THE DETERMINATION OF ERGOTAMINE IN PREPARATIONS CONTAINING ERGOTAMINE TARTRATE AND CYCLIZINE HYDROCHLORIDE

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Benzhydrol, an impurity in some samples of cyclizine hydrochloride, interferes with the colorimetric estimation of ergotamine in preparations containing ergotamine tartrate and cyclizine hydrochloride. A method for overcoming this difficulty is described. The inhibition of colour development has been utilised to provide a method for the detection and estimation of benzhydrol in cyclizine hydrochloride.

The use of ergotamine tartrate for the treatment of migraine has resulted in the clinical use of compound tablets containing, besides ergotamine tartrate, a stimulant such as caffeine or a drug to combat sickness or both. In this connection we became interested in the assay of granules, containing ergotamine tartrate and cyclizine hydrochloride. It was found that when powdered granules were shaken with 1 per cent tartaric acid the resulting extract could be assayed colorimetrically for ergotamine using dimethylaminobenzaldehyde solution B.P.: this procedure was adopted for routine work. Some batches of granules, however, gave unexpectedly low results for ergotamine content and we were satisfied that the ergotamine tartrate had been put into the granules and that the assay procedure was faulty. It was discovered that solutions of ergotamine tartrate and cyclizine hydrochloride in 1 per cent tartaric acid gave satisfactory assay results when freshly prepared, but underwent a gradual change when heated on a steam bath and after 30 min. gave no colour with the official reagent. Solutions of ergotamine tartrate alone were unaffected by this treatment. It was decided therefore to avoid heating during the assay of the granules but, despite this, low results for the ergotamine content were still occasionally obtained. We concluded that some samples of cyclizine hydrochloride contained an impurity which interfered with the colour reaction.

To test this, a solution of ergotamine tartrate in 1 per cent tartaric acid was divided into eight equal portions. To each was added the appropriate amount of cyclizine hydrochloride to afford a solution similar to that used in the assay of the granules, a different batch of cyclizine hydrochloride being used for each solution. All solutions were then assayed colorimetrically for ergotamine. The results showed clearly that some samples of cyclizine hydrochloride interfered with the colour reaction and some did not.

Attempts made to isolate the interfering substance by fractional crystallisation were unsuccessful. When, however, an aqueous solution of "impure" cyclizine hydrochloride was distilled at atmospheric pressure a cloudy distillate was obtained which, after standing for several days,

deposited colourless needles, of m.p. 65°. This material strongly interfered with the ergot colour reactions (Table I). It was identified as benzhydrol; its ultra-violet absorption curve was superimposable on that of an authentic specimen determined under identical conditions and it gave no depression in m.p. on admixture with an authentic specimen.

Once identified, the impurity was easily removed from the tartaric acid extract of the granules by ether extraction and the ergotamine was then quantitatively recovered during the assay.

TABLE I

INFLUENCE OF BENZHYDROL ON COLORIMETRIC ASSAY OF ERGOTAMINE TARTRATE

Weight of benzhydrol in 50 ml. of 1 per cent tartaric acid (mg.)	Per cent recovery of ergotamine tartrate by assay
—	100
0·31	90·3
0·77	70·9
1·08	60·0
1·51	49·3
2·11	38·2
2·32	37·2
3·09	30·3
4·22	24·3
7·24	20·2

Each assay was performed on a 0·00754 per cent w/v solution of ergotamine tartrate in aqueous 1 per cent tartaric acid solution.

This work provides the basis for a qualitative and quantitative test for the presence of benzhydrol in samples of cyclizine hydrochloride.

The following method was evolved for the determination of ergotamine in granules containing 87·7 per cent cyclizine hydrochloride and 3·5 per cent ergotamine tartrate.

Method

Transfer an accurately weighed quantity of about 0·4 g. of finely powdered granules to a 100 ml. graduated flask, add aqueous 10 per cent w/v tartaric acid solution (75 ml.) and shake mechanically for 30 min. Adjust the volume to 100 ml. with the tartaric acid solution, mix thoroughly and filter through a 9 cm. Whatman No. 1 filter paper rejecting the first 25 ml. of filtrate.

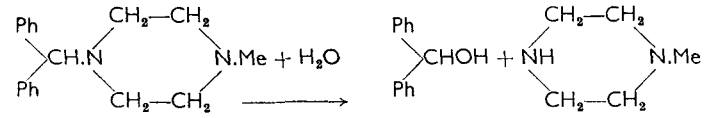
Transfer 50 ml. of the filtrate to a 100 ml. separating funnel, add ether (25 ml.) and shake thoroughly. Allow to stand for 5 min. and transfer the aqueous layer to a 100ml. graduated flask. Wash the ether layer with four successive 10 ml. portions of the tartaric acid solution adding the washings to the bulk of the solution in the 100 ml. flask. Adjust the contents of the flask to 100 ml. with the tartaric acid solution and mix. Transfer a 5 ml. aliquot of the solution to a 25 ml. flask and slowly add dimethylaminobenzaldehyde solution B.P. (10 ml.); mix thoroughly and set aside for 15 min. Determine the ergotamine tartrate content by comparing the extinction of this solution at 550 mμ with that obtained by treating a standard ergotamine tartrate solution in an identical manner.

Estimation of Benzhydrol in Cyclizine Hydrochloride

The inhibition of colour development in this reaction has been used as a test for benzhydrol in production batches of cyclizine hydrochloride. A calibration curve, constructed from data in Table I, has been used tentatively for quantitative estimation. Thus a sample which lowered the ergotamine recovery by 20 per cent was estimated to contain 0·45 per cent of benzhydrol. As a check measurement of the extinction at 225 mμ and 204 mμ of an ether extractive of this sample gave the benzhydrol content as 0·425 per cent. A second sample, which showed no significant inhibition of the colour reaction, was found to contain 0·009 per cent of benzhydrol by this criterion.

Discussion

All batches of cyclizine hydrochloride used, complied fully with the B.P.C. 1959 monograph. The isolation of benzhydrol led us to consider the possible source of this impurity. This can arise firstly by hydrolysis of diphenylmethyl halide used in manufacture or secondly by 'hydrolysis' of cyclizine in the following manner.



Cyclizine Benzhydrol *N*-Methylpiperazine

Pertinently, when cyclizine hydrochloride, free from interfering agent, was dissolved in water and the solution boiled under reflux for some hours, both benzhydrol and benzophenone could be recovered from the aqueous solution by distillation. The latter no doubt had been formed by oxidation of benzhydrol. Neither benzophenone nor methylpiperazine interfered with the ergot colour reaction.

Benzhydrol inhibits the colour reaction with lysergic acid in a similar manner and we have no doubt that it would do the same with other ergot alkaloids. Some observations have an interesting bearing on the mechanism whereby benzhydrol inhibits this colour reaction of ergotamine. A solution of ergotamine tartrate and cyclizine hydrochloride (giving a satisfactory colour reaction) gave practically no colour after heating on a steam bath for 30 min. The same treatment of ergotamine tartrate solution alone or of cyclizine hydrochloride alone, led to no such colour inhibition. This suggests that "hydrolysis" of cyclizine—normally slow, is accelerated in some way by the ergotamine. It was established that a solution of benzhydrol in 1 per cent tartaric acid gave no colour reaction with dimethylaminobenzaldehyde solution.

The tentative method has shown that while some samples of cyclizine hydrochloride contain benzhydrol as an impurity the maximum content corresponds to no more than 0·5 per cent.

Discussion

The paper was presented by Mr. Caws. The following points were made in the discussion.

It was necessary to increase the concentration of tartaric acid solution to 10 per cent to prevent loss of ergotamine in the ether layer during extraction. The benzhydrol had a low toxicity and did not interfere with the pharmacological effect of ergotamine. Cyclizine was comparatively stable in aqueous solution, and the rate of hydrolysis was slow: tablets would not be affected to any extent.