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THE CAUSTIC HYDROLYSIS AND THE PROPANOL EXTRACTION OF VARIOUS HARDWOODS. I.

Since the discovery of p-hydroxybenzoic acid in Populus tremuloides, our attention has been turned in that direction. In the first three project reports for 1932 the emphasis has been on the presence and amounts of p-hydroxybenzoic acid. It has been found to be present in the extract of the wood itself, this belongs in and has been reported under project 809-13. The acid has also been found in the ether extract of the hydrolyzed wood, both by a caustic and acid hydrolysis. The greatest amounts of the acid both in the percent of the extract as well as actual weight has been found in the extract from a caustic hydrolysis.

In 1932, report No. 2, a caustic hydrolysis of spruce sawdust was run to determine whether or not the acid is present in that particular softwood. As reported, no appreciable amount of, if any, acid was present. The question then arose as to what hardwoods might also contain p-hydroxybenzoic acid. At first only a few were selected to be tried, namely paper birch and red alder. No p-hydroxybenzoic acid was found in either. Other hardwoods were then hydrolyzed and a concentrated study took form. Since the ether extract of the hydrolyzed wood was available, it was decided to quantitatively determine all the knowns present, namely: vanillin, syringaldehyde, vanillic acid, syringic acid, coumaric acid, ferulic acid, and p-hydroxybenzoic acid. All six of these knowns do not exist in every extract but those that do were quantitatively determined.

At the same time, since the sawdusts of the various hardwoods were available, a sample was extracted with a solution of 75% propanol. By these methods we hoped to obtain as much information as possible on small scale experiments.

In all, thirty four (34) different hardwoods including Populus tremuloides were studied. The various samples were obtained from a number of sources. Mr. Peckham supplied us with some samples, others were obtained from Dr. Isenberg. Dr. Wise, Dr. Joranson and Miss Barbara Reeder also contributed several samples.

The procedure followed for the caustic hydrolysis was the same for each wood. A sample consisting of 100 grams oven dried weight was stirred and boiled under reflux for 8 hours in a solution of 120 grams of sodium hydroxide dissolved in 3000 cc. of water. The mixture was filtered, and the sawdust was washed with water. The filtrate plus the initial washing were acidified with dilute sulfuric acid. This acidified filtrate was then extracted thoroughly with ether using a large separatory funnel. The ether solution was then dried with anhydrous sodium sulfate and concentrated to a small volume. The ether solution was then brought to a volume of 100 cc. and weighed. A solids determination was run on each extract to determine the amount and percent of the extract.

The sawdust was washed with dilute acetic acid, followed with an ethanol wash and finally an ether wash. These washings were discarded. The sawdust was then left to air dry, weighed and bottled. A moisture content was run on each bottled sample and the amount of recovered sawdust was determined.

Since we are only interested in the ether extract of the hydrolysis, the aqueous solutions were discarded. From past experiences we have found that only a possible insignificant trace of the phenolic materials are left in the aqueous solution after an ether extraction.

The ether extracts were chromatographed using two developers. The developers were butanol-2% aqueous ammonia and butanol, pyridine, water (10:3:3). The results of the chromatograms were put in the form of a chart and will follow the report.

The technique used to determine quantitatively the amount of each unknown has been described before. A known amount of the sample was streaked on the paper. To determine vanillin and syringaldehyde, the developer used was n-butyl ether saturated with water. For the acids, the paper was developed with benzene saturated with formic acid. These developers were selected because of the excellent separation between the desired materials. The other materials present do not separate sufficiently well to use these developers for introductory chromatograms.

Each paper is also spotted with the extract as well as the knowns along side the streak. The paper is then cut and the strip is sprayed with a reagent to determine the position of the material. The paper containing the aldehydes is sprayed with 2,4-dinitrophenylhydrazine spray and the paper containing the acids is sprayed with bis-diazotized benzidine. The band containing the material is then cut from the chromatogram and eluted with 95% ethanol in a micro Soxhlet extractor. The ethanol solution is treated with 5 cc. of 0.2% alcoholic potassium hydroxide, and the volume is brought to 50 cc. in a

volumetric flask. The optical density of the solution is then read using the spectrophotometer. The optical density is read at the wave length for the peak of the specific material being read. The optical density is then converted to mg./liter by multiplying with a conversion factor determined from a straight line graph of concentrations against optical density.

The wave length used as well as the conversion factors are as follows:

	Wave Length, mmu	Conversion factor
Vanillin	352	5.33
Syringaldehyde	368	6.76
Vanillic acid	298	13.1
Syringic acid	300	16.4
p-Hydroxybenzoic acid	278	8.30
Ferulic acid	332	14.3
Coumaric acid	336	7.7

This technique worked very fine in every case with one exception. In every extract vanillic acid is present. In those extracts that contain ferulic acid also, the problem of separating the two arose. Using the developer, benzene saturated with formic acid, the paper is left in the tank for about 15 hours by which time the solvent front is off the paper. The reference values given are references in regard to vanillic acid. The Rv values are as follows:

p-hydroxybenzoic acid - 0.27

syringic acid - 0.77

Vanillic acid - 1.00

Ferulic acid - 1.04

In the developer, 10:3:3, the Rf of vanillic acid is 0.44 and ferulic is 0.46. Other developers were tried; but none satisfactorily separated all of the acids. The problem then arose as to how to read the concentrations of two materials from one solution.

A solution of each acid was prepared. The solution containing 10 mg./liter of the acid was treated with the potassium hydroxide solution. Both solutions' ultraviolet curves were read on the spectrophotometer. The curves were both plotted on a sheet of graph paper (Fig. I). It was noted at once that at the peak of ferulic acid (332mμ) the optical density of vanillic acid is practically zero. Since the optical densities of two materials are supposed to be additive, it seemed like an easy solution. With optical density readings at both wave lengths of 298 and 332 mμ, the concentration of ferulic acid could be read directly from the optical density at 332. By converting this value of ferulic acid to its optical density at 298 mμ and subtracting this value from the density read at 298 mμ would leave the density of the vanillic acid present. A series of solutions were prepared mixing known amounts of the two acids. By following the above methods it was found that with these two acids, the densities are not additive. However, a definite trend appeared which indicated a conversion factor was necessary to determine

the correct results. The equation finally arrived at is as follows:

D_1 - Optical Density at 298

D_2 - Optical Density at 332

F - mg./liter of ferulic acid

V - mg./liter of vanillic acid

$D_2 \times 14.3 \times 0.75 = D_2 \times 10.7 = F$

$[D_1 - (\frac{F \times 0.82}{18.2})] \times 13.1 = [D_1 - (F \times .045)] \times 13.1 = V$

The conversion factor 18.2 is the factor for converting the optical density to mg./liters for ferulic acid at the wave length of 298 mu. The other two factors, 0.75 and 0.82 were found to be necessary to constantly arrive at the correct results. The only possible reason for these factors is that in the case of these two acids, their optical densities are not additive and correction factors must be applied.

A series of known solutions were read with the following results:

Sample contained mg./liter		D_1	D_2	Calculated mg./liter	
Ferulic	Vanillic			Ferulic	Vanillic
10*	- - -	.560	.700	10.01	- - -
8	2	.520	.755	8.1	2.03
6	4	.570	.555	5.96	3.96
4	6	.640	.365	3.9	6.09
2	8	.708	.193	2.12	8.03
- - -	10*	.772	.035	- - -	10.1
2	2	.272	.208	2.2	2.2
4	4	.491	.389	4.12	4.01
8	8	.960	.760	8.13	7.8
2	4	.432	.205	2.19	4.36
8	6	.806	.750	8.02	5.83

* where only one acid was used no conversion factor was necessary.

In obtaining a workable method for the determination of two compounds from a single solution, the formation of the potassium salt of the compound was desired since this method was employed for the determination of all the other compounds. However, several other methods were attempted in order to obtain the best results.

A known sample of ferulic acid was dissolved in 95% ethanol and the ultraviolet curve was determined. It was found, however, that on repeated readings of the same sample in the following days that the peak shifted and the densities increased. Apparently a reaction was slowly taking place in the ethanol, possibly an esterification. Since the material obviously is not stable in ethanol, this solution was discarded.

Another sample of ferulic acid was dissolved in dioxane. The ultraviolet curve remained steady for two successive days; however, when a sample was boiled and cooled, the optical density dropped tremendously. The peak remained constant, but with the lower optical density, correct determination would be impossible.

The sample previously mentioned as being dissolved in ethanol and treated with potassium hydroxide was read for five successive days. The U.V. curve (Fig. 1) remained constant throughout this period.

A final check was made by spotting two papers, each containing 4 mg./liter of ferulic acid and 4 mg/liter of vanillic acid.. One paper was eluted with 95% ethanol and the other was eluted with ethanol plus a little

alcoholic potassium hydroxide. Both solutions were made up to standard and read on the spectrophotometer. Results:

1. Eluted with ethanol: Ferulic acid - 4.14 mg./l Vanillic acid- 4.24 mg./l
2. Ethanol + KOH: Ferulic acid - 3.8 mg./l Vanillic acid- 4.4 mg./l

From the many tests conducted, it becomes quite apparent that the method to be used can be the same as used for the other compounds. The elution with ethanol does not affect the ferulic acid; evidently any esterification that takes place is a slow process and boiling in ethanol does not hasten the reaction.

A chart follows giving all the data. From the data several very interesting and important facts should be noted.

Before any conclusions are mentioned, it should be noted that only 34 different hardwoods are reported here, any conclusions can only apply to these woods. Also the information concerning the various woods cannot readily be used as comparative data. The data must apply to each individual wood. In the conclusions, comparisons will be stated; however, many varying factors exist which must be remembered. The age of the various trees is unknown in most cases. Some of the trees were freshly cut, others were cut or ground and stored for many years. These factors may or may not affect the results, this is not known as of now. Also in some instances possibly only the sapwood is used. The only positive sample of sapwood was the Populus alba; however, some samples consisted of blocks and pieces of wood and were definitely not a uniform sample of the tree.

First of all, it must be noted that p-hydroxybenzoic acid in large amounts only is found in the Populus species. Some of the acid has been found in the Salix eriocephala, but only in a small amount. Every one of the Populus species contains large amounts of the acid exceeding ten percent of the extract. It should also be noted that the Salix eriocephala belongs to the same family as the Populus, namely the Salicaceae family.

The presence of ferulic acid is also of interest. There is no clear cut division of this acid; however, the species containing the largest amounts of p-hydroxybenzoic acid do not contain ferulic acid. The Populus species that contain both acids are the lowest in p-hydroxybenzoic acid.

Also in regard to ferulic acid, although the acid is found in Acer saccharum, the other Acer species tested do not seem to contain the acid.

The amount of syringic acid present should also be noted. With the exception of the Populus tremuloides and the Populus grandidentata, the Populus species contain the lowest yield of syringic acid of any of the woods studied up to this time.

The presence of coumaric acid is without any explanation or pattern from the experiments conducted. The four woods containing this acid belong to four different species and three different families. The Tilia heterophylla belong to the Tiliaceae family. The Platanus occidentalis belongs to the

Platanaceae family. The Populus tremula and the Salix eriocephala both belong to the Salicaceae family. Other samples belonging to the Tiliaceae and the Silicaceae families were included in this group of woods and did not contain any coumaric acid.

Vanillic acid is present in every wood in this report. The amounts of the acid vary with no significant pattern.

Vanillin and syringaldehyde are also present in every wood reported here. In most cases the yield of syringaldehyde exceeds that of vanillin with the vanillin-syringaldehyde ratio as low as 1 : 2.6. In several woods the ratio is 1 : 1 and in four samples the yield of vanillin exceeds that of syringaldehyde. Of these thirty-four different woods, however, only Acer negundo really stands out as far as the vanillin yield is concerned. The yield of syringaldehyde is about average, but the vanillin yield is such as to have a ratio of 1 : 0.36.

In regard to the table of results of the chromatograms, I should like to mention that the unknown spots, especially those from the propanol extracts, from the different woods need not necessarily be the same material. The chromatograms from the ether extracts of the caustic hydrolyzed woods gave in most cases, clean clear cut spots. The chromatograms from the 75% propanol extracts on the other hand streaked quite badly making accurate reading almost impossible.

Each wood sample was also extracted with 75% propanol. This solvent was used because some time ago after a series of extractions on Aspen sawdust (Project 809-13 report number 7) it was found to yield the greatest amount of extract. Whether other solvents would extract other woods more completely we

CAUSTIC HYDROLYSIS OF HARDWOODS

SYRINGALDEHYDE		RATIO V:S	VANILLIC ACID		SYRINGIC ACID		FERULIC ACID		p-HYDROXYBENZOIC ACID		p-COUMARIC ACID		RECOVERED SAWDUST
Mg.in Ext.	% of Ext.		Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg.in Ext.	% of Ext.	gms.
96.6	4.2	1:1.5	87.4	3.8	54.6	2.4	None	---	840.0	36.8	None	---	63.2
38.5	2.6	1:2.6	14.6	1.0	44.5	3.0	21.4	1.5	None	---	None	---	68.8
49.2	2.3	1:1.3	74.4	3.5	83.7	3.9	30.6	1.4	None	---	None	---	75.4
33.0	2.4	1:1.9	36.5	2.6	59.0	4.2	53.0	3.8	None	---	None	---	67.6
19.0	1.2	1:1	42.5	2.7	50.0	3.2	51.0	3.3	None	---	None	---	63.2
24.0	2.1	1:0.95	21.0	1.8	42.5	3.7	37.0	3.2	None	---	None	---	74.8
33.0	2.2	1:1.4	34.0	2.2	43.0	2.9	58.0	3.9	None	---	None	---	80.0
23.0	1.9	1:1.5	29.5	2.6	77.0	6.4	69.0	5.8	None	---	None	---	73.1
44.0	4.5	1:1.3	22.8	2.3	36.3	3.7	40.0	4.1	None	---	None	---	78.8
48.0	5.6	1:2.5	18.3	2.1	35.8	4.2	None	---	None	---	None	---	73.8
54.0	2.6	1:2.2	49.5	2.4	36.5	1.8	None	---	923.0	45.2	None	---	73.4
41.5	5.1	1:1.3	23.3	2.8	41.0	5.0	38.3	4.7	None	---	None	---	73.1
55.0	5.1	1:1.3	23.3	2.2	62.5	5.8	23.8	2.2	None	---	None	---	73.0
32.5	2.1	1:1.1	18.8	1.8	26.3	2.5	14.5	1.3	None	---	None	---	64.6
22.0	3.1	1:1	14.0	1.9	21.0	2.9	9.3	1.3	None	---	None	---	73.9

WOOD		ETHER EXTRACT		VANILLIN		SYRINGALDEHYDE		RATIO V:S	VANILLIC ACID
(100 gm.)	Scientific Name	gms.	Code No.	Mg. in Ext.	% of Ext.	Mg.in Ext.	% of Ext.		Mg. in Ext.
QUAKING ASPEN (A)	Populus tremuloides	2.28	1359-95-B	61.3	2.7	96.6	4.2	1:1.5	87.4
PAPER BIRCH	Betula papyrifera	1.46	1359-124-A	14.6	1.0	38.5	2.6	1:2.6	14.6
RED ALDER	Alnus rubra	2.13	1359-129-A	37.8	1.8	49.2	2.3	1:1.3	74.4
WHITE OAK	Quercus alba	1.40	1359-145-A	18.0	1.3	33.0	2.4	1:1.9	36.5
OVERCUP OAK	Quercus lyrata	1.55	1359-153-A	19.0	1.2	19.0	1.2	1:1	42.5
WATER OAK	Quercus nigra	1.16	1527-39-A	26.0	2.2	24.0	2.1	1:0.95	21.0
BLACKJACK OAK	Quercus marilandica	1.48	1527-30-A	24.0	1.6	33.0	2.2	1:1.4	34.0
SO. RED OAK	Quercus falcata	1.20	1527-47-A	16.0	1.3	23.0	1.9	1:1.5	29.5
BLACK GUM	Nyssa sylvatica	0.98	1527-12-A	33.0	3.4	44.0	4.5	1:1.3	22.8
SWEET GUM	Liquidamber styrauflua	0.86	1527-21-A	19.0	2.2	48.0	5.6	1:2.5	18.3
COTTONWOOD	Populus deltoides	2.05	1527-54-A	24.0	1.2	54.0	2.6	1:2.2	49.5
YELLOW POPLAR	Liriodendron tulipifera	0.82	1527-61-A	31.0	3.8	41.5	5.1	1:1.3	23.3
MAGNOLIA	Magnolia grandiflora	1.07	1527-68-A	40.5	3.8	55.0	5.1	1:1.3	23.3
WHITE BAY	Magnolia viriginiana	1.05	1527-75-A	31.0	2.0	32.5	2.1	1:1.1	18.8
YELLOW BIRCH	Betula lutea	0.72	1527-81-A	22.5	3.1	22.0	3.1	1:1	14.0

A - Data taken from Project 1932 Report #1
B - only Sapwood used

CAUSTIC HYDROLYSIS OF HARDWOODS (cont'd)

SYRINGALDEHYDE		RATIO V:S	VANILLIC ACID		SYRINGIC ACID		FERULIC ACID		p-HYDROXYBENZOIC ACID		p-COUMARIC ACID		RECOVERED SAWDUST
Mg. in Ext.	% of Ext.		Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.	gms.
48.5	5.4	1:2.6	18.0	2.0	21.5	2.4	None	---	None	---	None	---	73.4
60.5	3.6	1:2	20.0	1.2	50.0	3.0	27.0	1.6	None	---	None	---	65.8
59.5	5.6	1:1.9	37.5	3.5	42.0	3.9	None	---	None	---	None	---	73.3
37.5	3.7	1:1	13.0	1.2	40.5	4.0	34.8	3.4	None	---	None	---	72.5
25.5	1.6	1:1.1	22.0	1.4	6.0	0.4	22.0	1.4	290.0	17.9	None	---	69.4
36.0	4.1	1:1.6	11.3	1.3	29.5	3.4	14.5	1.7	None	---	None	---	67.4
24.0	1.5	1:1.2	32.0	2.1	54.5	3.5	81.0	5.2	None	---	33.5	2.2	65.2
35.0	2.8	1:1.04	39.0	3.1	65.5	5.1	None	---	None	---	None	---	67.1
42.5	1.7	1:1	29.5	1.1	27.0	1.0	None	---	805.0	31.1	None	---	66.7
38.0	3.2	1:1	15.0	1.3	28.5	2.4	49.5	4.2	342.0	29.2	None	---	63.0
32.5	2.7	1:1	38.0	3.2	59.0	5.0	None	---	None	---	None	---	81.4
33.5	3.8	1:0.36	45.0	5.1	47.0	5.4	None	---	None	---	None	---	67.3
34.5	2.1	1:1.2	34.0	2.1	74.5	4.6	66.0	4.0	None	---	None	---	66.4
37.0	6.4	1:2.3	13.0	2.2	7.0	1.2	25.8	4.4	None	---	62.5	10.8	74.3
24.0	1.9	1:0.6	13.0	1.0	13.5	1.1	16.5	1.3	233.5	18.4	33.5	2.6	62.6
26.0	1.8	1:1	13.5	0.9	9.5	0.7	6.0	0.4	163.0	11.4	None	---	64.2
22.0	1.7	1:0.6	9.0	0.7	30.0	2.3	45.5	3.5	26.5	2.1	38.0	2.9	66.1
89.5	8.1	1:1.1	24.5	2.2	46.5	4.2	60.0	5.4	None	---	None	---	64.5
30.5	1.9	1:1.1	14.0	0.9	29.5	1.9	35.0	2.2	None	---	None	---	64.6

WOOD		ETHER EXTRACT		VANILLIN		SYRINGALDEHYDE		RATIO V:S	VANILLI ACII
(100 gm.)	Scientific Name	gms.	Code No.	Mg. in Ext.	% of Ext.	Mg. in Ext.	% of Ext.		Mg. in Ext.
EUCALYPTUS	Eucalyptus regnans	0.90	1527-87-A	18.5	2.1	48.5	5.4	1:2.6	18.0
BLACK ASH	Fraxinus nigra	1.66	1527-93-A	30.5	1.8	60.5	3.6	1:2	20.0
SOFT MAPLE	Acer rubrum	1.07	1527-99-A	31.5	2.9	59.5	5.6	1:1.9	37.5
HARD MAPLE	Acer saccharum	1.02	1527-105-A	36.5	3.6	37.5	3.7	1:1	13.0
EUROPEAN(B) WHITE POPLAR	Populus alba	1.62	1527-117-A	23.0	1.4	25.5	1.6	1:1.1	22.0
IRONWOOD	Carpinus caroliniana	0.87	1527-123-A	22.5	2.6	36.0	4.1	1:1.6	11.3
WHITE BASSWOOD	Tilia heterophylla	1.55	1527-129-A	20.0	1.3	24.0	1.5	1:1.2	32.0
SILVER MAPLE	Acer saccharinum	1.27	1527-135-A	34.5	2.7	35.0	2.8	1:1.04	39.0
NO. BLACK COTTONWOOD	Populus trichocarpa	2.59	1527-149-A	42.5	1.7	42.5	1.7	1:1	29.5
BIG TOOTH ASPEN	Populus grandidentata	1.17	1527-155-A	38.0	3.2	38.0	3.2	1:1	15.0
RED MAPLE	Acer rubrum	1.17	1527-111-A	32.0	2.7	32.5	2.7	1:1	38.0
BOX ELDER	Acer negundo	0.88	1555-12-A	92.5	10.5	33.5	3.8	1:0.36	45.0
EASTERN RED OAK	Quercus borealis	1.63	1555-18-A	29.0	1.8	34.5	2.1	1:1.2	34.0
AMERICAN SYCAMORE	Platanus occidentalis	0.58	1555-30-A	15.8	2.7	37.0	6.4	1:2.3	13.0
EUROPEAN POPLAR	Populus tremula	1.27	1555-36-A	41.5	3.3	24.0	1.9	1:0.6	13.0
BALSAM POPLAR	Populus tacamahaca	1.43	1555-42-A	26.0	1.8	26.0	1.8	1:1	13.5
DIAMOND WILLOW	Salix eriocephala	1.29	1555-54-A	34.0	2.6	22.0	1.7	1:0.6	9.0
BEECH	Fagus grandifolia	1.11	1555-24-A	80.5	7.3	89.5	8.1	1:1.1	24.5
BASSWOOD	Tilia americana	1.56	1555-48-A	28.0	1.8	30.5	1.9	1:1.1	14.0

do not know. Our main purpose here is a comparison using identical conditions.

In each case, a sample of 200 grams of oven dried wood sawdust was packed in a large Soxhlet extractor and extracted for a period of twenty-four hours. This extraction period was eight hours a day for three days. The sawdust was left covered with the solvent overnight during this period, so actually the extraction time is much longer.

All the propanol used was pressed from the sawdust and concentrated to a small volume using the laboratory circulating evaporator. A solids determination was run on each extract. The sawdust was air dried and bottled, after a sample was removed for a moisture determination.

Each extract was chromatographed using both butanol-2% aqueous ammonia and the butanol-pyridine-water developers. The results of these chromatograms will follow. The extracts did not chromatograph cleanly; rather they streaked very badly making accurate readings next to impossible. Also none of the spots could be matched with any known compounds with only the exception of p-hydroxybenzoic acid in the extract of all the Populus family.

A table will follow showing the yields of extracts from the various woods.

This report containing the 34 different woods does not conclude this series. Other Populus species will be run when obtained as well as woods such as weeping willow, black willow, chestnut, hickory and many others.

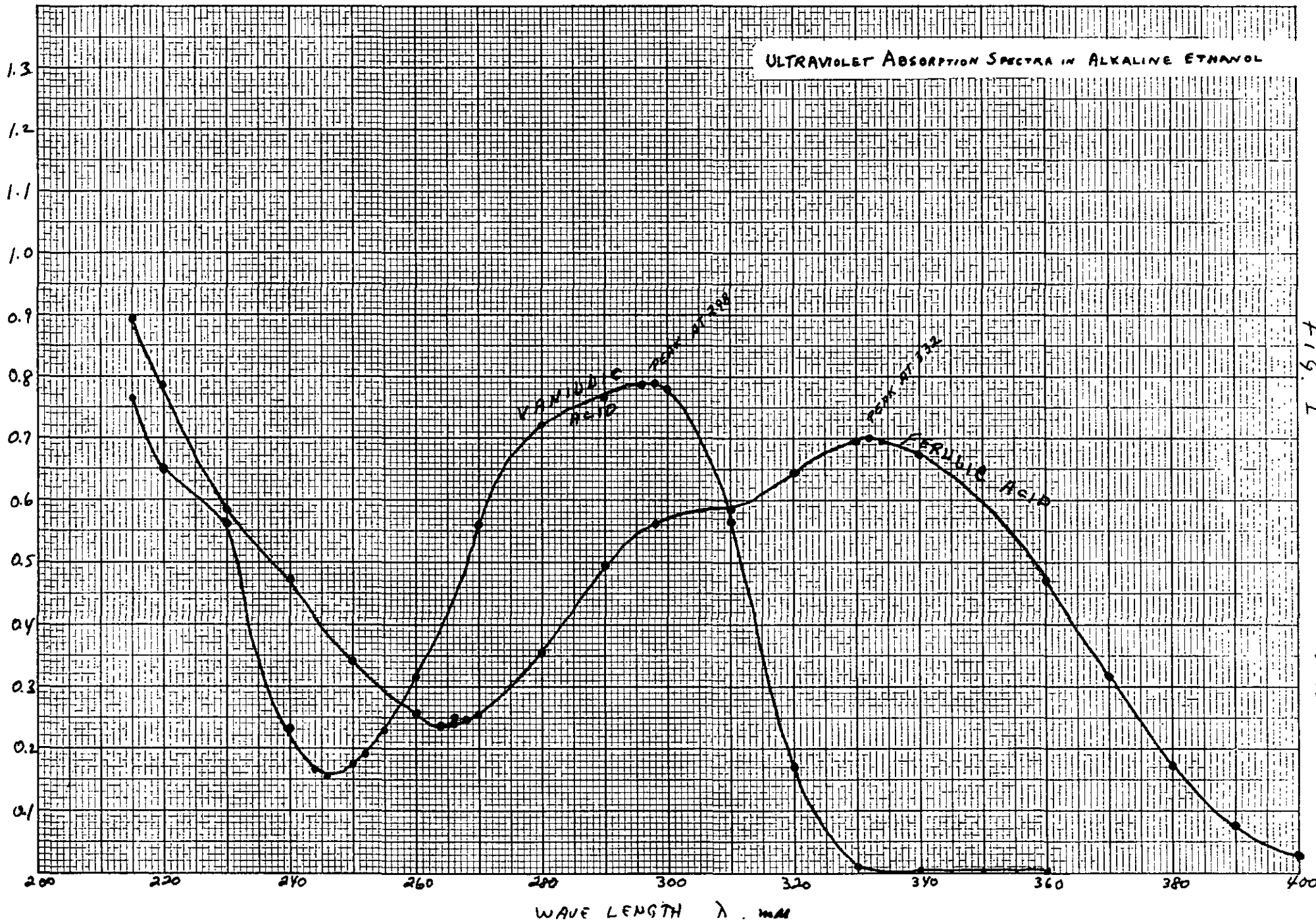

FIG. I

Project 4932
October 22, 1956

75% PROPANOL EXTRACTION OF HARDWOODS

Wood 200 g. O.D. used	Scientific Name	Propanol Extract Grams	Propanol Extract Per cent	Sawdust Recovered Grams O.D.
Quaking Aspen	Populus tremuloides	8.24	4.12	---
White Oak	Quercus alba	12.80	6.40	185.0
Overcup Oak	Quercus lyrata	12.80	6.40	187.0
Water Oak	Quercus nigra	3.72	1.86	194.4
Blackjack Oak	Quercus marilandica	5.44	2.72	185.0
So. Red Oak	Quercus falcata	8.22	4.11	191.0
East Red Oak	Quercus borealis	12.40	6.20	186.3
Black Gum	Nyssa sylvatica	2.57	1.29	189.4
Sweet Gum	Liquidamber styrauflua	1.96	0.98	192.0
Cottonwood	Populus deltoides	4.26	2.13	200.0
No. Black Cottonwood	Populus trichocarpa	4.65	2.33	183.0
Bigtooth Aspen	Populus grandidentata	6.83	3.42	196.0
Eur. White Poplar	Populus alba	6.75	3.38	182.0
European Poplar	Populus tremula	9.20	4.60	190.3
Balsam Poplar	Populus tacamahaca	7.00	3.50	188.1
Yellow Poplar	Liriodendron tulipifera	3.25	1.63	193.8
Magnolia	Magnolia grandiflora	2.96	1.48	196.0
White Bay	Magnolia viriginiana	3.38	1.69	187.6
Yellow Birch	Betula lutea	2.48	1.24	193.1
Eucalyptus	Eucalyptus regnana	1.29	0.65	197.1
Black Ash	Fraxinus nigra	6.83	3.42	194.0
Soft Maple	Acer rubrum	4.83	2.42	191.2
Hard Maple	Acer saccharum	4.00	2.00	190.3
Red Maple	Acer rubrum	5.37	2.68	191.3
Silver Maple	Acer saccharinum	9.00	4.50	184.0
Box Elder	Acer negundo	6.16	3.08	194.0
Ironwood	Carpinus caroliniana	4.02	2.01	196.0
White Basswood	Tilia heterophylla	9.28	4.64	192.0
Basswood	Tilia americana	8.53	4.27	190.6
American Sycamore	Platanus occidentalis	3.43	1.72	189.0
Diamond Willow	Salix eriocephala	11.04	5.52	188.2
Beech	Fagus grandifolia	3.63	1.82	194.4

#4

'igtooth Aspen	Red Maple	Box Elder	Eastern Red Oak	American Sycamore	European Poplar	Balsam Poplar	Diamond Willow	Beech	Basswood	
opulus ididentata	Acer rubrum	Acer negundo	Quercus borealis	Platanus occidentalis	Populus tremula	Populus tacamahaca	Salix eriocephala	Fagus grandiflora	Tilia americana	Remarks
	X	X	X	X	X	X	X	X	X	Positive to $FeCl_3$
			X					X		
X	X	X	X	X	X	X	X	X	X	Maule--Identified as syringic acid.
X	X	X	X	X	X	X	X	X	X	Identified as vanillic acid.
X		X	X	X	X	X	X	X	X	Both p-hydroxybenzoic acid and ferulic acid at this Rf.
X	X	X	X	X	X	X	X	X	X	Pos. Maule and 2.4.
X	X	X	X	X	X	X	X	X	X	Identified as syringaldehyde.
X	X	X	X	X	X	X	X	X	X	Identified as vanillin.
		X						X		Pos. Maule and 2.4.
		X								Positive to $FeCl_3$
X	X	X	X	X	X	X	X	X	X	
X	X	X			X	X	X		X	

#3

RESULTS OF ETHER EXTRACTS FROM CAUSTIC HYDROLYSIS OF HARDWOODS—DEVELOPED WITH BUTANOL 2% AQUEOUS AMMONIA

ite ay	Yellow Birch	Eucalyptus	Black Ash	Soft Maple	Hard Maple	European White Poplar	Iron-wood	White Basswood	Silver Maple	Northern Black Cottonwood	Bigtooth Aspen
olia iniana	Betuca lutea	Eucalyptus regnans	Fraxinus nigra	Acer rubrum	Acer saccharum	Populus alba	Carpinus caroliniana	Tilia heterophylla	Acer saccharinum	Populus trichocarpa	Populus grandidentata
X	X	X	X	X	X	X	X	X	X	X	
			X							X	
X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X
X	X		X		X	X	X	X		X	X
X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X
			X								
			X								
X	X	X	X	X	X	X	X	X	X	X	X
	X		X	X	X	X		X	X	X	X

#2

CHROMATOGRAPHIC RESULTS OF ETHER EXTRACTS FROM CAUSTIC HYDROLYSIS OF HA

ıck	Southern Red Oak	Black Gum	Sweet Gum	Cotton-wood	Yellow Poplar	Magnolia	White Bay	Yellow Birch	Eucalyptus	Black Ash	Soft Maple	Hard Maple	Europea White I
ıus idica	Quercus falcata	Nyssa sylvatica	Liquidamber styraufiua	Populus deltoides	Lirio- dendron tulipifera	Magnolia grandiflora	Magnolia virginiana	Betuca lutea	Eucalyptus regnans	Fraxinus nigra	Acer rubrum	Acer saccharum	Popul alba
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X									X			
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X		X	X	X	X	X		X		X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X									X			
										X			
	X	X	X	X	X	X	X	X	X	X	X	X	X
	X			X	X			X		X	X	X	X

#1

Rf	Aspen *Populus tremuloides*	Paper Birch *Betula papyrifera*	Red Alder *Alnus rubra*	White Oak *Quercus alba*	Overcup Oak *Quercus lyrata*	Water Oak *Quercus nigra*	Blackjack Oak *Quercus marilandica*	Southern Red Oak *Quercus falcata*	Black Gum *Nyssa sylvatica*	Sweet Gum *Liquidamber styrauflua*	Cotton-wood *Populus deltoides*	Yellow Poplar *Lirio-dendron tulipi*
0.00	X	X	X	X	X	X	X	X	X	X	X	X
0.01				X	X	X	X	X				
0.08	X	X	X	X	X	X	X	X	X	X	X	X
0.10	X	X	X	X	X	X	X	X	X	X	X	X
0.12	X	X	X	X	X	X	X	X	X		X	X
0.27				X	X	X	X	X	X	X	X	X
0.38	X	X	X	X	X	X	X	X	X	X	X	X
0.44	X	X	X	X	X	X	X	X	X	X	X	X
0.62		X	X	X	X	X	X	X				
0.72			X									
0.79												
0.82	X	X	X	X	X	X	X	X	X	X	X	X
0.91				X	X	X	X	X			X	X

#4

Red Maple	Box Elder	Eastern Red Oak	American Sycamore	European Poplar	Balsam Poplar	Diamond Willow	Beech	Basswood	
Acer rubrum	*Acer negundo*	*Quercus borealis*	*Platanus occidentalis*	*Populus tremula*	*Populus tacamahaca*	*Salix eriocephala*	*Fagus grandiflora*	*Tilia americana*	
X		X							Positive to $FeCl_3$
		X					X		
				X	X	X		X	
		X					X		
		X				X			
X	X	X	X	X	X	X	X	X	Syringic acid
X	X	X	X	X	X	X	X	X	Vanillic acid
	X	X	X	X	X	X	X	X	Ferulic acid
			X^{B}	$X^{A\ B}$	X^{A}	$X^{A\ B}$			A=p-hydroxybenzoic acid B=coumaric acid
X	X	X	X	X	X	X	X	X	Pos. to 2.4 and Maule
X	X	X	X	X	X	X	X	X	Pos. to 2.4 and Maule

#3

CHROMATOGRAPHIC RESULTS OF ETHER EXTRACT FROM CAUSTIC HYDROLYSIS OF HARDWOODS--DEVELOPED

Black Gum	Sweet Gum	Cotton-wood	Yellow Poplar	Magnolia	White Bay	Yellow Birch	Eucalyptus	Black Ash	Soft Maple	Hard Maple	European White Poplar	Iron-wood
Nyssa aquatica	*Liquidamber styrauflua*	*Populus deltoides*	*Liriodendron tulipifera*	*Magnolia grandiflora*	*Magnolia virginiana*	*Betuca lutea*	*Eucalyptus regnans*	*Fraxinus nigra*	*Acer rubrum*	*Acer saccharum*	*Populus alba*	*Carpinus caroliniana*
								X				X
X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X
X			X	X	X	X		X		X	X	X
								X				
		X^A									X^A	
X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X

#2

EXTRACT FROM CAUSTIC HYDROLYSIS OF HARDWOODS--DEVELOPED WITH BUTANOL, PYRIDINE, WATER (10:3:3)

ucalyptus	Black Ash	Soft Maple	Hard Maple	European White Poplar	Iron-wood	White Basswood	Silver Maple	Northern Black Cottonwood	Bigtooth Aspen	Red Maple	Box Elder	E R C b
ucalyptus regnans	*Fraxinus nigra*	*Acer rubrum*	*Acer saccharum*	*Populus alba*	*Carpinus caroliniana*	*Tilia heterophylla*	*Acer saccharinum*	*Populus trichocarpa*	*Populus grandidentata*	*Acer rubrum*	*Acer negundo*	
	X				X	X	X	X		X		
						X	X	X				
								X				
X	X	X	X	X	X	X	X	X	X	X	X	
X	X	X	X	X	X	X	X	X	X	X	X	
	X		X	X	X	X			X		X	
	X											
				X[A]		X[B]		X[A]	X[A]			
X	X	X	X	X	X	X	X	X	X	X	X	
X	X	X	X	X	X	X	X	X	X	X	X	

#1

Rf	Aspen Populus tremuloides	Paper Birch Betula papyrifera	Red Alder Alnus rubra	White Oak Quercus alba	Overcup Oak Quercus lyrata	Water Oak Quercus nigra	Blackjack Oak Quercus marilandica	Southern Red Oak Quercus falcata	Black Gum Nyssa sylvatica	Sweet Gum Liquidamber styrauflua	Cotton-wood Populus deltoide
0.01				X	X	X	X	X			
0.09			X								
0.12	X		X								
0.18	X	X	X								
0.27		X	X								
0.38	X	X	X	X	X	X	X	X	X	X	X
0.44	X	X	X	X	X	X	X	X	X	X	X
0.46		X	X	X	X	X	X	X	X		
0.52	X										
0.56	X[A]										X[A]
0.76	X	X	X	X	X	X	X	X	X	X	X
0.83	X	X	X	X	X	X	X	X	X	X	X

#4

BALSAM POPLAR	DIAMOND WILLOW	BEECH	BASSWOOD	REMARKS
Populus camahaca	*Salix eriocephala*	*Fagus grandifolia*	*Tilia americana*	Positive to
X	X	X	X	$FeCL_3$
	X	X	X	$FeCL_3$
				$FeCL_3$
				$FeCL_3$
				$FeCL_3$
				" MAULE
				" MAULE
		X	X	
X				$FeCL_3$
	X			
	X			
X	X	X	X	" MAULE, 2.4
				" MAULE, 2.4
		X	X	2.4

#3

)PANOL EXTRACTS DEVELOPED WITH BUTANOL-PYRIDINE-WATER (10:3:3)

HARD MAPLE	RED MAPLE	IRONWOOD	WHITE BASSWOOD	SILVER MAPLE	NO. BLACK COTTONWOOD	BIG TOOTH ASPEN	EUROPEAN WHITE POPLAR	BOX ELDER	EASTERN RED OAK	AMERICAN SYCAMORE	EURO POP
Acer saccharum	Acer rubrum	Carpinus caroliniana	Tilia heterophylla	Acer saccharinum	Populus trichocarpa	Populus grandidentata	Populus alba	Acer negundo	Quercus borealis	Platanus occidentalis	Popul tremul
X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X					X		
									X		
									X		
								X	X	X	
X	X	X	X	X			X				X
					X	X					
							X	X	X	X	X
X	X	X	X	X	X	X					
							X	X	X	X	X
X	X	X	X	X	X	X					

#2

CHROMATOGRAPHIC RESULTS OF 75% PROPANOL EXTRACTS DEVELOPED WITH I

SWEET GUM	YELLOW POPLAR	MAGNOLIA	WHITE BAY	COTTONWOOD	YELLOW BIRCH	EUCALYPTUS	BLACK ASH	SOFT MAPLE	HARD MAPLE	RED MAPLE	IRONWOOD	I
Liquidamber styraciflua	Liriodendron tulipifera	Magnolia grandiflora	Magnolia virginiana	Populus deltoides	Betula lutea	Regnans	Fraxinus nigra	Acer rubrum	Acer saccharum	Acer rubrum	Carpinus caroliniana	h
	X	X	X	X	X	X	X	X	X	X	X	
	X	X		X			X	X	X	X	X	
							X					
						X						
							X					
							X					
				X			X	X	X	X	X	
X				X			X					
X	X	X	X	X	X		X	X	X	X	X	
X	X	X	X	X	X		X	X	X	X	X	

#1

RF	ASPEN	WHITE OAK	OVERCUP OAK	BLACKJACK OAK	WATER OAK	SO. RED OAK	BLACK GUM	SWEET GUM	YELLOW POPLAR	MAGNOLI
	Populus tremuloides	Quercus alba	Quercus lyrata	Quercus marilandica	Quercus nigra	Quercus falcata	Nyssa sylvatica	Liquidamber styraciflua	Liriodendron tulipifera	Magnoli grandif
.00	X	X	X	X	X	X			X	X
.02		X	X	X	X	X			X	X
.05		X	X			X				
.07	X									
.10		X	X							
.13	X	X	X							
.25		X	X	X	X	X				
.32						X				
.38	X	X	X			X				
.45		X	X	X	X	X				
.56	X							X		
.63		X	X				X			
.69	X									
.70										
.80		X	X	X	X	X	X	X	X	X
.85	X									
.91		X	X	X	X	X	X	X	X	

EAN POPLAR	BOX ELDER	EASTERN RED OAK	AMERICAN SYCAMORE	EUROPEAN POPLAR	BALSAM POPLAR	DIAMOND WILLOW	BEECH	BASSWOOD	REMARKS
us	Acer negundo	Quercus borealis	Platanus occidentalis	Populus tremula	Populus tacamahaca	Salix eriocephala	Fagus grandifolia	Tilia americana	Positive to
		X	X	X	X	X		X	$FeCl_3$
	X	X	X			X	X		$FeCl_3$
		X						X	
		X							" MAULE
		X		X	X				$FeCl_3$
	X		X	X					Flourescent
							X		
	X		X	X	X	X	X	X	
	X		X			X			
	X	X	X	X	X	X	X	X	2.4
			X				X		2.4

NOL EXTRACTS DEVELOPED WITH BUTANOL 2% AQUEOUS AMMONIA

HARD MAPLE	RED MAPLE	IRONWOOD	WHITE BASSWOOD	SILVER MAPLE	NO. BLACK COTTONWOOD	BIG TOOTH ASPEN	EUROPEAN WHITE POPLAR	BOX ELDER	EASTERN RED OAK	AMERICAN SYCAMORE	EUROPEAN POPLAR
Acer ccharum	Acer rubrum	Carpinus caroliniana	Tilia heterophylla	Acer saccharinum	Populus trichocarpa	Populus grandidentata	Populus alba	Acer negundo	Quercus borealis	Platanus occidentalis	Populus tremula
X	X	X	X	X	X	X	X		X	X	X
X	X	X	X	X	X	X		X	X	X	
			X	X					X		
									X		
					X	X	X		X		X
								X		X	X
X	X	X	X	X	X	X	X	X		X	X
								X		X	
X	X	X	X	X				X	X	X	X
										X	

#3

CHROMATOGRAPHIC RESULTS OF 75% PROPANOL EXTRACTS DEVELOPED WITH BUTANOL :

ET M	YELLOW POPLAR	MAGNOLIA	WHITE BAY	COTTONWOOD	YELLOW BIRCH	EUCALYPTUS	BLACK ASH	SOFT MAPLE	HARD MAPLE	RED MAPLE	IRONWOOD	WHITE BASSWOO
idamber aciflua	*Liriodendron tulipifera*	*Magnolia grandiflora*	*Magnolia virginiana*	*Populus deltoides*	*Betula lutea*	*Regnans*	*Fraxinus nigra*	*Acer rubrum*	*Acer saccharum*	*Acer rubrum*	*Carpinus caroliniana*	*Tilia heterophy*
X	X	X	X	X	X	X	X	X	X	X	X	X
X			X	X	X		X	X	X	X	X	X
												X
							X					
				X			X					
				X			X					
							X					
X			X	X	X	X	X	X	X	X	X	X
	X	X					X					
X	X	X	X		X		X	X	X	X	X	X
X			X		X		X					

#1

RF	ASPEN	WHITE OAK	OVERCUP OAK	BLACKJACK OAK	WATER OAK	SO.RED OAK	BLACK GUM	SWEET GUM	YELLOW POPLAR	MAGNOLIA
	Populus tremuloides	Quercus alba	Quercus lyrata	Quercus marilandica	Quercus nigra	Quercus falcata	Nyssa sylvatica	Liquidamber styraciflua	Liriodendron tulipifera	Magnolia grandiflora
.00	X	X	X	X	X	X		X	X	X
.02		X	X	X	X	X		X		
.05										
.08	X	X	X			X				
.10	X	X	X	X	X	X				
.21										
.28	X									
.30		X	X							
.42	X	X	X	X	X		X	X		
.68	X									
.70		X	X	X	X	X			X	X
.79	X									
.81		X	X	X	X	X	X	X	X	X
.95				X	X			X		

PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR The Institute of Paper Chemist
REPORT NO. 4
DATE April 3, 1961
NOTE BOOK 1739 and 1834
PAGE TO
SIGNED Irwin A. Pearl

Irwin A. Pearl

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Dr. Pearl (2)
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Notebook 1739
Pages 157-159

Notebook 1834
Pages 24-28
30-43
48-52
56

Methylation Studies on Salireposide and Related Glucosides

In Report No. 3 the attempted methylation of salireposide by means of methyl iodide and silver oxide was reported and it was demonstrated that the free phenolic group of salireposide was responsible for the anomalous results obtained. It was further pointed out that for structure studies, the phenolic group would have to be methylated first by some methylating agent such as diazomethane so that the glucose moiety could be methylated satisfactorily by methyl iodide and silver oxide. In accordance with this reasoning salireposide was submitted to methylation by diazomethane.

PREPARATION OF DIAZOMETHANE--A solution of 2.5 grams of potassium hydroxide in 4 ml. of water and 12.5 ml. of ethanol was placed in a small flask fitted with a dropping funnel and vertical spiral condenser attached to a larger flask surrounded by an ice bath. The reaction flask was placed in a warm water bath at 65° and a solution of 10.7 grams DIAZALD (N-methyl-N-nitroso-p-toluenesulfonamide obtained from Aldrich Chemical Co., Inc. of Milwaukee, Wisconsin) in 65 ml. of ether was added dropwise through the dropping funnel. A canary yellow distillate was collected in the cold receiver. After all the DIAZALD solution was added 40 ml. more ether were added to drive over all the diazomethane. The clear yellow solution of diazomethane in ether was employed for methylation reactions.

PRELIMINARY REACTION OF SALIREPOSIDE WITH DIAZOMETHANE -- A solution of 1.0 gram of salireposide in 30 ml. of tetrahydrofuran containing a little ethanol was treated with one-fifth of the diazomethane solution prepared above. Reaction was very slow, and nitrogen was evolved slowly. The reaction flask was closed with a small inverted Erlenmyer flask and allowed to stand overnight. By the next morning, all diazomethane had disappeared, and a small precipitate of polymethylene was present. The solvent was removed by evaporation under reduced pressure to leave an oil which solidified when covered with water. The material was spotted on paper and sprayed with both silver and diazotized para-nitroaniline reagents. All spots were positive to both sprays indicating that no methylation of the phenolic hydroxyl group took place whatsoever.

Because the related glucoside of hydroquinone, arbutin, was available in quantity commercially, further development studies were performed on arbutin which also possessed a free phenolic hydroxyl group.

PRELIMINARY REACTION OF ARBUTIN WITH DIAZOMETHANE -- A solution of 2.0 grams of arbutin in 50 ml. of tetrahydrofuran containing 15 ml. of ethanol was treated with four-fifths of the diazomethane solution prepared above. Nitrogen was evolved slowly. The reaction flask was closed with a small inverted Erlenmyer flask and allowed to stand. After three days, all diazomethane had disappeared, and the solution became colorless. The reaction mixture was evaporated to dryness in a rotating evaporator, and the residual syrup was dissolved in ethanol. Paper chromatography of the ethanol solution along with arbutin indicated two spots with the silver spray reagent and only one spot of the original arbutin with the diazotized para-nitroaniline spray. The second spot with the silver spray was relatively small, and it appeared that this methylation was only partially successful.

The inability to methylate preferentially the phenolic hydroxyl group of either salireposide or arbutin led to experiments on the complete methylation of these glucosides with diazomethane by employing boron trifluoride-etherate as a catalyst for activation of the methylation of alcoholic hydroxyl groups as suggested by Johnson and co-workers (1,2).

REACTION OF ARBUTIN WITH DIAZOMETHANE AND BORON TRIFLUORIDE-ETHERATE -- A suspension of 1.0 gram arbutin in 100 ml. of tetrahydrofuran was treated with 2 drops of redistilled boron trifluoride-etherate and then dropwise with stirring with an ether solution of diazomethane prepared from 5 grams (0.25 mole) of DIAZALD. Gas was evolved immediately, and the diazomethane was consumed with the rapid evolution of nitrogen as fast as the solution was added. When all the diazomethane solution had been added, the tetrahydrofuran solution was filtered to remove any undissolved arbutin and possibly any polymethylene polymer, and the clear colorless filtrate was evaporated to dryness in a rotating evaporator. A yellowish pleasant smelling oil remained. This was spotted on paper along with arbutin and developed in the 10:3:3 butanol-pyridine-water (BPW) developer. The chromatogram was sprayed with diazotized para-nitroaniline reagent (DpNA). The arbutin gave a strong blueish lavender spot while the methylation mixture gave a similar smaller spot at the same R_f plus another blueish lavender spot with higher R_f. This would indicate that the methylation mixture contains unchanged arbutin plus partially methylated arbutin with methoxyl on the glucose moiety and not on the phenolic hydroxyl group.

About one-third of this syrupy methylation mixture was covered with 6 ml. of 2N hydrochloric acid and 5 ml. of methanol and boiled under reflux for 1.5 hours. The methanol was removed under reduced pressure, and the resulting aqueous solution was extracted with ether. The aqueous solution was treated

with 0.33 gram of silver carbonate to remove any residual hydrochloric acid and then spotted on paper along with known methylated glucoses. The paper was developed in 9:2:2 ethyl acetate-acetic acid-water (EAW) and sprayed with the para-anisidine reagent. The chromatogram revealed a strong glucose spot, moderately strong monomethyl glucose spot, weak dimethyl glucose spot, and very weak trimethyl glucose spot. Thus, it appears that under the conditions of methylation of this experiment, some of the glucose hydroxyls were methylated before all the phenolic hydroxyls in the arbutin were methylated. The boron trifluoride-etherate catalyzes the methylation of the phenolic hydroxyl as well as that of the alcoholic hydroxyls so that both methylate simultaneously.

The next experiment was planned to eliminate the ethanol from the reaction mixture, eliminate the boron trifluoride-etherate catalyst, keep the temperature down, and bring all arbutin into solution before the diazomethane was added. An ethereal solution of diazomethane was prepared free of ethanol by employing 3 grams of potassium hydroxide in 5 ml. of water plus 1 ml. of carbitol and 5 ml. of ether and treating with a solution of 10 grams of DIAZALD in 70 ml. of ether in the manner described previously. This diazomethane solution was added portionwise to a solution of 1 gram of arbutin in 100 ml. of tetrahydrofuran containing 2 ml. of water. After about one-fifth of the diazomethane solution was added, a sort of end point was reached at which point the diazomethane remained in excess and did not react. The methylation was interrupted at this point, and the mixture was chromatographed in the EAW developer and sprayed with DpNA. Several blueish lavender spots appeared along with the spot for the original arbutin indicating methylation of alcoholic hydroxyl groups before the phenolic hydroxyl group was methylated. Thus, under these conditions, even without boron trifluoride-etherate, the alcoholic groups were methylated. However, the extent of reaction was much less in this experiment. Therefore, another experiment was

planned with diazomethane prepared in the normal manner in the presence of ethanol, a relatively small amount of boron-trifluoride catalyst, a large excess of diazomethane, and no water in the reaction mixture. Dioxane was employed as the solvent because arbutin was completely soluble in this solvent.

An ethereal solution of diazomethane was prepared in the usual manner from 21.5 grams of DIAZALD. This was added portionwise to a filtered cold solution of 1.0 grams of arbutin in 100 ml. of dry dioxane containing one drop of boron trifluoride-etherate. As each 5 ml. of the diazomethane solution was added, nitrogen was evolved, and a colorless solution remained. After three-fourths of the diazomethane solution had been added, the yellow color of the diazomethane persisted, and nitrogen evolution almost ceased. At this point another drop of boron trifluoride-etherate was added which caused immediate decolorization and nitrogen evolution. The balance of the diazomethane solution was decolorized as fast as it was added leaving a clear colorless reaction mixture. The ether was removed by evaporation under reduced pressure to less than 100 ml. The resulting solution was spotted in duplicate on paper and developed with EAW. Chromatograms were sprayed with modified silver and DpNA reagents. Both chromatograms were identical indicating unchanged arbutin and three methylated products. All products still retained the unmethylated phenolic hydroxyl as evidenced by the fact that each gave a colored spot with DpNA reagent.

Since it was obvious that methylation was proceeding to some extent, the reaction mixture was cooled to 0° and treated portionwise with more diazomethane solution prepared from 21.5 grams of DIAZALD. After all the diazomethane was added and reaction was complete, the ether was removed under reduced pressure, and the residual solution was spotted on paper and chromatographed in EAW as before. Spraying of the chromatograms with both silver and DpNA reagents indicated that all the original arbutin had disappeared from the reaction mixture,

and only the three reaction products noted earlier were present. The methylated product with the lowest R_f value gave a much weaker spot than the two methylated products with the higher R_f values. If these three spots represent various degrees of methylation of arbutin, then only one, two, and three hydroxyl groups were being methylated. Otherwise there should be four spots. Thus, it appears that one of the hydroxyl groups of the glucose moiety must be involved with the protection of the phenolic hydroxyl group from methylation.

The reaction mixture was subject to another identical methylation with diazomethane from 21.5 grams of DIAZALD. Chromatographic results indicated that the methylation product giving the uppermost spot was almost gone from the reaction mixture, and the two remaining components were present in about equal amounts. At this point a few milligrams of the methylation mixture was boiled under reflux with 10 ml. of water containing 1 ml. of 6N hydrochloric acid for one hour. The pale yellow solution was freed of hydrochloric acid by means of silver carbonate, and the excess silver carbonate and silver chloride were removed by filtration. The neutral yellow filtrate was chromatographed in EAW along with a mixture of known methylated glucoses, and the chromatograms were sprayed with para-anisidine hydrochloride and heated. Spots were obtained only for dimethyl glucose and trimethyl glucose. Trace spots were obtained for monomethyl and tetramethyl glucoses, but no spot whatsoever was obtained for unmethylated glucose. Thus, it appears that after three methylations, only dimethyl and trimethyl arbutins exist in the methylation mixture. Obviously one hydroxyl of the four on the glucose moiety of arbutin is resistant to methylation, and the maximum number of methyl groups introducible on the glucose of arbutin by this procedure is three.

The reaction mixture was diluted with a little dioxane and methylated a fourth time with diazomethane from 21.5 grams of DIAZALD and employing boron trifluoride-etherate to keep the reaction active. Chromatographic analysis

indicated progressive methylation, and the methylation was repeated a fifth and a sixth time. After the sixth methylation, it was impossible to resolve the remaining elongated spot on the chromatogram by the EAW developer. Chromatography in the 63:10:27 butanol-acetic acid-water system (BAW) indicated the presence of trimethyl arbutin with a small amount of dimethyl arbutin. Hydrolysis followed by purification of the hydrolyzate and chromatography in EAW indicated that the chief component of the hydrolyzate was a trimethyl glucose, a little dimethyl glucose, and a trace of tetramethyl glucose. Further chromatograms of the hydrolyzate along with authentic samples of 3,4,6-trimethyl glucose, 2,3,4-trimethyl glucose, and 2,3,6-trimethyl glucose in both EAW and BPW developers and spraying with para-anisidine reagent indicated that the trimethyl glucose obtained by hydrolyzing an exhaustively methylated arbutin is either 2,4,6- or 2,3,6-trimethyl glucose. In other words, either the 3- or the 4-hydroxyl group of the glucose moiety and the phenolic hydroxyl of arbutin are not methylated with diazomethane under the conditions employed in this experiment. This is indeed an unexpected result, and if confirmed, indicates that in arbutin probably the phenolic hydroxyl and the hydroxyl of the glucose moiety in either positions 3 or 4 are somehow involved in a co-ordinate covalent bond in some manner so as to prevent methylation with diazomethane of these two hydroxyl groups. While unexpected, nevertheless, conformation considerations indicate that the pheholic hydroxyl group and the hydroxyl group at position 3 of the glucose moiety are in favorable spacial positions to react while the 2,4, and 6 hydroxyl groups of the glucose moiety are not.

In these studies previously reported 2,3,6-trimethyl glucose and 2,4,6-trimethyl glucose were chromatographed along with 2,3,4-trimethyl glucose prepared by hydrolysis of authentic 2,3,4-trimethyl levoglucosan (obtained from Dr. N. K. Richtmyer of National Institutes of Health). The 2,3,6- and 2,4,6-

trimethyl glucoses give pink spots with the para-anisidine spray reagent and the 2,3,4-trimethylglucose gives an orange spot turning to brick red upon standing.

FURTHER METHYLATIONS OF SALIREPOSIDE WITH DIAZOMETHANE--Having carried through the methylations study on arbutin as far as possible, attention was again turned to the methylation of salireposide. The material recovered from the preliminary diazomethane methylation reaction described earlier was dissolved in dioxane and methylated with diazomethane solution prepared from 21.5 grams of DIAZALD in the presence of catalytic amounts of boron trifluoride-etherate. The methylation was repeated three times with paper chromatography of the methylation reaction mixture after every methylation. After four methylations, no change appeared in the spots obtained on paper chromatograms. Paper chromatograms in EAW indicated one elongated spot and those in BAW indicated two spots. Hydrolysis of the reaction product as described under the arbutin experiment, and chromatography of the hydrolyzate indicated only two products--amonomethyl glucose and dimethyl glucose. This would be expected from the analogy with arbutin because in the case of salireposide, one of the hydroxyl groups of the glucose moiety is already benzoylated, leaving only two hydroxyl groups available for methylation if one hydroxyl group is rendered unmethylatable due to involvement with the phenolic hydroxyl group. If one of the hydroxyl groups of the glucose moiety in salireposide is tied up with the phenolic hydroxyl group as in the case of arbutin, the problem of locating the benzoylated hydroxyl group in salireposide resolves itself into the identification of the dimethyl glucose obtained in this experiment as 2,4-, 2,6-, or 4,6-dimethyl glucose. At the moment, the identity of this particular dimethyl glucose has not been determined because of the unavailability of authentic dimethyl glucoses for comparison either chromatographically or by other physical means. Experiments along this line are planned and will be reported at a later date.

METHYLATIONS OF PHENYL-α-GLUCOSIDE WITH DIAZOMETHANE -- Before leaving the matter of diazomethane methylation of glucosides it was desired to methylate a glucoside not possessing a phenolic hydroxyl group to determine whether all the hydroxyls of the glucose moiety were amenable to methylation by diazomethane in the presence of boron trifluoride-etherate. Accordingly, 1.0 gram of phenyl-α-glucoside was dissolved in 100 ml. of dry dioxane, cooled to 0°, treated with 2 drops of boron trifluoride-etherate, and then portionwise with an ethereal solution of diazomethane prepared from 21.5 grams of DIAZALD. The methylation proceeded in the same manner outlined under arbutin, and when the presence of diazomethane persisted, another drop of boron trifluoride-etherate was added. After all diazomethane was added and consumed, the mixture was concentrated under reduced pressure to about 50 ml. A sample was hydrolyzed with hydrochloric acid, and the clarified hydrolyzate was chromatographed in the EAW developer and sprayed with para-anisidine reagent. After the first methylation, the chromatogram of the hydrolyzate indicated some unmethylated starting material, considerable monomethyl and dimethyl product, and little trimethyl product, and no tetramethyl product.

The methylation mixture was remethylated under the same conditions. This process was repeated a total of seven times making eight methylations in all. After each methylation, the ether was removed, and a small sample of the methylation mixture was hydrolyzed as above and chromatographed.

After the second methylation a little unmethylated material still remained; at least two monomethylated products were present; two dimethyl products; considerable trimethylated product; and some tetramethylated product.

After the third methylation no unmethylated product was present; only a trace of monomethylated material still persisted, while most of the product

was in the dimethylated and trimethylated state with a little tetramethylated product.

The methylation reaction proceeded in much the same manner through all methylations with the monomethylated and dimethylated products disappearing in the manner described for the unmethylated material. After the eighth methylation, the reaction mixture consisted primarily of trimethyl- and tetramethyl-phenyl-α-glucoside and a small amount of dimethylphenyl-α-glucoside. Further methylations, no doubt, would eventually bring the reaction product to a completely methylated material, namely 2,3,4,6-tetramethyl phenyl-α-glucoside.

For our purposes at the moment this was not necessary. The essential fact was that with phenyl-α-glucoside, tetramethylation does take place to a considerable extent, there being no fundamental hindrance to such methylation. However, in the cases of arbutin and salireposide, hindrance to complete methylation of the glucose hydroxyls is offered by the presence of a phenolic hydroxyl group. This, in turn, also prevents methylation of the phenolic hydroxyl group in both substances. Seven methylations of arbutin with diazomethane brought the methylated materials to a stage where they contain a maximum of three methyl groups. This means that one hydroxyl group, possibly the 3-hydroxyl, remains unmethylated. In the case of salireposide, the maximum methylation that was reached was dimethylation because of the hindrance mentioned above and the presence of a benzoyl group on another hydroxyl of the glucose moiety.

While these discoveries add considerable to our knowledge of methylation and might add even more if pursued further, it is evident that a great deal more work will have to be performed to finally clear up the structure of salireposide. Success in this direction may come by isolating pure crystalline materials from the completely methylated salireposide and identification of the methylated glucose obtained from hydrolysis of this material. There are

indications that the methylated reaction mixture will yield crystals.

In the case of the methylated arbutin reaction mixture, crystals are already depositing, and these may well be the pure trimethyl arbutin from which could be obtained a known trimethyl glucose. This would enable one to designate the particular hydroxyl group in the arbutin molecule that resists methylation because of some influence of the phenolic hydroxyl group in the aglucone.

More experiments along these lines will be performed and reported in the future.

iap/jaf

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PROJECT REPORT FORM

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CAUSTIC HYDROLYSIS OF ASPEN PHLOEM AND XYLEM

Three samples were given us by Mr. J. Mugg. These samples were:

1. Water soluble materials from a methanol extraction of aspen phloem.
2. Water soluble materials from a methanol extraction of aspen soft xylem.
3. Water soluble materials from a methanol extraction of aspen year old zylem.

The above descriptions of the samples are all that is known concerning them. We do not know what, if anything, has been done to the samples, nor how they were obtained. The samples appeared to be very dilute, so no solids determination was run.

To each of the aqueous samples, 25 cc. of 4% sodium hydroxide were added. The solutions were then boiled under reflux for a period of 8 hours. When cool, the solutions were acidified with dilute sulfuric acid and were extracted with ether.

The ether solutions were dried with anhydrous sodium sulfate and concentrated tp a small volume. The 3 ether solutions were then chromatographed for acids, using benzene saturated with formic acid as the developer. The chromatogram was sprayed with bis-diazotized benzidine. The following results were obtained.

1. Phloem - Rv- 0.00; 0.26; 0.37; 0.72; 0.77; 1.0

2. Soft xylem - Rv- 0.00; 0.26; 0.37; 0.77; 1.0

3. Year old xylem - Rv- 0.00; 0.26; 0.37; 0.77; 1.0

The Rv value is the position of the material in reference to vanillic acid. Known acids were spotted against the sample with the following results:

p-Hydroxybenzoic acid - Rv 0.26

Coumaric acid - Rv 0.37

Syringic acid - Rv 0.77

Vanillic acid - Rv 1.0

The material in the phloem with the Rv 0.72 may possibly be sinapic acid. The spot was too faint for any satisfactory comparison.

The interesting thing to note concerning these three samples is as follows. In the phloem, coumaric acid is about 6 to 8 times as heavy as p-hydroxybenzoic acid. In the soft xylem, coumaric acid is about 4 to 5 times as heavy as the p-hydroxybenzoic acid. In the year-old xylem, a shift seemed to occur and the yield of p-hydroxybenzoic acid was about 4 to 5 times greater than coumaric acid.

We do not know if there is a definite trend as to the ratio of coumaric and p-hydroxybenzoic acid since, as stated before, we know nothing concerning these samples. However, from past hydrolysis of aspen sawdust, we know that coumaric acid has never been found in any of our samples.

Dlb/ms

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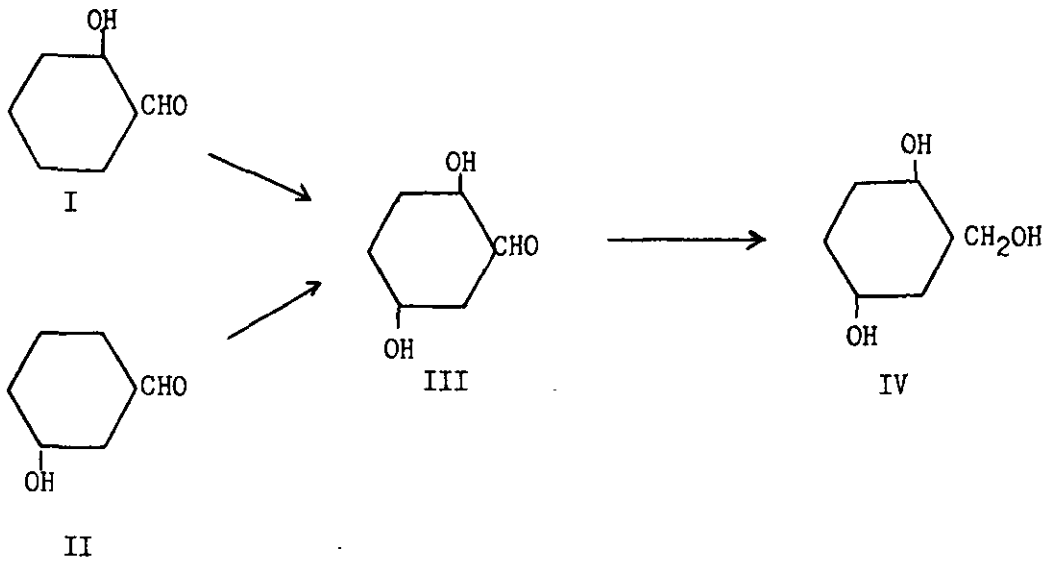
EXPERIMENTS ON GENTISYL ALCOHOL

In connection with our chromatographic experiments on the glucosides of Populus tremuloides bark it was desired to check many of our extracts for gentisyl alcohol. Because salireposide was found in abundance in the extract of this bark and because salireposide was shown to be a benzoylated glucoside of gentisyl alcohol (see Report No. 3) it was suspected that Populus tremuloides bark extracts would contain gentisyl alcohol in addition to salicyl alcohol, the aglucone of both salicin and tremuloidin. The present report describes experiments on the preparation of gentisyl alcohol and the chromatography of gentisyl alcohol along with several bark extracts.

Gentisyl alcohol (IV) was prepared by the hydrogenation of gentisyl aldehyde (III) which in turn was prepared by the oxidation of either salicylaldehyde (I) or m-hydroxybenzaldehyde (II) with potassium persulfate.



PREPARATION OF GENTISYL ALDEHYDE (III)--The first preparation of gentisyl aldehyde followed the procedure described by Hodgson and Beard (1). A solution of 12.5 grams of sodium hydroxide in 200 ml. of water was treated at room temperature with 30.5 grams of m-hydroxybenzaldehyde. This solution was placed in a 2-liter beaker and stirred vigorously while a solution of 75 grams of potassium persulfate in 900 ml. of water and 100 ml. of 40% sodium hydroxide solution were added from separate dropping funnels over a period of 90 minutes. The temperature was maintained at 30-35°, and the sodium hydroxide solution was added just fast enough to keep the reaction mixture on the alkaline side. After all reactants were added, the mixture was allowed to stand 40 hours at room temperature. The brown reaction mixture was acidified with dilute hydrochloric acid to Congo red and extracted three times with 300 ml. each of ether. The water layer was then strongly acidified with 175 ml. of concentrated hydrochloric acid, filtered to remove the brown precipitate, and extracted four times with 250 ml. of ether each. The ether extract was dried, and the ether was removed by distillation. The residue was boiled with benzene, and the benzene was decanted from the tarry material. Evaporation of the benzene yielded a yellow oil which crystallized to yield about 1.0 gram of yellow needles melting at 90-95°. The yield of crude product was very poor and the procedure was not repeated. Instead, the earlier procedure of Neubauer and Flatow (2) was tried.

A solution of 62 grams of salicylaldehyde in 400 ml. of water containing 25 grams of sodium hydroxide at 25° was treated alternately every five minutes with 20 ml. of 40% sodium hydroxide solution and 150 ml. of 10% potassium persulfate solution beginning with the sodium hydroxide solution. Addition was stopped after 200 ml. of sodium hydroxide solution and 1500 ml. of potassium persulfate solution had been added. The temperature was

maintained between 25° and 35°. After addition ceased, the brown solution was allowed to stand at room temperature for 27 hours. Concentrated hydrochloric acid was added dropwise to the Congo red end point, and the solution was filtered and extracted three times, each with 500 ml. of ether. The aqueous solution was strongly acidified with 350 ml. of concentrated hydrochloric acid, heated on the steam bath to 70°, and allowed to cool to room temperature. The mixture was filtered, and the clear filtrate was extracted four times with 500 ml. of ether each. The ether extract was concentrated by distillation to 500 ml. and then evaporated to dryness in a rotating evaporator. The tarry residue was extracted by vigorously shaking with 250 ml. of benzene. The benzene was evaporated spontaneously at room temperature to yield 15 grams of pale yellow crystals melting at 89-93°. Several recrystallizations from benzene yielded pure gentisyl aldehyde melting at 97-99°. The yield of purified material was 6 grams. The melting point of the pure product was in agreement with that reported by previous investigators (1,2).

PREPARATION OF GENTISYL ALCOHOL (IV)--The hydrogenation procedure of Birkinshaw, Brachen and Raistrick (3) was employed for the preparation of gentisyl alcohol from gentisyl aldehyde. A solution of 1.0 gram of gentisyl aldehyde in 15 ml. of ethanol was treated with 0.1 gram of 10% palladium on charcoal and hydrogenated at 31° at an original pressure of 37.5 pounds per square inch. After one-half hour the pressure remained steady at 36.0 pounds per square inch. After one hour, the reaction was stopped, the catalyst was removed by filtration, and the filtrate was evaporated in a rotating evaporator to yield a pale colored oil which darkened on standing. The oil was dissolved in boiling chloroform which yielded white crystals of gentisyl alcohol on standing. The crystals were filtered and found to melt at 98-100°. This melting point agrees with that reported by Brack (4)

(4) and by Engel and Brzeski (5) who isolated gentisyl alcohol from penicillin broths and recrystallized their products from chloroform. The yield of pure gentisyl alcohol in this experiment was 0.45 grams.

Several similar hydrogenation experiments employing crude gentisyl aldehyde did not give the desired results.

CHROMATOGRAPHIC EXPERIMENTS WITH GENTISYL ALCOHOL--Both gentisyl alcohol and gentisyl aldehyde were chromatographed in the 10:3:3 butanol-pyridine-water and the 9:2:2 ethyl acetate-acetic acid-water developers at 20°. The developed chromatograms were sprayed with the modified silver and diazotized para-nitroaniline reagents. Gentisyl alcohol had a BPW $\underline{R}_f$ 0.77 and an EAW $\underline{R}_f$ of 0.79. Gentisyl aldehyde had a BPW $\underline{R}_f$ of 0.84 and an EAW $\underline{R}_f$ of 0.92. Both compounds gave black spots when immersed in the silver nitrate solution even before treatment with ethanolic alkaline solution. The alkaline spray produced immediate intense black spots.

The diazotized para-nitroaniline spray gave reddish brown spots for the two gentisyl compounds quite unlike the purple spots with salireposide and debenzoylated salireposide.

The immediate silver spots obtained with the two gentisyl compounds were also obtained with hydroquinone, but were not obtained with salireposide or with debenzoylated salireposide. Thus, this immediate spot with silver nitrate even before treatment with alkali must be due to the two free phenolic hydroxyl groups in gentisyl alcohol, gentisyl

aldehyde and hydroquinone.

A comparison spot with salicyl alcohol indicated that salicyl alcohol did not give a spot with the modified silver spray, but gave a bright red spot with the diazotized para-nitroaniline spray. The R_f in the 8:2:1 ethyl acetate-pyridine-water developer was identical with that of gentisyl alcohol.

Further chromatographic studies on gentisyl alcohol and related compounds will be given in future reports in connection with the examination of specific extracts.

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THE CAUSTIC HYDROLYSIS AND THE PROPANOL EXTRACTION OF VARIOUS HARDWOODS. II.

In Project 1932, Report No. 4, a number of hardwood samples were hydrolyzed with sodium hydroxide and also extracted with 75% propanol. The resulting extracts were all chromatographed and all the known materials that were present were quantitatively determined. This report is a continuation of Report No. 4.

In an attempt to include as many of the pulpwoods as possible, another twelve different species have been obtained. These wood samples were handled in identically the same manner as described in Report No. 4. Each sample was hydrolyzed with sodium hydroxide, acidified and extracted with ether. The extract was chromatographed and all known compounds were quantitatively measured.

Each wood was also extracted with 75% propanol. The extracts were also chromatographed.

The results of the caustic hydrolysis of the various hardwoods have been compiled into a table which will follow. As far as the results are concerned, there isn't much to call any attention to. The yields of vanillin and syringaldehyde appear normal in all cases. p-Hydroxybenzoic acid is still found in a large amount in all members of the Salicaceae family. Vanillic and syringic acids appear in all the extracts. Ferulic acid is also present in all with the exception of the Weeping Willow, Salix babylonica.

One wood does stand out, however, and that is the Catalpa, (Catalpa speciosa). This is the only hardwood of the now 46 different species hydrolyzed that contains p-hydroxybenzoic acid and does not belong to the Salicaceae family. This extract also contains coumaric acid, the lightest yields of vanillic and syringic acids in this group of woods, and an enormous amount of ferulic acid. The yield of vanillin and syringaldehyde, though higher than the other woods in this report, are not far above normal. From all appearances, the results derived from Catalpa indicates that further work should be done on it.

Each of the extracts were also chromatographed. The results of the chromatograms have been compiled in table form and will also follow.

A sample of each of the twelve different hardwoods were extracted with 75% propanol. The propanol solutions were concentrated to a small volume. A solids determination was run on each extract. The extracts were also chromatographed. The results of the extracts as well as the chromatographic results will follow.

At this time it has been found that some of the ether extracts from the hydrolysis of the woods contain p-hydroxybenzaldehyde. In any of the cases were the p-hydroxybenzaldehyde is present, the vanillin yield is incorrect. Both vanillin and p-hydroxybenzaldehyde move approximately at the same rate in the n-butyl ether saturated with water developer. In the future, each of the 46 extracts will be chromatographed for p-hydroxy-

benzaldehyde and in every case where it is found the correction will be made. These corrections will be reported in a future report.

It has also been found that coumaric acid has been overlooked in the extract from Populus tremuloides. Several other extracts will also be rechecked for any possible presence of coumaric acid. These corrections will also be made in a future report.

Two more hardwoods are planned to be included in this study. Neither are at present available; but both have been promised to us. The one is Swamp Cottonwood (Populus heterophylla) and the other is Chosenia bracteosa, both of these woods belong to the Salicaceae family. The results for these woods will also be reported in a future report.

db/mrb

75% PROPANOL EXTRACTION OF HARDWOODS

Wood		Propanol Extract		Sawdust Recovered
200 g. O.D. Used	Scientific Name	Grams	Per Cent	Grams, O.D.
Willow Oak	Quercus phellos	6.14	3.07	191.6
Scarlet Oak	Quercus coccinea	12.54	6.27	189.5
Black Oak	Quercus velutina	13.16	6.58	185.3
Turkey Oak	Quercus catesbael	9.50	4.75	191.5
American Elm	Ulmus americana	4.36	2.18	195.3
Black Willow	Salix nigra	5.82	2.91	190.0
Weeping Willow	Salix babylonica	10.55	5.28	189.9
Lombardy Poplar	Populus nigra	7.61	3.81	193.1
Catalpa	Catalpa speciosa	10.16	5.08	190.8
Chestnut	Castanea dentata	16.30	8.15	188.7
Shagbark Hickory	Carya ovata	9.95	4.98	188.6
Butternut	Juglans cinerea	18.92	9.46	180.2

CAUSTIC HYDROLYSIS OF HARDWOODS

fic	Ether Extract g.	Code No.	Vanillin Mg. in Ext.	Vanillin % of Ext.	Syringaldehyde Mg. in Ext.	Syringaldehyde % of Ext.	Ratio V:S	Vanillic Acid Mg. in Ext.	Vanillic Acid % of Ext.	Syringic Acid Mg. in Ext.	Syringic Acid % of Ext.	Ferulic Acid Mg. in Ext.	Ferulic Acid % of Ext.	p-Hydroxybenzoic Acid Mg. in Ext.	p-Hydroxybenzoic Acid % of Ext.	P-Coumaric Acid Mg. in Ext.	P-Coumaric Acid % of Ext.	Recovered Sawdust
hellos	0.44	1555-60-A	58.8	13.4	57.3	13.0	1:1	13.5	3.1	20.0	4.5	29.5	6.7	None	--	None	--	66.8
occinea	1.26	1555-67-A	45.0	3.6	65.0	5.2	1:1.4	26.0	2.1	53.5	4.2	65.0	5.2	None	--	None	--	63.0
elutina	2.07	1555-73-A	39.0	1.9	54.0	2.6	1:1.4	38.0	1.8	74.0	3.6	72.5	3.5	None	--	None	--	63.7
atesbael	0.79	1555-79-A	34.5	4.4	49.5	6.2	1:1.4	26.5	3.4	53.5	6.8	56.5	7.2	None	--	None	--	68.0
ra	1.32	1555-93-A	37.0	2.8	41.6	3.2	1:1.2	24.5	1.9	39.5	3.0	48.5	3.7	647.0	48.9	None	--	67.6
icana	0.91	1555-85-A	37.5	4.1	40.5	4.5	1:1.1	20.5	2.3	53.0	5.8	56.0	6.2	None	--	None	--	67.2
igra	2.00	1555-99-A	30.5	1.5	34.5	1.7	1:1.1	30.5	1.5	39.5	2.0	50.0	2.5	743.0	37.2	None	--	67.2
eciosa	1.68	1555-105-A	49.0	2.9	96.0	5.7	1:1.96	69.0	4.1	79.0	4.7	315.0	18.8	25.0	1.5	38.0	2.3	66.0
entata	1.74	1555-111-A	34.0	2.0	34.0	2.0	1:1	20.5	1.2	47.0	2.7	54.5	3.1	None	--	None	--	61.7
a	1.10	1555-117-A	22.5	2.0	25.0	2.3	1:1.1	18.5	1.7	45.1	4.1	27.5	2.5	None	--	None	--	63.4
a	1.69	1555-123-A	32.0	1.9	34.0	2.0	1:1.1	29.0	1.7	32.5	1.9	None	--	446.0	26.4	None	--	64.4
nerea	2.84	1555-129-A	23.0	0.8	24.0	0.8	1:1	19.0	0.7	57.0	2.0	31.0	1.1	None	--	None	--	57.3

CAUSTIC HYDROLYSIS OF HARDWOODS

(100 g.)	Scientific Name	Ether Extract g.	Code No.	Vanillin Mg. in Ext.	Vanillin % of Ext.	Syringaldehyde Mg. in Ext.	Syringaldehyde % of Ext.	Ratio V:S	Vanillic Acid Mg. in Ext.	Vanillic Acid % of Ext.	Syringic Acid Mg. in Ext.	Syringic Acid % of Ext.	Ferulic Acid Mg. in Ext.	Ferulic Acid % of Ext.	p-Hydroxybenzoic Acid Mg. in Ext.
Willow Oak	Quercus phellos	0.44	1555-60-A	58.8	13.4	57.3	13.0	1:1	13.5	3.1	20.0	4.5	29.5	6.7	None
Scarlet Oak	Quercus coccinea	1.26	1555-67-A	45.0	3.6	65.0	5.2	1:1.4	26.0	2.1	53.5	4.2	65.0	5.2	None
Black Oak	Quercus velutina	2.07	1555-73-A	39.0	1.9	54.0	2.6	1:1.4	38.0	1.8	74.0	3.6	72.5	3.5	None
Turkey Oak	Quercus catesbael	0.79	1555-79-A	34.5	4.4	49.5	6.2	1:1.4	26.5	3.4	53.5	6.8	56.5	7.2	None
Black Willow	Salix nigra	1.32	1555-93-A	37.0	2.8	41.6	3.2	1:1.2	24.5	1.9	39.5	3.0	48.5	3.7	647.0
American Elm	Ulmus americana	0.91	1555-85-A	37.5	4.1	40.5	4.5	1:1.1	20.5	2.3	53.0	5.8	56.0	6.2	None
Lombardy Poplar	Populus nigra	2.00	1555-99-A	30.5	1.5	34.5	1.7	1:1.1	30.5	1.5	39.5	2.0	50.0	2.5	743.0
Catalpa	Catalpa speciosa	1.68	1555-105-A	49.0	2.9	96.0	5.7	1:1.96	69.0	4.1	79.0	4.7	315.0	18.8	25.0
Chestnut	Castanea dentata	1.74	1555-111-A	34.0	2.0	34.0	2.0	1:1	20.5	1.2	47.0	2.7	54.5	3.1	None
Shagbark Hickory	Carya ovata	1.10	1555-117-A	22.5	2.0	25.0	2.3	1:1.1	18.5	1.7	45.1	4.1	27.5	2.5	None
Weeping Willow	Salix babylonica	1.69	1555-123-A	32.0	1.9	34.0	2.0	1:1.1	29.0	1.7	32.5	1.9	None	--	446.0
Butternut	Juglans cinerea	2.84	1555-129-A	23.0	0.8	24.0	0.8	1:1	19.0	0.7	57.0	2.0	31.0	1.1	None

RESULTS OF ETHER EXTRACTS FROM CAUSTIC HYDROLYSIS--DEVELOPED WITH BUTANOL-2% AQUEOUS AMMONIA

el	Black Willow Salix nigra	American Elm Ulmus americana	Lombardy Poplar Populus nigra	Catalpa Catalpa speciosa	Chestnut Castanea dentata	Shagbark Hickory Carya ovata	Weeping Willow Salix babylonica	Butternut Juglans cinerea	Remarks
			X	X		X		X	Positive to $FeCl_3$
								X	
	X	X	X	X	X	X	X	X	Mäule - Syringic Acid
	X	X	X	X	X	X	X	X	Vanillic Acid
	X	X	X	X	X	X	X	X	p-Hydroxybenzoic Acid and Ferulic Acid
	X	X	X	X	X	X	X	X	Positive Mäule α 2,4
	X	X	X	X	X	X	X	X	Syringaldehyde
	X	X	X	X	X	X	X	X	Vanillin
						X			
	X	X	X	X	X	X	X	X	
	X	X	X	X	X	X	X	X	

CHROMATOGRAPHIC RESULTS OF ETHER EXTRACTS FROM CAUSTIC HYDROLYSIS--DEVELOPED WITH BUTANOL-2% AQ

R_f	Willow Oak	Scarlet Oak	Black Oak	Turkey Oak	Black Willow	American Elm	Lombardy Poplar	Catalpa	Chestnut	Shagbark Hickory	Weeping Willow
	Quercus phellos	*Quercus coccinea*	*Quercus velutina*	*Quercus catesbael*	*Salix nigra*	*Ulmus americana*	*Populus nigra*	*Catalpa speciosa*	*Castanea dentata*	*Carya ovata*	*Salix babylonica*
0.00		X	X	X			X	X		X	
0.01			X								
0.08	X	X	X	X	X	X	X	X	X	X	X
0.10	X	X	X	X	X	X	X	X	X	X	X
0.12	X	X	X	X	X	X	X	X	X	X	X
0.27	X	X	X	X	X	X	X	X	X	X	X
0.38	X	X	X	X	X	X	X	X	X	X	X
0.44	X	X	X	X	X	X	X	X	X	X	X
0.62											
0.72											
0.79										X	
0.82	X	X	X	X	X	X	X	X	X	X	X
0.91	X	X	X	X	X	X	X	X	X	X	X

CAUSTIC HYDROLYSIS--DEVELOPED WITH BUTANOL-PYRIDINE-WATER (10:3:3)

an	Lombardy Poplar	Catalpa	Chestnut	Shagbark Hickory	Weeping Willow	Butternut	
ana	Populus nigra	Catalpa speciosa	Castanea dentata	Carya ovata	Salix babylonica	Juglans cinerea	Remarks
			X			X	Positive to $FeCl_3$
			X			X	
	X	X	X	X	X	X	Syringic Acid
	X	X	X	X	X	X	Vanillic Acid
	X	X	X	X		X	Ferulic Acid
		X					
	X A	X A,B			X A		p-Hydroxybenzoic Acid - A Coumaric Acid = B
	X	X	X	X	X	X	Positive Mäule α 2, 4
	X	X	X	X	X	X	Positive Mäule α 2,4

CHROMATOGRAPHIC RESULTS OF ETHER EXTRACTS FROM CAUSTIC HYDROLYSIS--DEV.

R_f	Willow Oak	Scarlet Oak	Black Oak	Turkey Oak	Black Willow	American Elm	Lombardy Poplar	Catalpa
	Quercus phellos	*Quercus coccinea*	*Quercus velutina*	*Quercus catesbael*	*Salix nigra*	*Ulmus americana*	*Populus nigra*	*Catalpa species*
0.01		X	X	X				
0.09		X	X	X				
0.12								
0.18								
0.27								
0.38	X	X	X	X	X	X	X	X
0.44	X	X	X	X	X	X	X	X
0.46	X	X	X	X	X	X	X	X
0.52			X					X
0.56					X A		X A	X A,E
0.76	X	X	X	X	X	X	X	X
0.83	X	X	X	X	X	X	X	X

:TIVES OF HARDWOODS DEVELOPED WITH BUTANOL 2% AQUEOUS AMMONIA

ı	Lombardy Poplar	Catalpa	Chestnut	Shagbark Hickory	Weeping Willow	Butternut	Remarks
ıa	Populus nigra	Catalpa speciosa	Castanea dentata	Carya ovata	Salix babylonica	Juglans cinerea	Positive To
	X	X	X	X	X	X	$FeCl_3$
	X		X	X		X	$FeCl_3$
			X	X	X		
	X	X	X			X	Mäule
	X				X		$FeCl_3$
						X	
		X		X			
			X		X		
	X						
	X	X	X	X	X	X	2,4
	X		X	X		X	2,4

CHROMATOGRAPHIC RESULTS OF 75% PROPANOL EXTRACTIVES OF HARDWOODS DEVEL

R_f	Willow Oak	Scarlet Oak	Black Oak	Turkey Oak	Black Willow	American Elm	Lombardy Poplar	Catalpa
	Quercus phellos	*Quercus coccinea*	*Quercus velutina*	*Quercus catesbael*	*Salix nigra*	*Ulmus americana*	*Populus nigra*	*Catalpa speciosa*
0.00	X	X	X	X		X	X	X
0.02	X	X	X	X	X		X	
0.05			X	X				
0.08			X			X	X	X
0.10					X		X	
0.21	X		X					
0.28								X
0.30								
0.42	X	X	X	X		X		
0.68								
0.70	X	X					X	
0.79								
0.81	X	X	X	X	X	X	X	X
0.95	X	X	X	X	X	X	X	

RDWOODS DEVELOPED WITH BUTANOL-PYRIDINE-WATER (10:3:3)

ombardy Poplar	Catalpa	Chestnut	Shagbark Hickory	Weeping Willow	Butternut	Remarks
opulus nigra	*Catalpa speciosa*	*Castanea dentata*	*Carya ovata*	*Salix babylonica*	*Juglans cinerea*	Positive to
X	X	X	X	X	X	$FeCl_3$
X	X	X			X	$FeCl_3$
						$FeCl_3$
						$FeCl_3$
						$FeCl_3$
						$FeCl_3$
					X	Mäule
		X			X	Mäule
		X				
X				X		$FeCl_3$
	X			X		
		X			X	Mäule, 2,4
X	X	X	X	X	X	Mäule, 2,4
X	X	X	X	X	X	2,4

CHROMATOGRAPHIC RESULTS OF 75% PROPANOL EXTRACTIVES OF HARDWOODS DEVELOPE

R_f	Willow Oak *Quercus phellos*	Scarlet Oak *Quercus coccinea*	Black Oak *Quercus velutina*	Turkey Oak *Quercus catesbael*	Black Willow *Salix nigra*	American Elm *Ulmus americana*	Lombardy Poplar *Populus nigra*	Catalp *Catalp specio*
0.00	X	X	X	X	X	X	X	X
0.02	X	X	X	X			X	X
0.05								
0.07								
0.10				X		X		
0.13								
0.25	X	X	X	X				
0.32								
0.38				X				
0.45		X	X	X				
0.56					X		X	
0.63								X
0.69								
0.70								
0.80	X	X	X	X				
0.85	X	X	X	X	X	X	X	X
0.91				X	X		X	X

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PROJECT NO. 1932
COOPERATOR IPC
REPORT NO. 6
DATE April 7, 1961
NOTE BOOK 1834
PAGE 10-23 and ~~TO~~ 44-46
SIGNED Irwin A. Pearl

Irwin A. Pearl

FURTHER STUDIES ON THE HOT WATER EXTRACT OF POPULUS TREMULOIDES BARK

The isolation of salireposide from the bark of the American quaking aspen (Populus tremuloides) was reported in Project Report No. 2 and 3. In these reported studies, salireposide was isolated by fractional concentration of the hot water extract. Because Sakai, Tsurumi, Eno and Inukai (1) isolated salireposide from the hot water extract of the bark of Salix purpurea by extraction with ether containing a little ethanol and because Fujikawa and Tokuoka (2) isolated salireposide from the hot water extract of the bark of Salix koriyanagi by ethyl acetate extraction, it was decided to apply these non-aqueous extractions to some aqueous residues left after isolation of tremuloidin, salireposide, and salicin from the hot water extracts of Populus tremuloides.

The experiment reported in the present report employed the aqueous residue from the isolation of salireposide and tremuloidin from the large scale hot water extract of evaporated ethanol extract of fresh Populus tremuloides bark described on page 29 of Project Report No. 1. The aqueous residue had been evaporated to dryness in a rotating evaporator and was stored at room temperature as a semisolid syrup for a period of 18 months.

The semisolid yellow syrup was covered with water and shaken occasionally until solution was complete. The resulting turbid solution was placed in a one-liter flask and extracted with ether containing a little ethanol in an air-agitated liquid-liquid extractor for several days. A yellow

extract was obtained, and a heavy oily layer separated in this yellow ether extract. This heavy oily layer was insoluble in water, but soluble in ethyl acetate. It was dissolved in ethyl acetate after separation from the ether layer. The subsequent fractionation of the ether extract is pictured in the flow sheet of Figure 1 in which all fractions are identified.

The aqueous residue left after ether-ethanol extraction was placed in a non-agitated liquid-liquid extractor and was extracted continuously with ethyl acetate. The fractionation of the ethyl acetate extract is pictured in the flow sheet of Figure 2 in which all fractions are identified.

Several of the crystalline fractions were subjected to mixed melting points with authentic tremuloidin, salireposide, and salicin. On the basis of these experiments the following unequivocal identifications were made for the fractions of Figures 1 and 2:

Items (II), (II-A), and (III-A) are tremuloidin; item (V-A) is anhydrous salireposide; and item (V-C-1) is salicin.

These identifications were confirmed by paper chromatography.

In addition to the above identifications, most of the fractions of Figures 1 and 2 were spotted on paper along with tremuloidin, salicin, and salireposide and developed in duplicate in 10:3:3 butanol-pyridine-water at 20°. The papers were sprayed with the modified silver reagent (Ag), and with the diazotized para-nitroaniline reagent (DpNA). Results are given below.

ITEM (III)--This ethanol solution contained small amounts of tremuloidin and salireposide and a number of non-glucoside materials such as salicyl alcohol, syringic acid and p-hydroxybenzoic acid.

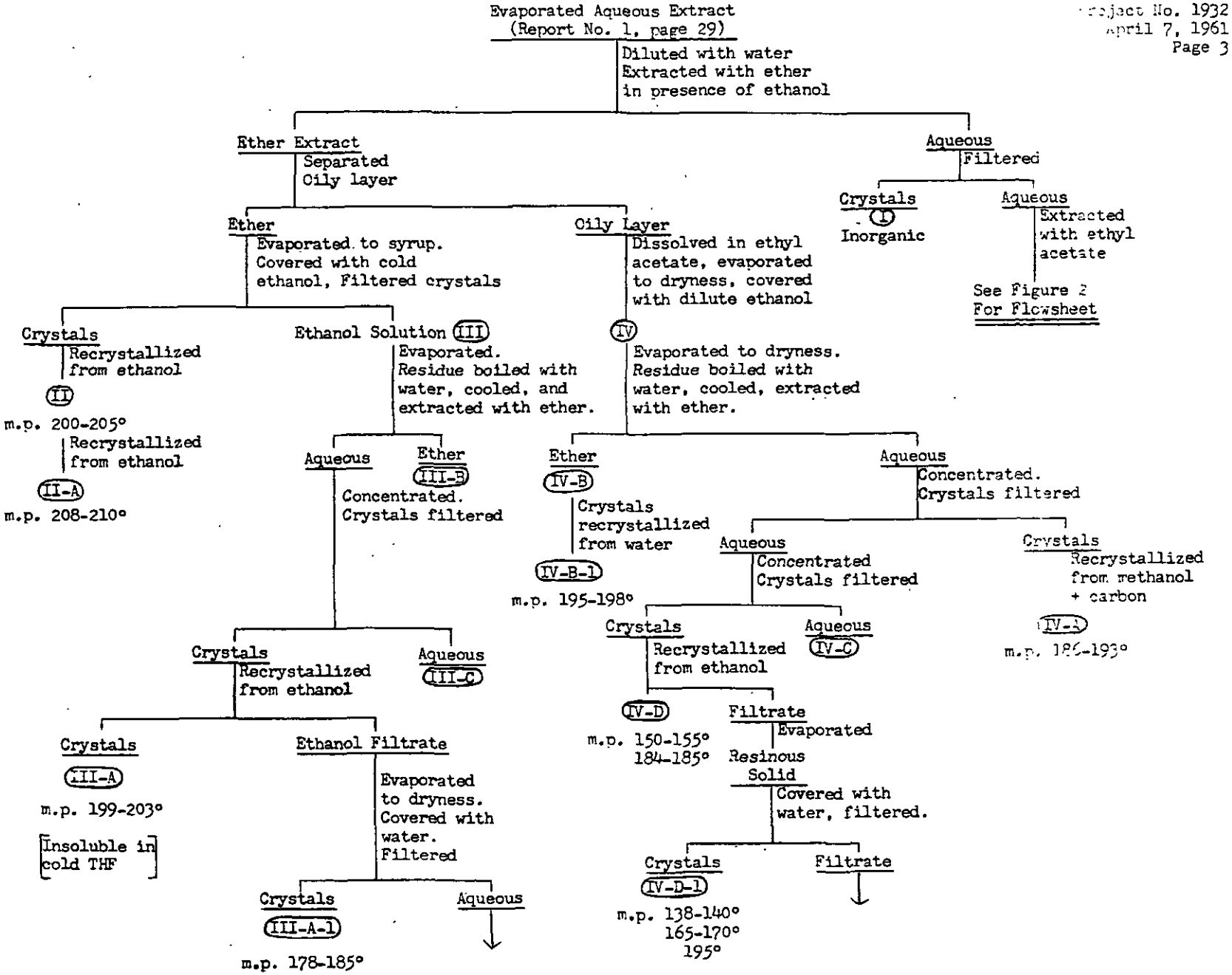


Figure 1

Fractionation of Aqueous Residue After Isolation of Tremuloidin and Salireposide From Hot Water Extract of Populus tremuloides Bark Extractives

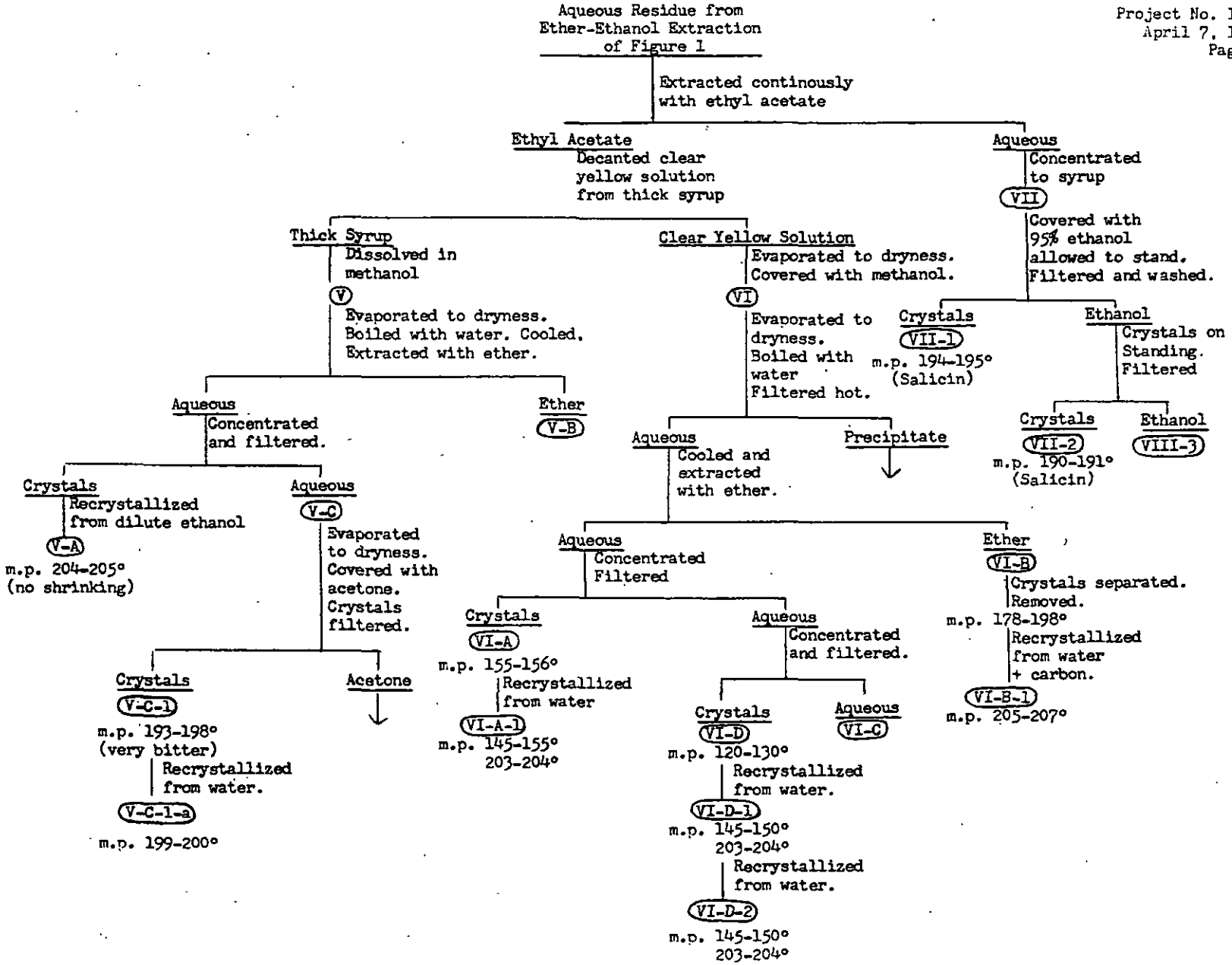


Figure 2

Further Fractionation of Aqueous Residue After Isolation of Tremuloidin and Salireposide From Hot Water Extract of *Populus tremuloides* Bark Extractives

ITEM (III-A-1)--The Ag spray indicated only tremuloidin, but the positive DpNA pink spot at approximately the same R_f value indicated a trace of salicyl alcohol. This probably accounts for the crude melting point of this fraction.

ITEM (III-B)--An instantaneous spot with Ag before alkali treatment at R_f 0.83 indicates gentisyl alcohol (see Report No. 5). In addition, this ether solution gave spots for syringic acid, salicyl alcohol, vanillic acid and unidentified compounds with R_f 0.20 (pink DpNA) and R_f 0.58 (salmon DpNA).

ITEM (IV-A)--This crystalline fraction gave no spots with the Ag reagent and gave a violet spot with pink ring at R_f 0.91. No identification was made.

ITEM (IV-B)--This ether solution gave an instantaneous Ag spot at R_f 0.83 indicating gentisyl alcohol. The DpNA spray indicated gentisyl alcohol and vanillic acid along with unidentified spots at 0.51 (buff) and, 0.77 (violet and salmon).

ITEM (IV-D)--Ag-sprayed chromatogram indicated these crystals to be about half and half tremuloidin and salireposide. The DpNA-sprayed chromatogram indicated only the salireposide.

ITEM (IV-D-1)--The Ag-sprayed chromatogram indicated about three-fourths tremuloidin and one-fourth salireposide. The DpNA chromatogram indicated salireposide and some salicyl alcohol impurity.

ITEM (V-B)--This ether extract contained gentisyl alcohol as indicated by the instantaneous silver spot. The DpNA spray indicated gentisyl alcohol and a purple spot at R_f 0.80.

ITEM (V-C)--This crude aqueous solution contained salicin and small amounts of two sugars at R_f's 0.22 and 0.26. The DpNA spray indicated spots at the following R_f's: 0.09 (violet), 0.52 (violet), 0.66 (orange), 0.73 (purple),

and 0.77 (gray).

ITEM (VI-A)--This crystalline material appeared to be salireposide with a trace of salicyl alcohol.

ITEM (VI-B)--This ether extract contained gentisyl alcohol and several unidentified spots with the following R_f's as indicated by DpNA: 0.37 (violet), 0.51 (buff), 0.77 (violet and salmon), 0.83 (violet with gray center). The crystalline compound melting at 205-207° (Item VI-B-1) which separated from this solution has not been identified.

ITEM (VI-C)--This aqueous solution contained salireposide and unknown silver-reducing material from R_f 0.65 to R_f 0.85. The DpNA reagent indicated unidentified spots at the following R_f's: 0.67 (salmon) and 0.82 (salmon and pink).

ITEM (VI-D)--Chromatography of this crude material indicated only salireposide, but the melting point indicates considerable impurities.

Further work on individual fractions was held in abeyance pending further identifications of unknown compounds.

In another experiment the final concentrated aqueous residue from a similar hot water extraction of evaporated ethanol extract of fresh Populus tremuloides bark [Fraction (E) of Figure 2 of Project Report 1] was submitted to continuous extraction with ethyl acetate.

This concentrated Fraction (E) was covered with 250 ml. of water and agitated. Some crystalline material did not dissolve. This was filtered and recrystallized from water. The aqueous filtrate was placed in a non-agitated liquid-liquid extractor and extracted continuously with ethyl

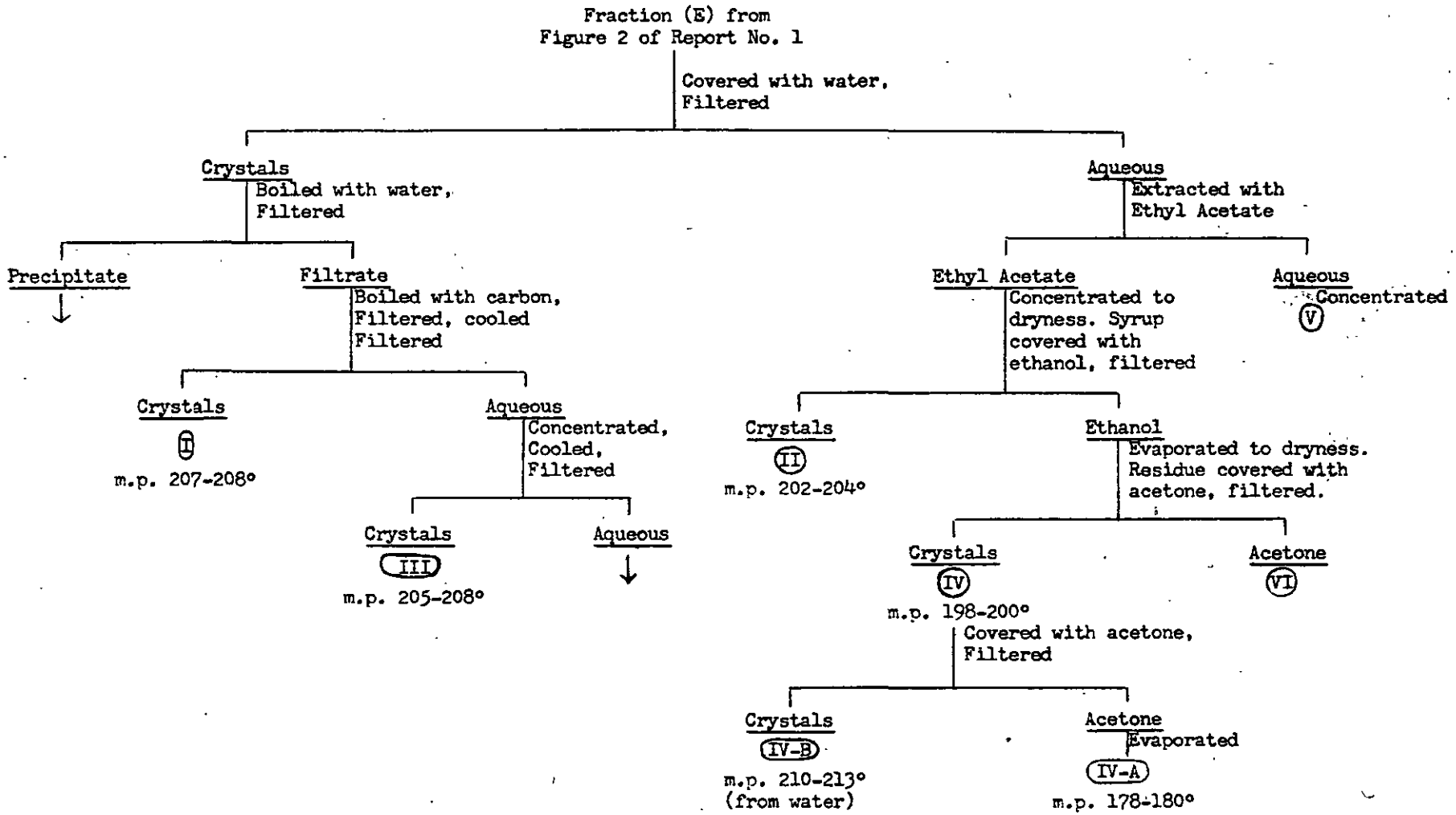
acetate. The fractionation of this experiment is pictured in Figure III in which all fractions are identified.

Paper chromatography and mixed melting points indicate that Items I, II, and III are tremuloidin. Other fractions are impure mixtures, and identification of individual components was not pursued further at this time.

The two experiments reported herein indicate that tremuloidin and salireposide can be obtained from Populus tremuloides bark extracts by extraction with ethyl acetate and ether-ethanol even when they are not obtainable by crystallization procedures described in earlier reports.

Figure 3

Ethyl Acetate Fractionation of Aqueous Residue after Glucoside Isolation from Hot Water Extract of Populus tremuloides Bark Extractives



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Dr. Pearl (2)
Mr. Beyer

PROJECT NO. 1932
COOPERATOR Institute
REPORT NO. 7
DATE January 10, 1957
NOTE BOOK 1555
PAGE 134-144 TO 146-147
SIGNED Donald L. Beyer

THE ETHER EXTRACTION AND CAUSTIC HYDROLYSIS OF WHITE WATER FROM ASPEN

In connection with the work previously reported concerning the presence of p-hydroxybenzoic acid in the Populus species, it was decided to include several experiments involving groundwood white water in order to see if the p-hydroxybenzoic acid is present.

A sample of 10.7 liters of white water was obtained from the groundwood pit of Kimberly-Clark at Kimberly, Wisconsin on November 26, 1956. The sample was given to us by Mr. George Saunders.

Mr. Saunders informed us that the white water had been filtered. Also approximately 1200-1300 grams of O. D. pulp was filtered from the water. The wood being ground was mixed poplar including Populus tremuloides, Populus grandidentata, and Populus tacamahaca.

A solids determination was run on the white water sample and was found to be 0.13 gram/100 grams of water. The entire sample contains 14 grams of solids. The white water sample was concentrated in the laboratory circulating evaporatory to a volume of 760 cc.

The concentrated white water was divided into two equal parts. The one fraction was treated with several cubic centimeters of sulfuric acid, to be certain that the solution was acidic, and was then extracted continuously with ether using the air-agitated extractor. The extraction

continued for 24 hours, after which all the ether used was dried and concentrated to a volume of 100 cc., 74 grams. The solution, which carries our number 1555-135-A, has a total solids of 0.98 gram/100 grams. The total extract is 0.73 grams or 10.4% of the solids of the white water sample.

The second fraction was treated with 20 grams of sodium hydroxide. The solution was boiled under reflux for 8 hours. The solution was then acidified with dilute sulfuric acid and extracted continuously with ether for 24 hours. The ether concentrated to a volume of 100 cc., 75 grams. This extract, which carries our number 1555-136-A, had a total solids of 3.27 grams/100 grams. The total extract amounted to 2.45 grams or 35.0% of the white water solids.

The two extracts were chromatographed using various developers, with the following results.

Developed with butanol 2% aqueous ammonia.

1555-135-A Rf - 0.00; 0.08; 0.10; 0.12; 0.14; 0.25; 0.31; 0.78; 0.84

1555-136-A Rf - 0.00; 0.08; 0.10; 0.12; 0.14; 0.25; 0.31; 0.78; 0.84; 0.88

Developed with butanol, pyridine, water (10:3:3)

1555-135-A Rf - 0 0.37; 0.44; 0.56; 0.82; 0.85

1555-136-A Rf - 0.04; 0.18; 0.37; 0.44; 0.56; 0.82; 0.85

Developed with benzene saturated with formic acid.

1555-135-A Rv - 0.00; 0.02; 0.10; 0.27; 0.41; 0.77; 1.00

1555-136-A Rv - 0.00; 0.02; 0.27; 0.37; 0.41; 0.77; 1.00

Rv 0.27 is p-Hydroxybenzoic acid.

Rv 0.37 is Coumaric acid.

Rv 0.41 is believed to be dihydrocourmaric acid.

Rv 0.77 is syringic acid.

Rv 1.00 is vanillic acid.

No known aldehydes were found in either extract.

A sample of the extract of 1555-135-A was chromatographed using benzene saturated with formic acid as the developer. The compound Rv 0.41 was eluted from the strip of paper with 95% ethanol and treated with alcoholic potassium hydroxide. The ultra violet curve for the unknown was run along with an ultra violet curve for known dihydrocourmaric acid. The curves matched sufficiently well to establish the identity of the unknown as dihydrocoumaric acid. The two curves will follow.

The five known acids were determined quantitatively by the chromatographic and spectrophometric method that has been mentioned frequently in past reports. The sample was chromatographed, the strips were cut and eluted with 95% ethanol, treated with alcoholic potassium hydroxide, diluted to 50 ml. volume, and read at the proper wave length on the spectrophotometer. The results of these determinations follow:

	1555-135-A		1555-136-A	
	mg.	% of ext.	mg.	% of ext.
p-Hydroxybenzoic acid	2.5	0.34	32.5	1.3
Coumaric acid	--	--	66.0	2.7
Dihydrocoumaric acid	64.5	8.80	47.0	1.9
Syringic acid	7.3	1.00	23.5	1.0
Vanillic acid	10.8	1.50	23.5	1.0

In order to learn what the source of dihydrocoumaric acid might be, a 100 grams sample of O. D. Populus tremuloides was extracted in a soxhlet with water for a period of 24 hours. The water solution was then concentrated to a small volume and extracted with ether continuously for 24 hours. The ether solution was concentrated to a volume of 100 cc., 73 grams. The total solids were 0.43 gram/100 grams and the extract amounted to 0.3 gram. The extract was chromatographed and only p-hydroxybenzoic acid was identifiable.

Chromatographic results were:

Developed with butanol 2% aqueous ammonia. Rf - 0.00; 0.10; 0.79

Developed with 10:3:3 butanol, pyridine, water. Rf - 0.03; 0.20; 0.24; 0.56; 0.80.

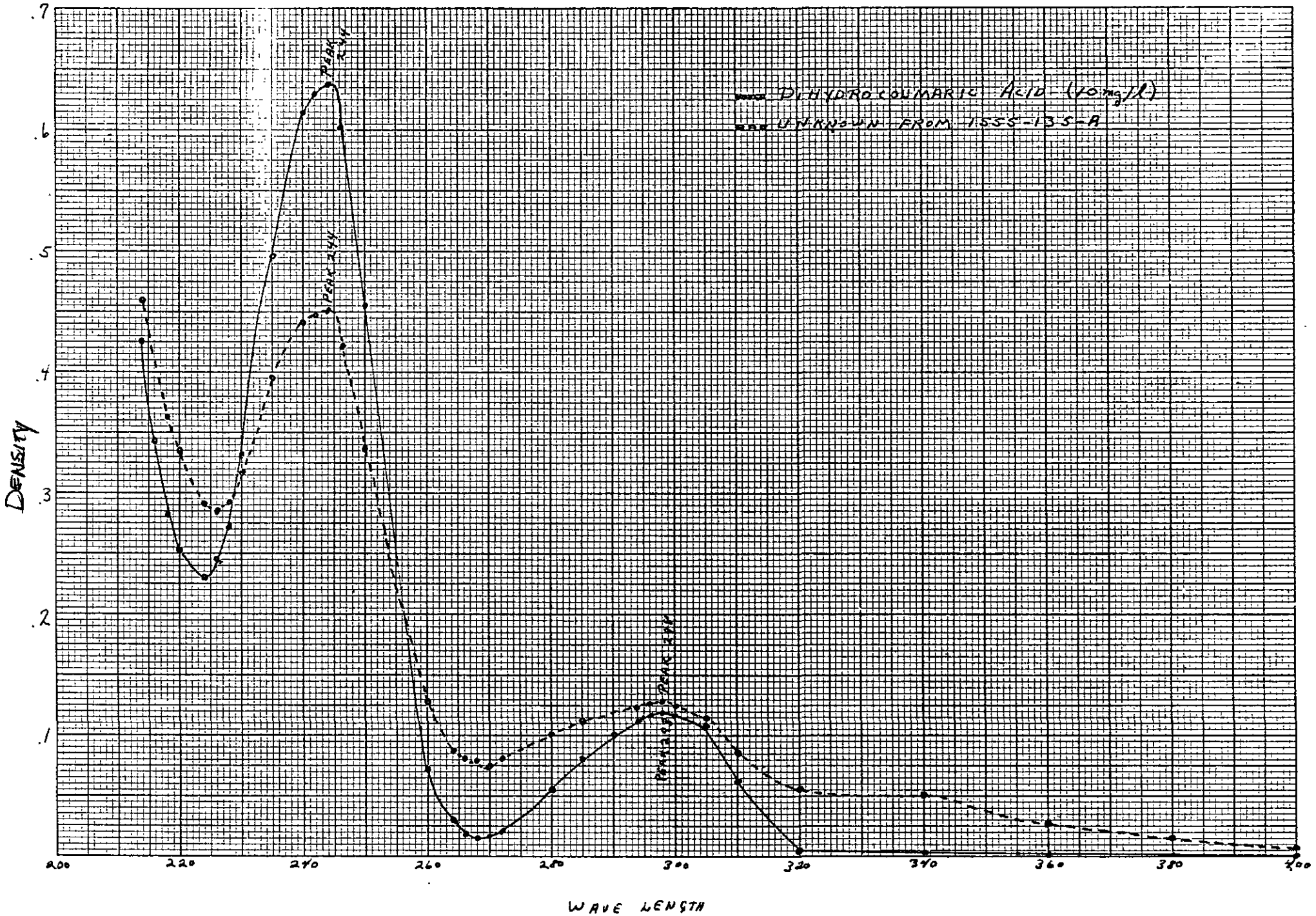
Developed with benzene saturated with formic acid. Rv - 0.00; 0.27.

p-Hydroxybenzoic acid has Rf 0.10 in butanol, 0.56 in pyridine, and Rv 0.27 in benzene-formic acid.

The p-hydroxybenzoic acid has determined quantitatively and 2.0 mg. were found in the extract, this is only 0.7% of the extract.

In none of the work done on Populus tremuloides has any dihydrocourmaric acid been found. From the data known I feel certain that the presence of dihydrocoumaric acid is not due to the Populus tremuloides. Whether the acid is present in the other two woods making up the mixed aspen used in the Kimberly mill is not known. Neither the Populus granditentata nor the Populus tacamahaca have been worked on. The possibility also remains that some other species of wood might have been in the supply used at the time the sample was taken. A duplication of the above results should be had from another sample of white water before any definite conclusions can be made.

dlb/mv


DIHYDROCOUMARIC ACID (10 mg/l)
UNKNOWN FROM 1555-135-A
PEAK 244
PEAK 244
PEAK 294
DENSITY
.7
.6
.5
.4
.3
.2
.1
200
220
240
260
280
300
320
340
360
380
400
WAVE LENGTH

PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR IPC
REPORT NO. 7
DATE April 10, 1961
NOTE BOOK 1834
PAGE 67-69 ~~and~~ and 116-130
SIGNED Irwin A. Pearl
Irwin A. Pearl

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STUDIES ON THE GLUCOSIDES OF POPULUS GRANDIDENTA BARK

In Report No. 2 preliminary studies on the bark of bigtooth aspen (Populus grandidentata), and on page 6 of that report, the preparation of an ethanol extract of brown bark and the parparation of smooth green bark dust were described. The present report describes further experiments with these materials.

The concentrated ethanol extract of the brown bark of Populus grandidentata described on page 6 of Report No. 2 was evaporated to dryness in a rotating evaporator under diminished pressure to leave 274 grams of residue containing considerable oily material. The dark brown mixture was covered with 4 liters of water, heated one hour on the steam bath with occasional shaking, and allowed to stand at room temperature overnight. The mixture was filtered through Celite, and the residue was boiled with 3 liters of water, cooled, and filtered through Celite. The preliminary fractionation of this material is pictured in Figures 1, 2, and 3 in which all fractions are identified.

PRELIMINARY CHROMATOGRAPHY OF FRACTIONS

Several of the fractions identified in Figures 1, 2, and 3 were chromatographed in the 10:3:3 butanol-pyridine-water (BPW) system in duplicate. The chromatograms were sprayed with the modified silver (Ag) and diazotized para-nitroaniline (DpNA) reagents. Chromatograms sprayed

with DpNA were examined under ultraviolet light for fluorescence. Results are given below.

ITEM (B-1)--This white crystalline material melting at 199-201° gave a pale violet spot at R_f 0.83 which fluoresced blue-white in ultraviolet light. The silver spray gave no spot.

ITEM (C-II)--This ether solution was mixture of many compounds giving colors with DpNA R_f's as follows: 0.52 (violet), 0.67 (violet), 0.83 (violet), 0.88 (pink), and 0.91 (orange). Those at 0.67 and 0.83 fluoresced under ultraviolet light. The Ag spray gave only a faint spot at R_f 0.83.

ITEM (D-2)--This ethyl acetate solution was a mixture of many compounds giving colors with DpNA R_f's as follows: 0.39 (blue), 0.56 (violet), 0.63 (purple), 0.76 (yellow), 0.83 (violet), and 0.90 (orange). The spot at 0.56 fluoresced white, and the spot at 0.76 fluoresced very strong blue-white under ultraviolet light. The Ag spray indicated spots at R_f's 0.56, 0.76, and 0.83.

ITEM (E-1)--This white crystalline solid melting at 196-197° gives no spot whatsoever with DpNA and gave a single spot for salicin with the Ag spray.

ITEM (E-2)--This tetrahydrofuran solution of the materials extracted by the second ethyl acetate extraction was a mixture of materials giving colored spots with DpNA with R_f's as follows: 0.52 (pink), 0.62 (violet), 0.67 (violet), and 0.83 (violet). Spots at R_f's 0.62 and 0.70 fluoresced strong blue-white under ultraviolet light. Spots were located at the following R_f's with the Ag spray: 0.20, 0.23, 0.63, 0.67, and 0.70.

CRAIG MACHINE FRACTIONATION OF FRACTION (C-I)

The main ethyl acetate extract after removal of ether-soluble material and concentration to 120 ml. [Fraction C-I] was placed in the first three tubes of the large automatic Craig counter-current distribution machine. The other tubes were filled with water which had been saturated with ethyl acetate. The upper phase for the counter-current distribution was ethyl acetate which had been saturated with water. After 100 transfers at 20°, the experiment was monitored by chromatographing every fifth tube on paper in the 8:2:1 ethyl acetate-pyridine-water (EPW) developer and spraying the chromatograms with both DpNA and Ag. Results for the DpNA spray are given in Table I and for the Ag spray are given in Table II.

[Table I and II]

On the basis of the diazotized para-nitroaniline-sprayed chromatograms, the tubes were combined into fractions as noted in Table III. The combined fractions were evaporated to small volumes in a rotating evaporator. Solids determinations were made on the concentrated solutions, and the yields are included in Table III. After concentration, Fractions C-I-E and C-I-F deposited crystalline material. These crystalline solids were filtered and weighed and are included in Table III as Fractions C-I-E-1 and C-I-F-1, respectively.

TABLE I

CHROMATOGRAPHY OF CRAIG MACHINE FRACTIONATION IN ETHYL ACETATE-PYRIDINE-WATER AND SPRAYING WITH DIAZOTIZED PARA-NITROANILINE

Tube	R_f[a]
1	0.34, 0.46, 0.55
5	0.34, 0.46, 0.55
10	0.46, 0.55, 0.61
15	0.55, 0.61
20	0.61
25	0.61
30	0.61
35	0.76
40	0.76
45	0.76
50	0.76, 0.81
55	0.76, 0.81
60	0.76, 0.81
65	0.76, 0.81
70	0.76, 0.81, 0.88
75	0.81, 0.88
80	0.81, 0.88
85	0.81, 0.88
90	
95	

[a] The color of the spots at the various R_f values were as follows: 0.34, pink; 0.46, violet; 0.55, red; 0.61, violet; 0.76, light violet; 0.81, light tan; and 0.88, light tan.

TABLE II

CHROMATOGRAPHY OF CRAIG MACHINE FRACTIONATION IN ETHYL ACETATE-PYRIDINE-WATER AND SPRAYING WITH MODIFIED SILVER REAGENT

Tube	R_f
1	0.07, 0.12, 0.34, 0.46, 0.55
5	0.07, 0.12, 0.34, 0.46, 0.55, 0.61
10	0.46, 0.55, 0.61
15	0.61
20	0.61
25	0.61
30	0.61
35	0.76
40	0.76
45	0.76
50	0.76
55	0.76, 0.81
60	0.76, 0.81
65	0.76, 0.81
70	0.76, 0.81, 0.88
75	
80	
85	
90	
95	

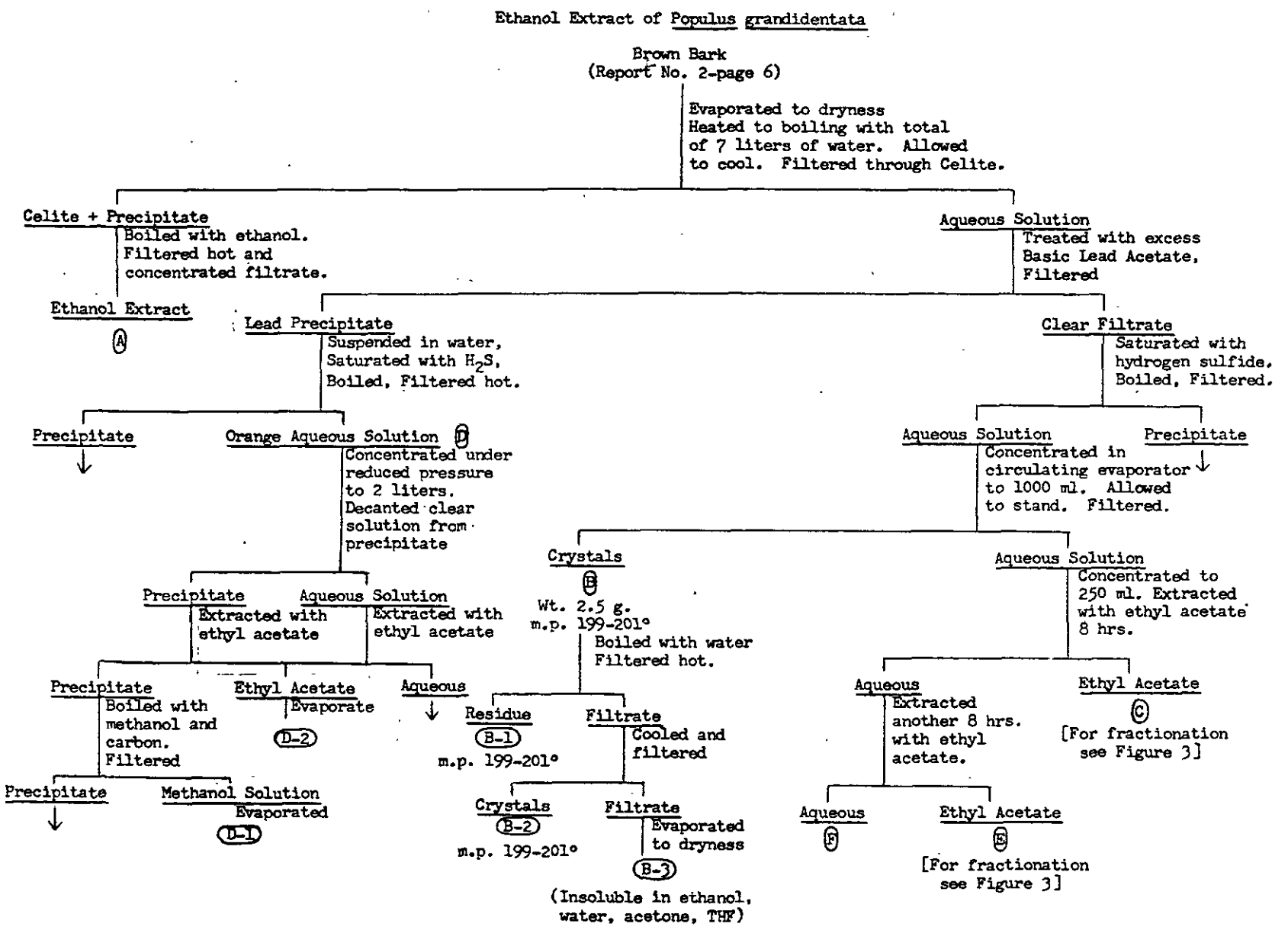

Figure 1

Preliminary Fractionation of Hot Water Extractives of Populus grandidentata
Brown Bark Ethanol Extract

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Page 6

Figure 2

Fractionation of First Ethyl Acetate Extract (Fraction C of Figure 1)

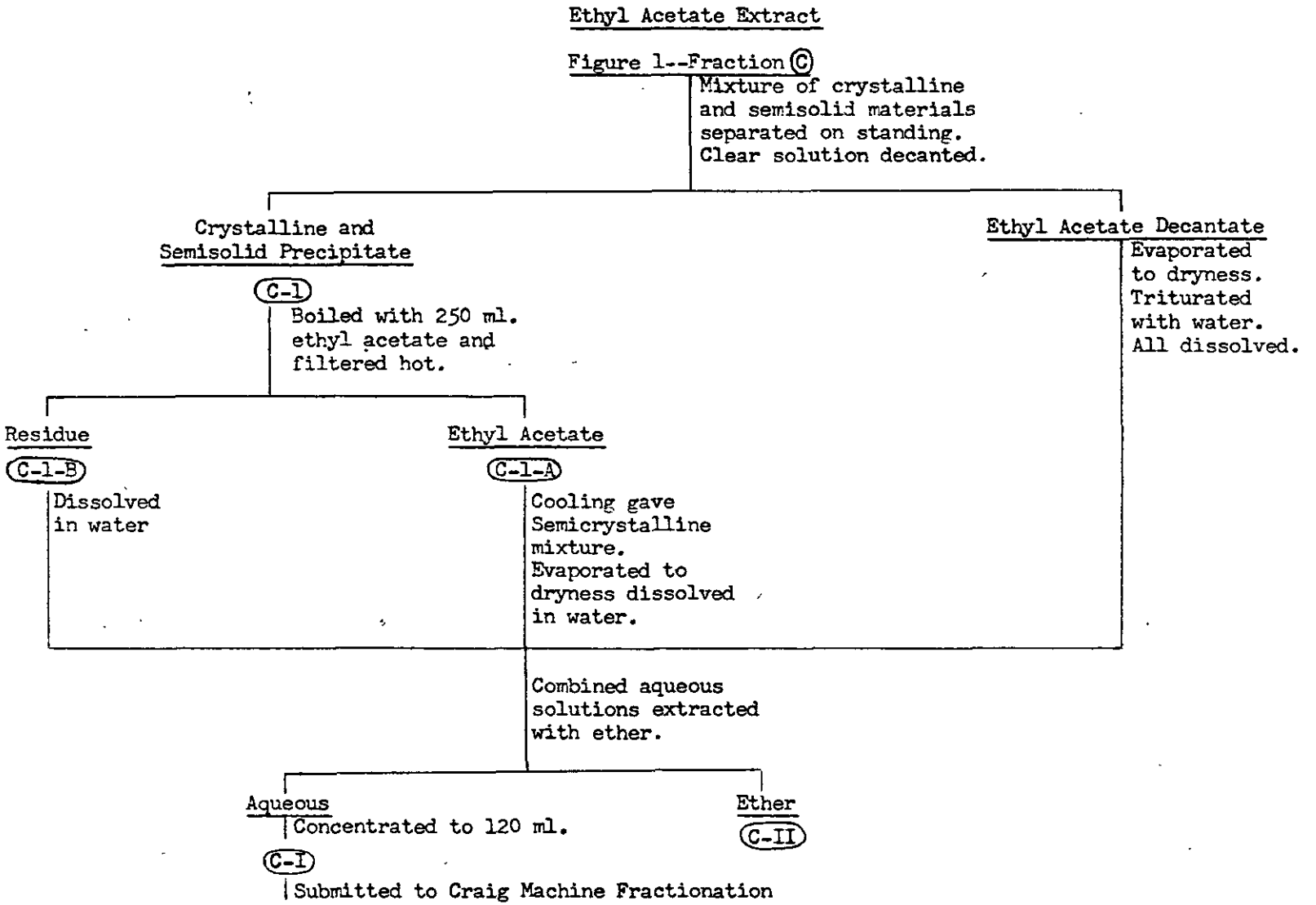


Figure 3

Fractionation of Second Ethyl Acetate Extract (Fraction E of Figure 1)

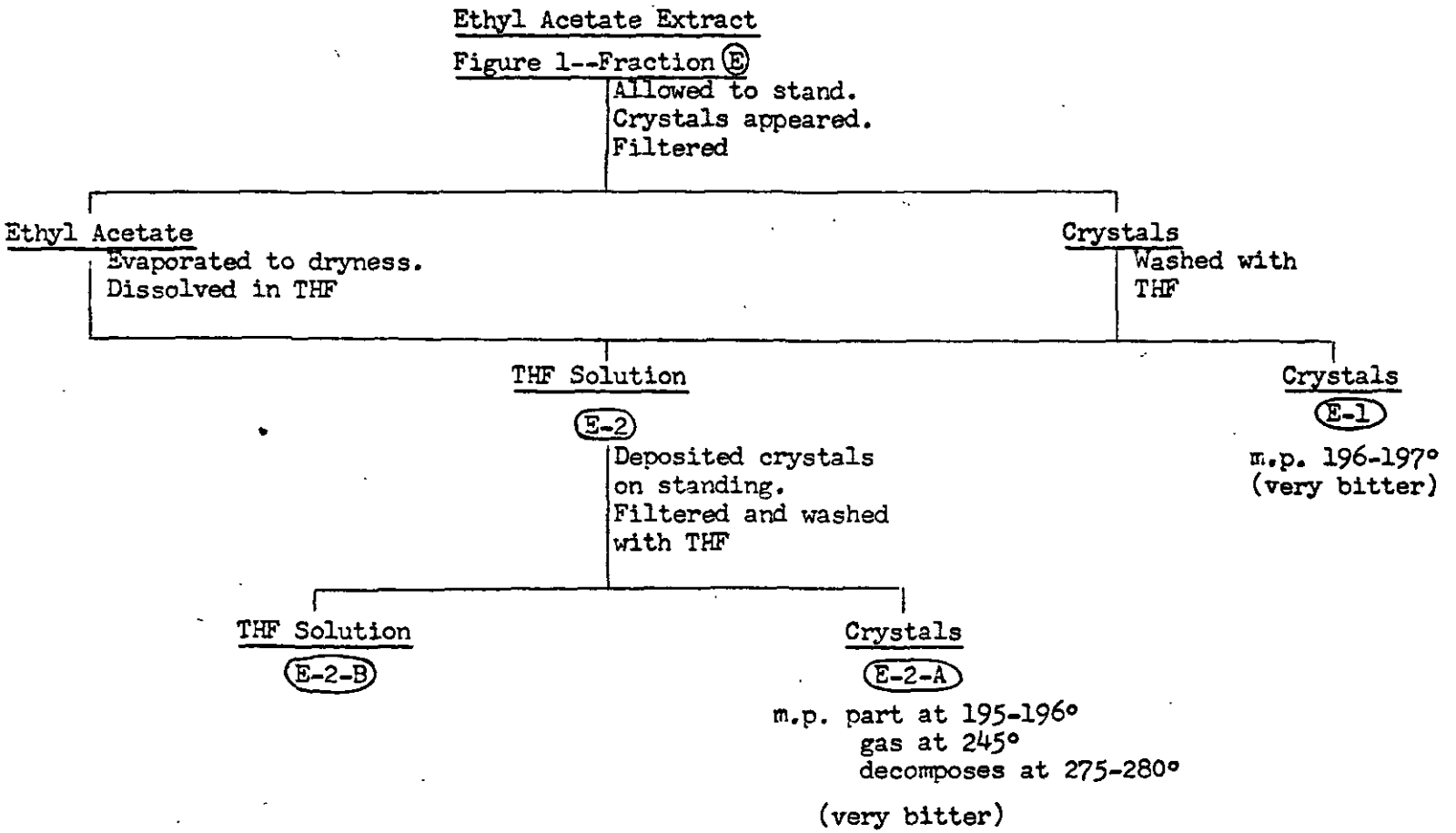


TABLE III

PRELIMINARY CRAIG MACHINE FRACTIONATION OF FRACTION (C-1)

Fraction	Tubes	Yield, g.
C-I-A	0 - 7	9.26
C-I-B	8 - 18	4.23
C-I-C	19 - 32	2.05
C-I-D	33 - 47	1.66
C-I-E	48 - 68	3.30
C-I-E-1[a]	48 - 68	2.00
C-I-F	69 - 88	1.31
C-I-F-1[b]	69 - 88	1.40
C-I-G	89 - 99	0.12
		25.33

Further fractionations of individual fractions from the Craig Machine are pictured in Figures 4 through 10. These figures identify all fractions.

Preliminary Chromatography of Individual Fractions

Individual fractions noted in Figures 4 through 10 were subjected to preliminary chromatographic evaluation in the BPW system. Chromatograms were sprayed with the DpNA and Ag reagents. Salicin and salireposide were employed as reference materials. Results are noted in Table IV.

---

[a] Crystalline product separated from fraction C-I-E. Crystals shrunk at 138-145° and finally melted at 200-220°.

[b] Crystalline product separated from fraction C-I-F. Crystals melted at 190-193°.

TABLE IV

PAPER CHROMATOGRAPHY OF INDIVIDUAL FRACTIONS

Fraction	Comments
C-I-A-1	Ag spot for salicin.
C-I-A-2	DpNA spots at R_f 0.47 (pink with purple tail) and 0.83 (pink).
C-I-A-3-a	Ag spot for salicin.
C-I-A-3-b	Ag spot for salicin and at R_f 0.11. DpNA spot at 0.48 (purple).
C-I-B-1	Ag spot at R_f 0.00.
C-I-B-2	DpNA spots at R_f 0.47 (pink with purple tail) and 0.83 (pink).
C-I-B-3	Ag spot at R_f 0.11 and very strong at R_f 0.58. Very strong purple DpNA spot at R_f 0.58.
C-I-C-1	Ag spot at R_f 0.11.
C-I-C-2	Ag spot at R_f 0.58. Strong purple DpNA spot at R_f 0.58.
C-I-D-1	Nothing.
C-I-D-2	DpNA gave long brownish lavender spot with center at R_f 0.77.
C-I-E-1-a	Strong salireposide spot with DpNA.
C-I-E-1-b	Salireposide spot with DpNA.
C-I-E-1-c	DpNA gave strong purple salireposide spot and a weak pink spot at R_f 0.79.
C-I-E-1-d	DpNA gave strong purple salireposide spot and a weak pink spot at R_f 0.79.
C-I-E-1-e	DpNa gave strong purple salireposide spot.
C-I-E-1-f	DpNa gave purple salireposide spot.

While the above work was in progress on the fractionation of materials from the brown bark of Populus grandidentata other similar work was in progress on the hot water extractives of the green bark of the same tree. The latter work is reported in Progress Report 8 and reports the isolation of a new glucoside from this bark. Further experiments on

individual fractions identified in the present report were held in abeyance pending the results obtained on the green bark extractives.

Figure 4

Fractionation of Craig Machine Fraction C-I-A

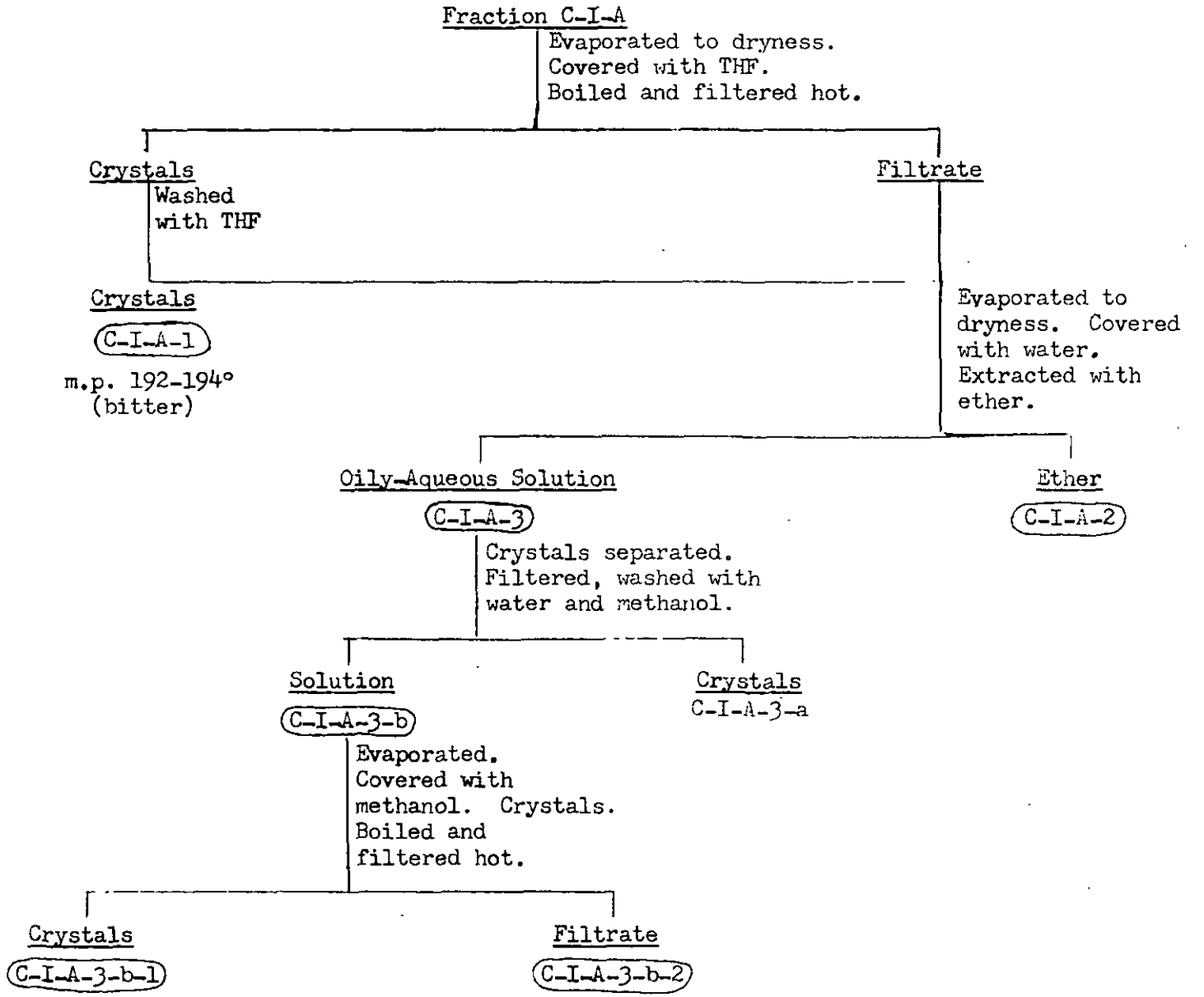


Figure 5

Fractionation of Craig Machine Fraction C-I-B

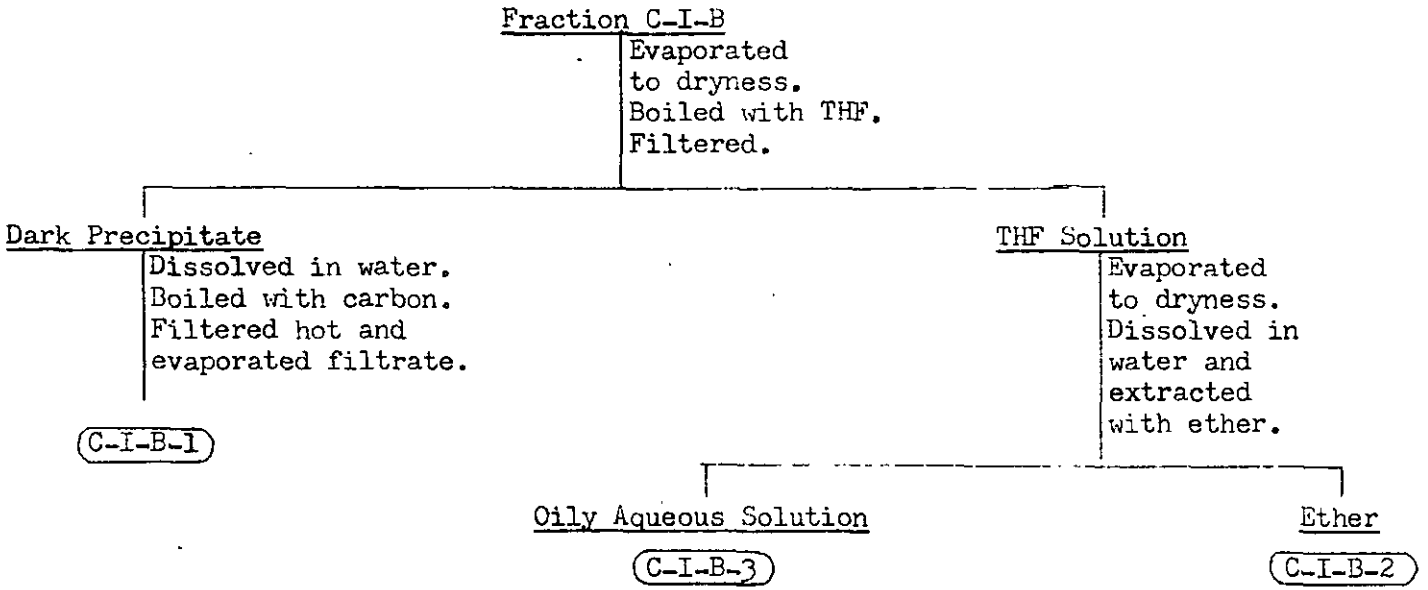


Figure 6

Fractionation of Craig Machine Fraction C-I-C

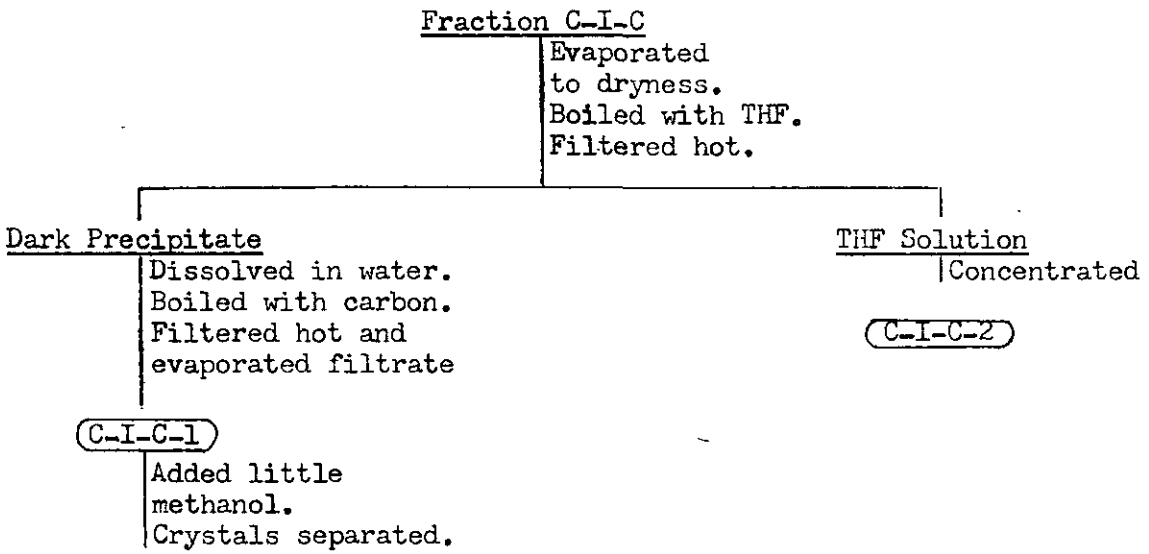


Figure 7

Fractionation of Craig Machine Fraction C-I-D

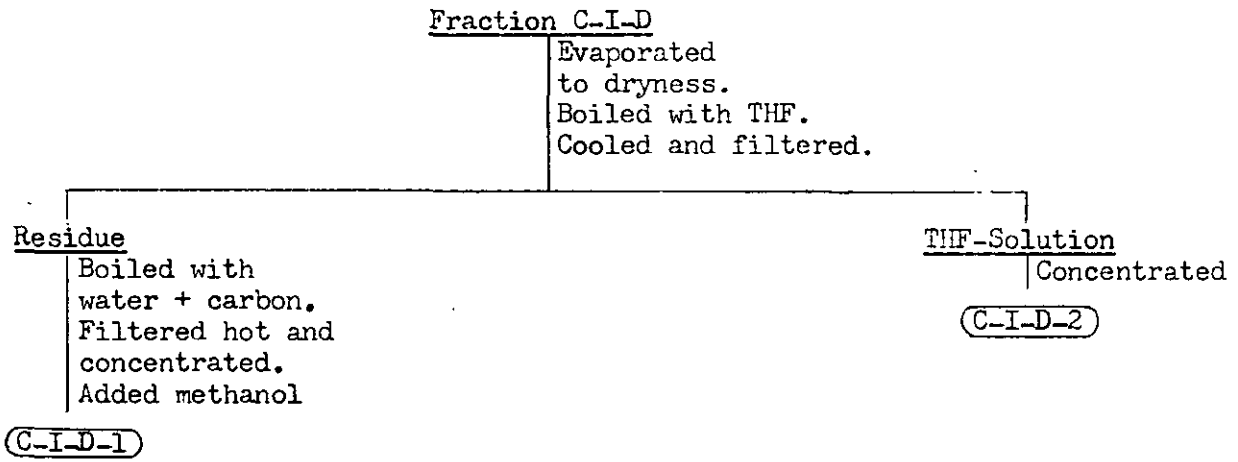


Figure 8

Fractionation of Craig Machine Fraction C-I-E

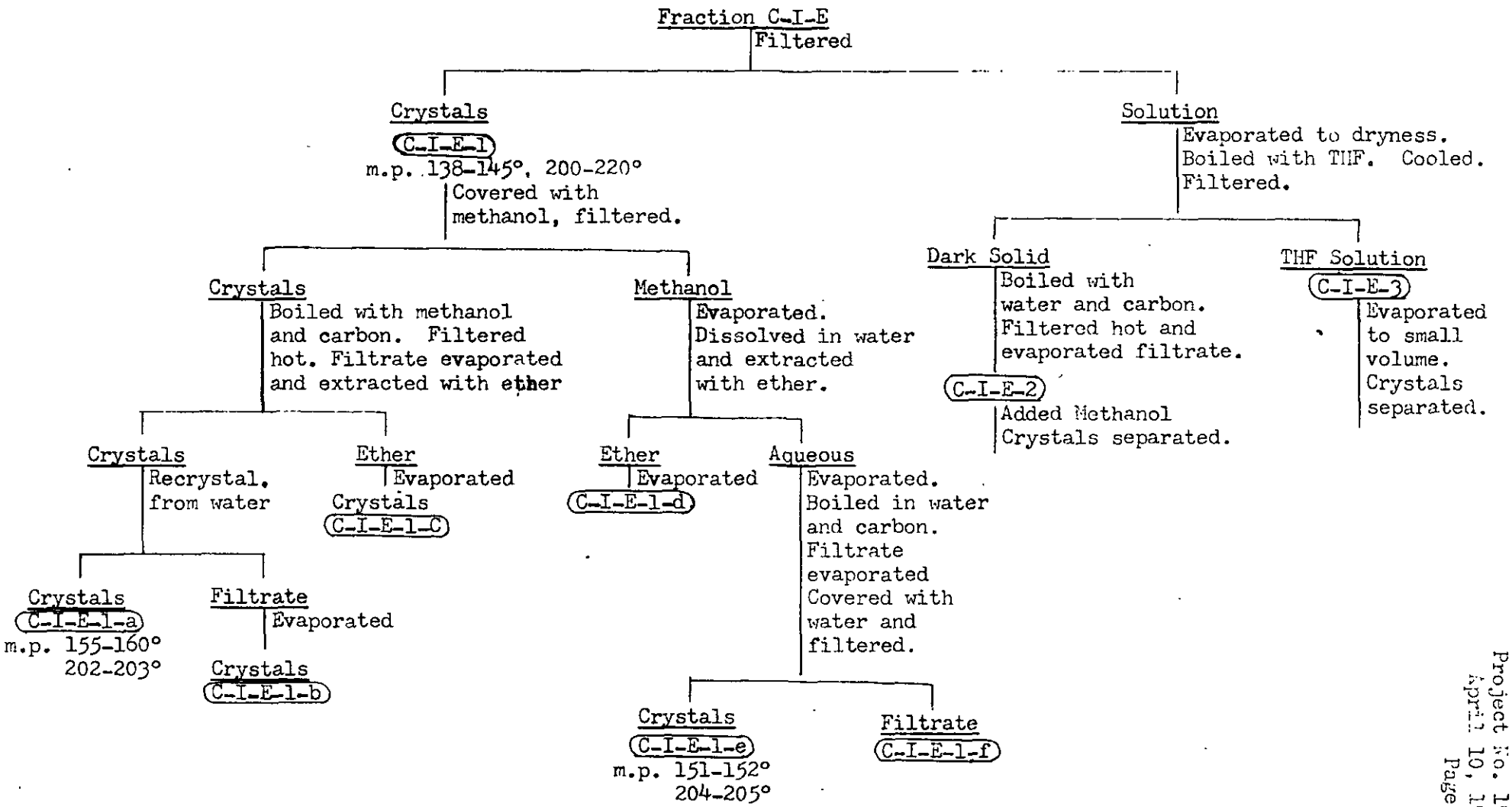


Figure 9

Fractionation of Craig Machine Fraction C-I-F

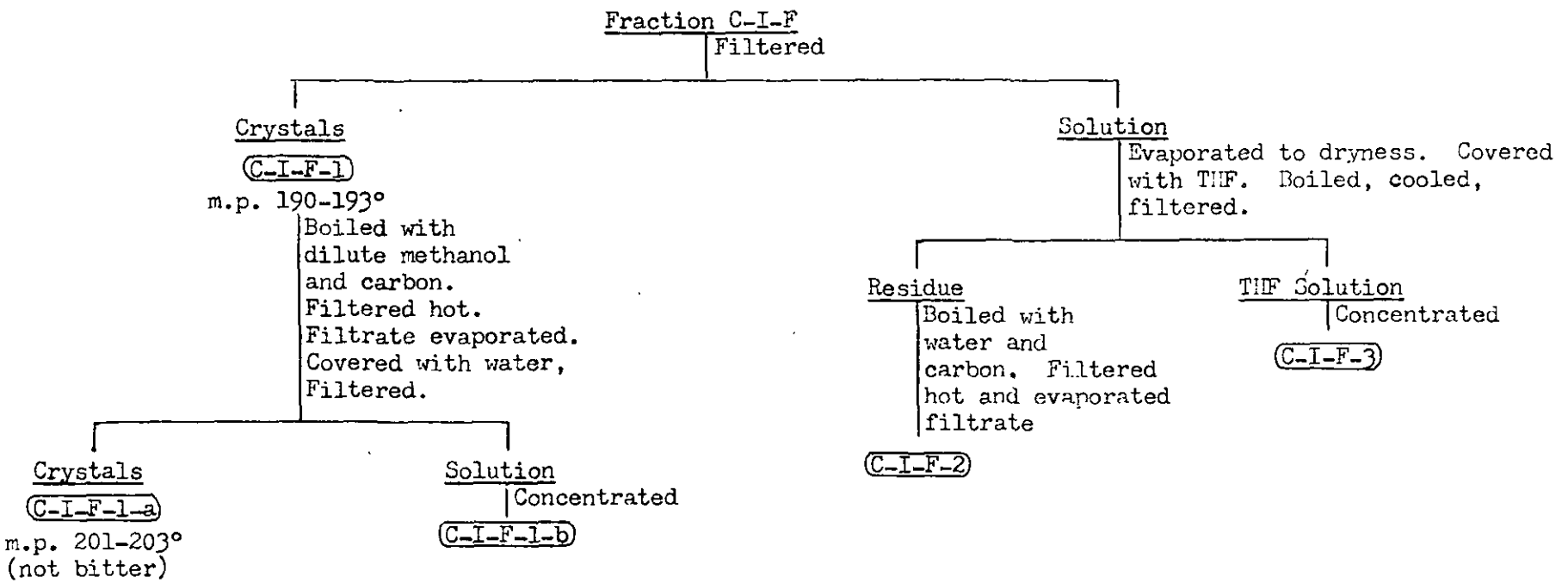
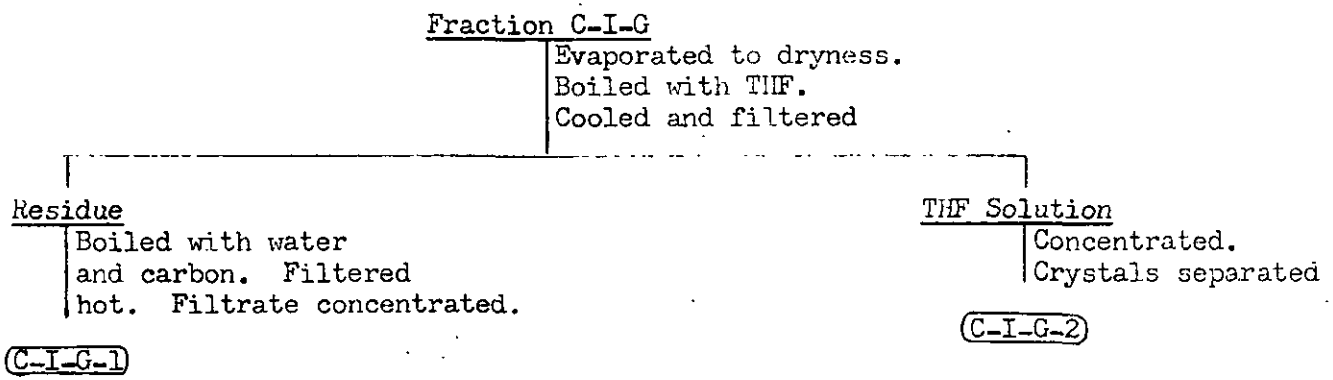


Figure 10

Fractionation of Craig Machine Fraction C-I-G



PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR Institute
REPORT NO. 8
DATE March 6, 1957
NOTE BOOK 1555
PAGE 148 TO 159
SIGNED Donald L. Beyer

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THE CAUSTIC HYDROLYSIS AND THE PROPANOL EXTRACTION OF VARIOUS HARDWOODS. III.

(ADDITIONAL DATA AND CORRECTIONS OF PREVIOUS REPORTS).

In Project 1932, reports numbers 4 and 6 described the experimental work and reported the data accumulated from the caustic hydrolysis of forty-six different hardwoods. Since that time, it has been found that p-hydroxybenzaldehyde is present in some of the extracts. Also coumaric acid wasn't found until we were nearing the end of the series and consequently all the extracts were not checked for it. In this report, these two materials are quantitatively determined in the extracts containing them.

In the past reports, the developer used for the separation of the aldehydes was n-butyl ether saturated with water. This developer very satisfactorily separated vanillin and syringaldehyde, however, p-hydroxybenzaldehyde moves identically as does vanillin. Since this is the case, all of the vanillin results from any extract containing p-hydroxybenzaldehyde are incorrect. To accomplish a complete separation of the aldehydes a new developer was used. This developer was heptane, n-butyl ether, water (6:1:1). This developer is a modification of the petroleum ether, n-butyl ether, water developer used by Stone and Blundell.

All of the forty-six extracts were chromatographed in both the heptane, n-butyl ether and water developer and also in the benzene saturated

with formic acid developer. Those extracts containing either of the compounds that we were looking for, were removed and brought up to a volume of 100 ml. Since a considerable period of time had elapsed since the extracts were obtained, and since the extracts were ether solutions, the concentrations had no doubt changed. To account for any changes and to avoid errors, the extracts were diluted to 100 ml. and weighed. A total solid determination was run on each extract and the total solids present in each extract was calculated. The quantitative determinations of p-hydroxybenzaldehyde, vanillin, and coumaric acid were run on this solution and the milligrams of material present in the extract was calculated. Since the total solids present in the original extract is known, it becomes an easy task to convert the data to include the entire original extract. The data reported here will be based on the total original extract.

The following extracts contained either p-hydroxybenzaldehyde or coumaric acid or both. This data should be incorporated with that from report no. 4 and no. 6.

It is interesting to note that p-hydroxybenzaldehyde was found only in woods which also contained coumaric acid. It is possible that enzymatic oxidation of coumaric acid is responsible for the formation of p-hydroxybenzaldehyde.

dlb/jw

ADDITIONS AND CORRECTIONS FOR THE CAUSTIC HYDROLYSIS OF HARDWOODS.

Wood		Code no.	Vanillin mg.	Vanillin %	p-Hydroxybenzaldehyde mg.	p-Hydroxybenzaldehyde %	Coumaric Acid mg.	Coumaric Acid %
Quaking Aspen	Populus tremuloides	1359-95-B	44.1	1.9	22.9	1.0	54.2	2.4
Red Alder	Alnus rubra	1359-129-A	---	---	---	---	15.7	0.7
Sweet Gum	Liquidamber styraciflua	1527-21-A	---	---	---	---	12.5	1.4
Cottonwood	Populus deltoides	1527-54-A	---	---	---	---	11.3	0.5
Black Ash	Fraxinus nigra	1527-93-A	---	---	---	---	23.6	1.4
White Basswood	Tilia heterophylla	1527-129-A	19.9	1.3	8.6	0.6	33.5	2.2
European Poplar	Populus tremula	1555-36-A	22.5	1.8	21.8	1.7	33.5	2.6
Diamond Willow	Salix eriocephala	1555-54-A	18.9	1.5	20.0	1.6	38.0	2.9

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102-105, 109

PROJECT NO. 1932
COOPERATOR Institute of Paper Chemistry
REPORT NO. 8
DATE April 18, 1961
NOTE BOOK 1895
PAGE 0 TO
SIGNED Irwin A. Pearl

Irwin A. Pearl

FURTHER STUDIES ON THE GLUCOSIDES OF POPULUS GRANDIDENTATA

In Report No. 7 studies were reported on the brown bark extractives of a Populus grandidentata tree which had been described on page 6 of Report No. 2. The present report describes similar experiments on the greenish smooth bark obtained from the same tree. The experiments are concerned with the hot water extractives and in particular, with the glucoside content of these extractives.

LARGE SCALE HOT WATER EXTRACTION OF POPULUS GRANDIDENTATA GREEN BARK

Three thousand grams of the air-dried Wiley milled Populus grandidentata green smooth bark described on page 6 of Report No. 2, containing 2750 grams of oven dry bark solids was covered with 11 liters of water and heated with stirring almost to boiling for 2 hours. The mixture was filtered through cloth and squeezed as dry as possible to yield 6 liters of filtrate. The residue was boiled in two equal batches, each with 8 liters of water, and both batches were filtered again and squeezed dry to yield a total of 17 liters of filtrate making a combined total of 23 liters of filtered hot water extract. The residual bark dust was air dried to give 2557 grams of extracted bark dust containing 8.0% moisture or 2350 grams of oven dry solids.

Preliminary processing of the hot water extract of this experiment is pictured in the flow sheet of Figure 1. Further fractionation of the ethyl acetate extract is pictured in the flow sheet of Figure 2. All fractions are identified.

Preliminary Paper Chromatography of Fractions

Most of the fractions of Figures 1 and 2 were chromatographed on paper and developed in the 10:3:3 butanol-pyridine-water (BPW) developer. Chromatograms were sprayed with diazotized para-nitroaniline (DpNA) and modified silver (Ag) reagents. Unknown materials were compared with salicin, salireposide, and tremuloidin. In these studies salireposide gave a purple spot with DpNA and a silver spot at R_f 0.79; salicin gave only a silver spot at R_f 0.62; and tremulidin gave only a silver spot at R_f 0.86. Results of the chromatograms are given below.

Fraction (A-1) -- This ether solution gave spots with Ag at 0.47, 0.80, 0.85, and 0.93. Spots with DpNA were obtained at 0.38 (purple), 0.59 (violet), 0.70 (purple), 0.75 (pink), 0.78 (purple), and 0.86 (red).

Fraction (A-2-a) -- This crystalline fraction gave a violet spot with DpNA at 0.81 and an Ag spot at the same position. Did not appear to be salireposide.

Fraction (A-2-a-1) -- These crystals gave the same chromatogram as fraction (A-2-a). Did not appear to be salireposide.

Fraction (A-2-a-1-a) -- These crystals gave a purple spot with DpNA at 0.79 and a spot with Ag at the same R_f. Appeared to be identical with salireposide.

Fraction (A-2-a-2-a) -- These crystals gave a violet spot with DpNA at 0.83 and a spot with Ag at the same R_f. Quite different from salireposide.

Fraction (A-2-b-1) -- These crystals gave chromatograms identical with those of salireposide.

Fraction (A-2-b-2) -- This aqueous solution gave spots with Ag at 0.47, 0.62, 0.70, 0.79, 0.86, and 0.93. Spots with DpNA were obtained at 0.64 (violet), 0.71 (purple), 0.79 (purple), and 0.86 (red).

Fraction (A-2-b-2-I) -- This ether solution gave spots with Ag at 0.76 and 0.85. With DpNA, spots were obtained at 0.78 (purple) and 0.85 (violet).

Fraction (A-2-b-2-II) -- This ether solution gave spots with Ag at 0.61, 0.74, and 0.80, and with DpNA at 0.73 (purple) and 0.82 (violet).

Fraction (A-2-b-2-III) -- This ether solution gave a spot with Ag at 0.79 and a spot with DpNA at the same R_f (purple). Appeared to be the same as salireposide. A faint pink spot was obtained with DpNA at 0.82.

Fraction (A-2-b-a-IV) -- This ether solution gave spots with Ag at 0.62 and 0.73. DpNA gave spots at 0.64 (violet). 0.69 (purple), 0.77 (purple), and 0.82 (pink).

Fraction (A-2-b-2-V) -- This ethyl acetate solution gave spots with Ag at 0.57 and 0.69 and a trace spot at 0.42. Spots were obtained with DpNA at 0.55 (violet) and 0.66 (purple).

Fraction (A-2-b-2-VI) -- This concentrated aqueous solution gave spots with Ag at 0.16, 0.20, 0.42, and 0.57 with a trace spot at 0.69. Spots with DpNA were obtained at 0.48 (violet), 0.58 (purple), and 0.67 (violet).

Fraction (B) -- This ether extract gave spots with Ag at 0.47, 0.62, 0.70, and 0.75 and with DpNA at 0.62 (purple), 0.64 (purple), 0.69 (pink), 0.78 (purple), and 0.86 (pink).

Fraction (C) -- This aqueous solution gave spots with Ag at 0.03, 0.09, 0.17, 0.21, 0.46, and 0.61. With DpNA, spots were obtained at 0.51 (violet) and 0.62 (purple).

Fraction (C) was also spotted separately and developed along with known sugars in three different developing systems--8:2:1 ethyl acetate-pyridine-water; 9:2:2 ethyl acetate-acetic acid water; and 10:3:3 butanol-pyridine-water. Chromatograms were sprayed with the aniline hydrogen phthalate and p-anisidine sprays. The fraction showed strong spots for glucose and mannose and a weak spot for galactose along with oligo-saccharide material. No spots for either arabinose or xylose were obtained.

Thus, the above chromatograms indicated the presence of a great many possible glucosidic compounds unaccounted for by those already identified in the aqueous extracts of Populus species. The first such compound to be studies was the crystalline material melting between 200° and 206° comprising fractions (A-2-a), (A-2-a-1), (A-2-a-2), and (A-2-a-2-a).

Optical Rotation Studies on New Compound

Optical rotations were taken on three fractions in methanol at 30°. Results are shown in Table I.

TABLE I

OPTICAL ROTATIONS OF NEW COMPOUND IN METHANOL

Fraction	m.p., °C.	Concentration, %	Specific Rotation $[\alpha]_D$ at 30°
(A-2-a-1)	200-201	4.38	-64.6°
(A-2-a-2)	205-206	1.5	-61.5°
(A-2-a-2-a)	204-205	5.28	-66.3°

The optical rotation data of Table I are similar to those for the glucoside salicin, but the higher melting point and different chromatographic behavior of this new compound indicate that this must be a different material. Because it was found on the bark of Populus grandidentata it was named GRANDIDENTATIN.

CHEMICAL STUDIES ON GRANDIDENTATIN

The three fractions noted in Table I were combined and boiled with dilute methanol in the presence of decolorizing carbon. The hot solution was filtered, and the colorless filtrate was evaporated somewhat under reduced pressure to remove the ethanol. Colorless crystals separated. These were recrystallized by suspending in 10 ml. of water, heating to boiling, adding 6 ml. of ethanol to effect solution, and allowing to stand at room temperature over night. Colorless prisms separated. These were filtered and allowed to dry. They melted at 201-202°. Analysis by Huffman Microanalytical Laboratories indicated: Carbon, 56.87, 57.08; Hydrogen, 6.74, 6.86.

Several fractions reported in Report No. 7 on the extractives of the brown bark of Populus grandidentata appeared to be the new glucoside, grandidentatin. Specific rotations were determined on Fractions B-1 and B-2 of Figure 1 of Report No. 7. Specific rotations in methanol at 30° were as follows: (Figure 1, Report No. 7, B-1) $[\alpha]_D^{30}$ -60.0° (c = 4.45); and (Figure 1, Report No. 7, B-2) $[\alpha]_D^{30}$ -64.9° (c = 4.31).

These two fractions were combined and recrystallized from dilute ethanol in the presence of decolorizing carbon to give colorless crystals melting at 201-202° and not depressing a mixed melting point with the grandidentatin obtained from the present experiment. During the melting point determination of colorless clear crystals of grandidentatin, at about 140-150° the clear crystals became opaque without shrinking or melting. This phenomenon was noted earlier and indicated for Fraction A-2-a-2 in Figure 2.

Acetylation of Grandidentatin

A mixture of 0.3 gram of recrystallized grandidentatin, 3.0 ml.

of pyridine, and 2.5 ml. of acetic anhydride was allowed to stand in a stoppered flask at room temperature 16 hours. The mixture was poured in 50 ml. of cold water, and the oil which separated soon solidified. This solid was filtered, washed with water, and allowed to dry. The crude product weighed 0.42 grams and melted at 165-168°. The crude acetate was recrystallized from 5 ml. of absolute ethanol to give colorless needles melting at 167-169°. Analysis indicated: Carbon, 58.58, 58.79; Hydrogen, 5.85, 5.92.

The specific rotation of grandidentatin acetate was determined in chloroform solution at 30° and found to be $[\alpha]_D^{30}$ -8.37° (c = 3.64).

Molecular weight determinations by the micromethod of Rast and Pregel employing camphor indicated values of 651, 541, and 525 for grandidentatin acetate.

Hydrolysis of Grandidentatin with Acid

The first studies on the elucidation of the structure of grandidentatin were concerned with the products of acid hydrolysis. A suspension of 0.2 gram grandidentatin in 25 ml. of normal sulfuric acid was boiled under reflux for one hour. After 30 minutes of boiling, a clear colorless solution resulted. The clear solution was cooled to give a turbid solution with a phenolic odor. The mixture was extracted once with 25 mL.of ether, and the ether was washed with water, dried with sodium sulfate, and evaporated to dryness under reduced pressure to yield 0.023 gram of crystalline residue with a phenolic odor. Paper chromatography of the ether extract and the residual aqueous solution along with glucose and grandidentatin indicated that the hydrolysis was incomplete. Glucose was found in the hydrolyzate, but most of the original grandidentatin remained there, too. Accordingly, the

solution was boiled under reflux an additional 4 hours, extracted with ether as before, and chromatographed in the BPW developer. The chromatograms were sprayed with DpNA, Ag, p-anisidine, and Mäule reagents. The ether extract gave two spots with DpNA. These were a bright blue spot fading to gray at R_f 0.68 and a rose red spot at R_f 0.95. The silver spray indicated glucose in the aqueous hydrolyzate, and this was confirmed by the p-anisidine spray and by reduction of Fehling Solution.

Thus it appears that grandidentatin is a glucoside which yields glucose and two phenolic compounds upon hydrolysis.

The acid aqueous hydrolysis mixture after the several tests described above, was passed through a column of Duolite A-6 anion-exchange resin, and the column was washed thoroughly with water. The combined effluent and washings having a pH of 10.9 were treated with a drop of dilute sulfuric acid to drop the pH to 4.6 and concentrated to 65 ml. in a rotating evaporator. This solution was made to 100 ml. volume with absolute ethanol and spotted on paper for quantitative glucose determination by chromatography and spectrophotometry as described by Timell, Glaudemans, and Currie (1) and modified by Dubey and co-workers (2). The yield of glucose was 40 milligrams.

The bright blue spot fading to gray at R_f 0.68 given by the ether extract of the acid hydrolysis mixture was reminiscent of that obtained with p-coumaric acid. Accordingly, an attempt was made to isolate the material responsible for this spot. The first experiment along this line was an alkaline hydrolysis of grandidentatin by the procedure employed by Rabate (3) for the similar hydrolysis of salireposide.

Hydrolysis of Grandidentatin with Barium Hydroxide

A mixture of 0.4 grams grandidentatin and 8 ml. of saturated

barium hydroxide solution was diluted with 2 ml. of water and boiled under reflux for 5 minutes. As soon as the barium hydroxide solution came in contact with the grandidentatin a yellow solution resulted. A colorless precipitate began to separate, and the yellow color became much paler. After 5 minutes no further reaction appeared to take place so boiling was discontinued, and the reaction mixture was cooled and filtered with suction. The precipitate was washed with water and appeared to be a barium salt. The precipitate was suspended in 20 ml. of water and treated dropwise with 5% sulfuric acid until a slight excess was present as determined by Congo Red paper. At this point colorless crystals seemed to be mixed with precipitated barium sulfate. The mixture was extracted with ether, and the ether was evaporated to yield a crystalline solid melting at 214-216° with gas evolution. A mixed melting point with authentic p-coumaric acid melting at 215-216° was not depressed. Both samples gave identical blue spots when chromatographed and sprayed with DpNA, and both samples gave identical infrared absorption spectra. Therefore, the ether-soluble portion of the alkaline hydrolyzate of grandidentatin is p-coumaric acid, and grandidentatin must be a p-coumaroyl ester of a parent glucoside as yet unknown. The red spot originally found on the DpNA sprayed chromatogram at R_f 0.95 indicated that this parent glucoside contained a phenolic aglucone.

To get at the nature of this parent glucoside, the aqueous filtrate from the barium salt obtained above was carefully treated with just enough 5% sulfuric acid to completely remove the barium as barium sulfate. A pinch of barium carbonate was added to the mixture to insure the neutrality of the solution, and the barium sulfate and barium carbonate were removed by filtration. The clear, colorless filtrate which had a bitter taste was concentrated in a rotating vacuum evaporator. A syrup resulted, and this

syrup could not be induced to crystallize. Chromatography indicated more than one material present. The mixture was diluted with a little water, stirred with 2 grams of Amberilite IR-120 cation-exchange resin, and filtered. The resin was washed with water, and the filtrate was found to be free of barium. The filtrate was extracted with ether, and the ether was evaporated to give more crystalline p-coumaric acid melting at 213-214°. The aqueous solution was concentrated in a rotating vacuum evaporator to yield a colorless syrup which would not crystallize. This syrup of de-p-coumaroylated grandidentatin was chromatographed along with salicin in the 8:2:1 ethyl acetate-pyridine-water (EPW) developer, and the chromatograms were sprayed with the Ag and DpNA spray reagents. Like salicin, the de-p-coumaroylated grandidentatin gave no spot with DpNA indicating no free phenolic group. A spot was obtained with the Ag spray at R_f 0.65, while salicin gave a spot at R_f 0.76.

The hydrolysis of grandidentatin with barium hydroxide was repeated several times to obtain larger quantities of de-p-coumaroylated grandidentatin. All products obtained were syrups, and none would crystallize.

Acetylation of De-p-coumaroylated Grandidentatin

The colorless syrup of de-p-coumaroylated grandidentatin obtained above was covered with 3 ml. of pyridine and 2 ml. of acetic anhydride and allowed to stand in a stoppered flask for 16 hours. The mixture was poured into water and after a short time, colorless needles separated. These were filtered and washed with water to give a crude product melting at 120-121°. This was recrystallized from dilute ethanol to give fine glistening needles of de-p-coumaroylated grandidentatin acetate melting at 121-122°. Analysis indicated: Carbon, 53.90, 53.68; Hydrogen, 6.55, 6.41.

The specific rotation of de-p-coumaroylated grandidentatin acetate was determined in chloroform at 30° and found to be $[\alpha]_D^{30}$ -58.2° (c =2.2).

Molecular weight determinations were made on de-p-coumaroylated grandidentatin acetate by the Rast-Pregel micromethod with camphor. The molecular weight found was 480.

Enzymatic Hydrolyses of De-p-coumaroylated Grandidentatin

Enzymatic hydrolysis was attempted for the isolation of the aglucone of grandidentatin. The first experiment employed cellulose as the hydrolytic enzyme. A solution of 0.2 grams of grandidentatin in 100 ml. of water was obtained by boiling in distilled water and cooling to 30°. This solution was treated with 100 ml. of 0.01M sodium acetate solution buffered to pH 5.6 and containing 120 mg. of cellulose (obtained from Dr. Dillingham). The resulting mixture gave no test with Fehling solution. After standing overnight, a sample still gave no reducing test with Fehling solution. After four days, the mixture became slightly turbid, but no reducing test with Fehling solution could be obtained. After eight days and no test with Fehling solution, the mixture was filtered, and the filtrate was treated with 0.2 gram of emulsin (obtained from Mr. Dickey). After standing at room temperature for several days, the mixture gave a reducing test with Fehling solution indicating that hydrolysis had taken place. Accordingly, a fresh sample of de-p-coumaroylated grandidentatin was submitted to hydrolysis by emulsin.

Hydrolysis of De-p-coumaroylated Grandidentatin by Emulsin

In the first experiment with emulsin about 10 mg. of the syrupy

de-p-coumaroylated grandidentatin was dissolved in 5 ml. of water and treated with 0.1 gram of emulsin. After 1.5 hours the hydrolysis seemed to be complete as evidenced by the disappearance of the starting material on a chromatogram of the hydrolyzate and the appearance of glucose in its place. In addition a red spot with DpNA at BPW R_f 0.83 was obtained. After standing 16 hours the emulsin hydrolyzate was filtered and extracted twice with ether. The ether solution was dried with anhydrous sodium sulfate and allowed to evaporate to dryness. The residue consisted of a few mg. of a colorless crystalline material melting at 81-84° with a distinctive phenolic odor which gave a red spot when treated on paper with the DpNA spray reagent. This could be the aglucone of the de-p-coumaroylated grandidentatin, but further work proved this not to be the case.

Since the enzymatic hydrolysis with emulsin of the small sample appeared to have worked so well a larger sample of 0.35 gram was subjected to emulsin hydrolysis by solution in 15 ml. of water, treating with 0.2 gram of emulsin, and allowing to stand at room temperature. After four hours, the hydrolyzate showed a strong rose red spot on paper when sprayed with DpNA. The mixture was extracted with ether until the aqueous solution did not show the red spot with the DpNA reagent. The ether extract was dried with sodium sulfate and allowed to evaporate. A yield of 20 mg. of colorless material with a phenolic odor was obtained. This yield of 20 mg. was very small compared with the 100 to 150 mg. yield expected.

Hydrolysis of De-p-coumaroylated Grandidentatin by β-Glucosidase

Being suspicious that this phenolic material giving the red spot with DpNA might be a secondary product of the hydrolysis produced by the

action of an oxidase in the emulsin preparation rather than a primary hydrolytic product produced by the β-glucosidase present in the emulsin preparation, another enzymatic hydrolysis was carried out with pure β-glucosidase obtained from Nutritional Biochemicals Co. De-p-coumaroylated grandidentatin was prepared in the regular manner from 0.40 grams of grandidentatin by means of barium hydroxide hydrolysis. The syrupy product was dissolved in 100 ml. of 0.1 M sodium acetate solution buffered to pH 5 and treated with 0.1 gram of pure β-glucosidase. Even after two days the hydrolyzate failed to give a test for the phenolic compound which gives a red spot with DpNA spray even though all the original de-p-coumaroylated grandidentatin had disappeared, and a very strong test for glucose was obtained. The hydrolyzate was extracted with ether, but nothing was obtained. These facts seem to confirm the suspicion that the red spot material is a secondary product, and that the primary product of the hydrolysis other than glucose is a non-phenolic material not extracted by ether under the conditions of the experiment. These results are consistent with the infrared spectrum of the acetate of the de-p-coumaroylated grandidentatin in that this shows no characteristic bands for aromatic materials, nor in fact, does it show a single C=C band. Thus, it appears that we are dealing with an aliphatic or alicyclic aglucone, and more subtle attempts will have to be made to isolate it from the hydrolyzate. Experiments along this line are under way and will be reported in a later report.

At this point it appears that grandidentatin is a complex glucoside composed of p-coumaric acid, glucose, and an aglucone that is nonaromatic in character. The change in physical appearance of the crystals during melting point determination suggested that the compound might be a hydrate. Accordingly, a weighed sample was dried in an Abderhalden drying apparatus at 100° under diminished pressure. The moisture content of the sample was 3.23%.

Grandidentatin contains no methoxyl groups. Carbon, hydrogen, and moisture analyses indicate compound with a formula $C_{24}H_{34}O_{10}.H_2O$ having a molecular weight of 500.

Based on a molecular weight of 500 an 0.2 gram sample of grandidentatin should produce 71 mg. of glucose upon acid hydrolysis. The experiment described earlier in this report yielded 40 mg. However, in this experiment, some glucose was lost in chromatography and Fehling solution tests. Therefore, it appears that there is only one glucose moiety in the grandidentatin molecule.

Methylation of Grandidentatin

Preliminary experiments were made in an attempt to learn more about the location of the p-coumaroyl group in grandidentatin by use of the methylation procedure employed earlier (see Report No. 1) for the determination of the benzoyl substitution on tremuloidin.

A solution of 0.4 gram of grandidentatin in 10 ml. of absolute methanol was placed in the methylation apparatus described in Report No. 1. The mixture was treated with 6 ml. of methyl iodide, and with stirring and heating under reflux, was treated portionwise with 3 grams of freshly prepared silver oxide over a period of 3 hours. The mixture was filtered, and the precipitate was washed with methanol. The filtrate and washings were evaporated to dryness in a rotating vacuum evaporator. The methylation was repeated two times to give a syrupy product which was completely soluble in methyl iodide. Therefore, no methanol was employed in the fourth methylation. During the fourth methylation the silver oxide did not appear to change to silver iodide during the procedure. The reaction mixture from this methylation was filtered, and the precipitate was washed with methanol. The clear filtrate and washings were evaporated to a syrup. This methylated grandidentatin

was very resistant to hydrolysis by acid. However, hydrolysis was accomplished by boiling 10 mg. of methylated grandidentatin with excess 1 N hydrochloric acid under reflux for 8 hours. The hydrolyzate was concentrated directly to dryness in a rotating vacuum evaporator, and the residue was extracted with water. The water was concentrated to 2 ml. and chromatographed in the EPW developer along with a known mixture of methylated glucoses. A spot was obtained only for a tri-O-methyl glucose. The fact that a tri-O-methyl glucose was obtained instead of a tetra-O-methyl glucose indicates that one of the hydroxyl groups of the glucose was probably substituted by the p-coumaroyl group in the original grandidentatin. This is strong evidence that the p-coumaroyl group in the original grandidentatin is present on the glucose moiety and not on the aglucone. Further chromatograms of the hydrolyzate together with 2,3,4-tri-O-methyl glucose, 2,3,6-tri-O-methyl glucose, and 3,4,6-tri-O-methyl glucose in BPW developer and spraying with the p-anisidine reagent indicated that the color and R_f of the tri-O-methyl glucose from the hydrolyzate were identical with those of 3,4,6-tri-O-methyl glucose and quite different from those of the two other tri-O-methyl glucoses. This is evidence that grandidentatin is a glucoside that has a p-coumaroyl group on the 2-O-position of the glucose. However, further work will have to be done to confirm this evidence. Actual isolation of the methylated glucose followed by melting point, mixed melting point, and infrared spectra will be made.

Attempts to crystallize the methylated glucose were unsuccessful. On the chance that failure to crystallize were due to incomplete methylation, the product was methylated two more times to yield a colorless methylation product as a clear syrup. Attempts were made to purify the methylated products by Magnesol column chromatography. Only syrupy material could be obtained by all procedures.

Although it is impossible at this time to write the exact formulation for grandidentatin, the facts discussed in this report enable us to speculate concerning its structure. A glucoside of an alicyclic alcohol containing three hydroxymethyl groups and with p-coumaroyl substitution on the glucose is consistent with all the chemical data and elementary analyses. Such a structure for grandidentatin is pictured in Figure 3. Of course, the exact grouping of the atoms in the aglucone are purely speculative, and the position of the p-coumaroyl group is assumed to be 2. Figure 3 also pictures the structures of analogous grandidentatin acetate and the acetate of de-p-coumaroylated grandidentatin along with calculated and determined analytical values. Infrared absorption spectra are pictured in Figures 4 and 5.

iap/jaf

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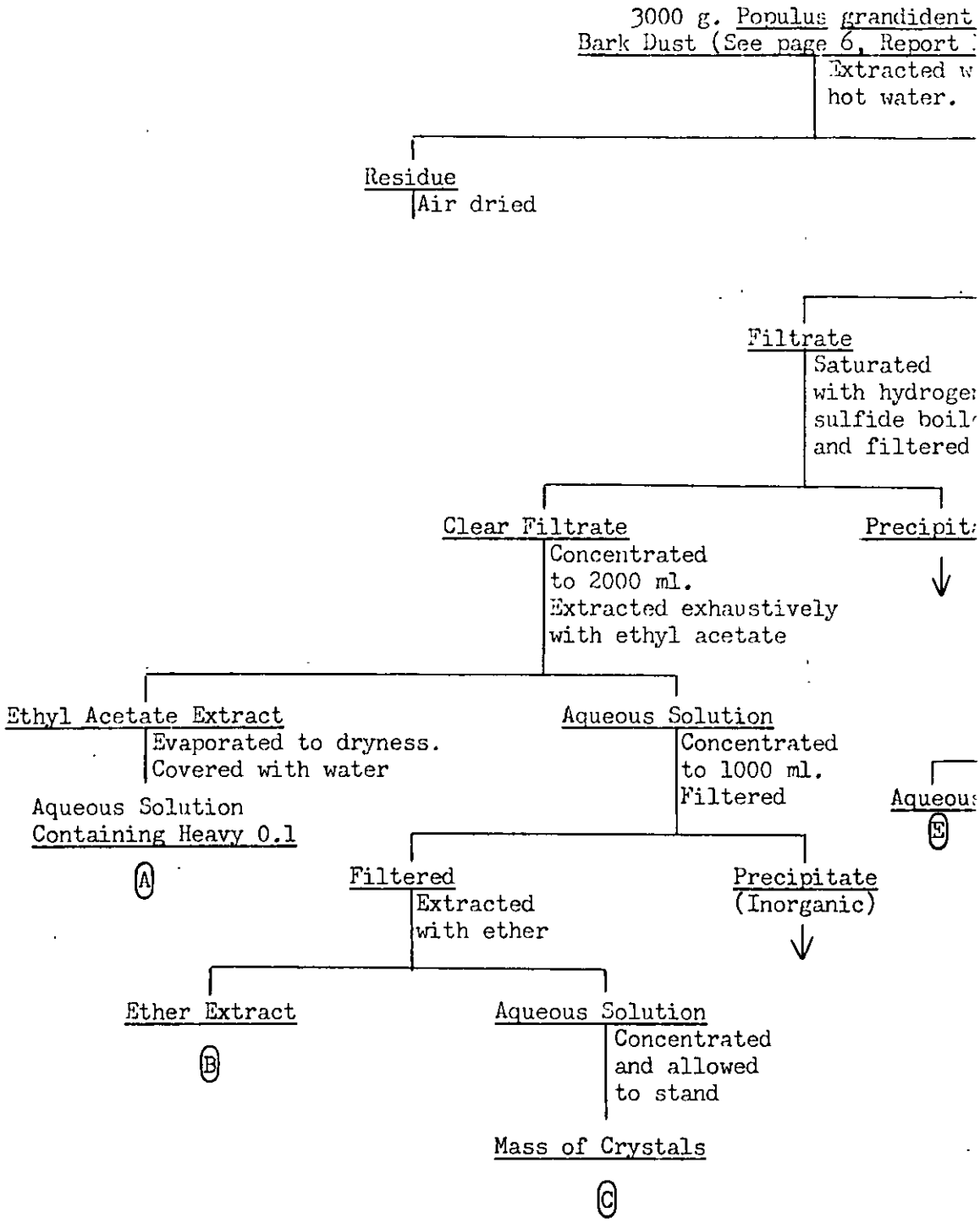
1. T. E. Timell, C. P. J. Glaudemans, and A. L. Currie, Anal. Chem. 28, 1916 (1956)

2. G. A. Dubey, B. F. Lueck, L. P. Hughes, G. Look, and A. J. Wiley. Personal communication from G. A. Dubey.

3. J. Rabate, Bull. Soc. Chim. Biol. 17, 328 (1935)

Figure 1

Preliminary Fractionation of the Large Scale Hot Water Extraction of *Populus grandidentata* Green Bark


3000 g. Populus grandident
Bark Dust (See page 6, Report
Extracted w
hot water.
Residue
Air dried
Filtrate
Saturated
with hydroge
sulfide boil
and filtered
Clear Filtrate
Concentrated
to 2000 ml.
Extracted exhaustively
with ethyl acetate
Precipit
Ethyl Acetate Extract
Evaporated to dryness.
Covered with water
Aqueous Solution
Containing Heavy 0.1
A
Aqueous Solution
Concentrated
to 1000 ml.
Filtered
Aqueou
E
Filtered
Extracted
with ether
Precipitate
(Inorganic)
Ether Extract
B
Aqueous Solution
Concentrated
and allowed
to stand
Mass of Crystals
C

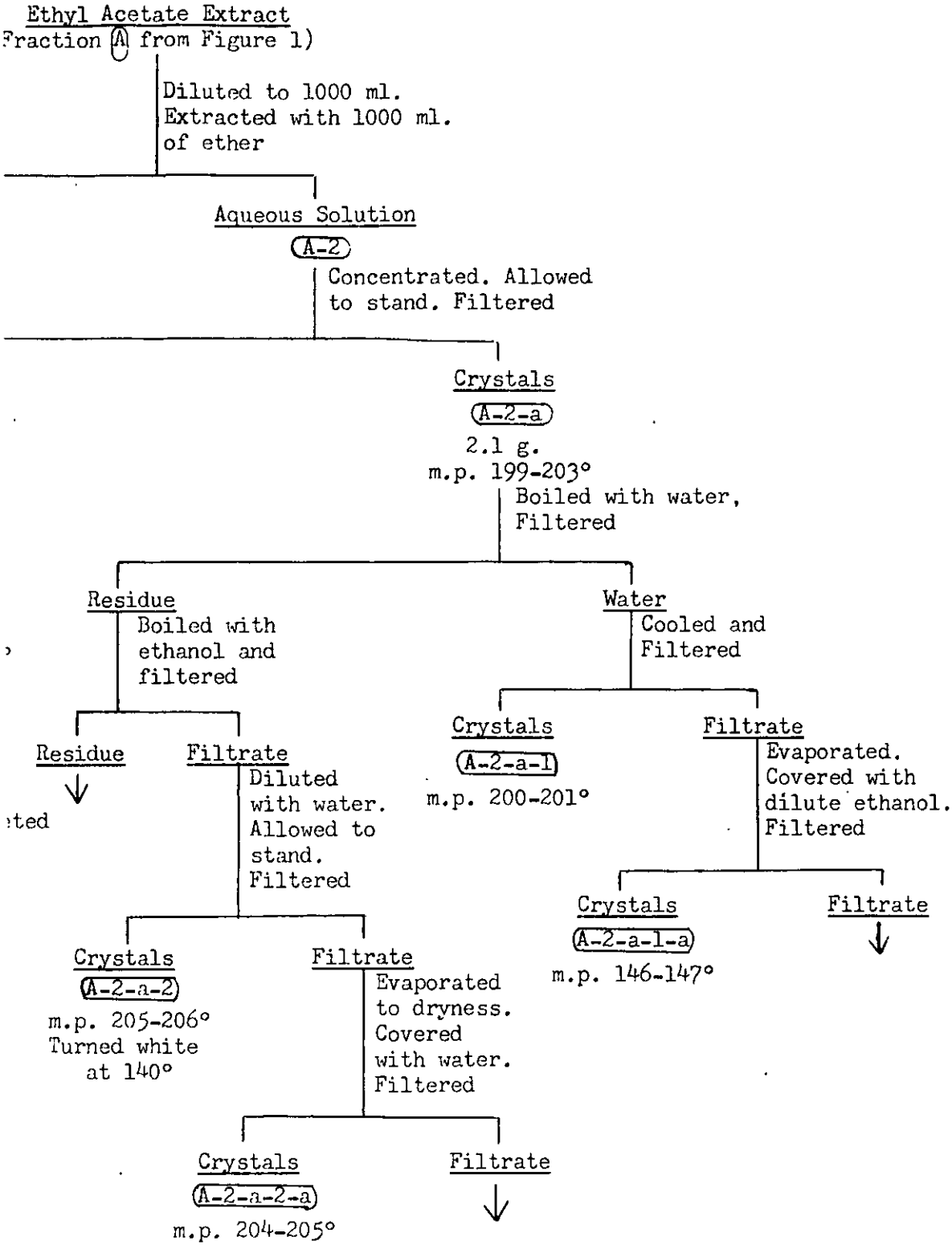


Figure 2

Fractionation of the Ethyl Acetate Extract of the Hot Water Extractives of Populus grandidentata Green Bark

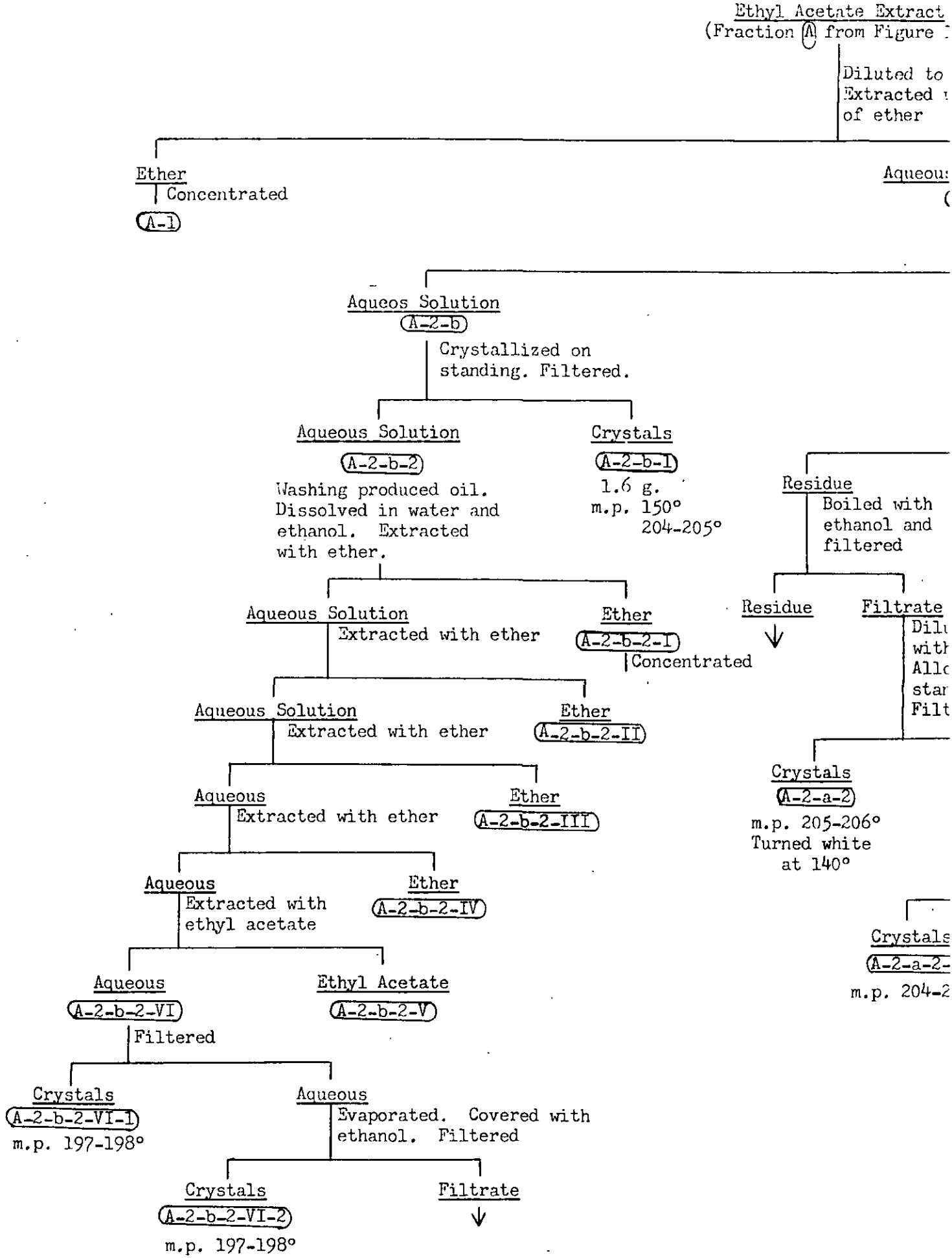
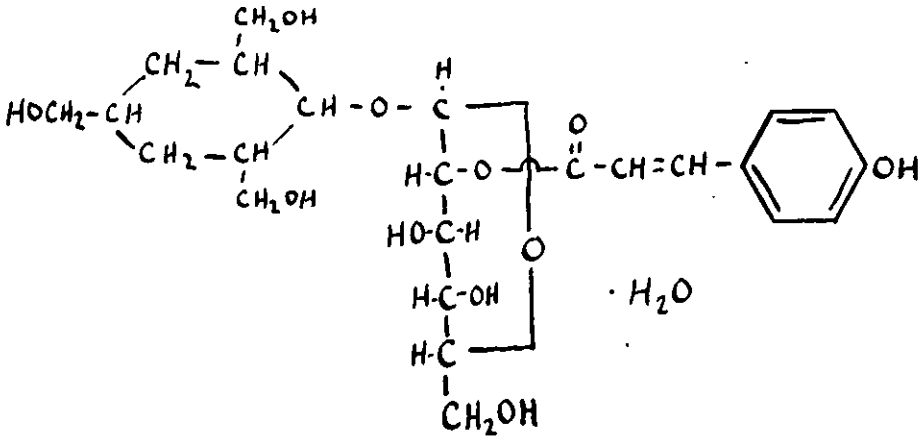

Ethyl Acetate Extract
(Fraction A from Figure
Diluted to
Extracted
of ether
Ether
Concentrated
A-1
Aqueou
(
Aqueos Solution
A-2-b
Crystallized on
standing. Filtered.
Aqueous Solution
A-2-b-2
Washing produced oil.
Dissolved in water and
ethanol. Extracted
with ether.
Crystals
A-2-b-1
1.6 g.
m.p. 150°
204-205°
Residue
Boiled with
ethanol and
filtered
Residue
Filtrate
Dilu
with
Allo
star
Filt
Aqueous Solution
Extracted with ether
Ether
A-2-b-2-I
Concentrated
Crystals
A-2-a-2
m.p. 205-206°
Turned white
at 140°
Aqueous Solution
Extracted with ether
Ether
A-2-b-2-II
Aqueous
Extracted with ether
Ether
A-2-b-2-III
Aqueous
Extracted with
ethyl acetate
Ether
A-2-b-2-IV
Crystals
A-2-a-2-
m.p. 204-2
Aqueous
A-2-b-2-VI
Ethyl Acetate
A-2-b-2-V
Filtered
Crystals
A-2-b-2-VI-1
m.p. 197-198°
Aqueous
Evaporated. Covered with
ethanol. Filtered
Crystals
A-2-b-2-VI-2
m.p. 197-198°
Filtrate

Figure 3

Structural Formulas (Suggested)

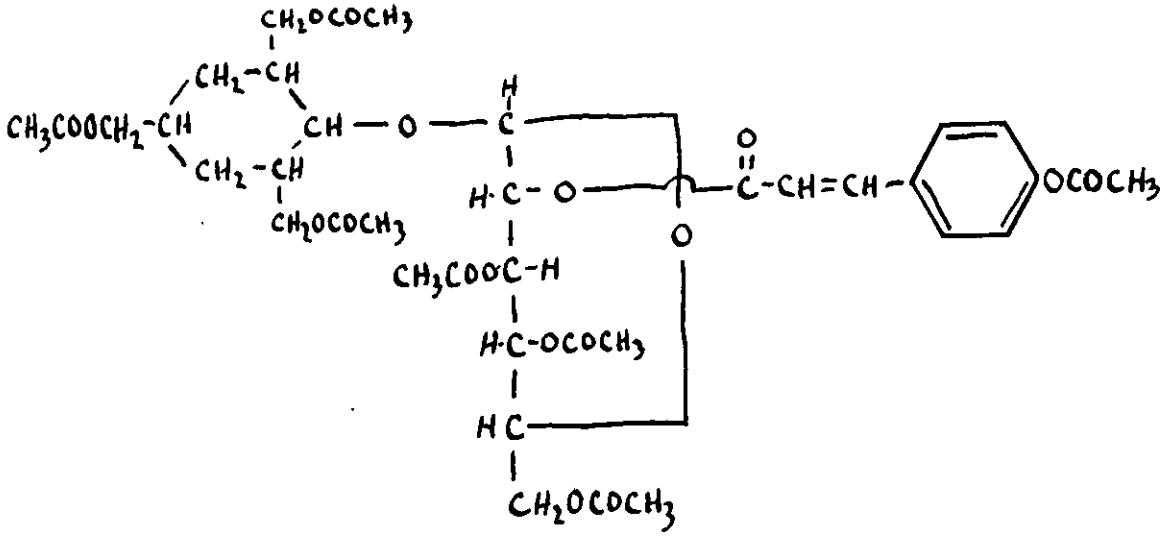


Grandidentatin

$C_{20}H_{34}O_{10}.H_2O$ (Molecular weight, 500)

Calculated for: C, 57.6; H, 7.2; H_2O, 3.48.

Found: C, 56.87, 57.08; H, 6.74, 6.86; H_2O, 3.23.



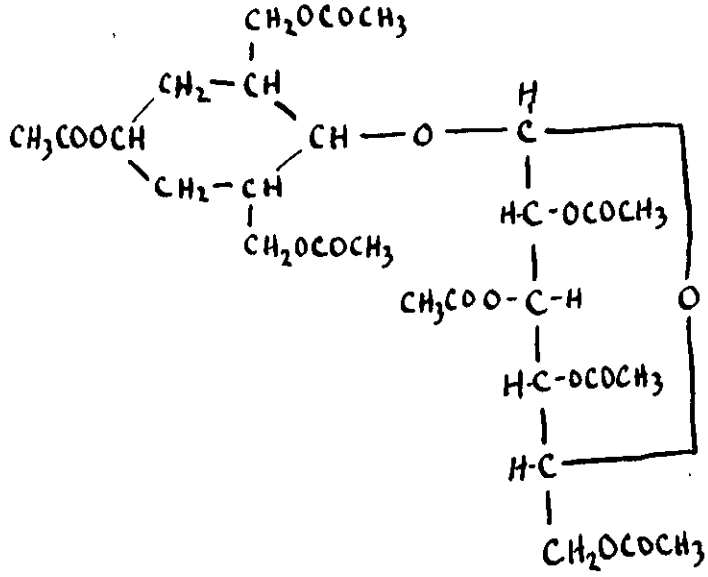
Grandidentatin Acetate

$C_{38}H_{48}O_{17}$ (Molecular weight, 776)

Calculated for: C, 58.7; H, 6.2

Found: C, 58.58, 58.79; H, 5.85, 5.92

Figure 3 (Continued)



Acetate of De-p-coumaroylated Grandidentatin

$C_{29}H_{42}O_{16}$ (Molecular weight, 646)

Calculated for:C, 53.87, H, 6.50.

Found: C, 53.90, 53.68; H, 6.55, 6.41.

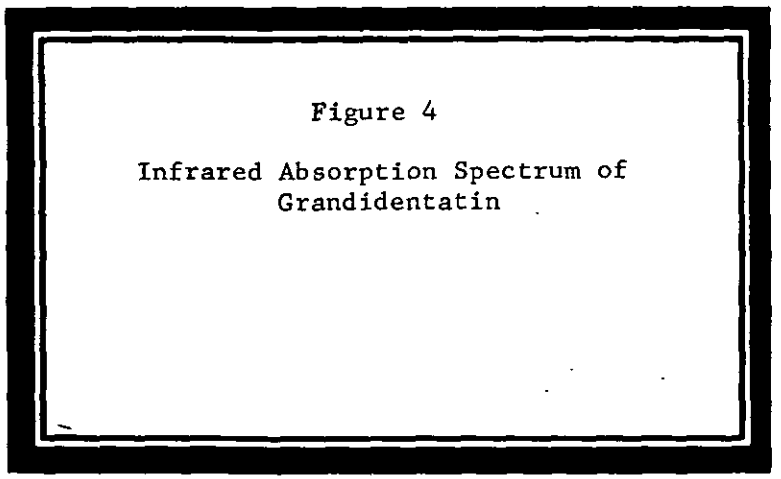


Figure 4

Infrared Absorption Spectrum of
Grandidentatin

Fig. #4

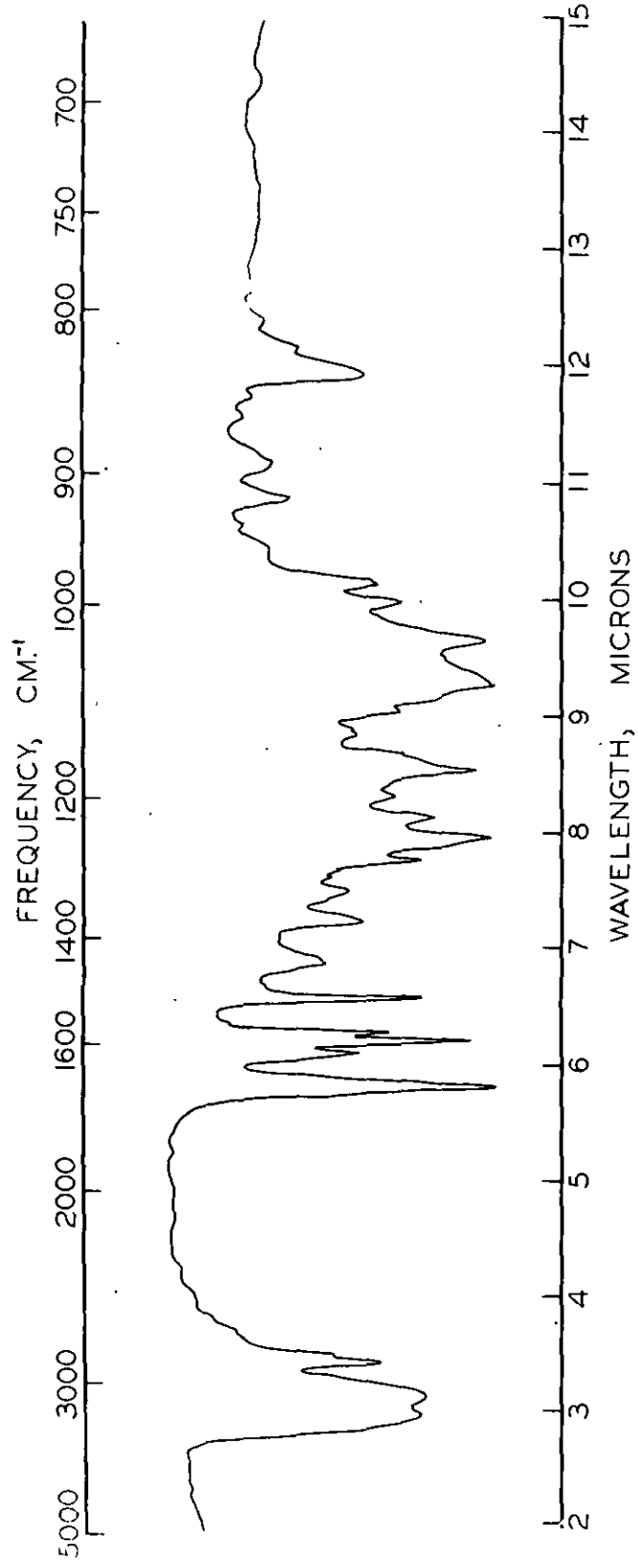

FREQUENCY, CM.⁻¹
5000
3000
2000
1600
1400
1200
1000
900
800
750
700
WAVELENGTH, MICRONS
2
3
4
5
6
7
8
9
10
11
12
13
14
15

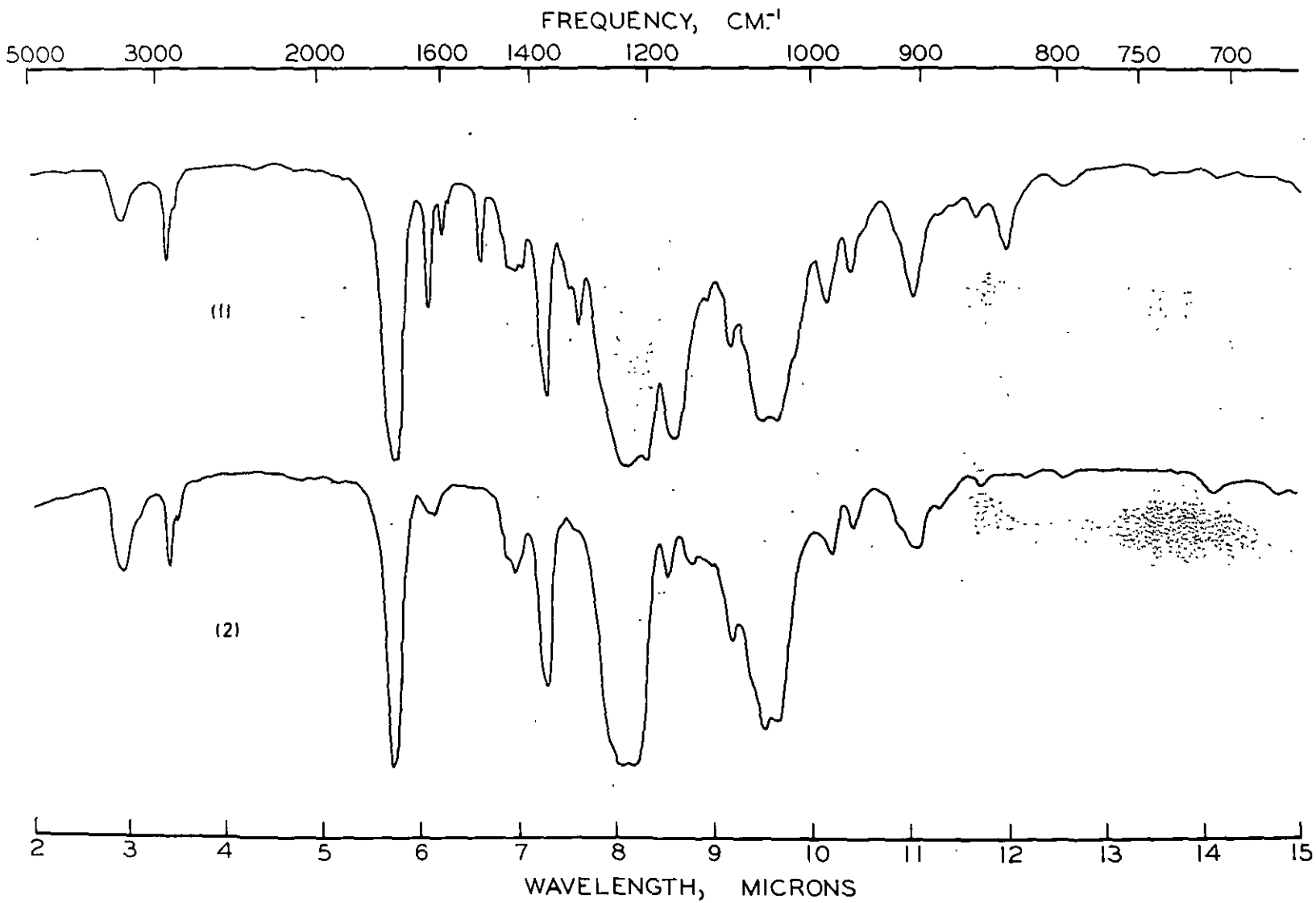
Figure 5

Infrared Absorption Spectra

1. Grandidentatin Acetate

2. De-p-coumaroylated Grandidentatin Acetate

FIG. #5


FREQUENCY, CM.⁻¹
5000
3000
2000
1600
1400
1200
1000
900
800
750
700
(1)
(2)
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH, MICRONS

PROJECT REPORT FORM

Copies to: Files
Dr. Pearl (2)
Mr. Beyer
Reading Copy

PROJECT NO. 1932
COOPERATOR Institute
REPORT NO. 9
DATE August 1, 1957
NOTE BOOK 1607
PAGE 7 TO 16
SIGNED Donald L. Beyer

Donald L. Beyer

THE ETHANOL EXTRACTION OF ASPEN BARK

In previous reports, both in 1932 and in 809-13, the presence of p-hydroxybenzoic acid has been mentioned in almost every connection with Aspen wood. This acid has been found in the extracts of the wood, in the spent liquors of all pulping processes, and in the ether extract from any hydrolyzed wood sample. However, when a sample of Populus tremuloides bark was hydrolyzed, (see report 809-13 number 25) the amount of p-hydroxybenzoic acid present was very small and a very large concentration of p-coumaric acid was found present. With this in mind and with the possibility of finding other compounds probably not present in the wood itself, we decided to obtain some bark extract.

Upon request, we received three bags of mixed mill run Aspen bark from Mr. Bill Nelson of the Green Bay Pulp and Paper Company. The bark was broken into small pieces and packed tightly in three five-gallon pickle jars. The jars were then filled with 95% ethanol and left stand for one week. After standing a week, the ethanol was decanted off and the jars were filled with fresh solvent. After a second week, the solvent was again changed. The extraction was continued for a three-week period with three changes of solvent. All the solvent used was concentrated to a small volume and bottled. The starting weight of bark on the O.D. basis was calculated to be 29.62 lbs. The extract was given our number 1607-10-A and amounted to a volume of 8390 ml., 7937 grams. The total solids were 19.5 gm/100gm. Two small samples of the dilute ethanol had previous been

evaporated to dryness, these samples amounted to a total of 22.5 grams of solids. The total extract amounted to 3.46 lbs. 11.68% based on the starting O.D. bark.

A 7 gram sample of the bark extract was dissolved in cold 4% sodium hydroxide. The caustic solution was extracted with ether. The ether was evaporated to dryness leaving a neutral fraction of 0.13 grams, approximately 2.0%. The aqueous solution was then acidified with dilute sulfuric acid and again extracted with ether. The ether solution was concentrated to a volume of 50 ml., 37.3 grams. The total solids were 3.29 gms/100 gms. The total solids amounted to 1.23 grams, 17.6%.

The ether solution was chromatographed with the following results:

Developed with butanol 2% aqueous ammonia

Benzidine ---- Rf 0.00; 0.03; 0.08; 0.10; 0.12; 0.20; 0.83

2,4 ---------- Rf 0.20

Mäule -------- Rf 0.08

$FeCl_3$ -------- Rf 0.00

Rf - 0.20 is the main spot, appears to be about 60% of the sample.

Developed with butanol, pyridine, water (10:3:3)

Benzidine ---- Rf 0.00; 0.04; 0.38; 0.44; 0.56; 0.82

2,4 ---------- Rf 0.56

Mäule -------- Rf 0.38

$FeCl_3$ -------- Rf 0.00

Rf - 0.56 is the main spot.

By comparison with the above developers as well as comparison with Benzene saturated with formic acid, the following acids matched. No known aldehydes were found.

acid	BuOH	10:3:3	Benzene-formic acid
Vanillic acid	0.10	0.44	matched with authentic
Syringic acid	0.08	0.38	matched with authentic
p-Hydroxybenzoic acid	0.12	0.56	matched with authentic
p-Coumaric	0.20	0.56	matched with authentic

It should be noted that the main spot compares identically with p-coumaric acid. However, from the chromatograms, it was noted that the spot also showed a positive test with 2,4-dinitrophenylhydrazine. The authentic p-coumaric acid does not give a positive test. Consequently, there must be an unknown material, carbonyl in structure also present with the same Rf values as p-coumaric acid. When the benzene saturated with formic acid developer is used, the spot corresponding to p-coumaric acid does not show a positive test to 2,4-dinitrophenylhydrazine.

A quantitative determination of these acids will be reported further on in this report.

Another sample of bark was obtained from Miss Barbara Reeder. This sample of bark was peeled from a Populus tremuloides. The following memorandum accompanied the bark sample. "Populus tremuloides was felled on May 23, 1957 and the bark was peeled off. Location - Ripco farm about 12 miles S.W. of Eagle River Park site # 1. South side of road in clearing. Height - 57 ft. Base diameter - 24.3 x 25.5 cm. Age - 35 years. Code no. T - 56."

As before the bark was broken into small pieces and packed into three five-gallon pickle jars. The bark was covered with 95% ethanol. The solvent was changed three times during a six-week period. The bark to be extracted was calculated to amount to 32.19 lbs. The ethanol solution was concentrated to a

volume of 8395 ml., 8395 grams T.S. - 26.63 gm/100 gm. This extract carried our number 1607-14-A. The total extracted solids amounted to 4.93 lbs., 15.31% based on the O.D. weight of the bark.

A 13.3 grams sample of the extract was dissolved in cold 4% sodium hydroxide and was extracted with ether. The ether was evaporated to dryness leaving 0.7 grams 5.3% of neutrals. The aqueous solution was acidified with dilute sulfuric acid and extracted with ether. The ether was concentrated to a volume of 50 ml., 38 grams. T.S. 8.65 gm/100 gm. The total extracted solids amounted to 3.29 grams, 24.7%.

The ether solution was chromatographed with the following results:

Developed with butanol 2% aqueous ammonia.

Benzidine ---	Rf	0.00;	0.03;	0.08;	0.10;	0.12;	0.20;	0.38;	0.44; 0.72; 0.83
2,4 ---------	Rf						0.20;	0.38;	0.44
Mäule -------	Rf			0.08				0.38	
$FeCl_3$ -------	Rf								
Fl. ---------	Rf					0.12			

Rf - 0.20 is the main product.

Developed with butanol, pyridine, water (10:3:3)

Benzidine ---	Rf	0.00;	0.04;	0.19;	0.38;	0.44;	0.56;	0.78; 0.84
2,4 ---------	Rf						0.56;	0.78; 0.84
Mäule -------	Rf				0.38			0.78
Fl. ---------	Rf					0.44		

Rf - 0.56 is the main product.

By comparison, the presence of vanillic, syringic, p-hydroxybenzoic, and p-coumaric acids was established as before. The presence of ferulic acid was also

established by the fluorescent comparison with the authentic. This acid was also found in the extract from the mill run Aspen bark. In this extract, the presence of vanillin, syringaldehyde, and p-hydroxybenzaldehyde was found by comparison with the authentic. The comparison was found more pronounced by using the developer, heptane, n-butyl ether, water (6:1:1).

All of the known compounds found in each of the ether extracts were chromatographed in known amounts and were quantitatively determined by means of the Beckman spectrophotometer. This method has been described many times in the past reports, both in this project 1932 as well as for project 809-13. The results of this quantitative determination were as follows:

Compound	7 gm. from ext. of mill run Aspen			13.3 gm. from ext. of Populus tremuloides		
	mg.	% of ext.	% of 1607-10-A	mg.	% of ext.	% of 1607-14-A
Vanillin	None	----	----	2.0	0.06	----
Syringaldehyde	None	----	----	1.5	0.05	----
p-Hydroxybenzaldehyde	None	----	----	2.0	0.06	----
Vanillic acid	25.8	2.1	0.35	40.8	1.2	0.31
Syringic acid	16.0	1.3	0.23	32.8	1.0	0.25
Ferulic acid	46.0	3.7	0.66	86.8	2.6	0.65
p-Hydroxybenzoic acid	19.0	1.5	0.27	31.0	0.9	0.23
p-Coumaric acid	166.8	13.6	2.38	534.5	16.2	4.00

dlb/tb

PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR Institute of Paper Chemistry
REPORT NO. 9
DATE April 21, 1961
NOTE BOOK 1895
PAGE 60-64 67 TO 69, 79-82
SIGNED Irwin A. Pearl

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PRELIMINARY STUDIES ON THE LEAVES OF SOME POPULUS SPECIES

Extraction of Populus Grandidentata Leaves

In Report No. 8 the isolation of small quantities of a new glucoside, grandidentatin, from the bark of the bigtooth aspen (Populus grandidentata) was described. Because some of the glucosides of several Salix and Populus species occur in both the bark and the leaves of these species, it was thought that the leaves of bigtooth aspen might be a better source of grandidentatin and enable us to isolate larger amounts of the new glucoside for structural studies.

Accordingly 3240 grams of bigtooth aspen leaves were obtained from Dr. Joranson. These leaves were collected on July 21, 1960 from the ravine behind the Institute from a tree 13 years old, 35 feet in height and 4 inches in diameter at breast height. The code number for the tree was G-25-60. The leaves contained 54% moisture giving an oven dry weight of 1490 grams for the leaves. The leaves were divided into two equal batches. Each batch was digested with 11 liters of boiling water and filtered through cloth with squeezing. The leaves were then digested again in two batches, each with 10 liters of boiling water and squeezed through cloth. The leaves were allowed to air dry.

The entire combined filtrate was stirred with excess basic lead acetate and allowed to stand. The mixture was filtered, and the clear filtrate was deleaded in the normal manner with hydrogen sulfide. The resulting clear aqueous solution was concentrated in a laboratory circulating

vacuum evaporator. Shortly after evaporation began, crystals separated in the concentrated liquor. Concentration was continued until the concentrated liquor became thick with precipitated crystals. The crystals were filtered and washed with water. The crystals weighed 10.0 grams and melted at 180-181°. Recrystallization from water gave crystals melting at 180-181° and not depressing the melting point of a mixture with authentic synthetic populin. The crystals were chromatographed in the 10:3:3 butanol-pyridine-water (BPW) developer alongside populin, and both materials gave spots with the modified silver spray at the same R_f. Neither gave spots with diazotized p-nitroaniline (DpNA) spray reagent. Both gave identical infrared absorption spectra. The specific rotation of the unknown material in 80% acetone was $[\alpha]_D^{30}$ -28.8° (c = 3.36) and that for the authentic populin was $[\alpha]_D^{30}$ -29.0 Therefore, the unknown crystalline material was identified as populin.

The complete fractionation of this experiment is pictured in the flow sheet of Figure 1. All fractions are identified. Individual fractions were chromatographed in BPW and sprayed with both DpNA and modified silver reagents. Data for individual fractions are noted below.

Fraction (B)--These crystals melting at 178-179° proved to be more populin by chromatography and mixed melting point with authentic populin.

Fraction (C)--These bitter crystals proved to be crude salicin.

Fraction (C-1)--These pure crystals proved to be pure salicin by chromatography and mixed melting point with authentic salicin.

Fraction (C-2)--Chromatography indicated these bitter crystals to be pure salicin, but the melting point of 193-194° indicated that they were somewhat impure.

Fraction (C-3)--This ethanolic mother liquor gave no spot with the silver spray, but gave the following spots with DpNA: R_f 0.54 (violet),

0.65 (red-violet), and 0.85 (pink).

Fraction (D)--Spots were obtained with the modified silver spray at R_f's 0.48, 0.60, 0.72, 0.85. The DpNA spray gave the following spots: 0.64 (red-violet), 0.79 (violet), heavy 0.86 (purple surrounded by red-violet). No spots for salireposide or grandidentatin could be found.

Fraction (E)--Chromatography of this residual aqueous solution indicated a number of sugars by the silver spray and one material with R_f 0.56. The DpNA spray indicated only one material with a red-violet spot at R_f 0.64.

Extraction of Populus Tremuloides Leaves

The finding of populin in the leaves of Populus grandidentata was very unexpected because earlier studies (see Reports No. 3 and 4) led us to believe that the barks of the two American species, Populus tremuloides and Populus grandidentata might not contain this glucoside, and we assumed that the glucoside content of the leaves of a particular species would resemble the glucoside content of the bark of the same species. At the time that populin was found in the leaves of Populus grandidentata it had not been found in the bark of this species, but it had not been proven absent. On the other hand, all tests made on the bark of Populus tremuloides indicated that populin was not present in any amount whatsoever. Because of the possibility of populin being present in the leaves of a species and absent in the bark of the same species, an experiment was made on the leaves of the quaking aspen (Populus tremuloides).

A sample of 2250 grams of quaking aspen leaves was received from Dr. Joranson. These leaves were collected on July 25, 1960 from the ravine behind the Sulphite Pulp Manufacturers' Research League building from a

tree 11 years old, 35 feet in height, and 4 inches in diameter at breast height. The code number of the tree was T-75-60. The leaves contained 61.3% moisture giving an oven dry weight of 870 grams for the leaves. These leaves were extracted with boiling water in the same manner described for the bigtooth aspen leaves. Concentration of the clarified aqueous extract gave crystals of populin again in the case of these quaking aspen leaves as well as in the case of the bigtooth aspen leaves although the amount was somewhat less. The complete fractionation of this experiment is given in the Flow sheet of Figure 2 in which all fractions are identified. Individual fractions were chromatographed in BPW and sprayed with both DpNA and modified silver spray reagents. Data for individual fractions are noted below.

Fractions (A), (B), and (C)--These three crystalline fractions gave no spot whatsoever with DpNA and a spot with the modified silver spray corresponding with populin. Mixed melting point determinations and infrared absorption spectra indicated that these three fractions were populin.

Fraction (D)--This ethyl acetate extract gave spots with the modified silver spray at R_f's 0.49, 0.59, 0.72, and 0.84. The DpNA spray gave the following spots: 0.57 (violet), 0.64 (red-violet), 0.72 (purple), 0.79 (violet), and heavy 0.86 (purple surrounded by red-violet).

Fraction (E)--This aqueous residue gave only spots for sugars with the modified silver spray and no spots whatsoever with the DpNA spray reagent.

Thus, although no populin has ever been found in the hot water extractives of the bark of the American quaking aspen (Populus tremuloides), the leaves of this species provide a good source for the glucoside.

The leaves of the two Populus species of this report, after hot water extraction and drying, were subjected to ethanol extraction by repeated

soaking with 95% ethanol at room temperature and concentrating all combined extracts. These concentrated extracts are identified as Fraction (G) in both Figure 1 and Figure 2.

iap/rjw

Figure 1

FRACTIONATION OF HOT WATER EXTRACTIVES OF BIGTOOTH ASPEN LEAVES

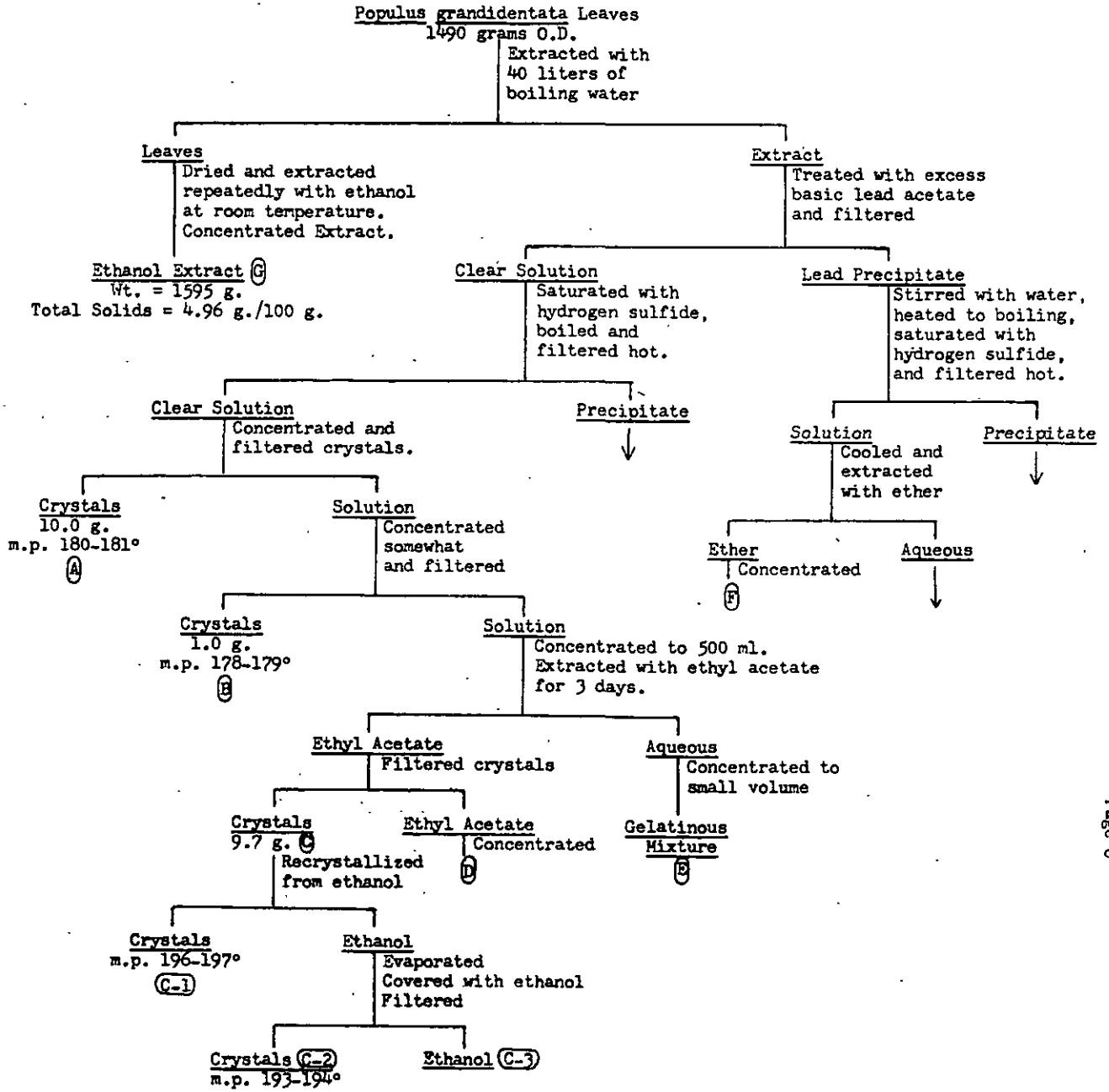
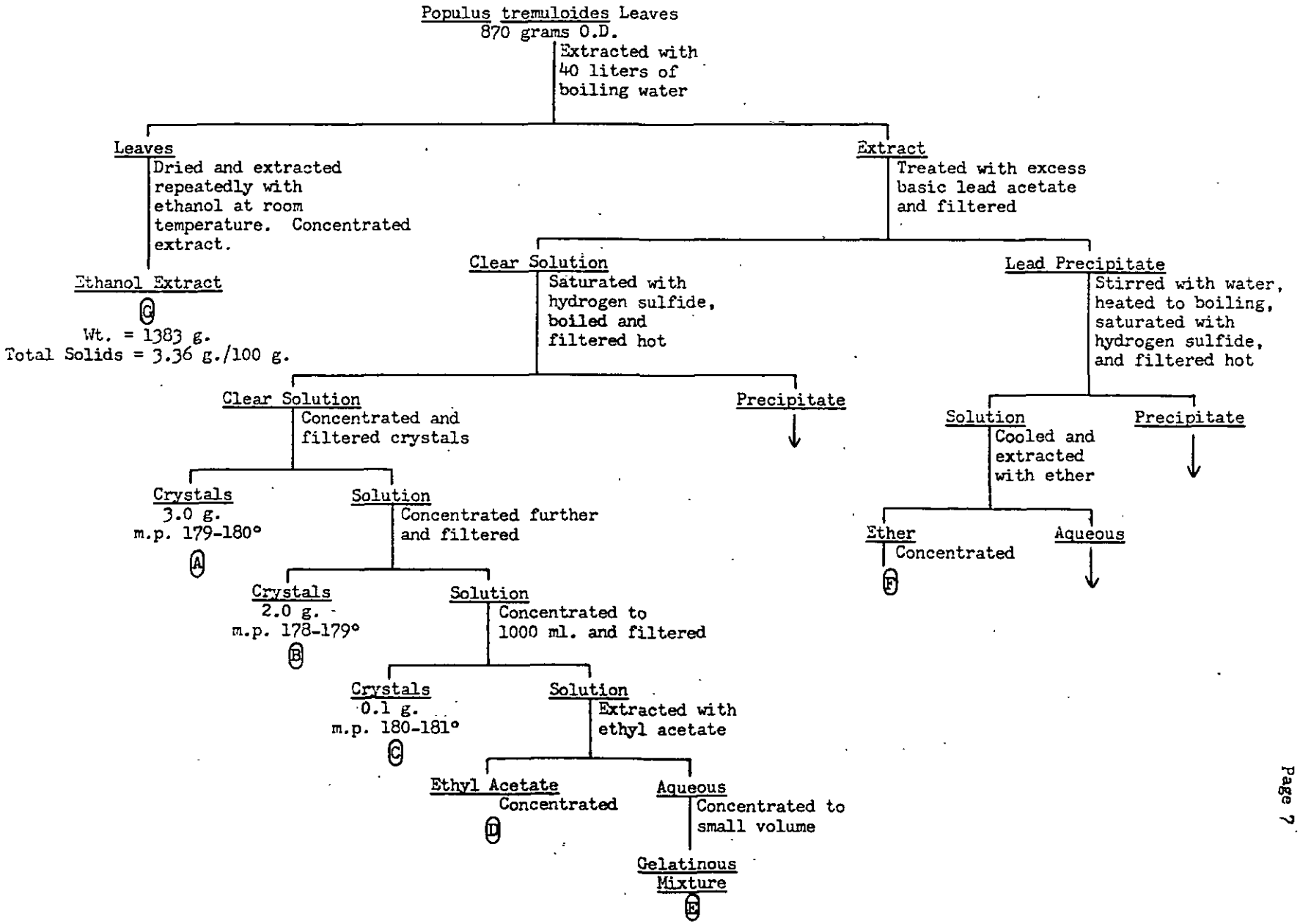


Figure 2

FRACTION OF HOT WATER EXTRACTIVES OF QUAKING ASPEN LEAVES



PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR Institute
REPORT NO. 10
DATE September 23, 1957
NOTE BOOK 1642
PAGE TO
SIGNED Donald L. Beyer

Donald L. Beyer

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Pages: 47-50
53-60
72-73
79-80

THE CAUSTIC HYDROLYSIS AND THE PROPANOL EXTRACTION OF VARIOUS HARDWOODS. IV.

Three new hardwoods have been obtained. These woods have been cut into sawdust and have been hydrolyzed with sodium hydroxide. Another sample of the sawdust has been extracted with 75% propanol. The procedure followed is the same as reported in the three previous reports concerning the hydrolysis and extraction of hardwoods. (Reports No. 4, 6, and 8). The data obtained is to be an addition to that already obtained in the previous three reports.

The three new woods are as follows. The first is Tupelo Gum (Nyssa aquatica) obtained from Mr. Dickey. Mr. Dickey received the wood from the Union Bag and Camp Paper Corporation of Savannah, Georgia. The second wood is Fraser Magnolia (Magnolia fraseri) also received from Dickey. This originally was obtained from the West Virginia Pulp and Paper Company, Covington, Virginia. The third wood is Lilac (Syringa vulgaris), this was also given to us by Mr. Dickey who cut the sample locally.

The three woods were hydrolyzed, and the ether-soluble phenols were chromatographed, and known compounds were determined quantitatively by using the Beckman spectrophotometer. This method is also fully described in the previous reports.

None of these woods contained any p-hydroxybenzaldehyde, p-hydroxybenzoic acid, nor p-coumaric acid. The yields of vanillin, syringaldehyde, vanillic acid, syringic acid, and ferulic acid along with other data follows.

Common name	Tupelo gum	Fraser magnolia	Lilac
Scientific name	Nyssa aquatica	Magnolia fraseri	Syringa vulgaris
Ether ext. (phenols)	2.82 gm. 2.82%	1.69 gm. 1.69%	3.24 gm. 3.24%
Code No.	1642-47-A	1642-53-A	1642-57-A
Vanillin	27.0 mg. 1.0%	16.5 mg. 1.0%	20.0 mg. 0.6%
Syringaldehyde	46.0 mg. 1.6%	38.0 mg. 2.2%	29.0 mg. 0.9%
Vanillic acid	43.5 mg. 1.5%	37.5 mg. 2.2%	38.5 mg. 1.2%
Syringic acid	60.0 mg. 2.1%	48.5 mg. 2.9%	70.0 mg. 2.2%
Ferulic acid	68.0 mg. 2.4%	46.0 mg. 2.7%	61.0 mg. 1.9%
Recovered sawdust	63.4 gm. 63.4%	74.6 gm. 74.6%	67.5 gm. 67.5%

The chromatographic results of these three woods follows.

DEVELOPED WITH BUTANOL 2% AQUEOUS AMMONIA.

Rf	Tupelo gum 1642-47-A	Fraser magnolia 1642-53-A	Lilac 1642-57-A	Remarks
0.00	x	x	x	
0.01	x		x	
0.08	x	x	x	Syringic acid
0.10	x	x	x	Vanillic acid
0.12	x	x	x	Ferulic acid (Flourescent)
0.27	x	x	x	Positive 2,4-D
0.38	x	x	x	Syringaldehyde
0.44	x	x	x	Vanillin
0.82	x	x	x	
0.91	x	x		

DEVELOPED WITH BUTANOL—PYRINDINE- WATER (10:3:3)

Rf	Tupelo gum 1642-47-A	Fraser magnolia 1642-53-A	Lilac 1642-57-A	Remarks
0.01	x	x	x	
0.09	x		x	
0.12	x			
0.38	x	x	x	Syringic acid
0.44	x	x	x	Vanillic & Ferulic acids
0.76	x	x	x	Positive 2,4-D & Mäule
0.83	x	x	x	Positive 2,4-D & Mäule

The three woods were also extracted with 75% propanol with the following results.

	Tupelo gum	Fraser magnolia	Lilac
Propanol extract	10.3 gm. 5.2%	5.19 gm. 2.6%	7.80 gm. 3.9%
Recovered sawdust	187 gm. 94%	188 gm. 94%	181.3 gms. 90.7%

dlb/tb

PROJECT REPORT FORM

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REPORT NO. 10
DATE April 29, 1961
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PAGE ____ TO ____
SIGNED Irwin A. Pearl

Irwin A. Pearl

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Pearl (2) — Notebook 1739: page 118
Reading Copy — Notebook 1895: Pages 15-21, 27-28, 34-37, 39, 41

FURTHER STUDIES ON THE EXTRACTIVES OF POPULUS TREMULOIDES BARK

In Project Report No. 1 the isolation of glucosides from the hot water extractives of Populus tremuloides bark and from the hot water extractives of the ethanol extractives of Populus tremuloides bark was reported. Report No. 1 also reported the isolation and identification of the new glucoside, tremuloidin. In Report No. 3 the identification of salireposide as one of the glucosides isolated from this species was reported, and in Report No. 4, studies on the structure of salireposide were described. These structure studies required large amounts of salireposide. In order to obtain more salireposide, all the ethanol extractives of Populus tremuloides bark on hand (this is the same bark extract employed on page 5 of Report No. 1 and described in detail in Report No. 9 of Don Beyer) was processed.

LARGE SCALE HOT WATER EXTRACTION OF FRESH POPULUS TREMULOIDES BARK EXTRACTIVES

Two thousand grams of the ethanol extractives of fresh Populus tremuloides bark after standing at room temperature for three years was evaporated to dryness in a rotating vacuum evaporator in a 5-liter flask to leave 533 grams of solids. The residue was covered with 4.5 liter of water, heated to boiling, and allowed to stand overnight. The cooled mixture was filtered through a wet paper, and the filtrate was purified by the basic lead acetate procedure as described in the past. The complete fractionation procedure employed in this experiment is pictured in the

flow sheets of Figures 1 and 2 in which all fractions are identified. In this experiment the water-insoluble extractives were also processed.

The individual fractions of Figures 1 and 2 were chromatographed in 10:3:3 butanol-pyridine-water (BPW), and the chromatograms were sprayed with the modified silver (Ag) and diazotized p-nitroaniline (DpNA) reagents Results are noted below along with other comments.

Fraction A-1--This material appeared as fine white fluffy needles melting sharply at 212-213°. The DpNA spray showed up no spots whatsoever, and the Ag spray indicated tremuloidin at R_f 0.85. Specific rotations were $[\alpha]_D^{25}$ -9.1° (80% acetone) and $[\alpha]_D^{22}$ +22.2 (pyridine). These rotations are somewhat more dextro-rotatory than previously reported rotations for tremuloidin.

Fractions B-1-a, B-2-a, B-3-a, and B-4-a--These crystalline fractions which precipitated from concentrated ether extracts all appeared to be tremuloidin. They all gave spots with Ag at 0.85 and no spots whatsoever with DpNA. Fraction B-1-a had an $[\alpha]_D^{29}$ -12.8° (c = 2.6 in 80% acetone) and Fraction B-2-a had an $[\alpha]_D^{26}$ +19.9° (c = 1.7 in pyridine) indicating both fractions were tremuloidin.

Fraction B-1-a-1--This methanol washing from the crystallized tremuloidin gave spots with Ag at 0.80 and 0.86 and with DpNA at 0.79 (purple) and 0.86 (violet).

Fraction B-2-a-1--This acetone washing from the crystallized tremuloidin gave a spot with Ag at 0.80 and spots with DpNA at 0.81 (purple) and 0.86 (pink).

Fraction B-3-a-1--This acetone washing from crystallized tremuloidin

gave a faint spot with Ag at 0.61 and a strong spot at 0.79. Spots were obtained with DpNA at 0.78 (purple) and 0.84 (pink).

Fraction B-4-a-1--This acetone washing from the crystallized tremuloidin gave spots with Ag at 0.62 and 0.79 and with DpNA at 0.77 (purple) and 0.83 (pink).

Fraction B-1-b--This evaporated ether solution gave spots with Ag at 0.76 and 0.85 and with DpNA at 0.58 (blue), 0.69 (blue), 0.79 (purple), and 0.86 (red).

Fraction B-2-b--This evaporated ether solution gave spots with Ag at 0.62, 0.75, and 0.90 and with DpNA at 0.80 (violet) and 0.87 (pink).

Fraction B-3-b--This evaporated ether solution gave spots with Ag at 0.61, 0.72, and 0.85 and with DpNA at 0.76 (purple) and 0.80 (pink).

Fraction B-4-b--This evaporated ether solution gave spots with Ag at 0.62 and 0.73 and with DpNA at 0.77 (purple) and 0.83 (pink).

Fraction B-5-a--This crystalline fraction obtained from the extracted aqueous solution gave only one spot with Ag at 0.79 and with DpNA at 0.78 (purple) indicating salireposide.

Fraction B-5-b--This aqueous solution left after extraction gave spots with Ag at 0.02, 0.04, 0.10, 0.18, 0.23, 0.46, 0.52, 0.61, and 0.76. With DpNA, spots were obtained at 0.51 (purple), 0.60 (pink) and 0.77 (purple).

Fractions E-2-2-a and E-2-3--These white crystalline materials gave spots only for p-coumaric acid at 0.56 (bright blue with DpNA). Mixed melting points with authentic p-coumaric acid were not depressed.

Further Fractionation of Individual Fractions

On the basis of the chromatography of individual fractions a number of fractions were combined to give composite samples as follows:

Composite (A)--Fractions B-1-a-1, B-2-a-1, B-3-a-1, and B-4-a-1.

Composite (B)--Fractions B-2-b, B-3-b, and B-4-b.

Fractionation of Composite A

Composite sample A in acetone was allowed to stand. After one week at room temperature, considerable crystalline precipitate separated. The complete fractionation of this composite sample is pictured in the flow sheet of Figure 3 in which all fractions are identified.

Chromatography of Fractions from Composite A

A number of the fractions of Figure 3 were chromatographed in BPW along with authentic salireposide, salicin, and tremuloidin. Chromatograms were sprayed with both Ag and DpNA reagents. Results are noted below.

Fraction A-1--This crystalline material appears to be almost pure salireposide with a trace of tremuloidin.

Fraction A-2--This crystalline material appears to be crude salicin with a trace of salireposide and a trace of phenolic material at R_f 0.50.

Fraction A-3--This crystalline material appears to be pure tremuloidin.

Fraction B-2--This crude fraction gives spots with Ag at 0.69, 0.81 (salireposide), and 0.88. With DpNA, it gives spots at 0.72 (pink), 0.81 (purple--salireposide), and 0.87 (pink).

Fraction B-2-a--This purified fraction still gives spots with Ag and DpNA for salireposide, but the rotation indicates that it is tremuloidin. The rotation of $[\alpha]_D^{29}$ -9.1° (c = 1.6 in 80% acetone) is the same as that

reported for Fraction A-1 of Figure 1 and is more dextro-rotatory than previously reported rotations for tremuloidin.

Fraction B-2-b-1--Chromatography indicates that these crystals are tremuloidin with a trace of salireposide.

Fraction B-2-b-2-a--This crystalline fraction appears to be pure tremuloidin by chromatography.

Fraction B-2-b-2-b--This crystalline fraction with the melting point characteristics of salireposide has a specific rotation $[\alpha]_D^{28}$ -27.3° (c = 2.2 in 80% acetone) which is not as great as that of pure salireposide (-35.6°). Chromatography indicates salireposide with a trace of tremuloidin or some glucoside with the same R_f as tremuloidin.

VARIOUS EXPERIMENTS ON PREVIOUSLY REPORTED FRACTIONS

During the course of our work on the glucosides of the barks of the several Populus species we have had the occasion to reinvestigate a particular fraction because of some new experimental finding. Some of these experiments which have not been recorded heretofore are reported in the present report.

Specific Rotation Studies

A number of fractions isolated in the past and suspected of being tremuloidin were found to give a purple color when spotted on paper (without chromatography) and sprayed with the DpNA reagent. In order to check the nature of these fractions, their specific rotations were determined. The solubility of authentic tremuloidin in acetone-water mixtures was determined, and it was found that 80% acetone was the best solvent for tremuloidin.

This solution was employed for specific rotation measurements of these fractions.

Craig machine Fraction F-1-a from Report No. 2--This fraction melting at 201-203° had a rotation $[\alpha]_D^{30}$ -11.1° (c = 3.38 in 80% acetone). Tremuloidin.

Fraction B-1-a of Donald A. Scott (notebook 1775, page 68)--This crystalline fraction from Populus grandidentata melted at 203-206° and had a rotation $[\alpha]_D^{30}$ -15° (c = 3.98 in 80% acetone). This appears to be tremuloidin.

Fraction G-4-b of Donald A. Scott (notebook 1775, page 97)--This crystalline fraction from Populus grandidentata melted at 196-203° and had a rotation $[\alpha]_D^{30}$ -12° (c = 3.33 in 80% acetone). This appears to be largely tremuloidin.

Salireposide acetate--The rotation of this compound was determined in chloroform and found to be $[\alpha]_D^{30}$ -9.8° (c = 4.60 in chloroform).

Purification of Tremuloidin

Many of the tremuloidin fractions isolated during the course of these studies had melting points somewhat lower than the 209-210° observed for the purest fractions obtainable. Although chromatography indicated no impurities, it was obvious that the products were not absolutely pure. An attempt was made to purify such a product by cellulose column chromatography.

A column of Whatman No. 1 cellulose powder was packed dry in a tube 20 mm. in diameter to a height of 150 mm. A solution of 0.2 gram of crude tremuloidin (Fraction E of Figure 4 in Project Report No. 1) melting at 200-201° in a minimum amount of warm 80% acetone was placed on top of the column, and the column was eluted with 9:2:2 ethyl acetate-acetic acid-water. One ml. fractions were collected in the eluate. The first ten fractions were

evaporated somewhat. Crystals were obtained in the first three fractions. These three fractions were each filtered, and the fine glistening needles washed with a little solvent mixture. Yields and melting points were as follows: 1, 0.035 gram (208-210°); 2, 0.005 gram (208-210°); and 3, 0.047 gram (208-211°). A total of 0.087 gram of pure tremuloidin was recovered from the 0.2 gram of crude starting material. Undoubtedly, evaporation of other fractions to dryness would have yielded more of the purified material, but this was not done in this experiment.

iap/rjw

Figure 1

LARGE SCALE HOT WATER EXTRACTION OF FRESH POPULUS TREMULOIDES BARK EXTRACTIVES

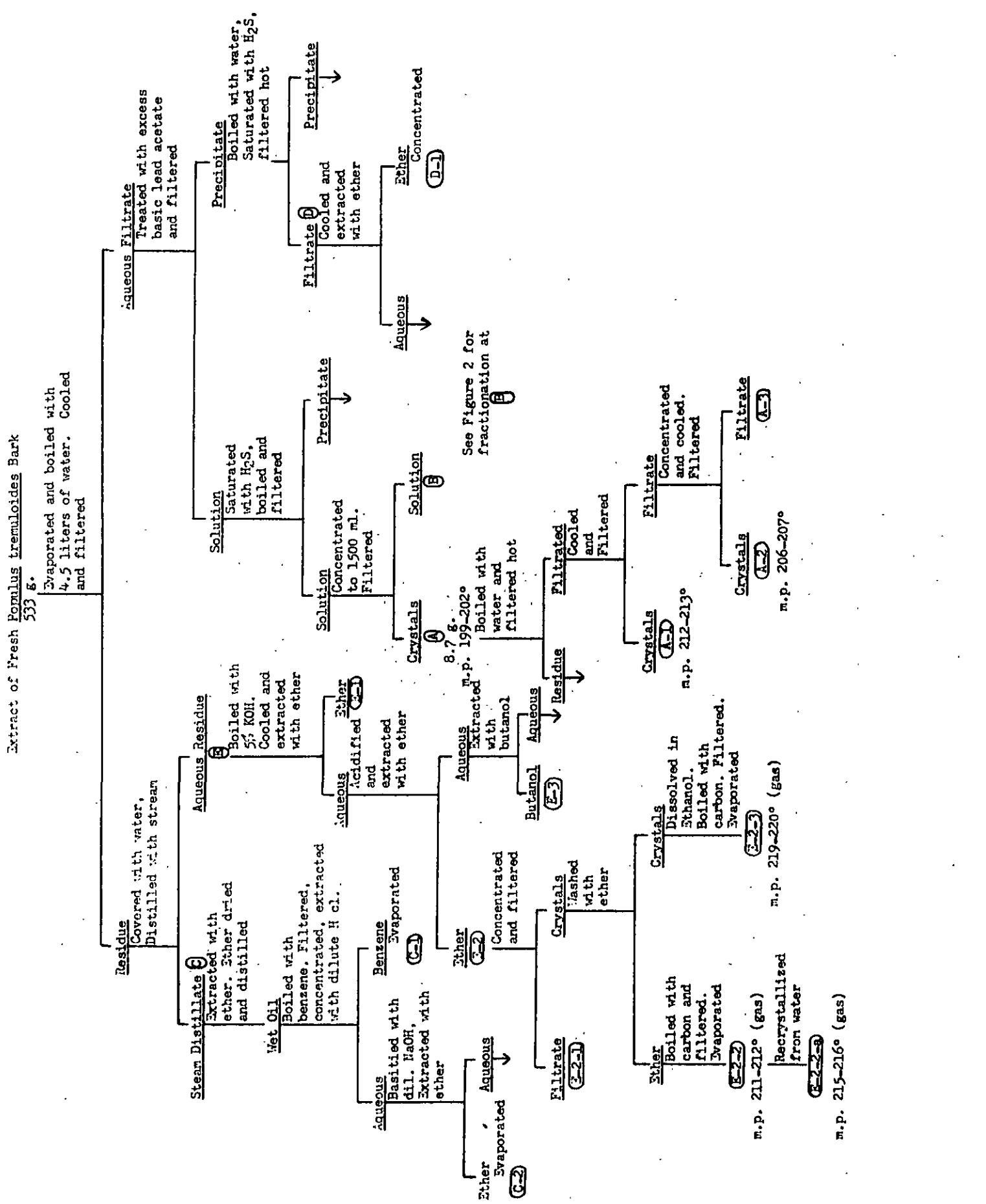


Figure 1

LARGE SCALE HOT WATER EXTRACTION OF FRESH POPULUS TREMULOIDES BARK EXTRACTIVES

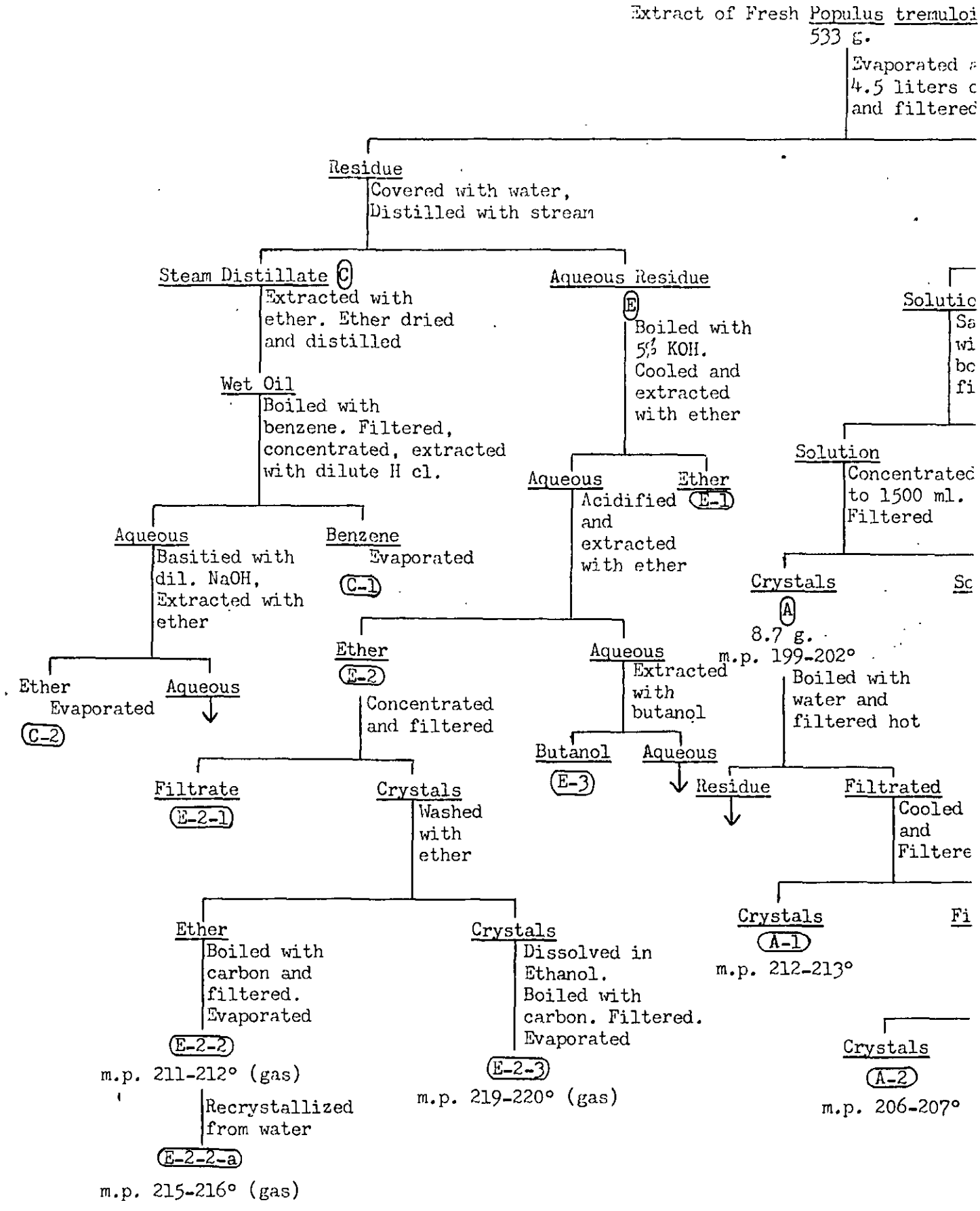
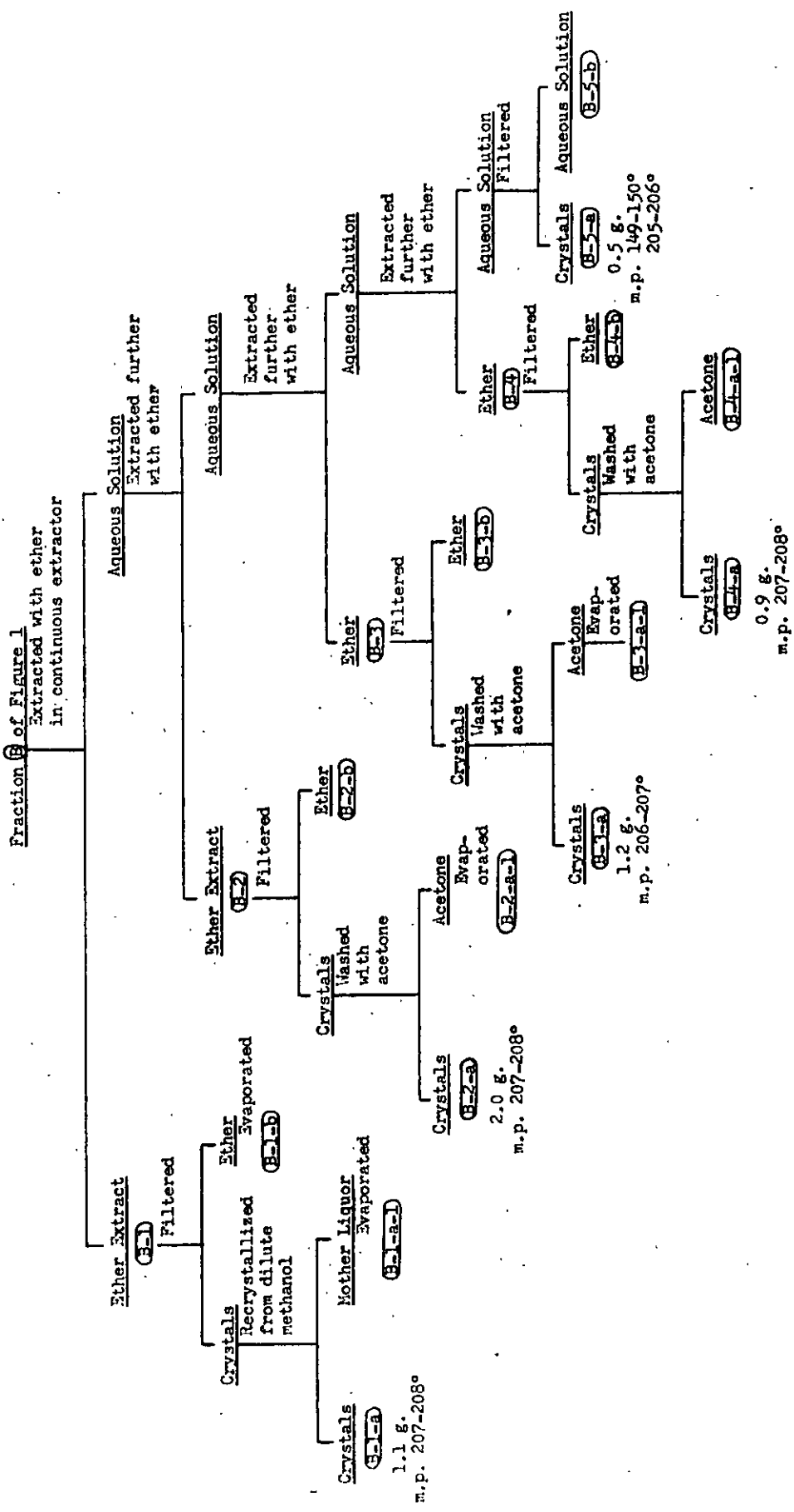

Extract of Fresh Populus tremuloi
533 g.
Evaporated
4.5 liters
and filtered
Residue
Covered with water,
Distilled with stream
Steam Distillate C
Extracted with ether. Ether dried and distilled
Wet Oil
Boiled with benzene. Filtered, concentrated, extracted with dilute H cl.
Aqueous
Basitied with dil. NaOH, Extracted with ether
Benzene
Evaporated
C-1
Ether
Evaporated
C-2
Aqueous
Aqueous Residue
E
Boiled with 5% KOH. Cooled and extracted with ether
Aqueous
Acidified and extracted with ether
Ether
E-1
Ether
E-2
Concentrated and filtered
Aqueous
Extracted with butanol
Butanol
E-3
Aqueous
Filtrate
E-2-1
Crystals
Washed with ether
Ether
Boiled with carbon and filtered. Evaporated
E-2-2
m.p. 211-212° (gas)
Recrystallized from water
E-2-2-a
m.p. 215-216° (gas)
Crystals
Dissolved in Ethanol. Boiled with carbon. Filtered. Evaporated
E-2-3
m.p. 219-220° (gas)
Solutio
Sa
wi
bc
fi
Solution
Concentrated to 1500 ml. Filtered
Crystals
A
8.7 g.
m.p. 199-202°
Sc
Boiled with water and filtered hot
Residue
Filtrated
Cooled and Filtere
Crystals
A-1
m.p. 212-213°
Fi
Crystals
A-2
m.p. 206-207°

Figure 2

Fractionation of Fraction (B) of Figure 1



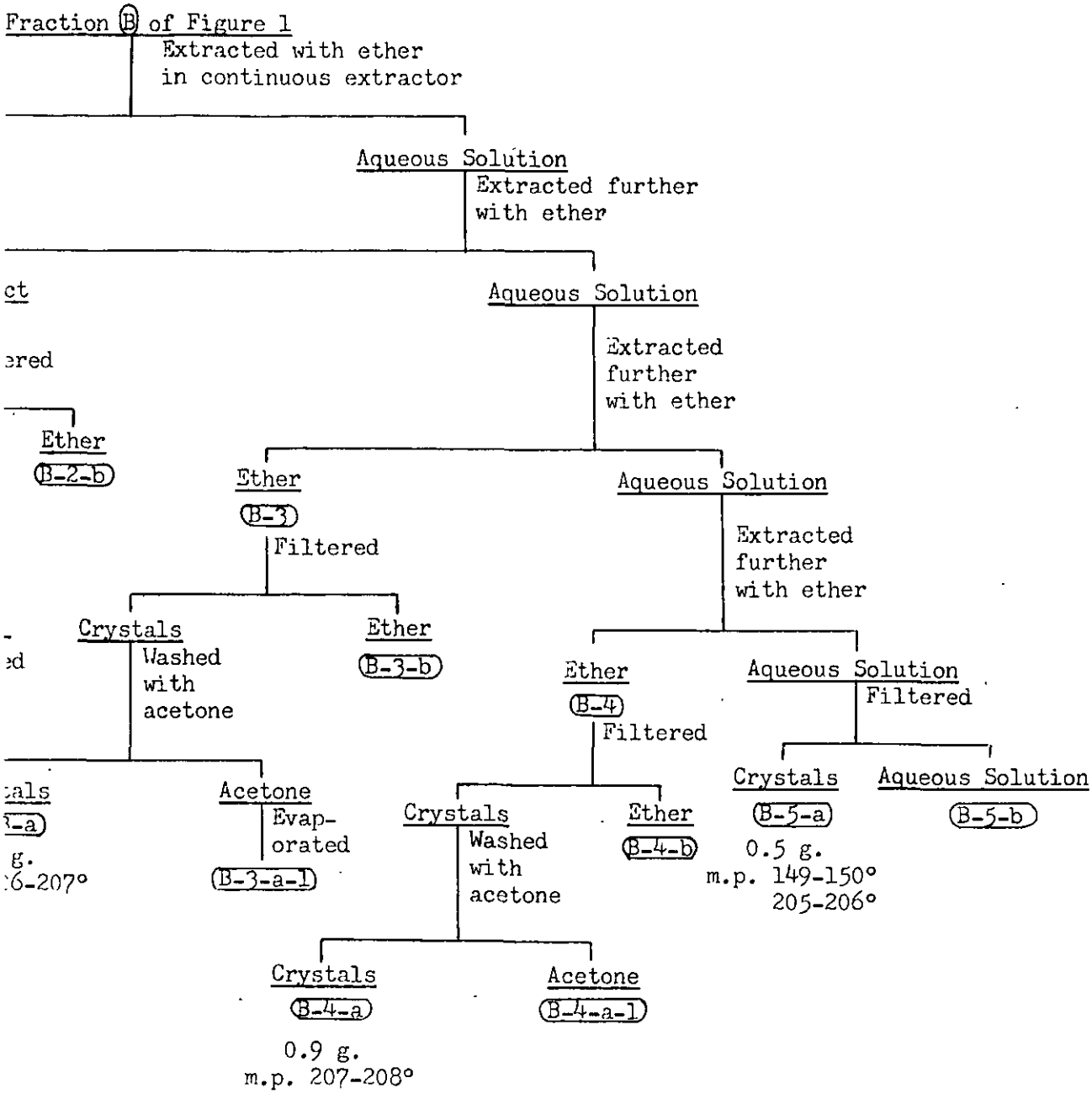
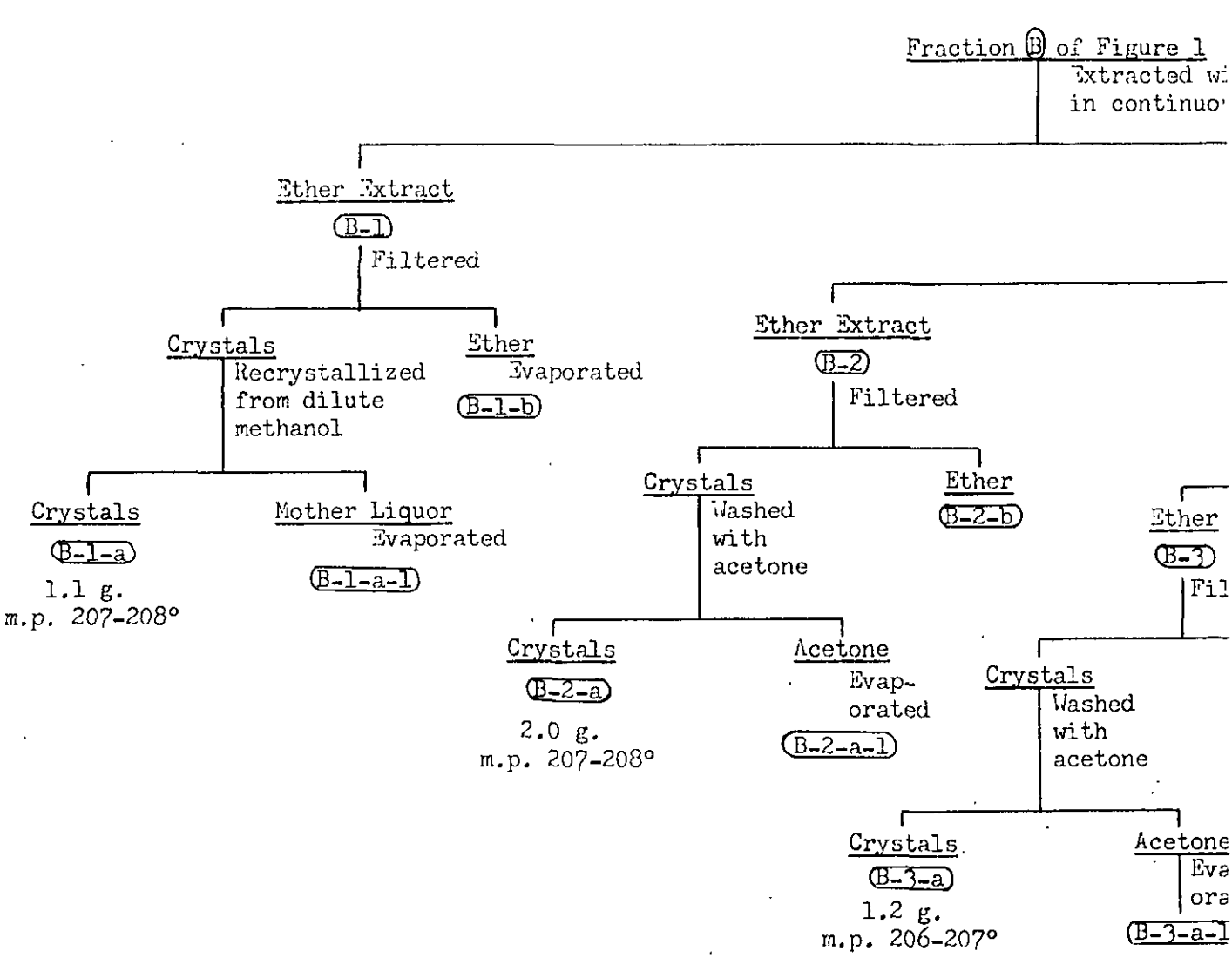


Figure 2
Fractionation of Fraction (B) of Figure 1


Fraction (B) of Figure 1
Extracted wi
in continuo
Ether Extract
(B-1)
Filtered
Crystals
Recrystallized
from dilute
methanol
Ether
Evaporated
(B-1-b)
Crystals
(B-1-a)
1.1 g.
m.p. 207-208°
Mother Liquor
Evaporated
(B-1-a-1)
Ether Extract
(B-2)
Filtered
Crystals
Washed
with
acetone
Ether
(B-2-b)
Crystals
(B-2-a)
2.0 g.
m.p. 207-208°
Acetone
Evap-
orated
(B-2-a-1)
Ether
(B-3)
Fil
Crystals
Washed
with
acetone
Crystals
(B-3-a)
1.2 g.
m.p. 206-207°
Acetone
Eva
ora
(B-3-a-1
Cry
(B
0.
m.p.

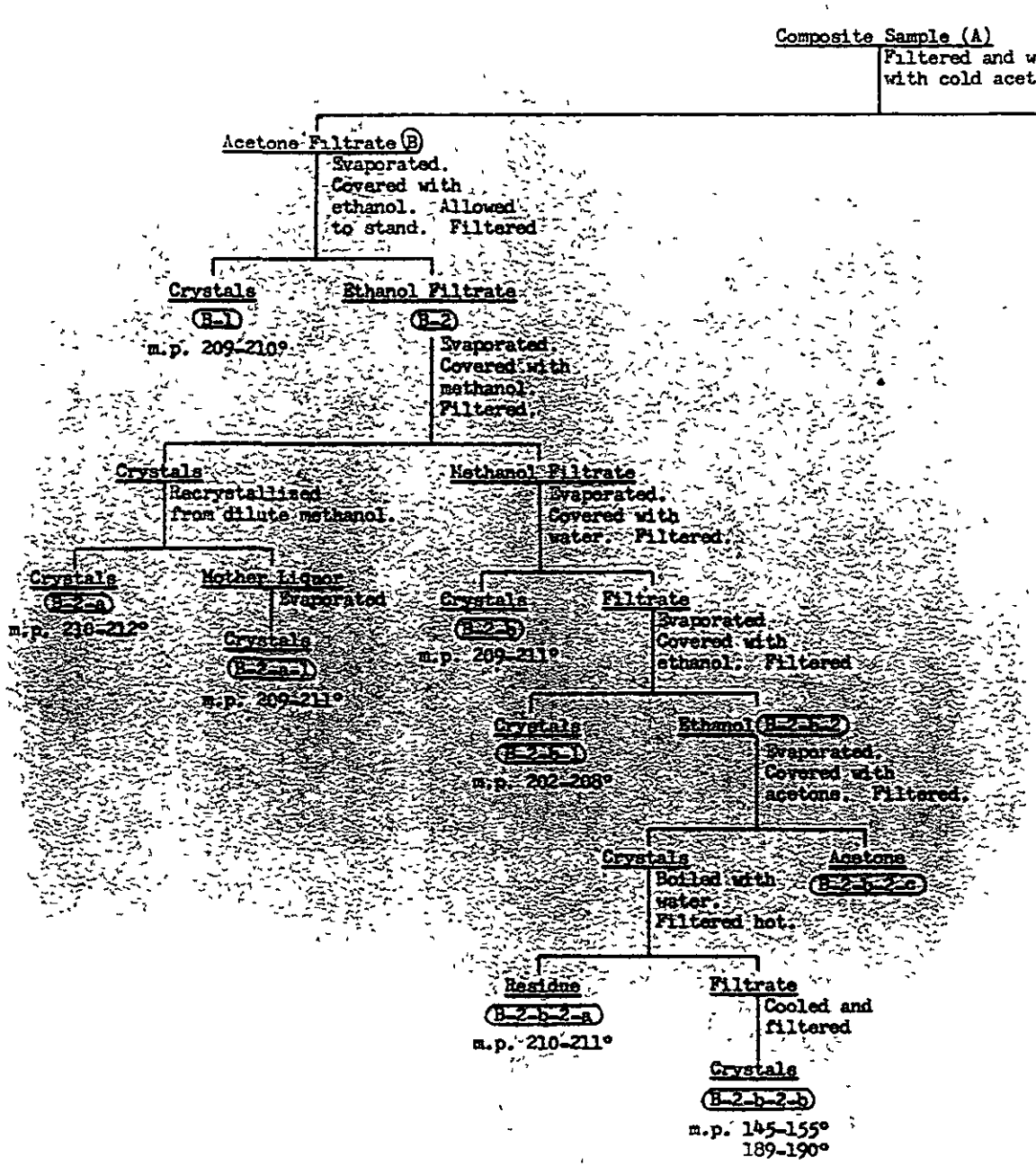
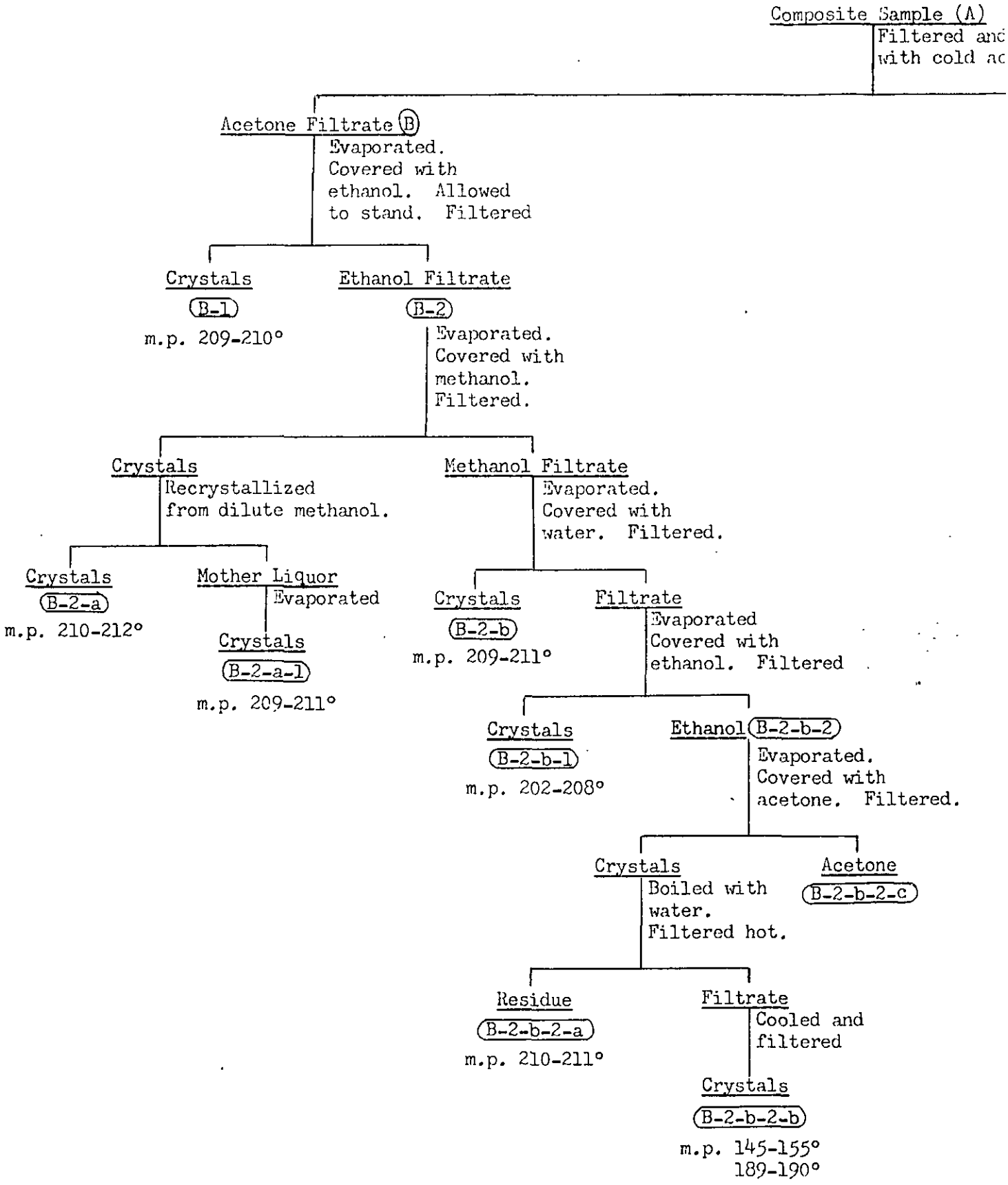


Figure 3

Fractionation of Composite Sample A of Populus Tremuloides Bark Extractives

Figure 3

Fractionation of Composite Sample A of Populus Tremuloides Bark Extractives


Composite Sample (A)
Filtered and
with cold ac
Acetone Filtrate (B)
Evaporated.
Covered with
ethanol. Allowed
to stand. Filtered
Crystals
(B-1)
m.p. 209-210°
Ethanol Filtrate
(B-2)
Evaporated.
Covered with
methanol.
Filtered.
Crystals
Recrystallized
from dilute methanol.
Methanol Filtrate
Evaporated.
Covered with
water. Filtered.
Crystals
(B-2-a)
m.p. 210-212°
Mother Liquor
Evaporated
Crystals
(B-2-a-1)
m.p. 209-211°
Crystals
(B-2-b)
m.p. 209-211°
Filtrate
Evaporated
Covered with
ethanol. Filtered
Crystals
(B-2-b-1)
m.p. 202-208°
Ethanol (B-2-b-2)
Evaporated.
Covered with
acetone. Filtered.
Crystals
Boiled with
water.
Filtered hot.
Acetone
(B-2-b-2-c)
Residue
(B-2-b-2-a)
m.p. 210-211°
Filtrate
Cooled and
filtered
Crystals
(B-2-b-2-b)
m.p. 145-155°
189-190°

PROJECT REPORT FORM

PROJECT NO. 1932
COOPERATOR Institute
REPORT NO. 11
DATE September 30, 1957
NOTE BOOK 1607 and 1642
PAGE ____ TO ____
SIGNED Donald L. Beyer
Donald L. Beyer

Copies to: Files
Dr. Pearl (2)
Mr. Beyer

Pages: 1607
17-159
1642
7-46
51-53
73-78
90-110

THE CAUSTIC HYDROLYSIS OF THE 75% PROPANOL EXTRACTS OF VARIOUS HARDWOODS.

In reports number 4, 6, 8, and 10 from Project 1932, a large number of various hardwoods have been hydrolyzed with sodium hydroxide as well as extracted with 75% propanol. In this report the extracts obtained from the propanol extractions of these hardwoods were hydrolyzed with sodium hydroxide.

In the future another series of hydrolysis will be performed on the propanol extracts of these hardwoods. This next hydrolysis will be an acid hydrolysis. A sample containing two grams of extract was used for the caustic hydrolysis and a similar sample will be used for the forthcoming acid hydrolysis. From the previous reports many of the extractions did not yield a four gram extract. Therefore, in order to have a sufficient starting sample, many of the hardwoods were again extracted with 75% propanol. In many cases the original wood previously reported was extracted, however, in some cases there wasn't enough untreated sawdust available to obtain the necessary amount of extract. Whenever this problem arose, new wood had to be obtained and with the help of Dr. Joranson and Dr. Einspahr we were kept supplied with what we needed.

Since the complete data on the yields and the recovery of the sawdust has already been reported, these additional extractions will not be reported here in detail. I shall only mention the woods extracted, without data.

The woods that were extracted in varying amounts so as to obtain a sufficient amount of extract were:

Code No.	Common Name	Scientific Name
1607-22-A	Yellow birch	Betula lutea
1607-23-A	Hard maple	Acer saccharum
1607-24-A	Yellow poplar	Liriodendron tulipifera
1607-38-A	White Bay	Magnolia virginiana
1607-39-A	Black gum	Nyssa sylvatica
1607-40-A	Sweet gum	Liquidamber styrauflua
1607-46-A	Ironwood	Carpinus caroliniana
1607-48-A	Water Oak	Quercus nigra
1607-49-A	So. Red Oak	Quercus falcata
1607-50-A	Paper birch	Betula papyrifera
1607-108-A	Magnolia	Magnolia grandiflora
1642-51-A	White oak	Quercus alba
1642-52-A	Beech	Fagus grandifolia
1642-73-A	Catalpa	Catalpa speciosa
1642-77-A	Soft maple	Acer rubrum
1642-93-A	Red maple	Acer rubrum
1642-95-A	Black ash	Fraxinus nigra
1642-97-A	No. black cottonwood	Populus trichocarpa
1642-110-A	Box elder	Acer negundo

The caustic hydrolysis of the propanol extract of each of the hardwoods was done in the following identical manner. A sample of the propanol extract containing two grams of solids was evaporated to dryness using the

Rinco rotating evaporator. To the viscous residue were added 100 ml of a 4% sodium hydroxide solution. This solution was boiled under reflux for eight hours. The cool solution was then extracted with ether. This ether solution contained a neutral fraction. The aqueous solution was then acidified with dilute sulfuric acid and again was extracted with ether. This ether solution contained the phenolic fraction. After the acidification and the ether extraction, a tar-like insoluble solid remained. This material was dissolved in ethanol and bottled.

The phenolic fraction was the only fraction which was worked on. The ether solution was chromatographed to determine which of the eight known materials were present. A complete chart showing the chromatographic results will follow. All of the known compounds that were found present in each of the fractions were chromatographed quantitatively, located, eluted, and read on the Beckman spectrophotometer. This procedure has been reported previously in many of the other reports.

A complete table will follow showing all the data and results accumulated.

A Summary of Results

As in the caustic hydrolysis of the wood, each product contains vanillin, syringaldehyde, vanillic acid, and syringic acid. However, in regard to the vanillin- syringaldehyde ratio, unlike the general trend of the hydrolyses of wood, the yield of vanillin is primarily greater than the syringaldehyde. The vanillic and syringic acids seem to have no relationship to each other. This was also true to the hydrolysis of the wood. Some woods

are higher in vanillic acid, others have a greater syringic acid yield. There was also no relationship between the hydrolysis of the wood and the hydrolysis of the extract. Some woods followed the pattern where either vanillic or syringic acid was greater in either hydrolysis, while others were reversed.

The presence of p-hydroxybenzoic acid was again found in all woods belonging to the Populus and the Salix genera. Previously, the only other wood found containing p-hydroxybenzoic acid was catalpa. Again catalpa contained the acid, however, the hydrolysis of the extract also showed some p-hydroxybenzoic acid in American sycamore, Platanus occidentalis.

From the data obtained from the hydrolysis of wood, p-hydroxybenzaldehyde was found only in four of the hardwoods in this group. Each of these four again showed the presence of p-hydroxybenzaldehyde. These woods are: Populus tremuloides, White basswood; Tilia heterophylla, European poplar; Populus tremula, and Diamond willow, Salix eriocephala.

Coumaric acid had been found in nine of the hardwoods in this group when the wood was hydrolyzed. From the data obtained from the hydrolysis of the propanol extract, only four woods contained the p-coumaric acid. The one wood, red alder, Alnus rubra was not included in this report, since no wood was available. The other four woods which contained no p-coumaric acid in the hydrolysis of the extract were: Populus tremuloides; Sweet gum, Liquidamber styraciflua; Cottonwood, Populus deltoides; and Black ash, Fraxinus nigra. The four woods containing the p-coumaric acid were: White basswood, Tilia heterophylla; European poplar, Populus tremula; Diamond willow, Salix eriocephala;

and American sycamore, Platanus occidentalis. It should be noted that three of these four woods also contain the p-hydroxybenzaldehyde.

The many hardwoods which yielded ferulic acid after the caustic hydrolysis of the wood in most cases also showed the presence of ferulic acid after the hydrolysis of the propanol extract. However, ferulic acid was absent in the products of nine of the woods which had previously showed the acid. In the case of yellow birch, Betula lutea, paper birch Betula papyrifera, ironwood, Carpinus caroliniana, black ash, Fraxinus nigra, and butternut, Juglans cinerea the yield of ferulic acid found in the hydrolyzed product from the wood was very small, less than 1.5% of the extract. The other four woods however had quite large yields previously, these woods were: Populus grandidentata (4.2%); hard maple, Acer saccharum (3.4%); scarlet oak, Quercus coccinea (5.2%); and chestnut, Castanea dentata (3.1%).

It should be pointed out, that in this series of hydrolysis where a two gram sample is used as the starting material the ether extract is very small and dilute. The possibility must exist that a small concentration of any of these compounds could be missed which might account for some of the absent materials.

As previously mentioned, red alder was omitted from this study since no wood was available. Eucalyptus regnans was also eliminated from this study for the same reason. Both of these woods are expected in the near future and the series will then include them also. Any new woods which might be obtained will also be added and reported at that time.

It should be noted that the laboratory work required to complete this series was done in most part by Miss Sue Lee. The final laboratory work as well as most of the quantitative determination of the known compounds was done by Miss Dawn Laskowski.

#1

CAUSTIC HYDROLYSIS OF THE PROPANOL E

Wood		1st Ether Extract (Neutrals)			2nd Ether Extract (Phenols)			Vanillin		Syring-Aldehyde	
Common Name	Scientific Name	Code No.	g.	%	Code No.	g.	%	mg.	%	mg.	
Aspen	Populus tremuloides	1607-17-A	0.28	14.0	1607-17-B	0.37	18.5	7.5	2.0	3.8	1
Big Tooth Aspen	Populus grandidentata	1607-41-A	0.23	11.5	1607-41-B	1.19	59.5	9.0	0.8	4.8	(
Cottonwood	Populus deltoides	1607-33-A	0.18	9.0	1607-33-B	0.26	13.0	4.8	1.5	2.8	:
European White Poplar	Populus alba	1607-91-A	0.33	16.5	1607-91-B	0.33	16.5	4.5	1.4	3.5	1
European Poplar	Populus tremula	1607-83-A	0.20	10.0	1607-83-B	0.74	37.0	3.8	0.5	2.8	(
No. Black Cottonwood	Populus trichocarpa	1607-149-A	0.43	21.5	1607-149-B	0.42	21.0	6.3	1.5	3.5	(
Lombardy Poplar	Populus nigra	1607-75-A	0.32	16.0	1607-75-B	0.65	32.5	3.8	0.6	2.5	(
Balsam Poplar	Populus tacamahaca	1607-113-A	0.61	30.5	1607-113-B	0.86	43.0	3.5	0.4	3.0	(
Diamond Willow	Salix eriocephala	1607-145-A	0.64	32.0	1607-145-B	0.70	35.0	3.3	0.5	2.5	(
Black Willow	Salix nigra	1607-103-A	0.26	13.0	1607-103-B	0.60	30.0	5.0	0.8	3.5	(
Weeping Willow	Salix babylonica	1607-71-A	0.33	16.5	1607-71-B	0.32	16.0	7.0	2.2	5.8	:
Overcup Oak	Quercus lyrata	1607-29-A	0.09	4.5	1607-29-B	0.33	16.5	3.5	1.1	3.5	:
White Oak	Quercus alba	1642-81-A	0.19	9.5	1642-81-B	0.48	24.0	1.5	0.3	2.8	(
Willow Oak	Quercus phellos	1607-157-A	0.17	8.5	1607-157-B	0.38	19.0	3.3	0.9	3.8	:
Black Oak	Quercus velutina	1607-115-A	0.07	3.5	1607-115-B	0.39	19.5	4.0	1.0	2.5	(
Turkey Oak	Quercus catesbae	1607-55-A	0.12	6.0	1607-55-B	0.34	17.0	4.3	1.3	3.5	:
Scarlet Oak	Quercus coccinea	1607-65-A	0.04	2.0	1607-65-B	0.17	8.5	5.0	2.9	7.8	:
Blackjack Oak	Quercus marilandica	1607-51-A	0.22	11.0	1607-51-B	0.37	18.5	7.0	1.9	4.5	
East. Red Oak	Quercus borealis	1607-139-A	0.18	9.0	1607-139-B	1.05	52.5	4.3	0.4	4.8	(
Water Oak	Quercus nigra	1607-153-A	0.20	10.0	1607-153-B	0.50	25.0	6.0	1.2	7.0	
So. Red Oak	Quercus falcata	1642-7-A	0.11	5.5	1642-7-B	0.62	31.0	5.0	0.8	6.0	
Black Ash	Fraxinus nigra	1642-11-A	0.07	3.5	1642-11-B	0.77	38.5	6.3	0.8	15.3	:
Silver Maple	Acer saccharinum	1607-95-A	0.08	3.0	1607-95-B	0.28	14.0	4.5	1.6	4.8	
Soft Maple	Acer rubrum	1607-59-A	0.52	26.0	1607-59-B	0.25	12.5	9.0	3.6	9.0	
Hard Maple	Acer saccharum	1642-19-A	0.14	7.0	1642-19-B	0.51	25.5	7.8	1.5	9.3	
Red Maple	Acer rubrum	1607-127-A	0.19	9.5	1607-127-B	0.42	21.0	5.5	1.3	5.8	
Box Elder	Acer negundo	1607-25-A	0.38	19.0	1607-25-B	0.29	14.5	7.3	2.5	3.8	
Basswood	Tilia americana	1607-99-A	0.23	11.5	1607-99-B	0.87	43.5	3.3	0.4	2.5	
White Basswood	Tilia heterophylla	1607-137-A	0.18	9.0	1607-137-B	0.70	35.0	6.3	1.8	2.5	
American Elm	Ulmus americana	1607-63-A	0.22	11.0	1607-63-B	0.44	22.0	6.8	1.5	3.5	
Shagbark Hickory	Carya ovata	1607-109-A	0.14	7.0	1607-109-B	0.37	18.5	4.3	1.2	3.3	
Butternut	Juglans cinerea	1607-121-A	0.25	12.5	1607-121-B	0.51	25.5	2.5	0.5	2.0	
Chestnut	Castanea dentata	1607-79-A	0.012	0.6	1607-79-B	0.29	14.5	4.0	1.4	4.3	
Black Gum	Nyssa sylvatica	1642-35-A	0.27	13.5	1642-35-B	0.50	25.0	9.5	1.9	5.8	
Sweet Gum	Liquidamber styrauflua	1642-98-A	0.07	3.5	1642-98-B	0.21	10.5	3.0	1.4	2.8	
Yellow Poplar	Liriodendron tulipifera	1642-27-A	0.36	18.0	1642-27-B	0.44	22.0	4.3	1.0	4.3	
Magnolia	Magnolia grandiflora	1642-15-A	0.20	10.0	1642-15-B	0.43	21.5	6.5	1.5	5.3	
White Bay	Magnolia virginiana	1607-133-A	0.29	14.5	1607-133-B	0.39	19.5	4.8	1.2	3.3	
Yellow Birch	Betula lutea	1607-87-A	0.60	30.0	1607-87-B	0.33	16.5	3.8	1.2	4.5	
Paper Birch	Betula papyrifera	1642-43-A	0.25	12.5	1642-43-B	1.13	56.5	4.0	0.4	2.0	

#2

IE PROPANOL EXTRACT OF HARDWOODS

Syring-Aldehyde		p- Hydroxy-Benzaldehyde		Vanillic Acid		Syringic Acid		p-Hydroxy-Benzoic Acid		p-Coumaric Acid		Ferulic Acid		Insoluble Tar		
mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	Code No.	Gm.	%
3.8	1.0	6.3	1.7	12.5	3.4	17.8	4.8	63.5	17.2	None	---	None	---	1607-18-A	0.36	18.0
4.8	0.4	None	---	8.5	0.7	8.8	0.7	81.3	6.8	None	---	None	---	1607-42-A	0.48	24.0
2.8	1.1	None	---	9.8	3.5	12.3	4.7	27.0	10.4	None	---	None	---	1607-34-A	0.17	8.5
3.5	1.1	None	---	5.0	1.5	5.5	1.7	32.3	10.0	None	---	3.8	1.2	1607-92-A	0.27	13.5
2.8	0.4	9.5	1.3	6.0	0.8	7.0	1.0	41.0	5.5	12.8	1.7	7.5	1.0	1607-84-A	0.22	11.0
3.5	0.8	None	---	16.0	3.8	9.5	2.3	120.5	28.7	None	---	None	---	1607-150-A	0.45	22.5
2.5	0.4	None	---	13.3	2.0	6.3	1.0	42.5	6.5	None	---	18.8	2.9	1607-76-A	0.43	21.5
3.0	0.3	None	---	8.5	1.0	14.3	1.7	33.0	3.8	None	---	8.8	1.0	1607-114-A	0.38	19.0
2.5	0.4	9.3	1.2	7.3	1.0	4.8	0.7	6.8	1.0	13.5	1.9	11.3	1.6	1607-146-A	0.43	21.5
3.5	0.6	None	---	7.0	1.2	5.3	0.9	68.3	11.4	None	---	14.3	2.4	1607-104-A	0.25	12.5
5.8	1.8	None	---	5.8	1.8	5.5	1.7	19.0	6.0	None	---	None	---	1607-72-A	0.09	4.5
3.5	1.1	None	---	12.8	3.9	15.8	4.8	None	---	None	---	8.0	2.4	1607-30-A	0.24	12.0
2.8	0.6	None	---	7.8	1.6	10.8	2.3	None	---	None	---	7.3	1.5	1642-82-A	0.57	28.5
3.8	1.0	None	---	6.8	1.8	6.5	1.7	None	---	None	---	2.8	0.7	1607-158-A	0.24	12.0
2.5	0.6	None	---	5.5	1.4	19.5	5.0	None	---	None	---	5.0	1.3	1607-116-A	0.15	7.5
3.5	1.0	None	---	5.0	1.5	17.8	5.2	None	---	None	---	4.5	1.3	1607-56-A	0.21	10.5
7.8	4.5	None	---	13.5	7.9	15.8	9.3	None	---	None	---	None	---	1607-66-A	0.11	5.5
4.5	1.2	None	---	14.3	3.9	9.3	2.5	None	---	None	---	18.5	5.0	1607-52-A	0.33	16.5
4.8	0.5	None	---	13.3	1.3	19.3	1.8	None	---	None	---	12.3	1.1	1607-140-A	0.38	19.0
7.0	1.4	None	---	6.8	1.4	9.8	2.0	None	---	None	---	11.5	2.3	1607-154-A	0.98	49.0
6.0	1.0	None	---	10.5	1.7	8.8	1.4	None	---	None	---	8.0	1.3	1642-8-A	1.15	57.5
15.3	2.0	None	---	22.0	3.0	14.0	1.8	None	---	None	---	None	---	1642-12-A	0.42	21.0
4.8	1.7	None	---	12.5	4.5	20.0	7.1	None	---	None	---	None	---	1607-96-A	0.33	16.5
9.0	3.6	None	---	12.5	5.0	17.8	7.1	None	---	None	---	None	---	1607-60-A	1.27	63.5
9.3	1.8	None	---	17.3	3.4	13.8	2.7	None	---	None	---	None	---	1642-20-A	0.72	36.0
5.8	1.4	None	---	14.0	3.3	11.8	2.8	None	---	None	---	None	---	1607-128-A	0.97	48.5
3.8	1.3	None	---	23.8	8.2	10.5	3.6	None	---	None	---	None	---	1607-26-A	0.39	19.5
2.5	0.3	None	---	11.2	1.3	13.3	1.5	None	---	None	---	18.0	2.1	1607-100-A	0.49	24.5
2.5	0.8	4.5	1.3	19.3	2.8	17.5	2.5	None	---	13.5	1.9	25.0	3.6	1607-138-A	0.46	23.0
3.5	0.8	None	---	15.3	3.5	11.0	2.5	None	---	None	---	22.5	5.1	1607-64-A	0.52	26.0
3.3	1.0	None	---	10.8	2.9	21.0	5.7	None	---	None	---	6.0	1.6	1607-110-A	0.29	14.5
2.0	0.4	None	---	7.5	1.5	7.3	1.4	None	---	None	---	None	---	1607-122-A	0.08	4.0
4.3	1.5	None	---	10.0	3.4	5.5	1.9	None	---	None	---	None	--	1607-80-A	0.19	9.5
5.8	1.2	None	---	15.0	3.0	17.3	3.5	None	---	None	---	25.3	5.1	1642-36-A	0.24	12.0
2.8	1.3	None	---	7.5	3.6	7.8	3.7	None	---	None	---	None	---	1642-99-A	0.16	8.0
4.3	1.0	None	---	11.5	2.6	12.0	2.7	None	---	None	---	8.8	2.0	1642-28-A	0.89	44.5
5.3	1.2	None	---	11.0	2.6	14.5	3.4	None	---	None	---	9.0	2.1	1642-16-A	1.00	50.0
3.3	0.8	None	---	10.0	2.8	14.5	3.7	None	---	None	---	5.0	1.3	1607-134-A	0.68	34.0
4.5	1.4	None	---	18.5	5.6	17.0	5.3	None	---	None	---	None	---	1607-88-A	0.46	23.0
7.0	0.6	None	---	6.3	0.6	27.3	2.4	None	---	None	---	None	---	1642-44-A	0.43	21.5

#3

Balsam Poplar	Populus tacamahaca	1607-113-A	0.61	30.5	1607-113-B	0.86	43.0	3.5	0.4	3.0	0
Diamond Willow	Salix eriocephala	1607-145-A	0.64	32.0	1607-145-B	0.70	35.0	3.3	0.5	2.5	0
Black Willow	Salix nigra	1607-103-A	0.26	13.0	1607-103-B	0.60	30.0	5.0	0.8	3.5	0
Weeping Willow	Salix babylonica	1607-71-A	0.33	16.5	1607-71-B	0.32	16.0	7.0	2.2	5.8	1
Overcup Oak	Quercus lyrata	1607-29-A	0.09	4.5	1607-29-B	0.33	16.5	3.5	1.1	3.5	1
White Oak	Quercus alba	1642-81-A	0.19	9.5	1642-81-B	0.48	24.0	1.5	0.3	2.8	0
Willow Oak	Quercus phellos	1607-157-A	0.17	8.5	1607-157-B	0.38	19.0	3.3	0.9	3.8	1
Black Oak	Quercus velutina	1607-115-A	0.07	3.5	1607-115-B	0.39	19.5	4.0	1.0	2.5	0
Turkey Oak	Quercus catesbae	1607-55-A	0.12	6.0	1607-55-B	0.34	17.0	4.3	1.3	3.5	1
Scarlet Oak	Quercus coccinea	1607-65-A	0.04	2.0	1607-65-B	0.17	8.5	5.0	2.9	7.8	4
Blackjack Oak	Quercus marilandica	1607-51-A	0.22	11.0	1607-51-B	0.37	18.5	7.0	1.9	4.5	1
East. Red Oak	Quercus borealis	1607-139-A	0.18	9.0	1607-139-B	1.05	52.5	4.3	0.4	4.8	0
Water Oak	Quercus nigra	1607-153-A	0.20	10.0	1607-153-B	0.50	25.0	6.0	1.2	7.0	1
So. Red Oak	Quercus falcata	1642-7-A	0.11	5.5	1642-7-B	0.62	31.0	5.0	0.8	6.0	1
Black Ash	Froxinus nigra	1642-11-A	0.07	3.5	1642-11-B	0.77	38.5	6.3	0.8	15.3	2
Silver Maple	Acer saccharinum	1607-95-A	0.08	3.0	1607-95-B	0.28	14.0	4.5	1.6	4.8	1
Soft Maple	Acer rubrum	1607-59-A	0.52	26.0	1607-59-B	0.25	12.5	9.0	3.6	9.0	1
Hard Maple	Acer saccharum	1642-19-A	0.14	7.0	1642-19-B	0.51	25.5	7.8	1.5	9.3	1
Red Maple	Acer rubrum	1607-127-A	0.19	9.5	1607-127-B	0.42	21.0	5.5	1.3	5.8	
Box Elder	Acer negundo	1607-25-A	0.38	19.0	1607-25-B	0.29	14.5	7.3	2.5	3.8	
Basswood	Tilia americana	1607-99-A	0.23	11.5	1607-99-B	0.87	43.5	3.3	0.4	2.5	
White Basswood	Tilia heterophylla	1607-137-A	0.18	9.0	1607-137-B	0.70	35.0	6.3	1.8	2.5	
American Elm	Ulmus americana	1607-63-A	0.22	11.0	1607-63-B	0.44	22.0	6.8	1.5	3.5	
Shagbark Hickory	Carya ovata	1607-109-A	0.14	7.0	1607-109-B	0.37	18.5	4.3	1.2	3.3	
Butternut	Juglans cinerea	1607-121-A	0.25	12.5	1607-121-B	0.51	25.5	2.5	0.5	2.0	
Chestnut	Castanea dentata	1607-79-A	0.012	0.6	1607-79-B	0.29	14.5	4.0	1.4	4.3	
Black Gum	Nyssa sylvatica	1642-35-A	0.27	13.5	1642-35-B	0.50	25.0	9.5	1.9	5.8	
Sweet Gum	Liquidamber styrauflua	1642-98-A	0.07	3.5	1642-98-B	0.21	10.5	3.0	1.4	2.8	
Yellow Poplar	Liriodendron tulipifera	1642-27-A	0.36	18.0	1642-27-B	0.44	22.0	4.3	1.0	4.3	
Magnolia	Magnolia grandiflora	1642-15-A	0.20	10.0	1642-15-B	0.43	21.5	6.5	1.5	5.3	
White Bay	Magnolia virginiana	1607-133-A	0.29	14.5	1607-133-B	0.39	19.5	4.8	1.2	3.3	
Yellow Birch	Betula lutea	1607-87-A	0.60	30.0	1607-87-B	0.33	16.5	3.8	1.2	4.5	
Paper Birch	Betula papyrifera	1642-43-A	0.35	17.5	1642-43-B	1.13	56.5	4.3	0.4	7.0	
Beech	Fagus grandifolia	1642-23-A	0.24	12.0	1642-23-B	0.68	34.0	11.3	1.7	17.3	
Am. Sycamore	Platanus occidentalis	1607-125-A	0.30	15.0	1607-125-B	0.59	29.5	3.5	0.6	3.0	
Ironwood	Carpinus caroliniana	1642-31-A	0.16	8.0	1642-31-B	0.32	16.0	3.0	1.0	2.8	
Lilac	Syringa vulgaris	1642-90-A	0.49	24.5	1642-90-B	0.57	28.5	6.0	1.1	2.5	
Catalpa	Catalpa speciosa	1642-39-A	0.22	11.0	1642-39-B	0.56	28.0	6.0	1.1	6.8	
Fraser Magnolia	Magnolia fraseri	1642-106-A	0.12	6.0	1642-106-B	0.44	22.0	4.5	1.0	6.0	
Tupelo Gum	Nyssa aquatica	1642-102-A	0.12	6.0	1642-102-B	0.23	11.5	3.5	1.5	3.3	

3.0	0.3	None	---	8.5	1.0	14.3	1.7	33.0	3.8	None	---	8.8	1.0	1607-114-A	0.38	19.0
2.5	0.4	9.3	1.2	7.3	1.0	4.8	0.7	6.8	1.0	13.5	1.9	11.3	1.6	1607-146-A	0.43	21.5
3.5	0.6	None	---	7.0	1.2	5.3	0.9	68.3	11.4	None	---	14.3	2.4	1607-104-A	0.25	12.5
5.8	1.8	None	---	5.8	1.8	5.5	1.7	19.0	6.0	None	---	None	---	1607-72-A	0.09	4.5
3.5	1.1	None	---	12.8	3.9	15.8	4.8	None	---	None	---	8.0	2.4	1607-30-A	0.24	12.0
2.8	0.6	None	---	7.8	1.6	10.8	2.3	None	---	None	---	7.3	1.5	1642-82-A	0.57	28.5
3.8	1.0	None	---	6.8	1.8	6.5	1.7	None	---	None	---	2.8	0.7	1607-158-A	0.24	12.0
2.5	0.6	None	---	5.5	1.4	19.5	5.0	None	---	None	---	5.0	1.3	1607-116-A	0.15	7.5
3.5	1.0	None	---	5.0	1.5	17.8	5.2	None	---	None	---	4.5	1.3	1607-56-A	0.21	10.5
7.8	4.5	None	---	13.5	7.9	15.8	9.3	None	---	None	---	None	---	1607-66-A	0.11	5.5
4.5	1.2	None	---	14.3	3.9	9.3	2.5	None	---	None	---	18.5	5.0	1607-52-A	0.33	16.5
4.8	0.5	None	---	13.3	1.3	19.3	1.8	None	---	None	---	12.3	1.1	1607-140-A	0.38	19.0
7.0	1.4	None	---	6.8	1.4	9.8	2.0	None	---	None	---	11.5	2.3	1607-154-A	0.98	49.0
6.0	1.0	None	---	10.5	1.7	8.8	1.4	None	---	None	---	8.0	1.3	1642-8-A	1.15	57.5
15.3	2.0	None	---	22.0	3.0	14.0	1.8	None	---	None	---	None	---	1642-12-A	0.42	21.0
4.8	1.7	None	---	12.5	4.5	20.0	7.1	None	---	None	---	None	---	1607-96-A	0.33	16.5
9.0	3.6	None	---	12.5	5.0	17.8	7.1	None	---	None	---	None	---	1607-60-A	1.27	63.5
9.3	1.8	None	---	17.3	3.4	13.8	2.7	None	---	None	---	None	---	1642-20-A	0.72	36.0
5.8	1.4	None	---	14.0	3.3	11.8	2.8	None	---	None	---	None	---	1607-128-A	0.97	48.5
3.8	1.3	None	---	23.8	8.2	10.5	3.6	None	---	None	---	None	---	1607-26-A	0.39	19.5
2.5	0.3	None	---	11.2	1.3	13.3	1.5	None	---	None	---	18.0	2.1	1607-100-A	0.49	24.5
2.5	0.8	4.5	1.3	19.3	2.8	17.5	2.5	None	---	13.5	1.9	25.0	3.6	1607-138-A	0.46	23.0
3.5	0.8	None	---	15.3	3.5	11.0	2.5	None	---	None	---	22.5	5.1	1607-64-A	0.52	26.0
3.3	1.0	None	---	10.8	2.9	21.0	5.7	None	---	None	---	6.0	1.6	1607-110-A	0.29	14.5
2.0	0.4	None	---	7.5	1.5	7.3	1.4	None	---	None	---	None	---	1607-122-A	0.08	4.0
4.3	1.5	None	---	10.0	3.4	5.5	1.9	None	---	None	---	None	---	1607-80-A	0.19	9.5
5.8	1.2	None	---	15.0	3.0	17.3	3.5	None	---	None	---	25.3	5.1	1642-36-A	0.24	12.0
2.8	1.3	None	---	7.5	3.6	7.8	3.7	None	---	None	---	None	---	1642-99-A	0.16	8.0
4.3	1.0	None	---	11.5	2.6	12.0	2.7	None	---	None	---	8.8	2.0	1642-28-A	0.89	44.5
5.3	1.2	None	---	11.0	2.6	14.5	3.4	None	---	None	---	9.0	2.1	1642-16-A	1.00	50.0
3.3	0.8	None	---	10.0	2.8	14.5	3.7	None	---	None	---	5.0	1.3	1607-134-A	0.68	34.0
4.5	1.4	None	---	18.5	5.6	17.0	5.3	None	---	None	---	None	---	1607-88-A	0.46	23.0
7.0	0.6	None	---	6.3	0.6	27.3	2.4	None	---	None	---	None	---	1642-44-A	0.43	21.5
17.3	2.5	None	---	22.3	3.3	16.3	2.4	None	---	None	---	34.3	5.0	1642-24-A	0.63	31.5
3.0	0.5	None	---	34.3	5.8	16.5	2.8	3.3	0.6	70.8	12.0	68.5	11.6	1607-126-A	0.49	24.5
2.8	0.9	None	---	10.3	3.2	8.8	2.8	None	---	None	---	None	---	1642-72-A	0.97	48.5
2.5	0.4	None	---	16.0	2.8	11.0	1.9	None	---	None	---	7.0	1.2	1642-91-A	0.72	36.0
6.8	1.2	None	---	30.8	5.5	12.5	2.2	7.5	1.3	2.8	0.5	102.0	18.2	1642-40-A	0.35	17.5
6.0	1.4	None	---	14.5	3.3	21.0	4.8	None	---	None	---	15.5	3.5	1642-107-A	1.14	57.0
3.3	1.4	None	---	16.5	7.2	12.3	5.3	None	---	None	---	15.3	6.7	1642-103-A	0.62	31.0

#4

CHROMATOGRAPHIC RESULTS - 2nd ETHER EXTRACT DEVELOPI

Rf Value	Populus tremuloides	Populus grandidentata	Populus deltoides	Populus alba	Populus tremula	Populus trichocarpa	Populus nigra	Populus tacamahaca	Salix eriocephala	Salix nigra	Salix babylonica	Quercus lyrata	Quercus alba	Quercus phellos	Quercus velutina	Quercus catesbael	Quercus coccinea	Quercus marilandica	Quercus borealis	Quercus nigra	Quercus falcata	Fraxinus nigra	Acer saccharinum	Acer rubrum	Acer saccharum	Acer rubrum	Acer negundo	Tilia americana
	Aspen	Big Tooth Aspen	Cottonwood	Eu. White Poplar	European Poplar	Northern Black Cottonwood	Lombardy Poplar	Balsam Poplar	Diamond Willow	Black Willow	Weeping Willow	Overcup Oak	White Oak	Willow Oak	Black Oak	Turkey Oak	Scarlet Oak	Blackjack Oak	East. Red Oak	Water Oak	So. Red Oak	Black Ash	Silver Maple	Soft Maple	Hard Maple	Red Maple	Box Elder	Basswood
.00	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.03						X			X													X			X			
.08	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.10	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.12	X	X	X	XF	R&C	X	XF	XF	F&C	XF	X	F	F	F	F	F		F	F	F	F							F
.14	X									X	X						X										X	
.16											X						X					X						
.27	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.38	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.44	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.69		X		X	X	X		X									X											
.76											X														X			
.80	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
.90						X		X			X		X				X			X		X					X	

EXTRACT DEVELOPED WITH BUTANOL 2% AQUEOUS AMMONIA

Acer rubrum	Acer saccharum	Acer rubrum	Acer negundo	Tilia americana	Tilia heterophylla	Ulmus americana	Carya ovata	Juglans cinerea	Castanea dentata	Nyssa sylvatica	Liquidamber styraciflua	Liriodendron tulipifera	Magnolia grandiflora	Magnolia virginiana	Betula lutea	Betula papyrifera	Fagus grandifolia	Platanus occidentalis	Carpinus caroliniana	Syringa vulgaris	Catalpa speciosa	Magnolia fraseri	Nyssa aquatica	
Soft Maple	Hard Maple	Red Maple	Box Elder	Basswood	White Basswood	American Elm	Shagbark Hickory	Butternut	Chestnut	Black Gum	Sweet Gum	Yellow Poplar	Magnolia	White Bay	Yellow Birch	Paper Birch	Beech	Am. Sycamore	Ironwood	Lilac	Catalpa	Fraser Magnolia	Tupelo Gum	
X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
	X																X							
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Syringic Acid
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Vanillic Acid
				F	RC	F	F			F		F	F	F			F	X RC		F	X RC	F	F	P-Hydroxybenzoic F-Ferulic C-Coumaric
			X			X			X						X			X			X			
									X						X		X							
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Syringaldehyde
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Vanillin
										X			X				X							
	X														X	X								
X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X		X	X	X	X	X	
			X		X		X	X	X	X			X	X	X			X		X				

CHROMATOGRAPHIC RESULTS - 2nd ETHER EXTRACT DEVELOPED W.

Rf Values	Aspen	Big Tooth Aspen	Cottonwood	Eu. White Poplar	European Poplar	Northern Black Cottonwood	Lombardy Poplar	Balsam Poplar	Diamond Willow	Black Willow	Weeping Willow	Overcup Oak	White Oak	Willow Oak	Black Oak	Turkey Oak	Scarlet Oak	Blackjack Oak	East. Red Oak	Water Oak	So. Red Oak	Black Ash	Silver Maple	Soft Maple	Hard Maple	Red Maple	Box Elder	Basswood
Populus tremuloides																												
Populus grandidentata																												
Populus deltoides																												
Populus alba																												
Populus tremula																												
Populus trichocarpa																												
Populus nigra																												
Populus tacamahaca																												
Salix eriocephala																												
Salix nigra																												
Salix babylonica																												
Quercus lyrata																												
Quercus alba																												
Quercus phellos																												
Quercus velutina																												
Quercus gatesbael																												
Quercus coccinea																												
Quercus marilandica																												
Quercus borealis																												
Quercus nigra																												
Quercus falcata																												
Froxinus nigra																												
Acer saccharinum																												
Acer rubrum																												
Acer saccharum																												
Acer rubrum																												
Acer negundo																												
Tilia americana																												
.00					X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X		X	X		
.02								X	X			X	X	X	X	X	X		X	X	X	X			X	X		
.06						X			X						X	X		X	X	X	X	X			X	X		
.10						X		X			X	X			X					X	X	X						
.15					X	X	X		X		X		X	X		X			X		X	X				X		
.38	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.44	X	X	X	XF	XF	X	XF	XF	XF	XF	X	XF	XF	XF	XF	XF	X	XF	XF	XF	XF	X	X	X	X	X	X	XF
.56	X	X	X	X	XC	X	X	X	XC	X																		
.70						X			X		X															X		
.78	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
.81									X																			
.84	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ACT DEVELOPED WITH BUTANOL PYRIDINE WATER (10:3:3)

Acer saccharinum	Acer rubrum	Acer saccharum	Acer rubrum	Acer negundo	Tilia americana	Tilia heterophylla	Ulmus americana	Carya ovata	Juglans cinerea	Castanea dentata	Nyssa sylvatica	Liquidamber styraciflua	Liriodendron tulipifera	Magnolia grandiflora	Magnolia virginiana	Betula lutea	Betula papyrifera	Fagus grandifolia	Platanus occidentalis	Carpinus caroliniana	Syringa vulgaris	Catalpa speciosa	Magnolia fraseri	Nyssa aquatica	
Soft Maple																									
Hard Maple		X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Red Maple		X	X			X			X	X								X	X	X		X			
Box Elder		X	X			X			X										X	X		X			
Basswood									X					X				X							
White Basswood			X			X	X				X		X	X			X	X	X	X	X				
American Elm			X			X	X																		
Shagbark Hickory																									
Butternut																									
Chestnut																									
Black Gum																									
Sweet Gum																									
Yellow Poplar																									
Magnolia																									
White Bay																									
Yellow Birch																									
Paper Birch																									
Beech																									
Am. Sycamore																									
Ironwood																									
Lilac																									
Catalpa																									
Fraser Magnolia																									
Tupelo Gum																									

Syringic Acid
Vanillic Acid
F-Ferulic Acid
P-Hydroxybenzoic
C-Coumaric