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PINACEAE

MONOCARBOXYLIC AND DICARBOXYLIC ACIDS FROM *PSEUDOTSUGA MENZIESII* BARK*

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Abstract—The benzene-soluble, *n*-hexane-insoluble extract from the bark of *Pseudotsuga menziesii* (Mirb) Franco was found to contain a number of *n*-fatty acids and α,ω-dicarboxylic acids ranging in chain length from 16 to 28 and 16 to 24 carbons, respectively

INTRODUCTION

THE WHOLE bark of Douglas-fir [*Pseudotsuga menziesii* (Mirb) Franco] yields wax-like fractions which are prepared by successively extracting the bark with *n*-hexane and with benzene [1] The chemical properties of the two 'wax' fractions have been investigated[1] and several of their major chemical components have been identified by classical chemical techniques [1–3] Kurth[3] reported the presence of lignoceric acid, a C_{20} dicarboxylic acid, several hydroxy acids, lignoceryl alcohol, and 'phytosterol' in the benzene-soluble, hexane-insoluble fraction ('benzene wax')

By utilizing TLC and GLC, we have applied a different and more thorough approach to the analysis of the lipid constituents of the 'benzene wax' This communication presents the first of our results and reports the identities of individual *n*-fatty acids, and of dicarboxylic acids The presence of other families of compounds is also reported

RESULTS AND DISCUSSION

The acids recovered from saponified 'benzene wax' could be resolved into three major families by TLC of their methyl esters *Spot* 1 (R_f 0 74) migrated nearly the same distance as authentic methyl lignocerate (R_f 0 76) suggesting that it was a mixture of methyl esters of non-oxygenated fatty acids, *spot* 2 (R_f 0 54) migrated similarly to authentic dimethyl octadecanedioate (R_f 0 57) suggesting a mixture of dimethyl esters of dicarboxylic acids; *spot* 3 (R_f 0 17) was not similar to other standards in our possession, but further investigation suggests that it contains the methyl esters of the hydroxy acids observed by Kurth [3] We will report on *spot* 3 in a future paper TLC of the neutral compounds from saponified 'benzene wax' yielded two major components which could be the aliphatic alcohols and 'phytosterols' observed by Kurth,[3] and will be reported on later

GLC resolved the compounds of TLC *spot* 1 into a number of fatty acid methyl esters (Table 1), identifications of which were based on studies with four GLC liquid phases The ester of greatest abundance was methyl lignocerate, but substantial amounts of several other esters were present (Table 1) Several of the GLC components of *spot* 1 remain unidentified

* Part II in the series "Douglas-fir Bark Extractives" For Part I see M L LAVER, H H FANG and H AFT, *Phytochem* **10,** 3292 (1971)

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[3] E F KURTH, *Tappi* **50,** 253 (1967)

TABLE 1 MONOCARBOXYLIC ACIDS* IN DOUGLAS-FIR BARK 'BENZENE WAX'

Acid†	Retention time SE-30, 215°	% Relative abundance
Palmitic	1 3	0 9
(Unident, sat'd)	1 6	0 3
(C_{18} unsat'd)	2 2	3 1
Stearic	2 4	0 4
(Unident, unsat'd)	3 1	0 8
	3 8	trace
	4 5‡	0 4
	4 5‡	3 3
Arachidic	4 5	2 5
n-Heneicosanoic	6 4	trace
Behenic	8 9	22 3
n-Tricosanoic	12 1	0 7
Lignoceric	17 0	51 7
n-Pentacosanoic	23 0	0 5
Cerotic	31 7	12 7
(*n*-Heptacosanoic)	—§	trace
(Montanic)	59 3	0 3
		99 9

* Identified by GLC of methyl esters in TLC *spot* 1

† Named compounds in parentheses, '(—)', denote tenative identifications

‡ These peaks were resolved from methyl arachidate at 195°

§ Observed in a temperature-programmed run on SE-30, and isothermally on Apiezon-L

The dimethyl esters identified in TLC *spot* 2 are listed, with their relative abundances, in Table 2 Initial identifications were based on GLC studies made with three liquid phases: SE-30, Apiezon-L, and Reoplex-400 Besides the C_{20} compound reported by Kurth,[3] we found substantial amounts of other dimethyl esters.

Verification of the identities of the *spot* 2 dimethyl esters was obtained by GLC–MS We

TABLE 2 DICARBOXYLIC ACIDS* IN DOUGLAS-FIR BARK 'BENZENE WAX'

Acid	% Relative abundance†
Hexadecanedioic	36 3
Octadec-?-enedioic‡	24 8§
Octadecanedioic	14 6
Eicosanedioic	14 3
Docosanedioic	8 7
Tetracosanedioic	1 3
	100 0

* Identified by GLC of dimethyl esters in TLC Spot 2 and verified by tandem GLC–MS

† Estimated from SE-30 data

‡ Location of double bond not established

§ Includes small, unresolved shoulder

examined the high ends of the mass spectra of authentic dimethyl esters and of the GLC peaks in *spot* 2 for those *m/e* ion peaks characteristic of dimethyl esters of dicarboxylic acids· M, M-31, M-64, M-73, M-92 and M-105 [4–6] We positively identified all of these *m/e* peaks for each of our authentic compounds except for C_{22}, where the peak believed to be M could not be accurately assigned For the C_{16} and C_{20} dimethyl esters of spot 2, we identified all the above ions, for C_{22}, all except M, and for C_{24}, only M-31 and M-73 The spectra of the $C_{18\ 0}$ and $C_{18\ 1}$ dimethyl esters were of mixtures of the two, since resolution was incomplete at the mass spectrometer However, M, M-31, M-64 and M-73 were identified for the $C_{18\ 0}$ dimethyl ester, and M = 340 *m/e* and (M–CH_3OH) = 308 *m/e* were identified, which indicates the presence of a $C_{18\ 1}$ dimethyl ester The MS taken at various points on the total-ionization peak showed that the unsaturated diester emerged from the GLC first This is consistent with the GLC studies No obvious signs of branching were noted in any of the spectra.

EXPERIMENTAL

Solvents All *n*-hexane (purified grade) and other solvents (reagent grade) were distilled once

Bark collection and solvent extraction Whole bark was stripped from the bottom end of a freshly-felled 58-yr-old Douglas-fir growing at Blackrock, Oregon The air-dried bark (moisture content 9 3%) was ground to pass a ½-in screen A portion (768 g dry wt) was extracted in a Soxhlet (52 hr) first with *n*-hexane (8 l), then the residue was extracted with benzene The resultant benzene solution was filtered while hot and concentrated *in vacuo* at 30° or less, the final traces of benzene were removed by freeze-drying, leaving the 'benzene wax', a light-brown wax-like solid (20 0 g)

Saponification and separation of acids from neutrals The 'benzene wax' was saponified (10% NaOH) by gentle refluxing (3 75 hr), cooled (0°) acidified, (glacial HOAc) and extracted with benzene The acids in the benzene solution were separated from the neutral compounds by extracting with 5% NaOH The benzene solution, containing neutral compounds, was washed (dil HOAc, followed by H_2O,) dried (Na_2SO_4) and concentrated *in vacuo* at 30° or less for qualitative TLC The acids were recovered by extraction (benzene) from the acidified NaOH extract Methyl esters of these acids were prepared (MeOH–HCl) for further separation by TLC

TLC Qualitative and preparative TLC of the neutrals and of the methyl esters was conducted on 500 μ layers of Silica Gel G with *n*-hexane–Et_2O (7 3 v/v) Spots were detected[7] with 0 2% 2′,7′-dichlorofluorescein and with I_2 vapors, in addition, reactions of the methyl esters and of the neutral compounds after TLC with the following steroid detection reagents were noted 85% phosphoric acid–H_2O (1 1 v/v), Liebermann–Burchard reagent, $SbCl_3$–glacial HOAc (1 1 w/w) Tentative identification of TLC components was made by comparison with simultaneously developed selected authentic compounds

GLC GLC was performed on Gas-Chrom Q (100/120 mesh) with (a) 1 5 m 4 75% SE-30, (b) 0 75 m 8 25% Apiezon-L, (c) 0 9 m 7 4% Reoplex-400, and (d) 1 8 m 11 1% EGSS-X Identification of GLC peaks was made by comparing $\log_{10}$ retention time of the 'unknown' peaks with a plot of chain- length vs $\log_{10}$ retention time prepared from authentic compounds Standard compounds were also added to the unknown samples for peak enhancement studies Peaks due to unsaturated compounds were located by comparing GLC's before and after treatment with bromine [8] Methyl ester abundance percentages for each TLC spot were estimated from GLC peak areas

GLC–MS Mass spectra (70 eV) of *spot* 2 peaks were obtained by tandem GLC (SE-30) with rapid-scan MS using an Atlas MAT CH-4 Nier type

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Key Word Index—Pseudotsuga menziesii, Pinaceae, Douglas-fir, *n*-fatty acids, α,ω-dicarboxylic acids