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(Lignin & Wood Study)  
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# PROJECT REPORT FORM

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COOPERATOR Institute  
REPORT NO. 21  
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NOTE BOOK 1683 and 1731  
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SIGNED *Donald L. Beyer*  
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## THE CAUSTIC HYDROLYSIS OF THE 75% PROPANOL EXTRACTS OF VARIOUS HARDWOODS. II.

In Project 1932, Report No. 11, the 75% propanol extractives of the different hardwoods were hydrolyzed with caustic. The extracts were extracted with ether to obtain a neutral fraction, then acidified and extracted again to obtain a phenolic fraction. The phenolic fraction was chromatographed and the known materials were determined quantitatively.

Since the time that report had been written, five new woods have been received. These woods have been extracted with 75% propanol and the sawdust has been hydrolyzed with caustic (Report No. 20). Also, since that time, two woods have been obtained on which some work had been done, but the supply was too small to enable us to obtain a sufficient amount of the propanol extract for this study.

The 75% propanol extracts from these seven woods were hydrolyzed with caustic following the same procedure as in Report No. 11 (2.0 grams of solids were used as the starting sample). The seven woods to be reported here are:

Red Alder	<u>Alnus rubra</u>
Eucalyptus	<u>Eucalyptus regnans</u>
Ailanthus	<u>Ailanthus altissima</u>
White Ash	<u>Fraxinus americana</u>

River Birch	<u>Betula nigra</u>
Chestnut Oak	<u>Quercus montana</u>
Black Cherry	<u>Prunus serotina</u>

The data on these seven woods is recorded in the following table:

<u>Alnus rubra</u>	<u>Eucalyptus regnans</u>	<u>Allanthus altissima</u>	<u>Fraxinus americana</u>	<u>Betula nigra</u>	<u>Quercus montana</u>	<u>Prunus serotina</u>
0.15 gm. 7.5%	0.04 gm. 2.0%	0.07 gm. 3.5%	0.05 gm. 2.5%	0.07 gm. 3.5%	0.03 gm. 1.5%	0.14 gm. 7.0%
0.33 gm. 16.5%	0.14 gm. 7.0%	0.19 gm. 9.5%	0.15 gm. 7.5%	0.19 gm. 9.5%	0.16 gm. 8.0%	0.30 gm. 15.0%
0.69 gm. 39.5%	1.24 gm. 62.0%	1.28 gm. 64.0%	0.69 gm. 34.5%	1.12 gm. 56.0%	1.16 gm. 58.0%	1.18 gm. 59.0%
3.3 mg. 1.0%	3.8 mg. 2.7%	3.3 mg. 1.7%	3.0 mg. 2.0%	2.0 mg. 1.1%	3.3 mg. 2.1%	2.5 mg. 0.8%
3.8 mg. 1.2%	6.0 mg. 4.3%	2.5 mg. 1.3%	3.0 mg. 2.0%	2.5 mg. 1.3%	2.8 mg. 1.8%	2.8 mg. 0.9%
none	none	none	none	none	none	13.5 mg. 4.5%
15.3 mg. 4.6%	8.8 mg. 6.3%	9.0 mg. 4.8%	14.3 mg. 9.5%	15.8 mg. 8.3%	7.0 mg. 4.4%	8.3 mg. 6.0%
23.5 mg. 7.1%	9.0 mg. 6.4%	9.8 mg. 5.2%	13.0 mg. 8.7%	23.8 mg. 12.5%	7.8 mg. 4.9%	10.3 mg. 7.4%
17.0 mg. 5.2%	none	4.5 mg. 2.4%	8.3 mg. 5.5%	none	8.5 mg. 5.3%	none
none	none	3.0 mg. 1.6%	none	none	none	17.5 mg. 12.5%
3.5 mg. 1.1%	none	none	none	none	none	2.8 mg. 2.0%

terminations:

	<u>Alnus rubra</u>	<u>Eucalyptus regnans</u>	<u>Ailanthus altissima</u>	<u>Fraxinus americana</u>	<u>Betula nigra</u>	<u>Quercus mont.</u>
1st Ether Extract (Neutrals) Yield	0.15 gm. 7.5%	0.04 gm. 2.0%	0.07 gm. 3.5%	0.05 gm. 2.5%	0.07 gm. 3.5%	0.03 gm. 1.
2nd Ether Extract (Phenols) Yield	0.33 gm. 16.5%	0.14 gm. 7.0%	0.19 gm. 9.5%	0.15 gm. 7.5%	0.19 gm. 9.5%	0.16 gm. 8.
Insoluble Tars	0.69 gm. 39.5%	1.24 gm. 62.0%	1.28 gm. 64.0%	0.69 gm. 34.5%	1.12 gm. 56.0%	1.16 gm. 58.
Quantitative determinations:						
Vanillin	3.3 mg. 1.0%	3.8 mg. 2.7%	3.3 mg. 1.7%	3.0 mg. 2.0%	2.0 mg. 1.1%	3.3 mg. 2.
Syringaldehyde	3.8 mg. 1.2%	6.0 mg. 4.3%	2.5 mg. 1.3%	3.0 mg. 2.0%	2.5 mg. 1.3%	2.8 mg. 1.
p-Hydroxybenzaldehyde	none ---	none ---	none ---	none ---	none ---	none ---
Vanillic Acid	15.3 mg. 4.6%	8.8 mg. 6.3%	9.0 mg. 4.8%	14.3 mg. 9.5%	15.8 mg. 8.3%	7.0 mg. 4.
Syringic Acid	23.5 mg. 7.1%	9.0 mg. 6.4%	9.8 mg. 5.2%	13.0 mg. 8.7%	23.8 mg. 12.5%	7.8 mg. 4.
Ferulic Acid	17.0 mg. 5.2%	none ---	4.5 mg. 2.4%	8.3 mg. 5.5%	none ---	8.5 mg. 5.
p-Hydroxybenzoic Acid	none ---	none ---	3.0 mg. 1.6%	none ---	none ---	none ---
p-Coumaric Acid	3.5 mg. 1.1%	none ---	none ---	none ---	none ---	none ---

The ether extracts containing the phenols were chromatographed. The results of these chromatograms follow. The Rf values used correspond to the values used in Report No. 11.

Developed with butanol-2% aqueous ammonia.

Rf- .00; .03; .08; .10; .12; .14; .16; .27; .38; .44; .69; .76; .80; .90

<u>Alnus rubra</u>	X	X	X	X		X	X	X		X
<u>Eucalyptus regnans</u>	X	X	X			X	X	X		X
<u>Ailanthus altissima</u>	X	X	X	X		X	X	X		X
<u>Fraxinus americana</u>	X	X	X	X		X	X	X		X
<u>Betula nigra</u>	X	X	X	X		X	X	X	X	X
<u>Quercus montana</u>	X	X	X	X		X	X	X		X
<u>Prunus serotina</u>	X	X	X	X	X	X	X	X	X	X

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

Rf- .00; .02; .06; .10; .15; .38; .44; .56; .70; .78; .81; .84

<u>Alnus rubra</u>	X				X	X	X		X	X
<u>Eucalyptus regnans</u>					X	X			X	X
<u>Ailanthus altissima</u>					X	X	X		X	X
<u>Fraxinus americana</u>	X				X	X			X	X
<u>Betula nigra</u>	X		X		X	X			X	X
<u>Quercus montana</u>	X	X		X	X	X			X	X
<u>Prunus serotina</u>	X	X		X	X	X	X	X	X	X

The addition of the above results will now bring this series up to date. As new woods are obtained, future reports will be written.

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PAGE 77-80 TO 142-150  
SIGNED *Donald L. Beyer*  
Donald L. Beyer

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

(ADDITIONAL DATA AND CORRECTIONS OF PREVIOUS REPORTS)

Recently, it has been found that acetovanillone and acetosyringone are present in many of the hydrolyzed mixtures of wood. Since these two compounds are completely hidden on the chromatograms by vanillin and syringaldehyde, it is possible that they might exist in the extracts of the many hardwoods heretofore hydrolyzed. The presence of either or both of these compounds would change the results reported for either vanillin, syringaldehyde, or both. However, since the yields of these two aldehydes are very small, and since there are so many variables present which could slightly change the yields, it is believed that the presence of any other compound would not appreciably effect the data already reported.

It does seem important, however, to have the qualitative data as to which extracts might contain either acetovanillone or acetosyringone. In order to obtain the qualitative data, all the ether extracts of the various hardwoods were chromatographed. The technique employed is that previously reported in Project 809-13, Report No. 37. The papers were treated with a sodium bisulfite solution at the top to hold back the aldehydes. The papers were then developed with a solution of heptane, n-butyl ether, water (6:1:1). Several papers were chromatographed for each sample to enable spraying with 2,4-dinitrophenylhydrazine, diazotized p-nitroaniline, and also bis-diazotized benzidine. The authentic compounds were chromatographed along side the extracts to enable comparison to be made. The following qualitative results were obtained.

<u>Scientific Name</u>	<u>Code No.</u>	<u>Acetovanillone</u>	<u>Acetosyringone</u>
<u>Salix nigra</u>	1555-93-A	yes	yes
<u>Salix babylonica</u>	1555-123-A	yes	yes
<u>Salix eriocephala</u>	1555-54-A	no	yes
<u>Populus tremuloides</u>	1359-95-B	no	yes
<u>Populus grandidentata</u>	1527-155-A	yes	yes
<u>Populus tacamahaca</u>	1555-42-A	no	yes
<u>Populus deltoides</u>	1527-54-A	yes	yes
<u>Populus trichocarpa</u>	1527-149-A	yes	yes
<u>Populus alba</u>	1527-117-A	no	yes
<u>Populus nigra</u>	1555-99-A	yes	yes
<u>Populus tremula</u>	1555-36-A	no	yes
<u>Juglans cinerea</u>	1555-129-A	no	yes
<u>Carya ovata</u>	1555-117-A	no	yes
<u>Betula lutea</u>	1527-81-A	yes	yes
<u>Betula papyrifera</u>	1359-124-A	yes	yes
<u>Betula nigra</u>	1712-134-A	no	yes
<u>Alnus rubra</u>	1359-129-A	yes	yes
<u>Carpinus caroliniana</u>	1527-123-A	no	yes
<u>Fagus grandifolia</u>	1555-24-A	yes	yes
<u>Castanea dentata</u>	1555-111-A	yes	yes
<u>Quercus alba</u>	1359-145-A	yes	yes
<u>Quercus lyrata</u>	1359-153-A	yes	yes
<u>Quercus montana</u>	1731-89-A	yes	yes
<u>Quercus borealis</u>	1555-18-A	no	yes
<u>Quercus velutina</u>	1555-73-A	yes	yes
<u>Quercus falcata</u>	1527-47-A	no	yes

<u>Quercus coccinea</u>	1555-67-A	yes	yes
<u>Quercus marilandica</u>	1527-30-A	yes	yes
<u>Quercus catesbaei</u>	1555-79-A	no	yes
<u>Quercus phellos</u>	1555-60-A	yes	yes
<u>Quercus nigra</u>	1527-39-A	yes	yes
<u>Ulmus americana</u>	1555-85-A	yes	yes
<u>Magnolia grandiflora</u>	1527-68-A	yes	yes
<u>Magnolia virginiana</u>	1527-75-A	yes	yes
<u>Magnolia fraseri</u>	1642-53-A	yes	yes
<u>Liriodendron tulipifera</u>	1527-61-A	yes	yes
<u>Liquidambar styraciflua</u>	1527-21-A	yes	yes
<u>Platanus occidentalis</u>	1555-30-A	yes	yes
<u>Prunus serotina</u>	1731-64-A	yes	yes
<u>Ailanthus altissima</u>	1712-138-A	no	yes
<u>Acer saccharum</u>	1527-105-A	yes	yes
<u>Acer rubrum</u>	1527-99-A	yes	yes
<u>Acer saccharinum</u>	1527-135-A	yes	yes
<u>Acer negundo</u>	1555-12-A	yes	yes
<u>Tilia americana</u>	1555-48-A	yes	yes
<u>Tilia heterophylla</u>	1527-129-A	no	yes
<u>Nyssa aquatica</u>	1642-47-A	yes	yes
<u>Nyssa sylvatica</u>	1527-12-A	yes	yes
<u>Fraxinus americana</u>	1712-149-A	no	yes
<u>Fraxinus nigra</u>	1527-93-A	yes	yes
<u>Syringa vulgaris</u>	1642-57-A	yes	yes
<u>Catalpa speciosa</u>	1555-105-A	no	yes
<u>Eucalyptus regnans</u>	1527-87-A	yes	yes

It should be noted that acetosyringone is present in every one of the ether extracts. Acetovanillone is found in most of the extracts. As mentioned before, it did not seem practical to go back and make quantitative corrections for all the samples; however, several samples were selected to be corrected.

We selected eight samples, these samples had changed in volume since first prepared due to the evaporation of the ether. All the samples were diluted to their original volume, which in most cases was 100 ml. and in several cases 50 ml., and total solids were run. From the original data we have the total extract in grams for each sample. Also, from the quantitative data we have the density readings for vanillin and syringaldehyde.

The first step was to chromatograph the present solution, spotting ✓ 0.1 ml. of sample on the sodium bisulfite treated paper. The strips containing acetovanillone and acetosyringone were processed and diluted to 50 ml. From the density reading of this sample we can calculate the mg./l. concentration of the sample to read:

$$D \cdot F = \text{mg./l.}$$

D = density reading  
F = factor used for the compound  
being read

The next equation will give the mg. of material in the present sample.

$$\frac{\text{mg./l.} \cdot 10 \cdot V}{20} = \text{mg. in A} \quad \begin{array}{l} V = \text{volume in ml. for sample} \\ A = \text{present sample} \end{array}$$

Once the yield is found for the present sample, the yield can be found for the original sample by employing a simple proportion.

$$\frac{\text{mg. material in A} - \text{total ext. of A}}{\text{mg. material in B} - \text{total ext. of B}} \quad B = \text{original sample}$$

$$\text{mg. material in B} = \frac{\text{mg. material in A} \cdot \text{total ext. of B}}{\text{total ext. of A}}$$

Once the yield of the compounds has been found for the original sample, the density reading corresponding to this yield can be calculated.

$$\text{mg./l. conc.} = \frac{\text{mg. material in B} \cdot 20}{10 \cdot V}$$

$$D = \frac{\text{mg./l. conc.}}{F}$$

Once the density reading of the compound that corresponds to the original sample is determined it becomes easy to merely subtract that reading from the original density reading for either vanillin or syringaldehyde, whichever the case may be, and from the resulting density figure calculate the yield of vanillin or syringaldehyde.

The results of the eight samples selected were as follows.

dlb/geh

Name	<u>Magnolia</u> <u>fraseri</u>	<u>Syringa</u> <u>vulgaris</u>	<u>Prunus</u> <u>serotina</u>	<u>Quercus</u> <u>montana</u>	<u>Salix</u> <u>babylonica</u>	<u>Betula</u> <u>nigra</u>	<u>Ailanthus</u> <u>altissima</u>	<u>Fraxinus</u> <u>americana</u>
Code no.	1642-53-A	1642-57-A	1731-64-A	1731-89-A	1555-123-A	1712-134-A	1712-138-A	1712-149-A
Original report no.	10	10	20	20	6	20	20	20
Wt. of wood used (g.)	100	100	100	100	100	50	50	50
Original data:								
Vanillin (mg.)	16.5	20.0	11.0	13.5	32.0	9.8	10.5	4.8
(% of ext.)	1.0	0.6	0.8	1.4	1.9	1.4	1.4	1.9
Syringaldehyde (mg.)	38.0	29.0	18.5	17.0	34.0	14.8	15.5	5.0
(% of ext.)	2.2	0.9	1.4	1.7	2.0	2.0	2.1	2.0
Corrected data:								
Vanillin (mg.)	7.5	11.5	6.0	8.0	26.5	9.8	10.5	4.8
(% of ext.)	0.4	0.4	0.4	0.8	1.6	1.2	1.4	1.9
Syringaldehyde (mg.)	22.0	19.5	12.0	9.0	22.5	7.8	9.5	2.8
(% of ext.)	1.3	0.6	0.8	0.9	1.3	1.1	1.3	1.1
Acetovanillone (mg.)	11.3	11.0	6.9	6.7	6.1	none	none	none
(% of ext.)	0.7	0.3	0.5	0.7	0.4	--	--	--
Acetosyringone (mg.)	19.8	11.6	8.0	10.3	14.0	8.8	7.3	2.8
(% of ext.)	1.2	0.4	0.6	1.0	0.8	1.2	1.0	1.1

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SIGNED Donald L. Beyer  
Donald L. Beyer

## THE CAUSTIC HYDROLYSIS OF VARIOUS PALMS

In project 1932 report number 13, a sample of Sabal palmetto was hydrolyzed with caustic. From this experiment, it was found that the ether extract of the hydrolyzed mixture contained a very large amount of p-hydroxybenzoic acid. To ascertain whether or not large yields of p-hydroxybenzoic acid can be obtained from other palms, we requested samples of various palms from several sources. Following our requests, the various palms received were as follows:

From the U. S. Plant Introduction Station, Savannah, Georgia

Sabal palmetto - cabbage palm - petiole

Sabal palmetto - cabbage palm - leaves

Butia capitata - (Cocos australia) - butia palm - petiole

Butia capitata - (Cocos australia) - butia palm - leaves

Rhapidophyllum hystrix - needle palm - petiole

Rhapidophyllum hystrix - needle palm - leaves

From the Fairchild Tropical Gardens, Miami, Florida

Butia bonneti - petiole and several pieces of the trunk.

Serenoa repens - saw palmetto - petiole

Arenga pinnata - sugar palm - petiole

Nanhorapa richardiana - petiole

From the U. S. Department of Agriculture, Agricultural Research Service, Plant Quarantine Branch, Washington D. C. (Shipped from Florida)

Opsiandra maya - petiole

Ptychosperma sp. - petiole  
Pseudophoenix saonae - petiole  
Nannorhops ritchiana - petiole  
Cocos nucifera - coconut palm - petiole  
Phoenix pusilla - petiole  
Cocos coronata - petiole  
Corypha talliera - petiole  
Livistona saribus - petiole  
Phoenix dactylifera - data palm - petiole  
Hyophorbe amaricaulis - bottle palm - petiole and leaves  
Acrocomia armentalis - Cuban acrocomia - petiole  
Inodes causiarum - Puerto Rico Hat Palm - petiole  
Arecastrum romanzoffianum - Queen palm - petiole  
Linoma alba, var aurea - petiole  
Pseudophoenix sargenti - Sargent cherry palm - petiole  
Elaeis guineensis - African oil palm - petiole  
Latania commersonii - petiole  
Roystonea regia - Cuban royal palm - petiole

Each of these samples was air dried, cut into small pieces and then ground into sawdust in the Wiley Mill. The ground sawdust was air dried further and then bagged. Moistures were taken on all bagged samples.

The hydrolysis procedure was the same for every sample. A 50 gram O.D. sample was stirred and heated to boiling in a mixture of 60 grams of sodium hydroxide in 1500 ml. of water for 8 hours. The mixture was then filtered and the solid residue was washed with water. The filtrate was acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a volume of 100 ml. and weighed. A total solids was determined on each sample

so that the total ether extract could be calculated.

The ether solution was then chromatographed. Two developers were used for the qualitative chromatograms. These developers were, butanol-2% aqueous ammonia and butanol - pyridine - water (10:3:3). The developed chromatograms were then sprayed with the following sprays: bis-diazotized benzidine; 2,4-dinitrophenylhydrazine, and the Mäule reagents. Several papers were also sprayed with diazotized p-nitroaniline. In all cases where diazotized p-nitroaniline was used, the results were identical to that of the bis-diazotized benzidine. Both sprays showed the same spots. The diazotized p-nitroaniline showed more brilliant colors to some of the spots, however these materials were known. The colors for the various unknown materials were not at all distinctive. A table of chromatographic results will follow. Several of the Rf values recorded on the table are identified as being two compounds. Naturally, the presence of two materials cannot be determined from a single chromatogram, however, the presence of these compounds were determined from papers developed with other developers.

Finally, the ether extract of each sample was chromatographed quantitatively so as to determine the yields of all the known compounds present. The aldehydes include vanillin, syringaldehyde and p-hydroxybenzaldehyde. However, since acetovanillone and acetosyringone are present, a double set of chromatograms must be used. The technique for determining the yield of these four compounds was reported in 1932 report no. 22. Vanillin, syringaldehyde, acetovanillone, and acetosyringone are present in every sample. There is no pattern found in the yields. However, in most cases, the largest concentration is that of acetosyringone. All yields are small, in only a few isolated cases do the yields of any of these compounds amount to one percent or more of the ether extractives.

p-Hydroxybenzaldehyde was found in about half of the samples. This material also was present in only a small concentration. The p-hydroxybenzaldehyde is not listed on the chromatographic data chart. This compound has the same Rf value as vanillin in both the developers used for qualitative work. To separate the compounds for quantitative yields, the developer used is heptane, n-butyl ether and water (6:1:1). On a regular chromatogram, syringaldehyde and acetosyringone occupy a spot about one quarter the way down, the p-hydroxybenzaldehyde is found about half way down and the vanillin and acetovanillone are together about three-quarters way down. This is for a fifteen hour development. From a second paper, with an area treated with 15% sodium bisulfite to hold back the aldehydes, the acetovanillone and acetosyringone are easily separated.

To quantitatively determine the yield of the acids, the ether extract is developed with a benzene solution saturated with formic acid. Every sample contained vanillic acid, syringic acid and p-hydroxybenzoic acid. The yield of vanillic and syringic acid seemed very uniform. In most samples both acids were found to be about 1.5% to 2.5% of the total ether extractives.

The p-hydroxybenzoic acid was the main product in every sample. The yield of this acid varied considerably, ranging from 4% of the extractives from the leaves of Sabal palmetto to 81.3% of the extractives from the trunk of Sabal palmetto. The yield from the leaves of all the species was low, while the yield from the petiole and trunk was high, averaging between 30 to 60 percent of the extractives. The highest yields obtained were from the trunk of Sabal palmetto and the petiole of Livistona saribus. The yield of p-hydroxybenzoic acid based on the weight of the O.D. sawdust was about 3% in both of these species.

Ferulic acid was found in every extract with the exception of the petiole and the leaves of Sabal palmetto. The yield of ferulic acid was also quite uniform

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averaging about two to three percent of the ether extractives.

p-Coumaric acid was found in most of the species. The yield of p-coumaric acid was the lowest of all the acids averaging about one percent of the ether extractives.

A chart follows showing all of the data accumulated from the hydrolysis of the various palms.

dlb/bvs

## CAUSTIC HYDROLYSIS OF THE SAWDUST OF VARIOUS TREES

WOOD (50 gm. sample)		Ether Extract				QUANTITATIVE DETERMINATION OF KUMIC COMPOUNDS																		
Scientific name	type	Code No.	Gm.	%	Vanillin mg.	%	Syringaldehyde mg.	%	p-Hydroxybenzaldehyde mg.	%	Acetovanillone mg.	%	Acetosyringone mg.	%	Vanillic acid mg.	%	Syringic acid mg.	%	o-Hydroxybenzoic acid mg.	%	o-Coumaric acid mg.	%	Ferulic acid mg.	%
<i>Sabal palmetto</i> (A)	trunk	1689-11-A	1.92	3.8	16.5	0.9*	25.5	1.3*	0.3	0.5	---	---	---	---	25.3	1.3	59.8	3.1	1557.5	81.3	none	---	37.5	2.2
<i>Sabal palmetto</i>	petiole	1731-131-A	1.66	3.3	6.5	0.4	24.3	1.5	5.0	0.3	5.8	0.3	18.0	1.1	21.0	1.3	21.3	1.3	191.0	11.5	none	---	none	---
<i>Sabal palmetto</i>	leaves	1731-135-A	4.09	8.2	4.3	0.1	16.8	0.4	none	---	9.0	0.2	22.0	0.5	19.3	0.5	22.5	0.6	162.0	4.0	none	---	none	---
<i>Butia capitata</i>	petiole	1731-139-A	1.31	2.6	7.5	0.6	13.3	1.0	none	---	6.3	0.5	11.0	0.9	23.5	1.8	41.8	3.2	612.0	46.7	17.3	1.3	21.5	1.6
<i>Butia capitata</i>	leaves	1731-143-A	3.82	7.6	2.8	0.1	5.3	0.1	none	---	5.5	0.1	6.5	0.2	19.3	0.5	23.8	0.6	244.0	6.4	44.3	1.2	14.5	0.4
<i>Cocos nucifera</i>	petiole	1762-43-A	1.40	2.8	4.5	0.3	5.5	0.4	none	---	4.0	0.3	8.5	0.6	33.5	2.4	38.5	2.8	355.0	25.4	36.0	2.6	38.5	2.8
<i>Cocos coronata</i>	petiole	1762-59-A	1.44	2.9	5.0	0.3	4.0	0.3	none	---	11.5	0.8	6.5	0.5	48.0	3.3	47.5	3.3	300.0	20.8	22.0	1.5	36.0	2.5
<i>Rhaphidophyllum hystrix</i>	petiole	1731-147-A	2.13	4.3	15.3	0.7	25.3	1.2	none	---	15.5	0.7	29.8	1.1	40.3	1.9	36.0	1.7	955.0	44.8	20.5	1.0	48.8	2.3
<i>Rhaphidophyllum hystrix</i>	leaves	1731-151-A	3.94	7.9	9.8	0.3	16.0	0.4	none	---	8.5	0.2	36.5	0.9	50.5	1.3	48.5	1.2	1245.0	31.6	78.0	2.0	44.3	1.1
<i>Butia boerhavi</i>	petiole	1762-22-A	1.58	3.2	3.5	0.2	5.0	0.3	6.0	0.4	7.0	0.4	8.5	0.5	21.5	1.3	38.5	2.4	1137.0	72.0	none	---	24.5	1.6
<i>Butia boerhavi</i>	trunk	1762-138-A	1.57	3.1	5.0	0.3	4.2	0.3	none	---	10.9	0.7	10.0	0.6	45.9	2.9	60.1	3.8	379.0	24.2	none	---	40.9	2.6
<i>Serenoa repens</i>	petiole	1762-18-A	1.88	3.8	6.0	0.3	15.0	0.8	12.0	0.6	11.0	0.6	17.0	0.9	29.0	1.5	19.5	1.0	1162.0	61.8	none	---	35.5	1.9
<i>Arenga pinnata</i>	petiole	1762-39-A	0.84	1.7	9.5	1.1	8.5	1.0	4.5	0.5	5.5	0.7	11.5	1.4	21.5	2.6	22.0	2.6	465.0	55.4	none	---	26.0	3.1
<i>Xanthorrhoea richardiana</i>	petiole	1762-35-A	1.46	2.9	8.5	0.6	5.0	0.3	6.5	0.4	4.0	0.3	11.0	0.8	17.5	1.2	22.0	1.5	535.0	36.6	88.0	6.0	33.5	1.2
<i>Xanthorrhoea richardiana</i>	petiole	1762-51-A	1.75	3.5	8.5	0.5	5.5	0.3	none	---	2.5	0.1	7.0	0.4	24.5	1.4	34.0	1.9	597.0	34.1	56.5	3.5	45.5	2.6
<i>Ocotelea naya</i>	petiole	1731-155-A	1.41	2.8	2.3	0.2	3.0	0.2	none	---	4.8	0.3	7.8	0.2	24.8	1.8	13.5	1.0	185.0	13.1	8.8	0.6	24.8	1.8
<i>Psychosperma sp.</i>	petiole	1762-14-A	1.33	2.7	6.5	0.5	11.0	0.8	8.5	0.6	7.0	0.5	5.5	0.4	25.0	1.9	23.0	1.7	585.0	44.0	26.0	2.0	27.5	2.1
<i>Pseudophoenix saundersii</i>	petiole	1762-47-A	1.26	2.5	3.0	0.2	2.5	0.2	none	---	7.5	0.6	7.0	0.6	27.0	2.1	23.0	1.9	300.0	23.8	16.5	1.3	44.5	3.5
<i>Pseudophoenix sargentii</i>	petiole	1762-122-A	1.17	2.3	5.0	0.4	5.0	0.4	9.0	0.8	4.5	0.4	8.0	0.7	19.5	1.7	26.0	2.2	378.0	32.3	6.5	0.6	39.5	3.4
<i>Phoenix nassella</i>	petiole	1762-55-A	1.46	2.9	9.5	0.6	6.5	0.4	none	---	4.0	0.3	9.5	0.7	36.5	2.5	34.5	2.4	618.0	42.3	31.5	2.2	44.5	2.5
<i>Phoenix lactiflora</i>	petiole	1762-73-A	0.94	1.9	3.0	0.3	5.5	0.6	none	---	5.0	0.5	8.0	0.9	22.5	2.4	21.5	2.3	408.0	43.4	9.5	1.0	25.5	2.7
<i>Corypha tallera</i>	petiole	1762-65-A	1.26	2.5	2.5	0.2	5.5	0.4	none	---	6.0	0.5	4.5	0.4	33.0	2.6	35.5	2.9	478.0	37.9	none	---	32.3	2.5
<i>Nivistora saribus</i>	petiole	1762-69-A	2.96	5.0	8.5	0.3	4.5	0.2	none	---	5.0	0.2	10.0	0.4	44.5	1.8	43.5	1.8	1573.0	63.4	none	---	36.5	1.5
<i>Euphorbia americana</i>	petiole	1762-33-A	1.78	3.6	11.5	0.6	10.0	0.6	9.5	0.5	6.5	0.4	5.5	0.3	57.0	3.2	30.5	1.7	565.0	31.7	9.0	0.5	46.5	2.6
<i>Euphorbia americana</i>	leaves	1762-106-A	1.54	3.9	6.5	0.3	12.0	0.6	none	---	4.0	0.2	5.5	0.3	15.0	0.8	10.5	0.5	136.0	7.0	none	---	11.0	0.6
<i>Asrocopia arserialis</i>	petiole	1762-98-A	1.18	2.4	8.5	0.7	7.0	0.6	5.5	0.5	3.0	0.3	13.0	1.1	26.0	2.2	42.5	3.6	305.0	25.8	20.5	1.7	23.0	2.0
<i>Indes castanea</i>	petiole	1762-102-A	1.78	3.6	9.0	0.5	9.5	0.5	17.5	1.0	8.0	0.5	29.0	1.6	25.0	1.4	42.0	2.4	1120.0	63.4	10.5	0.6	30.5	1.7
<i>Arecastrum romanoffianum</i>	petiole	1762-110-A	0.80	1.6	8.0	1.0	13.0	1.6	5.5	0.7	5.0	0.6	9.5	1.2	25.0	3.1	26.0	3.2	312.0	30.0	12.5	1.6	36.0	4.5
<i>Alpinia alba</i> , var. <i>aurea</i>	petiole	1762-118-A	2.00	4.0	10.5	0.5	4.0	0.2	16.0	0.8	6.0	0.3	8.5	0.4	38.0	1.9	22.0	1.1	872.0	43.6	18.5	0.9	55.5	2.8
<i>Alpinia guineensis</i>	petiole	1762-126-A	1.80	3.9	7.0	0.4	15.0	0.8	7.0	0.4	5.5	0.3	15.0	0.8	30.0	1.7	45.0	2.5	1013.0	56.3	12.5	0.7	44.5	2.5
<i>Latania comersonii</i>	petiole	1762-130-A	1.66	3.3	4.5	0.3	4.5	0.3	10.5	0.6	4.0	0.2	11.0	0.7	40.0	2.4	42.5	2.6	739.0	44.5	27.5	1.7	48.0	2.9
<i>Borstenia regia</i>	petiole	1762-134-A	1.11	2.2	6.5	0.6	4.5	0.4	none	---	6.0	0.5	10.0	0.9	26.0	2.3	20.5	2.7	450.0	40.5	19.0	1.7	32.0	2.9

A - Data taken from project 1932 report # 13

\* - Uncorrected values - Acetovanillone and acetosyringone very likely present.

CHROMATOGRAPHIC RESULTS

Developed with Butanol 2% Aqueous Ammonia

Developed with Butanol-Pyridine-Water (10:3:3)

	Rf	Developed with Butanol 2% Aqueous Ammonia													Developed with Butanol-Pyridine-Water (10:3:3)									
		0.00	0.05	0.10	0.12	0.14	0.27	0.38	0.44	0.60	0.76	0.82	0.88	Rf	0.00	0.04	0.09	0.12	0.18	0.27	0.38	0.44	0.56	0.72
<i>Sabal palmetto</i> (A)	trunk	X	X	X	Xa		X	X	X			X		X			X		X	Xa	X	X	X	
<i>Sabal palmetto</i>	petiole	X	Y	X	X		X	X	X	X	X			X	X	X		X	X	X	X	X	X	
<i>Sabal palmetto</i>	leaves	X	X	X	X		X	X	X	X	X			X	X	X		X	X	X	X	X	Y	
<i>Eulia capitata</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Eulia capitata</i>	leaves	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Cocos nucifera</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Cocos coronata</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Rhapidophyllum hystrix</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Rhapidophyllum hystrix</i>	leaves	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Lutia bonnetii</i>	petiole	X	X	X	X		X	X	X	X	X			X	X	X		X	X	X	X	X	X	
<i>Lutia bonnetii</i>	trunk	X	Y	X	Xa		X	Y	Y	Y	X			X	X	X		X	X	Xa	X	X	X	
<i>Serenoa repens</i>	petiole	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Arenga pinnata</i>	petiole	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Nannorhiza richardiana</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Nannorhiza richardiana</i>	petiole	X	X	X	Xa	X	Y	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Opalandra saxa</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		Y	X	X	Xa	X	X	
<i>Ptychosperma sp.</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Pseudophoenix saonae</i>	petiole	X	X	X	Xa	Y	X	X	X	X	X			X	X	X		X	X	Ya	X	X	X	
<i>Pseudophoenix saonae</i>	petiole	Y	X	X	Xa	X	Y	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Phoenix ouallia</i>	petiole	X	X	X	Xa	Y	Y	X	X	Y	Y			X	X	X		X	X	Y	Xa	X	X	
<i>Phoenix lactylifera</i>	petiole	Y	X	X	Xa	X	X	X	Y	Y	X			X	X	X		X	X	Xa	X	X	X	
<i>Corypha talliera</i>	petiole	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Livistona saribus</i>	petiole	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Lyophorbe amaricanalis</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Lyophorbe amaricanalis</i>	leaves	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Acrocomia urundinacea</i>	petiole	X	X	X	Xa	X	X	X	Y	Y	X			X	X	X		X	X	Xa	X	Y	X	
<i>Inodes canariensis</i> (P)	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Arecastrum romanzoffianum</i>	petiole	X	X	X	Xa		X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Linum alba, var. aurea</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		Y	Xa	X	X	X	X	
<i>Elaeis guineensis</i>	petiole	X	X	X	Xa	X	X	Y	X	X	X			X	X	X		X	X	Xa	X	X	X	
<i>Lantanja comersonii</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		Y	Xa	X	X	X	X	
<i>Ravstonoa regia</i>	petiole	X	X	X	Xa	X	X	X	X	X	X			X	X	X		X	X	Xa	X	X	X	

A- Taken from 1932 report #13  
 F- One more spot seen:  
 BuOH - Rf - 0.80  
 Py. - Rf - 0.74  
 Purple to PDB  
 Purple to PNA

Syringic acid  
 Vanillic acid  
 p-Coumaric acid  
 p-Hydroxybenzoic acid  
 o-Ferulic acid  
 Acetovanillone  
 Ferulic acid  
 Syringaldehyde and Acetosyringone  
 Acetovanillone  
 Ferulic acid  
 Syringic acid  
 p-Hydroxybenzoic acid  
 p-Coumaric acid  
 X - ferulic acid  
 Y - ferulic acid  
 Vanillic acid  
 p-Hydroxybenzoic acid  
 Syringaldehyde, Aceto-syringone, and other compounds  
 p-Hydroxybenzoic acid  
 p-Coumaric acid  
 X - ferulic acid  
 Y - ferulic acid  
 Vanillic acid  
 p-Hydroxybenzoic acid  
 Syringic acid  
 and other compounds  
 Vanillic, Acetosyringone and other compounds

# PROJECT REPORT FORM

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Dr. Pearl (2)  
Mr. Beyer

PROJECT NO. 1932  
COOPERATOR Institute  
REPORT NO. 24  
DATE April 20, 1959  
NOTE BOOK 1762  
PAGE 7 TO 13  
SIGNED *Donald L. Beyer*  
Donald L. Beyer

## ETHANOL EXTRACTION OF CATALPA SPECIOSA BARK. II.

In Project 1932, Report Number 18, an ethanol extraction of Catalpa speciosa was reported. The ethanol extract was hydrolyzed with caustic yielding ferulic acid as the main component. The work to be reported here is a repeat of that reported in Project Report Number 18, so as to verify the results previous obtained.

On October 20, 1958 several branches of Catalpa speciosa were obtained for us by Dr. Joranson. The branches were cut from a tree in Appleton. The branches were barked and the bark was air dried. The air dried bark was then ground in the Wiley mill and air dried further. The dried ground bark was then bagged and a moisture was taken.

A 200 gram O.D. sample of the bark was extracted in a Soxhlet with absolute ethanol for a period of 32 hours (4 days). A solid was noticed to have separated from the ethanol solution. This solid was filtered and amounted to 4.0 grams 2.0%. The solid was recrystallized from ethanol yielding a waxy appearing tan solid, m.p. 79-81°. The solid was stored in ethanol and given our number 1762-8-B.

The clear ethanol extract was given our number 1762-8-A and was found to contain 18.8 grams of extract 9.4%.

The ground bark, still wet with ethanol, was covered with 700 ml. of water and allowed to stand for a day. The bark was then extracted in the Soxhlet for an additional 8 hours with absolute ethanol. The ethanol was about 75%. The ground bark was sucked as dry as possible and the solvent was bottled as sample 1862-9-A and contained 13.3 grams of extract, 6.7%.

The ground bark was then extracted with water in the Soxhlet for another 16 hours (2 days). The water solution was concentrated and bottled as sample 1762-10-A and contained 3.33 grams of extract 1.7%.

The total extractive yield from the Catalpa bark was as follows:

Absolute ethanol ext.	1762-8-A	18.8 grams	9.4%
Solid ppt.	1762-8-B	4.0 grams	2.0%
.75% EtOH extract.	1762-9-A	13.3 grams	6.7%
Water extract	1762-10-A	<u>3.3</u> grams	1.7%
		39.4 grams	19.8%

A sample of the absolute ethanol extract (1762-8-A) containing 10 grams of extract was evaporated to dryness. The residue was dissolved in a solution of 200 ml. of 4% sodium hydroxide. The solution was stirred and boiled under reflux for 8 hours.

The caustic solution, when cool, was extracted with ether. The ether solution was concentrated to a small volume and bottled as sample 1762-11-A. This neutral sample amounted to 0.55 grams of extract, 5.5%.

The aqueous solution was then acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and bottled as sample 1762-11-B. This sample containing the phenolic materials amounted to 5.6 grams, 56.0%.

Sample 1762-11-B.

This sample was chromatographed with the following results.

Developed with butanol 2% aqueous ammonia.

Benzidine - $R_f$	0.00; 0.02; 0.08; 0.10; 0.12; 0.14; 0.38; 0.44; 0.60; 0.80
2,4-D. ---- $R_f$	0.38; 0.44
Mäule ---- $R_f$	0.08 0.38
Fluorescent $R_f$	0.12

By comparison-- $R_f$  0.08 is syringic acid;  $R_f$  0.10 is vanillic acid;  
 $R_f$  0.12 is p-hydroxybenzoic acid and also ferulic acid when the spot is fluorescent;  
 $R_f$  0.14 is p-coumaric acid;  $R_f$  0.38 is syringaldehyde and acetosyringone;  $R_f$  0.44  
 is vanillin; and  $R_f$  0.60 is acetovanillone.

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

Benzidine -- $R_f$	0.15; 0.22; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D ----- $R_f$	0.78; 0.84
Mäule ----- $R_f$	0.38 0.78
Fluorescent- $R_f$	0.44

By comparison-- $R_f$  0.38 is syringic acid;  $R_f$  0.44 is vanillic acid and  
 also ferulic acid when fluorescent;  $R_f$  0.56 is both p-hydroxybenzoic and p-coumaric  
 acids;  $R_f$  0.78 is syringaldehyde and acetosyringone while  $R_f$  0.84 is vanillin  
 and acetovanillone.

The known compounds were chromatographed quantitatively to determine  
 the yields. The results were as follows:

Vanillin	-----	5.5 mg. in sample	0.1% of extract.
Syringaldehyde	-----	5.0 mg.	0.1%
Acetovanillone	-----	8.0 mg.	0.1%
Acetosyringone	-----	5.5 mg.	0.1%
Vanillic acid	-----	405.0 mg.	7.2%
Ferulic acid	-----	1070.0 mg.	19.1%
Syringic acid	-----	230.0 mg.	4.1%

p-Coumaric acid	----- 31.0 mg.	0.6%
p-Hydroxybenzoic acid	----- 83.5 mg.	1.5%

The yield of ferulic acid, although a little smaller than that reported in Project Report Number 18, is still the main component.

The fraction 1762-11-B was fractionated and ferulic acid was isolated. The acid was identified by a melting point and a mixed melting point with authentic ferulic acid and also by a matching comparison of the Ultraviolet curve. This work-up was previously reported in Project 809-13 Report Number 38.

dlb/tab

# PROJECT REPORT FORM

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 Dr. Pearl (2)  
 Mr. Beyer

✓ PROJECT NO. 1932  
 COOPERATOR Institute  
 REPORT NO. 25  
 DATE April 20, 1959  
 NOTE BOOK 1762  
 PAGE 26 TO 34  
 SIGNED *Donald L. Beyer*  
 Donald L. Beyer

## CAUSTIC HYDROLYSIS AND NITROBENZENE OXIDATION OF HYDROLYZED POPULUS TREMULOIDES SAWDUST

One of the first caustic hydrolyses of Populus tremuloides sawdust was reported in project 809-13 report number 29. The sawdust after having been hydrolyzed was bottled and stored under our number 1093-115-B. This sample of sawdust was hydrolyzed for a second time to see what known phenolic compounds might be obtained.

The previously hydrolyzed sawdust (50 grams O.D.) of Populus tremuloides was stirred and boiled under reflux with 1500 ml. of water and 60 grams of sodium hydroxide for 8 hours. The cool mixture was filtered and the sawdust was washed with water. The filtrate was acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and bottled as sample 1762-26-A. The ether extract amounted to 0.22 grams, 0.4%.

The sample was chromatographed with the following results.

Developed with butanol-2% aqueous ammonia.

Benzidine --- $R_f$	0.08; 0.10; 0.12; 0.38; 0.44; 0.69; 0.88
2,4-D. ----- $R_f$	0.38; 0.44
Måule ----- $R_f$	0.08 0.38
Fluorescent - $R_f$	nothing.

By comparison-- $R_f$  0.08 is syringic acid;  $R_f$  0.10 is vanillic acid,  
 $R_f$  0.12 is p-hydroxybenzoic acid;  $R_f$  0.38 is syringaldehyde and  $R_f$  0.44 is vanillin.

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

Benzidine -- $R_f$	0.09; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D. ----- $R_f$	0.78; 0.84
Måule ----- $R_f$	0.38 0.78

By comparison-- $R_f$  0.38 is syringic acid;  $R_f$  0.44 is vanillic acid;  
 $R_f$  0.56 is p-hydroxybenzoic acid;  $R_f$  0.78 is syringaldehyde and  $R_f$  0.84 is vanillin.

The sawdust was washed with acetic acid, ethanol and ether, then left to air dry. When dry the sawdust was bottled and labeled 1762-26-B. The O.D. yield was 44.4 grams.

The ether extract was chromatographed quantitatively to determine the yields of the known compounds. The results were as follows.

Vanillic acid	-----	17.8 mg. in sample	8.1% of extract.
Syringic acid	-----	23.0 mg.	10.5%
p-Hydroxybenzoic acid-		11.3 mg.	5.1%
Vanillin	-----	17.3 mg.	7.9%
Syringaldehyde	-----	19.5 mg.	8.9%

Three sawdust samples of Populus tremuloides which had previously been hydrolyzed were oxidized with nitrobenzene according to the procedure of Stone and Blundell, Anal. Chem. 23:771, 1951.

The three samples were:

1. Populus tremuloides wholewood--hydrolysis report 50 in 809-13 Report No. 25.
2. Populus tremuloides 100 mesh wood--hydrolysis reported in 809-13 Report No. 29.
3. Populus tremuloides 100 mesh wood--hydrolysis reported in 809-13 Report No. 29.

The code numbers of the sawdust were: 1 - 1218-150-B; 2 - 1093-111-B; and 3 - 1093-119-B.

Each sawdust sample was run in duplicate. A sample of exactly 0.05 grams of sawdust was covered with approximately 1.5 grams of 2N sodium hydroxide. The amount of sodium hydroxide solution was carefully weighed for each sample. The mixture was then heated in a small bomb at 170° for 2 hours. When cool, the mixture was filtered. The oxidation mixture was then chromatographed both qualitatively and quantitatively. The quantitative determinations were only for vanillin and syringaldehyde. The results showed poor comparisons for the duplicate runs.

Wholewood--1218-150-B	a - Vanillin - 2.0 grams/100 g.
	b - Vanillin - 1.12 grams/100 g.
	a - Syringaldehyde - 2.37 grams/100 g.
	b - Syringaldehyde - 5.55 grams/100 g.
100 mesh wood --1093-111-B	a - Vanillin - 1.68 grams/100 g.
	b - Vanillin - 0.85 grams/100 g.
	a - Syringaldehyde - 2.14 grams/100 g.
	b - Syringaldehyde - 1.65 grams/100 g.
100 mesh wood--1093-119-B	a - Vanillin - 2.57 grams/100 g.
	b - Vanillin - 2.90 grams/100 g.
	a - Syringaldehyde - 6.87 grams/100 g.
	b - Syringaldehyde - 6.82 grams/100 g.

The qualitative chromatograms for acids showed syringic acid present in all three oxidation mixtures. Only the oxidation of the wholewood-1218-150-B contained a trace of p-hydroxybenzoic and p-coumaric acids. None of the samples showed any vanillic acid.

dlb/tab

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PROJECT NO. 1932  
COOPERATOR Institute  
REPORT NO. 26  
DATE April 21, 1959  
NOTE BOOK 1762  
PAGE 10  
SIGNED *Donald L. Beyer*  
Donald L. Beyer

## THE STUDY OF KNOWN COMPOUNDS FROM SABAL PALMETTO AFTER A KRAFT COOK

Upon our request, two 60 pounds bolts of Sabal palmetto were sent to us on January 2, 1959 (and received on January 6, 1959) by the Collier Company, Everglades, Florida. One bolt was sent to the pulp lab, where it was barked and chipped. The chips were put on a 4-mesh screen. The chips remaining on the screen were bagged and kept in the cold room. These chips were given our number 1762-64-A.

Other samples collected, bagged and stored were:

1762-64-B The fines that passed through the 4-mesh screen.

1762-64-C Long stringy fibers and other large pieces removed from the chips.

1762-64-D The bark.

The kraft cook was run for us by Mr. Daniel Bowers of the pulp lab. The following is his notebook data.

"The chips were cooked using the Kraft cooking process in the pulp lab using the 10 pound stationary digester. Cooking conditions are as follows:

Digester charge, chips wt. grams O.D.	3951
Active alkali NaOH as Na <sub>2</sub> O, %	22.5
Sulfidity Na <sub>2</sub> S as Na <sub>2</sub> O, %	25
Water ratio cc./grams O.D.	6.64
Time to maximum temp., minutes	60

Time at maximum temp., minutes	.30
Maximum temp., °C.	172
Digester pressure at max. temp., lb.	104
Yield, per cent of total	32.2
Permanganate number (25 ml. basis)	16.3

A beater run was made. The No. 3 Valley beater used--5500 g. bpl.

<u>Int., min.</u>	<u>SRF, cc.</u>
0	880
5	840
10	790
15	690
20	570
25	440

Strength data for beater run.

<u>Int.</u>	<u>Basic wt.</u>	<u>Caliper</u>	<u>Apparent density</u>	<u>Burst</u>	<u>Tear</u>	<u>Tensile</u>
0 min.						
5 min.						
10 min.	Sheets would not come off of the sheet mold wire on the 0, 5, and 10 min. intervals.					
15 min.	48.0	4.5	10.7	52.1	1.31	17.7
20 min.	46.4	4.2	11.0	60.1	1.47	21.0
25 min.	47.7	4.0	11.9	71.3	1.38	22.8

This concludes Mr. Bowers' data.

The liquor obtained from the above kraft cook of Sabal palmetto amounted to approximately five gallons. The total solids were 12.1 g./100 g.

A liquor sample containing 100 grams of solids was acidified with dilute sulfuric acid and extracted continuously with ether using the air-agitated extractor for a period of about 4 hours at which time the extraction was stopped because of the formation of a very heavy unbreakable emulsion.

Another liquor sample containing 100 grams of solids was acidified with dilute sulfuric acid. The acidified solution was milky gray in appearance. The acidified liquor was filtered through a celite pad. The filtration was very slow. The filtrate was then extracted continuously with ether using the air-agitated extractor for a period of 24 hours. The entire celite pad containing the filtered residue was extracted with ether in a Soxhlet extractor for 16 hours. The ether used for both extractions was combined and concentrated to a small volume. Needle-like crystals separated from the concentrated ether solution. The crystals were filtered and recrystallized from acetone, m.p. 117-118°. The crystals burned with the odor of sulfur dioxide. A sample of the crystals was burned on a silver coin which formed silver sulfide. The solid appeared to be sulfur.

The ether solution was then evaporated to dryness and was redissolved in cold acetone. The final volume was 100 ml., 84 grams and contained 10.05 grams of extract solids. The sample was given our number 1762-95-A.

The sample was chromatographed qualitatively.

Developed with butanol 2% aqueous ammonia.

Benzidine - $R_f$	0.00; 0.01; 0.08; 0.10; 0.12; 0.14; 0.38; 0.44; 0.60; 0.83; 0.90
PNA ----- $R_f$	0.00; 0.08; 0.10; 0.12; 0.14; 0.38; 0.44; 0.60; 0.83; 0.90
2,4-D ----- $R_f$	0.38; 0.44; 0.60
Mäule ----- $R_f$	0.08 0.38
Fluorescent $R_f$	Nothing

(PNA is diazotized p-nitroaniline)

By comparison-- $R_f$  0.08 is syringic acid;  $R_f$  0.10 is vanillic acid;  $R_f$  0.12 is p-hydroxybenzoic acid;  $R_f$  0.14 is p-coumaric acid;  $R_f$  0.38 is syringaldehyde and acetosyringone;  $R_f$  0.44 is vanillin and  $R_f$  0.60 is acetovanillone.

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

Benzidine - $R_f$	0.03; 0.09; 0.14; 0.23; 0.38; 0.44; 0.56; 0.78; 0.84
PNA ----- $R_f$	0.03; 0.09; 0.14; 0.23; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D ----- $R_f$	0.78; 0.84
Mäule ----- $R_f$	0.38 0.78
Fluorescent $R_f$	Nothing

By comparison-- $R_f$  0.38 is syringic acid;  $R_f$  0.44 is vanillic acid;  $R_f$  0.56 is p-hydroxybenzoic and p-coumaric acids;  $R_f$  0.78 is syringaldehyde and acetosyringone and  $R_f$  0.84 is vanillin and acetovanillone.

The known compounds were chromatographed quantitatively to determine the yields.

Compounds	mg. in sample	% of extract
Vanillin	19.0	0.2
Syringaldehyde	22.5	0.2
Acetovanillone	41.5	0.4
Acetosyringone	160.0	1.6
Vanillic acid	295.0	2.9
Syringic acid	290.0	2.9
p-Coumaric acid	30.0	0.3
p-Hydroxybenzoic acid	1935.0	19.3

As expected, p-hydroxybenzoic acid is the main component from the ether extraction of the kraft liquor.

Calculated yield of p-hydroxybenzoic acid based on starting weight of wood.

3951 grams of O.D. wood used in cook.

Water ratio was 6.64:1 which would be 26,235 cc.

Liquor received: 1000 ml. amounted to 1060 grams.

Total liquor in grams would then be 27,809 grams.

Total solids were 12.1 g./100 g. or 3365 grams for entire cooking liquor.

100 grams of solids yielded 1935 mg. of p-hydroxybenzoic acid.

Entire cooking liquor derived from the 3951 grams of wood would then contain 65.11 grams of p-hydroxybenzoic acid, or 1.65% based on the weight of the wood.

dlb/tab

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Donald L. Beyer

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## THE STUDY OF THE KNOWN COMPOUNDS FROM THE CAUSTIC HYDROLYSIS OF A COCONUT

In Project 1932, Report Number 23, the caustic hydrolysis of many palms were reported. Among these palms was Cocos nucifera the coconut palm. The question arose as to whether or not p-hydroxybenzoic acid and the other phenolic compounds would be present in the fruit of the coconut palm.

A coconut was purchased from the Krambo Food Store. The coconut was broken and separated into three parts. The outer shell was left to air dry and was given our number 1762-87-A. The brown fibrous material, hereafter referred to as the inner shell, was cut away from the white coconut meat. Both the inner shell and the coconut meat was left to air dry.

The outer shell was ground to a fine powder in the micro-pulverizer. The powdered shell was bottled and a moisture was taken.

The white coconut meat was cut into small pieces and wax extracted in a Soxhlet with ether for several days. The coconut meat was then air dried and ground to a coarse powder with a pestle in a mortar, yield was 65 grams. The dried meat was given our number 1762-87-C. The ether used in the extraction was evaporated to dryness leaving 117 grams of an oily extract. This oil was given our number 1762-87-B.

The inner shell was also extracted with ether. The ether was evaporated to dryness leaving 17.4 grams of an oily extract which solidified to a waxy solid. This extract was bottled and stored as sample 1762-87-D. The inner shell was air dried and ground to a powder. The yield was 9 grams and carried the number 1762-87-E.

Each of the three powdered coconut samples was hydrolyzed with caustic. A 50-gram sample of both the powdered shell (1762-87-A) and the coconut meat (1762-87-C) was stirred and boiled under reflux for 8 hours in a solution of 60 grams of sodium hydroxide in 1500 ml. of water. For the inner shell, the mixture consisted of 9 grams of the powdered inner shell (1762-87-E), 11 grams of sodium hydroxide and 270 ml. of water.

The hydrolyzed mixtures were filtered, and the residues were washed with water. The filtrates were acidified with dilute sulfuric acid, and the acidified mixtures were extracted with ether. The ether solutions were concentrated to small volumes and total solids were taken. The yield of extracts will follow in a chart.

The three extracts were qualitatively chromatographed. Results will follow.

The three extracts were then chromatographed quantitatively to determine the yields of the known compounds.

	Outer Shell 1762-87-A	White Meat 1762-87-C	Inner Shell* 1762-87-E
Ether ext. yield	2.29 g. 4.6%	1.30 g. 2.6%	1.39 g. 2.8%
Code no.	1762-88-A	1762-152-A	1762-158-A
Vanillin	12.0 mg. 0.6%	none	none
Syringaldehyde	5.0 mg. 0.2%	none	none
Acetovanillone	6.0 mg. 0.3%	none	none
Acetosyringone	6.5 mg. 0.3%	none	none
Vanillic acid	42.0 mg. 1.8%	29.5 mg. 2.3%	41.6 mg. 3.0%
Syringic acid	54.0 mg. 2.4%	26.0 mg. 2.0%	52.7 mg. 3.8%
Ferulic acid	16.5 mg. 0.8%	none	22.2 mg. 1.6%
p-Hydroxybenzoic acid	878.0 mg. 38.3%	30.0 mg. 2.3%	77.7 mg. 5.6%

\*Inner shell data based on starting weight of 50 g.

#### Chromatographic Data

Developed with butanol 2% aqueous ammonia.

#### 1762-88-A (Extract from outer shell)

BDB -- Rf 0.00; 0.08; 0.10; 0.12; 0.38; 0.44; 0.60; 0.88  
 PNA -- Rf 0.00; 0.08; 0.10; 0.12; 0.38; 0.44; 0.60  
 2,4-D. -- Rf 0.38; 0.44; 0.60  
 Maule -- Rf 0.08 0.38  
 Fl. -- Rf 0.12

BDB - bis-diazotized benzidine; PNA diazotized p-nitro aniline.

#### 1762-152-A (Extract from white coconut meat)

BDB -- Rf 0.00; 0.08; 0.10; 0.12; 0.58; 0.88  
 Maule -- Rf 0.08

1762-158-A (Extract from inner shell)

HDB	-- Rf	0.00; 0.08; 0.10; 0.12; 0.88
Mäule	-- Rf	0.08
Fl.	-- Rf	0.12

By comparison - Rf 0.08 is syringic acid; Rf 0.10 is vanillic acid and also ferulic acid when fluorescent; Rf 0.12 is p-hydroxybenzoic acid, Rf 0.38 is syringaldehyde and acetosyringone, Rf 0.44 is vanillin and Rf 0.60 is acetovanillone. Rf - 0.58 was a white waxy appearing spot.

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

1762-88-A

HDB	---- Rf	0.00; 0.04; 0.14; 0.23; 0.38; 0.44; 0.56; 0.78; 0.84
PNA	---- Rf	0.00; 0.14; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D.	---- Rf	0.78; 0.84
Mäule	---- Rf	0.38 0.78
Fl.	---- Rf	0.44

1762-152-A

HDB	-- Rf	0.04; 0.17; 0.38; 0.44; 0.56; 0.76; 0.86
Mäule	-- Rf	0.38

1762-158-A

HDB	-- Rf	0.18; 0.38; 0.44; 0.56; 0.86
Mäule	-- Rf	0.38
Fl.	-- Rf	0.44

By comparison - Rf 0.38 is syringic acid, Rf 0.44 is vanillic acid and also ferulic acid when fluorescent; Rf 0.56 is p-hydroxybenzoic acid; Rf 0.78 is syringaldehyde and acetosyringone; Rf 0.84 is vanillin and acetovanillone. Rf 0.17 and 0.76 found in sample 1762-152-A are waxy materials.

dlb/mah

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REPORT NO. 28  
DATE May 12, 1959 Typed 5-13-59  
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Donald L. Beyer

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## THE CAUSTIC HYDROLYSIS OF VARIOUS BAMBOOS

On February 6, 1959 we received three samples of bamboo from the U. S. Plant Introduction Station, Route 4, Savannah, Georgia. The three samples were entered as follows:

Our Number	Name	U. S. Plant Intro. Station No.
1762-92-A	<u>Phyllostachys vivax</u>	PI No. 82047
1762-92-B	<u>Phyllostachys bambusoides</u>	PI No. 12180
1762-92-C	<u>Phyllostachys viridis</u>	PI No. 77257

All three species were about three years old. The samples we received were all taken from the lower six feet of the culms.

The three samples were ground in the Wiley Mill, air dried, bagged, and a moisture determination was run.

The three samples were hydrolyzed with caustic. A 50 gram o.d. sample of the bamboo was boiled under reflux for 8 hours in a solution of 60 grams of sodium hydroxide and 1500 ml. water. The cool mixture was filtered and the solid was washed with water. The filtrate was acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and weighed. A total solids was run to obtain the yield of total extractives. The ether solution was then chromatographed both qualitatively and quantitatively. The results, combined in a table, will follow.

	<u>Phyllostachys</u> <u>vivax</u>	<u>Phyllostachys</u> <u>bambusoides</u>	<u>Phyllostachys</u> <u>viridis</u>			
Ether extract						
Code No.	1762-114-A	1804-11-A	1804-7-A			
Yield, g.	2.22	1.85	2.00			
Yield, %	4.4	3.7	4.0			
Quantitative determination:						
	mg.	%	mg.	%	mg.	%
Vanillin	27.5	1.2	23.0	1.2	13.5	0.7
Syringaldehyde	13.0	0.6	14.0	0.8	25.0	1.3
p-Hydroxybenzaldehyde	38.5	1.7	32.0	1.7	14.0	0.7
Acetovanillone	8.0	0.4	7.0	0.4	7.5	0.4
Acetosyringone	41.5	1.9	18.5	1.0	19.5	1.0
Vanillic acid	43.0	2.0	64.0	3.4	57.0	2.8
Syringic acid	85.5	3.9	84.5	4.6	68.0	3.4
Ferulic acid	117.0	5.3	82.0	4.4	106.5	5.3
p-Hydroxybenzoic acid	35.0	1.6	60.0	3.2	75.0	3.8
p-Coumaric acid	575.0	25.9	560.0	30.3	710.0	35.5

The main significant fact from the above data is the very high yield of p-coumaric acid found in the ether extractives of bamboo.

The ether extract (1762-114-A) obtained from Phyllostachys vivax was evaporated to dryness. The residue was boiled in water and filtered. Upon cooling, a gummy solid separated. The clear solution was decanted off and cooled further. A yellow solids separated which was filtered, m.p. 203-204°. The clear solution was left to cool further in the refrigerator. Lighter yellow crystals separated, m.p. 205-206°. Both samples were mixed with authentic p-coumaric acid (m.p. 213°) and melted.

m.m.p. with sample melting at 203-204° was 208-212°.

m.m.p. with sample melting at 205-206° was 211-212°.

Both samples were chromatographed alongside of the authentic p-coumaric acid. The spots matched identically on papers developed with butanol-2% aqueous ammonia, butanol - pyridine - water (10:3:3), and also with benzene saturated with formic acid. The papers were sprayed with diazotized p-nitroaniline and the resulting greenish-black color also matched in all cases.

QUALITATIVE DATA

Developed with butanol 2% aqueous ammonia

Rf value	<u>Phyllostachys vivax</u>	<u>Phyllostachys bambusoides</u>	<u>Phyllostachys viridis</u>
0.00	X	X	X
0.08	X	X	X
0.10	X	X	X
0.12	X	X	X
0.14	X	X	X
0.38	X	X	X
0.44	X	X	X
0.60	X	X	X
0.84	X	X	X

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

0.04	X		
0.12	X	X	X
0.27	X	X	X
0.34	X	X	X
0.38	X	X	X
0.44	X	X	X
0.56	X	X	X
0.78	X	X	X
0.84	X	X	X

The known compounds were identified at the following Rf values.

	BuOH 2% aq. amm.	BuOH-Py-H <sub>2</sub> O
	Rf	Rf
Syringic acid	0.08	0.38
Vanillic acid	0.10	0.44
Ferulic acid	0.12	0.44
p-Hydroxybenzoic acid	0.12	0.56
p-Coumaric acid	0.14	0.56
Syringaldehyde	0.38	0.78
Acetosyringone	0.38	0.78
Vanillin	0.44	0.84
p-Hydroxybenzaldehyde	0.44	0.84
Acetovanillone	0.60	0.84

The materials that are found at the same Rf values reported above are easily separated using the developers employed in the quantitative chromatographic procedure.

dlb/mk

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DATE July 6, 1959  
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Donald L. Beyer

## THE COMPLETE DATA ON POPULUS HETEROPHYLLA

On January 15, 1959 we received a 100 pound bolt of swamp cottonwood, Populus heterophylla, from the U. S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Delta Research Center, Stoneville, Miss. (see letter to Mr. Don Theisen from Mr. J. S. McKnight dated January 8, 1959).

The bolt we received had a large center section of wetwood. Together with the bolt, a bag of 20 pounds of bark was also sent.

The bolt was cut by the pulp lab. Half the bolt was saved. The other half was barked and an inner bark of about 1/16 to 1/8 of an inch was removed and kept separate. The wood was then cut, separating the sapwood from the wetwood. All the samples were either cut into sawdust or ground in the Wiley mill, air dried, bagged, and a moisture was taken.

1762-63-A Sapwood cut into sawdust—moisture 6.2%

1762-63-B Wetwood cut into sawdust—moisture 3.3%

1762-63-C Inner bark ground into sawdust.

1762-63-D Outer bark ground into sawdust.

1762-63-E The 20 pound bag of additional bark ground into sawdust.

### 75% Propanol Extraction of Populus heterophylla.

A sample of 214 grams (200 g. O.D.) of the sapwood - 1762-63-A was extracted in a Soxhlet with 75% propanol for 32 hours (4 days). The propanol was concentrated to a small volume and was found to contain 5.61 grams of extract, 2.8%.

The sawdust was air dried, bottled, and a moisture was taken. The yield of the recovered sawdust was 189 grams.

A sample of 200 grams of O. D. Populus heterophylla wetwood was extracted in a Soxhlet with 75% propanol for 24 hours (3 days). The propanol was concentrated to a small volume and was found to contain 4.07 grams of extract, 2.0%.

The sawdust was air dried and a moisture was taken. The O. D. yield was 191.7 grams.

A second extraction was made, extracting 150 grams of O.D. wetwood with 75% propanol. The yield of this extraction, which continued for 24 hours, amounted to 3.4 grams, 2.3%.

#### NaOH Hydrolysis of the Populus heterophylla sawdust.

A 50 grams O.D. sample of both the Populus heterophylla sapwood and the wetwood was hydrolysed with sodium hydroxide. The sawdust sample was treated with 60 grams of sodium hydroxide and 1500 ml. of water. The mixture was boiled under reflux for 8 hours. The reaction mixture was then filtered and the sawdust residue was washed with water. The filtrate was acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and bottled. The sawdust was washed with acetic acid, ethanol, and finally ether. When air dry the sawdust was weighed and bottled. The following data was obtained from the two hydrolysis.

	Sapwood	Wetwood
Ether extract:		
Code No.	1804-23-A	1804-27-A
Yield, grams.	1.58	1.35
Yield, per cent	3.16	2.70

Quantitative determination:	mg.	%	mg.	%
Vanillin	34.0	2.2	34.5	2.6
Syringaldehyde	21.5	1.4	16.5	1.2
Acetovanillone	4.0	0.3	5.5	0.4
Acetosyringone	22.0	1.4	5.5	0.4
Vanillic Acid	29.0	1.8	16.5	1.2
Syringic Acid	29.5	1.9	44.0	3.3
p-Hydroxybenzoic acid	759.0	41.7	735.0	54.5

The chromatographic results were essentially the same for both extracts:

Developed with butanol-2% aqueous ammonia.

Rf	Sapwood	Wetwood	
0.00	X	X	
0.08	X	X	Syringic acid
0.10	X	X	Vanillic acid
0.12	X	X	p-Hydroxybenzoic acid
0.38	X	X	Syringaldehyde and acetosyringone
0.44	X	X	Vanillin
0.60	X	X	Acetovanillone
0.69	X		
0.88	X	X	

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

Rf	Sapwood	Wetwood	
0.18	X	X	
0.27	X	X	
0.38	X	X	Syringic acid
0.44	X	X	Vanillic acid
0.56	X	X	p-Hydroxybenzoic acid
0.78	X	X	Syringaldehyde and acetosyringone
0.84	X	X	Vanillin and acetovanillone

NaOH Hydrolysis of the 75% Propanol Extract of Populus heterophylla sapwood and wetwood.

A sample of the 75% propanol extract of both the sapwood and the wetwood, containing two grams of solids, was evaporated to dryness. The residue was covered with 100 ml. of 4% sodium hydroxide solution and the solution was boiled under reflux for 8 hours. When cool, the caustic solution was extracted with ether. The ether solution was evaporated to dryness leaving a neutral residue. The aqueous solution was then acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and bottled. The following data were obtained.

	Sapwood	Wetwood
Neutral extract:		
Code No.	1804-32-A	1804-47-A
Yield, grams	0.37	0.37
Yield, per cent	18.5	18.5 (based on the 2.0 grams of starting sample)
Ether extract:		
Code No.	1804-32-B	1804-47-B
Yield, grams	0.46	0.22
Yield, per cent	23.0	11.0
Insoluble tars:		
Yield, grams	0.70	1.05
Yield, per cent	35.0	52.5

Quantitative determinations of ether extract

	mg.	%	mg.	%
Vanillin	4.8	1.0	5.0	2.3
Syringaldehyde	2.0	0.4	5.0	2.3
Acetovanillone	none	---	2.8	1.3
Acetosyringone	2.8	0.6	2.5	1.1
Vanillic acid	4.4	1.0	9.5	4.3
Syringic acid	8.3	1.8	6.5	3.0
Ferulic acid	4.8	1.0	7.0	3.2
p-Hydroxybenzoic acid	55.3	12.1	54.5	24.8

The chromatographic results were almost identical.

Developed with butanol 2% aqueous ammonia

Rf	Sapwood 1804-32-B	Wetwood 1804-47-B	
0.00	X	X	
0.08	X	X	Syringic acid
0.10	X	X	Vanillic acid
0.12	X	X	p-Hydroxybenzoic and ferulic acids.
0.38	X	X	Syringaldehyde and acetosyringone
0.44	X	X	Vanillin
0.60		X	Acetovanillone
0.80	X	X	

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

Rf	Sapwood 1804-32-B	Wetwood 1804-47-B	
0.00	X	X	
0.38	X	X	Syringic acid
0.44	X	X	Vanillic and ferulic acids
0.56	X	X	p-Hydroxybenzoic acid
0.78	X	X	Syringaldehyde and acetosyringone
0.84	X	X	Vanillin and acetovanillone

H<sub>2</sub>SO<sub>4</sub> Hydrolysis of the 75% Propanol Extract of Populus heterophylla sapwood and wetwood.

A sample of the 75% propanol extract of both the sapwood and the wetwood, containing 2.0 grams of solids, was evaporated to dryness. The residue was covered with 100 ml. of 0.5N sulfuric acid, and the mixture was boiled under reflux for 4 hours. When cool, 50 mg. of ribose were added to the mixture. The mixture was extracted with ether. The ether extract was concentrated to a small volume and bottled. The aqueous solution was evaporated to remove all traces of ether and then passed through a small column packed with Duolite A-7 anion resin. The effluent was concentrated to a known small volume and bottled.

As before, the ether solution was chromatographed quantitatively for the known materials present. The aqueous sample was chromatographed quantitatively for sugars. In order to compare the yield of sugars present in the hydrolyzed solution, another sample of the 75% propanol solution was evaporated to dryness and the residue was boiled in 100 ml. of water for 1 hour. The aqueous solution was filtered and concentrated to a known volume and bottled. A 50 mg. amount of ribose was also added to the water solution after boiling. This ribose sample is used as a standard in the quantitative procedure. The following data was obtained from the acid hydrolysis.

	Sapwood	Wetwood
Ether extract:		
Code No.	1804-36-A	1804-51-A
Yield, grams	0.64	0.26
Yield, per cent	32.0	13.0
Insoluble tars:		
Yield, grams	1.04	1.26
Yield, per cent	52.0	63.0

Quantitative determination--phenols:

	mg.	%	mg.	%
Vanillin	1.7	0.3	2.0	0.7
Syringaldehyde	2.0	0.3	3.5	1.3
Acetovanillone	1.4	0.2	1.3	0.5
Acetosyringone	2.0	0.3	2.5	0.9
Vanillic acid	11.7	1.8	9.8	3.8
Syringic acid	7.2	1.1	10.3	4.0
p-Hydroxybenzoic acid	19.4	3.0	12.8	4.9

Quantitative determination--sugars:

	mg.	mg.
Galactose: Before hydrolysis	19	none
After hydrolysis	58	14
Glucose: Before hydrolysis	117	none
After hydrolysis	153	36
Mannose: Before hydrolysis	10	none
After hydrolysis	18	10

There was no arabinose, xylose, or rhamnose found in any of the samples.  
 Also for the phenols, there was no ferulic or p-coumaric acids found in any sample.

The chromatographic results for the two ether extracts were identical.

Developed with butanol 2% aqueous ammonia.

BDB----Rf - 0.00; 0.08; 0.10; 0.12; 0.38; 0.44; 0.60; 0.88  
 2,4-D.-Rf - 0.38; 0.44; 0.60  
 Mäule--Rf - 0.08; 0.38

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

EDB----Rf - 0.00; 0.38; 0.44; 0.56; 0.78; 0.84  
 2,4-D.-Rf - 0.78; 0.84  
 Mäule--Rf - 0.38; 0.78

Ethanol Extraction of Populus heterophylla Bark.

A sample of 400 grams of O.D. bark sawdust was extracted in a Soxhlet with absolute ethanol for a period of 28 hours (4 days). The ethanol solution was then concentrated to a small volume and bottled. The extractives amounted to 44.7 grams, 11.2%. The ethanol solution was given our number 1804-31-A and was given to Dr. Pearl.

dlb/tab

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PROJECT NO. 1932  
COOPERATOR Institute  
REPORT NO. 30  
DATE July 6, 1959  
NOTE BOOK 1804  
PAGE 15 TO 22  
SIGNED *Donald L. Beyer*  
Donald L. Beyer

## THE CAUSTIC HYDROLYSIS OF SABAL PALMETTO SAWDUST AND BARK

In Project 1932 Report Number 23, the caustic hydrolyses of many palms were reported. However, the data for Sabal palmetto was incomplete, since no quantitative data was available for either acetovanillone or acetosyringone. It was during the time that the palms were being hydrolyzed that the presence of acetovanillone and acetosyringone was found. However, the extract from Sabal palmetto was lost and consequently it was impossible to obtain the additional data.

In Project 1932 Report Number 26, referred to our receiving two bolts of Sabal palmetto from the Collier Company, Everglades, Fla. A small section from one bolt was cut using a circle saw in such a way as to remove the bark from the inner wood. Both the bark and the inner wood were dried and ground into sawdust using the Wiley mill.

Both the bark and the inner wood sawdust were hydrolyzed with caustic. The procedure followed that of Report number 23. A 50 gram O. D. sample was treated with 60 grams of C.P. sodium hydroxide and 1500 ml. of water. The mixture was boiled under reflux for 8 hours. When cool, the mixture was filtered and the residue was washed with water. The filtrate was acidified with dilute sulfuric acid and extracted with ether. The ether solution was concentrated to a small volume and bottled.

The data obtained from the two hydrolyses follow.

	Inner Wood	Bark
Ether extract:		
Code no.	1804-15-A	1804-19-A
Yield, grams	1.98	2.78
Yield, per cent	4.0	5.6

Quantitative determinations:

	mg.	%	mg.	%
Vanillin	9.0	0.5	8.5	0.3
Syringaldehyde	26.5	1.3	2.5	0.1
p-Hydroxybenzaldehyde	5.5	0.3	none	---
Acetovanillone	6.0	0.3	23.5	0.8
Acetosyringone	9.5	0.5	9.5	0.3
Vanillic acid	40.0	2.0	68.0	2.4
Syringic acid	53.5	2.7	20.5	0.8
Ferulic acid	34.0	1.7	89.0	3.2
p-Hydroxybenzoic acid	1337.0	67.5	1343.0	48.3

The qualitative chromatographic data for both samples were identical.

Developed with butanol 2% aqueous ammonia

BDB .... Rf - 0.00; 0.08; 0.10; 0.12; 0.27; 0.38; 0.44; 0.60; 0.88  
 2,4-D... Rf - 0.38; 0.44; 0.60  
 Male .. Rf - 0.08; 0.38  
 Fluorescent Rf - 0.12

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

BDB ..... Rf - 0.00; 0.04; 0.12; 0.18; 0.27; 0.38; 0.44; 0.56; 0.78; 0.84  
 2,4-D ... Rf - 0.78; 0.84  
 Male ... Rf - 0.38; 0.78  
 Fluorescent Rf - 0.44

dlb/bvs

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COOPERATOR Institute  
REPORT NO. 31  
DATE July 22, 1959  
NOTE BOOK 1804  
PAGE 59 TO 75  
SIGNED Donald L. Beyer  
Donald L. Beyer

## THE COMPLETE DATA FOR PIN CHERRY (PRUNUS PENNSYLVANIA)

In the past reports of project 1932, many different hardwoods have been processed. There have been five different experiments run on each of the wood samples that we received. These experiments were: the caustic hydrolysis of the sawdust; the 75% propanol extraction of the sawdust; the caustic hydrolysis of the 75% propanol extract; the sulfuric acid hydrolysis of the 75% propanol extract, and a water extraction of the 75% propanol extract. Now as new wood samples are received, we will conduct these five experiments on the wood and the resulting data for the individual wood will be reported.

On May 25, 1959 we received a bolt of Prunus pennsylvanica (Pin Cherry) from the Hammermill Paper Co., Erie, Pennsylvania. (See letter of C. H. Alder dated May 15, 1959.) The wood was barked and cut into sawdust. The bark was bottled and stored under our number 1804-44-B. The sawdust was air dried and bagged, identified with our number 1804-44-A.

A 50 gram O.D. sample of the sawdust was hydrolyzed with sodium hydroxide. The resulting ether extract amounted to 0.70 grams, 1.4%. The recovered sawdust amounted to 30.2 grams O.D. The ether extract was labeled 1804-57-A and the recovered sawdust was 1804-57-B.

The ether extract was then chromatographed both qualitatively and quantitatively. Qualitative results:

Developed with butanol-2% aqueous ammonia.

BDB.....	Rf -	0.00; 0.08; 0.10; 0.12; 0.14; 0.38; 0.44; 0.60; 0.82
2,4D.....	Rf -	0.38; 0.44; 0.60
Mäule....	Rf -	0.08 0.38
Fluorescent.	Rf -	0.12

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

BDB.....	Rf -	0.01; 0.09; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D....	Rf -	0.78; 0.84
Mäule....	Rf -	0.38; 0.78
Fluorescent.	Rf -	0.44

Quantitative results:

Acetovanillone.....	4.0 mg.	0.6 % of extract
Acetosyringone.....	6.5 mg.	0.9 %
Vanillin.....	9.0 mg.	1.3 %
Syringaldehyde.....	10.5 mg.	1.5 %
p-Hydroxybenzaldehyde.....	16.0 mg.	2.3 %
Vanillic acid.....	32.0 mg.	4.6 %
Syringic acid.....	42.5 mg.	6.1 %
Ferulic acid.....	18.5 mg.	2.6 %
p-Coumaric acid.....	15.5 mg.	2.2 %
p-Hydroxybenzoic acid.....	19.5 mg.	2.8 %

A 200 gram O.D. sample of the sawdust was extracted in a Soxhlet with 75% propanol for a period of 24 hours (3 days). The propanol solution was concentrated to a small volume and bottle as 1804-62-A. The total extractives amounted to 5.25 grams, 2.6%. A second extraction was necessary to accumulate sufficient extractives for the various hydrolysis. This time only a 100 gram O.D. sample was extracted with 75% propanol. The yield of extractives amounted to 2.26 grams, 2.3%.

A sample of the 75% propanol extract containing 2.0 grams of solids was evaporated to dryness. The residue was then dissolved in a solution of sodium hydroxide and hydrolyzed. The caustic solution was extracted with ether. The neutral fraction obtained amounted to 0.19 grams, 9.5%. The aqueous solution was acidified and extracted again with ether. This ether extract (1804-63-B) amounted to 0.48 grams, 24.0%. The insoluble tars that were formed upon acidification amounted to 1.12 grams, 56%.

The ether extract, 1804-63-B was chromatographed both quantitatively and qualitatively.

Qualitative results:

Developed with butanol-2% aqueous ammonia.

BDB.....	Rf -	0.00; 0.08; 0.10; 0.12; 0.14; 0.38; 0.44; 0.60; 0.80
2,4-D....	Rf -	0.38; 0.44; 0.60
Mäule....	Rf -	0.08; 0.38
Fluorescent.	Rf -	0.12

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

BDB.....	Rf -	0.00; 0.06; 0.15; 0.38; 0.44; 0.56; 0.78; 0.84
2,4-D....	Rf -	0.78; 0.84
Mäule....	Rf -	0.38 0.78
Fluorescent.	Rf -	0.44

Quantitative results:

Acetovanillone.....	3.0 mg.	0.6 %
Acetosyringone.....	3.5 mg.	0.7 %
Vanillin.....	3.8 mg.	0.8 %
Syringaldehyde.....	3.8 mg.	0.8 %
Vanillic acid.....	10.3 mg.	2.2 %
Syringic acid.....	25.0 mg.	5.2 %
Ferulic acid.....	6.3 mg.	1.3 %
p-Coumaric acid.....	16.8 mg.	3.5 %
p-Hydroxybenzoic acid.....	11.3 mg.	2.4 %

Another sample of the 75% propanol extract containing 2.0 grams of solids was evaporated to dryness and then hydrolyzed with sulfuric acid. The hydrolyzed solution was extracted with ether. The ether solution (1804-69-A) contained 0.44 grams of solids, 22.0%. The insoluble tars that remained amounted to 1.16 grams 58.0%.

The aqueous solution was then passed a column packed with Duolite A -7 anion resin. The effluent was concentrated and bottled as 1804-69-B. This aqueous solution contains the sugars.

Another sample of the 75% propanol extract containing 2.0 grams of solids was evaporated to dryness. The residue was then boiled in water, filtered, concentrated, and filtered again. The final solution was labeled 1804-74-A and contains the sugars before hydrolysis.

The ether extract, 1804-69-A, was chromatographed both quantitatively and qualitatively.

Qualitative results:

Developed with butanol-2% aqueous ammonia

BDB.....	Rf -	0.00; 0.08; 0.10; 0.12; 0.31; 0.38; 0.44; 0.52; 0.60; 0.79; 0.88
2,4-D.....	Rf -	0.38; 0.44 0.60
Mäule.....	Rf -	0.08; 0.38

No fluorescent spots. Rf 0.31 and 0.52 were red spots to BDB similar to the spots found in Black Cherry

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

BDB.....	Rf -	0.38; 0.44; 0.78; 0.84
2,4-D.....	Rf -	0.78; 0.84
Mäule.....	Rf -	0.38 0.78

No fluorescent spots. The red spots appeared to be combined with other compounds at Rf 0.84.

Quantitative results:

Acetovanillone.....	1.8 mg.	0.4 %
Acetosyringone.....	2.8 mg.	0.6 %
Vanillin .....	1.8 mg.	0.4 %
Syringaldehyde.....	2.5 mg.	0.6 %
Vanillic acid.....	11.3 mg.	2.6 %
Syringic acid.....	7.0 mg.	1.6 %
p-Hydroxybenzoic acid.....	4.8 mg.	1.1 %

The sugar determinations showed the following results:

	Before hydrolysis	After hydrolysis
Galactose .....	14 mg.	26 mg.
Glucose.....	65 mg.	158 mg.
Mannose .....	8 mg.	31 mg.
Arabinose.....	none	15 mg.
Xylose.....	none	11 mg.
Rhamnose.....	none	10 mg.

The above data completes the work on Prunus pennsylvanica. As other wood samples are received the same hydrolysis will be run and the data reported.

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PROJECT NO. 1932  
COOPERATOR IPC  
REPORT NO. 32  
DATE September 9, 1959  
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PAGE \_\_\_\_\_ TO \_\_\_\_\_  
SIGNED *Patricia F. McCoy*  
Patricia F. McCoy

## STABLE DIAZO SALTS FOR CHROMATOGRAPHIC SPRAY REAGENTS

Diazotized amine spray reagents are routinely employed in the location of phenolic materials on paper chromatograms. It was recently learned that the stable diazo salts of many aromatic compounds are commercially produced and can be easily obtained. Thirty one of these dyestuff intermediates were received from Koppers Company, Antara Chemicals, and E. I. du Pont de Nemours and Company, Inc. Twenty phenolic compounds related to wood chemistry were sprayed with these diazo salts and their color reactions are reported in this paper.

### Experimental Procedure:

Whatman No. 1 paper was spotted with four per cent solutions of the twenty phenolic compounds. The spots were exposed to ammonia vapor and immediately sprayed with 0.05% water solutions of the diazo salts. The papers were air dried for half an hour, then sprayed with saturated sodium carbonate and allowed to dry. The colors of the spots were recorded before and after the sodium carbonate spray. For exact comparison, Ridgway's Color Standards and Color Nomenclature was used as a reference. In the tables to follow, the apparent color is listed first, followed by the color name given in the above book. It should be noted that these colors are not indefinitely stable, and for a suitable comparison, should be recorded when the paper has dried thirty to sixty minutes.

The following stable salts of diazotized aromatic amines were obtained from three dye companies.

Koppers Company, Incorporated

Fast Orange GC Salt	m-dichloroaniline
Fast Ponceau L Salt	4-nitro-o-toluidine (5-nitro-2-aminotoluene)
Fast Red AL Salt	Alpha -aminoanthraquinone
Fast Red GL Salt	3-nitro-4-aminotoluene
Fast Red 3GL Salt	4-chloro-2-nitroaniline
Fast Red TR Salt	5-chloro-2-aminotoluene
Fast Scarlet GE Salt	2,5-dichloroaniline
Fast Scarlet R Salt	4-nitro-2-aminoanisole
Fast Blue VB Salt	4-amino-4'-methoxydiphenylamine
Fast Red KB Salt Supra	4-chloro-2-aminotoluene
Fast Bordeaux GP Salt	2-amino-3-nitroanisole
Fast Blue B Salt	Ortho-dianisidine

Antara Chemicals (A sales division of General Aniline and Film Corporation)

Fast Black Salt K	4-amino-2,5-dimethoxy-4'-nitroazobenzene
Fast Blue Salt BBN	4-benzamido-2,5-diethoxyaniline
Fast Bordeaux Salt BD	4-amino-2,5-dimethoxybenzotrile
Fast Brown Salt V	2-amino-2'-chloro-4-methoxy-5-methyl-4'-nitroazobenzene
Fast Corinth Salt V Conc.	4-amino-2,4'-dimethyl-5-methoxy-2'-nitroazobenzene
Fast Garnet Salt GBC New	4-amino-2,3-dimethylazobenzene
Fast Orange Salt GR	o-nitroaniline
Fast Red Salt GG	p-nitroaniline

Fast Red Salt ITRN	N,N-diethyl-3-amino-4-methoxybenzenesulfonamide
Fast Red Salt PDC	N-n-butyl-3-amino-4-methoxybenzenesulfonamide
Fast Red Salt RCN	2-amino-4-chloroanisole
Fast Red Salt RL	3-nitro-2-aminotoluene
Fast Scarlet Salt G	4-nitro-2-aminotoluene
Fast Violet Salt BN	4-benzamido-2-methoxy-5-methylaniline
Fast Yellow Salt GC	o-chloroaniline
Variamine Blue Salt RT	4-aminodiphenylamine
Variamine Blue Salt FG	4-amino-3-methoxydiphenylamine
Fast Orange Salt RDN	2-chloro-5-trifluoromethylaniline

E. I. du Pont de Nemours & Company, Inc.

Du Pont Naphthanil Diazo Blue B	Ortho-dianisidine
Du Pont Naphthanil Diazo Scarlet 2G	2,5-dichloroaniline
Du Pont Naphthanil Diazo Red 3G	4-chloro-2-nitroaniline
Du Pont Naphthanil Diazo Scarlet R	4-nitro-2-aminoanisole
Du Pont Naphthanil Diazo Red AL	alpha-amino-anthraquinone

Twenty phenolic compounds were tested with the above diazotized salts, they are:

1. Acetovanillone
2. Caffeic Acid
3. p-Coumaric Acid
4. 2-5 Dihydroxybenzoic Acid
5. 3-5 Dihydroxybenzoic Acid
6. Ferulic Acid
7. 2-5 Dihydroxyacetophenone
8. p-Hydroxybenzaldehyde
11. p-Hydroxyphenylacetic Acid
12. Quinic Acid
13. Protocatechuic Acid
14. Salicyl Alcohol
15. Syringaldehyde
16. Syringic Acid
17. Vanillic Acid
18. Vanillin

- |                                      |                    |
|--------------------------------------|--------------------|
| 9. p-Hydroxybenzoic Acid             | 19. Vanillin       |
| 10. 2-Hydroxy-5-Methoxy Benzoic Acid | 20. Acetosyringone |

Phenolic Compounds	m-dichloroaniline		4-nitro-o-toluidine		alpha-aminoanthraquinone	
	B	A	B	A	B	A
Acetovanillone	l. yellow	salmon Orange-pink	l. salmon Orange Pink	salmon Shrimp Pink	l. violet Pale Purplish Vinous	l. pink Pale Vinaceous Pink
Caffeic Acid	purple-brown Vinous Gray	dark green- brown Deep Grayish Olive	blue gray Dark Plumbago Slate	green brown Mouse Gray	green brown Mouse Gray	green-brown Andover Green
p-Coumaric Acid	l. cream	coral Coral Pink	orange brown Pinkish Cinnamon	rust L. Russet Vinous	rose Purplish Vinous	rose Purplish Vinous
2-5 Dihydroxybenzoic Acid	v. l. yellow	l. brown l. Drab	l. salmon Shell Pink	olive brown Drab	l. yellow Pale Olive Buff	olive brown Grayish Olive
3-5 Dihydroxybenzoic Acid	gold Apricot Yellow	bright yellow Lemon Chrome	orange Zinc Orange	gold Light Orange Yellow	orange Bittersweet Orange	orange Bittersweet Orange
Ferulic Acid	l. cream	l. purple l. Vinaceous Gray	rust Vinous Pink	purple L. Purplish Vinous	purple brown Livid Brown	purple brown Vinous Drab
2-5 Dihydroxy-acetophenone	green-yellow Chalcedony Yellow	yellow green Citron Green	yellow green Citron Green	yellow green Citron Green	yellow green Citron Green	yellow green Grape Green
p-Hydroxybenzaldehyde	l. peach Light Buff	l. yellow gold Pale Yellow Orange	peach Cream Buff	l. peach Pale Cinnamon Pink	l. coral Shell Pink	l. coral Tilleul Buff

The color names from Ridgway's Color Standards and Color Nomenclature are capitalized  
 B = before sodium carbonate spray      l. or L = light      surr. = surrounding

Phenolic Compounds	m-dichloroaniline		4-nitro-o-toluidine		alpha-aminoanthraquinone	
	B	A	B	A	B	A
1. p-Hydroxybenzoic Acid	1. yellow Marguerite Yellow	gold Buff Yellow	yellow Colonial Buff	orange Orange Buff	coral Jasper Pink	brown Vinous Fawn
2. 2-Hydroxy-5-Methoxy Benzoic Acid	1. salmon Salmon Color	1. rose Pale Purplish Vinaceous	1. peach Pale Ochraceous Buff	cream Pale Pinkish Buff	gray	white
3. p-Hydroxyphenyl-acetic Acid	yellow Naphthalene Yellow	coral Coral Pink	yellow Naphthalene Yellow	salmon Orange Pink	pink L. Purplish Vinaceous	pink L. Purplish Vinaceous
4. Quinic Acid	-	white	-	white	-	white
5. Protocatechuic Acid	1. rust L. Ochraceous Salmon	1. brown L. Cinnamon Drab	1. red brown L. Congo Pink	salmon Salmon Buff	1. yel. brown Naples Yellow	1. yel. brown Olive Buff
6. Salicyl Alcohol	yel. brown c 1. yel. surrounding	yel. surr. by gold Pinard Yel. surr. by Apricot Yel.	1. gold brown	gold Orange Buff	red Etruscan Red	red brown Russet Vinaceous
7. Syringaldehyde	1. gold rim	1. green brown coral rim Pale Olive Buff c Coral Pink	c 1. gold Cream Buff	1. yel. c a little salmon pink	1. red brown Pale Brownish Vinaceous	1. red brown L. Vinaceous Fawn

The color names from Ridgway's Color Standards and Color Nomenclature are capitalized  
 B = before sodium carbonate spray      1. or L = Light      surr. = surrounding  
 A = after sodium carbonate spray      yel. = Yellow      c = with

Phenolic Compounds	m-dichloroaniline		4-nitro-o-toluidine		alpha-aminoanthraquinone	
	B	A	B	A	B	A
16. Syringic Acid	gold Ochraceous Buff	orange Strawberry Pink	orange Ochraceous Buff	dark pink Geranium Pink	brown Russet Vinaceous	brown Vinaceous Brown
17. Vanillic Acid	yellow Naphthalene Yellow	orange Ochraceous Buff	l. yellow green Straw Yellow	orange Bittersweet Pink	red brown Russet Vinaceous	brown Vinaceous Brown
18. Vanillin	yellow gold Maize Yellow	pink c brown rim Seashell Pink	peach Pale Ochraceous Salmon	salmon rim Bittersweet Pink	l. rose Pale Purplish Vinaceous	l. brown L. Vinaceous Fawn
19. Vanilil	cream Cream Color	bright yellow gold L. Cadmium	cream Cream Color	bright yellow gold Primuline Yellow	l. orange gold Ochraceous Buff	bright yellow gold Primuline Yellow
20. Acetosyringone	yellow Deep Colorial Buff	yellow brown Honey Yellow	yellow gold Mustard Yellow	yellow brown Honey Yellow	orange gold Ochraceous Buff	yellow brown Honey Yellow

The color names from Ridgway's Color Standards and Color Nomenclature are capitalized.

B = before sodium carbonate spray      l. or L = light      surr. = surrounding  
 A = after sodium carbonate spray      yel. = yellow      c = with

Phenolic Compounds	3-nitro-4-aminotoluene		4-chloro-2-nitroaniline		5-chloro-2-aminotoluene	
	B	A	B	A	B	A
1. Acetovanillone	l. brown Tilleul Buff	coral Coral Pink	l. yel. brown Cartridge Buff	pink Corinthian Pink	pink Shrimp Pink	l. orange Orange Pink
2. Caffeic Acid	purple brown Vinous Gray	green brown Grayish Olive	dark red brown Cinnamon Drab	green brown Drab	brown Mouse Gray	moss green Citrine Drab
3. p-Coumaric Acid	orange brown Avellaneous	salmon L. Congo Pink	rust Pinkish Cinnamon	rosé Vinous Lilac	l. yellow Barta Yellow	orange Bittersweet Pink
4. 2,5-Dihydroxybenzoic Acid	l. yel. with white ring & pink ring	brown with white surr. ring Drab	white	l. olive Olive Buff	pink Shrimp Pink	v. l. brown
5. 3,5-Dihydroxybenzoic Acid	orange Carrot Red	orange Carrot Red	orange Bittersweet Orange	orange Apricot Orange	orange Orange Ruofus	brown orange Raw Sienna
6. Ferulic Acid	pink brown Vinous Fawn	pink brown Vinous Fawn	rust Rufous	rust Cacao Brown	yel. brown Dark Olive Buff	rose L. Purplish Vinaceous
7. 2,5-Dihydroxy-acetophenone	green yel. Chalcedony Yellow	yel. green Citron Green	yel. green Citron Green	yel. green Citron Green	yel. green Citron Green	yel. green Citron Green

The color names from Ridgway's Color Standards and Color Nomenclature are capitalized.

B = before sodium carbonate spray      L. or L. = light      surr. = surrounding  
 A = after sodium carbonate spray      yel. = yellow      c = with

Phenolic Compounds	3-nitro-4-aminotoluene		4-chloro-2-nitroaniline		5-chloro-2-aminotoluene	
	B	A	B	A	B	A
3. p-Hydroxybenzaldehyde	1. red brown L. Pinkish Cinnamon	1. rust Seashell Pink	1. peach surr. with 1.yel.	1. orange Salmon Color	cream Cartridge Buff	cream Cartridge Buff
7. p-Hydroxybenzoic Acid	orange brown Pinkish Cinnamon	orange brown Apricot Buff	yellow Cream Color	orange Flesh Ocher	1. cream Ivory Yellow	yellow gold Pale Orange Yellow
10. 2-Hydroxy-5-Methoxy Benzoic Acid	blue inside c pink rim Perula Blue c Shell Pink	pink L. Congo Pink	blue rim with pink surr. Russian Blue	rose-purple L. Purplish Vinaceous	peach Cartridge Buff	pink Pale Congo Pink
11. p-Hydroxyphenyl-acetic Acid	1. orange brown Pinkish Cinnamon	pink Corinthian Pink	yellow Straw Yellow	rose Deep Rose Pink	1. yellow Marguerite Yellow	yellow with orange rim
12. Quinic Acid	rose brown	1. brown	white	white	white	1. pink
13. Protocatechuic Acid	1. brown with white rim	1. yellow brown	salmon L. Ochraceous Salmon	brown L. Drab	brown Vinaceous Buff	1. brown
14. Salicyl Alcohol	red with yellow rim L. Jasper Red	orange brown c orange surr. Apricot Buff	salmon pink Coral Pink	orange c salmon surr. Ochraceous Buff Flesh Color	yellow brown Buff Cream Buff	gold with pink rim

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Phenolic Compounds	3-nitro-4-aminotoluene		4-chloro-2-nitroaniline		5-chloro-2-aminotoluene	
	B	A	B	A	B	A
15. Syringaldehyde	pink with white rim Chatenay Pink	yellow with 1. pink rim	white	mosa green Water Green	1. yellow brown Cream Buff	v.l. orange A Cream Buff
16. Syringic Acid	red L. Jasper Red	rose Purplish Vinaceous	pink Jasper Pink	purple Argyle Purple	1. yel. brown Cream Buff	red orange Bittersweet Orange
17. Vanillic Acid	orange Salmon Color	brown orange Congo Pink	salmon L. Ochraceous Salmon	pink Pinkish Vinaceous	yellow Colonial Buff	orange Orange-Pink
18. Vanillin	l. brown Pallid Brown- ish Drab	salmon Flesh Pink	gold L. Ochraceous Buff	pink Pinkish Vinaceous	1. yel. brown Cream Buff	orange Apricot Buff
19. Vanillin	l. yellow orange Maize Yellow	bright yellow gold Primuline Yellow	yellow Straw Yellow	bright yellow gold Strontian Yellow	yellow brown Colonial Buff	bright yellow Strontian Yellow
20. Acetosyringone	yellow orange Buff Yellow	yellow brown Honey Yellow	yellow Straw Color	yellow brown Old Gold	yellow brown (dark) Olive Ocher	yellow brown (dark) Olive Ocher

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Phenolic Compounds	2,5-dichloroaniline		4-nitro-2-aminoanisole		4-amino-4'-methoxydiphenylamine	
	B	A	B	A	B	A
1. Acetovanillone	l.cream Naples Yellow	pink Jasper Pink	olive brown Dark Olive Buff	salmon pink Coral Pink	l.brown Tilleul Buff	pink Safrano Pink
2. Caffeic Acid	brown L.Cinnamon Drab	brown Drab	dark violet Dark Violet	dark purple Dark Vinaceous Gray	dark blue green Hortense Blue	brown Drab
3. p-Coumaric Acid	orange Ochraceous Orange	rose-purple Vinaceous Lilac	orange brown Apricot Orange	rose Deep Purplish Vinaceous	red brown Russet	purple Purplish Lilac
4. 2,5-Dihydroxybenzoic Acid	l.brown Tilleul Buff	l.gray green Pale Olive Gray	coral Salmon Color	l.gray	yel. brown Olive Buff	yel. brn Olive
5. 3,5-Dihydroxybenzoic Acid	orange Ochraceous Orange	gold L.Cadmium	bright red orange Ruofus	orange Orange	dark violet Matthew's Purple	dark purple Bishop's Purple
5. Ferulic Acid	orange brown L.Ochraceous Buff	purple Argyle Purple	red brown Cinnamon Ruofus	violet L.Vinaceous Purple	violet brown Vinaceous Drab	violet Deep Soft Bluish Violet
7. 2,5-Dihydroxy-acetophenone	yel.green Citron Green	yel.green Citron Green	yel.green Citron Green	yel.green Citron Green	chartreuse Lemon Yellow	yel.green Citron Green

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Phenolic Compounds	2,5-dichloroaniline		4-nitro-2-aminoanisole		4-amino-4'-methoxydiphenylamine	
	B	A	B	A	B	A
3. p-Hydroxybenzaldehyde	l.yel.brown Naples Yellow	orange Flesh Ocher	cream Naples Yellow	salmon pink Coral Pink	brown orange Salmon Buff	orange Salmon Color
2. p-Hydroxybenzoic Acid	gold Mustard Yellow	orange Apricot Buff	yel.brown Amber Yellow	orange Mikado Orange	orange brown L.Ochraceous Salmon	orange Salmon Color
10. 2-Hydroxy-5-Methoxy Benzoic Acid	red brown Vinous Pawn	l.purple	gray blue Violet Plumbeaceous	rose Rhodonite Pink	l.purple	v.l.brown
11. p-Hydroxyphenyl-acetic Acid	gold Primaline Yellow	orange red Alizarine Pink	brown orange Yellow Ocher	orange with purple surr.	orange brown L.Ochraceous Salmon	pink Alizarine Pink
12. Quinic Acid	white	white	white	brown	white	v.l.violet
13. Protocatechuic Acid	orange brown L.Vinaceous Cinnamon	l.brown	yellow brown Naples Yellow	pink with white surr.	brown	v.l.brown
14. Salicyl Alcohol	yellow brown Mustard Yellow	gold with purple rim	gold with yellow rim	orange Cadmium Orange	yellow brown c gold surr.	orange red surr. by purple Orange Vinaceous

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Phenolic Compounds	4-chloro-2-aminotoluene		2-amino-3-nitroanisole	
	B	A	B	A
Acetovanillone	v.l.brown Cartridge Buff	v.l.brown Cartridge Buff	l.yel.brown Tilleul Buff	pink Safrano Pink
Caffeic Acid	green brown Dark Olive Buff	green brown Grayish Olive	gray green Storm Gray	moss green Deep Olive Green
β-Coumaric Acid	orange brown Vineaceous Buff	pink Orange Pink	yel.brown Cinnamon	rose brown Purplish Vineaceous
2,5-Dihydroxybenzoic Acid	l.cream Ivory Yellow	brown Drab	l.yellow with pink rim	l.gray green Tea Green
3,5-Dihydroxybenzoic Acid	orange Orange Buff	golden brown Yellow Ocher	dark red orange Dragon's Blood Red	dark red orange Dragon's Blood Red
Ferulic Acid	l.orange brown Tilleul Buff	purple L. Pinkish Lilac	brown Brownish Drab	purple L. Vineaceous Purple
2,5-Dihydroxyacetophenone	yel.green Citron Green	yel.green Citron Green	yel.green Citron Green	yel.green Citron Green

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Phenolic Compounds	4-chloro-2-aminotoluene		2-amino-3-nitroanisole	
	B	A	B	A
9. p-Hydroxybenzaldehyde	cream Pale Pinkish Buff	cream Pale Pinkish Buff	1.pink brown Seashell Pink	v.l.tan
10. p-Hydroxybenzoic Acid	1.yellow Massicot Yellow	gold Apricot Yellow	gold Mustard Yellow	orange Ochraceous Salmon
10. 2-Hydroxy-5-Methoxy Benzoic Acid	1.pink Pale Pinkish Cinnamon	v.l.pink	coral pink Coral Pink	1.violet
11. p-Hydroxyphenyl-acetic Acid	yellow Barium Yellow	salmon Shrimp Pink	gold Mustard Yellow	rose Jasper Pink
12. Quinic Acid	white	white	white	v.l.violet
13. Protocatechuic Acid	gold Mustard Yellow	golden brown Old Gold	1.brown	green finner rim c white surr.
14. Salicyl Alcohol	orange brown Warm Buff	golden brown c yellow surr. Avellaneous	purple surr. by brown	yellow orange c orange surr. Ochraceous Salmon

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Phenolic Compounds	4-chloro-2-aminotoluene		2-amino-3-nitroanisole	
	B	A	B	A
15. Syringaldehyde	l.yellow	moss green Water Green	peach	v.l.yellow
16. Syringic Acid	yellow Ivory Yellow	pink Pale Rhodonite Pink	purple brown L.Brownish Drab	purple Deep Purplish Vinaceous
17. Vanillic Acid	yellow Marguerite Yellow	orange Orange Buff	gold Chamois	orange brown Ferruginous
18. Vanillin	yellow Marguerite Yellow	orange brown L.Pinkish Cinnamon	l.gold Cartridge Buff	pink Salmon Color
19. Vanillin	yellow Marguerite Yellow	bright yellow gold Strontian Yellow	peach Pale Salmon Color	bright yellow gold Strontian Yellow
20. Acetosyringone	l.yellow Cream Color	yellow brown Olive Ocher	red orange rim Jasper Red	yellow brown Honey Yellow

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Phenolic Compounds	4-amino-2,5-dimethoxy-4-nitroazobenzene		4-benzamido-2,5-diethoxyaniline		4-amino-2,5-dimethoxybenzonitrile	
	B	A	B	A	B	A
1. Acetovanillone	l. violet Pale Lilac	l. violet Pale Lilac	l. brown Tilleul Buff	orange pink Safrano Pink	v.l. peach Pale Ochraceous Salmon	v.l. peach Pale Ochraceous Salmon
2. Caffeic Acid	brown Cinnamon Drab	gray brown L. Drab	brown Saccardo's Umber	red brown L. Purple Drab	brown L. Drab	green brown Serpentine Green
3. p-Coumaric Acid	purple Deep Purplish Vinaceous	violet Deep Dull Lavender	gold brown Cinnamon Buff	rose pink Venetian Pink	orange Ochraceous Salmon	violet Lavender
4. 2,5-Dihydroxybenzoic Acid	l. gold L. Ochraceous Salmon	l. brown gold Vinaceous Buff	l. yellow c purple rim	l. green brown Smoke Gray	white moss green	Serpentine Green
5. 3,5-Dihydroxybenzoic Acid	red violet Lobelia Violet	blue violet Pale Bluish Violet	rose c white rim Auricula Purple	rose L. Vinaceous Lilac	rose Vinaceous	orange Apricot Buff
6. Ferulic Acid	violet L. Hyssop Violet	purple gray Vinaceous Gray	orange Ochraceous Orange	purple Vinaceous Lilac	purple Persian Lilac	blue L. Alice Blue
7. 2,5-Dihydroxyacetophenone	yellow Pale Greenish Yellow	brown-yellow Chartreuse Yellow	yel. green Pale Green Yellow	yel. green Oil Yellow	yellow Naples Yellow	moss green Serpentine Green

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Phenolic Compounds	4-amino-2,5-dimethoxy-4' nitroazobenzene		4-benzamido-2,5-diethoxyaniline		4-amino-2,5-dimethoxybenzonitrile	
	B	A	B	A	B	A
3. p-Hydroxybenzaldehyde	1. pink brown Vivaceous Fawn	brown Avellaneous	1. orange brown L. Vivaceous Cinnamon	pink Hydrangea Pink	1. gold brown Vivaceous Cinnamon	coral Coral Pink
7. p-Hydroxybenzoic Acid	pink Chatenay Pink	violet Deep Dull Lavender	1. cream	white	yellow Cream Buff	coral Coral Pink
10. 2-Hydroxy-5-Methoxy Benzoic Acid	1. brown	white rim	1. yellow Sulphur Yellow	white	1. blue c orange rim Palid Methyl Blue	1. pink-brown Seashell Pink
11. p-Hydroxyphenyl-acetic Acid	pink	1. brown	white	white	yel. brown Vivaceous Cinnamon	purple Daphne Pink
12. Quinic Acid	1. yellow	1. brown	white	white	white	v.l. yellow
13. Protocatechuic acid	yel. brown Cinnamon Buff	brown	yellow c purple rim	1. yellow	1. orange brown Vivaceous Buff	1. green brown Olive Buff
14. Salicyl Alcohol	rose L. Corinthian Red	blue L. Dull Glaucous Blue	golden brown Chamois	orange. Apricot Buff	orange brown c yellow surr.	orange with fushia outside Shrimp Pink with Eosin Pink
15. Syringaldehyde	yel. c rose surr. Amber Yellow c L. Purplish Vln.	olive brown Buffy Brown	1. cream Tilleul Buff	1. yel. brown Smoke Gray	1. orange Pale Flesh Color	1. purple violet Grayish Lavender

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Phenolic Compounds	4-amino-2,5-dimethoxy-4-nitroazobenzene		4-benzamido-2,5-diethoxyaniline		4-amino-2,5-dimethoxybenzotrile	
	B	A	B	A	B	A
16. Syringic Acid	violet Lavender Blue	sky blue L. Dull Glaucous Blue	gold Naples Yellow	rose Cameo Pink	orange pink Carrot Red	violet Amparo Purple
17. Vanillic Acid	rose Purplish Lilac	gray blue Russian Blue	white with gold rim	orange rim	gold Buff Yellow	red purple Tyrian Pink
18. Vanillin	rose Purplish Lilac	gray blue Russian Blue	white with gold rim	orange rim	gold Buff Yellow	red purple Tyrian Pink
19. Vanillin	l. brown	gold Wax Yellow	l. brown Vinous Buff	golden brown Lemon Chrome	l. brown Vinous Buff	golden brown Lemon Chrome
20. Acetosyringone	yel. brown Cinnamon Buff	l. brown Vinous Buff	l. brown Vinous Buff	l. brown Vinous Buff	l.yel.brown L. Buff	l.yel.brown L. Buff

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Phenolic Compounds	2-amino-2'-chloro-4-methoxy-5-methyl-4'-nitroazobenzene		4-amino-2,4'-dimethyl-5-methoxy-2'-nitroazobenzene		4-amino-2,3'-dimethylazobenzene	
	B	A	B	A	B	A
1. Acetovanillone	1. violet Pale Lilac	1. violet Pale Lilac	pink Pale Cinnamon Pink	1. purple Dull Lavender	1. brown Tilleul Buff	pink Hydrangea Pink
2. Caffeic Acid	1. yellow green Old Gold	brown Buffy Brown	blue green Olive Gray	brown Drab	1. green brown Olive Buff	green brown Vetiver Green
3. p-Coumaric Acid	1. blue brown Ecru Drab	1. brown L. Vinaceous Fawn	gold brown Cinnamon Buff	pink Venetian Pink	orange L. Ochraceous Orange	violet Lavender
4. 2,5-Dihydroxybenzoic Acid	1. yellow Cream Color	1. brown Avellaneous	pink Pale Vinaceous Pink	1. yel. brown Pale Olive	white c salmon edge	1. yel. brown Pale Olive Buff
5. 3,5-Dihydroxybenzoic Acid	purple L. Brownish Vinaceous	purple Pale Purple Drab	purple Argyle Purple	purple Argyle Purple	salmon-red L. Corinthian Red	salmon-pink Pinkish Vinaceous
6. Ferulic Acid	blue L. Glaucous Blue	gray-blue c yellow rim Pallid Purplish Gray	purple Deep Vinaceous Gray	blue gray Pale Violet Gray	yellow brown Cinnamon Buff	gray brown Smoke Gray
7. 2,5-Dihydroxyacetophenone	yellow Naples Yellow	moss green Grayish Olive	yel. brown Naples Yellow	yel. brown Naples Yellow	green yel. Citron Yellow	yel. green Citron Green

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Phenolic Compounds	2-amino-2'-chloro-4-methoxy-5-methyl-4'-nitroazobenzene		4-amino-2,4'-dimethyl-5-methoxy-2'-nitroazobenzene		4-amino-2',3'-dimethylazobenzene	
	B	A	B	A	B	A
8. p-Hydroxybenzaldehyde	1. yellow	1. yellow	pink brown Vineaceous Pink	brown Drab	peach Pale Cinnamon Pink	peach Pale Cinnamon Pink
9. p-Hydroxybenzoic Acid	1. peach	1. yellow	salmon Salmon Buff	orange brown Vineaceous Fawn	1. yellow Pale Vinaceous Pink	pink Pale Vinaceous Pink
10. 2-Hydroxy-5-Methoxy Benzoic Acid	light cream	1. salmon Seashell Pink	1. purple Pale Purplish Vineaceous	1. brown L. Vinaceous Fawn	peach	white
11. p-Hydroxyphenyl-acetic Acid	1. cream	light gold Pale Ochraceous	v.l.peach	pink with blue gray rim	1. yellow	white
12. Quinic Acid	v.l.yellow	v.l.yellow	v.l.peach	1. brown	white	white
13. Protocatechuic Acid	1. yel. brown Cream Buff	1. brown Avellaneous	purple L.Purplish Vineaceous	brown Avellaneous	peach	yellow brown Pale Olive Buff
14. Salicyl Alcohol	yel. green Deep Olive Buff	golden brown Chamois	orange brown Vineaceous Brown	maroon $\bar{c}$ blue violet rim Dark Vinaceous	brown yel. Mustard Yel.	orange Ochraceous Salmon

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Phenolic Compounds	2-amino-2'-chloro-4-methoxy-5-methyl-4'-nitroazobenzene		4-amino-2,4'-dimethyl-5-methoxy-2'-nitroazobenzene		4-amino-2',3'-dimethylazobenzene	
	B	A	B	A	B	A
15. Syringaldehyde	white	l. cream	yel. brown rust surr. Mustard Yel. C Cinnamon	green brown Buffy Brown	white	yellow Chartreuse Yellow
16. Syringic Acid	white	l. peach Pale Ochraceous Salmon	purple Argyle Purple	blue L. Alice Blue	white	gray Gray
17. Vanillic Acid	white	yellow	orange L. Congo Pink	violet blue Violet Plumbaceous	l. gold	pink Pale Brownish Vinaceous
18. Vanillin	pale peach	yellow	orange L. Congo Pink	violet blue Violet Plumbaceous	l. gold	pink Pale Brownish Vinaceous
19. Vanillin	l. gold	bright yel. gold Strontian Yellow	pink brown Vinaceous Cinnamon	yel. brown Olive Ocher	yel. brown	gold Strontian Yellow
20. Acetosyringone	white	l. yel. brown L. Buff	dark red pink Congo Pink	brown Vinaceous Buff	white	l. beige Olive Buff

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Phenolic Compounds	o-nitroaniline		p-nitroaniline		N,N-diethyl-3-amino-4-methoxy-benzensulfonamide	
	B	A	B	A	B	A
1. Acetovanillone	l. brown Drab	rose Jasper Pink	l. brown brown	purple Argyle Purple	yel. brown Olive Buff	pink Pale Congo Pink
2. Caffeic Acid	dark red brown Vineaceous Drab	green brown Drab	brown Tawny Olive	dark brown Army Brown	brown	green brown Drab
3. p-Coumaric Acid	orange brown L.Ochraceous Salmon	l. red brown L. Cinnamon Drab	orange brown L.Ochraceous Salmon	blue gray L.Olive Gray	orange yel. Amber Yellow	pink Pinkish Vinaceous
4. 2,5-Dihydroxybenzoic Acid	orange brown L.Ochraceous Salmon	green L. Olive Gray	v.l. yellow	l. brown Olive Buff	v.l. pink Pale Flesh Color	l. green brown Water Green
5. 3,5-Dihydroxybenzoic Acid	orange Ochraceous Orange	orange Zinc Orange	orange Yel. Ocher	orange Ochraceous Orange	orange Ochraceous Salmon	gold Antimony Yellow
6. Ferulic Acid	pink Congo Pink	green brown Drab	l. green brown Gray Green	l. gray green Mineral Gray	orange brown	purple Argyle Purple
7. 2,5-Dihydroxy-acetophenone	yel. green L.Yel. Green	yel. green Citron Green	yel. green L. V. Green	yel. green Citron Green	yel. green Citron Green	yel. green Citron Green

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Phenolic Compounds	o-nitroaniline		p-nitroaniline		N,N-diethyl-3-amino-4-methoxy-benzensulfonamide	
	B	A	B	A	B	A
8. p-Hydroxybenzaldehyde	l. gold	orange Salmon Color	peach	rose Purplish Vinous	l. peach Cream Buff	l. orange L. Ochraceous Salmon
9. p-Hydroxybenzoic Acid	l. gold	orange Apricot Buff	yellow	rose Purplish Vinous	l. peach Cream Buff	gold Ochraceous Buff
10. 2-Hydroxy-5-Methoxy-Benzoic Acid	blue gray Plumbaceous	rose Deep Vinaceous	salmon Salmon Color	white	gray blue c orange rim Sky Gray	white
11. p-Hydroxyphenyl-acetic Acid	yel. brown Pinkish Cinnamon	rose Deep Vinous	yellow	purple c violet rim Lobelia Violet	yel. brown Colonial Buff	orange brown Salmon Buff
12. Quinic Acid	v.l. peach	pink Pale Vinaceous	"	l. yellow	white	white
13. Protocatechuic Acid	red brown Buff Pink	l. brown Pale Drab Gray	pink Flesh Color	purple brown Vinous Gray	orange brown Vinous Buff	l. brown Vinous Buff
14. Salicyl Alcohol	orange with yellow rim Apricot Buff	orange with yellow rim Apricot Buff	golden brown Warm Buff	orange c rose surr. Congo Pink c Daphne Pink	orange Vinous Buff	orange brown c yellow surr. Ochraceous Buff

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Phenolic Compounds	o-nitroaniline		p-nitroaniline		N,N-diethyl-3-amino-4-methoxybenzenesulfonamide	
	pink Flesh Pink	rose Deep Purplish Vivaceous	orange Flesh Pink	yellow c blue rim		
15. Syringaldehyde	salmon pink L.Coral Red	rose Deep Purplish Vivaceous	v.bright red orange L.Coral Red	violet Deep Dull Bluish Violet	1. yel. brown Pale Olive Buff orange brown Warm Buff	1. yel. brown Pale Olive Buff bright rose red Corinthian Red
16. Syringic Acid	orange c yel. & green rims Flesh Pink	orange Vivaceous Orange	yel. gold Apricot Yel.	violet Saccardo's Violet	yellowish green Citron Green	orange Flesh Ocher
18. Vanillin	orange t yel. & green rims Flesh Pink	orange Vivaceous Orange	yel. gold Apricot Yel.	violet Saccardo's Violet	yel. green Citron Green	yel. orange Flesh Ocher
19. Vanillin	1. brown	gold Primuline Yellow	1. brown	golden brown Old Gold	yel. brown	gold Primuline Yellow
20. Acetosyringone	1. yel. brown Pale Olive Buff	1. yel. brown Pale Olive Buff	1. yel. brown	1. yel. brown	1. yel. brown Cartridge Buff	yellow brown Olive Buff

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 A = after sodium carbonate spray      yel. = yellow      c = with

Phenolic Compounds	N-n-butyl-3-amino-4-methoxybenzenesulfonamide		2-amino-4-chloroanisole		3-nitro-2-aminotoluene	
	B	A	B	A	B	A
1. Acetovanillone	yel. brown Olive Buff	pink Pale Congo Pink	l. brown Vinous Buff	salmon Orange Pink	l. brown Vinous Buff	purple Vinous Lilac
2. Caffeic Acid	gray blue L. Gray	green brown Drab	gray brown Drab	moss green Grayish Olive	yel. brown Honey Yellow	brown Cinnamon Drab
3. p-Coumaric Acid	orange yel. Amber Yel.	pink Pinkish Vinous	yel. brown Vinous Buff	pink Corinthian Pink	orange brown Vinous Buff	purple brown L. Purple Drab
4. 2,5-Dihydroxybenzoic Acid	v.l. pink Pale Flesh Color	l. green brown Water Green	pink Pale Vinous Lilac	green brown L. Drab	gray with orange rim	brown L. Drab
5. 3,5-Dihydroxybenzoic Acid	orange Ochraceous Salmon	gold Antimony Yellow	orange Carrot Red	orange Capucine Orange	bright orange Flame Orange	bright orange Flame Orange
6. Ferulic Acid	orange brown L. Ochraceous Salmon	purple Argyle Purple	purple brown L. Vinous Gray	purple violet Purplish Lilac	red brown Cinnamon	brown Drab
7. 2,5-Dihydroxyacetophenone	yel. green Citron Green	yel. green Citron Green	yel. green Citron Green	yel. green Vetiver Green	pea green $\bar{c}$ pink rim Citron Green	yel. green Vetiver Green

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Phenolic Compounds	N-n-butyl-3-amino-4-methoxybenzenesulfonamide		2-amino-4-chloroanisole		3-nitro-2-aminotoluene	
	B	A	B	A	B	A
8. p-Hydroxybenzaldehyde	l. peach Cream Buff	l. orange L. Ochraceous Salmon	l. red brown Pale Pinkish Cinnamon	white	purple inner ring c̄ gold outside	rose brown Brownish Vinaceous
9. p-Hydroxybenzoic Acid	peach	gold Ochraceous Buff	l. yel. brown Cream Buff	l. yel. brown Cream Buff	gold Amber Yellow	orange c̄ purple rim Dark Vinaceous
10. 2-Hydroxy-5-Methoxy-Benzolic Acid	v. l. gray blue c̄ orange rim Sky Gray	white	gray	white	gray with orange rim	l. red brown Vinaceous Pink
11. p-Hydroxyphenyl-acetic Acid	yellow Cream Buff	orange brown Salmon Buff	yel. brown Colonial Buff	orange brown Salmon Buff	gold Amber Yellow	purple Amparo Purple
12. Quinic Acid	white	white	white	white	white c̄ yellow rim	l. brown Pale Pinkish Buff
13. Protocatechuic Acid	orange brown Vinaceous Buff	orange brown Vinaceous Buff	orange brown L. Pinkish Cinnamon	brown Drab	red brown Pinkish Cinnamon	purple brown L. Drab
14. Salicyl Alcohol	orange brown c̄ yel. surr. Vinaceous Buff	orange Ochraceous Buff	orange brown c̄ yel. surr. L. Pinkish Cinnamon	gold with orange surr.	red brown c̄ yel. rim Pinkish Cinnamon	orange c̄ purple surr. Deep Purplish Vinaceous
15. Syringaldehyde	l. yellow brown Pale Olive Buff	l. yellow brown Pale Olive Buff	white	l. brown Olive Buff	l. yellow	yellow Primrose Yellow

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Phenolic Compounds	N-n-butyl-3-amino-4-methoxybenzenesulfonamide		2-amino-4-chloroanisole		3-nitro-2-aminotoluene	
	B	A	B	A	B	A
16. Syringic Acid	orange Warm Buff	bright red pink Corinthian Red	orange Apricot Buff	bright red pink Corinthian Red	orange Ochraceous Buff	violet Blanc's Violet
17. Vanillic Acid	yel. green Citron Green	orange Flesh Ocher	yellow	orange Salmon Color	green yel. Amber Yel.	purple L. Vinaceous Purple
18. Vanillin	yel. green Citron Green	orange Flesh Ocher	yel. brown Cream Buff	orange Salmon Color	green yel. Amber Yel.	purple L. Vinaceous Purple
19. Vanillin	l.yel.brown Cartridge Buff	gold Primuline Yel.	l. yellow	bright yel. Strontian Yel.	l. brown	bright yel. Strontian Yellow
20. Acetosyringone	l.yel.brown Cartridge Buff	yel.brown Olive Buff	orange brown Pinkish Cinnamon	yel. brown Honey Yel.	golden yel. Buff Yellow	golden brown Honey Yellow

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Phenolic Compounds	4-nitro-2-aminotoluene		4-benzamido-2-methoxy-5-methylaniline		o-chloroaniline	
	B	A	B	A	B	A
Acetovanillone	cream Ivory Yel.	pink Shrimp Pink	l. brown Tilleul Buff	l. brown Tilleul Buff	cream Ivory Yel.	orange Flesh Color
Caffeic Acid	brown Drab	brown Drab	green brown Smoke Gray	brown Drab	purple brown L. Drab	brown Drab
p-Coumaric Acid	yel. green Olive Buff	pink Shrimp Pink	yellow Ivory Yel.	light brown Tilleul Buff	gold brown Vinous Buff	pink Alizarine Pink
2,5-Dihydroxybenzoic Acid	cream Ivory Yel.	brown Drab	yel. with pink rim	brown Drab	l. pink brown Tilleul Buff	gray brown Drab
3,5-Dihydroxybenzoic Acid	orange Antimony Yel.	golden brown Olive Ocher	white with pink rim	salmon L. Vinaceous Buff	orange Ochraceous Buff	gold Light Cadmium
Ferulic Acid	l. orange brown Cartridge Buff	purple L. Vinaceous Lilac	golden brown Drab Gray	purple Dull Purplish Vinaceous	orange brown Light Drab	purple L. Vinaceous Lilac
2,5-Dihydroxy-acetophenone	yel. green Citron Green	pea green Vetiver Green	yel. green Citron Green	pea green Vetiver Green	yel. green Citron Green	dark pea green Grayish Olive

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Phenolic Compounds	4-nitro-2-aminotoluene		4-benzamido-2-methoxy-5-methylaniline		o-chloroaniline	
	B	A	B	A	B	A
p-Hydroxybenzaldehyde	cream Ivory Yel.	peach Pale Vinateous Lilac	l. peach	pink brown Avellaneous	peach Pale Cinnamon Pink	peach Pale Cinnamon Pink
p-Hydroxybenzoic Acid	l. yellow	gold Antimony Yellow	white	white	yel. gold Amber Yel.	gold Ochraceous Buff
2-Hydroxy-5-Methoxy-Benzoic Acid	white with orange rim	pink Pale Ochraceous Salmon	white	white	gray with salmon rim	salmon L.Ochraceous Salmon
1. p-Hydroxyphenyl-acetic Acid	yellow	pink Venetian Pink	v.l. pink	v.l. yellow Ivory Yel.	yellow	salmon Flesh Color
2. Quinic Acid	white	white	white	white	white	white
3. Protocatechuic Acid	golden brown L.Ochraceous Buff	brown Drab	golden brown L.Ochraceous Buff	brown Drab	salmon Ochraceous Salmon	brown Drab
4. Salicyl Alcohol	red brown yellow surr. L.Ochraceous Buff	yellow brown orange surr.	l. brown surr. by yellow L.Ochraceous Buff	gray brown surr. by orange Drab with Orange	salmon surr. by yellow Ochraceous Salmon	gold Apricot Yellow

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Phenolic Compounds	4-nitro-2-aminotoluene		4-benzamido-2-methoxy-5-methylaniline		o-chloroaniline	
	B	A	B	A	B	A
15. Syringaldehyde	l. cream	gray green Olive Buff	v.l.gold	olive brown Olive Buff	l. salmon	l. gray green Water Green
16. Syringic Acid	yellow Barium Yel.	salmon pink Orange Vinous	l. yellow Amber Yel.	pink Pale Laelia Pink	gold Amber Yel.	salmon pink Orange Vinaceous
17. Vanillic Acid	yellow Barium Yel.	orange Capucine Orange	v.l.yellow	l. peach Cartridge Buff	yel. brown Barium Yel.	orange Orange Buff
18. Vanillin	yellow Barium Yel.	orange Capucine Orange	l. yellow	l. brown Vinous Buff	yel. brown	orange Orange Buff
19. Vanillin	yellow Barium Yel.	bright yellow Strontian Yellow	yellow Barium Yel.	bright yellow Strontian Yellow	l. yellow	bright yellow Strontian Yellow
20. Acetosyringone	yellow Straw Yel.	yel. brown Honey Yel.	yel. C. salmon surr. Pale Flesh Color surr.	yel. brown Honey Yel.	gold Naples Yel.	yel. brown Honey Yel.

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Phenolic Compounds	4-aminodiphenylamine		4-amino-3-methoxy-diphenylamine		2-chloro-5-trifluoro-methylaniline	
	B.	A	B	A	B	A
. Acetovanillone	l. pink	l. yellow Ivory Yel.	v.l. orange brown Tilleul Buff	v.l. orange brown Tilleul Buff	l. yellow	pink Chatenay Pink
. Caffeic Acid	brown Drab	brown Drab	moss green Deep Olive Buff	brown Drab	brown Drab	brown Drab
. p-Coumaric Acid	v.l. purple	l. cream Ivory Yel.	cream Ivory Yel.	v.l. cream	yellow	pink Flesh Pink
. 2,5-Dihydroxybenzoic Acid	white	brown Drab	yel. brown Cartridge Buff	brown Drab	v.l. tan	l. gray green Olive Buff
. 3,5-Dihydroxybenzoic Acid	white	pink brown L. Cinnamon Drab	yellow Cream Buff	pink brown L. Cinnamon Drab	gold Apricot Yel.	gold Wax Yellow
. Ferulic Acid	white	yellow Marguerite Yellow	white	green Chrysolite Green	l. yel. brown Cream Buff	rose pink Pale Purplish Vineaceous
. 2,5-Dihydroxy-acetophenone	yel. green Citron Green	olive green Grayish Olive	yel. green Citron Green	yel. green Citron Green	yel. green Reed Yel.	yel. green Reed Yel.

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Phenolic Compounds	4-aminodiphenylamine		4-amino-3-methoxy-diphenylamine		2-chloro-5-trifluoromethylaniline	
	B	A	B	A	B	A
p-Hydroxybenzaldehyde	l. pink Ecrú Drab	l. pink Ecrú Drab	cream with rose rim Phlox Pink	violet Deep Dull Lavender	brown Avellaneous	gray brown Drab Gray
p-Hydroxybenzoic Acid	white	white	yellow Cartridge Buff	white	brown surr. by rose	pink Salmon Buff
0. 2-Hydroxy-5-Methoxy-Benzoic Acid	white	white	yellow L. Buff	white	l. green gray c̄ pink surr. Pale Olive Buff c Tilleul Buff	pink Shell Pink
1. p-Hydroxyphenyl-acetic Acid	white	orange brown Cartridge Buff	yellow Ivory Yel.	white	yellow Naphthalene Yellow	l. yel. brown Cream Color
2. Quinic Acid	white	l. gray	white	white	-	white
3. Protocatechuic Acid	l. brown	brown Drab	gold Chamois	brown Avellaneous	pink brown L. Pinkish Cinnamon	brown Avellaneous
4. Salicyl Alcohol	l. brown	olive brown Dark Olive Buff	peach Cartridge Buff	brown Avellaneous	gold with Salmon rim	gold with yellow rim

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Phenolic Compounds	4-aminodiphenylamine		4-amino-3-methoxy-diphenylamine		2-chloro-5-trifluoromethylamine	
15. Syringaldehyde	l. pink	moss green L. Turtle Green	pink Pale Vinaceous Pink	moss green Chrysolite Green	v.l.yellow green Pale Olive Buff	v.l.yellow green Pale Olive Buff
16. Syringic Acid	white	v.l.pink	v.l.yellow	-	yellow Naphthalene Yellow	salmon pink Jasper Pink
17. Vanillic Acid	white	white	v.l.yellow	-	l.yellow	orange Orange Pink
18. Vanillin	l.pink	brown Drab	yellow Cream Color	brown Avellaneous	l. peach	pink Coral Pink
19. Vanillin	l.yellow	bright yel. gold Strontian Yellow	yellow Naphthalene Yellow	bright yel. gold Strontian Yellow	yellow Strontian Yellow	yellow brown Olive Ocher
20. Acetosyringone	l. pink	yel.brown Olive Ocher	pink Vinaceous Buff	yel.brown Honey Yel.	yellow Colonial Buff	yel.brown Ivory Yellow

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Phenolic Compounds	ortho dianisidine	
	B	A
1. acetovanillone	brown Wood Brown	red brown Vinaceous Fawn
2. Caffeic Acid	dark blue green Deep Grayish Blue Green	dark brown c̄ green surr. Clove brown c̄ Tea Green
3. p-Coumaric Acid	brown with red brown rim Avellaneous c̄ Orange Cinnamon	rose brown c̄ purple surr. L. Brownish Drab c̄ Purple Drab
4. 2,5-Dihydroxy-benzoic Acid	l. olive green c̄ pink surr. Deep Olive Buff c̄ Vin. Buff	l. olive green Deep Olive Buff
5. 3,5-Dihydroxy-benzoic Acid	purple Slate Purple	purple Veronia Purple
6. Ferulic Acid	orange brown Cinnamon Drab	blue violet Lobelia Violet
7. 2,5-Dihydroxy-acetophenone	l. olive brown Deep Olive Buff	yellow green Citron Green

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Phenolic Compounds	ortho dianisidine	
	B	A
8. p-Hydroxy-benzaldehyde	pink brown Buff Pink	pink brown Buff Pink
9. p-Hydroxybenzoic Acid	brown Avellaneous	orange L. Ochraceous Orange
10. 2-Hydroxy-5-Methoxy Benzoic Acid	l. purple Pale Purple Drab	v.l. brown Vinous Buff
11. p-Hydroxyphenyl-acetic Acid	gray brown c̄ yel. brown rim	red brown Vinous Fawn
12. Quinic Acid	l. violet Lavender Gray	white
13. Protocatechuic Acid	pink brown Pale Vinaceous Fawn	l. pink brown L. Vinaceous Fawn
14. Salicyl Alcohol	brown c̄ gold surr. Clay Color c̄ Primitine Yell.	orange brown c̄ fushia surr. Rufous with Old Rose

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 A = after sodium carbonate spray    yel. = yellow    c̄ = with

Phenolic Compounds	ortho diarsidine	
	B	A
15. Syringaldehyde	orange brown L. Pinkish Cinnamon	brown Avellaneous
16. Syringic Acid	orange brown Vineaceous Cinnamon	purple Veronia Purple
17. Vanillic Acid	yellow brown Cinnamon Buff	red brown Ocher Red
18. Vanillin	gray brown Drab	red brown Ocher Red
19. Vanillin	red brown Vineaceous Buff	bright yel. gold Primuline Yellow
20. Acetosyringone	pink brown Buff Pink	l. brown Vineaceous Buff

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In some cases, the color of the spots was not dark or distinct enough for specific identification (Ridgway's Color Standards and Color Nomenclature) and the color name is not given. Also, some of the phenolic compounds appeared to reject part of the diazo sprays which resulted in different colored rings or areas of color. In this instance, both colors are reported.

A comparison was made between the p-nitroaniline spray reagent regularly used in this laboratory and the stabilized diazo salt obtained from Antara Chemicals. The standard phenolic compounds were sprayed with both reagents and the colors of the spots were compared. In all instances the colors were identical but the normal background color (probably due to coupling of the diazo salt with some unchanged p-nitroaniline) obtained by the older method was absent.

To determine the effect of concentration of the phenolic materials upon color spectrophotometric determinations were done. p-Hydroxybenzoic acid, vanillic acid and syringic acid were each made up in three different concentrations and 0.3 ml. were applied to Whatman No. 1 paper. The spots were exposed to ammonia and sprayed with the diazo salts as previously described. They were then analyzed in the General Electric Recording Spectrophotometer. In all cases, the spectrophotometric curves were identical showing that the color itself did not change in hue when concentrations were increased or decreased.

Summary:

The stabilized diazo salts reported in this paper have proven to be a satisfactory and simple method for the identification of many phenolic compounds. They may be kept in the dry form for several years and need only to be added to water thus eliminating complex mixing. These salts should be very useful in the identification of phenolic compounds.

Reference:

Ridgway, Robert. Color Standards and Color Nomenclature. 1st ed. Washington, D. C., 1912. Published by the author.

pfm/jt