EXTRACTIVES FROM GRAND FIR [*ABIES GRANDIS* (DOUGL.) LINDL.] BARK[1]

I. H. Rogers
Department of the Environment, Canadian Forestry Service, Western Forest Products Laboratory, Vancouver, British Columbia

and

D. Grierson
Chemistry Department, University of British Columbia, Vancouver, British Columbia

(Received 23 December 1971)

ABSTRACT

The neutral fraction of the petroleum-ether-soluble bark extract contains a homologous series of normal alkanes (C_{19} to C_{42}), free β-sitosterol, behenyl and lignoceryl alcohols, esters of these three compounds with arachidic, behenic, and lignoceric acids, and a major fraction comprising a mixture of behenyl and lignoceryl ferulates. The triterpene lactones cyclograndisolide and epicyclograndisolide are minor components.

INTRODUCTION

As part of a continuing program of background research at the Western Forest Products Laboratory into the composition of the extractives of western Canadian wood species, we have examined the bark of a true fir, grand fir [*Abies grandis* (Dougl.) Lindl.]. Such studies may eventually assist in commercial exploitation of bark residues by virtue of the discovery of extractives with pharmacological application or of mixtures of extractives with sales potential for waxes, adhesives, etc. Compounds present as bark extractives probably play a significant role in the selection of suitable hosts by attacking bark beetles. These substances may help a tree to withstand severe climatic conditions, and their composition is also of interest to chemotaxonomists.[2]

Previous workers have isolated and structurally identified the triterpene abieslactone [I] present in the barks of amabilis fir [*Abies amabilis* (Dougl.) Forb.] and of *A. mariesii* Masters native to the mountains of northern Japan (Uyeo et al. 1968). It recently has been shown that structure I is incorrect (Kutney et al. 1971) in terms of the location of the double bond. As there are nine species of true firs native to Canada and the United States, and these are divisible into five taxa for botanical reasons (Smedman et al. 1969), it was of chemosystematic interest to examine the triterpene fraction of *A. grandis* bark, which is not in the same botanical subgroup as *A. amabilis*. The triterpene fraction of the petroleum-ether-soluble bark extract from *A. grandis* was donated to a group in the chemistry department of the University of British Columbia. They have reported (Allen et al. 1971) on the presence of two new triterpenes, cyclograndisolide [II a] and epicyclograndisolide [II b]. The present paper describes the chemical composition of the remainder of the petroleum-ether soluble neutrals fraction of *A. grandis* bark, after removal of the triterpene fraction. Preliminary work on this extract has been described (Rogers and Grierson 1969).

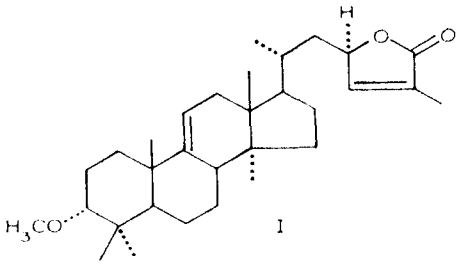
RESULTS

Hydrocarbons

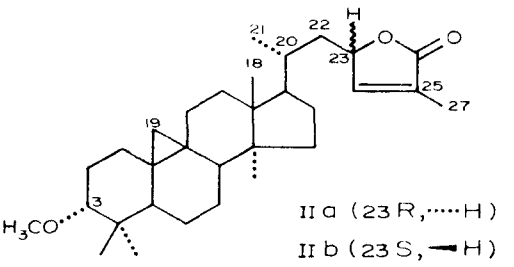
Normal and branched-chain alkanes were separated by the urea-inclusion-complex

[1]D. Grierson acknowledges with gratitude the financial support of a National Research Council of Canada summer studentship during the period of this research.

[2]The referees have pointed out, and the authors agree, that the results here reported are of limited value to chemotaxonomists because the bark from only one tree was examined and tree-to-tree variability was therefore not evaluated.



I



II a (23 R, ····H)
II b (23 S, —H)

technique using "active" urea (Rosenstein and Gorin 1957). Members of the homologous series of normal paraffins from C_{19} to C_{42} inclusive were detected with the weighted center of the distribution at C_{31}. Gas liquid chromatography (g.l.c.) of the branched-chain fraction showed four poorly defined unidentified peaks.

Wax esters

Recrystallization of the crude wax esters from acetone yielded a white wax. Methanolysis followed by acetylation of the unsaponifiable portion and analysis showed the presence of behenyl and lignoceryl alcohols in the ratio 1:2. The wax-acid methyl ester fraction was composed of arachidic (3%), behenic, (37%) and lignoceric (60%) acids. Esters of unsaturated wax acids were also undoubtedly present as oils, but were removed in the process of recrystallization.

Sterol esters

We were unable to separate fully the wax-ester fraction from the steroid esters by either chromatography or fractional recrystallization. Saponification and additional purification yielded β-sitosterol accompanied by the ubiquitous campesterol. No other sterols were detected. Once again only normal alkanoic acids were found, the composition of this fraction being palmitic (12%), stearic (2.5%), arachidic (6.1%), behenic (44%), and lignoceric (34%). Undoubtedly a portion of the latter two acids was contributed by the wax ester contaminant.

Wax alcohols

The free wax alcohols were identified by g.l.c. analysis of their acetate derivatives. The components present were behenyl (15%), lignoceryl (80%), and ceryl alcohols (5%).

Free sterols

Only β-sitosterol was isolated. Its identity was confirmed by m.p. and mixed m.p., quantitative i.r. (infrared spectroscopy) spectral comparison with an authentic standard, and from the n.m.r. (nuclear magnetic resonance spectroscopy) and mass spectral data.

Wax alcohol ferulates

By far the most plentiful component (circa 50%) of the neutral fraction of the extract was composed of a mixture of two alcohol ferulates. Saponification followed by g.l.c. analysis of the acetate derivatives identified behenyl (44%) and lignoceryl alcohols (56%). Ferulic acid was identified by m. and m.m.p. and quantitative i.r. spectral comparisons with an authentic standard. The mass-spectral fragmentation pattern and n.m.r. data were in harmony with this assignment. Wax alcohol ferulates have previously been isolated from the barks of other conifers, including members of the genera *Pseudotsuga, Larix, Abies,* and *Pinus* (Rowe et al. 1969). Since they have also been observed in two hardwood species, they are apparently widely distributed in tree barks.

EXPERIMENTAL

Preparative layer chromatography plates were prepared from silica gel G (E Merck)

TABLE 1. *Column chromatographic separation of brown wax from* A. grandis *bark*

	Fraction solvent volume (ml)	Weight (mg)	Components
A	petroleum ether (200)	690	hydrocarbons
B	" " (100)	228	hydrocarbons + wax esters
C	" " (200)	263	wax esters
D	" " (200)	450	wax + sterol esters
E	" " (500)	994	sterol esters + 2 unknowns
F	petroleum ether/benzene (9:1)(500) " " " (4:1)(400)	826	unidentified mixture
G	" " " (7:3)(400) " " " (1:1)(300)	1444	wax alcohols + triterpenes
H	" " " (2:3)(400)	909	wax alcohols + β-sitosterol
I	" " " (2:3)(300)	1016	β-sitosterol + wax alcohol ferulates
J	benzene (400)	1925	β-sitosterol + ferulic esters + 2 unknowns
K	ether (500) methanol (500)	5004	ferulic esters
	Total recovery	13.75 g	
	Total applied	26.70 g	

and either chloroform or methylene dichloride was used as developing solvent. The n.m.r. spectra were measured in deuteriochloroform with a Varian HA 100 spectrometer. Signal positions are quoted in terms of the Tiers' τ scale relative to tetramethylsilane as internal standard. Mass spectra were recorded at 70 ev on an AEI MS9 double-focusing mass spectrometer. Infrared spectra were prepared from KBr disks and were recorded on a Perkin Elmer 521 spectrophotometer with positions of absorption maxima quoted in wave numbers (cm^{-1}). Melting points were measured in a Mettler FPI automatic apparatus and are uncorrected. The g.l.c. analyses were performed on a Hewlett-Packard model 7620-A instrument equipped with an HP 3370-A electronic integrator. The columns were 6′ × ⅛″ O.D. stainless steel packed with either 3% JXR on 60–80 mesh Gas-Chrom Q or 10% EGSS-X on 100–200 mesh Gas-Chrom P.

Wax alcohol fractions were converted into their acetate derivatives by reaction with acetic anhydride/pyridine (1:1) at room temperature for 24 hr. Ice water was added to destroy excess reagent. The crude acetates were filtered off and washed through a short column of alumina in chloroform solution. The recovered acetates were analyzed by g.l.c. (3% JXR) and compared with the acetates of purchased standard alcohols (K and K labs.).

Preparation and fractionation of crude extract

Whole bark from a healthy 100-year-old specimen of *A. grandis*, growing on the University of British Columbia campus, was air-dried and ground in a Wiley mill to pass a 3-mm screen. A sample of the bark (61.7 grams oven-dry weight) was extracted for 24 hr in an all-glass soxhlet apparatus with petroleum ether (65–110°). A brown wax was recovered (1.480 g, yield 2.4%). This process was repeated several times on a larger scale. In a typical separation, a sample of wax (26.7 g) was applied to a column of deactivated alumina (400 g, activity III). Elution commenced with petroleum ether (65–110°), and fractions were recovered as summarized in Table 1. Free resin and fatty acids, and possibly other highly polar compounds, were irreversibly adsorbed on the alumina.

Hydrocarbon fraction

A portion of mixed fractions A and B (600 mg) was saponified for 8 hr in 5N KOH/ethanol under reflux conditions. The unsaponifiable fraction (327 mg) was applied to a column of 22% $AgNO_3$/silicic acid (15 g). Flushing with 100 ml petroleum ether yielded 116 mg of white, wax-like hydrocarbons. Treatment with 1 g "active" urea (Rosenstein and Gorin 1957) in benzene solution for three weeks at room temperature afforded a white crystalline complex, which was filtered, washed with cold benzene, and dried. The recovered washings contained the branched-chain alkanes.

The urea complex was twice treated with boiling toluene, filtered, washed with hot toluene, and the filtrate evaporated to recover the normal paraffins. Both fractions were dissolved in warm petroleum ether and washed through short columns of alumina (activity III). Yields of recovered normal and branched alkanes were respectively 42 mg and 57 mg.

Analysis of the normal alkanes by g.l.c. (3% JXR) and comparison with a purchased standard (#19254, Applied Science Labs.) indicated the presence of 24 members of the homologous series from C_{19} to C_{42} inclusive, with the weighted center of the distribution at C_{31}. The branched alkane fraction showed four unidentified peaks.

Wax ester fraction

Fraction C, a yellow wax, was recrystallized from acetone; negative Liebermann-Burchard test. $\nu_{max.}$ 1730 and 1250 (ester), 722 and 712 (long-chain methylene). Methanolysis (282 mg) in 5N KOH/methanol (6 ml) under refluxing conditions for 4 hr, followed by addition of excess 4% HCl/methanol and further refluxing for 90 min, yielded a crude product (123 mg) which was separated on a column of alumina (15 g, activity III). Elution with petroleum ether yielded a fatty acid methyl ester fraction (44 mg). Benzene/petroleum ether (3:1) recovered a wax alcohol fraction (27 mg). Analysis of the acetates (3% JXR) showed C_{22} and C_{24} in ratio 1:2.

The methyl ester fraction ($\nu_{max.}$ 1730 and 1255) was analyzed by g.l.c. (10% EGSS-X). Comparison with a purchased standard (K 107, Applied Science Labs.) showed the normal alkanoic acids C_{20} (3%), C_{22} (37%) and C_{24} (60%).

Sterol ester fraction

Fraction D gave a positive Liebermann-Burchard test. Fractional recrystallization failed to remove the wax ester contaminant. Accordingly the impure ester (238 mg) was saponified for 2 hr in 10% KOH/ethanol. Workup by partition of the reaction products between ether and the aqueous phase yielded a mixture of wax alcohols and sterols (78 mg) and a fatty acid fraction containing some wax alcohol (127 mg).

Separation of the sterols from wax alcohols was accomplished by preparative t.l.c. (CH_2 Cl_2). White crystals from MeOH, m. 139–140°C. $\nu_{max.}$ 3430 and 1050 (OH), 1640 and 795 (trisubstituted double bond). Identical with β-sitosterol standard on quantitative i.r. comparison. Diagnostic species at m/e 414 (M), 399 (M-15), 396 (M-18), 381 (M-33), 329 (M-85), 315 (M-99), 273 (M-141) and 255 (M-159); other abundant fragments at m/e 303, 289, 231, 213, 159, 149, 145 and 133. Electron bombardment at 13 ev showed campesterol (M^+ 400) mixed with β-sitosterol (M^+ 414).

The fatty acid fraction, after removal of unsaponifiables, was esterified with diazomethane and analyzed by g.l.c. (10% EGSS-X). Only normal alkanoic acids were detected; C_{16} (12%), C_{18} (2.5%), C_{20} (6.1%), C_{22} (44%) and C_{24} (34%).

Triterpene lactones

Fraction G consisted of a mixture of wax alcohols with two triterpene lactones of closely similar R_f. The layer chromatography comparison with an authentic standard showed neither of them to be abies-lactone. Attempts to separate the mixture on columns of deactivated alumina were

only partially successful. This material was further investigated in the chemistry department, University of British Columbia (Allen et al. 1971).

Wax alcohols

A pure wax alcohol fraction was available from a previous column separation of the crude bark extract on deactivated alumina and from fraction H by preparative t.l.c. White, waxlike crystals from acetone, m.p. 75–6°C. $\nu_{max.}$ 3400 and 1050 (OH). The n.m.r. 6.35 (triplet, CH_2OH), 8.75 (singlet, CH_3), 9.15 (triplet, CH_3). Major fragment at m/e 336 (M-18). No parent ion. Gas liquid chromatography analysis (3% JXR) showed C_{22} (15%), C_{24} (80%) and C_{26} (5%).

Free sterol

β-sitosterol was recovered from fractions H, I and J by preparative t.l.c. ($CHCl_3$). White crystals from MeOH, m.p. 139–140°C. $\nu_{max.}$ 3430 and 1050 (OH), 1640 and 795 (trisubstituted double bond). Identical with an authentic standard on quantitative i.r. spectral comparison. The n.m.r. 4.70 (1H, multiplet, olefinic), 6.50 (IH, multiplet, H–$\underset{|}{C}$–OH), 8.17 (singlet, OH), 9.01 and 9.34 (CH_3). Anal. Calcd. for $C_{29}H_{50}O$:M^+ 414.386147. Found:414.386934.

Phenolic acid ester

Fraction K yielded white, waxy crystals, m.p. 78°C from ethyl acetate. $\nu_{max.}$ 3550 (OH), 1705 and 1260 (ester), 1625 (conjugated double bond), 970 and 1310 (trans disubstituted double bond), 1595, 1585, 1500 and 1450 (aromatic).

Saponification (0.230 g) in 1.25 N NaOH/ethanol under reflux conditions for 2 hr yielded wax alcohols (53 mg) and a phenolic acid fraction. The wax alcohols gave white crystals, m.p. 74–6°, from petroleum ether. $\mu_{max.}$ 3500–3300 (OH). Major fragment at m/e 336 (M-18). The acetate derivative analyzed by g.l.c. (3% JXR) for C_{22} (44%) and C_{24} (56%).

The phenolic acid was purified by vacuum sublimation (twice at 135°C at 0.07 mm Hg). Yellow needles, m. and mixed m.p. 169–171°C from hot water. $\lambda_{max.}$ 234 nm (ϵ 7050), $\lambda_{shldr.}$ 296 nm (ϵ 6530) and $\lambda_{max.}$ 324 mm (ϵ 8430). $\nu_{max.}$ 3500–3250 (OH), 1690 (α,β unsaturated COOH), 1660, 1620 and 970 (conjugated trans double bond), 1600, 1587, 1500 and 1450 (aromatic). Quantitative comparison with authentic ferulic acid gave superimposable spectra. The n.m.r. (deuterioacetone) showed a pair of one proton doublets centered at 2.44 and 3.68 derived from the olefinic protons with the coupling constant ($J = 16$ Hz) indicative of a trans double bond, a one proton singlet at 2.76 (aromatic C_2), a pair of one proton doublets centered at 2.92 and 3.18 (aromatic C_6 and C_5, respectively) and a three proton singlet at 6.13 (OCH_3). Anal. Calcd. for $C_{10}H_{10}O_4$: C 61.85; H 5.19. Found: C 61·87; H 5·18.

REFERENCES

ALLEN, F. H., J. P. KUTNEY, J. TROTTER, AND N. D WESTCOTT. 1971. The structures and absolute stereochemistry of cyclograndisolide and epicyclograndisolide, novel triterpene lactones from *Abies grandis*. Tetrahedron Lett. 283–286.

KUTNEY, J. P., N. D. WESTCOTT, F. H. ALLEN, N. W. ISAACS, O. KENNARD, AND W. D. MOTHERWELL. 1971. The structure of abieslactone. Tetrahedron Lett. 3463–3466.

ROGERS, I. H., AND D. GRIERSON. 1969. Extractives from the bark of grand fir [*Abies grandis* (Dougl.) Lindl.]. Can. Dep. Fish. For., Bi-Mo. Res. Notes **25**(4):33–34.

ROSENSTEIN, L. AND M. H. GORIN. 1957. Treatment of a mixture of saturated and unsaturated fatty acids with expanded urea. U. S. Patent 2, 800, 466.

ROWE, J. W., C. L. BOWER, AND E. R. WAGNER. 1969. Extractives of jack pine bark: occurrence of cis- and trans- pinosylvin dimethyl ether and ferulic acid esters. Phytochem. **8**:235–241.

SMEDMAN, L. A., K. SNAJBERK, E. ZAVARIN, AND T. R. MON. 1969. Oxygenated monoterpenoids and sesquiterpenoid hydrocarbons of the cortical turpentine from different *Abies* species. Phytochem. **8**:1471–1479.

UYEO, S., J. OKADA, S. MATSUNAGA, AND J. W. ROWE. 1968. The structure and the stereochemistry of abieslactone. Tetrahedron **24**: 2859–2880.