THE EPHEDRINE ASSAY OF CHINESE EPHEDRA.*

BY C. T. FENG AND B. E. READ.

As set forth in a recent note we have shown that Chinese Ma Huang is obtained from various species of Ephedra, *E. equisetina*, Bunge; *E. sinica*, Stapf; and *E. mahuang*, Liu. So far there has been no differentiation made between the various samples of this drug which occur upon the Chinese market. However, irrespective of the possible variation in the drug samples we consider it is essential that there be made a critical statement of the methods of assay and the results as found and studied by us over a long period of time. It has been pointed out by Chou that ephedrine is a stronger base than ammonia, turning it out from its salts; hence it is a matter for critical study to show how suitable the U. S. P. belladonna assay method may be as used by various workers. The chemical laboratory of the American Medical Association has stated that when ephedrine is shaken out with ammonia and chloroform there is a reaction so that the hydrochloride is obtained instead of the free alkaloid.

In examining methods for separating ephedrine and pseudo-ephedrine hydrochloride we showed clearly that the use of ammonia to liberate the alkaloidal bases was open to question. Ammonia, when added to the extraction in limited amount sufficient only to make the fluids quite alkaline to litmus, did not produce the pure alkaloid. We have now studied the effects of the addition of molecular equivalents of strong ammonia and find that when the ammonia is present in very large excess the alkaloids are set free.

Hot and cold methods of extraction were made, also direct alkalinization of the powdered drug with potassium carbonate or soda ash, and adsorption by fuller's earth, which confirmed in a general way the high figures obtainable by these various methods.

ASSAY METHODS USED.

Method I. Hot Extraction with Acetic Acid.—One hundred Gm. of Chinese Ma Huang were prepared in No. 15 powder and macerated with 150 cc. boiling 3 *N* acetic acid, and stood one whole night. It was then successively treated for four nights with 200-cc. portions of alcohol 95%, containing 0, 12.5, 5 and 2.5 cc. of glacial acetic acid, respectively. The marc was evaporated to a thick syrup. The syrup was extracted with 200-cc. water and filtered. The clear water extract was made alkaline with ammonia and then treated with 20 Gm. of soda ash and shaken out with four portions of chloroform, 75, 50, 25 and 25 cc. The chloroformic extracts were evaporated to dryness and titrated against 0.1 *N* hydrochloric acid. There were used 58 cc. of 0.1 *N* HCl, which are equivalent to 0.959% of pure ephedrine alkaloid.

The titrated solution was evaporated to dryness, redissolved in water, made up to a standard volume and quantitatively tested by, (*a*) the Biuret color method, (*b*) Physiological test, (see later paragraph). The Biuret assay yielded a color the equivalent of 0.563% pure ephedrine, the remainder of the alkaloid was assumed to be pseudo-ephedrine. When this work was first done the refined

* From the Department of Pharmacology, Peking Union Medical College, Peking, China.
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methods for assaying mixtures of the two alkaloids had not been developed. Reference to these methods will show that so long as ephedrine is present to 66% or more of the mixture there is no change in the results deduced.

This same method repeated with more finely powdered samples of Ma Huang yielded slightly greater amounts, and the ephedrine was proportionately greater in the final extract of the mixed bases. The dried chloroform residues required 60.1 cc. of 0.1 *N* hydrochloric acid, which is equivalent to 0.993% of bases estimated as pure ephedrine. The Biuret assay showed 0.614% of ephedrine.

In order to check these figures repeated observations have been made of the actual yields of pure ephedrine hydrochloride from the end-titration mixtures. Ten average samples yielded 0.135 Gm. pure crystalline ephedrine hydrochloride for every ten cc. of the 0.1 *N* hydrochloric acid used. The theoretical equivalent is 0.2016. Hence we are able to obtain about 66% of the total alkaloidal residues as pure ephedrine hydrochloride. Without further treatment with strong acids, which cause the formation of isomers we are able to obtain by chloroform solution and recrystallization from the mother liquors about 11% pure pseudo-ephedrine hydrochloride.

Method II. Hot Extraction with Hydrochloric Acid.—One hundred Gm. of dried Ma Huang powder were mixed with 100 cc. of water and 50 cc. 8 *N* hydrochloric acid, thoroughly mixed and macerated 5 hours.

There were added 350 cc. of alcohol 95%, the whole was mixed, raised to boiling temperature, and macerated over night on a warm radiator. It was filtered hot the following day. There were, further, two like additions of alcohol, the whole thoroughly mixed, boiled, macerated 2 to 6 hours, and filtered hot. The marc was finally rinsed with 5 successive portions of 100 cc. alcohol, and the whole well pressed each time. The combined extracts were evaporated, *in vacuo*, to dryness.

The dry residue was extracted with boiling water, 75-, 50- and 50-cc. portions, respectively, and then rinsed and pressed five times with 10 cc. of water.

To the watery extract was added 75 Gm. potassium carbonate and chloroform extraction was made with 100-, 75-, 50- and 25-cc. portions. The chloroformic extract was filtered and heated to dryness at low temperature. The residue was titrated with 0.1 *N* hydrochloric acid. There were used 82.98 cc., which is the equivalent of 1.37% of alkaloid in terms of pure ephedrine base.

The titrated solution was reëxtracted with chloroform and retitrated, requiring 80.03 cc. of 0.1 *N* hydrochloric acid, which is equivalent to 1.32%.

Method III. Direct Alkalinization.—Seeing that such an unusually large yield was obtained by this last method, we took the same sample of the crude drug and applied the direct alkalinization method which has been found to give good yields.

One hundred Gm. of the powdered crude drug were thoroughly mixed with 100 cc. of water and 50 Gm. of potassium carbonate, and stood for 5 hours. It was then shaken out with four successive portions of chloroform of 500 cc. each to which was added 20, 10, 5 and 0 parts of concentrated ammonia. The chloroform extracts were evaporated down to 20 cc. and 4 cc. of ether were added to form a solution lighter than water. Acid extraction was now made with successive portions of 0.5 *N* hydrochloric acid and water making a total volume of the acid

extracts and rinsings of 150 cc. which were washed three times with 5 cc. of a mixture of 1 part ether and 2 parts chloroform.

To the acid extract was added 50 Gm. potassium carbonate. It was shaken out with 75-, 50-, 50- and 25-cc. portions of chloroform. The chloroform solution was filtered and air dried. The residue was softened with 2 cc. of neutral absolute alcohol, and titrated with 0.1 *N* hydrochloric acid, and required 67.8 cc., which is equivalent to 1.12% of alkaloids calculated as pure ephedrine.

When further extracted with potash and chloroform and retitrated with acid the percentage of alkaloid present was 1.00%. These results were well checked by further estimations using the same and slightly varied procedures.

Method IV. Extraction with Cold Acids.—It was found that decoctions of Ma Huang made by boiling different samples of the drug in distilled water, were always faintly acid to litmus. This fact and the success obtained with cold alkaline extraction made us carefully examine our original acid extractions with large bulks of material, and it is quite plain that simple extraction with cold acids do not get out all the alkaloid, as shown in the following experiment. Portions of four Kg. were macerated over night with 4 liters of water and 2 liters of 8 *N* acetic or sulphuric acid. To the former was then added 16 liters of alcohol 95%, and to the latter 16 liters of water. They were macerated 1 night, and then drained. The marc was again treated with 16 liters of solvent and 1 liter of their respective acids, macerated 5 hours, drained and well pressed. The acetic acid extracts were treated with sodium carbonate to reduce the acidity almost to neutrality point and then evaporated to 2 liters each and after adding 100 cc. 8 *N* acetic acid they were filtered. To the filtrates were added 350 Gm. of sodium carbonate so that they were strongly alkaline. The precipitated alkaloid was filtered off and was taken up in chloroform, three lots of 300 cc., carefully filtered and evaporated, and added to the chloroform extractions of the mother liquors. In all 2100 cc. of chloroform were used. The dried chloroform residue was titrated with 73.1 cc. of 1 *N* hydrochloric acid, which is equivalent to *0.302%* of alkaloid.

The sulphuric acid extract was not evaporated down like the acetic acid one, on account of its tendency to change the ephedrine. The final bulk of 32 liters made alkaline with 2 kilos of sodium carbonate were shaken out in a similar way to the acetic acid method, and yielded a basic residue equivalent to 0.283% as calculated from the acid titration.

Method V.—Repeating Method I, we prepared from the acid alcohol extracts a water extract, and then used fuller's earth as an adsorption agent. Twenty Gm. of fuller's earth (Lloyd's reagent) were added and after shaking it well was filtered and washed with distilled water. The fuller's earth was then treated with 20 cc. saturated potassium carbonate solution and the whole shaken out with four successive portions of 40 cc. of chloroform. The chloroform was evaporated to dryness and the residue titrated with 0.1 *N* hydrochloric acid.

The titrated solution was further tested as in Method I.

It yielded 0.941% and 0.961% of total alkaloid. Tested by the Biuret method there was present in both samples 0.512% pure ephedrine base.

Method VI. Physiological Assay.—While it has been repeatedly shown in these laboratories that large repeated doses of ephedrine do not show like effects upon the blood pressure, little has been done to establish a method for biological assay.

It should also be emphasized that therapeutic doses repeated at intervals of a few minutes are likely to give negative results and embarrass the heart. Contraction of the turbinates, and relief of bronchial spasm in asthma, show negative results from quickly repeated doses of the drug. This prolonged action of the drug has made it so superior to adrenalin for certain conditions, but in considering the biological assay of ephedrine by its effect on the blood pressure, it is this very difference which has to be carefully allowed for. In the earlier publications upon ephedrine relatively large doses were used to demonstrate its blood pressor effect. We have found after a very large number of trials that a much smaller dosage can be used to show an appreciable rise in blood pressure. Small doses if repeated at sufficiently long intervals show a like effect accurate enough for assay purposes, as demonstrated in the following experiment conducted on a dog weighing 6.2 kilos. This experiment was carried out to demonstrate the possibilities of physiological assay and to confirm the results obtained by chemical assay. The animal was luminalized and arranged for an ordinary blood pressure tracing with a canula and burette attached to the femoral vein so that there was no reflex stimulation of the blood pressure from subsequent manipulation of the vessels. A standard solution of pure ephedrine hydrochloride was made, 1 cc. of which represented 1 mg. of the drug. The solutions obtained from the chemical assay of Ma Huang were similarly diluted so that 1 cc. contained 1 mg. of the theoretical alkaloids present.

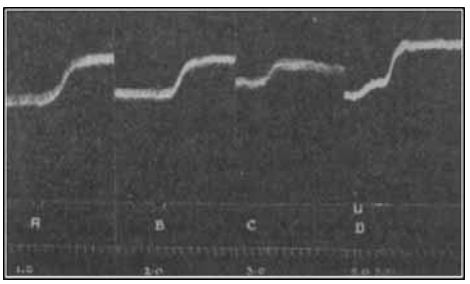

Fig. 1.—Blood pressure raising effects of ephedrine and Ma Huang extracts.

A. Physiological assay of theoretical content of one mg. alkaloid.

B. Effect of one mg. pure ephedrine hydrochloride.

C. Effect of half the amount of unknown.

D. Double the amount of unknown.

The small dose of 1 mg. was introduced into the femoral vein, it being flushed in with 1.5 cc. of saline. The figure (Fig. 1) shows the marked effect of the unknown A, and the like effect one hour later of an equal amount of the pure standard B. These injections were repeated at hourly intervals with an exactly similar result, after which as shown at C and D of the effect of half and double the amount of the unknown, respectively, was produced, also at hourly intervals.

Method VII. Ammonia Chloroform Assay.—The very poor assay results and the erroneous melting points reported by some workers led us to make a special study of this method.

A strong aqueous solution of ephedrine hydrochloride was taken and shaken out with two portions of 100-cc. chloroform after the whole had been treated with strong ammonia in sufficient quantity to make the solutions strongly alkaline to litmus. Separation was made in exactly the same way as usual except there was no filtration of the chloroform and separation was made one minute after shaking. Starting with about 15 Gm. of the salt, the two chloroform shakings when evaporated yielded 2.2 and 0.5 Gm. These residues were washed with ether and dried. They gave melting points of 210–212° and 212–214° C. (uncorrected). The remain-

der of the salt left in the aqueous portion, over 12 Gm., was dried, and melted at 210–212°.

This was repeated with a 20-Gm. mixture of 16 Gm. of ephedrine hydrochloride and 4 Gm. of pseudo-ephedrine hydrochloride, dissolved in 100 cc. of water. But there was used an excess of strong ammonia 100 cc. NH_4OH (15 *N*) more than equal to its equivalent weight for replacement of the total amount of the alkaloid. The whole was shaken out with two portions of 100 cc. of chloroform.

Weight of alkaloid obtained from first batch of $CHCl_3$	15.1 Gm.
Weight of alkaloid obtained from second batch of $CHCl_3$	1.3 Gm.
Total	16.4 Gm.

16.4 Gm. of the alkaloids is equivalent to 20.02 Gm. of the hydrochloride salts.

The combined residues were titrated with 1 *N* hydrochloric acid, and 99.0 cc. were used. This is equivalent to 16.34 Gm. alkaloids, or 19.95 Gm. of the salts originally used. The titrated fluid was evaporated to dryness, washed with ether, dried and found to weigh just under 20 Gm.

The assumption that the two isomers undergo no change throughout these processes was checked by taking the final weighed residues and washing thoroughly with 300-cc. chloroform. Pseudo-ephedrine hydrochloride is known to be soluble in chloroform while the ephedrine salt is only very slightly so, 0.0253%. There were dissolved by the chloroform 4.1 Gm. of the salts, presumably the pseudo-ephedrine portion, 15.8 Gm. were left undissolved.

DISCUSSION.

These results confirm the high results obtained by Schoetzow and Needham and others and emphasize the need for hot extraction, and the use of strong bases like soda and potash for liberating the alkaloids.

The results with ammonia explain much that has been obscure about the small yields of some workers and the melting points of the alkaloids so obtained. Table I shows the results of many assays.

There is room for considerable work upon methods of extraction of the alkaloids. Hot acid extraction we have known to produce a change in the proportions of the isomers, though not of the total alkaloidal content.

TABLE OF RESULTS OF VARIOUS EPHEDRA ASSAYS.

Chen.	Chow.	Neilsen, *et al.*	Schoetzow and Needham.	Masucci and Suto.	Read and Feng.	
0.018	0.26	0.2	0.403	0.305	I	0.976
0.091		0.9	0.632	0.515	II	1.32
			0.863		III	1.12
					IV	0.292
					V	0.95

SUMMARY.

1. Various methods of assay for ephedra have been tried out, showing that when fully extracted the total yield of alkaloids may exceed 1 per cent.

2. The ammonia chloroform method has been critically studied and it is shown that a large excess of ammonia is necessary for complete liberation of the alkaloids.

3. A demonstration of physiological assay suggests another method for confirming the chemical methods used.

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THE QUANTITY OF ALKALOID IN HYOSCYAMUS AND A NEW METHOD FOR ITS EVALUATION.

BY H. R. WATKINS AND S. PALKIN.

In a recent paper on automatic devices for extracting powdered materials, striking results are shown with hyoscyamus.[1] Further work has shown that in some cases the new method gives yields of alkaloid in this drug more than three times as great as those obtained by Pharmacopœial (IX and X) procedures. The data on samples of hyoscyamus in Table I illustrate this point.

TABLE I.

TOTAL ALKALOID IN HYOSCYAMUS BY U. S. P. AND AUTOMATIC EXTRACTOR METHODS.

Sample.	Total alkaloid. U. S. P. method. Per cent.	Total alkaloid. Automatic extractor method. Per cent.	Sample.	Total alkaloid. U. S. P. method. Per cent.	Total alkaloid. Automatic extractor method. Per cent.
H-N	0.026	0.118	4A	0.104	0.200
	0.023	0.123		0.102	0.202
		0.121	5A	0.059	0.230
		0.124		0.056	0.234
C-9031	0.042[a]	0.172	6A	0.068	0.198
	0.038	0.177		0.084[a]	
P-11216	0.099[a]	0.164	7A	0.061	0.191
	0.103	0.164		0.052	0.191
1A	0.056	0.177	8A	0.175	0.315
	0.064	0.181		0.142	0.321
2A	0.069	0.215	9A	0.055	0.177
		0.210		0.050	0.181
3A	0.123	0.200			
	0.122	0.200			

[a] Assays by U. S. P. X Method; all other assays in this column by U. S. P. IX Method, prior to January 1, 1926.

[1] Palkin and Watkins, *Ind. Eng. Chem.*, 19, 535 (1927).