

THE SYNTHESIS OF METHONIUM COMPOUNDS, THEIR ISOLATION FROM URINE, AND THEIR PHOTOMETRIC DETERMINATION

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In consequence of the observation made by W. D. M. Paton that octamethylene-bis(trimethylammonium) chloride is remarkably effective in causing neuromuscular block, a number of simple bisquaternary ammonium salts have been synthesized. They are all polymethylene bis(trimethylammonium) di-iodides of the general type $I[(CH_3)_3N.(CH_2)_nN(CH_3)_3]I$ where n is 2–13 and 18.

Investigations on the pharmacological actions of these compounds have already been published (Paton and Zaimis, 1948a, b; 1949). They will be referred to in this paper as "methonium" compounds, a name which has been approved by the British Pharmacopoeia Commission (e.g. "decamethonium di-iodide," "hexamethonium di-iodide" etc.). This series has also been independently studied by Barlow and Ing (1948a, b).

The purpose of this paper is to present: (a) a method of synthesis of the methonium di-iodides and their characterization, (b) a method of their isolation from urine, and (c) a photometric procedure for their determination in urine.

General method for the synthesis of the methonium di-iodides

A solution of the appropriate diamine dihydrochloride (1 mol.) and sodium hydroxide (9 mol.) in methyl alcohol was boiled under reflux with methyl iodide (9 mol.) for 4 hours. The neutral solution was filtered from sodium chloride and evaporated to dryness. The residue was treated with boiling acetone to dissolve the sodium iodide and the insoluble residue collected and washed with cold acetone. The di-iodide was then crystallized from methyl or ethyl alcohol with separation from traces of sodium chloride if still present.

The diamines (with the exception of ethylenediamine, propylenediamine, and octadecamethylenediamine which were already available) were prepared by reduction of the appropriate nitriles with sodium and dry ethanol (Mendius method). The diamines were isolated as dihydrochlorides. Their melting points are collected in Table I with the analyses where they are new or where their melting points differ from those given in the literature.

The methonium di-iodides are white crystalline solids, not markedly hygroscopic, very soluble in water, forming neutral solutions; soluble in warm methyl and ethyl alcohol but less so in cold, insoluble in acetone. They are stable in aqueous solution and can be sterilized by heat.

If saturated aqueous sodium picrate solution is added to solutions of the methonium di-iodides, methonium dipicrates are precipitated as sparingly soluble crystalline salts.

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TABLE I
POLYMETHYLENE DIAMINES DIHYDROCHLORIDES, HCl, $\text{H}_2\text{N}(\text{CH}_2)_n\text{NH}_2$, HCl

Compound	n	m.p.	m.p.(lit.)	Analyses			
				Found		Calculated	
				C	H	C	H
Tetramethylenediamine ..	4	298° (dec.)	290° (dec.) ¹ >290° ²				
Pentamethylenediamine ..	5	248°	225–230° ³ 242–243° ³ 255° ⁴				
Hexamethylenediamine ..	6	247–248°	248° ^{5,6}				
Heptamethylenediamine ..	7	279–280°	250° (dec.) ⁶	41.1	10	41.4	9.8
Octamethylenediamine ..	8	282°	284° (dec.) ⁷ 272–274° ⁸				
Enneamethylenediamine ..	9	305–306°	Not recorded ⁹	46.2	10.6	46.5	10.4
Decamethylenediamine ..	10	307–308°	309–310° ⁷				
Undecamethylenediamine	11	310° (dec.)	254–255° ¹⁰	50.4	11.1	50.9	10.8
Dodecamethylenediamine	12	314–316°	250° ¹¹	52.5	11.3	52.8	11.1
Tridecamethylenediamine	13	320° (dec.)		54.3	11.5	54.2	11.3

¹ Naegeli and Lendorff (1932); ² Yoshimura (1910); ³ Meisenheimer (1921); ⁴ Kanewskaja (1927); ⁵ Curtius and Clemm (1900); ⁶ v. Braun and Müller (1905); ⁷ Kumagai *et al.* (1929); ⁸ Steller (1900); ⁹ Solonina (1897); ¹⁰ v. Braun and Danziger (1912); ¹¹ v. Braun (1909).

The melting points of the di-iodides and dipicrates are collected in Table II together with the analyses of the di-iodides.

Isolation of methonium salts from urine

From biological tests made with urine excreted after the intravenous administration of methonium compounds in man and animals, evidence was obtained that the urine possessed marked biological activity. It was, therefore, of interest to investigate the nature of the compound or compounds excreted.

The method used for the isolation of the excreted compound consisted in the treatment of the urine with ammonium reineckate solution, removal of the reineckate precipitate by filtration, and subsequent double decomposition with the isolation of a methonium salt, easily identified by its melting point. Fortunately normal urine constituents do not interfere with the method. Human urine gives no precipitate with ammonium reineckate. Creatinine reineckate may separate from cat urine, but as it is relatively water-soluble it may be removed by washing. Rabbit urine, if left for more than half an hour with ammonium reineckate, may give a dark precipitate which is not soluble in acetone.

Procedure.—Pairs of rabbits were kept in metabolism cages. Hexamethonium di-iodide (50 mg.) was injected intravenously and the excreted urine was collected over a period of 24 hours after the injection.

The urine collected in each experiment from four animals was filtered and treated with excess of saturated ammonium reineckate solution. (The saturated solution was made by stirring 80 g. of ammonium reineckate with 1,000 ml. of water for 3 hours and then filtering. The saturated solution should be stored in the refrigerator.) The urine thus treated was allowed to stand for half an hour with occasional shaking. A precipitate was formed

TABLE II
METHONIUM DI-IODIDES, $I[(CH_3)_3N(CH_2)_nN(CH_3)_3]I$

Compound	n	m.p.	m.p.(lit.)	Analyses						Dipicrates	
				Found			Calculated			m.p.	m.p.(lit.)
				C	H	N	C	H	N		
Dimethonium	2	265° (dec.)	250° (dec.) ¹	24.3	5.9	6.8	24.1	5.5	7.0	>275° (dec.)	262 ^{2a}
Trimethonium	3	305° (dec.)	305–308° (dec.) ¹							282° (dec.)	284–286° (dec.) ³
Tetramethonium	4	292–293°									297–298° (dec.)
Pentamethonium	5	304°	268–273° (dec.) ⁵	29.3	6.8	6.4	29.8	6.4	6.3	263°	285° (dec.) ⁴
Hexamethonium	6	275°	270° ⁶	31.4	6.4	6.0	31.6	6.6	6.1	233–234°	
Heptamethonium	7	240°	242° ⁷							196–197°	
Octamethonium	8	260–261°		34.4	7.0	5.7	34.8	7.0	5.8	176°	
Enneamethonium	9	219°		36.1	7.3	5.7	36.2	7.1	5.6	163–164°	
Decamethonium	10	238°	231° ⁷	37.5	7.5	5.4	37.5	7.5	5.4	145–146°	
Undecamethonium	11	214°		38.6	8.0		38.8	7.7		150–151°	
Dodecamethonium	12	216°		40.0	7.9		39.6	7.8		152°	
Tridecamethonium	13	207°		40.7	7.8	5.1	41.1	8.0	5.1	149–150°	
Octadecamethonium	18	162°				4.3			4.5		

¹ Skrap and Philippi (1911), ² Schneider (1895), ³ Dudley, Rosenheim, and Stirling (1926), ⁴ Willstätter and Heubner (1907), ⁵ v. Braun (1911a), ⁶ v. Braun (1910), ⁷ v. Braun (1911b).

which was filtered by suction through a sintered glass funnel (No. 3), covered with a 3 mm. layer of celite (519A). Any excess of ammonium reineckate was removed by washing the precipitate with distilled water. Without removal from the funnel, the precipitate, while still slightly damp, was covered with acetone and left for several minutes for solution to be effected and stirred with a rod if necessary. The reddish-pink acetone solution was filtered into a clean Buchner flask and fresh amounts of acetone added to the celite layer until the filtrate was colourless. To the coloured solution a slight excess of a saturated aqueous solution of silver sulphate was added and the silver reineckate precipitate was removed by filtration. To the filtrate, barium iodide (a 4 per cent (w/v) solution) was added dropwise, the precipitate formed being shaken thoroughly after every few drops. The flask and the contents were kept warm during the simultaneous precipitation of barium sulphate and silver iodide. Great care was taken to avoid any excess of barium iodide solution. When precipitation of the sulphate was complete the mixture was filtered and the filtrate evaporated to dryness under reduced pressure. Any trace of water was removed from the residue by two additions of a few ml. of methyl alcohol and evaporation to dryness under reduced pressure. Finally the portion soluble in methyl alcohol was recovered by evaporation of the solution to dryness under reduced pressure and treated in one of the two following ways:

(i) If the crude residue was dissolved in 2–3 ml. of boiling methyl alcohol a crystalline di-iodide separated on cooling. After recrystallization from methyl alcohol colourless crystals were obtained melting at 275°; yield 64 mg.

(ii) When the crude residue in 1 ml. of water was mixed with 3.5 ml. of saturated sodium picrate solution a precipitate was formed. The picrate crystallized from water as yellow needles melting at 233°; yield 100 mg.

Examination of the mother liquors of the picrate failed to reveal any other substance. A similar result was obtained in six experiments with rabbits, in one experiment with a pair of cats, and in one experiment with eight rats. The cats received the same dose of hexamethonium di-iodide as the rabbits, but the rats received only 10 mg. each. All the injections were given intravenously. The substances isolated have all the characteristics of hexamethonium di-iodide or hexamethonium dipicrate according to the method used.

To find the percentage loss in the process of isolation, known amounts of hexamethonium were added to normal rabbit urine and subsequently isolated by the above method. The recovery as picrate was about 55 per cent. Apparently about 60 per cent of the injected compound is excreted in the urine unchanged during the first 24 hours after the injection.

The fact that no metabolite has been found in the urine does not necessarily indicate that all the injected material is excreted unchanged. Possibly some small percentage of the compound is changed in the body, but no evidence of a possible demethylation was found. Detoxication appears to depend on renal excretion and this may extend over more than one day, since evidence was obtained that the second day's urine contained about 10 per cent of the amount injected.

Details of the rate of the excretion of these compounds in man and animals will be published at a later date.

Photometric estimation of methonium salts in urine

It has been shown in the previous chemical process of isolation that methonium ions can be precipitated as methonium reineckates, $[(CH_3)_3N(CH_2)_nN(CH_3)_3]$, $2(NH_3)_2Cr(SCN)_4$; these salts are insoluble in water and give a reddish-pink colour on solution in acetone. The intensity of colour produced is proportional to the

methonium ions. It was thought that this colour reaction might be useful for their estimation in urine and a photometric procedure has been worked out for this purpose.

Procedure.—The 24 hours' filtered urine from animals (rabbits, cats, or rats) which had received hexamethonium di-iodide intravenously was treated with saturated ammonium reineckate solution and the precipitate dissolved in acetone in the manner already described. Great care must be taken that all traces of ammonium reineckate solution are *thoroughly* removed and that the coloured acetone solution is really clear. This can be achieved, if necessary, by refiltering the acetone solution through a clean sintered glass funnel covered again with a layer of celite.

The coloured acetone solution was transferred to a graduated cylinder, measured, and the optical densities read using a Chance green (OG_1) filter and a Hilger Biochem Absorptiometer. The colour is stable for several hours and its intensity is unaffected by a small percentage of water. The maximum absorption occurs at $525\text{ m}\mu$.

The concentration of hexamethonium reineckate was determined by inspection of a previously constructed standard curve. In order to obtain such a curve a known amount of hexamethonium di-iodide was dissolved in the urine of the appropriate animal and the solution treated in the manner described above. Finally, the acetone solution of the reineckate was transferred to a graduated cylinder and sufficient acetone added to make a final solution containing 1.5 mg. hexamethonium reineckate in 1 ml. of acetone. From this standard solution different dilutions were made and the corresponding densities determined and plotted against the equivalent mg. per ml. acetone. As is indicated in Fig. 1

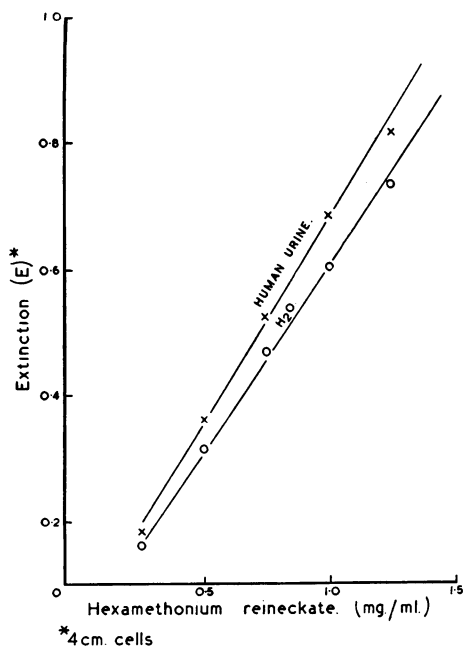


FIG. 1.—Calibration curve for hexamethonium reineckate. Constructed as described in the text.

the curve follows Beer's Law from a concentration of 0.25 to 1.25 mg. per ml. For amounts outside this range the reading has to be repeated with more or less concentrated solutions as necessary.

In using such a standard curve it is important to remember that there are always factors apart from concentration which influence the final colour density. It is advisable that the determination of an unknown solution should be carried out under the same conditions as the standard and the calibration curve of the standard should be made with the urine of the same animal.

The limitation of the photoelectric determination is that more than 10 mg. of methonium salt must be present in the urine for an adequate determination. This consideration makes the technique suitable for the determination of hexamethonium and pentamethonium salts excreted in human urine but not for the excretion of decamethonium salts where the total dose injected does not usually exceed 3 to 5 mg.

It seemed desirable to investigate possible extensions of the method to other substances. Experiments of a purely preliminary character showed the possibility of estimating substances like *d*-tubocurarine chloride, flaxedil [tri-(diethylaminoethoxy)-benzene triethyl iodide], quinine, and tetraethylammonium iodide. It appears from these experiments that the method may have applications to other basic compounds which are precipitated with ammonium reineckate.

Accuracy of method

Several experiments were carried out to test the accuracy of the method. In these experiments standard curves were constructed on different days. The results were analysed statistically. Table III shows the analysis of variance for hexamethonium di-iodide dissolved in water.

TABLE III

	Degrees of freedom	Sum of squares	Mean square	Variance ratio	P
Between doses	5	1.6697	0.33394	327.1	<0.001
Linearity	1	1.6643	1.6643	1,630.1	<0.001
Departures	4	0.0054	0.00135	1.32	>0.05
Within doses	24	0.0245	0.001021		
Total	29	1.6942			

The error introduced into an estimate of potency of an unknown solution calculated by reference to a standard curve is derived from two sources: (i) the error of estimation in a single observation, the order of which is given by the "within doses" term of the above analysis, i.e. a variance of 0.001 or a standard deviation of approximately 5 per cent; (ii) the error in the determination of the slope of the standard curve. The analysis shows, however, that the ratio of variance due to departure from linearity to variance attributable to linearity is so very small that error due to this factor can be neglected.

Analyses were made for determinations with the compound dissolved in human urine (Fig. 1) and in cat and rabbit urine, with similar results. There were no significant differences between the slopes of the three standard curves for the drug dissolved in urine, but it should be noted that the standard curve for the compound dissolved in water is less steep.

The soundness of the method was checked by parallel biological tests and chemical determinations. The results were consistent and in good agreement.

SUMMARY

1. A general method of preparing a series of polymethylene bisquaternary ammonium di-iodides having 2-13 and 18 methylene groups is described. They are referred to as "methonium" compounds.

2. After intravenous injection of a methonium salt in rabbits, cats, and rats the unchanged compound was isolated from the urine.

3. A photoelectric method is described for the estimation of methonium compounds in urine. The photoelectric determination depends on the precipitation of the methonium ions as reineckates and estimation of these reineckates in acetone solution.

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