A STUDY OF THE FACTORS INFLUENCING THE YIELD OF ASCARIDOLE IN CHENOPODIUM AMBROSIOIDES L. var. ANTHELMINTICUM

BY

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TABLE OF CONTENTS

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INTRODUCTION AND GENERAL HISTORY

The oil extracted from Chenopodium plants known as Oleum Chenopodii or more commonly as American Wormseed Oil has been used extensively as a vermifuge for more than a hundred years. According to Pauly,[1] who has made a most intensive survey of the literature, the American Indians were acquainted with the antholmintic properties of the plant, using its juices as a vermifuge. Early settlers in this country soon became familiar with the drug and its uses.

By 1820 the oil had acquired sufficient importance as to be adopted by the U. S. Pharmacopeia, and it has been listed as an official drug since that time.

Pauly[1] found evidence of importation of the oil into Germany from Baltimore as early as 1823. However, its use abroad must have declined since it was reintroduced into Germany in 1881.

Expanding use of the drug in this country was interrupted by the introduction of inferior grades of oils to the market. Many early writers believed that these undesirable oils were the products of Western farmers who

were attempting a cultivation of wormseed plants. These Western oils fell into disrepute with the medical profession and this may have affected the entire industry.

According to Hall and Migdor[2], chenopodium oil was introduced into veterinary medicine by the French in 1896, although there was undoubtedly some used previous to that time.

Bruening, in 1906, made the first recorded pharmacological study of the oil on Ascaris lumbricoides. He found the oil to be quite effective in killing the worm. The main constituent of the oil was found to give analagous results and was called ascaridole. Many other investigators studied the pharmacology of the oil in succeeding years. It was generally accepted that ascaridole is the active principle of the oil, although this was questioned by Hall and Hamilton[3]. However, the work of Smillie and Pessoa[4] seems to have definitely proved that ascaridole is the essential constituent.

Meanwhile the anthelmintic properties of the oil were tested on other worm infestations. The Rockefeller Sanitary Commission and the International Health Board united in 1915 in an active campaign against hookworm infestations. Among the many remedies that had been used, thymol was preferred until 1917. The supply of thymol was affected by the World War and in an intensive search for a substitute, chenopodium oil was tested in hospitals and field studies. The results obtained from these tests conducted in the Orient and also in Brazil indicated that chenopodium oil was slightly

more efficient than thymol, cheaper and easier to administer. According to Ashford[5] 13,000 cases were treated in the Orient and 73% were cured in two administrations.

The same author records that although carbon tetrachloride, introduced by Hall in 1921, is increasing in use for hookworm treatments, it is found to be most felicitous when mixed with chenopodium oil. The use of halogenated hydrocarbons has also displaced it to some extent in veterinary medicine, yet it remains as a most valuable weapon to combat certain parasites common to animal.

A member of the Rockefeller Institute remarked to Pauly[1] that chenopodium oil will never reach its maximum use until a standard oil, or preferably pure ascaridole, is produced on a commercial scale.

PART I

THE DETERMINATION OF ASCARIDOLE IN OIL OF CHENOPODIUM

The necessity of finding an accurate and convenient method for the determination of ascaridole in Chenopodium oils has been recognized by pharmacologists for years. Medical literature contains numerous references to cases of poisoning produced by administration of the drug and in most instances it is thought that overdosages was the cause. Since ascaridole is known to be the essential constituent oil and since it is subject to deterioration, the exact quantity present should be known before administration. In 1927[6] the League of Nations considered the problem to be of sufficient importance as to request two German chemists to make a careful study of some of the suggested assay methods.

Possibly the first quantitative method was that suggested by Wirth[7] in 1920. A solution of 50% potassium hydroxide in 50% aldehyde free alcohol was used as reagents for a colorimetric assay. Langer[8] in 1921 proposed another colorimetric method using phenolphthalein. An assay based upon the specific gravity or optical rotation of the oil was suggested by Parry[9] at about the same time. It was assumed that no change occurs when ascaridole is mixed with the hydrocarbon fraction. Nelson[10] remarked that

any method based upon physical constants is unreliable, since ascaridole is subject to deterioration upon standing and the physical constants are not changed. Munch and Heindollar[11] have recently published results showing good agreement between specific gravity and ascaridole content of oils.

The U. S. Pharmacopoeia adopted as official a method devised by Nelson[12] which takes advantage of the solubility of ascaridole in 60% acetic acid, the hydrocarbon fraction being insoluble. Paget[13] pointed out that this method is unreliable since ascaridole is readily converted by steam into its glycol and glycol anhydride, both of which are soluble in the acetic acid solution, as is ascaridole, and that they would be determined as such. The present author further showed that in the mixture a partition effect occurs since the ascaridole is soluble both in the acetic acid and in the hydrocarbon fraction.

Paget[13] introduced a titration method using dilute $TiCl_3$ solutions as a reducing agent and by use of an empirical factor he was able to obtain reproducible results. This method will be discussed later in more detail.

Knaffl-Lenz and Hofmann[6], at the request of the League of Nations, studied the Paget method and found that the empirical factor employed was affected by the concentration of HCl used in the $TiCl_3$ solutions. These results

were confirmed by a previous study, conducted in the local laboratory, in which it was pointed out that the variations are slight unless an enormous excess of HCl is added.[14]

A thorough study of biological assay methods made by Knaffl-Lenz and Hofmann[6] on mice, worms and fish convinced them that such methods could not be used. Munch and Reindollar[15] were likewise unable to obtain satisfactory results from biological assay methods.

Knaffl-Lenz and Hofmann[6] then proposed a colorimetric assay based upon the red color produced by the action of concentrated HCl on alcoholic solutions of ascaridole. This method will be discussed later.

A new iodimetric method was offered by Cocking and Hymas[16], which will also be described in some detail.

At the time the present study was undertaken the Paget method was thought to be the most accurate and useful one. Details of the method are: 10 cc. of an approximately 1% solution of chenopodium oil in 95% ethyl alcohol is introduced into a flask and about 50 cc. of 0.02 N $TiCl_3$ solution (prepared according to Knecht and Hibbard) is added while keeping the contents of the flask in an atmosphere of carbon dioxide. The flask is then stoppered with a Bunsen valve and heated two minutes to boiling and the contents titrated back in a carbon dioxide atmosphere with ferric alum using 1 cc. of 5%

potassium thiocyanate as an indicator. Paget found that 1 gram of ascaridole would reduce 1.277 grams of titanium trichloride.

This method was thoroughly tested on oils prepared by admixture of weighed amounts of ascaridole and cymene and satisfactory results were obtained. The results were submitted in a report made to the Association of Official Agricultural Chemists in 1930[4]. Further confirmation of the applicability and accuracy of the method was requested and the following experiments were made. Some of the results of these latter experiments were included in a final report made to the A. O. A. C. in 1931[17], at which time the method was adopted as an official assay.

A Study of the Paget Assay

Effect of Adulterants

In order to test the reliability of the Paget assay on adulterated oils the following solutions were prepared: Samples 1 and 2 were made by mixing a commercial oil with carbon tetrachloride. The commercial oil contained 90% ascaridole as determined by both the Paget and Nelson methods. Sample 3 was a solution of the stock oil and cineol and 4 was composed of stock oil and 95% ethyl alcohol. A comparison was made of results obtained by

both the Paget and Nelson assays. The results are listed in Table I.

TABLE I.

Ascaridole found by Nelson and Paget Assays

	Percent of Ascaridole			
Assay M	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
Nelson	43.0	65.0	60.5	61.0
Paget #I	44.9	66.7	60.9	62.5
#II	44.4	66.1	59.8	62.9
#III	44.3	68.3	60.0	63.1
Paget Av.	44.5	67.0	60.4	62.8
As made	45.0	67.5	60.0	63.0

In the determinations of samples 1 and 2 by the Nelson assay, which method consists of shaking 10 cc. of oil with 60% acetic acid in a 110 cc. cassia flask with the neck holding 10 cc. graduated in tenths and reading the volume of the undissolved (non ascaridole ?)fraction after standing, it was necessary to invert the flask in order to read the volumes of undissolved oil. The carbon tetrachloride mixture has a gravity greater than that of the acetic acid solution.

It is obvious that the Nelson assay fails to discriminate between ascaridole and other adulterants soluble in the acetic acid solution. Schimmel and Company have frequently found cineol present in chenopodium oils and a Maryland buyer has personally reported the presence of as

much as 10% alcohol in oils handled.

The Effect of Concentration of $TiCl_3$

Paget had found that the empirical factor proposed for this assay was influenced by the concentration of titanium trichloride solutions used for titration. More ascaridole was reduced by using 0.1 N than by the specified 0.02 N solutions, the difference amounting to 10% for pure ascaridole. Since the concentration of commercial titanous chloride solutions used for preparation of the standard reagent is variable (in two instances materials purchased as 15% $TiCl_3$ solutions were found to contain more than 30%) and since the standard solution may change on long standing, it was desirable to ascertain if the assays would be appreciably affected by variations of concentration. The results in Table II were obtained by titrating samples of pure ascaridole which had been redistilled five times, and having the following physical constants: boiling point 85°C at 8 mm., optical rotation = -2°, refractive index = 1.4745 and specific gravity 1.0029 at 20°/20°C.

The excess of $TiCl_3$ present in individual titrations was kept approximately constant, the maximum deviation being 2.5 milligrams per 10 milligrams of ascaridole present in the aliquots.

Effect of Concentration of $TiCl_3$ on the Determination of Ascaridole

Number of Determination.	Normality of $TiCl_3$	Percent of Ascaridole determined	Percent of Ascaridole present	% Error
Average of 3	0.032	99.2	100.0	-.8
Average of 9	0.010	100.3	100.0	+.3
Average of 7	0.009	99.6	100.0	-.4

These values indicate that with these limits the concentration of the $TiCl_3$ used has little effect on the assay of ascaridole, the maximum deviation amounting to less than 1%.

The Effect of an Excess of $TiCl_3$

It was thought that the excess of $TiCl_3$ solution used in titration might have an influence on the results obtained by this assay. Accordingly a series of alcoholic solutions containing varying amounts of ascaridole were prepared. The same volume of $TiCl_3$ solution was added to 10 cc. aliquots of each sample and the assays completed in the usual manner. Later two other alcoholic solutions containing approximately 1 gram and 0.1 gram of ascaridole per 100 cc. were titrated using different volumes of the titanium solution in each instance. The data listed in Table III are those obtained from the 10 cc. aliquots used.

TABLE III

The Effect of an Excess of $TiCl_3$ on the Determination of Ascaridole

Sample No.	Normality of $TiCl_3$	cc. of $TiCl_3$	Gms. of $TiCl_3$ in excess	Gms. of ascaridole determined	Gms. of ascaridole present	% error
1	0.0370	40.0	0.1662	0.0104	0.0108	-3.7
2	"	"	.1533	.0208	.0205	+1.5
3	"	"	.1387	.0318	.0322	-1.2
4	"	"	.1241	.0435	.0422	+3.1
5	"	"	.1073	.0566	.0558	+1.4
6	"	"	.0818	.0640	.0637	+0.5
7	"	"	.0708	.0736	.0751	-2.0
8	"	"	.0657	.0725	.0798	-0.9
9	"	"	.0474	.0900	.0943	-0.5
10	0.0410	20	.0343	.0978	.0981	-0.3
11	"	30	.1110	.1005	.0981	+2.4
12	"	40	.1840	.1060	.0981	+8.1
13	0.0410	10	.0661	.0109	.0110	-0.9
14	"	20	.1467	.0105	.0110	-4.5

The data in the above table indicates that the errors are not appreciable unless a very large excess of titanium trichloride is added to the alcoholic solution of oil. The errors produced by a large excess of $TiCl_3$ added to a small amount of ascaridole (nos. 1 and 14) are not so large as those produced by addition of approximately the same excess to more concentrated ascaridole solutions (Sample No. 12).

The Influence of the Solvent

Paget suggested the use of 95% ethyl alcohol as a solvent for this assay since both the oil and the standard titanium solution are soluble in it. He showed that

approximate results could be obtained if no solvent was used, the error amounting to about 8% for pure ascaridole.

Since ethyl alcohol had been suggested as a suitable solvent for extraction of chenopodium oil from the herb it was necessary to determine if it would affect the results appreciably. Varying amounts of 95% alcohol were added to the same volume of a titanium trichloride solution and the solutions heated for two minutes to boiling and titrated back with ferric alum as usual. The number of grams of $TiCl_3$ "oxidized" by the alcohol in each instance is presented in the table below.

Volume of 95% ethyl alcohol (cc)	Total amount of $TiCl_3$ removed (grams)	Amount of $TiCl_3$ removed per cc. of alcohol.(gms.)
10	.0016	.0002
20	.0034	.0002
40	.0069	.0002
100	.0307	.0003

The effect of the alcohol upon the results obtained by the usual assay of oils is very small since slightly less than 10 cc. of alcohol is present in the specified 10 cc. aliquots of a 1% solution of oil. This effect may be eliminated by the use of blank determinations. In titration of large volumes of alcoholic solutions containing small amounts of ascaridole this correction for the influence of the solvent must be made.

The Determination of Small Amounts of Ascaridole in Aqueous Solutions

In succeeding studies it was desirable to extract chenopodium oils from the plant by steam distillation. The limited capacity of the still used, and the resulting small amounts of oil in the distillate would not permit a successful separation of the oil for analysis in most instances (it was necessary to determine if the small amounts of ascaridole on the aqueous distillates could be accurately assayed.)

A sample of pure ascaridole weighing 1.237 grams was made up to volume with distilled water in a 100 cc. volumetric flask. After violent shaking 10 cc. aliquots were quickly withdrawn with a pipette, the pipette allowed to drain into the titrating flask, rinsed with 10 cc. of 95% ethyl alcohol and the contents of the flask titrated as usual. The amount of ascaridole found to be present in the 10 cc. aliquots was 0.1250 and 0.1248 grams respectively or showing an average error of + 1.07%.

Another sample containing 11.004 grams of ascaridole in a liter of aqueous solution was prepared and, after shaking, a 10 cc. aliquot was weighed in a 100 cc. volumetric flask, made up to volume with 95% ethyl alcohol and 10 cc. aliquots of this alcoholic solution titrated

as usual. The amount of ascaridole found in two aliquots was 0.0108 and 0.0111 grams respectively, representing an average error of 1.35%.

Apparently the method is applicable to the determination of small amounts of ascaridole present in water mixtures. The solubility of ascaridole was exceeded in both instances yet the method of obtaining samples of the oil-water mixture did not introduce appreciable errors.

A Study of the Knaffl-Lenz Hofmann Assay

As has been mentioned previously, Knaffl-Lenz and Hofmann[6] proposed, in 1929, a new colorimetric method for the determination of ascaridole. In this assay 1 cc. of concentrated hydrochloric acid is added to a 1% solution os oil in alcohol and the mixture allowed to stand for at least six hours. The samples are then compared in a colorimeter with a standard solution of 100% ascaridole treated in the same manner.

Determinations were made upon seven oils using this assay method. Oils 5, 6, and 7 were samples of commercial "wormseed" oils whose ascaridole content had been frequently checked by the Paget method. Oils 1, 2, 3 and 4 were samples of adulterated oils that were used in

the previous study of the Paget method. Three distinct assays were made upon each oil, the time of reaction being 6, 12 and 24 hours. It was found necessary to dilute each mixture with 5 cc. of alcohol in order to obtain a sufficient volume for use in a Dubosque colorimeter. Comparisons were made with a pure sample of ascaridole as a standard, treated in the same way as the samples. The results obtained are presented in Table IV together with the values found by using the Paget assay.

TABLE IV

Method	Time (hrs.)	Per Cent Ascaridole Found in Samples 1	2	3	4	5	6	7
Colorimeter	6	54.0	62.0	57.4	61.0	67.8	73.5	68.2
"	12	45.5	67.7	56.7	61.0	68.6	56.0	62.7
"	24	44.5	65.7	60.4	55.8	68.6	59.2	63.6
Paget (Av.of 3)	-	44.5	67.0	60.2	62.8	74.3	60.0	65.4

The results obtained by this assay show some variation in the percentage of ascaridole found. Accurate readings were difficult to obtain due to the fact that dark insoluble oil droplets were present in every mixture after standing the prescribed time.

The presence of carbon tetrachloride in samples

numbers 1 and 2 and cineol in number 3 did not influence the results obtained. The authors found that turpenol alone, of a number of adulterants tested, gave a slight added color to the solutions.

While this method has possibilities it was not suitable for use in the type of investigations to be discussed later and was given no further study. There is a possibility that acetic acid would be a better reagent than hydrochloric acid since it has been observed that a red color is produced by ascaridole upon long standing in 60% acetic acid solutions.

A Study of the Cocking Hyman Assay

Although Knaffl-Lenz and Hofmann and also Bodendorff had independently decided that an iodometric method for the determination of ascaridole was not practical, Cocking and Hymas, in 1930 proposed the following assay: 3 cc. of potassium iodide solution (83% $\frac{W}{V}$) in a stoppered tube of 60 cc. capacity, be mixed with 5 cc. of concentrated hydrochloric acid (31.8%) and 10 cc. of glacial acetic acid and immediately cooled to -3°C.(limit of 0° - 3°). After cooling, 5 cc of an approximately 5% solution of oil in 90% acetic acid is added, the tube stoppered and allowed to stand for 5 minutes. The time of standing may

be extended to 10 minutes provided the temperature does not exceed 10°C. The contents of the tube are then titrated with N/10 thiosulfate. A blank is carried out under identical conditions except that the final solution is diluted with 10 cc. of water before titrating. An empirical factor was used, the relation being such that 1 cc. of N/10 thiosulfate is equivalent to 0.00665 grams of ascaridole.

Determinations by this method were made upon the seven oils used for the colorimetric assay, and in addition, oil number 8, a sample of pure ascaridole

Preliminary tests using pure ascaridole gave consistently low results. Variation of the amount of hydrochloric acid present and the time and temperature of the reaction showed no effect. The amount of ascaridole present in the reaction mixture was changed by reducing the size of the aliquots and higher results were obtained. The results of these assays are shown in Table V.

TABLE V

Variation of Cocking Hymas Assay with Size of Aliquot

Volume of Aliquot (cc.)	Amount of Ascaridole found (%)
5 cc. (as prescribed)	89.4
3 cc	93.7
2 cc	96.8
1 cc	101.4

Since most of the oils analyzed contained less than 75% ascaridole, two assays were run on each sample using 5 cc. and 3 cc. aliquots, respectively. These results appear in Table VI, together with values obtained by the Paget assay.

TABLE VI

Comparison of the Cocking & Hymas Assay With the Paget Assay

Method	Vol. of Sample cc.	Ascaridole found in Samples (per cent) 1	2	3	4	5	6	7	8
Iodometric	5	45.0	62.6	65.6	59.1	72.4	56.6	61.7	89.4
"	3	44.6	67.9	57.4	56.8	73.6	66.3	68.0	93.7
Paget	-	44.5	67.0	60.2	62.8	74.3	59.8	65.4	100.0

The results obtained from the iodometric assay were not in good agreement with those from the Paget method. If a higher factor is employed, as might be indicated from the results obtained on pure ascaridole, the analyses of the normal oils would be invalidated. Although a criticism of the method is not warranted from these few analyses, it may be concluded quite definitely that the assay has no advantage over that proposed by Paget. In the former method the empirical factor used may be influenced in several ways. According to the authors there is evidence that three reactions may occur in sequence. There is likely a normal peroxide liberation of iodine from the acidified potassium iodide solution, followed by another unexplainable liberation

of iodine and then re-absorption of iodine after its liberation. The Paget factor seems to be affected only by a marked change in the concentration of hydrochloric acid of the titanium trichloride solution and by a large excess of the reagent.

The Determination of Ascaridole by Specific Gravity

Parry[9] outlined a method for the estimation of ascaridole in chenopodium oils based upon the assumption that the specific gravity of ascaridole and an average value for the gravity of the low boiling hydrocarbon fraction are additive. Using 1.005 (25/25°C.) as the value for the specific gravity of ascaridole and 0.8466 (25/25°C.) as the mean value for that of the hydrocarbon fraction, the per cent of ascaridole in an oil would be equal to:

$$\frac{\text{Specific gravity of oil } (25/25^{\circ}\text{C}) - 0.8466}{0.1584}$$

Apparently little use has been made of this proposed assay other than as a means of comparison with the results obtained by other methods. Paget[13] noted that the method would be of no value in the determination of ascaridole in oils adulterated with liquids of high density. Nelson[10] pointed out that the results would be influenced by variations in the composition of the

hydrocarbon fraction and also by the presence of high boiling decomposition products.

Recently, Munch and Reindollar[11] made a study of the relationship between the physical constants and ascaridole content of chenopodium oils. They found good agreement between the percentages of ascaridole as calculated from the specific gravity and that determined by some other unstated method. Reference to previous articles by the authors would indicate that the method used was the Nelson assay.

Although the rather obvious discrepancies of any method based upon physical constants had been noted it was thought advisable to investigate the assay in some detail.

The specific gravities of a number of oils used in the study of Paget's method had been determined and these values, and the percentages of ascaridole calculated from them, were tabulated together with the corresponding values from the Paget assay. They are listed in the table under the caption "Original Analyses". Since most of these determinations had been made some years previous to the time of tabulation, it was thought advisable to redetermine the specific gravities and ascaridole content of these oils which had been preserved. These values are tabulated in Table VII under the headinf "Final Analyses".

TABLE VII.

Relation Between Ascaridole Content and Specific Gravity

ORIGINAL ANALYSIS					FINAL ANALYSIS			
			Per Cent of Ascaridole				Per Cent of Ascaridole	
No. of oil	Age	Sp.Gr. 25/25° C.	From Sp.Gr.	Paget Assay	Time of standing (yrs.)	Sp.Gr. 25/25° C.	From Sp.Gr.	Paget Assay
1	1 mo.	0.958	70.5	69.0	-	-	-	-
2	"	0.948	64.0	63.0	-	-	-	-
3	"	0.969	77.1	74.9	-	-	-	-
4	"	0.979	83.8	81.0	2½ "	1.009	102.5	76.7
5	"	0.968	76.8	79.9	"	0.992	91.8	77.6
6	"	0.963	73.5	77.8	"	0.971	78.4	74.1
7	"	0.984	86.9	80.9	"	0.994	93.0	79.1
8	"	0.978	82.9	77.9	"	1.020	109.4	76.5
9	"	1.004	99.6	99.7	"	1.014	105.7	93.7
10	"	0.973	79.9	60.6	"	1.003	101.9	57.9
11	4 mo.	0.960	77.0	75.4	1½ "	1.019	108.8	65.3
12	"	0.972	79.1	76.7	"	1.018	108.2	64.7
13	"	0.975	81.2	78.9	"	1.030	115.6	61.2
14	1 yr.	0.984	86.7	87.1	4½	1.000	96.8	84.7
15	1 yr.	0.978	83.1	66.8	3	.983	86.1	59.2
16	2 "	0.967	76.2	64.9	2	-	-	-
17	2 "	0.971	78.8	62.4	2	0.974	80.4	63.6
18	3 "	0.973	79.9	80.0	4½	1.011	103.8	66.2
19	?	0.962	75.0	67.8	2	0.973	79.7	60.7
20	?	0.987	88.4	77.1	2	0.991	90.1	79.1
21	?	0.962	77.7	74.0	2	0.988	89.5	82.7
22	-	-	-	79.8	3	1.027	113.9	63.3
23		-	-	90.0	3½	.996	94.3	83.3
24		-	-	94.7	2	1.006	100.6	80.0
25		-	-	63.8	2	0.948	63.9	54.4
26		-	-	97.1	2	1.005	100.0	95.6
27		-	-	78.6	3	0.998	95.5	80.6

The data presented in Table XII for original analyses of oils numbers 1 to 13 inclusive, in most instances show good agreement between the values for the ascaridole content as calculated from the specific gravity and those determined by the Paget assay. The greatest deviation occurs in the values for oil number 10 which had evidently suffered some decomposition during steam distillation. It will be noted, however, that these determinations had been made upon recently distilled oils, number 14 being the one exception. The majority of the older oils had much higher gravities than would be indicated by their ascaridole content, as shown by the data for oils numbers 15 to 18 inclusive.

A much greater divergence in the results is displayed by the data presented under the heading "Final Analysis", for determinations made after the original oils had been allowed to stand for periods of from one and a half to four and a half years. In some instances the specific gravities exceed that of pure ascaridole and consequently the calculated percentages of ascaridole are more than 100%.

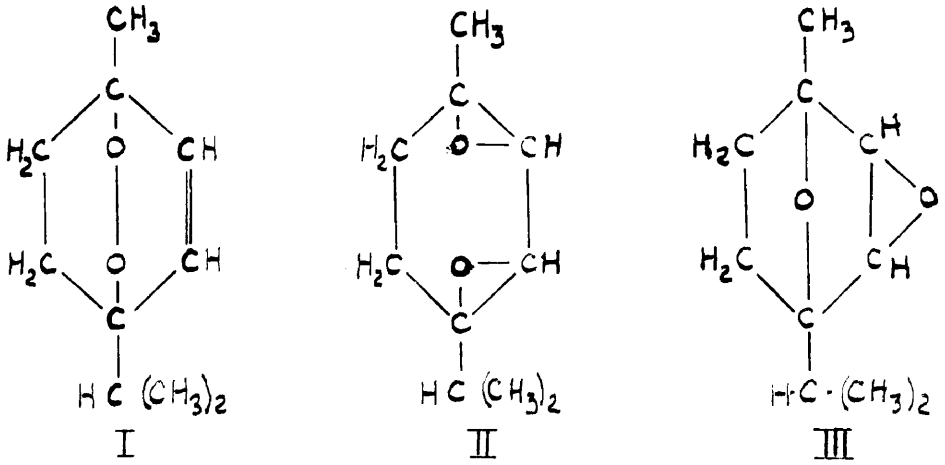
It may be concluded from these results that an estimation of ascaridole based upon the specific gravity of the oil is applicable only to freshly distilled oils,

which have not been decomposed during the distillation process. Such a decomposition would not be detectable by use of the Nelson assay, since the products formed by rearrangement are soluble in 60% acetic acid and this may explain the good agreement found by Munch and Reindollar between the ascaridole content and the specific gravity of chenopodium oils.

Determination of Oils

The fact that the specific gravities of chenopodium oils are increased with age apparently has been noted by but one investigator. Garrigues[18] observed such an increase in an oil which, judging from his data, was very deficient in ascaridole. Schimmel and Company[19] reported a decrease in the density of old oils. Nelson[10] found no significant differences in the amount of ascaridole calculated from the specific gravities of one year old oils and that determined by fractional distillation. Since the results obtained by the previous experiments are in marked contradiction to those reported by other investigators, a further study was made of this apparent decomposition. The fact that the ascaridole content of many of the oils had decreased upon standing suggested that ascaridole itself had suffered some change. Schimmel and Company[20] had reported that ascaridole rearranges upon heating it to

150°C. On the basis of a study of the glycols formed by hydration of this rearrangement product, Nelson[21] proposed the structure represented by II as being that of the anhydride formed by heating ascaridole (I).



I II III

He suggested that this so-called glycol anhydride would likely be an unstable dioxide and that it might rearrange to form a more stable compound (III).

Thomas and Dobbe[22], in heating the conversion product of this rearrangement with ammonia under pressure, obtained an oxamino compound instead of a dioxydiamino compound, which would indicate that the structure of the glycol anhydride would be (III) rather than II.

Recently Richter and Presting[23] have expressed some doubt as to the structure of the anhydride proposed by Nelson. They are not convinced that a homogenous product is obtained when ascaridole is heated, although

no separation has been made by distillation. They report the density of the product to be 1.0239 at 18/4°C., which corresponds to that found by other investigators.

Regardless of the homogeneity of the product obtained, it is certain that its specific gravity is greater than that of ascaridole. One would expect an increase in the specific gravity of chenopodium oils if there had been a conversion of ascaridole to a rearrangement product of the type found upon heating.

However, the fact that the specific gravity of oil number 13 (Table VII) exceeds that of the anhydride indicates that some change other than a rearrangement of ascaridole to the anhydride. Nelson[24] had found a small amount of dextro camphor in one sample of year old oil but was uncertain as to whether or not it had been formed ageing. Since the density of this compound is less than one, it could not be responsible for the increases in gravity noted.

One possibility would be that there was a further change of the rearrangement product of ascaridole to a glycol isolated by Nelson[24] and reported by him as having a specific gravity of 1.0981 (20/20°C.).

Since there was less than ten grams of most of the oils available, respectively, a separation of their constituents by fractional distillation was not possible.

However, it was felt that a further study of the change of specific gravities and ascaridole content of the oils might be worth while. Accordingly the increase in specific gravity and the change in ascaridole content of most of the oils was computed and tabulated in Table VIII.

A consideration of this data indicated that the increase of specific gravity in many instances was not comparable to the decrease in the concentration of ascaridole. Also oils numbers 17, 18, 20 and 21 showed an increase in the percentage of ascaridole present. One explanation of this phenomena would be a loss of low boiling hydrocarbons by volatilization during standing, since at the time of the original analyses there was no indication that the oils would be used again, and they were, for the most part, preserved in bottles stoppered with corks.

In an effort to determine if there had been a loss of hydrocarbons, fractional distillations under reduced pressure were made when there was a sufficient quantity of oil available. It was found necessary to analyze the low boiling fractions for ascaridole and to determine the amount of hydrocarbons present by difference.

The amount of low boiling material present in the remainder of the oils was determined by a modification of

the Nelson method. Five cubic centimeters of the oils were shaken with 60% acetic acid in Babcock fat testing bottles, and the volumes of the insoluble layers which separated upon standing were assumed to represent the amount of hydrocarbons present. A comparison of the values determined by this method and by fractional distillation, respectively, of the same oils, showed very good agreement.

Using these values and those for the amount of ascaridole present, the percentage of decomposition product was determined by difference. However, the percentages of the decomposition products found would not be a true indication of the extent of deterioration of the oils during standing, since there was evidence that some of the oils had undergone some change previous to the time of the original analysis.

An approximation of the amount of decomposition product was obtained by subtracting the percentage of ascaridole as determined by titration from the amount calculated from gravity. The original amount of hydrocarbons present were then calculated by difference, i.e., 100% - (percent of ascaridole + percent of decomposition product). It was then possible to estimate the change that had occurred in the composition of individual oils during the period of standing.

These final values are listed in Table VIII in

the order of their deterioration as indicated by the increase in the amount of decomposition material formed. The types of containers in which they were preserved during the period of standing are also listed in this table.

TABLE VIII

No. of oil	Orig. % Asc.	De-crease in Sp.Gr. $25/25^{\circ}$	Net change in the percentage of Ascari-dole	Hydro-carbon	Decomp. product	Time of stand-ing (yrs.)	Type of Container
13	79	.055	-18	-12	+30	1½	clear glass
11	75	.050	-10	-17	+27	"	"
12	77	.046	-12	-14	+26	"	"
8	78	.042	-2	-16	+18	2½	brown "
10	60	.035	-2	-14	+16	"	" "
19	68	.011	-7	-8	+15	2	" "
4	81	.030	-4	-10	+14	2½	" "
5	80	.024	-2	-10	+12	2½	" "
15	83	.005	-8	-2	+10	3	clear "
6	78	.008	-4	-5	+9	2½	brown "
18	60	.058	+6	-15	+9	4½	clear "
14	87	.016	-3	-7	+9	4½	brown "
9	99.7	.010	-6	-0.3	+6	2½	" "
21	74	.026	+9	-14	+5	2	" "
7	81	.010	-2	-6	+4	2½	" "
17	62	.003	+2	-3	+1	2	clear "
20	88	.004	+2	-3	+1	2	" "

Consideration of this data leaves one in doubt as to the nature of the change that occurs in the oils upon standing. The marked decrease in the percentage of hydrocarbons present in some oils suggests that the loss may have been due to

their polymerization rather than volatilization. In direct contradiction to this is the fact, previously mentioned, that four oils, numbers 17, 18, 20 and 21 displayed an increase in the percentage of ascaridole present. Also oil number 9 which was a "high test" oil from a commercial still had very little hydrocarbon originally present (0.3%) and the marked increase in its specific gravity and decrease in ascaridole content could not have been affected to any extent by a change in the amount of hydrocarbons present. Furthermore, there is no relation between the increase in specific gravity and the amount of decomposition material formed. This would indicate that the product formed is not the same in all oils.

Some evidence of this non-homogeneity of products was obtained by a determination of the specific gravities and ascaridole content of the high boiling fractions and residues left from the previous distillation of a few oils made in order to determine the amount of hydrocarbons present. The specific gravity (25/25°C.) of the decomposition product in fractions that contained ascaridole was calculated by assuming the specific gravity of ascaridole to be 1.005. These values for different oils seem to fall into two distinct groups, the range of the first being from 1.015 to 1.048 and the second being

1.030 to 1.040. The latter values approximate those reported for the specific gravity of the anhydride of ascaridole. In one instance the specific gravity of the intermediate fraction between the boiling point of the low boiling hydrocarbons and that of ascaridole had a very high specific gravity. Analysis for ascaridole and subsequent calculation of the specific gravity of the other product present showed that the value was 1.114 which would correspond to the specific gravity of the glycol isolated by Nelson. This high gravity fraction was obtained from oil number 13, which had the highest specific gravity of all of the oils.

The most likely explanation for these results is that in many instances three distinct changes has occurred, namely, decomposition or rearrangement of ascaridole, loss of hydrocarbons by volatilization and decomposition or polymerization of the hydrocarbons.

An effect of light on the decomposition of the oils is indicated by a comparison of the amount of deterioration with the type of container in which the oil had been preserved. The apparent discrepancy shown by oils numbers 17, 15 and 18 which had been kept in clear glass bottles for periods of two, three and four years respectively, and had not changed markedly, is explainable by the fact that at the time of the original analyses these oils had already deteriorated to some

extent. Oil number 15 had been exposed to light for a year and oil number 18 for three years previous to these first determinations. Oil number 17 was secured from a commercial firm and was more than two years old when received.

Possibly the most significant conclusion to be drawn from these results is that the problem warrants further study. It is planned to repeat the experiment under controlled conditions using appreciable amounts of materials and preventing any possible loss of the hydrocarbon fraction by volatilization.

SUMMARY OF PART I.

A study of the Paget Method for the determination of ascaridole in chenopodium oil demonstrated that:

1. The method may be used to determine the amount of ascaridole in oils adulterated with carbon tetrachloride, cineol or alcohol.

2. The accuracy of the method is not appreciably affected by the concentration of the titanium trichloride solution or by the use of an excess of this reagent within reasonable limits.

3. A correction must be made when a large excess of alcohol is present in the aliquots titrated.

4. Very small amounts of ascaridole in aqueous solution may be accurately determined.

The Knaffl-Lenz Hofmann Colorimetric Assay was found to be a fairly reliable method for the estimation of ascaridole. The formation of an insoluble product by action of the reagent with the oils serves to invalidate the results.

The Cocking-Hymas Iodometric Assay was found to give low values for oils of high ascaridole content. The method has no advantage over the Paget assay in regards to utility or convenience. A determination of the ascaridole content of oils by specific gravity is only reliable when freshly

distilled and undecomposed oils are used.

A marked increase in the specific gravity of oils after standing for long periods of time was noted, which is contrary to previous opinion. There was an indication of a decomposition of ascaridole in some instances, but there may also have been a polymerization of the hydrocarbons present.

The decomposition of the oils was apparently increased by exposure to light.

HISTORY OF PRODUCTION OF OIL IN MARYLAND

The origin of the chenopodium or wormseed industry in Maryland has been investigated by Mueller[25]. His source of information was an interview with a Mr. Frank Thomas of Westminster, Maryland, whose father was one of the pioneers in the development of the industry.

According to Mr. Thomas the cultivation of the plant was introduced in Carroll County, near Westminster in about 1840. The names of the original producers are unknown. Many of the older inhabitants of the district can recall the time when it was not unusual for a man to ride horseback to Baltimore drug houses, a distance of about twenty miles, with a few small bottles of wormseed oil in his pocket.

At that time the oil was obtained from the plant by cooking the herb with water in an iron soap kettle fitted with a soap stone top. After some time the oil was skimmed from the surface and poured into bottles. Later a still was developed using the same type of pot and cover but equipped with a water cooled coil. The modern steam still, which was introduced by Klee Brothers in about 1908, will be discussed later.

The growth of the industry was very slow up until recent years. Since cultivation of the weed entails

considerable labor and expense most farmers were content to cultivate but small acreages. By 1870 the total production of oil for one year was about 2000 pounds obtained from some fifty acres. By 1900 the production had increased markedly. The oil was sold by the producer or local buyer to the more important New York drug houses or brokers. Germany had become the principal foreign importer and redistributed the oil in Europe. The foreign trade collapsed in 1914 and was revived again in 1917 when other importers were found. Increasing demands for the oil during the war caused a phenomenal rise in price and an immediate response in greater production. By 1921 the average production was about 12,000 pounds. Although the price of oil receded sharply after the war, production was increased. Possibly the maximum amount of oil was obtained in 1928 when a local buyer estimated that 100,000 pounds were obtained in the district. This large production served to saturate the market and depress the prices. The following years, 1929, the acreage was reduced and a prolonged drought further decreased the oil output to about 22,000 pounds. This severe drop in production stimulated prices for a short time but in the following year the acreage was further reduced and only about 8000 pounds of oil were marketed.

Throughout its history, the industry has been confined to a rather limited area in Carroll County. The area extends from the Frederick road to Westminster and from

Sykesville to Mount Airy. Attempts have been made to grow the plant in other parts of the State, but few have been successful. These attempts have not been confined to Maryland. As noted previously, efforts have been made from time to time to cultivate the herb in the middle western states. In recent years Hogstad[26] at South Dakota produced a satisfactory oil in 1923 but was unsuccessful with a 1924 crop. Konantz[27] reported that he obtained a satisfactory oil from plants raised in Illinois, but no results were given on a second year's crop. Pauly[1] was able to produce oil from Wisconsin plants by distillation of the seeds alone, but was not able to obtain useable oils from the entire plant.

Recently successful cultivation of plants have been reported in India, Java, and the Dutch East Indies.

Since this industry has been almost entirely confined to this State and because it had increased to an appreciable size, the Agricultural Experiment Station of the University of Maryland, in 1928, decided to sponsor this study.

PART II.

A STUDY OF THE FACTORS INFLUENCING THE YIELD OF ASCARIDOLE FROM CHENOPODIUM PLANTS

In order to discuss the study made of various factors that may determine the production of ascaridole in chenopodium plants it will be necessary to briefly outline methods of cultivation practiced by the Maryland, or more particularly, the Carroll County grower.

The plants are, for the most part, grown from seed selected by the individual planter and sown in seed beds the latter part of February, or as soon as the weather conditions are favorable. It is necessary, according to the growers, to locate these beds in freshly cleared woodland in order to obtain the best results. Sometimes a type of mulch is placed over the beds and removed when the plants are large enough to need no further protection.

By the latter part of May or the first of June the plants are usually large enough to be transplanted in the fields. Most of the growers use a transplanting machine which places the plants about eighteen inches apart in rows which are three feet apart.

Fertilizers are applied in most instances, but the

mixtures used depend upon the personal opinion of the individual planter. Most of the mixtures used, however, contain a fairly high concentration of phosphorus.

The herb apparently requires about the same tillage as corn, and three or four cultivations are made during the season.

The time of harvest is determined by the maturity of the plant as judged by the color of the seeds. When about three-fourths of the seeds are dark brown or black in color, the plant is harvested by means of reconverted binders for the most part, although the herb is cut by hand by a few individual planters.

After cutting, the plants are allowed to "cure" for several days and then steam distilled.

A review of the methods used in cultivation of the chenopodium plant would indicate that the most likely problems to be considered in an effect to increase the yield of ascaridole would be the selection of seeds, the fertilization of the herb, the time of harvest and the methods of distillation. Accordingly, individual studies were made of these phases in the production of oil from the plant. Since ascaridole is the essential constituent of the oil and since it is believed that chenopodium oils will be eventually marketed at prices depending upon their ascaridole content, the results of

the following studies are expressed in terms of ascaridole and not of chenopodium oil.

The Estimation of Ascaridole in Chenopodium Plants

As a prerequisite to the study of the various factors that influence the yield of ascaridole, a satisfactory method of extracting the material from the plant had to be determined.

The previous study of the Paget assay had indicated that it was sufficiently accurate for the determination of small amounts of ascaridole in aqueous solution, and it was in a study of the efficiency of steam distillation as compared to other methods of extraction of the plant. Hock[28] had extracted small samples of ground herb with various organic solvents and, upon analysis of the extracts, had found the highest percentage of ascaridole was obtained when 95% ethyl alcohol was used.

Accordingly, ten gram samples of various parts of plant material were shaken for one hour with 200 cc. of 95% ethyl alcohol and the ascaridole content determined by titration. Duplicate samples of the material were steam distilled and the distillates assayed for ascaridole. In every instance the values obtained by alcohol extraction

were much greater than corresponding results from steam distillation. A slight difference in values might be expected but the fact that the heavy woody part of the plant, which yielded but a trace of ascaridole by steam distillation, showed an appreciable amount of ascaridole in the alcoholic extract, indicated that the alcohol was dissolving some constituent of the plant other than ascaridole, which was capable of oxidizing the titanium trichloride.

Later Pauly found appreciable amounts of potassium nitrate in alcoholic extracts of chenopodium plants. The same material was likewise isolated in alcoholic extracts of the herb by a graduate student in the local laboratory. As a matter of interest, weighed amounts of potassium nitrate were dissolved in alcohol and titrated with the titanium solutions by the method used for ascaridole. It was found that approximately three moles of $TiCl_3$ are equivalent to one mole of KNO_3. It is apparent from these results that alcohol was dissolving potassium nitrate from the plant and hence cannot be used as an extraction agent for the oil.

One hundred gram samples of finely ground, whole plant material were then extracted for twenty-four hours with 250 cc. respectively, of the solvents listed below, and aliquots titrated as usual. Since the titanium

trichloride is insoluble in both ether and petroleum ether, it was found necessary to evaporate these aliquots to a small volume and take them up in alcohol for titration with the standard solution. At the same time duplicate samples were steam distilled from a Kjeldahl flask and the aqueous distillates assayed for ascaridole. The results obtained are listed in Table IX.

TABLE IX

The Extraction of Ascaridole by Various Solvents

Solvent	Average Percent of Ascaridole
Ethyl ether	0.57
Petroleum ether	.56
Chloroform	.65
Steam distillation	.53

It was found impossible to obtain uniform results by titration of the aliquots of the chloroform extraction and the percentage given represents the average of widely diverging values. Rejecting this value, the agreement is good between the amount of ascaridole extracted by steam distillation and by other solvents. The average deviation from the mean of the values determined by steam distillation would indicate that the average value listed may be in error

to $\pm$ .02%.

In succeeding experiments it was desirable to steam distill larger quantities of herb than would be possible in the small glass apparatus. A copper still having a capacity of about five pounds of chenopodium plants was used for these experiments. Steam was obtained from a laboratory line and the pressure varied from 1 to 3 pounds per square inch.

Duplicate samples of herb grown in plots that were treated with the same fertilizer mixture and cured for the same length of time were steam distilled. Ten cubic centimeter aliquots of the distillates were quickly withdrawn from the collection bottle after thorough shaking, weighed in a 100 cc. volumetric flask and made up to volume with ethyl alcohol. Ten cc. aliquots of the alcoholic solution were then titrated as usual. From the per cent of the ascaridole found in the distillate and weight of the distillate the total amount of ascaridole present in the plant was calculated. The value obtained and the weight of the plant material distilled were used to calculate the per cent of ascaridole in the plant and thence the amount of ascaridole in the herb.

From the results obtained by these experiments the average deviation of the mean values for the amount of ascaridole in the plant was found to be 8%. Approximately

classification as a distinct variety of a definite species.

The plants cultivated in Carroll County, however, seem to be of two types. Mueller[25] remarked upon these types, noting that on one type the stems were green, while on the other they were red. Nock remarked upon two rather distinct types of plants, the most striking difference being one of size. He determined the ascaridole content of the two types and found the smaller plant contained more ascaridole. Most of the wormseed growers seemed to prefer the smaller plant believing, which usually bears more fruit, to be the superior plant.

It was thought to be worth while to attempt to confirm Nock's results.

EXPERIMENTAL

Samples of each of the two types of plants were obtained from the same fertilizer plot at College Park, allowed to cure and steam distilled. The aqueous extracts were analyzed for ascaridole and the percent of ascaridole in the herb computed both on wet and on dry basis. The following year, 1929, the experiment was repeated with plants selected from fields in Carroll County. Seeds were selected from each type of these plants and sown in separate green house beds. From the plants grown, fifty of each type were transplanted in a field on the Experiment Station grounds in June, 1930. No fertilizer was applied to these plants. At the time of maturity only six plants could be found that were typical of the smaller "variety" (hereafter designated as type B). These six plants together with six of the larger (type A) plant were cut when mature and allowed to cure for one month.

Dr. J. B. S. Norton, of the Experiment Station Staff, very kindly offered to examine specimens of each type. He found the larger plant, type A, had more erect branches and was greener than type B. The buds and flowers of type A were usually longer than wide, and the

average width of the seeds was less than 1 mm. In type B the stems were reddish, the buds and flowers wider than long and the seeds averaged 1 mm. or more in width. The distribution of glandular hairs was approximately the same in each type.

When the plants had cured three of each type was steam distilled as usual. Oil from the distillate was separated and analyzed and the ascaridole content of the residual aqueous layer likewise determined. The results in this instant are based upon the air dry weight of the plants and appear with those previously mentioned in Table X.

TABLE X

Source of Material	Plant Type	% Asc. in separated oil	Per Cent Ascaridole Wet basis	Per Cent Ascaridole Dry basis	Total Asc. (Grams)
College Park ('28)	A	-	0.54	1.79	20
College Park ('28)	B	-	0.66	2.18	21
College Park ('28)	A	-	.78	2.25	11
Woodbine ('30)	B	-	1.25	3.67	13
Woodbine ('30)	A	54	-	0.65	8
College Park ('31)	B	69	-	0.63	7

These results show that in two instances the smaller plant contained a larger percentage of ascaridole. The values obtained from the 1931 College Park plants are practically checks, the difference being less than the

experimental error. One possible explanation for these values may be the long period of curing to which they were subjected. Plants of type A do not reach maturity as soon as those of type B and since they were always cut at the same time the larger plants were less mature than the earlier. The prolonged curing of the 1931 crop may have allowed the production of ascaridole to proceed to a maximum value. If, however, the type B plants were slightly past maturity there may have been a loss of fruit and leaves due to shattering, which would account for the low yield obtained. The difference in the ascaridole content of the separated oils can not be considered significant on the basis of one determination.

Although the ascaridole content of type B plants is greater than type A, the total amount of ascaridole produced is not enhanced to any great extent due to the differences in size of the plant, as shown in the final column of Table X. However, there is a distinct advantage in handling a smaller bulk of material if the same yield of ascaridole may be obtained.

The Source of Oil in the Plant

Wirth[7] conducted the first recorded microchemical study of chenopodium plants in an effort to locate the source of oil in the herb. He found the essential oil is contained in glandular hairs, most of which are found on the upper half of the ovary. Hogstad in a more extensive study confirmed this fact but also found the hairs on the stems, especially on less mature types, and on the leaves and the embrynic flow tips. They were absent on the woody basal section as would be expected.

Kinantz[27] conducted separat steam distillations upon 100 pound samples of seeds, stalk and entire plant material. He found that no oil was present in the stalk, but obtained 438 cc. of an oil containing 85% ascaridole from the seeds, and 282 cc. of oil assaying 80% from the entire plant.

Since a method was available capable of determining with a fair degree of accuracy small amounts of ascaridole, it was decided to attempt a further investigation of the source of ascaridole, rather than oil, in the various parts of the plant. The experiment was devised to include a study of the distribution of ascaridole in the two types of plants in a further attempt to investigate the phase of

the problem.

EXPERIMENTAL

Three plants of the type A variety and four of type B, remaining from the previous study on these types, were separated into four constituents, namely, fruit, seed stems, leaves and stalk. Twenty gram samples of each constituent were steam distilled and the distillates analyzed as usual. (The amount of stem material from the type B plants was only 8 grams, which was distilled as usual. The amount of leaf material from the same plants was 2 grams and was not distilled. The value used in Table X for the percent of ascaridole is the one obtained from leaf material of type A herb.) The percent of ascaridole was calculated in each instance and multiplied by the corresponding weight of plant material. The sum of the resulting yields of ascaridole in the plant constituents was obtained for each plant type and used to calculate the total percentage of ascaridole in the plants. These values are compared to those obtained by distillation of entire herb in the previous experiment and are listed in Table XI.

TABLE XI

Distribution of Ascaridole in the Plant

Part of Plant	Type A (3 plants) Wt. of Material Gms.	Ascaridole %	Total Amt. Asc. Gms.	Type B (4 plants) Wt. of Material Gms.	Ascaridole %	Amt. Asc. in part Gms.
Stems	24	0.25	.06	8.0	0.27	.02
Leaves	33	.52	.17	2.0	.52	.01
Fruit	236	1.08	2.55	57.	1.12	.64
Stalk	221	.03	.06	40.	.03	.01
Totals	514		2.84	107		.66
Percent ascaridole in plant from summation of parts =			0.55			0.62
Percent ascaridole from distillation of entire plant =			.65			.63

These results would confirm the prevalent opinion that most of the ascaridole occurs in the fruit and that little is present in the stalk. The relatively small amounts found in the seed stems and leaves indicate that they are of little importance in the total contribution of ascaridole in the plant.

Since the differences in the ascaridole content of the fruit and stems of the two types of plants, respectively, are of no significance, the selection of a plant type having an abundance of seeds is indicated. Type B plant having 53% by weight of seeds would seem to be favored over type A.

The difference in the percent of ascaridole in plants of type A as determined by the distillation of parts of the plant and of the total herb, respectively, however, does not permit any definite conclusions to be drawn from this study.

The Time of Harvest and Curing

It is common knowledge among the wormseed growers in the Carroll County producing district that in order to obtain a marketable oil the plants must be allowed to mature before harvesting. This fact was most decidedly confirmed in a practical way by the introduction of the modern steam distillation equipment in 1908 by the Klee Brothers of near Westminster, Maryland. Mr. Henry Klee, the surviving member of the firm, relates that the first year the still was used the amount of oil obtained from the herb corresponded to an average yield of 100 pounds per acre, almost twice the usual production. The oil, however, was of low specific gravity and could not be sold. Representatives from New York drug concerns inspected the still but could offer no explanation for the low gravity. The following year the herb was allowed to grow approximately two weeks longer before harvest, whereupon less oil was obtained, but the gravity was within the specified range.

Hogstad[26], in a study of western oils, steam distilled the plants at various stages of growth throughout the season and observed that the maximum amount of oil is obtained just previous to or at the time of pollination, but that this oil is decidedly deficient in ascaridole and consisted chiefly of cymene. He also noted that air drying tended to increase the ascaridole content. Since he was never able to obtain an oil which would meet the requirements of the U. S. Pharmacopoeia it was thought advisable to investigate this apparent change in more detail.

EXPERIMENTAL

Twelve five-pound samples of chenopodium plants from the same fertilizer plot at College Park in 1928 were cut at intervals of two weeks. The samples were weighed immediately after cutting and in each instance six of them were steam distilled immediately, the other six being allowed to cure in the laboratory for four days before distillation. The distillates were analyzed for ascaridole and the results in each case were expressed in terms of percent of ascaridole based upon the wet (uncured) weight of plant material. These results are listed below in Table XII.

TABLE XII

Changes in Ascaridole Content of Plant With Age and Curing

Date of Cutting	Percent of Ascaridole based upon wet weight		Remarks
	Fresh herb	Cured herb	
Aug. 14	0.61	0.77	Early blossoming stage
28	0.73	1.35	Late blossoming stage
Sept. 11	0.58	1.34	Past maturity (likely loss due to shattering

The experiments were repeated in 1930, except that only cured plants were distilled. The results are in Table XIII.

TABLE XIII

Changes in Ascaridole Content with Age

Date of Cutting	Percent of Ascaridole based upon wet weight	Remarks
Aug. 20	0.29	Early blossoming
" 27	1.04	Late "
Sept. 4	1.18	Fully developed seeds, few dark
Sept.11	1.45	Maturity (most seeds dark)

The data from Table XII indicates that there is a decided gain in the ascaridole content of the chenopodium

plant during curing as noted by other investigators. However, previous experiments would not indicate whether this gain was due to a chemical change in the plant or to loss by volatilization of the low boiling constituents of the oil. The fact that Hogstad noted a predominance of p-cymene in the oil at the early stages of growth and suggested a synthesis of ascaridole from the cymene during the growth would not necessarily apply to the change occurring during curing. Since the ascaridole content of the plants at College Park was based upon the wet weight the results obtained could not be influenced by any loss of cymene or other low boiling compounds by volatilization during the curing process. These results would indicate that there is an actual synthesis of ascaridole during curing.

The results from both tables agree with those obtained by other investigators by indicating an increase in the ascaridole content of the herb during the period of growth. The fact that no increase was noted in the final period of the 1928 experiments is likely due to a loss by shattering in the well matured plants. The wormseed planter is cognizant of this fact and tries to cut the herb before it is completely matured.

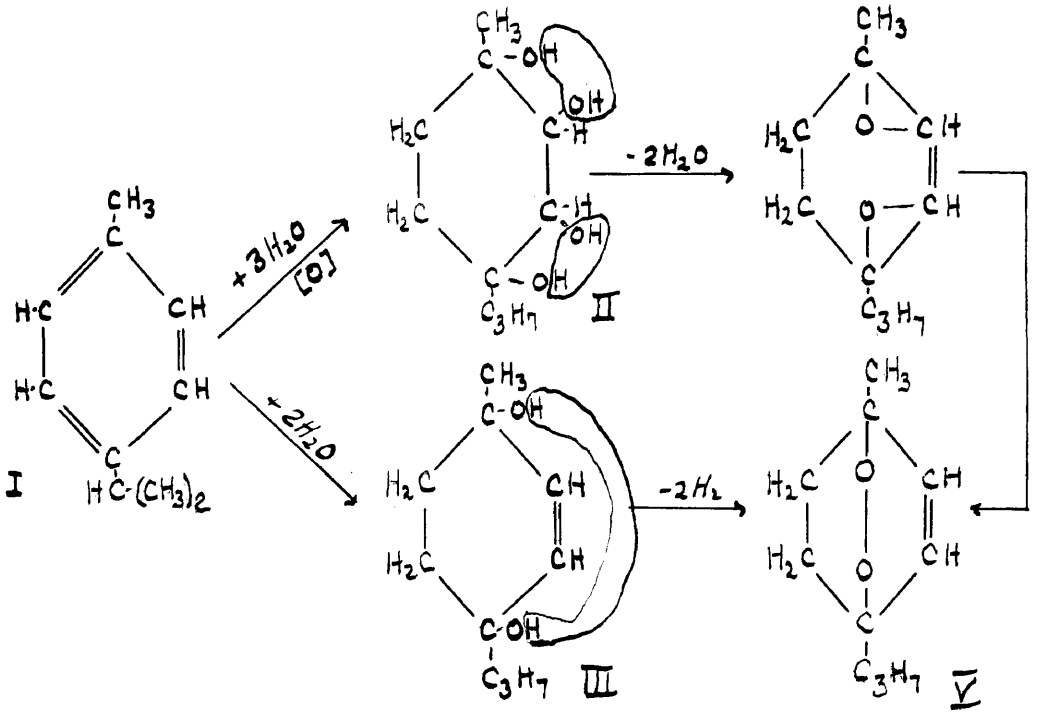
Following the suggestion of Hogstad an attempt was made to secure further evidence of a chemical change

in the oil during the growth of the plant. A commercial planter in the wormseed district of Carroll County was asked to cut a number of plants during the time of pollination and to distill the oil from them immediately after cutting. Due to a misunderstanding the herb was cut at the proper time, but allowed to cure for some four weeks before distillation. The oil obtained was of high quality, containing 70.2% ascaridole as determined by the Paget assay and 63.5% by the Nelson assay. The specific gravity of the oil was 0.963 at 25/25°C. Since no record was made of the weight of plant material cut, the quantitative aspects of this procedure are in doubt. However, the accidental experiment does indicate that the process of ascaridole synthesis in the plant either during growth or curing is likely of the same type.

Accepting the meager evidence presented that ascaridole is synthesized from cymene, one is confronted with the problem of how this synthesis might occur. Of the several products isolated from the rearrangement of ascaridole, two seem to be the most likely intermediate compounds which might be formed in the transition from cymene to ascaridole. One of these isolated by Nelson and termed by him an erythrite of ascaridole would seem to be 1,2,3,4 tetra hydroxy menthane (formula II). Analyses agreed with this formula and oxidation gave the expected

product an dihydroxy- methylisopropyladipic acid which, however, differed from previous acids of this type identified by Wallach. The other product was made by Richter and Priesting[23] by incomplete hydrogenation of ascaridole with colloidal palladium and corresponded to para 2 menthene 1,4 diol (formula III).

The addition of 6 hydrogen and 4 oxygen atoms, corresponding to 3 molecules of H_2O and one atom of oxygen to cymene (formula I) would be required to form Nelson's erthyrite (formula II). This compound might form ascaridole anhydride by loss of 2 molecules of water and then rearrange to ascaridole (formula V). If the menthene diol compound (formula III) is formed from cymene the addition of hydrogen and oxygen corresponding to 2 molecules of water would be necessary. A loss of 2 hydrogen atoms from this diol would form the peroxide.



In either event the reactions would seem to require some enzyme. In the formation of the erthyrite a enzyme of the so-called hydroxidase type would be necessary. The succeeding reactions, those of loss of water, and rearrangement respectively, would not likely require an enzyme. A hydroxidase would also be indicated for the synthesis of the menthene diol (III). However, the removal of H_2 from this compound would seem to require an additional catalyst. Of these two suggested reactions the latter seems to be the more likely one.

In the process of curing, some reaction other than simple dehydration followed by rearrangement, must certainly occur since other investigators have always obtained an oil deficient in ascaridole from unmature plants whether cured or uncured. The fact that an oil containing 70% ascaridole was obtained from very unmature plants simply by prolonged standing would indicate that mere loss of water is not a satisfactory explanation for the curing process.

FERTILIZER STUDIES

Although it has been recognized for many years that chenopodium plants require fertilizer treatment in order to produce appreciable yields of a satisfactory oil, no one seems to have investigated this problem in any detail. Schimmel and Company[27], in 1925, mention that the yield of dried seeds produced by herb grown in the Dutch East Indies had been doubled by the use of phosphate fertilizers. Dyson[28], in a brief review of the industry, states that phosphate is essential to the growth of the plant.

A survey of the Carroll County district showed that in most instances, there is no definite fertilizer treatment used. The selection of the analyses and the rate of application are quite variable among the individual planters. At the request of individual planters of the Carroll County district the University of Maryland Experiment Station sponsored an investigation of this phase of the industry. Nock[29], in 1927, conducted a series of experimental plots at Woodbine, in the Carroll County district.

In an attempt to secure additional information in regards to the effect of fertilizer treatment, the present

study was undertaken in 1928. The same general plan followed by Nock was used in these experiments.

Experimental Plots

Fertilizer plots were maintained for a period of two years upon a farm near Woodbine in Carroll County and for one year at College Park. The plants were obtained from the seed beds of an established grower, set out by machine and cultivated with the regular crop. Those at College Park were planted by hand at the specified distribution of eighteen inches apart in rows that were three feet apart. Their cultivation was identical with that used in general practice.

Within three to four weeks after planting the fertilizers were applied by hand. The experiments included four series of plots. In each of the first three series the number of units of one of the three elemental constituents of the fertilizer was increased, by increments of two, the number of units of the other two kept constant. One of the analyses, recommended by Nock as being especially felicitous to ascaridole production, having the analysis 4-8-4 was used exclusively in the fourth series, which was a study of the effect of rate of application. The ingredients used for the

analyses were; ammonium sulfate, muriate of potash and nitrate of soda.

Occasional observations were made during the growing season to determine if any marked differences in the size or maturity of the plants in the different plots could be noted.

The plants were harvested when the majority of the seeds were dark brown in color. At this stage of growth the maximum amount of ascaridole is obtained as has been discussed previously. Since the plots at Woodbine were about one twenty-fifth of an acre in size, it was not expedient to harvest and weigh all of the herb. Accordingly every fifth row was cut in each plot for samples for weighing. From the material from each row representative plants were selected for distillation. The plots at College Park were much smaller, each containing 63 plants, and the total material from the plots was weighed and samples selected as before for distillation.

The plants were stored in a light, warm laboratory where they were allowed to cure for four to five days. They were then steam distilled in the five pound copper still, previously described, until no more oil drops were observed in the distillate. Since the steam pressure

could not be adjusted very accurately, the time of distillation varied from 30 to 50 minutes. Ten cubic centimer samples of the distillate were weighed in a 100 cc volumetric flask, made up to volume with 95% ethyl alcohol and aliquote titrated in the usual manner.

This procedure was varied with College Park plots and those of Woodbine in 1929. A separation of oil from distillate was made in the former instance with separatory funnels, the oils weighed and assayed and the aqueous layer likewise analyzed. In the 1929 distillations a simple receiving device consisting of a vertical glass tube with a stopcock at the bottom and an upright side arm of small bore tubing sealed about three inches above the stopcock was used. The distillate was collected in a funnel attached to the smaller tube through which it passed into the larger tube, and the oil separated out on top. By adjusting the stopcock at the bottom of the larger tube it was possible to drain off the aqueous layer at the same ratio as the distillate was collected from the still. Separations of oil by this device were more successful as will be shown later.

The percent of ascaridole found in the distillate multiplied by the weight of distillate gave the amount of ascaridole in the samples used. The percentage of ascaridole in the plant was then determined by dividing

the weight of ascaridole obtained multiplied by a hundred, by the uncured weight of the plant.

From the number of plants cut in each plot and their total wet weight, the weight of 100 plants for each plot was computed. This method would serve to decrease any errors introduced by loss of unequal distribution of plants in any plot.

The yield of ascaridole on the basis of 100 plants was obtained by multiplying the weight of plant material by the percent of ascaridole.

A discussion of the errors involved will be reserved for individual consideration of each series of plots.

Results

The data secured from the three sets of plots are listed in Tables XIV, XV and XVI. The results obtained by a progressive increase of two units of nitrogen, phosphorus and potash respectively, are tabulated in each table in that order. The fourth series shows the effect of increasing the rate of application of the 4-8-4 analysis by increments of two hundred pounds. The results obtained by using no fertilizer on a plot (No.1) at College Park are also included in Table XIV.

In every instance the percentages of ascaridole

are calculated on the basis of wet or uncured weight of the plant. The weight of plant material is expressed in terms of the calculated weight of one hundred plants and the yields of ascaridole in pounds produced by one hundred plants.

TABLE XIV

Yield of Ascaridole from College Park Plots (1928)

Plot No.	Fertilizer Analysis	Rate of application lbs./acre	Percent Ascaridole in Plants	Weight of herb lbs.per 100 plants	Wt. of Ascaridole lbs.per 100 plants
1	-	-	0.64	236	1.5
2	2-8-4	600	0.82	217	1.8
3	4-8-4	"	0.85	238	2.0
4	6-8-4	"	0.72	254	1.8
5	4-6-4	"	1.01	230	2.3
6	4-10-4	"	0.80	261	2.1
7	4-12-4	"	0.85	260	2.2
8	4-8-2	"	0.76	266	2.0
9	4-8-6	"	0.68	286	1.9
10	4-8-8	"	0.93	214	2.0
11	4-8-10	"	1.13	170	1.9
12	4-8-4	200	0.98	159	1.6
13	4-8-4	400	1.02	168	1.7
14	4-8-4	800	0.60	171	1.0
15	4-8-4	1000	0.77	182	1.4

TABLE XV

Yield of Ascaridole from Woodbine Plots
(1928)

Plot No.	Fertilizer Analysis	Rate of application lbs./acre	Percent Ascaridole in Plants	Weight of herb lbs.per 100 plants	Wt. of Ascaridole lbs.per 100 plants
2	2-8-4	600	1.45	223	3.2
3	4-8-4	"	0.90	259	2.3
4	6-8-4	"	1.13	267	3.0
5	4-6-4	"	1.71	261	4.5
6	4-10-4	"	1.10	325	3.6
7	4-12-4	"	1.45	293	4.3
8	4-8-2	"	0.88	254	2.2
9	4-8-6	"	0.77	258	2.0
10	4-8-8	"	1.10	304	3.3
11	4-8-10	"	1.20	303	3.6
12	4-8-4	200	1.54	245	3.7
13	4-8-4	400	1.37	289	4.0
14	4-8-4	800	1.50	266	4.0
15	4-8-4	1000	1.64	306	5.0

TABLE XVI

Yield of Ascaridole from Woodbine Plots
(1929)

Plot No.	Fertilizer Analysis	Rate of application lbs./acre	Percent Ascaridole in Plants	Weight of herb lbs.per 100 plants	Wt. of Ascaridole lbs.per 100 plants
2	2-8-4	600	0.25	63	0.16
3	4-8-4	"	.34	92	.31
4	6-8-4	"	.36	57	.20
5	4-6-4	"	.44	101	.44
6	4-10-4	"	.36	121	.44
7	4-12-4	"	.36	129	.46
8	4-8-2	"	.36	115	.41
9	4-8-6	"	.36	131	.41
10	4-8-8	"	.38	136	.52
11	4-8-10	"	.51	110	.56
12	4-8-4	200	.47	80	.38
13	4-8-4	400	.44	97	.43
14	4-8-4	800	.42	90	.38
15	4-8-4	1000	.38	126	.48

DISCUSSION OF RESULTS

The Effect of Fertilizer Treatment

Consideration of the data presented in Table XIV shows that the unfertilized plot (No. 1) produced an appreciable amount of plant material, but that the yield of ascaridole was low by comparison to adjacent plots. The values for this plot cannot be compared to the results obtained from plots numbers 10 to 15 respectively, since they were all apparently affected by a soil difference in the field. It is obvious that increased yields of ascaridole are obtained by use of fertilizers.

The Effect of Nitrogen

A progressive increase of two units of nitrogen from 2 to 6 in the fertilizer treatment at College Park resulted in a rather uniform increase in the amount of plant material formed. Corresponding plots at Woodbine in 1928 likewise showed a definite increase in the amount of herb produced when the concentration of nitrogen was increased from 2 to 4 units, but an additional two units of nitrogen had little effect. The nitrogen series at Woodbine in 1929 was not considered since these plots were most markedly affected by a severe drought.

Approximately one-fourth of the plants were killed and the others were decidedly stunted.

The effect of nitrogen on the production of ascaridole may seem to be indefinite as shown by the variation of the results obtained from 1938 plots at Woodbine and College Park respectively. However, the differences in the yields from individual plots of this series at College Park are within the limits of experimental error and may be considered to be checks. Plots treated with two and six units of nitrogen, respectively, at Woodbine also showed no difference on the production of ascaridole.

It is concluded that an increase in the concentration of nitrogen has no effect upon the yield of ascaridole obtained.

The Effect of Phosphorus

If plot number 3, treated with a 4-8-4 analysis, is considered a member of this series, a slight increase in the amount of herb produced at College Park is noted by an increase in the number of units of phosphorus from two to four. An addition of two more units of phosphorus produced a marked increase in the yield of plant material. However, no difference was obtained when the concentration was further increased to twelve units.

Practically the same trend is found in the 1928 Woodbine series. The yield of herb from plots treated with six and eight units of phosphorus respectively was practically the same while that obtained by treatment with ten units was considerably higher. A decrease is noted in the yield of material when twelve units were applied. Omitting plot number 3 of the 1929 Woodbine series, which was affected by drought, a pronounced increase again is noted in the yield produced by application of a 4-10-4 analysis as compared to a 4-6-4 analysis.

The yields of ascaridole produced by plots in this series at College Park may be considered identical in every instance. Those from the 1928 Woodbine plots are decidedly irregular and the only conclusion possible is that phosphorus does not increase the production of ascaridole. The values obtained from plots in 1929 at Woodbine show no differences beyond the limits of errors.

Phosphorus seems to increase the production of herb but apparently does not favor the yield of ascaridole. There is a possibility that it may, by increasing the size of the plant, retard to some extent the maturity of the herb.

As has been discussed under the subject of "Time of Harvest", the stage of maturity of the plant when

harvested is a decided factor in the production of ascaridole. It had been noted throughout the growing season that plants in plots numbers 6 and 7 were always larger than those in any other and they may have matured later.

The Effect of Potash

Since two plots in this series, numbers 10 and 11, were affected by a soil difference, no interpretation of the results obtained for the production of plant material is attempted. The data from the 1928 Woodbine plots indicates that the only appreciable increase in plant material is obtained when the concentration of potash is increased from six to eight units. Values for the three lower concentrations and for the two higher, respectively, are almost identical. The results from the 1929 Woodbine series, excluding those from plots numbers 3 and 11, both of which were affected by drought, show a slight upward trend.

The only significant fact demonstrated by the data for the yields of ascaridole in the potash series at College Park is that the amount of this material produced by the plants in the "affected" soil area (plots numbers 10 and 11) was practically identical with that obtained

from plots containing a much larger amount of herb. Since the plots in this deficient soil area were treated with highly concentrated potash fertilizers, it may be assumed that this constituent of the analysis is felicitous to the production of ascaridole in the plant. This assumption is confirmed by the results obtained from the 1928 Woodbine plots. Although no significant change is shown by an increase of the concentration of potash from two units to six, a decided increase in ascaridole was obtained when two additional units were applied. A further increase of two units of potash produced no marked change. Practically the same trend is noted in the data from the 1929 Woodbine plots. At the lower concentrations the yields of ascaridole obtained were identical (excluding plot no. 3 for previously stated reasons). Although the yields are very small by comparison with the results from previous years' plots, the increase in the amount of ascaridole obtained by treatment with a 4-8-8 analysis over that obtained by use of a 4-8-6 mixture is significant. The further increase in the value for the succeeding plot in the series is within the limit of errors and not significant.

These results would indicate that potash has little effect on the production of plant material, but must

certainly favor the synthesis of ascaridole in the plant. There is a distinct upward trend shown by the values for the percentages of ascaridole in the plant for the series in each of the three sets of plots.

The Effect of Rate of Application

The data for the College Park series concerned with the rate of application of the 4-8-4 mixture is not considered because of the soil difference indicated. The results of the 1928 Woodbine plots for this series are most inconsistent since the production of plant material is increased by an increased rate of application from two hundred pounds per acre to four hundred, while two successive increments of two hundred pounds effected but a slight rise above the yield of the plot treated with the lowest rate. The highest yield, however, was obtained when a rate of one thousand pounds per acre was used. Comparable results were obtained from the 1929 Woodbine plots in that the highest yield of plant material was again produced by the plot treated with the highest rate of application.

The effect of the rate of application on the yield of ascaridole approximates its effect on the production of the herb. No appreciable difference is noted in the series

until the maximum rate of application was used. The 1929 Woodbine series show the same effect.

Summary

Nitrogen was found to be a factor in the production of plant material, but an increase in its concentration from two to six units had no effect upon the production of ascaridole. A highly concentrated phosphorus fertilizer enhanced the yield of plant material but did not increase the yield of ascaridole. Increasing the concentration of potash above six units increases to some extent the production of herb and decidedly increases the yield of ascaridole. The rate of application shows little effect in either the yield of plant material or ascaridole until used at the rate of one thousand pounds per acre.

The most practical fertilizer treatment, which is indicated by these results, would be a 4-8-8 analysis.

METHOD OF DISTILLATION

The methods employed in the distillation of chenopodium ambrosioides have been studied by several investigators. Since the inception of the modern steam distillation plant in 1908, Schimmel and Company[19] have made numerous recommendations as to the proper practice necessary to secure oils that would meet pharmaceutical requirements. They found that prolonged boiling of ascaridole with water caused a decomposition of the ascaridole.

Several investigators have contended that the poor quality of oils obtained from Western plants has been due to faulty distillation methods.

Konantz[30] remarked that the oil obtained by Wirth[7] from herb grown in Michigan was undoubtedly low in ascaridole content because of prolonged distillation with low steam pressure. Nelson[10] had found that the amount of decomposition products (as determined by fractional distillation) in oils obtained from commercial stills was not more than 2.3% if high steam pressures were employed. He recommended high steam pressures, a warm condenser and a rapid distillation.

Russell[31] obtained more oil from a rapid distillation with a warm condenser than from a slow distillation with a

cold condenser. In the former instance the oil was of a superior quality. He found no difference in the quality or yield of oil distilled from herb at pressures of from 80 to 100 pounds per square inch but low gravity oils were obtained at 40 to 60 pounds pressure.

As a result of these studies distillation methods have been fairly well standardized throughout the production district. The commercial distiller employs steam pressures of approximately 90 to 100 pounds and the average time of distillation is from fifteen to thirty minutes.

Although this phase of the chenopodium industry seemed to have been investigated most thoroughly, it was thought worthwhile to inspect the various types of stills used in the producing district and to determine if possible the efficiency of their operation.

EXPERIMENTAL

Visits were made to various distillation units during the period of operation. Since numerous descriptions and photographs of these stills appear in the literature, only a brief outline of the process will be mentioned.

The cured herb is packed tightly in iron retorts

of from 200 to 400 pound capacity. Hinged iron covers, balanced with counterweights are tightly clamped on the retorts by means of wing bolts. Steam generated from a small boiler is introduced near the bottom of the retorts and the vapors condensed by conducting them through some thirty feet of iron pipe placed horizontally in a trough and cooled with running water. The condensate is collected in various types of metal barrels of approximately 50 gallon capacity. After the barrel is fairly well filled, the oil separating out on top, a stop-cock at the bottom is opened and the water layer, in most instances, allowed to run off at a rate approximately equal to the flow of the distillate into the receiver. At least three of the stills in the district, however, are equipped so that a recovery of the oil lost in the waste liquor may be effected. In these stills the aqueous layer is collected and returned by means of a steam siphon to a retort, and redistilled. An appreciable amount of oil is secured from the waste liquor as will be shown.

Nock had reported an efficiency of 95% for one of the larger stills in the district. This result was obtained by analyzing the oil obtained by distillation and the waste liquor and assuming the total amount of ascaridole to be that available. The amount of ascaridole present in

the recovered oil divided by the total amount available would give the approximate efficiency of the still.

This experiment was repeated on the same still in 1929. A redistillation of the aqueous layer from the first distillation was made and the oil recovered was analyzed. The results are listed in Table XVII.

TABLE XVII

Lbs. of herb	Distillation	Amt. of oil separated lbs.	% Asc. in oil	Amt. Asc. separated lbs.	Amt. aq. layer lbs.	Amt. of asc. in aq. layer %	Amt. Asc. in aq. layer lbs.
3690	1st	44	65	29	498	0.30	15
-	2nd	7	86	6	82	.65	5
	Total	51		35			

From data in the above table the amount of ascaridole available in the herb can be obtained by two methods. By the first the amount of ascaridole in the separated oil after the first distillation (29 pounds) plus the ascaridole found in the water layer (15 pounds) would equal to 44 pounds, or by the second method the total ascaridole recovered by both distillations (35 pounds) plus the ascaridole in the residue from the second distillation (5 pounds) would be equal to 40 pounds. It is thought that

the most significant error in the first method was in the value for the pounds of distillate which was obtained by calculation from an estimated volume. The volume of the residue from the second distillations could be determined with much greater accuracy. The most likely source of error in the second method would be due to deterioration of the oil, which will be discussed below. The efficiency, therefore, was calculated on the basis of the average of the above two values (42 pounds) and was equal to 83%.

An additional check of the efficiency was obtained by distilling a representative sample of the herb used in the above experiment in the small laboratory still and assaying the distillate. The analysis showed the plant to contain 1.08% ascaridole which multiplied by the weight of herb used in the large still (3690 pounds) would indicate that 40 pounds of ascaridole was available. This would indicate an efficiency of 87% for the large still.

A number of other stills in the district were visited and samples of oils and the discarded aqueous layer obtained, in most instances. The results are expressed in terms of percent of ascaridole found, listed below.

TABLE XVIII.

Year	Still	Amount of Ascaridole (percent) in Oil from 1st distillation	Water layer from 1st distillation	Oil from 2nd distillation	Water layer from 2nd distillation
1928	A	65	0.30	86.	0.65
1931	A	-	-	95.	-
1930	B	81	.51	99.7	0.31
1931	C	-	-	98.0	-
1931	C	-	-	96.2	-
1929	D	77	.26		
1929	E	78	.36		
1929	F	64	.30		

The most significant data in this table is that expressed for the ascaridole content of oils recovered by redistillation. In four instances practically pure ascaridole was obtained. Munch and Reindollar[30] in 1931 remarked upon the high quality of such oils stating that they are termed "high test" oil of wormseed.

At about the same time Pauly[1], as a result of laboratory experiments, had found that practically pure ascaridole was obtained by redistillation of waste liquors from distillation. He refluxed pure ascaridole with water for 8 hours and noted that the deterioration amounted to only 11 percent. However, since one-half of his material was lost by escape of vapors from the condenser, these results may not be reliable. He contended that the main factor in distillation is not the decomposition of ascaridole

by steam but its loss due to its solubility in water. He found the solubility of ascaridole at room temperature to be about 0.3 grams per 100 cc.

The values in the above table for ascaridole content of the aqueous layers seem to support Pauly's conclusion since in most instances they approximate the solubility of ascaridole in water. However, the oil obtained by redistillation for still A in 1928, had evidently decomposed appreciably, since it assayed 100% ascaridole by the Nelson method (it was completely soluble in 60% acetic acid). The specific gravity at 25/25°C was 1.007, indicating that very little low boiling constituents were present. The sample of oil from still F likewise had suffered decomposition since its specific gravity was 0.973 indicating an 80% ascaridole content. The specific gravity corresponded to the ascaridole content of all of the other oils.

The three large stills, A, B, and C, are quite similar in their general plan except that the retorts of A are exposed to the air, while those of B and C are embedded. The retort used for redistillation, however, is above the surface in each instance. It was thought that possibly the oils in still A would require longer exposure to steam due to loss of heat by radiation through the walls of the retort and that this might account for the deterioration

noted in the redistilled 1928 oil. Although the oil from the first distillation showed no deterioration, yet it may have been in an unstable condition due to heat. This conclusion was invalidated by the very fine oil obtained the succeeding year. No significant differences in the operation of the stills were observed.

The two values listed for recovered oils from still C represent samples obtained from the ends of the condenser by rapid and slow distillation respectively. The oil obtained by rapid distillation contained 98% ascaridole. It was, however, reddish in color likely due to the presence of iron oxide dissolved from the retort. Less rapid distillation resulted in a slight drop in ascaridole content.

These results show that an appreciable recovery of ascaridole is affected by redistillation of the waste liquors from the first distillation. The efficiency of a still equipped for this recovery of oil was found to be approximately 85%.

Analysis of the discarded water layers from several stills showed that the amount of ascaridole lost is practically the same in all instances and corresponds to the reported solubility of ascaridole.

SUMMARY OF PART II

The ascaridole content of two types of chenopodium plants cultivated in Carroll County was determined. The smaller type of plant was found to yield the higher percentage of ascaridole. However, this may be due to a difference in the rate of maturity of the plants.

A determination of the ascaridole content of constituent parts of the herb was made. The greatest percentage of ascaridole was found to be present in the fruit.

An increase in the ascaridole content of plants after curing was noted. The maximum amount of ascaridole was found in mature plants. A suggested mechanism for the synthesis of ascaridole in the plant was made.

A study was made of the effect of fertilizer treatment upon the yield of ascaridole in chenopodium plants. An increased concentration of nitrogen improved the yield of plant material to some extent but did not increase the production of ascaridole. A marked increase in plant material was obtained by the use of concentrated phosphorus analyses, but the yield of ascaridole was not affected. High concentrations of potash increased both the yields of ascaridole and plant material. A 4-8-8 analysis is recommended for treatment of the herb. An

increase in the rate of application of a 4-8-4 analysis showed no effect on either the production of ascaridole or of plant material until the value of one thousand pounds per acre was employed.

A study of a modern steam distillation plant in Carroll County showed its efficiency, based upon the recovery of chenopodium oil from the plant to be 85%. Redistillation of the separated water layer is recommended. The most significant loss of ascaridole was found to be due to its solubility in water. There was evidence of some decomposition of ascaridole, however, in two instances.

BIBLIOGRAPHY

1. Pauly, H. J. - Ph.D. Thesis - Univ. of Wisconsin (1931).
2. Hall and Wigdor - J. Amer. Vet. Med. Assn. 69,195 (1926).
3. Hall and Hamilton - J. Pharmacol. 11, 231 (1918).
4. Smillie and Pesson - J. Pharmacol. 24, 359 (1925).
5. Ashford, B. K. - Chemistry in Medicine, p. 655, Chemical Foundation (1928).
6. Knaffl-Lenz and Hoffman - Arch. Pharm. u. Ber. 267, 117 (1929).
7. Wirth, E. H. - J. Amer. Pharm. Assn. 9, 127 (1920).
8. Langer, A. - Pharm. Weekblad, 58, 1392 (1921).
9. Parry, C. F. - Chemistry of the Essential Oils, Vol. I. p. 535 (1921).
10. Nelson, E. K. - J. A. C. S. 42, 1207 (1920).
11. Munch and Reindollar - J. Amer. Pharm. Assn. 20, 203. (1931).
12. Nelson, E. K. - J. Amer. Pharm. Assn. 10, 836 (1921).
13. Paget, H. - Analyst, 51, 170 (1926).
14. Broughton and Weiland - J. Assn. Off. Agr. Chem. 13, 334 (1930).
15. Munch - Bioassays, Williams and Wilkins, Baltimore (1931).
16. Cocking and Hymas - Analyst 55,180 (1930).
17. Broughton, L. B. - J. Assn. Off. Agr. Chem. 14, 341 (1931).
18. Garrigues, S. S. - Amer. J. Pharm. 26, 404 (1854).
19. Schimmel and Co. - Annual Reports, April (1908).
20. Schimmel and Co. - Annual Reports, April (1908).
21. Nelson, E. K. - J. A. C. S. 35, 84 (1913).

22. Thoms and Dobke - Summ. in Pharm. Zentralk. 71, 601 (1930).

23. Richter and Presting - Ber. 64, 878 (1931).

24. Nelson, E. K. - J. A. C. S. 33, 1404 (1911).

25. Mueller, M.R. - Travel Reports, Bureau of Plant Industry (1919).

26. Hogstad, A. - Amer. J. Pharm. 96, 809 (1924).

27. Schimmel and Co. - Annual Reports (1925).

28. Dyson, G. N. - Perf. and Ess. Oil Rec. 21, 293 (1930).

29. Nock, A. - Master's Thesis, Univ. of Md. (1928).

30. Russell, G. A. - J. Amer. Pharm. Assn. 11, 255 (1922).

31. Munch and Reindollar - J. Amer. Pharm. Assn. 20, 443 (1931).

REPORT ON CHENOPODIUM OIL.

THE DETERMINATION OF ASCARIDOLE IN CHENOPODIUM OIL.

By L. B. BROUGHTON, *Associate Referee*, and G. S. WEILAND (Chemistry Department, University of Maryland, College Park, Md.).

Chenopodium or American wormseed oil has acquired special importance during the last ten years from its use in the campaign against hookworm.

It is now known from the work of Schimmel & Co.[1] and Nelson[2] that the chief components of the oil are (a) ascaridole (Formula I), which is present to the extent of 60–75 per cent; and (b) a mixture of terpenes with *p*-cymene, *l*-limonene and probably *a*-terpinene, collectively known as the hydrocarbon fraction.

The work of Smillie and Pessoa[3] no longer leaves any doubt that the organic peroxide ascaridole is the sole component of the oil that exhibits anthelminthic action against hookworm and roundworm, the parasites for which the oil is generally used.

Since the establishment of this fact, many attempts have been made to develop a means of ascertaining the quantity of ascaridole present in an oil. Color reactions that take place on heating chenopodium oil with other substances have been described and recommended for qualitative information. Wirth[4], in 1920, stated that a 50 per cent solution of potassium hydroxide in 50 per cent aldehyde-free alcohol was suitable for a microchemical reagent for chenopodium oils. Langer[5], in 1921, proposed the use of phenolphthalein, taking advantage of the red color produced. As a quantitative measurement, this color reaction is only fairly satisfactory. The intensity of the red coloration is proportional to the ascaridole content only if it is heated uniformly at a temperature of 155°C. With oils of at least 60 per cent ascaridole content, the experimental conditions are easy to check, but not with those of lower content. The lower boiling portions prevent the heating of the oil to this temperature in a given time, so that the red color is not apparent at first, and if heating is continued the lower fractions boil off. Knaffl-Lenz and Hofmann[6] attempted to perfect a biological method, using worms, fish and mice, with unsatisfactory results. These authors, however, have proposed a color reaction with hydrochloric acid that shows some merit.

Of the quantitative methods, two have been proposed. In 1921 Nelson[7] published an assay method based on the fact that the hydro-

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[1] Schimmel & Co., Report, April, 1908.
[2] *J. Am. Chem. Soc.*, **33**, 1404 (1911); **34**, 351 (1913); **42**, 1204 (1920).
[3] *J. Pharmacol.*, **24**, 359 (1924).
[4] *J. Am. Pharm. Assoc.*, **9**, 127 (1920).
[5] *Pharm. Ztg.*, **66**, 191 (1921).
[6] *Arch. Pharm.*, **2**, 117 (1929).
[7] *J. Am. Pharm. Assoc.*, **10**, 836 (1921).

carbon fraction of the oil is insoluble in 60 per cent acetic acid and that the ascaridole is miscible with this solvent; and in 1926 Paget[1] proposed a method, taking advantage of the oxidizing property of the organic peroxide ascaridole. It is with these two methods that this study is principally concerned.

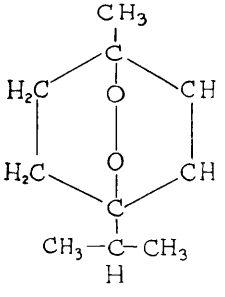
Until 1921 the United States Pharmacopeia stated that wormseed oil should be judged by its physical constants and specified that it have a specific gravity of 0.955–0.980 at 25°C. and an optical rotation of −4° to −10°, and that it be soluble in eight volumes of 70 per cent alcohol and have a refractive index of 1.4723–1.4770 at 20°C.

Nelson[2] pointed out that an estimate of the ascaridole in an oil, based on purely physical constants, was not reliable, since ascaridole is unstable under certain conditions and the oil may deteriorate without changing the physical constants. He then proposed the solubility method, details of which are as follows:

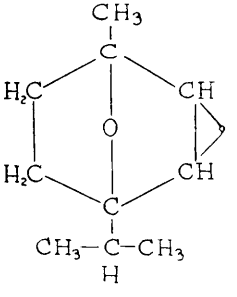
> Ten cubic centimeters of chenopodium oil is agitated thoroughly in a cassia flask, the neck of which holds 10 cc. and is graduated in tenths, with 60 per cent acetic acid. The flask is then filled to the mark with 60 per cent acetic acid and allowed to stand or carefully centrifuged. The volume of the undissolved oil deducted from ten, multiplied by ten, gives the volume percentage of ascaridole in the sample.

This method was adopted by the U. S. P. as official.

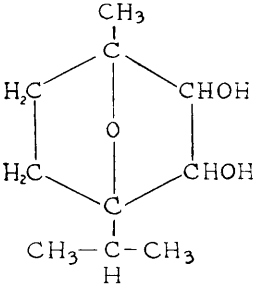
In his report on the use of the Nelson method, Paget[3] pointed out the fact that ascaridole readily reverts to the inactive form, ascaridole glycol anhydride, Formula II, by an intromolecular change and that this product is readily hydrated to form ascaridole glycol (Formula III).



FORMULA I



FORMULA II



FORMULA III

Paget further stated that he found evidence of this change taking place by the application of either dry heat or steam. Since the oil is extracted commercially by steam distillation, it seems likely that such a change would occur. The anhydride and glycol are soluble in 60 per

[1] *Analyst*, **51**, 170 (1925).
[2] *J. Am. Pharm. Assoc.*, **10**, 836 (1921).
[3] Loc. cit.

cent acetic acid and therefore would be determined as ascaridole by the Nelson method. Paget also referred to the adulteration of the oil with substances such as cineole, which is soluble in acetic acid.

Previous work by Wallach[1] demonstrated the possibility of the reduction of ascaridole with hydrogen in the presence of palladium. The reduction product was 1–4 terpin, corresponding to the addition of four atoms of hydrogen. Based on this fact, Paget sought to find a suitable reducing agent for the peroxide. He finally selected titanium trichloride ($TiCl_3$) as the most satisfactory. From the constitution of the peroxide, reduction should correspond to the addition of four atoms of hydrogen, but the amount used was about one-third of this; assuming the addition of but two hydrogens, the amount of $TiCl_3$ used was about three-fourths of the theoretical amount. Examination of the reduction products by this author did not show the presence of the anhydride or of the glycol. Hence, Paget was forced to rest the determination on the empirical factor that one gram of ascaridole is reduced by 1.2770 grams of $TiCl_3$. This figure was the mean of a number of results varying from 1.240 to 1.3040. Details of the method as described by him are as follows:

Titanous chloride solution was prepared and standardized as described by Knecht and Hibbert[2], 66 cc. of the commercial 15 per cent solution being made up to 2250 cc. One gram of chenopodium oil was diluted with 96 per cent alcohol to 100 cc. and to 10 cc. of this solution in a flask, through which a current of carbon dioxide was passing, an excess of titanous chloride was added; the flask was then closed with a Bunsen valve, and its contents were heated almost to boiling for 1 or 2 minutes. If the pale violet color of the titanous chloride disappeared, more was added to insure the presence of an excess. The formation of a precipitate of titanic oxide during heating did not interfere with the determination. About 1 cc. of 5 per cent potassium thiocyanate solution was then added, and the solution was titrated back with a standard solution of iron alum until a permanent faint red color was obtained. The amount of iron used, calculated in terms of titanous chloride, gave by difference the quantity of the titanous chloride oxidized.

STUDY OF THE PAGET METHOD.

Since the determination of ascaridole by the Paget method is based upon an empirical factor, it was first essential to verify its value. A quantity of ascaridole, designated as oil A, was obtained, redistilled five times, and that portion giving the following constants: boiling point 85°C. at 5 mm., optical rotation = −2°, refractive index = 1.4745, and specific gravity 1.0029, was selected as a standard for the factor. This oil showed a value of 100 per cent ascaridole by the Nelson method, being completely soluble in 60 per cent acetic acid at 25°C.

In a preliminary study of the standardization of this method, it was noted that the concentration and volume of hydrochloric acid, added to the titanous chloride in preparing the standard solution as recommended

[1] *Ann.*, **60**, 392 (1912).
[2] New Reduction Method in Volumetric Analysis, 1925.

by Knecht and Hibbert, affected the value of the empirical factor obtained. (This observation was noted by Knafll-Lenz and Hofmann and reported by these authors, *loc. cit.*)

To test the effect of different concentrations of hydrochloric acid used in the titanium trichloride reagent, the following solutions were prepared: Solution No. 1 contained 22 cc. of hydrochloric acid per liter of $TiCl_3$ solution; solution No. 2 was prepared as per Paget's directions, 44 cc. of concentrated hydrochloric acid per liter; and solution No. 3 contained 88 cc. per liter volume. The acid employed was 36.5 per cent. These solutions were standardized against a 1 per cent alcoholic solution of the standard oil A. The determinations were carried out following Paget's directions. The time of heating was one and one-half minutes in each case. The results are given in Table 1.

TABLE 1.

Effect of hydrochloric acid on the standardization of $TiCl_3$.

NO.	HCl PER LITER	ACID NORMALITY OF SOLUTION	NORMALITY OF $TiCl_3$	FACTOR ($TiCl_3$ OXIDIZED PER 1 GRAM OF ASCARIDOLE)
	cc.			*grams*
I	22	0.284	0.032	1.295
II	44	0.568	0.033	1.283
III	88	1.136	0.034	1.186

The data in Table 1 show very clearly the influence of concentrated hydrochloric acid on the reduction of the peroxide, and the value of the factor for $TiCl_3$. If it is assumed that two atoms of hydrogen are involved in the reduction of the compound, two molecules of titanous trichloride would be required; or for each gram of ascaridole 1.8378 grams of $TiCl_3$ would be necessary. Hydrochloric acid is necessary to stabilize the $TiCl_3$ reagent. However, it seems to change part of the ascaridole, whether by molecular rearrangement or by actual reduction, so that the empirical factor for $TiCl_3$ is dependent upon the concentration of hydrochloric acid used in preparing the reagent, as the above findings confirm.

Owing to the factor variation shown in Table 1, the preparation and standardization of the titanous chloride used in these studies were as follows:

Two hundred cubic centimeters of titanous chloride solution (15–20 per cent) was boiled with 400 cc. of concentrated hydrochloric acid (36.5 per cent) in a flask, cooled, and diluted to 9 liters. It was kept in a storage bottle, and the bottle was completely filled. The outlet of the bottle was fitted with a rubber stopper through which a piece of glass tubing was inserted. The tube was bent downwards, and a pinch cock

was placed at the rubber connection. Glass tubing formed the connection to the bottom of the buret. The stopper in the storage bottle had two holes, one leading to the top of the buret and the other to a bottle containing alkaline pyrogallol solution and from thence to a Kipp hydrogen generator. The apparatus was air-tight, and the pyrogallol solution was changed frequently to prevent oxidation of the $TiCl_3$. With these precautions a solution of approximately 0.02 *N* $TiCl_3$ was obtained.

With the standard solution adjusted as described, samples of oil A, approximately 1 cc., were introduced by a pipet into a clean, dry, weighed 100 cc. volumetric flask; the flask and oil were reweighed, and the weight of the oil was recorded. The flask containing the oil was then filled to the mark with 96 per cent alcohol, and the total weight was taken. Next, a 10 cc. aliquot was removed, and the weight was obtained by difference. The aliquot was immediately placed in the titrating flask under carbon dioxide, and the procedure described by Paget was followed. Sixteen determinations were made upon samples of the standard oil A. The results are listed in Table 2.

TABLE 2.

Value of the $TiCl_3$ factor.

SAMPLE NO.	$TiCl_3$ TO REDUCE ONE GRAM OF ASCARIDOLE
	grams
1	1.288
2	1.290
3	1.287
4	1.285
5	1.305
6	1.294
7	1.281
8	1.298
9	1.264
10	1.271
11	1.277
12	1.292
13	1.284
14	1.275
15	1.282
16	1.277
Average Factor	1.284
Paget Factor	1.277
Difference	0.007

Table 2 gives the number of grams of titanous trichloride required to reduce one gram of the standard oil A used in establishing the value of the Paget factor. The minimum value was 1.264, the maximum 1.305. The average is 1.284, which checks within 0.007 of the Paget factor; the variation is doubtless due to the difference in the strength of the hydrochloric acid used by the author of the method and that used in preparing the solution for these studies.

COMPARISON OF NELSON AND PAGET METHODS.

The factor value in the Paget method having been established, attention was directed to the accuracy of the Nelson and Paget methods in determining the percentage of ascaridole under varying conditions of concentration. For this work a quantity of commercial oil was obtained from E. W. Pickett, a producer of Carroll County, Maryland. This oil, referred to later as oil B, had a gravity of 0.9840 at 25°C. It contained 88 per cent ascaridole by the Nelson solubility assay and 91.78 per cent by the Paget method. This oil was distilled under a pressure of 8–10 mm., and the following data were obtained:

TEMPERATURE RANGE	OIL
°C.	cc.
65–95	349
94–101	538
Above 101	517

The fraction boiling between 65°–95°C., known to be largely cymene, is designated as oil C; the higher boiling fraction (above 101°C.), as oil D; and the middle fraction (94–101), known to be largely ascaridole, as oil E. In addition to these oils, oils F and G were obtained from plants grown at College Park, Maryland; H is a sample obtained from Baltimore, and I, J, and K were obtained from distillers in Carroll County, Maryland.

The percentage of ascaridole in these oils was determined by the two methods, data for which are listed in Table 3.

TABLE 3.

Ascaridole in commercial oils.

	OIL	NELSON ASSAY	PAGET ASSAY
		per cent	*per cent*
Group I	A	100.00	100.00
	E	100.00	100.00
Group II	D	98.00	93.19
	F	70.00	67.81
	G	70.00	66.81
	H	75.00	59.95
	I	70.00	68.95
	K	75.00	74.90
Group III	B	88.00	91.78
	C	35.00	45.49
	J	62.00	63.00

Oils A and E represent samples of ascaridole of the highest purity. Oil A was used to establish the value of the Paget factor. Oil E was prepared with the greatest care, and based on the factor 1.284 is 100 per

cent pure by the Paget method. Both of these oils were completely soluble in 60 per cent acetic acid, or gave 100 per cent purity by the Nelson method. The remaining oils in this list fall into two classes: Oils D, F, G, H, I, K (Group II) and B, C and J (Group III). Group II shows a higher percentage of ascaridole by the Nelson method than by the Paget assay, and the reverse is noted in Group III.

Paget pointed out that the Nelson method is based on the fact that the hydrocarbon fraction of chenopodium oil is insoluble in 60 per cent acetic acid and that the ascaridole is miscible with this reagent, but that ascaridole readily forms ascaridole glycol anhydride by an intromolecular change and that this product is readily hydrated to form ascaridole glycol, both of which are soluble in 60 per cent acetic acid. He further stated that he had found evidence of this change taking place by the application of either dry heat or steam.

Since the oils in Group II were obtained by the usual method of steam distillation of the herb, it is apparent that a fraction of the ascaridole would be converted into its tautomeric forms, ascaridole glycol anhydride and ascaridole glycol, and being soluble in 60 per cent acetic acid accounts for the high values by the Nelson method.

To confirm this theory further, portions of oils I, J and K were heated to 115°C. under vacuum and then allowed to stand in clear glass-stoppered bottles from February to May, when determinations were again made. Results of this experiment are given in Table 4.

TABLE 4.

Ascaridole as shown by Paget and Nelson methods.

OILS	FEBRUARY		MAY	
	Nelson	Paget	Nelson	Paget
	per cent	*per cent*	*per cent*	*per cent*
I	70.00	68.95	70.00	67.03
J	62.00	63.00	62.00	61.22
K	75.00	74.90	75.00	69.27

The slightly lower figures obtained by the Paget method indicate the deterioration of the oil due to intermolecular change, whereas the same values obtained by the Nelson method at the end of the experimental period showed no deterioration of the oil.

Oils B, C and J, or Group III, present a little different problem. Here a lower percentage of ascaridole is recorded by the Nelson method than by the Paget. Oil C is the first fraction obtained from the distillation of oil B; it consists largely of cymene. When the Nelson method is applied under such conditions, ascaridole and cymene, and ascaridole and 60 per cent acetic acid are found to be miscible with one another

in all proportions, but cymene and 60 per cent acetic acid are only partially miscible. If, therefore, to an oil containing cymene, as is the case with American wormseed oil, 60 per cent acetic acid is added, ascaridole distributes itself between the two liquid layers, and two conjugate ternary solutions, each consisting of ascaridole, cymene and acetic acid, are thereby produced. These two solutions are in equilibrium with each other, and the composition of each will depend upon the concentration of the three components and the temperature of the mixture. In the Nelson method, therefore, the cymene layer should always retain some ascaridole, and the amount would depend upon the concentration of the cymene in the oil. It is on this assumption that the low results obtained by the Nelson method are accounted for in oils B, C and J.

To verify the above assumption further, oil E, the purified middle fraction from B, was used in a series of dilution experiments with highly purified cymene, the principal constituent of the hydrocarbon fraction of chenopodium oil. Oil E was completely soluble in 60 per cent acetic acid and titrated 100 per cent ascaridole by the Paget method, when the factor 1.284 was used. The purest cymene available for these studies contained 10.95 per cent ascaridole by the Paget method and 7.00 per cent by the Nelson assay. This was redistilled until no ascaridole was detected by either method. Nine samples of these two oils were prepared by weighing each component, the ascaridole being varied from 10 to 90 per cent at approximately 10 per cent intervals. The results of this study are listed in Table 5.

TABLE 5.

Ascaridole in samples adulterated with cymene.

SAMPLE NO.	AS MADE	NELSON ASSAY	ERROR— NELSON ASSAY	PAGET ASSAY	ERROR— PAGET ASSAY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	9.86	9.00	8.70−	9.85	0.10−
2	19.98	18.00	9.99−	19.99	0.05+
3	29.44	24.00	18.60−	29.62	0.82+
4	39.71	30.00	24.45−	39.26	1.10−
5	49.88	38.00	23.90−	50.55	1.34+
6	59.74	46.00	23.00−	59.22	0.87−
7	69.78	55.00	19.76−	68.99	1.13+
8	79.56	70.00	12.10−	79.63	0.09+
9	89.34	88.00	2.00−	88.41	1.05−

Consideration of the data in Table 5 shows the accuracy of the Paget method in measuring the active constituent in chenopodium oil. The maximum error recorded is within 2 per cent of the exact amount present. The percentage deviation by the Nelson assay is recorded as 2–24 per cent. Repeated determinations gave the same values. The separation in every determination was sharp, and the two layers were quite clear. The points of maximum and minimum deviation are shown more clearly in the graph.

The graph shows the Nelson method to be a three component extraction phenomenon. The appearance of two minima in the curve expressing the results is obvious, since 60 per cent acetic acid dissolved completely 100 per cent ascaridole, and when there is no ascaridole at all none can be lost; it follows that the error in the extraction must approach zero for both very high and very low percentages of ascaridole. The location of the maximum error and the slope of the error curve naturally depend on the slope of the isotherum for the ternary system in question.

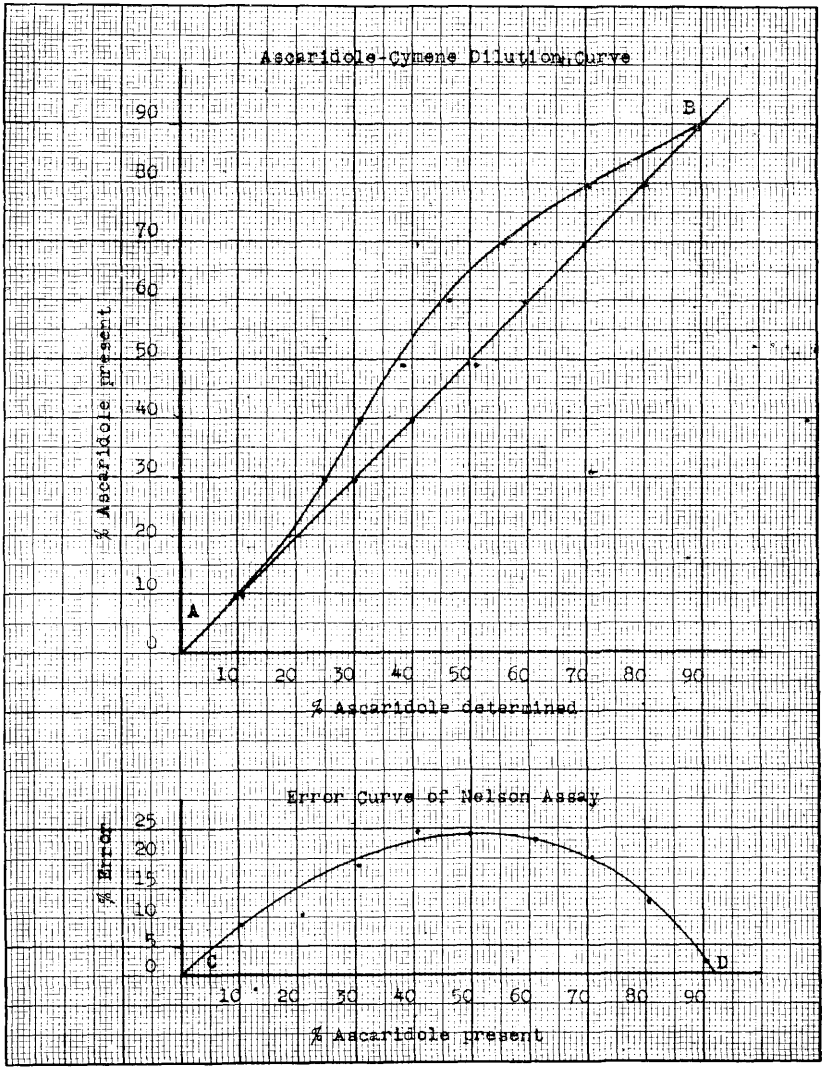


FIGURE 1.

SUMMARY.

An examination of the literature reveals that a number of qualitative and quantitative methods have been proposed for the estimation of ascaridole in oil of chenopodium.

Of the quantitative methods, the Nelson extraction and the Paget reduction methods have been studied. A third method, depending on the color reaction between hydrochloric acid and ascaridole, has been proposed by Knafll-Lenz and Hofmann. Preliminary studies, not reported, indicate that it warrants consideration.

Studies confirming the factor proposed by Paget in the reduction method reveal that the exact value of the factor is dependent upon the concentration and quantity of hydrochloric acid used in preparing the titanous chloride solution.

The factor value obtained in these studies with highly purified ascaridole was 1.284, a value higher by 0.007 than that proposed by the author of the reduction method.

When the Nelson extraction method and the Paget reduction assay were compared, they gave identical values with two highly purified samples of ascaridole.

When these methods were used on fourteen other oils of varying composition, two sets of data were obtained. In the first, the Nelson method gave higher results than the Paget assay; in the second, the reverse was recorded. The high results obtained in the first group by the extraction method is accounted for by the presence in the oil of ascaridole glycol anhydride and ascaridole glycol. In the second group the low results by this same method are accounted for by the presence of *p*-cymene, the principal constituent of the hydrocarbon fraction of American wormseed oil. This is confirmed by a series of dilution studies with highly purified ascaridole and cymene.

These studies show that the Paget method is reliable under the conditions outlined, and that the Nelson method is not reliable as an indicator of the percentage of ascaridole contained in an oil. The Paget method differentiates between the ascaridole and its glycol and anhydride on the one hand, and is not influenced by the presence of the hydrocarbons in chenopodium oil on the other. Very small samples are required for its use, and it is accurate within the limits of experimental error allowable for analytical methods.

RECOMMENDATIONS[1].

It is recommended—

(1) That the titanium trichloride method be further studied collaboratively.

(2) That the Knafll-Lenz and Hofmann colorimetric method be studied as a possible tentative assay.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 13, 66 (1930).

Method of Distillation

The methods employed in the distillation of chenopodium ambrosioides have been studied in detail by Schimmel and Company (19), Konantz (30), Wirth (7), Nelson (10) and Russell (31). It is the opinion that the poor quality of oils obtained from Western plants has been due to the faulty distillation methods.

Konantz (30) remarked that the oil obtained by Wirth from herb grown in Michigan was undoubtedly low in ascaridole content because of prolonged distillation with low steam pressure. Nelson(10) found that the amount of decomposition products (as determined by fractional distillation) in oils obtained from commercial stills was not more than 2.3% if high steam pressures were employed. He recommended high steam pressures, a warm condenser and a rapid distillation.

Russell (30) obtained more oil from a rapid distillation with a warm condenser than from a slow distillation with a cold condenser. In the former instance the oil was of a superior quality. He found no difference in the quality or yield of oil distilled from herb at a pressure of from 80 to 100 pounds per square inch, but low gravity oils were obtained at 40 to 60 pounds pressure.

As a result of these studies distillation methods have been generally standardized throughout the production district of Maryland. The commercial distiller employs steam pressure of approximately 90 to 100 pounds and the average time of distillation is from fifteen to thirty minutes. The cured herb is packed tightly

in iron retorts of from 200 to 400 pound capacity. Hinged iron covers, balanced with counterweights are tightly clamped on the retorts by means of wing bolts. Steam generated from a small boiler is introduced near the bottom of the retorts and the vapors condensed by conducting them through some thirty feet of iron pipe placed horizontally in a trough and cooled with running water. The condensate is collected in various types of metal barrels of approximately fifty gallon capacity. After the barrel is fairly well filled, the oil separating out on top, a stopcock at the bottom is opened and the water layer, in most instances, allowed to run off at a rate approximately equal to the flow of the distillate into the receiver.

Regardless of the mode of handling and the time of setting of the distillate, the water layer always contains a quantity of oil which if not recovered by the addition of salt, thereby increasing the gravity of the aqueous fraction and affecting a further separation of the oil and water, an appreciable quantity of valuable oil is lost.

Recovery of Oil by Distillation

A survey of the wormseed district of Maryland revealed that only a few distillers were making an effort to separate the oil from the emulsion that is drawn from the distillate reservoirs. Three of the stills in the district, however, are equipped so that a recovery of the oil lost in the waste liquor may be affected. In these stills the aqueous layer is collected and returned by means of a steam siphon to a retort and redistilled. An appreciable

amount of oil is secured from the waste liquor as is shown by a record taken on one of the stills equipped for redistillation of the waste liquor. For this record 3690 pounds of the cured herb was used. Representative samples of this herb by distillation method assayed 1.08 percent ascaridole, giving a total of 39.84 pounds of ascaridole available for recovery. Distillation of the herb gave 44 pounds of oil that assayed 65 percent equivalent to 29 pounds of ascaridole. The waste liquor was then steam distilled and an additional 7 pounds of oil were recovered assaying 86 percent ascaridole equivalent to 6 pounds of ascaridole or a total of 51 pounds of oil recovered containing 35 pounds of ascaridole. Analysis showed the herb to contain 39.84 pounds of ascaridole, 35 pounds were recovered or the still by the double distillation method was found to be 86.7 percent efficient. These findings are given in Table ( ) below.

Table ( ).

Pounds of Herb	Distillation	Pounds of oil Recovered	Percent of Ascaridole in Oil	Pounds of Ascaridole Recovered
3690	Distillation of herb	44	65.	28.60
	Distillation of liquor	7	85.	5.95
Total		51	67.74	34.55
Pounds of Ascaridole in Herb by Analysis				39.84
Efficiency of still 34.55 ÷ 39.84 =				86.7%

The above table shows that by distilling the waste liquor the yield of oil is increased from 44 to 51 pounds making available 34.55 pounds of ascaridole recovered against 28.60 pounds obtained from a single distillation, or an increase in the recovered ascaridole of 15 percent. The cost of the distillation of the waste liquor is comparatively nothing after the initial equipment is installed. In one plant this was done at a cost not exceeding one hundred dollars.

A point of interest in the above table is the high percentage of ascaridole in the seven pounds of oil obtained by distilling the waste liquor. As shown the first oil obtained assayed 65 percent of ascaridole and the second 85 percent. The difference in the ascaridole content of the two oils is due to the solubility of the active constituent in water over that of the hydrocarbon fraction. Pauly ( ) has shown that the solubility of ascaridole in water to be .3 grams per 100 cc.

In support of the above findings a number of stills in the district were visited and samples of oil and the discarded aqueous layer were obtained for examination. The results in Table ( ) are expressed in terms of percent of ascaridole found.

Table ( )

Percent of Ascaridole in Oils obtained from first and second distillation

Year	Still	Oil from 1st distillation	Water layer from 1st distillation	Oil from second distillation
1928	A	65.0	.30	86.0
1931	A	-	-	95.0
1930	B	81.0	.61	99.7
1931	C	-	-	98.0
1931	C	-	-	96.2
1929	D	77.0	.26	-
1929	E	78.0	.36	-
1929	F	64.0	.30	-

Stills A, B and C of the above table are equipped for distillation of the waste liquor, or water fraction after the oil from the initial distillation of the herb had been removed. It is significant to note the high quality of oil obtained. Munch and Reindollar ( ) have remarked upon the high quality of certain oils obtained from the Maryland district; and Pauly ( ) found that practically pure ascaridole was obtained by redistillation of waste liquors from the distillation of the herb.

Stills D, E and F of the above table were not equipped for redistillation. Examination of the waste liquor shows an average loss of .3 pounds of ascaridole for each 100 pounds of waste liquor not redistilled. This emphasizes the value of redistillation to

obtain not only a high quality oil but an increase in the total oil recovered by fifteen percent.

Tests on the Efficiency of Stills.

Many investigators have studied the process of steam distillation in an effort to improve the quality and yield of the oil obtained. As a result of these studies together with the observations of the commercial distillers the process of distillation is fairly uniform through out the producing district. Steam pressures of from ninety to a hundred pounds per square inch are used and the condensors are kept fairly warm.

There is, however, a loss of oil in the discarded waste water from the receiving barrels , a fact recognized by several of the distillers in the district. As previously mentioned, in a few stills a recovery of some oil is made by redistillation of this waste liquor. An investigation was made to determine the amount of ascaridole lost in the waste water of several commercial stills and to find the efficiency of one of the more advanced types of stills .

Composite samples of waste liquors from five stills in Carroll County were analyzed for ascaridole. Two of the stills(A and B) were equipped for redistillation and samples of the waste water after the second distillation were also assayed. The data appears in table ( )

	Amount of ascaridole (%) in	
Still	Waste from 1st. distillation	Water from 2nd. distillation
A	0.34	0.26
B	.51	.31
C	.26	--
D	.36	--
E	.30	--

Since a considerable volume of water is discarded after the first distillation these data show that there is an appreciable loss of ascaridole in the waste liquor. The rather high values for the amount of ascaridole in the final waste for stil s A and B do not represent such a great loss since the volume of discarded liquor in this instance is much smaller than that from the original distillation.

Most of these values approximate the solubility of ascaridole in water as determined by Pauley and this fact suggests that little improvement can be made in the method of separation. The advantage of redistillation is shown more clearly in the following experiment in which a known weight of cured plant material was steam distilled, the oil separated and analyzed for ascaridole. The waste liquor was also analyzed then redistilled. The oil recovered by redistillation and the final waste liquor was likewise assayed. Having determined the weight of the separated oil and the waste water in each instance, the amount of ascaridole present was computed.in terms of pounds. The data appears n table ( ).

Amount of herb (lbs.)	Distillation	Amount of ascaridole (lbs.) in Separated Oil	Water layer
3690	first	29	15
----	second	6	5

From the data in the above table the actual amount of ascaridole available in the herb can be determined by two methods. By the first, the amount of ascaridole in the separated oil after the first distillation 29 lbs.) plus the amount found in the water layer(15 lbs.) would be qual to 44 pounds, representinf the total amount of ascaridole availble n the plants. By the second methodthe total amount of ascaridole t ecovered by both distillations (35 lbs.) plus the amount in the waste fter the second distillation (5) pounds would be equal to 40 pounds.

The most likely error in the first method was in the determination of the weight of the waste liquor which was calculated from an estimated volume. An appreciable error in the second method was likely due to deterioation of the ascaridole during redistillation .

The efficiency was calculated on the basis of the average of the above two values for the amount of ascaridole present (42 ~~pounds~~ 165) and the amount actually recovered(35 lbs.) and was equal to 83%. The efficiency of the single distillation process based on the average value for the ascaridole present and the amount recovered from the first distillation (29 lbs.) was 69%.

An additional check of the efficiency of the still was made by distilling a representative sample of the herb used in the above experiment in a small laboratory still and analyzing the distillate for ascaridole. The assay showed the plant to contain 1.08% ascaridole, which multiplied by the weight of the herb used in the large still (3690 lbs.) would indicate that 40 pounds of ascaridole were available in the herb. The efficiency of the still was then found to be 87%.

Besides the economy effected by the redistillation process as shown by the above experiments, the fact that a superior quality of oil is obtained adds to its value. This was noted by Munch and Reindollar in 1931 and by the present authors at about the same time. Three samples of redistilled oil collected in 1930 and 1931 from different stills (respectively), assayed 95.0%, 99.7% and 98%. ascaridole. The significance of this phase of the redistillation process will be discussed later.

The Oil Extraction Process

Steam distillation has always been employed as the method of xtraction of oil from the plants. The original "pot" still consisted f an iron kettle set over a firebox. The top was fitted with a soap stone ver with a hole in the center over which a soap stone cap was placed. T e cap was connected to a horizontal condenser consisting of a series of on pipes forming a coil.

The herb was placed in the pot together with water and steam rated by heating the pot extracted the oil from the plants. The vapors e condensed in the coil which was cooled by running water and the stillate collected in champagne bottles. When the bottles were filled e contents were poured into barrels and the oil skimmed from the top e capacity of these stills was about three pounds of oil per day from ch pot.

This type of still has been entirely displaced bythe modern st am still introduced by the Klee brothers of near Westminister in 1908 herb is tightly packed in iron retorts of from two to four hundred nds capacity. Hinged iron covers balanced by counter weights are clamped urely by means of wing bolts and steam generated by a vertical steam ler is introduced near the bottem of the retort. The vapors are ducted through some thirty feet of iron pipes placed horizontally in rough and cooled with running water.

The condensed vapors are collected from the ends of the condens es in various types of metal containers. In most of these receivers the uid first strikes a metal shelf which prevents agitation of the separated l layer. When the container, which is usually of fifty gallon capacity, nearly filled, a faucet at the bottem is opened and the water layer

permitted to run off at a rate approximately equal to the flow of distillate into the receiver. At least three of the stills in the district ,however, are so equipped that a recovery of the oil present in the waste liquor is effected. In these stills the water layer is collected in a sunken reservoir and returned to a retort by means of a steam siphon for redistillation. An appreciable amount of oil is recovered from this waste liquor as will be shown later.

Distillation is complete when the distillate from the ends of the condensor pipes is practically free of oil drops. The time of distillation varies from fifteen to thirty minutes according to the quality of the herb, tightness of packing and steam pressures employed. The oil is drawn off from the receiver, filtered and allowed to stand in a smaller container for some time thus permitting more water to separate and finally poured into tin cans for shipment.

The waste herb is removed from the retorts by chain slings and placed in stacks for future removal.

There are today about thirty stills of this type in operation in Carroll County. Producers who do not own a still pay from thirty - five to fifty cents per pound of oil obtained to have their herb distilled.