THE DETERMINATION OF BENACTYZINE

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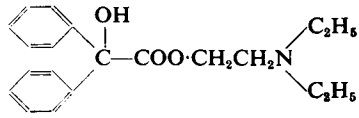
Benactyzine hydrochloride has been used in Denmark for the treatment of emotional and physical tension[1] and has been recently tested in this country[2–4]. It is usually administered orally, and tablets are marketed here under the trade name Suavitil. Benactyzine is 2-diethylaminoethyl benzilate, and has the structure



Protection of the drug from hydrolysis is of importance in the preparation of tablets and solutions for injection, as neither the benzilic acid nor the ester fragment of the molecule show any activity[5]. Analytical methods must therefore be capable of distinguishing benactyzine from the benzilic acid and 2-diethylaminoethanol produced on hydrolysis.

A. Solid Benactyzine Hydrochloride

Benactyzine hydrochloride shows typical benzenoid absorption in the ultra-violet region. Benzilic acid, as would be expected, has a similar absorption spectrum and the extinction coefficients of both compounds at their maxima are given in Table I.

The infra-red absorption curves (2000 to 700 cm.$^{-1}$) for both compounds as 0·5 per cent. w/v solutions in purified bromoform are given in Figure 1.

Benactyzine hydrochloride may be estimated by means of the strong carbonyl absorption peak at 1743 cm.$^{-1}$ and chloroform solutions containing 0·2 to 0·6 per cent. w/v with a cell path of 0·8 mm. give a rectilinear calibration. Benzilic acid shows strong absorption at the same wave number, so that for quantitative purposes absorption at 1743 cm.$^{-1}$ is not specific for benactyzine hydrochloride; any appreciable amounts of benzilic acid can, however, be detected from a qualitative examination of the spectrum in bromoform, particularly in the 1700 cm.$^{-1}$ region.

Conventional methods can be used to determine the ionisable chlorine and the total nitrogen content of benactyzine hydrochloride, but attempts to devise a gravimetric assay similar to that used for phenadoxone in the British Pharmacopœia proved unsuccessful. Picric acid failed to give a satisfactory precipitate from aqueous solution, and precipitation of the reineckate or picrolonate was not strictly quantitative.

The most useful procedure is based on complete hydrolysis of the benactyzine with strong sodium hydroxide, with subsequent steam distillation of the diethylaminoethanol produced and its titration with

TABLE I

SPECTROSCOPIC CHARACTERISTICS OF BENACTYZINE HYDROCHLORIDE AND BENZILIC ACID

Solvent	Wavelength of maximum mμ	Benactyzine hydrochloride		Benzilic acid	
		E (1 per cent. 1 cm.)	Molecular extinction	*E* (1 per cent. 1 cm.)	Molecular extinction
Ethanol	252½	10·9	397	16·8	383
	258½	12·4	451	19·7	450
	264½	9·8	357	15·3	349
0·1 N HCl in ethanol	252½	10·8	393	—	—
	258½	12·4	451	—	—
	264½	9·7	353	—	—
Water	251½	11·1	403	—	—
	258	13·0	473	—	—
	263 (inflexion)	10·5	382	—	—

standard acid. The method is rapid and reasonably specific, and benactyzine can be adequately characterised by this assay and ultra-violet absorption measurements. The assay described in Section B for pharmaceutical preparations is specific, but due to the techniques involved it is insufficiently accurate for control of the pure material.

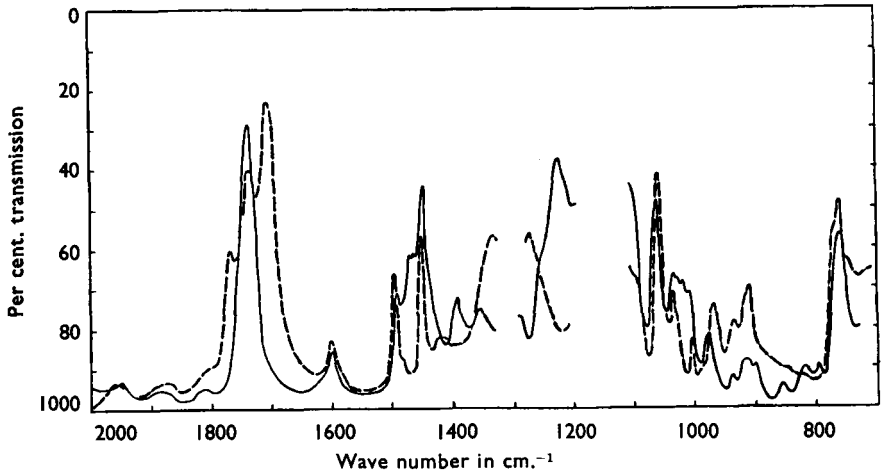


FIG. 1

FIG. 1. Infra-red absorption spectra of —— benactyzine hydrochloride (0·512 per cent. w/v in bromoform) and — — — benzilic acid (0·498 per cent. w/v in bromoform) both in 0·99 mm. cell using a sodium chloride prism.

EXPERIMENTAL

A sample of benactyzine hydrochloride was recrystallised twice from *iso*propanol and dried *in vacuo*. Analysis of the purified material gave C, 66·4; H, 7·1; N, 3·83; ionisable chlorine, 9·75 per cent.; ($C_{20}H_{25}O_3N.HCl$ requires C, 66·0; H, 7·2; N, 3·85; ionisable chlorine, 9·75 per cent.) m.pt. 182° C.

This material was used to establish the ultra-violet extinction coefficients given in Table I, the infra-red absorption spectrum shown in Figure 1

and for the recovery experiments in Section B. When tested by the recommended procedure the sample assayed 100·0 per cent. ± 0·2 per cent. Six specimens of commercial quality assayed between 99·0 and 100·0 per cent.

PROCEDURE

Reagents

1. *Boric acid solution.* Dissolve 20 g. boric acid A.R. in 1 litre of water.
2. *Indicator solution.* Dissolve 0·1 g. bromocresol green and 0·02 g. methyl red in 100 ml. 95 per cent. ethanol.

Transfer about 0·5 g., accurately weighed to a steam distillation apparatus. Add 20 ml. water and 20 ml. sodium hydroxide solution (20 per cent. w/v). Steam distil rapidly, collecting the distillate in 5 ml. of boric acid solution. When distillation of the volatile base is complete (about 100 ml. of distillate), titrate with 0·05 N hydrochloric acid, using 2–3 drops of the indicator solution. Make a blank titration and subtract the result from the sample titration.

One ml. 0·05N hydrochloric acid is equivalent to 0·01820 g. $C_{20}H_{25}O_3N.HCl$.

B. SOLUTIONS AND TABLETS

As a rule benactyzine is injected in solutions containing 5 mg. per ml. or taken in tablets containing 1 mg. For analysis of these products the methods outlined for the solid material are insufficiently sensitive and non-specific. Our attempts to adapt the sensitive sulphuric acid colour reaction for benzilic acid described by Snell and Snell[6] failed, since with benactyzine the intensity of the colour was not reproducible. Ultraviolet spectroscopy appeared to be the only promising method for the products envisaged, provided that it could be made specific by separating benactyzine from its hydrolysis products and other sources of irrelevant absorption.

Benzilic acid can be quantitatively extracted with ether from an acidified solution, leaving the benactyzine in the aqueous layer. After making alkaline with sodium bicarbonate, the benactyzine can be extracted with ether, the solvent removed and the residue dissolved in ethanolic hydrochloric acid for spectroscopic determination. Two solvent extractions completely remove the benzilic acid, but three extractions are necessary to recover all the benactyzine.

This procedure has been successfully applied to aqueous solutions and permits the determination of both benactyzine and any benzilic acid produced by hydrolysis. The official bacteriostatics used in solutions for injection do not interfere with the analysis of benactyzine, but their presence usually prevents the determination of any free benzilic acid.

Tablets are extracted with hydrochloric acid, and an aliquot of the filtered solution is assayed for benactyzine, as described for aqueous solutions. Any small amount of irrelevant absorption derived from the tablet excipients is removed by the acid ether extractions and does not interfere with the benactyzine assay. Determination of benzilic acid is

not a satisfactory measure of decomposition in tablets, and it is essential to assay the benactyzine. The chief source of error in the procedure is extraneous absorption from the apparatus.

Typical results obtained with solutions and tablets of known composition are shown in Table II.

TABLE II

ANALYSIS OF TABLETS AND SOLUTIONS OF BENACTYZINE HYDROCHLORIDE

Preparation	Benzilic acid added	Benactyzine hydrochloride added	Benactyzine hydrochloride found
	mg./ml.	mg./ml.	mg./ml.
Aqueous solution ..	0·48	0·0	0·0
	0·00	4·1	4·1
	0·24	4·1	4·1
	0·97	3·9	3·9
	0·00	*5·1	5·0
	mg./tablet	mg./tablet	mg./tablet
Tablets	—	0·00	0·02
	—	1·03	1·01
	0·10	0·92	0·88
	—	1·01	1·00
	—	1·00	1·02

* 0·2 per cent. of chlorocresol added.

PROCEDURE

Apparatus. All apparatus must be completely free from grease, and well washed with redistilled water.

Reagents

1. *Redistilled water.* Used throughout.
2. *N hydrochloric acid.* Dilute 45 ml. of hydrochloric acid A.R. to 500 ml. with water.
3. 0·5*N hydrochloric acid.* Dilute 22·5 ml. of hydrochloric acid A.R. to 500 ml. with water.
4. 0·1*N hydrochloric acid in ethanol.* Dilute 0·90 ml. of hydrochloric acid A.R. to 100 ml. with ethanol.
5. *Ether.* Anæsthetic ether redistilled in an all glass apparatus.

AQUEOUS SOLUTIONS. Transfer a suitable aliquot containing about 40 mg. of benactyzine hydrochloride to a separator, and dilute to 10 ml. with water. Add 10 ml. of N hydrochloric acid, and extract with 40 ml. and 25 ml. portions of ether. Wash the combined ether extracts with 2 × 5 ml. portions of 0·5N hydrochloric acid, and add the washings to the main aqueous solution. Reject the ether extracts*. To the solution add cautiously with swirling 1·4 g. of sodium bicarbonate A.R.; the solution should now be alkaline to litmus paper. Extract immediately with 50 ml., 30 ml. and 15 ml. portions of ether. Wash the combined ether extracts with 2 × 10 ml. of water containing a little sodium bicarbonate A.R. Filter the ethereal solution through a plug of cotton wool, and remove the solvent by distillation. Dissolve the residue in 100·0 ml. of cold 0·1N hydrochloric acid in ethanol and measure the

* In the absence of interfering substances these ether extracts can be evaporated, the residue being dissolved in ethanol and the benzilic acid estimated spectroscopically.

optical density of the solution in a 1 cm. cell at the maximum at 258½ mμ. Make a blank estimation omitting the benactyzine and correct the optical density of the sample solution*. Calculate the benactyzine hydrochloride content of the sample. The *E* (1 per cent. 1 cm.) of pure benactyzine hydrochloride at 258½ mμ is 12·4.

TABLETS. Weigh 30 tablets and powder finely. Transfer a weight containing about 25 mg. of benactyzine hydrochloride to a glass stoppered flask, and add 50·0 ml. of 0·5N hydrochloric acid. Mix by swirling, and

TABLE III

STABILITY OF BENACTYZINE HYDROCHLORIDE IN AQUEOUS SOLUTION (0·5 PER CENT. W/V AT 37° C.)

Solution	Time in days	Percentage of original concentration		
		Benactyzine hydrochloride	Benzilic acid calculated as benactyzine HCl	Total
Distilled water	9	89	7	96
	21	87	11	98
	53	85	17	102
	125	70	30	100
‡ Buffer pH 6·3	3	6	94	100
‡ Buffer pH 6·1	5	20	82	102
‡ Buffer pH 4·9	3	83	14	97
	7	70	29	99
	14	52	48	100
	27	30	68	98
‡ Buffer pH 3·5	3	99	2	101
	7	94	4	98
	14	94	8	102
	27	85	14	99
	38	82	20	102
	95	59	39	98
Citric acid pH 2·8 ..	10	94	1	95
	21	96	5	101
	43	92	9	101
	74	88	12	100
Citric acid pH 2·1 ..	10	98	2	100
	21	95	6	101
	43	92	9	101
	74	83	14	97

‡ Phosphate/citrate buffer solutions (Vogel, *Quantitative Inorganic Analysis*, 1945, 809).

set aside for five minutes. Stopper, shake mechanically for ten minutes and filter. Transfer 20·0 ml. of the filtrate to a separator. Continue as in Section A from "extract with 40 ml. and 25 ml. portions of ether . . .", dissolving the residue in 25·0 ml. 0·1N hydrochloric acid in ethanol.

Multiply the result by the appropriate correction factor to allow for the increase in volume of the 0·5N hydrochloric acid due to solution of the tablet excipients†.

C. STABILITY OF AQUEOUS SOLUTIONS AND TABLETS

Benactyzine is unstable in neutral or alkaline aqueous solution, but as the pH is lowered the stability progressively improves. Solutions with a pH below 3 show small losses (about 10 per cent.) when stored for three

* The blank should not exceed 5 per cent. of the sample absorption.
† For the tablets used in our experiments the correction factor is 1·04.

months at 37° C. Typical results for solutions of different pH are shown in Table III and are plotted graphically in Figure 2. The sums of the amounts of benactyzine and hydrolysed benactyzine (calculated from the free benzilic acid) show good agreement with the original benactyzine concentration, indicating that simple hydrolysis has occurred.

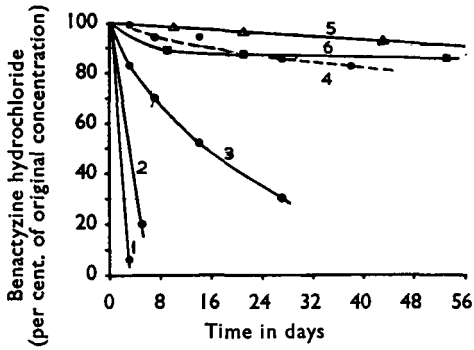


FIG. 2

FIG. 2. Decomposition of benactyzine hydrochloride in aqueous solution (0·5 per cent. w/v). 1. Buffer pH 6·3. 2. Buffer pH 6·1. 3. Buffer pH 4·9. 4. Buffer pH 3·5. 5. Citric acid pH 2·8 and 2·1. 6. Distilled water. Initial pH 5·5.

Trial batches of sugar coated tablets prepared with an excipient of starch and lactose underwent considerable hydrolysis on storage at 37° C., but when 1 per cent. of tartaric acid was added to the excipient the tablets showed good stability. Typical assay results obtained after storing stable and unstable tablets are recorded in Table IV.

TABLE IV

STABILITY OF BENACTYZINE HYDROCHLORIDE IN TABLETS

Storage time	Benactyzine hydrochloride mg./tablet			
	Unstabilised tablets		Stabilised tablets	
	Room temperature	37° C.	Room temperature	37° C.
Initial	0·98	—	0·98	0·98
2 weeks	0·91	0·88	1·00	0·97
1 month	0·93	0·83	0·97	0·99
2 months	0·88	0·70	0·95	0·98
3 months	0·87	0·72	0·97	0·98
6 months	0·92	0·74	0·98	0·96

SUMMARY

1. Infra-red and ultra-violet absorption spectra are presented for benactyzine hydrochloride.

2. An assay for benactyzine is proposed, based on hydrolysis and steam distillation of diethylaminoethanol.

3. Ultra-violet spectroscopic methods have been successfully applied to the determination of benactyzine hydrochloride in its pharmaceutical preparations.

4. Results are presented of stability tests on aqueous solutions and tablets of benactyzine hydrochloride.

The authors are indebted to Mr. W. H. C. Shaw for the infra-red examination, to Mr. J. A. Edwards for carrying out the microanalysis and to Mrs. M. F. Goodchild for technical assistance.

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DISCUSSION

The paper was presented by MR. J. L. PHILLIPS.

The CHAIRMAN said he could not follow why the method for procaine hydrochloride could not be applied to benactyzine. Was it because an insufficient quantity was present in the material under examination?

MR. L. BREALEY (Nottingham) said that difficulty had been encountered in using the described method with benactyzine tablets. The E (1 per cent. 1 cm.) of pure benactyzine was only about 20. That meant that any other material extracted interfered seriously with the assay and gave high results. It had been found impossible to eliminate the interference completely but it had been possible to make the method work satisfactorily if a three-point correction were used. It was an easy correction to apply because the benactyzine had three very sharp peaks, and a formula had been worked out using the values on the peaks and results had been greatly improved. Certainly all extraneous absorption had been found to be perfectly linear, but it was appreciable and should be corrected. What precautions did the authors take, as there must be a number of ways to eliminate the interference?

MISS A. E. ROBINSON (London) said that she also had encountered relatively high optical density of the blank determination using a variety of solvents. However, by using ether it had been possible to obtain a blank with an optical density of less than 0·01 in a 1 cm. cuvette at wavelengths ranging from 220 to 300 mμ. Unlike the authors, no extraneous absorption had been encountered.

DR. W. MITCHELL (London) asked what was the pH of a simple aqueous solution of the drug. He noted that the stability seemed to be greater between about 4 and 6·3.

DR. A. H. BECKETT (London) said he was worried about the accumulation of extraneous absorption from the apparatus. He suggested that in the determination of the solid materials, titration in non-aqueous media in the presence of mercuric acetate would prove to be convenient and rapid.

DR. G. E. FOSTER (Dartford) asked whether the authors had considered adding a certain amount of water and distilling rather than using steam distillation.

MR. C. A. JOHNSON (Nottingham) said it seemed a little unwise to use a method based on hydrolysis in the case of a material which was apparently so prone to it. Non-aqueous titration or spectrophotometric methods seemed preferable. The authors found a melting point of 182° C. but he had found that with repeated recrystallisation he could not achieve a

melting point higher than 180° C. It was possible that the soda glass tube was bringing about a certain amount of decomposition and lowering the melting point.

MR. J. L. PHILLIPS, in reply, said that the method suggested by the Chairman could not be carried out due to insufficient quantity of the drug being present. When the method was originally used there was a great deal of difficulty with high blanks. Redistilled ether was used, and it was also found that by using one set of perfectly clean separators, kept for this determination only, the difficulty of high absorption could be surmounted. It had not been found necessary to use a three-point correction. The pH of an aqueous solution of benactyzine hydrochloride was about 6. The difficulty about titration in non-aqueous solvents was that the dose was 1 mg. and titration would not be sufficiently sensitive for pharmaceutical preparations. The melting point was found to be 182° C. by the B.P. method and was sufficiently characteristic.