SHORT COMMUNICATION

Stereospecific Analysis of *Onosmodium hispidissimum* Mack. Seed Oil Triglycerides[1]

S.L. MacKenzie[a,*], **E.M. Giblin**[a] and **G. Mazza**[b]
[a]Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, S7N 0W9, Canada and
[b]Agriculture Canada Research Station, Morden, Manitoba, R0G 1J0, Canada

The objective of the study was to determine the fatty acid composition of *Onosmodium hispidissimum* Mack. seed oil and the stereospecific distribution of γ-linolenic and stearidonic acids in the seed oil triglycerides. The seed oil contained about 20% γ-linolenic acid and about 8% stearidonic acid. About 90% of both γ-linolenic acid and stearidonic acids were esterified to the *sn*-2 and *sn*-3 positions.

KEY WORDS: γ-Linolenic acid, *Onosmodium*, stearidonic acid, stereospecific analysis.

γ-Linolenic acid (GLA) is an essential fatty acid and a precursor of prostaglandin E_1 and its derivatives. In the last decade, there has been considerable interest in plant seed oils containing GLA as potential dietary sources to ameliorate clinical symptoms of a variety of conditions including rheumatoid arthritis, eczema, high blood pressure and multiple sclerosis (1–4).

GLA is found in the seed oils of the Boraginaceae, Aceraceae and Ranunculaceae families. Most well known are borage (*Borago officinalis*), evening primrose (*Oenothera* spp.) and blackcurrant (*Ribes nigrum*) which typically contain, respectively, 24–25 (5), 7–14 (6) and 19% GLA (7) in the seed oil. GLA accumulates predominantly in the *sn*-3 position of evening primrose and blackcurrant oil triglycerides (8) but in the *sn*-2 position of borage (9). However, Muhkerjee and Kiewitt (9) concluded that GLA is preferentially esterified at the *sn*-1 and *sn*-3 positions of evening primrose seed oil. Stearidonic acid (Δ 6, 9, 12, 15 18:4) is distributed in blackcurrant seed oil similarly to GLA (8).

Evidence from studies on peanut oil (10) and synthetic triglycerides (11) indicates that absorption of fatty acids, especially unusual ones, is a function of stereospecific distribution and of the nature of the adjacent fatty acids. Furthermore, Horrobin (12) maintains that "the precise positions of the individual fatty acids and the nature of their companions on the triglyceride molecule are clearly very important determinants of biological activity." Lawson and Hughes (8) suggested that "a high linoleic acid or high linoleic:oleic acid ratio in the 2-position may facilitate GLA absorption."

Onosmodium hispidissimum Mack. (false gromwell) is a perennial plant of the Boraginacea. It is native to southern Manitoba, southern Ontario and the Eastern United States. Seeds of *Onosmodium* species constitute a rich natural source of GLA (13,14) and also of stearidonic acid (15). In previous studies, the overall fatty acid composition was determined but triglyceride species have not been identified, and the stereospecific distribution of GLA remains unknown.

This report extends a previous study (13) and present information on the stereospecific distribution of GLA and stearidonic acids in *O. hispidissimum* seed oil.

EXPERIMENTAL PROCEDURES

Seed. Mature seeds of *O. hispidissimum* Mack. were harvested in late September 1990 from field-grown plants established at the Agriculture Canada Research Station (Morden, Manitoba, Canada) in 1988 and 1989. The seeds were kept in nonrefrigerated storage at 20 ± 1°C for approximately 3 mon prior to analysis.

Extraction. Ten grams of seed were boiled with 12.5 mL isopropanol for 10 min. The seeds were ground using a Brinkman Model 1-PT10-35 homogenizer (Brinkman Instruments Inc., Rexdale, Ontario, Canada) at top speed for 2 min. After adding another 12.5 mL isopropanol, the seeds were again ground for 2 min. After adding 12.5 mL dichloromethane and 5 mL water, the mixture was allowed to stand for 1 h and then filtered through a glass-fiber filter. The precipitate was washed with 25 mL dichloromethane/isopropanol/water (1:2:0.6, vol/vol/vol). Fifty mL dichloromethane and 50 mL M KCl in 0.2M H_3PO_4 were added, and the organic phase was separated. The residual aqueous phase was backwashed with 20 mL dichloromethane, and the organic phase was separated. The combined organic phases were dried over anhydrous magnesium sulfate.

To determine total oil recovery, the sample was adjusted to 30 mL by rotary evaporation and solvent addition. A 10-mL aliquot was filtered through a 0.22-μm nylon filter, and the filter was washed with 5 mL hexane. Two mL chloroform/benzene/methanol (CBM; 1:1:1; vol/vol/vol) was added, and the whole was evaporated to dryness with dry nitrogen. CBM (2 mL) was added to the remaining 20 mL, and the whole was concentrated to about 2 mL in preparation for fractionation.

Column chromatography. A 1.2-cm i.d. × 45 cm column was filled with 30 g silicic acid (Biorad minus 325 mesh; Biorad Laboratories, Ltd., Mississauga, Ontario, Canada). The column was prewashed with, successively, 500 mL each of hexane, 50% hexane/ether, ether, methanol and hexane (16).

Sample (about 2 mL) application was followed by 2 × mL rinses with hexane. The column was then eluted successively with 6 × 100 mL aliquots of each of hexane, hexane/ether (98:2, vol/vol), hexane/ether (95:5, vol/vol), hexane/ether (85:15, vol/vol), ether and methanol. Eluant fractions were concentrated to about 10 mL and filtered through a 0.45-μm nylon mesh, and the filter was rinsed with hexane or the corresponding elution solvent. CBM (2 mL) was added, and the fractions were evaporated to dryness in a stream of dry nitrogen. After determining the mass, each sample was dissolved in hexane or meth-

[1]This paper is NRCC No. 36484.

*To whom correspondence should be addressed at National Research Council, Plant Biotechnology Institute, 110 Gymnasium Road, Saskatoon, SK, Canada, S7N 0W9.

TABLE 1

Fatty Acid Composition of *Onosmodium hispidissimum* Seed Oil

	Composition (mol%)[a]									
	16:0	18:0	18:1	18:2	γ-18:3	α-18:3	18:4	20:1	22:1	?
mol%	6.5	2.5	13.5	18.2	20.1	26.8	8.1	1.8	0.2	0.2
SD	0.2	0.1	0.2	0.1	0.6	0.5	0.2	0.0	0.04	0.05

[a]n = 4.

anol to a concentration of 10 mg/mL in preparation for thin-layer chromatography (TLC).

TLC. About 200 μg of each sample was spotted on Whatman type 6 Silica gel 60A plates (Maidstone, England). Plates were developed with benzene/diethyl-ether/ethyl acetate/acetic acid (80:10:10:0.2, by vol). Lipids were detected by spraying with 0.2% dichlorofluorescein in ethanol and observed under ultraviolet light.

Fatty acid analysis. After adding 200 μg C17:0 as an internal standard, a 100-μL aliquot of each fraction was evaporated to dryness and transmethylated by heating for 3 h at 80°C in 0.5 mL 3N methanolic HCl. On cooling to room temperature, 0.5 mL 0.9% NaCl was added, and the fatty acid methyl esters were extracted twice with 250 μL hexane. The combined extracts were assayed in a Hewlett-Packard Model 5890 gas chromatograph fitted with a DB23 column (30 m × 0.32 mm i.d.) (Palo Alto, CA). The injector was operated in the split mode at a ratio of 80:1. The oven temperature was programmed as follows: 180°C for 1 min; 4°C/min to 240°C; hold for 8 min. Injector and detector temperatures were 250°C. The carrier gas was helium at a flow rate of 22 cm/s at 150°C. Data were acquired and computed with a Hewlett-Packard Model 3392 A integrator.

Stereospecific analysis. The stereospecific distribution of the triglyceride fraction was determined by the method of Takagi and Ando (17). The 1- and 2-monoglycerides formed by reaction with the Grignard reagent were resolved by TLC. The 2-monoglycerides were assayed as the methyl esters. 1-Monoacyl glycerols were reacted with 3,5-dinitrophenyl isocyanate, and the *sn*-1 and *sn*-3 monoglyceride derivatives were resolved on a chiral column (Sumichiral OA-4100; Rose Scientific, Edmonton, Alberta, Canada). Each fraction was assayed as the methyl esters.

RESULTS AND DISCUSSION

Fatty acid composition. The oil constituted about 19.5% of the seed weight. The fatty acid composition is shown in Table 1. Mass spectrometric analysis indicated that the two unidentified components that eluted after 20:1 are fatty acids but the spectra could not be unambiguously interpreted. The seed oil content is slightly greater and the oil GLA content lower than previously recorded (13). In addition, the present analyses indicate slightly greater 18:0 and α-18:3 contents but slightly lesser values for 18:2. No significance can be attributed to these differences.

A distinct separation of monoglycerides and polar lipids was not achieved with the silicic acid column and the protocol described. Individual triglyceride fractions contained as much as 25% GLA and 41% stearidonic acid (data not shown). The proportions of linolenic and stearidonic acids in several triglyceride fractions indicate that these fatty acids must be incorporated into more than one position in the glyceride structure. The two unidentified components mentioned earlier were located exclusively in the diglyceride fraction.

The compositions of the triglycerides, diglycerides and monoglycerides plus polar lipids are shown in Table 2. The diacylglyceride (DAG) and monoacylglyceride (MAG) fractions contain significantly less GLA (14.8 and 13.2%, respectively) than does the triacylglyceride (TAG) fraction (21.3%). Similarly, the proportion of stearidonic acid in the triglycerides is much greater than in the other two fractions.

Stereospecific distribution. The stereospecific distribution of the seed oil triglyceride fatty acids is presented in Table 3. Palmitic and stearic acids are predominantly located in the *sn*-1 position. Lawson and Hughes (8) reported a similar finding for other GLA-containing seed oils. Oleic and linoleic acids were evenly distributed among all three positions. α-Linolenic and eicosenoic acids were distributed mostly (82 and 87%, respectively) in the *sn*-1 and *sn*-3 positions. Erucic acids, although present in small amounts, was found exclusively in the *sn*-2 and *sn*-3 positions. GLA was distributed mainly between the *sn*-2 and *sn*-3 positions (89% of total) but predominantly (50%) in the former. In this respect, *Onosmodium* seed triglycerides resemble those of borage (8) rather than those of other GLA-containing seed oils. As was observed for blackcurrant seed oil (8), stearidonic acid was distributed similarly to GLA.

The percentage distribution of the various fatty acids among the three triglyceride positions reflects the selectivity of the corresponding acyltransferases. Thus the glycerol-3-phosphate acyltransferase appears to have a preference for saturated acyl-CoA substrates but can also readily accept 18:1, 18:2, α-18:3 and 20:1 CoAs. The much reduced proportion of stearidonic acid and the zero incorporation of erucic acid in the *sn*-1 position relative to the other two positions suggest a low affinity for the corresponding CoAs. The lysophosphatidic acid acyltransferase has a reduced affinity for 16:0, 18:0, α-18:3 and 20:1,

TABLE 2

Fatty Acid Analysis of *Onosmodium hispidissimum* Lipids[a]

		Composition (mol%)									
	% of oil	16:0	18:0	18:1	18:2	γ18:3	α18:3	18:4	20:1	22:1	?	?
TAGs	92.0	6.4	2.4	14.1	17.9	21.0	27.0	8.4	1.8	0.2	—	—
DAGs	3.6	8.4	4.3	22.4	25.9	14.7	15.7	3.5	1.9	0.4	1.7	1.3
MAGs + polar lipids	4.2	17.8	5.1	17.0	29.5	13.2	13.5	2.9	1.2	—	—	—

[a]—Denotes not detected. Abbreviations: TAGs, triacylglycerols; DAGs, diacylglycerols; MAGs, monoacylglycerols.

TABLE 3

Stereospecific Distribution of *Onosmodium hispidissimum* Seed Oil Triglyceride Fatty Acids

	mol% of total fatty acids			Percentage distribution of each fatty acid		
Fatty acid	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
16:0	15.2	3.7	4.6	65	16	19
18:0	5.4	1.9	2.1	57	20	22
18:1	16.7	14.5	11.7	39	34	27
18:2	16.0	18.4	15.8	32	37	31
γ18:3	6.3	29.8	22.8	11	50	39
α18:3	31.6	13.3	27.5	44	18	38
18:4	1.7	9.4	9.4	8	46	46
20:1	2.6	0.7	2.1	48	13	39
22:1	0.0	0.5	0.8	0	38	62

while the DAG acyl transferase has a distinct preference for 22:1 CoA but does not discriminate strongly against any of the mono- or polyunsaturates.

REFERENCES

1. Wolf, R.B., R. Kleiman and R.E. England, *J. Am. Oil Chem. Soc. 60:*1858 (1983)
2. Passwater, R.A., *Evening Primrose Oil,* Keats Publishing Co., New Canaan, 1981.
3. Gunstone, F.D., *Progr. Lipid Res. 31:*145 (1992).
4. Horrobin, D.F., *Ibid. 31:*163 (1992).
5. Tetenyi, P., I. Hethelyi, T. Okuda and S. Imre, *Herba Hung. 13:*61 (1974).
6. Hudson, B.J.F., *J. Am. Oil Chem. Soc. 61:*540 (1984).
7. Traitler, H., H. Winter, U. Richli and Y. Ingenbleek, *Lipids 19:*923 (1984).
8. Lawson, L.D., and B.G. Hughes, *Ibid. 23:*313 (1988).
9. Muhkerjee, K.D., and I. Kiewitt, *J. Agric. Food Chem. 35:*1009 (1987).
10. Myher, J.J., A. Marai, A. Kuksis and D. Kritchevsky, *Lipids 12:*775 (1977).
11. Mattson, F.H., G.A. Nolen and M.R. Webb, *J. Nutr. 109:*1682 (1979).
12. Horrobin, D.F., in *Dietary Omega-3 and Omega-6 Fatty Acids,* NATO ASI Ser., Ser. A, 171, Plenum Press, New York, 1989, pp. 297–307.
13. Mazza, G., and H.H. Marshall, *Can. Inst. Food Sci. Technol. J. 21:*558 (1983).
14. Gross, A.T.H., and D.G. Dorrell, *Can. J. Plant Sci. 56:*659 (1976).
15. Craig, B.M., and M.K. Bhatty, *J. Am. Oil Chem. Soc. 41:*209 (1964).
16. Christie, W.W., in *Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids,* 2nd edn., Pergamon Press, Oxford, 1982.
17. Takagi, T., and Y. Ando, *Lipids 26:*542 (1991).

[Received December 2, 1992; accepted April 19, 1993]