The Seed Fatty Acid Composition and the Distribution of Δ5-Olefinic Acids in the Triacylglycerols of Some Taxares (*Cephalotaxus* and *Podocarpus*)

Robert L. Wolff[a,*], Frédérique Pédrono[a], Anne M. Marpeau[b], and Frank D. Gunstone[c]

[a]ISTAB, Laboratoire de Lipochimie Alimentaire, [b]Institut du Pin, Laboratoire des Substances Végétales, Université Bordeaux 1, Talence, France, and [c]Scottish Crop Research Institute, Dundee, Scotland

ABSTRACT: The fatty acid compositions of the seeds from four *Cephalotaxus* species or varieties (plum yews; Cephalotaxaceae) and two *Podocarpus* species (podocarps; Podocarpaceae) have been established. These compositions were compared with those previously published for some Taxaceae species (*Taxus* and *Torreya*). Cephalotaxaceae, Podocarpaceae, and Taxaceae belong to the Taxares suborder. Δ5-Olefinic acids are present in the seed lipids from all species analyzed. In *Cephalotaxus, Podocarpus,* and *Torreya*, the prominent Δ5-olefinic acid that occurs is the trienoic acid 5,11,14-20:3 (sciadonic) acid, comprising from 6.7 to 26.4% of total fatty acids. In these species, the Δ5,11 structure is largely favored over the Δ5,9 structure: the 5,9-18:2 (taxoleic) and 5,9,12-18:3 (pinolenic) acids are at the limit of detection, in contrast to *Taxus* and most Pinaceae species, where these two Δ5-olefinic acids generally predominate. 14-Methylhexadecanoic acid, an habitual though minor component of Pinaceae and *Ginkgo biloba* seed lipids, could not be detected in *Cephalotaxus* species studied here and was tentatively identified in trace amounts only in one *Podocarpus* species. In addition to sciadonic acid, *Cephalotaxus* and *Podocarpus* seeds are characterized by unusually high amounts of 11,14-20:2 acid, in the range of 3.1–12.0%. This contrasts with most of the 170 species of conifers analyzed so far (from the families Pinaceae, Cupressaceae, Taxodiaceae, Taxaceae, and Sciadopityaceae, which belong to the Pinares suborder), where this acid is generally ≤2%. A close resemblance between *Torreya grandis* and three of the *Cephalotaxus* species analyzed might be indicative of some phyletic relationship between the families Cephalotaxaceae and Taxaceae. ^{13}C nuclear magnetic resonance spectroscopy of the seed oils from *C. drupaceae* and *P. andinus* has shown that Δ5-olefinic acids are apparently excluded from the internal position of triacylglycerols, which is a characteristic common to all Coniferales species analyzed so far, and consequently of great antiquity.

Paper no. J8951 in *JAOCS 76*, 469–473 (April 1999).

KEY WORDS: *Cephalotaxus*, conifers, fatty acid composition, Δ5-olefinic acids, *Podocarpus*, seed lipids, sciadonic acid, Taxares, taxonomy, *Torreya*.

*To whom correspondence should be addressed at ISTAB, Laboratoire de Lipochimie Alimentaire, Université Bordeaux 1, Allée des Facultés, 33405 Talence Cedex, France. E-mail: r.wolff@istab.u-bordeaux.fr

The conifers comprise about 560–600 extant species (1,2) [but *ca*. 20,000 species in the Jurassic (2)], and it is estimated that one tree out of two is a conifer in the Northern Hemisphere (2). This emphasizes the present and past importance of conifers in the vegetable kingdom, particularly among higher plants. These trees are of great antiquity: it is assumed that they are derived from Progymnospermae that disappeared *ca*. 350 million yr ago.

The order Coniferales is sometimes divided into two suborders, Taxineae and Pinineae, or Taxares and Pinares, respectively (1). The latter have an obvious cone (Phanerostrobilares), whereas the former have not such an obvious structure (Aphanerostrobilares). Most studies of the seed oil fatty acid compositions have been devoted to species belonging to the Pinares (Pinaceae, Taxodiaceae, Cupressaceae, and Sciadopityaceae), and it is now well established that all species of this suborder are characterized by the systematic presence of several Δ5-unsaturated polymethylene-interrupted fatty acids (Δ5-UPIFA, or Δ5-olefinic acids) in their seed lipids. Seven possible structures have been established: 5,9-18:2 (taxoleic) acid, 5,11-18:2 acid, 5,9,12-18:2 (pinolenic) acid, 5,9,12,15-18:4 (coniferonic) acid, 5,11-20:2 acid, 5,11,14-20:3 (sciadonic) acid, and 5,11,14,17-20:4 (juniperonic) acid. These acids seldom occur all together in a given species, and their profile, together with that of more common fatty acids, has been used as a valuable chemotaxonomic tool for the differentiation of several conifer families, genera, and even sections (3–6).

The Taxares include the families Phyllocladaceae, Taxaceae, Podocarpaceae, and Cephalotaxaceae, but only a few species from this suborder have been studied with regard to their seed oil fatty acid composition [some *Taxus* and *Torreya* species (Taxaceae) (7–9), and two *Podocarpus* species (8)]. Here too, the systematic presence of Δ5-UPIFA in the seed lipids seems to be the rule. No data have been reported for Cephalotaxaceae seed lipids. This family is particularly interesting, because "obscurity is probably the only aspect of the generic and family affinities of these undoubtedly ancient plants about which we can be totally sure" (10). For some authors, the Cephalotaxaceae family comprises two genera,

Amentotaxus and *Cephalotaxus* (plum yews) (10). For others (2), it contains only one genus, *Cephalotaxus*, with *Amentotaxus* being grouped together with the remainder of taxads into the single family Taxaceae. This uncertain position of Cephalotaxaceae has led some authors to regard the distinction between Taxares and Pinares doubtful (10).

In the present study, we have established the fatty acid composition of the seeds from four species or varieties of *Cephalotaxus*. These trees are entirely Old World in origin, ranging from the eastern Himalayas and from Korea and Japan south through China to Taiwan, Thailand, and Malaysia. However, they are also cultivated as ornamental trees. Apparently, there is no consensus regarding the number of species in this genus. Whereas some authors consider that there are only two species with a few varieties (2), others admit the existence of about six species (10). We examine here *C. fortunei* (China), *C. drupaceae* (China, Japan, and Korea), and two of its varieties, *C. harringtonia* (*C. drupaceae pedunculata*, a horticultural variety) and *C. drupaceae sinensis* (a spontaneous wild Chinese variety).

The Podocarpaceae family contains 17 genera, of which *Podocarpus* (podocarps), a genus of nearly 100 species, is spread throughout the Southern Hemisphere, and northward to the West Indies, Mexico, southern China, and southern Japan. With regard to the kariology, *Podocarpus* species are particularly heterogeneous, with chromosome base numbers ranging from $n = 10$ to $n = 19$. Affinities with other families of conifers are still unclear but seem to be ancient, and it has been suggested that Podocarpaceae have been separated from other conifer families not only for an appreciable period of geologic time but also by their geographic position. Possible interfamily links suggested appear to be with the Taxaceae, perhaps *via* the Phyllocladaceae (10). We examine here *P. andinus* (southern and central Andes, Chile, and Argentina, but that may be cultivated in temperate climates) and *P. nagi* (distribution unknown by the authors, but at least found in Japan).

EXPERIMENTAL PROCEDURES

Seeds. *Cephalotaxus* and *Podocarpus* seeds were purchased from Sandeman Seeds (Pulborough, Great Britain) and Lawyer Nursery, Inc. (Plains, MT), and kept in a refrigerator until use. They were manually dehulled prior to oil extraction.

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 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-capped 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 water bath. FAME were extracted twice with 2 mL hexane, and water (2 mL) was 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 chain lengths (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 seeds from *Cephalotaxus* species or varieties are rich in oil, in the range of 45 to 65% on a weight basis (Table 1). The most abundant fatty acid is 9-18:1 acid in *C. drupaceae*, *C. fortunei*, and *C. sinensis* (41%), followed by 9,12-18:2 acid (28%), but the latter acid predominates in *C. harringtonia* (41%) (in this species, 9-18:1 acid accounts for 25%) (Table 1). Globally, the fatty acid compositions of the seeds from *C. drupaceae*, *C. fortunei*, and *C. sinensis* are much alike, whereas that of *C. harringtonia* is significantly different. The differences in the contents of 9-18:1 and 9,12-18:2 acids are reflected in the contents of products metabolically derived:

TABLE 1
Oil Content and Fatty Acid Composition of the Seeds from Some Taxares Species of the *Cephalotaxus* and *Podocarpus* Genera

	ECL[a]	*C. drupaceae*	*C. fortunei*	*C. harringtonia*	*C. sinensis*	*P. andinus*	*P. nagi*
Oil content[b]		65.5	63.5	46.2	54.7	67.3	30.8
Fatty acid[c]							
16:0	16.00	5.65	5.99	8.48	6.10	5.09	5.26
16:1	16.26	0.06	0.07	0.06	0.07	Trace[d]	0.06
br-17:0[e]	16.72	—[f]	—	—	—	—	0.05
17:0	17.01	0.05	0.05	0.07	0.05	0.07	0.08
17:1	17.28	0.03	0.03	0.03	0.03	Trace	0.04
18:0	18.00	2.59	2.53	1.98	2.61	3.91	1.16
18:1 Δ9	18.22	44.03	44.34	25.18	44.02	32.55	12.64
18:1 Δ11	18.32	0.54	0.54	0.62	0.53	0.20	0.57
18:2 Δ9,12	18.70	28.26	29.30	41.37	28.85	32.04	40.26
18:3 Δ9,12,15	19.37	0.35	0.36	0.51	0.38	0.18	0.22
20:0	20.00	0.06	0.06	0.05	0.08	0.29	0.10
20:1 Δ11	20.20	2.46	2.31	1.14	2.52	2.15	0.91
20:2 Δ11,14	20.69	3.55	3.08	4.65	3.21	5.99	12.02
22:0	22.00	0.14	0.13	0.21	0.06	—	—
18:2 Δ5,9	18.44	0.04	0.02	Trace	0.01	—	Trace
18:3 Δ5,9,12	18.91	0.03	0.03	0.10	0.04	0.03	0.03
20:2 Δ5,11	20.37	0.83	0.83	0.34	0.83	0.63	0.12
20:3 Δ5,11,14	20.83	9.93	10.17	13.75	9.68	16.65	26.39
20:4 Δ5,11,14,17	21.49	0.13	0.14	0.20	0.16	0.14	—
Others		1.27	0.02	1.26	0.73	0.08	0.09
∑Δ5[g]		10.96	11.19	14.39	10.72	17.45	26.54

[a]Equivalent chain length on a 30-m DB-Wax capillary column (J&W Scientific, Folsom, CA), calculated according to (13), with 16:0, 18:0, and 20:0 acid methyl esters as standards.
[b]Weight (%) of the dehulled seeds (typically 10 g).
[c]Data are the means of analyses of two fatty acid methyl ester preparations.
[d]Trace amounts (peak visible on the chromatogram, but not taken into account by the integrator).
[e]Branched 17:0 (14-methylhexadecanoic) acid.
[f]Not detected or not reported.
[g]Sum of Δ5-olefinic acids.

11-20:1, 11,14-20:2, 5,11-20:2, and 5,11,14-20:3 acids. Fatty acids derived from 9,12-18:2 acid are higher in *C. harringtonia* and lower in the three other species or varieties, whereas the contrary is observed for products derived from 9-18:1 acid.

Surprisingly, the average composition of the seeds from *C. drupaceae*, *C. fortunei*, and *C. sinensis* closely resembles that of *Torreya grandis* (9), a Taxaceae, even in the details (Fig. 1). In particular, they are characterized by low levels of acids with 18 carbon atoms and a Δ5,9 dienoic structure (i.e., 5,9-18:2 and 5,9,12-18:3 acids) that are otherwise abundant in *Taxus* species and most Pinaceae species. This resemblance may be a coincidence. However, it may also be indicative of some phylogenic link between Cephalotaxaceae and Taxaceae *via T. grandis* (or one of its ancestors). Note that the chromosome base numbers are different: $n = 12$ for *Cephalotaxus* and $n = 11$ for *Torreya* (but $n = 12$ for *Taxus*) (10).

A peculiar feature of the seed fatty acid composition of *Cephalotaxus* species, as well as of *T. grandis*, is the unusual abundance of 11,14-20:2 acid, the elongation product of linoleic acid. It is higher than 3% of total fatty acids. From a compilation of our database on conifer seed lipids (more than 170 species analyzed), it appears that the 11,14-20:2 acid reaches at most 2% in all other conifers, and is most often less than 1%, despite a level of 9,12-18:2 acid that may be higher than in the species studied here.

It has been suggested that 14-methylhexadecanoic (14-MHD) acid, which was recently formally identified in *Ginkgo biloba* (15) and all Pinaceae seeds analyzed so far (16), but

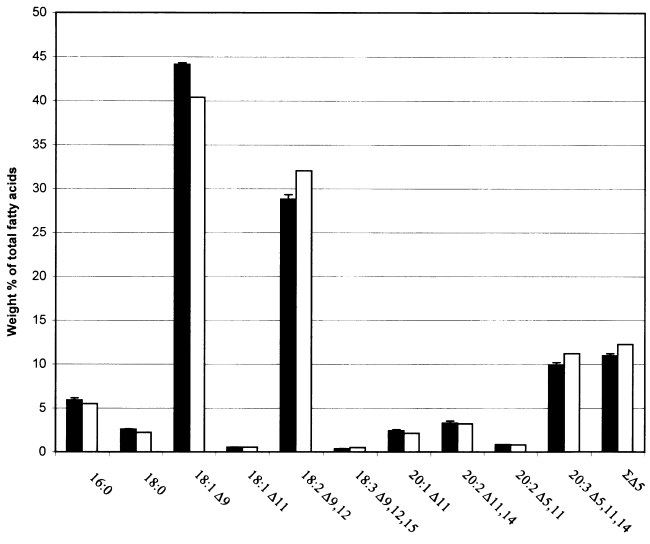

FIG. 1. Comparison of the mean fatty acid composition of the seeds from *Cephalotaxus drupaceae, C. fortunei*, and *C. sinensis* (Cephalotaxaceae) (black bars) with that of *Torreya grandis* (Taxaceae; Ref. 9) (white bars). Fatty acids accounting for less than 0.3% are not presented.

that seldom occurs in Angiosperm seeds, could be used as a chemometric marker for the taxonomy of conifers. On routine chromatograms, we occasionally observed a bump at the place where 14-MHD was expected to elute, but in too small an amount to be taken into account by the integrator. To verify the presence of 14-MHD, the saturated acid fraction was isolated by argentation thin-layer chromatography (Ag-TLC) from FAME prepared from *C. drupaceae* seeds, concentrated *ca*. 5 times, and reanalyzed by GLC. No peaks could be detected between 16:0 and 17:0 acid methyl esters, under conditions where a component representing less than 0.005% should have been detected. The absence of 14-MHD acid from Cephalotaxaceae seeds, as well as from Taxaceae seeds (9), would indicate that the presence or absence of 14-MHD acid in the seed lipids might be a supplementary criterion to justify the separation of the two suborders Taxares (thus including Cephalotaxaceae) and Pinares.

With regard to *C. harringtonia*, sometimes considered a horticultural variety of *C. drupaceae*, its fatty acid composition is so different from that of the parent that one might perhaps consider it a true species, and not only a variety. On the other hand, *C. drupaceae* and *C. fortunei*, considered to be good species, have kept in common the same fatty acid composition.

When considering *Podocarpus* species, it is obvious from Table 1 that the two species analyzed here differ one from another both by their seed oil content and their fatty acid composition. This is not surprising, owing to the heterogeneity of this family. However, some characteristics are common not only to the two species but also to the *Cephalotaxus* species. The 5,9-18:2 and 5,9,12-18:3 acids are absent, and Δ5-UPIFA with 20 carbon atoms and a Δ5,11-dienoic structure predominate, especially the 5,11,14-20:3 acid, which reaches 26.4% in *P. nagi* seeds. *Podocarpus* species are also characterized by exceptionally high levels of 11,14-20:2 acid, in the range of 6 to 12%. The particular abundance of 11,14-20:2 and 5,11,14-20:3 acids in *P. nagi* seed oil was first recognized by Ito *et al.* (17) and Takagi (18), who established their structures by chemical means. Later, Takagi and Itabashi (8) confirmed this characteristic by capillary GLC, and our results are in good agreement with data from the latter authors.

14-MHD acid was absent from *P. andinus*, even after isolation of the saturated fatty acids by Ag-TLC and re-analysis of the concentrated fraction, but it represented *ca*. 0.05% in *P. nagi*. Here too, the genus *Podocarpus* appears heterogeneous, and the two species analyzed might not be representative of the whole genus. It is too early to try to generalize our data for podocarps; it is necessary to accumulate more data from other species. According to Sahni (19), who compared the structural morphology of the seeds, "the genera *Taxus*, *Torreya*, and *Cephalotaxus* are structurally so distinct from the podocarps and other conifers, that they deserve the rank of a separate phylum, Taxales," in the sense of a group equal in rank with the Coniferales. From this study and previous analyses of *Torreya* and *Taxus* seed lipids (9), some analogies are apparent between the three genera, particularly the absence of 14-MHD acid. With regard to Δ5-UPIFA, *Taxus* is completely different from *Torreya* [preponderance of the Δ5,9 dienoic structure in the former and of the Δ5,11 dienoic arrangement in the latter (9)], which in turn is quite close (at least in *T. grandis*) to several *Cephalotaxus* species.

^{13}C NMR spectroscopy of the oils from *P. andinus* and *C. drupaceae*, based on the study of signals for the acyl carbon atoms (C-1), showed a signal at 173.0 ppm that relates to all the Δ5-olefinic acids in the α chain, but there was no evidence of the signal that would be expected for Δ5-olefinic acids in the β position. However, signals corresponding to less than 3% may not be detected. Consequently, the Δ5-olefinic acids are essentially esterified to the external positions of triacylglycerols (TAG). The practical exclusion of Δ5-olefinic acids from the internal position of TAG is thus a characteristic apparently common to most Taxares [Taxaceae (8,9), Cephalotaxaceae (this study), and Podocarpaceae (8, this study)] and also to all other Pinares analyzed so far (8,14, 20–22). This clearly means that the peculiar stereospecific esterification of Δ5-olefinic acids to TAG in Coniferophyte seeds is a feature of great antiquity (late Carboniferous, or Permian, *ca*. 280–300 million yr).

REFERENCES

1. Keng, H., A New Scheme of Classification of the Conifers, *Taxon 24*:289–292 (1975).
2. Debazac, E.F., *Manuel des Conifères*, Ecole Nationale des Eaux et Forêts, Nancy, 1964, pp. 147–162.
3. Wolff, R.L., L.G. Deluc, A.M. Marpeau, and B. Comps, Chemotaxonomic Differentiation of Conifer Families and Genera Based on the Seed Oil Fatty Acid Compositions. Multivariate Analyses, *Trees 12*:57–65 (1997).
4. Wolff, R.L., B. Comps, A.M. Marpeau, and L.G. Deluc, Taxonomy of *Pinus* Species Based on the Seed Oil Fatty Acid Compositions, *Ibid. 12*:113–118 (1997).
5. Wolff, R.L., B. Comps, L.G. Deluc, and A.M. Marpeau, Fatty Acids of the Seeds from Pine Species of the *Ponderosa-Banksiana* and *Halepensis* Sections. The Peculiar Taxonomic Position of *Pinus pinaster*, *J. Am. Oil Chem. Soc. 75*:45–50 (1998).
6. Wolff, R.L., Clarification on the Taxonomic Position of *Sciadopitys verticillata* Among Coniferophytes Based on the Seed Oil Fatty Acid Compositions, *Ibid. 75*:757–758 (1998).
7. Madrigal, R.V., and C.R. Smith Jr., *Taxus baccata* Seed Oil: A New Source of *cis*-5,*cis*-9-Octadecadienoic Acid, *Lipids 10*:502–504 (1975).
8. Takagi, T., and Y. Itabashi, *cis*-5 Olefinic Unusual Fatty Acids in Seed Lipids of Gymnospermae and Their Distribution in Triacylglycerols, *Lipids 17*:716–723 (1982).
9. Wolff, R.L., F. Pédrono, A.M. Marpeau, F.D. Gunstone, and W.W. Christie, The Seed Fatty Acid Composition and the Distribution of Δ5-Olefinic Acids in the Triacylglycerols of Some Taxaceae (*Taxus* and *Torreya), J. Am. Oil Chem. Soc. 75*:1637–1641 (1998).
10. Page, C.N., in *The Families and Genera of Vascular Plants*, edited by K. Kubitski, *Pteridophytes and Gymnosperms*, Vol. 1, edited by K.U. Kramer and P.S. Green, Springer-Verlag Berlin Heidelberg New York, 1990, pp. 282–361.
11. Folch, J., M. Lees, and G.M. Sloane-Stanley, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).

12. Morrison, W.R., and L.M. Smith, Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Trifluoride, *J. Lipid Res. 5*:600–608 (1964).
13. Berdeaux, O., and R.L. Wolff, Gas-Liquid Chromatography–Mass Spectrometry of the 4,4-Dimethyloxazoline Derivatives of Δ5-Unsaturated Polymethylene-Interrupted Fatty Acids from Conifer Seed Oils, *J. Am. Oil Chem. Soc. 73*:1323–1326 (1996).
14. Gunstone, F.D., and R.L. Wolff, Conifer Seed Oils: Distribution of Δ5-Olefinic Acids Between α and β Chains by ^{13}C Nuclear Magnetic Resonance Spectroscopy, *Ibid. 73*:1611–1613 (1996).
15. Hierro, M.T.G., G. Robertson, W.W. Christie, and Y.G. Joh, The Fatty Acid Composition of the Seeds of *Ginkgo biloba*, *Ibid. 73*:575–579 (1996).
16. Wolff, R.L., W.W. Christie, and D. Coakley, The Unusual Occurrence of 14-Methylhexadecanoic Acid in Pinaceae Seed Oils Among Plants, *Lipids 32*:971–973 (1997).
17. Ito, S., Y. Koyama, and Y. Toyama, The Separation and Structure Determination of an Eicosatrienoic and an Eicosadienoic Acid in Nagi Seed Oil, *Bull. Chem. Soc. Japan 34*:1439–1444 (1963).
18. Takagi, T., 5,11,14-Eicosatrienoic Acid in *Podocarpus nagi* Seed Oil, *J. Am. Oil Chem. Soc. 41*:516–519 (1964).
19. Sahni, B., On Certain Archaic Features in the Seeds of *Taxus baccata*, with Remarks on the Antiquity of the Taxinae, *An. Bot. 34*:117–133 (1920).
20. Blaise, P., V. Tropini, M. Farines, and R.L. Wolff, Positional Distribution of Δ5-Acids in Triacylglycerols from Conifer Seeds As Determined by Partial Chemical Cleavage, *J. Am. Oil Chem. Soc. 74*:165–168 (1997).
21. Wolff, R.L., E. Dareville, and J.C. Martin, Positional Distribution of Δ5-Olefinic Acids in Triacylglycerols from Conifer Seed Oils: General and Specific Enrichment in the *sn*-3 Position, *Ibid. 74*:515–523 (1997).
22. Gunstone, F.D., S. Seth, and R.L. Wolff, The Distribution of Δ5 Polyene Acids in Some Pine Seed Oils Between the α- and β-Chains by ^{13}C-NMR Spectroscopy, *Chem. Phys. Lipids 78*:89–96 (1995).

[Received July 20, 1998; accepted November 8, 1998]