

# THE SPECTROPHOTOMETRIC DETERMINATION OF DIMETHYLTUBOCURARINE AND TUBOCURARINE

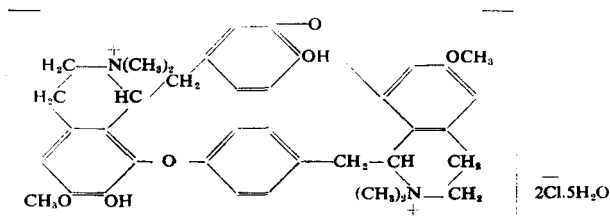
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DIMETHYLTUBOCURARINE iodide has been introduced into medicine as a compound with the curare action of tubocurarine chloride, but with about 3 times the potency of the latter. It has accordingly become of importance to have some means of ascertaining whether complete methylation of tubocurarine has taken place. The method of assay at one time in use in this laboratory was to dissolve the sample in absolute ethanol and determine the  $E_{280}^*$  on a spectrophotometer,  $E_{280}$  for pure dimethyltubocurarine iodide being taken to be  $74 \pm 1.5$ . A number of preparations were examined and it was found that  $E_{280}$  varied between 73.2 and 76.6, although the majority of the values lay between the limits  $74 \pm 1.5$ . It was decided to investigate the reason for the variation of the results outside these limits.

Tubocurarine chloride possesses the following formula :



Dimethyltubocurarine is prepared by methylating the phenolic hydroxyl groups. It was thought that, as tubocurarine had two phenolic hydroxyl groups, the spectrum in alkaline solution should be different from that in acid and neutral solutions, whilst that of dimethyltubocurarine should not alter with change in pH, and should be essentially similar in shape to that of tubocurarine in acid and neutral solution. That this is actually the case is shown in Figure 1. This graph indicates that the spectral differences between dimethyltubocurarine and tubocurarine are greatest in alkaline solution, the degree of alkalinity chosen being 0.1N sodium hydroxide. It will be seen that the absorption maximum for tubocurarine in 0.1N sodium hydroxide is at 292  $m\mu$  and that for dimethyltubocurarine lies at 280  $m\mu$ . Also  $E_{292}$  for tubocurarine chloride was found to be about 160, whilst the corresponding value for dimethyltubocurarine iodide was about 20. The difference in position of the maxima is attributed to ionisation of the phenolic hydroxyl groups of tubocurarine in alkaline

\* Throughout the paper  $E_x$  is used to indicate  $E \frac{1 \text{ cm.}}{1 \text{ per cent.}}$  at wave-length  $x \text{ m}\mu$ .

solution. These spectral differences appeared to be sufficiently great to be used as a basis for working out a method for the assay of tubocurarine in the dimethyl compound.

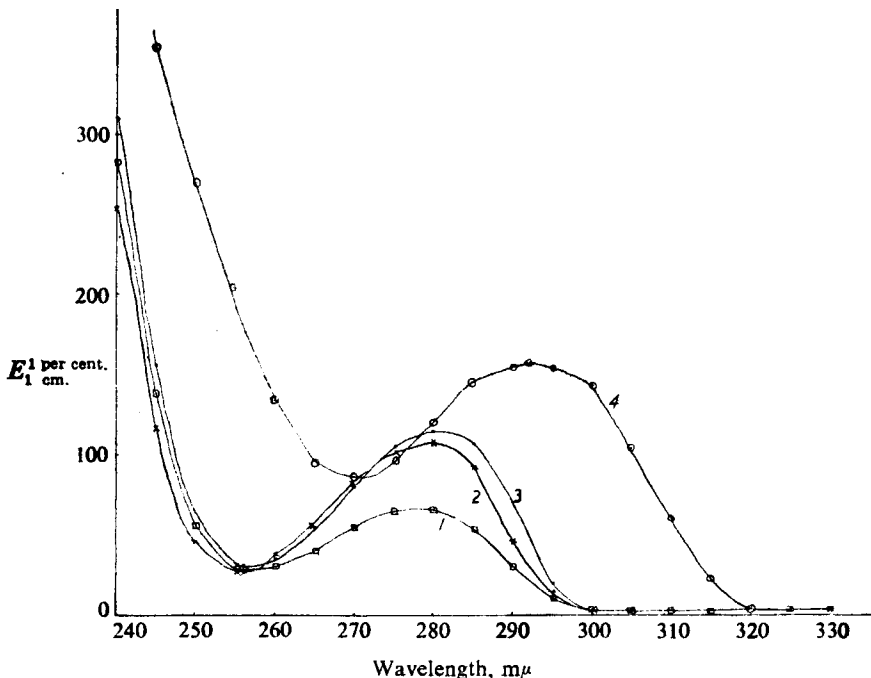


FIG. 1. Absorption spectra.

1. Dimethyltubocurarine iodide in 0.1N hydrochloric acid, absolute ethanol and 0.1N sodium hydroxide.
2. Tubocurarine chloride in 0.1N hydrochloric acid.
3. Tubocurarine chloride in absolute ethanol.
4. Tubocurarine chloride in 0.1N sodium hydroxide.

#### PREPARATION OF STANDARDS

Dimethyltubocurarine iodide is very much less soluble in absolute ethanol than is tubocurarine chloride. No change in the absorption spectrum of dimethyltubocurarine iodide occurred after three crystallisations from absolute ethanol, from which it was assumed to be free from tubocurarine. Tubocurarine chloride was also recrystallised from absolute ethanol until no change took place in the absorption spectrum. One recrystallisation was found to be sufficient.

#### DRYING EXPERIMENTS

Dimethyltubocurarine iodide is known to exist as the trihydrate and tubocurarine chloride as the pentahydrate. As the state of hydration of the preparations produced as standards was unknown, it was decided to attempt to dehydrate them by heating them *in vacuo* over phosphorus

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pentoxide as drying agent. It was found to be necessary to heat dimethyltubocurarine iodide at 110°C. for 2 hours in order to obtain a preparation which, when tested spectrophotometrically, gave the required increase in  $E_{280}$  in 0.1N sodium hydroxide. An average value of  $E_{280}$  for dimethyltubocurarine iodide before drying was found to be 67.3, and after drying it increased to about 71.3, an increase of 5.6 per cent. (theoretical 5.6 per cent.). In spite of the theoretical increase in  $E_{280}$  the drying process was not considered satisfactory as a slight discoloration took place at the same time. This was attributed to decomposition of the iodide. Attempts at drying tubocurarine chloride *in vacuo* at 110°C. for 2 hours led to an increase in  $E_{292}$  from 152 to 165, corresponding to a loss of 7.88 per cent. (theoretical 11.5 per cent.).

From these results it was decided that drying at 110°C. under high vacuum was not a reliable method of producing the anhydrous preparations. The Karl Fischer method of determining water appeared to offer an alternative approach to the problem. This method proved to be entirely satisfactory. A sample of tubocurarine chloride after recrystallisation until no further spectral changes took place gave a moisture content of 11.9 per cent. This slightly high value was attributed to adsorbed moisture in excess of water of crystallisation. A series of determinations on dimethyltubocurarine iodide samples gave values slightly greater than 5.6 per cent. Table I gives  $E_{280}$  and  $E_{292}$  on purified specimens of dimethyltubocurarine iodide and tubocurarine chloride calculated to the anhydrous salts after allowing for moisture determined by the Karl Fischer method.

TABLE I  
SPECTROPHOTOMETRIC DATA FOR SAMPLES OF DIMETHYLTUBOCURARINE  
AND TUBOCURARINE CALCULATED TO THE ANHYDROUS SALTS

	$E_{280}$	$E_{292}$
<i>Dimethyltubocurarine iodide</i> :—		
Original sample ... ..	75.5	20.9
1st recrystallisation ... ..	74.3	19.7
2nd     ,,     ... ..	73.2	18.4
3rd     ,,     ... ..	73.2	18.2
<i>Tubocurarine chloride</i> :—		
Original sample	131	174
1st recrystallisation ... ..	130	171

In order to obtain maximum accuracy in the determination of  $E_{292}$  for dimethyltubocurarine iodide the following procedure was adopted: 70 mg. was dissolved with warming in 100 ml. of water, the solution was cooled, 20 ml. of N sodium hydroxide was added and the volume made up to 200 ml. with water. The optical density of this solution was determined at 292  $m\mu$  in a 1 cm. cell against 0.1N sodium hydroxide as a blank, and  $E_{292}$  calculated. The solution was diluted 5 times, suf-

ficient sodium hydroxide being added to maintain the alkalinity at 0.1N. and the density at 280  $m\mu$  determined as before. From this,  $E_{280}$  was calculated. It was found that  $E_{292}$  values obtained in this manner were much more reproducible, but slightly lower than if the same solution were used to determine both E values.

#### GRAPHICAL SOLUTION

Using the values  $E_{292} = 18.4$  and 171 for anhydrous dimethyltubocurarine iodide and anhydrous tubocurarine chloride, values of  $E_{292}$  were calculated for mixtures containing 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 per cent. of anhydrous tubocurarine chloride on the assumption that the laws of absorption would be obeyed. Table II illustrates these results together with experimentally determined values on anhydrous mixtures.

TABLE II  
MEASUREMENT OF TUBOCURARINE CONTENT BY  $E_{292}$

Tubocurarine chloride per cent.	Tubocurarine iodide per cent.	$E_{292}$ theory	$E_{292}$ experimental
0.0	0.0	18.4	18.4
0.5	0.6	19.2	19.0
1.0	1.3	19.9	19.9
2.0	2.5	21.4	21.2
3.0	3.8	23.0	22.9
4.0	5.0	24.5	24.8
5.0	6.2	26.0	26.2

It will be seen that the experimentally determined values are in good agreement with the theoretical values. Although the  $E_{292}$  mixture values alter by only 1.5 for 1.0 per cent. of tubocurarine chloride, it was found experimentally that the density readings of the solutions altered by 0.05. The estimated error in the determination of  $E_{292}$  was  $\pm 0.2$ .

#### RECOMMENDED METHOD OF ASSAY

Weigh 70 mg. and dissolve in distilled water, warming on a water-bath to effect solution. Cool, transfer to a 200 ml. graduated flask, add 20 ml. of N sodium hydroxide and make up to 200 ml. with water. Determine the optical density at 292  $m\mu$  on a spectrophotometer using 0.1N sodium hydroxide as a blank. Dilute the solution 1 to 5, adding sufficient sodium hydroxide to the dilution to maintain the 0.1N alkalinity. Determine the optical density of this solution at 280  $m\mu$  using the same blank as before. Calculate  $E_{280}$  and  $E_{292}$ . Determine moisture on the sample by the Karl Fischer method. Calculate  $E_{292}$  to the anhydrous sample and from Table II or a prepared graph read

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off the percentage of tubocurarine iodide in the anhydrous mixture. Let  $x$  = percentage of tubocurarine iodide read on graph, then tubocurarine

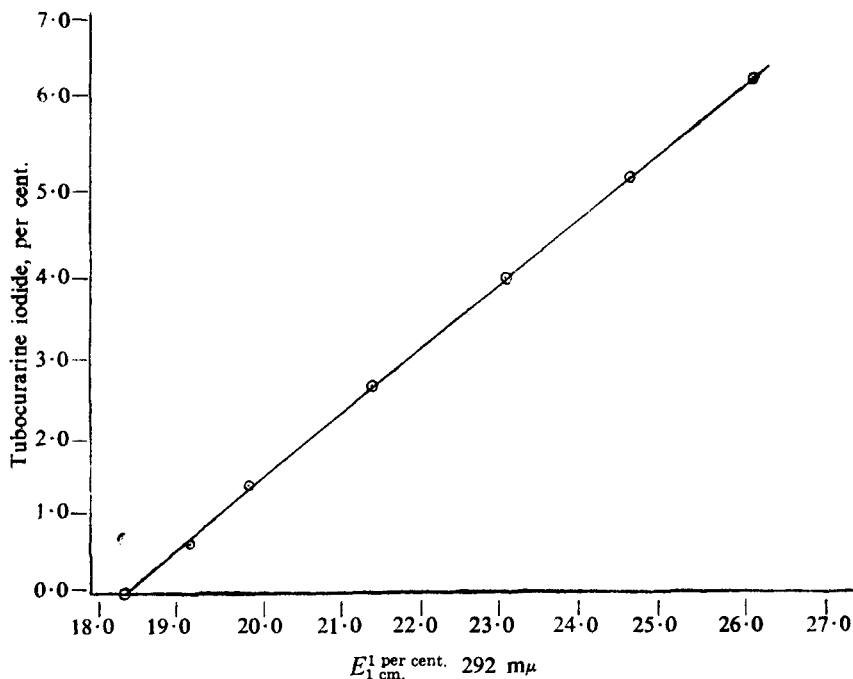


FIG. 2. Percentage of tubocurarine iodide in mixtures of anhydrous tubocurarine iodide and anhydrous dimethyltubocurarine iodide.

iodide: dimethyltubocurarine iodide =  $x : 100 - x$ , or tubocurarine iodide =  $\left(\frac{x}{100 - x}\right)$  dimethyltubocurarine iodide. Let actual anhydrous dimethyltubocurarine iodide content be  $y$  per cent., then anhydrous tubocurarine iodide will be  $\left(\frac{x}{100 - x}\right) y$  per cent.

$$\text{For a mixture } E_{280} = 0.732y + 1.03y \left(\frac{x}{100 - x}\right)$$

Here  $x$  and  $E_{280}$  are known, hence  $y$  can be calculated. From this the content of anhydrous tubocurarine iodide is given by  $\left(\frac{x}{100 - x}\right) y$ . Percentage of anhydrous dimethyltubocurarine iodide  $\times 1.062 =$  percentage of dimethyltubocurarine iodide trihydrate.

*Assay of Commercial Samples.* Four preparations of dimethyltubocurarine iodide were examined by this method with the results shown in Table III.

It will be seen from Table III that the sum total of moisture, anhydrous

TABLE III

Sample	Moisture (Karl Fischer) per cent.	Dimethyltubocurarine Iodide, anhydrous per cent.	Tubocurarine Iodide, anhydrous per cent.	Total per cent.
A ... ..	5.6	94.3	0.8	100.7
B ... ..	6.1	94.4	nil	100.5
C ... ..	5.8	93.0	0.8	99.6
D ... ..	6.3	93.0	nil	99.3

dimethyltubocurarine iodide, and anhydrous tubocurarine iodide in the case of each sample comes within the limits  $100 \pm 0.7$  per cent.

### SUMMARY

1. The absorption spectra of tubocurarine chloride and of dimethyltubocurarine iodide have been examined in acid, neutral and alkaline solution.

2. The absorption spectrum of tubocurarine chloride in alkaline solution differs from the spectrum in acid or neutral solution and also from the spectrum of dimethyltubocurarine iodide in acid, neutral or alkaline solution.

3. A method has been worked out for the determination of tubocurarine iodide in amounts varying from 0.5 to 6.0 per cent. in the presence of dimethyltubocurarine iodide.

The spectrophotometric measurements in this investigation were carried out with a Beckman quartz spectrophotometer having a hydrogen discharge tube as ultra-violet source.

The author wishes to thank Dr. N. Evers and Mr. Wilfred Smith for suggestions and criticisms and the Directors of Allen and Hanburys Ltd., for permission to publish this paper.

### DISCUSSION

The paper was presented by DR. N. EVERS.

The CHAIRMAN asked for information on the stability of the compounds, in particular what were the optimum conditions for preparing sterile solutions for injection.

DR. G. FOSTER (Dartford) asked whether any alternative methods of estimation of dimethyltubocurarine iodide had been tried, such as a determination of methoxyl groups.

DR. EVERS, in reply, said that he understood the substance was reasonably stable at the pH at which it was dissolved. The results of chemical assays, using, *e.g.*, Millon's reagent, had not been satisfactory.

MR. C. J. EASTLAND (London), also in reply, confirmed that solutions were stable at their natural pH (6.4) and such solutions could be autoclaved.