The oil boiling above 150° C. constituted about half of the total. The presence of caryophyllene was indicated by odor and physical constants, but we were unable to isolate any derivatives. The characteristic odor of the oil seems to reside in this upper fraction.

SUMMARY.

1. The fresh leaves gave 0.05 per cent of yellowish brown oil whose constants are recorded.

2. The oil contains about 20 per cent of terpenes, a considerable amount of cineol and smaller quantities of esters and alcohols, but no aldehydes nor ketones. Acetic acid and small amounts of unidentified acids and lactones are also present.

3. The chief constituents are high boiling and may include caryophyllene.

A COMPARATIVE STUDY OF THE THREE RECOGNIZED ASSAYS FOR OIL OF CHENOPODIUM.*

BY WILLIAM F. REINDOLLAR.[1]

Chenopodium or American Wormseed Oil has long been employed as an anthelmintic against round worms in man and stock animals, and more recently has come into use as an agent in the treatment of hookworm.

The principal constituents of the oil are ascaridole, an organic peroxide occurring to the extent of 60–80 per cent, and a hydrocarbon fraction containing cymene, limonene, sylvestrene, phellandrene, etc. Of these components it has been shown that ascaridole alone exhibits anthelmintic action (1), hence should be used as the basis for evaluating the oil. As ascaridole readily reverts to an inactive form, ascaridole glycol anhydride when distilled with steam, as in its removal from the plant, it is highly desirable to employ a method which distinguishes between these two substances.

Assay processes for wormseed oil are given in the Official and Tentative Methods of the Association of Official Agricultural Chemists (2), and in the British (3) and United States Pharmacopœias (4). The first two procedures determine ascaridole, while the last named measures the acetic-acid soluble fraction of the oil. The A. O. A. C. method is a modification of one first proposed by Humphrey Paget (5), and which involves the reduction of ascaridole by titanous chloride; the British Pharmacopœia has adopted the Cocking and Hymas (6) procedure based on the liberation of iodine from potassium iodide by the organic peroxide ascaridole; while the U. S. P. employs the Nelson (7) method which depends on the differential solubility of the organic peroxide and hydrocarbon fractions in 60 per cent acetic acid.

Although Broughton and Weiland (8) have demonstrated the merits and limitations of the A. O. A. C. procedure and pointed out its superiority over the method official in the U. S. P. X (9), it was not adopted by either the British or

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[1] From the Laboratories of the Bureau of Chemistry of the State of Maryland Department of Health.

American Pharmacopœias in their recent revisions. Investigators are not in agreement concerning the British method. Broughton (10) felt that, in spite of the number of factors which influence its accuracy, the method was worthy of further study, Dafert and Capesius (11) found it simple and accurate, while Bodendorff (12) stated that the development of an exact quantitative method for ascaridole based upon iodometric procedure is highly improbable.

In view of the fact that three recognized procedures exist for evaluating wormseed oil, and that two of them are employed to determine the legality of the product in certain areas where it is sold, it would seem highly desirable that the three assays yield consistent results when applied to the same oil. In order to learn how good an agreement might be obtained under such circumstances, this investigation was undertaken.

EXPERIMENTAL.

Reagents.—Pure crystallized ferrous ammonium sulfate, whose iron content had been determined, was employed as a primary standard in preparing the volumetric ferric iron solution and in standardizing the titanous chloride solution. Freshly prepared 0.1 *N* sodium thiosulfate, standardized by titration against standard dichromate solution, served as the basic volumetric reagent in the British Pharmacopœial assay. Other reagents were prepared from C.P. chemicals as described in the three procedures.

Samples.—Ten samples of wormseed oil were selected for examination. These oils varied in age from eight years to about six months. Five samples were the so-called normal oils, the other five were produced by redistillation of the condensed steam employed in the distillation of the normal product and are termed by the producers "high test" oils. Their ascaridole content usually falls between 85 and 99 per cent. All of these samples were authentic, having been collected from the stills at the time of production.

Procedure.—The procedures were carried out as described in the three aforementioned texts, little or no difficulty being encountered. By employing a salt-ice mixture the temperature of −3° C. required for reaction in the British Pharmacopœial method is readily reached and maintained. Five blanks run on the reagents at different times during this assay varied from 0.35 cc. to 0.62 cc. with an average of 0.46 cc. 0.1 *N* sodium thiosulfate. This variation is one of the weak points of the British method. In the U. S. P. procedure a period of one-half hour was permitted to elapse for the separation of the oily and aqueous layers. The A. O. A. C. method presents no difficulties once the unstable titanous chloride solution has been standardized and properly preserved from access of air. The results obtained by each method together with specific gravity and solubility data are presented in Table I.

TABLE I.

Lab. No.	Date of Distillation.	Type of Oil.	Specific Gravity.	Solubility 70% Alcohol.	A. O. A. C.	B. P.	U. S. P.
1-996	1931	Hi Test	...	1.5	88.8	88.8	97.5
1-997	1931	Normal	0.991	2.0	73.9	73.4	69.4
3-1417	1933	Normal	0.990	2.0	73.4	75.7	70.9
3-1430	1933	Hi Test	1.007	1.5	93.9	90.0	100.0
5-1264	1935	Hi Test	1.007	1.5	93.6	91.9	100.0
5-1267	1935	Normal	0.978	2.0	66.8	68.2	55.1
8-1213	1938	Hi Test	1.002	1.5	90.7	88.8	100.0
8-1214	1938	Normal	0.989	1.5	77.0	77.4	67.1
8-1216	1938	Normal	0.990	2.0	78.3	79.8	71.6
8-1217	1938	Hi Test	1.007	1.5	97.1	93.0	100.0

An inspection of these data show that results obtained by either the A. O. A. C. or B. P. method are fairly concordant and that each differs considerably from those obtained by the U. S. P. process. Values obtained by this last-named assay are higher than the other two in the high per-

centage range and distinctly lower in the normal percentage range. It, therefore, becomes necessary in reporting wormseed oil data to specify the analytical procedure employed.

A comparison of the analyses of these oils, made just after distillation, with the recently prepared data reveals some interesting facts. Apparently specific gravity and alcohol solubility increase with age while there is a distinct loss in ascaridole when determined by the A. O. A. C. method. Although aging modifies these three factors the correlation existing among them (13) remains unaffected. The values obtained in the original and recent analyses are presented in Table II.

TABLE II.

Lab. No.	Date of Distillation.	Type of Oil.	Specific Gravity. Early.	Specific Gravity. Recent.	Solubility 70% Alcohol. Early.	Solubility 70% Alcohol. Recent.	Ascaridole (A. O. A. C.). Early.	Ascaridole (A. O. A. C.). Recent.
1-996	1931	Hi Test	1.001	...	1.5	1.5	98.8	88.8
1-997	1931	Normal	0.966	0.991	4.0	1.5	84.3	73.9
3-1417	1933	Normal	0.966	0.990	5.0	2.0	79.6	73.4
3-1430	1933	Hi Test	1.001	1.007	2.0	1.5	97.5	93.9
5-1264	1935	Hi Test	0.999	1.007	2.0	1.5	98.4	93.6
5-1267	1935	Normal	0.951	0.978	6.0	2.0	69.9	66.8
8-1213	1938	Hi Test	0.993	1.002	1.5	1.5	95.6	90.7
8-1214	1938	Normal	0.958	0.989	5.5	1.5	81.3	77.0
8-1216	1938	Normal	0.959	0.990	5.5	2.0	82.9	78.3
8-1217	1938	Hi Test	0.994	1.007	1.5	1.5	98.5	97.1

SUMMARY.

A comparative study of the A. O. A. C., B. P. and U. S. P. assays for wormseed oil made upon authentic samples shows that the two former methods yield results concordant with each other but at variance with those obtained by the U. S. P. procedure.

Although aging of wormseed oil results in an increase in density and alcohol-solubility and a decrease in ascaridole, the general relationship existing among these factors remains unchanged.

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On September 15, 1939, the Winthrop Chemical Company, Inc., opened a new Professional Service Office in Baltimore, Maryland, Suite 1626, Baltimore Trust Building. This office will be in charge of Mr. E. E. Dungan.

Druggists, physicians, dentists and hospitals located in Maryland, Delaware, Virginia, the District of Columbia, and sections of New Jersey, Pennsylvania and North Carolina, are cordially invited to avail themselves of the facilities of this office. All information concerning the scientific and clinical phases of Winthrop products is obtainable at the Baltimore office within a minimum interval of time.