The Occurrence of 6,9,12,15-Octadecatetraenoic Acid in *Echium plantagineum* Seed Oil

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Abstract

The seed oil of *Echium plantagineum*, a member of the borage family, has been shown to contain two polyunsaturated fatty acids not commonly found in vegetable oils: all-*cis*-6,9,12-octadecatrienoic acid and all-*cis*-6,9,12,15-octadecatetraenoic acid. Prior to their discovery in the Boraginaceae, nonconjugated tetraenoid acids were not known to occur in oils of higher plants.

Introduction

RESULTS OBTAINED in a continuing screening program at this Laboratory indicated the presence of small amounts of a nonconjugated C_{18}-tetraenoid acid in seed oils of the Boraginaceae or borage family. *Anchusa capensis* was reported to contain 4.4% of this tetraene, and 2.1% of the same component was reported in *Cynoglossum amabile* (5). Varying amounts of C_{18}-tetraenoid acid have been found in a number of other borage species (7). Gas-liquid chromatographic (GLC) analyses of these oils showed that the tetraene was accompanied by a nonconjugated C_{18}-trienoid acid different from the common linolenic (9,12,15-octadecatrienoic) acid. While the work reported in our paper was underway, we learned that Craig and Bhatty (3) had obtained results similar to ours in work on seed oils from other genera in the borage family.

Seed oil of *Echium plantagineum* (Fig. 1) was selected as a representative of this group for more detailed characterization of the unusual components. *E. plantagineum* is a hairy branching annual from 1½ to 3 ft high. It produces numerous blue-violet flowers along one side of an elongated flower stalk. This plant is native to southern Europe and thrives in areas having warm, dry summers.

Results and Discussion

GLC analysis (10) of *E. plantagineum* seed oil in the form of methyl esters yielded the chromatogram depicted in Figure 2. Peak *f* had equivalent chain length (ECL) 19.7 which agreed with that of the common linolenic (all-*cis*-9,12,15-octadecatrienoic) acid (10). This was preceded by smaller peak *e* having ECL 17.4. This ECL value was in agreement with that observed by Miwa et al. (10) for all-*cis*-6,9,12-octadecatrienoic acid. Peak *g*, the last to emerge, was surmised to be the octadecatetraenoic acid demonstrated in related oils by alkali isomerization (5).

Echium oil was converted to methyl esters and subjected to countercurrent distribution in the conventional hexane-acetonitrile system (11). The two trienes were obtained as a single peak between 400 and 600 transfers (Fig. 3). The tetraene was obtained as a slower moving component after 600–800 transfers. The UV and IR spectra of these fractions indicated that the double bonds were all-*cis* and that there was no conjugation. Alkali isomerization by the standard AOCS method (1) proved that the two fractions were trienoid and tetraenoid, respectively, and that they had the common methylene-interrupted spacing of double bonds. More widely separated double bonds cannot be conjugated by alkali (4) as required for determination by ultraviolet spectroscopy according to the AOCS method.

Positions of double bonds were established by Von Rudloff's permanganate-periodate oxidation method (13). Since the triene mixture yielded C_3 and C_6 monobasic acids together with C_6 and C_9 dibasic acids, the trienes were methyl esters of all-*cis*-9,12,15-octadecatrienoic and all-*cis*-6,9,12-octadecatrienoic acids. The tetraene yielded propionic and hexanedioic acids as oxidation products, indicating that it was methyl all-*cis*-6,9,12,15-octadecatetraenoate.

The NMR spectra of the respective concentrates corroborated the structures indicated by oxidative degradation and alkali isomerization. In the case of the tetraene, terminal unsaturation in the 15-position was indicated by a sharp triplet at 9.02 τ ($J = 7.5$ cps) as in linolenic acid (12). Integration of the triplet at 7.22 τ indicated the presence of six protons on carbons flanked by olefinic linkages and confirmed methylene-interrupted spacing of double bonds. The presence of 6 protons on carbons α to the carboxyl or to an unsaturated carbon was indicated by a peak at 7.8 τ. A double bond in the 3-position was not ruled out solely by the oxidative cleavage products obtained, but it was by the absence of the NMR peaks at 6.9–7.15 τ that would be associated with a methylene group flanked by a carboxyl group and a double bond (2). Similar considerations were applied to NMR spectrum of the triene concentrate: Δ^3-unsaturation was again excluded by the absence of peaks in the 6.9–7.15 τ region.

For some time a *conjugated* C_{18}-tetraenoid fatty acid—9,11,13,15-octadecatetraenoic acid—has been known as a constituent of *Parinarium laurinum* oil and of oils from several species of *Impatiens* (6).

FIG. 1. Experimental planting of *Echium plantagineum* in the Snake River Valley near Wawawai, Washington. These plants have attained a height of 14–16 in.

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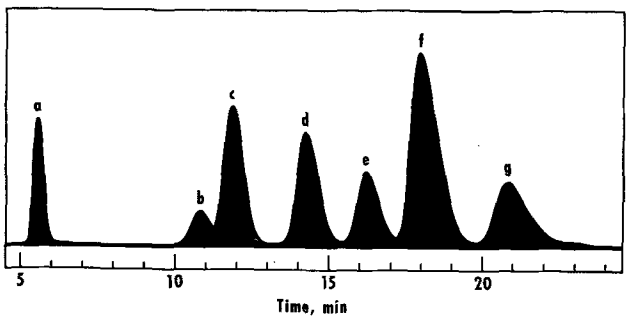

FIG. 2. GLC analysis of *Echium plantagineum* seed oil, using an R-446 column at 200C. Presumed identity and amounts of components: (*a*) C_{16} sat., 7.4%; (*b*) C_{18} sat., 3.7%; (*c*) C_{18} monoene, 17.1%; (*d*) C_{18} diene, 14.9%; (*e*) C_{18} "unusual" triene, 9.7%; (*f*) C_{18} triene, 33.6%; (*g*) C_{18} tetraene, 13.1%.

Non-conjugated acids with four double bonds have not been observed previously in seed oils of higher plants, although they have long been known in fish oils. Matic (9) as well as Klenk and Brockerhoff (8) have characterized all-*cis*-6,9,12,15-octadecatetraenoic acid from fish oils.

Experimental

IR spectra were determined with an Infracord Model 137 spectrophotometer on 1% carbon tetrachloride solutions. NMR spectra were determined with a Varian A-60 NMR spectrometer on 20% carbon tetrachloride solutions containing 0.5% tetramethylsilane. UV spectra were determined in ethanol solution with a Beckman DU spectrophotometer. GLC analysis was carried out as described by Miwa and co-workers (10) unless otherwise noted.

Preparation of Methyl Esters. Coarsely ground seeds of *Echium plantagineum* (50.3 g) were extracted overnight in a Soxhlet apparatus with petroleum ether (30–60C). The bulk of the solvent was removed under a nitrogen atmosphere and the remainder *in vacuo* with a rotating evaporator. The oil obtained (7.3 g) was saponified by refluxing with 150 ml 1 *N* ethanolic potassium hydroxide under nitrogen. Unsaponifiables were removed by extracting the resulting soap solution with petroleum ether-ethyl ether (1:1). Free acids (6.1 g) were obtained by acidification with HCl and extraction with ethyl ether. These acids were esterified with methanol containing 1% H_2SO_4. The result of the GLC analysis of these esters is given in the caption of Figure 2.

Countercurrent Distribution of Methyl Esters. Countercurrent distribution (CCD) of the *Echium* methyl esters was carried out in a 200-tube Post apparatus, using as the solvent system mutually saturated hexane and acetonitrile. A 40-ml portion of lower phase was placed in each of the 200 tubes. The methyl esters to be distributed were divided evenly among the first four tubes. The automatic operation of the instrument introduced 10 ml of equilibrated upper phase to tube 0 at every transfer stage. As hexane layers progressed past tube 200, they were decanted into a fraction collector, two upper phases per tube. The wt distribution curve obtained by evaporating *in vacuo* contents of selected tubes decanted from transfers 400–850 are indicated in Figure 2. GLC analyses indicated the larger of the peaks (transfers 460–560) contained 74.8% linolenate and 25.2% of the unusual triene. The smaller peak (transfers 640–800) contained C_{18} tetraene of 98–99% purity, according to GLC analysis.

Alkali Isomerization of Triene and Tetraene. Alkali isomerizations were carried out by the standard AOCS methods for a triene and for a tetraene, respectively (1). The results indicated the triene fraction to be essentially 100% of the two trienes combined, and the tetraene fraction to be 96.3% pure.

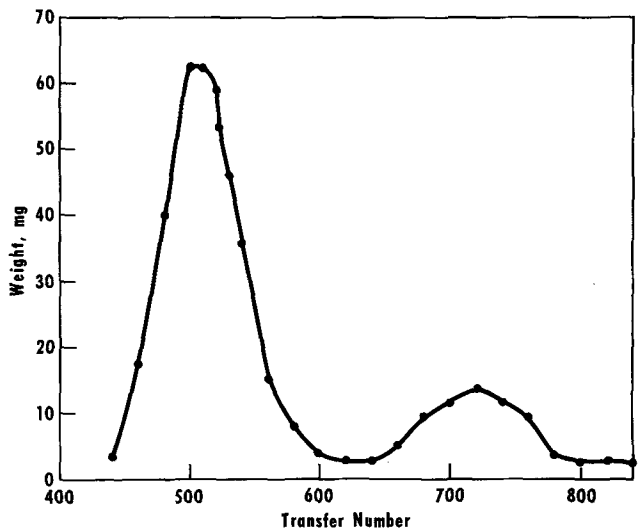

FIG. 3. Countercurrent distribution of *Echium plantagineum* methyl esters.

Oxidative Degradation of Triene Fraction. Triene concentrate (0.108 g) was oxidized by Von Rudloff's permanganate-periodate procedure (13). The cleavage products isolated and identified by GLC were: C_3 and C_6 monobasic acids together with C_6 and C_9 dibasic acids. The dicarboxylic acids were converted to methyl esters by esterification with 1% methanolic sulfuric acid, and were analyzed by GLC according to the procedure of Miwa and co-workers (10). The monocarboxylic acids were handled similarly, except that GLC analysis was carried out on the free acids. Because of uncertainties as to detector response to cleavage products of such widely varying chain length and oxygen content, it was felt that the GLC analysis of the triene concentrate from the CCD afforded the best measure of the relative amounts of the two isomers.

Oxidative Degradation of the Tetraene Concentrate. Tetraene concentrate (0.100 g) was oxidized by permanganate-periodate (13). The cleavage products isolated were propionic and adipic acids. These cleavage products were analyzed as described for the triene concentrate.

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