Fatty Acid Composition of Some Pine Seed Oils

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ABSTRACT: The fatty acid composition of seeds from seven species of the genus *Pinus* (*P. pinaster, P. griffithii, P. pinea, P. koraiensis, P. sylvestris, P. mughus,* and *P. nigra*) was established. Pine seeds are rich in oil (31–68% by weight) and contain several unusual polymethylene-interrupted unsaturated fatty acids with a *cis*-5 ethylenic bond. These are the *cis*-5,*cis*-9 18:2, *cis*-5,*cis*-9,*cis*-12 18:3, *cis*-5,*cis*-11 20:2, and *cis*-5,*cis*-11,*cis*-14 20:3 acids, with a trace of *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid. Their percentage relative to total fatty acids varies from a low of 3.1% (*P. pinea*) to a high of 30.3% (*P. sylvestris*), depending on the species. The major *cis*-5 double bond-containing acid is generally the *cis*-5,*cis*-9,*cis*-12 18:3 acid (pinolenic acid). In all species, linoleic acid represents approximately one-half the total fatty acids, whereas the content of oleic acid varies in the range 14–36% inversely to the sum of fatty acids containing a *cis*-5 ethylenic bond. The easily available seeds from *P. koraiensis* appear to be a good source of pinolenic acid: their oil content is *ca.* 65%, and pinolenic represents about 15% of total fatty acids. These values appear to be rather constant. *Pinus pinaster,* which is grown on several thousand acres in the southwest of France, is an interesting source of *cis*-5,*cis*-11,*cis*-14 20:3 acid (7% in the oil, which is *ca.* 35% of the dehulled seed weight), an acid sharing in common three double bonds with arachidonic acid. Apparently, *P. sylvestris* seed oil contains the highest level of *cis*-5 double bond-containing acids among pine seed oils that have ever been analyzed.

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KEY WORDS: *cis*-5 Olefinic acids, equivalent chainlengths, fatty acid composition, pine seed oil.

Pine seeds that are consumed by humans are those from *Pinus koraiensis* (China), *P. pinea* (Europe), *P. sibirica* (Siberia), *P. monophylla* (California), and probably some others. The fact that these seeds are seldom consumed may explain why little is known about their fatty acid composition. However, it has been shown in a few cases (1) that pine seeds contain polymethylene-interrupted unsaturated acids with an ethylenic bond in the *cis*-5 position. This seems to be a general feature of Conifer seeds and leaves (1,2), although such acids also may occur in great abundance in many other plants. These acids may be of physiological or biochemical relevance. Berger and German (3) have demonstrated that the *cis*-5,*cis*-11,*cis*-14 20:3 acid from the seeds of *Biota orientalis* can replace arachidonic acid in hepatic phosphatidylinositol of rats. We made a similar observation with *cis*-5,*trans*-9 18:2 acid, the endogenous desaturation product of dietary elaidic acid (R.L. Wolff, unpublished results). This emphasizes the importance of the *cis*-5 ethylenic bond in the metabolism of fatty acids, particularly for the acylation of phospholipids.

As we needed a sufficient quantity of such an oil for biochemical experiments, we undertook the study of the fatty acid composition of seeds from several pine species available in France. Edible pine seeds that are commercialized in France are those from *P. koraiensis* (imported from China) and *P. pinea* (imported from Spain, Portugal, Italy, and Turkey). Other pine seeds are only used for forest planting. In this study, we give the detailed fatty acid composition of seeds from seven pine species, some of which were purchased from different commercial sources. The percentage of *cis*-5 double bond-containing acids largely varies from one species to another but is quite constant for a given species.

EXPERIMENTAL PROCEDURES

Seeds and standards. Seeds from *P. nigra, P. griffithii, P. sylvestris,* and *P. mughus* were obtained from the French National Office for Forests (O.N.F., Supt, France). Seeds from *P. pinaster* were from a local pine seed seller in the Landes. Seeds from *P. pinea* were collected in the southeast of France (Toulon). Imported seeds from *P. koraiensis* were purchased in supermarkets from Bordeaux and Toulon. Two samples of *P. koraiensis* seed oil were kind gifts from the NOF Corporation (Tokyo, Japan) and the Bertin Society (Lagny-le-Sec, France). A sample of oil extracted from seeds of *P. pinea* imported from Spain was also given by the latter. The following fatty acid methyl esters (FAME) were purchased from the Sigma Chemical Company (St. Louis, MO): *cis*-5 20:1, *cis*-11 20:1, *cis*-11,*cis*-14 20:2, and *cis*-11,*cis*-14,*cis*-17 20:3 acids. The *cis*-5 18:1 acid was generously donated by Dr. Svennson (Pharmacia, Stockholm, Sweden). *Taxus baccata* seed oil, which was used as a source of *cis*-5,*cis*-9 18:2 acid (4), was prepared as described previously (5). Seeds from *Chamaecyparis lawsoniana* (Cupressaceae), a source of *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids (1), were also from O.N.F.

Oil extraction. The oil from the seeds was extracted mainly according to Folch *et al.* (6). After having manually removed the shells, when this was possible, the seeds were

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crushed in a mortar. An aliquot (10 g) was dispersed in 66 mL of methanol with an Ultra-Turrax T-25 (Janke & Kunkel GmbH and Co. KG, Staufen, Germany) equipped with an S-25N shaft. Chloroform (133 mL) was added, and the suspension was dispersed a second time with the Ultra-Turrax. The suspension was then filtered on paper in a separatory funnel. The vessels and the residue on the filter were rinsed with several portions (total: 25 mL) of a chloroform/methanol (2:1, vol/vol) mixture. The clear filtrate was thoroughly mixed with 45 mL of a 1% (wt/vol) aqueous solution of KCl and allowed to stand for about 2 h. The lower phase was drained, the solvents were removed in a rotary evaporator at 50°C, and the oil was weighed.

FAME preparation. FAME were prepared according to Morrison and Smith (7). To two drops of oil, introduced in a Teflon-lined screw-capping tube, was added 1.5 mL of a methanolic solution of BF_3 (12%, wt/vol), and the mixture was homogenized with 1.5 mL of benzene. The tubes were tightly capped, and the reaction was allowed to proceed for 1 h in a boiling water bath. FAME were extracted twice with 2 mL hexane, with water (2 mL) being added to the mixture.

Gas–liquid chromatography (GLC). FAME were analyzed in a Carlo Erba HRGC chromatograph (Carlo Erba, Milano, Italy) equipped with a fused-silica capillary column (CP-Sil 88, 50 m × 0.22 mm i.d., 0.2 µm film; Chrompack, Middelburg, The Netherlands). The oven was held at 170°C, while the injector (split mode) and the flame-ionization detector were maintained at 250°C. The inlet pressure of the carrier gas (helium) was 100 kPa. Quantitative data were given by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Peak identification. Unusual polymethylene-interrupted fatty acids were identified by comparison of their equivalent chainlengths (ECL) with those calculated with authentic related standards. ECL were determined according to Ackman (8) with 16:0, 18:0, and 20:0 acid methyl esters as reference components. Calculations were realized according to Sebedio and Ackman (9) or Wolff (5). FAME prepared with *T. baccata*, *C. lawsoniana*, and *P. koraiensis* seed oils were used as secondary standards for the location of *cis*-5 double bond-containing acids. The number of double bonds was confirmed by fractionation of FAME by thin-layer chromatography on silver-impregnated silicic acid (5).

RESULTS AND DISCUSSION

Table 1 gives the experimental ECL of fatty acids, containing a *cis*-5 ethylenic bond, present in pine seed oils. They are compared with ECL calculated as follows. For polymethylene-interrupted dienoic acids, the ECL is obtained by adding the fractional chainlengths (FCL; FCL = ECL − base value) of the corresponding monoenoic acids and the base value. For example, the ECL of *cis*-5,*cis*-9 18:2 acid is the sum of the FCL of *cis*-5 18:1 and *cis*-9 18:1 acids plus the base value 18.00, that is 0.44 + 0.67 + 18.00 = 19.11. For trienoic acids, the ECL are obtained by adding the FCL of the corresponding dienoic acids and the base value and by subtracting the FCL of the monoenoic acid having an ethylenic bond common to the two dienoic acids, which is counted twice. For example, the ECL of *cis*-5,*cis*-9,*cis*-12 18:3 acid (pinolenic acid) is the sum of the FCL of *cis*-5,*cis*-9 18:2 and *cis*-9,*cis*-12 18:2 acids, plus the base value 18.00, minus the FCL of *cis*-9 18:1 acid: 1.10 + 1.57 + 18.00 − 0.67 = 20.00. For tetraenes, ECL are calculated by adding the FCL of the two corresponding trienes and the base value and subtracting the FCL of the common diene. For example, the ECL of *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid is the sum of the base value 18.00, the FCL of *cis*-5,*cis*-9,*cis*-12 18:3 (2.02) and *cis*-9,*cis*-12,*cis*-15 18:3 (2.62) acids, minus the FCL of *cis*-9,*cis*-12 18:2 acid (1.57), and the result is 21.07. The agreement between experimental and calculated values is excellent (Table 1). Furthermore, these identifications are supported by the comparison of our quantitative data for the seeds of *P. koraiensis* with those of Takagi and Itabashi (1), which are fundamentally identical. We also have calculated in the same way the theoretical ECL of *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid, which has been characterized in the leaves of some pine species (2) and Cupressaceae seeds (1). No peak was detected at the place where this acid is expected to elute. On the other hand, a trace component (less than 0.08%) was located at the place where *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid, also present in pine leaves (2) and Cupressaceae seeds, elutes. These two tetraenes were present in *C. lawsoniana* seeds. A typical chromatogram of FAME prepared with the oil extracted from pine seeds is given in Figure 1.

Pine seeds are rich in oil: their content varies from 31 to 68% (Table 2). All pine seeds analyzed in the present study contain in significant quantities four fatty acids with a *cis*-5

TABLE 1
Experimental and Calculated Chromatographic Retention Data for Methyl Esters of Fatty Acids Containing a *cis*-5 Ethylenic Bond and of Related Authentic Standards

Fatty acid structure[a]	ECL[b]	
	Experimental	Calculated
5-18:1	18.44	—
9-18:1	18.67	—
5,9-18:2	19.10	19.11
9,12-18:2	19.57	—
5,9,12-18:3	20.02	20.00
9,12,15-18:3	20.62	—
5,9,12,15-18:4	21.09	21.07
5-20: 1	20.40	—
11-20: 1	20.59	—
5,11-20:2	21.02	20.99
11,14-20:2	21.50	—
5,11,14-20:3	21.94	21.93
11,14,17-20:3	22.52	—
5,11,14,17-20:4	22.97	22.97

[a]Double bonds in the *cis* configuration. Authentic standards were 5-18:1, 5-20:1, 11-20:1, 11,14-20.2, and 11,14,17-20:3 acid methyl esters.
[b]Equivalent chainlengths, experimentally determined on a CP-Sil 88 column (Chrompack, Middleburg, The Netherlands) under conditions described in the legend of Figure 1 or calculated as described in the text.

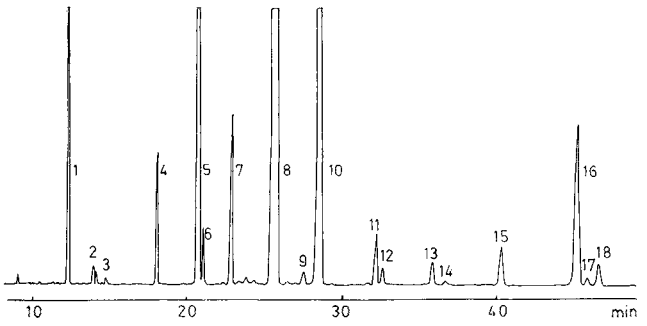

FIG. 1. Typical chromatogram of fatty acid methyl esters prepared from the oil extracted from *Pinus sylvestris* seeds. Analysis on a CP-Sil 88 capillary column (50 m × 0.22 mm i.d., 0.2 μm film; Chrompack, Middelburg, The Netherlands) operated isothermally at 170°C with an inlet carrier gas (helium) pressure of 100 kPa. Peak identification: **1**, 16:0; **2**, 16:1; **3**, 17:0; **4**, 18:0; **5**, 9-18:1; **6**, 11-18:1; **7**, 5,9-18:2; **8**, 9,12-18:2; **9**, unknown; **10**, 5,9,12-18:3; **11**, 11-20:1; **12**, 9,12,15 18:3; **13**, 5,11-20:2; **14**, 5,9,12,15 18:4; **15**, 11,14-20:2; **16**, 5,11,14 20:3; **17**, 22:0; **18**, unknown. Double bonds are in the *cis* configuration.

ethylenic bond (Table 2). Their structures are *cis*-5,*cis*-9 18:2, *cis*-5,*cis*-9,*cis*-12 18:3, *cis*-5,*cis*-11 20:2, and *cis*-5,*cis*-11,*cis*-14 20:3 acids. The amounts of these acids vary widely from one species to another. Their sums range from a low of 3.1% in *P. pinea* seed oil to a high of 30.3% in *P. sylvestris* seed oil (Table 2). *Pinus sylvestris, P. mughus, P. nigra,* and *P. griffithii* display the highest levels of *cis*-5,*cis*-9,*cis*-12 18:3 acid (18.9–21.8%), whereas *P. pinea* has the lowest (0.4%). Similar high levels of pinolenic acid were found in the seeds of *P. densiflora, P. thunbergii,* and *P. pentaphylla* (1). *Pinus sylvestris* and *P. pinaster* contain more than 5% *cis*-5,*cis*-11,*cis*-14 20:3 acid. This acid is interesting for biochemical or physiological studies because it closely resembles arachidonic acid, with three double bonds common to the two fatty acids. Except in *P. nigra*, where it reaches 3.6%, the *cis*-5,*cis*-9 18:2 acid is generally lower than 3% of total fatty acids. The *cis*-5,*cis*-11 20:2 acid is a minor component in all species (less than 0.8%). Apart from these unusual fatty acids, pine seeds contain the more common oleic (*cis*-9 18:1) and linoleic (*cis*-9,*cis*-12 18:2) acids in great abundance. The latter is relatively constant, ranging from *ca.* 45 up to 56%, whereas the former varies from 14.4 to 36.3% and is inversely related to the amount of *cis*-5 double bond-containing acids. Pine seed oils also contain small amounts of *cis*-11,*cis*-14 20:2 acid (0.5–1.0%) and *cis*-9,*cis*-12,*cis*-15 18:3 acid (0.2–1.3%).

Repeated analyses of different samples of oil from *P. koraiensis* and *P. pinea* seeds show their fatty acid compositions to be fairly constant and independent from their origin. For example, the total content of *cis*-5 fatty acids in *P. koraiensis* seeds varies in the narrow range 17.3–17.7% (four samples from different sources; R.L. Wolff, results not shown). *Pinus koraiensis* seeds from China are an interesting source of *cis*-5,*cis*-9,*cis*-12 18:3 acid because they are commercialized already dehulled on a ton-scale. Their oil content is high (more than 65%), and the percentage of pinolenic acid is constant (14–15%). On the other hand, the seeds from *P. pinaster* appear to be a good source of *cis*-5,*cis*-11,*cis*-14 20:3 acid (*ca.* 7%). In France, *P. pinaster* is grown on several thousand acres of former sandy moors, called the Landes, in the southwest part of the country. Harvesting on an industrial or semi-industrial scale seems feasible. Consequently, seeds from *P. koraiensis* and *P. pinaster* appear to be a convenient starting material for the production of oils that contain polyunsaturated acids with a *cis*-5 ethylenic bond with either 18 or 20 carbon atoms. Incidentally, *P. koraiensis* and *P. pinea* seed oils are produced industrially in France on a small scale for some cosmetic applications. Although we are not aware of the possibility of harvesting *P. sylvestris* seeds, this species seems to be the most widespread in Europe (from Scotland to Norway and eastward to Mongolia). Perhaps, this species might be the best pine seed source of *cis*-5 polyunsaturated fatty acids (*ca.* 30% of total fatty acids).

TABLE 2
Fatty Acid Composition (as weight percent) and Oil Content of Seeds from Different Pine Species

	Pinus						
Fatty acid[a]	*pinaster*	*pinea*	*koraiensis*	*nigra*	*griffithii*	*mughus*	*sylvestris*
16:0	3.60	5.55	4.20	4.16	4.51	3.27	3.36
16:1[b]	0.24	0.16	0.10	0.33	0.27	0.30	0.35
17:0	0.05	0.05	0.03	0.05	0.05	0.05	0.05
18:0	2.39	3.20	1.82	1.94	2.62	1.60	1.78
9-18:1	17.87	36.34	24.06	17.73	17.04	18.02	14.36
11-18:1	0.22	2.03	1.46	0.72	0.58	0.51	0.77
5,9-18:2	0.74	0.14	1.80	3.62	2.35	2.84	2.70
9,12-18:2	55.85	47.19	48.38	45.00	46.29	45.81	44.84
5,9,12-18:3	7.13	0.35	14.92	18.89	21.78	19.55	21.65
9,12,15-18:3	1.30	0.63	0.17	0.62	0.32	0.33	0.38
Unknown component[c]	0.27	0.49	0.20	0.17	0.21	0.22	0.20
11-20:1	1.01	0.74	1.03	0.97	0.81	1.37	1.14
5,11-20:2	0.76	0.14	0.10	0.34	0.19	0.51	0.50
11,14-20:2	0.85	0.51	0.49	0.87	0.73	0.84	0.99
5,11,14-20:3	7.09	2.47	0.90	3.44	1.53	3.73	5.46
Others	0.63	0.01	0.34	1.15	0.72	1.05	1.47
Sum Δ5[d]	15.72	3.10	17.72	26.29	25.85	26.63	30.31
Oil content	36	43.5	67	31[e]	49	35[e]	32[e]

[a]All ethylenic bonds in the *cis* configuration.
[b]Sum of two isomers.
[c]Unknown component eluting just before 20:0 acid but distinct from it (20:0 acid was not distinguishable from *cis*-5,*cis*-9,*cis*-12 18:3 acid).
[d]The total contents of Δ5 ethylenic-containing acids have since been confirmed by ^{13}C nuclear magnetic resonance (Gunstone, F.D., S. Seth and R.L. Wolff, unpublished data).
[e]Seeds that were not dehulled. Results as weight percent.

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