313. *Palladous Chloride as a Reagent for the Isolation and Estimation of Purine Derivatives, and as an Oxidising Agent.*

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During a study of certain aspects of the chemistry of the nucleic acids, the difficulties attending the isolation and purification of small amounts of purine derivatives became increasingly evident. The m. p.'s of these compounds are in many cases unsatisfactory as criteria of purity, and frequently analyses alone can give the required information. The following difficulties occur in the isolation of purine derivatives. Their solubilities in aqueous solutions vary widely. Their isolation by formation of salts with acids is at times impracticable, as with xanthine, or is undesirable, *e.g.*, in the case of the nucleosides, which are readily hydrolysed by acids. The conversion of purines into metallic salts requires the presence of definite acidic groups. The preparation of the free purines from salts of either type may be troublesome.

With these facts in view, a number of hitherto untried reagents were investigated. The chief requisites were that they should easily form sparingly soluble precipitates with purine derivatives when added to their aqueous solutions, and that these precipitates should be readily converted into the free purines. The results obtained with palladous chloride were so satisfactory in these respects that its possibilities were explored more fully. An exhaustive study has not been attempted, but the directions in which the reagent may be of use are indicated.

Palladous chloride effected almost quantitative precipitation of all those purines which were tested, and the free purine derivatives were readily isolated from the co-ordination complexes after their decomposition by metallic silver. The sparing solubility of the

complexes has been utilised to estimate certain purines, and the formation of sparingly soluble complexes has been observed with a large number of nitrogenous substances.

Palladous chloride may have applications in effecting the separation of purines from each other by taking advantage of the effect of hydrogen-ion concentration on the solubility of the complexes produced.

Palladous chloride may be used as a clean and convenient oxidising agent of a variety of compounds, and it has the special advantage that the amount of oxygen absorbed may readily be estimated by collecting and weighing the metallic palladium formed in the corresponding reduction. Examples of such oxidations are given.

EXPERIMENTAL.

Formation of Sparingly Soluble Palladous Chloride Complexes.—From time to time the conversion of various substances into Pd-containing complexes has been recorded. Gutbier and collaborators (*Ber.*, 1905, **38**, 2105, 2107; 1906, **39**, 1292, 4134; *Z. anorg. Chem.*, 1905, **47**, 23; 1916, **95**, 129), especially, have made a systematic study of many cyclic and aliphatic bases, but the conditions employed by them differ markedly from those now described. The formation of sparingly sol. complexes with purine derivatives has not hitherto been recorded. The present results are in Table I.

TABLE I.

Positive.	
Caffeine	Cryptopine
Theobromine	Papaverine
Theophylline	Xanthaline
1-Methylxanthine	Narcotine
3-Methylxanthine	Thebaine
7-Methylxanthine	Dihydrothebaine
Xanthine	Apomorphine
Hypoxanthine	3 : 4 : 6 : 7-Tetramethoxyaporphine
Adenine	2′ - Nitro - 3′ : 4′ : 6 - trimethoxy - 1 - benzyl-3 : 4-dihydro*iso*quinoline
Xanthosine	Trimethylamine
Adenosine	Pyridine*
Guanosine	Quinoline*
2 : 6-Dichloro-7-methylpurine	*iso*Quinoline*
Strychnine †	*sym.*-Dimethylurea
Brucine	
Negative.	
Allantoin	Dihydrocodeinone
Urea	Hydroxycodeinone
Monomethylurea	Atropine
Ammonia (chloride)	Nicotine
Biuret	Ephedrine
3 - Methyl - 4 : 5 - diamino - 2 : 6-dihydroxypyrimidine and its 5-formyl deriv.	Cotarnine
	m-Methoxy-β-phenylethylamine
	6-Methoxy-2-methyltetrahydro*iso*-quinoline.
Tetrahydrobrucine	Benzamidine
Brucidine	

* Gutbier *et al* (*loc. cit.*) have prepared complexes from these substances.

† Also recorded by Wagenaar (*Pharm. Weekbl.*, 1930, **66**, 1073).

3 Drops of a 2% solution of $PdCl_2$ in extremely dil. HCl aq. were added to 5 mg. of the substance under examination in 5 c.c. of H_2O at 100°. The purine complexes all separated immediately, and their solubilities were of the same order as that of the compound with theobromine. With several alkaloids, no ppt. formed until the solution was cooled.

The cryst. forms of the $PdCl_2$ complexes of some purine derivs. are characteristic, and may be used after some experience to facilitate the identification of the compounds. The purine complexes darken above 250°, but as a rule do not melt.

Effect of Hydrogen-ion Concentration on the Precipitation of Purine Complexes.—No separation of purine complexes took place in strongly acid solutions. Table II gives the p_H at which pptn. began, as shown by the formation of a faint cloudiness, and also that at which the pptn. was complete. The tests were made as follows: 2 mg. of the substance were dissolved in cold H_2O and the p_H adjusted to the required value by the addition of the requisite amount of HCl aq., which had been previously determined with another sample. The final volume was 2·0 c.c. Alternatively, in the case of tubes at p_H 1, the substance was dissolved directly in 2 c.c. of 0·1*N*-HCl. A series of tubes having been thus prepared at room temp. for testing one substance, 1 drop (0·04 c.c.) of 2% $PdCl_2$ aq. was added to each. In all cases, pptn. was rapid when the reaction was not much more acid than the p_H of total pptn., but required several mins., and became progressively less complete, as the reaction of the tubes became more acid than this value.

TABLE II.

	p_H at which pptn.			p_H at which pptn.	
Substance.	begins.	is complete.	Substance.	begins.	is complete.
Adenine	0·8(?) or < 0·8	1·0	Xanthosine ...	1·8	2·5
Adenosine ...	1·3	1·8	Guanosine ...	1·6	2·0
Hypoxanthine	1·0	1·4	Caffeine	1·4	2·0

The separation of the complexes is hastened by heating, but the p_H values are not altered.

Constitution of Complexes.—The complexes with caffeine and theobromine were dried at 110° and analysed, Pd being pptd. with CH_2O in NaOH aq. (Found, for caffeine complex: Pd, 19·4. $2C_8H_{10}O_2N_4,PdCl_2$ requires Pd, 18·9%. Found, for theobromine complex: Pd, 20·4. $2C_7H_8O_2N_4,PdCl_2$ requires Pd, 19·8%).

Estimation of Caffeine and Theobromine by Precipitation as the Palladous Chloride Complex.—Caffeine. 4% $PdCl_2$ aq. (2·5 c.c.) was added to a hot aq. solution of caffeine (0·1110 g. in 50 c.c.), and the mixture heated on the water-bath until pptn. was complete. When cold, the complex was collected, washed with H_2O (20 c.c.), and dried at 110° (0·1506 g., corresponding to a recovery of 93·1% of the caffeine used).

Theobromine. In a similar manner, 0·1427 g. gave 0·2080 g. of complex, coresponding to 97·8% recovery.

Recovery of Purine from Palladous Chloride Complexes.—A suspension of the complex and an excess of freshly pptd. Ag in hot H_2O was warmed on the water-bath for 10—15 mins., and filtered. The filtrate contained all the purine derivative, which remained after evapn. of the H_2O.

Palladous Chloride as an Oxidising Agent.

During the foregoing work, it was observed that alloxan is immediately oxidised by a hot solution of $PdCl_2$, which is reduced to black amorphous Pd. Other compounds were therefore examined, and those which reduced $PdCl_2$ were: Piperidine,* cinchonine,* quinine, apomorphine, narcotine, cinnamic acids,* alloxan, uric acid,* catechol, quinol, resorcinol (slowly), benzaldehyde (slowly). The reactions proceed in distinctly acid solution, but are progressively inhibited by increasing concns. of HCl. The results obtained suggest that reduction of $PdCl_2$ might in some cases be made the basis of a simple method of estimating aqueous solutions of the substances.

Quantitative conversion of piperidine into pyridine. A solution of piperidine (0·15 g.) in 2% $PdCl_2$ (50 c.c.) in very dil. HCl aq. was boiled under reflux for 1 hr.; a ppt. of Pd gradually separated and was collected (see below). The filtrate was then basified with NaOH and distilled, the first portion of the distillate being collected in cold satd. picric acid aq. A copious cryst. ppt. of pyridine picrate separated, and after cryst. from water formed yellow needles, m. p. 162—164°, not depressed by admixture with an authentic specimen of m. p. 162—164°. Piperidine picrate melts at 145—147°.

The quant. oxidation of piperidine to pyridine requires the production of 0·56 g. of Pd; when the metal was collected from the reaction mixture, washed, and dried at 110°, it weighed 0·53 g., indicating about 95% conversion.

Oxidation of uric acid. The wt. of Pd formed by reducing $PdCl_2$ with uric acid appears to bear a fairly constant ratio to the weight of uric acid used, provided that the concn. of HCl remain low. Solutions of uric acid in H_2O (50 c.c.) were boiled for 30 mins. with 2% $PdCl_2$ (5 c.c.) and the pptd. Pd was collected and dried at 100°. Some results are given below:

Uric acid, mg.	24·5	28·6	55·9	47·4
Pd, mg.	25·1	29·9	59·8	51·0
Ratio	1·02	1·04	1·07	1·07

In order to investigate the course taken by this oxidation, a mixture of uric acid (0·25 g.) and 2% $PdCl_2$ aq. (20 c.c.) was boiled gently until all the Pd had been pptd., and then cooled. Next day the Pd and the excess of uric acid were filtered off, and the solution was concentrated to 2 c.c. The crystals (A) (0·09 g.) which separated were collected, and the filtrate (B) was examined as described below.

(A). After recrystn. from H_2O, the crystals, m. p. 212—215°, were identified as parabanic acid (Found: N, by micro-Kjeldahl, 24·9. Calc. for $C_3H_2O_3N_2$: N, 24·6%). The aq. solution was acid to litmus and yielded a Ag salt when mixed with ammoniacal $AgNO_3$. The NH_4 salt separated at once when a drop of NH_3 aq. (*d* 0·880) was added to an alc. solution. A cold aq. solution gave no ppt. with mixed $CaCl_2$ aq. and NaOAc aq., but this mixture yielded a heavy ppt. of CaC_2O_4 when heated for 30 mins. in a water-bath. The substance was not oxidised by boiling $PdCl_2$ aq. These reactions are characteristic of parabanic acid; alloxan is readily oxidised by $PdCl_2$.

(B). On mixing a drop of the filtrate B with a drop of conc. HNO_3, crystals of urea nitrate separated. The remainder of the filtrate B was mixed with

* A detailed examination of the oxidation of these compounds is given below.

$CaCl_2$ aq. and NaOAc aq., and the ppt. collected and dried at 100° for 2 hrs. This material reduced cold acid $KMnO_4$ and a Kjeldahl estimation of N was negative, suggesting that it was calcium oxalate, mesoxalate, or a mixture of these (Found: Ca, as CaO, 25·4, 25·6. Calc. for C_2O_4Ca,H_2O: Ca, 27·4%; for $C_3H_2O_6Ca,3H_2O$: Ca, 17·5%). These analyses indicate that the Ca salts are a mixture of about 80% oxalate and 20% mesoxalate, since CaC_2O_4,H_2O is stable at 100°, and Freund (*Ber.*, 1884, **17**, 780) states that calcium mesoxalate is a dihydroxymalonate trihydrate when dried at 100°.

The products of the oxidation of uric acid by $PdCl_2$ are therefore parabanic acid, urea, oxalic and mesoxalic acids, and the reaction follows the normal course of acid oxidations of uric acid. Careful search yielded no allantoin, which is not oxidised by $PdCl_2$.

Oxidation of 3 : 4-*methylenedioxycinnamic acid.* A solution of the acid (0·1 g.) in 2% $PdCl_2$ aq. (14 c.c.) was heated in the water-bath for 2 hrs., and extracted thoroughly with Et_2O. After being dried and distilled, the extract left a cryst. residue, m. p. 210—230°, which was recrystallised from dil. EtOH and identified as piperonylic acid, m. p. 230—232°, not depressed on admixture with authentic material.

Oxidation of cinchonine. A solution of cinchonine (1 g.) and 2% $PdCl_2$ aq. (60 c.c.) was boiled for 3 hrs., and the Pd (0·65 g.) collected. The filtrate was made alkaline with NH_3 aq., and the solid (A; 0·41 g.) collected. The filtrate was evaporated to dryness in a vac. desiccator, the residue dissolved in H_2O, filtered, and mixed with satd. picric acid aq. A bulky ppt. of a picrate (B; 0·72 g.) separated.

(A). This product has not been definitely identified. In its behaviour, and from the analyses, it resembles most closely a mixture of cinchonine, or an isomeride thereof, and α-hydroxycinchonine, m. p. 252°, which is formed by the addition of H_2O to the double bond of cinchonine under the influence of hot dil. mineral acids (Jungfleisch and Leger, *Compt. rend.*, 1889, **108**, 952). The substance (A) melted at 242—254°, and dissolved easily in dil. acids and EtOH. Three recrystns. from 50% EtOH yielded colourless plates, m. p. 257—261° [Found: C, 75·5, 75·7; H, 7·9, 8·0; N, 9·1; *M* (Rast), 309. Calc. for $C_{19}H_{22}ON_2$ (cinchonine), C, 77·5; H, 7·5; N, 9·5%; *M*, 294. Calc. for $C_{19}H_{24}O_2N_2$, C, 73·1; H, 7·7; N, 9·0%; *M*, 312]. In HCl aq., this substance had $[\alpha]_{5461}$ − 261°. It decolorised cold acid $KMnO_4$ about 10 times more slowly than cinchonine, indicating the presence of only small amounts of unsatd. material, and was very slowly oxidised by hot $PdCl_2$ aq. It was not attacked by a large excess of semicarbazide at 100°. CHI_3 could be detected by its odour when the substance was warmed with NaOI; this reaction is given by α-hydroxycinchonine by reason of the group $CH_3{\cdot}CH(OH)$–.

(B). The picrate formed coarse yellow irregular plates, m. p. 150—167° (Found: N, 13·9. $2C_{18}H_{20}O_3N_2,3C_6H_3O_7N_3$ requires N, 13·9%). The m. p. was not altered by recrystn. from EtOH, in which the picrate was sparingly sol. It was evidently the *picrate* of cinchotenine, in which the vinyl group of cinchonine has been oxidised to carboxyl and $H{\cdot}CO_2H$. There was no depression of m. p. of a mixture with the picrate, m. p. 149—165°, of authentic cinchotenine (Skraup, *Annalen*, 1879, **197**, 376).

The wt. of Pd collected after the oxidation was about 30% in excess of that required by the weight of cinchotenine actually isolated as picrate without any special precautions being taken to ensure a max. yield. This supports the view that the material (A) was not formed as the result of an oxidation.

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