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value in making bio-assays of the chlorophyll preparations which are beginning to appear on the market.

5. Chlorophyll extracts apparently slow the action of the heart, at the same time increasing the force of the contraction, suggesting a digitalis action. The heart action (in the frog) appears to assume a dicrotic character with large doses.

6. The spectrum of chlorophyll resembles that of blood.

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May 25, 1927.

# THE OCCURRENCE AND ALKALOIDAL CONTENT OF VARIOUS EPHEDRA SPECIES II.

# BY C. NIELSEN AND H. McCAUSLAND.

In THIS JOURNAL, Vol. XVI, No. 4, April 1927, we reported on the occurrence and alkaloidal content of several Ephedra species. We are now in a position to report on two species which were briefly mentioned in that article, and which we have since obtained and analyzed for alkaloidal content.

#### EPHEDRA TRIFURCA, TORREY.

The specimens of this plant were gathered for us by Frank A. Thackery of the United States Department of Agriculture, Bureau of Plant Industry, Sells, Arizona. The species was identified by Dean J. J. Thornber of the University of Arizona. The plants were received in October and were in full bloom. Figure 1

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shows a female specimen, Figs. 2 and 3 branches of male specimens of the plant. Most of the staminate catkins had fallen off in the shipment, but the illustrations show a few. The species answer the description in the textbooks. The shrub is erect, 1.5 to 5 feet high with spinous, straight, green or yellowish green, opposite or fascicled branches. Leaves inconspicuous, scale-like, white, distinctly sheath-

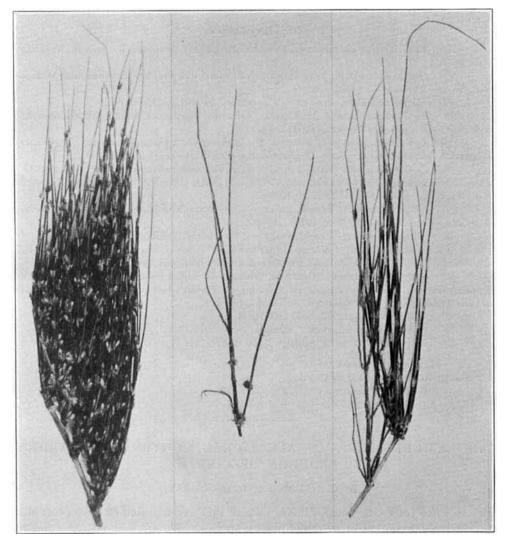


Fig. 1.—Ephedra Trifurca, Fig. 2.—Ephedra Trifurca, Fig. 3.—Ephedra Trifurca, Torrey, Female Plant. Torrey, Branch of Male Plant. Torrey, Branch of Male Plant.

ing the stem, at length splitting to the base. Staminate and ovulate catkins on separate plants, almost sessile. Ovulate catkins of 8 to 10 whorls of bracts; bracts rather large, very thin, scaly, scarious, round-cordate, clawed, sometimes with pinkish red centers. Fruit solitary, slender-ovate, four-sided. In the staminate catkins the imbricate bracts are very much smaller. Stamens protruding, bright May 1928 AMERICAN PHARMACEUTICAL ASSOCIATION

yellow, united into columns or clusters of three or more. The size of the staminate catkin is that of a small pea, the ovulate is 3 to 5 times larger.

*Ephedra trifurca* torrey grows in Arizona, Colorado, California, Texas and Mexico.

The branches and the catkins were analyzed separately. No alkaloids were found in either. The analytical method used was the same as the one we used in determining the total alkaloidal content in Chinese Ma-Huang, as follows:

Fifteen Gm. of the powdered drug are macerated with 150 cc. of a mixture of 3 parts of ether and 1 part of chloroform. After ten minutes maceration, 5 cc. of 10% ammonia solution and 0.5 Gm. of anhydrous sodium carbonate are added and the mixture is well shaken during a period of several hours. The mixture is then allowed to settle and the liquid filtered through a covered filter. Seventy-five cc. of the filtrate, corresponding to 7.5 Gm. of drug, are shaken with three portions of water acidified with dilute sulphuric acid, using 15, 10 and 10 cc. The combined acid washings are neutralized with ammonia, and 5 Gm. of anhydrous sodium carbonate are added to the neutralized liquor, stirring until dissolved. This alkaline solution is shaken with four portions of ether, using 35, 30, 25 and 20 cc. The combined ether washings are filtered and evaporated with gentle heat to about 3 cc. The residue is removed from the heater and the remaining solvent blown off. The final residue is dissolved in 2 cc. of neutral methyl alcohol,

and diluted with about 40 cc. of distilled water free from CO<sub>2</sub>. This solution is titrated with N/50 H<sub>2</sub>SO<sub>4</sub>, using a suitable indicator, such as methyl red, brom-cresol green or brom-thymol blue. One cc. of N/50 sulphuric acid is equivalent to 0.0033 Gm. of Ephedra alkaloids.

### EPHEDRA VULGARIS, RICH.

### (Ephedra distachya Linn.)

This variety grows in the mountains of Switzerland and Tyrol. It is commonly known as *Ephedra vulgaris*, and is probably the species to which the literature has referred as *Ephedra subaris* yor *Helpetica*. It is not identified

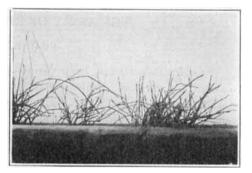


Fig. 4.—Ephedra Vulgaris, Rich (E. distachya, first-year growth from seeds—Linue).

vulgaris var. Helvetica. It is not identical with Ephedra Helvetica, C. A. Meyer. Chen and Kao suggested that this might be the European form of one of the varieties of Ma-Huang which grows in China and contains ephedrine and pseudo-ephedrine, but that this variety as found in Europe contains pseudoephedrine only. We obtained fresh seeds of this European variety from H. Correvon, Chène-Bourg prês Genève, Switzerland. These were planted in the open early in the spring of 1927 in a mixture of clay soil, leaf mold and sand. The plants grew quite well, although they did not reach more than 6 to 8 inches in height. Late in the fall when they had reached their maximum of growth a number of selected specimens were plucked, leaving the roots in the ground. The slender plants were dried at a low temperature, powdered and analyzed for alkaloidal content. There was no alkaloid present in this first-year growth.

The plants were taken indoors before the frost and a second growth is now under way (Fig. 4). We hope to obtain more vigorous growth next summer, when the test for alkaloids will be repeated. If this variety contains ephedrine alkaloids when grown in its native soil, it is possible that the transplantation was

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responsible for the absence of alkaloids in the rather delicate first-year growth. A similar instance is known to have occurred with other plants. *Hyoscyamus mulicus*, a native of Egypt, for instance, is rich in hyoscyamine (1%); but when it was transplanted from the wilderness to the botanical gardens near Cairo, it was found to be free from alkaloids.

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## THE ASSAY OF EPHEDRA VULGARIS.\*

## BY J. B. WILLIAMS.

Ephedra vulgaris has been variously reported as containing from about 0.2% to about 1% alkaloid. During the past year seven samples of the drug have been assayed by the writer using the method of the U. S. P. X for belladonna, but collecting the final chloroform solution in a tared flask, drying at a temperature of about 50° C. for one or two hours and weighing the alkaloidal residue before titrating. The results obtained in terms of total alkaloid were as follows:

No.	Gravi- metric.	Volu- metric.	No.	Gravi- metric.	Volu- metric.	No.	Gravi- metric.	Volu- metric.
1	1.42	1.25	3	0.93		6	1.21	0.86
	1.43	1.24		0.94			$0.74^{1}$	$0.55^{1}$
	1.42		4	1.17	1.01		0.84	0.66
	$1.37^{1}$	$1.15^{1}$		1.18	1.01		0.87	
							$0.73^{1}$	$0.56^{1}$
2	0.71	0.548	5	1.11	1.04		1.02	0.90
	0.64	0.495		1.13	1.09		0.99	
	0.74		6	$0.705^{1}$	$0.502^{1}$		$0.88^{1}$	$0.70^{1}$
	0.661	0.481		$0.655^{1}$	$0.472^{i}$			

No. 7. Titrated without drying, volumetric only, 1.02, 1.01, 1.15, 1.16.

<sup>1</sup> Alkaloid dried over night; others dried for one or two hours.

The factor used for the volumetric estimations was 1 cc. N/10 acid = 0.0165 Gm. alkaloid, using methyl-red indicator. The above results indicate that the alkaloid is quite volatile, although there is also more or less decomposition of the alkaloid in chloroform solution with the formation of hydrochloride. This, however, apparently does not take place under the conditions of the assay, as is indicated by

<sup>\*</sup> Scientific Section, A. PH. A., St. Louis meeting, 1927.