Vernonia anthelmintica (L.) Willd. Extraction of Oil or Trivernolin from the Seed

C. F. KREWSON and W. E. SCOTT, Eastern Regional Research Laboratory, 2 Philadelphia, Pennsylvania

Abstract

Vernonia anthelmintica (L.), Willd. (ironweed) seed from Pakistan or India produced 22-28% oil rich in trivernolin, the glycereide of 12,13-epoxyoleic acid, when the highly active lipolytic enzyme was properly controlled. Trivernolin was obtained from the seed as a major end-product without isolation of the oil from the extract in yields of 55-60% of the wt of the oil present. Control of lipolysis was achieved, either by a rapid extraction technique where whole seed was flaked as a slurry made of seed and extracting solvent (petroleum naphtha, bp 35-59°C), or by autoclaving the seed prior to flaking or grinding. An improved quality oil was produced by removal of the major portion of the unsaponifiable material; this upgraded the oil by increasing the epoxy lipid content, decreasing the iodine value (I.V.) and reducing the amount of color and odor.

Introduction

Developmental research on V. anthelmintica at this laboratory is a part of an extensive USDA project which seeks new cash crops especially to relieve or replace those in surplus. The seed of this species of Vernonia, native to India and Pakistan, yields 22-28% oil containing about 70-75% vernolie (12,13-epoxyoleic) acid (Fig. 1) combined as glycereides. A previous report (1) described the epoxy fatty components isolated from the seed oil and presented a comprehensive review of the literature on this species of Vernonia. V. anthelmintica is not now a large commercial crop and considerable agronomic development will be required to make growing on a large scale feasible. However, good quality seed has been grown in this country during the 1963 season at a number of locations. This report deals with the production of trivernolin-rich oil from V. anthelmintica seed and with a simple procedure for obtaining pure trivernolin as a major product without isolation of the oil from the extract; the report also presents a method for the removal of the unsaponifiable material which usually amounts to ca. 6-7% (1,2) of the wt of the oil, thereby improving the quality of the oil.

Experimental Procedures and Results

Materials and Equipment. The Vernonia seed used in these investigations was from India or Pakistan, supplied in part through the courtesy of Quentin Jones, Crops Res. Div., USDA; a portion was purchased from Herbst Brothers, Seedsmen, Inc., New York, N. Y. Analytical data on seed samples and on the prepared products were obtained as previously described (1).

In the small-scale laboratory experiments, either a Waring Blendor or a "FitzMill" was used for comminuting the Vernonia seed. The W. J. Fitzpatrick Comminuting Machine (Chicago, Ill.) was equipped with a No. 2 screen (½ in. diam holes). In the FitzMill, dry seed was comminuted and immediately placed in the solvent (n-hexane). Seed was pulverized in the Waring Blendor in the presence of solvent (petroleum naphtha, p.n., bp 35–59°C). In both procedures, extracts were decanted through a suction filter equipped with a No. K-5 (Republic-Seitz Filter Corp., Newark, N. J.) filter pad. Solvent was removed by a rotating evaporator under reduced pressure.

In the larger-scale experiments using from 10-67.6 lb Vernonia seed with p.n. as the solvent, the seed was flaked in a Model NSP mill (Lauhoff Rolling Mill Corp., Detroit, Mich.). When whole dry Vernonia seed was flaked it was fed to the rolling mill by a Vibro-Flow Feeder (Type FMO 10) manufactured by the Syntron Company of Homer City, Pa.; the rate of feed was ca. 1-2 lb/min. When solvent-wetted Vernonia seed was flaked the slurry was fed manually to the rolling mill at the rate of ca. 2 lb/min. In either case flaked seed dropped into a 40-gal receiver partially filled with solvent. Cakes of dry ice were placed over the flaking rollers for the purpose of creating a safe working atmosphere with p.n. After flaking, sufficient solvent was added to bring the ratio to about 2-3 gal/lb seed for a first extract. The mixtures were stirred for ca. 3 min, the mares allowed to settle, and the supernatant extracts pumped off through a Sparkler filter press equipped with No. K-5 filter pads to 20-gal evaporators for removal of solvent under reduced pressure.

To clarify dark colored oils or trivernolin, as much as 1% Dareo G-60 and 2% Filtrol No. 4 based on the quantity of seed extracted was added during the stirring process in the extraction of flaked seed. When mares were used for experimental animal feeding the clarification step was added after the initial filtration of extracts and washings and a second filtration performed to remove the adsorbents from the miscella.

Where enzyme activity was controlled by autoclaving, the whole seed was treated at optimum conditions (3) of ca. 15 psi, 120-125°C, for 30-45 min.

A. Small-Scale Laboratory Extractions. The purpose of these small-scale extractions was to supplement the rapid extraction technique previously described (1) to find out if this procedure for the con-

\[
\text{d-}(+)\text{-cis-12,13-epoxy-cis-9-octadecenoic acid (12,13-epoxyoleic)} \\
\text{C}_{18}\text{H}_{32}\text{O}_{3}
\]

Fig. 1. Vernolic acid formula.
Experiment 1. For oil using eight 3.6-l portions of n-hexane at 4°C to pass a screen with 2-mm diam holes; finer screens as previously described, the seed was ground in a Wiley mill for larger-scale operations. In the work previously described, lipolytic activity in Vernonia seed might be feasible for extraction. In the work previously described, the seed was ground in a Wiley mill to pass a screen with 2-mm diam holes; finer screens could not be used because of the oily character of the seed and the overheating of the mill. The free fatty acid content of the oil was low (1.9%) if the extraction was quickly completed (less than 90 min). However, this procedure failed to extract all of the oil; for example, only 20.7% compared to an analytical figure of 23.9% for one oil. When the marc was dried, reground to pass 0.5-mm diam holes and exhaustively extracted in a Soxhlet apparatus an additional 2.9% of oil was obtained. In replicas of this experiment where the time consumed in grinding and extracting was increased to 3 hr the free fatty acid content of the oils produced was increased from 1.9-3.3%. Two-hr extraction periods in a Soxhlet have produced oils with a free fatty acid content of 8% and higher. Two experiments (Table I) are representative of optimal trials with the FitzMill preparation of seed for extraction, experiment 1 for oil and experiment 2 for trivernolin. In experiment 1, V. anthelmintica seed (417.3 g mfc, oil 26.4%) and n-hexane were used; the product was an almost water-white liquid, Gardner No. less than one. This yield compares favorably with the 11.7% previously reported (1). A second crop (8.2 g) of trivernolin of lower purity (90.8%) was obtained by conch of the mother-liquor with 45.4 g in a rotating evaporator; the yield was 10.9% (purity 97.4%) based on the dry wt of seed used; the product was an almost water-white liquid, Gardner No. less than one. This yield compares favorably with the 11.7% previously reported (1). A second crop (8.2 g) of trivernolin of lower purity (90.8%) was obtained by conch of the mother-liquor to 0.9 liters and chilling to -20°C. Complete removal of solvent produced a residue of 34.0 g; total solids extracted was 87.6 g (21.0%). Only 2.6 g (less than 1%) oil was obtained by 3 additional extractions of the marc at 4°C using 0.9-liter portions of solvent. The three Waring Blendor experiments presented in Table I serve to illustrate: that optimum extraction occurred at the temp range 4-8°C with respect to quantity and quality of oil, and that at the lower temp enzyme activity was reduced to a min and extreme haste in extraction procedures was unnecessary when seed was comminuted wetted with the solvent.

**TABLE I**

<table>
<thead>
<tr>
<th>Extract No.</th>
<th>Quantity</th>
<th>Free fatty acids (FFA)</th>
<th>Oxirane oxygen</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>2.0</td>
<td>6.6</td>
<td>5.04</td>
<td>97.4</td>
</tr>
<tr>
<td>4-5</td>
<td>2.0</td>
<td>6.9</td>
<td>4.90</td>
<td>98.9</td>
</tr>
<tr>
<td>6-8</td>
<td>2.0</td>
<td>6.8</td>
<td>4.60</td>
<td>92.2</td>
</tr>
<tr>
<td>Total</td>
<td>87.6</td>
<td>21.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Crop No. | Quantity | Free fatty acids (FFA) | Oxirane oxygen | Purity |
1. | 45.4 | 19.9 | 6.6 | 5.04 | 97.4 |
2. | 8.2 | 2.0 | 6.7 | 4.90 | 98.9 |
Residue: | 34.5 | 2.1 | 6.3 | 4.60 | 92.2 |
Total: | 87.6 | 21.0 | | |

In experiment 2 (Table I), the same quantity of seed was prepared and extracted as in experiment 1 except that 9.0 g (ca. 10% of the wt of oil present in the seed) of Filterol No. 4 and 4.5 g Darco G-60 was stirred with the 3.6-l solvent-seed mixture in preparation of the initial extract. Also, the marc was extracted only twice with 0.9-l portions of cold n-hexane and these two were added to the initial extract and the combination was then evaporated to 1.5 liters. This concentrate was chilled to -20°C with mechanical stirring to obtain trivernolin. In 2 hr the trivernolin crystals were removed at -20°C on a filter plate and washed 3 times with minimal quantities of cold solvent. The trivernolin was dried to a constant wt of 454.4 g in a rotating evaporator; the yield was 10.9% (purity 97.4%) based on the dry wt of seed used; the product was an almost water-white liquid, Gardner No. less than one. This yield compares favorably with the 11.7% previously reported (1). A second crop (8.2 g) of trivernolin of lower purity (90.8%) was obtained by conch of the mother-liquor to 0.9 liters and chilling to -20°C. Complete removal of solvent produced a residue of 34.0 g; total solids extracted was 87.6 g (21.0%). Only 2.6 g (less than 1%) oil was obtained by 3 additional extractions of the marc at 4°C using 0.9-liter portions of solvent.

The three Waring Blendor experiments presented in Table II serve to illustrate: that optimum extraction occurred at the temp range 4-8°C with respect to quantity and quality of oil, and that at the lower temp enzyme activity was reduced to a min and extreme haste in extraction procedures was unnecessary when seed was comminuted wetted with the solvent.

**CRUDE TRIVERNOLIN SOLUTION**

**CRUDE TRIVERNOLIN**

**FIG. 3. Isolation and purification of trivernolin.**