XV.—*Researches on Kryptophanic Acid, the normal free acid of Human Urine.*

By J. L. W. THUDICHUM, M.D.

CONTENTS.

1. Mode of isolating kryptophanic acid from fresh human urine by lime and alcohol process.
2. Purification by lead acetate process.
3. Purification by copper acetate process.
4. Mode of obtaining kryptophanic acid from urinary extracts after removal of all products of decomposition of urochrome by sulphuric acid.
5. Mode of obtaining kryptophanic acid from fresh urine, without intervention of heat.
6. Chemical properties of kryptophanic acid.
7. Absorption of oxygen by kryptophanic acid in alkaline solution.
8. Kryptophanates of the alkalis.
9. Kryptophanate of lead.
10. Basic lead kryptophanate.
11. Kryptophanate of copper (with alcohol).
12. Kryptophanate of copper (without alcohol).
13. Kryptophanate of magnesium.
14. Calcium kryptophanates.
15. Kryptophanate of baryum.
16. Acid baryum kryptophanate.
17. Kryptophanates of cobalt.
18. Silver-salts.
19. Theoretical considerations concerning kryptophanic acid.

1. *Mode of isolating Kryptophanic Acid from fresh Human Urine by Lime and Alcohol process.*

The urine is treated with milk of lime to alkalinity, filtered and evaporated. After filtration from the gypsum, it is acidified with acetic acid, and evaporated to crystallisation. After some standing, the syrup is filtered from the salt cake, and is now ready for treatment with alcohol. Of this syrup one volume is mixed with four volumes of alcohol of 95 per cent., or five volumes of 90 per cent., and shaken in a stoppered bottle. A voluminous, flaky, adhesive, dark precipitate is formed, which speedily settles in the bottom of the fluid. The liquor is decanted, the precipitate shaken with some fresh strong alcohol, and the alcohol again decanted. The bottle and precipitate

are now slightly warmed, when the latter gives out much alcohol, which is poured away.

The impure calcium salt is now dissolved in a small quantity of water, filtered through a calico bag with the aid of pressure (it soon clogs any paper filter), and again precipitated with four volumes of strong alcohol. The precipitate is collected in a calico bag, and freed from alcohol by pressure. It is now free from the soluble urinary ingredients. By repeating the solution in water and precipitation with alcohol a few times more, almost pure calcium kryptophanate can be obtained. But it is preferable to adopt one or other of the following processes of purification.

2. *Purification by Lead Acetate.*

The crude calcium salt, above described, is dissolved in water, and mixed with a large quantity of nearly saturated watery solution of neutral lead acetate. The mixture is shaken in a stoppered bottle, and filtered. The filtrate is nearly colourless; a dark-coloured voluminous precipitate of basic salt remains on the filter. The latter is washed with some saturated lead solution. The united filtrates, containing all the neutral lead kryptophanate in solution, are mixed with five or six volumes of alcohol of 95 per cent., whereupon perfectly white lead kryptophanate is deposited in flakes. They are washed with alcohol, slightly with water (for washing with much water produces basic salt), then with alcohol again, lastly, with ether, and dried *in vacuo*, during which they assume a slightly yellowish colour on the surface. The precipitate may also simply be washed with water, to remove lead acetate, the basic kryptophanate being also insoluble in water as well as in alcohol. By decomposition with the exact amount of sulphuric acid required, the free acid is obtained, and best transformed into baryum salt by baryta-water in excess and a current of carbonic acid. The baryum salt is precipitated by alcohol, redissolved in water, and again treated with lead acetate in excess. The filtrate with alcohol yields pure white kryptophanate. (The insoluble part is basic salt, and can be used for obtaining the free acid by decomposition with an equivalent quantity of dilute sulphuric acid.)

3. *Purification by Copper Acetate.*

To the solution of the crude calcium salt an excess of copper acetate is added. A voluminous dirty green precipitate ensues, and a greenish-blue solution forms, which is separated by filtration. To this latter five or six volumes of alcohol of 95 per cent. are added, which produce a voluminous greenish-blue flaky precipitate. This is filtered and washed with alcohol, and dried *in vacuo* over sulphuric acid. The mother-liquor must remain blue with excess of copper acetate, in order to obtain all kryptophanic acid as copper salt, free from calcium. This copper salt is soluble in water, while moist with alcohol, but insoluble in water after it has been dried. Its solution, before the addition of alcohol, must not be heated, as in that case a change takes place, as evidenced by the formation of an insoluble greenish-red precipitate. In order to obtain the free acid, the copper salt must be decomposed with sulphuretted hydrogen.

4. *Mode of obtaining Kryptophanic Acid from Urinary extracts after removal of all products of decomposition of Urochrome by Sulphuric Acid.*

In the foregoing process applied to fresh urine the urochrome remains undecomposed in the alcoholic solution. Kryptophanic acid is therefore not a product of urochrome, as is also evident from many other circumstances. In the process now to be described, kryptophanic acid is obtained in large amount from the extracts from which urochrome products have been entirely removed.

The extracts containing great quantities of ammonium sulphate are mixed with excess of milk of lime, filtered through cloths, and boiled to drive out all ammonia. They are then acidified with acetic acid, and evaporated to crystallisation. The filtered extract is treated with alcohol like the extracts from fresh urine. The precipitate contains much more potassium chloride than that from fresh urine, and requires special care to be purified from this by one or other of the above processes. It also contains iron, which follows the acid into nearly all its preparations. This is best removed by adding to the solution, freed from calcium by ammonia and ammonium carbonate, a sufficient amount of ammonium sulphide, and filter-

ing immediately. By evaporation the alkali and sulphide are removed, and the solution of the ammonium-salt is ready for the application of lead acetate.

5. *Mode of obtaining Kryptophanic Acid from fresh Urine without the intervention of Heat.*

The dilute free acid and its acid salts are little affected by heat and air, but its neutral and alkaline salts become dark in solution, or in the wet state. To avoid this objectionable effect the following process may be adopted. The filtered fresh urine is treated with lead acetate as long as this reagent produces a precipitate. An excess of neutral lead salt is to be carefully avoided. Experience shows that to every litre of the fresh urine of healthy men 40 cubic centimetres of a solution saturated at 9.5° C. of lead acetate should be added, and the precipitate produced thereby will be sulphate and phosphate exclusively, with only a trace of organic salt. (From 1 litre of average urine 6.2 grm. of mixed lead salts are obtained.) To the filtrate more acetate and some ammonia are added. The precipitate is collected in a calico-bag, pressed, washed with water, pressed again, and then decomposed with sulphuric acid in slight excess. It is yellow, and gives the spectrum of urochrome, being a broad absorption band at the beginning of blue. The filtrate is treated with baryum carbonate and a little baryta water. The solution of baryum kryptophanate is now mixed with five volumes of nearly absolute alcohol, whereupon the kryptophanate is precipitated, the urochrome remaining in solution. The baryum kryptophanate is dissolved in water, and again precipitated with lead acetate; the precipitate, after filtration, is digested with a sufficiency of saturated solution of lead acetate and the solution filtered. The filtrate is treated with five volumes of nearly absolute alcohol, whereupon lead kryptophanate falls down in white flakes which should be washed with alcohol, a little water, alcohol again, lastly with ether, and dried *in vacuo*.

6. *Properties of Kryptophanic Acid.*

It is a transparent, amorphous, gummy, solid mass, almost or entirely colourless. It is soluble in water in all proportions; less soluble in alcohol. Alcohol produces a precipitate in

the water solution, and after this has deposited, ether produces a new precipitate in the mixture. It has a freely acid taste. It decomposes the carbonates of alkalis and earths with effervescence, forming watery solutions of the salts. Its solution forms, with lead acetate, a copious thick white precipitate; with mercury acetate a similar precipitate; with silver nitrate the precipitate is slight.

The lead acetate precipitate forms a clear solution with excess of acetate. The solution of the free acid is not precipitated by mercury dichloride, nor by copper acetate.

The aqueous solutions of its earthy salts show the following reactions:—

They are precipitated by excess of alcohol. On being heated they fuse and become dark, ultimately dry, and may be powdered, after which they are very stable. Boiled with a great excess of alkaline copper solution they reduce the oxyde and form sub-oxyde, which remains in solution, but is deposited if the mixture is concentrated and air excluded. The alkaline solution fluoresces blue.

The watery solutions of the earthy salts are precipitated copiously white by lead acetate, and are soluble in excess of acetate; by mercury acetate, white; by mercury nitrate, voluminous white; the ordinary estimation of urea by this reagent is thus shown to be liable to error and to require a correction for kryptophanic acid, which has probably increased all values obtained for urea by from 5 to 10 per cent.; by silver nitrate, white voluminous. All these precipitates are slightly soluble in water, insoluble in alcohol; they are soluble in nitric acid.

Iodine, dissolved in an iodide, on being added to a solution of kryptophanic acid or of a kryptophanate, produces immediately an iodo-kryptophanic acid, in which one or several atoms of hydrogen are replaced by iodine. The liquid immediately contains hydriodic acid. Thus, on addition of tincture of iodine to fresh urine, the iodine is at first precipitated, as if the tincture had been added to water, but is almost immediately redissolved by substituting hydrogen in kryptophanic acid. This reaction was the subject of a lively debate in France some years ago.

Bromine added to kryptophanic acid, or the solutions of its salts in water, immediately produces a brominated acid, in which one or more atoms of hydrogen are replaced by bromine.

The reaction with bromine is perhaps neater than that with iodine, and the product less liable to change and decomposition.

The kryptophanates on being heated, emit acid vapours, but no urinary smell whatever is perceived, like that emitted by omicholine. They then leave a quantity of charcoal, which requires prolonged heating before it is entirely consumed. Kryptophanic acid prevents the precipitation of ferric oxide from solutions containing excess of caustic alkali. Like oxalic acid, it holds prussian blue in solution, in the presence of free hydrochloric acid.

An ammoniacal silver nitrate solution becomes immediately very dark on addition of ammonium kryptophanate. On standing it becomes apparently black (being red when diluted), and deposits metallic silver as a black powder.

Nitrate of silver and excess of nitric acid, added to a solution of kryptophanic acid containing urea (to urine with little urochrome and much Kr, such as is discharged in certain conditions of the brain, intermittent spasmodic disease), is reduced on standing, and the glass is covered with a silver mirror.

7. *Absorption of Oxygen by Crude Kryptophanic Acid in Alkaline Solution.*

A quantity of crude kryptophanate of calcium was dissolved in water to 20 c.c., and enclosed in a graduated tube over mercury; 47 c.c. of air were now allowed to enter the tube, and after that 14 c.c. of concentrated caustic potash solution were added. After three weeks of standing, the air had diminished to 38 c.c., so that 9 c.c. of oxygen had been absorbed, equal to 19.1 per cent. of the air employed.

When highly purified, neither the free kryptophanic acid, nor its baryum salt with excess of baryta water, seem to absorb pure oxygen over mercury.

8. *Kryptophanates of the Alkalies.*

These salts are very soluble in water.

The sodium-salt is not precipitated from its aqueous solution by alcohol.

9. *Kryptophanate of Lead*, $C_5H_7PbNO_5$.

Crude calcium kryptophanate, precipitated by alcohol, was mixed with excess of a saturated solution of lead acetate, and filtered from the precipitate of basic kryptophanate.

The solution was mixed with five volumes of alcohol of 95 per cent. The precipitate of kryptophanate, a white flaky mass, thereby produced, was washed with alcohol, water, alcohol, and ether, in succession, and dried *in vacuo*. It darkened during the washing, and ultimately became pale yellowish. When quite dry it had shrunk very much, and acquired a dark colour.

Analysis (dried *in vacuo*) :—

- I. ·3996 grm., with H_2SO_4 and ignition, yielded ·3104 $PbSO_4$, equal to 53·07 per cent. Pb.

The formula $C_5H_7NPbO_5 + aq.$, requires 53·62 per cent. Pb.

The above lead salt which had been kept over sulphuric acid, was now further dried at 105° .

(Exposed to the air this salt absorbed nearly the whole of its water, and on again drying lost it again.)

Analysis of the salt dry at 105° :

- I. ·5900 yielded ·4820 grm. $PbSO_4$, which, allowing ·0008 for correction, gives 55·72 per cent. Pb.
 II. Combustion—1·3905 burned with CuO , etc., gave ·2363 H_2O , and ·800 CO_2 , equal to 1·89 per cent. H, and 15·69 per cent. C.
 III. Nitrogen determination—·7708 grm. burned gave gas equal to 21·48 c.c. normal (*i.e.*, at 0° , 760 mm. and dry). After oxygen and pyrogallate of potassium, it gave gas = 13·97 c.c. normal, therefore nitrogen = 17·73 c.c., or 0·2226 grm., or 2·89 per cent.
 IV. ·5847 gave ·4760 $PbSO_4$, equal to 55·73 per cent. Pb.

Calculated.			Found.			
			I.	II.	III.	IV.
5 C	60	16·30	—	15·69	—	—
7 H	7	1·90	—	1·89	—	—
Pb.....	207	56·25	55·72	—	—	55·73
N	14	3·80	—	—	2·89	—
5 O	80	21·75				
	<hr/>	<hr/>				
	363	100·00				

10. *Basic Lead Kryptophanate.*

When the neutral salt is washed with water for a long time it loses one-third of its acid, and there remains behind a salt having the composition $2(\text{C}_{10}\text{H}_{14}\text{Pb}_2\text{N}_2\text{O}_{10})\text{PbO}$.

Theory.				Found.		
				I.	II.	III.
20 C		240	14.16	13.05	—	—
28 H		28	1.65	1.75	—	—
5 Pb		1035	61.06	—	61.2	60.61
4 N		56				
21 O		336				
		<hr/>				
		1695				

Note.—Analyses 1 and 2 on the same preparation. Analysis 3 on sample prepared in another operation.

11. *Kryptophanate of Copper (with alcohol).*

Prepared by adding a great excess of copper acetate to a solution of the calcium or sodium salt, filtering and precipitating the filtrate with much alcohol, washing with alcohol, and drying in the steam-oven, powdering, drying *in vacuo*. Prepared in this way the salt is a compound of kryptophanate with alcohol.

Analyses :—

I. Combustion with oxide of copper, a little potassium chlorate, and copper turnings.

1.2771 burned gave .5305 H_2O , and 1.3555 CO_2 equal to 4.62 per cent. H., and 28.95 per cent. C.

II. .4885 burned as I, gave .2100 H_2O , and .5290 CO_2 equal to 4.67 per cent. H., and 29.53 per cent. C.

III. .7235 burned and treated with HNO_3 gave .2192 CuO , equal to 24.20 per cent. Cu.

IV. .4470 gave CuO .1384, equal to 24.67 per cent. Cu.

V. In this analysis 24.18 per cent. Cu were obtained.

	Found.				
	I.	II.	III.	IV.	V.
C	28·95	29·53	—	—	—
H	4·62	4·67	—	—	—
Cu	—	—	24·20	24·67	24·18

The analyses lead to either $2(\text{C}_5\text{H}_7\text{CuNO}_5) + \text{C}_2\text{H}_6\text{O}$, or $2(\text{C}_5\text{H}_7\text{NCuO}_5) + \text{C}_2\text{H}_6\text{O} + \text{H}_2\text{O}$.

12. *Kryptophanate of Copper (without alcohol), $\text{C}_5\text{H}_7\text{CuNO}_5$.*

If the foregoing salt is exposed to moist air, and then dried *in vacuo*, it loses *alcohol*, and at the same time changes in colour, becoming very dark green.

Analysed in that state it yields results corresponding with the formula $\text{C}_5\text{H}_7\text{NCuO}_5$.

Analyses:—

I. Combustion with copper oxide, potassium chlorate, and Cu.

·2859 gave ·2840 CO_2 , equal to 27·09 per cent. C (water not weighed).

II. Nitrogen determination—·4016 gave ·0255 gm. of N., equal to 6·35 per cent.

III. ·1752 gm. gave ·0603 CuO, equal to 27·50 per cent. Cu.

IV. Dried at 120° ·4077 left ·1455 CuO, equal to 28·82 per cent. Cu.

Calculated.			Found.			
			I.	II.	III.	IV.
C_5	60·	26·72	27·09	—	—	—
H_7	7·	3·12	—	—	—	—
Cu	63·5	28·29	—	—	27·50	28·82
N	14·	6·24	—	6·35	—	—
O_5	80·	35·63				
	224·5	100·00				

Dry Distillation of Copper Salt.—8·25 gm. of this salt was subjected to dry distillation, and yielded, first water, which was removed from the receiver; then, on stronger heating, a white heavy vapour came over, which was alkaline, smelled of cyanide, and of tobacco, and crystallised in white crystals on cooling. It

was perhaps cyanide and cyanate of ammonium, effervesced with platinum tetrachloride, and gave a crystalline salt; it was accompanied with a dark red oil, which, when mixed with HCl and PtCl_4 reduced the platinum, and gave a rather black solution. The oil was more soluble in ether than the crystals, so that a separation could be thereby effected.

13. *Kryptophanate of Magnesium.*

Free acid, prepared by the decomposition of wet lead-salt by means of an equivalent of dilute sulphuric acid, was treated with an excess of magnesia, filtered, and the filtrate evaporated on the water-bath.

The salt formed a mass like treacle, which was dried up, powdered, and dried at 110° to 120° .

Analyses:—

- I. .5363 grm. ignited gave .1023 grm. MgO equal to 11.45 per cent. Mg .
- II. Dried at 125° for some time, .508 grm. salt left after combustion .100 grm. MgO , equal to 11.8 per cent. Mg .
- III. .4653 grm. gave .0893 MgO , equal to 11.51 per cent. Mg .
- IV. .5232 grm. gave .03484 grm. nitrogen, equal to 6.66 per cent. N .

Calculated.			Found.			
			I.	II.	III.	IV.
10 C.	120	29.56	—	—	—	—
14 H	14	—	—	—	—	—
2 Mg	48	11.83	11.45	11.81	11.51	—
2 N	28	6.90	—	—	—	6.66
10 O.	160					
2 Aq	36					
	<hr/>					
	406					

Dried at from 140° to 160° it lost an atom of water, and became $\text{C}_{10}\text{H}_{14}\text{Mg}_2\text{N}_2\text{O}_{10} + \text{Aq}$.

Theory.		Found.	
		I.	II.
30.93	C	31.01	—
4.12	H	4.78	—
12.37	Mg	—	12.12

- I. Combustion ·3650 grm. gave ·1570 grm. H_2O , and ·415 CO_2 , equal to 4·78 per cent. H., and 31·01 per cent. C.
 II. ·4690 grm. gave ·0948 grm. MgO , equal to 12·12 per cent. Mg.

14. *Calcium Kryptophanates.*

Some free acid was made by decomposing lead salt with an equivalent of dilute H_2SO_4 (a little baryta water was added to remove a slight excess of sulphuric acid). An excess of milk of lime, prepared from lime which had been washed, was added to the acid, and the mixture boiled and filtered. To the filtrate about an equal volume of strong alcohol was added, and a precipitate obtained. This was dried in the water-bath, further dried at 110° , and analysed.

- I. ·6242 grm. gave ·3152 grm. CaSO_4 , equal to 14·85 per cent. Ca.

Further dried at 170° .

- II. ·2845 grm. gave ·1465 grm. CaSO_4 , equal to 14·92 per cent. Ca.
 III. ·6240 grm. burned with lead chromate gave ·7542 CO_2 equal to 33·14 per cent. C.

The filtrate was evaporated to dryness, and the lime-salt powdered and dried at 135° .

Analysis:—

- IV. ·348 grm. gave ·2002 grm. CaSO_4 , equal to 16·94 per cent.

Theory.			Found.			
			I.	II.	III.	IV.
10 C	120	32·88	—	—	33·14	—
13 H	13	—	—	—	—	—
$\frac{3}{2}$ Ca	60	16·43	14·85	14·92	—	16·95
2 N	28					
9 O	144					
	<hr/>					
	365					

When kryptophanic acid is boiled with excess of milk of lime, there is produced a salt containing $\frac{3}{2}$ of Ca to 1 of acid. This salt has considerable stability, but appears to be very slowly

attacked by the CO_2 of the air. If, however, the salt be dried up and then powdered, one-third of the calcium passes into carbonate, and the dibasic salt $\text{C}_{10}\text{H}_{14}\text{CaN}_2\text{O}_9$ is produced (just as in the case of the Ba salt).

15. *Kryptophanate of Baryum.*

The magnesium salt was precipitated boiling with slight excess of hydrate of baryum, filtered, and evaporated nearly to dryness in the water-bath; refiltered, and the filtrate dried in the water-bath. It formed a perfectly translucent red-brown varnish. Dried at 110° .

Analysis :—

I. .445 grm. gave .338 grm. BaSO_4 , equal to 44.66 per cent. Ba.

$\text{C}_{10}\text{H}_{14}\text{Ba}_2\text{N}_2\text{O}_{10} + \text{Aq.}$ requires 44.62 per cent. Ba.

This salt took up CO_2 of the air, and formed a deposit of BaCO_3 . The solution on evaporation to dryness left a residue, which dried at 110° , contained 36.28 per cent. Ba.

It would also seem that there is a baryta-salt of the formula $\text{C}_{10}\text{H}_{15}\text{Ba}_3\text{N}_2\text{O}_{10}$.

16. *Metamorphosis of Baryum Kryptophanate into an Acid Salt by boiling with water.*

15.5 grammes of copper kryptophanate (containing a certain quantity of alcohol) was decomposed with hydrogen sulphide and filtered. The filtrate was boiled for a long time with baryum carbonate and some baryum hydrate, and gave off a smell of ammonia. It was subsequently filtered. The precipitate on the filter contained much carbonate, and probably an organic product of decomposition. The filtrate was mixed with a large quantity of 98 per cent. alcohol, whereupon a pale yellowish white baryum salt was precipitated. This salt was washed with alcohol of 98 per cent. (the wash-alcohol was strongly alkaline), dissolved in water, and the aqueous solution boiled. Thereupon ammonia was again perceptible, and an abundance of baryum carbonate precipitated. This was removed by filtration, the filtrate evaporated to dryness at 110° and powdered. It

formed a gummy mass, offering no difficulty to comminution. The total amount of baryum salt obtained was 4·377 grms.

Analyses :—

- I. ·5170 grm. burned with lead chromate and copper turnings gave ·1800 H₂O and ·5090 CO₂, equal to 3·87 per cent. H., and 26·94 per cent. C.
- II. ·4025 grm. gave ·02391 grm. nitrogen, equal to 5·94 per cent. N.
- III. ·2700 grm. dried at 110°, and burned, left ·1458 BaSO₄, equal to 31·75 per cent. Ba.

These data lead to the formula C₁₀H₁₄BaN₂O₉ :

Calculated.				Found.		
				I.	II.	III.
10 C	120	27·09		26·84	—	—
14 H	14	3·16		3·87	—	—
Ba	137	30·93		—	—	31·75
2 N	28	6·32		—	5·94	—
9 O	144	32·50		—	—	—
	<hr/>	<hr/>				
	443	100·00				

The reaction by which this salt is produced is the loss of baryum oxide. Thus :

This salt gave a white precipitate with silver nitrate, soluble in nitric acid. It gave a white precipitate with lead acetate soluble in acetic acid. It gave no reaction with copper acetate, zinc chloride, or calcium chloride. With corrosive sublimate it gave a white precipitate soluble in HNO₃; with mercuric nitrate the same; with mercurous nitrate a precipitate which apparently white at first became quickly dark. The solid baryum salt, moistened with strong sulphuric acid, appeared to give a double sulphate and kryptophanate of baryum.

17. *Kryptophanates of Cobalt.*

A solution of kryptophanic acid (some of the same sample as that employed for magnesium salt) was treated with cobalt carbonate; effervescence ensued in the cold and more on boiling. A red solution was obtained and precipitated by two volumes of alcohol of 94 per cent. A pale pink precipitate ensued, which was filtered off. On drying in the steam-bath it shrunk, then fused like the calcium and other salts, and soaked the paper like fat (of this the greater portion dissolved in warm water with red colour as before). It ultimately became hard, and then was white rose coloured in the interior of the lumps. It was powdered and dried at 100° to 110°.

Determination of Co.—0.834 grm. burned and reduced in H atmosphere gave .014 grm. or 16.78 per cent Co. The formula $C_{10}H_{16}CoN_2O_{10}$ (M.W. 382.8) requires 15.36 per cent. Co.

The formula $C_{10}H_{14}CoN_2O_9$ requires 16.19 per cent. Co.

Solution in Alcohol.—Was of a rose red colour, and on evaporation in platinum dish, became indigo blue wherever it dried, and was ultimately a deep blue hard mass. It was redissolved in water, when it again became red; filtered from slight precipitate and again evaporated. Dried at 110°. On heating it swelled up greatly, gave out stinking gas, and left residue of cobalt and carbon. This had to be treated with nitric acid to deflagrate all carbon, after which the cobalt was reduced in hydrogen atmosphere.

Determination of Cobalt.—2.897 grm. left after the before mentioned treatment .08 grm. Co or 27.7 per cent. Co. The formula $C_{10}H_{14}Co_2N_2O_{10}$ (M.W. 439.6) requires 26.7 per cent. Co. $C_{10}H_{12}Co_2N_2O_9$ requires 27.9 per cent. Co.

18. *Silver-salts.*

When a solution of a tetrabasic kryptophanate, as for instance the magnesium salt, $C_{10}H_{14}Mg_2N_2O_{10}$, is mixed with a solution of silver nitrate, there is produced a dark grey precipitate, which either has not a definite composition or else suffers decomposition spontaneously or on washing. The following determinations of silver in different specimens of the precipitate will serve to illustrate the inconstancy of its composition after having been washed and dried.

			Percentage of silver.
Precipitate <i>a</i>			77·2
Do. <i>b</i>			60·08
Do. <i>c</i>			56·56

Precipitate *a* was prepared by using a *very small proportion* of silver nitrate to precipitate the magnesium kryptophanate. It was washed, dried in the steam-bath, and finally dried at 100° to 110°

Precipitate *b* was washed six times with a small quantity of water and dried in vacuo.

Precipitate *c* was very little washed, and then pressed and dried in vacuo for several days.

Thus it appears that by dint of washing decomposition of the silver-salt is effected, so as to give a salt richer in silver than the original precipitate.

Precipitate *c*, which may be regarded as the least altered specimen of the precipitated silver-salt, roughly approximates in composition to the formula $C_{10}H_{14}Ag_4N_2O_{10} + 2 \text{ Aq.}$, corresponding with the magnesium-salt from which it was formed. The following are the details of its preparation and analysis:—

3 gram. of silver nitrate were dissolved in water and precipitated with 8 c.c. of very concentrated solution of tetrabasic magnesium kryptophanate. The dark-coloured precipitate, after slight washing, pressing, and drying *in vacuo* for several days, weighed 1·603 gram.

I. ·8455 gram. burnt with copper oxide, etc., gave ·4055 gram. of CO_2 (water not weighed).

II. ·4300 gram. gave ·2432 gram. of metallic silver (by ignition after moistening with nitric acid).

The formula $C_{10}H_{14}Ag_4N_2O_{10} + 2 \text{ Aq.}$ requires

Calculated.			Found.
10 C	120	15·2	13·08
18 H	18	—	—
4 Ag	432	54·7	56·56
2 N	28		
12 O	192		
<hr/>			
790			

(It will be understood that the insufficiency of the washing would occasion the carbon to be too low, and the ignited residue to be too high for the real quantity of silver.)

The interpretation which is to be put on the data given by this examination of the silver-salt appears to be the following. The tetrabasic silver-salt is very perishable, and breaks up into tribasic silver-salt and silver oxide. By washing, tribasic silver-salt is more or less perfectly dissolved out, and silver oxide accordingly accumulates in the precipitate.

The stable silver-salt of kryptophanic acid appears to be the tribasic salt, and is formed by double decomposition, when either a dibasic or a tribasic kryptophanate is added to a solution of silver-nitrate. It is white, and appears to be rather soluble in water.

a. Some silver-salt obtained by double decomposition between dibasic calcium-salt and silver nitrate, was washed and dried in vacuo. .1110 grm. gave .0570 grm. of metallic silver, or 51.35 per cent. Ag.

b. Some silver-salt prepared from dibasic baryum-salt and silver-nitrate, and dried in vacuo, gave 52.80 per cent. Ag.

c. Another quantity of silver-salt made from a specimen of calcium kryptophanate, believed to be the tribasic salt, had the following history :—

Calcium kryptophanate, purified twice by resolution in water, and precipitation by alcohol, was boiled with animal charcoal to decolorize it, and then mixed with silver nitrate as long as a precipitate was produced. The precipitate, white at first, became slightly coloured grey. It was washed, ultimately boiled with alcohol, and dried at 100° to 110°. At 130° it became brownish on surface.

Analyses :—

- I. .2785 grm. left .1479 grm. or 53.1 per cent. Ag.
- II. .3722 grm. left .2005 grm. or 53.86 per cent. Ag.
- III. 52.8 per cent. Ag. found.
- IV. .2048 grm. burned with CuO, Cu, and KClO₃, yielded .150 of CO₂ and .046 H₂O, equal to .0409 grm., or 19.97 per cent. C., and .0051 grm. or 2.49 per cent. of H.
- V. .2656 grm. burned with CuO and Cu gave gas = 16.1 c.c. at 12.5° and 641.2 mm. (dry), equal to 13. c.c. absolute. Corrected with O for NO this became 12. c.c. absolute, equal to .01507 grm., or 5.7 per cent. N (roughly).

Calculated.			Found.						
			a.	b.	I.	II.	III.	IV.	V.
10 C	120	19.08	—	—	—	—	—	19.97	—
13 H	13	2.07	—	—	—	—	—	2.49	—
3 Ag	324	51.53	51.35	52.80	53.1	53.8	52.8	—	—
2 N	28	4.45	—	—	—	—	—	—	5.7
9 O	144								
		<hr/>							
		629							

19. Theoretical considerations on Kryptophanic Acid.

Kryptophanic acid is written in much of the foregoing as a dibasic acid of the formula $C_5H_9NO_5$. But it is evident that it may be considered as tetrabasic, and to have the formula $C_{10}H_{18}N_2O_{10}$. In that case the metallic salts will have the general formula $C_{10}H_{14}M'_4N_2O_{10}$.

Examples:—

Lead-salt	$C_{10}H_{14}Pb_2N_2O_{10}$.
Do. hydrated.....	$C_{10}H_{14}Pb_2N_2O_{10} + 2Aq.$
Basic	$2(C_{10}H_{14}Pb_2N_2O_{10})PbO.$
Copper salt	$C_{10}H_{14}Cu_2N_2O_{10}$.
Do. with alcohol	$C_{10}H_{14}Cu_2N_2O_{10} + C_2H_6O.$
Magnesium salt.....	$C_{10}H_{14}Mg_2N_2O_{10} + Aq.$
Do. dihydrate	$C_{10}H_{14}Mg_2N_2O_{10} + 2Aq.$
Baryum salt	$C_{10}H_{14}Ba_2N_2O_{10} + Aq.$
Do. tribasic	$C_{10}H_{13}ba^I_3N_2O_{10}$.
Do. acid.....	$C_{10}H_{14}BaN_2O_9$.
Calcium salt	$C_{10}H_{13}ca^I_3N_2O_9$.
Do. acid.....	$C_{10}H_{14}CaN_2O_9$.
Cobalt-salt, acid.....	$C_{10}H_{14}CoN_2O_9$.
Do. basic	$C_{10}H_{12}Co_2N_2O_9$.
Silver-salt	$C_{10}H_{13}Ag_3N_2O_9$.

It gives me great pleasure to acknowledge the important aid which I have received from my friend Mr. J. A. Wanklyn in the latter part of this research, more particularly in the elucidation of the changes of basicity of several salts.

My thanks are also due to my assistant Mr. C. G. Stewart, for the care and assiduity with which he prepared for me large quantities of the primary material.

The foregoing research has been conducted by me for the Medical Department of the Privy Council, at the Pathological Laboratory of St. Thomas's Hospital.