

## SCIENTIFIC SECTION

### DIFFERENTIAL CHARACTERS OF THREE COMMON CHINESE SPECIES OF EPHEDRA.

BY J. C. LIU AND B. E. READ.\*

Received for publication, December 18, 1928.

In a previous communication to THIS JOURNAL (3), upon the botanical sources of ephedrine and pseudoephedrine, the botanical characteristics of *Ephedra sinica*, Stapf and *E. equisetina*, Bge. were described, and we stated that "the material collected and sold as Mahuang consists chiefly of *Ephedra sinica*, Stapf." This is true for the north of China, to which our investigations hitherto have been confined. Conditions have now enabled us to investigate the Central China markets and we find a third species of Ephedra, *Ephedra distachya*, L., that is collected and sold in that area. This is a well-known plant, the botanical description of which can be found in authoritative books. It was collected in Shansi by Smith (5), and we have many samples from the Hankow market, and the mountain areas west of Chihli, which assure us that this species is important and probably is the chief source of supply of Mahuang for Central China.

Seeing that the drug Mahuang appears upon the market without flowers, fruit or leaves (excepting bracts), we are presenting the chief points of differences in these three species of Ephedra, so that their pharmacognostic character may be readily observed and the necessary distinctions be made.

If the drug appears complete with its main stems and roots, gross inspection will be sufficient to indicate which of the species has been collected.

More often incomplete plants are available; just the stems or chopped stems are used, having been freed from the useless roots and woody material. These stems may be complete enough to readily indicate their origin by the length of their internodes, by the roughness of their surfaces and by the degree of roundness, but more often one finds the material so finely chopped that a short histological study is necessary. We herewith give the chief points of difference with simple stains.

A good deal of Mahuang is sold at the ports in powder form. A microscopic study may be helpful in showing the large number of wood cells present in *E. equisetina*; subsequently a few simple chemical tests give the desired distinctions. Complete chemical analyses are being undertaken in these laboratories, a report of which will be published separately; simple ash determinations are here given for diagnostic purposes.

Commercially the present relative importance of the three species probably stands in the following order, *E. sinica*, *E. distachya* and *E. equisetina*. In 1885 the Customs returns show that Mahuang was exported in quantity from the following places: Newchwang 966 piculs, Tientsin 213 piculs, Hankow 66 piculs, Ningpo 61 piculs. Newchwang and Tientsin material would be chiefly *Ephedra sinica*, Hankow and Ningpo would be *Ephedra distachya*, about a tenth of the total. This is

---

\* Department of Pharmacology, Peking Union Medical College, Peking, China.

not to say that *E. equisetina* is scarce; there is much of it, and with the great demand during the last few years much of it has been collected, which freed from the woody stems we have shown has an unusually high content of ephedrine. *E. distachya* we have analyzed and found similar to *E. sinica* in containing both ephedrine and pseudoephedrine in the proportion of about four to one, but the total alkaloid we found to be less than *Ephedra sinica* by about 30 per cent.

The large export of Mahuang from Tientsin during the past three years has been reported upon in detail (4). With the disturbed conditions prevailing it is impossible to say how this would be in normal times. To offset the difficulties encountered with shipping at Tientsin there has been systematic collection of the wild plant by the International Famine Relief Association at Tatungfu, North Shansi, so that the supply has been well kept up. In Central China railway and shipping facilities are improving and supplies may pass down the Yangtse, in which case larger amounts of *E. distachya* are likely to appear on the market.

#### I. GROSS STRUCTURE.

The appearance of the flowers and fruits are quite characteristic for the species examined, which would be the natural basis for differentiation botanically. However, the drug is collected after the flowering and fruiting season and we have scarcely ever seen a single fruit or flower in twenty-five tons of the drug coming from various places. Special collections made by us or for us at other seasons of the year may bear flowers or fruit, but the latter is so easily shaken off it is hard to transport intact specimens.

Hence a macroscopic study of the drug is limited to an examination of the whole plant for size and habit, the stems and the minute leaves. We have drawn up in Table I the differential characteristics of these three species of *Ephedra*, and from it one can readily pick out the essential differences in length of internode, surface of stem, shape of stem and appearance of the leaf; see Table V.

TABLE I.—MACROSCOPIC STUDIES OF THREE CHINESE EPHEDRAS.

	<i>E. sinica.</i>	<i>E. distachya.</i>	<i>E. equisetina.</i>
(1) Habit	(a) Herbaceous (b) Not branching (or only occasionally so near base)	(a) Slightly woody (b) Branching, from upper and lower parts of main stem	(a) Very woody (b) Very much branching
(2) Stems			
(a) Length	About 30 cm.	About 37 cm.	1/4 to 2 meters
(b) Color (when dried)	Ashy greyish green	Greenish yellow (not ashy)	Ashy yellowish green
(c) Surface	Slightly rough	Rough	Very smooth
(d) Shape	Ancipital (flat)	Roundish round	Round
(e) Thickness	Broad 1.5 to 2.1 mm., thick 1.07 to 1.13 mm.	1.6 to 2.1 mm.	1.27 to 1.55 mm.
(3) Internodes			
(a) Length	3 to 6 cm.	2.5 to 6 cm.	1 to 2.5 cm.
(b) Average length	3.54 cm.	3.27 cm.	1.92 cm.
(c) Average weight	21.3 Gm.	27.2 Gm.	13.1 cm.
(4) Leaves (all minute)			
(a) Length	4 mm.	Similar to <i>E. sinica</i>	2 mm.
(b) Color	Reddish brown base, white lamina		Brownish purple, lamina dark
(c) Position	Tips subulate, recurved	Often fissioned at base	Close to stem

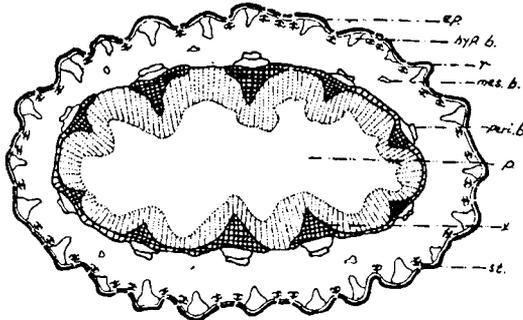


Fig. 1.—*Ephedra sinica*, Stapf.

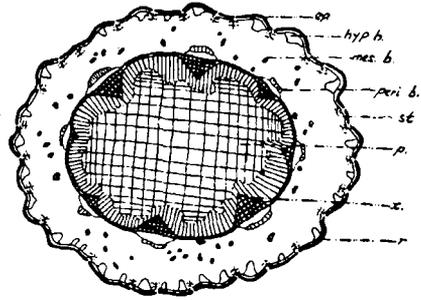


Fig. 3.—*Ephedra equisetina*, Bge.

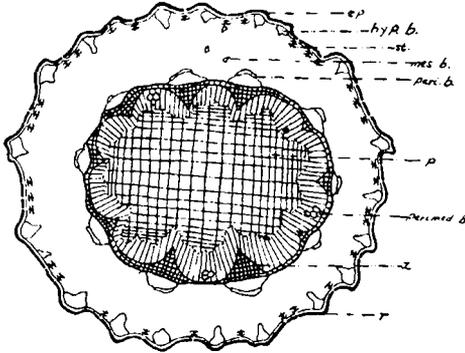


Fig. 2.—*Ephedra distachya*, L.

Figs. 1, 2 and 3.—Camera lucida pictures of cross-sections of 3 Chinese *Ephedras*, showing (1) the general round or flattish shape (2) the angulate or undulate outline; and (3) the lignification, represented by heavy straight lines. *ep.*, epidermis; *hyp. b.*, hypodermal bast; *mes. b.*, mesocortical bast; *peri. b.*, pericyclic bast; *perimed. b.*, perimedullary bast; *p.*, pith; *x.*, xylem; *st.*, stomata; *r.*, ridges.

Fig. 1.—In *E. sinica*, neither pith nor any bast is lignified, perimedullary absent, and mesocortical basts few or absent.

Fig. 2.—In *E. distachya*, pith and perimedullary basts present and lignified; mesocortical basts present or absent.

Fig. 3.—In *E. equisetina*, pith and all, except hypodermal basts, lignified; perimedullary absent, and mesocortical basts numerous.

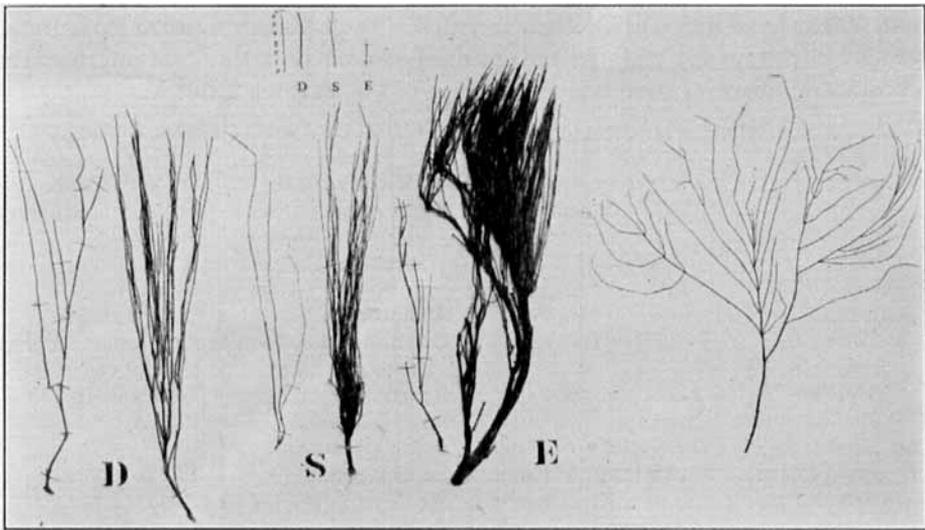


Fig. 4.—Gross appearance of the three common Chinese species of *Ephedra*. D, *Ephedra distachya*, L. (notice the branching); S, *E. sinica*, Stapf (notice the simple habit and the recurved tip of leaf); E, *E. equisetina*, Bunge, upper part of plant (notice the branching; the woody stem; the short internode and the tiny leaves). D and S complete plant.

Fig. 5.—*Ephedra distachya*, L. (spread out to show branching). Cultivated in the garden of the Peking Union Medical College.

II. HISTOLOGICAL STRUCTURES.

A. *Brief History.*—Efforts to identify the species of *Ephedra* through histological means have been attempted by various botanists. The result, so far, has not been satisfactory. In his monograph on *Ephedra* (6), Dr. Stapf in 1889 made the following summary of the situation up to that time. "Details of the anatomical structure of the stem of *Ephedras* are scattered in a great number of special treatises and bulletins, notably in De Bary's "Comparative Anatomy of the Phanerogams and Ferns." Bertrand (1) gave a very clear description of the same. In conclusion, Bertrand says that "the anatomical structures of the different species suggest only quite insufficient and unreliable characteristics for classification. In view of the large amount of research material at my disposal, I believed that the matter might be undertaken afresh. The final conclusion was, however, the same."

Graham (2) in 1907 took the number of vascular bundles in the second internode from the apical bud, and the presence or absence of different tissues and cell contents in some 16 species of *Ephedra*, for the purpose of classification. Although we agree with him that the number of vascular bundles in the second internode, from the apex, of *E. distachya* is constant, namely 8, such fortunate regularity does not exist, however, in the other two oriental species, *E. sinica* and *E. equisetina* Bge., which Graham did not examine, as seen from the following tables. Again after the plant has been collected inland, dried, packed and shipped frequently from one side of the globe to the other, it is impossible to decide with certainty whether the second internode on the crude drug is actually the second internode from the apical bud.

B. *Method for Differentiating Three Chinese Ephedra Species Based on Lignification.*—The histology of *Ephedra* has been given by Graham (2) and other authorities (6). In this article we are not concerned with the details of the structures, except the pith and the four groups of bast or stereoms; namely, the hypodermal, the mesocortical (intercortical), the pericyclic and the perimedullary, named in their order of occurrence from the periphery to the center. In applying one or two drops each of phloroglucin and concentrated hydrochloric acid to cross-sections of *Ephedra* which can be made easily by free-hand cutting, the lignified tissues will be stained red, while cellulose cells remain unchanged. It was observed that some of the tissues mentioned above are lignified differently in the three species of *Ephedra* in question. Incorporating this feature with a few other simple observations, the three species of *Ephedra* can be easily differentiated. The results of our observations are drawn up in Tables II, III and IV, and are given in detail in Figs. 1, 2 and 3.

TABLE II.—MICROSCOPIC STUDIES OF *Ephedra sinica*, STAFF.

Number of vascular bundles	Plant internodes.	Male fl. state.		Fem. fl. state.		Red fr. state.		
	1.	8	8	10	8	10	6-7	8
	2.	9	8	10	10	10	10	10
	3.	10	8	10	10	10	10	8
	4.	10	10	10	10	10	10	8
	5.	10	10	10	9	11	10	9
	6.	9	10		10	11	10	10
	7.	10				12 ?	10	8
	8.	10				10-12 ?	10	
	9.						10	



No. of ridges	30	24	25	31	24	24	34	23
Stomata	DO NOT STAIN CLEARLY							
Lignified bast								
Hypodermal	—	—	—	—	—	—	—	—
Mesocortical	+	+	+	+	+	+	+	+
Pericyclic	+	+	+	+	+	+	+	+
Perimedullary	0	0	0	0	0	0	0	0
Pith lignified	+	+	+	+	+	+	+	+
Diameters (mm.)	1.5 × 1.6	1.5	1.5 × 1.4	1.4	1.2	1.4	1.3	1.6 × 1.4

### III. CHEMICAL ANALYSIS.

While we are making a separate report of our chemical analyses it is considered of value here to give the results for total ash and acid insoluble ash which show such a big difference for the two commoner commercial species *E. sinica* and *E. distachya*. The roughness of the surface of the stems led us to suppose that certain mineral constituents would be present in larger amount in these two species which show under a low power lens sand-like particles upon the stem. Mr. Mei Ping-fu has kindly performed the analyses reported in Table V; the results are the average of at least three determinations of each species. It is clear that the earlier analyses reported in THIS JOURNAL, 14 (1925), 189, from these laboratories were done upon *E. sinica* for the insoluble fraction amounted to 18.78 per cent of the ash. At that time Mahuang was identified by the obsolete term *Ephedra vulgaris*, and we had no detailed information to hand concerning the various botanical species upon the Chinese market. For the sake of convenience we give in Table V the essential differences between the three species.

TABLE V.—ESSENTIAL DIFFERENCES OF THREE CHINESE EPHEDRA.

Plant observations.	A. Macroscopic.		
	<i>E. sinica</i> .	<i>E. distachya</i> .	<i>E. equisetina</i> .
Length of internode	3-6 cm.	(2) 2.5-6 cm.	1-2.5 cm.
Leaf	4 mm. long; tip recurved	4 mm. long; often fissured to base	2 mm. long; close to node, dark lamina
Shape of stem	Flattish	Roundish round	Round
Surface of stem	Roughish	Rough	Very smooth
	B. Microscopic.		
General outline.	Angulate.	Angulate.	Undulate.
Number of ridges	(19), 22-23, (26-30)	(19), 22-23, (26-28)	23-25, 31-34
Number of bundles	6-10	8 (constant) (never seen below)	6-8 (10)
(2nd internode)	(Not constant)	8 in any internode	(Not constant)
Stomata	(30) 35-45 (60) clearly stained	31-41 (51) fairly clearly stained	Usually not clearly stained
Lignified bast			
Hypodermal	—	—	—
Mesocortical	—(0)	—(0)	++
Pericyclic	—	—	++
Perimedullary	0	++	0
Pith lignified	—	++	++
Diameters	1.07-1.13 × 1.5-2.1	1.6-2.1	1.27-1.55
Differences between the 2 diameters	0.6-0.8 mm.	0.1-0.2 mm.	0-0.1 mm.

C. *Analytic.*

Ephedra.			
Total ash in dried material	9.77	8.20	6.55
Acid insoluble ash in dried material	2.23	0.42	0.28

## REMARKS.

1. It is interesting to note from the histological tables that whether the surface of the stem is rough or smooth does not depend upon the number, but the acuteness of the ridges, each of which is supported within by a hypodermal stereom. Thus, if there is any difference at all between the number of ridges to the stem, that of *E. equisetina*, although it is the smoothest of the three, is on an average higher than in the other two species.

2. The number of stomata is of little significance for identification purposes, although the partial or complete response to the phloroglucin stain is suggestive.

3. The differences in lignification are not to be considered a matter of chance. Any one familiar with the habit and the habitat of the three species of *Ephedra* can easily see why the tissues of *E. equisetina* are more lignified than the other two species. It is not only a taller plant, bearing cauline branches on a woody stem, but it is also generally found on rocky beds on high cliffs. Therefore it needs strength for its support.

## CONCLUSIONS.

1. *Ephedra sinica* is distinguished by (1) its herbaceous character; (2) its ancipital branches (shown by the big difference between the long and the short diameters); (3) its whitish and recurved leaf-scales; (4) its cellulose pith and basts; (5) the presence or absence of cellulose mesocortical basts; (6) the absence of perimedullary basts; and (7) the high percentage of acid insoluble ash.

2. *Ephedra distachya* is distinguished by (1) its roundish and rough branches; (2) its lignified pith; (3) its cellulose basts, except the perimedullary, which when present are lignified; (4) the presence or absence of cellulose mesocortical basts; (5) high ash value but small insoluble fraction.

3. *Ephedra equisetina* is distinguished by (1) its woody main stems; (2) its very smooth and round branches; (3) its short internodes, less than 2 cm. long; (4) its very short and close leaf-scales; (5) its lignified pith and all the basts except the hypodermal; (6) the presence of numerous mesocortical basts; (7) the absence of perimedullary basts; and (8) low ash values.

## BIBLIOGRAPHY.

- (1) Bertrand, "Anatomie des Gnétacées et des Conifères," *Ann. d. Sc. Nat.* (1874), Ser. V., tom. xx., 19.
- (2) R. J. D. Graham, "On the Histology of the Ephedreae with Special Reference to the Value of Histology for Systematic Purposes," *Trans. Roy. Soc. Edinburgh*, 46 (1908), 203.
- (3) B. E. Read and J. C. Liu, "Chinese Botanical Sources of Ephedrine and Pseudoephedrine," *Jour. A. Ph. A.*, 17 (1928), 339.
- (4) B. E. Read, *Chinese Economic Journal* (Oct. 1928), 817.
- (5) H. Smith, "Plantæ Sinenses," Sweden, 17 (1927), 7.
- (6) O. Stapf, "Die Gattung der Ephedreae," *Denkschr. math.-naturw.*, Wien, 56 (1889).