

Dissolve about 0.3 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed, in 200 cc. of water; heat to boiling and add 10 cc. of diluted nitric acid followed by sufficient silver nitrate to precipitate all the chloride; allow to stand for six hours; transfer to a tared Gooch crucible; wash well with hot water; then with cold water and dry at 120° C., cool in a desiccator and weigh: the chloride (Cl⁻) calculated from the silver chloride weighed is not less than 17.30 nor more than 17.60 per cent. Transfer about 0.35 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed, to a 500-cc. Kjeldahl flask and determine the nitrogen content according to the method described in Medical War Manual No. 6, Laboratory Methods of the U. S. Army, page 221: the percentage of nitrogen corresponds to not less than 6.7 per cent nor more than 7.0 per cent. Incinerate 0.1 Gm. of hydroxyphenylmethylaminoethanol hydrochloride, accurately weighed: the ash is not more than 0.0001 Gm.

A COMPARISON OF THE CHEMICAL AND BIOLOGICAL ASSAYS OF OLEUM CHENOPODII.*

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In the lower portion of Carroll County, Maryland, is a narrow strip of land four miles wide and fifteen miles long which is known as the "oil belt" and which produces the greater part of the Oleum Chenopodii, or the American wormseed oil of commerce. Here the plants are grown, harvested and steam-distilled to obtain the volatile oil which occurs mainly in the seed, of which it constitutes three to three and a half per cent. Distillation is carried out during a period ranging from two to six weeks, in October and November depending upon the size of the crop, weather conditions, etc. The oil is distilled with live steam under pressure, allowed to separate from the condensed water, and is drawn off as "normal" oil of wormseed. The product is somewhat soluble in water and this, together with the fact that a portion remains mechanically suspended in the lower liquid, has led, in recent years, to the practice of collecting and redistilling the water. While this second distillation yields a much smaller quantity of oil, this redistilled product has been found to be much richer in ascaridol and of higher specific gravity than the normal oil. It is termed by the growers "high-test" oil of wormseed.

During the autumn of 1929 authentic samples of normal and high-test oil were collected at the stills. These were examined to determine if any relationship exists between the physical constants and/or ascaridol content, and certain pharmacological reactions. The following U. S. P. physical constants were determined:

- (a) Specific gravity at $\frac{25^{\circ}}{25^{\circ}}$ C.
- (b) Specific rotation.
- (c) Refractive index at 20° C.
- (d) Solubility of one volume of oil in 70% alcohol.

Although certain inaccuracies have been shown by Paget (1) to exist in the U. S. P. assay method for ascaridol, and these findings have been confirmed by Nelson (2) and Broughton (3), yet in view of its official character, and present status as an arbiter of quality, it was thought best to adhere to the Pharmacopœial

* Scientific Section, A. PH. A., Baltimore meeting, 1930.

assay. Ten normal and four high-test oils were examined and the results obtained are given in Table I.

Samples of these oils were tested within a week after collection, and after approximately five months, for their toxicity to gold fish, earth worms, blood worms and porcine ascarides. A measured volume of the oil was triturated in a mortar with talc and a measured volume of water, then filtered. The filtrate was assumed to represent the original concentration although in fact it was somewhat weaker. Measured volumes of this filtrate and of tap water were prepared to give various dilutions which were used for tests. In some experiments the procedure used by Knaffl-Lenz (4) was followed. A measured volume of oil was dissolved in 5 volumes of alcohol and vigorously shaken with water to obtain a cloudy emulsion. This emulsion was then diluted with tap water and tested at once.

In all experiments except on ascarides, the solutions were tested at room temperature. Because of their susceptibility to temperature, these solutions were placed in a series of jars in a tray containing water at 38° C. As a uniform procedure, 50 cc. of solution was allowed for each animal. In most experiments a total volume of 250 cc. was placed in a jar and 5 test animals added. After 15 minutes, 30 minutes, 45 minutes, 1, 2 and 3 hours, and after approximately 24 hours, the number of survivors were noted. The minimum lethal dose was se-

TABLE I.—SAMPLES OF WORMSEED OIL: 1929.

		Specific gravity, 25/25.	Ascaridol, %.	Specific rotation.	Refractive index at 20° C.	Volumes 70 % alcohol in which one vol. oil is soluble.
1	Normal	0.9616	59.8	-6.66°	1.4752	5
2	Normal	0.9593	58.0	-6.38°	1.4750	6
3	Normal	0.9549	50.0	-6.40°	1.4754	9
4	Normal	0.9622	59.9	-7.59°	1.4749	5
5	Normal	0.9715	62.2	-5.84°	1.4747	3
6	Normal	0.9629	66.6	-7.55°	1.4753	4
7	Normal	0.9510	55.8	-8.23°	1.4752	8
8	Normal	0.9748	64.3	-5.54°	1.4744	3
9	Normal	0.9749	72.8	-4.38°	1.4750	3
10	Normal	0.9765	75.6	-5.89°	1.4748	2
11	High-test	0.9883	87.6	-4.08°	1.4739	2
12	High-test	0.9978	99.0	-4.26°	1.4729	2
13	High-test	0.9993	100.0	-3.91°	1.4727	2
14	High-test	0.9959	98.2	-3.57°	1.4740	2

TABLE II.—THE TOXICITY OF OIL OF CHENOPODIUM.

Product no.	Ascaridol content, per cent.	Gold-fish.	Minimum Lethal Dose—Cc./Liter.		
			Earth-worms.	Blood-worms.	Porcine ascarides.
3	50.0	0.67	2.67	0.20	2.5
2	58.0	1.0	2.67	0.10	1.5
1	59.8	1.0	4.0	0.4	...
4	59.9	0.67	6.67	0.13	5.0
5	62.2	1.0	4.0	2.0	Over 5.0
6	66.6	1.0	2.0	0.4	5.0
0	74.9	0.4	4.0	1.0	2.5
11	87.6	2.0	2.5	0.4	2.5
12	99.0	0.67	2.5	0.1	1.5
13	100.0	1.0	2.0	0.1	5.0
Ethyl alcohol	..	20.0	60.0	40.0	175.0

lected as that concentration killing at least three of five animals within three hours. It is recognized that different values for the M. L. D. might be obtained by using a different period of survival. The M. L. D. of this series of oils is given in Table II. Values of ethyl alcohol are given for comparison.

It is evident that these toxicity figures fail to agree among themselves. No lethal doses have been found to agree with chemical analyses for the ascaridol content. Further work is under way in an effort to develop a biological assay which will bear some relationship to ascaridol content. In a similar investigation Knaffl-Lenz has also failed to obtain consistent results upon worms, fish or mice which bore any relation to results of chemical assay.

CONCLUSIONS.

1. The Pharmacopœial assay confirms the greater ascaridol content and higher specific gravity reported for "high-test" oil of chenopodium, as compared with "normal" oil.

2. No method of biological assay has been found which agrees with the chemical determination of ascaridol in either normal or high-test oil of chenopodium.

3. Goldfish, earthworms, bloodworms and porcine ascarides differed in susceptibility to different samples of oil of chenopodium of the same ascaridol content.

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THE VOLATILE OIL OF *PYCNANTHEMUM MITICANS*.*¹

BY HAROLD W. WERNER.

The plant *Pycnanthemum miticans* (Michx.) Pers. (*Koellia mutica* (Michx.) Britton) is a member of a genus represented in the United States by eighteen species, all of which, excepting *P. californicum* (1), are found east of the Mississippi River ranging from Canada down into Florida.

Like the other members of the genus, *P. miticans* is a perennial herb with opposite leaves and square stems. This species grows to be about 1 meter tall, and is more or less branched. The leaf blades are lanceolate, the floral ones whitened, thus giving the plant an attractive and distinctive appearance. The small white flowers, dotted with magenta, are terminally arranged in dense, conspicuously bracted corymbs.

Although oils produced in two consecutive years were investigated, results were parallel; therefore only the work on the oil of 1928 will be reported here.

The herb from which the oil was produced represents two cuttings made while the plants were in full bloom. The material was dried and the oil extracted by

* From thesis presented in partial fulfilment of the requirements for the Degree of Master of Science in Pharmacy, University of Florida, May 1929.

¹ Scientific Section, A. P. H. A., Rapid City, 1929.