is split off when it is distilled with barium hydroxide. Moreover, cocaine is the methyl ester of an alcohol, for when boiled with water, it is decomposed into methyl alcohol and benzoyl-ecgonine. If mineral acids, barium hydroxide or alkalies are substituted for water, hydrolytic cleavage is carried further with decomposition of benzoyl-ecgonine. In this way, ecgonine, benzoic acid and methyl alcohol are obtained. The following shows the structural formulae of cocaine and ecgonine, as proved by Willstätter.



Cocaine.



Ecgonine.

In the living organism, the alkaloid is said to be changed rapidly into ecgonine. The hydrolysis is thought to be due to the action of one or more specific enzymes.

The reagents especially sensitive to cocaine are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, phospho molybdic acid and picric acid. Pure concentrated sulphuric and nitric acids dissolve cocaine without colour.

EXPERIMENTAL

EXPERIMENTAL.

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

Experiments were first carried out to determine whether any cocaine could be extracted from acidic solution. This was done because a quantitative estimation of cocaine following the usual methods, in which the ether extract of the acidic solution was discarded, was not possible. Two methods were used in order to find out which would yield the best results.

Experiment 1.

Extraction of Cocaine from Solution.

0.25 grams of cocaine were dissolved in 50 cc. water, with the addition of 20 drops of 10% tartaric acid. This solution was extracted twice with ether, using 50 cc. each time. The ethereal layer was separated each time from the aqueous acid liquid and allowed to stand for two hours in a dry, loosely stoppered flask. It was then decanted from the few drops of water which had collected on the bottom of the flask through a dry

filter into a dry flask. The larger portion of the ether was distilled off on the steam bath, and when but 10 cc's remained, was poured into a weighed glass dish. The residual ether was evaporated off on the steam bath, and when it had all been driven off, the residue remaining in the glass dish was dried by standing over conc. sulphuric acid in a vacuum dessicator.

Wt. of cocaine recovered - .0053 grams.

The aqueous acid solution from above was made strongly alkaline with sodium carbonate solution, and extracted twice with ether, using 50 cc. each time. The ethereal extract was allowed to stand for two hours in a dry, loosely stoppered flask. The subsequent treatment was the same as above.

Wt. of cocaine recovered - .1343 grams.

Experiment 2.

The same procedure as outlined above was followed in this experiment, but petroleum ether was substituted for ether.

The results obtained were as follows:

	Weight of Cocaine Extracted.
From Acid Solution	.0053 grams.
From Alkaline Sol^{n}.	.1601 grams.

It can thus be seen that some cocaine is extracted from the acidic solution, but the amount is so small that in the following experiments, the ethereal extract of the solution made acidic with tartaric acid was discarded.

Another set of two experiments were carried out to find which of two methods suggested was most suitable to the work in hand.

Experiment 3.

Extraction of Cocaine by the Stas - Otto Process.

0.25 grams of cocaine were added to 200 grams of minced beef liver contained in a litre bolt head flask, 50 cc. of water were added, and well shaken. 250 cc of 95% alcohol - previously distilled over tartaric acid to remove basic substances - were added to the contents of the flask,

with the addition of 20 drops of 10% tartaric acid. The flask was connected to an upright condenser, and heated on the steam bath for twenty minutes. After being cooled, the contents of the flask were filtered through a fine cloth to remove fat, and the residue was washed with alcohol. Several hours were required for complete drainage. The filtrate was evaporated to a thin syrup in a glass dish upon the steam bath, 100 cc's of cold water were added, and the mixture thoroughly stirred. This was then filtered through a moistened, fluted filter, and the filtrate evaporated to near-dryness on the steam bath. The residue was thoroughly mixed with 100 cc's of alcohol, and again filtered, the filtrate being again evaporated to dryness. The resulting residue was dissolved in 50 cc's water, giving an acid solution of the cocaine in the form of the tartrate.

The acid solution was extracted directly with 50 cc. ether. After separation, the ether layer was discarded, and the aqueous portion made strongly alkaline with sodium carbonate solution.

The cocaine was extracted by shaking out three times with ether, using 50 cc's each time. The ethereal solution, washed with water, was allowed to stand for two hours in a dry, loosely stoppered flask, and then decanted through a dry filter. The ether was distilled to small bulk on the steam bath, and then evaporated on a weighed glass dish. The residue was dried over conc. sulphuric acid contained in a vacuum dessicator, and then weighed. The weight of the residue was 0.0774 grams, and behaved as cocaine towards the following reagents.

A. Iodo-potassium iodide.

A few drops of the reagent added to the residue dissolved in dilute hydrochloric acid produced a brown precipitate.

B. Potassium Permanganate.

The reagent was employed in the following way.

The residue obtained above was dissolved in dilute hydrochloric acid, evaporated to dryness on the steam bath, and the resulting residue was dis-

solved in a few drops of water. A few drops of potassium permanganate solution when added to the above solution produced a violet precipitate.

As a further test, the residue obtained in experiment 3 was rubbed on the tongue for a few minutes. Temporary anaesthesia occurred, lasting for a few minutes.

Experiment 4.

Extraction of Cocaine by the Petroleum Ether Method.

In this second method, the liver was extracted with very dilute tartaric acid, and the cocaine extracted from the acid solution by rendering the solution alkaline and extracting with petroleum ether.

The following trial was made. .25 grams of cocaine were added to 200 grams of minced beef liver contained in a litre bolt head flask, 50 cc's water were added, and the mixture well shaken. To the mixture was added 150 cc's of very dilute tartaric acid solution, and the whole warmed very gently on the steam bath for about fifteen hours. The contents of

the flask were then shaken up and filtered through a fine cloth, the residue being washed with water. Several hours were required for complete draining. The filtrate gave an acid reaction, and was of a deep red colour. It was shaken out with 50 cc's of petroleum ether - previously distilled over tartaric acid to remove basic substances - the acid solution run off, and the ether layer discarded. The acid solution was made strongly alkaline with sodium carbonate solution, and extracted three times with petroleum ether, using 100 cc's each time. The liquids were not shaken, but rocked gently in a large separatory funnel for five minutes - this in order to avoid the formation of a disagreeable emulsion which results when the liquids are roughly shaken together. The combined ethereal extracts were washed with a little water, and then allowed to stand for about two hours, in a dry, loosely stoppered flask. The liquid was then decanted from the drops of water which had collected on the bottom of the flask through a dry filter. The filtrate was distilled to small volume, and then evaporated

to dryness in a weighed glass dish. The residue was dried in a vacuum dessicator. It had a waxy appearance, and weighed 0.0691 grams. It behaved as cocaine towards the above-mentioned reagents.

It can be seen from these experiments that the two methods are about equal with regard to the amount of cocaine extracted. The first method was followed in all the subsequent experiments. Although the second process is somewhat shorter, the first was chosen because there was a lesser volume of liquid to extract, and the emulsion formed on shaking the liquids could be broken quite easily.

Experiment 5.

Decomposition of Cocaine at Ordinary Temperatures.

200 grams of minced beef liver were placed into each of seven flasks, of one litre capacity. To the contents of each flask, 0.25 grams of cocaine were added, with the addition of 50 cc's distilled water, and each flask was well shaken. They were allowed to stand at ordinary room temperature for various periods of time, the cocaine then being es-

timated by the Stas - Otto process.

The residue obtained in each case was a yellowish, waxy substance with a faint, aromatic odor. The residues did not seem to be pure cocaine, and they were therefore purified in the following manner.

The residue was treated with 25 cc's of dilute hydrochloric acid, and the solution filtered. The filtrate was extracted with 25 cc's of ether, and the aqueous liquid drawn off, the ether extract being discarded. The aqueous acid liquid was made strongly alkaline with sodium carbonate solution, and extracted three times with ether, using 25 cc's each time. The combined ethereal extracts were then treated as in the Stas - Otto process, already outlined. The results obtained are given in the following table.

Period of Standing.	Weight of Cocaine Recovered.
0 Days	0.0773 grams.
0 Days	0.0755 grams.
3 Days	0.0592 grams.

3 Days	0.0590 grams.
5 Days	0.0545 grams.
7 Days	0.0480 grams.
7 Days	0.0476 grams.
10 Days	0.0425 grams.
15 Days	0.0281 grams.
20 Days	0.0043 grams.

The results, as shown in the above table, indicate that the cocaine was gradually decomposed the longer it was in contact with the putrefying liver. The cocaine could be detected with certainty fifteen days after exposure to the decomposing material, by making use of the potassium iodide test, and the anaesthizing action of the residue upon the tongue. After twenty days, however, the presence of cocaine in the residue could not be determined with certainty. In all these experiments, cocaine was known to be the only alkaloid present, and therefore it was quite easy to detect its presence.

Experiment 6.

Decomposition of Cocaine at Low Temperatures.

This set of experiments was carried out similar to those above, except that the flasks were kept at temperatures below freezing. The results are as follows.

Period of Standing.	Weight of Cocaine Recovered.
15 Days	0.0710 grams.
30 Days	0.0653 grams.

These figures show that the decomposition of cocaine is very considerably slower in the cold than at ordinary temperatures, due, no doubt, to the fact that no putrefaction takes place in the cold.

SUMMARY

SUMMARY.

1. The presence of cocaine in putrefying matter cannot be detected with certainty after fifteen days.

2. The decomposition of cocaine in organic matter is greatly lessened at low temperatures.

BIBLIOGRAPHY

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1. H. W. Glasenap, J. C. S., 1895, A., ii, 336;
from Chem.Centr., 1894, 2, 220.

2. L. P. J. Palet, Chem. Abstr., 13, (1919), 3203;
from Anales soc. quim. Argentina,
7 (1919), 22 - 26.

3. Sonnie - Morett, Journ. de pharm. et de chem;
28, 390 - 93.

4. W. Wiechowski, J. C. S., 1901, A., ii, 615;
from Arch. exp. Path. Pharm.,
46, 155,- 162.

5. Madelaine Magnette, J. Pharm. Belg., 7, 757-67,
781 - 3, 797 - 805, 821-3,(1925),

6. H. Popp, Chemiker - Zeitung 16, (1901),
288, 306, 316, 325, 788.