**Alchornea cordifolia Seed Oil: A Rich Source of a New C₂₀ Epoxide, (+)cis-14,15-epoxy-cis-11-eicosenoic Acid**

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**ABSTRACT**

A C₂₀ homolog of vernolic acid has been found at the 50% level in *Alchornea cordifolia*, Euphorbiaceae, seed oil. This new acid, (+)cis-14,15-epoxy-cis-11-eicosenoic (alchomoic) acid, was isolated by high-pressure liquid chromatography and characterized by mass spectrometry, nuclear magnetic resonance and infrared spectroscopy, optical rotary dispersion, and ozonolysis-gas-chromatography.

**INTRODUCTION**

Epoxide fatty acids have long been known to be seed oil constituents (1,2). Those found have all been 18-carbon acids with the epoxy group located in one of three positions: Δ9, Δ12, or Δ15; two stereoisomers are known for both the Δ9 and Δ12 positions. We now report a new epoxy acid, (+)cis-14,15-epoxy-cis-11-eicosenoic (alchomoic) acid, which makes up over 50% of the acyl groups of the seed oil from *Alchornea cordifolia* (Schum. & Thonn.) Muell.-Arg., a Euphorbiaceous plant collected in Ghana.

**EXPERIMENTAL PROCEDURES**

Oil, 46% (dry basis), was extracted from the ground seed with petroleum ether using a Butt extractor. Oxirane oxygen determination with HBr, infrared (IR), nuclear magnetic resonance (NMR), and gas chromatography-mass spectrometry (GC-MS) analyses were accomplished as previously described (3-5).

Thin layer chromatography (TLC) of the seed oil was performed on plates spread with 250 μ layers of Silica Gel G. Hexane-diethyl ether (70:30) was the developing solvent. The oil was tested for epoxy groups on the TLC plate using the picric acid method of Fioriti and Sims (6).

Methyl esters were prepared from the oil by two procedures: (a) reaction with diazomethane, followed by transesterification with 0.1% sodium methoxide in methanol, and (b) saponification in 0.5 N NaOH in methanol followed by reaction with 10% BF₃ in methanol.

A Hewlett-Packard model 402 gas chromatograph was used for methyl ester analyses. The chromatograph was equipped with a 10-ft x 4-mm ID glass column packed with 5% LAC-2-R 446 and a 4-ft x 4-mm ID glass column packed with 5% Apiezon L. Analyses were made isothermally at 185°C.

Epoxy esters were separated from the un-oxygenated ones on 25 g of 60/200 mesh Hi-Flosil (Applied Science Laboratories, Inc., State College, PA) packed in a 15 mm ID column. The esters were eluted with hexane containing increasing amounts of diethyl ether. Progress of the separation was monitored by TLC. The two components of the epoxy ester fraction were separated with a Waters model ALC-201 liquid chromatograph equipped with a 30-cm x 1/4-in. C₁₈-Bondapack column. The elution solvent was acetonitrile-water (80:20) at 2 ml/min.

The epoxy esters were ozonized as previously described (7), and the ozonides were reduced directly in the gas chromatographic column. To accomplish this, a 6-ft x 2-mm glass column was packed with 5% Apiezon L except for the first 6 in. which were filled with 1% palladium on 42/60 mesh Chromosorb P (8). With hydrogen as the carrier gas, the ozonides were catalytically reduced and the products were subsequently separated by the column and then directed to a mass spectrometer.

**RESULTS AND DISCUSSION**

Large amounts of epoxy groups were indicated in *A. cordifolia* oil by its hydrogen bromide uptake (57%, calculated as epoxyoleic acid) and moderate IR absorption at 827 and 848 cm⁻¹. TLC of the oil showed components with the same migration characteristics as free vernolic acid, monovemoyl, divemoyl, and trivemoyl triglycerides, when compared to *Vernonia antieiniminctia* oil. These components gave an orange color when reacted with picric acid, characteristic of epoxy groups (6).

GC analysis of the base catalyzed esters (Table I) revealed two unusual components. The smaller of the two (2.3%) had equivalent chain lengths (ECLs) of 19.0 from the Apiezon L column and 22.9 from the LAC-2-R 446 column, identical to authentic methyl vernolate. The major fatty ester had ECLs of 21.1 (Apiezon L) and 25.2 (LAC-2-R 446). These ECLs are ca. 2 units greater than those of methyl vernolate and indicated a similar fatty ester with two more methylene units. The mass...
TABLE I

| Fatty Acid Composition of Alchornea cordifolia Seed oil, % by Gas Liquid Chromatography |
|-----------------------------------|----------------------------------|
| 14:1                              | 0.1                              |
| 15:0                              | 0.2                              |
| 16:0                              | 12.8                             |
| 17:1                              | 0.1                              |
| Vernolic acid                     | 2.3                              |
| Alchornoic acid                   | 51.2                             |

The spectrum of the smaller component was indistinguishable from authentic methyl vernolate (MW = 310), and the major component exhibited a molecular ion of m/e 338. These spectra were not definite enough to locate the epoxy groups (5), and so methoxy-hydroxy derivatives were formed from the epoxy acyl groups by BF3-methanol treatment (9). After silylation, these compounds give spectra which can be used to locate the original epoxy function (5) as illustrated in Figure 1. Here, the spectrum of derivatized methyl alchornoate and the structures of the ions, which locate the methoxy and trimethylsilyloxy groups, are shown. These intense ions locate the epoxy group in the 14,15 position. In addition, the position of the double bond at Δ11 is indicated by the abundance of ions with m/e 217. In silylated derivatives of diols or methoxy-hydroxy esters, the primary cleavage is between oxygenated carbon atoms unless there is a double bond located one methylene unit from the oxygenated carbons in which cleavage between the double bond and the oxygenated carbon becomes important (5). If the double bond was conjugated to the epoxy group or separated by more than one methylene group, the m/e 217 ion would be much less intense or not found at all.

The location of the olefinic group was substantiated by both ozonolysis and NMR. Ozonolysis of the intact epoxy-ester fraction produced three major fragments: C9 epoxide aldehyde, C11 aldehyde-ester, and C10 methyl ester (thermal degradation of the C11 aldehyde-ester). In addition to these major fragments, minor fragments (C9 epoxide aldehyde, C9 aldehyde-ester, and C8 methyl ester) were observed from methyl vernolate.

No absorption for trans olefins was detected in the IR spectrum (960 cm⁻¹) and, therefore, the double bonds are in the cis configuration.

NMR data also were consistent with the epoxy monoenoic structure for the major acyl group from A. cordifolia. Proton signals were observed at 0.98 (terminal methyl), 1.28-1.56 (chain methylenes), 2.036 (methylenes adjacent to both the epoxy and olefinic groups), 2.296 (Δ2 protons), 2.906 (cis-epoxy), 3.626 (methoxyl), and 5.426 (olefinic). The location of the methylene generating the signal at 2.036 was established, since both the epoxy and olefinic proton signals collapsed when irradiated at 2.036.

Optical rotary dispersion (ORD) of the pure cis-14,15-epoxy,cis-11-eicosenoate, isolated by high-pressure liquid chromatography (HPLC), showed specific rotations of [α]25 + 2.24, [α]50 + 2.43, [α]520 + 2.66, [α]480 + 3.07, [α]400 + 4.21, [α]360 + 4.51, [α]335 + 4.62, [α]320 + 4.35, [α]300 + 3.40, [α]280 + 1.52 (C. 2.38). The ORD spectrum has the same sign and shape as that of methyl vernolate (10). Therefore, the configuration of methyl alchor-
(14S,15R) is most likely the same as (+) methyl vernolate (12S,13R) (10).

Three other Euphorbiaceae species, Euphorbia lagascae (11), Cephalocroton cordofanus (12), and Cephalocroton puauschelli (13), have been reported to produce seed oils containing large amounts of vernolic acid. We speculated that, in the past, minor amounts of alchornoic acid could have been overlooked in the analysis of oils from these species. However, GC experiments designed to find alchornoic acid at levels less than 0.1% failed to detect methyl alchornoate in oils from these species. It appears that the biosynthetic production of alchornoic acid either results from epoxidation of a C18 acid and subsequent chain elongation, as indicated from the small amount of vernolic acid present, or from a C20 acid directly.

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REFERENCES


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