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Abstract

The chief component of *Vernonia anthelmintica* (L.) Willd. (ironweed) seed oil, trivernolin, and its minor components, 1,3-divernolin and vernolic (epoxyoleic) acid, have been produced in pound quantities of commercial grade quality (93–99% pure). Refinement of these epoxy components to chromatographically pure compounds has now been accomplished. Trivernolin has been the most difficult, vernolic acid the easiest, to purify. To upgrade trivernolin to about 100% purity, a combination of procedures was necessary including extraction, low temperature crystallizations, adsorbent treatments, and column chromatography, each monitored by thin-layer chromatography (TLC). Vernolic acid of 93.7% purity was highly refined by a single pass through a silicic acid column. Some physical characteristics of these epoxy compounds are given. None of the compounds were completely stable in storage as evaluated by chromatographic procedures.

Introduction

An extensive search is being made by USDA scientists to find new plants which the American farmer can grow economically for industrial purposes—plants that produce seeds containing unique oils which will be noncompetitive with those now in production; also, some of these new plants will serve as replacements for crops now in surplus. *Vernonia anthelmintica* (L). Willd. seed is unique in that it yields an epoxy fatty oil and therefore has a potential industrial market. This seed contains from 20–27% oil rich in vernolic (epoxyoleic) acid (70–75%) combined chiefly as the single glyceride, trivernolin. The extracted marc also has potential value in animal feed.

The present work deals with the purification of the epoxy components of *Vernonia* oil for the purpose of obtaining chromatographically pure trivernolin, 1,3-divernolin and vernolic acid. Some physical properties of these compounds are reported.

Experimental Procedures

Materials and Methods. The epoxy components were prepared as previously described (1), the trivernolin 96.6% pure based upon oxirane oxygen value, the 1,3-divernolin ca. 99% pure, and the vernolic acid 93.7% pure. The melting points reported were made with a Kofler micro melting point apparatus. The apparatus was installed in a cold room at 4C to obtain the trivernolin melting points.

Oxirane oxygen estimations were made by the Durbetaki method (2).

Silicic acid columns were prepared by pouring petroleum naphtha (p.n.) (bp 35–59C) slurry mixtures containing 80% silicic acid, Mallinckrodt's analytical reagent, 100 mesh powder, and 20% Johns-Manville's High-Flo Supercel into 1- by 18-in. glass columns.

The techniques of TLC followed throughout this investigation were similar to those described by Stahl (3). Glass plates, 20 x 20 cm were coated with a thin layer (0.25 mm) of Silica Gel G manufactured by E. Merck A. G., Darmstadt, Germany. The plates were activated by heating in an oven for 2 hr at 105C. One hundred or 200 μg of sample were used in 10 μl chloroform solution ca. 2 cm from the edge of the plates, the chromatograms were run by the ascending technique for 20–30 min at room temp, and the solvent fronts were allowed to reach a height of 100–125 mm. Trivernolin was chromatographed using a mixture of petroleum ether (bp 35–45C) and ethyl ether in the ratio of 80:20 v/v; divernolin the same solvents at a ratio of 70:30 v/v. Vernolic acid required petroleum ether-ethyl ether-acetic acid in the ratio 75:25:2.0 v/v, and plates showing all three components were also developed with this solvent system. After spraying with a solution 1:1 of 0.2 M potassium permanganate in 50% (by wt) H_2SO_4 followed by heating in an oven at 175C for 30 min.

For GLC analysis, the pure trivernolin was converted to methyl vernolate by the method of Barford et al. (4). In the GLC examination of these esters, a column of Chromosorb W coated with 15% silicon rubber (SE-30) was used as in the procedure previously described by Herb et al. (5).

Results

Purification of Trivernolin. In the trivernolin prepared as described on page 337, column 2 (1) a residue appeared when the impure trivernolin was allowed to stand at room temp for several weeks. A 102 g sample containing this residual impurity was readily filtered through a heavy asbestos type pad (Seitz Republic, type K-5) leaving ca. 200 mg of brown-colored amorphous residue on the filter pad; this residue was discarded. A small portion of the filtered trivernolin, when submitted to TLC examination, disclosed the presence of an impurity which migrated faster than trivernolin, several trailing impurities and a substance remaining at the base-line. Working in a cold room at ca. 4C, the trivernolin, which quickly solidified, was crushed and mechanically stirred for 2 hr with 200 ml redistilled p.n. After settling, the supernatant liquid was decanted through a filter. The process was repeated four times and the extracts combined. About 2 g of light yellow oil insoluble in p.n. at 4C remained; its composition has not been determined.

The solution of combined extracts (ca. 1 liter) was mechanically stirred at −20C to precipitate trivernolin. The trivernolin was slurry-washed several times with cold solvent and filtered at −20C. Evaporation of mother-liquor and washing produced 2 g of yellow oil of unknown composition. A portion of the trivernolin was monitored by TLC which showed that all impurities had been removed except the material which did not migrate from the base-line. All but a small amount of this base-line impurity was removed by two additional recrystallizations from p.n. at −20C, each

[1] Presented at the AOCS Meeting, Atlanta, 1963.

[2] A laboratory of E. Utiliz. Res. and Dev. Div., ARS, USDA.

preceded by a carbon (Darco G-60) treatment. (The solubility of trivernolin in p.n. at −20C appeared to be ca. 1.3 g per liter.) The weight of trivernolin at this stage was 83 g. Three further recrystallizations from p.n. at −20C, using one carbon treatment, reduced the quantity of impure trivernolin to 66 g but failed to remove the trace of base-line material. This impurity was completely removed only after two fractionations through silicic acid columns in the following manner.

A. Ten g of the 66 g of trivernolin mentioned above was dissolved in 80 ml of p.n. and the sample placed in the usual fashion on a column containing 30 g silicic acid-HyFlo supercel mixture. Elution was accomplished with 50 ml portions of a 1:4 ether-p.n. mixture. Five fractions were obtained, weighing after solvent removal: 9.91,0.06,0.01 and 0.01 g, respectively.

B. Since the 9.91-g fraction above still contained a faint trace of the base-line impurity when examined by TLC, it was rechromatographed. Of the 9.91 g, 9.74 g was dissolved in 15 ml of p.n. and placed on a new column charged as described above. The eluting solvent was again the 1:4 ether-p.n. mixture. The amount used for fraction 1 was 150 ml, which yielded 7.65 g of pure trivernolin. A second fraction using 50 ml of the 1:4 mixture weighed 0.77 g, and three additional 50-ml fractions tailed off at 0.09,0.07, and 0.01 g, respectively. As shown in Table I, the oxirane oxygen value of the first fraction (7.65 g) was 5.175% (theory 5.176%) indicating its purity to be about 100% trivernolin. The iodine value, optical rotation, refractive index and melting point of this pure trivernolin are also given in Table I. A GLC analysis was made of methyl vernolate prepared from the pure trivernolin. The only impurity found was a trace (0.05%) of methyl oleate.

Purification of 1,3-Divernolin. The 1,3-divernolin previously described (1) was 99% pure based upon its oxirane oxygen content. Examination by TLC showed this material contained several types of impurities, several trailing, and a substance remaining at the base line. To remove these impurities a 10-g sample was dissolved in 90 ml of 1:1 ether-p.n. mixture. This faintly yellow colored solution was placed on a column (prepared from a p.n. slurry containing 50 g of the 80% silicic acid-20% supercel mixture. Four eluted fractions were made using one 100-ml and three 50-ml portions of 1:1 ether-p.n. mixtures. The yellow pigment remained at the top of the column appearing as a thin brown band. Evaporation of solvent from the fractions gave weights of 1,3-divernolin fractions of 9.16,0.43,0.04 and 0.02 g. During the elution process it was necessary to keep the column slightly warmed by a heat lamp placed at a distance of about 2 ft from the column to prevent precipitation of 1,3-divernolin and to obtain an adequate solvent flow rate. Neither 50 ml of ether, which eluted 0.06 g of solid (odiferous), nor 50 ml of absolute methanol, which eluted 0.06 g of solid, moved the pigment in the brown band mentioned above. Chloroform eluted a portion of this pigment, each of two 50-ml portions yielding 0.05 and 0.06 g of solids, respectively. The remainder of the brown pigment was soluble in a 1% NaOH solution. These trace materials have not been further examined.

Fraction I (9.18 g) from the silicic acid column contained a trace-impurity trailing the 1,3-divernolin and an impurity not migrating from the base-line when examined by TLC. This material (8.93 g) was recrystallized from 150 ml of acetone at −20C. One g carbon (Darco G-60) was used in this process prior to cooling and filtration. The 8.37 g of 1,3-divernolin obtained had an oxirane oxygen value of 4.99% (theory 4.93%) and still contained a trace of the impurity not migrating from the base-line as shown by TLC examination. Two slurry washes of the 8.37-g sample of 1,3-divernolin with p.n. at room temp followed by a single recrystallization (mechanically stirred) from 600 ml warm p.n. cooled to room temp gave 6.76 g TLC pure 1,3-divernolin. The purity based upon an oxirane oxygen value of 4.924% (theory 4.931%) was 99.9%. Table I gives additional information on the physical characteristics of this compound.

TABLE I

Properties of Trivernolin, 1,3-Divernolin and Vernolic Acid

Property	Trivernolin		1,3-Divernolin		Vernolic acid	
	Found	Theory	Found	Theory	Found	Theory
Oxirane oxygen (%)	5.175	5.176	4.924	4.931	5.341	5.397
Purity based on oxirane value (%)	100.0		99.9		99.0	
Iodine value (Wijs)	82.9	82.1	78.8	78.2	86.1	85.6
Optical rotation at 25C (dextro)	2.20 [a]		4.66 [b]		2.03 [a]	
Index of refraction						
25C	1.4756					
40C	1.4706				1.4628	
60C	1.4647		1.4639		1.4547	
Melting Point °C	15.0		57.0		32.5	

[a] *n*-Hexane.
[b] Benzene.

Purification of Vernolic Acid. The vernolic acid previously described (1) was 93.7% pure, based upon the oxirane oxygen value of 5.060% (theory, 5.397). Although this vernolic acid was the least pure of the epoxy compounds prepared, it was the easiest to purify. One treatment using a silicic acid column was effective in the removal of base-line impurities shown to be in the starting material by TLC.

A 10-g sample of vernolic acid was dissolved in 20 ml of p.n. and placed on a column containing 50 g silicic acid mixture prepared as described above. Elution was accomplished with four 50-ml p.n. fractions and three 150-ml ethyl ether fractions. The weights of solvent-free fractions obtained were 6.72, 0.30,0.12,0.10,2.52,0.17, and 0.04 g. The 2.52-g fraction was a yellow sweet-smelling oil whose composition has not been investigated. The first fraction (6.72 g) showed no impurities present upon TLC examination. The single column chromatographic treatment elevated the oxirane oxygen percentage of the vernolic acid from 5.060–5.341 (theory 5.391) and its mp from 29.8–32.5C. Additional properties of the vernolic acid are shown in Table I.

Stability of Trivernolin, 1,3-Divernolin and Vernolic Acid. It was of interest to determine the effect of storage on the highly purified epoxy compounds, particularly since TLC could detect the presence of small amounts of impurities not readily discernible by chemical means. Samples of each in stoppered glass vials were stored at ca. 25C in the laboratory and under nitrogen at −20C in a low-temp box. After 60 days at room temp, all of the epoxy compounds when examined by TLC showed changes in composition. The chromatograms demonstrated the presence of base-line impurities (Fig. 1, a–c) and in addition the trivernolin (Fig. 1, b) had the trailing impurity which previously had been removed by the crystallization steps of the purification scheme. The samples at −20C

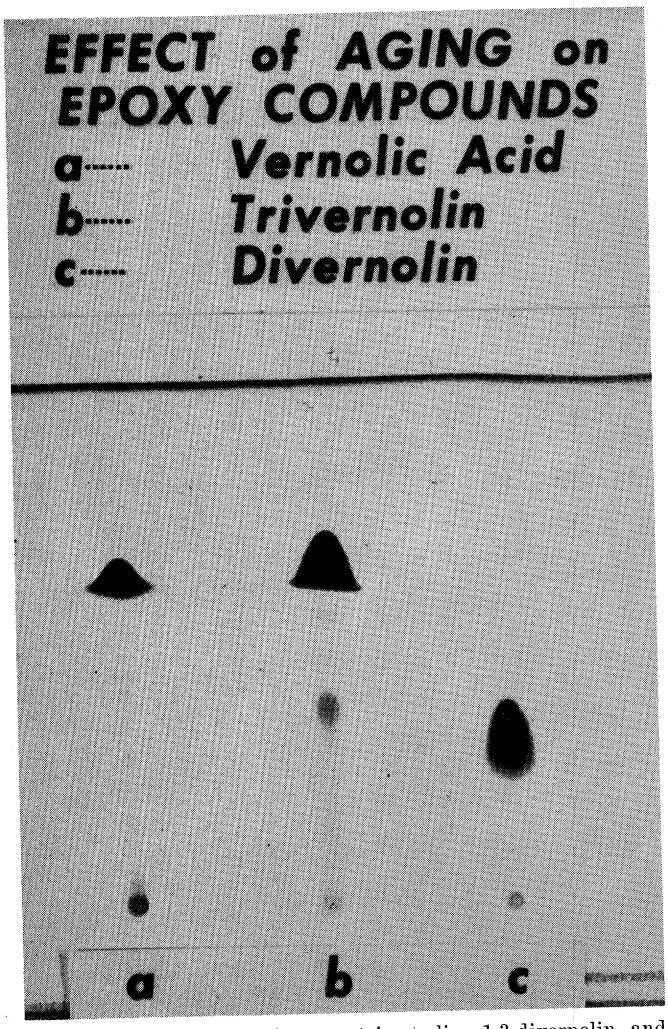

FIG. 1. Effect of aging on trivernolin, 1,3-divernolin and vernolic acid.

were examined by TLC (Fig. 2, a and b) after a period of 90 days' storage, and the chromatograms indicated slight changes in the compounds characterized by the reappearance of faint but definite base-line spots.

Discussion

Of the three epoxy components obtained from *V. anthelmintica* seed oil, trivernolin was the most difficult, vernolic acid the least difficult, to produce chromatographically pure. Purification of trivernolin was a tedious, time-consuming, TLC monitored process involving a) removal by filtration of impurities which precipitated out of liquid trivernolin on standing at room temp; b) exhaustive extraction of pulverized trivernolin at ca. 4C leaving about 2% impurities behind as cold p.n. insoluble material; c) repeated low-temp recrystallizations from p.n. with adsorbent treatments; and finally d) two passes through silicic acid columns under optimum conditions. 1,3-Divernolin was purified with less difficulty. High purity was obtained by one pass through a silicic acid column under optimum conditions, followed by one crystallization from acetone at −20C and two slurry washes at room temp with p.n., and completed by a recrystallization at room temp from a warm p.n. solution; progress followed by TLC monitoring. Only a single pass through a silicic acid column was required to purify the vernolic acid.

By chromatographic standards none of the three epoxy components of *V. anthelmintica* seed oil appeared to be completely stable. Trivernolin seemed to be most susceptible to degradation. Studies on the stability of both trivernolin-rich *V. anthelmintica* seed oil and purified trivernolin under a variety of storage conditions are in progress.

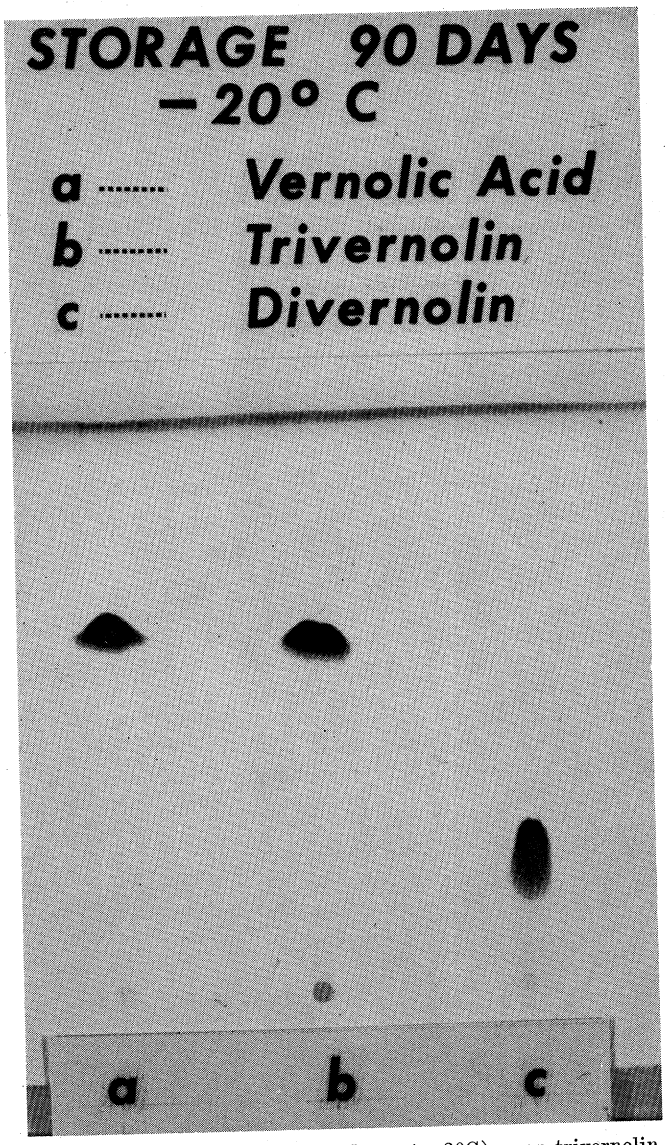

FIG. 2. Effect of storage (90 days at −20C) upon trivernolin, 1,3-divernolin and vernolic acid.

Vernolic acid impurities appear detectable by refractive index measurements. Further indication of this was demonstrated by aging the impure sample of vernolic acid (n_D^{40} 1.4641) for 120 days at ca. 25C; the value shifted to 1.4680.

As yet, no monovernolin has been detected in either *V. anthelmintica* seed oil or products prepared from the oil.

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