A Review of Tests for Ephedrine*

By Margaret Airston and Edward S. Brady, II

EPHEDRINE

The medicinal properties of the Chinese plant Ma Huang were discovered nearly five centuries ago, but it was not until the latter part of the nineteenth century that its active principle, ephedrine, was isolated by Nagai (1887) and Merck (1888). It was re-isolated in 1923 by Chen and Schmidt (1) and, after extensive research, was recognized and admitted to the British Pharmacopœia, the Chinese Pharmacopœia and the United States Pharmacopœia. Ephedrine was synthesized by Nagai and Kano in 1929; previously (1921) Spath had investigated a synthetic product and had shown it to be identical with the natural alkaloid (2). Other syntheses have been more recently studied and developed.

Structure.—Chemically the ephedrine base is $C_{10}H_{15}ON$, β-phenyl-β-hydroxy-α-methyl-ethyl-methylamine or α-hydroxy-β-methyl-aminopropyl-benzene. There are 2 *a*-symmetric carbon atoms which give rise to six stereoisomers, *d*-, *l*- and *dl*-ephedrine and *d*-, *l*- and *dl*-pseudo-ephedrine (3). The laevo form is the one official in the United States Pharmacopœia as the free alkaloid, the hydrochloride and the sulfate. The commercial title "Ephetonin" is applied to the racemic form, as the hydrochloride. Synthetic products which have a structure similar to that of ephedrine include Synephrine, Neo-synephrin, Benzedrine, Propadrine, Veritol, Suprifen, Cobefrin and Isalon (3).

Sources.—The varied sources of the first ephedrine samples and the introduction of the various synthetics under similar names have caused some confusion to the analyst. The present chief natural source, *Ephedra Sinica*, contains some seven closely related bases, in addition to ephedrine base, which may exist as *d*, *l* and racemic forms (3, 4, 11). The synthetic ephedrines first introduced were often mixtures of various proportions of *d*- and *l*- ephedrine. Thus the task of establishing identity tests for ephedrine, already difficult because of the chemical nature of the drug, became still more involved due to the lack of uniformity of samples to be tested. Even in recent literature there seems to be some confusion in nomenclature and tests, and it is the purpose of this paper to review and to attempt to clarify the tests for the identification of *l*-ephedrine and its salts.

Physical Tests.—Ephedrine (base) United States Pharmacopœia, Eleventh Revision, is described as an unctuous, almost colorless solid, or white to colorless crystals or granules. It is soluble in water, alcohol, ether, chloroform and liquid petrolatum, insoluble in petroleum ether. It is alkaline to litmus. Ephedrine hydrochloride and sulfate are described as fine, white, odorless crystals or powder. The rather wide range given for the melting points, particularly of the base, is due to the variation in moisture content. The sulfate is soluble in water and hot alcohol, more difficulty soluble in cold alcohol, while the hydrochloride is soluble in water and alcohol and insoluble in ether. Physical constants of ephedrine and its salts are tabulated in the chart below.

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Table I.—Physical Constants of Ephedrine and Its Salts

Form	Melting Point, °C.	Boiling Point, °C.	Specific Rotation	Source of Data
Base	34 to 40		As HCl, 0.5 Gm./10 cc. $-33.0°$ to $-35.5°$	U. S. P. XI (7)
Base	38.1	225	$-6.3°$ (3.5% in EtOH) $+11.2°$ (in water)	Henry (5)
Base	43	225	$-5.08°$ (3% in EtOH) $+14.95°$ (2% in water)	Rosenthaler (6)
HCl	216 to 220		$-33.0°$ to $-35.5°$	U. S. P. XI (7)
SO_4	240 to 247.5[a]		$-29.5°$ to $-32°$	U. S. P. XI (7)

[a] Omitted in the Second Supplement to the U. S. P. XI.

Moore and Tabern have made a thorough investigation of the melting points and solidification temperatures of ephedrine (8). Standards of ephedrine based on total alkalinity, specific rotation and congealing point have been adopted by the American Drug Manufacturer's Association (9).

Macrochemical Tests.—Derivatives of ephedrine are formed in most of the accepted analytical organic reactions. Heated with iodine in an alkaline medium (sodium hydroxide), iodoform is obtained. This reaction is the basis for a quantitative estimation. Benzoic acid may be obtained by oxidizing ephedrine in an alkaline hydroxide solution; distillation of ephedrine with potassium permanganate yields benzaldehyde and fatty amines (10). The odor of benzaldehyde was observed upon the mixing of a drop of 0.1% ephedrine solution with a drop of 10% potassium permanganate solution on a microscope slide, even without heating. Nitrosoephedrine is formed when ephedrine is allowed to react with sodium nitrite and hydrochloric acid. A diazo compound is formed upon nitration and subsequent reaction with zinc and sodium nitrite (10). Ephedrine may be converted into its stereoisomeride pseudo-ephedrine by boiling with 25% hydrochloric acid. This reaction is reversible, an equilibrium mixture of the two bases being formed in each case. Acylation produces the same result (5). Unlike other alkaloids, ephedrine base in chloroformic solution reacts to form the hydrochloride, which is the basis for a U. S. P. XI test, as follows: "Dissolve 0.05 Gm. of Ephedrine in 10 cc. of chloroform, allow the solution to stand over night in a closely covered vessel, and then evaporate it spontaneously: white crystals of ephedrine hydrochloride appear, which, when dissolved in water, respond to the identity tests for chloride."

Color Tests.—Color tests are the most generally used tests for the identification of ephedrine.

The U. S. P. XI test is as follows: "Dissolve 0.01 Gm. Ephedrine in 1 cc. distilled water with the aid of 1 or 2 drops of dilute hydrochloric acid and add 0.1 cc. of cupric sulfate T. S. followed by 1 cc. of sodium hydroxide solution (1 in 5): a reddish purple color develops. To the mixture add 1 cc. of ether and shake well: the ethereal layer is purple and the aqueous layer is blue." A study of this reaction with ephedrine and nineteen homologs was made by K. K. Chen (11).

In the Dènigés test, ephedrine is dissolved in 2 cc. of concentrated sulfuric acid (to a colorless solution), and three or four drops of a 40% formaldehyde solution are added. A pink color develops which darkens to red and finally to blood-red when heated on a water bath (16). Boil 0.01–2 Gm. ephedrine with 1 cc. of a mixture of equal parts of nitric and sulfuric acid until brown, add 10 cc. of water and sodium hydroxide until neutral (red color); extract with ether and evaporate this solution (cool); add 2 cc. acetone and 2 cc. sodium hydroxide (33° B.). The acetone layer will be red, the sodium hydroxide yellow (12).

The bromalid reaction yields a pink to red color in the upper layer of a mixture prepared by dissolving 0.1 Gm. ephedrine in 3 cc. of water, adding five drops of sodium hypobromite solution, then 1 cc. of sodium hydroxide (d. 1.33), 1 to 2 cc. pyridine and shaking. The red color appears in the upper layer upon heating (12).

Selenious acid in sulfuric acid with ephedrine yields an olive to red color. This reaction may be used to differentiate ephedrine from adrenaline, since adrenaline forms a red color with this reagent only upon heating. A further test for differentiation is the lack of any color change in an ephedrine solution upon the addition of sulfanilic acid and sodium nitrite, even when heated; adrenaline yields a rose color with these reagents (13).

A few drops of osmium tetraoxide reagent (3 cc. of 1% aqueous solution osmic tetraoxide and 2 drops of sodium hydroxide 36° B.) added to a crystal of *l*-ephedrine (base or hydrochloride) dissolved in 1 cc. of water forms an orange-colored precipitate instantly; if a small amount of this precipitate is heated with 5 cc. of pure hydrochloric acid a violet color is rapidly obtained. Performance of this test with the pseudo-ephedrines and their salts results in the slow

formation of a yellow precipitate which dissolves without change in hydrochloric acid to form a yellow solution (14).

Microchemical Tests.—Investigation of the reactions of ephedrine with the various alkaloidal precipitants began with widespread acceptance of ephedrine by the medical profession. Results were not entirely satisfactory, since ephedrine is slow to form crystalline precipitates and long standing is necessary in most cases. Thus the concentration of the drops vary greatly from test to test because of evaporation, depending upon temperature and surface of the drop. The results of a critical laboratory survey of these microchemical tests is here presented.

EXPERIMENTAL

Materials Used: Ephedrine hydrochloride, Parke, Davis & Co., ephedrine base, solutions of reagents as stated under each test. Most of the tests were performed using 1% solutions of the alkaloidal salt. When too large a volume of precipitate was formed at that concentration, the solutions were diluted to strengths varying from 0.5% to 0.10%. In less sensitive tests, successive drops of the 1% solution were evaporated upon the microscopic slide until a concentration of the alkaloid was obtained which gave a good test.

Technique.—The simple technique consists of placing a drop of the alkaloidal solution upon one end of a clean glass plate slide by means of a glass rod or dropper and spreading it slightly. The reagent, if liquid, is placed next to the alkaloidal drop in the same manner, not quite touching it, and the two are joined by drawing a platinum or glass capillary rod across the reagent drop and allowing it to flow into the alkaloidal drop (6). This produces zones of varied relative concentrations of alkaloid and reagent throughout the drop and provides some optimum zone where crystallization will be best. Where two or more drops are added to a test drop, the ones which cause no precipitation may be first mixed directly with the test drop. Solid reagents are added to the edge of the drop by means of a capillary glass rod or platinum wire. The solid may be made to adhere to the rod by breathing upon the rod or by dipping it in distilled water and shaking off the excess. No cover slip is used and the drop is examined through the microscope under low power magnification. 100×.

Gold Chloride.—Yellow oily droplets are at first observed upon the addition of gold chloride (1% aqueous solution) to a test drop containing ephedrine. Needles and blades are formed slowly upon standing, but in dilute drops these may be confused with reagent deposited through evaporation of the test drop. Though reported by many observers (15, 16), this test did not appear to be very reliable.

Platinic Chloride.—Pale yellow silky needles slowly appear at the periphery of the drop containing ephedrine upon standing after the addition of 1% platinic chloride solution. This is quite sensitive (1:10,000) but forms slowly (15) (Fig. 1).

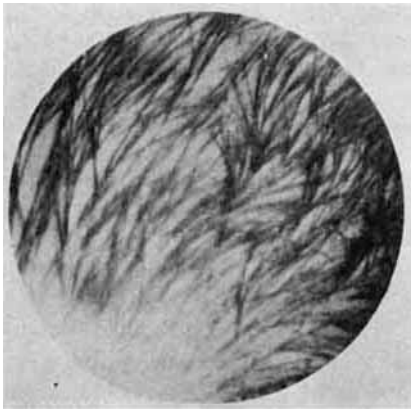

Fig. 1.—Ephedrine with Platinic Chloride T.S.

Krauts or Dragendorff's Reagent (Potassium Bismuth Iodide).—This reagent seems to present the best means for the micro-identification of ephedrine. Groups of red-brown branching needles and blades form rather rapidly upon the addition of this reagent (Fig. 2). This test was adopted by the Association of Official Agricultural Chemists (17). Ephetonin forms similar crystals (6); amorphous precipitates only were obtained by other observers (16).

Fig. 2.—Ephedrine with Dragendorff's Reagent.

Potassium Iodide repeatedly failed to yield any but reagent crystals in test drop to which it was added. Rhombs and prisms previously reported

(16) seemed to form with equal rapidity in all test drops, whether or not they contained ephedrine, due to crystallization of the reagent.

Picric Acid.—Yellow oily droplets formed upon the addition of a 1% aqueous solution of picric acid to a test drop containing ephedrine remain unchanged for so long a period (often 15 minutes) that a negative test has been reported in some instances (12). However, brown mossy growths which formed rosettes and burrs of light brown needles were observed in many concentrated test drops upon long standing. This crystalline growth is unlike any crystal formed by picric acid alone upon partial spontaneous evaporation of a test drop, and was successfully used to aid the more rapid formation of crystals in other ephedrine drops through seeding (Fig. 3). Ephetonin forms branching rods with this reagent (6).

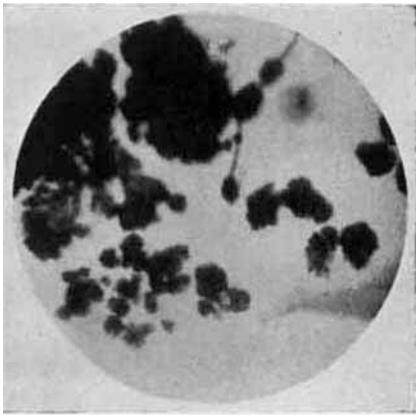

Fig. 3.—Ephedrine with Picric Acid T.S.

Potassium Thiocyanate.—Large irregular plates and prisms were formed upon the addition of potassium thiocyanate to an ephedrine test drop (Fig. 4). This has been reported by other observers (16), and may be used to differentiate ephedrine from ephetonin, which forms rectangular plates (6).

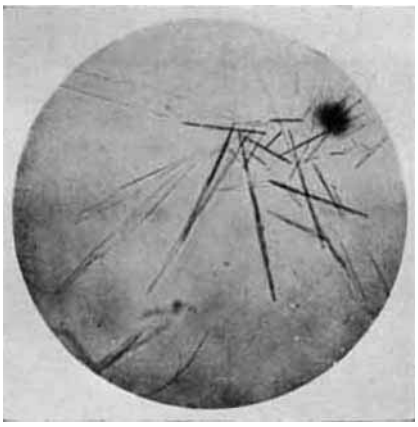

Fig. 4.—Ephedrine with Potassium Thiocyanate T.S.

Potassium Oxalate.—A small crystal of potassium oxalate placed at the edge of a test drop containing ephedrine hydrochloride forms straight light needles or prisms, singly and in groups (Fig. 5). Ephetonin forms thin rhombic coalescent crystals (18).

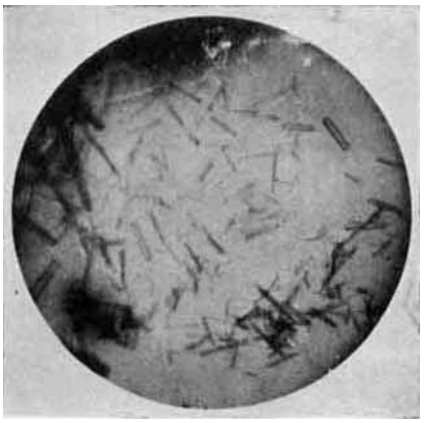

Fig. 5.—Ephedrine with Potassium Oxalate T.S.

Sodium Perchlorate.—Rectangular plates were formed when a fragment of this salt was added to an ephedrine test drop (Fig. 6) (6).

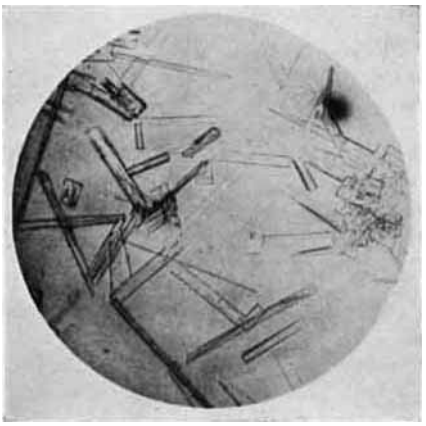

Fig. 6.—Ephedrine with Sodium Perchlorate T.S.

Sodium Vanadate produced with ephedrine a white granular precipitate only; ephetonin is reported to form spindle-shaped crystals and conglomerates with this reagent (18).

Salting Out.—Crystal formation due to salting out was suspected at the high concentrations required by some tests, but the addition of excess calcium chloride to a test drop of ephedrine hydrochloride formed irregular plates and blades, somewhat like those formed with potassium thiocyanate, but unlike others (Fig. 7). Amorphous precipitates are formed with Mayer's Reagent, Wagner's Reagent and phosphomolybdic acid; no tests were obtained with mercuric chloride, sodium nitroprusside, bismuth nitrate, ammonium molybdate, phospho-

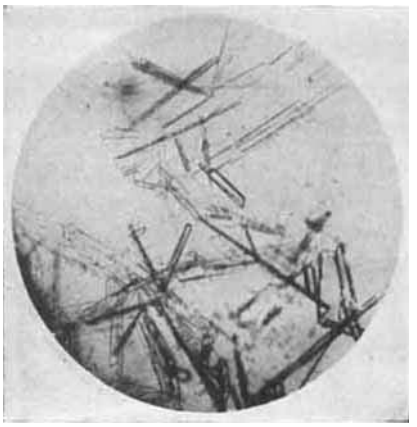

Fig. 7.—Ephedrine Salted out with Sodium Chloride.

tungstic acid and zinc-chlor-iodide. A nitrous acid tests was not successful, probably due to rapid dissipation of the acid at room temperature. Million's reagent, also reported as a precipitating reagent (15), formed clear solutions with samples tested, no crystalline precipitate being obtained even on long standing. The orange precipitate of the osmic acid color test (14) was examined microscopically but appeared to consist entirely of amorphous masses.

SUMMARY AND CONCLUSION

The authors have presented a review and discussion of tests for *l*-ephedrine and its salts and a laboratory survey of microchemical tests for this alkaloid, from which the following conclusions may be drawn:

1. Numerous acceptable tests for ephedrine have already been devised. Of these, the color and microscopic methods seem to offer the best means of identification.

2. The U. S. P. XI biuret reaction and the osmic tetraoxide reagent seem to give the best color tests.

3. Microcrystalline precipitates form slowly in ephedrine test drops, but identification may be made through the micro tests with Dragendorff's Reagent, platinic chloride reagent and potassium oxalate.

4. A combination of these tests may be used to distinguish *l*-ephedrine from ephetonin, the pseudo-ephedrine and adrenaline.

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A Study of the Composition of Precipitated Calcium Phosphate National Formulary VI*

By J. W. Millar

The formula given in National Formulary VI for Precipitated Calcium Phosphate is $Ca_3(PO_4)_2$ and the same formula is given in the French Codex for "Neutral Calcium Phosphate." The British Pharmacopœia gives no formula, but Bennett and Cocking (1) attribute the following formula to the salt of the British Pharmacopœia:

$$CaH_4(PO_4)_2 + 2[3Ca_3(PO_4)_2.Ca(OH)_2]$$

* Contribution from the Laboratories of the College of Pharmacy, University of California, Medical Center, San Francisco, California.