AROMATIC ALDEHYDES IN THE CROSSOSOMATACEAE

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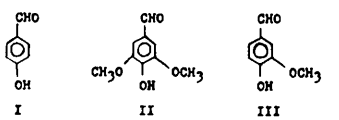
The desert shrubs *Crossosoma bigelovii* Wats. and *C. californicum* Nutt. exhibit atypical staining phenomena; their wood gives a strong Weisner-positive lignin reaction with phloroglucinol-HCl, but stains a very light pink with safranin. In this study, the wood of these species was compared with that of *Ulmus americana* L., *Prunus mexicana* S. Wats., *Bumelia lanuginosa* (Michx.) Pers., *Cercis canadensis* L., and *Juniperus virginiana* L. Lignin degradation products were obtained by alkaline cupric hydroxide oxidation, analyzed by thin-layer chromatography, and quantitated by spectrophotometric methods. Other species did not show the atypical staining of the Crossosoma spp. Although there was a difference in staining reaction, chromatographic separation of the aromatic aldehydes did not reveal major differences between woods of Crossosoma spp. and the other species. Quantitation by spectrophotometric methods indicated the presence, in Crossosoma, of syringaldehyde and vanillin, the aromatic aldehydes characteristic of angiosperms. The atypical staining may be due to extractives other than lignin or to the presence of other components which may block the chemical reaction responsible for fixation of safranin.

This study was undertaken to investigate some of the degradation products of the lignin of *Crossosoma bigelovii* Wats. and *C. californicum* Nutt. Crossosoma is a desert shrub limited to the southwestern United States, adjacent Mexico, and islands off the California shores. Because of atypical staining phenomena, it was suspected that the wood might possess some unusual chemical features. Wood of this genus gives a strong Weisner-positive lignin reaction with phloroglucinol-HCl, but stains weakly with safranin, the usual stain for lignified cell walls. Walls of the xylem cells also stain strongly with lacmoid, a specific stain for callose (1), and staining with Toluidine blue O results in blue cell walls. Weisner-positive lignins usually stain blue-green to green with Toluidine blue O (2).

Lignin studies of a particular species yield only a limited amount of information since the structure of the lignin still remains uncertain. However, most workers agree that lignin can be described as a complex, three dimensional aromatic polymer of phenylpropanoid units (3-6). To date, in spite of much work, no better definition has been found than that of Brauns and Brauns (3) which states that lignin is "that wood constituent which when oxidized with nitrobenzene, yields vanillin in the case of coniferous woods, vanillin and syringaldehyde in the case of woods of dicotyledons, and p-hydroxybenzaldehyde, vanillin, and syringaldehyde in the case of the monocotyledons."



I p-hydroxybenzaldehyde

II syringaldehyde

III vanillin

Because of its nature, lignin cannot be extracted in an intact fashion. Various techniques have been developed to investigate the nature of the polymer but each has its own advantages and limitations. Although alkaline nitrobenzene oxidations have been used to a great extent to degrade lignin, alkaline copper hydroxide oxidations yield a reaction mixture which contains fewer compounds interfering with chromatography (7). For this reason, the latter procedure was adopted with some modifications for this problem.

MATERIALS AND METHODS

In this investigation the lignin degradation products of *C. bigelovii* and *C. californicum* were compared to those of some hardwoods and one softwood. Samples of hardwoods *Ulmus americana* L., *Prunus*

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mexicana S. Wats., *Bumelia lanuginosa* (Michx.) Pers., *Cercis canadensis* L., and the softwood *Juniperus virginiana* L. were collected from the Oklahoma State University Ecology Preserve. Stems of each species were immediately killed and fixed in Craf III for histological examination and voucher specimens were preserved. Wood of *C. californicum* was obtained from a growing plant, and wood of *C. bigelovii* was taken from a dried specimen from Arizona (Richardson, 148). This specimen was previously preserved in 70% ethanol and killed and fixed in Craf III.

Histological studies

Transverse, radial and tangential sections of all samples were cut at about 20 μ on a sliding microtome, stained with safranin and counterstained with Delafield's hematoxylin (8). Some sections were also stained with an iron mordanted safranin (9) and results were compared.

Preparation of plant material for degradation studies

The bark was removed and wood samples of each species were converted to shavings which were extracted with 80% ethanol under reflux until extracts were colorless. Extract was filtered through nylon cloth, allowed to air dry and ground to a powder to pass a 40-mesh screen in a Wiley Mill. The powder was extracted exhaustively in a Soxhlet extractor for 48 hr with water followed by ethanol/benzene (1:1, v/v) and then benzene for 48 hr. Once the powder was air dried, it was used as a source of lignin and referred to as extracted wood meal.

Oxidation of extracted wood meal

Alkaline copper hydroxide oxidations were carried out with each sample. To 300 mg of wood meal, 1.3 g $Cu(OH)_2$ and 10 ml of 2 N NaOH were added. The mixture was sealed in a 22 ml Parr bomb and immersed in an oil bath at 175 C for 3 hr. The bomb was cooled in running tap water, the reaction mixture was filtered through filter paper, and the residue in the filter paper was washed in 5 volumes of hot water. The combined filtrate and washings were acidified with 2 N HCl to a pH between 2 and 3 and extracted with 3 - 25 ml portions of chloroform in a separatory funnel. The chloroform extract was then evaporated to dryness in a rotary evacuator and the residue containing the benzaldehydes was taken up in 1 ml of absolute ethanol.

Chromatography

Thin layer plates were banded with 0.25 mm of Kieselgel G nach Stahl (10) and allowed to dry overnight. They were activated in an oven for 1 hr at 120 C and spotted with 25 ul of each sample. Authentic samples of p-hydroxybenzaldehyde (Eastman Organic Chemicals), syringaldehyde (Mann Research Laboratories), and vanillin (Matheson, Coleman and Bell) were used as standards. Although p-hydroxybenzaldehyde has not been reported to occur in angiosperm and gymnosperm lignin, it was included since some lignins yield this product upon degradation. Plates were developed in a Chromatotank containing a benzene/acetic acid solvent (9:1) for 1 hr. In order to obtain better separation, they were allowed to dry and then redeveloped. Once sprayed with a 0.4% solution of 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl they were compared with R_F values of authentic samples (Table 1).

TABLE 1. *Characteristics of aromatic aldehydes on Kieselgel G thin layer plates.*

Aldehyde	Chromatographic Color with DNPH	R_F [a]	Spectral max[b]
p-hydroxybenzaldehyde	brown-red	.34	339 mμ
syringaldehyde	burnt sienna	.63	370 mμ
vanillin	brick-red	.74	352 mμ

[a] Solvent: benzene/acetic acid (9:1 v/v)
[b] Solvent: 95% ethanol with 5 drops 1.25 N ethanolic KOH per 10 ml.

Isolation and quantification of aromatic aldehydes

Chromatographic bands corresponding to the aromatic aldehydes were located under (long wave) ultraviolet light. Unsprayed bands were scraped into centrifuge tubes and 10 ml of 95% aqueous ethanol were added. The tubes were shaken by hand and centrifuged at 3,500 rpm for 10 min. The supernatant solution was decanted and 5 drops of 1.25 N KOH in 95% aqueous ethanol were added. A standard curve from authentic samples was prepared for each aldehyde. Measurements were made with a Perkin-Elmer 202 spectrophotometer at wavelengths of maximum absorption of the ionized form of each aldehyde (Table 1). A straight line relationship was found between the absorbance and concentration for each of the authentic samples. The slopes of these lines were determined and used for calculation of the aldehyde concentrations.

RESULTS AND DISCUSSION

Hardwoods and the softwood did not show the atypical staining of Crossosomataceae. Lignified cell walls stained a brilliant red with both Johansen's and Gray and Pickle's safranin. These results indicate that there is a difference in the staining reaction between the *Crossosoma* spp. and the other species studied, but chromatographic separation of the aromatic aldehydes did not exhibits any major differences. Both species of *Crossosoma* possess chromatographic fractions similar to the other species (Fig. 1). Table 2 shows the results of these experiments.

Quantitative data also emphasize similarities. *Crossosoma* contained syringaldehyde and vanillin in proportions similar to those of the other species (Table 2). The techniques of quantitation are not entirely accurate since the aromatic aldehydes are converted, in part, to their corresponding

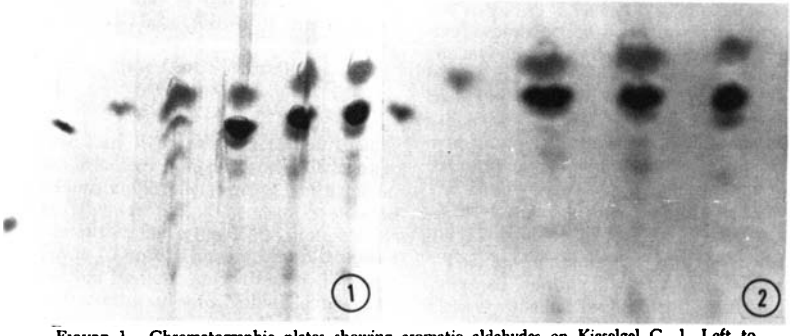

FIGURE 1. Chromatographic plates showing aromatic aldehydes on Kieselgel G. 1. Left to right, the first three spots represent authentic samples of p-hydroxybensaldehyde, syringaldehyde, and vanillin; next are samples *Juniperus virginiana*, *Prunus mexicana*, *Bumelia lanuginosa*, and *Ulmus americana*. 2. Left to right, syringaldehyde, vanillin, *Crossosoma bigelovii*, *Crossosoma californicum*, and *Cercis canadensis*.

TABLE 2. *Aromatic aldehydes from alkaline cupric hydroxide oxidation.*

Species	Thin layer[a] I	II	III	Relative yields[b] II	III
Crossosoma bigelovii	–	+	–	23	47
Crossosoma californicum	–	+	+	34	43
Cercis canadensis	–	+	+	38	38
Prunus mexicana	–	+	+	48	21
Bumelia lanuginosa	–	+	+	43	63
Ulmus americana	–	+	+	73	53
Juniperus virginiana	–	+	+	—	61

[a] Kieselgel G using DNPH
[b] Grams x 10-9 from 300-mg samples of pre-extracted wood meal.

acids during alkaline oxidation (7, 11).

Lignin degradation by alkaline cupric hydroxide oxidation did not reveal any different chemical features in the Crossosomataceae. The atypical staining may be due to extractives other than lignin or to the presence of other components that may be blocking the chemical reaction which causes the fixation of safranin.

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