Fatty Acid Composition of Some Ranunculaceae Seed Oils

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The fatty acid composition of seed oils of eight Ranunculaceae was determinated in order to characterize new sources of gamma-linolenic acid. Fatty acids were identified as fatty acid methyl esters (FAME) by capillary gas-liquid chromatography (GC) and capillary GC-Fourier transform infrared spectroscopy (FTIR). For trienic FAME the use of a cyanopropyl-polysiloxane stationary phase (CP-Sil 88) allowed the separation with high resolution of methyl ester of columbinic acid (trans-5,*cis*-9,*cis*-12 18:3) and gamma-linolenic acid (*cis*-6,*cis*-9,*cis*-12 18:3).

The results confirmed the presence of columbinic acid in *Thalictrum* seed oils, and that of gamma-linolenic acid in *Anemone* and related species seed oils. The taxonomic subdivision of Ranunculaceae into sub-families and tribes, which resulted from morphological considerations, did not account for the above results.

Introduction

Species of the botanical family Ranunculaceae are herbaceous plants bearing dry fruits such as achenes and follicles. About fifty genera and two thousands species are comprised in this family. Numerous members are spread over all continents, but the main growing area is located between temperate and cold regions of the northern hemisphere.

Fatty acid composition of twenty eight species seed oils have been already reported (Bagby *et al.*, 1962; Ferlay *et al.*, 1993; Kaufmann and Barve, 1965; Rankoff *et al.*, 1971; Smith *et al.*, 1968; Spencer *et al.*, 1970; Takagi *et al.*, 1983; Tsevegsüren and Aitzetmüller, 1993; Ustun *et al.*, 1990; Viano and Gaydou, 1984; Vioque *et al.*, 1994; Wu *et al.*, 1980). Among these, six species belonging to the genus *Anemone* displayed fair amounts of gamma-linolenic acid (GLA) or *cis*-6,*cis*-9,*cis*-12 18:3, in their lipids (Smith *et al.*, 1968, Tsevegsüren and Aitzetmüller, 1993). Eight other species of the *Aquilegia* and *Thalictrum* genera contained columbinic acid (COL) or *trans*-5,*cis*-9,*cis*-12 18:3 (Bagby *et al.*, 1962; Kaufmann and Barve, 1965; Spencer *et al.*, 1970; Takagi *et al.*, 1983; Wu *et al.*, 1980).

In the course of investigations upon new sources of GLA (Ucciani, 1995), we have determinated the composition of fatty acids of eight Ranunculaceae seed oils, the species involved having never been reported earlier. The results obtained have been tentatively brought together with the taxonomy of this botanical family.

Materials and Methods

Ranunculaceae-, borage-, pine seeds were collected in gardens and natural sites (1994, september, region of Marseille, south of France). Columbine (*Aquilegia vulgaris*) seeds were purchased in seed shop (Vilmorin, Marseille, France).

The seeds (150 mg) were crushed twice in hexane (50 ml), solvent of analytical grade purchased from SDS, Peypin, France.The suspension was filtered off and the solvent was evaporated under vacuum. The recovered oils (9.5–24.5 weight % of the dried seeds) were converted into fatty acid methyl esters (FAME) by methanolysis in homogen medium (Cecchi *et al.*, 1985).

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GC analyses of FAME were carried out on a Carlo Erba 4130 chromatograph, equipped with a flame ionization detector and a split injector (Carlo Erba, Milano, Italy) Helium was the carrier gas (inlet pressure 120kPa). Injector and detector temperatures were maintained at 250°C. Two fused-silica capillary columns (Chrompack, Middleburg, The Netherlands) were used: CP-Wax 52 CB (crossbond Carbowax), 30m x 0.25mm i.d., 0.15μm film thickness, 180°C isothermal, and CP-Sil 88 (cyanopropylpolysiloxane), 50m x 0.25 i.d., 0.20μm film thickness,160°C isothermal until the C18 trienes were eluted. The temperature was then increased at a rate of 10°C/min to 190°C and held until completion.

Equivalent chain lengths (ECL) were calculated according to Ackman (1972), with 16:0, 18:0, 20:0 and 22:0 as standards.

Infrared spectra were obtained from the oil in CCl_4 solution on an Acculab 4 spectrophotometer (Beckman instruments, Fullerstone, CA) and by GC-FTIR of the FAME using a Carlo Erba HR-GC 5300 chromatograph coupled with a Nicolet 20 SXB spectrophotometer. The temperatures of the light-pipe and transfer line were maintained at 300°C. The chromatographic conditions were identical to those used within CP-Wax analyses (see above). Spectrometric conditions were : mercury-cadmium-tellurium detector, scale 6000–400 cm^{-1}, cooling by liquefied nitrogen.The spectrum resolution was about 8 cm^{-1}.

Results and Discussion

The species studied in this paper are presented in Table I. Oil content in weight % of dried seeds, iodine value and IR absorption are also given in this Table.

All the oils exhibited high iodine values except the *C. pubescens*, one. This corresponds to a large amount of polyunsaturated fatty acids. *Thalictrum* oils showed a fair IR absorption at 968 cm-^{1} due to C-H bending of trans disubstituted unconjugated carbon-carbon double bond. Other absorptions in the spectrum were common to all seed oils.

FAME prepared by methanolysis of the oils (Cecchi *et al.*, 1985) were analyzed by GC. They were identified by comparison of their ECL with that of standards and of the FAME obtained from borage, pine and columbine seed oils. GLA, pinolenic acid (PIN) or *cis*-5,*cis*-9,*cis*-12 18:3 and COL were known to occur in these oils (Ucciani *et al.* 1992, Takagi *et al*,. 1983). Such analysis was first performed on CP-Wax 52 CB capillary column, then on a longer and more polar CP-Sil 88. Additional analysis by GC-FTIR was realized in order to locate the peaks corresponding to trans-unsaturated FAME. The results obtained by these methods are listed in Table II.

On CP-Wax column, FAME of *trans*-5 and *cis*-9 18:1 were not separated and *trans*-5,*cis*-9 18:2 was eluted just before methyl linoleate. Trienic FAME with Δ5 or Δ6 were co-eluted before methyl lin-

Table I. Species studies, seed oil content and oil characterstics.

Species	Seed oil content*	Oil IV^{+}	Oil IR^{++}
Anemone hortensis L.	14	142	–
Clematis flammula L.	9.5	139	–
Consolida pubescens (DC.) Soo (= *Delphinium pubescens* DC.)	23	118	–
Hepatica nobilis Miller (= *Anemone hepatica* L.)	20.5	147	–
Ranunculus parviflorus L.	24.5	141	–
Thalictrum flavum L.	15.5	165	968
Thalictrum morisonii C. C. Gmelin (= *T. Exaltatum* Gaudin)	19.5	172	968

* In wt % dried seed; $^{+}$ Iodine value; $^{++}$ In cm^{-1}.

Table II. Chromatographic retention data of FAME.

Equivalent chain length CP Wax 52 at 180 °C[1]	Equivalent chain length CP-Sil 88 at 160 °C[2]	CG-FTIR[3]	Fatty acid structure[4]
16.00	16.00	–	16:0
16.27	16.63	–	*c*-9 16:1
18.00	18.00	–	18:0
18.24	18.31	+	*t*-5 18:1
18.23	18.57	–	*c*-9 18:1
18.29	18.66	–	*c*-11 18:1
18.45	18.89	+	*t*-5, *c*-9 18:2
18.65	19.45	–	*c*-9,*c*-12 18:2
18.90	19.77	+	*t*-5,*c*-9,*c*-12 18:3
18.92	19.87	–	*c*-5,*c*-9,*c*-12 18:3
18.92	20.08	–	*c*-6,*c*-9,*c*-12 18:3
19.27	20.65	–	*c*-9,*c*-12,*c*-15 18:3
20.00	20.00	–	20:0
20.17	20.27	+	*t*-5 20:1
20.18	20.57	–	*c*-11 20:1

[1] Peyronel *et al.*, 1984; [2] Wolff, 1994 and reference therein; [3] +: absorption at 968 cm^{-1}; [4] *c: cis, t: trans.*

Table III. Fatty acid composition of Ranunculaceae seed oils.

Species	Fatty acids (% of total acids)[+]								
	16:0	16:1	18:0	18:1[1]	18:2	COL*	GLA*	LIN*	20–24
A. hortensis	12.5	0.2	2.3	9.8	68.8	–	4.6	0.7	1.0
C. flammula	10.2	0.5	3.8	16.5	63.1	–	–	3.6	2.1
C. pubescens	3.1	0.2	1.4	41.2	41.8	–	–	1.6	10.5[2]
H. nobilis	8.7	0.2	2.5	12.6	66.4	–	8.2	0.8	0.5
R. aquatilis	9.8	3.3[3]	2.1	19.5	24.2	–	–	40.6	0.4
R. parviflorus	10.6	2.1	3.1	23.2	41.7	–	–	18.7	0.5
T. flavum	4.3	2.0	3.9	27.3[4]	26.8[5]	34.8	–	0.3	0.6
T. morisinii	4.9	2.6	3.0	26.9[6]	26.2[7]	41.9	–	0.2	0.2

[+] *c: cis, t: trans*; * COL: *t*-5,*c*-9,*c*-12 18:3, GLA: *c*-6,*c*-9,*c*-12 18:3, LIN: *c*-9,*c*-12,*c*-15 18:3; [1] 0.2–0.5% *c*-11 18:1; [2] 9.5% 20:1; [3] 2.1% 16:2; [4] 9.4% *t*-5 18:1; [5] 6.0% *t*-5,*c*-9 18:2; [6] 10.2% *t*-5 18:1; [7] 4.5% *t*-5,*c*-9 18:2.

olenate. Consequently this kind of stationnary phase could not be recommanded for identification of uncommon trienoic acids as those of Ranunculaceae seed oils. On the other hand, the CP-Sil 88 column allowed a good separation of the three octadecatrienoic (Aitztmüller and Tsevegsüren, 1993). Under our operating conditions for this column, a difference of ECL of 0.10 allowed a base line resolution of peaks. So it came possible to locate and identify unambiguously at once methyl ester of COL, its isomer PIN, GLA and linolenic acid (LIN). Concerning the trans Δ5 configurations, an additional proof was brought by the IR spectrum obtained by GC-FTIR with the 968 cm^{-1} band. Thus we could establish the fatty acid composition of the eight Ranunculaceae seed oils. The results, Table III, showed that GLA was contained in the oils of *A. hortensis* and *H. nobilis* (formerly *Anemone hepatica*) (4.6 and 8.2% of total fatty acid).

The two species of the genus *Thalictrum*, *T. flavum* and *T. morisonii*, exhibited a fair amount of COL (34.8 and 41.9% of total fatty acids). They also contained noticeable quantities of 18:1 and 18:2 acids with a Δ5 unsaturation. The four other species did not contain any fatty acids with a Δ5 or Δ6 double bond.Fatty acids with chain lenght of 20–24 carbon atoms were determinated in significant amount only in *C. pubescens* 9.5 % of total fatty acid of 20:1. Our results were in good agreement with those of literature (Aitzetmüller and Tsevegsüren, 1993 and 1994; Bagby *et al.*, 1962; Ferlay *et al.*, 1993; Kaufmann and Barve, 1965; Rankoff *et al.*, 1971; Smith *et al.*, 1968; Spencer *et al.*, 1970; Takagi *et al.*, 1983; Tsvegsüren and Aitzetmüller, 1993; Ustun *et al.*, 1990; Viano and Gaydou, 1984; Vioque *et al.*, 1994; Wu *et al.*, 1980).

The presence in the same botanical family of both uncommon trienoic compounds as metabolites, i.e. GLA and COL, rose a question of plant physiology. So we should believe that taxonomy of Ranunculaceae was involved. Such occurence of unsual fatty acids was well known for instance in the case of Apiaceae with petroselinic acid, and Brassicaceae with erucic acid (Badami and Patil, 1981).

According to Heywood (1985) Ranunculaceae would be divided in two sub-families, each of which comprising several tribes. From the literature as well as from our results, we could see that COL was present into two genera : *Aquilegia*, tribe of Helleboreae, sub-family of Helleboroideae and *Thalictrum*, tribe of Anemoneae, subfamily of Ranunculoideae. As for GLA it was found only in the genus *Anemone*, which belongs to the same tribe as *Thalictrum*.. We could conclude therefore at a discordance between taxonomy and chemical composition. The reason was likely that the grounds of taxonomy were morphological and not biochemical considerations.

Subject to additional works, the Ranunculaceae family displays any interest in but few cases, such as in some species of the genus *Anemone*. Nevertheless it would be interesting to revisit the composition of some Ranunculaceae seed oils, in order to precise wether GLA or COL is involved.

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