The Seed Fatty Acid Composition and the Distribution of Δ5-Olefinic Acids in the Triacylglycerols of Some Taxaceae (*Taxus* and *Torreya*)

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ABSTRACT: The fatty acid compositions of the seeds from three *Taxus* (yew) species and one *Torreya* species belonging to the Taxaceae family [*Taxus cuspidata* (Japanese yew), *T. chinensis* (Chinese yew), *T. baccata* (English yew), and *Torreya grandis* (Chinese nutmeg yew)] have been established. These compositions were compared with those previously published for *T. canadensis* (Canadian yew) and *Torreya nucifera*. In *Taxus* species, as well as in *Torreya* species, Δ5-olefinic acids are present in the seed lipids from all species analyzed. In *Taxus*, 5,9-18:2 (taxoleic) acid is the prominent Δ5-olefinic acid. It represents between 9.5 and 16.2% of total fatty acids. Other Δ5-olefinic acids that occur in low amounts are 5,9,12-18:3 (<3.5%), 5,11-20:2 (<0.3%), 5,11,14-20:3 (<2.2%), and 5,11,14,17-20:4 (<0.3%) acids. In *Torreya* species, the major Δ5-olefinic acid is 5,11,14-20:3 (sciadonic) acid (between 6.7 and 11.2%). In contrast to *Taxus* species, the 5,9-18:2 and 5,9,12-18:3 acids are scarce in *Torreya* species: less than 0.1%. Also, the 9,12,15-18:3 acid content is significantly lower in *Torreya* than in *Taxus*. The prominence of taxoleic acid among Δ5-olefinic acids in the seed lipids is a unique characteristic of the genus *Taxus* that isolates it from all other Coniferophytes analyzed so far. However, this feature is not shared by other Taxaceae species, such as *Torreya*, and with regard to their seed fatty acid compositions, the family Taxaceae appears particularly heterogeneous. Our observations favor the hypothesis that in Gymnosperm seeds, there might exist two Δ5-desaturases, one specific for unsaturated acids with a Δ9-ethylenic bond (active in *Taxus* but not in *Torreya*), and the other specific for unsaturated acids with a Δ11-ethylenic bond (active in *Torreya* but not in *Taxus*). Our data also highlight the importance of the elongase(s) in the metabolism of fatty acids in Gymnosperm seeds. 14-Methylhexadecanoic acid, a habitual component of Pinaceae and *Ginkgo biloba* seed lipids, could not be detected in the Taxaceae studied here. ^{13}C nuclear magnetic resonance spectroscopy of the oils from both genera has confirmed that Δ5-olefinic acids are apparently excluded from the internal position of triacylglycerols, which is a characteristic common to all Gymnosperm species analyzed so far, and consequently of great antiquity in their life history.

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Among Coniferophytes, Taxaceae are united by having an aril, and thus lacking a cone-like inflorescence in the female sex. This difference is likely to be primitive rather than derived, and some authors have regarded the Taxaceae as the nearest relatives (along with *Ginkgo biloba*) of the extinct Cordaitales, from which they would have differentiated 225–245 million years ago (1). The Taxaceae contain a few genera only: *Taxus*, *Torreya*, *Austrotaxus*, and *Pseudotaxus* (= *Nothotaxus*) (1). *Amentotaxus* is sometimes added to these genera (2), or included into the Cephalotaxaceae (1).

Taxus (yews) [etymologically, the name "yew" comes from Old English, "iw," probably derived from Gaulish (Keltic?) "evos," "if," or "iveteau" in French, "Eibe" in German] are a genus of perhaps seven to eight somewhat ill-defined species (1), scattered mostly in temperate climates of the Northern Hemisphere, frequently in small and local populations. Some authors even consider that the genus *Taxus* contains a single collective species with closely related geographic forms (2).

The fatty acid composition of *T. baccata* (English yew) seeds was first reported by Madrigal and Smith (3). Those from *T. cuspidata* (Japanese yew) and *T. canadensis* (Canadian yew) were described later by Takagi and Itabashi (4,5). Some precision has been added more recently for *T. baccata* seed fatty acids and triacylglycerol (TAG) structure by Wolff *et al*. (6–8) and Gunstone *et al*. (9).

In all these species, the major Δ5-olefinic acid was shown, by several analytical means, to be the 5,9-18:2 acid for which the name "taxoleic" was proposed (8). Multivariate analyses of the seed fatty acid compositions of 82 conifer species, including the three *Taxus* species mentioned above, indicated that the *Taxus* species formed a homogenous group that was completely isolated from all other conifer families (10), whereas *T. nucifera* was excluded from this group.

In the present study, we have analyzed the seed lipids from

another *Taxus* species, i.e., *T. chinensis* (Chinese yew), and reinvestigated the fatty acid composition of *T. baccata* and *T. cuspidata* to complete our knowledge on this genus and verify whether the prominence of taxoleic acid among Δ5-olefinic acids in the seed lipids is truly a characteristic of the family Taxaceae.

Torreya is a Taxaceae genus with only four species. *Torreya grandis*, analyzed in the present study, is endemic to a restricted area of China, whereas *T. nucifera*, analyzed by Takagi and Itabashi (4), grows in Japan. The two other species [*T. californica* (nutmeg yew) and *T. taxifolia* (stinking cedar)] are North American (California and western Florida, respectively). Like *Taxus*, *Torreya* are dioecious and the female bears fleshy arils. However, they differ in that only *Taxus* contain the poisonous alkaloid taxine (in the seeds, wood, and needles, but not in the aril) and also in their kariology: the chromosome base numbers are $x = 12$ for *Taxus* and $x = 11$ for *Torreya* (1). Incidentally, one should note that the seeds from *T. nucifera* are edible, and were used locally in Japan for the production of an edible oil known as "kaya" oil. The seeds are still used to prepare special foods in local mountain areas, such as "senbei" (Japanese crackers of wheat flour) and "miso" (bean paste) (Takagi, T., personal communication).

EXPERIMENTAL PROCEDURES

Seeds. *Taxus* seeds were purchased from Sandeman Seeds (Pulborough, Great Britain) or from Vilmorin (La Ménitré, France) and kept in a refrigerator until use. *Torreya grandis* seeds were purchased from Lawyer Nursery, Inc. (Plains, MT) and analyzed immediately after removal of the shell.

Oil extraction. The oil from the seeds was extracted mainly according to Folch *et al*. (11). The seeds (*ca.* 20 g) were ground in a household electric grinder. An aliquot (10 g) of the resulting homogenate was dispersed in 50 mL of 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. 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.

Fatty acid methyl ester (FAME) preparation. FAME were prepared according to Morrison and Smith (12). Two drops of oil introduced in a Teflon-lined screw-cap 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 1 h in a boiling waterbath. FAME were extracted twice with 2 mL of hexane, with water (2 mL) being added to the mixture. The pooled upper organic phases were dried over anhydrous Na_2SO_4. The FAME preparations were made in duplicate.

Gas–liquid chromatography (GLC). FAME were analyzed by GLC in a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy) equipped with a fused-silica DB Wax capillary column (30 m × 0.32 mm i.d., 0.5 μm film; J&W Scientific, Folsom, CA). The oven temperature 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. Quantitative data were calculated by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Peak identification. Δ5-Olefinic acids were identified by their equivalent chainlengths (ECL) (13). The use of ECL for identification was supported by identification through GLC–mass spectrometry of appropriate fatty acid derivatives (13).

^{13}C nuclear magnetic resonance (NMR) spectroscopy. The NMR spectra were obtained as previously described by Gunstone and Wolff (14). ^{13}C NMR spectra were obtained on a Bruker AM 300 spectrometer (Karlsruhe, Germany) at a frequency of 75.47 MHz. Samples were prepared in 5-mm tubes with approximately 100 mg of oil in 0.5 mL deuteriochloroform that contained tetramethylsilane as reference and $Cr(acac)_3$ as relaxation agent at a concentration of 0.025 M. Spectra were acquired by the NOE-suppressed, inverse-gated, proton-decoupled technique while employing a 90° excitation pulse, a 5-s pulse delay, and a sweep width of 20 kHz (32 K data points). The 90° pulse width was 3.9 μs. The number of scans was 1200 per spectrum.

RESULTS AND DISCUSSION

The oil contents of *Taxus* seeds (undehulled) varied between *ca*. 16 and 30% (Table 1) whereas those of *Torreya* species (dehulled) were both around 50%. The saturated acids (mostly 16:0 and 18:0) were less than 8% in all Taxaceae species. 14-Methylhexadecanoic (*anteiso*-17:0; 14-MHD) acid, which is a common component of Pinaceae (15) seeds, could not be detected on routine chromatograms in *Taxus* or in *Torreya*. The highest unsaturated acid content in *Taxus* was 9-18:1 acid, though in *T. chinensis*, its amount was practically equal to that of 9,12-18:2 acid. The latter acid was the second-most important component in *Taxus*, as in *T. grandis*, but it was the most prominent unsaturated acid in *T. nucifera*, where it accounted for more than 50% of total fatty acids. With regard to *T. baccata* and *T. cuspidata*, our present results are in excellent agreement with data published earlier (3,6). α-Linolenic acid was significantly higher in *Taxus* (1.5–2.1%) than in *Torreya* (0.2–0.5%). Other minor fatty acids (not included in Table 1) were 17:1, 11,14,17-20:3, and 7,11,14-20:3 [bishomopinolenic (16)] acids, from trace amounts to 0.3%. The 22:0 acid could not be detected, even as a trace.

14-MHD acid was recently formally identified in *G. biloba* (17) and all Pinaceae seeds analyzed so far (15); however, it seldom occurs in Angiosperm seeds, and we could not detect it even as a trace in either *T. baccata* or *T. grandis* seeds. For this purpose, the saturated acid fractions were isolated by ar-

TABLE 1
Oil Content and Fatty Acid Composition of the Seeds from Some Taxaceae Species of the *Taxus* and *Torreya* Genera

	ECL[a]	*T. baccata* (this study)[b]	*T. cuspidata* (this study)	*T. canadensis* (5)	*T. chinensis* (this study)	*T. grandis* (this study)	*T. nucifera* (5)
Oil content[c]		23.1	15.6	30.4	26.9	51.7	49.7
Fatty acid[d]							
14:0	14.00	trace[e]	trace	0.05	0.07	0.02	trace
16:0	16.00	3.03	3.18	2.35	3.23	5.49	6.03
16:1	16.26	0.06	trace	trace	0.09	0.05	trace
17:0	17.02	0.05	0.06	trace	0.05	0.05	trace
18:0	18.00	2.47	0.87	1.85	1.00	2.22	2.51
18:1 Δ9	18.22	54.78	39.21	46.77	34.31	40.39	30.35
18:1 Δ11	18.32	0.33	0.62	0.17	0.54	0.54	0.57
18:2 Δ9,12	18.70	23.08	29.35	27.93	34.22	32.05	51.26
18:3 Δ9,12,15	19.37	1.27	2.00	1.53	2.09	0.50	0.23
20:0	20.00	0.06	0.06	trace	0.06	0.07	—[f]
20:1 Δ11	20.20	1.33	1.49	1.53	1.46	2.12	0.28
20:2 Δ11,14	20.69	0.60	0.65	0.63	0.70	3.21	0.98
18:2 Δ5,9	18.44	9.50	16.16	13.65	16.08	trace	—
18:3 Δ5,9,12	18.91	0.33	2.66	1.48	3.31	0.04	0.08
18:4 Δ5,9,12,15	19.59	trace	0.25	—	0.28	—	—
20:2 (5,11	20.37	0.27	0.21	trace	trace	0.82	0.79
20:3 (5,11,14	20.83	1.64	2.16	1.95	2.13	11.20	6.68
20:4 (5,11,14,17	21.49	0.28	0.08	—	trace	0.19	—
Others		0.89	0.99	0.11	0.38	1.04	0.24
∑Δ5[g]		12.02	21.52	17.08	21.80	12.25	7.55

[a]Equivalent chainlength (ECL) on a 30-m DB Wax capillary column (J&W Scientific, Folsom, CA), calculated according to Reference 13, with 16:0, 18:0, and 20:0 acid methyl esters as standards.
[b]References in parentheses: data for Reference 5 apply to purified neutral lipids, whereas other data are for total lipids.
[c]Weight % of the seed; undehulled for *Taxus* species, dehulled for *Torreya grandis*, status not certain for *T. nucifera*.
[d]Data from this study are the means of analyses of two fatty acid methyl ester preparations.
[e]Trace amounts (peak visible on the chromatogram, but not taken into account by the integrator).
[f]Not detected or or not reported.
[g]Sum of Δ5-olefinic acids.

gentation thin-layer chromatography, concentrated *ca.* five times, and reanalyzed by GLC. No peaks could be detected between 16:0 and 17:0 acid methyl esters, in conditions where a component representing less than 0.005% should have been detected. Alternatively, the saturated acids were isolated by high-performance liquid chromatography in the silver-ion mode and analyzed as picolinyl esters by GLC-mass spectrometry in conditions described elsewhere (18). Here too, 14-MHD was not detectable. It should be noted that 14-MHD acid has beeen reported to be present in the needle lipids of *T. baccata* at a level of 0.2% of total fatty acids, which is considerably less than in the leaves of most Pinaceae species (19). The absence of 14-MHD acid in Taxaceae seeds is interesting, because it is generally considered that the two orders Pinidae and Taxidae, though probably both issued from the extinct Cordaitales, have evolved independently for over 225 million years. Thus, the presence or absence of 14-MHD acid in the seed lipids might be a good supplementary criterion to justify the separation of the two orders Taxidae and Pinidae.

The greatest difference between *Taxus* and *Torreya* was in the distribution profile of Δ5-olefinic acids. In *Taxus*, the main Δ5-olefinic acid was 5,9-18:2 acid, in the range 9.7 to 16.2%. This acid was absent from *Torreya*, as was 5,9,12-18:3 (pinolenic) acid, which accounted for 0.3–3.3% in *Taxus* but less than 0.1% in *Torreya*. The 5,11-20:2 acid was slightly higher in *Torreya* (*ca.* 0.8%) than in *Taxus* (≤0.2%), which cannot be explained by the relative abundance of its precursor, 11-20:1 acid. In *Torreya*, the major Δ5-olefinic acid was 5,11,14-20:3 (sciadonic) acid (6.7 to 11.2%), but this acid was a minor component in *Taxus* (less than 2.2%). Incidentally, and according to K. Aitzetmüller (personal communication), the level of 5,9-18:2 acid would be higher in *T. baccata* var. *fastigiata* than in our own specimen (variety not certain, but systematically obtained from seeds collected either from wild or ornamental trees purchased from different sources): 12.8 vs. 9.5%, the former value being closer to that reported by Madrigal and Smith (3) (origin uncertain, perhaps from United States). Globally, but not systematically, the sum of Δ5-olefinic acids was higher in *Taxus* than in *Torreya*, with a predominance of C_{18} Δ5-olefinic acids in the former species as compared to a predominance of C_{20} Δ5-olefinic acids in the latter species. However, an alternative interpretation can be made, which is based not on the chainlength but on the ethylenic bond arrangement. The 5,9 ethylenic bond structure is favored over the 5,11 dienoic structure in *Taxus*, in contrast to *Torreya*. From unpublished data (Wolff, R.L., F. Pédrono, A.M. Marpeau, W.W. Christie, and K. Aitzetmüller) we favor

the second point of view. In *Taxus*, there would exist an active Δ5-desaturase specific for Δ9-unsaturated acids with a low elongase activity, whereas the Δ9-specific Δ5-desaturase would be practically inactive in *Torreya*; this, in turn, would present relatively high activities of the elongase and of a Δ11-specific Δ5-desaturase. Generally, in Gymnosperm seeds, the Δ9-specific Δ5-desaturase only accepts unsaturated fatty acids with 18 carbon atoms as substrates, whereas the Δ11-specific Δ5-desaturase accepts unsaturated fatty acids with 20 carbon atoms as substrates, with the exception of Ephedraceae and *G. biloba* [their seed lipids contain both the 5,9- and the 5,11-18:2 acids (Wolff, R.L., F. Pédrono, A.M. Marpeau, W.W. Christie, and K. Aitzetmüller, unpublished data; Wolff, R.L., W.W. Christie, and A.M. Marpeau, submitted for publication)].

With regard to the taxonomy of Taxaceae, the fatty acid compositions of *T. chinensis* and *T. cuspidata* are hardly distinguishable, and this observation (despite the differences in oil contents) might indicate that the two species are indeed only two geographical varieties (China and Japan). On the other hand, *T. baccata* and *T. cuspidata* should be regarded as true species. The two *Torreya* species are quite distinct with regard to their fatty acid compositions (despite a rather similar oil content). With respect to the taxonomic and phylogenic relationships between *Taxus* and *Torreya* based on morphologic, anatomic, and physiologic criterions, it is clear that these two genera are completely distinct, if one admits that the seed fatty acid compositions may be used as valuable chemometric markers for the taxonomy of Coniferophytes. Originally (20), Taxaceae, Cephalotaxaceae, Podocarpaceae, and Ginkgoaceae were grouped into a tribe, the Taxares. We also analyzed the seed lipids from some species of Cephalotaxaceae and Podocarpaceae (Wolff, R.L., F. Pédrono, A.M. Marpeau, and F.D. Gunstone, unpublished results), and we have observed that their seed Δ5-olefinic acid composition closely resembled that of *Torreya* species, with a predominance of the 5,11 dienoic structure. This could indicate that *Taxus* species have evolved independently from *Torreya* species for a very long time. In this regard, yews appear to have a unique fatty acid composition among Gymnosperms, with taxoleic acid being the prominent Δ5-olefinic acid. In Pinaceae, 5,9,12-18:3 acid generally (but not systematically) predominates, juniperonic (5,11,14,17-20:4) acid is a major Δ5-olefinic acid in Cupressaceae and Taxodiaceae, and sciadonic (5,11,14-20:3) acid is the prominent Δ5-olefinic acid in Sciadopityaceae [with the single extant species *Sciadopitys verticillata*) (21)].

It is too early to propose whether these two genera should be ranked as distinct though related families, instead of simple genera. There is an evident need to analyze the other genera belonging to the Taxaceae family before making a decision.

With regard to the distribution of Δ5-olefinic acids in TAG, Takagi and Itabashi (5), using partial chemical cleavage with ethylmagnesium bromide, showed that they were low in the 2-position in *T. cuspidata and T. nucifera* seed TAG through the analysis of 2-monoacylglycerols. Additionally, Gunstone *et al*. (9) could not observe Δ5-olefinic acids in the 2-position in *T. baccata* seed oil by ^{13}C NMR spectroscopy. In the latter case, this was confirmed by partial chemical cleavage (7) and by the complete stereochemical analysis of the three positions of purified TAG (8), where it was shown that Δ5-olefinic acids were almost exclusively esterified to the *sn*-3 position (>90%).

The ^{13}C NMR results for *T. chinensis* and *T. grandis* seed oils are based on a study of signals for the acyl carbon atoms (C-1), of which there are three. Two of these (173.2 and 172.8 ppm) represent all the common saturated and unsaturated acids in the α (*sn*-1 and -3) and β (*sn*-2) positions. The additional signal at 173.0 ppm relates to all the Δ5-olefinic acids in the α chain; there is no evidence of the fourth signal which would be expected for Δ5-olefinic acids in the β position. However, it must be remembered that signals corresponding to less than 3% may not be detected. The results are given in Table 2.

These observations are in good agreement with data by Takagi and Itabashi (5) for *T. cuspidata* and *T. nucifera*. The practical exclusion of Δ5-olefinic acids from the internal position of TAG is thus a characteristic apparently common to all Taxaceae, and also to all other Coniferophytes analyzed so far (22). This clearly means that the peculiar stereospecific acylation of Δ5-olefinic acids to TAG in Coniferophyte seeds is a feature of great antiquity, that was probably present in the common ancestors of Taxales and Coniferales, the extinct Cordaitales. It should also be emphasized that the chainlength has no influence on this peculiar distribution, as it occurs in both *Taxus* (preponderance of C_{18} Δ5-olefinic acids) and *Torreya* (preponderance of C_{20} Δ5-olefinic acids).

This peculiar distribution contrasts with that found in those rare Angiosperms containing Δ5-olefinic acids. However, the number of examples is limited. In *Limnanthes alba*, almost 51% of TAG contain three Δ5-olefinic acids (23), whereas in *Dioscoreophyllum cumminsii* (a Menispermaceae) (24), it is likely that such TAG are preponderant because in these species, the 5-18:1 acid represents as much as 85% of total fatty acids (the 5-18:1 acid is seldom encountered in Gymnosperms). Thus, the mechanism of acylation of TAG by Δ5-olefinic acids in Gymnosperm seeds would be fundamentally different from that occurring in Angiosperm seeds, which might indicate that TAG biosynthesis in Angiosperm seeds is

TABLE 2
Distribution of Δ5-Olefinic and Other Acids (saturated, Δ9, Δ11, etc.) Between the α (*sn*-1 and -3) and β (*sn*-2) Positions in the Seed Triacylglycerols from Two Taxaceae Species

	Torreya grandis		*Taxus chinensis*	
	α (%)	β (%)	α (%)	β (%)
Δ5-Acyl chains	12.9[a]	—	21.0[a]	—
Other acyl chains	51.7	35.4	41.8[b]	34.7

[a]Δ5-Acids by gas–liquid chromatography are 12.3 and 21.8%, respectively (see Table 1).
[b]Also an unidentified signal (not Δ5-acids, of 2.5%).

not directly derived from that presented by Gymnosperm seeds.

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