J. Pharm. Pharmac., 1967, **19**, 257–261 Received November 14, 1966

Quantitative determination of physostigmine in solution

F. W. TEARE AND D. W. TAYLOR

A chemical assay is described for the indirect determination of physostigmine (eserine) in solution. The method involves a chemical reaction between methylamine, a hydrolysis product of physostigmine, and carbon disulphide to form a dithiocarbamic acid which is subsequently titrated with a standard aqueous sodium hydroxide solution. The alkaline hydrolysis of the physostigmine sample is carried out at room temperature using a closed system. The difference in the amount of methylamine found in a physostigmine hydrochloride solution before hydrolysis (blank) and after hydrolysis (sample) is indicative of the amount of intact alkaloid present. This indirect determination of methylamine makes it possible for the first time, to determine conveniently the amount of the alkaloid in solution in the presence of its degradation products.

AQUEOUS solutions of physostigmine (eserine) salts are known to decompose, gradually turning red on standing at room temperature. It has been well established that the first decomposition products are colourless eseroline, methylamine, and carbon dioxide. This decomposition is followed by rapid oxidation of eseroline to the red-coloured rubreserine, and more slowly to eserine blue, eserine brown and possibly other products (Ellis, 1943). Since pharmacological activity is lost during the first stage of decomposition to eseroline (Ellis, Krayer & Plachte, 1943), one should be able to determine the pharmacological activity of a physostigmine solution at any given time, if the amount of alkaloid present can be accurately determined.

Previous attempts to determine the alkaloid include spectrophotometric measurements of rubreserine (Ellis, 1943; Hellberg, 1947, 1949) or methylamine (Hellberg, 1947, 1949), titrimetric determinations of the alkaloid (Hellberg, 1947; Chatten, 1955; United States Pharmacopeia, 17th Revision) or methylamine (Hellberg, 1947), polarography (Parrak, Mohelska & Machovicova, 1961; Parrak & Radejova, 1962), and the determination of pharmacological activity (Ellis & others, 1943). These methods have not proved entirely satisfactory because of the lack of specificity either for physostigmine or one of the decomposition products, the inability to account for some decomposition products before analysis, or too laborious or inconvenient a procedure.

We have attempted to develop a method for the quantitative measurement of physostigmine in solution, directly or indirectly, and preferably in the presence of its degradation products.

The method presented determines the alkaloid by indirectly measuring the methylamine present before and after alkaline hydrolysis of equal aliquots of a solution of physostigmine hydrochloride. The methylamine is allowed to react with carbon disulphide to form a dithiocarbamic acid which is titrated with standard aqueous sodium hydroxide.

From the Faculty of Pharmacy, University of Toronto, Toronto 5, Canada.

Experimental

Materials. Carbon disulphide C.P., hydrochloric acid C.P., methylamine hydrochloride, physostigmine (Mann Research Laboratories, N.Y.), potassium hydrogen phthalate, sodium hydroxide, acetonitrile, 2-propanol, hydrochloric acid 4N, sodium hydroxide 4N and 0·2N*, methylamine hydrochloride solution (1·0 g of methylamine hydrochloride dissolved in water and diluted to 250 ml). All chemicals are of analytical reagent grade unless otherwise indicated.

Apparatus. Metrohm Potentiograph E336 (automatic recording titrator), micro combination glass-saturated calomel electrode, 5 ml piston burette, 50 ml round bottom flasks and 60 ml dropping funnels both with ground glass joints (24/40), and 5 ml microburettes.

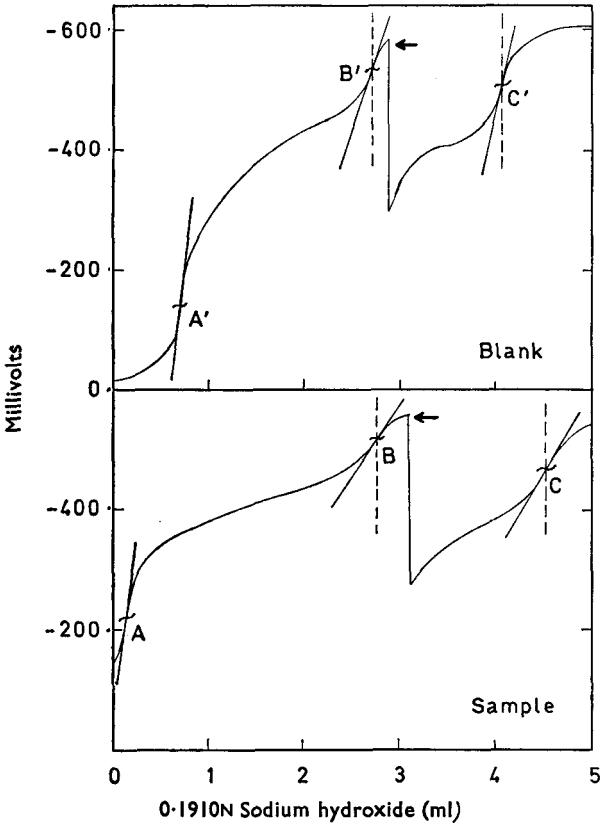

FIG. 1. Titration curves of non-hydrolysed (blank) and hydrolysed (sample) physostigmine hydrochloride solutions with sodium hydroxide. End points A′ and A, B′ and B indicate neutralization points of excess hydrochloric acid and hydrochloric acid salts of organic bases, respectively. Just after end points B′ and B at the arrows, carbon disulphide is added to react with methylamine present to form a dithiocarbamic acid which is neutralized at points C′ and C.

* Standardized against potassium hydrogen phthalate dried at 120° for 1 hr.

PROCEDURE

Dissolve an accurately weighed sample of physostigmine (about 250 mg) in about 0·5 ml of 4N hydrochloric acid and dilute to 50 ml with water. This provides nine 5·0 ml aliquots each equivalent to 24·7 mg of the alkaloid base. Place 5·0 ml aliquot of this solution in a 50 ml round bottom flask and add 4N sodium hydroxide (1·0 ml). Close the flask immediately with a stoppered dropping funnel containing 2-propanol (5 ml) and 4N hydrochloric acid (1 ml) and stir vigorously for 2 hr to allow for complete hydrolysis of the alkaloid. Add slowly the slight excess of acid in the alcohol in the funnel, stirring to ensure complete trapping of the methylamine vapour released on hydrolysis. Add a further 10 ml of 2-propanol and 5·0 ml of the methylamine hydrochloride solution via the funnel, followed by a further 10 ml of 2-propanol. Then remove the funnel and add 15 ml of acetonitrile to the contents of the flask. Adjust the pH, if necessary, to ensure that the titration curve falls within the confines of the recorder chart. Titrate the solution with sodium hydroxide (0·2N) to a point just past the second potentiometric end point (B) at which add carbon disulphide (2 ml) (Fig. 1). Allow 10 min for the reaction then titrate to the third potentiometric end point (C).

The amount of standard sodium hydroxide consumed between the second (B) and third (C) end points is a direct measure of the dithiocarbamic acid produced, and an indirect measure of the methylamine released as a result of the hydrolysis of the physostigmine and the added solution of methylamine hydrochloride.

Make a blank titration using a 5·0 ml aliquot of the same physostigmine hydrochloride solution and treating it exactly as the sample omitting the hydrolysis with 4N sodium hydroxide.

Results and discussion

The procedure described is a modification of that developed by Critchfield & Johnson (1956) for the determination of primary and secondary aliphatic amines in the presence of tertiary amines which do not react with carbon disulphide to form a dithiocarbamic acid.

A few modifications to the original method were necessary. To obtain an adequate separation between the end points B and C, B′ and C′ (Fig. 1), a 5·0 ml aliquot of the methylamine hydrochloride solution was added. To sharpen the end points, the less polar solvent system of 2-propanol (50%) and acetonitrile (30%) was used. To insure complete hydrolysis of the physostigmine (*ca.* 0·1 m-equiv.) in the 5·0 ml aliquots, 4N sodium hydroxide (1 ml *ca.* 4 m-equiv.) and a reaction period of 2 hr were required. The closed system used was necessary to prevent loss of methylamine.

Fig. 1 shows typical titration curves which indicate the three potentiometric end points obtained, and the time of carbon disulphide addition.

The results of five sample and three blank determinations made on the 50·0 ml of physostigmine hydrochloride solution are shown in Table 1.

CALCULATIONS

From the stoichiometric relationships one mole of sodium hydroxide is equivalent to one mole of physostigmine. On a weight basis each mole of sodium hydroxide is equivalent to 275·34 g of the alkaloid. Therefore, each ml of 0·2N sodium hydroxide is equivalent to 55·068 mg of alkaloid.

The 0·480 ml difference between the mean values of the sodium hydroxide (0·1910N) required for the sample and blank determinations (Table 1) is equivalent to 25·24 mg of physostigmine. The amount of alkaloid represents 102·2% of the amount originally dissolved in the solution.

Another aqueous solution of physostigmine hydrochloride (5·0 ml equivalent to 25·9 mg of base) was analysed similarly, except that in the blank determinations 5·0 ml of deionized water was used instead of a non-hydrolysed aliquot of the same physostigmine hydrochloride solution. Calculations showed that 100·2% of the original physostigmine in the solution was found (Table 1).

TABLE 1. INDIRECT DETERMINATION OF METHYLAMINE IN ALKALINE-HYDROLYSED PHYSOSTIGMINE SAMPLES AND CONTROL SOLUTIONS OF NON-HYDROLYSED PHYSOSTIGMINE AND WATER

Volume (ml) of sodium hydroxide (0·1910N) consumed by dithiocarbamic acid[1]			
Determinations of		Determinations of	
Sample 1	Blanks[2]	Sample 2	Blanks[3]
1·845	1·380	1·915	1·370
1·910	1·371	1·820	1·385
1·875	1·390	1·905	1·380
1·855		1·890	1·385
1·850		1·870	1·390
		1·905	
		1·860	
		1·860	
		1·875	
Mean = 1·867	1·387	1·878	1·382
s.d. = 0·0245	0·0061	0·0297	0·0076

[1] Dithiocarbamic acid resulting from the methylamine and carbon disulphide interactions.
[2] Containing physostigmine (see text).
[3] Containing water instead of physostigmine.

The close agreement between the two sets of determinations demonstrates the lack of reactivity of the nitrogen in the urethane side-chain of physostigmine with carbon disulphide, and thereby underlines the specificity of the entire method for the analysis of the alkaloid under the conditions outlined.

Work is in progress to ascertain whether this method, or some modification of it, can be used for the determination of physostigmine salicylate or other salts in aqueous solutions alone or in the presence of certain additives such as preservatives and antioxidants.

Acknowledgements. The authors wish to thank the National Research Council of Canada, The Canadian Foundation for the Advancement of Pharmacy, and the University of Toronto for grants.

References

Chatten, L. G. (1955). *J. Pharm. Pharmac.*, **7**, 586–590.
Critchfield, F. E. & Johnson, J. B. (1956). *Analyt. Chem.*, **28**, 430–436.
Ellis, S. (1943). *J. Pharmac. exp. Ther.*, **79**, 364–372.
Ellis, S., Krayer, O. & Plachte, F. L. (1943). *Ibid.*, **79**, 309–319.
Hellberg, H. (1947). *Svensk farm. Tidskr.*, **51**, 560–570.
Hellberg, H. (1949). *Ibid.*, **53**, 637–643.
Parrak, V., Mohelska, O. & Machovicova, F. (1961). *Pharm. Zentralhalle, Dtl.*, **100**, 503–511.
Parrak, V. & Radejova, E. (1962). *Chem. Zvesti*, **16**, 380–386.
United States Pharmacopeia, 17th revision (1965), p. 466, Easton, Pa.: Mack Publishing Co.