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THE LEAF LIPIDS OF SOME CONIFER SPECIES

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Abstract—Conifer leaf lipids contain, in addition to the fatty acids found in angiosperms, a series of polyunsaturated acids with Δ^5 olefinic unsaturation All the species contain Δ^5 C_{20} acids and the members of the family, Pinaceae, contain, in addition a series of C_{18} Δ^5 acids Significant amounts of a saturated C_{17} branched-chain acid were present in many of the species The distribution of polyunsaturated acids among certain lipid classes was investigated and it was found that C_{16} and C_{18} polyunsaturated acids with $\omega 3$ unsaturation are concentrated in the galactosyl diglycerides

INTRODUCTION

IT HAS been established that the major polyunsaturated acids present in angiosperm leaf lipids are linolenic (18 $3\omega 3$) and linoleic (18 $2\omega 6$) acids Many angiosperm species have present, in addition, varying proportions of hexadeca-7,10,13-trienoic (16 $3\omega 3$) acid[1] and, in two families, Boraginaceae and Caryophyllaceae, significant amounts of γ-linolenic (18.$3\omega 6$) and octadeca-6,9,12,15-tetraenoic (18·$4\omega 3$) acids have been found [2,3] All these acids are of the methylene-interrupted type. Neither non-methylene-interrupted nor C_{20} polyunsaturated acids have been reported as constituents of angiosperm leaf lipids

The presence of C_{20} and C_{22} methylene-interrupted polyunsaturated acids have been found in the lipids of lower plants[4–6] and also unusual non-methylene-interrupted C_{20} have been found in the leaf lipids of Gymnosperms and Equisetales.[4,7] During a survey of leaf lipids it was found that the leaves of *Picea abies* contained unusual C_{18} and C_{20} non-methylene-interrupted polyunsaturated acids and an investigation was undertaken of the leaf lipids of a range of conifer species to find the distribution of these unusual acids among conifers and also to compare the distribution of polyunsaturated acids among certain classes of conifer lipids.

RESULTS AND DISCUSSION

The fatty acid compositions of the total lipids from the leaves of 34 conifer species are shown in Table 1. The conifer species are similar to angiosperms in that the major constituent fatty acids are linolenic, linoleic and palmitic acids. All the conifer species studied contained small amounts of 16 $3\omega 3$, a lower homologue of linolenic acid The conifer leaf lipids differ from those of angiosperms in having present various C_{20} polyunsaturated acids including non-methylene-interrupted polyunsaturated acids with Δ^5 olefinic unsaturation. In addition, all the members of the Pinaceae studied contained a series of C_{18} Δ^5 polyunsaturated acids, 18 $2,\Delta^{5,9}$ 18 $3,\Delta^{5,9,12}$ and 18 $4\Delta^{5,9,12,15}$. This series of C_{18} acids has an additional Δ^5 olefinic bond inserted in the common plant acids, 18 $1\omega 9$, 18 $2\omega 6$, 18·$3\omega 3$ It

[1] G R JAMIESON and E H REID, *Phytochem.* **10**, 1837 (1971)
[2] G R. JAMIESON and E H REID, *Phytochem.* **8**, 1489 (1969)
[3] G R JAMIESON and E H REID, *Phytochem* **10**, 1575 (1971)
[4] G. R. JAMIESON, *Ph D. Thesis*, University of London (1970)
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TABLE 1 FATTY ACID COMPOSITION OF

	12 0	13 0	14 0	15 0 br	15 0	16 0	16 1 ω7+9	16 1 ω13t	16 2 ω6	16 3 ω3	17 0 br	17 0
Pinaceae												
Abies procera	0 1	tr	0 9	0 7	0 5	12 9	0 3	0 7	tr	0 1	3 8	0 3
A grandis	0 2	tr	1 1	0 6	0 5	12 6	0 3	0 9	tr	0 2	3 1	0 3
A concolor	1 0	0 2	1 2	1 1	0 4	10 1	0 7	1·3	tr	0 2	4 5	0 4
A amabilis	0 6	tr	1 6	0 8	0 2	9 7	0 7	0 9	tr	0 2	4 2	0 4
A nordmanniana	0 9	0 1	1 6	0 3	1 1	10 1	0 4	1 1	tr	1 3	4 1	0 4
A veitchii	1 5	0 3	2 6	1 2	0 4	13 1	0 2	1 3	0 2	0 8	4 8	0 4
A alba	0 2	tr	1 1	0 6	tr	9 8	0 1	0 9	tr	1 0	2 8	tr
Pseudotsuga menzeii	0 3	tr	1 5	0 6	0 5	13 3	0 4	0 7	tr	3 5	2 9	0 2
Picea abies	1 4	tr	4 4	tr	0 1	12·5	0 2	0 7	0 1	0 9	2 1	0 2
P sitchensis	2 3	0 1	3 5	tr	0 1	17 6	0 5	0 7	0 1	1 5	1 3	0 2
P obovata	1 1	0 1	2 5	0 1	0 1	12 0	0 2	0 5	0 3	1 5	3 5	0 3
P engelmannii	4 7	tr	3 7	0 2	0 2	14 2	1 2	0 9	0 2	1 4	2 4	0 5
P orientalis	4 0	tr	3 1	0 1	0 2	15 5	0 2	0 9	0 2	1 1	2 7	0 6
P mariana	4 0	0 1	2 9	0 3	0 3	13 8	0 2	1 0	0 1	1 2	2 6	0 3
Tsuga heterophylla	0 2	tr	1 7	0 2	0 4	15 9	0 5	1 0	tr	0 2	2 4	0 2
T canadensis	0 3	tr	1 5	0 1	0 3	12 2	0 2	0 7	tr	0 3	1 3	0 2
Larix decidua	0 2	tr	1 4	—	0 2	13 5	1 4	1 6	0 6	4 3	1 1	0 3
L leptolepis	0 2	tr	1 9	—	0 2	15 8	0 5	1 9	0 2	4 8	0 7	0 4
Cedrus deodara	5 0	0 2	2 3	—	0 3	15 7	0 2	0 7	0 2	4 2	1 6	0 4
Pinus sylvestris	1 7	tr	4 8	—	0 2	12 0	0 3	0 4	0 2	1 2	0 5	tr
P contorta	2 4	tr	2 3	—	0 1	11 0	0 1	0 3	0 1	1 0	0 2	0 1
P nigra	8 7	0 2	7 9	—	0 2	11 2	0 7	0 8	0 2	4 0	0 3	0 4
Taxodiaceae												
Sequoia gigantea	1 9	0 4	1 5	—	0 1	16 0	0 1	0 5	tr	3 1	0 1	0 1
Cupressaceae												
Thuja plicata	0 8	0 1	1 5	—	0 1	19 3	0 3	0 6	0 4	2 1	0 1	0 4
Chaemaecyparis pisifera	0 3	tr	0 6	—	0 1	14 9	0 5	0 2	0 2	1 6	0 3	0 4
C thyoides	0 6	tr	2 5	—	0 2	12 3	0 2	0 4	0 1	1 6	0 3	0 5
C lawsoniana	2 0	tr	3 9	—	0 1	15 8	0 4	0 4	tr	1 9	0 1	0 3
Cupressus sempervirens	1 8	0 1	8 9	—	0 1	14 7	0 8	0 4	tr	3 3	0 4	0 3
C nootkatensis	0 5	tr	1 5	—	0 2	18 9	0 1	0 3	tr	2 1	0 1	0 2
Cryptomeria saponica	0 5	0 4	1 8	—	0 1	15 4	1 8	1 1	tr	2 5	1 1	0 3
Juniperus communis	0 5	tr	1 7	—	0 3	18 4	0 3	1 0	0 2	4 1	0 2	0 4
J virginiana	3 7	0 2	4 7	—	0 1	16 0	0 1	0 3	tr	1 2	0 4	0 2
Taxaceae												
Taxa baccata	0 1	tr	1 4	—	0 3	15 3	0 3	0 4	tr	5 8	0 2	0 3

was also found that when one member of a genus contained these C_{18} Δ^5 acids then all the members studied had similar amounts of these acids The presence of Δ^5 acids in plant tissues is unusual but various acids of this type have been found recently in seed oils,[8–12] in *Dictyostelium discoideum* lipids[13] in pine bark,[14] and in the leaf lipids of Equisetales [2,7]

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[14] J W ROWE and J H SCROGGINS, *J Am Chem Soc* **29**, 1554 (1964)

THE TOTAL LIPIDS OF CONIFER LEAVES

18:0	18 1	18 2 $\Delta^{5,9}$	18 2 $\omega 6$	18 3 $\Delta^{5,9,12}$	18 3 $\omega 3$	18 4 $\Delta^{5,9,12,15}$	20 0 20 1	20.2 $\omega 6$	20 3 $\Delta^{5,11,14}$	20 3 $\omega 6$	20:4 $\Delta^{5,11,14,17}$	22 0
2 9	11 3	0 8	16 7	3 3	34 4	4 1	1 1	0 4	2 0	0 5	1 1	1 1
2 7	14 7	1 3	12 4	2 0	38 1	3 5	0 7	0 2	2 0	0 3	1 1	1 2
0 8	4 5	1 1	13 8	4 3	38 1	3 9	0 8	0 7	5 6	0 3	2 6	2 4
1 1	6 0	1 8	12 7	3 7	42 5	4 4	0 4	0 4	3 4	0 5	2 0	1 8
0 5	6 6	1 1	11 9	2 2	44 4	3 3	0 5	0 6	3 4	1 0	1 4	1 7
1 2	7 8	1 5	9 5	2 8	31 4	7 8	0 5	0 6	3 4	0 8	3 9	2 4
1 0	6 1	1 1	10 8	2 1	50 9	3 4	0 3	0 3	3 3	0 5	2 9	0 8
1·0	8 1	0 7	11 9	1 6	40 8	2 8	0 8	0 2	4 5	0 3	2 1	1 1
0 5	5 0	0 9	11 6	4 2	43 7	5 8	0 5	tr	2 4	0 3	0 9	1 6
1 0	11 3	1 4	11 0	3 5	35 1	4 7	0 5	0 1	1 6	0 2	0 6	1 2
0 6	5 4	0 5	14 5	4 9	37 7	3 9	0 4	0 5	4 5	0 4	2 3	2 2
0 9	3 8	0 8	13 9	3 3	37 4	3 7	0 5	0 3	3 1	0 4	0 8	1 5
1 1	3 3	0 8	12 8	4 2	37 2	4 3	0 9	0 2	3 7	0 2	0 8	1 8
1 2	3 7	0 5	13 3	4 2	34 5	4 2	1 2	0 3	4 6	0 2	2 2	2 7
1 6	4 4	0 3	9 0	1 8	49 7	2 4	1 5	0 3	2 7	0 6	1 9	1 1
2 2	2 9	0 1	11 4	1 1	51 2	0 7	1 2	0 4	6 1	0 3	3 2	2 1
1 6	3 8	0 9	12 0	2 5	44 0	2 6	0 5	0 3	3 2	0 2	1 1	2 7
1 2	4 0	1 1	10 8	2 7	46 0	4 0	0 4	0 2	2 5	0 6	0 9	1 6
0 5	3 6	0 5	9 9	1 3	43 3	2 6	0 4	0 2	3·4	0 4	1 9	1·2
0 7	7 2	0 2	15 2	4 3	34 1	3 2	0 3	1 6	8 3	1 1	2 0	0 5
1 2	17 6	0 2	22 0	2 2	31 0	1·7	0 6	0 6	3 5	0 2	1 0	0 6
0 9	2 7	0 4	8 2	1 9	35 0	1 9	0 9	0 7	6 7	0 6	1 3	4 3
1 1	1 7	—	16 1	—	43 4	—	1 1	0 5	6·3	0 2	3 7	2 1
1·4	2 2	—	15 6	—	45 0	—	1 6	0 2	3·5	0 1	3 5	1 2
1 4	1 1	—	15 4	—	49 6	—	1 3	0·3	5 9	0 2	2 8	2 9
1 2	1 6	—	12 4	—	44 0	—	2 6	0 6	8 7	0·3	7 7	2 2
1 2	8 4	—	15 8	—	39 4	—	0 7	0 7	4 4	0·3	3 5	0 8
1 2	2 9	—	16 1	—	40 4	—	0 6	0 3	4 1	0 2	1 8	1 6
1 3	3 6	—	23 2	—	33 6	—	0 9	0 7	7·3	0·2	2 9	2 7
0 9	2 1	—	15 4	—	45 6	—	0 9	0 2	4 2	0 4	2 1	3 0
1 0	2 7	—	3 8	—	51 3	—	0 2	0 1	2 0	0·1	1 4	0 3
1 6	3 6	—	14 0	—	34 4	—	1 5	0 5	9 1	0 1	4 3	4 0
1 2	8 2	—	11 3	—	44 5	—	0 9	0 5	2 7	0 3	3 8	2 4

Many of the conifer species studied contained considerable proportions of a saturated C_{17} branched-chain acid and from the chromatographic behaviour of this acid it is probably the *anteiso* isomer [15] The distribution of this acid among the different genera is shown in Fig. 1. Smaller amounts of a similar C_{15} acid were found in four of the genera studied These branched-chain acids are found only in trace amounts in angiosperm leaf lipids.

It has been shown[2] that, in the leaf lipids of angiosperm species, there are variations in the proportions of polyunsaturated acids during the growing season of a plant and a study

[15] G R. JAMIESON, in *Topics in Lipid Chemistry* (edited by F D GUNSTONE), Vol. 1, Logos Press, London (1970)

TABLE 2. FATTY ACID COMPOSITION OF

	12 0	14 0	16 0	16 1 ω7 + 9	16 1 ω13t	16 2 ω6	16 3 ω3	17 0 br	18 0	18 1
Picea abies										
Total	1 5	4 1	12 5	0 2	0 6	0 1	0 7	2 1	0 6	5 0
MGDG	0 2	0 7	2 0	0 1	—	0 1	3 5	0 3	0 6	1 2
DGDG	0 8	2 0	11 7	tr	—	0 2	1 6	2 0	1 7	1 7
Polar	0 4	1 0	18 0	0 1	0 9	tr	tr	3 1	2 0	7 0
Pinus sylvestris										
Total	1 7	4 8	12 0	0 3	0 4	0 2	1 2	0 5	0 7	7 2
TG	2 4	3 4	20 2	0 4	—	tr	0 4	3 2	1 7	12 0
DG	2 2	4 0	36 7	0 4	—	0 1	8 4	1 2	1 0	7 6
MGDG	0 6	0 9	4 9	0 3	—	0 6	12 7	0 1	0·5	1 8
DGDG	1 5	2 4	10 5	0 1	—	0 3	0 7	0 7	0 8	1 4
Polar	1 0	1 5	21 0	0 2	1 3	0 4	0 7	1 1	1 2	3 8
Larix decidua										
Total	0 6	1 0	13 8	0 3	1 4	0 1	3 1	2 3	1 1	4 3
TG	1 1	1 3	15 0	0 6	—	tr	0 2	3 7	1 3	8 5
DG	1 0	1 0	29 7	0 6	—	0 1	7 9	1 6	0 9	6 5
MGDG	0 1	0 2	2 0	0 2	—	tr	12 7	0 7	0 2	0 5
DGDG	0 4	0 2	10 4	0 2	—	0 2	2 8	1 6	1 1	0 7
Polar	0 3	0 8	15 8	0 3	2 9	0 2	0 8	1 8	2 1	7 4
Taxa baccata										
Total	0 1	1 8	17 3	0 3	0 6	tr	6 1	0 3	1 0	7 6
TG	0 6	2 1	18 3	0 5	—	tr	0 5	0 8	1 8	16 4
DG	0 8	2 3	24 2	0 6	—	0 2	6 8	0 5	1 2	5 4
MGDG	tr	0 4	4 5	0 2	—	tr	15 9	0 1	0 5	2 0
DGDG	0 1	1 4	10 6	0 2	—	0 1	1 8	0 2	0 8	1 8
Polar	0 4	1 6	19 8	0 2	1 2	0 2	0 7	1 0	1 7	6 4

tr—trace; TG—triglycerides; DG—diglycerides, MGDG—monogalactosyl diglycerides DGDG—digalactosyl diglycerides

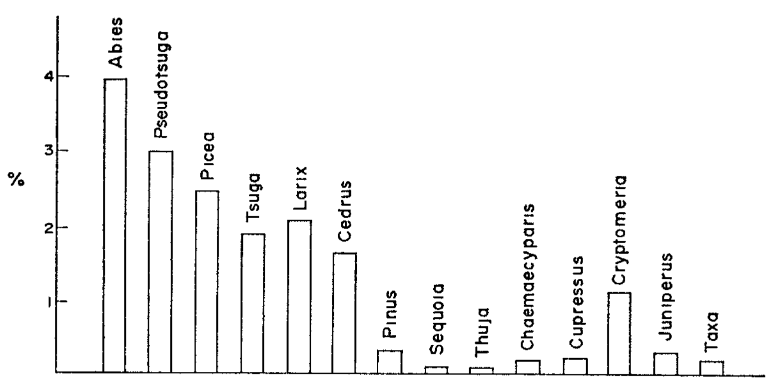

FIG 1 DISTRIBUTION OF 17 0 *anteiso* AMONG CONIFER GENERA

DIFFERENT LIPID CLASSES OF CONIFER LEAVES

18 2 $\Delta^{5,9}$	18 2 $\omega 6$	18 3 $\Delta^{5,9,12}$	18 3 $\omega 3$	18 4 $\Delta^{5,9,12,15}$	20 0 20 1	20 2 $\omega 6$	20 3 $\Delta^{5,11,14}$	20 3 $\omega 6$	20 4 $\Delta^{5,11,14,17}$	22 0 22 1
0 7	12 2	4 9	41 8	6 6	0 5	tr	2 8	0 2	1 1	1 7
0 3	5 1	1 4	77 6	6 9	tr	tr	tr	tr	tr	tr
0 2	6 3	0 3	68 30	2 4	tr	tr	0 2	tr	0 1	0 8
0 8	25 0	3 3	25 8	4 0	0 2	0 1	5 1	0 4	0 8	2 0
0 2	15 2	4 3	34 1	3 2	0 3	1 6	8 3	1 1	2 0	0 5
0 2	13 5	1 6	26 8	1 7	1 8	0 6	6 7	0 8	1 2	1 6
1 6	4 7	1 9	21 6	3 9	0 8	0 2	2 1	0 6	0 8	0 2
4 8	3 7	1 5	54 8	12 8	tr	tr	tr	tr	tr	tr
3 9	5 1	1 2	62 8	5 7	1 0	0 1	1 2	tr	0 6	tr
1 6	19 6	3 3	18 0	4 6	1 9	3 5	8 5	3 6	2 2	1 0
0 3	19 8	4 5	38 6	2 8	0 7	0 4	2 1	0 6	1 0	1 2
0 5	26 2	13 6	19 3	1 7	1 4	0 5	2 8	0 3	0 4	1 6
0 8	10 7	2 1	29 7	3 2	0 8	0 2	1 2	0 6	0 6	0 8
0 1	2 9	0 6	71 0	8 8	tr	tr	tr	tr	tr	tr
tr	4 0	0 4	71 6	1 6	1 2	0 1	0 7	0 1	1 3	1 4
0 6	32 2	6 1	17 2	1 8	0 9	1 1	4 5	0 8	1 4	1 0
—	16 4	—	37 9	—	0 8	0 4	2 8	0 4	4 2	2 0
—	19 6	—	28 1	—	1 4	0 5	3 1	0 4	3 1	2 8
—	5 3	—	47 3	—	1 0	0 2	1 6	0 4	1 8	0 4
—	4 3	—	71·9	—	0·2	tr	tr	tr	tr	tr
—	6 7	—	72 7	—	0·4	0 2	1 3	0 1	1 6	tr
—	27 6	—	19 8	—	1 3	1 0	6 1	0 8	7 1	3·1

was made of the fatty acid composition of the leaf lipids of larch (*Larix decidua*) from mid-April to the end of October, 1970. The variations in the proportions of 16.3ω3 + 18·3ω3 and 18.2ω6 are shown in Fig. 2. These variations are similar to those obtained for angiosperm species. There was a rapid increase in the proportion of ω3 acids during the early part of the growing season with a subsequent levelling out in the middle part of the season. This increase was accompanied by a corresponding decrease in the ω6 acids which also level out in mid-season. Throughout the growing season there was little variation in the proportions of the Δ^5 C_{18} and C_{20} acids.

The fatty acid compositions of several lipid classes from 4 conifer species are shown in Table 2. It is found that the C_{16} and C_{18} polyunsaturated ω3 acids are concentrated in the galactosyl diglycerides accounting for 78 7–92 5% and 68 8–76 0% of the total acids of the monogalactosyl and digalactosyl diglycerides respectively. These total amounts of ω3 acids are slightly lower than those found in the galactosyl diglycerides from angiosperm species.[1] Although 20 $4\Delta^{5,11,14,17}$ is an ω3 acid it is found only in trace amounts in the monogalactosyl diglycerides and both the Δ^5 C_{20} acids are preferentially concentrated in the polar lipids. This is similar to the distribution of these Δ^5 C_{20} acids in the lipid classes of *Equisetum arvense*.[4]

The distribution of fatty acids among the different lipid classes of conifers is similar to

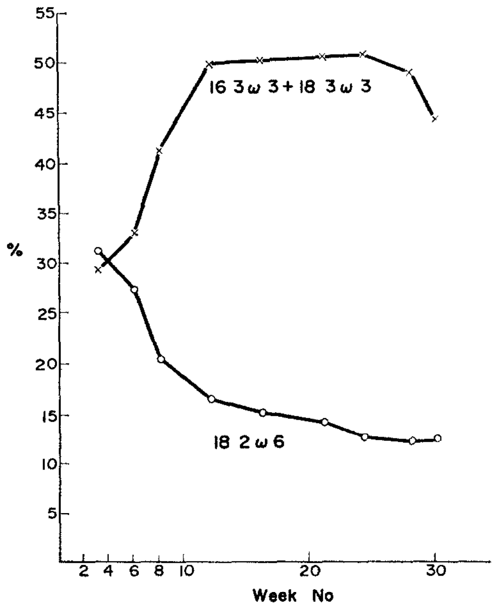

Fig 2 Variations in the proportions of ω3 and ω6 acids during the growing season of *Larix decidua*

that of angiosperms as follows: (i) 16·3ω3 is preferentially concentrated in the monogalactosyl diglycerides and it would appear that, in this lipid class, 18 3ω3 is replaced in part by the lower homologue; (ii) the diglyceride fraction contains a considerable amount of 16 3ω3, (iii) the trans acid, 16 1ω13t, is found only in the polar lipid fraction; (iv) the highest proportions of saturated acids are found in the triglyceride and diglyceride fractions; (v) the MGDG fraction has the highest degree of unsaturation (Table 3)

Table 3 Degree of unsaturation of the fatty acids of different lipid classes

	Average double bonds per mole fatty acid				
	MGDG	DGDG	Polar	TG	DG
Conifers					
Picea abies	2 9	2 3	1 8	—	—
Pinus sylvestris	2 7	2 4	1 6	1 6	1 4
Larix decidua	3 0	2 7	1 7	1 8	1 7
Taxa baccata	2 7	2 5	1 7	1 7	1 9
Mean	2 8	2 5	1 7	1 7	1 7
Pteridophyta					
Equisetum arvense[4]	2 7	2 2	1·7	—	—
Angiosperms					
Containing 16 3ω3[1]	2 9	2 7	1 9	1 8	1 5
Containing 18 4ω3[2,3]	3 1	2 7	1 7	—	—

EXPERIMENTAL

Samples of *Picea abies*, *Pinus sylvestris*, *Larix decidua* and *Taxa baccata* were collected in the surrounding districts of Paisley. Samples of the other conifer species were obtained from the Forestry Commission at Inverness and the Lael Forest, Wester Ross. Lipids were extracted and separated into classes by methods described.[3,4] The acids were tentatively identified using chromatographic and degradative methods already published.[4] The saturated C_{15} and C_{17} branched-chain acids were tentatively identified using chromatographic methods and tables of ECL values [15]

GLC analyses were carried out on a PE 800 chromatograph with polyester coated open tubular columns of different polarity.

Acknowledgements—Thanks are due to Mr W. Gray Kerr, Forestry Commission, Inverness, and Mr. W F. Sutherland, Forestry Commission, Lael Forest, for supplying identified samples of many conifer species

Key Word Index—Gymnospermae; chemotaxonomy; leaf lipids, Δ^5 fatty acids; galactosyl diglycerides.