Phytochemistry, 1976, Vol 15, pp. 2009–2010. Pergamon Press. Printed in England.

CONDENSED TANNINS FROM THE BARKS OF *ALNUS RUBRA* AND *PSEUDOTSUGA MENZIESII*

JOSEPH J. KARCHESY*†, PATRICIA M. LOVELAND*†, MURRAY L. LAVER‡
DOUGLAS F. BAROFSKY§ and ELISABETH BAROFSKY§
*Department of Forest Products, Oregon State University Corvallis, OR 97331 U.S.A.;
§Oregon Graduate Center, 19600 N.W. Walker Road, Beaverton, OR 97005 U.S.A.

(*Revised received* 8 *June* 1976)

Key Word Index—*Alnus rubra*; Betulaceae; *Pseudotsuga menziesii*; Pinaceae; bark; tannin; field desorption mass spectrometry.

Plants. Alnus rubra Bong. Voucher specimen 139987, Herbarium, Department of Botany, Oregon State University, Corvallis, OR 97331 U.S.A. *Source.* Freshly cut tree (12 inch diam. at breast height) in McDonald Forest, Benton County, Oregon, U.S.A. *Previous work.* On bark tannins [1,2]. *Pseudotsuga menziesii* (Mirb.) Franco. Voucher specimen 142702, Herbarium, Department of Botany, Oregon State University, Corvallis, OR 97331 U.S.A. *Source.* Freshly cut tree (*ca* 150 yr old) in McDonald Forest, Benton County, Oregon, U.S.A. *Previous work.* On bark tannins [3, 4].

Present work. The nature of the condensed tannin of *Alnus rubra* Bong. (red alder) bark was determined as a polymer of epicatechin in the course of the identification of suspected precursors [5] to the known staining phenomenon of this species. The condensed tannin of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) bark was studied as part of a continuing program in regard to utilization of an abundant waste material [6–9] and found to be a polymer of catechin and epicatechin.

Consistent with a catechin-type hydroxylation pattern, both condensed tannins showed λ_{max}^{EtOH} 280 nm and demonstrated appropriate color reactions [10] (red with vanillin–toluene–*p*–sulfonic acid reagent, black with ammoniacal silver nitrate reagent, and pink with toluene–*p*–sulfonic acid reagent). Alkali fusion yielded phloroglucinol and protocatechuic acid [11] in both cases. Treatment of both condensed tannins with propan-2-ol–3N-HCl under pressure [12] gave cyanidin chloride. Methylation of both condensed tannins yielded a product which gave a negative response to ferric chloride–potassium ferricyanide reagent but exhibited an hydroxyl absorbance at $\nu_{max}^{CHCl_3}$ 3580 cm^{-1} which subsequently disappeared on acetylation. Both methylated condensed tannins gave 5,7,3′,4′-tetra-*O*-methylcyanidin chloride [13] on treatment with propan-2-ol–3N-HCl under pressure [12].

Thioglycolysis and subsequent permethylation [14,15] of the methylated condensed tannins gave differing results, however. The methylated condensed tannin from red alder gave only the epicatechin thioglycolate derivative, methyl 2,3-*cis*-3,4-*trans*-(3-hydroxy-5,7,3′,4′-tetramethoxyflavan-4-ylthio)acetate, which was in every way identical to a synthetic sample prepared from (−)-epicatechin [16]. TLC analyses showed no spot which migrated the same distance as authentic methyl 2,3-*trans*-(3-hydroxy-5,7,3′,4′-tetramethoxyflavan-4-ylthio)-acetate [17], the corresponding catechin derivative. No other mobile degradation products were observed.

On the other hand, the methylated condensed tannin from Douglas fir yielded methyl 2,3-*cis*-3,4-*trans*-(3-hydroxy-5,7,3′,4′-tetramethoxyflavan-4-ylthio)acetate and methyl 2,3-*trans*-(3-hydroxy-5,7,3′,4′-tetramethoxyflavan-4-ylthio)acetate in approximately a 3:1 ratio.

We conclude from the above observations that the condensed tannin from red alder is based on epicatechin units while the condensed tannin from Douglas fir consists of both catechin and epicatechin units in an undetermined sequence. The condensed tannin from Douglas-fir is thus similar to that from western hemlock which is also composed of catechin and epicatechin units but in approximately a 1:1 ratio [15]. The red alder condensed tannin resembles that described from heather [14], but rather than the ether interflavanoid linkages proposed for heather condensed tannin we favor the concept of a carbon–carbon interflavanoid linkage from C-4 of one unit to C-6 or C-8 of another unit. This follows from a consideration of the most likely electrophilic substitution pathway for a catechin-type nucleus in condensed tannin formation [18, 19]. Malan and Roux [18] have, in addition, suggested that the B ring is nonfunctional as a nucleophile in condensed tannin formation. In agreement with this suggestion, our studies of red alder and Douglas fir condensed tannins by field desorption mass spectrometry (FDMS)-pyrolysis indicate that the B rings are not involved in the interflavanoid linkage because of their ease of fission under FDMS-pyrolysis conditions.

FDMS-pyrolysis (pyrolysis of compounds directly on the emitter surface in the mass spectrometer) has proven to be a supplemental structural tool in previous studies of phenolic compounds [5,20,21]. In these studies FDMS-pyrolysis of monomeric flavanoids yielded strong transient peaks which corresponded to the molecular weight of the B ring fragment and the A ring fragment [5,22]. For example, (+)-catechin and (−)-epicatechin, each of which contain a phloroglucinol A ring and a catechol B ring, both gave peaks at *m/e* 126 (phloroglucinol) and *m/e* 110 (catechol). FDMS-pyrolysis of the condensed tannins in the present study, however, yielded only an *m/e* 110 peak and no *m/e* 126 or higher mass

† Present addresses: JJK, Department of Chemistry, University of Oregon, Eugene, OR 97403 U.S.A.; PML, Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331 U.S.A.

‡ To whom correspondence should be addressed.

peak. It is believed that the *m/e* 110 peak arose from catechol which was produced by cleavage of the B ring on pyrolysis. Catechin has long been known to produce catechol on pyrolysis [23]. Presumably the absence of an *m/e* 126 peak was due to involvement of the A rings in interflavanoid linkages. The energy required (as indicated by the current in mA supplied to the emitter) to produce the peaks at *m/e* 110 was essentially the same for the monomeric catechins and the polymeric condensed tannins. This is further indication that the B rings in the condensed tannins are not involved in interflavanoid linkages.

EXPERIMENTAL

Tannin isolation. Alnus rubra Bong. air-dried bark (1.0 kg) was percolated at room temp. with *n*-hexane (4 l.), Et_2O (4 l.) and Me_2CO (4 l.) consecutively. The Me_2CO extract yielded a tan-colored, spongy solid (93.1 g). A part (5.0 g) of the Me_2CO-soluble solids was shaken with water (500 ml) and the resulting emulsion was extracted with EtOAc (250 ml, repeated 6 times). The aqueous fraction was concentrated to a sirup, Me_2CO was added and re-evaporated to yield a reddish tan-colored, spongy solid (1.9 g). The solid was applied to a dry column [24] of cellulose (300 g) and developed with H_2O (one column length). Cores were taken from the column and those portions (R_f 0.00–0.51) giving a magenta color with vanillin–toluene–*p*-sulfonic acid reagent [10] were eluted with MeOH. MeOH was evaporated to yield a light-brown solid (190 mg). The solid was dissolved in a minimum amount of MeOH and precipitated by pouring into Et_2O (100 ml) (repeated once). The final ppt. was dried at room temp under reduced pressure to yield a reddish-brown solid (105 mg). This is defined as the Me_2CO-soluble condensed tannin fraction; UV λ_{max}^{EtOH} 280 nm. *Pseudotsuga menziesii* (Mirb.) Franco air-dried bark was soaked for several days at room temperature in Me_2CO–H_2O (2:1) (repeated 2 ×). Me_2CO was removed and the remaining aqueous suspension was extracted with Et_2O (×4), then filtered through glass wool to remove the sticky, red solids which remained. The aq soln was freeze-dried, dissolved in a minimum of MeOH, and precipitated into EtOAc (repeated once). The ppt. was dissolved in water, filtered and the filtrate was freeze-dried to give a light brown powder. Condensed tannin was further fractionated by dry column chromatography and solvent precipitation as described above for the *Alnus rubra* Bong. condensed tannin.

Condensed tannin characterization. Alkali microfusion products [11] were identified by TLC [Si gel G using $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:4)] and FDMS (*m/e* 110 and 126). Anthocyanidins [12] were identified by comparison with authentic compounds (paper chromatography and UV). Methylation of the condensed tannins was accomplished by Me_2SO_4–K_2CO_3 in Me_2CO–MeOH (5:1). The tan-colored products gave a negative response to ferric chloride–potassium ferricyanide reagent and exhibited $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 2940, 1610, 1600, 1520, 1470, 1460, 1447, 1420, 1162, 1146, 1130, 1030. Acetylation of the methylated condensed tannins (C_5H_5N–acetic anhydride) gave light yellow solids; $\nu_{max}^{CHCl_3}$ cm^{-1}: 2935, 1745, 1605, 1598, 1512, 1460, 1453, 1440, 1415, 1370, 1155, 1140, 1125, 1023. The procedure of Sears and Casebier [15] for the thioglycolic acid degradation of the methylated condensed tannins was followed. Thioglycolysis products were methylated with CH_2N_2 in Et_2O, and were isolated by preparative TLC [Si gel G, C_6H_6–Me_2CO (9:1)]. Identification was made by comparison (mp, TLC, UV, IR, NMR, MS) with authentic thioglycolate derivatives of epicatechin and catechin synthesized according to published procedures [16,17].

Field desorption mass spectrometry. The field anode was an 8 μm wire activated by Barofsky and Barofsky's method [25]. The accelerating voltages were +1.8 kV applied to the field anode and −8.2 kV applied to the cathode. Emitter heating currents were varied between 0 and 16 mA. Approximately 10^{-8} g of sample was deposited in each case on the FD emitter by the microliter syringe technique [26].

REFERENCES

1. Peacock, J. C. (1900) *Am. J. Pharm.* **72,** 429.
2. Kurth, E. F. and Becker, E. L. (1953) *Tappi* **36,** 461.
3. Holmes, G. W. and Kurth, E. F. (1961) *Tappi* **44,** 893.
4. Dassler, H. G. and Wang, D. (1963) *Flora* **153,** 326.
5. Karchesy, J. J. (1974) Ph.D. Thesis, Oregon State University, Corvallis OR, 97331 U.S.A.
6. Laver, M. L., Fang, H. H.-L., and Aft, H. (1971) *Phytochemistry* **10,** 3292.
7. Loveland, P. M. and Laver, M. L. (1972) *Phytochemistry* **11,** 430.
8. Loveland, P. M. and Laver, M. L. (1972) *Phytochemistry* **11,** 3080.
9. Laver, M. L., Chen, C.-H., Zerrudo, J. V. and Lai, Y.-C.L. (1974) *Phytochemistry* **13,** 1891.
10. Roux, D. G. and Maihs, A. E. (1960) *J. Chromatog.* **4,** 65.
11. Roux, D. G. (1958) *J. Am. Leather Chem. Assoc.* **53,** 384.
12. Pigman, W., Anderson, E., Fischer, R., Buchanan, M. and Browning, B. L. (1953) *Tappi* **36,** 4.
13. Jurd, L. (1969) *Phytochemistry* **8,** 2421.
14. Betts, M. J., Brown, B. R., Brown, P. E. and Pike, W. T. (1967) *Chem. Commun.* 1110; Brown, B. R. and Shaw, M. R. (1974) *J. Chem. Soc.* Perkin I 2036.
15. Sears, K. D. and Casebier, R. L. (1970) *Phytochemistry* **9,** 1589.
16. Betts, M. J., Brown, B. R. and Shaw, M. R. (1969) *J. Chem. Soc.* (*C*) 1178.
17. Sears, K. D. and Casebier, R. L. (1968) *Chem. Commun.* 1437.
18. Malan, E. and Roux, D. G. (1975) *Phytochemistry* **14,** 1835.
19. Although this type of linkage may predominate, there is not sufficient evidence to conclude that the entire polymer is linked in this fashion. Other types of additional interflavanoid linkages, such as the ketal linkage found in proanthocyanidin A2 cannot be ruled out at present. [Jacques, D., Haslam, E., Bedford, G. R. and Greatbanks, D. (1973) *J. Chem. Soc. Chem. Commun.* 518; (1974) *J. Chem. Soc.* Perkin I 2663].
20. Karchesy, J. J., Laver, M. L., Barofsky, D. F. and Barofsky, E. (1974) Abstracts, 167th National Meeting of the American Chemical Society, Los Angeles, CA, CELL No. 45.
21. Karchesy, J. J., Laver, M. L., Barofsky, D. F. and Barofsky, E. (1974) *J. Chem. Soc. Chem. Commun.* 649.
22. Karchesy, J. J., Laver, M. L., Barofsky, D. F. and Barofsky, E., unpublished work.
23. Freudenberg, K. and Weinges, K. (1962) in *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.) p. 198. MacMillan, New York.
24. Loev, B. and Goodman, M. M. (1967) *Chem. Ind.* 2026.
25. Barofsky, D. F. and Barofsky, E. (1974) *Int. J. Mass Spectrom. Ion Phys.* **14,** 3.
26. Becky, H. D., Heindrichs, A. and Winkler, H. U. (1970) *Int. J. Mass Spectrom. Ion Phys.* **3,** App. 9–11.