

NRC Publications Archive Archives des publications du CNRC

The heartwood extractives of *Pinus banksiana* Lamb Von Rudloff, E.; Sato, A.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1139/v63-317>

Canadian Journal of Chemistry, 41, 9, pp. 2165-2174, 1963-09

NRC Publications Archive Record / Notice des Archives des publications du CNRC :

<https://nrc-publications.canada.ca/eng/view/object/?id=df95058c-7653-4f15-bc53-7de8a452279e>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=df95058c-7653-4f15-bc53-7de8a452279e>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

THE HEARTWOOD EXTRACTIVES OF *PINUS BANKSIANA* LAMB.¹

E. VON RUDLOFF AND A. SATO²

National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan

Received April 22, 1963

ABSTRACT

The heartwood of jack pine was analyzed by various chromatographic techniques and countercurrent distribution. The major components were found to be isopimaric, abietic, dehydroabietic, and neobietic acids, as well as glycerides of oleic, linoleic, and linolenic acids. Smaller amounts of pimaric, sandaracopimaric, myristic, palmitic, stearic, palmitoleic, and four unidentified acids were recorded, as well as fatty acid esters of β -sitosterol, α -terpineol, and four unidentified volatile alcohols. Of the phenols, pinocembrin, pinobanksin, and pinosylvin monomethyl ether predominated. Pinosylvin was isolated and several minor constituents including lignan-like compounds were detected in the acetone extract. The methanol extract was composed mainly of polymeric lignan-like fractions. From one of these a trimer, $C_{60}H_{66}O_{24}$, m.p. 186–188° C, $[\alpha]_D +3.2^\circ$, was isolated. The small amount of steam-volatile material consisted mainly of α - and β -pinene, benzoic acid, and α -terpineol. Traces of methyl benzoate, camphene, limonene, β -phellandrene, *cis-p*-menthan-8-ol, and nine unidentified trace components were recorded.

INTRODUCTION

Jack pine (*Pinus banksiana* Lamb.) is a medium- to large-sized conifer found from Nova Scotia to northern British Columbia and the MacKenzie River valley (1). This pine is used extensively in the lumber industry and in Kraft pulping. In 1931 Hibbert and Phillips (2) made a thorough study of the resins found in green and seasoned jack pine wood and reported the presence of abietic, pimaric, oleic, and linoleic acids as well as smaller amounts of linolenic acid, saturated fatty acids, resenes, phytosterol, and α -pinene. More recently, Buchanan *et al.* (3) confirmed that oleic and linoleic acids are the major fatty acids, and found, in addition to the above, small amounts of lignoceric acid, these fatty acids occurring mainly as triglycerides. Erdtman (4) isolated from the heartwood the two flavanones pinocembrin and pinobanksin and the hydroxystilbene pinosylvin monomethyl ether. Lindstedt and Misiorny (5) confirmed the presence of these three polyphenols by paper chromatography and found also pinosylvin and three unidentified minor constituents. Haagen-Smit *et al.* (6) investigated the volatile oil from the oleoresin of jack pine and reported the major constituents to be *dl*- and *l*- α -pinene (ca. 85%) and *l*- β -pinene (ca. 10%).

These various studies have shown that the composition of the wood extractives of jack pine is very complex and that separation into individual components or groups of related compounds by the conventional techniques is very tedious and only partially successful. Furthermore, procedures which involve heating in alkaline or acidic media undoubtedly lead to some isomerization and polymerization (2). No complete analysis of the heartwood extractives with more modern techniques appears to have been published. In the present study an attempt was made to obtain as comprehensive as possible an analysis by employing countercurrent distribution (7) and chromatographic techniques.

The heartwood was milled and extracted first with acetone and then with methanol. These extracts were analyzed as shown in the flow sheet (Fig. 1). In a separate experiment the steam-volatile components (0.05%) were recovered and analyzed by gas-liquid chromatography (GLC) (8, 9). The major components were found to be α - and β -pinene,

¹Issued as N.R.C. No. 7445.

²National Research Council of Canada Postdoctoral Fellow, 1961–1963.

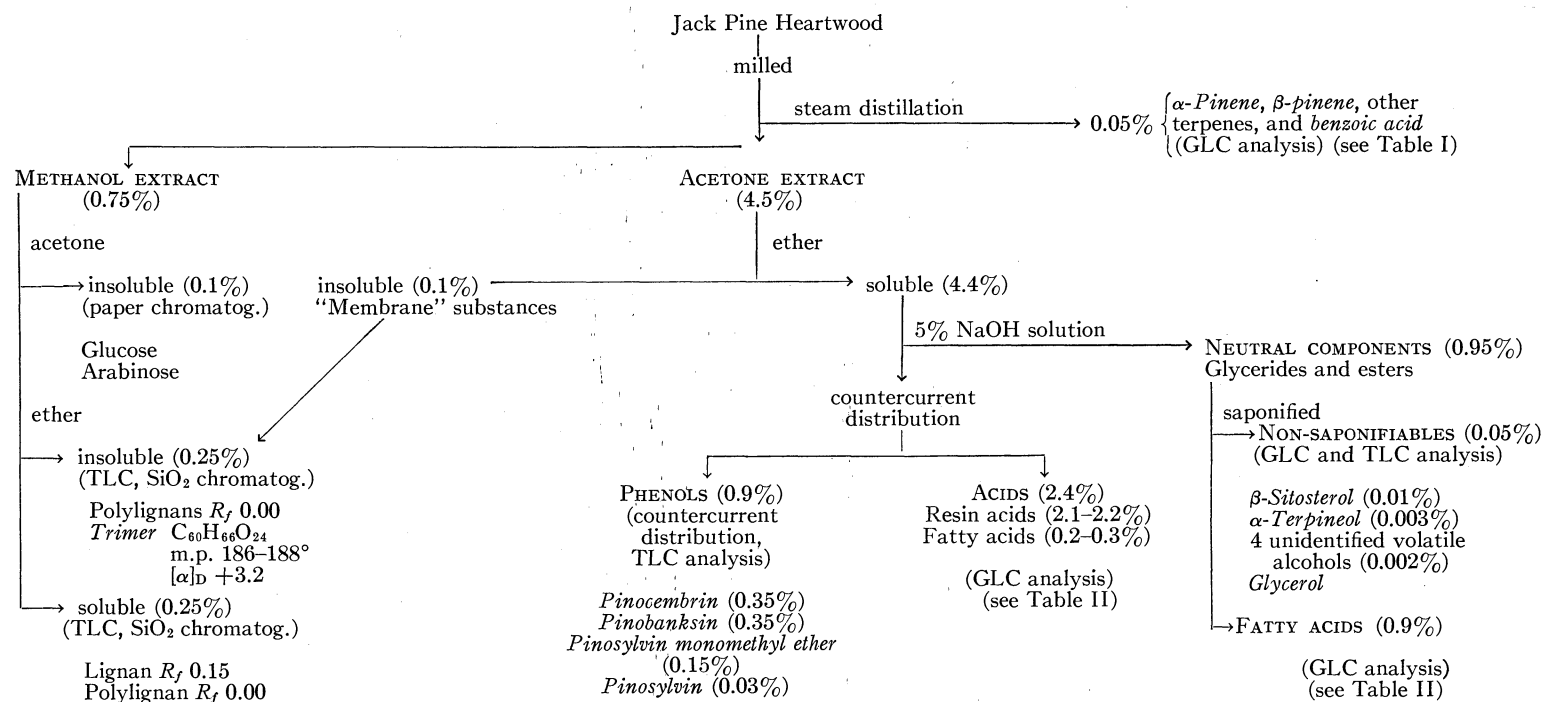


FIG. 1. Fractionation scheme for the wood extractives of jack pine.

confirming the findings of Haagen-Smit *et al.* (6). Thus, jack pine could be used for turpentine production, although the yield is low. In addition to the pinenes, smaller amounts of α -terpineol and benzoic acid were isolated, and camphene, limonene, β -phellandrene, *cis*- p -menthan-8-ol, and methyl benzoate were tentatively identified by retention characteristics (see Table I). Nine unidentified trace components were also

TABLE I
Composition of steam-volatile fraction from jack pine wood

Compound	Percent	RRT*
(a) Hydrocarbons†		
α -Pinene	46	0.29
(Camphene)	2	0.42
β -Pinene	28	0.55
(? Myrcene)	1	0.78
(Limonene)	1	1.00
(β -Phellandrene)	1	1.05
(? γ -Terpinene)	Trace	1.43
Unidentified	Trace	1.70
(b) Oxygenated‡		
Unidentified	0.5	0.90
Unidentified	1.5	1.05
(<i>cis</i> - p -Menthan-8-ol)	2	1.33
Unidentified	1.5	1.65
Unidentified	3	1.86
Unidentified	3	2.06
α -Terpineol	7.5	2.24
Unidentified	2	2.47
(Methyl benzoate)	Trace	1.57
(c) After treatment with diazomethane		
Methyl benzoate	10	1.57

NOTE: Names in parentheses refer to compounds identified by retention times only.
*RRT = relative retention time on 6 ft \times 1/4 in. polyethylene glycol column (PEG 20 M).

†Hydrocarbons at 65° C (limonene = 1.00).

‡Oxygenated monoterpenes at 120° C (camphor = 1.00).

recorded in the monoterpene range, but α -terpinyl acetate, propionate, or sesquiterpenoid constituents were not detected.

The acetone-soluble portion (4.5%) of the wood was divided into petrol- and ether-soluble material. Only a small amount (see Fig. 1) was insoluble in these two solvents (so-called "membrane" substances (4)). Initially, fractionation into neutral, phenolic, and acidic constituents was attempted by extraction with aqueous alkali of different strength. However, the separation into distinct groups of compounds was incomplete, and the material was, therefore, divided only into neutral and total acidic material. The neutral components were almost exclusively esters, and after saponification β -sitosterol, α -terpineol, four unidentified steam-volatile alcohols, and a mixture of long-chain fatty acids were isolated (see Fig. 1). Glycerol, the major non-saponifiable constituent, was identified by GLC as the triacetate. The fatty acid mixture was analyzed qualitatively and quantitatively by GLC (10) after conversion to the methyl esters. The quantitative data (see Table II) confirm that oleic and linoleic acids are the major acidic constituents of the glycerides (3), but appreciable amounts of linolenic acid were also recorded. Lignoceric acid was not detected; instead a number of minor acids, including three unidentified ones, were recorded in the C₁₄ to C₂₀ range. When an aliquot of the neutral material was chromatographed on a column of silicic acid, a partial separation of the triglycerides from the β -sitosteryl esters was obtained, some of the latter being obtained as waxy material melting in the 65 to 80° range (see Experimental).

TABLE II

Composition of the resin and fatty acid mixtures and relative retention times (methyl arachidate = 1.00) of methyl esters on various GLC columns

Methyl ester	Composition		Relative retention times			
	Combined acids (%)	Free acids (%)	GLC column:*			
			A	B	C	D
Benzoate	—	—	0.015	—	—	—
Laurate	—	—	0.04	—	—	—
Myristate	Trace	—	0.13	—	0.16	0.13
Palmitate	4	—	0.20	0.20	0.30	0.25
Palmitoleate	Trace	—	0.185	0.24	0.32	0.29
Unidentified	Trace	1	0.27	—	—	—
Stearate	2	—	0.45	0.46	0.50	0.50
Oleate	27	3	0.41	0.49	0.58	0.525
Linoleate	38	2	0.37	0.53	0.63	0.62
Linolenate	22	1.5	0.34	0.55	0.68	0.75
Unidentified	Trace	—	0.53	—	—	—
Unidentified	5	—	0.72	—	—	—
Behenate	—	—	2.30	—	—	2.01
Lignocerate	0	—	4.75	—	—	3.98
Arachidate	0	—	1.00	1.00	1.00	1.00
(? Palustrate)	—	0.5	—	—	—	—
Pimarate	—	4	0.81	0.93	0.71	1.27
Sandaracopimarate	—	2	0.87	0.99	0.80	1.53
Isopimarate	—	33	1.06	1.30	0.94	2.02
Levopimarate	—	0	1.20	1.83	1.28	1.96
Dehydroabietate	—	15	1.20	1.80	1.29	3.14
Abietate	—	24	1.50	2.04	1.50	3.14
Neoabietate	—	11	1.76	—	—	3.80

*A: Apiezon N (5% on Anakrom ABS 60-70 mesh) at 210° C. B: Neopentyl glycol adipate polyester (1% on Chromosorb W 60-80 mesh) at 190° C. C: QF-1 fluorinated silicone polymer (5% on Anakrom ABS) at 185° C. D: Polyethylene glycol 20 M (3% on Anakrom ABS) at 190° C.

Initially the phenolic (and acidic) fractions were analyzed by paper chromatography, using Lindstedt's technique (5, 11). However, it was found that the material from jack pine streaked and that R_f values fluctuated excessively. Employing different solvent systems did not give improved results and thin-layer chromatography (TLC) (12) was investigated. Using silica-gel G and chloroform-acetic acid (9:1) or toluene-dioxane-water (1:1:1) satisfactory separation and well-defined spots were obtained with a variety of polyphenols, acids, and lignans (see Table III). R_f values were reproducible within narrow limits when measured relative to a reference compound (e.g. chrysin). This technique also gave satisfactory results for the analysis of the phenolic heartwood constituents of jack pine (cf. Fig. 2). However, resin and fatty acids were not resolved sufficiently. Since methyl esters of fatty acids are conveniently and accurately analyzed by GLC (cf. e.g. ref. 10), this technique was employed for identification and estimation of acidic constituents. Hudy (13) has reported satisfactory separation of the methyl esters of a number of resin acids using Apiezon N and polyester GLC columns. However, the use of temperatures of 225° C and higher resulted in a partial isomerization of methyl levopimarate and palustrate to methyl abietate and dehydroabietate. In the present study, other liquid phases were investigated, and by employing a higher ratio of solid support to liquid phase, operating temperatures were reduced sufficiently to reduce isomerization to a negligibly small amount. Relative retention times (RRT) were measured with respect to methyl arachidate (see Table II), which was also used as internal standard for the quantitative data. The results obtained show the major acidic constituents to be isopimaric

TABLE III

 R_f values of lignans, polyphenols, β -sitosterol, and some acids on thin-layer chromatograms*

Compound	Chloroform - acetic acid (9:1 v/v)	Toluene-dioxane-water (1:1:1)	Color with acidic KMnO_4 reagent
Polylignan (unknown 1)	0.00	0.00	Brown
Olivil	0.05	0.06	Violet-brown
Isolariciresinol	0.09	0.11	Pink
Secoisolariciresinol	0.10	0.17	Brown
Unknown 2	0.15	0.21	Brown
Lariciresinol	0.18	0.23	Brown
Unknown 3	0.32	0.42	Brown
Pinosylvin	0.37	0.46	Brown
α -Conidendrin	0.49	0.38	Pink
Pinoresinol	0.49	0.42	Grey-brown
Unknown 4	0.50	0.46	Brown
Pinobanksin	0.56	0.59	Yellow
Chrysin	0.57	0.61	Yellow
Unknown 5	0.60	0.50	Brown
Pinocembrin	0.68	0.69	Yellow
Pinosylvin monomethyl ether	0.71	0.72	Yellow-brown
β -Sitosterol	0.76	0.56	Brown
Abietic acid	0.82	0.68	Brown
Stearic acid	0.83		Weak
Oleic acid	0.85	0.51	Light yellow
Pinostrobin	0.84	0.64	Yellow
Pinosylvin dimethyl ether	0.86	0.67	Yellow-brown
Benzoic acid	0.86		None

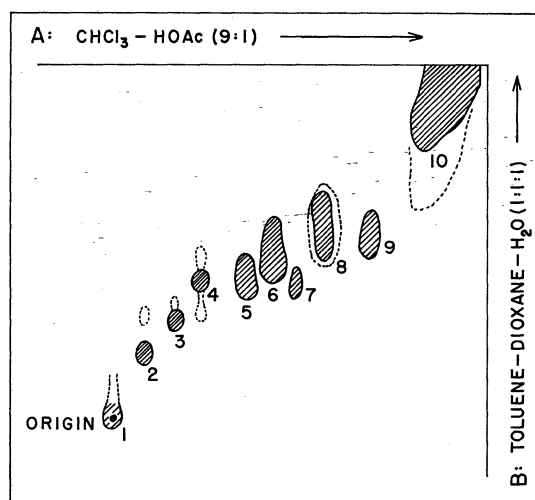
*Thirty minutes ascending, on silica gel G (250 μ thick).

FIG. 2. Two-dimensional thin-layer chromatogram of phenolic and acidic jack pine heartwood extractives.

- | | |
|----------------------------------|--------------------------------|
| 1. Unknown 1 (polylignan) | 6. Pinobanksin |
| 2. Unknown 2 (lignan R_f 0.15) | 7. Unknown 5 |
| 3. Unknown 3 | 8. Pinocembrin |
| 4. Pinosylvin | 9. Pinosylvin monomethyl ether |
| 5. Unknown 4 | 10. Resin acids etc. |

and abietic acids, with lesser amounts of dehydroabietic, neoabietic, pimaric, sandaracopimaric, and unsaturated fatty acids also being recorded (see Table II). A fairly large

peak of RRT 1.00 was also recorded, and, since no arachidic acid was detected in the mixture of acids from the triglycerides, this component could be palustric acid (13).

Countercurrent distribution (7) was found to be a suitable method for separating the phenolic components from resin and fatty acids. The major phenolic constituents were fractionated sufficiently to permit estimation and isolation when 200 transfers were employed. Pinocebrin and pinobanksin were found to be the major polyphenols, smaller amounts of pinosylvin monomethyl ether and pinosylvin also being isolated (see Fig. 1). TLC indicated that five other minor constituents were present. Of these, two (R_f 0.00 and 0.15) were lignan-like constituents found in larger amounts in the methanol extract (see below). No pinosylvin dimethyl ether, dihydropinosylvins, or chrysin was detected. These findings are in good agreement with the work of Erdtman (4) and Lindstedt and Misiorny (5). The unidentified compound F described by the latter authors may have been benzoic acid, but their compounds D and E could not be correlated with any of the above minor components. The ether-insoluble portion of the acetone extract ("membrane" substances) had R_f 0.00 (TLC) and was very similar to the major fraction obtained from the methanol extract.

The methanol extract (0.75%) was divided into ether-soluble and -insoluble material. The former was chromatographed on silicic acid and elution with chloroform gave a trace of the compound with R_f 0.15. Its infrared spectrum was very similar to that of lariciresinol (R_f 0.18), but the melting point (135.5–137° C) and molecular weight (573) differed from those of this lignan. Elution of the chromatogram with more polar solvents did not yield other crystalline components. All fractions appeared to contain lignan-like material of higher molecular weight. The ether-insoluble portion was taken up in hot acetone and the insoluble carbohydrate material was filtered off. Paper chromatography of the latter gave spots corresponding to glucose and arabinose. Erdtman (14) reported arabinose to be a constituent of jack pine and other pine species. The acetone-soluble portion was fractionated by crystallization and column chromatography, when several sharp-melting, amorphous fractions were obtained. One of these (m.p. 164–165° C, R_f 0.00) gave on recrystallization a small amount of a polylignan, $C_{60}H_{66}O_{24}$, m.p. 186–188° C, $[\alpha]_D +3.2^\circ$, $\lambda_{\max}^{\text{MeOH}}$ 281 $m\mu$, which had an infrared spectrum resembling very closely that of lariciresinol. Acetylation gave an acetate, m.p. 101–102.5° C, $[\alpha]_D -1.0^\circ$, but methylation gave intractable material. It is noteworthy that the oxygen content of this polylignan is 4 atoms per phenylpropanoid unit as compared with 3 in lignans of the lariciresinol type. Recently Freudenberg (15), in discussing a soluble oligomeric lignan-like material which Brauns (16) found in spruce wood (native lignin), pointed out that alcoholic solvents may react with lignin during isolation and that alcohols or sugars may add to dehydration polymerizates during lignin formation. Similarly, the higher oxygen content of the polylignan isolated in the present study suggests that the material may be the product of the addition of methanol to a reactive lignan-like compound. Two other fractions having similar properties, but with melting points of 124–125° C and 148–151° C, were also isolated. The nature and the source of these polylignans will be investigated further.

Benzoic acid and its methyl ester are not considered to be typical pine heartwood extractives (14). It is possible that these are artefacts, produced for example by oxidative degradation of the pinosylvins or flavanones. From a crude sample of pinobanksin, which had been standing several months, benzoic acid was isolated. However, benzoic acid was found in the steam-volatile fraction, and if it is a degradation product, it must have been produced during the milling or steam distillation of the wood.

EXPERIMENTAL

Melting points were determined with a Leitz hot-stage microscope. Countercurrent distribution was carried out with a modified 100-tube E.C. Apparatus Co. instrument (20-ml volume lower phase, 5- to 30-ml variable upper phase for each tube). Gas-liquid chromatograms were obtained with an instrument of conventional design (10), having a thermal conductivity cell as detector and using helium as carrier gas. A 1-mv Honeywell recorder was used with a chart speed of 0.5 in./min. Columns were made of 6 ft \times 1/4 in. (6 mm) O.D. copper or stainless steel tubing. The liquid phase was applied to the solid support (Chromosorb W 60-80 mesh) by means of the tray method in the ratio of 1 to 6, unless otherwise stated. Individual fractions were collected at the heated outlet in glass traps which were externally cooled. Infrared spectra were recorded with a Perkin-Elmer Model 21 double-beam spectrophotometer, liquids being mounted as films between sodium chloride plates and solids as KBr disks. Molecular weights were determined by means of a Mechrolab Model 301 osmometer.

Paper-partition Chromatography (PPC)

Initially, Lindstedt's technique (5, 11) was used for the separation of phenols. However, it was found that with benzene-ligroin-methanol (50:50:1 v/v) saturated with water as solvent R_f values fluctuated and spots streaked frequently even when the paper (Whatman No. 4) was allowed to equilibrate with the solvent in the chamber for 8 hours. R_f values (measured at the top of the spot) for pinosylvin, its monomethyl ether, pinobanksin, pinocembrin, and chrysin varied from 0.08 to 0.15, 0.71 to 0.84, 0.13 to 0.21, 0.35 to 0.44, and 0.15 to 0.25 respectively in different runs. The spray reagent was diazotized benzidine and ultraviolet fluorescence or absorption was also used. With some of the phenolic fractions from jack pine heartwood extractives excessive streaking was obtained. Other solvent systems, including *n*-butanol - acetic acid - water (4:1:1.8), chloroform - acetic acid - water (13:6:1), benzene-ligroin-methanol (5:5:1), and benzene-ligroin-acetic acid - methanol - water (5:5:1:1:5, upper phase), were tried without marked improvement.

Sugars were chromatographed on Whatman No. 1 paper using *n*-butanol - acetic acid - water (4:1:5, upper phase) and *n*-butanol-pyridine-water (3:3:1) as solvent and aniline phthalate as spray reagent. The R_f values for glucose, arabinose, and xylose were 0.12 and 0.16, 0.18 and 0.21, and 0.19 and 0.27 respectively.

Thin-layer Chromatography (TLC)

To solve the above difficulties with phenolic constituents TLC according to Stahl (12) was tried. Well-defined spots and good resolution were obtained on silica gel G (Merck, Darmstadt, Germany) and either chloroform - acetic acid (9:1) or toluene-dioxane-water (1:1:1, upper phase) as developing solvent. Phenolic compounds were detected by use of ultraviolet light, diazotized benzidine, or $\text{FeCl}_3\text{-K}_3\text{Fe}(\text{CN})_6$ (1% aqueous solutions, 1:1) spray. Equally good separation was obtained with some lignans and the technique could also be used for the detection of resin acids, fatty acids, β -sitosterol, and benzoic acid. The spray reagents used were: 0.1 M KMnO_4 and 10% H_2SO_4 (1:3, heated 5 minutes at 105° C), rhodamine B (0.05% in 10% aqueous acetone), and antimony trichloride (0.05% in chloroform). The glass plates were 5 \times 20 cm or 13 \times 13 cm in dimension, the support was applied in about 250- μ thickness and development time was about 30 minutes, when the solvent had travelled about 10 cm. R_f values for individual compounds varied by about 0.05 unit between different runs, but when measured with respect to a reference compound (e.g. chrysin) they were constant to within ± 0.02 unit. The R_f values of lignans, polyphenols, some acids, and β -sitosterol, as well as those of the unidentified components of the jack pine heartwood extractives, are shown in Table III. A two-dimensional chromatogram obtained with phenolic and acidic components from the jack pine wood extract is shown in Fig. 2.

Gas-Liquid Chromatography (GLC)

Steam-volatile components were analyzed by the GLC technique described by one of us for the analysis of terpenes (8) and essential oils (9, 17). Monoterpene hydrocarbons were analyzed at 65° C (flow rate 120 ml/min) on polyethylene glycol 20 M (PEG 20 M) and adipate polyester (APEG, NGA) columns. Relative retention times (RRT) were measured with respect to limonene. Oxygenated monoterpenes and methyl benzoate were chromatographed on the same columns at 120° C and RRT values were measured with respect to camphor. When separation was incomplete, analysis was also carried out on ethylene glycol bis(propionitrile) or QF-1 fluorinated silicone polymer columns. The percentage composition was obtained by the triangulation method, the error being $\pm(1-3)\%$ for large- to medium-sized peaks and $\pm(3-10)\%$ for medium- to small-sized ones.

Free fatty acids were methylated with freshly prepared diazomethane and the resulting methyl esters were analyzed by GLC at 160-190° C on Apiezon N (5% on Anakrom ABS, 60-70 mesh), QF-1 (5% on Anakrom ABS 60-70 mesh), neopentyl glycol adipate polyester (NGA, 1% on Chromosorb W, 60-80 mesh), and polyethylene glycol (PEG 20 M, 3% on Anakrom 60-70 mesh) columns. Resin acids were similarly methylated, but in the presence of some methanol in order to ensure complete esterification. A mixture of abietic acid (48%) and arachidic acid (52%) methylated in this manner gave peaks (at 185° to 210° C) which had corresponding areas ($\pm(2-3)\%$) on the Apiezon N, QF-1, and SE-30 columns. On the polyester and

polyethylene glycol columns the area of the peak recorded for methyl abietate was 5 to 15% smaller than the theoretical value. Therefore, all quantitative data were calculated from data recorded on the former columns. For methyl esters of fatty acids correction factors for acids of different chain length were required. Taking methyl arachidate as 1.00, the factors for stearate, oleate, linoleate, and palmitate were 0.95, 0.96, 0.96, and 0.90 (± 0.02) respectively. All retention times were measured with respect to methyl arachidate and the various RRT values obtained are shown in Table II.

Extraction of the Wood and Preliminary Fractionation

The logs of jack pine wood came from the Candle Lake district of Northern Saskatchewan.* The bark and sapwood was removed and the heartwood was milled in a hammer and a Wiley mill. The air-dried wood (5.0 kg) was extracted with acetone (15 liters) in a Soxhlet extractor for 30 to 36 hours. Excess solvent was removed by evaporation *in vacuo* on a rotatory evaporator. The residual wood meal was extracted similarly with methanol.

The brown, viscous residue from the acetone extract (226 g, 4.5%) was poured with stirring into petrol (b.p. 40–60° C; 2 liters), and after decantation of the petrol solution and washing with fresh solvent (2 × 250 ml), the insoluble residue (80 g, 35.4%) was taken up in a minimum of acetone and poured similarly into ether (2 liters). The ethereal solution was filtered to remove the insoluble precipitate ("membrane" substances, 4.3 g, 1.9%).

When small aliquots of the petrol and ether solutions were extracted successively with saturated sodium bicarbonate, 5% sodium carbonate, and 5% sodium hydroxide solution, the separation into neutral, phenolic, and acidic components was incomplete (TLC analysis). The insoluble resin acid salt layer (4), after acidification and chromatography on silicic acid gave a number of fractions with sharp melting points (0.5 to 1.5° spread) in the 70 to 90° C range. Subsequent analysis showed these to be mixed crystals of resin acids, fatty acids, and esters (see also below). A trace (1.5 mg) of a white, crystalline compound, m.p. 199.5–200° C, was isolated from the first fraction eluted with chloroform. The infrared spectrum had strong adsorption bands at 3400, 1730, 1268, 1200, 1080, 1065, and 700 (triplet) cm^{-1} and medium-intensity ones at 1600, 1508, 1453, 1023, 968, 880, 800, and 752 cm^{-1} , indicating that the compound may be an aromatic ester having free hydroxyl groups. This compound was not isolated again by the techniques described below.

In subsequent work the petrol- and ether-soluble material was extracted exhaustively with aqueous sodium hydroxide solution to remove all acidic and phenolic components. The ether layer was washed with water, dried (Na_2SO_4), and evaporated to dryness to give the neutral constituents (47.8 g, 21.2%; 15.2% from the petrol-soluble portion). The aqueous alkaline solutions and washings were combined, acidified, and extracted with ether to give the combined acidic and phenolic constituents (171.2 g, 75.7%; 46.3% from the petrol-soluble portion).

Steam-volatile Components

Fresh wood meal (200 g) was steam-distilled for 2 hours and the distillate was extracted with ether. After drying, the solvent was evaporated on a steam bath, using a small flask having a long neck. The residual oil (0.10 g, 0.05%) contained about 3% ether and had n_D^{25} 1.4680, $[\alpha]_D^{25}$ -18.2° (c , 1.2, CHCl_3). Aliquots (1–10 μl) were analyzed by GLC for monoterpenes as described above. The average composition is shown in Table I. The identity of α -pinene, β -pinene, and α -terpineol was confirmed by isolation and comparison of infrared spectra. The optical rotation of both pinene fractions was negative.

GLC analysis at higher temperatures (120–180° C) showed no peaks corresponding to α -terpinyl acetate or propionate, nor were any peaks in the sesquiterpene range recorded. When the steam-volatile product was treated with diazomethane a substantial peak (about 10%) corresponding to methyl benzoate (RRT 1.57) was observed. The presence of free benzoic acid was confirmed when steam distillation of the acidic portion of the wood extract gave crystalline benzoic acid, m.p. 117.5–120° C, which was undepressed on admixture with an authentic sample (m.p. 120–121° C). Steam distillation of the neutral portion of the acetone extract gave a very small amount of an oil similar in composition to the one obtained by direct steam distillation of the wood.

Countercurrent Distribution

An aliquot of the acetone extract (4.0 g) was distributed equally between the first four tubes of the apparatus, using 70% aqueous ethanol equilibrated with petrol (b.p. 60–70° C) as lower and upper phase (20 ml each per tube). After applying 100 transfers, the contents of each tube were withdrawn, the solvent evaporated, and the residual material weighed. By plotting weight against tube number the distribution curve was obtained. Tubes 0 to 25 contained about 20% of the extract, which was distributed in two overlapping peaks. Tubes 26 to 68 were practically devoid of material, whereas tubes 69 to 95 contained the balance of the extract. From tubes 79 and 82 crystalline material of m.p. 87–88° C and 73–74° C was obtained. The infrared spectra resembled those of β -sitosterol esters, with free acids as contaminants. Removal of the acidic material (resin acids, GLC analysis) gave waxy ester fractions, m.p. 70–74° and 67–72° C respectively, which were saponified. β -Sitosterol, m.p. 135–137° C (TLC R_f 0.76), was isolated from the non-saponifiable portion, whereas GLC analysis showed the saponifiable portion to contain a mixture of C_{18}

*Identified and supplied by Dr. W. B. Denyer, Department of Forest Pathology, Forestry Department, Saskatoon.

fatty acids (see also below). TLC analysis of the material in tubes 0 to 25 showed it to be composed of pinocembrin, pinobanksin, pinosylvin monomethyl ether, and several unidentified phenolic components.

From this experiment it was concluded that a fairly low number of transfers would suffice to separate the phenolic constituents completely from resin acids and neutral components, but that a very high number of transfers would be required to obtain fractionation into individual components. Since the neutral and acidic components were analyzed more conveniently by other techniques, only the phenolic constituents were fractionated by this means (see below).

Neutral Components

The infrared spectrum of the neutral portion indicated that it was composed mainly of esters. An aliquot (20 g) was saponified by heating under reflux with sodium hydroxide solution (5% in 80% aqueous ethanol) for 2 hours. After diluting with water, the non-saponifiables (0.2 g) were extracted with ether. GLC analysis showed the presence of five volatile components with RRT (camphor = 1.00, PEG 20 M column at 120° C) values of 0.88, 1.31, 1.90, 2.08, and 2.24 in the area ratios of 2:5:1:1:24. These RRT values correspond to those of some of the oxygenated terpenes found in the steam-volatile fraction (Table I). The component with RRT 2.24 was isolated and its infrared spectrum agreed in all respects with that of α -terpineol. The peak at RRT 1.31 may correspond to *cis-p*-menthan-8-ol. TLC analysis showed the non-volatile component to be β -sitosterol (R_f 0.76). The non-saponifiable material was steam-distilled and the volatiles were extracted with ether. On concentrating the ether solution a small amount of crystalline β -sitosterol, m.p. 136–137° C, was obtained. The soluble material was fractionated by GLC and the major component with RRT 2.24 was isolated pure in small amounts. The infrared spectrum agreed with that of α -terpineol in all respects. From the non-volatile portion (0.12 g) crystalline β -sitosterol, m.p. 135–136° C, $[\alpha]_D^{25}$ -24.2° (*c*, 2.5, CHCl_3), was obtained after recrystallization from ethanol. TLC analysis gave a single spot of R_f 0.76. The acidic components (17 g) were recovered from the saponification mixture in the usual manner. After methylation with diazomethane these were analyzed by GLC. The average composition is shown in Table II. The residual aqueous solution was deionized by treatment with ion-exchange resin and evaporated to a sirupy residue (3 g). An aliquot was acetylated and the ether-soluble product was analyzed by GLC (PEG 20 M column at 160° C), when a peak corresponding to glycerol triacetate, RRT 7.07 (camphor = 1.00), was recorded.

An aliquot (1.0 g) of the neutral portion was chromatographed on a column of silicic acid (25 g). Elution with chloroform gave two waxy fractions (mg amounts) having m.p. 67–72° C and 78.5–82° C respectively. The infrared spectra resembled that of β -sitosteryl stearate. Saponification gave β -sitosterol (TLC R_f 0.75) and a mixture of oleic, linoleic, and stearic acids (GLC).

Acidic Constituents (77.6%)

The total acidic material was fractionated into phenolic and acidic components by countercurrent distribution. Applying 50 transfers with 75% aqueous ethanol-petrol (b.p. 60–70° C) as solvent pair, an aliquot (5 g) was resolved into phenols (1.3 g; tubes 0 to 18) and resin and fatty acids (3.6 g; tubes 26 to 49).

Aliquots of the free acids were taken up in a mixture of ether and methanol (1:1) and were methylated with excess diazomethane solution. The average composition of the mixture of methyl esters as determined by GLC analysis is shown in Table II.

The phenolic material was analyzed by two-dimensional TLC (see Fig. 2). This, together with earlier paper chromatograms, showed that the major components were most likely pinocembrin, pinobanksin, and pinosylvin monomethyl ether. Spots corresponding to pinosylvin and five unidentified minor components were also detected (see Table III). To obtain an estimate of the relative amounts and to isolate pure components, an aliquot (5 g) was fractionated in a 200-transfer countercurrent experiment, using 75% aqueous ethanol-petrol (b.p. 60–70° C) as solvent pair. The distribution curve showed two main peaks with maxima at tube 6 ($K = 0.031$) and tube 18 ($K = 0.099$) respectively, and shoulders at tubes 2, 9, and 28. Calculation of theoretical curves (7) gave values of about 35% for the two major components, about 20% for that giving a shoulder at tube 28, and about 3% each for the other two components. Using TLC, these were tentatively identified as pinobanksin, pinocembrin, pinosylvin monomethyl ether, pinosylvin, and an unknown respectively. From tubes 5 to 7, pinobanksin, m.p. 177–180° C (from ethanol), $[\alpha]_D^{25}$ -6.4° (*c*, 1.1, MeOH), was obtained; from tubes 16 to 20, pinocembrin, m.p. 203.5–204° C, $[\alpha]_D^{25}$ -16.9° (*c*, 1.0, CHCl_3); and from tubes 27 to 30, pinosylvin monomethyl ether, m.p. 112.5–114° C. When pinobanksin was crystallized from aqueous solvents, it was obtained as the monohydrate, m.p. 174.5° C. Found: C, 61.60; H, 4.75%. Calculated for $\text{C}_{15}\text{H}_{12}\text{O}_5 \cdot \text{H}_2\text{O}$: C, 62.10; H, 4.86%. Pinosylvin was obtained as mixed crystals with pinobanksin from tubes 1 to 3, m.p. 156–170° C. Column chromatography on silicic acid gave practically pure pinosylvin, m.p. 154–156° C. The identity of each compound was confirmed by mixed melting points and comparison of infrared spectra. Attempts to obtain the unidentified components (cf. Fig. 2) in the crystalline state failed, and it was not possible to obtain crude products which were free from the above major constituents. It is noteworthy that crude fractions of pinobanksin gave crystalline benzoic acid (sublimed at the top of test tubes) on standing for several months in the cold.

Ether-insoluble Material (1.9%)

The ether-insoluble portion of the acetone extract was a brown, amorphous material having R_f 0.0 (TLC). Using acetic acid - hydrochloric acid - water (30:3:1 v/v) as developing solvent a single spot of R_f 0.77 was

obtained, which gave an orange-brown color with diazotized benzidine. The infrared spectrum of the crude material was similar to that of the material R_f 0.0 (TLC) obtained from the methanol extract (see below).

Methanol Extract

The residue (9.0 g) from the methanol extract (0.75%) was extracted with ether and the ether-soluble portion (4.0 g) was fractionated by column chromatography on silicic acid. Elution with chloroform gave a very small amount (8 mg) of an amorphous compound, m.p. 135.5–137° C, having an infrared spectrum similar to that of lariciresinol (m.p. 169.5–170.5° C). The R_f value (TLC) was 0.15, i.e. that of unknown 2 (Fig. 2), which is close to that of lariciresinol (R_f 0.18). However, the molecular weight was found to be 573, which is about 1.6 times that of lariciresinol. Further elution of the chromatogram with more polar solvents did not produce fractions of sharp melting points.

The ether-insoluble portion (5.0 g) was taken up in hot acetone and the mixture was filtered hot. The insoluble residue (1.0 g) was taken up in water and analyzed by paper chromatography. Two spots, corresponding in R_f values (see above) to glucose and arabinose, were detected. The acetone solution was allowed to cool slowly overnight, when an amorphous precipitate, m.p. 164–165° C, was obtained. Recrystallization from ethanol gave a microcrystalline powder, compound A (61 mg), m.p. 186–188° C, $\lambda_{\text{max}}^{\text{MeOH}}$ 281 μ , $[\alpha]_{\text{D}}^{25}$ +3.2° (c, 1.2, dioxane). The infrared spectrum was similar to that of lariciresinol, but the R_f value (TLC) was 0.0. Found: C, 61.03; H, 5.65; CH_3O , 17.39%; mol. wt., 1167. Calculated for $\text{C}_{60}\text{H}_{66}\text{O}_{24}$: C, 61.53; H, 5.68; CH_3O , 15.3%; mol. wt., 1171.4. Acetylation with acetic anhydride in the presence of sodium acetate gave a compound, m.p. 101.5–102.5° C, $[\alpha]_{\text{D}}^{25}$ -1.0° (c, 1.0, acetone). Found: C, 61.84; H, 5.64; CH_3O , 11.42; AcO , 26.83%; mol. wt., 1558. Calculated for decaacetate: C, 60.8; H, 4.84; CH_3O , 11.4; AcO , 25.9%; mol. wt., 1581.41. Calculated for nonaacetate: C, 59.3; H, 4.91; CH_3O , 11.7; AcO , 26.6%; mol. wt., 1540.38. Methylation with dimethyl sulphate gave intractable, viscous material. Treatment with diazomethane gave an amorphous compound of mol. wt. 1460. The parent compound gave a single spot of R_f 0.86 on TLC analysis, using acetic acid - hydrochloric acid - water (30:3:1) as solvent. The acetone-soluble (cold) portion was chromatographed on silicic acid (30 g), eluting successively with ether, chloroform, 2-butanone, and methanol. Elution with 2-butanone gave an amorphous material (240 mg), compound B, m.p. 124–125° C; after precipitation with water from a solution in dioxane. Elution with 2-butanone-methanol (1:1) gave another amorphous fraction, compound C, m.p. 148–151° C, $[\alpha]_{\text{D}}^{25}$ +3.2° (c, 1, dioxane). Both compounds had R_f values of 0.0 (TLC) and had infrared spectra similar to those of compound A and lariciresinol.

ACKNOWLEDGMENTS

We wish to thank Dr. W. B. Denyer for procuring the jack pine wood, Professor H. Erdtman for samples of pine polyphenols, Professor K. Freudenberg for samples of lignans, and Dr. E. O. Edwards and the Hercules Powder Co., Wilmington, U.S.A., for samples of resin acids. Microanalyses were carried out by Mr. M. Mazurek and infrared spectra were recorded by Mr. W. C. Haid. The technical assistance of Mr. M. Granat is also gratefully acknowledged.

REFERENCES

1. CANADIAN DEPARTMENT OF FORESTRY. Native trees of Canada. 6th ed. Queen's Printer, Ottawa. 1961. p. 16.
2. H. HIBBERT and J. B. PHILLIPS. Can. J. Res. **4**, 1 (1931).
3. M. A. BUCHANAN, R. V. SINNETT, and J. A. JAPPE. Tappi, **42**, 578 (1959).
4. H. ERDTMAN. Svensk. Kem. Tidn. **56**, 95 (1946).
5. G. LINDSTEDT and A. MISIORNY. Acta Chem. Scand. **5**, 121 (1951).
6. A. J. HAAGEN-SMIT, C. T. REDEMANN, T. H. WANG, and N. T. MIROV. J. Am. Pharm. Assoc. **39**, 260 (1950).
7. L. C. CRAIG, D. CRAIG, and E. G. SCHEIBEL. In Technique of organic chemistry. Vol. III, Part I. Edited by A. Weissberger. Interscience Publishers Inc., New York. 1950. p. 286.
8. E. VON RUDLOFF. Can. J. Chem. **39**, 1190, 1200 (1961).
9. E. VON RUDLOFF. Phytochemistry, **1**, 195 (1962).
10. B. M. CRAIG and N. L. MURTY. J. Am. Oil Chemists' Soc. **36**, 1124 (1959).
11. G. LINDSTEDT. Acta Chem. Scand. **4**, 448 (1950).
12. E. STAHL. Z. Anal. Chem. **181**, 303 (1961).
13. J. A. HUDY. Anal. Chem. **31**, 1754 (1959).
14. H. ERDTMAN. In Progress in organic chemistry. Vol. 1. Edited by J. W. Cook. Butterworths Scientific Publications, London. 1952. pp. 22–63.
15. K. FREUDENBERG. In Fortschritte der Chemie organischer Naturstoffe. Edited by L. Zechmeister. Springer-Verlag, Wien. 1962. p. 41.
16. F. E. BRAUNS. J. Am. Chem. Soc. **61**, 2124 (1939).
17. E. VON RUDLOFF. Tappi, **45**, 181 (1962).