PIPERAZINE ADIPATE: A NEW ANTHELMINTIC AGENT

PART I. PHYSICOCHEMICAL PROPERTIES

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ALTHOUGH piperazine and its water-soluble salts have long been used medicinally in the treatment of gout and rheumatism because of their possible value in dissolving uric acid, the anthelmintic potentialities of piperazine and its derivatives have been realised only in recent years. Following the discovery of the value of diethylcarbamazine citrate[1] in the treatment of filariasis, attention has been turned to the evaluation of other piperazine derivatives against helminth infections. In 1951, Mouriquand, Roman and Coisnard[2] reported the successful use of piperazine hydrate in the treatment of threadworm infestation in children. Wider use of this preparation was not immediately forthcoming as piperazine hydrate is a strongly basic substance of uncertain keeping qualities in solution. A more satisfactory derivative of piperazine was studied the following year by Turpin, Cavier and Savaton-Pillet[3], who described the employment of the bisphenylacetate salt, which was administered orally with concomitant use of piperazine suppositories.

Piperazine bisphenylacetate, though representing an advance on piperazine hydrate from the standpoint of pharmaceutical presentation, was not considered by us a satisfactory salt for the following reasons: (i) it contains only 25 per cent. of piperazine, so that the dose required is large, (ii) the material has a distinct urinary odour which renders it unpalatable to many patients and (iii) its solubility characteristics present difficulty in its alternative formulation as a liquid preparation stable to storage over a wide temperature range. We consequently turned our attention to other salts of piperazine. In particular, we sought a derivative of low solubility as we thought that such a compound might undergo slower absorption in the gut than the readily soluble hydrate, citrate, etc., thus avoiding the neurotoxic effects accompanying the use of the more soluble piperazine compounds which have subsequently been discussed[4,5]. The search for such a compound proved difficult, in that with one exception, the sparingly soluble salts prepared had either too high a molecular weight to offer advantages over the bisphenyl acetate or else were too toxic for oral administration. Piperazine adipate[6] alone of the many salts examined proved able to satisfy the pharmaceutical requirements we considered necessary. It was consequently submitted to pharmacological investigation (Part II) which showed it to be a relatively non-toxic compound with a high margin of safety. Preliminary clinical trials[7,8] showed that piperazine adipate achieved complete eradication of threadworm infestation in man without the occurrence of any side effects and wider clinical usage has confirmed these qualities[9]. The salt was also examined, at our request, by The Cooper Technical Bureau for its

veterinary applications. This study[10] showed that piperazine adipate was an effective curative agent against a variety of helminth infections in domestic animals.

The main advantages of piperazine adipate over the hydrate and its more soluble salts, are (i) its anthelminitic effect, which is markedly greater than that produced by an equivalent weight of piperazine as hydrate (ii) its freedom from side effects in clinical use (iii) its stability on storage, (iv) its high piperazine content and (v) its pleasant acidulous taste and freedom from odour.

Piperazine adipate[6], the neutral salt of piperazine and adipic acid $C_4H_{10}N_2 \cdot C_6H_{10}O_4$, forms colourless prisms of melting point 256° to 257° C. (uncorr.), which are characterised by a defect insertion of minute crystals. It remains unchanged on exposure to air and even after heating at 100° C. for prolonged periods its melting point and characteristics remain unchanged. It is non-hygroscopic and separates from aqueous solutions without water of crystallisation.

Piperazine adipate dissolves rather slowly in water at room temperature and is soluble to the extent of only about 5 per cent., the temperature gradient of solubility being shown in Table I. It is essentially insoluble in the lower aliphatic alcohols (see Table I).

TABLE I

Solvent	Temp. ° C. (± 0·2° C.)	Solubility g./100 g. of solvent	Solvent	Temp. ° C. (± 0·2° C.)	s
Water	20·0	5·53	Methanol	25·0	0·02
	25·0	6·02	Ethanol (aq. 44 per cent.) ..	25·0	0·57
	30·0	6·61	Ethanol (99·5 per cent.) ..	25·0	very low
	37·0	7·49	*iso*Propanol	25·0	„
	46·0	8·65	Dioxan	25·0	„
	56·3	10·14			

Aqueous solutions have *p*H 5·45 at 25° C. over the concentration range of 0·2 to 0·01M (i.e., 0·23 to 4·6 per cent. w/v) and this value is only slightly affected by increases in ionic strength caused by addition of simple neutral salts.

The classical dissociation exponent (pK'_a) of piperazine has previously been determined by three groups of workers[11,12,13]. The thermodynamic exponents (pK_a at 25° C.), however, are only reported by Smith and Smith. We have, consequently, determined these constants *de novo*, the values obtained being given in Table II.

TABLE II

THE DISSOCIATION EXPONENTS OF PIPERAZINE AND OF ADIPIC ACID IN AQUEOUS SOLUTION

Compound	Ref.	Temp. ° C.	pKa_1	pKa_2
Piperazine ..	11	15	4·05†	8·34†
	12	25	—	9·8†
	13	25	5·32	9·70
	*	25	5·29	9·66
Adipic acid ..	14	25	4·43	5·41

* Current investigation. † Classical pK_a values only.

Consideration of this Table shows that, in solution, piperazine adipate should behave as a very weak monobasic acid and this is indeed found to be the case. Thus potentiometric titration with sodium hydroxide gives the sigmoid curve A (Fig. 1) normally shown by compounds of this type. Addition of a slight excess of a strong acid leads to complete ionisation of the piperazine with concomitant suppression of the ionisation of the adipic acid. Titration with alkali now gives rise to curve B (Fig. 1)

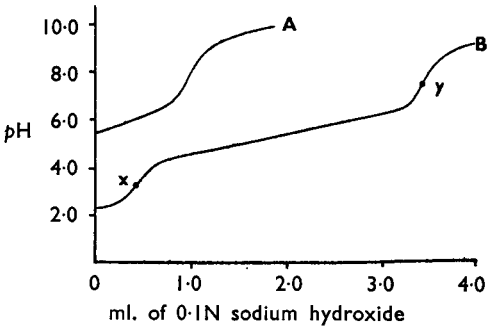

FIG. 1. Titration of 10 ml. aliquots of 0·01M piperazine adipate solution by standard alkali.
A. In the absence of added mineral acid
B. In the presence of 2·5 ml. of 0·1N hydrochloric acid.

in which the difference of titre corresponding to the two points of inflection x and y is exactly 3 times the titre of the original solution (*cf.* curve A). Piperazine adipate may consequently be readily estimated in this way, the tiration being unaffected by such electrolytic species as ammonium ions, alkali metal ions, or alternatively by the presence of weak acids of $pK_a < 4$ or of strong mineral acids. Extension of the method to the determination of piperazine in urine is under investigation.

EXPERIMENTAL

Practical notes

Potentiometric titrations were effected by means of a Muirhead direct-reading *p*H meter, with glass-calomel electrode assemblage incorporating a 3·5N potassium chloride bridge and capillary liquid junction. The linearity of response of the meter was checked by comparison with a standard potentiometer in order to ensure an instrumental accuracy of $\pm$ 0·01 *p*H unit. Alignment of the meter was carried out by means of 0·05M solutions of potassium hydrogen phthalate and sodium borate (borax), of *p*H 4·01 and 9·18, respectively, at 25° C. These solutions were prepared from the recrystallised A.R. salts, and accord with British Standard Specification 1647:1950. Piperazine hexahydrate and adipate were prepared from commercial and laboratory samples, respectively, by recrystallisation from water, the adipate to constant melting point after drying over phosphorous pentoxide. Solutions of the hexahydrate were

prepared as required, using carbon dioxide free water. Their concentrations were determined by titration against standard 0·1N hydrochloric acid, using bromophenol blue as indicator. B.D.H. volumetric solutions of 0·1N hydrochloric acid were employed as the standard acid. Solutions of sodium hydroxide were prepared as required from A.R. pellets, a strong solution being initially formed in order to remove carbonate by precipitation. These solutions were standardised by titration against the standard hydrochloric acid, using methyl red as indicator. Potassium chloride solutions used in ionic strength adjustments were checked for neutrality against methyl red and thymol blue indicators.

Solubility Determinations (Table I)

Saturated solutions of piperazine adipate were obtained by stirring suspensions of the salt for 24-hour periods in vessels immersed in water baths thermostatically controlled to $\pm$ 0·2° C. of the desired temperatures. The suspensions were rapidly filtered through sintered glass funnels and the saturated filtrates titrated in suitable aliquots, after dilution with water, by the standard alkali, the titrations being followed potentiometrically.

Determination of the Dissociation Exponents of Piperazine in Aqueous Solution at 25° C. (Table II)

The determination was carried out upon the dihydrochloride, also used by Smith and Smith[13], but in the present instance the salt was not isolated. 25 ml. aliquots of a 0·1M solution of the hexahydrate were mixed with 50 ml. of 0·1N hydrochloric acid solution and diluted to 250 ml. by the addition of water. The acid solutions so obtained were titrated (25 ml. aliquots diluted to 50 ml. with water or standard potassium chloride solution) with 0·1N sodium hydroxide. The titrations were carried out in a jacketted cell, the temperature of which was maintained at 25° C. $\pm$ 0·2° C. The pK'_a values so obtained were converted to thermodynamic values by application of the standard approximation of the Debye-Hückel relation, applicable within reasonable limits of error, where the ionic strength does not exceed 0·015.

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