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FATTY ACIDS OF *PINUS ELLIOTTII* TISSUES

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Abstract—The total fatty constituents of slash pine (*Pinus elliottii*) tissue cultures, seeds and seedlings were examined by GLC and MS. Qualitatively, the fatty acid composition of these tissues was very similar to that reported for other pine species. The fatty acid contents of the tissue cultures resembled that of the seedling tissues. In addition to the fatty acids common to botanical materials, Δ^5–C_{18} and –C_{20} nonmethylene-interrupted polyunsaturated acids were present in low relative abundances. The branched-chain C_{17} acid reported for several other *Pinus* species was confirmed as the *anteiso* isomer.

INTRODUCTION

GAS CHROMATOGRAPHIC retention times indicate that the most common fatty acids present in mature pine tree xylem are 16:0, 16Δ^1, 18:0, 18Δ^1, 18Δ^2, 18Δ^3, 20:0 and 22:0.[1–3] In addition to the more common saturated acids, a number of reports indicate the presence of a branched-chain C_{17} acid. Hemingway and Hillis[3,4] found that a branched acid was present in equal or greater amounts than palmioleic in *Pinus radiata* wood. More recently, Jamieson[5] observed that eleven of the conifer species studied contained considerable proportions of a saturated C^{17} branched-chain acid in leaf lipids which was assumed to be the *anteiso* isomer on the basis of GLC. McDonald and Porter[6] also reported a branched C_{17} in the bark lipids of *P. radiata*.

Also using GLC retention times, a number of reports suggested the presence of C_{18} and C_{20} acids with non methylene-interruped polyunsaturation containing Δ^5 bonds for *Pinus* species. A 18:3 $\Delta^{5,9,12}$ was reported in the wood of *P. radiata*[3] and a 20:3 $\Delta^{5,11,14}$ in the bark of the same species.[6] The leaf lipids of several *Pinus* species were all found to contain 18:2 $\Delta^{5,9}$, 18:3 $\Delta^{5,9,12}$ and 18:4 $\Delta^{5,9,12,15}$ which represents the common C_{18} series of fatty acids with an additional Δ^5 olefinic bond.[5] In addition, several polyunsaturated C_{20} acids containing the Δ^5 double bond have also been observed in pine leaves. Lehtenen *et al.*[7,8] reported the presence of small amounts of 18:2 $\Delta^{5,9}$ and 18:3 $\Delta^{5,9,12}$

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[7] ELOMAA, E., LEHTINEN, T. and ALHOJÄRVI, J. (1963) *Suomen Kemistilehti* **36B,** 52.

[8] LEHTINEN, T., ELOMAA, E. and ALHOJÄRVI, J. (1963) *Suomen Kemistilehti* **36B,** 154.

fatty acids in tall oil and pine seed extracts as well as 20:1 Δ^{11} and 20:3 $\Delta^{5,11,14}$ from the pine extracts.[9,10] All double bonds had the *cis*-configuration. Fatty acids with 16–20 carbon atoms containing the *cis*-Δ^5 olefinic bond have also been identified in other seed oils,[11–14] a slime mold,[15] and bacteria.[16]

In our continuing studies to characterize the slash pine tissue cultures grown in our laboratory, we have compared the fatty acid composition of tissue culture, seed, needle and stem tissues of *P. elliottii* with particular interest in the branched-chain and nonmethylene-interruped polyunsaturated acids.

RESULTS AND DISCUSSION

The tissue cultures employed in this study were originally initiated from an embryo explant of slash pine and have been maintained in germ-free cultures by periodic transfer for 2 yr on modified Brown and Lawrence media. The callus tissue culture is composed of parenchyma cells with infrequent occurrence of xylem elements. The cultures are characterized by a light brown and less often a green pigmentation in healthy actively growing tissues and by a very dark brown and highly osmiophilic pigmentation, reminiscent of injured pine cells in the senescing cultures.

TABLE 1. TOTAL EXTRACTABLE LIPIDS AND FATTY ACIDS FROM SLASH PINE TISSUE CULTURES, SEED, AND SEEDLING NEEDLE AND STEM TISSUES

Sample	Age (months)	Dry wt extracted (g)	Total extractable lipids (% of dry wt)
Tissue culture	1·5	6·40	4·01
Seeds	—	1·59	65·02
Needles	8–10	1·28	7·43
Stems	8–10	0·63	5·03

When compared to the seed, needle and stem tissues, the total lipid composition of the 6-week-old tissue grown in culture was low (4·01% of the dry tissue weight) and more closely resembled lipid levels found in the stem tissues (Table 1). This value is approximately

TABLE 2. FATTY ACID COMPOSITION OF THE

Tissue	12:0*	13:0	14:0	14:1	15:0	16:0	16:1 (Δ^7)	16:2 ($\Delta^{7,11}$)	16:3 ($\Delta^{7,11,14}$)	17:0	17:0 *anteiso*	18:0	18:1 (Δ^9)
Tissue culture	tr	—	4·3	—	0·2	22·1	0·8	0·4	tr	—	—	3·9	26·1
Seed	tr	—	0·1	tr	tr	6·2	0·1	tr	tr	tr	—	2·3	21·6
Leaf	0·4	tr	1·4	—	0·1	17·7	1·1	0·8	0·2	0·3	0·2	2·6	14·1
Stem	0·8	tr	1·0	tr	0·5	17·2	2·2	0·8	2·6	tr	tr	2·9	17·9

* Expressed as relative per cent (%) of the total fatty acids from gas chromatographic data using both packed and capillary columns. tr—Less than 0·1%.

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[13] TAKAGI, T. (1964) *J. Am. Oil Chem. Soc.* **41,** 516.
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[15] DAVIDOFF, F. and KORN, E. D. (1963) *J. Biol. Chem.* **238,** 3199.
[16] FULCO, A. J. and BLOCH, K. (1964) *J. Biol. Chem.* **239,** 993.

2-fold higher than other tissue cultures such as tobacco and soybean.[17] As one would expect, the seed tissues contained the highest total lipid composition (65·02%).

The total fatty acid constituents from each of the above tissue types obtained by alkaline hydrolysis of the total lipids were analyzed by GLC and MS (Table 2). The principal fatty acids from the tissues in this study were typical of those reported for higher plants[18] and consistent with previous reports of members of the Pinaceae. Qualitatively, the fatty acid composition of the tissue culture materials was very similar to that of the seed, needle and stem tissues. Each tissue type had both saturated and unsaturated acids ranging in carbon chain length from C_{12} to C_{22} with the even-numbered carbon chains predominant (Table 2). Because the fatty acid composition varies considerably from tissue to tissue within the same plant[19] and as the composition is also influenced by environmental conditions, the tissues of this study would not be expected to be quantitatively comparable. However, the principal fatty acids of the pine tissue cultures were C_{16}, $C_{18:1\Delta^9}$, and $C_{18:2\Delta^{9,12}}$ which quantitatively more closely resembled the stem and needle tissue acids. The predominant seed fatty acids were the unsaturated C_{18} isomers containing double bonds in the Δ^9, $\Delta^{6,9}$ and $\Delta^{5,9,12}$ positions with the linoleic acid representing almost half of the fatty acid components. The minor acids, particularly the unsaturated components of each tissue studied appeared to be characteristic of conifer species in general and more specifically the C_{18} and C_{20} polyunsaturated acids whose double bonds are separated by two methylene units and contain an olefinic bond at the Δ^5 position.[5–7] An unsaturated acid containing the Δ^5 double bond was a major component in only the seed tissues; $C_{18:3\Delta^{5,9,12}}$ at 16·9% of the total fatty acid fraction.

The MS employed in this study will not provide information on the position or the configuration of the double bonds. However, with knowledge derived from relative retention times and supported by electron impact data the position of the points of unsaturation and their stereochemistry can be determined with reasonable accuracy. Recent reports have suggested that systematic relationships exist between the GLC retention times, the chain length, and double bond position in the non methylene-interruped polyunsaturated methyl esters.[20] Similar relationships have been shown for the methylene-interrupted polyunsaturated esters.[21] Appropriate polyolefin standards were therefore employed using both packed and high resolution capillary columns to support the identifications made in this study.

TOTAL LIPIDS FROM SLASH PINE TISSUES

18:2 ($\Delta^{5,9}$)	18:2 ($\Delta^{9,12}$)	18:3 ($\Delta^{5,9,12}$)	18:3 ($\Delta^{9,12,15}$)	20:0	18:4 ($\Delta^{5,9,12,15}$)	20:1	20:2 ($\Delta^{11,14}$)	20:3 ($\Delta^{5,11,14}$)	20:4 ($\Delta^{5,11,14,17}$)	22:0
0·2	30·4	1·6	6·3	tr	0·2	tr	1·8	1·4	tr	tr
2·9	47·9	16·9	1·1	0·5	0·1	tr	0·5	tr	tr	tr
tr	24·8	tr	30·6	0·1	0·2	tr	1·4	1·9	0·2	1·5
tr	30·3	tr	12·9	7·6	—	tr	tr	0·9	tr	2·2

There were several compounds with retention times in the range for C_{20}–C_{22} acids, but MS of these compounds revealed that they were neither fatty acids esters nor the common

[17] Unpublished data.
[18] HITCHCOCK, C. and NICHOLS, B. W. (1971) *Plant Lipid Biochemistry*, p. 3, Academic Press, New York.
[19] WEETE, J. D., RIVERS, W. G. and WEBER, D. J. (1970) *Phytochem.* **9**, 2041.
[20] JAMIESON, G. R. and REID, E. H. (1971) *J. Chromatogr.* **61**, 346.
[21] ACKMAN, R. G. (1962) *Nature, Lond.* **194**, 970; *idem.* (1963) *J. Am. Oil Chem. Soc.* **40**, 558; *idem.* (1963) *J. Am. Oil Chem. Soc.* **40**, 564.

esters of resin acids. Molecular weights ranged from 342 to 370 with M-15 fragments which are characteristic of the loss of a methyl group common to several spectra.

The C_{17} branched-chain fatty acid characteristic of pine was not detected in the tissue culture and seed tissues which suggests that the branched acid may not be formed and/or accumulated in these tissues. This compound was present in both the stem (<0·1%) and needle (0·2%) tissues. The branched C_{17} acid has been reported for several conifer species and this communication confirms the *anteiso* isomer by MS. The M-29 fragment representing the loss of an ethyl group upon electron impact is characteristic of the typical 3-methyl branched acid and was observed in our analyses. A molecular ion at *m/e* 284 indicated no unsaturation. Although a branched-chain C_{15} acid has been reported for leaf lipids of several conifer species,[5] no other acids of this type were detected in the tissues of this study.

A major portion of the total fatty acid components isolated from each of the tissues examined were unsaturated and when compared, the tissue cultures more closely resembled the stem and needle tissues (Table 3). Using the relative concentrations of Δ^5 double bond containing acids as a marker, the tissue culture materials again closely resembled these tissues.

TABLE 3. UNSATURATED FATTY ACID RELATIONSHIPS BETWEEN SLASH PINE TISSUE CULTURE, SEED, SEEDLING NEEDLE AND STEM TISSUES

Source	Unsaturated acids as % of total fatty acids	Δ^5 Acids as % of total fatty acids	Δ^5 Acids as % of total unsaturated fatty acids
Tissue culture	73·1	7·5	10·2
Seed	91·1	19·9	21·8
Needle	76·2	8·2	10·7
Stem	67·6	5·9	8·7

We can conclude from these results that the slash pine tissue cultures employed in this study are composed of parenchyma cells which are typical with respect to the fatty acid composition of those found in healthy intact pine plants and that culturing in the manner described above does not result in serious alterations in these constituents. In a similar study, Weete[22] found quantitative variations when comparing the fatty acid composition of habituated tobacco tissue cultures and seedlings, but qualitatively the tissues were very similar. A significant application of the pine tissues cultures grown in axenic culture may be their use in elucidating the mechanisms involved in the formation of the Δ^5 double bond and particularly, the study of single pathogen-host interactions.

EXPERIMENTAL

Tissue preparation. Slash pine tissue cultures were maintained on modified Brown and Lawrence media in a Percival incubator at 22° under constant illumination as previously described.[23] Seeds were obtained from the field and the wings were removed prior to extraction. Stem and needle tissues were obtained from 8- to 10-month-old slash pine seedlings grown in a greenhouse.

Lipid extraction. All tissues except seeds were first homogenized in a Waring Blender in MeOH. The tissue was then collected by centrifugation and stirred for 1–2 hr with $CHCl_3$–MeOH (1:1). The tissue was collected and further extracted with $CHCl_3$ as before. Pine seeds were crushed and then extracted in a

[22] WEETE, J. D. (1971) *Lipids* **6**, 684.
[23] BROWN, C. L. and LAWRENCE, R. H. (1968) *Forest Sci.* **14**, 62.

micro-Soxhlet apparatus with the same series of solvents described above. The combined extracts of each tissue were reduced in vol. under N_2 and partitioned between *n*-hexane and a sat. NaCl solution. The *n*-hexane phase was washed with H_2O and dried under N_2 and used as the total lipid extract. The total fatty acid components were obtained by alkaline hydrolysis of the total lipids by the methods described by Wilde and Stewart.[24] Fatty acid methyl ester derivatives for GLC–MS analysis were prepared using BF_3–MeOH.[25]

Separation and identification. Chromatographic separation was achieved by use of Hewlett–Packard Model 5750 gas chromatograph equipped with either a 15 m × 0·24 mm stainless steel capillary column coated with Igepal CO-880 or a 2·75 m × 3 mm stainless steel column packed with 12% ethylene glycol succinate on a 60–80 mesh Gas-Chrom-P support. The capillary column was programmed from 80 to 170° at 4°/min while the packed column was operated isothermally at 195°. The injector port was maintained at 225° and the detector temp. at 250°. *ca.* 90% of the chromatographic effluent was allowed to simultaneously enter du Pont 21–491 double focusing mass spectrometer by means of a jet-type separator. The separator and transfer lines were held at 250°. The ion source was 200° with a filament current of 40 μA. All spectra were obtained at 70 eV with scan speeds of 2 sec/decade. Chromatographic standards were obtained from Applied Science Laboratories, College Park, Pennsylvania (U.S.A.).

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