The Unusual Occurrence of 14-Methylhexadecanoic Acid in Pinaceae Seed Oils Among Plants

Robert L. Wolff[a],*, William W. Christie[b], and David Coakley[b]

[a]ISTAB, Université Bordeaux 1, Talence, France, and [b]Scottish Crop Research Institute, Invergowrie, Dundee, Scotland

ABSTRACT: 14-Methylhexadecanoic (14-MHD) acid has been identified in a sample of pine seed oil (*Pinus contorta*) by gas–liquid chromatography–mass spectrometry of its picolinyl ester derivative. Its identification (through its equivalent chain length) and its distribution in four conifer families have been checked. It occurred only in Pinaceae, where it was found in 72 species belonging to the genera *Pinus*, *Abies*, *Cedrus*, *Tsuga*, *Pseudotsuga*, *Larix*, and *Picea*, in the range 0.02–1.15%. 14-MHD acid could not be detected in the lipids of Taxaceae (*Taxus baccata*), Cupressaceae (*Juniperus communis*), or Taxodiaceae (*Sciadopytis verticillata*), even after a 10-fold concentration of the saturated acid fraction isolated by argentation thin-layer chromatography. It is concluded that Pinaceae, along with *Ginkgo biloba* seed lipids, are major exceptions in the plant kingdom with regard to 14-MHD acid, which otherwise occurs almost exclusively in lipids of animals and microorganisms. The biosynthesis and metabolic role of 14-MHD acid, which otherwise also occur in wood and leaf lipids, remain unknown.
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It is generally assumed that methyl-branched saturated fatty acids are characteristic components of animal and microbial lipids, and that they generally do not occur in plant lipids. However, 14-methylhexadecanoic (14-MHD, or *anteiso*-17:0) acid was recently formally identified in *Ginkgo biloba* (maidenhair tree, a Gymnosperm) seed lipids by mass spectrometry (MS) of its picolinyl ester derivative (1). In an earlier study of this material and of other conifer seed lipids, Takagi and Itabashi (2) tentatively identified a 16:2n-6 acid that eluted during gas–liquid chromatography (GLC) on a Silar 5CP capillary column (commercial source not given in Ref. 2) between the 16:1 isomers and 17:0 acid, and which was present in higher amounts in *G. biloba* than in other Gymnosperm species. Because this is the place where 14-MHD acid is expected to elute, one may retrospectively surmise that the 16:2n-6 acid might have been confused with 14-MHD acid. However, this is not certain, because Jamieson and Reid (3) observed and tentatively identified both the 16:2n-6 and 14-MHD acids in the needle lipids of a large collection of conifer species. In addition to these acids, these authors also reported on the presence of *trans*-3-16:1 and 16:3n-3 acids that elute in the same region. 14-MHD acid was also formally identified by its mass spectrum in the sapwood of several North American pines (4).

In two systematic studies of conifer seed lipids, Wolff and Bayard (5) and Wolff *et al.* (6) did not mention any component eluting between the two close-eluting 16:1 isomers and 18:0 acid on a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands). Only recently was it recognized (7,8), through the use of a DB-Wax capillary column (J&W Scientific, Folsom, CA), that there existed an acid that was distinct from the 16:1 isomers and that eluted between them and 17:0 acid. This unknown component coeluted with the second-eluting 16:1 isomer on the CP-Sil 88 capillary column, and it was erroneously reported as a 16:1 acid in earlier publications (5,6). Later, it was observed that the unknown acid migrated along with the saturated acid fraction after silver-ion thin-layer chromatography (Ag-TLC) (7). In addition, it coeluted on both the CP-Sil 88 and the DB-Wax capillary columns with an authentic commercial standard of 14-MHD acid methyl ester (7), and it was then tentatively identified as 14-MHD acid. Although it is absent from Angiosperm lipids, we demonstrate here, by GLC–MS of its picolinyl ester derivative, that the unknown component is actually 14-MHD acid, and that it occurs in the seed oil of numerous conifer species, but exclusively of the Pinaceae family.

EXPERIMENTAL PROCEDURES

Samples. Fatty acid methyl esters (FAME), prepared in duplicate from the seed oil of several conifer species, were available from previous studies by one of us (5–8). The origin of the seeds, the extraction of oils, and the preparation of FAME have been described in detail elsewhere (5–8).

Analytical GLC. FAME were analyzed in a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy) equipped with a DB Wax column (30 m × 0.32 mm i.d., 0.5 μm film; J&W Scientific). The oven temperature was 190°C, and the inlet pressure of the carrier gas (helium) was 140 kPa. The injector (split mode) and the flame-ionization detector were maintained at 250°C. Quantitative data were calculated by an

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

Abbreviations: Ag-TLC, argentation thin-layer chromatography; FAME, fatty acid methyl ester; GLC, gas–liquid chromatography; 14-MHD acid, 14-methylhexadecanoic acid; MS, mass spectrometry.

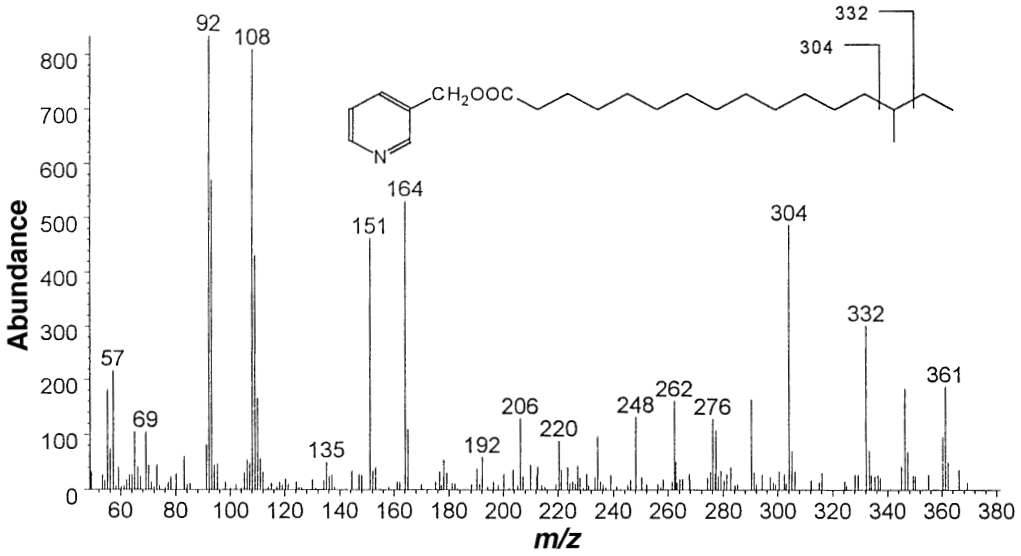

FIG. 1. Mass spectrum of picolinyl 14-methylhexadecanoate.

SP 4290 integrator (Spectra Physics, San Jose, CA). Identification of fatty acids was made as described previously (5,6).

GLC–MS. Methyl esters were hydrolyzed to the free fatty acids (9) before conversion to the picolinyl ester derivatives as described by Balazy and Nies (10). The derivatives were submitted to GLC–MS, with a Hewlett-Packard 5890 Series II plus gas chromatograph (Palo Alto, CA) attached to an HP model 5989 MS apparatus. The latter was used in the electron impact mode at 70 eV with a source temperature of 250˚C. The chromatograph was fitted with on-column injection, and equipped with a capillary column of fused silica coated with BPX-70 (50 m × 0.22 mm i.d., 0.25 µm film; SGE (UK) Ltd., Milton Keynes, United Kingdom). After holding the temperature at 80˚C for 3 min, the column was temperature-programmed at 20˚C/min to 160˚C, then at 2˚C/min to 260˚C, where it was held for 5 min. Helium was the carrier gas.

RESULTS AND DISCUSSION

Identification of 14-MHD. The methyl and picolinyl ester derivatives of the fatty acids were examined by GLC–MS, using a polar BPX-70 column for the separation. The spectrum of the picolinyl ester derivative is shown in Figure 1. The molecular ion is at *m/z* = 361, confirming the molecular weight, but the key diagnostic feature is a gap of 28 amu between *m/z* = 304 and 332. This represents cleavage on either side of the methyl branch point and is typical of cleavage expected of an *anteiso* derivative (11). The mass spectrum of the methyl ester was consistent with this interpretation (9).

Distribution of 14-MHD. As shown in Table 1, 14-MHD acid (identified on chromatograms by its equivalent chain length, 16.68) was present in all Pinaceae species analyzed. It was in the range 0.02–0.23% in *Pinus* species, 0.52–0.57 in *Tsuga* species, 1.15% in *Pseudotsuga menzieseii*, 0.17–0.26% in *Picea* species, 0.42–0.98% in *Cedrus* species, 0.57–0.87% in *Abies* species, and 0.36–0.42% in *Larix* species. Thus it seems that different species inside a given genus show some similarities with regard to their 14-MHD acid level. On the other hand, for species from other conifer families (Taxaceae, Cupressaceae, and Taxodiaceae; results not shown), 14-MHD acid was either absent or at the limit of detection. This was confirmed by GLC analysis after a 10-fold concentration of the saturated acid fractions prepared by Ag-TLC from the seeds of *Taxus baccata*, *Juniperus communis* and *Sciadopytis verticillata*, each of these species being representative of one of the preceding families (results not shown). Not even a trace of 14-MHD acid could be detected in these species, although 17:0 acid was clearly present (results not shown). It should be emphasized that 14-MHD acid was also very low in the needle lipids from these families (0.1–0.4%) as compared to Pinaceae (0.2–4.8%) (3). The reason why 14-MHD acid is present in the leaves and not in the seeds is unexplained.

This study has clearly demonstrated that 14-MHD acid is not limited to animals or microorganisms. It is a common constituent of the needle (3), wood (4), and seed (this study) lipids of Pinaceae. Though the amount of 14-MHD acid was low (most often less than 1%), it was present in the seed lipids from all of the 72 species studied here. However, no other branched-chain component was detected, to the contrary of some conifer leaf lipids where 12-methyltetradecanoic acid has been tentatively identified in trace amounts (3).

The peculiar distribution of 14-MHD acid in conifer seed lipids indicates that the presence of this constituent cannot be attributable to chance. This distribution precludes any possibility of contamination by microorganisms of some seed lots. However, to exclude this possibility definitively, we have purified the triacylglycerols from the crude seed oil of one species (*P. contorta*), and we have prepared FAME therefrom, because it is known that branched acids in microorganisms are esterified to polar lipids. We found that 14-MHD acid was present in triglyceride FAME at the same concentration as that in the original crude oil (result not shown).

TABLE 1
14-Methylhexadecanoic Acid (14-MHD) Content of Pinaceae Seed Lipids (wt% of total fatty acids, mean)

Species	14-MHD	Species	14-MHD	Species	14-MHD
Pinus banksiana	0.17	*P. laricio*	0.15	*P. abies*	0.20
P. contorta	0.23	*P. koekelare*	0.11	*P. sitchensis*	0.17
P. palustris	0.16	*P. nigra*	0.13	*P. glauca*	0.24
P. elliotii	0.17	*P. massoniana*	0.12	*Cedrus atlantica*	0.98
P. caribaea	0.18	*P. mughus*	0.07	*C. atlantica glauca*	0.76
P. echinata	0.16	*P. cembra*	0.09	*C. libani*	0.51
P. occidentalis	0.12	*P. peuce*	0.08	*C. deodara*	0.42
P. attenuata	0.11	*P. monticola*	0.12	*C. brevifolia*	0.70
P. muricata	0.16	*P. parviflora*	0.07	*Abies lasiocarpa*	0.70
P. radiata	0.11	*P. strobus*	0.06	*A. pinsapo*	0.65
P. taeda	0.15	*P. sibirica*	0.07	*A. balsamea*	0.57
P. pinaster	0.20	*P. koraiensis*	0.06	*A. cephalonica*	0.76
P. ponderosa	0.16	*P. griffithii*	0.15	*A. fraserii*	0.67
P. michoacana	0.15	*P. canariensis*	0.05	*A. equi-trojanii*	0.83
P. jeffreyi	0.16	*P. pinea*	0.08	*A. nordmanniana*	0.62
P. halepensis	0.11	*P. aristata*	0.09	*A. bornmulleriana*	0.71
P. brutia	0.09	*P. edulis*	0.02	*A. alba*	0.81
P. eldarica	0.07	*Tsuga canadensis*	0.52	*A. concolor*	0.73
P. sylvestris	0.11	*T. heterophylla*	0.57	*A. grandis*	0.83
P. resinosa	0.13	*Pseudotsuga menziesii*	1.15	*A. nobilis*	0.87
P. uncinata	0.16	*Picea engelmanii*	0.26	*A. lowiana*	0.75
P. thunbergii	0.12	*P. pungens glauca*	0.21	*Larix leptolepis*	0.38
P. salzmannii	0.13	*P. omorika*	0.17	*L. decidua*	0.42
P. pumilia	0.15	*P. orientalis*	0.22	*L. sibirica*	0.36

Ginkgo biloba is considered to be the most ancient living Gymnosperm fossil on the earth. Based on fossil records, it has existed for *ca.* 200 million years. If one hypothesizes that 14-MHD acid might be an evolutionary marker, then Pinaceae would be phylogenetically closer to *G. biloba* than any other conifer family, which have apparently lost the ability to synthesize 14-MHD acid. However, we are not aware of other dating techniques that could confirm this hypothesis. According to Hierro *et al.* (1), 14-MHD acid accounts for 0.86% of total fatty acids in *G. biloba* seed lipids. Wolff (unpublished data) found independently an even higher value, 1.40%. Takagi and Itabashi (2) found that the acid tentatively identified as 16:2n-6 acid (most probably 14-MHD acid) comprised 0.68% of the neutral lipids, and 1.54% of the polar lipids. Some *Cedrus* and *Abies* species, and *P. menziesii*, have 14-MHD acid contents in this range (Table 1).

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