

Condensed Tannins. Structure of the "Phenolic Acids"

RETENTION

By Peter E. Laks

Institute of Wood Research, Michigan Technological University, Houghton MI 49931, U.S.A.

and

Richard W. Hemingway

Southern Forest Experiment Station, 2500 Shreveport Highway, Pineville, Louisiana 71360, U.S.A.

Provided by INFOSe
NOTICE: This material
protected by copyright
(Title 17, U.S. Code)**Keywords**Polyphenol
Procyanidin
Bark extract
Pine tannin
Adhesives
Phenolic Acids
Loblolly pine
Pinus taeda L.*Condensed Tannins. Structure of the "Phenolic Acids"***Summary**

Conifer bark-derived condensed tannins can be used in formulating adhesives. Under some extraction conditions and during normal adhesive formulation, the tannin is exposed to strongly alkaline conditions. Alkaline rearrangement results in partial or total rearrangement of tannins of the procyanidin class to "phenolic acids" which have less phloroglucinol functionality. This makes the tannin less suitable for use in adhesives since it is the phloroglucinolic A-ring that gives tannins their characteristic high reactivity with formaldehyde and/or hydroxybenzyl alcohols. The extent of rearrangement depends on the pH and temperature of the solution, more severe conditions give a greater extent of reaction. The structure of the phenolic acids was determined using ^{13}C NMR.

Introduction

High yields of condensed tannins can be obtained by alkaline extraction of conifer barks. These bark extracts have been investigated extensively as components of phenolic adhesives (for example, see Voulgaridis *et al.* 1985). In contrast to tannin preparations obtained by neutral solvent extraction, condensed tannins extracted using aqueous alkali are acidic and contain carbonyl groups. Because of these characteristics, the term "phenolic acid" is used to describe tannins isolated in this way (Herrick 1980). It has not been clear whether the acidic functionality is an intrinsic property of the higher molecular weight tannins that can only be extracted under these forcing conditions, or if it is due to rearrangements in the tannin caused by the extraction with alkali.

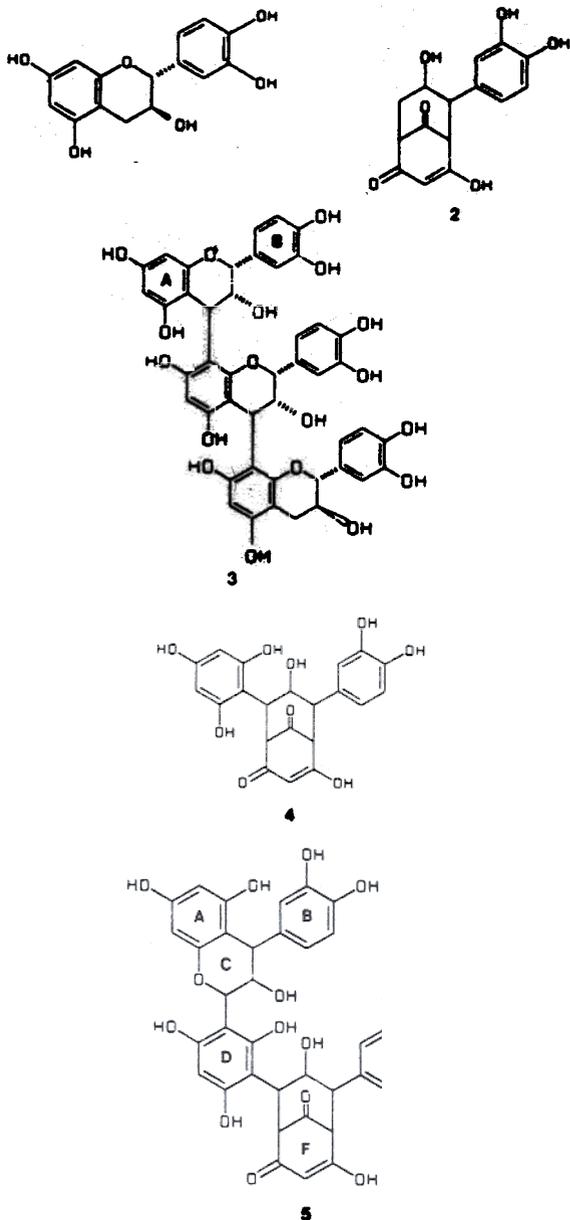
Previously, the only evidence for the nature of the phenolic acids was the observation by Sears *et al.* (1975) that catechin (1) rearranges to an enolic form of 6-(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9-bicyclo-[3.3.1]-nonatrione (2, catechinic acid) under severe alkaline conditions. However, rearrangements analogous to the formation of catechinic acid were observed recently when purified condensed tannins from southern pine inner bark (polymeric procyanidins, 3) were reacted with phloroglucinol under alkaline conditions (Laks *et al.* 1987a). In that study, phloroglucinol was used as a model for the phloroglucinolic A-ring of the tannin monomer units (the aromatic rings in the monomer are designated A and

B as shown in structure 3. Compounds 4 and 5 were isolated from the alkaline reaction of procyanidin condensed tannins with phloroglucinol. The former compound is the reaction product of single tannin upper units with phloroglucinol, while the latter is a dimer product. In both these structures, as well as catechinic acid (2), a phloroglucinol ring rearranges to a non-aromatic form containing an enol grouping. The latter is the source of the observed acidity of the phenolic acids.

Retention of the aromatic phloroglucinol A-rings in a tannin preparation is important if the material is to be used in wood adhesives, since it is these positions that give the tannin its high reactivity with formaldehyde and methylolphenols. Compared to the phloroglucinolic A-rings, Kiatgrajai *et al.* (1982) found the catechol-derived B-rings are relatively unreactive (also see McGraw and Hemingway 1982). Since the formation of catechinic acid residues destroys the aromaticity of the A-rings, it is important to know what effect exposure to alkali has on the number of phloroglucinol Ar-H positions available for reaction. This would be relevant not only to alkaline extractions, but also to adhesive formulations where high pH's are often used.

Materials and Methods

^1H and ^{13}C -nmr spectra were recorded using either a Varian FT-80A or XL-200 spectrometer. Con-



densified tannins were isolated from the phloem of loblolly pine trees as has been described previously (Hemingway *et al.* 1982). ^1H -nmr spectra (FT-80A) were taken on 50 mg of material, averaging 50 transients with a 8.11 s acquisition time. The tannin was initially dissolved in $\text{DMSO}-d_6$ to about two thirds of the final volume required in the sample tube, then D_2O added immediately before beginning acquisition. The D_2O was added to exchange with the hydroxyl protons so they would not interfere with the integration of the aromatic C-H signals.

1. Alkaline Rearrangements of Procyanidins

Purified loblolly pine tannin (1.0 g) was dissolved in 16 ml of deoxygenated water (prepared by repeated

freezing, exposure to vacuum and thawing). The pH of the solution was adjusted to either 8.0, 10.0 or 12.0 using solid NaOH while flooding the top of the solution with N_2 gas. The resulting solution was divided between two 10 ml reaction vials and sealed under an N_2 atmosphere. Reactions at 20° and 50°C were done in a thermostatically controlled water bath while boiling water was used for reactions at 100°C.

After the required reaction time, the higher temperature reactions were submerged in cold running water. All reaction solutions were then passed through a column (1 × 30 cm) containing 25 ml of Amberlite IR-120 (H^+ form) ion exchange resin. The column was eluted slowly with warm water until the eluant was clear. Freeze-drying the collected eluant gave a light-brown, low density solid (0.42 g, on average). The ion-exchange resin was used twice before discarding.

2. Alkaline Extraction of Pine Bark

Finely ground loblolly pine inner bark (30 g) was suspended in 400 ml of 1% NaOH in deoxygenated water and heated to 90°C for 1 hour with constant stirring. An atmosphere of N_2 was maintained over the solution during this time. After cooling to room temperature, ion-exchange resin (IR-120, H^+ form) was added until the pH was less than 7. Acetone (400 ml) was mixed into the neutralized solution and let stir for about 5 minutes. The suspension was then filtered and the residue washed further with acetone/water. The combined filtrates were evaporated to remove the acetone and the remaining water solution extracted with hexane (100 ml) and ethyl acetate (2 × 100 ml). The hexane and ethyl acetate layers containing fatty acids and low molecular weight phenolics were discarded, while the aqueous layer was freeze-dried. After dissolving in 1:1 methanol/water, the water-soluble materials were applied to a Sephadex LH-20 column (2.5 × 70 cm) packed and eluted overnight with the same solvent system. The eluting solvent was then changed to 1:1 acetone/water. Carbohydrates, low molecular weight phenolics and other bark extract components are washed off the column in the first step, while the higher molecular weight phenolics are retained by the LH-20 and only removed by the acetone/water solvent system. The materials eluted from the column by the acetone/water (phenolic acids) were isolated by evaporating and freeze-drying the eluant (yield -1.5 g).

Results and Discussion

To investigate the loss of phloroglucinol functionality during alkaline extraction or adhesive formulation, samples of tannin were subjected to different pH and temperature conditions for given reaction times. Loss of phloroglucinolic Ar-H functionality was moni-

by $^1\text{H-NMR}$. The protons on the A-rings give a broad signal centered on 5.8 ppm (δ from TMS). The broad signal due to the B-ring protons centered at 6.65 ppm was used as an internal standard, with the assumption that the B-rings do not participate in any reactions or rearrangements. This is a reasonable assumption since we did not find evidence for any reactions resulting in loss of catecholic functionality in our investigations on alkaline reactions of procyanidins (Laks, Hemingway and Conner 1987a; Laks and Hemingway 1987). A ratio of 0.356 (phloroglucinol signal/catechol signal) was obtained for unreacted tannin, reasonably close to the theoretical, calculated value of 0.370 for a tannin with an average molecular weight between 2,500 and 3,000, as reported by Karchesy and Hemingway (1980). The ratio from a rearranged tannin expressed as a percentage of the ratio in the tannin starting material indicates how much of the original phloroglucinol-hydrogen functionality is present. The data in Table 1 is presented in this form.

Table 1. Percentage of Phloroglucinol functionality remaining after 0.5 or 0.25 hours reaction time at pH 8, 10 and 12, and temperatures of 20°, 50° and 100°C.

pH	Temperature (°C)	Reaction Time (hrs.)	Ratio (A-ring/B-ring)	Percent A-ring Functionality Remaining
8	20	0.5	0.208	72
	50	0.5	0.155	53
	100	0.5	0.125	43
10	20	0.5	0.221	76
	50	0.5	0.161	55
	100	0.5	0.145	50
12	20	0.25	0.215	74
	50	0.25	0.136	47
	100	0.25	0.074	25

The extent of inter- and intramolecular reactions occurring in procyanidins at alkaline pH in the absence of an added nucleophile was determined in a series of reactions done at various temperatures, pH's and reaction times. After neutralization and decationization, ^{13}C and $^1\text{H-NMR}$ spectra were recorded for the products. Data is presented here for samples at pH 8.0 and 10.0 after a reaction time of 0.5 hours for each of the temperatures used (Table 1). In most cases, only about 50% of the original phloroglucinol functionality is left after reaction. The outer, accessible units of the tannin polymers are probably cleaving and rearranging first, followed by slower reaction of the inner, more sterically-hindered units. As would be expected, a higher temperature or longer reaction time results in a greater extent of rearrangement. There appears to be little difference between the products reacted at pH 8.0 and 10.0 for a given set of temperature/time conditions. Reactions at pH 12.0 result in a more rapid loss of phloroglucinol functionality, data from samples resulting from a reaction time of 0.25 hours are given.

The compounds isolated from the reaction of procyanidins with phloroglucinol (Laks *et al.* 1987a) proved to be valuable model compounds for interpreting the $^{13}\text{C-NMR}$ spectra of the alkaline-rearranged tannin. A summary of the $^{13}\text{C-NMR}$ spectra of these compounds is shown in Table 2. The spectrum of purified southern pine bark tannin after it has been exposed to NaOH (solution pH of 12) at 100°C for one hour, and "phenolic acids" obtained by extraction of southern pine bark with 1% NaOH are shown in Fig. 1 (spectra B and C, respectively). The carbon spectrum of the rearranged tannin appears to be complex with a number of signals between 30 and 110 ppm. However, virtually every signal can be assigned to structures found in the model compounds. A representative structure for alkali-rearranged procyanidins is shown in Fig. 2 with some of the important car-

Table 2. ^{13}C nmr chemical shifts for catechinic acid and its 8-phloroglucinol and 8-flavan adducts (from Laks *et al.* 1987a)

Compound	Ring	Carbon Number								
		2	3	4	5	6	8	9		
(2)	Bicyclic	59.2	211	106.5	192	64.2	54.4	67.3	37.7	211
	Catechol	132.5	116	144.8	145	117.4	122			
(4)	Bicyclic	50.7	206	106.4	188	61.5	45.6	71.2	37.2	206
	Catechol	131.7	116	145.0	145	117.4	122			
	Phloroglucinol	101.2 (104.8)	157	96.0 (97.8)	159	96.0 (97.8)	157			
(5)	Bicyclic (F)	50.7	206	106	188	61.3	45.5	70.4	36.7	206
	Catechol (B)	136.3	116	145	145	117.8	120.9			
	Catechol (E)	131.4	116	145	145	112.6	121.6			
	Phloro. (A)	99.7	157	97.1	157	95.5	157			
	Phloro. (D)	101.2	157	116.0	157	95.7	157			
	Pyran (C)		67.9	73.1	45.6					

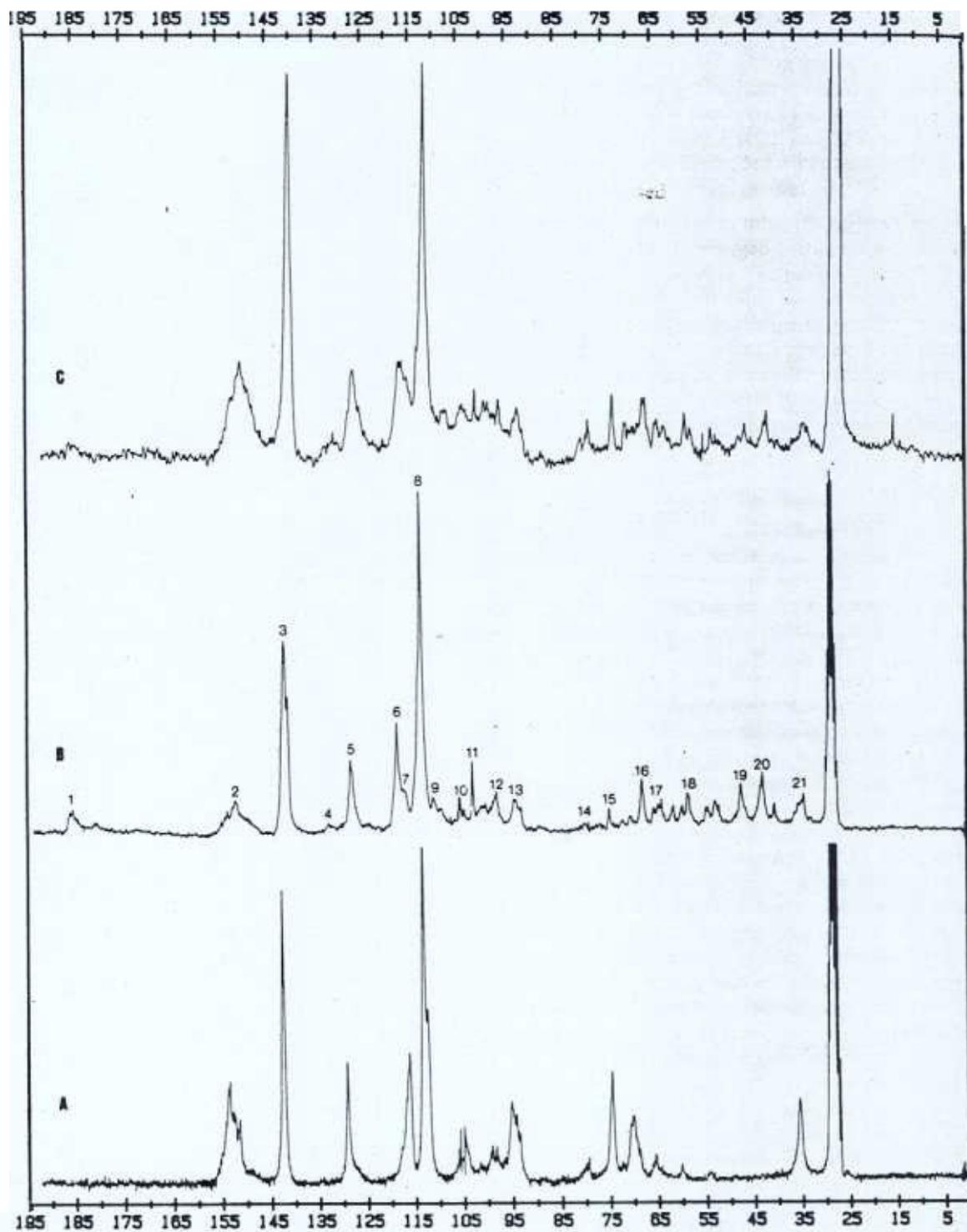


Fig. 1. ^{13}C -NMR spectra of purified procyanidins (A), alkali-rearranged procyanidins (pH 12, 50°C, 48 h.) (B), and tannin extracted with 1% NaOH at 90°C (C), all derived from southern pine bark.

bon species numbered and assigned to signals in Fig. 1, spectra B.

Some regular procyanidin structure is evident in the rearranged tannin and the tannin isolated by alkaline

extraction of bark. Signals from C-2 of the pyran ring of catechin and epicatechin monomers can be seen at 83 and 78 ppm, respectively. Comparison of the relative intensities of these signals shows there is more

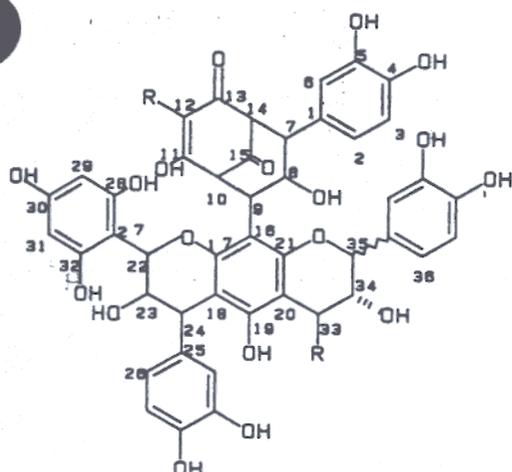


Fig. 2. The general structure of alkali-rearranged procyanidin condensed tannins (phenolic acids). R is H or a continuation of the same type of structure.

regular tannin structure in the phenolic acids obtained by extraction with 1% NaOH compared to the tannin heated to 100°C for one hour at pH 12.

Reasonably strong signals assignable to a structure like 4 are also apparent. Especially significant are the conjugated carbonyl and enol carbons centered on 189, the unsubstituted alkene carbon in the enol functionality at 105 and the bicyclic aliphatic carbons at 37, 45, 49, 62 and 71 ppm. Structures related to the upper unit of the dimer 5 are also evident. Signals can be assigned to the C-2, C-3 and C-4 (ring C) aliphatic carbons at 68, 71 and 45 ppm. The latter signal overlaps with carbons α to a catechol ring in structures like 4 and results in a relatively large absorbance at this chemical shift. Further evidence for structures like the upper unit of 5 in the rearranged tannin comes from signals at 136 (5, ring B, C-1) and 114 ppm (5, ring D, C-3). The substitution of a phloroglucinol ring, α to the catechol causes a characteristic change

Table 3. Assignment of ^{13}C NMR signals of alkali-rearranged procyanidins.

Signal (see Figure 1)	Chemical Shift	Representative Carbon(s) (see Figure 2)	Description
	206	15	Unconjugated carbonyls.
	189	11, 13	Conjugated carbonyls and enolic carbons.
	155	17, 19, 21, 28, 30, 32	Oxygenated, quaternary A-ring carbons.
3	145	4, 5	Oxygenated, quaternary B-ring carbons.
4	136	25	C-1 of B-ring when a phloroglucinol ring is in the α position.
5	131		C-1 of B-ring with non-phloroglucinol substituents in the α position.
6	122	2, 26	C-6 of B-rings in non-regular procyanidin structure.
7	119	36	C-6 of B-rings in regular procyanidin structure.
8	116	3, 6	C-2 and C-5 of B-rings.
9	114	27	C-1 of A-ring with an O-Aryl group in the α position.
10	108		C-6 and C-8 quaternary carbons of A-ring in regular procyanidin structure.*
11	105	12	= CR- of enol (R = H).
12	100-103	18, 20	Other quaternary carbons of A-rings.
13	95-98	29, 31	Tertiary carbons of A-rings.
14	83	35	C-2 of catechin pyran ring.*
15	78	35	C-2 of epicatechin pyran ring.*
16	70-71	8	Saturated, hydroxylated carbon of bicyclo-(3.3.1)-nonane ring.
17	66-68	22, 23, 34	Saturated, hydroxylated carbon of pyran rings or O-aryl substituted, tertiary carbon α to a phloroglucinol ring.
18	62	14	Tertiary carbon of bicyclo-(3.3.1)-nonane ring, β to catechol ring.
19	49	10	Tertiary carbon of bicyclo-(3.3.1)-nonane ring, β to phloroglucinol ring.
20	45	7, 24	Non-etherified tertiary carbon α to a catechol ring.
21	35-38	9, 33	Tertiary carbon of bicyclo-(3.3.1)-nonane or pyran ring α to a phloroglucinol ring.

*Carbon numbers refer to conventional flavonoid numbering system.

in the chemical shift of the catecholic C-1 (Laks *et al.* 1987a). Other signals assignable to catechinic acid-type structures are also present, particularly the ketone signal at 206 ppm. Table 3 summarizes how the signals in the alkali-rearranged tannin can be assigned.

Conclusion

Under alkaline conditions, procyanidin-type condensed tannins rearrange readily to structures lacking phloroglucinol functionality. These results indicate that in formulating tannin-based resins, particularly for cold-setting, laminating adhesives, exposure to alkaline conditions should be kept to a minimum. Heating a tannin solution at high pH is especially to be discouraged if high reactivity is desired.

References

- Hemingway, R.W., L.Y. Foo, and L.S. Porter. 1982. Linkage isomerism in trimeric and polymeric 2,3-*cis*-procyanidins. *J. Chem. Soc., Perkin I*, 1209-1216.
- Herrick, F.W. 1980. Chemistry and utilization of western hemlock bark extractives. *J. Agric. Food-Chem.* 28 (2): 228-237.
- Karchesy, J.J. and R.W. Hemingway. 1980. Loblolly pine bark polyflavanoids. *J. Agric. Food Chem.* 28 (2): 222-228.
- Kiatgrajai, P., J.D. Wellons, L. Gollob, and J.D. White. 1982. Kinetics of polymerization of (+)-catechin with formaldehyde. *J. Org. Chem.* 47, 2913-2917.
- Laks, P.E. and R.W. Hemingway. 1987. Condensed tannins: Base-catalysed reactions of polymeric procyanidins with phenylmethanethiol. Lability of the interflavanoid bond and pyran ring. *J. Chem. Soc., Perkin Trans. I*.
- Laks, P.E., R.W. Hemingway, and A.H. Conner. 1987a. Condensed tannins: Base-catalysed reactions of polymeric procyanidins with phloroglucinol. Intramolecular rearrangements. *J. Chem. Soc., Perkin Trans. I*.
- McGraw, G.W. and R.W. Hemingway. 1982. Electrophilic aromatic substitution of catechins: Bromination and benzylation. *J. Chem. Soc., Perkin Trans. I*, 973-978.
- Sears, K.D., R.L. Casebier, H.L. Hergert, G.H. Stout, and L.E. McCandlish. 1975. The structure of catechinic acid. A base rearrangement product of catechin. *J. Org. Chem.* 39, 3244-3247.
- Voulgaridis, E., A. Grigoriou, and C. Passialis. 1985. Investigations on bark extractives of *Pinus halepensis* Mill. *Holz Roh-Werkstoff* 43, 269-272.