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(8) Rosenthaler, *Chem. Investigation of Plants*, page 83.
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A CHEMICAL STUDY OF MA HUANG.*[1]

BY ALICE H. HAYDEN[2] AND C. B. JORDAN.

HISTORICAL.

Ma Huang presents one of the most interesting histories found in drug lore. Its antiquity, its varied rôles throughout the centuries and its recent spectacular rise to its present position in modern medicine weave a fascinating story for those interested in drugs and the relationship of pharmacognosy and chemistry to the development of medical science.

Early Chinese records (1) indicate that Ma Huang was known and used as early as the third century, B. C. The Chinese employed the drug to allay coughing, to promote sweating, to stimulate heart action, to relieve post-partem difficulties and to control fevers. Chen (2) reports that Ma Huang was usually prescribed with other crude drugs and made into a decoction and taken by the patient as such.

Ma Huang means astringent yellow. The taste is very astringent; this has been attributed to a high tannin content. There seems to be some doubt, however, as to just what is the exact application of the word "Huang" or "yellow." Some writers, Chen (3) and Nielsen (4), believe that the word yellow applies to the appearance of the dried stem, but Read (5) is of the opinion that the early literature refers to "Huang" as the color of the flower.

Ma Huang belongs to the seventh division of the Gymnospermous plants, the Gnetales. The exact botanical identity of this drug has been a subject of much confusion and discussion. The earlier investigators referred to it as *Ephedra vulgaris*, **var,** *helvetica*. However, recent investigations indicate that this term is now obsolete and should be dropped. The modern tendency is to consider Ma Huang as a generic term applying to various ephedrine-bearing species of Ephedra growing in China (5).

The drug is imported in large bales. There is no true grading of the product; oftentimes a single bale will contain several species of Ephedra. During the processes of collecting, drying, compressing and transporting, the plants become badly broken and most of the berries, flowers and bracts drop off so that a complete separation of the different species is practically impossible.

As far as can be ascertained, the first chemical investigation of the plant was carried out by Yamanshi, who, in 1885, isolated an alkaloid in an impure state. After the death of the discoverer, Nagai (1887) (6) with the assistance of Hori, continued the study, purified the product and named it ephedrine. It is interesting to note, however, that the term ephedrine was first used by Loew (1875) to desig-

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* Scientific Section, A. PH. A., Toronto meeting, 1932.

[1] An abstract based upon a thesis by Alice H. Hayden submitted to the Faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The original thesis is accompanied by a bibliography of over one thousand references.

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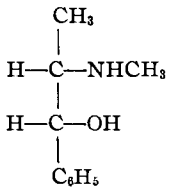
nate a red, amorphous powder obtained from the tannin of an American species of Ephedra (7). The name ephedrine has also been applied to an alkaloid obtained from *Ephedra monostachya*, but investigations show that this compound is not identical with the *l*-ephedrine obtained from Ma Huang. The word ephedrine is now used only in the sense in which Nagai employed it—to designate *l*-ephedrine:



The history of ephedrine is comparable with the histories of several other of our important drugs and chemicals. This compound, like cocaine, carbon tetrachloride and phenolphthalein, was known for a long time before its most valuable medicinal properties were realized and investigated. Miura (8) (1887) subjected Nagai's ephedrine to physiologic investigations and demonstrated its mydriatic effect. The greatest pharmacologic properties of the drug were overlooked owing to the fact that early workers failed to use anything but toxic doses. Consequently, ephedrine was little used for purposes other than ophthalmologic for many years. Some experiments demonstrating the essentially sympathomimetic effects of ephedrine were conducted as early as 1917 (9), but the real therapeutic possibilities of this compound were not recognized until 1923 when Chen re-isolated the alkaloid.

There have been many studies of Ma Huang and its alkaloids but, as far as we have been able to ascertain, no complete, systematic chemical examination of this ancient remedy has been executed. We are therefore submitting the following data regarding this drug.

GENERAL ANALYSES.

I. Determination of Volatile Constituents.

TABLE I.

Sample No.	Determination No.	Moisture in %. Sample.	Moisture in %. Average.	Other Volatile Constituents. Sample.	Other Volatile Constituents. Average.	Total Volatile Constituents. Sample.	Total Volatile Constituents. Average.
	1	3.62		1.07		4.69	
I	2	3.66	3.64	1.11	1.086	4.77	4.726
	3	3.64		1.08		4.72	
	1	3.71		1.11		4.82	
II	2	3.78	3.75	1.08	1.083	4.86	4.833
	3	3.76		1.06		4.82	
	1	4.02		1.21		5.23	
III	2	4.04	4.013	1.19	1.193	5.22	5.206
	3	3.98		1.18		5.17	
	1	4.26		1.24		5.50	
IV	2	4.30	4.293	1.18	1.21	5.48	5.503
	3	4.32		1.21		5.53	

II. Extraction of Samples with Various Solvents.

TABLE II.

Solvent.	Sample No.	Determination No.	Weight of Sample.	% Extractive. Individual.	Average.
Petroleum ether	I	1	9.78159	1.55	
		2	9.84735	1.59	1.57
Ether		1		1.36	
		2		1.34	1.35
Chloroform		1		0.588	
		2		0.572	0.58
Ethyl acetate		1		4.41	
		2		4.53	4.47
Ethyl alcohol		1		10.35	
		2		10.20	10.275
Water		1		9.46	
		2		9.25	9.355
Residue		1		72.47	
		2		72.16	72.315
					99.915
Petroleum ether	II	1	9.94740	1.64	
		2	9.64845	1.66	1.65
Ether		1		1.32	
		2		1.37	1.345
Chloroform		1		0.65	
		2		0.69	0.67
Ethyl acetate		1		4.81	
		2		4.63	4.72
Ethyl alcohol		1		11.28	
		2		11.17	11.225
Water		1		10.33	
		2		10.46	10.395
Residue		1		70.11	
		2		70.26	70.185
					100.19

Several American species were available to us, and, by way of comparison, the following extractions were made:

TABLE III.

Solvent.	Ma Huang. Individual.	Average.	*E. nevadensis.* Individual.	Average.	*E. antisyphilitica.* Individual.	Average.
Petroleum ether	1.68		1.72		0.98	
	1.64	1.66	1.74	1.73	0.96	0.97
Ether	0.93		0.82		1.01	
	0.87	0.90	0.93	0.87	1.64	1.32
Ethyl alcohol	17.04		14.09		12.61	
	16.83	16.93	.....	14.09	12.06	12.33
Water	9.70		4.07		3.52	
	9.25	9.47	.....	4.07	.....	3.52

III. Ash Determination.

TABLE IV.

Sample No.	Determination No.	Total Ash. Individual.	Average.
I	1	8.59	
	2	8.66	8.626
	3	8.63	
II	1	8.42	
	2	8.39	8.393
	3	8.37	
III	1	7.92	
	2	7.78	7.85

IV. Analysis of Ash.

TABLE V.

Analysis for	Determination No.	% of Ash. Individual.	Average.
Water-soluble ash	1	21.638	
	2	20.782	21.262
	3	21.365	
HCl-soluble ash	1	59.388	
	2	59.824	60.965
	3	63.684	
Insoluble ash	1	18.972	
	2	19.381	17.764
	3	14.940	

Liu and Read (10) have called attention to the high insoluble ash content of *E. sinica*. They have included this characteristic as one of the methods of differentiation of the several Chinese Ephedras: *E. sinica*, *E. distachya* and *E. equisetina*.

Chen (11) ran qualitative and quantitative tests on the ash. His tests showed the presence of chlorine, sulphur, phosphorus, calcium, potassium, sodium and small quantities of manganese and iron. We have found that magnesium is also present in the ash.

V. Crude Fibre Determination.[1]

TABLE VI.

Sample No.	Determination No.	Crude Fibre. Individual.	Average.
I	1	19.48	
	2	19.55	19.513
	3	19.51	
II	1	22.25	
	2	22.31	22.286
	3	22.30	
III	1	24.11	
	2	24.20	24.163
	3	24.16	

[1] U. S. P. Methods.

VI. Ether-Soluble Extractive.[1]

TABLE VII.

Sample. No.	Determination No.	Non-Volatile Ether. Individual.	Average.	Volatile Ether. Individual.	Average.
	1	7.31		0.67	
I	2	7.22	7.266	0.71	0.693
	3	7.27		0.70	
	1	6.75		0.73	
II	2	6.81	6.783	0.75	0.736
	3	6.79		0.73	
	1	7.93		0.55	
III	2	8.11	8.003	0.52	0.523
	3	7.97		0.50	

VII. Alcohol Extractives.[1]

TABLE VIII

Sample. No.	Determination No.	Alcohol. Individual.	Average.	Dilute Alcohol. Individual.	Average.
I	1	17.73		20.10	
	2	17.82	17.775	19.88	19.99
II	1	18.10		19.75	
	2	17.89	17.995	19.77	19.73
III	1	16.38		21.42	
	2	16.65	16.815	21.49	21.455

VIII. Water-Soluble Extractive.[1]

TABLE IX.

Sample. No.	Determination No.	Water Extract. Individual.	Average.
I	1	10.37	
	2	10.44	10.405
II	1	10.70	
	2	10.76	10.73
III	1	14.89	
	2	15.23	15.06

IX. Extractive Soluble in Purified Petroleum Benzin.[1]

TABLE X.

Sample. No.	Determination No.	Benzin Extract. Individual.	Average.
I	1	1.99	
	2	2.21	2.10
II	1	2.33	
	2	2.28	2.305
III	1	2.44	
	2	2.68	2.56

SPECIFIC ANALYSES.

Assays for Total Alkaloids.—Various optical, colorimetric, volumetric, gravimetric and biological assays have been devised for the determination of the total

[1] U. S. P. Methods.

alkaloids in ephedrine-bearing drugs and preparations. The yields obtained by different investigators are widely divergent. Generally, the earlier workers obtained lower results than the more recent investigators. Studies of the different assay processes have accounted for some of these variations, and from recent botanical studies, we now know that there are several species of Ephedra upon the market as Ma Huang and that some of these contain lower percentages of alkaloids than others. Some species also yield a higher percentage of *l*-ephedrine than do others. We have undertaken a special study of these methods in order to determine, if possible, the most accurate and satisfactory means of assay.

There are several factors to be taken into consideration in a study of this problem:

1. The alkaloid, ephedrine, has a melting point of 40° C. and is somewhat volatile.
2. Ephedrine is soluble in ether, chloroform, petroleum ether, alcohol and water.
3. The isomers, ephedrine and pseudoephedrine, are intraconvertible. A high total yield of alkaloids is not necessarily indicative of a high *l*-ephedrine content.
4. Chloroform reacts with ephedrine under certain conditions to form the hydrochloride. According to Peterson (12) this reaction is accompanied by the formation of benzaldehyde.

We have assayed two different samples of Ma Huang according to the following methods:

1. Assay of Ephedrine by U. S. P. IX Method for Belladonna Root (13).
2. Assay of Ephedrine by U. S. P. X Method for Belladonna Root (14).
3. J. B. Williams' (15) modification of the U. S. P. X Method.
4. Feng and Read (16) Direct Alkalinization Method.
5. Feng and Read (16) Hot Acid Extraction Method.
6. Paul and Glycart Assay (17).
7. Hsu's Method (18).
8. Barium Hydroxide Method.

The Barium Hydroxide Method of assay is as follows:

Dissolve 10 Gm. of barium hydroxide in 75 cc. of distilled water and mix thoroughly with 20 Gm. of the crude drug. Allow to macerate for two hours. Transfer the material to a percolator and pour on 100 cc. of an alcohol-ammonium chloride solution (2% ammonium chloride in diluted alcohol—95% alcohol 70, water 30), and allow to macerate for 12 hours. Allow the percolation to proceed at its normal rate until 500 cc. of the same menstruum have been added. Heat the percolate on a water-bath until the alcohol has been evaporated. Shake out the alkaloids with three successive portions of chloroform, using 50, 40 and 30 cc., respectively, and filter these extractions through a pledget of cotton moistened with chloroform. Filter the aqueous extract and rinse the marc with dilute ammonia. Add 10 cc. of stronger ammonia to the aqueous liquid and again extract with chloroform until a portion of the residue no longer gives a positive "Biuret" (19) test with dilute copper sulphate and sodium hydroxide. Combine the chloroformic extracts and allow to evaporate spontaneously. Dissolve the residue in a little neutral alcohol, add 20 cc. of 0.1*N* HCl and titrate the excess with 0.02*N* NaOH, methyl red being used as an indicator.

The following results were obtained using the different methods of assay for the two samples of Ma Huang:

Method.	Sample I. Individual.	Sample I. Average.	Sample II. Individual.	Sample II. Average.
U. S. P. IX Assay (13)	0.864		0.735	
	0.851	0.8575	0.721	0.728
U. S. P. X Assay (14)	0.854		0.722	
	0.841	0.8475	0.703	0.7125

Method.	Sample I. Individual.	Average.	Sample II. Individual.	Average.
Williams' Modification (15)	0.972		0.840	
U. S. P. X Method	0.961	0.966	0.819	0.8295
Feng and Read Direct	1.13		1.01	
Alkalinization Method (16)	1.26	1.195	0.969	0.9895
Feng and Read Hot Acid (16)	1.41		1.17	
Extraction Method	1.33	1.37	1.18	1.175
Paul and Glycart Assay (17)	1.10		0.878	
Titration Procedure No. 1	1.03	1.065	0.859	0.868
Titration Procedure No. 2	1.04		0.880	
	1.11	1.075	0.883	0.881
Hsu's Method (18)	1.88		1.61	
	1.83	1.855	1.65	1.63
Barium Hydroxide Method	2.10		1.83	
	2.04		1.88	
	2.02		1.79	
	2.11		1.86	
	2.14	2.062	1.85	1.842

TANNINS.

We have already pointed out that Ma Huang means astringent yellow, and that the "Ma" or "astringent" refers to the taste of the drug. The literature repeatedly emphasizes the astringent taste and many references state that this is due to the presence of a tannin.

The astringent taste is also perceptible in several of the American species of Ephedra, and it has been suggested that in the case of Ephedra nevadensis, the remedial action of the drug is probably due to a tannin (20).

Although tannin has generally been accepted as one of the constituents of Ma Huang, yet we find little information in literature regarding this constituent of the drug. We have, therefore, undertaken the problem of determining the kind and amount of tannin present in our samples.

Generic tests on the aqueous extract of the drug indicated the possibility of a tannin being present. The following tests were given:

Reagent.	Result.
Ferric chloride solution	Greenish black color
Gelatin solution	Precipitate
Copper acetate	Precipitate
Lead acetate	Precipitate
Potassium dichromate	Negative
Methylene blue	Precipitate
Ammonium hydroxide	Solution readily absorbs oxygen and darkens
Potassium ferricyanide and Ammonium hydroxide	Red-orange color
Fehling's solution	Reduction takes place
Basic lead acetate	Precipitate
Ammonium molybdate in concentrated Ammonium chloride	Yellow precipitate
Lime water	White precipitate
Iodine potassium iodide with Ammonium hydroxide	Green precipitate
Bromine water	Yellow precipitate

These tests seem to give quite conclusive evidence of the presence of a tannin. Our next step was an attempt to classify the tannin. We concluded that the tannin in Ma Huang belonged to the catechol group because it conformed to the following tests:

Reagent.	Result.
Iron alum	Greenish black color
Bromine water	Yellow precipitate
Concentrated sulphuric acid	A red ring forms at the point of junction
When boiled with acids	Deposits red coloring matter

The tests for pyrogallol tannins were negative. However, in order to prove still more conclusively that Ma Huang tannin was a member of the catechol group, we ran the following test: 50 cc. of the tannin solution were boiled for half an hour under a reflux condenser with 25 cc. of a mixture of 100 cc. of concentrated hydrochloric acid (diluted with an equal volume of water) and mixed with 150 cc. of 40% formaldehyde; 10 cc. of the filtrate from the above, mixed with 10 drops of 1% iron alum and 1 Gm. of solid sodium acetate gave no color.

We attempted to limit our tannin still further by running the different tests for the various tannins of the catechol group. The fact that our tannin was precipitated from its solution by the addition of lead acetate, immediately excluded the possibilities of either resorcinol or hydroquinone being present. Our tests for catechol and protocatechuic acid gave the following results:

Reagent.	Result.
Lead acetate	Precipitate (both)
Silver nitrate	Reduction takes place (catechol)
Fehling's solution	Reduction takes place (catechol)
Ferrous salts	Violet color (protocatechuic acid)
Ferric chloride plus sodium carbonate	Green color darkening upon addition of sodium carbonate
Ferric chloride plus sodium acetate	Green color darkening upon addition of sodium acetate

These results seem to indicate the possibility of either catechol or protocatechuic acid being present.

Since many tannins are substances of a glucosidal nature and occur in the plant in combination with a carbohydrate complex such as glucose, we endeavored to determine whether or not the tannin of Ma Huang was of this nature. We extracted the tannin from the crude drug by agitating the aqueous extract of the drug with ether and then saturating the aqueous solution with common salt and shaking with ethyl acetate. The ethyl acetate was evaporated and a yellowish brown, fluffy looking powder was obtained. Although this material looked much like a tannin, it did not conform to the usual tannin tests.

This material extracted with ethyl acetate was boiled under a reflux condenser with HCl (2%) for an hour. Upon cooling, a red amorphous powder separated out. If our original substance was a tannin, this red powder was probably a phlobaphene. After filtering the mixture, the filtrate was shaken with ether and the aqueous solution was boiled, neutralized with caustic soda and precipitated with basic

lead acetate. The solution was again filtered and any lead remaining in solution was removed by the addition of dilute sulphuric acid. The solution was once more filtered and the clear filtrate was heated to boiling with Fehling's solution but no reduction took place. This would seem to indicate that the tannin present in Ma Huang is not in glucosidal combination.

Our attempts to determine the amount of tannin present were not particularly successful. We were not able to obtain concordant results with different methods of analysis nor were our results with the same methods always consistent. All of our results, however, would indicate that the tannin content is above 10%.

SAPONINS.

Generic tests indicated that a saponin-like body was present in Ma Huang. We also noted that after extracting the drug with benzene, ether, chloroform and alcohol, the water extract foamed strongly upon heating. Some trouble with emulsions was also experienced with some of the assay processes. We attempted the separation of this constituent by extracting the crude drug with hot alcohol and subsequent precipitation with ether. We obtained a fluffy, flocculent, white precipitate which readily darkened and resinified after it had been filtered.

We carried out this isolation process again, this time purifying the saponin by dissolving it in water and treating it with lead acetate. The resulting lead salts were decomposed by treating with dilute sulphuric acid.

The purified saponins thus obtained, were found to conform to the following tests for saponins:

1. Aqueous extracts foamed readily when shaken.
2. Concentrated sulphuric acid produced a red color.
3. Concentrated sulphuric acid containing a little ferric chloride gave a bluish green color.
4. Upon hydrolysis, the substance yielded a reducing sugar.

Therefore we have isolated and definitely established the presence of a saponin as one of the constituents of Ma Huang.

GLUCOSIDES.

Some of our generic tests seemed to indicate the possibility of a glucoside being present, however, we could not be sure that these tests were due to strictly glucosides and not to glucosidal substances such as certain tannins, pigments or saponins.

In order to isolate any glucosides that might be present in the drug, we extracted the material with dilute alcohol and precipitated other materials by adding a solution of lead acetate. The lead was removed from the solution by treating it with hydrogen sulphide. The aqueous solution was evaporated and the residue was taken up with dilute alcohol. A few crystals separated out. Their solution was very bitter and did not reduce Fehling's solution until after the material had been hydrolyzed by treating with acid and refluxing for a period of ten hours. This crystalline material did not have a sharp melting point as it charred. The charring did not take place until the material had been heated to almost 200° C. A few crystals were fused with sodium and tested for the presence of nitrogen but no positive test was obtained.

From our experiments we concluded that a glucoside or a glucoside-like body was present as one of the constituents of Ma Huang.

SUMMARY AND CONCLUSIONS.

1. A proximate analysis of Ma Huang has been made.

2. A study of the assay methods of Ma Huang shows that there is much variation in the results obtained by using the different methods.

3. A new method of assay using barium hydroxide for liberating the alkaloids from the plant tissue has been developed and is recommended for the determination and isolation of the alkaloids.

4. Good species of Ma Huang should yield close to 2% total alkaloids.

5. A catechol tannin has been found to be present as one of the constituents of Ma Huang. This tannin may be catechol, protocatechuic acid or both.

6. A crystalline substance possessing glucosidal properties has been isolated.

7. A saponin has been isolated and determined as one of the constituents of Ma Huang.

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THE ASSAY OF PREPARATIONS CONTAINING PEPSIN OFFICIAL IN THE NATIONAL FORMULARY.*,[1]

BY GLENN L. JENKINS AND EDWARD M. HOSHALL.

INTRODUCTION.

The medicinal value of pharmaceutical preparations containing pepsin is commonly considered to be dependent upon the activity of this enzyme in the digestion of proteins. The present official method of assay of pepsin (1) based on the digestion of egg albumen has been shown to yield erroneous results due to numerous variable factors (2), (3). Methods have not been developed for the assay of preparations containing pepsin, consequently the only criterion of the quality of a

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[1] Scientific Section, A. PH. A., Toronto meeting, 1932.