by the well-known elaidinization catalysts NO_2, SO_2, Se, Te, etc. Catalysts producing extensive positional isomers (hydrogenation catalysts such as Ni, Pd, Pt, etc.) will yield a more complex mixture of geometric *and* positional isomers, and cannot be analyzed by this method.

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Vernonia anthelmintica (L.) Willd. Trivernolin, 1,3-Divernolin and Vernolic (Epoxyoleic) Acid from the Seed Oil[1]

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Abstract

The epoxy fatty acid components isolated from the seed oil of *V. anthelmintica*, Indian ironweed, where the seed had been allowed to undergo lipolysis after grinding, were trivernolin, 1,3-divernolin, and vernolic acid. By inactivation of the hydrolytic enzyme system present in the seed, oil containing more than 50% trivernolin may be obtained. This species has potentialities as a replacement crop for those now in surplus; its seed contains 20 to 26% of an oil rich in epoxyoleic (vernolic) acid combined as glycerides amounting to 70 to 75%.

Introduction

RECENT INTEREST in *Vernonia anthelmintica* (L.) Willd. centers upon the unique character of its seed oil giving the plant potentiality as a new oilseed crop which might replace those now in surplus. This species of *Vernonia,* native to India, has been difficult to grow for seed production, flowering in early fall in many locations only to be frost-killed before reaching seed maturity. Improved prospects for successful cultivation of the plant in the U.S. came in 1960: a new introduction from India produced seed in Nebraska, North Carolina, and Texas. Adaptation studies are in progress on a wider scale at a number of locations. This paper deals with the extraction of the oil from the seed and the isolation and identification of its constituents: trivernolin, 1,3-divernolin, and vernolic acid. These are required in quantity for developmental studies of their utility in plastic formulations and in the preparation of chemical derivatives.

The literature on *V. anthelmintica,* commonly called purple fleabane or Indian ironweed, is voluminous. The plant was well-known to the early Greeks and Theophrastus described it about 300 B.C. (1–3). Before Linnaeus' time more than a dozen Latin synonyms were used for its designation and in a preliminary search more than 70 vernacular terms have been encountered, mostly references to Indian tribal names. In India, *V. anthelmintica* is often confused with *Psoralia cordylifolia* L. (4). In 18th century literature it is described by Linnaeus (5), having received its present name in 1804, given to it by Karl Ludwig Willdenow (6). He was the first to place it in the genus *Vernonia,* named for 17th century English botanist, William Vernon. This genus is one of the largest in the family Compositae, with an estimated 400–600 species (7,8). There are many references to the medicinal (9–16) and early ones to the insecticidal (1,3) properties of *V. anthelmintica.* It is claimed to be anthelmintic, purgative, good for asthmatic and kidney disorders, hiccough, and inflammatory swellings and skin diseases, especially leucoderma and psoriasis. A comprehensive report on the earlier literature, rich in folk-lore, is in preparation. The following is a review of late 19th and 20th century contributions to the chemistry of this plant:

Lindley and Moore (8) mention the production of oil by pressing the seed. Dymock *et al.* (9) extracted the oil from the seed and claimed the presence of an alkaloid in an alcoholic extract which they named *Vernonine;* they also reported that the seed contained 7.7% ash, a figure in close agreement with 7.2% obtained here. Kesava-Menon (10) was among the first to record information on the properties of the seed oil and Bhaduri (17) stated that the seed contained a glycoside which he named *shomerjin.* Chopra *et al.* (11,12) found no alkaloid present in the seed but reported the presence of a bitter principle, presumably accountable for the medicinal properties. Vidyarthi (18) first announced the presence in the seed oil of a new acid which he named *vernolic* but he considered it to be a hydroxy-acid isomeric with ricinoleic. He also reported on the sterols found in the unsaponifiable material. The details of these studies on fatty acid composition appeared in other publications (19,20), Majumdar (13) worked on

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the unsaponifiable material obtained by ether extraction of the seed. He indicated the presence of two sterols, brassicasterol and stigmasterol, and also claimed the presence in the oil of a monohydroxyoleic acid along with stearic, palmitic, myristic, and oleic acids. Vidyarthi (20) gave quantitative figures for these acids which included linoleic acid in considerable proportion. The correct characterization of vernolic acid, the chief fatty acid component present in the seed oil, was made by Gunstone (21) in 1954, and confirmed by Smith and coworkers (22) who were the first to isolate this compound from *V. anthelmintica* seed oil. Gunstone identified it as *cis*-12,13-epoxy-*cis*-9-octadecenoic acid, the first naturally occurring epoxy fatty acid reported. Morris (23) established the asymmetric centers of this (+) acid as having the *D*-configuration and with coworkers (24) reported on the fatty acid composition of the oil. Their figures were in close agreement with Gunstone's (21), their prepared methyl esters (24) consisting chiefly of 78.5% epoxyoleic, 8.8% linoleic, 2.0% oleic, 2.7% palmitic, 1.3% stearic and about 6% hydroxy fatty acid esters which may have been formed in saponification; only traces of other fatty acids were reported. Earle *et al.* (25) have also given information on the fatty acid composition of the oil and Van Etten *et al.* (26) reported on the amino acid composition of the seed. Miwa and associates (27) presented information on the biosynthesis of epoxyoleic acid in the maturing seed. Riser *et al.* (28) compared *V. anthelmintica* seed oil and salts of vernolic acid with commercial controls and found them equal to or better than the latter as stabilizers for plasticized polyvinyl chloride.

Epoxy fatty acids have been found in other plants, epoxyoleic acid has been demonstrated in members of several familites (29–36), first isolated from kenaf seed oil (31) and its isomer, *cis*-9,10-epoxy-*cis*-12-octadecenoic acid, is a constituent of *Chrysanthemum coronarium* L. (33,37) and possibly other species (38). Mixtures of vernolic acid with its isomers have been synthesized, monoepoxyoctadecenoic, from linoleic by Swern and Dickel (39), and as 12,13-epoxy-9-octadecenoic acid by Pigulevskii and Naidenova (40). Osbond (41), emphasizing the structural relationship of these acids to linoleic, has recently reported on the synthesis of (±) vernolic acid. A relative of oleic acid, *cis*-9,10-epoxystearic acid, has been reported present in the mixed acids from uredospores of a wheat germ rust (42,43) and in the seed of *Tragopogon porrifolius* (44), its *trans*-isomer, in orujo oil (34). The acid structurally related to linolenic acid, 15,16-epoxyoctadec-9,12-dienoic, has been reported present in *Camelina sativa* (L.) Crantz (45). Gunstone (21), Smith *et al.* (33) and Morris *et al.* (23,38) have pointed out the possible biological importance of epoxy acids in lipid chemistry. Other seed oils have been shown to contain epoxy components (25,38,46,47) but these have not been identified. Evidence of the widespread occurrence of epoxy fatty acids is accumulating.

Figure 1 is a photograph of a drawing (16) of a *V. anthelmintica* plant at the flowering stage. This reproduction was used instead of an actual photograph because it shows more detail; it corresponds in development to a 4 to 5 months old plant grown from seed in this laboratory. Briefly described, it is an erect, robust, leafy annual, 3 to 5 ft high; leaves petioled about 3 to 8 inches long; florets, pale violet, about 3/16 inch in diameter; achenes (single-seeded fruit, here referred to as seed) greenish-brown, 3/16 to 1/4 inch long and marked with about 10 lighter colored longitudinal ridges—Figure 2 (10 × magnification).

FIG. 1. *Vernonia anthelmintica* (L.) Willd.

Materials and Methods

Seed used for these investigations was of Indian origin supplied by Quentin Jones of the Crops Research Division, Agricultural Research Service, U.S.D.A. Seed for small-scale experiments was obtained from larger batches (100 lb or more) by a series of passes through a Boerner-Sampler and ground in a Wiley mill (No. 1 with 3-inch blades) to pass a screen having round holes of 2 mm di. After initial extraction, if exhaustive removal of oil was desired, marcs were

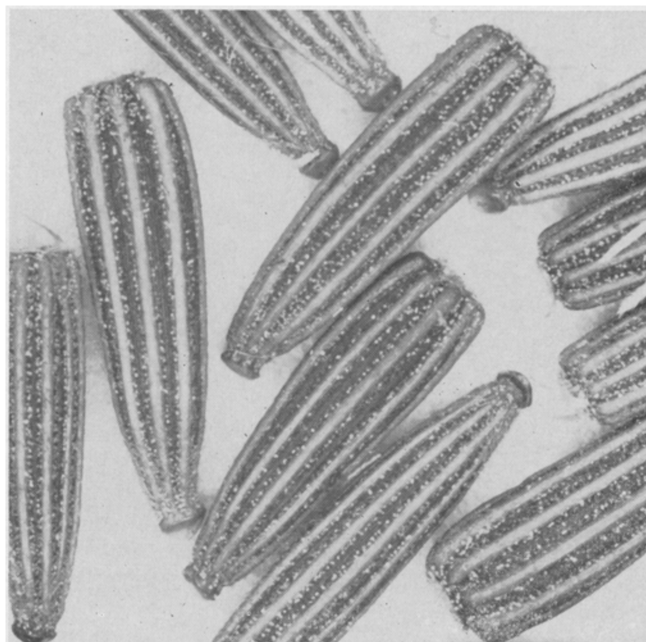

FIG. 2. *Vernonia anthelmintica* (L.) Willd. seed (10 × magnification).

dried and reground to pass holes of 0.5 mm di., then re-extracted. In Soxhlet extractions 100-g ground seed samples were used with a change of solvent after 2-hr operation to minimize heat effects; this was followed by 18-hr operational periods. Prolonged extraction removed small amounts of additional oil only if marcs were reground. Moisture determinations were completed in 3 hr on 70-g ground seed samples using toluene azeotropically; each sample was placed in a 1-l flask, 300 ml of toluene added, moisture-tube and condenser attached, and the mixture stirred magnetically during the distillation period.

Analytical data were obtained by AOCS procedures. In the Durbetaki (48) method for titrating oxirane oxygen, it was found advantageous to add 0.02 molar hydroquinone to prevent interfering side reactions with bromine; also the hydrogen bromide gas was diluted with nitrogen to prevent oxidation and suck-back.

Seed used in the pilot plant extraction was ground in a Ball and Jewel Shredder having 9-inch blades to pass a screen with 2 mm holes. A stainless steel extractor-evaporator was used to extract 225 lb. (212 lb, 96.1 kg, mfb) of ground seed. The seed was stored 26 days at room temperatures before extraction. A single batch method was used employing 1 gal of petroleum ether (bp 63–70C) for each lb of seed extracted. The extractor portion was equipped with a stainless steel, finely screened, 3-layer, false bottom, several layers of cheese-cloth having been placed between the screens and covering the top screen. The extractor section was loaded with the seed, closed, and the 833 l of solvent circulated by pump at 68C through the sample for 2 hr, followed by an overnight stand, a reheating and a recirculation of solvent the following morning. The warm extract was then pumped to the evaporator and the marc given 3 warm (68C) washes with 500- to 600-l portions of fresh solvent. The combined extract and washings were concentrated to about 20 l. The oil was obtained by complete removal of solvent under reduced pressure in a rotating evaporator. Two additional extractions were made using the procedure described.

In low-temperature separations involving epoxy components of the oil, it was advantageous to keep oil concentrations as low as 1 g per 15 ml of solvent and to scrape the walls of the container and stir during the initial stages of crystallization. In general, where solvent removal was necessary to obtain oil and oil products, it was done under vacuum with as little heat as possible with the aid of nitrogen. Samples were stored under nitrogen and maintained at about 5C. Trivernolin samples were preserved with about 0.03% hydroquinone.

Results

Four different accessions of *V. anthelmintica* seed of Indian origin were extracted by exhaustive Soxhlet technique using petroleum ether (bp 35–59C), the ground seed having been air-equilibrated from 2 to 26 days prior to extraction. The following characteristics obtained are compared in range to those of other investigators (10,11,17–22,25) shown in the second column:

Oil in seed, %	19.7– 26.3 (mfb)	14.2 – 26.9
Epoxyoleic acid in oil calc'd from oxirane oxygen value, %	68.7– 71.7	64.8 – 68.0
Iodine value (Wijs)	103.7–107.5	54.63–122.0
Free fatty acids in oil, as epoxyoleic acid, %	14.5– 38.6	26.4 – 34.3

TABLE I

Exhaustive Soxhlet Extraction of *V. anthelmintica* (L.) Willd. Seed Ground and Air-Equilibrated 4 Days

	Boiling range of petroleum ether, °C.			
	35–59		63–70	88–98
Oil in seed (moisture free basis), %	24.5	23.9 [a]	24.9	26.3
Epoxyoleic acid in oil (calc'd from oxirane oxygen), %	68.7	71.1	68.2	61.1
Free fatty acids in oil (as epoxyoleic acid), %	14.5	15.4	19.0	12.6
Iodine value of oil	107.3	103.7	101.9	96.7

[a] Whole seed stored one year before grinding.

These oils were viscous green liquids which solidified at 5C. The oil extracted in the pilot plant from a composite seed sample gave the following characteristics in addition to those listed above (in the second column are ranges reported by other investigators):

Specific gravity, 25C	0.9552	0.905 – 0.9731
Refractive index, 25C	1.4760	1.4742– 1.4860
Optical rotation, 25C	(+)2.5[a]	(−)10.7 –(+)9.8
Saponification value	179.3	164.9 – 209.9
Insoluble acids, %	93.0	91.6 ....
Water-soluble acids, as butyric, %	0.45	....
Reichert-Meissl value	3.57	7.88 ....
Unsaponifiable material,[b] %	6.7	1.68 – 7.9

[a] n-hexane. [b] $[\alpha]_D^{25} = (+)$ 18.4 (chloroform).

Preparatory to the pilot plant extraction 3 small-scale Soxhlet extractions were made on ground seed, air-equilibrated 4 days, to furnish criteria for a choice of petroleum ethers with different boiling ranges. From the results (Table I) one with boiling range 63–70C was selected. Data in this table also include an analysis on this same seed sample made 1 year later. The whole seed had been stored in a canvas bag at room temperatures under relatively dry conditions; the moisture content of the seed remained essentially unchanged (6.4 and 6.2%). This type of storage appears to have little effect upon the epoxy content; if anything, there was a slight increase on storage as evidenced by the oxirane and iodine values; also, there was no significant change in the free fatty acid content of the oil prepared from the stored seed.

The results of the pilot plant extraction are given in Table II. The oil obtained was green in color and stable for 6 months when stored at 5C under nitrogen. However, storage at room temperature for this length of time in a stoppered container without nitrogen protection seriously altered its epoxy content; the oxirane oxygen value dropped from 3.57 to 2.37%; also, when the oil was heated for 19 hr at 102C there was a reduction to 2.80%. 1,3-Divernolin (epoxyoleic acid diglyceride), previously thought to be stearin (10), precipitated from the oil on standing at room temperatures. The 1,3-divernolin was practically insoluble in petroleum ether at these temperatures and was purified by washing with this solvent and then recrystallizing several times from warm solvent (above 35C) in which it was readily soluble. Isolation of 1,3-divernolin on

TABLE II

Pilot Plant Extraction of *V. anthelmintica* (L.) Willd. Seed Ground and Air-Equilibrated 26 Days

(96.1 kg with petroleum ether, bp 63–70C)

		Extractions			
		1	2	3	Soxhlet analysis [b]
Oil yield [a]	kg	20.45	0.59	0.18	......
	%	21.2	0.60	0.20	23.3
Epoxyoleic acid in oil (calc'd from oxirane oxygen)	%	66.1	61.4	58.3	68.9
Free fatty acids in oil (as epoxyoleic acid)	%	38.3	42.8	41.0	18.9

[a] Total 23.2%, includes 1.2% in marc (Soxhlet analysis).
[b] Ground seed air-equilibrated 4 days.

a larger scale as described below was based upon this solubility characteristic.

1,3-Divernolin from Pilot Plant Extracted Oil. Half the oil obtained from Extract 1, Table II (10.2 kg) was dissolved in 40 l of warm (about 45C) p.e. (bp 63–70C) and the solution cooled to −18C. After standing at this temperature overnight, a precipitate was obtained which consisted chiefly of trivernolin, 1,3-divernolin, and vernolic acid. It was removed by filtration, washed with cold solvent and the filter cake dissolved in 24 l of fresh warm p.e. The solution was cooled and allowed to stand overnight at about 10C. The precipitate formed, chiefly 1,3-divernolin, was filtered off at this temperature, leaving a large proportion of trivernolin and vernolic acid in the mother liquor. This mother liquor was reserved for the preparation of trivernolin and vernolic acid described below. The filter cake was dissolved in about 20 l of solvent, the temperature maintained at about 45C while the solution was treated with a mixture of 16 g of each of carbon (Darco G-60), filter aid (Super-Cel), magnesium silicate (Florisil), and anhydrous sodium sulfate, and filtered before cooling below 35C. The clear filtrate was allowed to stand overnight at about 10C for precipitation of 1,3-divernolin. The 1,3-divernolin was filtered off and recrystallized from about 7 l of solvent, clarifying as previously described. The dried purified 1,3-divernolin obtained weighed 547 g, 5.37% of the oil, 1.14% of the seed on a moisture free basis; it melted at 55C and appeared to be 99.0% pure based upon oxirane oxygen value; it contained 0.15% FFA calculated as vernolic acid; theory for glycerol di-(epoxyoleic) ester, $C_{39}H_{68}O_7$, mol wt 648.97, oxirane oxygen 4.93%, I.V. 78.2, vernolic acid recovery 91.4%, carbon 72.18%, hydrogen 10.56%; found: oxirane oxygen 4.88%, I.V. 78.6, vernolic acid recovery from saponification 92.1%, carbon 72.00%, hydrogen 10.76%, $[\alpha]_D^{25}$ = (+) 4.64 (chloroform). The X-ray pattern for 1,3-divernolin showed a single phase with long spacing at 45.9 ± 0.2 A. The I.R. spectra for 1,3-divernolin are shown in Figure 3.

Vernolic Acid from Pilot Plant Extracted Oil. The mother liquor of about 26 l from the preparation of 1,3-divernolin above was cooled to −18C overnight. The precipitate was filtered off and washed at this temperature with cold solvent and redissolved in sufficient warm solvent to make 25.4 l of p.e. solution. Analysis showed that vernolic acid had not satisfactorily separated from trivernolin. A satisfactory partitioning technique follows: a 7-l aliquot (equivalent to 13.2 kg of seed, 2.81 kg of pilot plant extract having an acidity equivalent to 577 g of vernolic acid) was shaken with methanolic alkali solution. This phase contained NaOH equivalent to the FFA present (97.3 ml of 20 *N* alkali diluted to 1120 ml with water and sufficient methanol added to make 2800 ml; this gave a methanol content of 60%). Sharp partitioning occurred rapidly. The p.e. layer was reserved for the isolation of trivernolin described later. The aqueous phase was washed with 1 l of p.e. which was discarded. The aqueous phase was then shaken with a mixture of 6 l of p.e. and 224 ml of acetic acid (2 molar equivalents). Partitioning was satisfactory and the aqueous layer was discarded. The p.e. solution was washed 4 times with 3-l portions of 1% acetic acid solution followed by the same with water. It was dried over anhydrous sodium sulfate, clarified with 20 g of carbon, filtered and cooled to −20C. The vernolic acid was filtered off, washed at −20C with p.e. and the solvent removed to give 407 g of product, 14.5% of the oil, 3.1% of the seed on a moisture free basis. The freezing point of the vernolic acid was 29.8C and its purity was 93.7% based upon the oxirane oxygen value; theory for vernolic acid, $C_{18}H_{32}O_3$, mol wt 296.45, oxirane oxygen 5.40%, I.V. 85.6; found: 5.06%, 87.6, $[\alpha]_D^{25}$ = (+) 2.01 (n-hexane). The I. R. spectrum for vernolic acid is shown in Figure 3.

Trivernolin from Pilot Plant Extracted Oil. The p.e. layer reserved from the preparation of vernolic acid above was washed twice with 0.8-l portions of an aqueous solution (60% methanol containing 0.1 *M* sodium carbonate and sodium acetate) and 3 times with water. These washes contained only small amounts of lipoid material and so were discarded. The p.e. solution was clarified with a mixture of 12 g each of carbon, magnesium silicate, filter aid, and anhydrous sodium sulfate; it was filtered and diluted to 11 l with solvent, followed by overnight crystallization at −18C. After an additional low-temperature recrystallization the yield of trivernolin was 350 g, 12.5% of the oil, 2.7% of the seed on a moisture free basis. It melted at 25C, contained less than 0.1% FFA and had a purity of 95.6% based on oxirane oxygen value; theory for $C_{57}H_{98}O_9$, mol wt 927.40, oxirane oxygen 5.18%, I.V. 82.1; found: 4.95%, 82.7; $[\alpha]_D^{25}$ = (+) 2.01 (n-hexane.) See Figure 3.

Trivernolin from Enzyme Inactivated Seed. Experience in the pilot plant extraction suggested the presence of a hydrolytic enzyme system capable of rapidly converting the glycerides present to FFA after the seed is ground. This can be controlled by a) rapid extraction immediately after grinding, or b) heat treatment of the seed before grinding.

a) Rapid Extraction. Freshly ground seed, 132 g (6.2% moisture), was immediately extracted 5 times with 200-ml portions of hot p.e. (bp 35–59C) decanting each extract through an asbestos filter pad by suction filtration. The marc was finally washed onto the pad with hot solvent; the elapsed time from start of grinding to completion of extraction was 90 min. Extracts and wash were combined and the solvent evaporated to give 25.3 g of a light yellow oil, 20.7% yield on a moisture free basis, having a FFA content of 1.9%, oxirane oxygen value of 3.89%. A yield of 57% trivernolin from the oil was obtained following a separation as described under b).

b) Heat Inactivation. Most recently the enzyme in the seed has been inactivated by heat treatment prior to grinding. This is preferable if an oil containing trivernolin as the major component is desired. Whole seed (unground, moisture content 7.9%) was autoclaved about 35 min at 116C and 10 psi pressure. The seed was then ground and 2131 g (moisture 9.0%) was extracted with p.e. (bp 35–59C) 6 times at room temperatures using approximately 20 l. The light yellow oil weighed 410 g, 21.1% yield on a moisture free basis. Its oxirane oxygen value was 3.85%, I.V. 104.7 and FFA 1.0%. The trivernolin was obtained by adding 4 l of p.e. to dissolve 395 g oil. The solution stood overnight at about 3C and was filtered; only traces of solids were removed, indicating the absence of 1,3-divernolin. About 2 l of solvent were added to the filtrate to give a solvent:solute ratio of about 15:1. The trivernolin was crystallized at −20C, separated by filtration, washed with p.e. at −20C, and the solvent remaining on the filter cake evaporated off under vacuum. The yield of trivernolin (mp 25C, liquid at room temperatures) was 218 g, 55.2% of the oil, 11.7% of the seed on a mois-

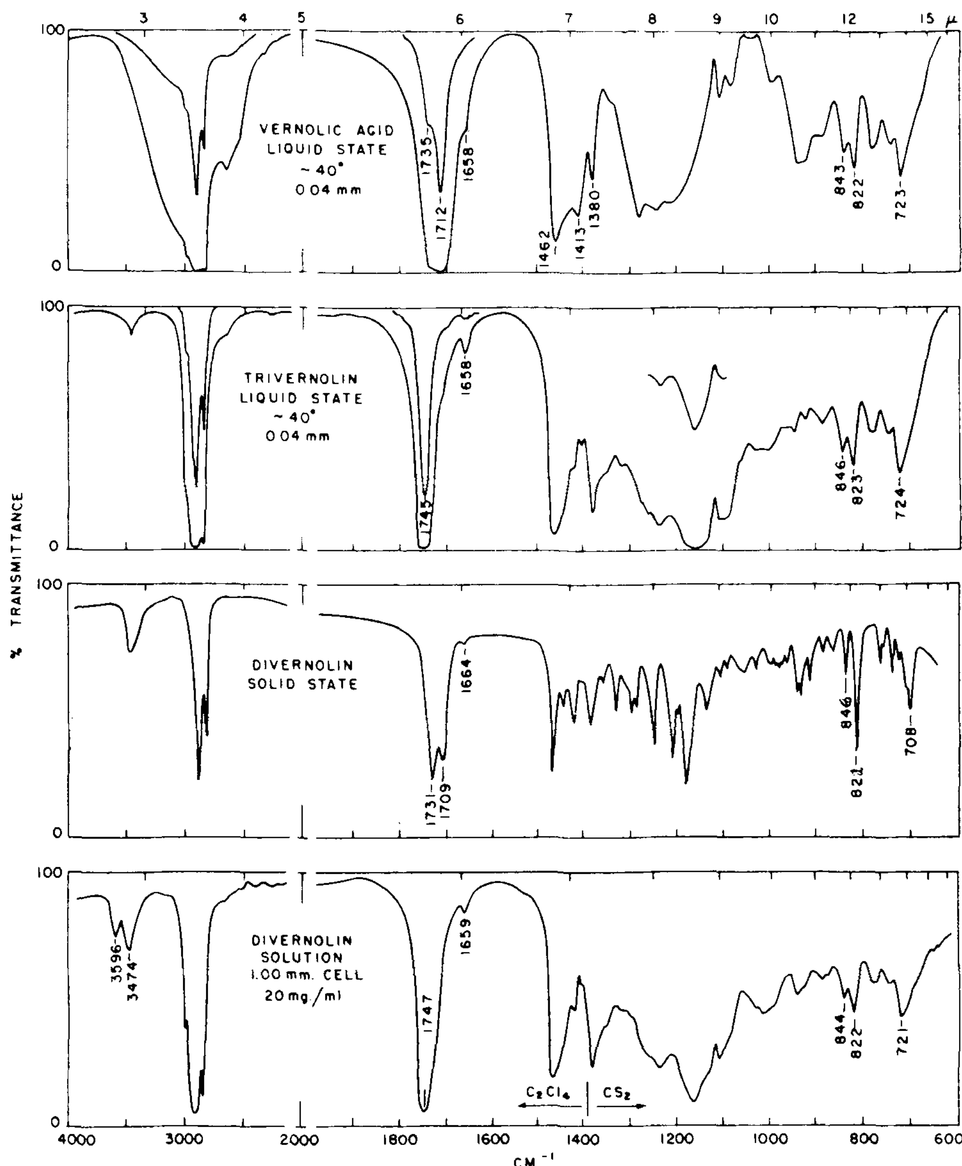

FIG. 3. Infrared spectra of vernolic acid, trivernolin, and 1,3-divernolin.

ture free basis; the FFA content was less than 0.2% and purity was 96.6% based on an oxirane value of 5.00%. From the combined mother liquor and wash, 140 g of solids were obtained by evaporation; these had an oxirane oxygen value of 2.13% which calculates to 55.3 g of vernolic acid (14.0% of the oil), potentially available from hydrolysis of these mixed esters.

Discussion

The high FFA (38.3–42.8%) of the oil obtained in the pilot plant extracts (Table II) prepared from ground seed which had been inadvertently stored 26 days awaiting late arrival of solvent was rather startling in view of the Soxhlet analysis value (19.0%) on the same seed sample air-equilibrated only 4 days.

Up to this time variance in the FFA content of *V. anthelmintica* seed oils was thought to be a characteristic of different seed accessions produced under a variety of growing conditions. This was the first evidence of the presence of a hydrolytic enzyme in the seed, highly active only after grinding, a fact which has now been confirmed with different accessions of seed.

Separation of 1,3-divernolin from trivernolin and vernolic acid depends upon abrupt changes in temperature of petroleum hydrocarbon solutions. 1,3-Divernolin becomes highly soluble above 32–35C and is partially soluble at 25C; it is almost completely insoluble at 18C. It is crystallized at about 3–12C since other lipoid components remain in solution in this temperature range and the 1,3-divernolin may be washed here where dilution is not critical. Trivernolin is highly soluble at 3C but crystallizes at about −4 to −15C. It is usually collected at −18 to −22C where the solubility is estimated to be only about 2 g per l. The temperature-solubility behavior of vernolic acid is similar to trivernolin; it was crystallized from p.e. by Smith *et al.* (22) at −10C, but in these investigations, at −20C to allow higher dilution and thorough washing; this is especially needed because the filter cake is highly solvated. If the FFA is under 2%, trivernolin crystallizes essentially free from acids in one crystallization and in several recrystallizations if the figure is as high as 8%. When the FFA content is higher it is necessary to separate trivernolin from vernolic acid by partitioning with alkali as previously described. Precursors of unsaponifiable material in the oil are soluble in p.e. at −20C; only 0.04% is found in trivernolin after crystallization from the oil, 17.5% in the mother liquor.

If oil of high trivernolin content and low FFA (2 to 5%) is desired, it can be obtained by immediate extraction of the seed after grinding. The quantity of FFA present in the oil will depend upon how rapidly these operations are performed. Oils of lower FFA can be prepared if the lipolytic enzyme in the seed is inactivated. Studies are in progress related to a) the control of this enzyme's activity (49) and its specificity in the hydrolysis of trivernolin and other glycerides, and b) the activity of the oxidative enzymes present in the seed.

Concerning the proton magnetic resonance spectrum of the trivernolin prepared as described above under the subject "Trivernolin from Enzyme Inactivated Seed," C. Y. Hopkins has stated in a communication: "The trivernolin spectrum is that of a pure monounsaturated triglyceride with the addition of the epoxy ring peak at about 7.2 τ."

If vernolic acid is the only product desired the method of handling the seed is not critical and direct saponification of the oil seems advisable, using the technique described by Smith *et al.* (22). Acetic acid appears to be a more satisfactory neutralizing agent for the soaps carrying the process out at pH 5 in the cold. Vernolic acid of about 95% purity on the basis of oxirane oxygen value is obtained with two crystallizations at −20C where the product is washed with cold solvent by slurry and decantation. No improvement in quality is obtained either by urea complex fractionation or by hexane-acetonitrile solvent partitioning or by a combination of both.

1,3-Divernolin appears to be present in the oil only under conditions where the enzyme is allowed to remain active after grinding the seed. At the present writing the optimum conditions for 1,3-divernolin production from *V. anthelmintica* are not known nor has any monoglyceride been isolated.

The analytical figures previously given were correct for either a 1,3- or 1,2-divernolin and supported the conclusion that the product formerly thought to be stearin (10) was a diglyceride. The compound's migration characteristics on thin-layer chromatography have been checked in comparison with 1-monopalmitin and 1,3-dipalmitin. Since it migrated to a position intermediate to these two standards as shown in Figure 4 it appeared to be a divernolin.

It is to be noted in Figure 3 that the I.R. spectra of a crystalline film and a dilute solution of 1,3-divernolin differ markedly. Probably the differences are to a large degree caused by a greater freedom to form rotational isomers in the solution state, a behavior common for long chain molecules. Thus, in the solid state the characteristic oxirane group bands in the 820–850 cm^{-1} region are sharper and more intense, numerous bands occur between 1100 and 1400 cm^{-1}, and the usual form of the CH_2 rocking band (at 721 cm^{-1} in the solution state) seems replaced by a band at 708 cm^{-1}. In the crystalline state the carbonyl band is split into two components at 1731 and 1709 cm^{-1}. The ratio of ester carbonyl groups to hydroxyl groups is 2 to 1, and apparently the 1709 cm^{-1} band can be explained as that of the portion of the carbonyls that is influenced by hydrogen bonding which conceivably could be by way of a 7-membered ring from the hydroxyl hydrogen, a 5-membered ring to the oxygen adjacent to a carbonyl, or various forms of intermolecular bonding.

Infrared, X-ray, and nuclear magnetic resonance data all supported the conclusion that the divernolin isolated was the 1,3-isomer. In solution spectra there was no I.R. band at 9.50 μ (Figure 3) as described for 1,2-diglycerides (50), but definitely one at 9.67 μ near 9.64 μ as described for 1,3-diglycerides. The OH stretching region of the divernolin was compared with a number of reference grade 1,2- and 1,3-diglycerides with a high-resolution grating spectrometer, and the pattern was like that of the 1,3-type. In X-ray analysis the 1,3-divernolin was shown to be of a single phase with a long spacing of 45.9 A, close to that of 45.2 A reported for 1,3-dilinolein (51). In two independent nuclear magnetic resonance studies one peak was found corresponding to the 4 magnetically equivalent protons on the 1- and 3-carbon atoms of the glyceryl unit. This peak was alike in the 1,3-divernolin and in the 1,3-dipalmitin. It was easily distinguished from a greater number of unequivalent glyceryl protons as in 1,2-dipalmitin. Furthermore, in the 1,3-divernolin there was no perceptible peaks from unesterified $-CH_2-O-$ or esterified $>HC-O-$ as in 1,2-diglycerides.

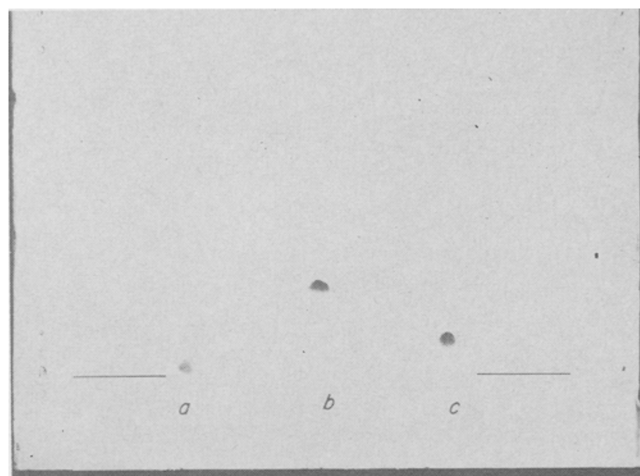

FIG. 4. Thin-layer chromatogram showing "a" 1-monopalmitin, "b" 1,3-dipalmitin, and "c" 1,3-divernolin. The chromatogram was developed with 20% diethyl ether and 1% acetic acid after spraying with 50% sulfuric acid. The reproduction was obtained by direct photocopy of the plate.

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The Nature of the C_{18} Polyethenoic Fatty Acids of Butter Fat [1,2]

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Abstract

Methyl C_{18} polyenoate concentrates were prepared from two samples of butter fat by low temperature crystallization and fractional distillation. The concentrates were fractionated on a silicic acid column and the resulting fractions were analyzed by ultraviolet and infrared spectrophotometric methods. About 42 and 30%, respectively, of the non-conjugated dienoate in the two samples were shown to have the *cis,trans* configuration. Fractions rich in dienoate and trienoate were prepared from the C_{18} polyene concentrates by silicic acid chromatography and the nature of these acids was studied by bromination, lipoxidase enzyme methods, and by alkali isomerization for varying periods of time. About 65 and 73%, respectively, of the non-conjugated dienoate in the two samples investigated were found to consist of linoleic acid while 79 and 71% of the trienoate were linolenic acid. Linoleic and linolenic acids were identified by preparing the characteristic tetra- and hexabromostearic acids. A *trans,trans* isomer of linoleic acid does not seem to be present in butter fat. A major proportion of the non-conjugated dienoic acid other than linoleic acid was found to have widely separated double bonds with *cis,trans* configuration. Occurrences of a C_{15} saturated acid, a branched-chain C_{17} saturated acid, and a heptadecenoic acid were indicated by gas chromatography.

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Introduction

BUTTER FAT is one the most complex of all animal fats, more than 25 fatty acids having been identified. However, the nature of the C_{18} polyethenoic acids, particularly the dienoic, has not been fully characterized. Eckstein (1) reported small amounts of linoleic and linolenic acids. Many workers were unable to find any linoleic acid (2–8), because of failure to isolate either a petroleum ether-insoluble tetrabromide or the tetrahydroxystearic acids, which are characteristic derivatives of linoleic acid. Green and Hilditch (4) and Brown (9) suggested that the octadecadienoic acids of butter fat are principally composed of geometric (*cis,trans* or *trans,cis*) isomers of linoleic acid. Later White and Brown (10) reported the isolation of petroleum ether-insoluble tetrabromides from C_{18} polyenoic acid concentrates of butter fat. On the basis of tetrabromide yield they reported that 66 and 75% of the octadecadienoic acids were linoleic acid. Shorland (8) reported that, while the octadecatrienoic acid is composed entirely of linolenic acid, the octadecadienoic acids from the New Zealand butter fat are principally composed of isomers of linoleic acid. In view of these divergent reports, it seemed important to reinvestigate the nature of the C_{18} polyethenoic acids of butter fat.

Experimental

Materials. The two specimens of summer butter fat used in the present investigation were kindly supplied by the Pickaway Dairy Co-operative Association Inc., Circleville, Ohio and the Department of Dairy Technology of the Ohio State University. One of these was received in the summer of 1956 (this will hereafter be referred to as SB-56) while the