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Journal of Wood Chemistry and Technology

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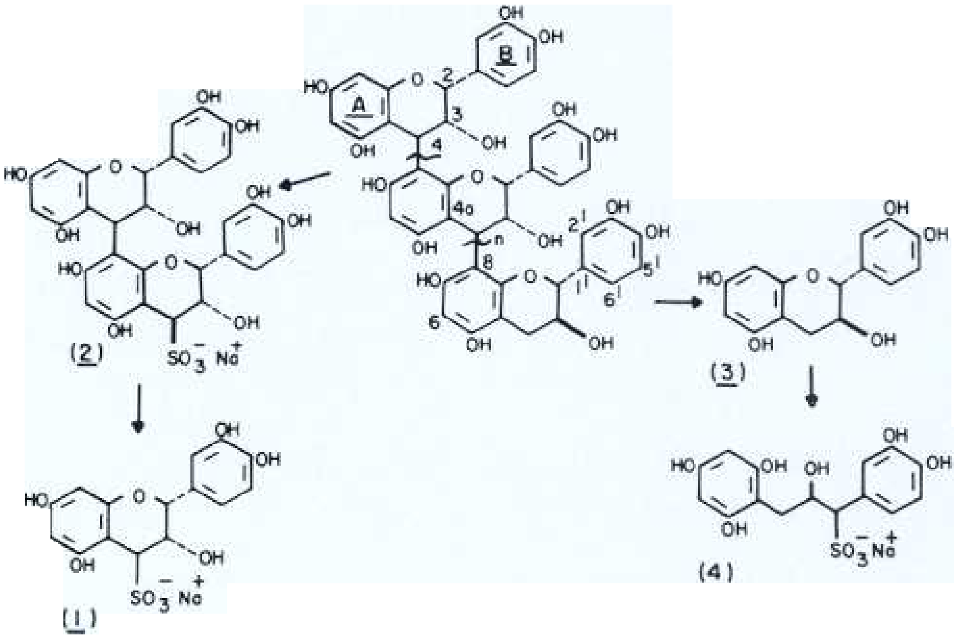
CONDENSED TANNINS: DESULFONATION OF HYDROXYBENZYLSULFONIC ACIDS RELATED TO PROANTHOCYANIDIN DERIVATIVES

Gerald W. McGraw
Department of Chemistry
Louisiana College
Pineville, Louisiana, 71360

Peter E. Laks[1] and Richard W. Hemingway[2]
Southern Forest Experiment Station
USDA-Forest Service
2500 Shreveport Highway
Pineville, Louisiana, 71360-5500

ABSTRACT

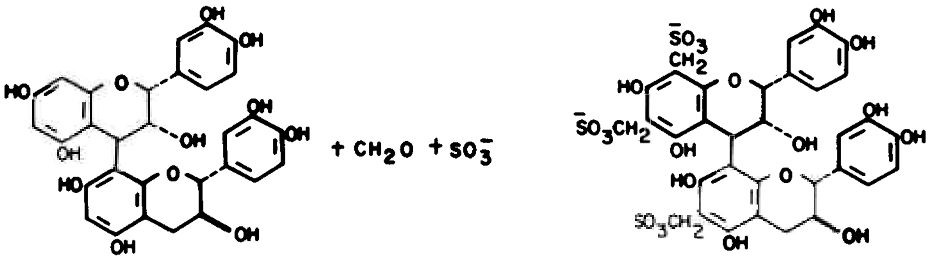
Studies on the desulfonation of 2,4,6-trihydroxybenzylsulfonic acid and sodium epicatechin-(4β)-sulfonate showed that sulfonates α to a phloroglucinol ring are good leaving groups at ambient temperature and pH greater than 8.0. In contrast, hydroxybenzylsulfonic acids with resorcinol or phenol hydroxyl functionality resist desulfonation even at pH 12 and 90°C. It was also not possible to make (2,4,6-trihydroxyphenyl), (4-hydroxyphenyl)-methane or (2,4,6-trihydroxyphenyl),(2,4-dihydroxyphenyl)-methane by slow addition of 2,4,6-trihydroxybenzylsulfonic acid to alkaline solutions of phenol or resorcinol. However, facile desulfonation of 2,4,6-trihydroxybenzylsulfonic acid derivatives permits the use of condensed tannins from most conifer barks as intermediates for the formulation of water-resistant, cold-setting, wood-laminating adhesives. Under typical adhesive formulation conditions, the sulfonic acid groups on tannin derivatives from conifer barks will be displaced, resulting in water-insoluble polymers.



SCHEME 1. Reaction of tannins with sulfite ion.

INTRODUCTION

Extraction of loblolly pine bark with water containing 4.0% sodium sulfite and 0.4% sodium carbonate (based on dry bark weight) gives a product containing condensed tannins at yields of 21-24% of the dry weight of bark with a cost of about $0.20/lb of extract.[3] In the course of this extraction, some condensed tannin interflavanoid bonds are cleaved. The quinone methides that result from this cleavage react with sulfite ions to produce sodium epicatechin-(4β) sulfonate (1) and oligomeric procyanidin-(4) sulfonates (2) from the polymer extender units, together with small amounts of catechin (3) and sodium 1-(3,4-dihydroxyphenyl)-2-hydroxy-3-(2,4,6-trihydroxyphenyl) propane-1-sulfonate (4) from ring-opening of the terminal flavan unit (Scheme 1).[4] Sulfonated tannins could be important raw materials for inexpensive, cold-setting, wood laminating adhesives. However, it is important to obtain a better



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SCHEME 2. Sulfomethylation of tannins

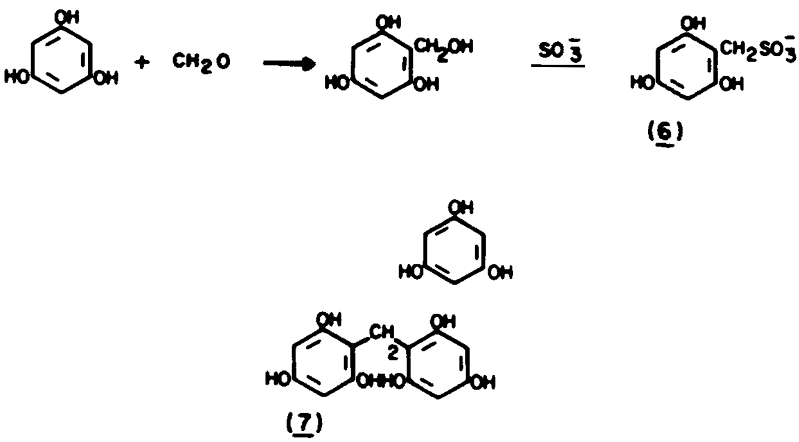
understanding of the conditions required to remove the sulfonate function at C-4 in compounds such as (1) and (2) to produce intermediates for adhesives that will form water-resistant gluelines.

Desulfonation reactions may also prove important in the use of sulfomethylated condensed tannin derivatives as resin intermediates. One of the most troublesome obstacles to using the condensed tannins from conifer barks is the tannins' extremely rapid rate of polymerization with formaldehyde.[5,6] It seems possible that sulfomethylated tannin intermediates might be made by reaction with formaldehyde in the presence of excess sulfite ion (Scheme 2). These derivatives (i.e., 5) would be expected to have improved water solubility and lower viscosity because the solution properties of condensed tannins are highly influenced by association effects.[7,8] The sulfonic acid function of a derivative such as (5) might then be removed with rapid polymerization of the tannin molecules in the formation of an adhesive. Here again, an understanding of the sulfonic acid function as a leaving group is essential. This study was made to explore factors influencing the desulfonation of hydroxybenzylsulfonic acids related to compounds (1) and (5).

RESULTS AND DISCUSSION

A. Sulfonation and Desulfonation of Phloroglucinol.

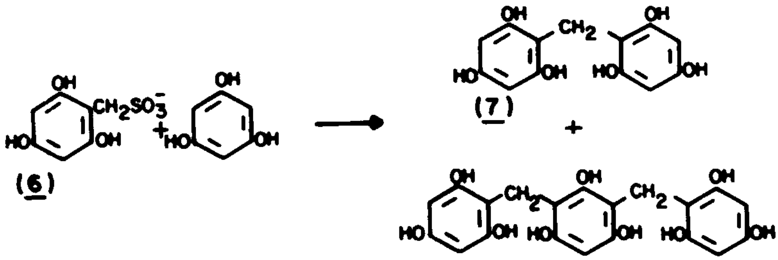
In order to make a model compound for the phloroglucinolic benzylsulfonic acid functions found in the tannin derivatives (1)



SCHEME 3. Sulfomethylation of phloroglucinol.

and (5), the sulfomethylation of phloroglucinol by reactions with formaldehyde and sulfite ion was examined under a wide variety of conditions. The highest yield of 2,4,6-trihydroxybenzylsulfonic acid (6), with minimal formation of di(2,4,6-trihydroxyphenyl) methane (7), was obtained by reaction of phloroglucinol, formaldehyde, and sulfite ion at mole ratios of 1:1:2, with a pH of 10.0, at ambient temperature (Scheme 3). The comparative ease with which (6) could be produced from phloroglucinol without encountering excessive condensation implies that a similar sulfomethylation of condensed tannins might be an effective route to a tannin derivative for use in formulation of cold-setting, phenolic adhesives. Prior to investigation of this reaction, it was necessary to learn more about reaction conditions required for desulfonation of compounds with 2,4,6-trihydroxybenzyl functionality.

To model these desulfonations, 2,4,6-trihydroxybenzylsulfonic acid (6) was reacted with phloroglucinol under a variety of pH and temperature conditions (Scheme 4). High yields of (7) were obtained from reactions at ambient temperature and pH 9.5 and above. Desulfonation of (6) and condensation to (7) also occurred at pH 8.0, but comparatively slowly. No reaction was observed at acidic pH. Reaction temperatures above ambient promoted the



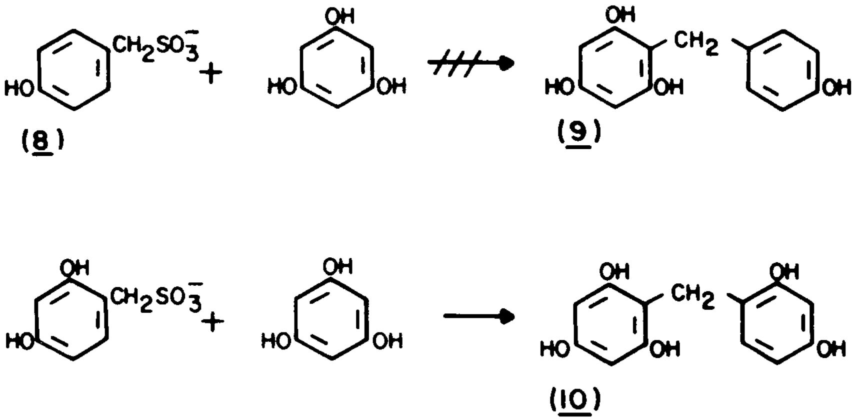
SCHEME 4. Reaction of 2,4,6-trihydroxybenzylsulfonic acid with phloroglucinol.

formation of higher molecular weight oligomers. On the basis of these results, one would expect polymerization of condensed tannin derivatives such as (1) or (5) with loss of the sulfonic acid functions at mild alkaline pH and low temperature. The viscosity of crude sulfite extracts of loblolly pine bark increases dramatically on addition of base. The rate of gelation is, however, sufficiently slow to permit use of these extracts in wood laminating adhesives.[9]

The importance of the phloroglucinol hydroxylation pattern in the desulfonation of (6) was determined by comparing its reactivity with that of *p*-hydroxybenzylsulfonic acid (8) and phloroglucinol (Scheme 5). *p*-Hydroxybenzylsulfonic acid was essentially unreactive under all conditions investigated, including pH levels of 11 to 13 at reflux temperature. Minor amounts of other products were noted on two-dimensional cellulose TLC plates, but (2,4,6-trihydroxyphenyl),(4-hydroxyphenyl)-methane (9) was not detected.

B. Sulfonation and Desulfonation of Resorcinol

Reaction of resorcinol with formaldehyde and excess sulfite ions gave a mixture of sulfomethylated products from condensation to dimeric and oligomeric products. Reaction of this mixture with phloroglucinol for 4 hours at pH 12 and 90 °C gave only low yields of the suspected (2,4,6-trihydroxy phenyl),(2,4-dihydroxyphenyl)methane (10). TLC and HPLC showed that large amounts of

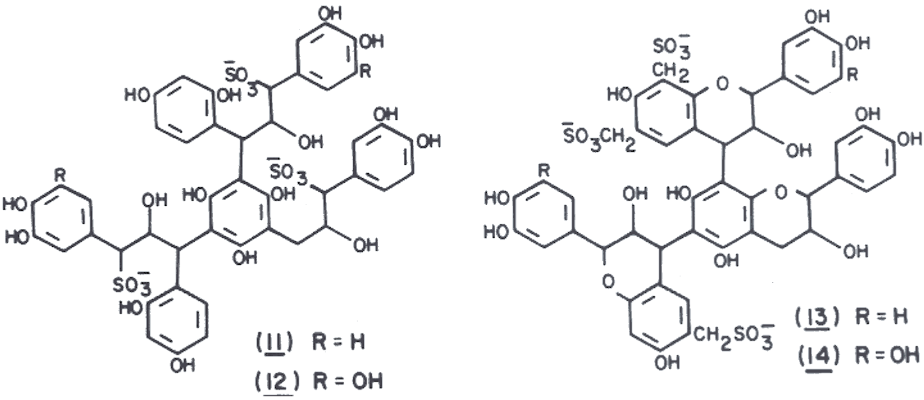


SCHEME 5. Reaction of phenolic and resorcinolic benzylsulfonic acids with phloroglucinol.

sulfonated derivatives remained unreacted. On the basis of these results, it can be predicted that sulfonic acid functions such as are found in (4), the pyran ring-opened profisetinidin and prorobinetinidin based C-2 sulfonated tannin derivatives (11 and 12) that are apparently produced from quebracho and wattle tannins[10] and their sulfomethylated derivatives (13 and 14), would require more severe desulfonation conditions than are needed for sulfonated conifer bark tannins (Scheme 6).

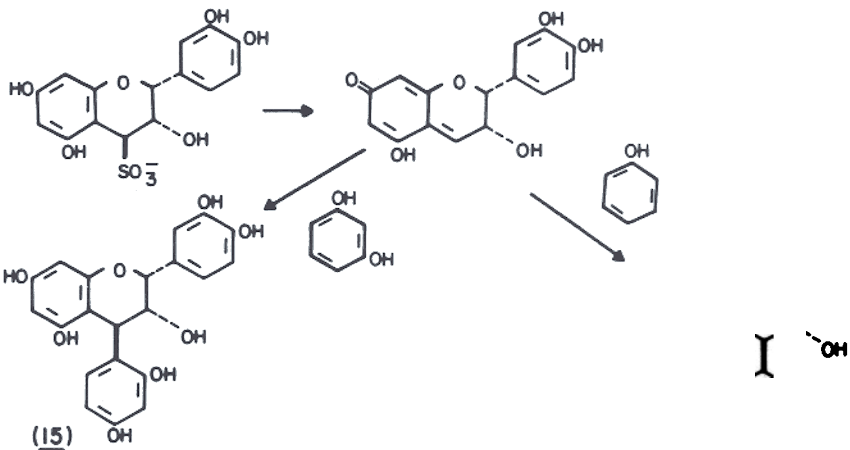
Previous work[11,12] has shown that more than 60% of the resorcinol normally used in cold-setting, wood-laminating adhesives can be replaced with tannin-resorcinol adducts related to (15) while exceeding requirements of the American Institute of Timber Construction. These tannin derivatives were made by reaction of condensed tannins (2 parts by weight) with resorcinol (1 part by weight) at about 120 °C in the presence of an acetic acid catalyst. It seemed possible that a similar phenol adduct (16) might be produced simply by slow addition of sulfite extracts from bark that contain significant proportions of (1) and (2) to alkaline solutions of phenol (Scheme 7). The high mole ratio of phenol forced by the slow addition might overcome differences in the nucleophilicity of the phenol and phloroglucinolic A-rings of the flavan derivatives.

C. Use of Desulfonation Reactions to Produce Phenol, Resorcinol, and Phloroglucinol Adducts



(11) R = H
(12) R = OH
(13) R = H
(14) R = OH

SCHEME 6. Sulfonated derivatives of quebracho and wattle tannins.



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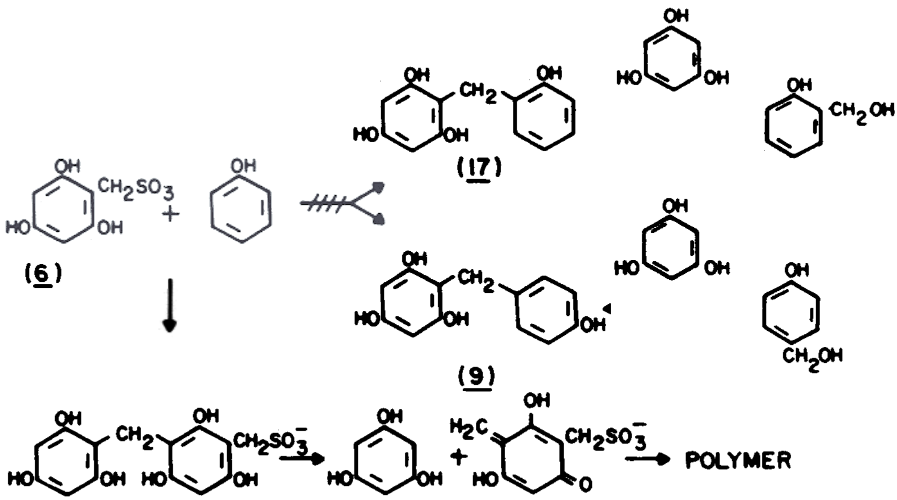
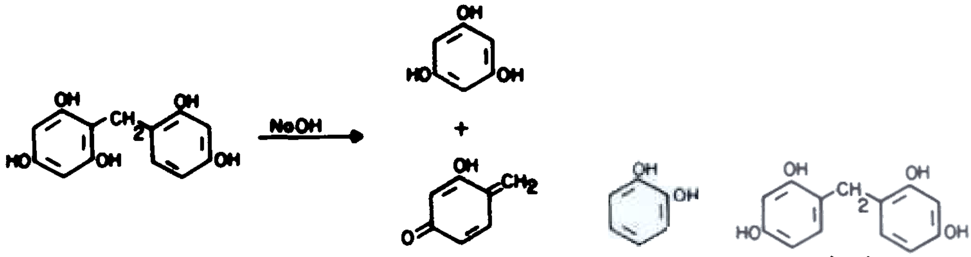
SCHEME 7. A possible route to tannin-phenol and tannin-resorcinol adducts.



SCHEME 8. Reaction of 2,4,6-trihydroxybenzylsulfonic acid with phenol.

To test this hypothesis, 2,4,6-trihydroxybenzylsulfonic acid (6) was added dropwise to phenol under a variety of conditions. However, no (2,4,6-trihydroxyphenyl),(2- or 4-hydroxyphenyl)methanes [(17) or (9)], could be isolated from the reaction products (Scheme 8). Instead, phloroglucinol and sulfonated derivatives of 2,4,6-trihydroxyphenylmethane oligomers were produced as determined by 2D-TLC. It was not possible, therefore, to overcome the comparatively high nucleophilicity of the phloroglucinolic ring by slow addition to phenol.

It was also not possible to make significant amounts of (2,4,6-trihydroxyphenyl), (2,4-dihydroxyphenyl)-methane (10) by direct reaction of 2,4,6-trihydroxybenzylsulfonic acid (6) with resorcinol. Again, small amounts of phloroglucinol and di(2,4,6-trihydroxyphenyl)-methane (7) were obtained, along with larger amounts of oligomeric 2,4,6-trihydroxybenzylsulfonate derivatives (TLC). When 2,4,6-trihydroxybenzylsulfonic acid was added dropwise to a solution of resorcinol at pH 10 at reflux, a significant amount of di(2,4-dihydroxyphenyl)-methane (18) was



(19) (18)

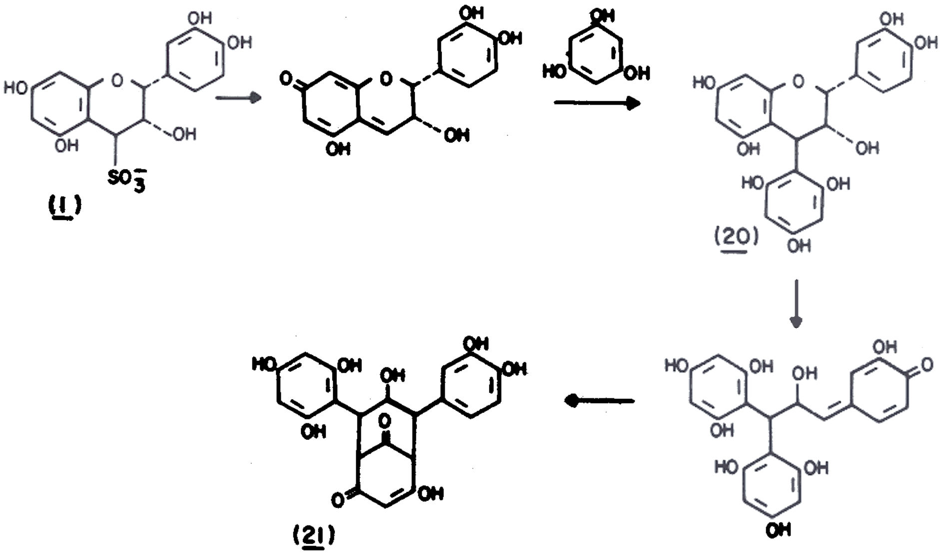
SCHEME 9 Formation of di(2,4-dihydroxyphenyl) methane from (2,4,6-trihydroxyphenyl), (2,4-dihydroxyphenyl) methane

produced in addition to the above-mentioned compounds. This compound could be formed from (10) by loss of phloroglucinol and reaction of the quinone methide (19) with resorcinol (Scheme 9). This observation is consistent with previous results showing that phloroglucinol was a good leaving group in alkaline solutions, particularly where two phloroglucinol rings are joined by a methine or methylene carbon.[13]

Sodium epicatechin-(4β)-sulfonate (1) was also reacted with phloroglucinol under a variety of conditions. When reactions were made in the pH range of 8-10 at ambient temperature, small amounts of epicatechin-(4β)phloroglucinol (20) were produced in the early stages of the reaction. However, at high pH and temperature, or after reaction times of over an hour at more mild conditions, an enolic form of 6-(2,4,6-trihydroxyphenyl)-8(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9-bicyclo-[3.3.1]-nonatrione (21) was the major reaction product (Scheme 10). This compound is also formed in reaction of polymeric procyanidins with phloroglucinol under alkaline conditions.[13]

CONCLUSIONS

The sulfonate function in sodium epicatechin-(4β)-sulfonate, like that in 2,4,6-trihydroxybenzylsulfonic acid, is a good leaving group in alkaline solution. Addition of condensed tannin



SCHEME 10. Reaction of epicatechin-(4B)-sulfonate with phloroglucinol.

sulfonate derivatives, such as (1) and (2), to solutions at pH >8.0 and ambient temperature will result in rapid loss of the sulfonate function and polymerization through the nucleophilic A-ring of procyanidin derivatives. If these products are to be used in applications such as cold-setting, wood-laminating adhesives, it is important to limit the severity of these conditions in order to restrict the extent of rearrangement of the phloroglucinol rings as occurs in the formation of (21).[13] If properly controlled, the tannins will carry sufficient phloroglucinol functionality to permit rapid crosslinking with PRF resols.[9]

Similar results can be expected in reactions of sulfomethylated condensed tannins of the procyanidin class (i.e., 5) based on the facile formation of di(2,4,6-trihydroxyphenyl) methane (7) from 2,4,6-trihydroxybenzylsulfonic acid and phloroglucinol. However, desulfonation of resorcinolic benzylsulfonic acid derivatives requires much more vigorous reaction conditions, and catecholic benzylsulfonate functions such as that in (4) would be expected to be stable under the usual conditions of resin formulation on the basis of the stability of *p*-hydroxylbenzylsulfonic acid at reflux temperture and pH 13. Therefore, approaches to the use of sulfonated polymeric procyanidins, which include most conifer bark tannins,[14] differ markedly from those for the profisetinidins (i.e., quebracho tannins)[15] and prorobinetinidins (i.e., wattle bark tannins).[16]

The facile desulfonation of procyanidin derivatives has been exploited recently in the formulation of cold-setting, wood-laminating adhesives. Condensed tannins isolated from *Pinus Taeda* bark by extraction with 4.0% sodium sulfite and 0.4% sodium carbonate have been used to replace 50% of the phenol-resorcinol-formaldehyde resin normally used in timber-laminating adhesives.[9] As would be expected from the above studies, the tannin-sulfonate derivatives were stable at room tempertures with neutral to slightly acidic pH. However, on addition of sodium hydroxide, the viscosity of the solutions increased to finally produce a hard water-insoluble gel, due to loss of the sulfonate function and repolymerization. When phenol-resorcinol-formaldehyde prepolymers and formaldehyde are added to alkaline solutions of these tannin derivatives, copolymerization between the PRF resin and the tannin can occur rapidly at room temperture. When either applied as mixtures with phenol-resorcinol-formaldehyde resins or as a two-component adhesive in Kreibich's "Honeymoon" system,[17] bond qualities exceeded the requirements of the American Institute of Timber Construction Standards AITC-107 and AITC-110.[9] These adhesives give bonds with high shear strength and high wood failure after vacuum-pressure water soak (AITC-110) and after 2 hours of boiling, suggesting sufficient crosslinking to overcome the inherent lability of the interflavanoid bonds in the tannins.

EXPERIMENTAL

Reaction of Pine Bark Tannins with Sulfite Ion

Condensed tannins from loblolly pine phloem[7,19] (2.5 g) were reacted with sodium bisulfite (4.5 g) in water (75 ml) at 105 °C overnight. The product was freeze-dried and separated on Sephadex LH-20 (2.4 cm x 90 cm) by elution with ethanol, and fractions (15 ml) were collected. Fractions 49 to 85 were combined and rechromatographed using the same separation conditions. Details of the isolation of compounds (2) and (4) will be described elsewhere but have been summarized.[4]

Sodium epicatechin-(4β)-sulfonate (1): Fractions 40 to 90 from the second column purification were combined to obtain a yellow-brown amorphous solid (0.4 g). Found: C, 43.02; H, 3.93; S, 7.88 $C_{15}H_{13}O_9SNa \cdot 1.5(H_2O)$ requires: C, 42.95; H, 4.06; S, 7.64%. ^{1}H-NMR ($d_6$acetone): 6.6-7.2 (3H,m,B-ring); 6.01(2H,s,A-ring); 5.50(1H,s,H-2); 4.58(1H,br-s,H-3); 4.13(1H,s,H-4). ^{13}C-NMR ($d_6$acetone-D_2O, 1:1): 157.3-158.5(A-ring C-O); 144.9,145.0(B-ring C-O); 131.2(C-1'); 119.6(C-6'); 116.4(C-5'); 115.3(C-2'); 99.1(C-6); 97.5(C-4a); 97.1(C-8);75.9(C-2); 66.8(C-3); 60.5(C-4). FAB-MS gave M+1 = 394; $C_{15}H_{13}O_9NaS$ +1 requires 394.

Sulfomethylation of Phloroglucinol

Phloroglucinol dihydrate (2g) was dissolved in methanol (15 ml). Formalin (37.8% solution, 1.0g) was stirred into the solution as it was held at room temperature over 5 minutes. Sodium sulfite (2.28 g) was then added as a solution in water (15 ml). Stirring was continued for 1.5 hours. The methanol was removed on a rotary evaporator and the aqueous solution was freeze-dried. The product was separated on a Sephadex LH-20 column (1.5 x 90 cm) by eluting with ethanol and collecting fractions (10 ml). The sulfomethylation reaction was carried out at several different pH values.

2,4,6-Trihydroxybenzylsulfonic acid (6): Fractions 33-49 were combined to give the product which crystallized (0.314 g) during evaporation of the ethanol. The crystals were washed with ethanol and dried; mp. 227-231 °C, decomposition. Found: C, 38.04; H, 3.41; S, 14.37%. $C_7H_8O_6S$ requires: C, 38.18; H, 3.64, S, 14.55%. ^{1}H-NMR (d_6-acetone-D_2O, 1:1): 6.06(2H,s,ArH); 4.18(2H,s,benzyl-CH_2). ^{13}C-NMR (d_6-acetone-D_2O, 1:1): 158.4(C-4); 157.8(C-2,C-6); 100.6(C-1); 97.1((C-3,C-5); 47.3(benzyl-CH_2). FAB-MS gave M+1=219; $C_7H_8O_6S$ +1 requires 219.

Some of the crystals (50 mg) were acetylated with dioxane-pyridine-acetic anhydride (0.5:1.0:1.0, v/v/v) overnight. The product was precipitated from water, washed with water, and dried under high vacuum. ^{1}H-NMR (d_6-DMSO): 6.84(2H,s,Ar-H); 3.72(2H,s,benzyl-CH_2); 2.21, 2.18(9H,s+s,ArOAc). ^{13}C-NMR

(d_6-DMSO): 168.7, 167.9(C=O); 149.6(C-2,C-6); 148.2(C-4); 118.2(C-1); 114.0(C-3,C-5); 46.2(benzyl-CH_2); 20.8, 20.7(ArO-Ac).

Di-(2,4,6-trihydroxyphenyl)methane (7): Fractions 24-32 were combined and the ethanol was removed under reduced pressure. The product was crystallized from methanol-water to give needles that decomposed to an amorphous solid at 64-68 °C. Found: C, 58.99; H, 4.77%. $C_{13}H_{12}O_6$ requires: C, 59.09; H, 4.55%. ^{1}H-NMR (d_6-acetone): 9.00 (4H, br-s, ArOH); 8.05 (2H,br-s,ArOH); 6.03 (4H,s,Ar-H); 3.72 (2H,s,CH_2). ^{13}C-NMR (d_6-acetone-D_2O, 1:1): 157.1(C-2,C-6); 155.9(C-4); 107.1(C-1); 96.6(C-3,C-5); 17.0(CH_2). FAB-MS gave M+1 = 265; $C_{13}H_{12}O_6$ +1 requires 265.

The amorphous residue from crystallization of (7) (50 mg) was acetylated with pyridine-acetic anhydride (1:1,v/v) overnight; poured into H_2O, and freeze-dried. The product was purified by prep-TLC (Si-gel, benzene-acetone, 8:2) by isolation of the band at R_F 0.44 and eluting with acetone. Evaporation of the acetone at reduced pressure gave an oil which, on standing, crystallized to needles, mp 148-149 °C. Found: C, 58.38; H, 4.86%. $C_{25}H_{12}O_{12}$ requires: C, 58.14; H, 4.65%. ^{1}H-NMR ($CDCl_3$): 6.83(4H,s,ArH); 3.67(2H,s,CH_2); 2.22, 2.11(18H,s+s,ArO-Ac). ^{13}C-NMR ($CDCl_3$): 168.5, 168.3(C=O); 149.6(C-2,C-6); 149.0(C-4); 121.4(C-1); 113.8(C-3,C-5); 29.7(benzyl-CH_2); 21.0, 20.6(ArO-Ac). FAB-MS gave M+1=517; $C_{25}H_{24}O_{12}$+1 requires 517.

Reaction of 2,4,6-Trihydroxybenzyl Sulfonic Acid with Phloroglucinol.

2,4,6-Trihydroxybenzyl sulfonic acid (11.6 mg, 0.048 mmoles) and phloroglucinol dihydrate (15.6 mg, 0.96 mmoles) were dissolved in 4 mL of pH 12 buffer and the pH adjusted with 6 N NaOH as necessary. The reaction vessel was purged with nitrogen, sealed, and placed in a water bath at the appropriate temperature. Aliquots were removed periodically for analysis by 2D-TLC [microcrystalline cellulose with tert-BuOH/HOAc/H_2O(3:1:1) followed by 6% HOAc]. The loss of sulfonate (R_f 0.28, 0.82) and phloroglucinol (R_f 0.56, 0.62) and the formation of di-(2,4,6-trihydroxyphenyl)methane(R_f 0.63, 0.32) were easily followed. Some samples were also analyzed by HPLC [Zorbax-CN,2.5% MeOH, 1.5 mL/min] showing the sulfonate (11.2 mL), phloroglucinol (15.0 mL) and the condensation product (20.2 mL).

A preparative scale reaction allowed for the isolation of the di-(2,4,6-trihydroxyphenyl)methane by column chromatography [Sephadex LH-20, eluting with EtOH-H_2O(1:1)] after separation from sulfonated materials by extraction into ethyl acetate.

Reaction of Phloroglucinol with p-Hydroxybenzylsulfonic Acid

p-Hydroxybenzylsulfonic acid (10 mg) was reacted with phloroglucinol (15.6 mg) in water (10 ml) at reaction pH of 11, 12, and 13 and temperatures of 50 °C, 90 °C and reflux. The reactions were monitored by two-dimensional cellulose TLC developed with *tert*-BuOH/HOAc/H_2O(3:1:1, v/v/v) and 6% acetic acid in the second dimension. In addition, reaction products were examined by HPLC (Zorbax-CN, 5% MeOH in water, 1.5 ml/min.).

p-Hydroxybenzylsulfonic Acid (8): *p*-Hydroxybenzyl alcohol (3 g) and sodium bisulfite (11.6 g) were dissolved in water (25 ml) and the reaction was heated under reflux for 2 hours, after which it was freeze-dried. The solid was dispersed in methanol and filtered to remove methanol-soluble materials (6.5 g). The methanol-insoluble products were separated by column chromatography on Sephadex LH-20 (1.5 x 90 cm), eluting with ethanol-water (1:1, v/v) and collecting 20 ml fractions. Fractions 22-31 were combined. Removal of solvent by rotary evaporation and freeze-drying gave an amorphous white solid (1.97 g). ^{1}H-NMR (d_6-acetone-D_2O): 7.21, 7.32(2H,d,ArH); 6.73, 6.84(2H,d,Ar-H); 3.95(2H,s,benzyl-CH_2). ^{13}C-NMR (d_6-acetone-D_2O): 158.6(C-4); 133.5(C-2,C-6); 124.8(C-1); 117.4(C-3,C-5); 58.1(benzyl-CH_2). FAB-MS gave M+1 = 187; $C_7H_8O_4S$ + 1 requires 187.

Reaction of Phloroglucinol with Resorcinolic Benzylsulfonic Acids

Resorcinol (4.4g) and sodium sulfite (12.6 g) were dissolved in water (50 ml) to which 37.8% formalin solution (3.2 g) was added at ambient temperature. The reaction vessel was purged with argon, sealed, and heated at 95 °C for 24 hours. The product was freeze-dried to obtain a light-brown solid. The product was extracted with ethyl acetate in a Soxhlet extractor to recover 0.23 g of ethyl acetate soluble material. The residue was then extracted with methanol to recover 4.78 g of methanol solubles. Column chromatography of the methanol-soluble fraction on Sephadex

LH-20, eluting with ethanol and collecting 15 ml fractions, gave an isolate in fractions 80-120 that appeared to contain a mixture of sulfonated resorcinol derivatives (0.24 g). Thin layer chromatography (microcrystalline cellulose, *tert*-BuOH/HOAc/H_2O(3:1:1)) indicated the mixture contained three sulfonated resorcinol drivatives with R 0.55, 0.45, and 0.17. Although difficult to purify, a small amount of the major sulfonated product (R 0.45) was isolated for structure determination by subjecting the mixture to chromatography over cellulose, eluting with *tert*-BuOH/HOAc/H_2O(3:1:1:). 1H-nmr(D_2O): 7.10-6.98(1H,m,ArH); 6.38-6.27(2H,m,ArH); 4.00 (2H,s,benzyl-CH2). 13C-nmr(D_2O-methanol-d4): 157.5 (C-1); 156.4(C-3); 133.9 (C-5); 111.9 (C-4); 108.7 (C-6); 104.2 (C-2); 51.9 (benzyl-CH2).

The mixture of sulfonated resorcinol derivatives (50 mg) and phloroglucinol dihydrate (71 mg) were dissolved in 15 ml of pH 12 buffer and NaOH was added to adjust the pH to 12.0. The reaction vessel was purged with nitrogen, sealed and placed in a bath at 90°C. After 4 hours, the vessel was removed from the bath and the contents neutralized. HPLC and TLC analyses showed major amounts of unreacted phloroglucinol and sulfomethylated resorcinol derivatives. Other products present in small proportions could not be isolated in sufficient purity or quantity to identify their structure.

Reaction of 2,4,6-trihydroxybenzylsulfonic Acid with Phenol

Phenol (20 mg) was dissolved in pH 12 buffer (4 ml) and the pH was readjusted to pH 12 with NaOH. 2,4,6-Trihydroxybenzylsulfonic acid (11.6 mg) was dissolved in a small amount of water and added dropwise to the phenol solution over a period of about 2 hours while the reaction mixture was held at 90 °C. After addition of the sulfonic acid, the reaction vial was purged with nitrogen, sealed, and returned to the water bath for an additional 3.5 hours of heating at 90 °C. Reaction products were examined by two-dimensional TLC [microcrystalline cellulose, *tert*-BuOH/HOAc/H_2O (3:1:1) and 6%HOAc] when one-half of the sulfonate had been added, after all the sulfonate had been added, and at the conclusion of heating. The reaction mixture was also examined by HPLC [Zorbox-CN, 2.5% MeOH] by comparison of retention volumes with (2,4,6-trihydroxyphenyl), (4-hydroxyphenyl)methane that was made by reaction of phloroglucinol with *p*-hydroxybenzyl alcohol.

Reaction of 2,4,6-Trihydroxybenzylsulfonic Acid with Resorcinol

Resorcinol (273 mg) was dissolved in buffer (pH 10), after which the pH was readjusted to pH 10 by addition of NaOH. 2,4,6-Trihydroxybenzylsulfonic acid (300 mg) was dissolved in water (20 ml) and added dropwise to the reaction mixture over a period of 1 hour. The solution was then refluxed for 3 hours. After cooling, the reaction mixture was neutralized, analyzed by HPLC and two-dimensional cellulose TLC, and then extracted with ethyl acetate. The ethyl acetate-soluble material was separated by chromatography on Sephadex LH-20, eluting with ethanol and collecting fractions (10 ml). Fractions 13-19 were combined and purified a second time by chromatograpy on Sephadex LH-20 by eluting with ethanol-water (1:1, v/v). Fractions 6-16 were combined.

Di-(2,4-dihydroxyphenyl)methane (18): The product was recovered as large orange-brown needles mp. 208 °C (22 mg) by crystallization from water. Found: C, 66.4; H, 5.4%. $C_{13}H_{12}O_4 \cdot 0.1H_2O$ requires C, 66.8; H, 5.2%. ^1H-NMR (d_6-acetone): 6.1-6.8(6H,m,Ar-H); 3.56(2H,s,benzyl-CH_2). ^{13}C-NMR (d_6-acetone-D_2O: 156.5(C-2); 155.5(C-4); 131.7(C-6); 120.1(C-1); 107.9(C-5); 103.5(C-3); 29.0(benzyl-CH_2).

A portion (15 mg) of (18) was acetylated with pyridine-acetic anhydride (1:1, v/v) overnight at ambient temperature. The product was precipitated from water to obtain a pale yellow oil that was dried under high vacuum. Preparative TLC (Si-gel, benzene-acetone, 9:1, v/v) gave the product at RF 0.55. ^1H-NMR ($CDCl_3$): 6.75-7.00(6H,m,ArH); 3.65(2H,s,benzyl-CH_2); 2.10,2.17(12H,s+s, ArOAc). ^{13}C-NMR ($CDCl_3$):130.9(C-6); 119.2(C-5); 116.1 (C-3); 30.0(benzyl-CH_2). The quaternary carbon atoms were not detectable with this small sample. FAB-MS gave M + 1 =401; $C_{21}H_{20}O_8$ + 1 requires 401.

(2,4,6-Trihydroxyphenyl),(2,4-dihydroxyphenyl)methane (10) Later eluted fractions gave a small amount of a light brown amorphous solid. ^1H-NMR (d_6-acetone): 6.2-7.0(5H,m,Ar-H); 3.73(2H,s,benzyl-CH_2).

Reaction of Epicatechin-(4β)-sulfonate with Phloroglucinol

Sodium epicatechin-(4β)-sulfonate (200 mg) and phloroglucinol (340 mg) were combined with sodium carbonate (400 mg) in water (50 ml). The reaction vial was purged with argon, sealed and heated at 105 °C for 2 hours. After the vial had cooled, the solution was acidified to pH 5 with acetic acid. The acidified solution was extracted with ethyl acetate to remove unreacted phloroglucinol. The aqueous phase was freeze-dried. The dried solid was dissolved in ethanol-water (70:30, v/v) and separated on Sephadex LH-20 (1.5 x 90 cm) by elution with ethanol-water (70:30, v/v) and collection of 15 ml fractions. Fractions 11-12 were combined to obtain a pale tan amorphous solid (110 mg). This was dissolved in ethanol-water (50:50, v/v) and separated by repeated chromatography on Sephadex LH-20 (1.5 x 90 cm) by elution with ethanol-water (50:50, v/v) to obtain a pure isolate of (21) (37 mg). To examine the reactions under milder conditions, sodium epicatechin-(4β)-sulfonate (5 mg) was combined with phloroglucinol (8.5 mg) and sodium carbonate (10 mg) in water (2 ml) in a series of sealed vials. Reactions under a variety of temperature and time conditions were monitored by two-dimensional cellulose TLC. Epicatechin-(4β)-phloroglucinol (20), which had been made and described earlier, was a significant product of reactions at ambient temperature over time periods of 10 minutes to 2 hours. Reactions over longer time periods or at higher pH or temperature gave (21).

6-(2,4,6-Trihydroxyphenyl)-8-(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9bicyclo[3.3.1]-nonatrione(21): This compound was identical with a product obtained from the reaction of polymeric procyanidins from loblolly pine bark with phloroglucinol at alkaline pH.[13] ^{1}H-NMR (d_4-methanol): 6.45-6.64(3H,m,catechol Ar-H); 5.96(2H,s,phloroglucinol Ar-H); 4.65(1H,dd,J = 5.0 and 11.0 Hz,H-7); 3.75(1H,dd,J = 3.6 and 5.0 Hz,H-6); 2.87(1H,dd,J = 4.6 and 11.0 Hz,H-β); 2.68-2.70(1H,d, 4.6 Hz,H-1); 2.55-2.75(1H,d,J = 3.6 Hz,H-5). ^{13}C-NMR (d_6-acetone-D_2O, 1:1 v/v): 206(bicyclic C-2, C-9); 188(bicyclic C-4); 159(phloroglucinol C-4); 157(phloroglucinol C-2,C-6); 145(catechol C-3,C-4); 131.7(catechol C-1); 122(catechol C-6); 117.4(catechol C-5); 116(catechol C-2); 106.4(bicyclic C-3); 101.2(phloroglucinol C-1); 97.8 96.0(phloroglucinol C-3, C-5); 71.2(bicyclic C-7); 61.5(bicyclic C-5); 50.7(bicyclic C-1); 45.6(bicyclic C-6); 37.2(bicyclic C-8). FAB-MS gave M + 1 = 415; $C_{21}H_{18}O_9$ + 1 requires 415.

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