THE DETECTION OF APOATROPINE AND BELLADONNINE IN ATROPINE

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The test for apoatropine in both the 1953 B.P. and the U.S.P. XIV depends on the formation of an immediate turbidity on adding ammonium hydroxide solution to an alkaloid solution of specified concentration. In the U.S.P. XIV, this is given as a test for "other alkaloids," and is supplemented by one with platinic chloride. Maeda[1] has criticised the ammonia test, claiming that no turbidity was produced in a sample of atropine sulphate which contained 3·9 per cent. of apoatropine, as measured by catalytic absorption of hydrogen. He recommended the adoption by the Japanese Pharmacopœia of a test with potassium permanganate solution, such as is given in both the 1953 B.P. and the U.S.P. XIV for detecting apohyoscine in hyoscine hydrobromide. It appeared desirable to compare the sensitivity of these tests for apoatropine, to investigate the possibility of using a bromine absorption method, and to extend the study to belladonnine, which is readily formed from apoatropine by dimerisation.

Apoatropine and Belladonnine

Apoatropine (under the name of atropamine) was isolated by Hesse[2] from a crude extract of *Atropa belladonna*, but was later considered by him to have been an artefact[3]. Belladonnine was reported by Hübschmann[4] as occurring in henbane berries. It is doubtful whether these compounds ever occur naturally in the plant, and they have not been found during the examination of about two hundred *Duboisia* extracts submitted to partition chromatography by the Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organisation at Canberra[5]. Apoatropine is formed by the action of heat whilst concentrating the alcoholic percolate of the leaf. It is removed with the hyoscine fraction during the *p*H separation of the bases, but is produced in greater proportion by the action of alkali during the racemisation of (−) hyoscyamine to atropine.

Maeda[1] found that dehydration to apoatropine proceeds at about one-seventh of the rate of alkali racemisation, resulting in from 5 to 7 per cent. of apoatropine in the crude atropine, and his figures have been confirmed by the extraction of apoatropine as a *p*H 7 fraction from many samples of racemised solutions. Atropine itself, in alcoholic solution, produces apoatropine when treated with alkali hydroxide or ethoxide.

Pure apoatropine was prepared by the method of Willstätter and Hug[6], as modified by Maeda[1]. The purified product melted at 62–3° C., and its hydrobromide at 234° C. It was converted to belladonnine by the method described by Küssner[7], the base becoming partly crystalline on standing, and being unaffected by potassium permanganate solution,

since the dimer, belladonnine, lacks the unsaturated linkage present in the side chain of apoatropine.

The solubility of the two bases in a mixture of 10 ml. of water and 4 ml. of solution of ammonia, corresponding to the B.P. test solution was, apoatropine 0·05 per cent. w/v, and belladonnine 0·002 per cent. w/v. The conversion of apoatropine to belladonnine in alkaline solution was followed by titrating with decinormal potassium permanganate solution a series of aliquots withdrawn from a solution of 1 per cent. w/v apoatropine and 0·2 per cent. potassium hydroxide in 50 per cent. ethanol, each ml. containing 10 mg. of base.

TABLE I

DECREASE IN $KMnO_4$ CONSUMED WITH TIME. APOATROPINE IN ALKALINE SOLUTION

Time, hours	0·1 N $KMnO_4$ per mg. bases	Per cent. dimerisation
0	0·55	0
2	0·36	34
4	0·23	58
24	0·14	75

The endpoint was taken to a pink colour permanent for 5 minutes, as in the B.P. test for apohyoscine.

Hydrolysis over the 24-hour period was measured by extracting tropic acid from the acidified solution with ether, and titrating after removing the solvent. It amounted to 3 per cent. of the original base. In acid solution, at *p*H 3, the permanganate titre fell much more slowly, showing 33 per cent. conversion to belladonnine after 7 days, whilst in an aqueous solution of the hydrobromide, at *p*H 5, it remained unchanged over the same period.

0·5 ml. of the original 1 per cent. solution, diluted to 10 ml. and treated with ammonia solution as in the B.P. test, gave no perceptible cloudiness, but, as the less soluble belladonnine was produced, a slight turbidity appeared in the 2-hour sample, and became marked after 4 hours.

Sensitivity of the B.P. Ammonia Test

A 1·5 per cent. w/v aqueous solution of pure atropine sulphate of m.pt. 195° C. gave no turbidity in the B.P. test, and no reaction with decinormal permanganate solution. To 10-ml. portions were added increasing amounts of apoatropine and belladonnine sulphates, and each was tested by adding 4 ml. of solution of ammonia. With apoatropine, there was no turbidity until from 3 to 4 per cent. of the amount of atropine sulphate was present, but this became marked when the apoatropine content reached 5 per cent., these figures being in accordance with the observed solubility of apoatropine (0·05 per cent. w/v), which is reached when this impurity in the sample amounts to 3·3 per cent. When the impurity was belladonnine, turbidity appeared when it reached 0·4 per cent. of the atropine sulphate, and became marked at 0·5 per cent. These figures are virtually the same in the U.S.P. test, which uses a 1·67 per cent. atropine sulphate solution, a slight opalescence appearing at 3·5 per cent., and a marked turbidity at 5 per cent., of apoatropine in the sample.

The Platinic Chloride Test

This U.S.P. XIV test was applied to similar solutions, prepared according to the official instructions, and proved somewhat more sensitive than the

ammonia test. Two per cent. of apoatropine in atropine sulphate was just detectable, whilst 3 per cent. gave a marked cloudiness. With belladonnine, 0·1 per cent. was not detectable, but the reaction with 0·2 per cent. was definite.

Sensitivity of the Permanganate Test

The B.P. test for readily oxidisable substances in hyoscine hydrobromide requires that when 1 drop of 0·1 N potassium permanganate solution is added to 5 ml. of a 1 per cent. w/v solution of hyoscine hydrobromide in water, the solution is not completely decolorised in 5 minutes. This test was applied to 5-ml. quantities of a 1 per cent. solution of atropine sulphate, containing added apoatropine. The observations are given in Table II.

TABLE II

PERMANGANATE TEST FOR APOATROPINE IN ATROPINE SULPHATE

Per cent. apoatropine in atropine sulphate	0·1 N $KMnO_4$, drops	Stability of colour
0	1	Permanent over 15 minutes
0·05	1	Almost fades
0·10	1 2	Fades Stable 5 minutes
0·50	5	Fades in 3 minutes

Titration with 0·1 N permanganate solution may be used as a means of estimating apoatropine in presence of atropine sulphate in a solution slightly acidified with sulphuric acid, although when much apoatropine is present, the solution develops a brownish colour which takes some time to disappear, and it is best to use another solution which has slightly less than the full amount of permanganate for comparison of the colour at the end-point. 20 mg. of apoatropine alone, dissolved in 1 ml. of dilute sulphuric acid, and diluted to 150 ml. required 10·9 ml. of 0·1 N permanganate to give a pink colour which did not fade for 5 minutes, whilst 4 mg. of apoatropine with 5 ml. of 1 per cent. w/v atropine sulphate and 0·5 ml. of dilute sulphuric acid in 150 ml. required 2·2 ml. of 0·1 N permanganate solution.

Bromine Absorption Test

When an aqueous solution of bromine is added to a solution of atropine sulphate an additive compound is precipitated, which is converted to a black iodine addition compound by potassium iodide. This reacts slowly with sodium thiosulphate, dissolving gradually on shaking, and the ultimate blank titre is the same whether there is atropine present or not. Apoatropine absorbs two equivalents of bromine to saturate the double bond, but each ml. of decinormal bromate-bromide solution is equivalent to only 1·35 mg. If a 0·1 g. sample of atropine sulphate containing 5 per cent. of apoatropine is brominated with acidified 0·1 N bromate-bromide solution, treated with potassium iodide, and titrated with decinormal sodium thiosulphate, 10 ml. of the bromate-bromide are necessary to give an excess of bromine in solution, and the difference between the blank and the actual titre is only 0·36 ml. The method is obviously impracticable.

Relative Toxicities

Maeda[1] gave the toxicity of apoatropine to mice as twenty times that of atropine, and regarded it as a dangerous impurity. Pure samples of the sulphates of atropine, apoatropine and belladonnine were administered to mice by intramuscular injection at the Department of Pharmacology of the University of Melbourne. The LD 50, in mg. per kilo, calculated as the bases, was found to be, atropine 230 mg.; apoatropine 35 mg.; belladonnine 50 mg.

Although these figures do not suggest that a small proportion of apoatropine or belladonnine in atropine would have a very injurious effect, it is apparent that, whilst the existing tests in the B.P. and U.S.P. are adequate for controlling the belladonnine content, they will allow considerable proportions of apoatropine to be present. It is quite possible to prepare commercial atropine and atropine sulphate which comply with the permanganate test for oxidisable impurities, and the introduction of this test, or a modification of it, might be considered.

Injection of Atropine Sulphate

One per cent. w/v solutions of atropine sulphate, with and without added apoatropine and belladonnine, were heated to 98–100° C. for 30 minutes. They were tested before and after heating with ammonia, platinic chloride, and 0·1 N permanganate.

Atropine sulphate solution alone gave a stable colour with 1 drop of permanganate before heating, and 3 drops afterwards. A sample containing 0·5 per cent. apoatropine required 4 drops, increased to 6 drops after heating, and one with 0·5 per cent. belladonnine, 1 drop before, and 3 drops after heating. A sample containing 4 per cent. of apoatropine showed no visible difference in its response to the ammonia and platinic chloride tests after sterilisation, and its titre of 1·1 ml. of 0·1 N permanganate solution was not appreciably altered. It is concluded that there is a very slight production of apoatropine from atropine during sterilisation, but no conversion to belladonnine.

This investigation has been confined to the product from natural (−) hyoscyamine. It would be of interest to compare the extent to which apoatropine and belladonnine are formed during the manufacture of the synthetic alkaloid.

SUMMARY

1. It is improbable that apoatropine and belladonnine occur naturally in the plant, but both are formed during the manufacture of atropine from natural sources.

2. The B.P. and U.S.P. ammonia tests, and the U.S.P. platinic chloride test are sensitive tests for belladonnine, but allow the presence of appreciable amounts of apoatropine.

3. Belladonnine does not react with potassium permanganate, but this reagent is a sensitive one for apoatropine, and is capable of quantitative application.

4. Apoatropine and belladonnine are both more toxic than atropine.

5. If apoatropine in amounts up to 3 or 4 per cent. is regarded as an undesirable impurity, a test with permanganate should be added to the B.P. monographs on Atropine and Atropine Sulphate.

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