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Chemotaxonomic significance of fatty acids and tocopherols in Boraginaceae

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Abstract

A collection of 45 accessions (36 species, 20 genera) of the family Boraginaceae was evaluated for oil content, fatty acid composition, tocopherol content and composition. All the accessions contained γ-linolenic acid, the lowest content (0.7%) being found in *Cerinthe major* L. and the highest (24.4%) in *Borago officinalis* L. Three tocopherol profiles were characterized by the extremes of more than 90% of α-, δ- and γ-tocopherol, respectively. Fatty acids and tocopherols were suggested to have potential chemotaxonomic value in this family.

Keywords: Boraginaceae; Chemotaxonomy; Fatty acids; Gamma-linolenic acid; Oil content; Tocopherols

1. Introduction

The family Boraginaceae Juss. is one of the best known sources of γ-linolenic acid (GLA; 18:3 $n-6$). This fatty acid is unusual in plants, but it is highly appreciated because of its nutritional and medical benefits (Horrobin, 1992). Whereas in other families of angiosperms (e.g. Onagraceae, Scrophulariaceae or Saxifragaceae) GLA is exclusively present in one or a reduced number of genera, it has been found in most of the species of Boraginaceae evaluated to date (Gunstone, 1992), being absent or at very low concentrations only in the tribes Cordioideae, Ehretioideae and Heliotropioideae (Kleiman, Earle, Wolf & Jones, 1964). Stearidonic acid (SDA; 18:4 $n-3$) is another fatty acid that is relatively uncommon in the plant kingdom, but occurring in the Boraginaceae (Hegnauer, 1989).

Several studies report on the fatty acid composition of the seed oil in a wide range of wild and cultivated species of the Boraginaceae (Kleiman et al., 1964; Miller, Earle & Wolff, 1968; Tétényi, 1974; Wolf, Kleiman & England, 1983; Tsevegsüren & Aitzetmüller, 1996). The concentrations of linoleic (18:2 $n-6$), α-linolenic acid (18:3 $n-3$), GLA, SDA and erucic acid (22:1 $n-9$) are of special chemotaxonomic importance within this family (Miller et al., 1968; Tétényi, 1974).

The tocopherols are efficient natural antioxidants showing in vivo (vitamin E) and in vitro activity. Goffman, Thies & Velasco (1999) have recently suggested that the tocopherols are compounds having important chemotaxonomic significance in the family Brassicaceae. No studies on the occurrence and variability of tocopherols in the Boraginaceae have been conducted yet. The objective of the present study was to evaluate the variability and chemotaxonomic significance of fatty acids and tocopherols in a collection of Boraginaceae.

2. Results and discussion

Table 1 presents the seed oil content, fatty acid composition, total tocopherol content and concentration of individual tocopherols of the 45 accessions analysed grouped at infrafamilial levels. The accessions were

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Table 1
Oil content (% seed weight), fatty acid composition (% of the total fatty acids), tocopherol content (mg kg^{-1} oil) and tocopherol composition (% of the total tocopherols) in 45 accessions (36 species) of the Boraginaceae

Species	Oil	Fatty acids[a]									Tocopherols[b]			
		x:0	16:0	18:0	18:1	18:2	18:3	γ18:3	18:4	*x*:1	Total	α-T	γ-T	δ-T
Subfam. *Heliotropioideae* Guerke														
Heliotropium arborescens L.	7.6	5.3	18.3	22.0	12.7	30.0	5.4	2.4	1.5	1.9	131	0.0	100.0	0.0
H. europaeum L.	7.8	3.1	11.3	9.7	15.7	54.4	2.3	1.7	0.5	0.4	231	37.3	62.7	0.0
Subfam. *Boraginoideae*, tribe *Eritrichieae* Benth. and Hook														
Amsinckia calycina (Moris) Chater	23.2	1.1	12.9	5.8	29.1	13.2	15.4	8.8	9.9	3.4	328	9.1	90.9	0.0
Eritrichium canum (Benth.) Kitam.	11.2	1.4	13.7	9.3	20.8	23.7	7.7	12.2	4.1	6.8	554	92.2	7.8	0.0
Lappula squarrosa (Retz.) Dumort	12.2	2.2	10.2	7.8	12.2	13.6	27.6	7.3	17.1	2.0	540	0.0	92.7	7.3
Subfam. *Boraginoideae*, tribe *Cynoglosseae* DC. in Meisner														
Cynoglossum amabile Stapf & Drumm	18.0	0.8	11.7	6.2	25.3	25.9	4.6	11.2	1.3	11.0	373	23.3	76.7	0.0
C. hungaricum Simk.	16.5	3.0	10.1	6.1	27.9	13.7	15.2	3.7	5.8	13.0	468	21.6	78.4	0.0
C. nervosum Hook	11.0	2.9	15.6	12.2	27.1	18.9	6.0	8.1	1.6	6.4	181	71.9	28.1	0.0
C. officinale L.	10.7	0.9	9.6	5.6	28.7	25.7	6.6	7.9	3.1	10.8	863	84.1	15.9	0.0
Lindelofia anchusoides (Lindl.) Lehm.	6.2	3.2	15.4	13.9	24.0	16.3	13.0	4.2	4.2	5.2	726	52.0	48.0	0.0
L. longiflora (Benth.) Baill.	10.2	1.3	14.2	7.2	27.1	20.6	9.1	9.4	3.1	7.0	176	100.0	0.0	0.0
L. longiflora (Benth.) Baill.	8.6	2.2	14.9	11.5	25.0	18.8	8.2	8.8	2.8	6.6	267	74.0	26.0	0.0
Omphalodes linifolia (L.) Moench	15.4	1.6	12.7	7.4	29.3	20.4	8.8	6.8	3.5	7.7	91	0.0	100.0	0.0
Solenanthus apenninus (L.) Fisch. & Mey	18.3	0.2	7.9	4.6	47.3	16.8	6.7	2.9	1.8	10.3	241	100.0	0.0	0.0
Subfam. *Boraginoideae*, tribe *Lithospermeae* (DC.) Guerke														
Alkanna orientalis (L.) Boiss.	27.6	0.4	6.1	4.1	14.5	23.6	35.6	11.4	3.5	0.8	841	0.0	97.4	2.6
Cerinthe major L.	19.7	1.9	15.5	8.4	31.8	19.6	16.7	1.5	0.5	3.4	537	0.0	95.7	4.3
C. major L.	15.4	0.7	12.3	7.1	30.3	16.6	28.0	0.7	0.3	3.5	668	0.0	96.9	3.1
C. minor L.	14.8	2.0	9.3	6.9	12.6	21.5	29.0	10.0	7.3	0.9	629	6.8	93.2	0.0
C. retorta Sibth. & Sm.	15.0	1.4	11.9	11.8	13.3	16.0	29.9	5.8	7.4	2.0	446	0.0	93.9	6.1
Echium simplex DC	15.9	1.8	12.0	8.2	16.4	22.9	17.6	16.2	3.4	1.3	605	8.4	91.6	0.0
E. vulgare L.	16.4	1.3	8.3	6.4	11.9	18.1	33.9	9.2	9.9	0.9	531	10.4	89.6	0.0
Lithospermum arvense L.	19.6	0.9	8.3	5.6	9.4	11.3	41.1	5.1	17.1	1.2	940	0.0	97.4	2.6
L. arvense L.	16.1	0.9	7.0	5.6	10.9	10.6	41.5	5.2	17.4	0.8	1184	0.0	97.3	2.7
L. officinale L.	11.8	0.5	6.9	7.2	12.3	17.2	29.1	12.3	13.3	1.1	483	0.0	100.0	0.0
L. officinale L.	11.4	1.1	9.2	6.5	10.9	16.0	30.8	15.3	9.2	0.9	729	6.8	93.2	0.0
L. officinale L.	14.8	0.9	7.7	5.3	11.6	20.1	29.9	16.6	7.0	0.8	425	0.0	100.0	0.0
Onosma arenarium W. & K.	18.0	0.5	8.2	5.6	14.6	26.8	27.3	7.9	6.8	2.0	593	12.7	87.3	0.0
Subfam. *Boraginoideae*, tribe *Trichodesmeae* Zak														
Caccinia macranthera O. Kuntze	12.1	2.6	12.4	6.6	28.6	26.2	9.5	5.5	2.5	5.0	602	8.4	85.2	6.3
Subfam. *Boraginoideae*, tribe *Boragineae*														
Anchusa arvensis (L.) M. Bieb.	25.8	0.0	7.9	4.1	20.4	24.3	16.3	15.2	4.7	6.5	1765	1.7	95.3	3.0
A. arvensis (L.) M. Bieb.	18.2	3.0	9.4	5.8	20.2	23.7	12.9	15.5	4.1	4.7	1416	0.0	97.3	2.7
A. azurea Mill.	13.0	2.3	11.3	7.2	20.8	38.1	2.1	10.2	0.5	6.8	1236	4.2	93.8	2.0
A. officinalis L.	19.0	0.8	9.3	4.6	19.3	32.4	12.7	12.9	2.6	4.9	1519	3.0	91.1	5.9
A. officinalis L.	21.3	0.0	8.6	5.6	21.7	25.4	15.6	12.8	3.8	5.9	1729	1.7	96.9	1.4
Borago officinalis L.	25.1	0.8	10.1	5.4	17.0	35.0	0.9	24.4	0.3	5.9	1267	0.0	11.9	88.1
B. officinalis L.	22.3	0.7	11.0	8.3	22.1	33.2	0.7	18.5	0.2	4.7	1019	0.0	9.6	90.4
Nonea lutea (Desr.) DC	27.5	0.6	13.9	7.2	24.5	29.3	9.4	9.0	2.6	3.0	1005	5.8	91.9	2.3
N. setosa Roem. & Schult.	24.2	0.6	11.7	6.7	18.8	34.0	11.3	10.5	3.0	3.3	1114	7.1	91.3	1.6
Pentaglottis sempervirens (L.) Bailey	24.6	0.0	10.8	5.3	25.9	26.7	3.9	22.1	1.2	3.7	814	29.6	66.4	4.0
Pulmonaria mollis Wulfen	25.5	0.6	10.2	6.3	17.6	28.2	10.5	19.4	3.6	3.0	964	6.2	51.9	38.1
P. obscura Dum.	24.3	4.9	14.1	13.1	16.9	22.2	9.4	14.3	3.6	1.5	346	15.2	68.3	16.5
Subfam. *Boraginoideae*, tribe *Myosotideae* Reichenb.														
Myosotis alpestris F.W. Schmidt	29.6	1.6	11.8	7.2	27.8	24.6	9.2	5.9	5.0	6.1	693	0.0	100.0	0.0
M. alpestris F.W. Schmidt	29.4	1.3	11.1	5.6	27.5	22.5	14.6	4.2	6.8	5.5	911	0.0	88.7	11.3
M. discolor Pers.	34.1	2.4	11.7	6.7	25.3	19.8	18.5	3.2	7.2	4.6	1062	1.8	88.4	9.8
M. sparsiflora Pohl	25.8	9.3	19.6	21.3	14.8	17.0	9.7	3.8	4.4	0.0	530	36.4	59.4	4.2
M. sylvatica (Ehrh.) Hoffm.	31.8	2.7	14.3	13.4	23.0	20.8	10.3	5.3	5.6	4.5	541	0.0	100.0	0.0

[a] *x*:0 = caprylic (8:0) + capric (10:0) + lauric (12:0) + myristic (14:0) acids; 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = alpha-linolenic acid; γ18:3 = gamma-linolenic acid; 18:4 = stearidonic acid; *x*:1 = eicosenoic (C20:1) + erucic (C22:1) + nervonic (C24:1) acids.

[b] = beta-; γ = gamma-; δ = delta-tocopherol.

highly variable for the fatty acid composition of the seed oil. Short-chain saturated fatty acids (8:0, 10:0, 12:0 and 14:0) were particularly abundant in *Myosotis sparsiflora* Pohl, where the sum of the four fatty acids accounted for 9.3% of the total fatty acids. This species also contained high levels of palmitic (16:0; 19.6%) and stearic acid (18:0; 21.3%). Similarly high concentrations of both fatty acids were only found in *Heliotropium arborescens* L. (18.3 and 22.0%, respectively).

GLA and SDA were present in all the accessions. GLA ranged from 0.7 (*Cerinthe major*) to 24.4% (*Borago officinalis*). Interestingly, the tribe Lithospermeae (subfam. Boraginoideae) included species with very low (*C. major*) and very high (*Lithospermum officinale* L., *Echium simplex* DC) GLA concentration. SDA concentration ranged from 0.2 (*Borago officinalis*) to 17.4% (*Lithospermum arvense*). The highest levels of this fatty acid were found in the tribes Lithospermeae and Eritrichieae, although both of them also contained species with relatively low concentrations (*Cerinthe major* and *Eritrichium canum* (Benth.) Kitam., respectively).

The chemotaxonomic value of the fatty acid composition of the seed oil in Boraginaceae is widely accepted (Hegnauer, 1989). According to Tétényi (1974), the fatty acid composition within this family follows some general rules at the tribal level. Although that author observed different criteria for infrafamilial classification, some of his findings are confirmed by the present study. For example, (1) the subfamily Heliotropioideae was characterized by low levels of α-linolenic, GLA, SDA and long-chain monounsaturated fatty acids; (2) the tribes Eritrichieae and Lithospermeae showed high concentrations of SDA; (3) the maximum levels of GLA were found in the tribe Boragineae (named Anchuseae by Tétényi) and (4) the maximum concentrations of long-chain monounsaturated fatty acids were present in the tribe Cynoglosseae.

Tocopherol composition was dominated by γ-tocopherol, which accounted for more than 50% of total tocopherols in 36 out of the 45 accessions analysed (Table 1). Very high levels of δ-tocopherol were only present in *Borago officinalis*. Relatively high values (>10%) of this tocopherol were also found in *Pulmonaria mollis* Wulfen (38.1%), *P. obscura* Dum. (16.5%) and *Myosotis alpestris* F.W. Schmidt (11.3%). β-tocopherol was only detected in *P. mollis* (3.8%, data not shown). These results reveal some trends that might be of taxonomic interest. Most relevant is that high concentrations of α-tocopherol (>50%) were almost exclusively restricted to the tribe Cynoglosseae, with the single exception of *Eritrichium canum* (tribe Eritrichieae). This species showed a tocopherol composition completely different to that of the other two analysed species of this tribe, i.e. *Amsinckia calycina* (Moris) Chater and *Lappula squarrosa* (Retz.) Dumort. Both of them contained more than 90% γ-tocopherol, compared with 92% α-tocopherol in *E. canum*. Interestingly, the differences in tocopherol composition were paralleled by differences in the fatty acid composition of the seed oil. Despite the high levels of SDA characteristic of this tribe (Tétényi, 1974), *E. canum* showed a low concentration of this fatty acid, i.e. 4.1 vs. 9.9 and 17.1% in *A. calycina* and *L. squarrosa*, respectively. *E. canum* was also different for other fatty acids considered of taxonomic significance, such as α-linolenic acid, GLA and long-chain monounsaturated fatty acids (Table 1). Brand (1931, cited by Al-Shehbaz, 1991) considered that *Eritrichium* is not a representative of the tribe, although that criterion was not supported by the author. In any case, the great differences for fatty acid and tocopherol composition cause some inconsistency in the classification of the three species within the same tribe.

The tribe Cynoglosseae included two main groups for tocopherol composition, characterized by high concentrations of α- and γ-tocopherol, respectively. Even within the genus *Cynoglossum* two of the analysed species, *C. amabile* Stapf and Drumm and *C. hungaricum* Simk., had high γ-tocopherol concentrations, while the other two, *C. nervosum* Hook and *C. officinale* L., exhibited high concentrations of α-tocopherol. Ivanov & Aitzetmüller (1998) analysed one accession of *C. officinale*, finding also a high α-tocopherol concentration (71% of the total tocopherol content). *Cynoglossum* is a cosmopolitan genus, divided by Riedl (1962) into four subgenera and seven sections. However, the generic limits and infrageneric classification of this genus are still controversial (Al-Shehbaz, 1991). The present results suggest that tocopherols might be helpful for a better infrageneric classification of *Cynoglossum*.

Other tribes were much more homogeneous for fatty acid and tocopherol composition. The tribe Lithospermeae showed a generalized high γ-tocopherol concentration. Also the fatty acid composition of the seed oil was relatively uniform; only *Cerinthe major*, with very low concentrations of GLA and SDA, was markedly different. The particularity of *C. major* within this tribe was already pointed out by Kleiman et al. (1964). The accessions analysed of the tribe Myosotideae provided another example for the potential chemotaxonomic value of tocopherols in the Boraginaceae. Three of the four species of *Myosotis* were relatively uniform for fatty acid and tocopherol composition. The fourth species, *M. sparsiflora*, was distinct for its fatty acid composition, showing higher concentrations of saturated fatty acids and absence of long-chain monounsaturated fatty acids. Simultaneously, this species had a considerably higher

concentration of α-tocopherol (36.4 vs. <2% in the other three species). The infrageneric classification of *Myosotis* is rather controversial (Al-Shehbaz, 1991). Riedl (1967) followed a separation of the genus into two subgenera. According to this classification, *M. sparsiflora* belongs to a different subgenus than the other three species analysed in the present study, although this classification has been questioned by Al-Shehbaz (1991). Nevertheless, the simultaneity of a different fatty acid and tocopherol profile in *M. sparsiflora*, together with the above mentioned infrageneric classification, suggest a possible taxonomic value of fatty acids and tocopherols for this monogeneric tribe.

In conclusion, the present study for the first time showed the infrafamilial variability for tocopherol content and composition in the Boraginaceae. The chemotaxonomic potential of fatty acids in this family was confirmed and that of tocopherols was suggested. Further studies, however, are needed to determine the degree to which tocopherols and fatty acids can contribute to delimit taxonomic classes within this family.

3. Experimental

Forty-five accessions of Boraginaceae were obtained from the Botanical Garden of the University of Göttingen. They were classified into subfamilies and tribes following the system used by Riedl (1967). Those genera not included in that work were classified according to Al-Shehbaz (1991) and Luque (1995).

The fatty acid composition of the seed oil was determined by gas–liquid chromatography of fatty acid methyl esters. Heptadecanoic acid (17:0) was used as an internal standard in order to quantify simultaneously the oil content. Fatty acid methyl esters were prepared following the procedure of Thies (1971) and analyzed on a Perkin Elmer gas chromatograph model 8600 (Perkin Elmer Corporation, Norwalk, CT) equipped with a fused silica capillary column FFAP, 25 m × 0.25 mm × 0.25 μm film thickness (Macherey and Nagel GmbH + Co KG, Düren). The oven, detector and injector temperatures were 200, 250 and 250°C, respectively. The carrier gas was hydrogen, at a pressure of 100 kPa. Two microliters of sample were injected, at a split rate of 1:70.

Tocopherol content was determined by high performance liquid chromatography as described by Thies (1997), with β-tocopherol as internal standard. The samples were first analysed without internal standard to check the presence of β-tocopherol. This tocopherol was used as internal standard in the samples without and as external standard in those samples containing β-tocopherol. Total tocopherol content was expressed as mg kg^{-1} oil. Individual tocopherols were expressed as % (w/w) of the total tocopherol content.

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