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Loblolly Pine Bark Polyflavanoids

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The inner bark of *Pinus taeda* L. contains (+)-catechin, the procyanidin B-1 (a C-4 to C-8 linked (−)-epicatechin to (+)-catechin dimer), and three polymeric procyanidins that have distinctly different solubility and chromatographic properties. An ethyl acetate soluble polymer (0.20% of bark, $\bar{M}n$ = 1200) was purified by chromatography on LH-20 Sephadex. A water-soluble tannin (6.3% of bark, $\bar{M}n$ = 2100) and an acetone/water-soluble tannin (3.7% of bark, $\bar{M}n$ = 2900) were purified by chromatography on cellulose columns. Despite differences in their physical properties, thiolysis with benzenethiol and ^{13}C-NMR spectra indicated that the three polymeric procyanidins were composed of C-4 to C-8 (or C-6) linked (−)-epicatechin upper units (chain extenders) and that the lower unit (chain initiator) was (+)-catechin.

Loblolly pine (*Pinus taeda* L.) is the principal commercial softwood of the southeastern United States, accounting for nearly one-half of the total southern pine inventory. At advanced ages, loblolly pine trees may attain diameters of 60 in. and heights of 150 ft. These trees typically are grown on a rotation age of 30–80 years, and the timber is used for a substantial part of the pulp and paper, lumber, plywood, and particle board produced in the United States (Koch, 1972). Large amounts of bark are harvested with the trees, most of which is burned for steam generation. However, loblolly pine bark is a rich source of polyflavanoids and the petroleum shortage of 1973 renewed interest in the possibilities for using these polymers to replace phenol in wood adhesives. Many approaches have been investigated, but none has been found commercially viable (Hemingway, 1978). More complete knowledge of the structure and properties of these polymers is needed if advances in using them are to be made.

Previous work on loblolly pine bark polyflavanoids concentrated on the water-soluble and alkali-soluble polymers in the outer bark (Hemingway and McGraw, 1976, 1977). Although results of the studies suggested polymers dominated by (−)-epicatechin units linked by C-4 to C-8 (or C-6) bonds, yields of thiolysis products were low (≤5%), and some of the analytical data did not fit well with that expected of a simple procyanidin-B type of polymer. During the conversion of inner to outer bark, several processes could cause differences in the chemistry of polymers from the two tissues. Outer-bark polymers of structures that differ from those found in the inner bark may be formed during death of phloem parenchyma, or by the cork cambium in much the same manner as occurs in heartwood formation (Hillis, 1977), or by the deposition of a secondary type of lignin in the outer bark (Hergert,

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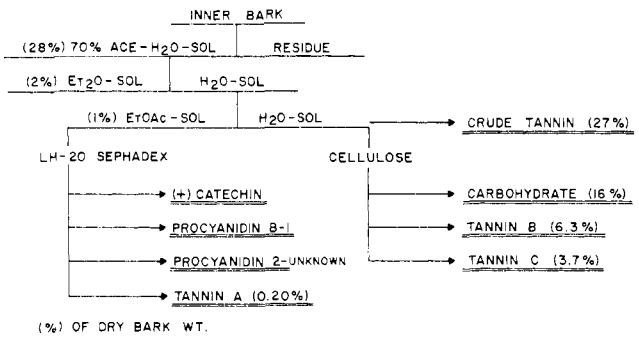

Figure 1. Scheme for isolation of polyflavanoids from loblolly pine bark. (Average yields from several isolations.)

1977). During suberization, wax or suberin polyestolides might combine with polyflavonoids, as in the cork of Douglas fir and white fir (Hergert and Kurth, 1952, 1953a; Hergert, 1958). In addition, secondary changes such as oxidation and further polymerization may occur during the many years that polyflavanoids are stored in the outer bark. All of these processes complicate the problem of isolation and elucidation of the structure of the polymers in the outer bark. Therefore, a study of the polyflavanoids in the inner bark was undertaken to provide a basis for understanding the more abundant outer-bark polymers.

EXPERIMENTAL SECTION

Mass spectra were obtained on a CEC Model 21-110 for electron impact and on a modified Hitachi-Perkin Elmer RMU-7 for field desorption (FD-MS). ^{1}H-NMR spectra were recorded at 60 MHz on a Perkin-Elmer R24B and at 100 MHz on a Varian XL-100. ^{13}C-NMR spectra were recorded at 25.2 MHz on a Varian XL-100. Elemental analyses were done by the University of Oregon Microanalytical Laboratory. A Waters Associates liquid chromatograph equipped with a series of Microstyragel columns was used for GPC (Hemingway and McGraw, 1977), and a Hewlett-Packard 7620A gas chromatograph fitted with $^1/_8$-in. by 4-ft columns packed with 3% OV-17 on 80–100 Chromosorb W was used for GLC (Colella, 1977). A Jasco DIP-181 polarimeter was used to measure optical rotations. Vapor pressure osmometry (VPO) of methylated tannins in $CHCl_3$ and acetone solutions was performed by Galbraith Laboratories.

Extraction of Inner Bark. Acetone–water (70:30) extracts of the inner bark of loblolly pine trees were prepared in several ways to find the best way of obtaining comparatively pure polyflavanoids that had little exposure to light or air. Particular care was necessary to limit enzymatic browning. The outer bark was removed in 1 ft^2 sections. The inner bark was stripped at the xylem cambium and immediately either frozen on dry ice or submerged in acetone–water. Extractions were made at ambient temperature in wide-mouth jars that were covered with aluminum foil and purged with argon. Extraction periods of several days to 2 weeks were examined. Some of the frozen inner-bark samples were dried under vacuum and ground to pass a 2-mm screen before extraction. Other frozen samples were broken into pieces of about 1 in.^{2} and extracted directly. Yields of water-soluble tannins were higher from the extraction of undried bark (Figure 1), but the ratios of various fractions did not vary significantly with extraction conditions.

Typically, 1.25 kg of inner bark (i.e., 500 g dry) was extracted with 12 L of 70% acetone. The acetone extract was filtered and reduced in volume under vacuum at 37 °C. Extraction of the resulting aqueous phase with diethyl ether (4 L total) removed a yellow oil (17.5 g) that was not

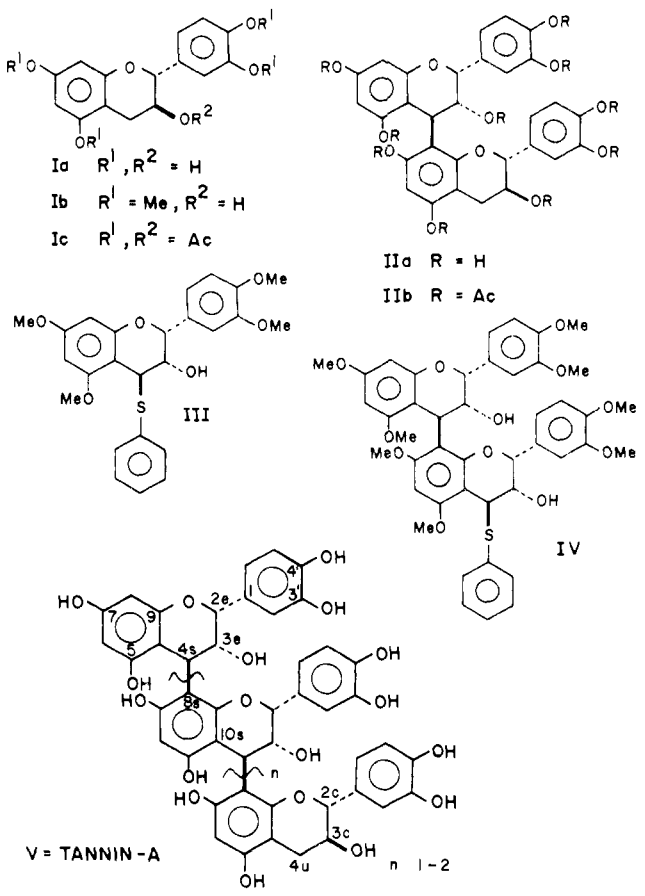

Figure 2. Compounds isolated from loblolly pine bark and tannin thiolysis products.

investigated further. The aqueous phase was then extracted with ethyl acetate (6 L total). The ethyl acetate extract was evaporated to near dryness under vacuum at 37 °C and redissolved in *t*-BuOH. A red-brown powder (5.9 g) was recovered after freeze-drying from *t*-BuOH. Freeze-drying the residual aqueous phase gave 139 g of the crude water-soluble tannin.

Isolation of (+)-Catechin, Procyanidin B-1, and Tannin-A. The ethyl acetate soluble material (3-g portions) was separated on a 2.5 × 100 cm LH-20 Sephadex column by elution with EtOH and collection of 15-mL fractions (Figure 1). Fractions 60–100 gave (+)-catechin (Ia) (Figure 2), mp 150–151 °C, sinter, 176–179 °C (174–175 °C; (Hergert and Kurth, 1953b), and M^+ = 290 amu (FD-MS). Treatment with ethereal diazomethane gave (−)-tetra-*O*-methylcatechin (Ib), mp 143 °C (143 °C; Weinges et al., 1969), and M^+ = 346.143 amu ($C_{19}H_{22}O_6$ requires 346.142), and $[\alpha]^{25}_D$ −13.2° (*c* 0.1, $CHCl_3$) {$[\alpha]^{18}_D$ −9.8° ($CHCl_3$); Clark-Lewis and Korytnyk, 1957; $[\alpha]_D$ −14.3 °C ($CHCl_3$); Thompson et al., 1972}. Acetylation (acetic anhydride–pyridine) afforded the pentaacetate (Ic), mp 130–131 °C (131 °C; Weinges et al., 1969), and $[\alpha]^{25}_D$ +35.5° (*c* 0.1, $CHCl_3$) {$[\alpha]_D$ +39.7° ($CHCl_3$); Thompson et al., 1972}. ^{1}H-NMR spectra of (+)-catechin (Ia) and its derivatives were identical with those of authentic samples and Thompson et al. (1972).

The combined fraction 125–160 gave procyanidin B-1 (IIa) as an off-white powder (100 mg) after freeze-drying from *t*-BuOH, and M^+ 589 amu (FD-MS). It gave cyanidin chloride on treatment with BuOH–HCl (PC). Thiolysis with benzenethiol (Brown and Shaw, 1974) yielded tetra-*O*-methylcatechin (Ib) and (+)-2,3-*cis*-3,4-*trans*-3′,4′5,7-tetra-*O*-methyl-4-phenylthioflavan-3-ol (III) (TLC and ^{1}H NMR) after methylation with diazomethane.

Chromatographic and ^{1}H-NMR spectral properties of the procyanidin (IIa) and its acetate (IIb) were identical with those reported by Weinges et al. (1968a) and Thompson et al. (1972). The optical rotation of the acetate $[\alpha]^{25}_{578}$ +109° (c 0.1, acetone) {$[\alpha]_{578}$ +110° (acetone); Weinges et al., 1968b} also indicated that the compound was procyanidin B-1. This compound was also identical with the major procyanidin obtained by reaction of the crude loblolly pine bark tannin with (+)-catechin (Fletcher et al., 1977). Other procyanidins, that are yet to be identified, were isolated by further elution with EtOH.

A red-brown material remained on the LH-20 Sephadex column after exhaustive elution with EtOH. Elution with MeOH removed all color from the column, and a brown powder (500 mg) was obtained by freeze-drying from *t*-BuOH. This isolate was designated as tannin-A. After drying at 80 °C and 0.01 mmHg for 24 h, elemental analysis showed C = 59.9%, H = 4.8%, and N < 0.10% ($C_{60}H_{50}O_{24}\cdot 3H_2O$ requires C = 59.6% and H = 4.7%).

Isolation of Tannin-B and Tannin-C. The crude water-soluble tannin (3 g) was dissolved in 40 mL of hot water and applied to 150 g of cellulose (Whatman CF II) packed in a nylon tube to make a column 3.25 by 80 cm. Elution with 60 mL of cold water gave 1.78 g of a carbohydrate-rich material (found C = 43.2%, H = 6.4%, and N < 0.15%) and 35% glucose on acid hydrolysis.

Elution with another 200 mL of cold water gave tannin-B, which was freeze-dried to obtain an off-white powder (0.70 g). Acid hydrolysis gave 2.4% reducing sugars (glucose). Elemental analysis after drying at 80 °C and 0.01 mmHg for 24 h showed C = 52.5%, H = 5.1%, and N < 0.10% ($C_{15}H_{12}O_6\cdot 3H_2O$ requires C = 52.6% and H = 5.3%).

All material was not recovered from the cellulose column by elution with water. Further elution with acetone–water (70:30) and freeze-drying gave an off-white powder (0.41 g) that was designated tannin-C. After drying at 80 °C and 0.01 mmHg for 24 h, elemental analysis showed C = 56.7%, H = 4.3%, and N < 0.10% ($C_{15}H_{12}O_6\cdot 1.5H_2O$ requires C = 57.1% and H = 4.8%).

Products from Benzenethiol Degradation. The crude water-soluble tannin (40 g) was dissolved in 700 mL of EtOH–H_2O (1:1), 20 mL of HOAc, and 40 mL of benzenethiol. The solution was heated at reflux under argon for 4 h (Brown and Shaw, 1974). After cooling, 500 mL of water was added and the solution was extracted with diethyl ether. The ether-soluble fraction was repeatedly methylated with ethereal diazomethane to a negative ferric chloride–potassium ferricyanide reaction. On concentration, 49.6 g of a yellow oil was obtained. TLC (Si-gel; benzene–acetone, 9:1) showed major products at R_f 0.70 (III), 0.44 (Ib), and 0.18 (IV), all of which gave red reactions with 40% formalin/H_2SO_4/H_2O (2:1:1) after they were heated to 110 °C. Chromatography with authentic tetra-*O*-methylepicatechin (R_f 0.52, green coloration with formalin–H_2SO_4) indicated the absence of this compound. Column chromatography (Si-gel) by elution with petroleum ether–acetone mixtures of increasing acetone concentration (up to 3:2) gave a pure fraction of compound III (Brown and Shaw, 1974). Other fractions that contained mixtures of compound Ib (R_f 0.44 TLC) and compound IV (R_f 0.18 TLC) were purified by preparative TLC.

Compound IB, (–)-tetra-*O*-methylcatechin, was crystallized four times from MeOH–Et_2O to obtain small needles, mp 143–144 °C (143 °C; Weinges et al., 1969), and $[\alpha]_D^{25}$ –1.0° (c 0.1, $CHCl_3$), {$[\alpha]_D^{18}$ –9.8° ($CHCl_3$); Clark-Lewis and Korytynk 1957} for the amorphous, but chromatographically pure material, and M^+ = 346.143 amu ($C_{19}H_{22}O_6$ requires 346.142). The ^{1}H-NMR spectrum is identical with that of an authentic sample and Thompson et al. (1972).

Compound III, (+)-2,3-*cis*-3,4-*trans*-3′,4′,5,7-tetra-*O*-methyl-4-phenylthioflavan-3-ol, was crystallized from benzene–acetone as needles, mp 208–210 °C, (208–210 °C; Brown and Shaw, 1974), and $[\alpha]_D^{25}$ +8.8° (c 0.1, $CHCl_3$) {$[\alpha]_D$ +8.6° ($CHCl_3$); Brown and Shaw, 1974}. The mass spectrum was consistent with that reported by Brown and Shaw, showing only a very small parent ion at 454 amu (<1%) with prominent fragment ions at 345 (100%), 327 (36%), 191 (79%), 167 (52%), and 151 (54%). By contrast, field-desorption MS exhibited a strong parent ion peak at 454 (100%) amu. Desulfurization of compound III with W-2 RaNi afforded tetra-*O*-methylepicatechin (MP, TLC, GLC, ^{1}H NMR, and MS).

Compound IV was tentatively assigned a structure of octa-*O*-methyl-4″-phenylthioprocyanidin B-2. While ^{1}H NMR indicated a thioether of a dimeric procyanidin, because of the broad spectra further information could not be obtained. Treatment of compound IV with W-2 RaNI afforded a second compound whose MS showed a parent ion at 690.268 (67%) ($C_{38}H_{42}O_{12}$ requires 690.268) consistent with the structure of a C-4 to C-8 (or C-6) linked procyanidin dimer. Major fragment ion peaks were observed at 479 (72%), 331 (55%), 299 (76%), 180 (42%), and 151 (100%) amu, which is similar to results obtained by Porter (1974). This series of peaks represents the expected stages of fragmentation involving retro-Diels-Alder fissions of the heterocyclic rings in both the upper and lower flavanoid units. In particular, the large peak at 331 amu represents the two phloroglucinol rings linked by the single C-4 carbon of the upper unit (Pelter et al., 1969). Thiolysis of this second compound with mercaptoacetic acid (Sears and Casebier, 1968) and analysis of the products by GLC showed that both the upper (after treatment with RaNI) and lower flavanoid units were tetra-*O*-methylepicatechin.

RESULTS AND DISCUSSION

Yields of Thiolysis Products. Previous work with acid-catalyzed benzenethiol degradation of polymeric procyanidins in an aqueous ethanol medium (Brown and Shaw, 1974) indicated that the optimum reaction period for degradation of procyanidin-based polymers is about 4 h. Therefore, tannins-A, -B, and -C were subjected to thiolysis by Brown and Shaw's method, and the yields of products were determined gravimetrically on isolates obtained by preparative TLC. Tannin-A gave tetra-*O*-methylcatechin (Ib), tetra-*O*-methyl-4-phenylthioepicatechin (III), and octamethyl-4″-phenylthioprocyanidin B-2 (IV) in mole ratios of 0.30, 2.00, and 0.70, respectively. Total yields of products recovered amounted only to 7.0% of the tannin. Similar 4-h thiolysis of tannin-B gave the same products in mole ratios of 1.0, 2.0, and 1.3, and the total yield amounted only to 10% of the tannin. Tannin-C gave only trace amounts of tetra-*O*-methylcatechin (Ib) and the two thioethers (III) and IV) were recovered in mole ratios of 2:1 and were 13% of the tannin.

Because these yields were so low, the quantitative GLC procedure of Colella (1977) was modified and used to determine the yields of the tetra-*O*-methylcatechin (Ib) and tetra-*O*-methylepicatechin obtained from tetra-*O*-methyl-4-phenylthioepicatechin (III) by desulfurization with W-2 RaNi. The crude tannin was heated with benzenethiol, and aliquots were analyzed after 1, 2, 4, 10, and 24 h (the latter gravimetrically by preparative TLC). Tetra-*O*-methylcatechin (Ib) was obtained in high proportions early in the degradation, and the yields of tetra-

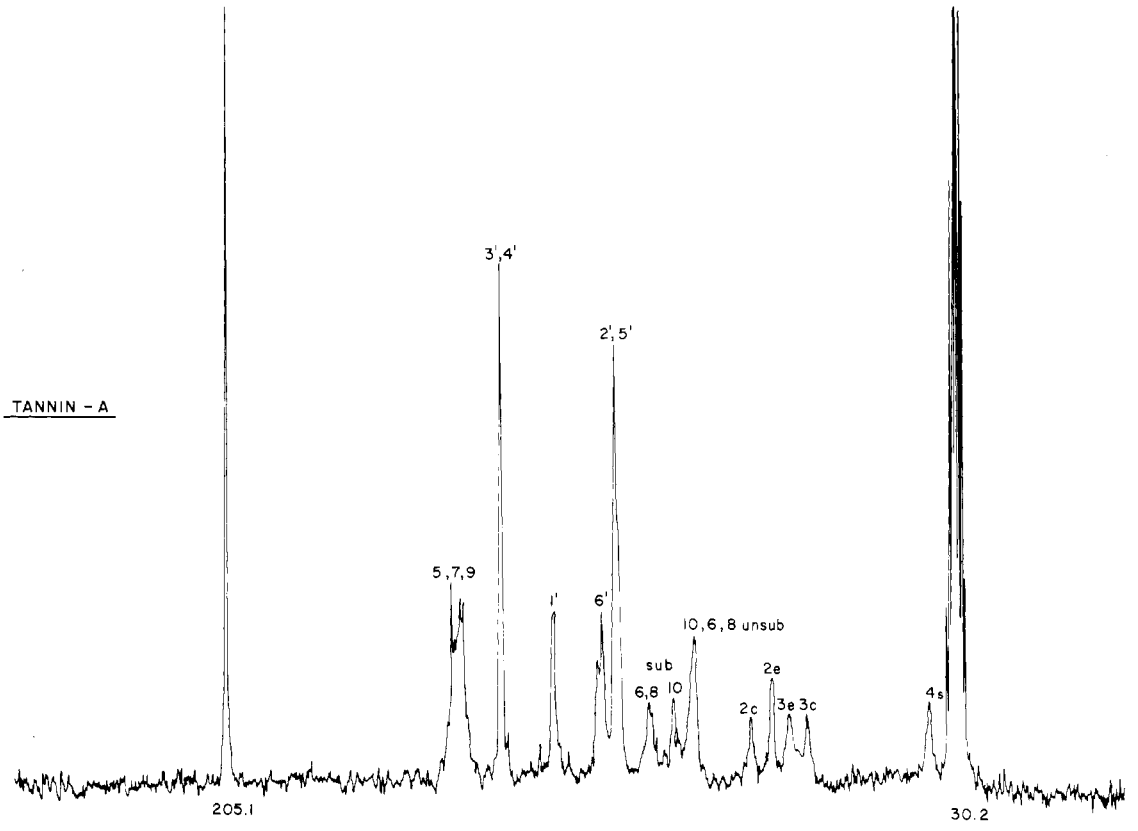

Figure 3. ^{13}C-NMR spectra of loblolly pine bark tannins in D_2O + acetone-d_6 at 30 °C.

O-methyl-4-phenylthioepicatechin (III) increased linearly as the reaction period increased. Since the results showed that thiolysis was far from complete after 24 h, tannin-B was reacted with benzenethiol for 60 h, and the products obtained after methylation with diazomethane were determined gravimetrically by preparative TLC. The yield of tetra-*O*-methylcatechin (Ib) increased to 6.8% and that of tetra-*O*-methyl-4-phenylthioepicatechin (III) to 37% of the tannin. Only small amounts of the dimeric thioether (IV) were detected. However, significant amounts of tetra-*O*-methylepicatechin (TLC and GLC) and tetra-*O*-methyl-4-phenylthiocatechin (^{1}H NMR) were indicated in these longer term degradation reactions.

Molecular Weight Distribution. The tannins-A, -B, and -C were dissolved in methanol or acetone–methanol and methylated with excess ethereal diazomethane repeatedly for several days until negative to the ferric chloride–potassium ferricyanide reaction. The methylated tannins were dissolved in a small amount of chloroform and precipitated from hexane to give white amorphous powders.

Estimates of the molecular weight distribution were obtained by GPC on Microstyragel columns (Hemingway and McGraw, 1977) by use of a calibration curve made from the elution volumes of tetra-*O*-methylcatechin, octa-*O*-methylprocyanidin B dimers, and standard polypropylenes and polystyrenes. Estimates of $\bar{M}n$ were also made by VPO. GPC of the methylated tannin-A indicated that it was a low-molecular-weight polymer ($\bar{M}n$ = 1200) and had a narrow molecular weight distribution ($\bar{M}w$ = 1450). VPO indicated an $\bar{M}n$ of 1250 for tannin-A. Tannin-B was of a much higher molecular weight ($\bar{M}n$ 2100), and the $\bar{M}w$ of 5900 showed that this isolate had a much broader molecular weight distribution than tannin-A. Tannin-C had an $\bar{M}n$ of 2900 and a $\bar{M}w$ of 14 000 as indicated by GPC.

^{13}C-NMR Spectra. The ^{13}C-NMR spectra of tannins-A, -B, and -C can be fully interpreted on the basis of C-4 to C-8 (or C-6) linked polymeric procyanidins in which the upper units (chain extenders) are (−)-epicatechin units and the lower terminal unit is (+)-catechin (V) (Schilling et al., 1973; Fletcher et al., 1977; Pelter et al., 1976; Wenkert and Gottlieb, 1977; Markham and Ternai, 1976; Chari et al., 1977; Arnone et al., 1977; and Hufford and Lasswell, 1978). The spectrum of tannin-A (Figure 3) showed a complex set of signals (δ, Me_4Si) at 154–157 ppm that can be assigned to the C-9, C-5, and C-7 carbons of the phloroglucinol A-ring. The resonance at 144 ppm is assigned to the C-3′ and C-4′ and that at 130–131 ppm to the C-1′ carbons of the catechol B-ring. Resonance at 119–120 ppm can be assigned to the C-6′ and that at 116 ppm to C-2′ and C-5′ of the B-ring. Resonance at 107.5 ppm can be assigned to the C-8 or C-6 carbons of the A-ring that are substituted and that at 102 ppm to the C-10 carbons adjacent to substituted C-4 carbons (Porter, 1979; Hufford and Lasswell, 1978). Resonance at 95–97 ppm can be assigned to the C-8 and C-6 carbons of the phloroglucinol A-ring that are not substituted and to the terminal C-10. The C-2 carbon of the terminal catechin unit appears at 81 ppm, while the C-2 carbons of the upper epicatechin units appear at 76 ppm. Resonance at 69.7 ppm is assigned to the C-3 carbon of the upper epicatechin units and that at 67.6 ppm to the C-3 of the terminal catechin unit. The substituted C-4 carbons appear at 36 ppm, and the C-4 of the terminal catechin unit would be buried in the acetone signal.

The spectra of tannin-B and tannin-C (Figures 4 and 5) are also consistent with those expected of an epicatechin-based polymeric procyanidin. The diminished resonance for the C-2 (81 ppm) and C-3 (67 ppm) carbons of the terminal catechin unit and relative increase in the intensity of substituted C-6 or C-8 carbons (107–108 ppm) as well as the C-10 carbon adjacent to substituted C-4 carbons (102 ppm) reflect the higher molecular weights of

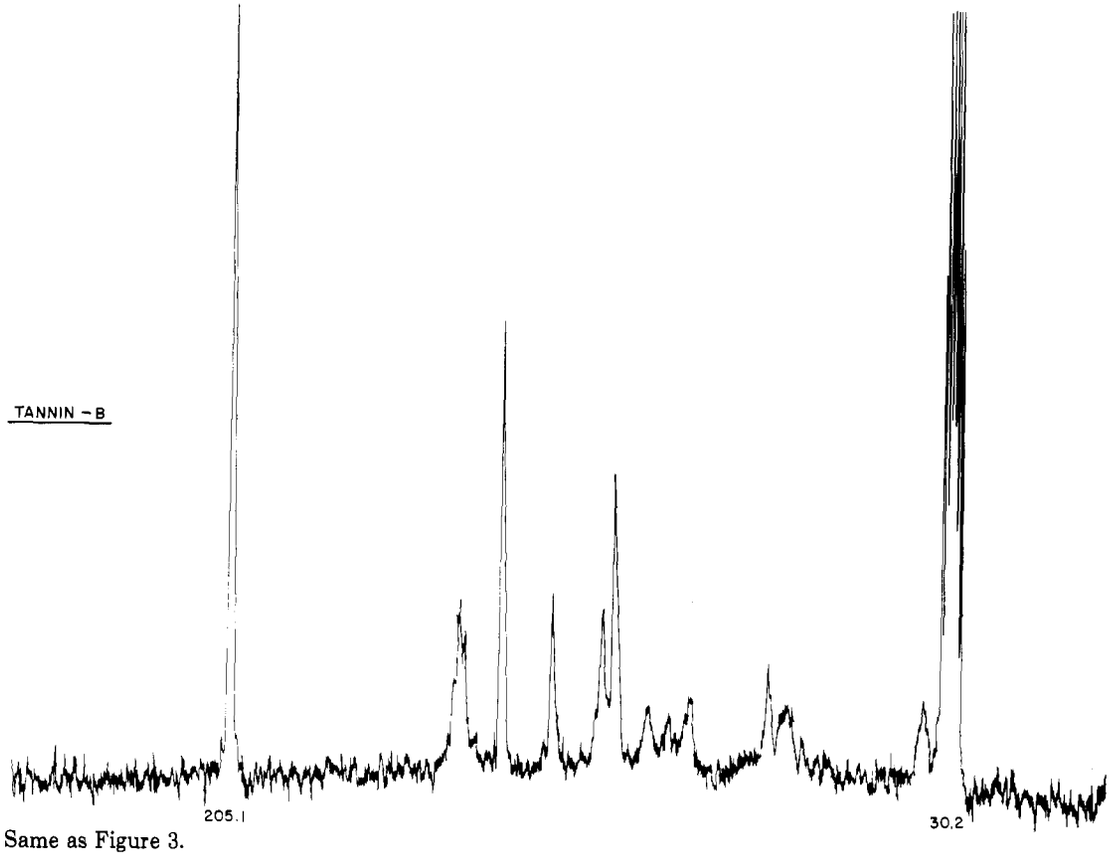

Figure 4. Same as Figure 3.

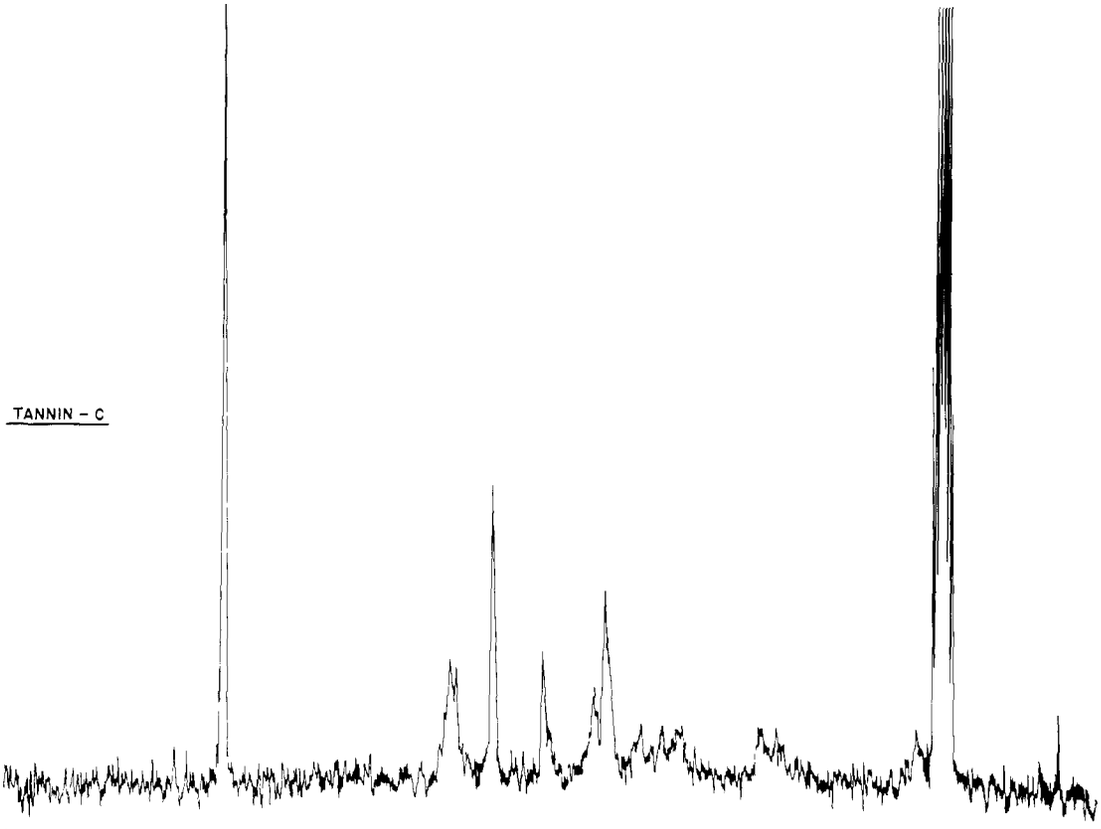

Figure 5. Same as Figure 3.

these polymers progressing from tannin-A to tannin-C.

The polyflavanoids of loblolly pine inner bark are excellent examples of the stereospecificity of the biosynthesis of procyanidins (Haslam et al., 1977; Jacques et al., 1977). Large amounts of (+)-catechin were found, and despite the high degree of concentration possible through chromatography on LH-20 Sephadex, no epicatechin was obtained. Therefore, the flavan-3-ol chain initiator (lower terminal unit) of the polymeric procyanidins would be expected to be (+)-catechin. Thiolysis products from the three tannins

and ^{13}C-NMR spectra showed that this was indeed the case. The major procyanidin found was the procyanidin B-1 (a (−)-epicatechin--C-4 to C-8--(+)-catechin dimer), and this compound was also the major product obtained from acid-catalyzed synthesis of procyanidins from loblolly pine bark tannins and (+)-catechin. Thus, the chain extenders (upper units) of the polymers would be expected to be derived from flavan carbocations of a (−)-epicatechin stereochemistry. Thiolysis products and ^{13}C-NMR spectra showed that the upper units of all three tannins were of a (−)-epicatechin stereochemistry.

Acid-catalyzed thiolysis with benzenethiol for 4 h, followed by methylation with diazomethane (Brown and Shaw, 1974) gave low yields of (−)-tetra-*O*-methylcatechin (Ib), (+)-2,3-*cis*-3,4-*trans*-tetra-*O*-methyl-4-phenylthioepicatechin (III) and a compound tentatively identified as the benzenethioether of octa-*O*-methylprocyanidin B-2 (IV) from the three tannins, despite the wide variation in their molecular weights. Examination of the yields of products obtained from the crude tannin over reactions of 1–24 h showed that thiolysis was far from complete after 24 h of heating. Even after 60 h, tannin-B gave only 6.8% of tetra-*O*-methylcatechin and 37% of tetra-*O*-methyl-4-phenylthioepicatechin. The tetra-*O*-methylepicatechin and tetra-*O*-methyl-4-phenylthiocatechin found in the long-term (10–60 h) thiolysis products appear to be the result of epimerization through B-ring inversion of (+)-catechin and 4-phenylthioepicatechin, respectively, since the ^{13}C-NMR spectra would have shown (+)-catechin units in the upper part of these polymers. Although the C-2 of catechin units was prominent in the ^{13}C-NMR spectrum of tannin-A, resonance at 81 ppm is barely detectable in the spectrum of tannin-B and does not appear in the spectrum of tannin-C. These results together with the results obtained from the thiolysis strongly suggest that no (+)-catechin units are present in the upper portion of the polymeric procyanidins of loblolly pine inner bark. Epimerization apparently does not occur with the use of toluene-α-thiol in absolute ethanol, as thiolysis periods of 120 h have been used to degrade sorghum tannins, and there were no reports of difficulties with epimerization (Gupta and Haslam, 1978).

Previous work on the thiolysis products obtained from loblolly pine bark polyflavanoids isolated from the outer bark indicated that the upper units of these polymers were composed of epicatechin and catechin moieties in approximate ratios of 5 to 1 (Hemingway and McGraw, 1976, 1977). While the optical rotation of the acetanilide derivative of the tetra-*O*-methylepicatechin thioglycolate showed the natural 2*R*,3*R* configuration, the optical rotation of the catechin thioglycolates has not been determined. Therefore, it is possible that the catechin moieties in these outer-bark polymers may have resulted from epimerization during the many years of storage in the outer bark. However, much higher proportions of catechin units are indicated in polymers from Douglas-fir bark (Karchesy et al., 1976) and western hemlock bark (Sears and Casebier, 1970), which have epicatechin to catechin ratios of 3:1 and 1:1. The optical rotations of the thioglycolates obtained from these polymers were not reported.

Quantitative yields of thiolysis products were not obtained, but all three tannins gave satisfactory elemental analyses for polymers of pentahydroxyflavanols (V) and treatment of all three polymers with HCl in BuOH or 2-PrOH gave only cyanidin chloride. The ^{1}H-NMR spectra of the tannins in the free phenolic form (D_2O + acetone-d_6) clearly showed resonances attributable to three B-ring protons for every A-ring proton. These spectra showed broad resonances in the region of the pyran ring protons but otherwise were devoid of any significant resonances, which could be attributed to impurities such as carbohydrates or aliphatics. The ^{1}H-NMR spectra of methylated (CH_2N_2) and acetylated (acetic anhydride–pyridine) tannins also were consistent with those expected of a polymeric procyanidin, but the presence of a small aromatic acetate indicated incomplete methylation. By far the most convincing result was the excellent agreement of the ^{13}C-NMR spectra with a C-4 to C-8 (or C-6) linked (−)-epicatechin polymer in which the lower unit was of a (+)-catechin stereochemistry.

The low yields of thiolysis products and particularly the low mole ratio of tetra-*O*-methylcatechin obtained from 4 h of thiolysis of tannin-A seemed unusual. Concurrent studies (McGraw and Hemingway, 1978) of the reaction of *o*- and *p*-hydroxybenzyl alcohols with catechin have shown that substitution occurs at both the C-6 and C-8 positions, and substantial proportions of the 6,8-dibenzylated catechin derivatives are formed when only 1 mol of benzyl alcohol has been added, and substantial proportions of unreacted (+)-catechin remain. It appears that substitution at either C-6 or C-8 with a benzyl alcohol substantially activates a second substitution of the A-ring. These observations led us to question whether the low yields of thiolysis products (particularly the yield of catechin from the lower terminal unit) and differing solubility properties among these tannins might be related to branching. Comparison of the ^{13}C-NMR spectra of the tannins with the shifts of the C-6 and C-8 benzylated pinocembrin derivatives (Hufford and Lasswell, 1978) indicated the resolution obtained in spectra has been inadequate to reach a conclusion as to how much C-4 to C-6 and C-8 linkage occurs. A similar problem exists when attempting to use ^{1}H-NMR spectra based on the A-ring proton shifts of methylated tannins (Hundt and Roux, 1978). Spectra of much higher resolution than we have obtained to date will be required to answer this question. We are pursuing other approaches for determining how much branching occurs in these polymers (McGraw and Hemingway, 1978).

Successful chemical utilization of loblolly pine bark polyflavanoids must take into account the differences in properties and molecular weight distributions of various isolates. One approach to simplifying problems of working with these high-molecular-weight polymers would be to reduce their molecular weight to those of small oligomers or monomeric phenols. The interflavanoid linkage of dimeric procyanidins was reportedly cleaved by hydrogenolysis over palladium on charcoal under mild reaction conditions to obtain high yields of diarylpropanoids (Jacques et al., 1974). However, attempts to use this reaction to reduce the molecular weight of the above described polymeric polyflavanoids from loblolly pine bark have been unsuccessful. Presumably, the interflavanoid linkage is not accessible to the catalyst. The catalyst is not inactivated since simple catalytic reduction of small molecular weight compounds was achieved in the presence of these tannins. We are continuing work on this approach.

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Chemistry and Utilization of Western Hemlock Bark Extractives

Franklin W. Herrick

A review is presented of research in North America during the past 30 years leading to the production of several types of water-soluble extracts from western hemlock bark. The objective of this work was to utilize the polyflavanoid components of the bark. These components were solubilized by treating ground bark with hot aqueous solutions of sodium bisulfite, sodium sulfite, ammonia, or sodium hydroxide. End-use research was directed to devising selective extraction processes to produce products that were effective and competitive with imported tannins in such markets as vegetable tanned leather, dispersants for oil-well drilling muds, and additives for boiler and cooling waters. Development work was conducted on the use of alkaline extracts of bark as components of phenol–formaldehyde resin adhesives for exterior-type plywood, particle boards, and laminated timbers. Chemical grouting systems based on the reaction of alkaline extracts and formaldehyde and heavy metal salts were tested under field conditions. Metal complexes (Fe, Zn, Cu, Mn) of the sulfonated extracts were found to be useful as agents to correct micronutrient deficiencies in agriculture. The chemical composition of western hemlock bark and the chemical structure of polyflavonoid derived extract products are discussed.

This review will deal specifically with western hemlock (*Tsuga heterophylla Raf. Sarg.*) bark since the development of commercial products by ITT Rayonier was largely based on hemlock bark. Our work on the utilization of western hemlock bark began in 1948 and was part of a revival of interest in tannins, waxes, and other useful products that might be obtained from the bark of the large conifer tree species native to the Pacific Northwest of the United States and Canada. Previous and contemporary research at the Oregon Forest Products Laboratory and

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