Conifer Seeds: Oil Content and Fatty Acid Composition

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ABSTRACT: The seed oils from twenty-five Conifer species (from four families—Pinaceae, Cupressaceae, Taxodiaceae, and Taxaceae) have been analyzed, and their fatty acid compositions were established by capillary gas–liquid chromatography on two columns with different polarities. The oil content of the seeds varied from less than 1% up to 50%. Conifer seed oils were characterized by the presence of several Δ5-unsaturated polymethylene-interrupted polyunsaturated fatty acids (Δ5-acids) with either 18 (*cis*-5,*cis*-9 18:2, *cis*-5,*cis*-9,*cis*-12 18:3, and *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acids) or 20 carbon atoms (*cis*-5,*cis*-11 20:2, *cis*-5,*cis*-11,*cis*-14 20:3, and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids). Pinaceae seed oils contained 17–31% of Δ5-acids, mainly with 18 carbon atoms. The 20-carbon acids present were structurally derived from 20:1n-9 and 20:2n-6 acids. Pinaceae seed oils were practically devoid of 18:3n-3 acid and did not contain either Δ5-18:4 or Δ5-20:4 acids. Several Pinaceae seeds had a Δ5-acid content higher than 50 mg/g of seed. The only Taxaceae seed oil studied *(Taxus baccata)* had a fatty acid composition related to those of Pinaceae seed oils. Cupressaceae seed oils differed from Pinaceae seed oils by the absence of Δ5-acids with 18 carbon atoms and high concentrations in 18:3n-3 acid and in Δ5-acids with 20 carbon atoms (Δ5-20:3 and Δ5-20:4 acids). Δ5-18:4 Acid was present in minute amounts. The highest level of Δ5-20:4 acid was found in *Juniperus communis* seed oil, but the best source of Δ5-acids among Cupressaceae was *Thuja occidentalis*. Taxodiaceae seed oils had more heterogeneous fatty acid compositions, but the distribution of Δ5-acids resembled that found in Cupressaceae seed oils. Except for *Sciadopytis verticillata*, other Taxodiaceae species are not interesting sources of Δ5-acids. The distribution profile of Δ5-acids among different Conifer families appeared to be linked to the occurrence of 18:3n-3 acid in the seed oils.
JAOCS 73, 765–771 (1996).

KEY WORDS: *cis*-5 Olefinic acids, conifer seeds, Cupressaceae, equivalent chainlengths, fatty acid composition, gas–liquid chromatography, oil content, Pinaceae, Taxaceae, Taxodiaceae.

A few recent studies have shown that Δ5-unsaturated polymethylene-interrupted fatty acids (Δ5-olefinic acids), present in Conifer seed oils, may have interesting biochemical and physiological properties. Berger and German (1) have observed that feeding mice a diet supplemented with *Biota orientalis* seed oil, which contains *cis*-5,*cis*-11,*cis*-14 20:3 and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids in substantial amounts, led to the replacement of arachidonic acid in hepatic phosphatidylinositol by the *cis*-5,*cis*-11,*cis*-14 20:3 acid. Based on these results and on his observations on the metabolic fate of the endogenous desaturation product of dietary elaidic (*trans*-9 18:1) acid, *cis*-5,*trans*-9 18:2 acid, Wolff (2) has put emphasis on the structural importance of a *cis*-5 ethylenic bond in fatty acids for the acylation of phospholipids.

Ikeda *et al.* (3), also using *B. orientalis* seed oil as a dietary supplement, observed a decrease in the concentration of rat serum total cholesterol, high-density lipoprotein cholesterol, triglycerides, and phospholipids as compared to a linoleic acid-enriched diet. Sugano (4) reported that pinolenic (*cis*-5,*cis*-9,*cis*-12 18:3) acid (probably from *Pinus koraiensis* seed oil) was hypocholesterolemic, that it stimulated aortic PG12 production and repressed platelet aggregation in relation to linoleic acid. Using the oils from *P. koraiensis* and *P. pinaster* seeds, the hypocholesterolemic effect of these oils on rats, with a remarkable lowering effect of *P. koraiensis* seed oil on serum triglycerides, could be confirmed (Bayard, C.C., and R.L. Wolff, unpublished results).

All Conifer seed oils used as dietary supplements in these experiments contained Δ5-olefinic acids in addition to the habitual 18:1n-9 and 18:2n-6 acids, and 18:3n-3 acid in some instances. It is thus reasonable to attribute the physiological effects of Conifer seed oils to Δ5-olefinic acids. These acids present a polymethylene-interrupted dienoic structure, combined in most instances with one or two supplementary ethylenic bonds arranged in a more classical methylene-interrupted dienoic acid structure. They may have 18 or 20 carbon atoms. According to literature data (5–9), six different Δ5-olefinic acids may occur in Conifer seed oils. These are the *cis*-5,*cis*-9 18:2, *cis*-5,*cis*-9,*cis*-12 18:3, *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4, *cis*-5,*cis*-11 20:2, *cis*-5,*cis*-11,*cis*-14 20:3, and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids. These two last acids have structures closely related to arachidonic (*cis*-5,*cis*-8,*cis*-11,*cis*-14 20:4) and eicosapentaenoic (*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 20:5) acids, with only the Δ8-ethylenic bond lacking in their structures. Δ5-Olefinic acids are frequently described as having "uncommon" structures. In fact, they have been observed in all Conifer seed oils analyzed until now. Considering the abundance of Conifers

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in the plant kingdom and the great number of species in this class, Δ5-olefinic acids should not be considered as uncommon acids, but rather as normal and characteristic components in Conifers.

The aim of the present study was to select potential sources of Δ5-olefinic acids that might be exploitable, at least on a scale compatible with the production of oils for biochemical, physiological, or nutritional experiments. For this purpose, we have determined the oil content and the fatty acid composition of seeds from twenty-five different Conifer species belonging to four families, Pinaceae, Taxaceae, Cupressaceae, and Taxodiaceae. A few species studied here were previously analyzed by other authors (5–8). Data for several species of the genus *Pinus* were reported previously (5,9). This systematic approach has led to the selection of a few species that may be interesting sources of Δ5-olefinic acids with either 18 or 20 carbon atoms. It also allowed distinction between two groups of Conifer families, based on major differences in their seed oil fatty acid compositions.

EXPERIMENTAL PROCEDURES

Seeds and standards. Conifer seeds were obtained from three French seed producers: the French National Office for Forests (O.N.F., Supt, France), the Vilmorin Society (La Ménitré, France), and the Versepuy Society (Le Puy-en-Velay, France). 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. Svensson (Pharmacia, Stockholm, Sweden).

Oil extraction. Oil from the seeds was extracted mainly according to Folch *et al.* (10). The seeds were finely ground in a household electric grinder. An aliquot (10 g) of the resulting powder was dispersed in 50 mL methanol with an Ultra-Turrax T-25 (Janke & Kunkel GmbH and Co. KG, Staufen, Germany) equipped with an S-25N shaft. Chloroform (100 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 35 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 (11). Two drops of oil introduced in a Teflon-lined screw-capping tube were dissolved in 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 one hour in a boiling waterbath. 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 4130 chromatograph (Carlo Erba, Milano, Italy). Two fused-silica capillary columns were used. A CP-Sil 88 column (50 m × 0.22 mm i.d., 0.2 μm film; Chrompack, Middelburg, The Netherlands) was operated at 170°C with an inlet pressure of the carrier gas (helium) of 120 kPa. The oven temperature for the DB Wax column (30 m × 0.32 mm i.d., 0.5 μm film; J&W Scientific, Folsom, CA) was 190°C, and the inlet pressure of helium was 140 kPa. The injector (split mode) and the flame-ionization detector were maintained at 250°C for both columns. Quantitative data were calculated by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Peak identification. Δ5-Unsaturated polymethylene-interrupted fatty acids were identified by comparison of their equivalent chainlengths (ECL) on the two capillary columns with those calculated with authentic related standards. Calculations were made according to Wolff and Bayard (9).

RESULTS AND DISCUSSION

Identification of Δ5-olefinic acids. In a previous study on pine seed oils (9), the Δ5-olefinic acids were identified by their ECL on the CP Sil 88 capillary column. These identifications were also supported by comparison of the fatty acid composition of *P. koraiensis* seed oil, which was previously published in the literature (5). Since the completion of the GLC study on pine seed oils, Gunstone *et al.* (12) have confirmed the overall Δ5-olefinic acid content of these oils by ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Moreover, the mass spectra of the 4,4-dimethyloxazoline derivatives of all fatty acids occurring in *P. pinaster*, *Chamaecyparis lawsoniana*, and *B. orientalis* seed oils have been established (Berdeaux, O., and R.L. Wolff, manuscript in preparation). In the present study, we have extended our calculations of ECL on the CP Sil 88 column to the DB Wax column (Table 1). As for the CP Sil 88 column (9), the agreement between experimental and calculated ECL is good. Consequently, both columns are quite well suited for the study of Conifer seed oils. The elution order of FAME on the DB Wax column is identical to that reported for a Silar 5CP column (5).

Another means to confirm our present results is to compare our values for some species that were previously studied by other authors and for which appropriate techniques were used to identify unambiguously the Δ5-olefinic acids (^{13}C NMR, GLC coupled with mass spectrometry, silver ion-impregnated thin-layer chromatography, chemical degradative procedures). Such comparisons are summarized in Table 2. *Taxus baccata* seed oil may be used as a standard for the location of *cis*-5,*cis*-9 18:2 acid (6), that from *Larix leptolepis* (= *L. kampferi*) for the location of *cis*-5,*cis*-9,*cis*-12 18:3 acid (8), that from *Sciadopytis verticillata* for the location of *cis*-11,*cis*-14 20:2 and *cis*-5,*cis*-11,*cis*-14 20:3 acids (5), and that from *B. orientalis* for the location of *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid (7).

The DB Wax column has some advantages over the CP Sil 88 column for the analysis of Conifer seed oils. On the latter, the *cis*-5,*cis*-9,*cis*-12 18:3 acid had an ECL of 20.02 (9), which precluded any possibility of separation from 20:0 acid,

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	18.16	—
9-18:1	18.23	—
5,9-18:2	18.43	18.39
9,12-18:2	18.69	—
5,9,12-18:3	18.91	18.89
9,12,15-18:3	19.37	—
5,9,12,15-18:4	19.59	19.59
5-20:1	20.12	—
11-20:1	20.21	—
5,11-20:2	20.37	20.33
11,14-20:2	20.69	—
5,11,14-20:3	20.83	20.85
11,14,17-20:3	21.33	—
5,11,14,17-20:4	21.49	21.47

[a]Double bonds are 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 DB Wax capillary column (J&W Scientific, Folsom, CA) operated under conditions described in the Experimental Procedures section or calculated according to Reference 9. For experimental data, $n = 6$. The coefficients of variation are less than 0.02 for all values.

at least under our experimental conditions. Moreover, the difference between the ECL of *cis*-9,*cis*-12,*cis*-15 18:3 acid and that of *cis*-11 20:1 acid on the CP Sil 88 column was small (9), and the two acids were poorly separated. When the content of 18:3n-3 acid is high, the *cis*-11 20:1 acid cannot be quantitated. None of these drawbacks occurred with the DB Wax column. However, great caution should be exercised when employing this column for the analysis of oils other than those from Conifer seeds and that may contain *trans*-5,*cis*-9,*cis*-12 18:3 (columbinic) or *cis*-6,*cis*-9,*cis*-12 18:3 (γ-linolenic) acids. In our hands, the ECL of these acids were 18.91 and 19.01 , respectively, identical or close to that of *cis*-5,*cis*-12,*cis*-15 acid (18.91, Table 1). However, the CP Sil 88 column is highly valuable to distinguish between these three octadecatrienoic acids. The ECL of columbinic, pinolenic, and γ-linolenic acids were 19.86, 20.02, and 20.20, respectively, under the conditions used in the present study. This led to a complete resolution of the three acids when they were mixed artificially (results not shown). In the present study, FAME prepared from Conifer seed oils were identified and quantitated on both the CP Sil 88 and the DB Wax capillary columns

Pinaceae seeds. The oil content and fatty acid compositions of Pinaceae seeds are summarized in Table 3. With the exception of *Cedrus atlantica*, all species analyzed contained 18:2n-6 acid as a main component. This acid frequently represented more than 40% of total fatty acids. This was previously observed in several species of the genus *Pinus* (5,9). Depending on the species, the second major fatty acid was either 18:1n-9 acid (the four *Abies* species and *C. atlantica*) or *cis*-5,*cis*-9,*cis*-12 18:3 acid (all other species). A second Δ5-olefinic acid with 18 carbon atoms (*cis*-5,*cis*-9 18:2 acid) was also present in all species, but it accounted for less than 6% in most instances. Another feature common to all Pinaceae seeds was the low level of 18:3n-3 acid (less than 0.6% of total fatty acids). This contrasted with Pinaceae leaf lipids, in which 18:3n-3 acid represented 30–50% of total fatty acids (13). Pinaceae leaf lipids also contained *cis*-5,*cis*-9 18:2 and *cis*-5,*cis*-9,*cis*-12 18:3 acids, but these acids were absent in leaf lipids from other Conifer families (13). The scarcity of 18:3n-3 acid in Pinaceae seeds may explain why its elongation product, *cis*-11,*cis*-14,*cis*-17 20:3 acid, was also absent. This also holds true for the Δ5-desaturation product of this acid, *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid, which could not be detected in any of the Pinaceae seed oils. This contrasted with Pinaceae leaf lipids, which contained small amounts of this eicosatetraenoic acid (13). On the other hand, 18:2n-6 acid was apparently elongated to *cis*-11,*cis*-14 20:2 acid, which was further desaturated to *cis*-5,*cis*-11,*cis*-14 20:3 acid. Both acids were present in small amounts in Pinaceae seed oils.

The abundance of Δ5-olefinic acids varied with the species in the range 15–29% of total fatty acids (Table 3), with *cis*-5,*cis*-9,*cis*-12 18:3 acid being always the main component. Combining the seed oil contents and the proportions of Δ5-olefinic acids in the oils allowed selection of the best potential sources of such acids. For example, *Tsuga canadensis* and *Picea abies* seeds contained more than 90 mg of Δ5-olefinic

TABLE 2
Content of Some Polyunsaturated Fatty Acids (wt% of total fatty acids) in Conifer Seed Oils. Comparison of Results Obtained in the Present Study with Literature Data

Fatty acid structure[a]	*Taxus baccata*		*Biota orientalis*		*Larix leptolepis*		*Sciadopytis verticillata*	
	Reference 6	Exp.[b]	Reference 7	Exp.	Reference 8	Exp.	Reference 5	Exp.
5,9-18:2	12.2	9.6	—[c]	trace[d]	2.7	2.2	trace	—
5,9,12-18:3	—	0.4	—	trace	24.9	25.8	trace	—
5,11-20:2	0.7	0.2	0.8	0.5	—	0.1	0.7	0.8
11,14-20:2	—	0.5	0.8	0.8	0.4[e]	0.4	5.0	4.6
11,14,17-20:3	—	—	0.4	0.6	—	—	0.2	0.2
5,11,14-20:3	1.2	1.5	4.3	3.3	0.8	0.5	15.0	15.1
5,11,14,17-20:4	—	0.2	11.3	9.0	—	—	1.8	2.0

[a]Double bonds are in the *cis* configuration. [b]Experimental data obtained in the present study. [c]Not reported. [d]Trace amounts. [e]Reported as 20:2 acid with no precisions on the structure.

TABLE 3
Fatty Acid Composition (wt%) and Oil Content of Some Pinaceae and Taxaceae Seeds

	16:0	16:1	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1	11,14-20:2	11,14,17-20:3
Tsuga canadensis	3.46	0.46	1.73	14.72	0.31	49.66	0.34	0.42	0.77	0.94	—[a]
Picea abies	2.78	0.20	1.49	13.41	1.55	49.89	0.21	0.24	0.31	0.58	—
P. sitchensis	2.94	0.17	1.22	16.23	0.99	48.01	0.41	0.31	0.31	0.47	—
Pseudotsuga menziesii	3.53	1.15	1.42	17.75	0.61	49.53	0.60	0.35	0.40	0.52	—
Larix leptolepis	2.62	0.38	1.36	18.38	1.11	45.53	0.35	0.31	0.50	0.39	—
L. decidua	2.80	0.43	1.46	18.76	0.97	43.10	0.56	0.23	0.40	0.35	—
Abies alba	4.12	0.81	1.66	25.82	0.32	42.71	0.52	0.39	0.59	0.28	—
A. concolor	4.10	0.73	1.55	37.43	0.43	37.17	0.47	0.56	0.76	0.26	—
A. nobilis	3.51	0.87	1.99	35.14	0.55	39.63	0.56	0.60	0.51	0.17	—
A. lowiana	3.30	0.75	1.58	24.55	0.39	45.16	0.35	0.49	0.85	0.58	—
Cedrus atlantica	5.23	0.98	2.87	41.67	0.43	29.04	0.42	1.07	0.67	0.36	—
Taxus baccata	3.17	0.06	2.46	56.00	0.31	22.81	1.68	trace	1.24	0.43	—

	22:0	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	5,11,14,17-20:4	Others	$\Sigma\Delta5$[c]	Oil content[d]	mg Δ5/g seeds[e]
T. canadensis	0.25	2.62	19.53	—	0.12	3.74	—	0.93	26.01	41.4	102
P. abies	0.19	3.25	24.67	—	0.05	0.94	—	0.24	28.91	33.9	93
P. sitchensis	0.19	2.10	25.75	—	0.07	0.66	—	0.17	28.58	30.6	83
P. menziesii	0.15	2.84	18.37	—	0.29	2.04	—	0.45	23.54	30.3	68
L. leptolepis	trace[b]	2.24	25.81	0.08	0.08	0.52	—	0.34	28.73	20.0	55
L. decidua	0.10	2.20	27.39	0.12	0.14	0.51	—	0.48	30.36	9.7	28
A. alba	0.30	6.24	12.47	—	0.24	2.04	—	1.49	20.99	37.7	75
A. concolor	0.34	5.56	8.78	—	0.24	1.12	—	0.50	15.70	41.4	62
A. nobilis	0.35	3.53	11.15	—	0.16	0.90	—	0.38	15.74	24.2	36
A. lowiana	0.27	5.66	13.32	—	0.20	1.84	—	0.71	21.02	16.6	33
C. atlantica	trace	5.60	10.54	—	0.14	0.69	—	0.29	16.97	44.2	71
T. baccata	—	9.57	0.36	—	0.22	1.50	0.17	0.02	11.82	23.1	27

[a]Not detected. [b]Trace amounts: peaks visible on the chromatograms but not taken into account by the integrator. [c]Sum of all fatty acids containing a Δ5-unsaturation. [d]wt%. [e]Total content of Δ5-unsaturated fatty acids in the seeds.

acids per g of seed. Although interesting, this content was still less than that determined for *P. koraiensis* seeds (*ca.* 110 mg/g, calculated from data in Ref. 9) but similar to that found in *P. sylvestris* seeds (9). However, *T. canadensis* (eastern hemlock) is abundant in the eastern regions of North America, from the Hudson Bay to North Carolina, and *P. abies* (Norwegian spruce) is a widespread species in northern countries of Europe and in northern regions of the former USSR. Both species are exploited for their wood, and it might be possible to harvest the cones when the trees are cut down.

Taxaceae seeds. We analyzed the seed oil of a single species of Taxaceae, *Taxus baccata* (Table 3). As compared to Pinaceae seed oils, *T. baccata* seed oil contained a higher level of 18:1n-9 acid and a lower content of 18:2n-6 acid. This also was observed with *T. canadensis*, but not with *T. cuspidata* or *Torreya nucifera* seed oils (5). The level of 18:3n-3 acid was low, though slightly higher than in Pinaceae seed oils. The main Δ5-olefinic acid was *cis*-5,*cis*-9 18:2 acid (almost 10% of total fatty acids). On the other hand, the *cis*-5,*cis*-9,*cis*-12 18:3 acid was rather low. These two features were apparently common to all *Taxus* species. *Cis*-5,*cis*-11 20:2 and *cis*-5,*cis*-11,*cis*-14 20:3 acids were present in the same ranges as in Pinaceae seed oils.

Cupressaceae seeds. Cupressaceae seeds had fatty acid compositions quite distinct from those of Pinaceae seeds (Table 4). First of all, they contained high amounts of α-linolenic acid, generally more than 20% of total fatty acids, whereas this acid was less than 0.6% in Pinaceae seeds. Second, they also contained the elongation product of α-linolenic acid, the *cis*-11,*cis*-14,*cis*-17 20:3 acid. Although present in low amounts in Cupressaceae seeds (less than 1.5% of total fatty acids), this acid was not present in Pinaceae seeds. Third and most important, they contained *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid, which was completely absent from Pinaceae seed oils. Finally, Cupressaceae seeds were practically devoid of *cis*-5,*cis*-9 18:2 and *cis*-5,*cis*-9,*cis*-12 18:3 acids, which were abundant in Pinaceae seeds. In summary, Pinaceae seed oils were distinct from Cupressaceae seed oils in that the former contained mainly Δ5-unsaturated polymethylene-interrupted acids with 18 carbon atoms, and the latter contained almost exclusively Δ5-unsaturated polymethylene-interrupted acids with 20 carbon atoms. In Pinaceae seeds, the Δ5-olefinic acids are probably formed *via* desaturation of 18:1n-9 and 18:2n-6 acids, whereas in Cupressaceae seeds, they are derived through Δ5-desaturation of the elongation products of 18:2n-6 and 18:3n-3 acids. Cupressaceae seeds also contained small amounts of *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid (generally less than 0.3%, except in *C. lawsoniana*, where it reached 2% of total fatty acids). Jamieson and Reid (13) observed that this acid was absent from Pinaceae leaf lipids, but that it occurred in all other Conifer families, including Cupressaceae.

Among the Cupressaceae seeds analyzed, those from *Thuja occidentalis* presented both a high oil content and high levels of *cis*-5,*cis*-11,*cis*-14 20:3 and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids. These two acids represented 5.8% of the seed weight, which is considerably higher than in *B. orientalis* seeds (2.1%). *Thuja occidentalis* (American arborvitae) is a widespread tree in the northeast regions of North America, but it is also widely grown in Europe as an ornamental tree. *Biota orientalis* is a common tree in China and Korea, and its seeds are harvested and sold in herb shops for pharmaceutical purposes (7). *Juniperus communis* seeds had the highest level of *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid (*ca.* 18% of total fatty acids) among Cupressaceae, but their oil content was rather low. However, juniper berries are harvested for several purposes (gin making, seasoning ingredient), and they might thus be an interesting source of such an acid. To our knowledge, the oil from the seeds of *Ephedra campylopoda* (Ephedraceae) is the only one that presents a higher proportion of *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid (21.9%) (14) than the seed oil from *J. communis*.

Taxodiaceae. Like Cupressaceae seed oils, Taxodiaceae seeds had only minute amounts of *cis*-5,*cis*-9 18:2 and *cis*-5,*cis*-9,*cis*-12 18:3 acids in their oils but a relatively high percentage of *cis*-5,*cis*-11,*cis*-14 20:3 and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids (Table 5). *Cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid was present in the seed oil of some species. Most Taxodiaceae were characterized by a high content of 18:3n-3 acid in their seed oil. With the exception of *Sciadopytis verticillata* (2.1%), the range for this acid was 17–45% of total fatty acids. In this respect, Taxodiaceae seeds resembled Cupressaceae seeds more than Pinaceae seeds. Linoleic acid was always present in higher amounts than oleic acid, a situation that also occurred in Cupressaceae and most Pinaceae seeds. Another point common to Cupressaceae and Taxodiaceae was the overall content of 16:0 and 16:1 acids, which were higher and lower, respectively, than in Pinaceae.

Most Taxodiaceae species grow in limited regions of the world, and they are seldom widespread trees. Except for *S. verticillata*, the oil content of their seeds (1–14%) and the total amount of Δ5-olefinic acids per g of seed (1.7–8 mg) were rather low, far less than in most Pinaceae or Cupressaceae seed oils. Consequently, Taxodiaceae seeds do not appear to be interesting sources of Δ5-olefinic acids.

Comments on the biosynthesis of Δ5-olefinic acids. Our observations on Conifer seed oils confirm and extend the observations of Takagi and Itabashi (5), who analyzed the seed oils of eighteen different Conifer species and of a few other authors who focussed their attention on a single species (6–8) or a single genus (9). The seed oils of all Conifer species analyzed contained Δ5-olefinic acids, which means that they contain a Δ5-desaturase. As shown in Figure 1 and as deduced from Conifer seed oil fatty acid compositions (5–9, this study), six fatty acids may be substrates for this desaturase, and six Δ5-olefinic acids can result from this reaction. Substrates and products of this desaturase are present in Conifer seed oils, but their distribution profile greatly depends on the family considered.

The differences we have noted between Pinaceae and Taxaceae on the one hand, and Cupressaceae and Taxodiaceae on the other hand, primarily come from the absence or the presence of 18:3n-3 acid in the seeds. In the first group, 18:3n-3

TABLE 4
Fatty Acid Composition (wt%) and Oil Content of Some Cupressaceae Seeds

	16:0	16:1	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1	11,14-20:2	11,14,17-20:3
Thuja occidentalis	5.20	—[a]	2.36	10.53	0.28	27.76	30.53	0.14	0.68	1.76	0.72
Biota orientalis	5.92	0.06	4.49	13.84		22.48	38.25	0.22	n.d.[b]	0.79	0.64
Juniperus communis	4.26	—	2.28	9.53	0.24	33.09	19.49	0.44	0.98	2.44	1.29
J. virginiana	5.25	—	3.27	14.43	0.25	26.31	28.14	0.13	0.88	1.32	0.66
Cupressus sempervirens	9.25	—	2.97	10.96	0.43	24.62	42.09	0.21	0.45	0.75	0.39
C. arizonica	6.46	trace	2.22	11.53	0.31	34.45	30.87	0.37	0.49	1.32	0.32
Calocedrus decurrens	9.07	0.05	3.28	20.27	0.43	26.84	33.13	0.12	0.63	0.85	0.92
Chamaecyparis lawsoniana	5.34	0.07	2.03	10.00	0.22	24.41	50.37	0.55	n.d.	0.19	0.10

	22:0	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	5,11,14,17-20:4	Others	$\Sigma\Delta5$[d]	Oil content[e]	mg Δ5/g seeds[f]
T. occidentalis	—	trace[c]	0.07	0.15	0.64	4.98	14.10	0.78	19.94	31.9	60
B. orientalis	trace	trace	trace	0.08	0.50	3.29	8.95	0.49	12.82	18.0	22
J. communis	0.16	—	0.08	0.07	0.29	7.21	17.99	0.26	25.64	11.9	29
J. virginiana	trace	—	0.04	0.04	0.68	9.08	9.45	0.07	19.29	17.2	32
C. sempervirens	0.12	—	0.06	0.11	0.16	3.47	3.72	0.24	7.52	11.1	8
C. arizonica	0.13	0.06	0.71	0.07	0.29	5.00	4.81	0.59	10.94	0.7	0.7
C. decurrens	trace	0.08	0.13	—	0.11	0.50	3.10	0.49	3.92	50.9	19
C. lawsoniana	0.13	0.11	0.29	2.05	0.42	0.50	0.74	2.26[g]	4.11	24.0	9

[a]Not detected. [b]Not determined [fatty acid methyl esters analyzed on the CP Sil 88 column (Chrompack, Middelburg, The Netherlands) only]. [c]Trace amounts: peaks visible on the chromatograms but not taken into account by the integrator. [d]Sum of all fatty acids containing a Δ5-unsaturation. [e]wt%. [f]Total content of Δ5-unsaturated fatty acids in the seeds. [g]Includes 1.70% of a component with an equivalent chain of 17.00 (17:0 acid?).

TABLE 5
Fatty Acid Composition (wt%) and Oil Content of Some Taxodiaceae Seeds

	16:0	16:1	18:0	9-18:1	11-18:1	9,12-18:2	9,12,15-18:3	20:0	11-20:1	11,14-20:2	11,14,17-20:3
Sciadopytis verticillata	3.35	trace[a]	2.10	21.29	0.37	46.30	2.12	0.26	1.35	4.59	0.24
Sequoia sempervirens	7.56	trace	2.42	14.38	0.27	48.35	17.40	0.82	0.72	1.00	0.19
Cryptomeria japonica	6.47	trace	2.96	10.20	0.27	24.05	45.67	0.38	0.72	0.67	0.64
Sequoiadendron gigantea	8.42	0.10	1.51	19.35	0.33	45.41	17.07	1.05	0.45	0.72	0.13
Taxodium distichum	7.92	0.11	2.68	7.98	0.60	29.22	28.36	0.51	n.d.[b]	1.06	1.81

	22:0	5,9-18:2	5,9,12-18:3	5,9,12,15-18:4	5,11-20:2	5,11,14-20:3	5,11,14,17-20:4	Others	$\Sigma\Delta5$[d]	Oil content[e]	mg Δ5/g seeds[f]
S. verticillata	trace	—[c]	—	—	0.77	15.11	2.04	0.11	17.92	37.1	63
S. sempervirens	0.56	0.06	0.42	0.06	0.20	3.92	1.60	0.07	6.26	13.8	8
C. japonica	0.19	0.06	0.38	0.09	0.39	1.48	4.76	0.62	7.16	12.1	8
S. gigantea	0.54	trace	0.18	0.06	0.15	3.50	0.80	0.23	4.69	11.7	5
T. distichum	0.23	trace	0.16	—	0.39	3.61	14.25	1.11	18.41	1.0	1.7

[a]Trace amounts: peaks visible on the chromatograms but not taken into account by the integrator. [b]Not determined (fatty acid methyl esters analyzed on the CP Sil 88 column only; see Table 4 for company source). [c]Not detected. [d]Sum of all fatty acids containing a Δ5-unsaturation. [e]wt%. [f]Total content of Δ5-unsaturated fatty acids in the seeds.

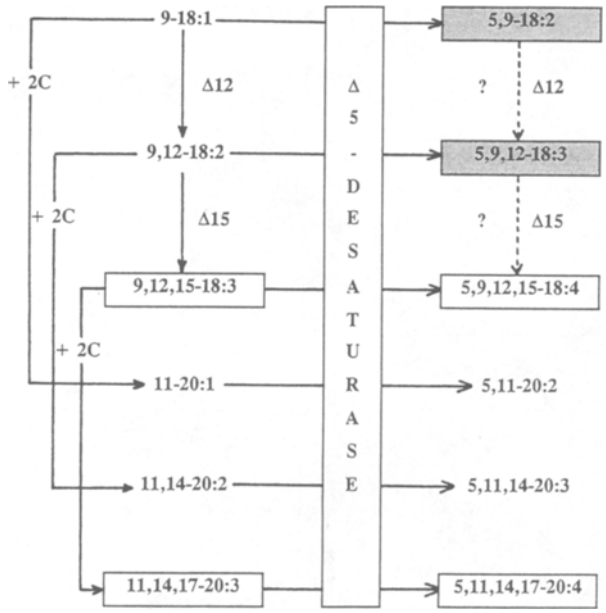

FIG. 1. Possible pathways for the biosynthesis of unsaturated fatty acids in Conifer seeds. Fatty acids in shaded boxes occur in noticeable amounts only in Pinaceae and Taxaceae seed oils. Those in open boxes are present in Cupressaceae and Taxodiaceae seed oils. Generally, fatty acids in shaded boxes do not occur together with those in open boxes. Other fatty acids are common to seed oils of all Conifer families.

acid is low and consequently, *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4, *cis*-11,*cis*-14,*cis*-17 20:3, and *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acids are lacking. On the other hand, Pinaceae seed oils are rich in *cis*-5,*cis*-9 18:2 and *cis*-5,*cis*-9,*cis*-12 18:3 acids, and this probably results from direct Δ5-desaturation of 18:1n-9 and 18:2n-6 acids, which are abundant in the seed oils. The fact that Pinaceae seed oils are generally rich in *cis*-5,*cis*-9,*cis*-12 18:3 acid with no *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid would indicate that the pathway shown on the right of Figure 1 (dotted arrows), thought theoretically possible, does not operate efficiently (or not at all) in Conifer seeds. The reactional sequence of desaturation by the Δ12- and Δ15-desaturases is probably the same as in most other vegetables, with 18:1n-9 and 18:2n-6 acids being their respective substrates (left pathway in Fig. 1).

In the second group (Cupressaceae and Taxodiaceae), 18:3n-3 acid is generally abundant in the seed oils, and it gives rise to *cis*-11,*cis*-14,*cis*-17 20:3 acid by elongation and then to *cis*-5,*cis*-11,*cis*-14,*cis*-17 20:4 acid *via* Δ5-desaturation. Direct Δ5-desaturation of 18:3n-3 acid leads to the formation of small amounts of *cis*-5,*cis*-9,*cis*-12,*cis*-15 18:4 acid. When 18:3n-3 acid is abundant, 18:1n-9 and 18:2n-6 acids are no more Δ5-desaturated, and *cis*-5,*cis*-9 18:2 and *cis*-5,*cis*-9,*cis*-12 18:3 acids are absent from the oils (or present in minute amounts).

In all Conifer families, independently from the presence or absence of 18:3n-3 acid, the elongation products of 18:1n-9 and 18:2n-6 acids, *cis*-11 20:1 and *cis*-11,*cis*-14 20:2 acids, are substrates of the Δ5-desaturase, and they give rise to *cis*-5,*cis*-11 20:1 and to *cis*-5,*cis*-11,*cis*-14 20:3 acids, respectively. These acids are found in the seed oils of all Conifer species. The *cis*-11 20:1 and *cis*-11,*cis*-14 20:2 acids are themselves minor components in all Conifer seed oils analyzed (5–9, this study).

The differences in the Δ5-olefinic acid profiles of seed oils among Conifer species would apparently depend on the activity of the Δ15-desaturase, but probably also on the substrate specificity of the Δ5-desaturase. Another point that needs further studies is the composition of the oils, i.e., their content in triglycerides, phospholipids, and glycolipids, which probably do not have the same fatty acid compositions.

ACKNOWLEDGMENT

The Vilmorin Society generously offered several samples of seeds from different Conifer species.

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[Received October 19, 1995; accepted February 20, 1996]