Seed Oil Fatty Acids of Loasaceae—A New Source of γ-Linolenic and Stearidonic Acids

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ABSTRACT: The pharmaceutically interesting Δ6-FA 18:3Δ6*c*, 9*c*,12*c* (γ-linolenic acid) and 18:4Δ6*c*,9*c*,12*c*,15*c* (stearidonic acid) appear to have evolved independently several times during plant phylogenetic evolution. They typically occur in "clusters" of a few closely related species or genera in about a dozen different plant families throughout the plant kingdom. A hitherto-unknown "cluster of occurrence" has now been discovered in the New World plant family Loasaceae. γ-Linolenic and stearidonic acids occur exclusively in representatives of the newly described genus *Nasa* at significance levels of between 3 and 10% each. *Nasa* had recently been separated from the older, more broadly circumscribed genus *Loasa*. The two Δ6-FA were not found in the closely related genus *Loasa* sensu stricto, nor in a number of other representatives of Loasaceae.

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KEY WORDS: *Blumenbachia, Caiophora, Gronovia*, γ-linolenic acid, *Loasa,* Loasaceae, *Mentzelia*, *Nasa*, seed oils, stearidonic acid.

Energy is stored in Loasaceae seeds usually in the form of seed oils. Literature data on Loasaceae seed oils are rather scarce, but oil contents between 20 and 47% of seed weight have been reported (1–4). Until recently, however, the FA composition of these seed oils had not been investigated in detail.

During work carried out in the former Institute for Chemistry and Physics of Lipids in Münster (Germany), now renamed the Institute for Lipid Research, in connection with preparations for the new Seed Oil FA (SOFA) database (5), we noted that the published scientific literature available until 1997 (when the SOFA database project started) contained only a single set of data—that of Coxworth (3) on *Mentzelia*—for the seed oil FA composition of a member of the Loasaceae. In the meantime, a limited amount of additional data, particularly on *Mentzelia* spp., was found hidden in the NCAUR "New Crops" database (6); however, to our knowledge, these have never really been discussed or published in a scientific journal. They are compiled here in Table 1.

TABLE 1
Data for Oil Content and FA Composition (unsaturated FA not specified) from the Published Literature and from the NCAUR[a] New Crops Database

Plant	Seed oil content (wt%)	FA (wt%)										Author (reference)
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	
Cevallia sinuata	21.7											Barclay and Earle (4)
C. sinuata	20.1	0.1	7.5	0.1	3.4	21.5	67.2	Trace	0.2			NCAUR (6)
Mentzelia adaerens	27.6											NCAUR (6)
M. aff. *albicaulis*	39.4	Trace	7.9	0.2	2.9	20.8	66.8	1.0	0.1	0.1		NCAUR (6)
M. albicaulis	34.5											Earle and Jones (2)
M. albicaulis	30.6											NCAUR (6)
M. albicaulis	32.4											NCAUR (6)
M. decapetala	35.4											NCAUR (6)
M. decapetala	40.1	Trace	6.2		3.0	21.2	59.5	7.4				NCAUR (6)
M. decapetala	37.0											Earle and Jones (2)
M. dispersa	46.5											Barclay and Earle (4)
M. dispersa	44.4											NCAUR (6)
M. involucrata	31.1											NCAUR (6)
M. lindleyi	33.0	0.2	11.2		3.0	20.3	56.8	1.3				Coxworth (3)
M. lindleyi	39.0	0.1	11.0	0.2	3.1	21.8	56.8	1.2	0.3	0.2	0.1	NCAUR (6)
M. nuda	39.3											Barclay and Earle (4)
M. nuda	37.2											NCAUR (6)
M. nuda	36.8											NCAUR (6)
M. speciosa	35.9	0.1	5.8	0.3	3.4	27.0	58.7	2.3	1.2			NCAUR (6)

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Because of the known chemotaxonomic and phylogenetic implications of the presence of unusual FA in seed oils (7,8), numerous analyses of the FA composition of Loasaceae seed samples were carried out in this institute in the years 1997–2002. Details of these results are presented here.

EXPERIMENTAL PROCEDURES

Most of the seeds of various species of Loasaceae were collected in the wild in South America by one of the authors (M.W.). A few additional seeds were obtained from botanical gardens. Seed of *Blumenbachia insignis* was from the Botanical Garden, University of Hamburg.

Seeds were crushed using a ball mill and extracted with heptane as described previously (7,9,10). The extracts were evaporated on a rotary evaporator, and FAME were prepared by transesterification with sodium methoxide in methanol by using the method of Schulte and Weber (11).

Capillary GLC was carried out on a Silar-5CP (Chrompack, Middelburg, The Netherlands) column as described for the standardized SOFA "fingerprints" (7,9,10) or on a DB-23 (Macherey-Nagel, Düren, Germany) capillary column as described below.

For analyses on the DB-23 columns, the ISO standard ISO 5509:2000 was followed. In brief, for the preparation of FAME, about 12 mg of oil was dissolved in 1 mL of petroleum ether. Then 25 μL of a solution of sodium methanolate in methanol (2 mol/L) was added, and the closed vial was agitated vigorously for 1 min. After this, about 20 mg of sodium hydrogen sulfate monohydrate (extra pure) was added. The closed vial was agitated again and then centrifuged at 5000 U/min for 1 min. The clear supernatant solution was transferred to another vial and was then ready for injection. GC conditions: DB-23 capillary column, 60 m long, 0.32 mm i.d., 0.2 μm film thickness; temperature program: column temperature from 155 up to 220°C at a rate of 1.5°C/min, then held isothermally for 10 min; injector temperature 250°C, FID 250°C; carrier gas: hydrogen at 36 cm/s, split 1:50, injection volume 1 μL.

The FAME peaks obtained were identified by comparison with standards and with FAME obtained from Boraginaceae seed oils of known composition. For a number of samples (*Nasa, Caiophora, Gronovia,* and *Blumenbachia*), parallel runs on a Silar-5CP column were carried out to check the retention and correct identification of γ-linolenic and stearidonic acids. Relative retention and ECL calculations were carried out on the chromatograms obtained from the Silar-5CP column, as used for our standardized SOFA fingerprints (7).

RESULTS

Data found in the published literature (5) and in the New Crops database (6), where unsaturated FA are not specified in detail, are compiled in Table 1.

The results of analyses carried out in our own laboratory are shown in Table 2. Loasaceae, including *Gronovia*, showed rather typical FA compositions, similar to those of edible oils and most oilseed plants. In the Loasaceae analyzed here, linolenic acid (18:3Δ9*c*,12*c*,15*c* or 18:3n-3) was the most prominent FA in two species of *Blumenbachia*, in all the investigated *Nasa* spp., and in *Caiophora* at levels of around 33–49%, indicating a rather high degree of unsaturation. In contrast, in *Gronovia*, which is considered a member of the subfamily Gronovioideae, and in *M. albescens*, from the subfamily Mentzelioideae, linoleic acid (18:2Δ9*c*,12*c* or 18:2n-6) was the major component, at 62–64% of total FA. This corresponds well with the NCAUR data on *Mentzelia* (Table 1). Oleic acid (18:1Δ9c or 18:1n-9), on the other hand, was rather high in most members of the subfamily Loasoideae, such as *Presliophytum, Blumenbachia,* and *Caiophora*, and particularly so in *Loasa* sensu stricto.

Figure 1 shows typical standardized capillary GC SOFA fingerprints (7) for a *Gronovia*, a *Nasa*, and a *Caiophora* species, representing three typical fingerprint patterns, as observed on a Silar-5CP column.

DISCUSSION

The taxonomic and phylogenetic implications of these findings will be discussed elsewhere. However, one should keep in mind that the chemotaxonomic significance of ordinary FA, such as linoleic acid (18:2Δ9*c*,12*c* or 18:2n-6) and linolenic acid (18:3Δ9*c*,12*c*,15*c* or 18:3n-3), is rather low. Nevertheless, both Tables 1 and 2 show that the differences in degree of seed oil unsaturation within Loasaceae are very large. *Gronovia*, in particular, is outstanding in that Δ15-desaturation is almost nonexistent (as in *Cevallia*).

The most significant deviation from normal FA patterns was the one found in all the *Nasa* spp. investigated, which contained the two Δ6-FA γ-linolenic acid (18:3Δ6*c*,9*c*,12*c* or 18:3n-6) and stearidonic acid (18:4Δ6*c*,9*c*,12*c*,15*c* or 18:4n-3) at levels between 3 and 10%.

The latter two FA do occur rather sporadically in plants (12), animals, and humans, and they may be important for pharmaceutical and nutrition purposes (12,13). In the plant kingdom, the two Δ6-FA are of chemotaxonomic significance. Stearidonic acid is the more significant of the two, because the occurrence of γ-linolenic acid alone is observed much more often. The distribution pattern of these two FA (14) seems to indicate that the Δ6-desaturase enzymes responsible for the biosynthesis of these FA may have evolved several times independently. The two Δ6-FA are found, alone or together, in "clusters" of a few closely related species only, within several plant families such as Ranunculaceae, Scrophulariaceae, Compositae, Primulaceae, Onagraceae, and several others (5). However, we do also know a few plant families (e.g., Asteliaceae, Grossulariaceae, and most subfamilies of Boraginaceae, except a few) in which all the members of the plant family or subfamily (that have been investigated so far) contain one or both of these polyunsaturated Δ6-FA (5,14,15).

We hope to determine whether the γ-linolenic acid cluster within Loasaceae (in *Nasa* spp.) is similar to that in Onagraceae (exclusively in all *Oenothera* spp., but not in all the other genera

TABLE 2
Oil Content and FA Composition (area % of total FA) of Loasaceae and *Gronovia*[a]

		Peak no. (Fig. 2)																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Species	Oil (%)	14:0	16:0	16:1Δ7	16:1Δ9	17:0	18:0	18:1Δ9	18:1Δ11	18:2	γ-18:3	18:3	18:4	20:0	20:1	22:0	24:0	24:1	Sum
Aosa rupestris	0.4	0.1	**12.7**	0.0	0.1	0.1	4.7	15.9	0.8	**42.4**	<0.1	21.6	<0.1	**0.5**	0.1	**0.3**	0.1	0.1	99.4
Blumenbachia hieronymii	0.4	0.1	8.5	0.0	0.2	0.1	2.2	**27.9**	0.6	24.8	<0.1	34.5	<0.1	0.2	0.0	0.1	0.5	0.3	99.8
B. insignis[b]	ND	0.1	8.2	<0.1	0.2	0.1	2.6	**28.7**	0.8	24.6	<0.1	33.9	<0.1	0.2	0.1	0.1	<0.1	<0.1	99.5
B. sylvestris	0.4	0.1	7.2	0.0	0.1	0.1	2.1	**40.5**	0.7	**36.9**	<0.1	11.3	<0.1	0.2	0.3	0.0	0.0	0.1	99.5
Caiophora andina	0.4	0.1	8.2	0.0	0.1	0.0	1.9	**34.6**	0.4	15.3	<0.1	**38.2**	<0.1	0.2	0.2	0.1	0.0	0.1	99.6
C. canarinoides	0.4	0.1	8.3	0.0	0.1	0.1	2.1	**30.1**	0.4	13.3	<0.1	44.5	<0.1	0.2	0.2	0.1	<0.1	0.1	99.4
C. cf. *sepiaria*	0.3	0.1	9.3	0.0	0.1	0.1	2.3	**34.1**	0.5	11.4	<0.1	**40.2**	<0.1	0.3	0.2	0.1	0.1	0.5	99.2
C. cirsiifolia 1[b]	ND	0.1	8.3	<0.1	0.1	0.1	2.2	**32.6**	0.4	11.8	<0.1	**42.0**	<0.1	0.3	0.4	0.1	0.1	<0.1	98.4
C. cirsiifolia 2	0.4	0.1	8.3	0.0	0.1	0.0	1.6	**32.3**	0.6	12.1	<0.1	**43.9**	<0.1	0.2	0.3	0.1	<0.1	0.2	99.6
C. sepiaria	0.4	0.1	8.9	0.0	0.1	0.0	2.0	**34.5**	0.5	9.7	<0.1	**42.9**	<0.1	0.2	0.3	0.1	<0.1	0.1	99.4
Gronovia scandens 1[b]	ND	0.1	**11.7**	<0.1	0.2	0.1	**8.5**	12.8	0.7	**63.6**	<0.1	0.5	<0.1	0.3	0.0	<0.1	<0.1	<0.1	98.3
G. scandens	ND	0.1	**11.7**	<0.1	0.2	0.1	**8.5**	13.0	0.7	**62.6**	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	97.1
Loasa acerifolia	0.3	0.1	**11.9**	0.0	0.1	0.1	2.9	**59.4**	0.0	10.4	<0.1	13.9	<0.1	0.3	0.2	0.1	0.1	0.1	99.5
L. bergii	0.4	0.1	8.4	0.0	0.2	0.0	2.0	**35.9**	0.6	24.4	<0.1	27.3	<0.1	0.2	0.3	0.1	0.0	0.1	99.5
L. nitida Casma	0.3	0.2	**12.8**	0.1	0.2	0.1	4.2	**55.3**	0.4	14.1	<0.1	11.8	<0.1	0.4	0.1	0.1	0.0	0.1	99.5
Mentzelia albescens	0.4	0.1	6.5	0.0	0.3	0.1	3.8	17.1	**1.7**	**64.7**	<0.1	3.0	<0.1	**0.9**	0.1	0.1	0.1	**0.3**	98.7
Nasa carunculata	0.3	0.1	6.3	0.0	0.1	0.1	5.1	14.5	0.5	10.4	**4.7**	**47.9**	**8.5**	0.3	0.1	0.1	0.1	0.1	98.8
N. cymbopetala	0.4	0.1	7.1	0.0	0.0	0.1	3.6	19.4	0.4	13.2	**7.9**	**42.9**	**3.7**	0.3	0.2	0.0	0.0	0.2	99.1
N. dyeri subsp. *australis*	0.3	0.1	8.1	0.0	0.1	0.2	4.6	17.5	0.6	6.8	**3.5**	**51.7**	**4.1**	0.4	0.3	0.1	0.2	0.2	98.4
N. hornii	0.4	0.1	7.5	0.0	0.1	0.1	3.4	15.7	0.7	17.4	**9.8**	**40.3**	**3.0**	0.3	0.2	0.1	0.1	**0.5**	99.1
N. magnifica	0.4	0.1	7.6	0.0	0.1	0.1	3.7	19.6	0.4	8.8	**8.1**	**42.9**	**6.8**	0.3	0.1	0.1	0.1	**0.3**	98.8
N. ranunculifolia 1[b]	ND	0.1	7.1	<0.1	0.1	0.1	2.9	16.8	0.6	15.2	**9.5**	**41.8**	**3.8**	0.3	0.1	0.1	<0.1	<0.1	98.5
N. ranunculifolia 2	ND	0.1	7.1	0.0	0.0	0.1	2.7	16.5	0.5	14.4	**9.8**	**42.3**	**4.3**	0.2	0.2	0.1	0.1	0.0	98.1
N. triphylla subsp. *elegans*	0.4	0.1	7.9	<0.1	0.1	0.1	4.6	17.1	0.5	10.1	**4.5**	**49.7**	**3.6**	0.4	0.3	0.1	0.1	0.1	99.0
N. cf. *magnifica*	0.4	0.1	8.3	0.0	0.1	0.1	3.1	12.9	0.5	11.4	**8.4**	**49.0**	**4.9**	0.2	0.1	0.0	0.0	0.2	99.0
N. vargasii	0.4	0.1	8.3	<0.1	0.1	0.0	3.6	16.3	0.5	21.7	**6.6**	**39.3**	**2.0**	0.3	0.1	0.1	0.1	0.1	99.1
Presliophytum heucheraefolium	0.3	0.1	**17.2**	0.1	**0.3**	0.2	4.2	**34.8**	1.0	**36.6**	<0.1	1.8	<0.1	**1.0**	0.2	**0.6**	**0.3**	**0.2**	98.3
Significance			**>10**		**>0.2**		**>7**	**>25**	**>1**	**>30**	**>1**	**>30**	**>1**	**>0.5**		**>0.2**	**>0.2**	**>0.2**	

[a]Values in boldface indicate significance. Values in boldface in the bottom row indicate the level of significance applied. ND, not determined.
[b]Silar-5CP column (Chrompack, Middelburg, The Netherlands).

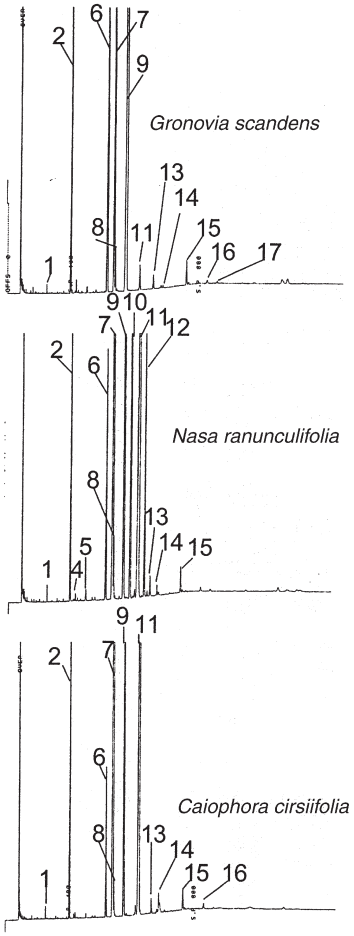

FIG. 1. Standardized seed oil FA "fingerprints" (7) of *Gronovia scandens, Nasa ranunculifolia,* and *Caiophora cirsiifolia* FAME, as obtained on a Silar-5CP column. Peak numbers for the main FA: 2 = 16:0; 6 = 18:0; 7 = 18:1Δ9*c*; 9 = 18:2Δ9*c*,12*c*; 10 = 18:3Δ6*c*,9*c*,12*c* (γ-linolenic acid); 11 = 18:3Δ9*c*,12*c*,15*c* (α-linolenic acid); 12 = 18:4Δ6*c*,9*c*,12*c*,15*c* (stearidonic acid). For minor FA see peak identification in Table 2.

there), in Scrophulariaceae (where γ-linolenic acid was found so far only in *Scrophularia* and a few closely related species), in Asteraceae [e.g., in *Saussurea* and *Youngia* (16,17)], or in Ranunculaceae [exclusively in *Anemone* and *Clematis* (9), but not in all the other genera]. In the latter four plant families, however, only γ-linolenic acid is found and the second Δ6-FA, stearidonic acid, is absent.

In the Primulaceae, on the other hand, both these Δ6-FA are found only in part of one out of five tribes, i.e., in part of the Primuleae (18,19). They occur in all *Primula* spp. and in *Dodecatheon* and *Soldanella*, but not in *Androsace* and *Douglasia* (which are also part of the Primuleae), and not in *Lysimachia, Anagallis, Cyclamen, Trientalis, Samolus,* and *Coris* (19). A similar situation could exist in other plant families, including the Loasaceae, which we discuss below.

The occurrence of the two Δ6-FA in part of the Loasaceae is certainly of academic interest only. However, if we assume independent evolution, then the biosynthetic pathways leading to these FA, or the enzymes involved in the key step of Δ6-desaturation, could still be quite different. For example, the amino acid sequence and possibly the temperature optimum, or other properties such as the substrate specificity (20), of the Δ6-desaturase found in South American *Nasa* or *Oenothera* could be quite different from the ones found in the many *Anemone* (10) or *Hackelia* and *Lappula* spp. (15) of the cold Altai or Tien-Shan mountains of Central Asia. Also, it is not known why γ-linolenic acid is found so much more often in the plant kingdom, with stearidonic acid being absent. In some cases this may simply depend on the availability of the precursor, linolenic acid. In other cases, however, linoleic acid may simply be the preferred substrate for a Δ6-desaturase enzyme with a slightly modified amino acid sequence (20). Moreover, a closer inspection of the data in Table 2 shows that the substrate preference of the *Nasa* Δ6-desaturase has also changed, presumably during the course of evolution. The precursor levels, at about 10.4 and 47.9% in *N. carunculata* vs. 10.1% and 49.7% in *N. triphylla* ssp. *elegans* are very similar, yet the level of stearidonic acid produced in *N. carunculata* is more than twice as high. This may indicate an increased 18:3 preference for the *N. carunculata* Δ6-desaturase. It has been shown recently (20) that changes in one or a few amino acid positions can have just such effects.

A better understanding of this, in turn, could be of interest for modern research projects in renewable resources that aim at the production of tailor-made fats by way of enzyme design and gene transfer, for example, into rapeseed.

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