# RESEARCH PAPERS

## PHOTOMETRIC DETERMINATION OF 2:4-DIAMINO-5-PHENYLTHIAZOLE

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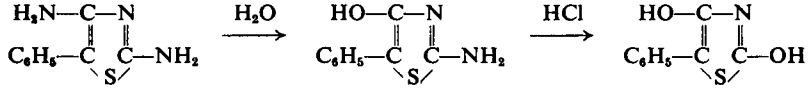
THE use of 2:4-diamino-5-phenylthiazole (amiphenazole, fenamizol, D.A.P.T.), as a morphine antagonist was reported by Shaw and Bentley in 1949[1] and 1952[2] and by Shaw and Shulman[3] in 1955. Amiphenazole has also been used by Shulman *et al.*[4] as a synergist with β-methyl-β-ethylglutarimide (4-methyl-4-ethyl-2:6-dioxopiperidine, bemegride, NP 13) in the treatment of barbiturate intoxication. In the early part of 1955 amiphenazole was studied by Canbäck, and others, in this laboratory[5–7]. During this work a rapid reliable method was required to investigate the stability of amiphenazole in pharmaceutical preparations.

From an analytical point of view, published papers offer little about amiphenazole. From a paper by Davies *et al.*[8], dealing with the synthesis and properties of 2:4-diaminothiazoles, it is known that amiphenazole is a monoacidic base [m.pt. of the picrate 189° to 191° C. (decomp.)]. Its soluble salts with halogen acids are white crystalline powders, stable to air. During the isolation of the free base, atmospheric oxidation readily produces tars. An aqueous solution of the hydrobromide or hydrochloride of amiphenazole with a slight excess of sodium hydrogen carbonate or ammonia solution deposits the base, which slowly becomes yellow and then brown. Amiphenazole is almost completely devoid of aromatic character; it neither forms Schiff's bases with aldehydes nor undergoes diazotisation, and it does not give the carbylamine reaction.[8]

In aqueous solution the halogen salts of amiphenazole slowly undergo hydrolysis, to which the 4-amino-group is extremely sensitive. By refluxing a 5 per cent. aqueous solution of the amiphenazole hydrobromide for 3 hours Davies *et al.*[8] prepared 2-amino-4-hydroxy-5-phenylthiazole. Finally, both amino-groups can be replaced by hydroxy-groups through acid hydrolysis (refluxing of the amiphenazole hydrochloride for 5 hours with diluted hydrochloric acid (1 + 2) gave 2:4-dihydroxy-5-phenylthiazole[8]).




2:4-diamino-5-phenylthiazole — 2-amino-4-hydroxy-5-phenylthiazole — 2:4-dihydroxy-5-phenylthiazole

An assay method was required for amiphenazole, specific enough to determine the parent compound in the presence of its hydrolysis products. During the introductory investigations, which will be briefly discussed

below, amiphenazole was found to give a sparingly soluble salt with Reinecke's acid. This reaction was applied to quantitative work.

## EXPERIMENTAL

As mentioned above, amiphenazole is a monoacidic base (pKa = 7·0)[9], which can be titrated in anhydrous acetic acid with acetous perchloric acid. This method also permits a direct titration of the hydrogen halides of amiphenazole after converting the halide salts into acetates by adding an excess of mercuric acetate dissolved in acetic acid[10]. This method has, however, two drawbacks: it is not applicable to aqueous solutions of amiphenazole and it is not specific. Efforts to extract the amiphenazole with ether or chloroform after adding an excess of ammonia solution resulted in yellow-coloured solutions of the base in the organic solvent. By the titration with acetous perchloric acid after evaporating the solvent in vacuum the visual end-point was diffuse and obscure. This trouble may be attributed to the presence of 2-amino-4-hydroxy-5-phenylthiazole formed during the extraction procedure. This compound is too weakly basic to be titrated with accuracy with acetous perchloric acid using a visual end-point.

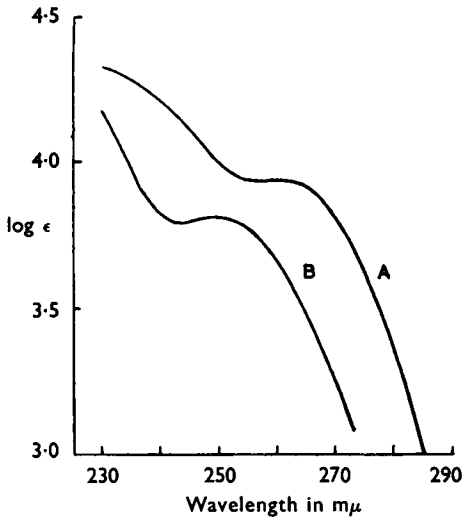



FIG. 1. Absorption curves of 2:4-diamino-5-phenylthiazole hydrochloride (A) and 2-amino-4-hydroxy-5-phenylthiazole (B) in aqueous solutions at pH 6.

The application of ultraviolet spectrophotometry to the determination of amiphenazole was also studied. The absorption curves of amiphenazole hydrochloride and 2-amino-4-hydroxy-5-phenylthiazole in aqueous solutions were recorded with a Beckman Model DU Spectrophotometer, and the effect of pH on these curves was studied. In Figure 1 the absorption curves at pH 6 are plotted. The absorption data, obtained by these measurements, did not give an ideal basis for a spectrophotometric determination of amiphenazole in presence of its hydrolysis products.

The reactions of amiphenazole with some colour forming reagents were also investigated. For instance amiphenazole may be coupled to diazotised *p*-aminoacetophenone in alkaline solution giving a yellow-coloured product. The efforts to apply this reaction to quantitative work were, however, not successful. Among tested reactions precipitation of amiphenazole by Reinecke's salt from aqueous solution was

found to be the most promising one. The precipitation of amiphenazole as reineckate was studied in detail and led to the photometric method of analysis described below.

*Photometric determination of amiphenazole as reineckate*

*Reagents.* Reinecke's salt, $NH_4[Cr(CNS)_4(NH_3)_2]\cdot H_2O$. Reinecke's salt reagent: A saturated aqueous solution, prepared by shaking 1 part of Reinecke's salt with 25 parts of distilled water. 2:4-Diamino-5-phenylthiazole hydrochloride, synthesised in this laboratory. The substance was estimated by "the acetous perchloric acid method" discussed above.

*Preparation and analysis of the 2:4-diamino-5-phenylthiazole reineckate*

To an aqueous solution of 2:4-diamino-5-phenylthiazole hydrochloride was added an excess of Reinecke's salt reagent. The amiphenazole reineckate, a light red, fine-grained precipitate, was collected in a G4 sintered glass crucible and washed with cold water. The precipitate was finally dried in vacuum over phosphorous pentoxide to a constant weight. Melting point: 163° to 165° C. (corr.).

The composition of the precipitate was checked by carefully charring the precipitate and then igniting the residue of chromium (III) oxide to a constant weight. 2:4-Diamino-5-phenylthiazole reineckate: $C_{13}H_{20}N_{10}S_5Cr$ Mol. wt. 510·64. The content of chromium (III) oxide found = 15·67 per cent.; calculated 14·89 per cent.

The solubility of the amiphenazole reineckate at 0° C. was also determined. Unsaturated and supersaturated aqueous solutions were shaken at a temperature of an ice-water mixture. The two methods gave similar results. Solubility: 1 part in 3000 parts of water at 0° C.

*Absorption data for the solution of amiphenazole reineckate in acetone*

The reineckate of amiphenazole is readily soluble in acetone, giving a red-coloured solution. The absorption curve of the acetone solution in the 470 to 580 m$\mu$ region is shown in Figure 2. The curve has a peak at 525 m$\mu$, a molecular extinction coefficient ($\epsilon$) = 106·3. The curve in Figure 2 is recorded with a Beckman Model DU Spectrophotometer.

The acetone solution of amiphenazole reineckate is stable for at least three hours at room temperature.

*Effect of various experimental conditions on the precipitation and isolation of the amiphenazole reineckate*

A quantity of a solution, containing about 30 mg. of 2:4-diamino-5-phenylthiazole hydrochloride, was measured into a small beaker and placed in an ice bath. When the solution had assumed the temperature of the bath, 5 ml. of Reinecke's salt reagent was added. The mixture was kept in the ice bath for a given time and then the precipitate collected in a G4 sintered glass crucible and washed. The reineckate was dissolved by pouring acetone on the precipitate. The solution was then slowly filtered and finally diluted to 25 ml. The extinction at 525 m$\mu$ was measured.

The following variables in the procedure were checked: (1) the time of cooling the ice bath after precipitation, (2) the washing procedure, and (3) the effect of variations in the concentration of the amiphenazole hydrochloride solution. The experiments showed, that after addition of the Reinecke's salt reagent the mixture should remain in the ice bath for at least 15 minutes. A prolongation of this time to two hours did not affect the result, while collecting the precipitate after only 5 minutes in the ice bath gave a slightly lower result.

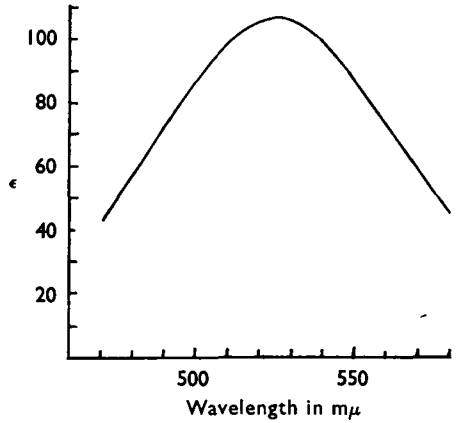



FIG. 2. Absorption curve of 2:4-diamino-5-phenylthiazole reineckate in acetone.

As shown above, the reineckate of amiphenazole is slightly soluble in water at 0° C. The effect of the solubility of the reineckate was checked by varying the washing procedure. After the precipitate had been collected in the sintered glass crucible, the beaker was rinsed with the wash liquor, which then was filtered through the crucible. The following wash liquors were used: (*a*), 2 × 2 ml. of water at room temperature (the water was however slightly cooled by rinsing the cold beaker), (*b*), 2 × 2 ml. of iced water, (*c*), 2 × 2 ml. of an iced, saturated, aqueous solution of amiphenazole reineckate, and (*d*), 2 × 5 ml. of the same wash liquor as in (*c*).

In all instances similar results were obtained. Even in variations (*a*) and (*b*) the wash water only dissolved negligible amounts of reineckate during the short time of washing. These experiments also showed that 2 × 2 ml. of wash liquor is sufficient. As the colour depends exclusively on the reineckate moiety it is essential that the precipitate is thoroughly washed.

Finally the effect of the concentration of the amiphenazole hydrochloride solution to be precipitated was checked. Solutions of about 30 mg. of the amiphenazole hydrochloride in 2, 5, 10 and 15 ml. of water were estimated. Similar results were obtained in all cases. The volume of the amiphenazole test solution taken may consequently vary between 2 and 15 ml. without any serious effects.

## PROCEDURE

Measure 2 to 15 ml. of solution, containing between 15 and 30 mg. of 2:4-diamino-5-phenylthiazole hydrochloride (or hydrobromide), into a small beaker and place it in an ice bath. When the solution is cooled to the temperature of the bath add 5 ml. of Reinecke's salt reagent, mix and allow to stand in the ice bath for 15 minutes. Collect the precipitate in a G4 sintered glass crucible, wash the precipitate twice with 2 ml. of iced water, rinsing the beaker, and dry by suction.

Dissolve the precipitate in acetone by drawing the solvent slowly through the filter under negative pressure and dilute to 25 ml. with acetone. Measure $E$ (1 cm.) at 525 m$\mu$ against water, and calculate the amount of 2:4-diamino-5-phenylthiazole hydrochloride present with the aid of a calibration curve.

The calibration curve is linear over the range 0 to at least 35 mg. of amiphenazole hydrochloride. The values in Table I have been obtained with a Beckman Model B Spectrophotometer.

TABLE I

THE RELATION BETWEEN $E$ (1 CM.) AT 525 M$\mu$ AND THE AMOUNT OF 2:4-DIAMINO-5-PHENYLTHIAZOLE HYDROCHLORIDE PRECIPITATED AS REINECKATE AND DISSOLVED IN 25 ML. OF ACETONE

| Amiphenazole hydrochloride mg. | Extinction against water |
|---|---|
| 12·4 | 0·240 |
| 18·1 | 0·360 |
| 20·9 | 0·415 |
| 24·1 | 0·475 |
| 27·8 | 0·550 |
| 34·8 | 0·678 |

## DISCUSSION

The deterioration of aqueous solutions of 2:4-diamino-5-phenylthiazole hydrochloride, which occurs during storage, depends on the replacement of the 4-amino-group by a hydroxyl group. The 2-amino-4-hydroxy-5-phenylthiazole, which is the hydrolysis product, forms no precipitate with Reinecke's salt and consequently does not affect the determination of amiphenazole. By the estimation of a saturated aqueous solution of 2-amino-4-hydroxy-5-phenylthiazole, to which 0·5 per cent. of amiphenazole hydrochloride was added, the calculated amount of amiphenazole was found.

Moderate amounts of glycerol and ethanol (e.g., 5 per cent. of glycerol and 15 per cent. of ethanol in a 1·5 per cent. solution of amiphenazole hydrochloride) or benzyl alcohol and propylene glycol (e.g., 2 per cent. of benzyl alcohol and 60 per cent. of propylene glycol in a 1·5 per cent. solution of amiphenazole hydrochloride) do not interfere with the determination of amiphenazole. On the other hand, the test solution must not contain polyethylene glycol. As mentioned above, amiphenazole is used in the treatment of barbiturate intoxication as a synergist with $\beta$-methyl-$\beta$-ethylglutarimide. The latter compound does not interfere with the determination of amiphenazole.

An investigation of the stability of solutions of amiphenazole hydrochloride for injection is under way in this laboratory and will be published later. However, some preliminary statements of the stability of aqueous solutions will be given here. At room temperature 2 or 3 per cent. decomposition of amiphenazole in aqueous solution was found after only one week. The hydrolysis proceeded during the storage and reached the figure of about 20 per cent. after five weeks. The solution then also contained light yellow crystals of 2-amino-4-hydroxy-5-phenylthiazole. On the other hand, a refrigerated solution was stable for five weeks.

## SUMMARY

1. A photometric method for the determination of 2:4-diamino-5-phenylthiazole (amiphenazole) hydrochloride (or hydrobromide) in aqueous solution has been described. It depends on the precipitation

of the compound as an insoluble reineckate. The hydrolysis products of amiphenazole have been found not to affect the determination of the compound.

2. Some other methods of determination of amiphenazole have been briefly discussed. Examples are, the titration with acetous perchloric acid and the application of ultra-violet spectrophotometry.

3. Some preliminary statements of the stability of amiphenazole in aqueous solution have been given.

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## References

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