I. OBSERVATIONS ON THE PHOSPHOTUNGSTATES OF CERTAIN BASES AND AMINO-ACIDS.

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INTRODUCTORY.

IN spite of the extensive use which is made of phosphotungstic acid as a precipitant for organic bases, comparatively few investigators have studied the individual salts of the acid or made any attempt to define the most suitable conditions for their preparation.

The observations recorded in this communication were made during an attempt to separate complex mixtures of bases and amino-acids by a process depending upon the relative solubilities of their phosphotungstates in various solvents.

Unfortunately the study is to a large extent incomplete, and, since there does not appear to be any likelihood of the opportunity arising whereby the work may be completed, it has been decided to publish the few results which have been obtained in the hope that they may either be of some slight practical utility, or that they may suggest more extensive investigations along similar lines.

Phosphotungstic acid was introduced as a precipitant for organic bases by Drechsel, and since that introduction its use for that purpose has become more and more extensive. References to the use of the acid are scattered so widely apart throughout the literature that it is difficult to review them at all completely. With regard to the acid itself, its preparation is described by Drechsel [1887], and also by Winterstein [1898]. The two methods are practically identical in detail and give a yield of from two to three parts of the acid from four parts of sodium tungstate and one part of sodium phosphate. The acid prepared by this method is usually somewhat impure and

it is necessary in such cases to subject it to a process of purification by ether treatment (Winterstein). Reference to the literature shows that a large series of phosphotungstic acids have been described, mainly by Kehrman and his co-workers [1887, 1888, 1892, etc.]. These acids possess different proportions of P_2O_5 and WO_3 . Apparently the acid prepared by the process referred to above, which is that sold commercially, forms salts of the type

(R being a monovalent base.)

Gulewitsch [1899] prepared and submitted to analysis pure preparations of arginine phosphotungstate and found that his analytical data satisfied the formula $(C_6H_{14}O_2N_4)_3, 2H_3PO_4, 24WO_3, 10H_2O$.

Similarly Barber [1906] in an investigation of the phosphotungstates of the simpler amino-acids gives a similar formula for:

In the case of the salts of aspartic acid and asparagine, however, he suggests formulae of a different type:

Wechsler [1911] confirmed the formula given by Gulewitsch for arginine phosphotungstate and found that a similar one, $(C_6H_9O_2N_3)_3, 2H_3PO_4, 24WO_3$, satisfied the analytical data for the histidine salt.

A considerable number of salts of phosphotungstic acid with various bases and amino-acids have been prepared during the course of this inquiry and in every preparation submitted to analysis the figures have agreed with the composition indicated by the formula:

With regard to the studies that have been made of the salts themselves there is little to review. Generally speaking they have been concerned with determinations and comparisons of the relative precipitability of various substances by the acid. Gulewitsch [1899] made a careful investigation of arginine phosphotungstate, and determined the conditions for its optimum precipitability. He pointed out the advantage of utilising a slight excess of the precipitant and of conducting the operation in a medium of 5 % sulphuric acid.

1. Betaine Phosphotungstate.

4. Cystine Phosphotungstate.

2. Adenine Phosphotungstate.

5. Arginine Phosphotungstate.

3. Histidine Phosphotungstate.

6. Urea Phosphotungstate.

Photomicrographs of some crystalline Phosphotungstates
(utilising 4 mm. objective and ocular 4. (Leitz.))

Barber [1906] investigated the phosphotungstates of several of the simpler amino-acids, and recorded their probable constitution, relative precipitability and their solubilities in water, alcohol and 50 % alcohol.

Levene and Beatty [1906] also studied the precipitability of several simple amino-acids.

Other investigations of a similar nature are most difficult to collate since they are scattered so widely throughout the mass of literature dealing with the individual bases themselves. The few that have been traced will be considered when the particular compound to which they refer is being discussed.

EXPERIMENTAL.

1. *Conditions under which maximum precipitation is obtained.*

From a consideration of first principles it is recognised that many factors will influence the precipitability of a substance. In the present case the most important of these factors are temperature, the concentration of base, and the degree of acidity of the medium in which the reaction is carried out.

The study of arginine phosphotungstate made by Gulewitsch [1899] included an experimental investigation of the conditions necessary for the optimum precipitation of that compound from a 0.02 % solution of arginine nitrate.

The results of his investigation demonstrated the advisability of using a slight excess of the precipitant and of operating in a medium of 5 % sulphuric acid. When the precipitation occurred in 2.5 or 7 % sulphuric acid, or when too great an excess of the phosphotungstic acid was used precipitation was either incomplete or the precipitate tended to redissolve.

The determinations of precipitability recorded in this paper were made by adopting a technique similar to that employed by Gulewitsch.

A series of numbered, stoppered, 50 cc. volumetric flasks was taken and into each was introduced a known volume of a dilute solution of the base under examination. A 20 % solution of sulphuric acid was then run in to each flask in an amount sufficient to render the contents of the flask of the required degree of acidity when made up to 50 cc. Distilled water was then added in an amount just sufficient to permit the further addition of the required amount of a 30 % solution of phosphotungstic acid in order to bring the volume of liquid in each flask up to 50 cc.

The flasks were then stoppered and shaken vigorously for half a minute,

after which they were allowed to stand at room temperature (19°) for 24 hours with occasional agitation.

The contents of the flasks were filtered and determinations of unprecipitated nitrogen made upon aliquot portions of the filtrates.

The following tables indicate the findings in the study of the precipitation of betaine and histidine from solutions of their salts.

TABLE I.

Precipitation of histidine from a dilute solution of histidine hydrochloride by phosphotungstic acid under varying conditions. Figures represent mg. nitrogen unprecipitated. (Temperature 19°.)

Strength of sulphuric acid present %	cc. 30 % phosphotungstic acid solution					
	0	5	10	15	20	30
0	11.58	—	—	—	5.21	6.00
2	—	—	—	—	3.99	—
3.5	—	—	—	—	3.90	—
5	—	7.96	5.25	3.60	3.65	3.50
7	—	7.96	5.25	4.05	4.00	3.60
9	—	—	—	—	4.37	—
11	—	—	—	—	4.57	—
18	—	—	—	—	5.20	6.33

TABLE II.

Precipitation of betaine from a dilute solution of betaine hydrochloride by phosphotungstic acid under varying conditions. (Temperature 19°.)

Strength of sulphuric acid %	cc. 30 % phosphotungstic acid solution					
	0	5	8	10	20	30
0	3.07	—	—	2.05	—	—
3.5	—	—	—	1.54	—	2.07
5	—	—	1.77	1.43	1.46	1.90
7	—	2.17	1.83	1.47	1.54	1.96
9	—	—	—	1.54	—	—
18	—	2.24	—	1.89	—	—

From a consideration of these figures it will be recognised that the conclusions are similar to those made for arginine by Gulewitsch, in that the most satisfactory precipitation of these two bases occurs when a slight excess of the reagent is used and when the operation is carried out in 5 % sulphuric acid. Gulewitsch recorded the different appearances of the precipitated arginine phosphotungstate obtained under varying conditions, for the beneficial influence of the slight excess of phosphotungstic acid is not as readily observed from the tables as it is from a study of the precipitates

themselves. Under conditions such as are indicated above the precipitates tend to become more distinctly crystalline and are therefore more readily filterable.

A convenient form in which to use the phosphotungstic acid is that of a 25—30 % aqueous solution. Many samples of the acid recently purchased [1916] have been somewhat impure, and it is wise never to employ an acid, which fails to dissolve in water to a clear solution, until it has been purified by the ether process sufficiently to pass this test.

Demjanowski [1912] did not find any appreciable difference in the precipitability of creatinine, carnosine and methylguanidine when he employed 2.5 % and 25 % solutions of the reagent, so apparently the strength of the latter is a comparatively unimportant matter.

Re-solution of the precipitate upon adding an excess of the precipitating acid does not always occur. Many phosphotungstates are extremely readily soluble in such excess, whilst others are not so influenced. The condition of the precipitate itself is governed by several factors. Upon standing amorphous precipitates often become slowly crystalline, whilst in many cases slow precipitation from a very dilute solution of the base results in the production of a crystalline precipitate direct.

A modification of the latter process is valuable for obtaining crystals of a relatively large size, if such are desired for any purpose.

The crystalline form of phosphotungstic acid precipitates when derived from the amorphous forms is usually that of masses of thin needles or of short rhombohedral prisms. When however the crystals are allowed to form slowly, as described above, by precipitation from very dilute solution or when the precipitates are slowly recrystallised from suitable solvents it is sometimes possible to obtain specimens which are characteristic of the particular salt.

Unfortunately the number which yield such characteristic crystals is small, and it is only very occasionally that the crystalline form of the salt is any guide to the recognition of the parent base. Certain of the phosphotungstates examined during the course of this investigation possessed colour and in certain cases this is helpful for purposes of identification.

2. *Preparation of the purified phosphotungstates.*

The phosphotungstates were in all cases prepared by the addition of a very slight excess of 30 % phosphotungstic acid to a solution of the base or its salt in 5 % sulphuric acid. After standing for 24 hours the precipitates

were filtered off and well washed with 5 % sulphuric acid. The products thus obtained were submitted to an initial purification by recrystallisation from hot water and the crystalline salts dried over sulphuric acid in a darkened and evacuated desiccator. The storage of the pure products should be carried out in the absence of light since many of them tend to assume a blue or violet colour if exposed to photochemical influences.

When prepared by such a process the phosphotungstates are in the large majority of cases well defined, crystalline products. A few of them are coloured but the greater number are colourless. The crystalline form of the salts varies with the conditions of crystallisation. If this is rapid the crystals are usually thrown out of solution in thick felted masses of needles. On the contrary slow crystallisation produces well formed crystals which are in some cases sufficiently large to be examined with a pocket-lens.

The choice of a solvent for recrystallisation of the phosphotungstates can be made from water, ethyl alcohol, methyl alcohol, acetone or mixtures of two or more of these.

The experience gained during the present research has led to the adoption of water or 50 % ethyl alcohol as being the most suitable solvents for the purpose. Even when recrystallised from the same solvent the crystalline form of an individual phosphotungstate may on different occasions exhibit differences, so that the identification of any particular salt by this means is a somewhat untrustworthy process.

3. *Analysis and composition of the purified phosphotungstates.*

The purified salts obtained by the process just described were in a number of cases submitted to analysis. Moisture was determined by the usual process of drying to constant weight at 110°. Nitrogen was estimated by Kjeldahl. In some cases determinations of tungsten oxide were carried out. Various procedures for conducting the latter analysis were tested, the best results being obtained by the method of Barber [1906], also used with good results by Wechsler [1911]. Many of the phosphotungstates were submitted to analysis after more than one recrystallisation and it was generally found that their composition remained unaffected by the purification. Apparently some compounds are so affected, for Barber [1906] states that upon recrystallisation of glutaminic acid phosphotungstate isomorphous mixtures of the salt and the free acid are obtained.

4. *Determinations of Solubility.*

Determinations of the solubility of the individual phosphotungstates in various solvents were carried out where the amount of material permitted. When this was not possible qualitative tests of this nature were made. The solubilities were estimated by determining the weight of salt dissolved in a known volume of solvent after they had remained in contact for 24 hours at room temperature with occasional agitation.

5. *Properties of the Individual Phosphotungstates.*(a) *Amino-acids.*

Glycine phosphotungstate, was studied by Barber [1906], who prepared it by the addition of 50 % phosphotungstic acid solution to a saturated solution of the amino-acid. He describes it as crystallising in microscopic whetstone-shaped crystals. On attempting to dissolve it in warm water he found that tungstic acid was thrown out of solution.

Preparations dried at 105° gave upon analysis figures which agreed with the formula, $(C_2H_5ON_2)_3, H_3PO_4, 12WO_3$. It normally occurs with water of crystallisation, approximately 5—6 molecules being present. Recrystallisation from hot alcohol results in glycine being deposited. Levene and Beatty [1906] record the precipitability of glycine by phosphotungstic acid.

Alanine phosphotungstate was described by Barber [1906] as crystallising in microscopic twin needles. It retains its composition after recrystallisation. Its analysis agrees with the formula $(C_3H_7O_2N)_3, H_3PO_4, 12WO_3, 4-5H_2O$. Levene and Beatty [1906] have studied its precipitability.

Leucine phosphotungstate. Barber [1906] could not prepare leucine phosphotungstate, and a similar failure was reported by Schulze and Winterstein [1901]. Levene and Beatty [1906], however, describe it as an oily product soluble in a small excess of the reagent.

Aspartic acid phosphotungstate. Barber [1906] states that it occurs in the form of stubby microscopic octahedra containing 24 molecules of water of crystallisation, and varying in composition, with the formula $(C_4H_7O_4N)_4, 2H_3PO_4, 20-22WO_3$. Its composition usually agrees most satisfactorily with $22WO_3$ and it is unchanged by recrystallisation. Levene and Beatty [1906] studied the precipitability of aspartic acid by phosphotungstic acid.

Glutaminic acid phosphotungstate is described by Barber [1906] as a flocculent mass which slowly becomes crystalline. Its composition as indicated

by the analytical figures indicates that it readily forms isomorphous mixtures with the free acid. Levene and Beatty [1906] describe it as slightly soluble in excess and they record its precipitability.

Cystine phosphotungstate was described by Winterstein [1901] who obtained a crystalline product. Plimmer [1913] has attempted to utilise the fact that cystine is completely precipitated from all but very dilute solutions by phosphotungstic acid as a basis for a method of separating cystine and tyrosine.

Cystine is readily precipitated from solution by means of phosphotungstic acid. The precipitate is usually of a pale cream colour and if thrown out of dilute solutions may be crystalline.

Upon recrystallisation from hot water, in which it is readily soluble, it separates out in very small rhombohedral tables of a pale sulphur yellow colour (Photograph 4). It does not alter in composition upon recrystallisation.

It usually contains 1.25 % water, equivalent to 4—5 molecules of water of crystallisation.

0.2041 g.; 17.6 cc. 0.01N NH_3 = 1.2 % N

0.2046 g.; 18.0 " " " = 1.2 % N.

Calculated for $(\text{C}_6\text{H}_{12}\text{O}_4\text{N}_2\text{S}_2)_3, 2\text{H}_3\text{PO}_4, 24\text{WO}_3, 4-5\text{H}_2\text{O}$ = 1.28 % N.

It is very soluble in alcohol, methyl alcohol and acetone.

Phenylalanine phosphotungstate was described in the first place by Schulze and Winterstein [1901] as a pale yellow oil which becomes crystalline upon standing. They state that it is not very soluble in cold water and that it is not precipitated from dilute solutions of the amino-acid. Levene and Beatty [1906] record a similar description of the compound. For this investigation it was prepared from a strong solution of phenylalanine in 5 % sulphuric acid. It separates initially as a lemon yellow oil, sometimes possessing characteristic odour. Upon standing the oil becomes crystalline, forming masses of thin plates and needles. It is readily recrystallised from hot water and after separating as an oil again soon crystallises. The crystals are short rhombohedral prisms which on account of their colour are fairly characteristic. It contains approximately four molecules of water of crystallisation.

Upon analysis it gave the following figures:

0.2071 g.; 16.0 cc. 0.01 N NH_3 = 1.1 % N

0.2065 g.; 15.5 " " " = 1.1 % N

0.1032 g.; 0.0855 g. WO_3 = 83.0 % WO_3 .

Calculated for $(\text{C}_9\text{H}_{11}\text{O}_2\text{N})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3, 4\text{H}_2\text{O}$, N = 1.22 %, WO_3 80.9 %.

Its composition is constant after repeated recrystallisation.

Tyrosine phosphotungstate was not obtained by Schulze and Winterstein [1901], nor by Barber [1906], moreover all attempts made during the course of this work to isolate the compound failed.

Histidine phosphotungstate. Wechsler [1911] has made an analysis of histidine phosphotungstate and considers that it is represented by the formula $(C_6H_5O_2N_3)_3, 2H_3PO_4, 24WO_3$.

It is readily prepared as a white microcrystalline powder. Upon precipitation from very dilute solutions of the base or by recrystallisation from water or dilute alcohol, it may be obtained in comparatively large, clear, well-formed crystals consisting of rhombohedral prisms. (Photograph 3.)

Wechsler describes the substance as anhydrous. Specimens prepared during this investigation, however, gave on analysis indications of the presence of five molecules of water of crystallisation. Its composition remains unaltered with purification.

0.2083 g.;	3.45 cc. 0.1 N NH_3	= 2.3 % N
0.2059 g.;	3.20 cc. „ „	= 2.2 % N
0.1959 g.;	0.1708 g. WO_3	= 87.2 % WO_3
0.2170 g.;	0.1885 g. „	= 86.9 % WO_3 .

Wechsler gives the following analysis of his product.

2.00	2.08	2.06 % N
86.4	89.8	— % WO_3 .

Calculated for $(C_6H_5O_2N_3)_3, 2H_3PO_4, 24WO_3$, N = 2.02 %, WO_3 = 89.3 %.

Calculated for $(C_6H_5O_2N_3)_3, 2H_3PO_4, 24WO_3, 5H_2O$, N = 1.98 %, WO_3 = 87.2 %.

The salt is very soluble in acetone and methyl alcohol but comparatively insoluble in water and alcohol.

Arginine phosphotungstate. Gulewitsch [1899] made a fairly complete study of this compound. Reference has already been made to his observations upon the conditions influencing its precipitation.

His preparations were submitted to analysis and gave the following data:

N	2.84	2.68 %
C	3.48	3.44 %
H	0.91	0.74 %.

From these figures he considered the substance was to be represented by

which requires C = 3.46, N = 2.6, H = 0.65.

Wechsler [1911] agrees with this formula since his preparations contained 2.64 % and 2.70 % nitrogen.

Several préparations made during the course of this work were analysed, the following results being typical of those obtained.

0.2025 g.; 3.20 cc. 0.1 N NH_3	= 2.2 % N
0.2096 g.; 4.15 cc. „ „	= 2.8 % N
0.2288 g.; 0.2014 g. WO_3	= 88.0 % WO_3
0.2394 g.; 0.2104 g. „	= 87.9 % WO_3 .

The substance contained water of crystallisation equivalent to 6—10 molecules.

Calculated for $(\text{C}_6\text{H}_{14}\text{O}_2\text{N}_2)_3, 2\text{H}_3\text{PO}_4, 24\text{WO}_3, 6\text{H}_2\text{O}$, N = 2.63 %, WO_3 = 87.2%.

The phosphotungstate prepared in the usual manner is a white amorphous powder. It may be readily obtained in the form of rhombohedral prisms of a comparatively large size by recrystallisation from water or dilute alcohol (Photograph 5). The salt tends to redissolve in the presence of a great excess of the reagent, a point also noted by Gulewitsch.

Lysine phosphotungstate is precipitated from great dilutions of the salts of the amino-acid as a white amorphous powder. It may be obtained in a crystalline condition by recrystallisation from dilute alcohol and usually separates out in large clusters of very fine needle-shaped crystals. It retains its composition although subjected to many recrystallisations and is anhydrous. Upon analysis it yields figures which satisfy the formula

0.2089 g.; 18.0 cc. 0.01 N NH_3 = 1.2 % N

0.2094 g.; 19.0 cc. „ „ = 1.3 % N.

Required for above formula 1.35 % N.

No other amino-acid phosphotungstates were investigated in this research. The solubilities of those whose other properties have been described are given in the table of solubilities at the end of the paper.

(b) *Amines.*

The basic nature possessed by representatives of this class of compound is responsible for the formation of a series of well-defined salts with phosphotungstic acid. Generally speaking they are precipitated from very dilute solutions of the parent base as heavy insoluble microcrystalline powders. One or two of them possess colour which renders their identification possible.

Trimethylamine phosphotungstate is precipitated from exceedingly dilute solutions of the base as a heavy white amorphous powder. It may be obtained

in the form of microscopic octahedra by recrystallisation from water. Its composition agrees with the formula $((\text{CH}_3)_3\text{N})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3$.

Analysis: 0.2011 g.; 18.9 cc. 0.01 *N* $\text{NH}_3 = 1.3\%$ N.

Calculated for above formula = 1.37 % N.

It is readily soluble in acetone but not appreciably soluble in the other solvents examined.

Isobutylamine phosphotungstate separates out as a heavy white flocculent precipitate from solutions of the base upon the addition of phosphotungstic acid. It is very soluble in acetone, but not greatly so in water or ethyl alcohol. It crystallises in large clear rhombohedral prisms.

Isoamylamine phosphotungstate is closely similar to the preceding compound in its crystalline appearance and solubilities.

Tri-isobutylamine, Tripropylamine and Tri-isoamylamine phosphotungstates are closely similar to each other. They are all precipitated from great dilution as heavy white, amorphous precipitates. They crystallise in microcrystalline rhombohedral prisms and tablets, and occasionally in fern-like aggregates closely resembling those assumed by ammonium chloride.

Their solubilities are similar, all being exceedingly soluble in acetone or dilute acetone, practically insoluble in water and ethyl alcohol and slightly soluble in methyl alcohol. The composition of two of these salts was investigated.

Tri-isobutylamine phosphotungstate.

0.4699 g. anhydrous salt; 4.4 cc. 0.1 *N* $\text{NH}_3 = 1.3\%$ N.

Required for $(\text{C}_4\text{H}_9\text{N})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3 = 1.23\%$ N.

Tri-isoamylamine phosphotungstate.

0.5832 g. anhydrous salt; 3.6 cc. 0.1 *N* $\text{NH}_3 = 1.4\%$ N.

Required for $(\text{C}_5\text{H}_{11}\text{N})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3 = 1.3\%$ N.

Putrescine and Cadaverine phosphotungstates. Putrescine and cadaverine are both precipitated from very dilute solution by phosphotungstic acid. The resulting salts are heavy white amorphous powders which can be obtained in a microcrystalline condition by recrystallisation from water or alcohol. The crystals are not characteristic, being as is the case with the majority of these salts, rhombohedral prisms.

They exhibit similar solubilities to one another. Acetone or dilute acetone readily dissolves them, but in water they are practically insoluble. Ethyl and methyl alcohol will take up a fair amount of the salts.

Phenylethylamine phosphotungstate. This compound closely resembles the

related phenylalanine phosphotungstate in many of its properties. It is not precipitated from dilute solutions of the base except on long standing. It separates out initially as a pale sulphur yellow oil which slowly becomes crystalline. On recrystallisation from water the coloured oil once again separates but the crystals which subsequently form are fairly large and well-formed. They usually appear as pale yellow prisms and tablets, closely resembling the salt of phenylalanine.

The analysis of the purified anhydrous salt gave the data:

$$0.3517 \text{ g.}; 3.6 \text{ cc. } 0.1 \text{ N } \text{NH}_3 = 1.4 \% \text{ N.}$$

$$\text{Required for } (\text{C}_8\text{H}_{11}\text{N})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3 = 1.3 \% \text{ N.}$$

Its composition is unaltered by repeated purification. Its solubilities are given in the table on p. 22.

p-Hydroxyphenylethylamine phosphotungstate is not precipitated from dilute solutions except on long standing. From concentrated solutions it is thrown out as a straw coloured microcrystalline powder, consisting of flat cubical plates. When deposition occurs slowly or when recrystallisation of the product is carried out with care the compound is readily obtained in the form of large orange-red octahedra, the colour of which resembles that of potassium dichromate.

The salt obtained by these methods is anhydrous and its composition corresponds to the formula $(\text{C}_8\text{H}_{11}\text{ON})_3, \text{H}_3\text{PO}_4, 12\text{WO}_3$.

$$0.2190 \text{ g.}; 2.05 \text{ cc. } 0.1 \text{ N } \text{NH}_3 = 1.3 \% \text{ N.}$$

$$\text{Required for above formula} = 1.28 \% \text{ N.}$$

It is not very soluble in cold water but readily in hot. It is fairly soluble in ethyl alcohol and very soluble in acetone. The crystalline form and colour of this salt are of help in its identification.

β -Iminazoly-ethylamine phosphotungstate. This compound is precipitated from great dilutions as is the corresponding amino-acid compound. It separates out as a white microcrystalline powder which often assumes a beautifully crystalline form upon standing. These crystals may also be obtained by recrystallisation from water or dilute alcohol, the salt separating from these solvents in the form of relatively large clear rhombohedra and prisms. Its composition agrees with the formula $(\text{C}_5\text{H}_9\text{N}_3)_3, 2\text{H}_3\text{PO}_4, 24\text{WO}_3$. Its solubilities and other properties are very similar to those of histidine phosphotungstate.

Indolethylamine phosphotungstate. This salt is precipitated from very dilute solutions of the base as a terra cotta coloured amorphous powder. Upon standing this undergoes change and becomes crystalline forming large

aggregates of needles possessed of a deep brownish purple colour. Upon recrystallisation the amorphous form usually separates out first, the transition to the crystalline form occurring sooner or later upon standing. The microscopical appearance of the crystals of the salt is characteristic.

Upon analysis the following figures for the determination of nitrogen were obtained.

$$0.1771 \text{ g.}; 3.5 \text{ cc. } 0.1 \text{ N } \text{NH}_3 = 2.8 \% \text{ N.}$$

$$\text{Required for } (\text{C}_{10}\text{H}_{12}\text{N}_2)_3, \text{H}_3\text{PO}_4, 12\text{WO}_3 = 2.5 \% \text{ N.}$$

The compound is very soluble in acetone and in diluted acetone, and fairly soluble in ethyl and methyl alcohol but is only very slightly soluble in cold water.

(c) *Purine Bases.*

The purine bases, generally speaking, are precipitated from very dilute solutions by phosphotungstic acid and the resulting salts are peculiar in being very slightly soluble, even in acetone. They crystallise in cubical plates and may in some cases be recognised by this microscopical appearance. Determinations of nitrogen by the Kjeldahl process do not yield accurate results since the purine nitrogen is not quantitatively accounted for by that method.

Adenine phosphotungstate is usually precipitated as a pale cream coloured powder. It is readily obtained in the form of clear sulphur-yellow cubical, or whetstone-shaped, crystals by careful recrystallisation from dilute alcohol or acetone (Photograph 2). It is very insoluble in water, and ethyl and methyl alcohols and only very slightly soluble in acetone. Funk [1915] has isolated adenine from the acetone-insoluble fraction of the phosphotungstates from autolysed yeast.

Analysis:

$$0.1050 \text{ g.}; 0.0899 \text{ g. } \text{WO}_3; \quad 85.6 \% \text{ WO}_3.$$

$$\text{Required for } (\text{C}_5\text{H}_5\text{N}_5)_3, \text{H}_3\text{PO}_4, 12\text{WO}_3; 86.0 \% \text{ WO}_3.$$

Guanine phosphotungstate is precipitated from extremely dilute solutions of guanine in the form of a buff coloured amorphous precipitate. Wechsler [1911] states that it possesses a brick red colour but the specimens prepared from pure guanine during this investigation were much paler in colour than he describes. Recrystallised from water or dilute alcohol it forms bunches of straw coloured, needle-shaped crystals or small cubical plates. Its properties are closely similar to those of the corresponding adenine salt.

Hypoxanthine phosphotungstate is precipitated as a pale cream coloured amorphous precipitate. It crystallises in small cubical plates possessing a

light straw colour. Its solubilities are very similar to those of adenine and guanine.

Xanthine phosphotungstate is very similar in its properties and its crystalline appearance to the hypoxanthine salt.

Uric acid phosphotungstate. The phosphotungstate of uric acid is referred to by Hammarsten [1909, p. 672] as a brick red coloured powder.

It is prepared by precipitation of a solution of sodium urate by phosphotungstic acid and forms a deep chocolate brown powder. It cannot be recrystallised from water or alcohol owing to formation of insoluble uric acid. If prepared from hot sodium urate solutions it may be obtained in a crystalline condition, the crystals being cubical plates and of a deep red brown colour.

(d) *Betaïnes and other Bases.*

The betaïnes form well defined crystalline salts with phosphotungstic acid as also do many of the naturally occurring bases. The representatives of both classes are numerous and only a few which were readily obtainable were examined.

Betaïne phosphotungstate. Betaïne is precipitated from very dilute solutions by phosphotungstic acid as a heavy white precipitate. The salt may be obtained in the form of large clear rhombohedral prisms by recrystallisation from water or dilute alcohol (Photograph 1). It is exceedingly soluble in acetone and in dilute acetone. Its composition satisfies the formula

0.3889 g.; 32.2 cc. 0.01 N NH_3 = 1.2 % N

0.3893 g.; 36.95 cc. „ „ = 1.3 % N

0.4658 g.; 0.4040 g. WO_3 = 86.7 % WO_3 .

Required for above formula N = 1.3 %, WO_3 = 86.2 %.

Repeated recrystallisation does not affect its composition.

Stachydrine phosphotungstate. Solutions of stachydrine salts yield a heavy, white granular precipitate with phosphotungstic acid. The salt after purification by recrystallisation forms clusters of needles and small rhombohedral prisms, often with cut edges. It is very soluble in acetone and dilute acetone. It is anhydrous and its composition agrees with the formula

Nicotinic acid phosphotungstate is very insoluble and is therefore precipitated from great dilutions of the base. It can be obtained in a crystalline condition forming small rhombohedral prisms. It is fairly soluble in acetone.

Choline phosphotungstate. Choline is precipitated from very dilute solutions (Gulewitsch states 1 in 20,000) by phosphotungstic acid. The initial precipitate is a heavy white amorphous substance. It can be obtained in a micro-crystalline form by recrystallisation from dilute alcohol. The crystals are minute rhombohedral prisms frequently possessing faceted edges.

Analysis of the purified substance:

0.3884 g.; 36.0 cc. 0.01 N NH_3 = 1.3 % N

0.3869 g.; 37.2 cc. " " = 1.4 % N.

Required for $(C_5H_{13}O_2N)_3, H_3PO_4, 12WO_3$ = 1.29 % N.

The salt remains unchanged in composition after more than one recrystallisation. It is readily soluble in acetone and dilute acetone but in other solvents is very insoluble.

Drummond and Funk [1914] record finding choline in the acetone-soluble fraction of the phosphotungstates from rice polishings.

Colamine (amino-ethyl alcohol) phosphotungstate. Barger [1914] records that colamine is not precipitated by phosphotungstic acid unless the solutions of the base are concentrated. This was found to be the case and the salt itself is very liable to re-solution in an excess of the reagent. Recrystallised from water or alcohol it forms beautiful clear rhombohedral crystals which often attain a relatively large size. The separation of choline and colamine by a method depending on the solubilities of their phosphotungstates is practicable. The latter salt is very soluble in water, alcohol or acetone.

Urea phosphotungstate. Urea is precipitated by phosphotungstic acid from its solutions, if these are not too dilute. The resulting white amorphous salt may be readily recrystallised from hot water in which it is very soluble. Large well-formed rhombohedral prisms are thus obtained which are easy to recognise (Photograph 6). The salt is readily soluble in ethyl and methyl alcohols and in acetone.

0.2002 g.; 3.4 cc. 0.1 N NH_3 = 2.3 % N

0.2071 g.; 4.01 cc. " " = 2.7 % N.

Required for $(CH_4ON_2)_3, H_3PO_4, 12WO_3$ = 2.74 % N.

Its composition is unaltered by repeated recrystallisation.

Creatinine phosphotungstate. Dilute solutions of creatinine do not always give a precipitate immediately upon the addition of phosphotungstic acid. Upon standing, however, the crystalline salt is in many cases slowly deposited. Hofmeister [1880] gives the limit of precipitability as 1 in 12,000 whilst Demjanowski [1912] obtained precipitation at a dilution of 1 in 25,000. Precipitated in this manner from dilute solutions of the base the phospho-

tungstate may be obtained in large well-formed crystals which are fairly characteristic. They usually consist of rhombohedra and rhombohedral prisms. When precipitated from more concentrated solutions the salt is thrown down as a heavy white precipitate consisting of microscopic needle-shaped crystals. The pure salt is anhydrous and is not very soluble in water or alcohol but is very soluble in various dilutions of acetone.

Analysis:

0.2090 g.; 5.91 cc. 0.1 N NH_3 = 4.0 % N

0.2070 g.; 5.59 cc. „ „ = 4.0 % N

0.1002 g.; 0.0865 g. WO_3 = 86.3 % WO_3 .

Required for $(\text{C}_4\text{H}_7\text{ON}_3)_3, \text{H}_3\text{PO}_4, 12\text{WO}_3$ = N 3.92 %, WO_3 86.7 %.

Its composition is unaltered by repeated recrystallisation.

Carnosine phosphotungstate. Carnosine is precipitated from very dilute solutions as the phosphotungstate in the form of a white heavy powder. It recrystallises in very minute whetstone- and needle-shaped crystals from water or dilute alcohol. Demjanowski [1912] describes its crystalline form and records its precipitability.

Analysis:

0.2074 g.; 3.47 cc. 0.1 N NH_3 = 2.3 % N

0.2082 g.; 3.60 cc. „ „ = 2.4 % N.

Required for $(\text{C}_9\text{H}_{14}\text{O}_3\text{N}_4)_3, 2\text{H}_3\text{PO}_4, 24\text{WO}_3$ = 2.6 % N.

Its composition is unaltered by purification. It is very soluble in acetone and dilute acetone.

Guanidine phosphotungstate is very insoluble in water and is precipitated from very dilute solutions of guanidine. When recrystallised from water or dilute alcohol it usually separates out again as a white microcrystalline powder consisting of minute spherulites of needles. Small rhombohedra are also sometimes obtained.

0.3904 g.; 11.49 cc. 0.1 N NH_3 = 4.1 % N

0.3879 g.; 11.36 cc. „ „ = 4.1 % N

0.3715 g.; 0.3461 g. WO_3 = 93.2 % WO_3 .

Required for $(\text{CH}_5\text{N}_3)_3, \text{H}_3\text{PO}_4, 12\text{WO}_3$ = 4.16 % N and 90.5 % WO_3 .

It is very soluble in acetone and mixtures of acetone and water.

Methylguanidine phosphotungstate is a heavy white powder which by recrystallisation from water or alcohol yields crystals consisting of small rhombohedral prisms with cut edges. Demjanowski [1912] describes a similar appearance for the salt. It is precipitated from very dilute solutions of methylguanidine salts.

(e) Inorganic Phosphotungstates.

A few inorganic salts of phosphotungstic acid are of importance from the point of view of this research, since the inorganic bases they contain are frequently present in tissue extracts or other material under investigation. The most important of these salts are the phosphotungstates of sodium, potassium and ammonium.

Ammonium, Sodium and Potassium phosphotungstates. Sodium phosphotungstate is very soluble in water and can only be obtained by concentration of its solutions almost to dryness.

Potassium phosphotungstate on the other hand is very slightly soluble in water and still less so in 5 % sulphuric acid, so that sodium and potassium may be almost quantitatively separated by this means. Ammonium phosphotungstate exhibits similar solubilities to the potassium salt, being very slightly soluble in most solvents. They are both difficult to obtain in a crystalline condition, usually occurring as very fine white precipitates which readily pass through filters.

Solubilities of the Phosphotungstates.

The solubilities in a number of solvents of all the phosphotungstates prepared during the course of this investigation have been determined, quantitatively where possible, and qualitatively where the amount of material was insufficient for the purpose, and the results are summarised in Table III.

The determinations were in all cases carried out at room temperature (18—20° C.) and are expressed in grams per 100 g. of solvent.

The figures given for the solubilities of certain of the phosphotungstates in 57 % acetone are taken from the paper by Wechsler [1911].

The original aim of the investigation, as has already been stated, was to attempt the separation of mixtures of bases by taking advantage of differences in the solubilities of their phosphotungstates. Wechsler compared the solubilities of a number of these salts in a solvent composed of four volumes of acetone to three of water and concluded that his results had a practical value. Reviewing the table of solubilities we see that wide variations in solubility are few.

The relatively great solubility of the majority of phosphotungstates in acetone is marked and this solubility becomes much greater if water is present.

The following series of solubility determinations in various dilutions of acetone were made upon a preparation of adenine phosphotungstate.

TABLE III

Phosphotungstate	Water	Ethyl alcohol	50% Ethyl alcohol	Methyl alcohol	Acetone	75 % Acetone	57 % Acetone	50 % Acetone	25 % Acetone	Remarks
Glycine ...	4.5	14.44	21.30	v.s.	v.s.	v.s.	—	v.s.	v.s.	—
Alanine ...	8.38	60.0	400.0	v.s.	v.s.	v.s.	—	v.s.	v.s.	—
Aspartic Acid ...	3.0	23.8	400.0	v.s.	v.s.	v.s.	—	v.s.	v.s.	—
Glutamic Acid ...	f.s.	f.s.	—	v.s.	v.s.	—	—	—	—	—
Cysteine ...	sl.s. cold, v.s. hot	sl.s. cold, v.s. hot	—	v.s.	v.s.	v.s.	—	v.s.	v.s.	—
Phenylalanine ...	f.s. cold	f.s.	—	v.s.	v.s.	v.s.	—	v.s.	v.s.	—
Histidine ...	0.4	8.8	v.s.	30.6	33.4	v.s.	160.0	v.s.	v.s.	Insoluble in ether, CHCl ₃ , CCl ₄
Arginine ...	0.2	3.0	v.s.	28.7	25.6	v.s.	120-130	v.s.	v.s.	—
Lyane ...	0.3	3.6	f.s.	9.6	12.6	v.s.	140	v.s.	v.s.	—
Trimethylamine ...	v.s.s.	v.s.s.	f.s.	f.s.	v.s.	v.s.	—	v.s.	v.s.	—
Isobutylamine ...	v.s.s.	sl.s.	f.s.	f.s.	v.s.	v.s.	—	v.s.	v.s.	—
Isoamylamine ...	v.s.s.	v.s.s.	f.s.	f.s.	v.s.	v.s.	—	v.s.	v.s.	—
Tripropylamine ...	v.s.s.	v.s.s.	f.s.	sl.s.	v.s.	v.s.	—	v.s.	v.s.	—
Triamylamine ...	v.s.s.	sl.s.	f.s.	sl.s.	v.s.	v.s.	—	v.s.	v.s.	—
Putrescine ...	v.s.s.	f.s.	—	f.s.	v.s.	v.s.	—	v.s.	v.s.	—
Cadaverine ...	v.s.s.	sl.s.	2	sl.s.	v.s.	v.s.	—	v.s.	v.s.	—
Phenylethylamine ...	sl.s.	f.s.	—	f.s.	v.s.	—	—	v.s.	—	—
p-Hydroxyphenylethylamine ...	sl.s.	f.s.	—	f.s.	v.s.	—	—	v.s.	—	—
β-Iminazoly-ethylamine ...	sl.s.	f.s.	—	f.s.	v.s.	—	—	v.s.	—	—
Indolethylamine ...	sl.s.	f.s.	—	f.s.	v.s.	—	—	v.s.	—	—
Adenine ...	0.05	0.05	0.5	v.s.	v.s.	19.3	10.5	9.4	0.1	—
Guanine ...	0.05	0.08	—	1.0	2.9	—	7.21	9.0	—	—
Xanthine ...	v.s.s.	v.s.s.	—	sl.s.	sl.s.	—	—	f.s.	—	—
Hypoxanthine ...	v.s.s.	v.s.s.	—	sl.s.	sl.s.	—	—	f.s.	—	—
Urea ...	v.s.	v.s.	v.s.	v.s.	v.s.	—	—	v.s.	—	—
Creatinine ...	0.2	0.3	2.7	0.6	8.0	v.s.	13.34	21.5	—	—
Carnosine ...	v.s.s.	sl.s.	—	f.s.	v.s.	v.s.	—	v.s.	—	—
Guanidine ...	0.2	0.4	19.5	29.2	9.9	v.s.	22.8	29.7	27.1	Insoluble in ether, CHCl ₃ , or CCl ₄
Methylguanidine ...	v.s.s.	sl.s.	—	sl.s.	f.s.	—	—	v.s.	—	—
Colamine ...	v.s.	v.s.	—	sl.s.	f.s.	—	—	v.s.	—	—
Betaine ...	0.1	0.1	2.8	1.2	30.5	v.s.	—	v.s.	—	—
Choline ...	0.02	0.02	0.5	0.6	115.0	37.0	—	v.s.	0.6	—
Stachydrine ...	0.05	0.03	0.8	—	23.0	v.s.	—	v.s.	3.4	—
Nicotinic acid ...	0.05	0.20	2.1	—	6.5	58.0	—	v.s.	3.5	—
Sodium ...	v.s.	v.s.	—	—	—	—	—	—	—	—
Potassium ...	0.3	0.3	—	4.5	0.3	—	—	v.s.	—	—
Ammonium ...	0.2	0.2	—	3.0	0.2	—	—	v.s.	—	—

v. = very. sl. = slight. f. = fairly.

TABLE IV.

% volume acetone	Solubility
100	2.9
80	15.4
75	19.3
60	12.3
60	9.4
30	2.0
25	0.1
10	0.07
0	0.05

The greatest solubility usually occurs, as in the above case, when acetone of about 90—75 % is used.

Acetone has already been used as a means of separating phosphotungstic acid precipitates into fractions, Funk [1915] and Drummond and Funk [1914]. The purines are usually found in the acetone-insoluble fractions, an observation which is in agreement with the solubility data recorded here.

The question of the practical utility of these results is an open one and it must be left so until experience has shown their value. The separation of sodium and potassium phosphotungstates, taking advantage of the great difference in their solubilities in water, the separation of the purine bases by the insolubility of their phosphotungstates in acetone, the separation of cystine and tyrosine, a process already examined by Plimmer [1913], and the separation of choline and colamine are a few of the more obvious applications of these results.

In the earlier stages of the work it was believed that the phosphotungstates of certain substances might be used as a help to the identification of the parent base. Unfortunately this hope is now much shaken. Few of the salts examined are sufficiently characteristic in their appearance to admit of easy recognition, whilst their composition renders the value of analytical data somewhat doubtful.

Certain phosphotungstates are fairly characteristic, particularly those which possess colour. Such for example are those of adenine, guanine, uric acid, *p*-hydroxyphenylethylamine, indolethylamine, phenylalanine, phenylethylamine and cystine. Others such as creatinine, urea, and colamine may in some cases be recognised by their crystalline form. Photomicrographs of a few of the crystalline phosphotungstates are given at the end of this paper (Plate I).

The recrystallisation of mixtures of purified phosphotungstates with the

object of identifying the individual components has yielded very disappointing results since the tendency to form mixed crystals is marked.

I must express my gratitude and indebtedness to Dr O. Rosenheim and Dr A. J. Ewins for their kindness in placing at my disposal pure specimens of many of the bases which have been examined during the course of this work.

SUMMARY.

1. A large number of the salts formed by amino-acids and nitrogenous bases with phosphotungstic acid have been prepared in a pure condition.
2. The composition, crystalline form and solubilities of these compounds have been examined.
3. In a few isolated cases the crystalline form or colour of the purified salt gives an indication as to the identity of the parent base, whilst separations based upon different solubilities of the salts may occasionally be made.
4. The optimum conditions for the precipitation of bases by phosphotungstic acid have been examined. The results confirm the observations of Gulewitsch that a slight excess of the precipitant operating in a medium of 5 % sulphuric acid yields the best results.

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