ISOLATION AND IDENTIFICATION
OF CERTAIN TOXIC COMPOUNDS
IN A FRACTION OF KRAFT MILL WASTE

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1962

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Date thesis is presented __August 11, 1961__

Typed by Lilah N. Potter

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INTRODUCTION

Increased utilization of our natural waters for
waste disposal has become an extremely important problem
to those concerned with the health and welfare of an
evergrowing population. The seriousness of this trend
is indicated in Oregon, where one Kraft pulp mill alone
can place 46,000 tons per day (14) of water contaminated
with chemical wastes in a stream. Although it has been
known for over a decade that this waste is a hazard to
Oregon's aquatic life, very little is known about the
chemical nature of this waste. Domestic use of water from
polluted streams may in time become hazardous to human
health.

Preliminary studies by McHugh (12), a member of
the Fish and Game Management of Oregon State University,
originally suggested that the steam distillable materials
in the waste from one modern Kraft mill, pulping only
Douglas fir, accounted for most of the toxicity of the
waste. At the suggestion of the above depart-
ment, a thorough investigation of the chemical composi-
tion of the steam distillable materials has been
initiated. The primary stages of that chemical

investigation will be reported in this thesis.

HISTORICAL

Although the Kraft process has been known for almost a century and the literature pertaining to this is voluminous, very little is known about the actual chemical reactions which occur during this process. It is very easy to understand why this problem still exists when the complexity of the starting material, i.e., Douglas fir chips, is considered and that the structure of the products from the reaction are based mostly on speculation.

Johnson and Cain (8) have shown that the volatile oil of Douglas fir wood is a mixture of (-)-α-pinene (I, 30%), (-)-camphene (II, 6%), (-)-limonene (III, 14%), (-)-α-terpineol (IV, 32.2%), a dark viscous oil (sodium carbonate extraction), and unidentified higher boiling compounds.
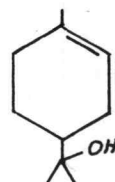
| I | II | III | IV |

Although they did not isolate or identify the components of the dark viscous oil, they suggested that it might contain one or more of the following acids; caproic

($C_6H_{12}O_2$), caprilic ($C_8H_{16}O_2$), capric ($C_{10}H_{20}O_2$), and lauric ($C_{12}H_{24}O_2$). Schorger (19) found that the oleoresin which collected in pockets in the heart wood contains chiefly (-)-α-pinene (I) and small amounts of (-)-limonene (III) and (-)-α-terpineol (IV), whereas the oleoresin which is obtained by scarification of the living sapwood contains (-)-α-pinene and (-)-β-pinene (V).

V
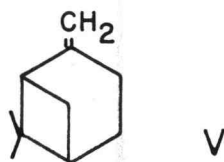
Since the bark, leaves, and twigs are not used in the Kraft process, the known composition of their volatile oils are not included.

Outside of the flavonone, taxifolin (VI), which Pew (15) isolated from the heart wood, the trisaccharide, melezitose (VII), which Hudson and Sherwood (7; 16, p. 455) isolated from the manna (exudate from insect-produced wounds), and the essential oils mentioned above, no other individual compounds have been reported in the literature.

VI
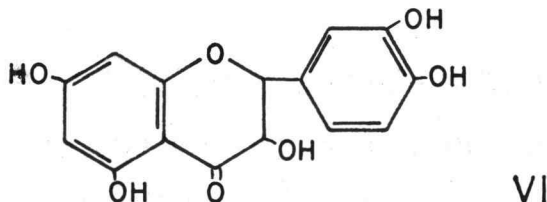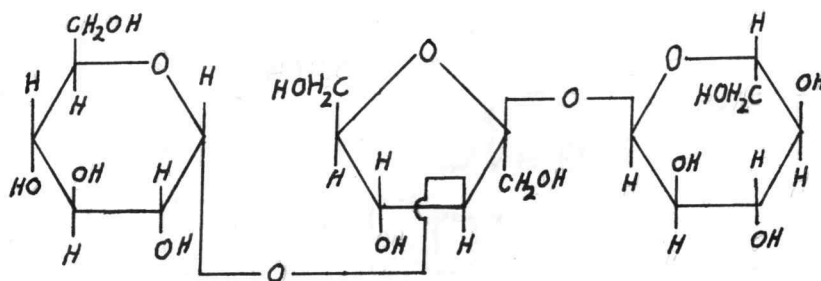
VII

Wise and Ratliff (22; 23, p. 388) have shown that
Douglas fir contains 28.4% lignin, 14.1% hemicellulose,
and 57.2% α-cellulose. The types of structural units
in the lignin fraction are suggested by the compounds
obtained by Fletcher and Harris (5) when they thermally
decomposed a Douglas fir lignin. They found acetone,
methanol, formic and acetic acids, C_{18}-C_{22} acids, phenol,
o-cresol, p-cresol, quaiacol (VIII), 2,4-xylenol (IX),
4-methyl quaiacol, 4-ethyl quaiacol, and neutral
material.
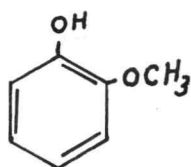
VIII
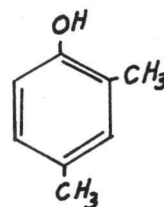
IX

McInnes and associates (13) have isolated α-ethoxypropio-
vanillone (X), vanillin (XI), and vanilloyl methyl ketone
(XII) from the ethanolysis of a Douglas fir lignin.
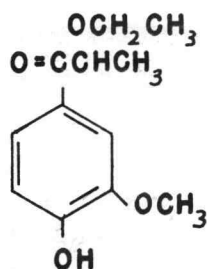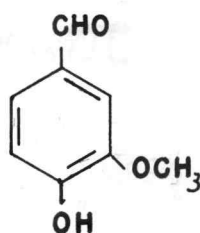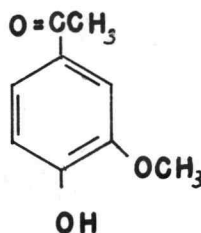
The literature pertaining to the by-products of
the Kraft process usually does not indicate where the
material was obtained, which tree or trees are used, or
the details of the Kraft plant operations. The blow off
gases from the digesters contain the volatile wood ex-
tractives. This volatile organic material which is known
as "Kraft turpentine" is collected and sold by some
mills. Although the crude "Kraft turpentine" (14) varies
with location, type of soil, age and type of tree, and
variations in the Kraft process, it normally contains
50 to 60% α-pinene, 15 to 20% β-pinene, 10 to 15% mono-
cyclic terpene hydrocarbons, and 10 to 15% sulfur com-
pounds and other impurities (23, p. 574).

The soluble and insoluble non-volatile materials
which remain in the sodium hydroxide-sodium sulfide

solution in the digesters are separated by filtration. The insoluble material, i.e., cellulose, is washed and used to make paper. The solution of non-volatile materials, usually called "black liquor", is concentrated in vacuo and used as a fuel or separated from the aqueous medium and sold. This material which is separated by acidification of the "black liquor" is a viscous black oil which is called "tall oil". The typical composition of "tall oil" (23, p. 611) is 40 to 50% resin acid, 40 to 50% fatty acids, and 10% neutral material. Sixty percent of the neutral fraction is composed of esters of fatty acids and the remainder is composed of sterols, higher alcohols, and hydrocarbons. Usually the fatty acids are composed essentially of oleic ($C_{18}H_{34}O_2$), linoleic ($C_{18}H_{32}O_2$), linolenic ($C_{18}H_{30}O_2$), and palmitic ($C_{16}H_{32}O_2$) acids. There are only two types of resin acids known at the present time (21, p. 170), abietic type (XIII) which contains two conjugated double bonds and pimaric type (XIV) which contains two nonconjugated double bonds.

XIII

XIV

COOH
|
CH$_2$
|
C=CH$_2$
|
COOH
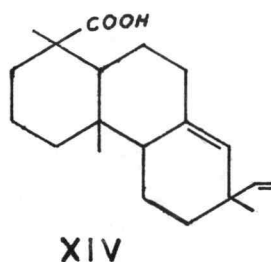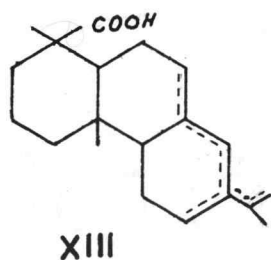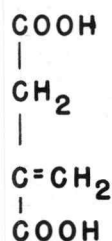
XV
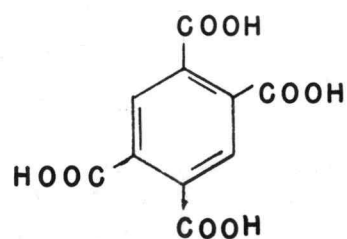
XVI

During the first world war, the government spon-
sored the development of three Kraft mills (18) because
White (18, p. 62) found that appreciable quantities of
acetone, methyl alcohol, and ammonia could be obtained
from the waste liquor by distilling it with lime. Two
of the plants never reached the operational stage and
the other one only lasted a few days.

Grangaard (6) has oxidized the aqueous waste with
oxygen at 140-170°C for 4 to 6 minutes and from this he
isolated and identified the following acids; acetic,
formic, fumaric, succinic, oxalic, malonic, lactic,
itaconic (XV), malic and pyromellitic (XVI).

Although the above data on Kraft mill waste has been reported, there are no references in the literature to the Kraft mill waste obtained from pulping of Douglas fir alone.

DISCUSSION

The principal aim of this project is to obtain detailed information about the chemical nature of certain fractions of Kraft mill waste which are known to contain organic compounds toxic to fish (see p. 1). Several of the compounds in the organic fraction of the effluent from one particular mill have been isolated and identified, others have been tentatively identified, and partial information has been obtained for two others. To simplify the discussion, this section will be divided into five parts.

1. The Basic Sample

One basic problem which arises in any investigation of this type is to obtain a sample as truly representative as possible. An ideal sample would be one representative of all Kraft mills at any time and a knowledge of its chemical nature would certainly be significant for stream pollution problems. However, earlier work on this problem (24) showed that the effluents from two different Kraft mills have significant differences in the distribution of toxicity when fractionated. Even the effluents from a single mill showed considerable variations over a period of years. Therefore, it appears

impossible to obtain an ideal sample and compromises are
necessary.

Since the chemical nature of Kraft turpentine
varies with the location, age and type of tree, the type
of soil in which the tree grows, the particular condi-
tions chosen for the Kraft cook (temperature and duration
of the cook, concentration of base and sulfide, etc.),
and probably the length of time the wood chips are stored
before use (14), the nature of the effluent will un-
doubtedly depend upon these factors. To be sure, the
engineering of the mill with respect to the reuse and
treatment of the waste waters before discharge will also
affect the nature of the effluents. Not all of these
variables can be controlled, but a certain degree of
uniformity can be achieved by using waste from a mill
which pulps only wood from one single species of tree.
The time variable factors can be in some degree averaged
by collecting the sample at random intervals over a
reasonable period of time.

A modern Kraft mill, which is probably reasonably
representative of many of those in the Pacific Northwest,
was chosen as a source of the sample. The mill pulps
only Douglas fir from the forest in this area and the
pulp produced by this mill is unbleached. The effluent
which was collected over a 3-month period (summer 1959)

was a sample of the so-called "foul condensate". This
material comes from the low vacuum stage of the evapor-
ator for condensation of the "weak black liquors" (see
experimental). Since it seems reasonable to assume that
the chemical makeup of the waste will remain quite con-
stant in a qualitative sense, the sample used should be
reasonably representative of those mills with similar
cooking and engineering design.

Even though the majority of the volatile wood
extractives are lost during the venting of the digestors
in the mill, the foul condensate could be expected to
contain small amounts of Kraft turpentine or products
derived therefrom. Low molecular weight materials from
lignin breakdown should also be present in the sample.
Although non-volatile products such as inorganic sub-
stances, salts of strong acids (sulfonated material) or
semi-polymeric lignin derivatives would not be expected
to be present, traces could be carried over in the
evaporator. Therefore, the sample was intially steam
distilled to remove all such substances.

2. The Presence of Benzene in the Waste

Quite unexpectedly, one 20-gallon batch of foul
condensate produced an unusually large amount of organic
material, predominantly boiling below 100°C. The major

component, benzene, was identified by gas phase chromatography and by comparison of its infrared spectrum with that of an authentic sample. Since the concentration of the organic material in the other batches of waste remained quite constant (0.48 g per gal), the large amount of benzene in the one sample must be considered an extraneous contaminant. Gas phase chromatography also showed the presence of trace amounts of acetone and ether in the volatile material.

3. The Minor Components

The organic residue from the steam distillate of the foul condensate was separated into three fractions by vacuum distillation. The first fraction (I, 5.4 g, b.p. 30-56°C (0.3 mm)) was further separated into three fractions (I-1, I-2, and I-3) by semi-preparative gas chromatography and the intermediate fraction, I-2, was similarly divided into two fractions (I-2A and I-2B). Fraction II (11.0 g, b.p. 56-95°C (0.3 mm)) was separated into five fractions by column chromatography (see Table I). Analysis by gas chromatography of fractions I-1, II-1, II-3, and II-4 showed that each of these fractions is a very complex mixture. Since only trace amounts of each of these fractions were available, no further work was attempted with them. Fraction III, the

TABLE I

Separation Diagram for Fractions I and II

Fraction I (5.4 g)
b.p. 30-56°C (0.3 mm)

gas chromatography

I-1 (40 mg)   I-2 (1.14 g)   I-3 (4.25 g)

gas chromatography

I-2A (630 mg)   I-2B (510 mg)

Fraction II (11.0 g)
b.p. 56-95°C (0.3 mm)

chromatographed 6.98 g
on silicic acid

II-1 (500 mg)   II-3 (308 mg)   II-5 (850 mg)

II-2 (4.25 g)   II-4 (263 mg)
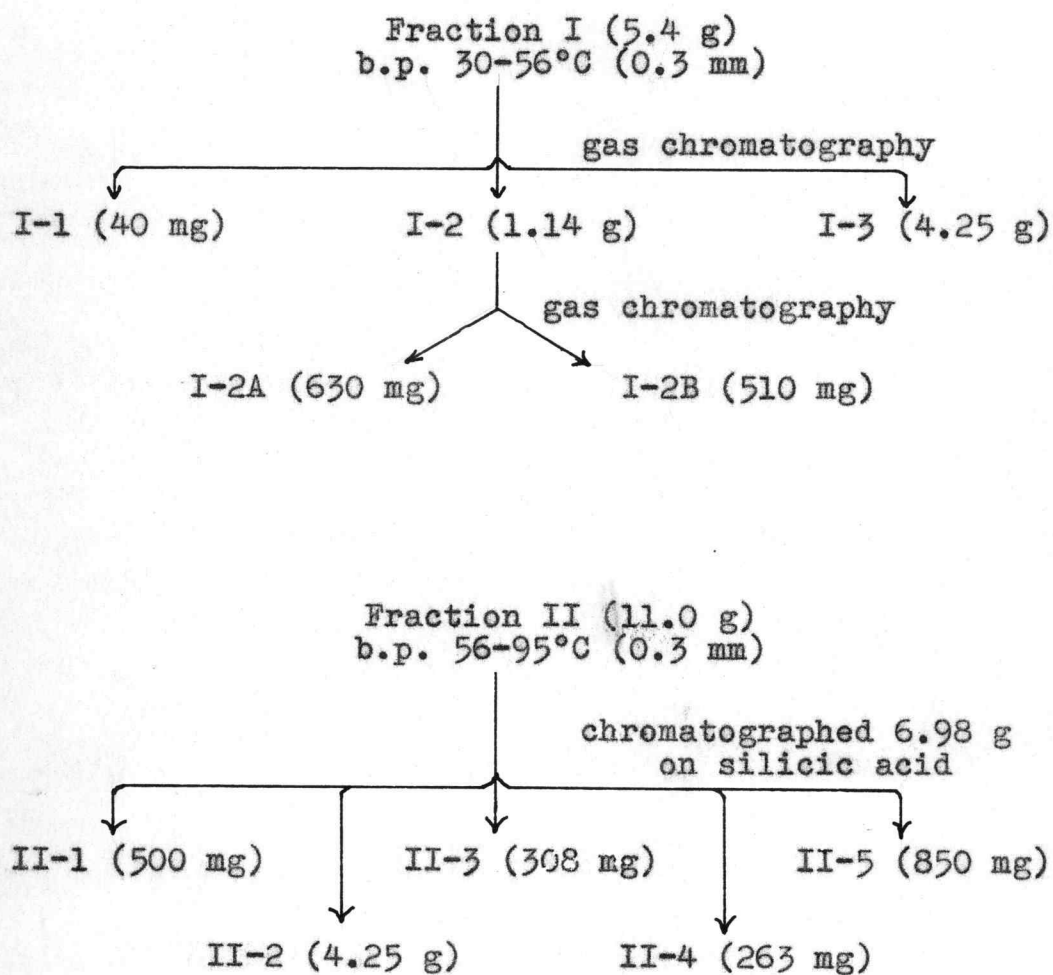
non-distillable material, was originally set aside for future investigation because it undoubtedly contains the most complex substances and time did not permit an extention of the investigation to this fraction. Future work is planned in this direction.

The first fraction of sufficient size to warrant a careful examination is I-2A. Gas chromatographic analysis showed that it is a very complex mixture. The average molecular weight and the ultraviolet and infrared spectra of the total mixture suggested the presence of terpenes or terpene-like materials. Since isolation and examination of individual constituents was out of the question, an attempt was made to identify components by gas chromatographic comparison with available terpenes known or suspected to be present in coniferous trees. The common practice is to add a small amount of the known to the mixture, and if the area under a given peak in the mixture is increased, it is assumed that the component is identical with the added substance. It is quite possible, of course, that two different substances will show identical behavior on gas chromatography, hence this means of identification is by no means absolute.

The degree of certainty attached to the identification can be greatly enhanced by using two columns with

different stationary phases. Since the relative reten-
tion time for any given component of the unknown need
not be the same for both columns, the success of this
technique depends upon whether or not the relative re-
tention time for a given component can be identified
with both columns. It seems quite reasonable to assume
that the relative amount of a compound in the mixture
will not vary; thus the areas under the various peaks
were used to identify the same peaks on the two chroma-
tograms.

The addition of various terpenes to the mixture
and analysis on the two columns led to the conclusion
that limonene, γ-terpinene, p-cymene and anisol are
present in the fraction I-2A. A variety of other ter-
penes were tested in similar fashion but could not be
effectively related to any component in the mixture.

Two other components of the mill waste were iso-
lated in relatively pure form though in too small quan-
tities to permit complete identification. The first of
these, I-2B, shows absorption in the infrared at 3400
and 1150 cm^{-1} (probably tertiary alcohol) and at
1680 cm^{-1} (α,β-unsaturated ketone). The ultraviolet
spectrum showed λ_{max} 238 ($E_{1\ cm}^{1\%}$ = 1170) and λ_{max} 280
($E_{1\ cm}^{1\%}$ = 230), thus indicating the presence of an
α,β-unsaturated ketone. The substance forms a bright

red 2,4-dinitrophenylhydrazone which melts above 300°C.
Since most mono-2,4-dinitrophenylhydrazones melt in the
range 100 to 250, it is assumed that this is a di-2,4-
dinitrophenylhydrazone. On this basis its analysis
corresponds to the formula $C_9H_{14}O_7$ for I-2B. Unfor-
tunately it would appear that no great significance can
be attached to the empirical formula since only a minute
amount of derivative (2.3 mg) was available for analysis
and it seems dubious that a polyfunctional substance of
that formula should be present in a steam distillate.

The second of the two components noted above,
II-5, is the only solid material isolated so far from
the mill waste. From an inspection of the infrared
spectrum of the unknown, it was concluded that the only
functional group present is hydroxyl. Compound II-5
forms a crystalline 3,5-dinitrobenzoate whose analysis
corresponds to an empirical formula $C_8H_{10}O_5$ if it is
assumed to be a double derivative. Since the alcohol
shows ultraviolet absorption at λ_{max} = 270 (ϵ 2150)
and decolorized cold, neutral potassium permanganate and
bromine water, this compound could have been a phenol.
However, II-5 in sodium hydroxide did not give the ultra-
violet spectrum expected on a phenolate ion. Since II-5
is only very slightly soluble in water, a majority of
the oxygen atoms must be in ether linkages. No

satisfactory structures can be written to accomodate these data.   Further investigations on this material are pending.

The infrared spectrum of fraction I-III suggested an ortho-substituted phenol (3480, 3050, 1610, 1605, 1590, 1503, 1365, and 750 cm^{-1}) with a methoxy group (2990, 2860, 2830, and 1260 cm^{-1}) as the ortho substituent.   Therefore, an infrared spectrum of an authentic sample of guaiacol was obtained and the two spectra were found to be identical.   The melting point of a mixture of their benzoates showed no depression.   Therefore the major compound in fraction I is guaiacol.

4.   The Major Product

The major product, II-2, bears no resemblance to any known lignin breakdown products or to any terpene known to be present in Douglas fir.   The structural analysis of this unusual compound will be discussed in detail.

The colorless oil, II-2, showed strong absorption in the infrared at 3400 and 1050 cm^{-1}, which is consistent with a primary alcohol.   The infrared spectrum also showed absorption at 1860(vw), 1760(vw), 1500(s), and 812(vs) cm^{-1} characteristic of para-disubstituted benzenes.   Absorption at 1625(vw), 984(m), 930(w), and

$895(vw)$ cm^{-1} suggested the presence of an unconjugated double bond. The ultraviolet spectrum (λ_{max} 273 (ϵ = 1000), λ_{max} 265 (ϵ = 1050), and λ_{max} 259 (ϵ = 850)) and the refractive index (n_D^{25}1.505) are in good agreement with the assumption of the presence of a benzene ring. Since the specific rotation is +38.1, II-2 must contain at least one asymmetric center.

Confirmation of the presence of a para-substituted benzene was obtained by oxidation of II-2 with potassium permanganate. A white crystalline acid was obtained and shown to be terephthalic acid by comparing its infrared spectrum with that of an authentic sample.

Since the analytical data for II-2 and its 3,5-dinitrobenzoate were in very good agreement with the formula $C_{12}H_{18}O$, the two alkyl groups must be saturated. This was confirmed by the following reactions; II-2 reacts with bromine via substitution and not addition (hydrogen bromide evolved); it did not add hydrogen catalytically at atmospheric pressure; and it failed to react with perbenzoic acid.

Taking into account all of the preceeding data, the following three carbon skeletons can be written;

A   B   C
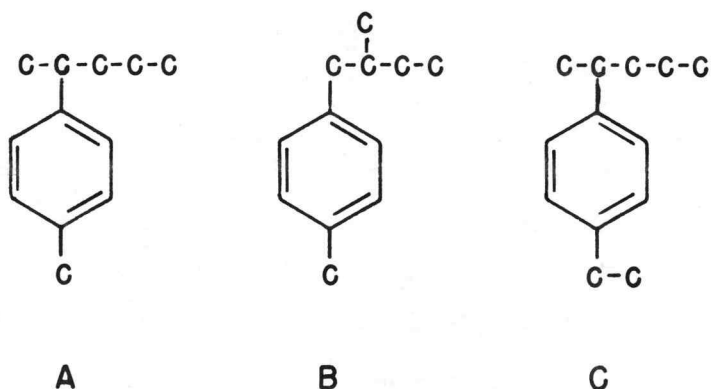
To distinguish between A or B and C, a selective oxida-
tion was attempted. For this purpose, II-2 was oxidized
(4) with dilute nitric acid. Though no single acidic
product could be isolated in pure form or in good yield,
examination by paper chromatography showed that p-toluic,
acetic, malonic, and oxalic acids were present. A small
amount of crude p-toluic acid was isolated and its in-
frared spectrum was identical to that of an authentic
sample.

Consideration of this data and the isoprene rule
suggested that the unknown compound was most probably
4-(p-tolyl)-1-pentanol. Since the melting point of the
3,5-dinitrobenzoate of II-2 is almost identical to the
reported melting point of the 3,5-dinitrobenzoate of
4-(p-tolyl)-1-pentanol (3), the simplest expedient seemed
to involve synthesis of this alcohol. This was accor-
dingly carried through.

A sample of 3-(p-toluoyl)-propionic acid (XVII)
was treated with methyl magnesium iodide and the
Grignard adduct lactonized spontaneously when treated
with dilute sulfuric acid. The crude 4-(p-tolyl)-4-
valerolactone (XVIII) was reduced via Martins modified
Clemmonsen reduction (11) to the expected acid, 4-(p-
tolyl)-pentanoic acid (XIX). Esterification and re-
duction of the ester with lithium aluminum hydride com-
pleted the synthesis. The synthetic material and II-2
had identical infrared spectra, and the melting point
of a mixture of the two 3,5-dinitrobenzoates showed no
depression. Therefore, II-2 is 4-(p-tolyl)-1-pentanol (XX).
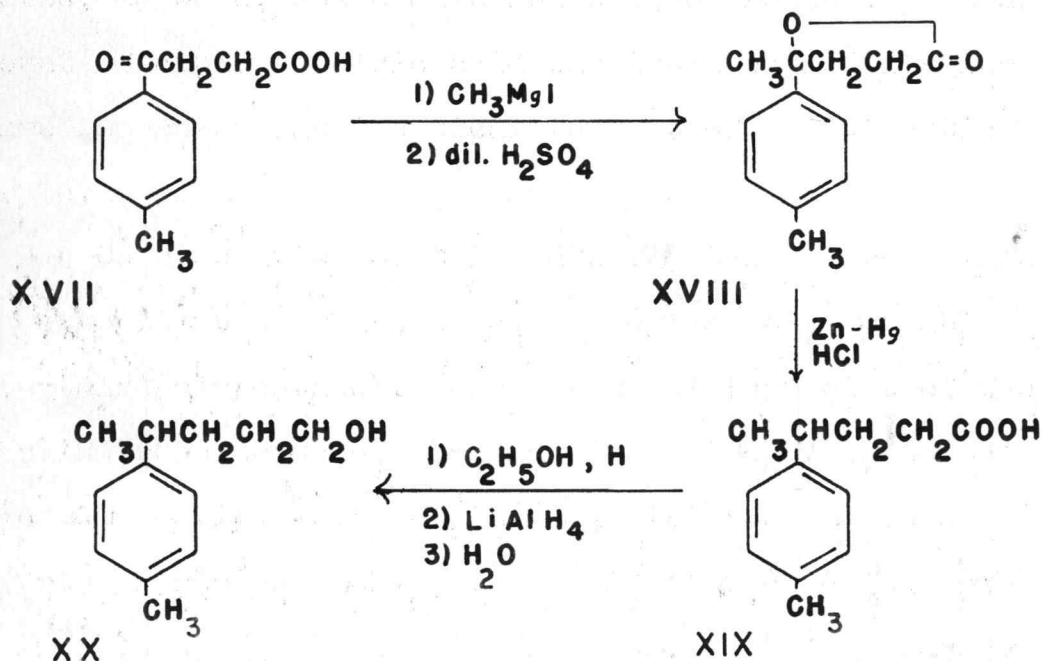
Since the alcohol (XX) from the mill waste is optically active and fits, as far as it goes, the isoprene rule, it may be assumed to have been derived from a sesquiterpene present in the wood. It is not unreasonable to suggest that one of the curcumenes, α, β, or γ curcumene (XXI, XXII, and XXIII, respectively) could be the precursor. Though none has been isolated from Douglas fir, γ-curcumene has been found in a coniferous species (1) and β- and γ-curcumenes both tend to form aromatic rings very easily.

$CH_3CHCH_2CH_2CH=C(CH_3)_3$

CH$_3$

XXI

$CH_3CHCH_2CH_2CH=C(CH_3)_3$

CH$_3$

XXII

$CH_3CHCH_2CH_2CH=C(CH_3)_3$

CH$_3$

XXIII
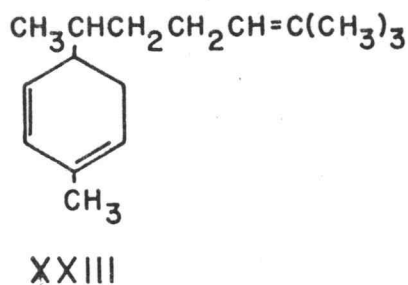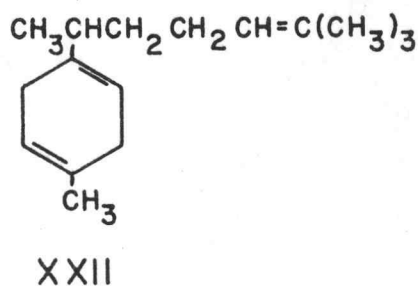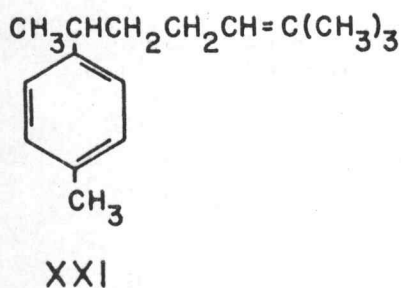
It is of no little interest that calculation of the molecular rotation for γ-phenyl-n-pentanol (XXIV) by Brewster's method (2) shows that the R-configuration has a plus rotation (calc. +40°).

$$CH_2CH_2CH_2OH$$
$$C_6H_5-\underset{H}{\overset{|}{C}}-CH_3$$
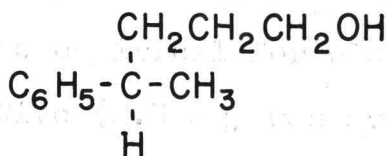
XXIX

Since the hydroxy group may be expected to have very little influence upon the molecular rotation, the calculated value is in good agreement with the molecular rotation (+38°) of 2-phenylpentane (10). Due to the fact that the p-tolyl group will be more polarizable than the phenyl, the 4-(p-tolyl)-1-pentanol should have a higher molecular rotation. Therefore, the molecular rotation of the isolated alcohol, +67, suggests very strongly that it is of high optical purity. Since the only naturally occurring curcumene to have the R-configuration at the specified carbon is γ-curcumene (1), the isolated alcohol could indeed have been derived from γ-curcumene. A search for this or closely related substances in Douglas fir might prove most enlightening on this point.

5. The Toxicity of the Components

The toxicity of the various fractions could not be determined by routine bioassay after each step simply because sufficient material was not available. Therefore, the toxicity tests were limited to the major fractions and these data are given in Table II.

Clearly 4-(p-tolyl)-1-pentanol is the most toxic compound isolated from the waste. This unusually toxic alcohol was found to equal cyanide ion in its ability to inhibit the cytochrome oxidase enzyme system in vitro (24). Despite this high toxicity, it is obvious from the data (Table II) that this substance can account for only a small fraction of the total toxicity of the waste. Since it has less than the average toxicity for the organic material in the waste, it is clear that a much more toxic compound or compounds must be present in the non-distillable fraction which has not yet been investigated.

TABLE II

Toxicities of the Constituents of Foul Condensate

Fraction	Concentration (ppm) to Kill x % in 48 hr		
	100%	50%	0%
Foul Condensate		8- 15	
Benzene	560		320
I	180	100	56
I-2	375	56	32
I-3	180	135	100
II-2	32	22	18

EXPERIMENTAL

Source of the Sample

The foul condensate (130 gal) was collected at random intervals over a 3-month period (summer 1959) from one particular mill. It produces unbleached pulp from Douglas fir only, using a 2½ hr cook at 170°C in a liquor containing 7½ lb base (as Na_2O) per cubic foot and 25% sulfidity. The digestors are vented periodically during the first hour. The filtrate from the pulp and the first-stage wash waters comprise "weak black liquors." These are concentrated in a vacuum evaporator process. The condensate from the low vacuum stage is foul condensate.

Isolation of Organic Constituents

The foul condensate was distilled in vacuo at 85-90°C in 5-gallon batches until about 350 ml of residue remained. The distillate was extracted with ether in a continuous liquid-liquid extractor for 24 hr. The ether layer was dried over anhydrous magnesium sulfate and the ether removed via distillation through an efficient Fenske column. The foul condensate, 130 gal, gave 63.4 g of black viscous oil. Routine bioassay showed the residual foul condensate and the aqueous layer after ether extraction to contain no important toxicity. They

were discarded.

Extraneous Volatile Components

From one particular 20-gallon sample there was
obtained 8.45 g of colorless liquid, b.p. 36-81°C,
257.5 g of colorless liquid, b.p. 81°C, and 9.0 g of
black viscous oil. An infrared spectrum of the large
fraction was identical with that of benzene. The first
fraction was chromatographed on a 6' x ¼" type "K" column
(75°C, flow 43, 15 psi). Four peaks were found (R_t 0.8,
1.4%; 3.2, 5.0%; 4.7, 2.5%; 6.0, 90.0%).* Comparison
with internal standards showed that peak 1 was ether,
peak 2 acetone, and peak 4 benzene.

Preliminary Separation

The organic constituents of the foul condensate
(63.4 g) were distilled through a semi-micro Claisen head
distillation apparatus. Three fractions were obtained.

Fraction I   b.p. 30-56°C (0.3 mm) 5.4 g light yellow oil

Fraction II b.p. 56-95°C (0.3 mm) 11.0 g light yellow oil

Fraction III   Residue   46.8 g black viscous
oil

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*Retention times (R_t) are given throughout in minutes.

1. Fraction I

Preliminary Separation

Fraction I above was separated into fractions by chromatography on an 8½' x ½" column (20% Reoplex 400 on Celite) at 170°C using He as carrier at 43 flow meter reading and 5 psi. Three fractions were obtained.

Fraction I-1 R_t 0-8 40 mg colorless liquid

Fraction I-2 R_t 8-33 1.14 g light brown liquid

Fraction I-3 R_t 33-42 4.25 g light brown liquid

Analysis of I-1

Fraction I-1 above was chromatographed on a 14' x 1/8" column (10% carbowax 20M on firebrick, 172°C, 15 psi). The chromatogram showed 18 peaks.

Separation of I-2

Fraction I-2 above was further separated into fractions by chromatography under the conditions described for preliminary separation. Two fractions were collected.

Fraction I-2A R_t 8-21 630 mg dark brown oil

Fraction I-2B R_t 21-26 510 mg light yellow oil

Analysis of Fraction I-2A

The fraction showed the following physical con-
stants: λ_{max} 267 ($E_{1\ cm}^{1\%}$ 6440), 263 (5200), 261 (7650),
258 (6440), 255 (5890), 252 (6150), 248 (4100), 242
(2680), 237 (1720); infrared bands at 2400, 3000, 2920,
1725, 1700, 1650, 1380 and 1145 cm^{-1}; average molecular
weight 165 ± 5 (commercial thermistor instrument).
Analysis via gas phase chromatography showed a complex
mixture. The use of internal standards using two differ-
ent columns, and the area under peaks to assign identity
to peaks in the unknown on the two columns gave the data
of Table III. β-Pinene, terpinolene, citronellol,
linalool, menthone, and menthofuran were also used as
comparison standards but their presence in the unknown
was not confirmed.

Analysis of I-2B

The fraction showed the following physical son-
stants: λ_{max} 238 ($E_{1\ cm}^{1\%}$ 1170) and 280 (230); infrared
bands at 3400, 2900, 1725, 1680, 1650 and 1150 cm^{-1};
molecular weight 235 ± 5 (commercial thermistor instru-
ment). Analysis via gas phase chromatography showed one
compound.

TABLE III

Gas Phase Chromatography of Fraction I-2A

Column A*				Column B**			
R_t	%***	Compd.	Known	R_t	%***	Compd.	Known
(min.)				(min.)			
2.1	trace	A		3.5	0.1	B(?)	
3.8	trace	B		5.9	0.8		
5.0	0.3	C		6.4	0.4	G	p-cymene
6.0	0.4	D		7.2	0.6	H	
6.7	1.4	E	(−)-limonene	8.9	3.2	L(?)	
8.0	0.2	F	(−)-terpinene	12.0	1.3	E or K	(−)-limonene
8.9	0.5	G	p-cymene	14.1	0.9	I	anisol
9.6	0.7	H		17.3	0.1	F	(−)-terpinene
12.1	0.9	I	anisol	19.0	2.1		
13.9	1.1	J		24.8	2.3		
19.0	1.4	K		30.8	1.1	J	
21.6	3.0	L		35.0	9.5		
25.0	7.7	M		45.7	9.8	M-R	
27.3	0.5	N		51.7	58.6		
31.0	30.2	O		60.4	8.0		
32.7	0.4	P					

TABLE III - Cont.

Column A*				Column B**			
R_t	%***	Compd.	Known	R_t	%***	Compd.	Known
(min.)				(min.)			
38.3	4.1	Q					
45.1	43.0	R					

*14' x 1/8", 10% carbowax 20M on firebrick, 133°, 15 psi
**12' x 1/8", 10% Graig polyester on firebrick, 108°, 15 psi
***% values based on area under peaks

2,4-Dinitrophenylhydrazone of I-2B

A solution containing 43 mg of 1-2B in ethanol
was mixed with 1.5 ml of solution containing 1.0 g of
2,4-dinitrophenylhydrazine in 30 ml of diglyme. Two
drops of concentrated HCl were added and the solution
allowed to stand 24 hr. The dark red precipitate (43 mg)
was isolated by filtration and dissolved in hot benzene-
chloroform. The insoluble 2,4-dinitrophenylhydrazine was
removed and the solution evaporated to dryness in vacuo.
The residue was recrystallized from nitroethane-toluene
giving 2.3 mg of fluffy orange crystals, m.p. > 300°C.

Anal. Found: C, 41.39; H, 3.76.

Analysis of I-3

The light brown oil, 4.25 g, was distilled giving
4.1 g of colorless liquid, b.p. 95°C (0.3 mm), with in-
frared bands at 3480, 3050, 2990, 2860, 2830, 1610, 1605,
1590, 1513, 1365, 1260 and 750 cm^{-1}. An authentic sample
of guaiacol gave an identical infrared spectrum.

Benzoates of I-3 and Guaiacol

One gram (8 mMoles) of I-3, mixed with 0.7 g
(5 mMoles) benzoyl chloride, was boiled over a low flame
for 5 min and poured, with stirring, into 10 ml of ice

water. After the precipitate had settled, the supernatant liquid was decanted and the residue washed with 5 ml of 5% sodium carbonate solution. The solid product was isolated by filtration and recrystallized from ethanol to give 0.9 g (80%) of the benzoate of I-3, m.p. 58°C. Guaiacyl benzoate was prepared by the same procedure and a mixture of the benzoates melted at 58°C.

2. Fraction II

Chromatography of II

A portion (6.98 g) of Fraction II was chromatographed on 200 g of silicic acid (dried at 110°C for 24 hr). The column was eluted with 2 liters of petroleum ether (b.p. 35-56°C), 2 liters of anhydrous benzene, 1 liter of carbon tetrachloride, 1 liter of chloroform, 1 liter of ether, and 1 liter of methanol. The eluate was collected in 40-ml samples. A total of 171 samples was collected and each evaporated and the residue weighed. A plot of residue weight versus tube number showed that essentially five fractions were separated.

Fraction II-1	500 mg	colorless liquid
Fraction II-2	4.25 g	light brown liquid
Fraction II-3	308 mg	dark oil
Fraction II-4	263 mg	dark oil
Fraction II-5	850 mg	dark oil

Analysis of II-1

Fraction II-1 above was chromatographed on a 6' x ¼" type "K" column (178°C, flow 43, 15 psi) giving 16 peaks.

Analysis of II-2

The light brown oil, II-2, (4.25 g), was re-chromatographed on 100 g of alumina (Act. III). The main body of the fraction was eluted in benzene and after removal of the solvent was distilled to give 2.6 g of colorless oil, b.p. 72-74°C (0.1 mm). This material showed the following physical constants: λ_{max} 237 (ϵ 100), 265 (105), and 259 (85); infrared bands at 3400, 2900, 1860, 1760, 1625, 1500, 1050, 984, 930, 895, 812 and 720 cm^{-1}; d^{23} 0.9635; n_D^{29} 1.505; $[\alpha]_D^{25}$ +38.1 (95% ethanol, 4.64 mg/ml).

Anal. Calcd. for $C_{12}H_{18}O$: C, 80.85; H, 10.18.
Found: C, 80.52; H, 10.03.

Bromination of II-2

A 5% solution of bromine in carbon tetrachloride was added to 3 mg of II-2 until a slight yellow color persisted. A test for hydrogen bromide evoluation (litmus) was positive. The solvent was removed and the

residue recrystallized from absolute ethanol. Three
milligrams of white crystals, m.p. 53-54°C, were obtained.

Attempted Hydrogenation of II-2

A solution containing 18.1 mg of II-2 in 1.5 ml
of absolute ethanol was hydrogenated over Adams catalyst
at 1 atm pressure for 8 hours. No hydrogen uptake was
detected.

Perbenzoic Acid Oxiation of II-2

A solution containing 27.6 mg (2 mMoles) of per-
benzoic acid and 31.0 mg (0.17 mMole) of II-2 in 4 ml
of chloroform was placed in an Erlenmeyer flask,
stoppered and allowed to stand 10 hr at room temperature.
The solution was extracted once with dilute sodium bi-
carbonate and the solvent evaporated _in vacuo_ to give a
light yellow oil. This product was transparent in the
infrared at 1240-1260 cm^{-1}.

Permanganate Oxidation of II-2

A well stirred solution containing 37.8 mg
(0.21 mMole) of II-2 and 250 mg (1.6 mMoles) of $KMnO_4$
was heated on a boiling water bath for 6 hours. After
the MnO_2 had been removed by filtration, the solution
was acidified with HCl and extracted with ether. The

ether extracts were dried over anhydrous magnesium sulfate and the ether evaporated in vacuo to give 28.2 mg (81%) of white crystals, sublimes at 250°C. Its infrared spectrum (KBr pellet) is identical to that of terphthalic acid.

Nitric Acid Oxidation of II-2

A solution containing 77.5 mg (0.44 mMole) of II-2 in 1.5 ml of 3.5 $\underline{N}$ HNO_3 was refluxed for 12 hr. Sufficient 1 $\underline{N}$ NaOH was added to dissolve the white precipitate. After the addition of some powdered zinc, a portion of solvent was removed via distillation. Unreacted zinc was separated, the filtrate acidified with HCl and extracted with ether. The ether extracts were dried over anhydrous magnesium sulfate and the ether evaporated in vacuo to give 29.9 mg of white crystalline solid, m.p. 158-166°C. Its infrared spectrum was very nearly identical to that of p-toluic acid, m.p. 178°C (4).

A sample of the ether extract (20 λ) was chromatographed on Whatman #4 paper using ethanol-ammonia-water (80:4:16) as solvent. Four spots were detected with bromophenol blue, R_f 0.73, 0.44, 0.26 and 0.00. The following four acids were chromatographed under the above conditions (9): p-toluic (R_f 0.72), acetic (0.43), malonic (0.25) and oxalic (0.00).

3,5-Dinitrobenzoate of II-2

A solution containing 100 mg (0.56 mMole) of II-2
and 200 mg (0.87 mMole) of 3,5-dinitrobenzoyl chloride
in 0.3 ml of anhydrous pyridine was warmed on a steam
bath for 10 minutes. This solution was then poured,
with stirring, into 4 ml of water. After the precipitate
had settled, the supernatant liquid was decanted, and
the residue washed with 1 ml of 2% sodium carbonate
solution. The solid was isolated and after recrystal-
lization from ethanol gave 50 mg (25%) of bright yellow
crystals, m.p. 78°C.

Anal. Calc'd for $C_{19}H_{20}O_6$: C, 61.28; H, 5.41.
Found: C, 61.28; H, 5.25.

Analysis of II-3 and II-4

Fraction II-3 and fraction II-4 above were
chromatographed on a 6' x ¼" column (20% Reoplex 400 on
firebrick, 200°C, flow 39, 15 psi). The chromatograms
showed 17 and 16 peaks, respectively.

Analysis of II-5

Upon standing, fraction II-5 deposited a small
amount of crystals. Crystallization of the entire
fraction from benzene-petroleum ether gave 50 mg of white

crystals with the following physical constants: λ_{max} 270
($E_{1\,cm}^{1\%}$ 11.6); infrared bands at 3400, 2940, 2880, 1625(w),
1470, 1445, 1420, 1380, 1370, 1170, 1125, 1010, 987, 980,
947, 900 and 835 cm^{-1}; m.p. 90-92°C. It decolorized
cold, neutral potassium permanganate, decolorized bromine
water, and gave no color reaction with ferric chloride.

3,5-Dinitrobenzoate of II-5

A solution containing 800 mg of fraction II-5 and
500 mg (2.68 mMoles) of 3,5-dinitrobenzoyl chloride in
2 ml of anhydrous pyridine was heated on a steam bath for
15 minutes. The resultant solution was poured, with
stirring, into 8 ml of water. The supernatant liquid was
decanted and the residue washed with 3 ml of 2% sodium
carbonate solution. The residue was isolated by filtra-
tion and recrystallized from ethanol giving 60 mg of
light pink solid, m.p. 170-171°C, $_{max}$ 230 ($E_{1\,cm}^{1\%}$ 774).

The 3,5-dinitrobenzoates of ethanol (m.p. 93°C),
sec. butanol (m.p. 75°C), and ethylene glycol (m.p.
169°C) were prepared by the above procedure. Their
molecular extinction coefficients (ϵ) at 230 mu are
21,400, 21,000 and 45,400. An estimated molecular weight
of II-5, based on twice the average ϵ (22,500) of these
knowns, is 190 $\pm$ 20.

Anal. Found: C, 45.60; H, 2.74.

Calc'd for $C_{21}H_{15}N_4O_{15}$: C, 44.8; H, 2.66.

$C_{22}H_{16}N_4O_{15}$: C, 45.83; H, 2.78.

$C_{23}H_{16}N_4O_{15}$: C, 46.9; H, 2.72.

3. Synthesis of 4-(p-tolyl)-1-pentanol

γ-(p-tolyl)-γ-valerolactone

A solution containing 38.1 g (0.25 mole) of methyl
iodide in anhydrous ether was added slowly to a stirred
solution containing 5.83 g (0.246 mole) of magnesium
turnings in anhydrous ether. The solution was stirred
for 15 min and a solution containing 23.6 g (0.123 mole)
of 3-(p-toluoyl)-propionic acid in anhydrous ether was
added at a rate which maintained a gentle boiling. The
solution was stirred an additional 2 hr and 300 ml of
dilute sulfuric acid was added slowly. The mixture was
stirred 2 hr longer, the ether layer separated and washed
with dilute sodium hydroxide. Dilute HCl was added to
the sodium hydroxide layer and 6 g (25%) of starting
material was recovered. The above ether layer was evapo-
rated in vacuo and the residue distilled to give 7 g
(40% based on acid used) of light brown oil, b.p. 98°C
(0.05 mm), infrared band at 1775 cm^{-1}. The literature
(17) gives a melting point of 56°C for this lactone.

4-(p-tolyl)-pentanoic acid

The above lactone (7 g, 37 mMoles) was reduced by heating with amalgamated zinc and hydrochloric acid according to the procedure of Martin (11) for 22 hours. At that time, the toluene layer was separated and extracted with dilute sodium hydroxide. The aqueous layer was acidified with dilute HCl and then extracted with ether. The ether layer was dried over anhydrous magnesium sulfate, the ether removed in vacuo, and the product distilled giving 2.7 g (38%) of light yellow oil, b.p. 115°C (1.5 mm) (3, 17).

Ethyl 4-(p-tolyl)-pentanoate

A solution containing 2.7 g (14 mMoles) of the above acid and one drop of concentrated HCl in 10 ml of absolute ethanol was refluxed for 2 hours. The solvent was evaporated in vacuo and the residue distilled, b.p. 144°C (9 mm) (3, 17), 2.31 g (78.5%) of colorless oil being obtained.

4-(p-tolyl)-1-pentanol

A solution containing 2.31 g (11 mMoles) of the above ester was added drop by drop to a well stirred solution containing 0.4649 g (12 mMoles) of lithium

aluminum hydride in anhydrous ether. The solution was stirred 15 min and moist ether added until reaction subsided. Sufficient concentrated sodium hydroxide was added to dissolve the aluminum hydroxide. The ether layer was separated and dried over anhydrous magnesium sulfate. The product, 1.28 g (62%) of colorless oil, was distilled, b.p. 72-74°C (0.1 mm). The literature (3) reports a boiling point of 151°C (16 mm) for this compound. Its infrared spectrum was identical with that of II-2.

4-(p-tolyl)-1-pentanyl 3,5-dinitrobenzoate

The 3,5-dinitrobenzoate of the above alcohol was prepared on a 0.1 mMole scale in 67% yield by the directions given earlier for preparation of the 3,5-dinitrobenzoate of II-2. This ester, recrystallized from ethanol, is a yellow solid, m.p. 78°C (literature m.p. 80°C (3)). A mixture of the 3,5-dinitrobenzoates of II-2 and 4-(p-tolyl)-1-pentanol melted at 78°C.

Toxicity of Constituents

Routine bioassays of constituents were carried out on guppies by George G. Chadwick, Fishery Research Biologist, O.S.U., and the results are tabulated in Table II.

SUMMARY

The organic residue in foul condensate from a typical Northwest paper mill, using only Douglas fir to produce unbleached pulp, was separated into three fractions by distillation. The two distillable fractions were further separated into several fractions by gas and elution chromatography. A few of the smaller fractions could not be investigated due to their complex nature.

Tentative identification of limonene, γ-terpinene, p-cymene, and anisol in one fraction was obtained by gas chromatographic comparison on two columns with different stationary phases.

Two compounds were isolated in relatively pure form though in quantities too small to permit complete identification. The spectral data from the first compound suggested the presence of tertiary hydroxyl and α,β-unsaturated ketone groups in the molecule. The tentative formula, $C_9H_{14}O_7$, derived from analysis of the 2,4-dinitrophenylhydrazone cannot be given much credence. The infrared spectrum of the second compound showed only the presence of hydroxyl groups and analysis of its 3,5-dinitrobenzoate indicates an empirical formula of $C_8H_{10}O_5$.

A relatively large amount (6.8%) of the total

residue was shown by infrared spectroscopy to be quaiacol. This was confirmed by the preparation of its benzoate ester and comparison with an authentic sample.

The largest single component of the waste (11.7%) isolated so far was shown by physical measurements to be an optically active aromatic substance bearing a primary alcohol on a side chain. Oxidative degradation to terphthalic acid and p-toluic acid and analysis of its 3,5-dinitrobenzoate suggested the formula 4-(p-tolyl)-1-pentanol for this molecule. This was confirmed via a five step synthesis starting with β-(p-toluoyl) propionic acid. This alcohol is toxic to fish at the level of 22 ppm and is an effective inhibitor for cytochrome oxidase in vitro. The high optical purity and adherence of the skeleton to the isoprene rule suggest that the alcohol is derived from a sesquiterpene, perhaps γ-curcumene.

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