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New Sources of γ -Linolenic Acid

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ABSTRACT

γ-Linolenic acid ($18:3^{\Delta 6,9,12}$) occurs in significant amounts in various species of plants surveyed. Of the species analyzed in this study, *Nonnea macrosperma*, with 5.1% γ-linolenic acid in the seed, is the richest source of this fatty acid. Other species in the same family (Boraginaceae) are also good sources: *Adelocaryum coelestinum*, *Alkanna froedinii*, *Alkanna orientalis* and *Brunnera orientalis*. *Scrophularia marilandica* (family Scrophulariaceae) seeds contain 37.9% oil, of which 9.6% is γ-linolenic acid. All species mentioned above are better sources, when the total amount of γ-linolenic acid in the seed is considered, than that used traditionally, Evening Primrose (*Oenothera biennis*, family Onagraceae). None of the other Onagraceae nor any of the *Ribes* (family Saxifragaceae) species analyzed are as rich in γ-linolenic acid as Evening Primrose. Octadecatetraenoic acid ($18:4^{\Delta 6,9,12,15}$) was found in significant amounts in most of the Boraginaceae and *Ribes* surveyed. The Onagraceae and Scrophulariaceae lack detectable amounts of this fatty acid.

INTRODUCTION

Recently, there has been much interest in γ-linolenic (all *cis*-6,9,12-octadecatrienoic) acid due to claims of its involvement in preventing or alleviating a wide variety of human diseases (1-4). The most common source of this fatty acid is Evening Primrose (*Oenothera biennis*) seed oil; however, several species of the Boraginaceae, Aceraceae and Ranunculaceae families are also known to contain significant amounts (5-9). In our search for new oilseed crops we have found additional sources of this fatty acid in 45 different species.

EXPERIMENTAL PROCEDURES

Oil content of the seeds analyzed was determined by Butt extraction (10) with petroleum ether as solvent, and is based on the dry weight unless otherwise noted. Oil was transesterified with 10% BF_3 in MeOH according to the method of Metcalfe et al. (11), and analyzed in a Hewlett Packard 7610 gas chromatograph using two columns simultaneously: a 10 ft × ¼ in glass column packed with 5% LAC-2-R 446 on Gas-Chrom Q, and a 4 ft × ¼ in glass column packed with 5% Apiezon L on Chromosorb W AW DMCS, at 185 C. If γ-linolenic acid was previously reported in the family, as in the Boraginaceae and Onagraceae, identification was made solely on the basis of equivalent chain lengths (ECL) with known saturated fatty acid methyl esters as standards (12). For the two remaining genera, one species of each was analyzed as follows.

A triene isomer standard was prepared from fatty acid methyl esters of *Echium plantagineum* L. The C_{18} tetraene ($18:4^{\Delta 6,9,12,15}$) methyl ester was isolated by high performance liquid chromatography (HPLC) on a Whatman Partisil M9 10/50 ODS-2 column with acetonitrile as solvent and a differential refractometer as detector. A 24-mg sample of the tetranene was then reduced with hydrazine (13) until trienes comprised ca. 40% of the total. The trienes were separated from the mixture under the same HPLC conditions as above and analyzed by gas chomatography (GC) using a 25-m SP-1000 glass capillary column, 0.25 mm id, at 180 C. Trienes with ECL of 18.95, 19.13, 19.21 and 19.29 were obtained. ECL of 18.95 and 19.29 were identified as γ- and α-linolenic acids, respectively, by comparison with standards. The other two trienes, presumably $C18:3^{\Delta 6,9,15}$ and $C18:3^{\Delta 6,12,15}$, were not identified.

Triene methyl esters from *Ribes alpinum* L. and *Scrophularia marilandica* L. were purified by HPLC as described above, and the two mixtures of α- and γ-linolenic were analyzed by glass capillary GC. Each produced two peaks with ECL of 18.93-18.95 and 19.29, representing the presumed γ-linolenic and α-linolenic. Verification of the C_6 double bond of γ-linolenic acid in the mixtures was made by ozonolysis and triphenylphosphine reduction (14) followed by GC-mass spectrometry (MS) (GC: 5% BDS in a 2m × 2mm glass column, programmed from 50 to 220 C at 5 C/min; MS by electron impact in a Kratos MS-30 mass spectrometer).

RESULTS AND DISCUSSION

Location of the Double Bonds

The C9 and C6 aldehyde esters of the ozonolysis products from the two *Ribes* and *Scrophularia* linolenic mixtures

TABLE I

Composition of Seed Oils from Four Families

Species	Total γ-linolenic acid (% of seed)	Oil (% by wt)	Fatty acids (area % by GC) 18:3$^{\Delta 6,9,12}$	16:0	16:1	18:0	18:1	18:2	18:3	18:4	20:0	20:1
Onagraceae												
Oenothera agrillicolla Mackenz.	1.7	25.1[a]	6.7	6.2	0.2	2.5	13.9	69.4	0.1		0.2	0.4
O. biennis L.	2.5	24.6	10.0	7.0	tr[b]	2.0	9.0	72.0				
O. brevipes Gray	0	18.9[a]		8.7	0.2	2.4	7.1	78.2	0.1		0.2	0.1
O. cardiophylla Torr.	0	35.6		11.2	0.1	2.2	6.4	77.7	0.2		0.3	0.1
O. clavaeformis Torr.	0	34.6		9.4	0.1	1.5	4.6	84.1	0.1		0.1	
O. depressa[c] Greene	1.7	26.3	6.3	7.3	0.1	2.0	8.2	69.8	0.2		0.3	0.2
O. drummondii Hook.	0.1	29.1	0.5	10.8	0.1	2.0	10.4	76.2				
O. elata Hbk.	1.6	24.4[a]	6.7	5.9	0.1	2.5	10.4	73.3	0.4		0.2	0.2
O. grandiflora Ait.	0.3	3.7[a]	9.3	7.7	0.1	1.7	9.0	67.4	0.2		0.3	0.1
O. hookeri Torr. & Gray	1.7	24.1[a]	7.0	6.3	0.1	2.4	9.3	74.0	0.2		0.4	0.1
O. laciniata Hill	0.7	18.5[a]	3.9	7.1	0.1	2.4	7.6	77.4	0.1		0.2	0.1
O. lamarckiana Ser.	2.3	28.3	8.2	9.2	0.1	2.1	12.3	62.2	0.2		0.4	0.2
O. leptocarpa Greene	0	28.5		7.9	tr	2.4	6.5	82.8	0.4		tr	
O. missouriensis Sims	0	20.8		6.5	0.3	3.0	16.3	71.5	0.2		0.6	0.1
O. odorata Jacq.	0.5	28.8	1.7	10.8	0.1	3.7	10.9	70.5	0.1		0.3	0.2
O. parviflora L.	1.9	26.2[a]	7.1	5.4	0.1	2.7	11.9	70.9	0.3		0.6	0.4
O. rhombipetala Nutt.	1.9	30.7	6.1	7.6	0.1	2.5	8.2	75.0	0.1		0.2	0.1
O. rosea Ait.	0	31.9		10.2	0.1	2.8	7.7	77.9	0.4		0.3	0.2
O. serrulata Nutt.	0.3	22.0	1.2	10.2	0.2	3.7	11.1	68.3	0.2		0.5	0.1
O. stricta Steud.	0.6	30.7	1.9	9.8	0.1	2.8	8.7	75.8	0.3		0.3	0.2
O. strigosa (Rydb.) Mackenz. & Bush	2.0	29.0[a]	7.0	5.8	0.1	2.6	10.6	72.6	0.2		0.4	0.2
O. tetragona Roth	1.8	30.8	5.9	6.8	0.1	2.3	6.6	73.9	0.3		0.2	tr
Boraginaceae												
Adelocaryum coelestinum[d] (Lindl.) Brand	2.7	21.5	12.4	11.0	0.2	3.1	31.2	14.1	4.7	3.7	0.7	4.6
Alkanna froedinii Rech.	4.6	47.0[a]	9.9	7.4	0.1	2.7	20.6	26.3	27.1	5.9		
Alkanna orientalis (L.) Boiss	2.8	22.9	12.4	5.8	0.1	2.6	15.3	26.1	31.7	4.9	tr	1.1
Amsinckia intermedia F. & M.	2.3	27.7	8.2	11.5		3.8	31.7	13.3	17.4	9.5	0.3	2.9
Amsinckia lunaris Macbr.	2.4	26.5	8.9	11.2		3.4	38.5	13.4	12.2	5.8	0.8	3.7
Anchusa strigosa Labill.	1.6	22.2	7.4	10.0	tr	2.1	38.0	38.0	tr			2.2
Brunnera orientalis (Schenk.) I. M. Jtn.	4.2	27.2	15.4	9.6	0.1	2.8	28.9	27.1	8.8	2.5	0.1	2.6
Cryptantha grayi (Macbr.) Jtn.	1.8	29.2	6.2	7.9	0.2	3.4	15.9	21.4	33.8	9.6	0.2	0.2
Cynoglossum nervosum[e] Benth. Exc. B. Clarke	1.9	24.2	7.8	10.1	0.4	1.9	32.7	21.7	7.0	3.1	1.6	4.7
Echium glomeratum Poir	1.1	17.1	6.6	8.0		2.3	13.2	15.3	44.6	9.1	0.9	
Hackelia floribunda[f] (Lehm.) Johnston	1.6	25.2	6.4	8.5	0.3	2.8	33.1	15.5	13.8	6.9	0.5	5.9
Nonnea macrosperma[g] Boiss & Heldr.	5.1	38.6	13.1	7.0	0.1	2.2	24.7	34.4	9.0			
Pectocarya platycarpa Munz & Johnst.	2.3	15.0	15.2	9.5	0.1	2.9	19.0	17.9	19.9	12.0	0.1	1.5
Trichodesma zeylanicum R. Br. (Burm. F.)	1.4	31.9	4.3	9.5	0.2	6.2	28.6	20.5	24.3	5.0	0.6	0.8
Scrophulariaceae												
Scrophularia canina L.	1.4	31.1	4.5	8.4	0.1	2.8	28.1	55.9	tr		tr	0.2
S. grayana Maxim.	1.6	43.4	3.8	6.4	0.1	1.8	16.3	71.0	0.5		tr	0.1
S. koraiensis Nakai	1.5	39.9	3.7	7.2	0.3	2.8	14.8	70.5	0.1		0.3	
S. lanceolata Pursh.	2.1	26.3	8.0	5.8	0.1	2.1	16.6	66.0	0.5		0.4	tr

TABLE I (continued)

Species	Total γ-linolenic acid (% of seed)	Oil (% by wt)	Fatty acids (area % by GC) 18:3Δ6,9,12	16:0	16:1	18:0	18:1	18:2	18:3	18:4	20:0	20:1
S. marilandica L.	3.6	37.9	9.6	7.2	0.6	2.5	14.9	62.6	0.6		0.9	0.2
S. michoniana Coss & Kral.	1.0	28.1	3.5	9.9	tr	2.6	28.1	55.8				0.1
Saxifragaceae												
Ribes alpinum L.	1.7	18.7	8.9	5.6	0.2	1.4	18.1	39.0	22.0	4.4	tr	0.1
R. mebrians Lindl.	0.6	17.7[a]	3.4	5.4	0.1	2.0	17.6	40.4	26.4	2.4		0.8
R. montigenum McClatchie	0.8	22.0[a]	3.7	5.3	0.1	1.5	22.3	32.5	28.9	3.3		0.5
R. orientale Desf.	0.2	11.7	1.9	6.8	0.2	1.4	18.1	49.1	19.6	0.9	tr	0.2

[a]Oil percentage calculated on fresh weight basis.
[b]tr=trace, <0.1%.
[c]Unknown fatty acids comprise 4.8% of the seed oil.
[d]Also 2.2% 22:0 and 7.4% 22:1.
[e]Also 0.7% 22:0 and 5.3% 22:1.
[f]Also 4.3% 22:1 and 1.5% 24:1.
[g]Unknown fatty acids comprise 8.8% of the seed oil.

were identified by comparison of their mass spectra with those published by Noble and Nawar (15). GC retention times were consistent with these identifications.

Analysis of the Oils

Of the 22 species of *Oenothera* analyzed, *O. biennis*, or Evening Primrose, seeds have the highest percentage of γ-linolenic acid at 2.5% (10% of the oil) (Table I); this species is the traditional source of γ-linolenic. *O. grandiflora* contains almost as much of this fatty acid in the seed oil, but the oil makes up less than 4% of the seed. Most other species of the genus also contain a significant amount of γ-linolenic; however, 6 have none at all. In fact, of the *Oenothera* surveyed, those that have the highest total oil content do not contain any γ-linolenic acid. None have a measurable amount of octadecatetraenoic acid.

Of the Boraginaceae surveyed, 5 are richer sources of γ-linolenic acid than Evening Primrose. For example, *Nonnea macrosperma* seeds contain 38.6% oil, and of that oil, 13.1% is γ-linolenic acid, yielding a total of 5.1% of the seed. Likewise, note *Alkanna froedinii, Brunnera orientalis, Alkanna orientalis* and *Adelocaryum coelestinum* (Table I). Additionally, many of these Borages have a large amount of octadecatetraenoic acid.

Scrophularia marilandica, with very high oil content (37.9%), has a substantial amount of γ-linolenic acid (9.6%); this seed is thus also a richer source of the fatty acid than Evening Primrose. None of the *Scrophularia* have any detectable tetraene. *Ribes alpinum*, with 8.9% γ-linolenic acid and 18.7% oil, is the best source of γ-linolenic acid in this genus. All *Ribes* surveyed contain a measurable amount of tetraene.

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