Behavior of Ellagitannins, Gallic Acid, and Ellagic Acid Under Alkaline Conditions

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Abstract: Examination of the rates of hydrolysis of different ellagitannins under conditions comparable with cold soda and alkaline-groundwood pulping processes showed that some ellagitannins are notably resistant to hydrolysis. The rate of hydrolysis was dependent upon the pH and temperature of the solution and particularly upon the structure of the compound. Decarboxylation of gallic and ellagic acids was a major reaction under conditions comparable with kraft and soda pulping processes. Gallic acid was much more stable than ellagic acid. There was little decarboxylation of gallic acid in NSSC pulping liquors. Ellagic acid was decarboxylated to high yields of 2,2′,3,3′,4,4′ hexahydroxydiphenyl under kraft and soda pulping conditions. There was less extensive but significant decarboxylation of ellagic acid under NSSC pulping conditions.

Keywords: *Eucalyptus* · Groundwood pulping · Neutral sulfite pulping · Soda pulping · Sulfate pulping · Reactions (chemical) · Hydrolysis · Decarboxylation · pH · Temperature · Carboxylic acids · Organic acids · Phenols · Tannins · Ellagic acid* · Ellagitannins* · Gallic acid

THE wood of *Eucalyptus* species is being used increasingly throughout the world for the manufacture of a variety of pulp and paper products. The heartwoods of most eucalypts contain appreciable quantities of ellagitannins, ellagic acid, and to a lesser extent gallic acid (*1*, *2*). These compounds cause manufacturing problems such as high chemical consumption during pulping (*3*, *4*), dark color of pulps (*4*, *5*), difficult burning properties of black liquors (*6*), and insoluble deposits which adhere strongly to metal surfaces (*7*).

The major compounds present in *Eucalyptus delegatensis* extractives (see Fig. 1) are ellagic acid, D-2 (either 2,3; 1,6 di-HHDP[1]-glucose [*8*] or 2,3; 4,6 di-HHDP-glucose [*9*]), D-1 (2,3 HHDP-glucose), and the ellagitannins of unknown structure D-13 and D-6 (*8*). There are also large amounts of gallic acid, D-4 (4,6 HHDP-glucose), and the unidentified ellagitannins D-3 (which yields gallic and ellagic acids and glucose on hydrolysis) and D-12 (see Fig. 2). There are small amounts of many other unidentified ellagitannins. The structures of D-1, D-2, and D-4 shown in Fig. 1 have been proposed by Seikel and Hillis (*8*). Hillis (*10*) has shown that under certain alkaline conditions the ellagitannins are much more chromogenic than their hydrolysis products gallic and ellagic acids. Although Seikel and Hillis (*8*) have demonstrated large differences in the ester hydrolysis rates of various ellagitannins in acidic solutions, there have been no studies of the rates of hydrolysis of these compounds under alkaline conditions. During kraft, soda, or NSSC pulping processes the ellagitannins are probably rapidly hydrolyzed. Baklien has demonstrated that ellagic acid is unstable in alkali over a wide range of pH and temperature conditions (*6*) and has suggested that decarboxylation of ellagic acid is a major reaction during soda pulping (*11*). However, both gallic and ellagic acids are rapidly oxidized in alkali under mild conditions, and it is possible that some of the ellagic acid degradation demonstrated by Baklien may have been caused by oxidation.

An understanding of the behavior of the ellagitannins, ellagic acid, and gallic acid in alkaline solutions should be of considerable assistance in an evaluation of how these compounds influence the properties of pulps and spent liquors. We have, therefore, examined the rates of hydrolysis of several ellagitannins under conditions comparable with cold soda and alkaline-groundwood pulping processes and have examined the reactions of gallic and ellagic acids under conditions comparable with kraft, soda, and NSSC pulping processes. We attempted, as far as possible, to carry out the reactions under oxygen-free conditions.

RESULTS AND DISCUSSION

Hydrolysis of Ellagitannins

The material used for the hydrolysis studies was a complex mixture of compounds (Fig. 2) typical of water soluble extracts from *Eucalyptus delegatensis*. The compounds examined were D-1 + D-4, D-2, D-3, D-6, and D-13.

The hydrolysis rates of the various ellagitannins differed considerably (Fig. 3). Compound D-2, which possibly contains an ester linkage with the anomeric glucose carbon, was the most sensitive to alkaline hydrolysis, just as it was under

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[1] HHDP = hexahydroxydiphenoyl.

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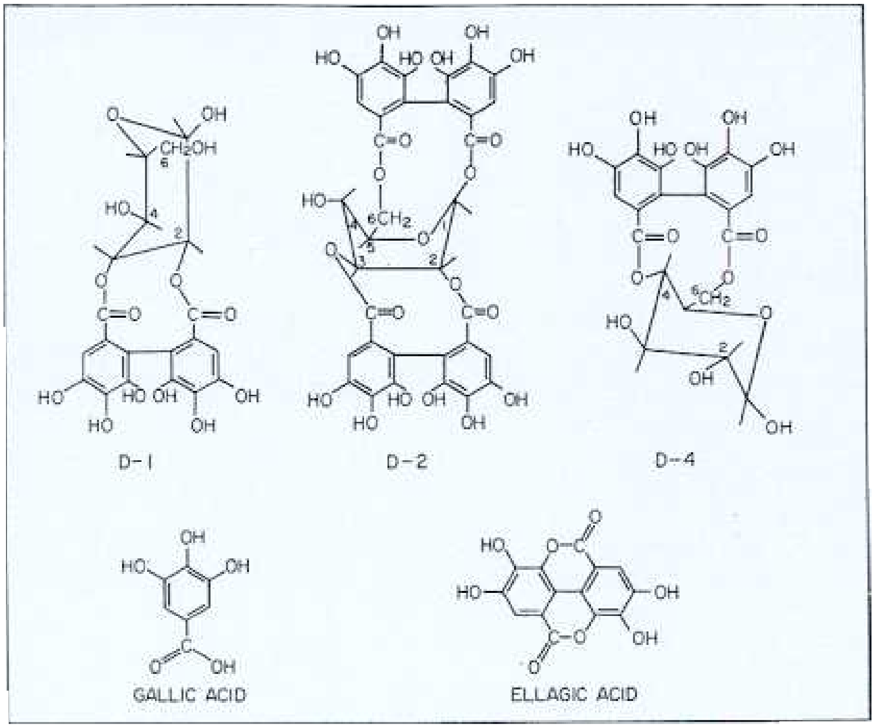

Fig. 1. Proposed structures of the ellagitannins D-1, D-2, and D-4 and hydrolysis products gallic and ellagic acids.

Table I. Chromatographic Properties of Ellagic Acid Reaction Products

	Ellagic acid	*IV*[a]	*V*[a]
R_f			
BAW	0.35	0.45	0.55
Forestal solvent[b]	0.35	0.40	0.40
6% Acetic acid	0.00	0.00	0.45
Color			
UV light	White-blue	Blue	Absorbs
UV + NH_3	Orange-yellow	Lemon-yellow	Blue
Barton's reagent[c]	Weak blue	Dark blue	Blue
Diazotized *p*-nitroaniline	Yellow-brown	Yellow-brown	Yellow
NSSC liquor	Weak yellow	Orange-pink	Orange-red

[a] IV is suspected 2,3,3′,4,4′ pentahydroxydiphenylmethylolid. V is 2,2′,3,3′,4,4′ hexahydroxydiphenyl.
[b] Acetic acid/conc. hydrochloric acid/water (30/3/10 vvv).
[c] 0.3% Ferric chloride/0.3% potassium ferricyanide (1/1 vv).

acidic conditions (*8*). Compound D-3, which contains a galloyl ester, was rapidly hydrolyzed under alkaline but was more stable under acidic conditions (*8*). Compounds D-1 + D-4 and then D-13 were the ellagitannins most resistant to hydrolysis while D-6 had an intermediate stability in alkali. Although hydrolysis of D-2 should yield D-1 (*8*), we did not observe a substantial increase in the amount of D-1 + D-4 under conditions where there was significant hydrolysis of D-2. This may have been because the amounts of D-2 were small compared with those of D-1 + D-4, or because there was a balancing rate of hydrolysis of D-4. Seikel and Hillis (*8*) found D-4 to be more easily hydrolyzed than D-13; our results were inconclusive.

The effect of temperature on the hydrolysis rate of D-2 at pH 8.2 is shown in Fig. 4. There was no significant hydrolysis of any of the ellagitannins at 0°C. Only 14% of D-2 remained after 3 hr at 50°C and it was essentially absent after 15 min at 100°C. Temperature also had a marked effect on the hydrolysis rate of the more stable ellagitannins D-1 + D-4 and D-13. At pH 9.8, they were unchanged after 3 hr at 0°C, 45% remained after 3 hr at 50°C, and they were rapidly hydrolyzed at 100°C. The effect of pH on the hydrolysis rate was marked (Fig. 5). After 3 hr at 50°C, 75% of D-6 remained at pH 8.2, 32% remained at pH 9.8, while it was essentially absent after 30 min at pH 13.1.

Because the ellagitannins D-13 and D-6 appear to be much more chromogenic than the other ellagitannins (*10*), conditions resulting in rapid hydrolysis of these compounds are especially important. Only treatments at 100°C at pH 8.2 and 9.8, or at 50°C and pH 13.1 resulted in rapid hydrolysis of D-13. Only 68% of D-6 was hydrolyzed after 3 hr at pH 9.8 and 50°C.

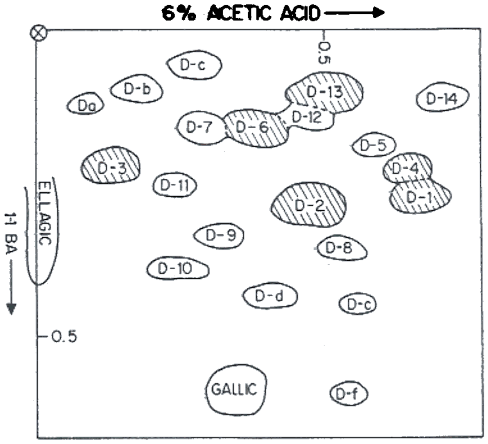

Fig. 2. Paper chromatogram of material used for hydrolysis studies. The compounds examined are in the hatched areas.

Decarboxylation of Gallic and Ellagic Acids

When gallic and ellagic acids were heated in alkaline solutions under conditions comparable with those of kraft, soda, and NSSC pulping processes, the reactions shown in Fig. 6 occurred.

Pyrogallol was identified by direct comparison with authentic material using paper and thin layer chromatography in a number of different solvents. Gas-liquid chromatography of the trimethylsilylether derivatives of the reaction product of gallic acid and pyrogallol gave peaks at matching retention times. The chromatographic properties of the reaction products of ellagic acid are summarized in Table I. Because large proportions of hexahydroxydiphenyl were produced under most conditions, this product was examined in some detail. The UV absorption spectra had a maximum at 259 nm, and the maxima of the acetate and methylated derivatives were 237 and 250 nm, respectively. The IR spectra of hexahydroxydiphenyl and pyrogallol were very similar as were those of the acetates of these two compounds. The methylated derivative had the same melting point as that reported in the literature for 2,2′,3,3′,4,4′ hexamethoxydiphenyl—123°C (*12*). Spots of hexahydroxydiphenyl on paper rapidly oxidized to purplish-brown products in air. Spots that had been exposed to ammonia vapor could not be extracted from the paper with cold or hot water but were slowly extracted with a hot 0.1*N* $NaHCO_3$ (pH 8.2) solution.

A blue fluorescent compound formed

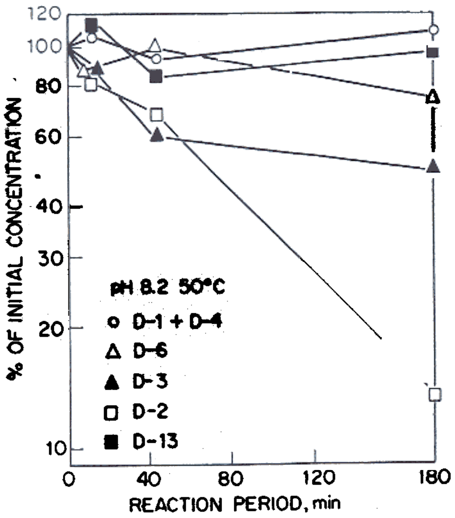

Fig. 3. Hydrolysis of ellagitannins at pH 8.2 and 50°C.

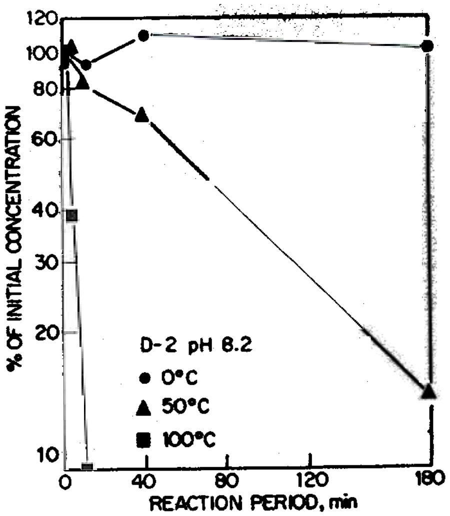

Fig. 4. Effect of temperature on hydrolysis of D-2 at pH 8.2.

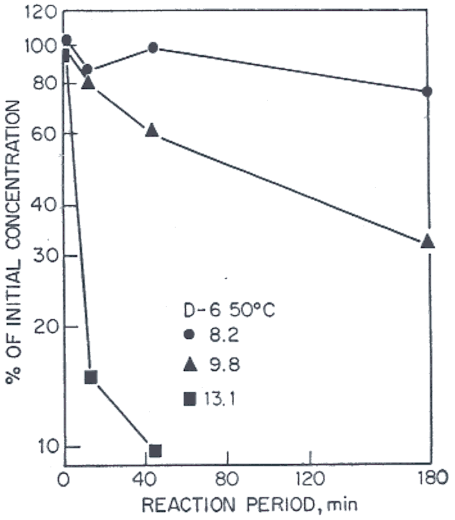

Fig. 5. Effect of pH on hydrolysis of D-6 at 50°C.

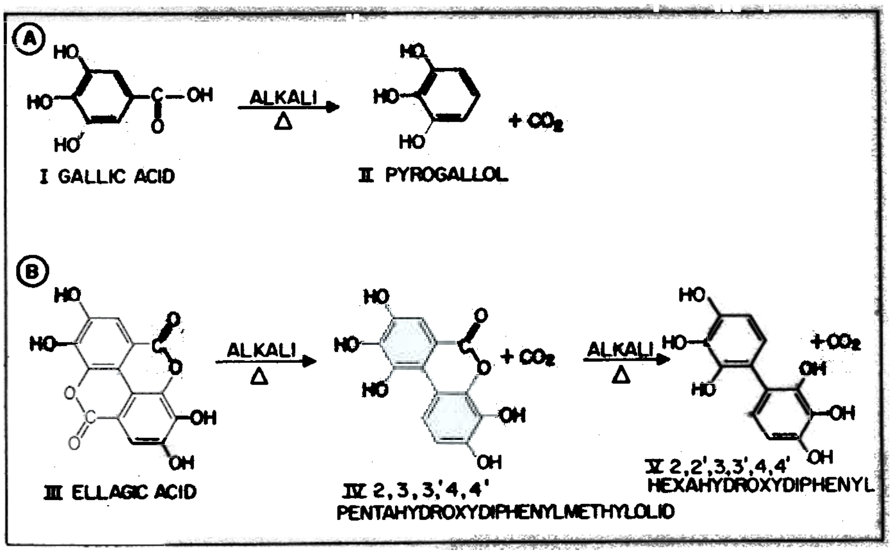

Fig. 6. Reactions of gallic and ellagic acids when heated in alkali in the absence of oxygen.

during the more mild treatments of ellagic acid. Paper chromatography of this compound (Table I) and thin layer chromatography of the methylated derivative gave R_f values similar to ellagic acid analogs. The UV spectra of this compound, its methylated derivative, and its trimethylsilyl ether collected by preparative gas-liquid chromatography were all consistent with those expected from 2,3,3',4,4' pentahydroxydiphenyl methylolid. We were unable to find any reports of the chromatographic, spectral, or physical properties of this compound and have as yet been unable to obtain sufficient material to obtain crystalline products.

Heat treatments in 0.5*N* NaOH and kraft pulping liquor gave very similar reaction products with gallic acid (Table II) and ellagic acid (Table III). Significant amounts of pyrogallol were found only after heating gallic acid for 2 hr at 160°C. On the other hand, ellagic acid was largely decarboxylated to hexahydroxydiphenyl after 30 min at 160°C. Even after heating at 130°C for 30 min, ellagic acid formed small amounts of hexahydroxydiphenyl and significant amounts of the suspected pentahydroxydiphenylmethylolid. Only a very small proportion of gallic acid was decarboxylated when it was heated in 0.5*N* Na_2CO_3 (Table II). The rate of decarboxylation of ellagic acid in 0.5*N* Na_2CO_3 was also lower than it was in 0.5*N* NaOH (Table III). We consistently observed smaller amounts of decarboxylation products when the acids were heated with NSSC pulping liquor (Tables II and III) than with Na_2CO_3. This was probably due to a rapid decrease in the pH of the solution during the reaction.

CONCLUSIONS

1. Compounds D-1 + D-4, D-13, and D-6 were all resistant to alkaline hydrolysis. It would be expected that these ellagitannins would not hydrolyze rapidly under the conditions of cold soda or alkaline-groundwood pulping and consequently they could contribute appreciably to the color of these pulps.

2. Ellagic acid was extensively decarboxylated to 2,2',3,3',4,4' hexahydroxydiphenyl during kraft and soda pulping. This compound rapidly oxidized to dark brown products which could contribute considerably to the dark color of pulps and could influence the properties of the spent liquors. Gallic acid was decarboxylated to pyrogallol during kraft and soda pulping but at a much lower rate than that of decarboxylation of ellagic acid. Decarboxylation was much less extensive when these acids were heated under NSSC pulping conditions.

EXPERIMENTAL

Hydrolysis of Ellagitannins

The aqueous liquor squeezed from fresh *Eucalyptus delegatensis* chips under high pressure was collected by Australian

Table II. Decarboxylation of Gallic Acid[a]

Reaction Temperature, 160°C

Reaction time, hr	*Gallic acid*	*Pyrogallol*
	0.5*N* NaOH (pH 13.5)	
2	++−+++	+−++
1	++++	Trace
0.5	++++	None
	Kraft Liquor (pH 13.1)	
2	++−+++	+−++
1	++++	Trace
0.5	++++	None
	0.5*N* Na_2CO_3 (pH 10.5)	
2	+++	+
1	++++	Trace
0.5	++++	None
	NSSC Liquor (pH 10.4)	
2	++++	Trace
	++++	Trace
0.5	++++	None

[a] Concentrations estimated by visual comparison of spot intensity on paper chromatograms.

Newsprint Mills Ltd., Boyer, Tasmania. The water solubles were freeze dried and extracted with acetone in a Soxhlet apparatus for 5 hr. The extract was redissolved in water and freeze dried to give a yellow powder in 24% yield of the original water solubles. The powder was examined by paper chromatography (PC) on Whatman No. 2 papers using 1-1 BA (*n*-butanol/27% acetic acid 1/1 vv) and then with 6% acetic acid (Fig. 2). The chromatograms were examined under shortwave UV light (254 nm) before and after exposure to ammonia vapor and then sprayed with either Barton's reagent (0.3% ferric chloride/0.3% potassium ferricyanide 1/1 vv) or with NSSC pulping liquor and allowed to oxidize in air (*10*).

The above ellagitannin mixture (100 mg) was dissolved in 10 ml of alkaline buffers (0.1*N* $NaHCO_3$, pH 8.2; 0.1*N* $NaHCO_3$ + 0.2*N* Na_2CO_3, pH 9.8; and 0.1*N* NaOH pH 13.1), a layer of mineral oil was applied to the top of the solution, and the reaction tube was continuously flushed with nitrogen to limit oxidation. Reactions were examined at 0, 50, and 100°C. After time periods of 5, 15, 45, and 180 min, 2-ml samples were added to HCl solutions and diluted to 4.0 ml. Three chromatograms were each spotted with 100 μl of these solutions. The chromatograms were developed with 1-1 BA and 6% acetic acid. One chromatogram for each condition was sprayed with NSSC pulping liquor to aid in the location of the spots. The remaining two chromatograms were examined under shortwave UV light and the spots representing D-1, D-2, D-3, D-4, D-6, and D-13 along with a blank spot were cut out.

The amount of ellagitannin on each spot was measured by an adaptation of the method of Swain and Hillis (*13*). A Folin-Denis reagent was prepared by heating under reflux 75 ml of distilled water, 10 g of sodium tungstate, 2 g of phosphomolybdic acid, and 5 ml of phosphoric acid for 2 hr, and the reagent was cooled and diluted to 100 ml. The sample spots were soaked in 3.0 ml of water for 2 hr, and 0.20 ml of the Folin-Denis reagent was added. After 3 min, 0.30 ml of saturated Na_2CO_3 was added. The solutions were stored overnight, and the absorbance at 750 nm was measured using the reagents added to the blank paper as a reference. Analysis of known amounts of ellagitannins showed a linear calibration curve up to an absorbance of 0.80. Compounds D-1 and D-4 could not be reproducibly separated, so the sum of their absorbance was considered.

Table III. Decarboxylation of Ellagic Acid[a]

Reaction temp., °C	Reaction time, hr	Ellagic acid	IV[b]	V[b]
		0.5*N* NaOH (pH 13.5)		
160		Trace–+	Trace	+++–++++
160	0.5	Trace–+	+	++
130	1	+	+	+–++
130	0.5	+	++	+
		Kraft Liquor (pH 13.1)		
160	1	Trace–+	Trace	+++–++++
160	0.5	Trace–+	++	++
130	1	Trace–+	++	++
130	0.5	+	++	+
		0.5*N* Na_2CO_3 (pH 10.5)		
160	1	Trace–+	Trace	+++–++++
160	0.5	Trace–+	+	++
130	1	++	+	+
130	0.5	+++	+	Trace
		NSSC Liquor (pH 10.4)		
160	1	++	+–++	Trace–+
160	0.5	+++	+	Trace
130	1	+++	+	Trace
130	0.5	++++	Trace–+	None

[a] Concentrations estimated by visual comparison of spot intensity on paper chromatograms.

[b] IV is suspected 2,3,3′,4,4′ pentahydroxydiphenylmethylolid. V is 2,2′,3′,4,4′ hexahydroxydiphenyl.

Decarboxylation of Gallic and Ellagic Acids

Gallic acid was available from stock supplies and ellagic acid was prepared from a magnesium-ellagic acid complex donated by Australian Paper Manufacturers Ltd. Acetate, methyl, and trimethylsilyl derivatives of the reaction products were prepared by standard methods.

Gallic or ellagic acid was dissolved or suspended (5 mg/ml) in 0.5*N* NaOH, 0.5*N* Na_2CO_3, a kraft pulping liquor (6.3 g Na_2S + 19.35 g NaOH + 350 ml of distilled water), and an NSSC pulping liquor (15 g Na_2SO_3 + 3.5 g Na_2CO_3 + 350 ml of distilled water), and the samples were continuously flushed with nitrogen to limit oxidation. The samples (5 ml) were then sealed in glass ampoules under nitrogen and heated to either 130 or 160°C for time periods of up to 2 hr in stainless steel bombs. About 15 min were required to come up to temperature and about 20 min were required to cool the samples. The ampoules were broken under HCl solutions.

The extent of decarboxylation was estimated by visual comparison of spot intensities on paper chromatograms. Gallic acid reactions were analyzed using 6% acetic acid where R_f values of gallic acid and pyrogallol were 0.35 and 0.56, respectively. Ellagic acid reactions were examined using either Forestal solvent (HCl/-acetic acid/water, 3/30/10 vvv) or BAW (*n*-butanol/acetic acid/water, 6/1/2 vvv) and 6% acetic acid in the second dimension. Chromatographic properties of the reaction products of ellagic acid are summarized in Table I. The chromatograms were examined under UV light before and after exposure to ammonia vapor and then sprayed with Barton's reagent.

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