Conifer Seed Oils: Distribution of Δ5 Acids Between α and β Chains by ^{13}C Nuclear Magnetic Resonance Spectroscopy

Frank D. Gunstone[a],* **and Robert L. Wolff**[b]

[a]School of Chemistry, The University, St. Andrews, Fife KY16 9ST, United Kingdom,
and [b]ISTAB, Université Bordeaux 1, Talence, France

ABSTRACT: Nine seed oils from three different conifer families, already examined by gas chromatography and known to contain diene, triene, and tetraene C_{18} and C_{20} Δ5 acids, have been reexamined by high-resolution ^{13}C nuclear magnetic resonance spectroscopy. The Δ5 acids are apparent only in the α-chains. This location is independent of chainlength, double- bond number, and the species considered and is probably a general factor of conifer seed oils. The spectra confirm the presence of oleic, linoleic, α-linolenic, and of Δ5 acids and give quantitative information about (total) n-6, n-3, and Δ5 acids that is in accord with that obtained by gas chromatography.

JAOCS 73, 1611–1613 (1996).

KEY WORDS: Conifer seed oil, Δ5 acids, distribution between α- and β-chains, ^{13}C nuclear magnetic resonance spectroscopy.

The chemical shifts of the acyl carbon atoms (C1) and, to a lesser extent, the C2 and glycerol carbon atoms in high-resolution ^{13}C nuclear magnetic resonance (NMR) spectra depend on whether the chain is α (attached to CH_2OH) or β (attached on CHOH) and also on the position of the double bond closest to the carboxyl group. The difference between such shifts for saturated acids and the common Δ9 acids (oleic, linoleic, linolenic) is small and can be exploited only when the high-resolution spectrum is collected over a long time (1,2). The difference increases as a double bond gets closer to the carboxyl group. Though less common, there are some important acids with unsaturation at Δ4 (docosahexaenoic), Δ5 (eicosapentaenoic, arachidonic), and Δ6 (stearidonic, γ-linolenic, petroselinic), and this has been exploited in several NMR studies. In particular, the distribution of such acids between the α and β chains in triacylglycerols and in phospholipids (3–9) has been determined.

Several nonmethylene-interrupted polyene acids have Δ5 unsaturation (10–13), and in an earlier paper (14) we reported on seven *Pinus* species. In a continuation of this study (11), one of us has reported on the C_{18} and C_{20} diene, triene, and tetraene acids with Δ5 unsaturation in 25 conifer species from four families (Pinaceae, Cupressaceae, Taxodiaceae, and Taxaceae). These acids have interesting biochemical and physiological properties, which are reviewed in Reference 12, and it is concluded in that paper that Δ5 olefinic acids should not be considered as uncommon acids but as normal and characteristic components of conifer seed oils.

EXPERIMENTAL PROCEDURES

The conifer seed oils were available from an earlier investigation by one of us (RLW). Details of their source, extraction, and fatty acid composition are given elsewhere (11). The NMR spectra were obtained as previously described by Gunstone and Seth (3).

^{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 (TMS) as reference and Cr(acac) 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 μ sec. The number of scans was usually 700–800 per spectrum.

RESULTS AND DISCUSSION

The ^{13}C NMR spectra of triacylglycerols usually show two signals for the three glycerol carbon atoms at about 68.9 and 62.1 ppm, corresponding to the one β and two α chains, respectively. Some, but not all, of the pine oils give three signals in this region. The additional signal at 62.14–62.16 ppm (0.05–0.06 ppm higher than that for the normal α-glycerol signal) corresponds to an α-carbon atom. From intensity values, it is possible, when there are three glycerol signals, to calculate the level of total Δ5 acids (Table 1). There is no evidence for a further signal around 68.9 ppm, which corresponds to a Δ5 chain in the β-position.

Normally, there are two acyl (C1) signals at around 173.2 (α) and 172.8 (β) ppm, but all pine oils examined showed a

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*To whom correspondence should be addressed at Scottish Crop Research Institute, Dundee DD2 5DA, United Kingdom.

TABLE 1
Total Content of Δ5 Acids by ^{13}C NMR and by Gas Chromatography (Ref. 11)[a]

Seed oil	NMR			
	Gl	C1	C2	GC
Larix decidua	31.1	30.4	31.4	30.4
L. leptolepis	30.3	30.2	29.5	28.7
Picea abies	30.3	28.0	30.6	28.9
P. sitchensis	29.7	30.1	29.5	28.6
Cedrus atlantica	—	16.6	16.4	17.0
Abies concolor	—	15.3	16.5	15.7
Sciadopitys verticillata	—	19.4	18.6	17.9
Thuja occidentalis	—	21.0	20.6	19.9
Juniperis virginiana	—	20.2	18.6	19.3

[a] NMR, nuclear magnetic resonance; GC, gas chromatography.

third signal at 173.01–173.07 (0.15–0.17 ppm below the usual α signal) due to the Δ5 acids. There is no evidence of a fourth signal for Δ5 acids in the region for β-chains. The intensity of the signal can be used to calculate the total Δ5 acids (Table 1).

The C1 (acyl) signals are generally used for NMR studies of this kind (1,3–9), though we have also used the C2 signal—especially for Δ5 acids (3,14). The C2 carbon atom is in a position γ to the double bond and (apart from the α or allylic carbon atom) is the signal most influenced by the double bond. In addition to the two expected signals, observed at 34.17 and 34.02 ppm, there is an α signal at 33.39–33.42 ppm, 0.61 units lower than the α-peak at 34.02 ppm. There was no evidence of a β-peak around 33.6 ppm. The content of Δ5 acids, based on intensity of the NMR signals, is given in Table 1. It is apparent from the results in this table that there is a general agreement on the levels of total Δ5 acids measured from the ^{13}C NMR spectra—both C1 and C2 signals—and the results obtained by gas chromatography (11). The results show clearly that the Δ5 acids are present only as α-chains, though it would be difficult to detect their presence in β chains at levels below about 3%.

The ω1–ω3 carbon atoms have signals with chemical shifts close to those given in Table 2. They can be used to distinguish between the three categories: saturated plus n-9, n-6, and n-3 acids. In these pine oils, each class may contain several members, and it is not possible to distinguish between them. For example, the n-6 acids include Δ9,12-18:2, Δ5,9,12-18:3, Δ11,14-20:2, and Δ5,11,14-20:3. The best quantitative data come from the ω2 signals for the following reasons: in the ω1 signals, the n-6 shift at 14.09 ppm is close to the shift for n-9/saturated acids at 14.13 ppm, and these two signals sometimes merge into one signal; in the ω3 cluster, there are only signals corresponding to the saturated/n-9 acids and n-6 acids because this carbon atom is olefinic for n-3 acids and appears in another region of the spectrum. This leaves the ω2 signals at about 22.7 (saturated and n-9), 22.6 (n-6), and 20.6 ppm (n-3): these are well-resolved and give quantitative results that agree with those measured by gas chromatography (Table 3).

TABLE 2
Chemical Shifts (ppm) for the ω1-3 Atoms in Saturated/n-9, n-6, and n-3 Acids

Atoms	Sat/n-9	n-6	n-3
ω1	14.13	14.09	14.29
ω2	22.71	22.60	20.57
ω3	31.95	31.55	olefinic

It is also possible to detect n-3 acids through the ^{1}H NMR spectra. The ω-CH_3 group, which normally has a signal at about 0.9 ppm, shows a second signal at about 1.0 ppm for n-3 acids. This is easily apparent in the ^{1}H spectra for *Thuja occidentalis* and *Juniperis virginiana* and is observed at low levels for the seed oils of *Cedrus atlantica, Abies concolor*, and *Sciadopitys verticillata.*

The allylic signals are listed in Table 4 and are as expected. The signals around 20.6 (ω2 for n-3 acids), 26.5 (C4 for Δ5 double bond), and 27.1–27.4 ppm are allylic to one double bond, while the signals at 25.5–25.7 ppm are allylic to two double bond (e.g., L11, Ln11 and 14, and corresponding positions in the Δ5 acids).

For the purpose of this discussion, the oils are classed into two groups: the six oils of *Larix decidua* to *Abies concolor* (see Table 1) and the three oils of *S. verticillata* to *J. virginiana* (Table 1).

The first group have 13 or 14 olefinic signals, which can be divided into five signals (130.16, 129.95, 129.68, 128.07, and 127.89 ppm) that correspond to oleate and linoleate with an overlap of L9 and O10, a further six signals (130.37, 130.26, 129.17, 128.75, 128.60, and 127.77 ppm) that correspond to those reported in our earlier paper (14) for Δ5,9,12-18:3, and small unidentified signals at 130.52 and 128.90 ppm. The smaller group of oils have ten signals (131.89, 130.16, 129.94, 129.67, 128.27, 128.21, 128.08, 127.90, 127.76, and 127.12 ppm) that correspond to oleate, linoleate, and linolenate with overlap of Ln9/L13 and L9/O10 and a further five signals (131.01, 130.08, 129.85, 128.33, 127.83 ppm) that are associated with the Δ5,11,14-20:3 and Δ5,11,14,17-20:3 acids. There should be more than five signals, but there must be some overlap of these signals with one another or with those for oleate, linoleate, and linolenate.

TABLE 3
Total Content of n-6 and n-3 Acids Based on the ω2 Signals

	n-6		n-3	
	NMR	GC[a]	NMR	GC[a]
Larix decidua	72.7	71.0	nd[b]	0.7
L. leptoleppis	73.7	71.8	nd	0.5
Picea abies	77.0	75.5	nd	0.2
P. sitchensis	74.5	74.3	nd	0.4
Cedrus atlantica	—	40.2	nd	0.4
Abies concolor	48.1	47.1	nd	0.5
Sciadopitys verticillata	66.2	61.4	4.1	4.1
Thuja occidentalis	36.0	32.9	44.1	44.8
Juniperus virginiana	37.4	35.4	37.4	37.6

[a] Gas chromatography (Ref. 11). See Table 1 for other abbreviation.
[b] nd = Not detected.

TABLE 4
Allylic signals (ppm)

Larix decidua	—	27.28	27.21	26.56	25.68	25.64	—
L. leptolepis	27.38	27.27	27.20	26.54	25.67	25.63	—
Picea abies	27.40	27.28	27.21	26.56	25.68	25.64	—
P. sitchensis	—	27.27	27.21	26.54	25.68	25.64	—
Cedrus atlantica[a]	27.38	—	27.20	26.53	25.67	25.62	—
Abies concolor	27.38	—	27.19	26.53	—	25.62	—
Sciadopitys verticillata[a]	—	—	27.20	26.51	25.64	25.54	—
Thuja occidentalis	—	27.20	27.14	26.50	25.63	25.53	20.56
Juniperus virginiana	—	27.22	27.16	26.51	25.64	25.54	20.57

[a]Also some additional signals in this region.

This study confirms the general conclusions of the gas-chromatographic examination (11), but more important, it demonstrates that the Δ5 acids—independent of their chain-length (C_{18} or C_{20}) and of their level of unsaturation (2–4 double bonds)—are to be found within the triacylglycerols only at the α position. The Δ5 acids were not found in the β position above the experimentally detectable level of about 3%. This is in line with our previous study of seven pine seed oils (14). This observation, combined with the fact that there is only one Δ5 acid per molecule in most triacylglycerols in pine seed oils (12), suggests that in conifer seed oils these acids are esterified to one α hydroxyl group, but not to both positions in a single molecule. This points to some unusual role for Δ5 unsaturation (or of the Δ5-desaturase) in the acylation (or desaturation) of glycerol esters in conifer seed oils.

ACKNOWLEDGMENTS

We thank Melanja Smith (St. Andrews) for expert technical assistance.

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[Received February 26, 1996; accepted July 15, 1996]