γ-Linolenic and Stearidonic Acids in Mongolian Boraginaceae[1]

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ABSTRACT: Seeds of nine Central Asian species of Boraginaceae were investigated for the first time for their oil content and for the fatty acid composition of their seed oils by capillary gas chromatography. Levels of γ-linolenic acid ranged from 6.6 to 13.0%, and levels of stearidonic acid ranged from 2.4 to 21.4% of total seed fatty acids. The seed oil of *Hackelia deflexa* exhibited the highest stearidonic acid content (21.4%) that has been found so far in nature. Other high contents of this fatty acid were in three *Lappula* species (17.2 to 18.1%). Seed oils of *Cynoglossum divaricatum* and *Amblynotus rupestris* contain considerable amounts of *cis*-11-eicosenoic (5.3 to 5.8%) and *cis*-13-docosenoic acid (7.0 to 9.7%) besides γ-linolenic (10.2 to 13.0%) and stearidonic acid (2.4 to 6.5%), which distinguish these oils from those of other Boraginaceae genera.
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KEY WORDS: *Amblynotus,* Boraginaceae, capillary gas chromatography, chemotaxonomy, *Cynoglossum, Hackelia, Lappula,* γ-linolenic acid, seed oil, stearidonic acid.

The fatty acids γ-linolenic acid (γ-Ln, 18:3n-6) and stearidonic acid (Sn, 18:4n-3) are metabolites of linoleic and α-linolenic acid. They are of considerable dietary, pharmaceutical, and biomedical importance (1,2) because they contain a Δ6 double bond. Comprehensive reviews on the occurrence of γ-Ln in plant seed oils were recently given by Gunstone (1) and Ucciani (3). Whereas γ-Ln acid has been found in seed oils of a range of plant families, e.g., in Onagraceae (4–6), Scrophulariaceae (6), Aceraceae (7), Moraceae (8), Liliaceae (9) and Ranunculaceae (10–12), the simultaneous occurrence of γ-Ln and Sn so far has been found only in seed oils of Boraginaceae (13–22) and Primulaceae (23), apart from *Ribes* (Saxifragaceae) (6,24).

The Boraginaceae, to which borage (*Borago*) belongs, is a large plant family (*ca.* 2500 species in 100 genera) and is widely distributed throughout the Northern Hemisphere. The family is widely known to herbalists and gardeners because it includes many ornamental plants, such as forget-me-not (*Myosotis*). In Mongolia (Central Asia), there are 38 species of Boraginaceae, which belong to 22 different genera (25). In continuation of our search for additional sources for the above physiologically active fatty acids and other unusual fatty acids, we have conducted a study on the seed oils of some of the less well-known species of Boraginaceae from Central Asia. This paper describes the fatty acid composition of seed oils from nine species of Boraginaceae (3 *Lappula,* 1 *Hackelia,* 3 *Myosotis,* 1 *Cynoglossum,* and 1 *Amblynotus*).

EXPERIMENTAL PROCEDURES

Materials. Plant seeds were collected at maturity from wild plants in Mongolia in the summer months of 1993 and 1994. Seeds of *Lappula myosotis, Hackelia deflexa, Myosotis sylvatica,* and *M. suaveolens* were collected from plants in the district of Ulan-Bator (Khandgait, Khurkhree) in July–August 1993. Seeds of *L. granulata* were collected from wild plants in Gobi-Gurvansaikhan (Baruunsaikhan), Umnegobi aimak, Bulgan sum region, Mongolia, in the middle of September 1994. Seeds of *L. intermedia* and *Cynoglossum divaricatum* were obtained from plants in Bulgan aimak, Bayangobi and Mogot sum region, Mongolia, in August 1994. Seeds of *Amblynotus rupestris* and *M. caespitosa* were gathered from plants in Khandgait, Ulan-Bator region, Mongolia, in June 1994. Voucher specimen have been deposited in the Botanical Institute of the Mongolian Academy of Sciences, Ulan-Bator. All chemicals used were of reagent grade.

Oil extraction. The seeds were cleaned from impurities and ground into powder with a coffee mill. The oils were extracted from crushed seeds with *n*-hexane in a Soxhlet extractor, followed by evaporation of the solvent. The oil contents in the seeds were determined gravimetrically.

Saponification and methyl ester preparation. Mixed fatty acid methyl esters (FAME) were obtained from the oils by saponification in 1.0 N methanolic KOH, followed by reaction with 20% BF_3/MeOH (26,27) or by transesterification with 0.2 M methanolic trimethylsulfonium hydroxide (28).

Thin-layer chromatography (TLC). TLC of the seed oils and of their FAME was carried out on glass plates (20 × 20 cm; Merck, Darmstadt, Germany) with a 0.25-mm layer of silica gel G. The plates were developed with *n*-hexane/diethyl ether (70:30, vol/vol or 80:20, vol/vol). Visualization was by spraying with phosphomolybdic acid (5% in ethanol) and heating at 100°C. Reference compounds were used to identify the spots.

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Capillary gas–liquid chromatography (GLC). Capillary GLC of FAME was carried out on two gas chromatographs [Hewlett-Packard HP 5890 (Palo Alto, CA) and Perkin-Elmer F22 (Überlingen, Germany], equipped with flame-ionization detectors and with fused-silica WCOT capillary columns (length 50 m, internal diameters 0.25 and 0.22 mm)—one with Silar 5 CP (Chrompack, Middelburg, The Netherlands) and one with BPX 70 (SGE, Weiterstadt, Germany). Chromatographic data were evaluated with a Chromato-Integrator D 2000 (Merck-Hitachi) and a Shimadzu Chromatopac C-R3A-Integrator (Tokyo, Japan). Identificaton of FAME was confirmed by chromatographic comparison with authentic standards and by calculation of equivalent chainlengths.

GLC conditions were different for the two columns: Silar 5 CP: The temperature was held at 165°C for 1 min, then programmed from 165 to 205°C at 1°C/min, then held at 205°C for *ca.* 60 min before cooling down the column to 165°C. The injector and detector temperatures were 230 and 260°C, respectively. These conditions correspond to those used for seed oil fatty acid "fingerprints" (27). BPX 70: The temperature was programmed from 100 to 240°C at 2°C/min and then maintained at 240°C for 32 min. The injector temperature was 260°C, and the detector temperature was 240°C. Nitrogen was used as a carrier gas for both. Flow rates were 1.13 mL N_2/min for the Silar 5 CP column and 0.69 mL N_2/min for the BPX 70 column.

Infrared absorption (IR). IR spectra were obtained from liquid films in sodium chloride cells with a Perkin-Elmer 781 Infrared Spectrophotometer (Beaconsfield, England).

RESULTS AND DISCUSSION

The results of the GLC assays of FAME and the oil contents of the air-dried seeds from nine Central Asian species of Boraginaceae are given in Table 1. The oil contents of the seeds range from high values (28.3%) in *M. sylvatica* to low values (4.6%) in *L. intermedia*. TLC of the seed oils and of their FAME did not indicate the presence of any oxygenated triglycerides or fatty acids. The IR spectra did not show any absorption for *trans* double bonds. A typical GLC separation of FAME of seed oils from *L. intermedia* and *A. rupestris* on column BPX 70 is shown in Figure 1.

The seed fatty acids of *Lappula* species (*L. myosotis*, *L. intermedia*, and *L. granulata*) investigated here include γ-Ln (6.7–7.1%) and Sn (17.2–17.7%) in the approximate ratio of 1:3. These results are in good agreement with those from *L. barbatum* (4% γ-Ln, 14% Sn), reported by Miller *et al.* (19), and from *L. redowskii* (5% γ-Ln, 17% Sn), found by Kleiman *et al.* (17).

Seed fatty acids of *H. deflexa* exhibited the highest Sn content (21.4%) found so far in nature, and they also contained γ-Ln (6.6%). This is a γ-Ln/Sn ratio of about 1:3 as in the closely related *Lappula* species. However, early studies on seed oils from *H. jessicae* by Miller *et al.* (19) and *H. floribunda* by Wolf *et al.* (6) reported levels on γ-Ln (6.4–9.8%) and Sn (6.9–8.0%) in an approximate ratio of 1:1.

Seed oils of *Myosotis* species (*M. sylvatica*, *M. suaveolens*, and *M. caespitosa*) contain γ-Ln (7.3–9.6%) and Sn (6.5–10.3%) in the approximate ratio of 1:1, whereas one lit-

TABLE 1
Content and Fatty Acid Composition of Seed Oils from Some Mongolian Boraginaceae

			Fatty acid methyl esters, peak area %									
Plants	Column used[a]	Oil content in wt%	8:0	12:0	14:0	16:0	16:1n-7	17:0	18:0	18:1n-9	18:1n-7	18:2n-6
Lappula myosotis	++	18.0		trace	0.1	5.9	0.1	0.1	1.9	13.3	0.7	12.9
L. intermedia	++	4.6	0.3			5.1	0.1		1.5	13.8	0.8	13.4
L. granulata	++	12.7	0.1		0.6	5.3	0.2		1.9	16.1	0.8	11.7
Hackelia deflexa	+	15.5		0.1	0.8	6.0	0.2	0.1	1.6	11.2	0.6	10.7
Myosotis sylvatica	+	28.3		0.2	1.3	9.4	1.0	0.3	4.3	24.3	0.8	22.3
M. suaveolens	+	24.3		0.1	0.3	6.3	0.2	0.2	2.3	21.7	0.6	26.3
M. caespitosa	++	27.4	0.1		trace	5.3	0.1	trace	1.9	20.9	0.5	26.7
Cynoglossum divaricatum	++	17.1	0.2			5.2	0.1	0.1	2.2	24.7	0.3	30.4
Amblynotus rupestris	++	7.1	0.3	0.2		7.6	0.1	0.1	3.2	18.7	0.4	24.2

	18:3n-6	18:3n-3	20:0	18:4n-3	20:1n-9	20:2n-6	22:0	22:1n-9	22:2n-6	24:0	24:1n-9	Others
L. myosotis	6.7	34.9	0.4	17.2	2.1	0.2	0.2	0.9	trace	0.1	0.9	1.3
L. intermedia	7.1	35.4		17.7	2.0	0.1	0.2	1.0		0.1	0.8	0.9
L. granulata	6.9	32.6		17.7	2.1	0.1	0.3	1.1	trace	0.2	0.9	1.4
H. deflexa	6.6	34.7	1.3	21.4	1.3	0.1	0.2	0.5		0.2	0.4	1.8
M. sylvatica	7.3	11.9	0.5	6.5	2.7	0.1	0.4	1.5	0.1	0.2	1.3	3.1
M. suaveolens	8.5	15.5	0.5	8.6	3.3	0.1	0.4	2.0	0.1	0.2	1.7	0.8
M. caespitosa	9.6	16.8		10.3	3.1	0.1	0.3	1.8	trace	0.1	1.6	0.7
C. divaricatum	10.2	5.4		2.4	5.3	0.3	0.6	9.7	0.1	0.1	1.7	1.0
A. rupestris	13.0	8.0		6.5	5.1	0.1	0.7	7.0	0.1	0.4	1.8	2.4

[a](+) Silar 5 CP/BF_3; (Chrompack, Middelburg, The Netherlands); (++) BPX-70/TMSH (SGE, Weiterstadt, Germany).

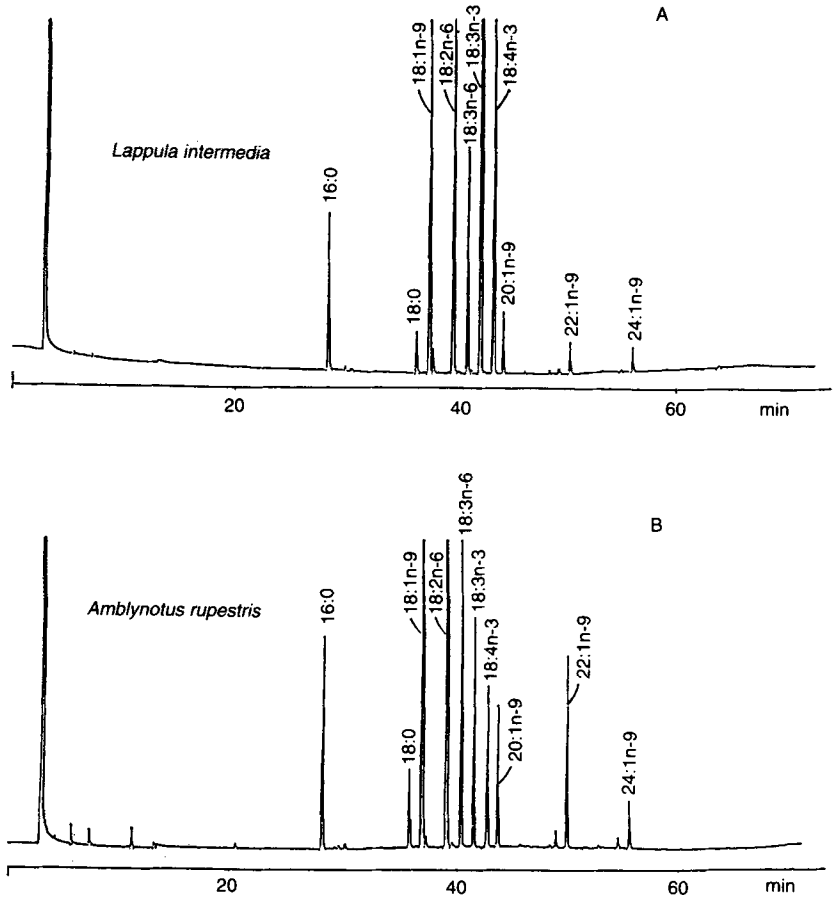

FIG. 1. Typical gas chromatograms of fatty acid methyl esters of seed oils from: A) *Lappula intermedia*; B) *Amblynotus rupestris*. BPX-70 (SGE, Weiterstadt, Germany) fused-silica capillary column (50 m, 0.22 mm i.d.) coated with 70% cyanopropyl equivalent modified silicone (0.25- micron film thickness); column temp. progr. from 100 to 240°C (30-min hold) at 2.0°C min^{-1}; carrier gas nitrogen 0.69 mL N_2 min^{-1}; inj. temp. 260°C, det. temp. 240°C, split 1:80.

erature reference (17) reported more Sn (12%) than γ-Ln (5%) in *M. sylvatica* seed oil.

The seed oil of *Cynoglossum divaricatum* (Cynoglosseae) contains more γ-Ln (10.2%) than Sn (2.4%), in a ratio of nearly 4:1. Moreover, there are considerable amounts of *cis*-11-eicosenoic (5.3%) and *cis*-13-docosenoic acid (9.7%), which may distinguish the genus *Cynoglossum* and possibly the whole tribe Cynoglosseae from the genera *Lappula*, *Myosotis* and *Hackelia*, which all belong to the tribe Eritrichieae.

Many references in the older literature deal with the analysis of seed oils of *Cynoglossum* species (6,13,17,19,20). Their content of γ-Ln and Sn seemed to be variable. However, they all contained *cis*-11-eicosenoic and *cis*-13-docosenoic acid in considerable amounts. The *Cynoglossum* species studied previously may fall into three categories with respect to the amounts of γ-Ln and Sn in their seed oils. One group of species [*C. amabile* (17) and *C. lanceolatum* (19)] is high in γ-Ln (11.0–13.0%) and low in Sn (0.7–0.8%) with 3.0–5.0% 20:1n-9 and 4.0–7.0% 22:1n-9. The second group of *Cynoglossum* species [*C. officinale* (17) and *C. nervosum* (19)] is intermediate in γ-Ln (6.0–7.8%) and Sn (3.0–3.1%) with 4.7–5.0% 20:1n-9 and 0.0–8.0% 22:1n-9. The third group of *Cynoglossum* species [*C. pictum* (17) and *C. creticum* (19)] is rather low in both γ-Ln (0.2–3.0%) and Sn (0.2–0.8%) with 6.0% 20:1n-9 and 8–12% 22:1n-9. For *C. zeylanicum* (20), no separate data were given on γ-Ln and Sn or 20:1n-9 and 22:1n-9. With respect to the relative amounts of γ-Ln and Sn present, *C. divaricatum* from Central Asia therefore appears to belong to the first group of *Cynoglossum* species.

The seed oil of *A. rupestris* contained γ-Ln (13%) and Sn (6.5%) in the ratio of 2:1. In addition to this, both *cis*-11-eicosenoic (5.1%) and *cis*-13-docosenoic acid (7.0%) are present. *Amblynotus rupestris* is therefore similar to *Cynoglossum* species in seed oil fatty acid composition, which may point to a closer relationship between these two genera. We were unable to find out to which tribe of Boraginaceae *Amblynotus* belongs. Also, in the literature, no information on the seed oil composition of a representative of genus *Amblynotus* was found.

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