respective standard deviations of the LD_{50}'s expressed in per cent (8).

Our results show that the potency in all cases was well within the limits of the standard deviations; hence there has been no deterioration of the extract. A preliminary experiment, conducted in July, 1940, two months after the manufacture of the extract and five months before the first assay given above, showed that 0.1 Gm. of the extract was equivalent to approximately nine U. S. P. XI Digitalis Units. The absence of change, even when diluted 1 to 10 with water, is in line with the finding by Straub (6) that aqueous solutions of convallaria extract in sealed ampuls retained their full strength for four years.

SUMMARY

The potency of a concentrated, purified, aqueous extract of the leaves of *Convallaria majalis* was determined by comparison with digitalis on frogs. The extract and a 10% aqueous dilution thereof were found to be stable at room temperature during the course of five months.

REFERENCES

(1) Büttner, *Münch. med. Wochschr.*, 83 (1936), 387.

(2) Hauke, G., *Deut. med. Wochschr.*, 63 (1937), 1047.

(3) Keseling, B., and Kamprad, W., *Münch. med. Wochschr.*, 84 (1937), 1778.

(4) Major, R., and Ledger, L., *Am. Heart J.*, 18 (1939), 444.

(5) Major, R., *Internat. Clin.*, 4 (1940), 6.

(6) Straub, W., *Münch. med. Wochschr.*, 83 (1936), 387.

(7) Kärber, G., *Arch. exp. Path. Pharmakol.*, 162 (1931), 480.

(8) Burn, J. H., "Biological Standardization," Oxford University Press, London, 1937, pp. 31, 38.

A Chemical Study of Oklahoma Plants

V. *Ephedra Nevadensis* Watson*

By Jess L. Beasley, Jr.,† and Loyd E. Harris‡

Fig. 1.—Habit photograph of *Ephedra Nevadensis*.[1]

Fig. 2.—*E. Nevadensis* showing staminate cones.[1]

Six species of Ephedra have been reported in North America, where they are found growing in the desert or semi-desert regions of Mexico, Colorado, Texas and Oklahoma (1). *Ephedra Nevadensis* was described by Watson (2) in 1879. Cross (3) reported in 1937 that collections of Ephedra were first made in Oklahoma, southwest of Hollis, in Harmon County, January 28, 1932.

Nagai (4) isolated the alkaloid ephedrine from *Ephedra vulgaris* in 1887. The use of this alkaloid in medicine has made it a drug of great importance during the past two decades. The chief sources of ephedrine are *Ephedra sinica* Stapf, *Ephedra equisetina* Bunge and other species of Ephedra (5)

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[1] By courtesy of Dr. G. L. Cross, Botany Department, University of Oklahoma.

in amounts varying from 1% to 2% of the air-dried plants. China supplies most of the drug material which is then extracted in the United States. Experiments on the cultivation of Asiatic Ephedra in the United States are being conducted. Hiner (6) has shown that there is a possibility of the growing of *Ephedra sinica*, on a commercial scale, in South Dakota.

Much attention has lately been given to the industrial synthesis of ephedrine and several patents have been granted, most of them originating in Germany (7).

Ephedra Nevadensis Wats. is found growing, in very limited numbers, in southwestern Oklahoma. It has been used as a treatment in kidney and venereal diseases by Indians and Mexicans. Some studies on the constituents of the plant found growing in California have been made. Terry (8) reported that no ephedrine or any alkaloid could be detected. Nielsen (9) and others reported the absence of ephedrine. Read and Feng (10) found nothing more than a possible trace of ephedrine; extracts did not give any rise in blood pressure.

No reports have been given on chemical studies of the Oklahoma plant. Since it is known that different cultural conditions, in some instances, will materially affect the nature of the chemical constituents of plants, it was believed worth while to make this study.

EXPERIMENTAL

The material used in this investigation was collected southwest of Hollis, Harmon County, Oklahoma, in early November, 1940. There had been no frosts or freezing weather up to that date. The green stems were the only part of the plant used for this examination. The material was allowed to dry in sacks, away from the sun and then reduced to a No. 20 powder before extraction.

Moisture and ash determinations were made on the powdered material, using the methods given in the United States Pharmacopœia, eleventh revision. The results are given in the following tables.

MOISTURE DETERMINATIONS

Weight of Sample, Gm.	Moisture, Per Cent
20	8.2
30	8.0
	8.1 Av.

ASH DETERMINATIONS

Weight of Sample, Gm.	Acid-Insoluble Ash, Per Cent	Total Ash, Per Cent
2.103	0.45	8.05
2.105	0.52	8.10

Extraction with Selective Solvents.—Samples of the ground material were extracted successively in a Soxhlet extractor with petroleum ether (b. p. 25–60° C.), anhydrous ether, chloroform, dehydrated alcohol and water. The residues remaining upon evaporation of the petroleum ether and ether were sticky and dark brown. The chloroform and alcohol extractions were gummy and dark green. The percentage of each extractive is listed in the following tabulation.

EXTRACTIVE CONSTITUENTS OBTAINED BY SELECTIVE SOLVENTS, PER CENT

Weight of Sample, Gm.	Petroleum Ether	Ether	Chloroform	Alcohol	Water
12.132	0.94	4.86	1.07	6.38	14.6
11.742	0.94	5.04	0.94	6.43	15.5

The residue from the alcohol extraction was treated with 50 cc. of water. (A 10-cc. portion of the aqueous solution was evaporated and calculations from this aliquot portion showed that two-thirds of the alcoholic residue had dissolved in water.) Drops of the amber-colored solution were tested on slides with the use of a microscope, using the following alkaloidal reagents: Wagner's reagent, potassium dichromate, picric acid, lead acetate, mercuric chloride and ammonium molybdate.

Positive results were obtained with ammonium molybdate and indications of precipitates were obtained with the first three of the reagents.

Tests for Ephedrine and Other Alkaloids.—A 20-Gm. sample was treated by the method outlined by Christensen and Hiner (6). The 250-cc. percolate was evaporated to 50 cc. Light, oily globules formed on top of the liquid and, when placed in a separatory funnel and left over night, a brown heavy oil-like material separated from the liquid. This oily liquid was drawn off, dried *in vacuo*, but did not result in a definite crystalline structure as shown under the microscope.

The ether-chloroform extraction, after separation of the oily material, was diluted to 100 cc. and divided into four parts. (*a*) One part was treated by the method of Chen (11), but a heavy clear oily liquid was obtained, which gradually darkened on exposure, where crystalline ephedrine hydrochloride should have appeared, had the alkaloid been present. (*b*) Another portion of it was treated in a similar manner, substituting sulfuric acid for hydrochloric acid, with similar results. (*c*) A third portion of the solution was tested by the method of Kelly (12) for the detection of small quantities of Ephedra alkaloids, with negative results. Color reactions for ephedrine as suggested by Pesez (13) also gave negative results.

SUMMARY

The green stems from the plant, *Ephedra Nevadensis* Wats., growing in southwestern Oklahoma, have been examined. In the preliminary analysis the percentages of moisture, total ash and acid-insoluble ash were determined. Samples of the powdered material were extracted by selective solvents. Tests for the presence of ephedrine were negative.

REFERENCES

(1) Pearson, H. H. W., "Gnetales," Cambridge, 1929; Wooton, E. O., and Standley, P. C., "Contr. U. S. Nat. Herb.," Vol. XIX, 1915, p. 38.

(2) Watson, *Proc. Am. Acad.*, 14 (1879), 299.

(3) Cross, G. L., *Proc. Oklahoma Acad. Sci.*, 17 (1937), 65.

(4) Nagai, N., *Pharm. Zeit.*, 32 (1887), 700; through "U. S. Disp.," Twenty-second edition, p. 425.

(5) "U. S. Pharmacopœia," Eleventh revision, p. 144.

(6) Hiner, L. D., and Christensen, B. V., JOUR. A. PH. A., 28 (1939), 199; Sievers, A. F., *Ibid.*, 27 (1938), 1221.

(7) Ger. Patents 527,620, 538,455, 547,174, 554,553, 585,164; Brit. Patents 297,385, 318,488, 365,535, 365,540, 365,541; U. S. Patent 186,727; Mansche, R. H. F., and Johnson, T. B., *J. Am. Chem. Soc.*, 51 (1929), 1906.

(8) Terry, R. E., JOUR. A. PH. A., 16 (1927), 397.

(9) Nielsen, C., *et al.*, *Ibid.*, 16 (1927), 288.

(10) Read, B. E., and Feng, C. T., *Proc. Soc. Exptl. Biol. Med.*, 24 (1927), 819.

(11) Chen, K. K., and Schmidt, Carl F., *J. Pharmacol.*, 24 (1925), 339; JOUR. A. PH. A., 14 (1925) 189.

(12) Kelly, J. W., *Am. J. Pharm.*, 109 (1937), 36.

(13) Pesez, M., *J. pharm. chim.*, 27 (1938), 120.

Effect of Picrotoxin on the Blood Potassium of Anesthetized Animals*

By Carlos E. Cardini and Maria E. Serantes

Picrotoxin strongly antagonizes the effects of narcotics; it increases respiratory tonus, restores consciousness, etc. Advantage has been taken of this action in the treatment of barbiturate poisoning. Maloney and collaborators (1), Rosenthal and Wallach (2), and Marshall and co-workers (3), have, among others, studied this question.

During anesthesia, changes appear in the titers of the inorganic constituents of the blood, the most prominent of these being a marked decrease in plasma potassium (Marenzi and Gerschman (4), 1933). It was considered of interest to study the narcosis-induced decrease in plasma potassium as affected by the suppression of narcosis with picrotoxin. It is also to be remembered that picrotoxin, along with its antinarcotic action, possesses convulsant properties capable by themselves of inducing variations in the plasma potassium of normal dogs.

EXPERIMENTAL

The anesthetic selected, chloralose, was administered intravenously to dogs at the rate of 10 ml. of an 8% solution per Kg. After deep anesthesia had been induced, a 0.5% solution of picrotoxin (Merck) in distilled water was injected intravenously, rapidly or gradually, in a dosage varying from 0.10 mg. to 1 mg./Kg. for rapid injections, from 0.25 to 0.35 mg./Kg. in prolonged administration, distributed over 10 to 20 min. Blood was obtained from the carotid artery at intervals noted in the curves. Potassium was determined by the method of Marenzi and Gerschman (4), 1932, using blood deproteinized with trichloroacetic acid. Potassium was determined directly in plasma and whole blood. The red cell potassium content was calculated from cell volume determinations and from plasma and whole blood analyses. Red cell concentration or contents is of very little significance even when water determinations and R. B. C. counts are available.

RESULTS

Rapid Administration.—Doses of 0.10 mg. and 0.12 mg./Kg. do not induce the disappearance of narcosis. Antagonistic action begins to be evident with doses above 0.15 mg. There is a latent period which for this dosage varies between 8 and 10 min. As a rule, in 20 to 30 min., convulsions begin to appear which last to the end of the experiment. The animal is conscious throughout the entire experiment, which lasts more than an hour. With a dose of 0.25 mg./Kg., consciousness is regained within 8 to 20 min., but rather severe convulsions appear 10 min. after the injection. With a dose of 0.50

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