

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

CHEMICAL UTILIZATION OF SOUTHERN PINE BARKS

Project 2753

Report Two

A Progress Report

to

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CHEMICAL UTILIZATION OF SOUTHERN PINE BARKS

SUMMARY

Fractionation and isolation studies initiated in Progress Report One were completed and reported. In addition to components reported in the earlier progress report, a number of new components occurring in the original extractives and after alkaline or acid hydrolysis were found. These include octanoic acid, decanoic acid, myristic acid, pentadecanoic acid, behenic acid, trans, trans-9,11-octadecadienoic acid, cis, cis, cis-5,11,14-eicosatrienoic acid, phenol, p-cresol, guaiacol, 4-ethylguaiacol, coumaric acid, ferulic acid, and succinic acid.

Large-scale studies on April samples of loblolly pine and slash pine barks showed no important differences from the March samples.

The effect of time of year upon the amount and nature of the extractives of both loblolly pine and slash pine bark samples was investigated by evaluation of the barks of bolts received each month for a year from essentially the same location. Although substantial differences were obtained in both the amount and nature of the extractives from different samples, no trends could be established. It appears that differences are due as much to individual samples as they are to the month of the year. A few unique results were obtained in several cases, and these should be repeated to determine whether the anomalies were significant or not.

The pinewood associated with the monthly samples of bark was chipped and submitted to turpentine evaluation. The turpentines obtained were analyzed for percentage composition of individual components, and some substantial differences were found between loblolly pine-derived turpentine and that obtained from the slash pines.

INTRODUCTION

Reference is made to the Introduction and Future Studies sections of Progress Report One. In accordance with the discussion in the Future Studies section, the present report is concerned primarily with the identification of unknown materials noted in Progress Report One, in completing several investigations reported in Progress Report One, and with the effect of month of the year upon the yield and nature of extractive components of loblolly and slash pine barks. In addition, the pinewood obtained with the monthly samples of pine bark was evaluated for yield and nature of turpentine, so that the effect of time of year on these factors was also determined and is reported herein. It was also noted that, during the comparison study of monthly samples of southern pine barks, a gradual evolution of procedure and knowledge took place in our laboratories. Many of the problems and unknowns present at the initiation of this investigation and noted in Progress Report One were solved and are reported in Progress Report Two.

As noted in Progress Report One, the many experiments reported in the present report included literally hundreds of individual qualitative and quantitative chromatograms, both paper and gas. Data for such qualitative and quantitative chromatograms including R_f values and colors of spots with various spray reagents of the many unknown compounds on paper chromatograms and retention times and peak heights on gas chromatograms are often included in reports. In the present instance, in the interest of brevity and budget, such data were usually not included. Instead, the chromatograms are usually discussed briefly. These data are on file, however, and if any of the cooperators are interested in specific results, we shall be happy to supply the original chromatograms.

EXPERIMENTAL AND DISCUSSION

CONTINUATION OF LARGE-SCALE INVESTIGATIONS OF MARCH LOBLOLLY AND SLASH PINE BARKS

Investigation of Ether Extractives (Fractions L-E and S-E)

These fractions are identified in Table XV on page 32 and Table XXXIV on page 64 of Progress Report One. The crude fractions were subjected to gas chromatography after treatment with REGISIL under the conditions detailed on pages 12 ff. of Progress Report One. No significant peaks were obtained indicating the absence of relatively low molecular weight compounds with hydroxyl or carboxyl groups.

Alkaline Hydrolysis of Ether Extractives

The ether extractives of both barks, Fractions L-E and S-E, were hydrolyzed in accordance with the general procedure described on page 35 of the first progress report, to yield ether-soluble neutral fractions L-E-N and S-E-N, ether-soluble acid fractions L-E-A and S-E-A, and water-soluble fractions L-E-W and S-E-W. The water-soluble fraction from loblolly pine bark was basified with sodium hydroxide solution and decationized by passing through a column of AMBERLITE IR-120 cation-exchange resin. The acid effluent of the column was treated with excess barium carbonate and filtered to remove sulfuric acid as barium sulfate (together with any other acids which might give insoluble barium salts). The precipitate was washed with water, and the combined filtrate and washings were passed again through the AMBERLITE IR-120 column. The effluent was concentrated to a small volume and dried by boiling with benzene under a water-separatory head. This left a water-free benzene solution of the water-soluble components, Fraction L-E-W. Quantitative data for this hydrolysis are given in Table I.

TABLE I

ALKALINE HYDROLYSIS OF ETHER-SOLUBLE FRACTIONS L-E AND S-E

Fraction	Yield, % ^a			
	Loblolly (L-E)		Slash (S-E)	
Ether-soluble neutrals	L-E-N	12.6	S-E-N	10.5
Ether-soluble acids	L-E-A	35.8	S-E-A	52.6
Water-solubles	L-E-W	2.1		

^aOn basis of solids in original ether-soluble fraction.

Evaluation of Alkaline Hydrolysis Fractions

Ether-soluble neutral fractions L-E-N and S-E-N. These two neutral fractions were submitted directly to gas chromatography under the standard conditions for neutral fractions described in detail on page 52 of Progress Report One. Under these conditions, the fraction was chromatographed directly on the FFAP column with the oven at 130°C. and programmed immediately to 265° at the rate of 6° per minute. Data for these chromatograms are given in Table II.

Quantitative isothermal gas chromatograms indicated that the tetra-cosanol yields were: L-E-N, 18.3% and S-E-N, 22.0%. Similarly, the hexacosanol yields were: L-E-N, 5.8% and S-E-N, 8.5%. Relative amounts of other components can be obtained from approximate ratio of peak heights.

TABLE II
 PROGRAMMED GAS CHROMATOGRAPHY OF NEUTRAL FRACTIONS L-E-N AND S-E-N
 UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units ^a		Identification ^b
		L-E-N	S-E-N	
1	0.8	8 (6)	15 (12)	
2	1.1	2 (2)	2 (2)	
3	4.7	2 (9)	2 (9)	
4	5.0	2 (10)	4 (20)	
5	6.1	2 (12)	2 (12)	
6	9.5	4 (38)	3 (28)	
7	11.3	1 (11)	1 (11)	
8	12.3	6 (74)	4 (49)	
9	14.0	1 (14)	1 (14)	
10	14.5	-	1 (15)	
11	15.4	1 (15)	2 (31)	
12	17.3	-	18 (312)	
13	18.3	1 (18)	3 (55)	Stearyl alcohol
14	20.7	1 (21)	2 (41)	Nonadecanol
15	23.7	2 (47)	2 (47)	Arachidyl alcohol
16	24.5	1 (25)	1 (25)	Uncosanol
17	26.6	7 (186)	6 (159)	Behenyl alcohol
18	28.0	2 (56)	4 (112)	Tricosanol
19	29.7	75 (2220)	76 (2260)	Tetracosanol
20	31.0	1 (31)	1 (31)	Pentacosanol
21	33.3	18 (600)	21 (700)	Hexacosanol

^aValues in parentheses are products of retention times and peak heights.

^bIdentifications were made by identity of retention times of authentic compounds under the same standard conditions. Positive identifications can be established only by isolation and subsequent infrared or mass spectroscopy. Thus, these are only tentative identifications.

Quantitative chromatograms indicated that approximately only one-half of the material submitted to gas chromatography was recovered in the components indicated by the 21 peaks of Table II. Some of each neutral fraction was chromatographed on thin-layer plates of silica gel and developed with 1:1 ethyl acetate - heptane. The developed plates were sprayed with 50% sulfuric acid and heated in an oven at 110°C. for 10 minutes. Approximately one-half of the material appeared as a purple spot at R_f 0.70 corresponding with the sitosterols, indicating that the nonvolatile components in the original neutrals were sterol in nature. Sterols decompose in stainless steel gas chromatographic columns and must be chromatographed in glass columns. At this time several neutral fractions obtained from the saponification of petroleum ether extractives also appeared to contain large amounts of sterols.

Ether-soluble acid fractions L-E-A and S-E-A. These two acid fractions were converted to their trimethylsilyl derivatives and submitted to gas chromatography under temperature-programmed conditions on a silicone column adopted as a standard procedure for acid fractions and described in detail on page 54 of Progress Report One. In accordance with these conditions, the fractions were treated with REGISIL and chromatographed on the SE-30 silicone column with the oven at 100°C. and programmed immediately upon injection to 240°C. at the rate of 6° per minute. Data for these chromatograms are given in Table III which includes actual yields rather than products of peak heights and retention times.

TABLE III
PROGRAMMED GAS CHROMATOGRAPHY OF ACID FRACTIONS L-E-A AND S-E-A
UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Yield, % ^a		Identification
		Loblolly L-E-A	Slash S-E-A	
1	1.8	0.20	0.20	
2	2.2	0.25	0.25	
3	3.1	0.15	0.15	
4	6.2	0.15	0.15	Undecanoic acid
5	9.4	0.15	0.15	
6	10.1	0.15	0.10	
7	11.8	0.70	0.25	
8	12.3	0.15	0.15	
9	14.2	0.15	0.10	
10	15.0	0.55	0.70	
11	15.5	0.80	0.70	Palmitic acid
12	18.0	10.00	9.00	Linoleic acid
13	18.5	2.65	3.00	Oleic acid
14	20.3	1.00	1.10	
15	21.2	1.95	5.35	^b
16	21.8	8.00	7.20	Arachidic acid
17	23.5	9.35	13.35	
18	25.3	8.65	11.35	^c Behenic acid
19	26.7	6.00	6.00	
20	28.3	13.00	13.00	^d Tetracosanoic acid
21	29.7	2.65	3.35	Pentacosanoic acid
22	31.7	6.85	6.65	Hexacosanoic acid
23	34.2	0.25	0.15	
Totals		73.75	82.40	

^aOn basis of solids in individual acid fractions.

^bRetention time corresponds with that of trans, trans-9,11-octadecadienoic acid (1).

^cRetention time corresponds with that of cis, cis, cis-5,11,14-eicosatrienoic acid (1).

^dRetention time corresponds with a 20-carbon chain acid containing 5 unsaturated bonds.

Water-soluble fraction L-E-W. This fraction in benzene was processed in exactly the same manner as were the last two ether-soluble acid fractions. The results of the programmed gas chromatography of this fraction after treatment with REGISIL are given in Table IV.

TABLE IV
PROGRAMMED GAS CHROMATOGRAPHY OF WATER-SOLUBLE FRACTION L-E-W

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1	2.2	100+	220+	Excess REGISIL
2	3.8	73	277	
3	4.6	56	258	Lactic acid
4	5.7	5	28	
5	6.3	3	19	
6	8.8	100+	880+	Glycerol
7	11.1	1	11	
8	11.7	2	23	
9	13.2	6	79	
10	31.7	15	460	

Thus, the gas chromatography of the individual fractions obtained by alkaline hydrolysis of the original ether-soluble fractions from both loblolly and slash pine barks are essentially similar to corresponding fractions obtained by the alkaline hydrolysis of the petroleum ether-soluble fractions L-PE and S-PE of Progress Report One. Apparently, the waxes in the bark could not be extracted completely by the original petroleum ether, but subsequent extraction with hot water expedited the extraction of these materials with ether.

Investigation of Ethanol Extractives (Fractions L-ETH and S-ETH)

These fractions are identified in Tables XV and XXXIV of Progress Report One. They were processed in much the same manner employed for the corresponding ether extractives.

Alkaline Hydrolysis of Ethanol Extractives

The ethanol extractives of both barks, Fractions L-ETH and S-ETH, were hydrolyzed with sodium hydroxide solution exactly as were the ether extractives to yield ether-soluble neutral fractions L-ETH-N and S-ETH-N and ether-soluble acid fractions L-ETH-A and S-ETH-A. Upon acidification of the alkaline hydrolysis mixtures in the present cases, heavy precipitates of ether-insoluble and water-insoluble material separated. These materials were separated and weighed as ether-insoluble acids L-ETH-IA and S-ETH-IA. Quantitative data for this alkaline hydrolysis are given in Table V.

TABLE V

ALKALINE HYDROLYSIS OF ETHANOL-SOLUBLE FRACTIONS L-ETH AND S-ETH

Fraction	Yield, % ^a	
	Loblolly (L-ETH)	Slash (S-ETH)
Ether-soluble neutrals	L-ETH-N 13.2	S-ETH-N 11.3
Ether-soluble acids	L-ETH-A 29.0	S-ETH-A 34.0
Ether-insoluble acids	L-ETH-IA 42.1	S-ETH-IA 41.5

^aOn basis of solids in original ethanol-soluble fraction.

Evaluation of Alkaline Hydrolysis Fractions

Ether-soluble neutral fractions L-ETH-N and S-ETH-N. These fractions were gas chromatographed directly in exactly the same manner used for the data of Table II. Results are given in Table VI.

TABLE VI
 PROGRAMMED GAS CHROMATOGRAPHY OF NEUTRAL FRACTIONS L-ETH-N and S-ETH-N
 UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units ^a		Identification ^b
		L-ETH-N	S-ETH-N	
1	2.2	3 (7)	4 (9)	1
2	4.1	1 (4)	1 (4)	3
3	4.8	1 (5)	1 (5)	4
4	5.3	1 (5)	1 (5)	5
5	8.0	11 (88)	4 (32)	6
6	9.5	1 (10)	1 (10)	7
7	10.2	5 (51)	4 (41)	8
8	11.6	1 (12)	1 (12)	9
9	11.9	1 (12)	1 (12)	10
10	12.6	2 (26)	2 (26)	11
11	14.2	1 (14)	7 (99)	12
12	15.1	1 (15)	1 (15)	13
13	16.9	1 (17)	2 (34)	14
14	19.4	2 (39)	2 (39)	15
15	21.7	4 (87)	5 (108)	17
16	23.2	1 (23)	1 (23)	18
17	24.9	12 (300)	12 (300)	19
18	29.4	2 (59)	2 (59)	21

^aValues in parentheses are products of retention times and peak heights.

^bCorresponding peaks in Table II.

Approximately 10% of both fractions actually chromatographed in these gas chromatographic analyses, leaving approximately 90% of the fractions unaccounted for. The neutral fractions were chromatographed on thin-layer plates of silica gel and found to contain large proportions of sterol or sterols. Nothing further was done with these components at this time.

Ether-soluble acid fractions L-ETH-A and S-ETH-A. These fractions were reacted with REGISIL and gas chromatographed in the same manner described for the data of Table III, and the results are given in Table VII.

Acid Hydrolysis of Ethanol Extractives

Approximately 4-gram samples of Fractions L-ETH and S-ETH were hydrolyzed by boiling 4 hours under reflux with N sulfuric acid. The mixtures were cooled and extracted with ether to give ether-soluble fractions L-ETH-E in 21% yield and S-ETH-E in 16% yield. Ether-insoluble, water-insoluble precipitates remained in both hydrolysis mixtures. These were filtered, dried, and weighed. In the case of the loblolly pine hydrolysis, the precipitate amounted to 69% of the original sample weight, leaving only 10% of the original sample in the aqueous solution. Similarly, the slash pine hydrolysis precipitate amounted to 71%, leaving only 13% of the original sample in the aqueous solution.

The aqueous solution from the loblolly experiment (Fraction L-ETH-W) was passed through a column of DUOLITE A-6 anion-exchange resin to remove acids and then concentrated under reduced pressure.

Evaluation of Acid Hydrolysis Fractions

Ether-soluble fractions L-ETH-E and S-ETH-E. These two fractions were treated with REGISIL and submitted to temperature-programmed gas chromatography under standard conditions as used for Tables III and VII. Results are given in Table VIII.

TABLE VII

PROGRAMMED GAS CHROMATOGRAPHY OF ACID FRACTIONS L-ETH-A AND S-ETH-A
UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units ^a		Identification ^b
		L-ETH-A	S-ETH-A	
1	2.0	100+ (200+)	100+ (200+)	Excess REGISIL
2	2.6	3 (8)	2 (5)	
3	3.5	2 (7)	2 (7)	
4	4.3	48 (202)	17 (73)	Phenol
5	5.4	5 (27)	2 (11)	p-Cresol
6	6.0	1 (6)	1 (6)	Guaiacol
7	6.7	1 (7)	1 (1)	
8	7.8	1 (8)		Octanoic acid
9	8.9	1 (9)	1 (9)	
10	10.3	1 (10)	3 (31)	
11	12.1	7 (85)	1 (12)	Decanoic acid
12	12.9	2 (26)	1 (13)	Vanillin
13	14.3	5 (72)	3 (43)	p-Hydroxybenzoic acid
14	14.7	1 (15)		
15	15.5	2 (31)	1 (16)	
16	15.9	1 (16)		
17	16.6	7 (116)	2 (33)	Vanillic acid
18	17.3	1 (17)		
19	17.7	29 (513)	24 (425)	
20	19.5	5 (98)	4 (78)	p-Coumaric acid
21	21.1	18 (381)	13 (274)	Ferulic acid
22	21.8	27 (590)	15 (328)	
23	22.5	1 (23)	2 (45)	
24	23.6	77 (1815)	43 (1015)	Linoleic and oleic acids
25	23.9	2 (48)	2 (48)	14
26	24.4	1 (24)	1 (24)	15
27	25.3	3 (76)	4 (101)	Arachidic acid
28	25.7	7 (180)	4 (103)	17
29	26.1	2 (52)	1 (26)	
30	26.7	2 (53)	1 (27)	Behenic acid
31	27.2	2 (54)	1 (27)	
32	28.8	4 (115)	2 (58)	19
33	30.5	4 (122)	2 (61)	Tetracosanoic acid
34	33.2	1 (33)		Pentacosanoic acid
35	36.3	2 (73)	1 (34)	Hexacosanoic acid

^aValues in parentheses are products of retention times and peak heights.

^bNumbers in this column are corresponding peaks in Table III.

TABLE VIII
 PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE FRACTIONS L-ETH-E and S-ETH-E
 UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units ^a		Identification ^b
		L-ETH-E	S-ETH-E	
1	2.0	100+ (200+)	100+ (200+)	1
2	2.6	2 (5)	3 (8)	2
3	3.5	2 (7)	14 (49)	3
4	4.3	2 (9)	2 (9)	4
5	5.4	1 (6)	2 (11)	5
6	6.0	1 (6)	30 (180)	6
7	6.7		1 (7)	7
8	7.8		4 (31)	8
9	8.9		2 (18)	9
10	10.3	1 (10)	7 (72)	10
11	12.1	3 (36)	3 (36)	11
12	12.9	1 (13)	1 (13)	12
13	14.3	3 (43)	4 (57)	13
14	16.6	2 (33)	3 (50)	17
15	17.7	15 (265)	27 (478)	19
16	19.5	5 (98)	4 (78)	20
17	21.1	1 (21)	8 (169)	21
18	22.5	2 (45)	12 (270)	23
19	23.6	3 (71)	28 (660)	24
20	24.4		3 (73)	26
21	25.3		7 (177)	27
22	25.7	1 (26)	11 (283)	28
23	26.1		1 (26)	29
24	26.7		1 (27)	30
25	27.2		2 (54)	31
26	28.8		5 (144)	32
27	30.5		2 (61)	33
28	33.2		1 (33)	34
29	36.3		1 (36)	35

^aValues in parentheses are products of retention times and peak heights.

^bNumbers in this column are corresponding peaks of Table VII.

The data of Table VIII indicate that the ether-soluble materials released by acid hydrolysis were very similar to those released by alkaline hydrolysis, especially for the slash pine fractions. Furthermore, the data of Tables VII and VIII suggest that the components responsible for the peaks of these chromatograms are essentially the same as those noted in Table III for the ether extractives, indicating incomplete extraction by the ether.

Water-soluble fraction L-ETH-W. This deionized aqueous solution from the acid hydrolysis of the ethanol extractives was chromatographed for sugars on paper and processed as described previously on page 58 of Progress Report One. Heavy concentrations of glucose and mannose were found along with traces of galactose, arabinose, xylose, and rhamnose.

ALKALINE EXTRACTION OF MARCH LOBLOLLY PINE BARK

Although the chief concern of the experimental program on this project was to investigate the nature of the components per se of these two barks, some attention was given to the extraction of the bark with hot alkaline solution because it was known that the yield of extractives under such conditions is much greater than with neutral solvents. The March loblolly pine bark was chosen for this experiment.

A sample of March loblolly pine bark containing 100 grams of oven-dry solids was placed in a 5-liter flask fitted with a reflux condenser and magnetic stirrer. The bark dust was covered with 3 liters of 4% sodium hydroxide solution, and the mixture was heated to boiling under reflux and magnetic stirring for 6 hours. The mixture was allowed to cool. The cool mixture was filtered, and the bark dust was washed well with water and allowed to air dry. The recovered dust contained 44 grams of air-dry solids indicating an extract amounting to 56% of the original bark solids.

The combined filtrate and washings were acidified to pH 1.0 with dilute sulfuric acid with stirring, and with continued stirring, the acid solution was treated with 25 grams of Celite filter aid. This mixture was filtered through a pad made of 25 grams of Celite. The mixture of Celite and precipitate was allowed to air dry, and the air-dried product contained 49 grams of air-dry precipitate. The filtrate was concentrated in a vacuum rotary evaporator.

Extractions of Alkaline Extractives

The Celite-precipitate mixture was placed in a Soxhlet apparatus and extracted exhaustively with heptane and then with ether. The concentrated aqueous filtrate was extracted in a liquid-liquid extractor first with heptane and then with ether. The heptane solutions were combined and concentrated to yield a solution (Fraction L-A-H) containing 1.9 grams of solids. The ether solutions were combined and concentrated to yield 7.6 grams of ether-soluble materials (Fraction L-A-E). The ether raffinate was Fraction L-A-W.

Evaluation of Fraction L-A-H

Gas chromatography. This heptane-soluble fraction was chromatographed directly on the FFAP column under the standard conditions employed for the data of Table II. Results are given in Table IX in which footnotes of Table II apply.

This fraction was also treated with REGISIL and gas chromatographed under the standard conditions employed for the data of Table III. Results are given in Table X in which footnotes of Table II apply.

TABLE IX

PROGRAMMED GAS CHROMATOGRAPHY OF HEPTANE-SOLUBLE FRACTION L-A-H
UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units	Identification
1	6.2	1 (6)	
2	9.3	22 (204)	
3	11.8	2 (24)	
4	13.0	1 (13)	Myristyl alcohol
5	13.7	3 (41)	
6	19.1	4 (76)	Tridecanoic acid
7	20.1	3 (60)	
8	20.5	2 (41)	
9	21.7	1 (22)	
10	23.7	6 (141)	Palmitic acid
11	25.1	1 (25)	
12	26.5	9 (237)	Oleic acid
13	27.6	4 (110)	Linoleic acid

TABLE X

PROGRAMMED GAS CHROMATOGRAPHY OF HEPTANE-SOLUBLE FRACTION L-A-H
AFTER TRIMETHYLSILYLATION UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units	Identification
1	4.1	1 (4)	
2	5.3	1 (5)	
3	6.5	3 (20)	
4	7.3	1 (7)	
5	9.5	2 (19)	Vanillin
6	10.9	2 (22)	
7	11.5	1 (12)	
8	11.8	1 (12)	Vanillic acid
9	15.3	2 (31)	Palmitic acid
10	17.3	4 (69)	Linoleic acid
11	17.8	10 (178)	Oleic acid
12	19.4	1 (19)	
13	20.8	10 (208)	Arachidic acid
14	22.2	12 (266)	
15	23.9	14 (335)	Behenic acid
16	24.9	3 (75)	

Paper chromatography. This fraction was chromatographed on paper, developed in both butanol saturated with 2% aqueous ammonia and with 10:3:3 butanol - pyridine - water. The developed chromatograms were sprayed with diazotized p-nitroaniline to give colored spots with phenolic compounds. Spots were obtained for small amounts of vanillic acid, ferulic acid, p-coumaric acid, and vanillin as well as for several unidentified compounds.

Evaluation of Fraction L-A-E

Gas chromatography. This ether-soluble fraction was treated with REGISIL and submitted to temperature-programmed gas chromatography under the standard conditions for such chromatography. However, suspected volatiles were lost with the excess reagent under these conditions. Accordingly, the treated sample was chromatographed on the same column with the oven at 70°C., maintained there for 10 minutes, and then programmed to 240° at a rate of 8° per minute. Other conditions were maintained the same as standard. Results are given in Table XI in which the footnotes of Table II apply.

The amount of phenolic acids in this fraction compared with similar fractions obtained by alkaline hydrolysis of individual extractives fractions suggests that some of these compounds were tied up with the unextractable portion of the bark when using neutral solvents, and were only liberated when the whole bark was treated with hot sodium hydroxide solution.

More work should be done on this subject matter. Large-scale experiments should be run, and the obtained alkaline extract should be fractionated on a column before subjecting it to gas chromatography. It appears that considerable material is available in the alkaline extract.

This completes the studies performed on the March loblolly and slash pine barks in this budgetary period.

TABLE XI

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE FRACTION L-A-E

Peak No.	Retention Time, min.	Peak Height, units	Identification
1	4.0	31 (124)	Phenol
2	4.6	53 (243)	
3	5.3	9 (48)	
4	5.8	2 (12)	
5	7.3	1 (7)	
6	8.2	3 (25)	p-Cresol
7	9.8	6 (59)	
8	10.4	6 (62)	
9	12.4	10 (124)	
10	13.8	3 (41)	
11	14.5	1 (15)	
12	15.6	2 (31)	
13	16.3	5 (81)	
14	17.7	3 (53)	
15	18.6	3 (56)	
16	19.2	4 (77)	Guaiacol Pyrocatechol
17	20.5	6 (123)	
18	21.6	6 (130)	
19	22.0	4 (88)	
20	22.9	2 (46)	
21	23.3	20 (466)	
22	23.8	76 (1810)	
23	24.7	4 (99)	
24	25.9	12 (310)	
25	27.6	16 (442)	
26	28.0	2 (56)	Vanillin p-Hydroxybenzoic acid
27	28.8	1 (29)	
28	29.8	2 (60)	
29	30.2	2 (60)	
30	31.1	3 (93)	
31	31.6	1 (32)	
32	32.5	3 (97)	
33	33.6	1 (34)	
34	34.3	1 (34)	
35	35.0	1 (35)	
36	35.9	1 (36)	Arachidic acid
37	38.7	1 (39)	
			Behenic acid

^aThis most important peak is the same as that obtained as peak no. 24 in Table XXXII of Progress Report One and described on page 60 of that same report. This compound has not been identified as yet.

COMPARISON OF MONTHLY SAMPLES OF LOBLOLLY AND SLASH PINE BARKS

Bark Samples

Every month for a year, starting in April of 1968, unbarked bolts of both loblolly and slash pine were received and processed. The bark samples origins were described on page 5 of Progress Report One.

Each pine bolt was processed in the same manner. Immediately upon receipt, the bolt was barked. The wood was chipped and frozen. A sample of the fresh chips was taken for moisture determination before bagging in polyethylene and freezing for future use. The bark was left to air dry and was then reduced to dust in a Wiley mill. The bark dust was air dried and bagged in polyethylene. Moisture determinations were made on the air-dry bark dust. Bolt sample data for the loblolly pine are given in Table XII and those for the slash pine are given in Table XIII.

It should be noted that some of the samples were delayed considerably enroute, thus introducing a variable that could not be controlled.

Bark Extractions

All bark dust samples were extracted in the same general manner as follows: A sample of bark dust containing 1000 grams of oven-dry bark was packed into a Soxhlet apparatus of 4000-ml. capacity and extracted exhaustively with petroleum ether (b.r. 30-60°C.). This extraction required 4 days of operation with 4 nights of standing in solvent. The petroleum ether extract was evaporated to dryness - finally under reduced pressure, and the residue was dissolved in tetrahydrofuran to give a clear concentrated solution for analysis.

TABLE XII
MONTHLY BOLT SAMPLES OF LOBLOLLY PINE

Month	Date Shipped	Date Received	Bark Moisture, % ^a	Wood Moisture, % ^b
April	4-3-68	4-12-68	8.1	49.5
May	5-6-68	5-10-68	10.0	55.8
June	6-4-68	6-10-68	11.2	51.9
July	7-2-68	7-15-68	12.3	51.1
August	8-7-68	8-15-68	9.9	53.0
September	8-30-68	9-11-68	11.3	39.8
October	10-1-68	10-25-68	8.6	52.3
November	11-7-68	11-15-68	7.3	52.5
December	12-3-68	12-11-68	5.4	50.1
January	12-31-68	1-24-69	6.2	51.2
February	1-31-69	2-12-69	7.1	47.0
March	2-28-69	3-10-69	6.6	
^c	2-27-68	3-7-68		57.3

^aBasis of airdry bark dust.

^bBasis of fresh wood chips before freezing.

^cOnly the wood was used from this March 1968 loblolly pine bolt.

TABLE XIII
MONTHLY BOLT SAMPLES OF SLASH PINE

Month	Date Shipped	Date Received	Bark Moisture, % ^a	Wood Moisture, % ^b
April	4-5-68	4-12-68	8.6	44.3
May	5-7-68	5-16-68	9.2	54.0
June	6-5-68	6-12-68	11.9	51.5
July	7-10-68	7-17-68	12.7	53.4
August	8-6-68	8-14-68	10.7	48.0
September	9-13-68	9-25-68	11.0	50.3
October	10-4-68	10-15-68	11.6	53.5
November	11-8-68	11-19-68	8.4	53.0
December	12-11-68	12-19-68	6.1	52.4
January	12-31-68	1-10-69	7.9	55.3
February	2-10-69	2-14-69	7.3	52.4
March	3-4-69	3-13-69	7.5	
^c	3-1-68	3-11-68		59.1

^aBasis of airdry bark dust.

^bBasis of fresh wood chips before freezing.

^cOnly the wood was used from this March 1968 slash pine bolt.

The extracted sawdust was removed from the Soxhlet apparatus quantitatively and allowed to air dry until all petroleum ether was evaporated. The dry dust was weighed, and the moisture determination made to calculate the oven-dry yield and the theoretical yield. All subsequent extractions and yields were based on an original bark dust sample of 1000 grams oven-dry.

The dry bark dust was placed in a 20-liter stainless steel bucket, covered with 14 liters of distilled water, heated to boiling, simmered for 1.5 hours, and filtered hot through a cotton cloth. The bark dust was squeezed as dry as possible and returned to the bucket for an identical processing with another 14 liters of water. The combined water extract was concentrated to approximately 3 liters in a large vacuum circulating evaporator. The turbid mixture was treated with 25.0 grams of Celite filter aid and filtered through a pad of 25.0 grams of Celite. The precipitate was washed with water, and the combined filtrate and washings were concentrated in a vacuum rotary evaporator to approximately 700 ml. Total solids were determined on the concentrates.

The concentrated aqueous solution was extracted exhaustively with ethyl acetate in a liquid-liquid extractor, and the ethyl acetate was concentrated for solids determination. The aqueous raffinate was evaporated somewhat to remove ethyl acetate, treated with a little toluene to prevent fermentation, and stored in the refrigerator.

The water-extracted bark dust was dried at room temperature, weighed, and sampled for moisture determination. This oven-dry dust was placed in the large Soxhlet extractor again and extracted with ethyl ether. The ether extract was concentrated for analysis.

All solvent was drained from the extractor, and the bark dust was extracted in the same apparatus with ethanol. The ethanol was concentrated for analysis. The bark dust was discarded.

The extractions described are pictured in Fig. 1. During the extraction processing all barks reacted essentially similar except the June loblolly pine bark. The wood from this bolt also gave results that could not be resolved with those obtained from other woods. Therefore, some doubt has arisen concerning the authentic identity of this bolt. If this project is renewed, another bolt of June loblolly pine will be obtained to check the results obtained with the present bark and wood.

The quantitative extraction data for the two barks are given in Tables XIV and XV.

The data of Table XIV for loblolly pine bark indicate a few trends. The petroleum ether extractives appear to decrease during the fall months from September to December. The water extractives also decrease during this time. The ether extractive yield is dependent on the petroleum ether and water extractive yields. This will be discussed more fully in connection with the discussion on these extracts. The June sample did not appear to belong.

No patterns appeared in the data of Table XV for the slash pine bark samples. Differences appeared to be specific to individual bolts of wood rather than to seasonal changes.

The extractives data for both loblolly and slash pine March barks are quite different from those reported for the pine barks obtained in large quantity the year before and reported in Progress Report One. The early pine barks were processed quite differently and also they were not obtained as individual bolts, but as bulk barks.

TABLE XIV
 EXTRACTION DATA FOR LOBLOLLY PINE BARKS

Month	Extract Yield, % ^a						Total
	Petroleum Ether	Water	Ether	Ethanol	Ethyl Acetate ^b		
April	2.80	8.35	1.34	0.78	1.82	21.8	
May	2.19	9.61	2.43	1.26	1.73	18.0	
June ^c	4.52	13.34	0.79	0.70	3.59	26.9	
July	2.09	8.74	1.51	1.30	1.84	21.0	
August	2.70	10.34	1.05	0.56	1.43	13.8	
September	1.48	7.66	1.07	0.38	1.06	14.0	
October	1.66	7.05	1.28	0.88	1.67	23.7	
November	1.76	7.06	1.27	1.02	1.07	15.2	
December	1.74	7.88	1.11	1.27	1.32	16.8	
January	2.03	7.56	1.53	1.71	1.70	22.5	
February	2.56	10.59	2.14	0.48	1.73	16.3	
March	3.01	11.08	1.21	1.32	2.80	25.3	

^aBased on the 1000 grams of starting bark dust.

^bBased on the weight of water extract.

^cAuthenticity of this sample is in doubt.

TABLE XV
EXTRACTION DATA FOR SLASH PINE BARKS

Month	Extract Yield, % ^a						Total
	Petroleum Ether	Water	Ether	Ethanol	Ethyl Acetate ^a	Ethyl Acetate ^b	
April	1.90	13.04	0.99	0.50	2.10	16.1	16.43
May	1.89	8.08	1.41	0.51	1.50	18.6	11.89
June	1.84	13.50	1.44	1.22	3.46	25.6	17.00
July	2.19	10.47	1.14	0.65	1.95	18.6	14.45
August	2.33	11.47	1.16	0.87	1.29	11.2	15.83
September	2.74	10.67	1.24	0.99	1.51	14.2	15.64
October	1.66	11.39	0.98	1.02	1.35	11.9	15.05
November	2.32	11.70	1.14	1.05	2.39	20.4	16.21
December	2.09	9.83	1.13	0.98	1.83	18.6	14.03
January	1.76	13.04	0.96	1.15	3.12	23.9	16.91
February	2.93	13.02	2.52	0.89	3.15	24.2	19.36
March	2.19	10.83	1.05	1.13	2.24	20.7	15.20

^aBased on the 1000 grams of starting bark dust.

^bBased on the weight of water extract.

Analysis of Petroleum Ether Extractives

Alkaline Hydrolysis of Petroleum Ether Extractives

Each petroleum ether extract was submitted to alkaline hydrolysis under the conditions adopted as standard and described in detail on page 51 of Progress Report One. Under these conditions loblolly pine barks yielded neutral fractions Lo-PE-N, ether-soluble acid fractions Lo-PE-A, and water-soluble, ether-insoluble fractions Lo-PE-W. Similarly, the slash pine barks yielded neutral fractions Sl-PE-N, ether-soluble acid fractions Sl-PE-A, and water-soluble, ether-insoluble fractions Sl-PE-W. The quantitative yields of the neutral and ether-soluble acid fractions are given in Table XVI for the loblolly pine barks and in Table XVII for the slash pine barks. Quantitative values for the water-soluble, ether-insoluble fractions are not included, because the processing involved precluded the obtainment of yields for the total fraction. Instead, yields of individual components will be noted in subsequent tables.

Gas Chromatography of Neutral Fractions Lo-PE-N and Sl-PE-N

These two neutral fractions were submitted directly to gas chromatography under the standard conditions for neutral fractions described in Progress Report One and used first in the report for the data of Table II. A total of 36 different peaks was obtained. Many of the peaks were missing in individual chromatograms. The complete results are reported in Table XVIII for the loblolly pine fractions and in Table XIX for the slash pine fractions.

TABLE XVI

ALKALINE HYDROLYSIS OF PETROLEUM ETHER EXTRACTIVES OF
LOBLOLLY PINE BARKS

Month	Neutral Fraction Lo-PE-N Yield, % ^a	Acid Fraction Lo-PE-A Yield, %
April	20.0	71.1
May	20.4	70.4
June	36.3	60.0
July	22.2	72.2
August	20.0	77.8
September	22.5	67.2
October	24.1	61.1
November	26.8	64.3
December	23.6	63.6
January	28.3	56.7
February	21.4	62.5
March	17.9	67.9

^aAll yields are on basis of solids in petroleum ether extractives.

TABLE XVII

ALKALINE HYDROLYSIS OF PETROLEUM ETHER EXTRACTIVES OF
SLASH PINE BARKS

Month	Neutral Fraction Sl-PE-N Yield, % ^a	Acid Fraction Sl-PE-A Yield, %
April	20.0	68.0
May	23.5	66.7
June	21.8	63.6
July	20.0	60.0
August	21.7	69.6
September	20.4	73.5
October	25.4	56.9
November	25.6	61.5
December	25.0	62.5
January	28.6	64.3
February	23.9	65.2
March	21.7	65.2

^aAll yields are on basis of solids in petroleum ether extractives.

TABLE XVIII
GAS CHROMATOGRAPHY OF NEUTRAL FRACTIONS Lo-PE-N FROM LOBLOLLY PINE BARKS

Peak No.	Retention Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	0.9	-	-	3 ^a	6	3	3	2	2	-	2	4	-
2	3.0	-	1	-	-	-	-	1	-	-	1	-	1
3 ^b	5.1	2	-	-	3	-	-	3	-	3	8	1	3
4	5.4	37	1	19	19	2	2	17	1	18	100+	2	90
5	6.0	-	1	2	-	-	-	1	-	-	-	1	-
6	6.3	12	7	2	5	2	2	6	1	7	39	6	26
7	6.9	1	1	1	1	-	-	1	1	2	4	1	2
8 ^b	7.4	1	1	5	1	-	-	1	1	1	3	1	2
9	9.2	5	4	1	2	2	2	2	-	1	1	1	-
10	9.6	-	-	1	-	-	-	4	2	10	4	8	2
11	10.8	25	1	1	8	-	2	3	-	3	7	-	5
12	11.6	19	3	1	9	1	4	8	3	7	21	2	5
13 ^b	12.2	1	-	-	2	-	-	2	-	1	4	-	2
14 ^b	12.9	2	-	2	1	-	-	2	1	2	2	1	1
15	13.3	1	1	1	1	-	-	-	2	-	5	-	1
16 ^b	14.1	2	-	1	1	-	1	1	1	1	1	-	1
17 ^b	14.9	1	-	2	-	-	-	-	-	2	1	-	1
18 ^b	15.9	2	1	8	-	-	-	-	-	-	1	-	1
19	18.0	-	-	15	1	1	-	2	-	1	1	-	-
20 ^b	19.9	-	2	100+	2	2	-	2	1	2	3	2	2
21 ^b	21.1	3	2	3	1	1	3	3	-	2	1	3	2
22	21.6	-	2	3	1	2	2	1	2	-	-	-	-
23	22.1	1	-	-	-	-	-	-	-	-	-	-	-
24 ^b	23.0	3	1	6	3	2	2	2	1	-	-	1	-
25 ^b	23.5	18	21	14	5	15	7	10	20	17	23	15	12

TABLE XVIII (continued)
 GAS CHROMATOGRAPHY OF NEUTRAL FRACTIONS Lo-PE-N FROM LOBLOLLY PINE BARKS

Peak No.	Retention Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
26	24.7	1	2	19	3	5	4	4	3	3	2	3	5
27 ^b	25.6	-	1	6	1	1	2	1	-	2	-	-	2
28 ^b	27.2	60	100+	100+	100+	100+	100+	100+	100+	73	100+	100+	100+
29	29.0	1	1	1	1	1	1	2	1	1	1	2	2
30	29.5	-	-	16	-	-	-	-	-	-	-	-	-
31 ^b	31.9	20	24	13	27	31	26	23	21	17	22	44	27
32	33.9	-	-	5	-	-	-	-	-	-	-	-	-
33	35.1	-	-	3	-	-	1	-	-	-	-	-	-
34	39.5	7	3	-	-	2	-	-	-	-	4	-	-
35	42.0	-	-	3	5	-	-	3	-	-	-	1	-
36	47.2	-	-	3	-	-	-	-	-	-	-	-	-

^aNumbers in table are peak heights for a standard injection and attenuation.

^bIdentification of peak numbers: 4, nonanol; 8, decanol; 14, myristyl alcohol; 16, pentadecanol; 17, possibly decanoic acid; 18, possibly undecanoic acid; 20, nonadecanol; 21, arachidyl alcohol; 25, behenyl alcohol; 27, oleic acid; 28, tetracosanol; and 31, hexacosanol.

TABLE XIX
GAS CHROMATOGRAPHY OF NEUTRAL FRACTIONS SL-PE-N FROM SLASH PINE BARKS

Peak No.	Retention Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	0.9	3 ^a	3	1	-	3	3	3	-	-	-	-	-
2	1.3	2	2	1	2	-	1	-	-	-	1	-	-
3	3.0	-	-	1	1	1	-	-	-	-	2	1	2
4	4.7	1	1	1	1	1	1	3	1	1	-	-	-
5	5.1	4	28	3	7	5	19	29	4 ⁿ	28	48	13	30
6 ^b	5.4	1	-	4	-	1	-	9	2	-	-	-	2
7	6.0	1	7	4	7	3	6	1	4	14	14	17	10
8	6.9	1	1	2	1	1	3	1	1	1	6	2	25
9 ^b	7.4	2	1	1	1	1	5	-	1	1	6	1	11
10	8.1	-	-	1	-	-	-	-	1	1	1	1	1
11 ^b	8.6	-	-	1	-	-	-	-	-	-	-	-	-
12	9.2	3	3	4	7	2	2	9	5	7	4	4	5
13	9.4	-	1	-	1	-	-	-	-	1	1	1	1
14	10.8	10	17	4	6	5	5	3	3	4	3	3	2
15	11.6	7	12	6	12	5	5	7	5	8	9	3	5
16 ^b	12.9	2	2	1	1	-	-	2	1	2	3	1	2
17	13.3	2	1	-	1	-	3	-	-	1	3	1	5
18 ^b	14.1	3	3	2	2	1	4	1	1	2	6	2	10
19 ^b	14.9	5	8	-	-	-	-	-	1	1	2	1	1
20 ^b	15.9	52	30	20	24	30	10	34	40	22	39	22	17
21	16.8	4	4	3	2	4	2	4	2	2	4	3	1
22	17.4	4	4	3	2	4	2	4	2	2	4	3	1
23	18.7	-	2	2	-	2	1	3	4	5	6	1	4
24 ^b	19.4	1	1	1	1	1	1	-	-	-	-	-	-
25	19.9	3	4	-	2	3	-	-	-	1	-	1	-

It should be noted that all the gas chromatograms obtained for the data of Tables XIX and XX were direct chromatograms on the FFAP column. The oven was at 130°C. at time of injection, and the temperature was programmed to 265°C. at the rate of 6° per minute. As discussed in detail on page 55 of Progress Report One, it appears that long-chain unsaturated fatty acids and fatty alcohols crack to some extent under these conditions. Thus, some of the low molecular weight material found in these tables are probably due to cracking of larger molecules.

The chief components of the neutral fractions of both bark experiments were tetracosanol and hexacosanol. In the case of the June loblolly pine bark, alkaline hydrolysis of the petroleum ether extractives yielded a substantial amount of nonadecanol. The neutral fractions were submitted to quantitative gas chromatography to give the results of Tables XX and XXI. Data are on basis of the solids in the neutral fractions.

TABLE XX

MAJOR COMPONENTS IN NEUTRAL FRACTIONS LO-PE-N FROM LOBLOLLY PINE BARKS

Month	Nonadecanol Yield, %	Tetracosanol Yield, %	Hexacosanol Yield, %
April		23.8	13.1
May		39.5	14.3
June	18.5	21.3	12.5
July		35.3	15.8
August		38.9	13.1
September		39.6	10.0
October		31.6	9.4
November		32.3	10.3
December		22.6	7.9
January		27.9	8.5
February		39.7	14.0
March		23.3	8.7

TABLE XXI

MAJOR COMPONENTS IN NEUTRAL FRACTIONS SL-E-N FROM SLASH PINE BARKS

Month	Tetracosanol Yield, %	Hexacosanol Yield, %
April	40.4	12.2
May	33.1	9.8
June	45.0	14.5
July	36.1	14.4
August	38.5	14.0
September	38.4	10.0
October	40.8	11.0
November	24.0	8.7
December	25.7	6.7
January	35.5	13.2
February	20.0	7.6
March	22.1	7.5

Gas Chromatography of Acid Fractions LO-PE-A and SL-PE-A

All acid fractions were treated with REGISIL and submitted to temperature-programmed gas chromatography under the standard conditions. The chromatograms were determined quantitatively, and the data are given in Tables XXII and XXIII as percentages of the individual acid fractions.

The data of Tables XXII and XXIII do not appear to fall into any pattern or suggest any trends. Although individual samples were obtained on a monthly basis from the same localities, the data indicate that differences between individual trees are probably greater than differences due to season. The results suggest that there are some general differences between the general extractive content of the two species barks. Although the important components are essentially the same, their quantitative distributions are somewhat different. Tetracosanoic acid is present in large amount after saponification of the petroleum

TABLE XXII
QUANTITATIVE GAS CHROMATOGRAPHY OF ACID FRACTIONS Lo-PE-A FROM LOBLOLLY PINE BARKS

Peak No.	Retention Time, min.	Apr., % ^a	May, %	June, %	July, %	Aug., %	Sept., %	Oct., %	Nov., %	Dec., %	Jan., %	Feb., %	Mar., %
1 ^b	6.4	0.15	0.15	0.10	0.05	0.05	0.05	0.10	0.10	0.15	0.10	0.15	0.10
2	11.9	0.20	0.30	0.10	0.35	0.05	0.10	0.20	0.20	0-25	0.25	0.10	0.15
3 ^b	12.9	0.60	0.30	0.10	0.35	0.25	0.25	0.35	0.20	0.35	0.10	0.15	0.20
4	13.3	0.60	0.35	0.45	0.45	0.20	0.30	0.15	0.10	0.15	0.10	0.15	0.10
5 ^b	14.7	5.80	4.00	2.20	2.85	1.60	2.00	1.25	1.10	2.85	2.20	1.45	2.75
6 ^b	16.7	7.40	7.20	7.20	8.40	7.60	10.00	10.00	10.10	13.40	12.60	14.00	14.00
7	17.7	37.00	23.80	13.00	20.70	11.10	8.55	3.10	11.90	21.30	22.20	4.40	33.00
8	19.0	0.80	2.80	4.00	2.00	3.40	1.60	3.60	0.40	2.20	1.60	2.00	5.00
9 ^c	19.6	6.00	5.35	13.35	9.05	16.95	8.25	16.00	10.65	13.35	4.00	5.05	2.65
10 ^b	20.3	7.35	15.60	22.40	15.05	8.45	12.75	14.25	8.00	9.60	11.20	7.20	14.15
11	20.8	-	-	-	0.65	6.65	5.35	4.00	2.65	-	-	13.35	-
12 ^d	21.6	4.15	6.00	12.25	8.80	10.00	9.60	9.35	6.65	3.35	2.80	5.35	2.40
13 ^b	23.1	4.65	6.05	3.60	9.60	10.00	10.25	10.00	9.35	7.05	8.15	8.50	3.60
14 ^e	24.5	0.95	3.60	2.00	3.60	2.65	4.65	4.65	4.80	2.40	2.15	6.00	0.55
15 ^b	26.1	7.45	9.45	4.65	10.80	11.05	15.75	14.00	16.00	14.65	19.35	18.00	8.65
16 ^b	27.7	0.55	0.55	0.40	0.15	0.40	0.55	0.10	0.50	-	1.25	1.85	-
17 ^b	30.3	4.15	2.65	2.00	4.80	5.35	4.25	5.35	3.00	8.65	11.35	10.10	5.35
Totals		87.80	88.15	87.80	97.65	95.75	94.25	96.45	86.60	99.70	99.40	97.80	92.65

^aBasis of solids in the instant acid fraction Lo-PE-A.

^bIdentification of peak numbers: 1, undecanoic acid; 3, pentadecanoic acid; 5, palmitic acid; 6, linoleic acid; 7, oleic acid; 10, arachidic acid; 11, abietic acid; 13, behenic acid; 15, tetracosanoic acid; 16, pentacosanoic acid; and 17, hexacosanoic acid.

^cRetention time corresponds with that of trans, trans-9,11-octadecadienoic acid (1).

^dRetention time corresponds with that of cis, cis, cis-5,11,14-eicosatrienoic acid (1).

^eRetention time corresponds with a 20-carbon chain acid containing 5 unsaturated bonds.

TABLE XXIII
QUANTITATIVE GAS CHROMATOGRAPHY OF ACID FRACTIONS SI-PE-A FROM SLASH PINE BARKS

Peak No.	Retention Time, min.	Apr., % ^a	May, %	June, %	July, %	Aug., %	Sept., %	Oct., %	Nov., %	Dec., %	Jan., %	Feb., %	Mar., %
1 ^b	6.4	0.10	0.05	-	0.10	0.05	-	-	0.10	0.15	0.20	0.10	0.20
2 ^b	9.2	0.10	0.20	-	-	-	-	-	0.10	-	0.15	0.10	0.10
3 ^b	10.8	-	-	-	0.70	-	-	-	0.10	-	0.10	-	0.10
4 ^b	12.9	0.35	0.25	0.15	0.30	-	-	0.50	0.10	-	0.20	-	0.40
5	13.3	0.45	0.35	0.45	0.40	0.20	0.25	0.20	0.10	0.10	0.20	0.10	-
6 ^b	14.7	2.65	2.60	1.80	2.00	3.90	0.45	1.20	1.25	1.15	3.20	2.00	4.25
7	15.7	0.25	0.25	0.15	0.30	0.25	-	-	-	0.10	0.10	-	0.70
8 ^b	16.7	12.80	10.50	12.00	9.30	12.00	7.00	14.60	15.00	14.00	14.50	8.60	11.20
9 ^b	17.7	8.40	12.10	11.10	7.60	22.50	2.40	8.30	5.60	12.20	16.00	11.20	22.80
10	19.0	3.00	2.00	3.40	4.00	2.90	3.00	2.00	3.00	3.00	1.00	4.00	3.20
11 ^c	19.6	14.65	12.00	13.35	15.15	10.25	34.50	14.65	11.80	12.00	8.30	24.60	18.00
12 ^b	20.3	13.35	13.35	13.65	12.35	10.00	12.00	9.05	18.80	17.35	8.90	12.40	10.15
13 ^b	20.8	-	-	-	-	-	1.35	0.40	-	-	-	5.10	-
14 ^d	21.6	8.65	8.55	8.00	10.15	7.20	13.35	12.00	4.40	5.35	6.90	11.00	9.35
15 ^b	23.1	8.40	8.55	10.00	9.35	7.20	7.35	8.40	7.60	7.35	9.30	5.05	4.65
16 ^e	24.5	3.35	3.60	5.35	4.15	2.70	2.95	2.80	3.30	2.65	1.85	2.90	1.60
17 ^b	26.1	13.05	10.80	12.75	14.15	12.30	9.35	13.60	14.60	10.80	12.50	7.60	8.15
18 ^b	27.7	0.35	0.25	0.40	0.80	0.05	-	-	-	-	-	-	-
19 ^b	30.3	6.25	4.65	5.35	6.65	6.60	3.45	6.55	7.30	4.00	6.90	4.15	3.20
Totals		96.15	90.05	97.90	97.45	98.10	97.40	94.25	93.15	90.20	90.30	98.90	98.05

^aBasis of solids in the instant acid fraction SI-PE-A.

^bIdentification of peak numbers: 1, undecanoic acid; 2, tridecanoic acid; 3, myristic acid; 4, pentadecanoic acid; 6, palmitic acid; 8, linoleic acid; 9, oleic acid; 12, arachidic acid; 13, abietic acid; 15, benzoic acid; 17, tetracosanoic acid; 18, pentacosanoic acid; and 19, hexacosanoic acid

^cRetention time corresponds with that of trans, trans-9,11-octadecadienoic acid (1).

^dRetention time corresponds with that of cis, cis, cis-5,11,14-eicosatrienoic acid (1).

^eRetention time corresponds with a 20-carbon chain acid containing 5 unsaturated bonds.

ether extractives of both species barks. Oleic acid comprises a larger proportion of the acids liberated from the loblolly pine extractives than those from the slash pine extractives. On the other hand, the isomer of linoleic acid, trans, trans-9,11-octadecadienoic acid, appears to be in larger proportion in the acids from the slash pine bark fraction than from the loblolly pine bark fraction. The other important components of both bark fractions are linoleic and arachidic acids. Behenic and hexacosanoic acids appear in quantity in individual samples of loblolly pine bark fractions.

From the results of Tables XIX through XXIII it is quite obvious that tetracosanyl tetracosanoate is a most important component of both loblolly pine bark and slash pine bark at all seasons of the year.

Gas Chromatography of Water-Soluble Fractions Lo-PE-W and Sl-PE-W

Since earlier chromatographic studies on these fractions had indicated that glycerol was the only product of importance in these fractions, all fractions were treated with REGISIL and chromatographed qualitatively under temperature-programmed conditions under which standard conditions, the peak heights were noted. Then, glycerol was determined quantitatively. Data for the two fractions appear in Tables XXIV and XXV. Yields of glycerol are given as percentages of the original petroleum ether extractives rather than the water-soluble portions because the processing procedure precluded solids determination on the water-soluble portions Lo-PE-W and Sl-PE-W.

The data of Tables XXIV and XXV indicate that the water-soluble fraction from the hydrolysis of the petroleum ether extractives of slash pine bark contain two components not found in the similar fraction obtained from the loblolly pine bark.

TABLE XXIV
GAS CHROMATOGRAPHY OF WATER-SOLUBLE FRACTIONS Lo-PE-W FROM LOBLOLLY PINE BARK

Peak No.	Retention Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	3.0	-	10 ^a	-	-	-	-	3	4	-	-	-	2
2 ^b	5.4	5	14	13	7	10	7	1	2	3	3	1	1
3	8.5	1	2	-	-	-	-	-	1	1	1	1	1
4 ^b	9.3	1	5	1	-	1	1	1	1	-	-	-	-
5	13.6	1	2	1	1	-	1	2	3	2	2	4	3
6 ^b	14.8	100+	100+	29	100+	54	71	44	100+	100+	100+	100+	100+
Quantitative glycerol, % ^c		3.68	2.18	0.42	1.02	0.54	0.66	0.48	1.08	1.96	2.72	0.86	3.20

^aNumbers in table are peak heights for a standard injection.

^bIdentification of peaks: 2, trimethylene glycol; 4, 2,6-di-tert-butyl-p-cresol; and 6, glycerol.

^cNumbers in this row are percentages based on solids of original petroleum ether extractives Lo-PE.

TABLE XXV
GAS CHROMATOGRAPHY OF WATER-SOLUBLE FRACTIONS SI-PE-W FROM SLASH PINE BARK

Peak No.	Retention Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	2.2	-	3 ^a	-	2	1	2	2	4	2	3	-	8
2	3.7	-	-	-	2	-	-	-	1	-	1	1	1
3 ^b	5.4	9	7	14	9	6	5	4	1	2	5	1	1
4	5.9	-	-	3	-	-	-	2	-	1	4	1	2
5 ^b	9.3	2	2	1	1	1	1	-	2	2	1	-	1
6	13.0	1	1	-	1	1	1	1	1	1	-	2	-
7	13.6	2	1	1	1	1	1	2	2	2	3	1	3
8 ^b	14.8	100+	100+	100+	33	72	50	81	87	100+	100+	100+	100+
Quantitative glycerol, %		1.08	1.12	1.04	0.36	0.66	0.54	0.74	0.66	0.82	1.70	0.88	2.38

^aNumbers in table are peak heights for a standard injection.

^bIdentification of peaks: 3, trimethylene glycol; 5, 2,6-di-tert-butyl-p-cresol; and 8, glycerol.

^cNumbers in this row are percentages based on solids of original petroleum ether extractives SI-PE.

In Progress Report One on page 56 we discussed the occurrence of 2,6-di-tert-butyl-p-cresol in water-soluble fractions. Since that time we have found that this compound is used as a stabilizer in the reagent-grade tetrahydrofuran employed as a solvent in our studies. This suggests another possible source for this compound in these fractions.

This concludes the investigations on the effect of time of year on the nature and amount of individual components in loblolly and slash pine barks. Although nothing definite was concluded regarding the effect of season on the components, the study resulted in the development of techniques for analyzing, fractionating, and evaluating the bark components. A number of components were indicated, but only a relatively few have been identified. Some of these unidentified components may be of interest. If this project is continued, these will be isolated in quantity and identified.

FURTHER STUDIES ON THE ETHYL ACETATE-SOLUBLE PORTION OF THE HOT WATER EXTRACTIVES

In Progress Report One the March barks of both loblolly and slash pine barks were submitted to large-scale extraction and fractionation. In that study, the hot water extracts were extracted with ethyl acetate, and the ethyl acetate-soluble fractions were then fractionated on polyamide columns to obtain subfractions for analysis. During the course of our study of monthly samples of both barks, considerable development of procedures took place, and some of these were applied to the important ethyl acetate-soluble materials. For these studies, the ethyl acetate-soluble fractions (see Tables XIV and XV) from the April and May loblolly pine barks and from the April slash pine bark were chosen. The May loblolly pine bark was taken to see if there was much difference on a monthly basis, but results indicated that no real difference was obtained.

Accordingly, this report will record only the data obtained for the April loblolly and slash pine bark-derived fractions. These ethyl acetate-soluble fractions are identified as Lo-EA and Sl-EA, respectively.

Preliminary Fractionation of Ethyl Acetate Extractives

Since it was found in earlier studies that more than two-thirds of the ethyl acetate-soluble fractions could not be chromatographed even as the trimethylsilyl derivatives, it was assumed that high molecular weight material was involved. Accordingly, an attempt was made to remove the low molecular weight material from the mass by preliminary extraction with ether. Therefore, the ethyl acetate extracts were evaporated to dryness under reduced pressure on a rotary evaporator, and the residues were extracted with boiling ether to yield ether-soluble fractions Lo-EA-E and Sl-EA-E. The ether-insoluble residues are Lo-EA-EI and Sl-EA-EI. The ether extracts represented approximately 20% of all samples.

Gas Chromatography of Ether-Soluble Fractions Lo-EA-E and Sl-EA-E

These fractions were treated with REGISIL and chromatographed under temperature-programmed conditions employed as standard for acid fractions, except that the column employed was the silicone SE-30 column described on page 12 of Progress Report One rather than the column described on page 54 of that report and employed in all determinations reported so far in this report. The results are given in Table XXVI which also includes isothermal chromatographic data on the same column for 100, 150, 180, 200, and 250°C.

TABLE XXVI

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE PORTIONS OF ETHYL ACETATE EXTRACTIVES
OF APRIL LOBLOLLY PINE BARK AND SLASH PINE BARK

Peak No.	Retention Time, min.	Peak Height, units Lo-EA-E	SI-EA-E	Isothermal Retention Times, min.				Identification
				100°	150°	180°	200° 250°	
1	1.7	20	100+	1.9				Excess REGISIL
2	2.1	1	-	2.3				
3	2.9	1	1	3.5				
4	3.3	1	1	4.1				
5	3.5	1	1	4.5	1.1			Valeric acid
6	4.3	5	15	6.2	1.5			Phenol
7	4.4	3	3		1.6			Lactic acid
8	5.6	1	2		2.0			p-Cresol
9	5.9	1	-		2.1			Guaiacol
10	8.0	5	4		3.5	1.4		Octanoic acid
11	8.5	1	1		3.9	1.5		Pyrocatechol
12	10.1	2	2		5.6	2.1		4-Ethylguaiacol
13	11.0	2	1		6.7	2.5		
14	11.8	14	8		8.3	2.8	1.4	Decanoic acid
15	12.5	1	5		9.5	3.1	1.6	Vanillin
16	13.0	1	-			3.3	1.8	
17	14.1	7	3			4.5	2.3	
18	14.4	2	1			4.8	2.5	
19	15.1	1	1			5.8	2.7	
20	15.8	2	4			6.2	3.0	
21	16.4	4	3			7.0	3.6	
22 ^a	17.6	21	33			9.0	4.5	
23	18.3	4	-				5.3	
24	18.7	2	1				5.7	
25	19.3	10	2				6.5	1.4

TABLE XXVI (continued)

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE PORTIONS OF ETHYL ACETATE EXTRACTIVES
OF APRIL LOBLOLLY PINE BARK AND SLASH PINE BARK

Peak No.	Retention Time, min.	Peak Height, units		Isothermal Retention Times, min.				Identification	
		Lo-EA-E	SI-EA-E	100°	150°	180°	200°		250°
26	19.9	2	4				7.1	1.5	p-Coumaric acid
27	20.3	4	1				8.1	1.6	Pentadecanoic acid
28	20.6	2	-				8.7	1.7	
29	21.6	28	10				10.8	1.9	Ferulic acid
30	22.4	3	1					2.1	
31	23.9	1	-					2.5	
32	24.8	1	2					2.9	Oleic acid
33 ^b	38.7	18	27					15.3	
34 ^b	42.7	11	5					19.2	Dihydroquercetin
35 ^b	45.5	9	2					21.8	Dihydromyricetin
36	48.6	1	-					23.5	

^aThis is the same component isolated in quantity previously as Peak 22 in Table XVII on page 36 of Progress Report One.

^bPeaks 33-35 were determined quantitatively by gas chromatography. A much larger excess of REGISIL was required for quantitative conversion of these flavanoid peaks to the desired volatile trimethyl derivative. The following percentage yields were obtained on the basis of the ether-soluble fractions: loblolly pine fraction Lo-EA-E: Peak 33, 12.25%; Peak 34, 15.00%; Peak 35, 11.75%. slash pine fraction SI-EA-E: Peak 33, 13.05%; Peak 34, 8.0%; Peak 35, 4.5%.

Acid Hydrolysis of Ether-Soluble and Ether-Insoluble Fractions

Fractions Lo-EA-E, Sl-EA-E, Lo-EA-EI, and Sl-EA-EI were all hydrolyzed by boiling 4 hours under reflux with 50 ml. of N sulfuric acid. The hydrolysis mixture was allowed to cool, and was then extracted thoroughly with ether to give the ether-soluble products Lo-EA-E-E, Sl-EA-E-E, Lo-EA-EI-E, and Sl-EA-EI-E. The aqueous acid raffinate was filtered to remove a little resinous solid and then deionized with DUOLITE A-6 anion-exchange resin. The remaining aqueous solutions were Lo-EA-E-W, Sl-EA-E-W, Lo-EA-EI-W, and Sl-EA-EI-W.

Gas chromatography of ether-soluble fractions. All ether-soluble fractions were submitted to temperature-programmed gas chromatography under the same conditions employed to obtain the data of Table XXVI. Essentially the same peaks were obtained for all fractions whether from the original ether-soluble portion of the ethyl acetate extractives or the ether-insoluble portion. Therefore, the data for all fractions are presented in Table XXVII and compared with the data of Table XXVI.

The data of Table XXVII when compared with those of Table XXVI suggest that acid hydrolysis changed the original ether-soluble portions and ether-insoluble portions very little, suggesting little if any glycosidic linkages.

Chromatography of water-soluble fractions. The water-soluble fractions were chromatographed for sugars on paper. Neither of the loblolly-derived fractions yielded any sugars whatsoever. The slash-derived fractions yielded a little glucose and traces of mannose and xylose.

TABLE XXVII

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE PORTIONS OF ACID-HYDROLYZED ETHYL ACETATE EXTRACTIVES
OF APRIL LOBLOLLY PINE AND SLASH PINE BARKS

Peak No.	Retention Time, min.	Peak Height, units			Identification ^a	
		From Ether-solubles	From ether-insolubles			
		Lo-EA-E-E	Sl-EA-E-E	Lo-EA-EI-E		
1	1.7	100+	100+	100+	1	Excess REGISIL
2	2.1	1	1	1	2	
3	2.9	4	3	4	3	
4	3.3	2	3	1	4	
5	4.3	9	8	2	6	Phenol
6	4.4	2	1	1	7	
7	5.6	6	6	2	5	p-Cresol
8	5.9	2	1	1	1	Guaiacol
9	7.0	10	1	2	2	
10	7.7	3	1	7	6	
11	8.0	1	1	1	1	Octanoic acid
12	8.5	9	6	4	7	Pyrocatechol
13	10.1	7	10	11	46	4-Ethylguaiacol
14	11.0	17	1	8	1	
15	11.8	11	8	3	3	Decanoic acid
16	12.5	1	1	1	1	Vanillin
17	13.0	1	1	1	1	
18	14.1	28	11	23	17	p-Hydroxybenzoic acid
19	14.4	2	12	-	35	
20	15.1	1	1	1	2	
21	15.8	1	8	2	3	
22	16.4	22	17	33	28	Vanillic acid
23	16.8	3	3	2	-	
24	17.6	77	90	18	34	
25	18.3	5	3	1	1	

TABLE XXVII (continued)
PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE PORTIONS OF ACID-HYDROLYZED ETHYL ACETATE EXTRACTIVES
OF APRIL LOBLOLLY PINE AND SLASH PINE BARKS

Peak No.	Retention Time, min.	Peak Height, units			Identification ^a
		From Ether-Solubles Lo-EA-E-E	From Ether-Insolubles Lo-EA-EI-E	From Ether-Insolubles Sl-EA-EI-E	
26	18.7	1	1	-	24 Myristic acid
27	19.3	1	1	-	25
28	19.9	12	3	3	26 p-Coumaric acid
29	20.3	15	1	-	27 Pentadecanoic acid
30	20.6	1	1	1	28
31	21.6	2	3	-	29 Ferulic acid
32	22.4	2	1	1	30
33	23.0	3	-	3	31
34	23.9	1	-	-	32
35	24.8	1	2	-	
36	25.4	1	-	-	
37	26.8	1	-	-	
38	32.4	1	-	-	
39	35.0	1	2	2	
40 ^b	38.7	2	1	2	33
41 ^b	42.7	20	14	15	34 Dihydroquercetin
42 ^b	45.5	15	8	10	35 Dihydromyricetin
43 ^b	48.6	1	2	5	36

^aNumbers in this column are corresponding peaks in Table XXVI.

^bPeaks 40-43 were determined quantitatively by gas chromatography to yield the following percentages on the basis of the individual fractions chromatographed:

Fraction:	Lo-EA-E-E	Sl-EA-E-E	Lo-EA-EI-E	Sl-EA-EI-E
Peak 40, %	1.0	2.0	1.4	1.0
Peak 41, %	12.0	13.25	10.0	6.25
Peak 42, %	8.5	7.25	6.5	4.0
Peak 43, %	0.5	1.25	3.5	1.5

Alkaline Hydrolysis of Ether-Soluble Fractions Lo-EA-E and Sl-EA-E

These two ether-soluble fractions from the ethyl acetate extractives were boiled under reflux with 4% sodium hydroxide for 4 hours and allowed to cool. The cool solutions were carefully acidified with dilute sulfuric acid and extracted thoroughly with ether. The yield of ether-soluble material, Fraction Lo-EA-E-A from the loblolly pine bark extract was 75% and that from slash pine bark, Fraction Sl-EA-E-A, was 67%.

Gas chromatography of ether-soluble alkaline hydrolysis fractions.

These two products were submitted to temperature-programmed gas chromatography under conditions identical with those employed for the data of Table XXVI. The same peaks were observed, but the peaks corresponding with flavonoid materials (33-35) were missing, indicating that these compounds were destroyed by the alkaline processing. The data for these chromatograms are given in Table XXVIII which reports only the peak heights obtained for the same standard injection used for Table XXVI and relates the peaks to those of Table XXVI.

The loss in yield upon alkaline hydrolysis approximately equaled the yields of the flavonoid compounds lost.

Alkaline Hydrolysis of Ether-Insoluble Fractions Lo-EA-EI and Sl-EA-EI

These ether-insoluble fractions were hydrolyzed in boiling sodium hydroxide solution in the same manner as were the ether-soluble fractions. The hydrolysis mixtures were cooled, acidified, and extracted as before to yield ether-soluble subfraction Lo-EA-EI-A in 33.0% yield and ether-soluble subfraction Sl-EA-EI-A in 14.8% yield. The acid aqueous raffinate was processed by ion-exchange and benzene dehydration as described in detail on page 63 of Progress Report One to give water-soluble fractions Lo-EA-EI-W and Sl-EA-EI-W.

TABLE XXVIII

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE ALKALINE HYDROLYSIS
FRACTIONS Lo-EA-E-A AND Sl-EA-E-A

Peak of Table XXVI	Peak Height, units		Identification
	Lo-EA-E-A	Sl-EA-E-A	
1	100+	100+	Excess REGISIL
2	1	1	
3	2	4	
4	7	12	
5	-	-	Valeric acid
6	4	4	Phenol
7	-	-	Lactic acid
8	2	4	p-Cresol
9	1	1	Guaiacol
10	2	4	Octanoic acid
11	1	1	Pyrocatechol
12	1	6	4-Ethylguaiacol
13	1	-	
14	6	10	Decanoic acid
15	-	2	Vanillin
16	1	-	
17	7	12	p-Hydroxybenzoic acid
18	2	4	
19	1	-	
20	1	5	
21	13	14	Vanillic acid
22	34	12	
23	1	4	
24	-	-	Myristic acid
25	5	10	
26	1	1	p-Coumaric acid
27	2	-	Pentadecanoic acid
28	1	1	
29	22	35	Ferulic acid
30	3	2	
31	4	2	

Gas chromatography of ether-soluble alkaline hydrolysis fractions.

These two ether-soluble products from the alkaline hydrolysis of the original ether-insoluble portions of the ethyl acetate extractives were chromatographed in exactly the same manner used for the corresponding fractions of Table XXVIII. In this instance, however, more peaks were noted than were obtained in either Table XXVIII or the original Table XXVI. The results obtained are tabulated in Table XXIX, and the peaks obtained are cross-referenced to those of Table XXVI.

The most noticeable result of Table XXIX is the fact that the flavonoid compounds of Peaks 33-35 of Table XXVI essentially disappeared, while the phenolic acid peaks 17, 21, and 31 increased considerably. Thus, it appears that the original ether-insoluble portion of the ethyl acetate extractives contained esters of phenolic acids and/or flavonoid components which degraded to phenolic acids upon treatment with boiling 4% sodium hydroxide solution.

Gas chromatography of water-soluble alkaline hydrolysis fractions.

These water-soluble fractions were treated with REGISIL and chromatographed under temperature-programmed conditions employed in the last few chromatograms. A large number of peaks, some in substantial amount, were noted, but only a few of them were identified. If work is continued, some of these more important peaks should be identified. The results of these chromatograms are given in Table XXX.

Recapitulation

This completes the reporting of the investigation so far of the ethyl acetate-soluble portion of the hot water extractives of loblolly pine and slash pine barks. During the course of this continuing study, several compounds were

TABLE XXIX

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE ALKALINE HYDROLYSIS
FRACTIONS Lo-EA-EI-A AND Sl-EA-EI-A

Peak No.	Retention Time, min.	Peak Height, units		Identification ^a	
		Lo-EA-EI-A	Sl-EA-EI-A		
1	1.7	100+	100+	1	Excess REGISIL
2	2.1	2	2	2	
3	2.9	3	3	3	
4	3.3	12	49	4	
5	4.3	35	35	6	Phenol
6	5.6	6	10	8	p-Cresol
7	5.9	2	1	9	Guaiacol
8	6.7	1	5		
9	7.4	1	1		
10	8.5	2	3	11	Pyrocatechol
11	9.7	1	1		
12	10.1	10	53	12	4-Ethylguaiacol
13	11.0	1	1	13	
14	11.8	2	5	14	Decanoic acid
15	12.5	1	2	15	Vanillin
16	13.0	1	1	16	
17	13.5	1	1		
18	14.1	10	20	17	p-Hydroxybenzoic acid
19	14.6	-	2		
20	15.1	3	-	19	
21	15.8	2	5	20	
22	16.4	24	36	21	Vanillic acid
23	16.7	1	1		
24	17.6	13	47	22	
25	18.3	2	5	23	
26	18.6	-	1		
27	19.3	4	16	25	
28	19.9	1	1	26	p-Coumaric acid
29	20.3	2	1	27	Pentadecanoic acid
30	20.6	-	1		
31	21.6	33	93	29	Ferulic acid
32	22.4	1	4	30	
33	23.9	1	3	31	
34	24.8	1	1	32	
35	25.7	1	1		
36	26.8	-	1		
37	27.2	-	1		
38	31.0	1	2		
39	38.7	2	1	33	
40	42.7	1	1	34	Dihydroquercetin
41	45.5	1	4	35	Dihydromyricetin

^aNumbers in this column refer to corresponding peak numbers in Table XXVI.

TABLE XXX

PROGRAMMED GAS CHROMATOGRAPHY OF WATER-SOLUBLE ALKALINE HYDROLYSIS
 FRACTIONS Lo-EA-EI-W AND Sl-EA-EI-A

Peak No.	Retention Time, min.	Peak Height, units		Identification
		Lo-EA-EI-W	Sl-EA-EI-W	
1	1.7	100+	100+	Excess REGISIL
2	2.3	2	3	
3	2.6	2	4	
4	3.1	8	100+	Lactic acid
5	4.0	100+	100+	
6	4.9	10	26	Oxalic acid
7	5.4	62	8	
8	6.1	1	1	Benzoic acid
9	6.6	-	1	
10	6.8	1	1	
11	7.8	1	7	
12	8.3	7	2	
13	8.9	4	5	Succinic acid
14	9.8	4	1	
15	10.4	5	8	
16	11.2	1	1	
17	11.9	8	14	
18	12.2	6	23	
19	12.8	1	1	
20	13.5	1	1	
21	14.3	18	1	
22	15.4	1	7	
23	16.0	2	22	
24	16.6	46	2	
25	17.2	2	3	
26	17.8	6	5	
27	18.1	1.	5	
28	18.5	2	1	
29	18.9	18	1	
30	19.7	3	3	
31	20.1	1	1	
32	20.6	1	3	
33	21.5	1	1	
34	22.1	49	1	
35	23.4	1	2	
36	24.0	-	1	
37	24.8	-	2	
38	24.4	2	1	
39	25.9	-	2	
40	26.7	2	-	
41	27.6	2	-	

identified which were not known at the time of Progress Report One. In Table XVII on page 36 of Progress Report One there appeared a listing of all the component peaks for the large-scale fractionation of the ethyl acetate-soluble portion, and the following discussion refers to this table.

In Table XVII of Progress Report One the following peaks have now been identified: 6, octanoic acid; 7, pyrocatechol; 11, decanoic acid; 13, vanillin; 16, p-hydroxybenzoic acid; 20, vanillic acid; 26, myristic acid; 30, p-coumaric acid; 34, ferulic acid; and 42, combined 18-carbon chain acids. Some of these peaks occur in greater quantity in the fractions of Table XVIII on page 38 of Progress Report One in which the peaks are cross-referenced to Table XVII. One such component is ferulic acid.

The very important component of Peak 22 of Table XVII is Peak Number 22 of Table XXVI of the present report and Peak Number 24 of Table XXVII of the present report. This component is one of the major components of these two pine barks and should be identified in future studies.

INVESTIGATION OF THE MONTHLY SAMPLES OF LOBLOLLY AND SLASH PINE WOODS FOR TURPENTINE YIELD AND COMPONENTS

Turpentine Yields

As noted earlier in this report, in connection with the monthly bark samples, the bolts of loblolly pine and slash pine were barked, chipped, and frozen immediately. Moisture determinations were made before freezing, and these are reported in Table XIII. The frozen chips were processed for turpentine yield determination by a modification of the procedure suggested by Drew and Pylant (2) in a recent study on the turpentine obtainable from the pulpwoods of the United States and Canada.

In accordance with this modified procedure, a sample of wood chips containing 500 grams of owendry wood was placed in a 5-liter round-bottom flask equipped with a thermometer reaching to the bottom of the flask. The chips were covered with 1800 ml. of reagent-grade ethylene glycol which was found to be chromatographically pure, and then treated with a solution of 30 grams of sodium hydroxide in 100 ml. of water. The flask was closed with a double water-separatory head system designed to allow for removal of water from the condensing vapors and also to collect condensed turpentine from these same vapors. The apparatus is pictured in Fig. 2.

The mixture was boiled under reflux for 2.5 hours during which time water was removed, and the original boiling temperature of 120°C. gradually rose to 128°C. The distilled turpentine collected in the calibrated collection tube. The yield was measured at 25°C., and assuming a specific gravity of 0.88, was converted to gallons per ton of owendry wood. Yields of turpentine from the monthly samples of both loblolly and slash pinewoods are given in Table XXXI.

It is obvious from the data of Table XXXI that there is little pattern in yield displayed by the monthly samples of these two barks. The lack of pattern is probably due to a number of factors among which are individual variations of trees, large differences in treatment of wood during transit including time and temperature variations, and experimental errors.

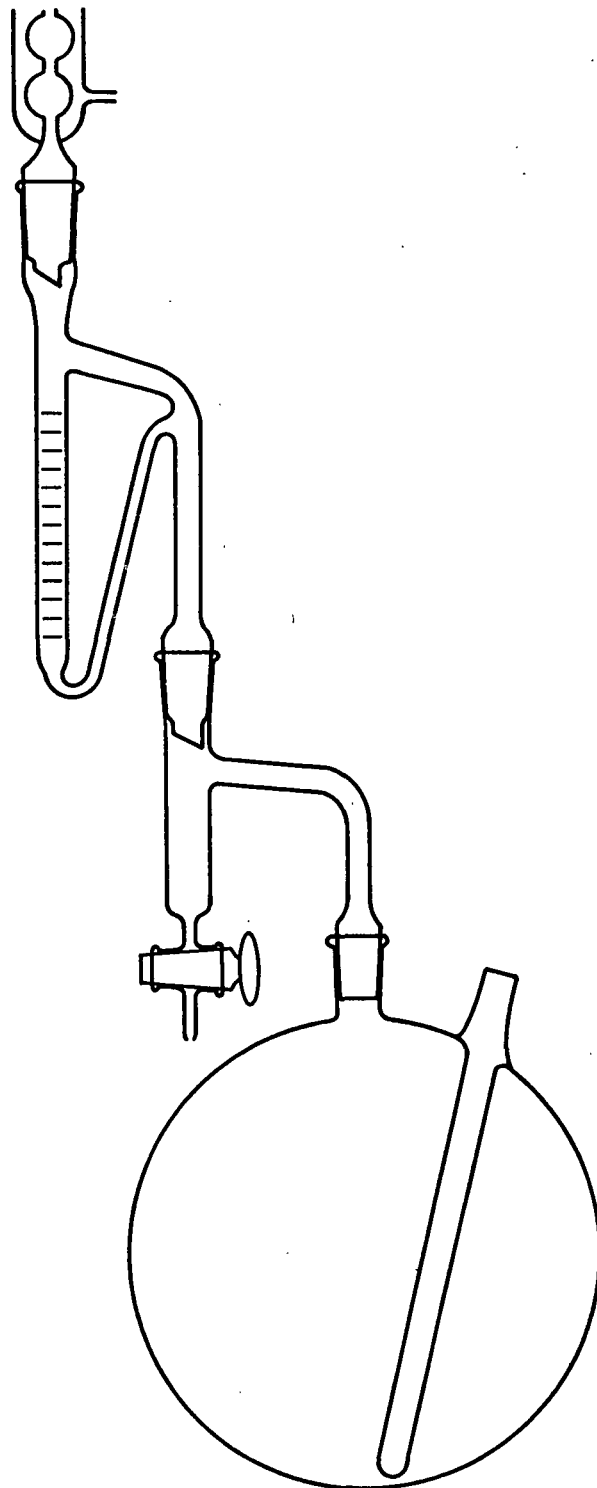


Figure 2. Apparatus Employed in the Recovery of Wood Turpentine

TABLE XXXI

TURPENTINE YIELDS FROM MONTHLY SAMPLES OF LOBLOLLY AND SLASH PINEWOODS

Month	<u>Turpentine Yield, gallons/ton o.d. wood</u>	
	Loblolly Pine	Slash Pine
April	0.86	0.77
May	0.77	0.81
June	0.58	0.77
July	0.58	0.62
August	0.67	0.67
September	0.86	0.62
October	0.72	0.67
November	0.72	1.68
December	1.54	1.10
January	0.62	0.77
February	0.82	0.72
March	1.10	1.10

Gas Chromatography of Individual Monthly Turpentines

Each monthly turpentine sample was chromatographed quantitatively in the Aerograph Model 202 on a 10-foot by 1/4-inch stainless steel column filled with 20% FFAP on 70-80 mesh Chromosorb W, DMCS. At injection, the oven was at 70°C. and programmed immediately to 250°C. at the rate of 4° per minute. The helium carrier gas flow rates were 75 ml. per minute. Data for the loblolly pine turpentines are given in Table XXXII and those for the slash pine turpentines are given in Table XXXIII.

Although no definite trends were developed by the data of Tables XXXII and XXXIII, several points of interest were noted. The light hydrocarbon fractions were present in substantial quantity in only the June loblolly pine-wood and in smaller quantities in only three other samples of loblolly pinewood.

TABLE XXXIII
GAS CHROMATOGRAPHY OF TURPENTINES OBTAINED FROM MONTHLY SAMPLES OF SLASH PINEMOODA

Peak No.	Ret. Time, min.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Identification
1	4.0	1.5	-	-	-	-	-	-	-	-	-	-	-	Light hydrocarbon
2	6.8	23.3	-	-	-	-	-	-	-	-	-	-	-	Light hydrocarbon
3	9.7	45.8	62.2	64.4	64.7	74.2	68.4	58.5	61.8	63.6	50.5	50.5	65.5	alpha-Pinene
4	10.7	0.7	0.9	1.6	0.7	0.5	1.1	2.2	0.8	0.9	0.5	1.1	1.1	Camphene
5	12.5	13.8	19.6	13.6	22.0	17.6	17.6	15.6	28.2	24.5	15.6	20.7	17.8	beta-Pinene
6	13.6	0.7	1.3	1.4	0.7	0.7	0.9	1.8	1.1	1.2	1.5	1.3	0.8	delta-3-Carene
7	14.0	-	-	0.3	-	-	-	0.6	0.1	0.1	0.7	0.2	-	alpha-Phellandrene
8	15.4	0.7	1.3	1.4	0.9	0.9	1.6	1.3	1.3	0.7	1.8	1.5	0.8	Limonene
9	15.8	4.8	6.7	10.8	0.6	0.5	3.6	15.1	3.6	4.9	17.6	12.7	3.9	beta-Phellandrene
10	16.6	-	-	-	0.1	0.05	-	-	-	-	-	-	-	gamma-Terpinene
11	17.6	-	-	0.1	-	-	-	0.05	-	0.05	0.05	0.1	-	p-Cymene
12	18.5	0.1	0.1	0.1	0.2	0.2	0.1	0.15	0.1	0.1	0.1	0.1	0.1	Terpinolene
13	25.5	0.3	0.3	0.3	0.3	0.1	0.4	0.3	0.1	0.2	0.3	0.3	0.3	Unknown
14	27.2	-	-	-	-	0.05	-	-	-	-	-	0.1	-	Unknown
15	28.7	-	0.1	1.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	-	0.1	alpha-Fenchol
16	29.6	-	-	0.3	-	-	0.1	-	-	-	-	-	-	Terpinene-4-ol
17	30.1	0.9	0.9	0.2	1.0	0.05	1.2	0.9	0.2	0.8	0.6	0.7	0.9	beta-Caryophyllene
18	32.1	1.1	2.1	3.4	3.7	4.5	3.0	2.1	1.7	1.6	4.2	3.2	2.2	4-Allylanisole
19	32.5	0.6	0.6	0.9	0.7	0.4	0.9	0.5	0.8	0.5	0.6	0.6	1.2	Borneol
20	34.1	-	0.1	0.1	0.1	0.05	0.1	0.05	-	0.1	0.05	-	-	alpha-Caryophyllene
21	36.3	-	0.1	0.1	0.1	0.05	0.1	0.1	-	0.1	0.05	0.2	0.1	trans-Anethole
22	37.0	-	-	-	-	0.05	-	0.05	0.1	-	0.05	-	-	Unknown
Totals		94.1	96.4	99.8	95.9	100.0	99.2	99.3	100.0	99.5	94.3	93.3	95.0	

^aData in table are yields in percent based on the solids in the individual samples. Solids were determined from volume and an assumed specific gravity of 0.88.

The June sample of loblolly pinewood was noted earlier in this report to be substantially different from the samples from the eleven other months, and it may not be only a matter of difference in month. In the case of the slash pinewood samples, only the April sample contained the light hydrocarbons in any quantity whatsoever, and the yield amounted to 25% of the total turpentine. This point deserves further investigation. In addition, the nature of these "light hydrocarbons" should be studied.

The ratio of beta-pinene to alpha-pinene was much greater in the March and April samples of loblolly pinewood than in all of the other samples of loblolly pine. In the slash pine series, the high ratio of these two pinenes was in December. This concentration of the desirable beta-pinene at certain times of the year is worthy of further investigation.

There are several differences in component content of the turpentine of the two pinewood species. In Table XXXII for loblolly pine, Peak Number 7 at 14.6 minutes' retention time is due to the presence in some of the samples of alpha-terpinene. In Table XXXIII for slash pine, Peak Number 7 occurs at 14.0 minutes, and this peak is due to alpha-phellandrene. In both series, only one peak is present, and the other component is absent.

In addition to the identified components in these turpentines, the loblolly pinewood-derived turpentines contained a component responsible for Peak Number 15 of Table XXXII. This component with a retention time of 27.9 minutes was absent in all slash pinewood-derived turpentines. Identification of this component would be interesting from the standpoint of taxonomy.

The recovery of components in most cases was essentially quantitative. In a few instances the recovery was down a few percent, and this is probably due to a difference in specific gravity from the assumed value of 0.88. Only 5 microliter injections of undiluted turpentines were employed. A very slight change in specific gravity would change the sum of the individual percentages by a few percent.

This completes the investigation to date on the turpentines obtained from the monthly samples of loblolly and slash pinewoods.

FUTURE STUDIES

The studies reported herein and in Progress Report One comprise a preliminary investigation of the components of the extractives of loblolly and slash pine barks. During this investigation, many of the original problems were solved, but some were left unsolved. These should be pursued. In addition, the preliminary investigation demonstrated some new avenues of research. Many individual compounds were isolated and identified. Many more were isolated, but not identified. Some of these unidentified components were major components, and continued work should be performed on these. Monitoring experiments demonstrated that some important components are still left to be separated pure so that they may be identified. Development work is left to be done on some aspects of the analytical separations. This applies to fatty acids, aromatic compounds, long-chain alcohols, and sterols.

Anomalous results should be checked, especially those associated with the June loblolly pine sample.

Development studies should be made on the larger scale production of some of the important components of these barks such as tetracosanoic acid, hexacosanoic acid, tetracosanol, hexacosanol, dihydroquercetin, and dihydro-myricetin. In addition, the very important component corresponding with Peak 22 of Table XVII, Peak 22 of Table XXVI, and Peak 24 of Table XXVII should be identified and its isolation in quantity investigated.

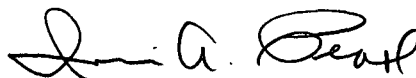
The components of alkaline hydrolysis of the ethyl acetate extractives should be elucidated. In addition, studies on the alkaline extraction of the whole bark should be initiated and the components liberated should be investigated. Preliminary experiments reported herein indicate that yields of products are much higher when the original bark is extracted with alkaline solution rather than with water.

It is hoped that this Group Project is continued so that these studies can be completed. If the project is continued, it might be advisable to include pertinent studies on two other important southern pines - shortleaf pine and longleaf pine.

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