THE DETERMINATION OF AMINO-COMPOUNDS OCCURRING AS IMPURITIES IN PHARMACEUTICAL CHEMICALS

PART II.

3-AMINO-4-HYDROXYPHENYLARSONIC ACID IN ACETARSOL

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IN connection with the manufacture of acetarsol, a quantitative method was required for the determination of traces of 3-amino-4-hydroxyphenylarsonic acid and it was thought that a suitable method might be based on the official limit test for this impurity. This depends upon diazotisation and coupling as does the test for arsanilic acid in tryparsamide and hence, in view of the sources of error revealed in the latter[1] and in the quantitative method[2] proposed by MacDonald and Reynolds, it was decided to make a thorough study of the conditions and reactions involved.

THE OFFICIAL TEST COLOUR STANDARD

The colour standard of the official test is not obtained directly from 3-amino-4-hydroxyphenylarsonic acid but from the product of hydrolysis of acetarsol with hydrochloric acid. Bonino[3], who describes a colour test for acetarsol involving heating with hydrochloric acid, suggests that an arsenoso-compound is first formed, whilst Phillips[4] found that acetarsol yielded 4-bromo-2-aminophenol when heated with hydrobromic

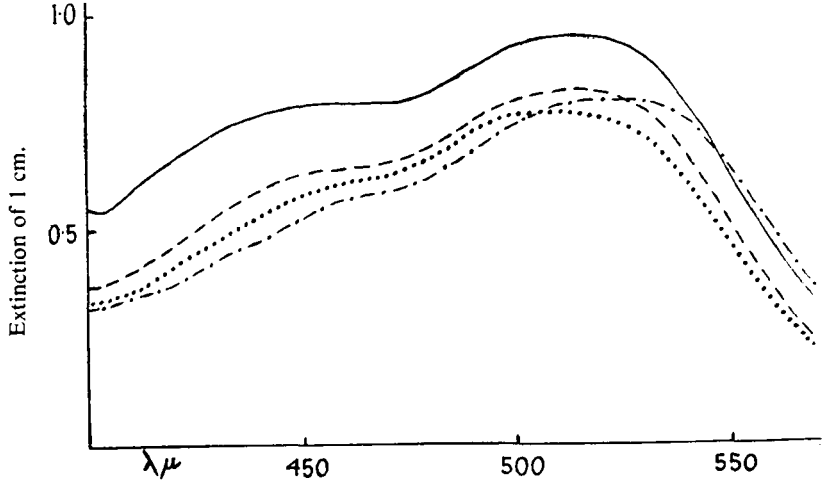

FIG. 1. Spectrophotometric absorption curves of β-naphthol azo-dyes
3-amino-4-hydroxyphenylarsonic acid (0·4 mg. = 1·7 micromol)
— — — hydrolysis product of acetarsol (0·4 mg. = 1·45 micromol)
····· 2-aminophenol (1·7 micromol)
·—·—· 4-chloro-2-aminophenol (1·7 micromol)

acid. Hence it appeared desirable to confirm that hydrolysis of acetarsol under the conditions specified in the official test does yield 3-amino-4-hydroxyphenylarsonic acid.

If the arsonic acid group were removed arsenate would be present, but none was detected by a test, using magnesia mixture, of known high sensitivity; more conclusive evidence was obtained from the spectrophotometric absorption curves of the azo-dyes obtained by diazotising and coupling with β-naphthol. From Figure 1 it is seen that the curve for the product of hydrolysis of acetarsol closely follows that for 3-amino-4-hydroxyphenylarsonic acid and the ratio of the colour intensities is approximately that of the molecular weights (mol. wt. of acetarsol/ mol. wt. of 3-amino-4-hydroxyphenylarsonic acid = 1/0·85). On the other hand the curves for 2-aminophenol and 4-chloro-2-aminophenol are somewhat different in shape and moreover the colour intensities are appreciably less than those for an equivalent weight of 3-amino-4-hydroxyphenylarsonic acid. Hence it was concluded that the latter is in fact the product of hydrolysis of acetarsol; however, since it was readily available and a simple method of purifying it by recrystallisation is described by Ehrlich and Bertheim[5], it was used in subsequent work because more convenient.

Optimum Conditions for Diazotisation and Coupling of 3-amino-4-hydroxyphenylarsonic Acid

Optimum conditions for diazotisation and coupling were determined in the usual way, an interesting feature of the results being the effect of *p*H on the rate of coupling and on the colour obtained. With decrease in alkalinity coupling became slower and the violet component of the colour increased gradually but at the point at which β-naphthol began to precipitate a sharp change to a definite violet occurred. These colour changes may be associated with the presence of a hydroxyl group in the dye molecule.

The following method embodies the optimum conditions, and a calibration curve obtained by this method is shown in Figure 3. To 5 ml. of solution containing not more than 0·25 mg. of 3-amino-4-hydroxyphenylarsonic acid add 5 ml. of N/1 hydrochloric acid, cool to about 5°C., add 2 ml. of freshly prepared 0·1 per cent. solution of sodium nitrite and mix. After 2 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath 5 minutes. Add 10 ml. of previously cooled β-naphthol solution (freshly prepared 5 per cent. solution of recrystallised β-naphthol in 2N sodium hydroxide) and mix. Leave in the ice-bath for 10 minutes, and then place in water at 20°C. for 5 minutes. Read the extinction of 2 cm. using an Ilford 604 filter. Subtract the value of the blank reading obtained in a test omitting the 3-amino-4-hydroxyphenylarsonic acid.

The Official Test—Coprecipitation

Since in the case of tryparsamide low results were obtained by precipitating the tryparsamide and applying the test for arsanilic acid to

the filtrate, it was expected that a similar effect would result with acetarsol. To determine the extent of the loss by coprecipitation in the official test, it was modified slightly as follows to adapt it for photoelectric absorptiometry. Furthermore, sodium carbonate was substituted for sodium hydroxide to avoid hydrolysis of the acetarsol since, in the case of chloracetylaminophenylarsonic acid, appreciable hydrolysis to arsanilic acid had been found to occur in sodium hydroxide solution. Dissolve 0·2 g. in a mixture of 1 ml. of N/1 sodium carbonate and 8 ml. of water. Add 1 ml. of 5N hydrochloric acid, mix, filter, using reduced pressure, to dryness, wash the precipitate with 1 ml. of water and use 1 ml. of water to wash the filter. Cool the mixed filtrate and washings, diazotise and couple with β-naphthol and read the extinction as already described.

This test was applied to a sample of acetarsol which contained about 0·06 per cent. of 3-amino-4-hydroxyphenylarsonic acid; the acetarsol filtered off was then again submitted to the test and this procedure repeated several times. The extinction values (corrected for reagent blank) obtained are shown in Figure 2 and it is seen that the extinction values tend to a constant value of about 0·075, which is shown later to be not entirely due to coprecipitation.

Consideration was then given to devising a test avoiding filtration, especially as it seemed unlikely that the acetarsol would enter into any chemical reactions during the test. Using the same material as used above, optimum conditions were determined and the following provisional test decided upon. Dissolve 0·2 g. in a mixture of 1 ml. of N/1 sodium carbonate and 8 ml. of water and add 1 ml. of 5N hydrochloric acid. Cool to about 5°C., add 2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrate and mix. Complete the test as already described.

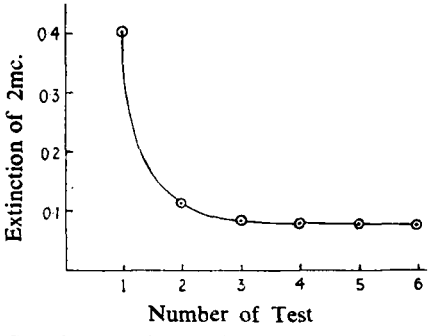

FIG. 2. Results obtained in successive tests by precipitation and filtration on the same material

Using this method an extinction of 0·44 was obtained, compared with 0·405 for the first value in the precipitation tests (Fig. 2). However, to put the test on a firm basis it was desirable to prepare a sample of pure acetarsol; in addition the constant extinction value of 0·075 reached in the precipitation series required explanation.

PURIFICATION OF ACETARSOL AND RECOVERY OF ADDED 3-AMINO-4-HYDROXYPHENYLARSONIC ACID

From Figure 2 it is seen that precipitation by acid from an alkaline solution is a relatively effective method of purifying acetarsol. Material from the same sample as used above was repeatedly submitted to this treatment, 7 precipitations being made at room temperature from sodium

carbonate solution by adding an exactly equivalent amount of diluted hydrochloric acid, followed by 4 precipitations from ice-cold sodium bicarbonate solution with ice-cold diluted hydrochloric acid. The last 2 specimens were tested by the method described above but using varying conditions for effecting solution and precipitation; identical results were obtained on both specimens and are shown in Table I.

TABLE I

	Conditions used to dissolve and precipitate acetarsol		Extinction Values
	Solution	Precipitation	
a	0·1 g. of sodium bicarbonate in 5 ml. of water at 5° C.	5 ml. of N/1 hydrochloric acid at 5° C.	0·025
b	As above but at room temperature	As above but at room temperature	0·04
c	1 ml. of N/1 sodium carbonate and 4 ml. of water at room temperature	As above	0·055
d	1 ml. of N/1 sodium hydroxide and 4 ml. of water at room temperature	As above	0·07

It was concluded that these two specimens of acetarsol were as pure as could be obtained by the reprecipitation method. Furthermore, it was clear that hydrolysis of acetarsol to 3-amino-4-hydroxyphenylarsonic acid occurs even under relatively mild alkaline conditions and probably also in acid solution. That hydrolysis occurs also in boiling water was shown by recrystallising the purified material from boiling water when the excellent crystals obtained were found to contain about twice as much amino-compound as the original material.

It was now possible to explain the constant value obtained in Figure 2. The extinction value of 0·075 would be made up of about 0·05 from amino-compound produced by hydrolysis during the test itself (Table I, c), and about 0·025 from amino-compound already present in the acetarsol, having been derived from acetarsol by hydrolysis under the conditions of solution and precipitation, and coprecipitated with it.

Calibration curves for 3-amino-4-hydroxyphenylarsonic acid (a) alone, prepared by the method already described, and (b) in the presence of acetarsol, prepared by the proposed method described later, are shown in Figure 3 and it is seen, as expected, that low recovery is obtained in the presence of acetarsol owing to coprecipitation. It is, therefore, necessary to use either internal standards or a calibration curve prepared with acetarsol; for the latter it is sufficient to use a specimen of only relatively low amino-compound content.

Proposed Methods

(a) Photoelectric absorptiometer. Prepare a calibration curve by the method described below using, instead of 5 ml. of water, 5 ml. of solution containing up to 0·25 mg. of 3-amino-4-hydroxyphenylarsonic acid; from each value subtract that obtained on the acetarsol alone. The determination on a sample is as follows. To 5 ml. of water at about 5°C. add 0·1 g. of sodium bicarbonate and 0·2 g. of acetarsol. Shake to dissolve and add 5 ml. of N/1 hydrochloric acid previously cooled to about 5°C. Add

2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrite and mix. After 3 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath for 5 minutes. Add 10 ml. of previously cooled β-naphthol solution (freshly prepared 5 per cent. solution of recrystallised β-naphthol in 2N sodium hydroxide) and mix. Leave in the ice-bath for 10 minutes and then place in water at 20°C. for 5 minutes. Read the extinction of 2 cm. using Ilford 604 filter and subtract the value of a reagent blank together with 0·025. Read the amount of 3-amino-4-hydroxyphenylarsonic acid from the calibration curve.

(b) Tintometer. Prepare a calibration curve relating red units and

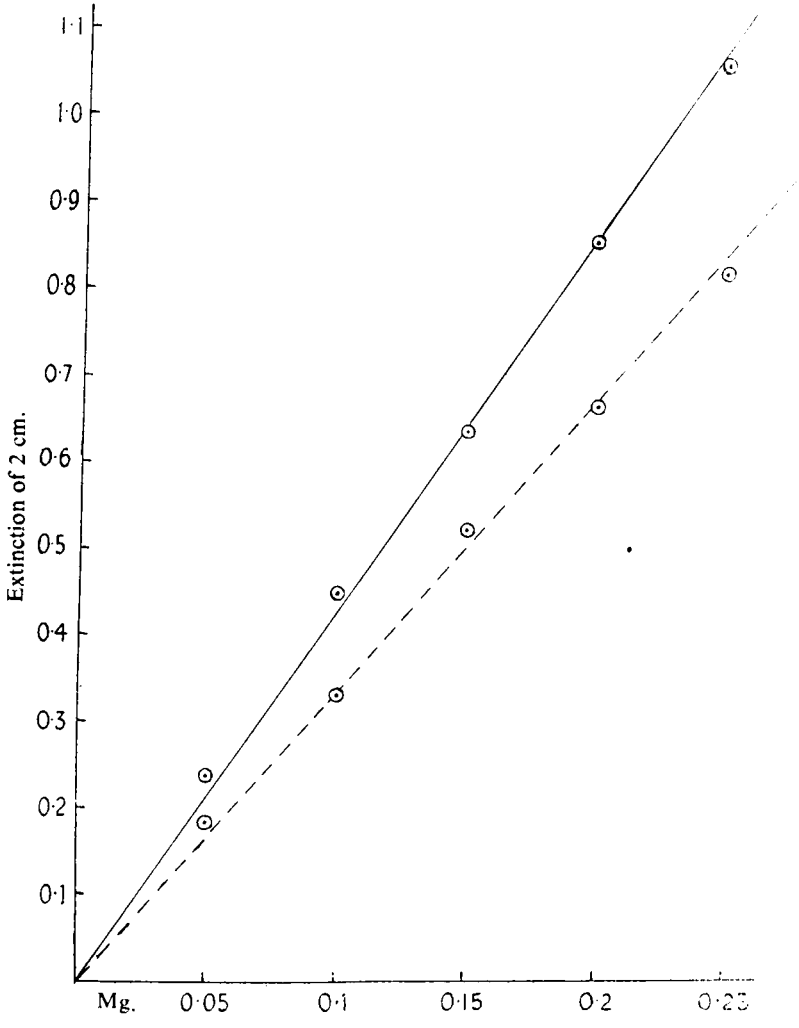

FIG. 3. Calibration curves for 3-amino-4-hydroxyphenylarsonic acid
——— alone
— — — in the presence of acetarsol

3-amino-4-hydroxyphenylarsonic acid by the method described under (a) above (using a 1 cm. cell 0·25 mg. gives about 5 red units). In testing samples, proceed as under *(a)* but subtract 0·1 red unit from the 1 cm. cell reading before reading off from the calibration curve.

(c) British Pharmacopœia Limit Test. The calculated official limit is 0·085 per cent. but, owing largely to the coprecipitation occurring, the actual limit is about 0·13 per cent. Thus the sample used in much of the above work contained by the proposed method 0·06 per cent., whereas by a method closely following the official test as regards preparation of solution for testing only about 0·04 per cent. was found. From Figure 3 it can be calculated that a sample of acetarsol containing 0·13 per cent. of 3-amino-4-hydroxyphenylarsonic acid would give, by the proposed test, a colour equal to that produced by 0·21 mg. of 3-amino-4-hydroxyphenylarsonic acid and hence a simple revised limit test with the same effective limit may be applied as follows. To 5 ml. of water at about 5°C. add 0·1 g. of sodium bicarbonate and 0·2 g. of acetarsol. Shake to dissolve and add 5 ml. of N/1 hydrochloric acid previously cooled to about 5°C. Add 2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrite and mix. After 3 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath 5 minutes. Add 10 ml. of previously cooled β-naphthol solution and mix. Leave in the ice-bath for 10 minutes and then place in water at 20°C. for 5 minutes. The red colour developed is not greater than that produced in the following way. To 5 ml. of a solution containing 0·21 mg. of 3-amino-4-hydroxyphenylarsonic acid add 5 ml. of N/1 hydrochloric acid and cool to about 5°C. Add 2 ml. of freshly prepared 0·1 per cent. sodium nitrite solution and complete the test as described above.

A solution of 3-amino-4-hydroxyphenylarsonic acid in N/2 hydrochloric acid containing 0·21 mg. in 10 ml. may be prepared by dissolving 0·0124 g. of acetarsol in a mixture of 21 ml. of hydrochloric acid and 21 ml. of water and boiling under a reflux condenser for 5 minutes followed by cooling and dilution with water to 500 ml.

SUMMARY

1. A method has been evolved for the determination of small amounts of 3-amino-4-hydroxyphenylarsonic acid in acetarsol.

2. The official limit test has been examined by photoelectric methods and the actual limit determined. A more satisfactory test is proposed.

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