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BY

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The Determination of Ascaridole in Chenopodium Oil.

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CHENOPODIUM or American wormseed oil was at one time extensively used as a general anthelmintic, but fell into disuse largely because of a number of cases of poisoning, due in most instances to over-dosage. In the last ten years it has acquired a special importance from its use on a large scale in campaigns against the hookworm, conducted, under the auspices of the International Health Board, in various tropical countries.

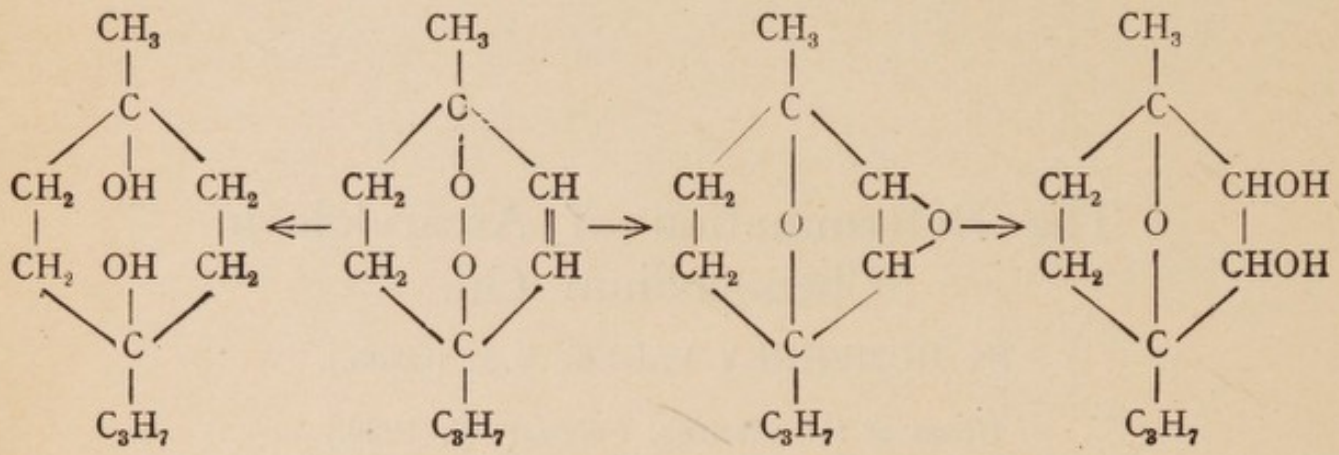
COMPOSITION AND CONSTANTS OF CHENOPODIUM OIL.—It is now known that the chief components of the oil are (*a*) ascaridole (Formula I), which is present to the extent of 60 to 75 per cent., and (*b*) a mixture of terpenes with *p*-cymene, which constitutes the residue, and which is here referred to as the hydrocarbon fraction. (Nelson, *J. Amer. Chem. Soc.*, 1911, **33**, 1404; 1913, **35**, 34; 1920, **42**, 1204; Henry and Paget, *J. Chem. Soc.*, 1921, **119**, 1715; 1925, **127**, 1649.)

The oil is recognised in the United States Pharmacopoeia (10th revision), which specifies the following constants for it:—Sp. gr., 0·955 to 0·980 at 25° C.; optical rotation, −4° to −10° in a 100 mm. tube at 25° C.; refractive index, 1·4723 to 1·4770 at 20° C.; and solubility, 1 volume in not less than 8 volumes of 70 per cent. alcohol.

Apart from the statement of these data, little has been done to develop a means of ascertaining the quantity of ascaridole present, although the problem has now become of importance, since Smillie and Pessoa's results (*J. Pharm. Exp. Ther.*, 1924, **24**, 359) no longer leave any doubt that ascaridole is the sole component of the oil which exhibits anthelmintic action against hookworm and roundworm, the parasites for which the oil is generally used.

THE U.S.P. METHOD OF DETERMINING ASCARIDOLE.—The hydrocarbon fraction is insoluble in 60 per cent. acetic acid, whilst ascaridole is miscible with this solvent, and it is on this fact that the method suggested by Nelson (*J. Amer. Pharm. Assoc.*, 1921, **10**, 836), and adopted by the United States Pharmacopoeia, for the determination of ascaridole is based. This author, however, has overlooked

the fact that ascaridole readily undergoes intramolecular change to ascaridole glycol anhydride (Formula II), and that this is easily hydrated, forming ascaridole glycol (Formula III).



IV I II III

These two changes occur in succession by the mere application to ascaridole of (*a*) dry heat, (*b*) steam, and it is clear that in the distillation with steam involved in the manufacture of the oil from the seed such changes must occur. In the course of work on the components of the oil the author has fractionally distilled many litres of it, and has invariably found evidence of such decomposition. The anhydride and the glycol are, like ascaridole, miscible with 60 per cent. acetic acid, and therefore rank as ascaridole when general tests of this kind are applied. Further, chenopodium oil commands a fairly high price, and is in consequence liable to adulteration, as in the case of the "synthetic" chenopodium oils to which Messrs. Schimmel and Co. have called attention in their Reports for 1919 and 1921, and one of the substances found in such oils—cineole—is also miscible with 60 per cent. acetic acid (see p. 7).

Quantitative Reduction of Ascaridole.—It is therefore desirable that a method should be found by which ascaridole itself can be determined. In the work already referred to, the only method which has been found satisfactory is the isolation of ascaridole by distillation under reduced pressure, but, as ascaridole is liable to explode on the application of heat, this does not constitute a desirable process for general use. Formula I shows that ascaridole is a peroxide, and, like these substances in general, it forms no solid derivatives by which it can be isolated. Attention was therefore directed to the possibility of its quantitative reduction. It has long been known that in presence of ferrous sulphate ascaridole is converted into the corresponding glycol (Formula III), but, as will be seen from Formulae I, II and III, this is not a true reduction, but merely involves intramolecular change and hydration. Wallach (*Annalen*, 1912, **392**, 60), however, has shown that hydrogen in presence of palladium reduces ascaridole to 1·4-terpin (Formula IV) by the addition of four atoms of hydrogen, and there therefore seemed a possibility of finding an agent which would effect such a change in a manner both quantitative

and measureable. Of the reducing agents tried, titanous chloride (or sulphate), which was employed as described by Knecht and Hibbert (*New Reduction Methods in Volumetric Analysis*, 1925), was found to be the most convenient for this purpose.

The products of this reduction are being examined, but the only information yet available is that they include neither ascaridole glycol nor its anhydride. If the reaction proceeds as in the catalytic hydrogenation described by Wallach, the amount of titanous chloride used should correspond to the addition of four atoms of hydrogen, but though the end of the titration is quite definite, the amount actually used is only one-third of this; even if only two atoms of hydrogen are added, the ethylene linkage not being reduced by titanous salts, the amount used is still only about three-quarters of the theoretical quantity. This point has been investigated by the use of carefully purified ascaridole in preliminary titration experiments. It appeared at first as if the reaction reached a definite equilibrium, but controlled variation of (1) the quantities of material, solvent and reagent used, (2) time of reaction, (3) nature of indicator, (4) the substitution of methylene blue for iron alum in determining the residual excess of reducing agent, and (5) the use of sodium hyposulphite in place of titanous salts, all failed to effect any material modification of the apparent end of the reaction first found (Table I). Until the products have been examined the process must rest on the empirical basis that one grm. of ascaridole is reduced by 1·2770 grms. of titanous chloride. This figure is taken, being the mean of results from several experiments varying from 1·2420 to 1·3040 grms.

While the author does not claim a high degree of accuracy for the process, it has the advantage over the existing general method of determination that, with the expenditure of a very small amount of the oil, it does give evidence of the amount of ascaridole really present.

The opportunity has been taken to determine to what extent the general methods already available are of value, and it is shown that with genuine samples of oil, they give quite useful results, but in case of adulterated oils, such as sample E, which was purchased in the open market as *Oleum chenopodii* U.S.P., they are quite useless.

Experimental.

Preparation and Constants of Ascaridole.—Ascaridole was obtained from chenopodium oil by repeated fractional distillation at 15 mm. pressure (*J. Chem. Soc.*, 1921, **119**, 1715), and isolation of the middle portion of a fraction boiling at 115° C. at 15 mm. It had the following constants:—$D_{15°}^{15°}$, 1·0111; $D_{25°}^{25°}$, 1·0050; α, −2·15° in a 1 dcm. tube; $[\alpha]_D^{25}$, −2·14°; $n_D^{20°}$, 1·4736; and on analysis gave: C = 71·18, H = 9·65 per cent. (ascaridole, $C_{10}H_{16}O_2$, requires: C = 71·43, H = 9·52 per cent.). This pure ascaridole is referred to later on as oil A.

The hydrocarbon fraction was obtained from the portion of the oil boiling below 115° C. at 15 mm. by repeated distillation, first at low pressure and finally over sodium at 760 mm. It then boiled between 170° and 185° C.; and had $D_{15°}^{15°}$, 0·8585; $D_{25°}^{25°}$, 0·8506; α, −18·21° in a 1 dcm. tube; $[\alpha]_D^{25}$, −21·41°; $n_D^{20°}$, 1·4862.

Of the five samples of chenopodium oil which were examined, oils B and C represented a commercial brand of ascaridole, the remaining three (D, E and F) being ordinary chenopodium oils of commerce, all of which fulfilled the requirements of the United States Pharmacopoeia (Rev. 10) as to specific gravity, optical rotation, and solubility in 70 per cent. alcohol and 60 per cent. acetic acid.

Determination by Means of Specific Gravity and Optical Rotation.—The specific gravity and specific rotation at 25° C. of ascaridole and the hydrocarbon fraction, were determined, and from these data the percentages of ascaridole have been calculated (from the specific gravities and specific rotations) in the oils B to F (*cf.* Parry, *Chemistry of the Essential Oils*, 1921, Vol. I, p. 535), on the assumption that no change occurs on admixture of ascaridole with the hydrocarbon fraction (Table II).

Determination by Solubility.—Nelson's method of determination was carried out by shaking 10 c.c. of each oil for 5 minutes in a cassia flask* with 60 per cent. acetic acid, the flask being then set aside at about 15° C. till separation was complete. All bubbles of oil were collected into the graduated neck, and from the volume of the insoluble hydrocarbons so obtained the apparent percentage of ascaridole was calculated. Oils B and C were completely soluble, except for a faint cloudiness in the solution; and though in every case the amount of ascaridole found was higher than that indicated by the specific gravity, reference to Table II, columns 4 and 6, will show that the results obtained by these two methods agreed well.

Determination by Distillation.—In order to check the results obtained above, each sample was separated by repeated distillation into four fractions:—(i) b.p. below 100° C. at 15 mm.; (ii) b.p. 100° to 110° C. at 15 mm.; (iii) b.p. 110° to 120° C. at 15 mm.; and (iv) the residue undistilled at 120° C. at 15 mm.

Experience gained during fractionation of considerable quantities of chenopodium oil has shown that of the fractions obtained by distilling about 250 c.c. at a time, (i) consists almost entirely of the hydrocarbons present in the oil, and (iii) of ascaridole, whilst (ii) is a mixture containing about 75 per cent. of ascaridole. The percentages shown in Table II, column 8, are arrived at on the basis of this assumption. In general, the previous results were confirmed (Table II), except in the case of Oil E, which was not easily separated into its constituents; on redistillation an ascaridole fraction was obtained from it representing, however, only about 38 per cent. of the oil. The quantity of Oil F available was insufficient for fractional distillation.

Determination by Means of Reducing Agents.—Experiments with sodium metabisulphite and with sulphurous acid proved these reagents to be inactive. Sodium hyposulphite was also found to be unsuitable, since, in addition to effecting the reduction of ascaridole, it also reacted with the mixture of hydrocarbons. Titanous chloride was found to be the most convenient reducing agent.

* These are special flasks having the neck gradnated in tenths of a c.c., which are used in the determination of cinnamic aldehyde in cassia oil ; they are obtainable from the usual dealers in chemical apparatus.

Titanous chloride solution was prepared and standardised as described by Knecht and Hibbert, 66 c.c. of the commercial 15 per cent. solution being made up to 2250 c.c. One grm. of chenopodium oil was diluted with 96 per cent. alcohol to 100 c.c., and to 10 c.c. of this in a flask, through which a current of carbon dioxide was passing, an excess of titanous chloride, about 50 c.c., was added; the flask was then closed with a Bunsen valve, and its contents heated almost to boiling for one or two minutes. If the pale violet colour of the titanous chloride disappeared, more was added to ensure the presence of an excess. The formation of a precipitate of titanic oxide during heating did not interfere with the determination. About 1 c.c. of 5 per cent. potassium thiocyanate was then added, and the solution titrated back with a standard solution of iron alum until a permanent faint red colour was obtained. The amount of iron used, calculated in terms of titanous chloride, gave by difference the quantity of titanous chloride oxidised.

For example, (i), 10 c.c. of a 1 per cent. solution of pure ascaridole were heated, under the conditions described, with 50 c.c. of $TiCl_3$ (= 0·003856 grm. per c.c.); it then required 15·9 c.c. of iron alum (= 0·001485 grm. per c.c.), equivalent to 0·0651 grm. of $TiCl_3$; therefore 0·1 grm. of ascaridole oxidised 0·1277 grm. of $TiCl_3$. (ii) 10 c.c. of a mixture, containing cymene, 18·3 per cent.; cineole, 27·4 per cent.; and ascaridole, 54·3 per cent., after being heated with 40 c.c. of $TiCl_3$, oxidised 18·1 c.c., or 0·0698 grm. $TiCl_3$, which is equivalent to 54·7 per cent. of ascaridole. (iii) 10 c.c. of a 1 per cent. solution of Oil F, after heating with 40 c.c. of $TiCl_3$, required 12·85 c.c. of iron alum, equivalent to 0·0527 grm. of $TiCl_3$; therefore 0·1015 grm. of $TiCl_3$ was oxidised, equivalent to 79·5 per cent. of ascaridole.

Solutions in alcohol of the hydrocarbon fraction, of ascaridole glycol or of its anhydride were found to have no oxidising action on titanous chloride under these conditions.

Modifications of the Reduction Method of Determination.—As has been stated above, attempts were made to modify this method of determining ascaridole, so as to obtain results in agreement with those to be expected if the reduction followed a simple course. When the solution was left in the cold in a current of carbon dioxide, the reaction was complete at the end of one hour, and the same point was reached when the solution was heated or boiled for one or fifteen minutes. The presence or absence of a solvent was without influence on the reaction, except that when no solvent was used the rate was slower and the end-point less definite. By increasing the concentration of the titanous chloride solution from $N/50$ to about $N/10$, a little more titanous chloride was oxidised, corresponding to about 10 per cent. of ascaridole, but, if the concentration was raised much beyond this, the reduction proceeded too vigorously. The addition of 10 c.c. of a 20 per cent. solution of Rochelle salt, did not further affect the result.

Variations in the conditions having failed to effect the object in view, the possibility of a re-oxidation of the primary reduction product by the ferric alum was considered. Methylene blue was tried, first as an indicator only, and then as an oxidising agent for the excess of titanous chloride; the end-point of the

titration was practically the same as before, but was less sharp, even when carried out at the boiling point. Sodium tungstate solution, when added to a solution of a titanous salt, forms a bright blue coloration which is destroyed on the addition of oxidising agents; the disappearance of the colour, however, was not sufficiently marked to allow of the use of this salt as an indicator. The determination of peroxides, such as hydrogen peroxide, by titanous chloride, is based on the production of an orange colour due to the formation of titanium trioxide, the disappearance of which coincides with the complete reduction of the peroxide. Under certain conditions this colour was produced with ascaridole, but it could not be made of use in a process of determination.

The results obtained by these modifications, which are given in Table I, below, display no advantage over the first reduction method described above.

TABLE I.

(Except where otherwise stated, 10 c.c. of a 1 per cent. solution of oil A was used.)

	Oil used.	Reducing agent and conditions.	Ascaridole found. Per Cent.
(1)	A	Left 65 min. cold with 60 c.c. $TiCl_3$ about N/50	97
(2)	A	Heated 1 min, with 60 c.c. $TiCl_3$,,	101
(3)	A	Boiled 10 min. with 50 c.c. $TiCl_3$,,	100
(4)	0·1675 grm. A	No solvent; heated 5 min. with 100 c.c. $TiCl_3$,,	108
(5)	A	Heated 1 min. with 10 c.c. $TiCl_3$ about N/10	110
(6)	A	As (2) using Rochelle salt	104
(7)	A	As (2) titrating back with methylene blue	104
(8)	0·0960 grm. A	No solvent; titrating back with methylene blue	109
(9)	0·1158 grm. A	Reduced by sodium hyposulphite	101

APPLICABILITY OF THE METHODS.—A comparison of the results obtained by the application of the methods of determination described to the oils examined is made in Table II. From these it will be seen that, omitting Oil E, (i), the specific gravity is a useful guide to the ascaridole content; (ii) solubility in 60 per cent. acetic acid, which is the most rapid, and the most convenient process for field work, is also of value, although for the reasons given above, the results obtained with it are always high. But for the detection of adulterated or seriously deteriorated oils, such as Oil E, it is necessary to have recourse to the reduction or distillation methods

TABLE II.

			PERCENTAGE OF ASCARIDOLE FOUND BY				
Oil used.	D^{25}_{25}	$[\alpha]^{25}_{D}$	D^{25}_{25}	$[\alpha]^{25}_{D}$	Solubility in 60 per cent. acetic acid.	Reduction by $TiCl_3$.	Distillation.
A	1·0050	−2·14°	100	100	100	100	100
B	1·0002	−2·21°	97	99	100	97	92
C	0·9995	−2·29°	96	98	100	96	90
D	0·9611	−5·83°	72	81	74	72	70
E	0·9586	−5·48°	70	83	73	48	45
F	0·9746	−5·57°	80	82	82	80	—

Further Examination of Oil E.—A comparison of the fractions obtained from Oil E with those obtained from a normal oil, such as Oil D, is given below.

Table III.

Oil used.	B.pt. below 100° C./15 mm.	B.pt. 100°–110° C./15 mm.	B.pt. 110°–120° C./15 mm.	B.pt. above 120° C./15 mm.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
D	21	7	66	6
E	28	9	38	25

On shaking the fraction boiling below 100° C. at 15 mm. with a 50 per cent. solution of resorcinol in water, 39 per cent. was dissolved; and on distilling the resorcinol solution with steam an oil was recovered smelling strongly of cineole and boiling at 175° to 180° C., in which cineole was definitely identified by its crystalline compound with resorcinol, melting at 82° C. By repeated distillation of that part of the oil which boiled above 110° C. at 15 mm., fraction 3 (b.pt. 110° to 120° C. at 15 mm.), was raised to 43 per cent.; this was almost completely volatile in steam, but the oil so recovered contained only 73 per cent. of ascaridole (reduction method).

From the oil undistilled at 120° C. at 15 mm. no fraction of constant boiling point could be isolated. Twenty-seven per cent. of this oil was not volatile in steam, and it yielded ascaridole α- and β-glycols, probably representing ascaridole glycol anhydride in the crude oil. The portion volatile in steam contained about 20 per cent. of ascaridole. It was gently heated at atmospheric pressure, until conversion of ascaridole into the non-volatile glycol anhydride took place, and again distilled with steam; the resulting volatile oil distilled mainly at 220° to 245° C. at 760 mm. without decomposition, and was not ascaridole or any of its derivatives or a high-boiling ester.

This sample was therefore not a genuine chenopodium oil. It was probably an inferior sample brought to apparent conformity with the U.S.P. standards by the addition of cineole and a high-boiling constituent.

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