2. Raffinose and 1^F-β-fructosylsucrose are also present, the former being mainly associated with the xylem and the latter with the 'soft xylem'.

3. A protein preparation from the 'soft phloem', when incubated with sucrose, produced glucose, fructose and 1^F-β-fructosylsucrose. The preparation also exhibited marked α- and β-glucosidase and α-galactosidase activities.

I thank Dr R. E. Kremers for many helpful discussions and encouragement; Dr D. Gross for useful advice; and the Institute of Paper Chemistry, Appleton, Wisconsin, U.S.A., for the facilities provided for this work. The Rhinelander Paper Company, Wisconsin, U.S.A., kindly allowed plant materials to be collected from their experimental forest.

REFERENCES

Albon, N., Bell, D. J., Blanchard, P. H., Gross, D. & Rundell, J. T. (1953). *J. chem. Soc.* p. 24.
Allen, P. J. & Bacon, J. S. D. (1956). *Biochem. J.* **63**, 200.
Andrews, P., Hough, L. & Powell, D. B. (1956). *Chem. & Ind.* p. 658.
Ash, A. S. F. & Reynolds, T. M. (1955). *Aust. J. Chem.* **8**, 276.
Bacon, J. S. D. (1954). *Biochem. J.* **57**, 320.
Bacon, J. S. D. (1959). *Biochem. J.* **73**, 507.
Bacon, J. S. D. & Bell, D. J. (1953). *J. chem. Soc.* p. 2528.
Bacon, J. S. D. & Edelman, J. (1951). *Biochem. J.* **48**, 114.
Barker, S. A., Bourne, E. J. & Carrington, T. R. (1954). *J. chem. Soc.* p. 2125.
Barker, S. A. & Carrington, T. R. (1953). *J. chem. Soc.* p. 3588.
Bois, E. & Chubb, W. O. (1942). *Canad. J. Res.* **20** B, 114.
Bradfield, A. E. & Flood, A. E. (1950). *Nature, Lond.*, **166**, 264.
Cerbulis, J. (1955). *Arch. Biochem. Biophys.* **58**, 406.
Conchie, J. & Levvy, G. A. (1955). *Biochem. J.* **61**, xvi.
Dedonder, R. (1952*a*). *Bull. Soc. Chim. biol., Paris*, **34**, 171.
Dedonder, R. (1952*b*). *Bull. Soc. Chim. biol., Paris*, **34**, 144, 157.
Feingold, D. S., Avigad, G. & Hestrin, S. (1956). *Biochem. J.* **64**, 351.
French, D. (1954). *Advanc. Carbohyd. Chem.* **9**, 149.
Gross, D. (1954). *Nature, Lond.*, **173**, 487.
Gross, D., Blanchard, P. H. & Bell, D. J. (1954). *J. chem. Soc.* p. 1727.
Hay, J. G. & Pridham, J. B. (1953). *Nature, Lond.*, **172**, 207.
Henderson, R. W., Morton, R. K. & Rawlinson, W. A. (1959). *Biochem. J.* **72**, 340.
Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
Jermyn, M. A. (1955). *Aust. J. biol. Sci.* **8**, 577.
Kremers, R. E. (1957). *T.A.P.P.I.*, **40**, 262.
Kritchevsky, G. & Anderson, A. B. (1955). *J. Amer. chem. Soc.* **77**, 3391.
Kuprevich, V. F. (1949). *Bot. Zh.* **34**, 613.
Meeuse, D. (1949). *New Phytol.* **48**, 125.
Meeuse, D. (1952). *Acta bot. neerl.* **1**, 216.
Pazur, J. H. (1952). *J. biol. Chem.* **199**, 217.
Pridham, J. B. (1956). *Analyt. Chem.* **28**, 1967.
Pridham, J. B. (1957). *Analyt. Chem.* **29**, 1167.
Pridham, J. B. (1958). *Nature, Lond.*, **182**, 1687.
Schlubach, H. H., Lübbers, H. & Borowski, H. (1955). *Liebigs Ann.* **595**, 229.
Sevag, M. G., Lackman, D. B. & Smollens, J. (1938). *J. biol. Chem.* **124**, 425.
Sosa, A. (1940). *Ann. Chim.* **14**, 5.
Stewart. C. M. (1957). *T.A.P.P.I.*, **40**, 244.
Sultze, R. (1956). Ph.D. Thesis: Institute of Paper Chemistry, Appleton, Wisconsin, U.S.A.
Sumner, J. B. & Myrbäck, K. (1950). *The Enzymes*. New York: Academic Press Inc.
Swain, T. (1953). *Biochem. J.* **53**, 201.
Zimmermann, M. H. (1957). *Plant Physiol.* **32**, 288.
Zimmermann, M. H. (1958). In *The Physiology of Forest Trees*, p. 381. Ed. by Thimann, K. V. New York: The Ronald Press Co.

Biochem. J. (1960) **76**, 17

Condensed Tannins

4. THE DISTRIBUTION AND DEPOSITION OF TANNINS IN THE HEARTWOODS OF *ACACIA MOLLISSIMA* AND *SCHINOPSIS* SPP.*

BY D. G. ROUX AND S. R. EVELYN
Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(*Received* 4 *September* 1959)

The pattern of the distribution of monomeric and polymeric leucofisetinidins, of tannins and non-tannins, and of the number-average molecular weight of the tannins in a cross-section of the heartwood of *Schinopsis quebracho-colorado* (syn. *S. lorentzii*) was examined by Roux (1958*b*) and by Roux & Evelyn (1958*b*). A possible mechanism for the formation of tannins in quebracho woods (*Schinopsis* spp.) has been discussed by White (1958). This work has now been extended to include the vertical distribution of heartwood components, and in the present study comparisons are also made

* Part 3: Roux & Maihs (1960).

with the radial and vertical distribution of tannin components in the heartwood of the black wattle tree (*Acacia mollissima*).

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were determined as described by Roux & Maihs (1960), with water as solvent. Molecular-weight estimations were performed by Mrs M. C. Bill of this Laboratory, using a modified Ray ebulliometer (Roux & Evelyn, 1958*a*, *b*), and tannin analyses by the hide-powder (shake) method were by Mrs M. C. Bill and Miss S. Whitelaw, also of this Laboratory. Infrared comparisons were by Dr J. R. Nunn, National Chemical Research Laboratories, C.S.I.R., Pretoria.

Origin of wood samples. Sections of the trunk of authentic specimens of *A. mollissima* were obtained by the Wattle Research Institute, Pietermaritzburg, from the experimental farm 'Bloemendal'. A complete trunk of *S. quebracho-colorado* was supplied by the Union Department of Forestry from the plantations at Hanglip, Northern Transvaal. Other cross-sections of quebracho trees (*Schinopsis balansae* and *S. quebracho-colorado*) were obtained from those sources in the Argentine described by Roux & Evelyn (1958*b*).

Examination of heartwood extractives of Schinopsis *spp.*

Isolation of (+)-catechin and gallic acid. Heartwoods (800 g.) from the upper portion of the trunk of either species of quebracho (heartwood diameter 3–6 cm.), or from the outer margin (1–3 cm. wide) of the heartwood area of the mature tree, were ground to fine mesh in a Wiley mill and extracted (three extractions of 2 l. each) with commercial ethyl acetate. The extracts were dried in a rotary evaporator and the solid (120 g.) was dissolved in methanol to give an 8% solution. The methanolic solution was streaked on Whatman no. 3, 22½ in. × 18½ in. paper-chromatographic sheets (5 ml., or 0·4 g. of extract/sheet) and the chromatograms were developed with 2% acetic acid. Under ultraviolet light a dark-mauve band (R_F 0·45) was located and cut accurately from each sheet. The bands were stripped in 70% ethanol and the solvent was removed under vacuum. Paper chromatography of the residue (5 g. from 100 sheets, or 40 g. of extracts) showed the presence of only two substances, suspected to be (+)-catechin and gallic acid. The components were separated into acidic and phenolic fractions by treatment with sodium hydrogen carbonate solution and ether extraction.

(+)-Catechin. The phenolic fraction was recrystallized twice from water (0·68 g.); m.p. and mixed m.p. with (+)-catechin, 175°. Infrared-absorption curves of this substance and of (+)-catechin were superimposable over the range 2·5–15 μ. The acetyl derivative was formed as described for (−)-leucofisetinidin (Roux & Evelyn, 1958*b*); m.p. and mixed m.p. with penta-acetyl-(+)-catechin, 131°.

Gallic acid. The acidic fraction was also recrystallized from water (0·56 g.); m.p. and mixed m.p. with gallic acid, 253° (decomp.). Infrared-absorption curves of this substance and of gallic acid were identical over the range 2·5–15 μ. The acetyl derivative was formed as above; m.p. and mixed m.p. with triacetylgallic acid, 171°.

Table 1. *Total extractives, non-tannin content, yields of anthocyanidins and average molecular weight of tannins present in vertical heartwood samples of* Schinopsis quebracho-colorado (*42 years old*)

In col. 5 and in the corresponding columns in Tables 2 and 3 the yields in parentheses are obtained under anhydrous conditions (Roux & Bill, 1959) and are estimated as fisetinidin.

Average height above ground of cross-section (in.)	Sampling position*	Extractives (%)	Non-tannins (% of extractives)	Conversion into fisetinidin (%)	Average mol.wt. of tannins
206·5	1†	37·7	22·3	8·5 (12·9)	726
104	02	39·1	—	6·5 (13·7)	619
	1†	36·6	16·3	5·3 (17·7)	1066
	2	34·4	—	6·7 (14·5)	948
37	04	21·8	—	5·0 (11·3)	592
	03	29·1	—	5·3 (9·1)	1021
	02	28·6	—	6·7 (12·7)	1176
	1†	33·4	13·8	6·0 (10·8)	1203
	2	34·6	—	6·9 (11·2)	939
	3	35·1	—	6·8 (12·7)	838
	4	21·3	—	3·9 (7·2)	696
8	05	29·8	—	6·5 (16·5)	689
	04	23·9	—	4·3 (11·2)	813
	03	20·1	—	4·8 (8·3)	909
	02	32·9	—	7·8 (10·5)	1127
	1†	31·1	16·8	5·2 (10·8)	1134
	2	23·2	—	5·1 (9·8)	906
	3	31·0	—	7·2 (8·0)	764
	4	13·2	—	3·5 (3·1)	681
(−)-7:3′:4′-Trihydroxyflavan-3:4-diol				21·3 (47·3)	290

* Heartwood deposition is irregular at each level as shown by the maximum and minimum diameters: (206·5 in.) 1, 0·8 in.; (104 in.) 2, 0·9 in.; (37 in.) 3·2, 2·5 in.; (8 in.) 5·2, 4·3 in.

† Central heartwood at each level as judged by annual rings.

Table 2. *Radial heartwood samples from the outer edge and centre of a commercial quebracho log reputed to be* Schinopsis balansae

The outer edge and centre of this log were separated by about 95 annual rings. Heartwood diameter, 9 in. The sample on the outer edge was taken at a point 5–10 annual rings inside from the sapwood/heartwood junction; hence the higher molecular weight compared with most edge samples in Table 1.

Sampling position	Extractives (%)	Non-tannins (% of extractives)	Conversion into fisetinidin (%)	Average mol.wt. of tannins
Outer edge	33·4	10·3	5·1 (10·6)	910
Centre	32·1	8·2	5·4 (10·4)	1784

Isolation of gallic acid from quebracho sapwoods. The sapwood (300 g.) of *S. quebracho-colorado* was extracted with methanol. The solutes (10 g.) were fractionated on cellulose sheets, and gallic acid (0·3 g.) was isolated by the method used for heartwood extractives above; m.p. and mixed m.p. with gallic acid, 253°.

Vertical sampling of heartwood of S. quebracho-colorado. The trunk of a 42-year-old (45 ft. tall) tree from Hanglip, Northern Transvaal, was cut 0·5 ft. from the ground (trunk diameter 8·5 in. and heartwood diameter 5·0 in.), and again at a height of 17·5 ft. from the ground (trunk diameter 4·5 in. and heartwood diameter 1 in.). Sections of the trunk 3·5 in. thick were cut at four different heights (Table 1). The heartwoods of each of these cross-sections were sampled radially by drilling as before (Roux & Evelyn, 1958*b*), and the radial samples at each height examined by chromatography for total leucofisetinidin content and for the average molecular weight of the tannins (Table 1).

Paper-chromatographic examination of radial samples of quebracho sapwood and heartwood. Cross-sections of relatively young (40–60-year) and more mature (120–150-year) samples of *S. balansae* and *S. quebracho-colorado* were examined by radial drilling and two-dimensional chromatography (see Roux & Evelyn, 1958*b*).

The colourless sapwoods of *Schinopsis* spp. contain gallic acid as main component, associated with hydrolysable tannic acids (see King & White, 1957*a*, *b*). In most instances traces of (+)-catechin and (−)-leucofisetinidin accompany these, but in one specimen (*S. quebracho-colorado*) both flavans were absent from the sapwood.

At the sapwood/heartwood junction, (−)-7:3′:4′-trihydroxyflavan-3:4-diol, (+)-catechin and gallic acid predominate, but other compounds and condensed tannins are present, usually in lower concentration.

Samples taken progressively inwards from the sapwood/heartwood junction to the central heartwood show a progressive reduction in concentration of (−)-7:3′:4′-trihydroxyflavan-3:4-diol (Roux, 1958*b*; Roux & Evelyn, 1958*a*) and of (+)-catechin (King & White, 1957*a*, *b*), while gallic acid remains relatively constant. In the relatively young specimens (wood diameter 5·5–9 in.) of both *Schinopsis* spp. the flavans persist into the central heartwood (see Roux, 1958*b*), a distance of 1·5–2·5 in. from the heartwood periphery, while in older specimens (wood diameter 13 in.) the flavans are present only in the narrow (0·5 in. wide) outer perimeter of the heartwood and decline rapidly in concentration over this band.

In all specimens examined the sapwood was about 1–1·5 in. wide and contained 24–26 annual rings.

Examination of sapwood islands within the heartwood of Schinopsis balansae. The cross-sectioning of the wood of a quebracho log, typical of those used for manufacturing commercial tanning extract, showed the presence of sapwood islands within the central heartwood. The major sapwood island lay within the annual rings 4–33 from the centre and the smaller ones within the rings 27–47. Paper-chromatographic examination of radial drillings from the largest sapwood island showed the predominant presence of gallic acid, (+)-catechin (trace) and other 'hydrolysable tannins' (see King & White, 1957*a*). The distribution of components was similar to that in some of the outer quebracho sapwoods examined. Extractives from heartwood surrounding these sapwood islands appeared similar in every way to central heartwood samples from a specimen of *S. quebracho-colorado* of about the same age.

Drillings from the centre, and from the outer edge of the log within the annual rings 90–100 from the centre, were extracted as before and the extracts examined for the average molecular weight of the tannins and total leucofisetinidin content (Table 2). Chromatographic examination of the extractives from the ridges on the outer edge of the heartwood of this log originating from the annual rings 105–110 showed the presence of (−)-leucofisetinidin and (+)-catechin in high concentration. Owing to the crude and therefore irregular trimming away of the sapwood, the sample used for molecular-weight estimation (annual rings 90–100) originated from 'older' heartwood than that used for chromatographic examination (annual rings 105–110), although both were obtained from the edge of the log. The monomeric flavans appeared to be absent from both samples used for molecular-weight estimation.

Examination of the heartwood tannins of Acacia mollissima

Radial sampling of black-wattle heartwood. The 4 in. cross-section of the trunk of a 10-year-old tree was sampled radially by drilling as indicated in Table 3, and the drillings were exhaustively (7 times for 24 hr. each) extracted with methanol. The residue remained light brown and the extraction of tannins was obviously incomplete. Further extraction with acetone–water mixture (1:1, v/v) or with sodium carbonate solution proved unsatisfactory as the tannins removed under these conditions were insoluble in the methanol–ether methylating medium. This resulted in incomplete methylation and subsequent insolubility in the solvent (benzene or acetone) used for molecular-weight estimation. The tannin residues in the wood still appeared to contain leucofisetinidins, as judged by the scarlet colour developed when the extracted drillings were treated with hydrochloric acid–propan-2-ol under the conditions of

Table 3. *Radial sampling of* 10*-year-old wood of* Acacia mollissima *of diameter* 7·25 *in.*

In col. 1, the location of the sample is within annual rings in parentheses.

Sampling position	Extractives (%)	Non-tannins (% of extractives)	Conversion into fisetinidin (%)	Average mol.wt. of tannins
	Samples by radial drilling			
05 (6–9)	2·7	—	(4·9)	—
04 (4–5)	8·1	—	(14·8)	836
03 (2–3)	6·4	—	(7·1)	1008
02 (1–2)	4·7	—	(1·1)	895
*1 (1)	4·3	—	(2·0)	993
2 (1–2)	7·8	—	(4·4)	1131
3 (2–3)	5·4	—	(6·7)	1161
4 (3–4)	4·4	—	(9·7)	893
5 (4–5)	6·2	—	(12·5)	776
6 (5–7)	2·2	—	(1·0)	—
	Samples by sectioning along annual rings			
*1 (heartwood centre)	5·5	48·6	2·0 (1·4)	
2	5·5	39·0	4·3 (3·4)	
3	5·5	39·5	6·4 (5·1)	1123†
4	6·0	41·4	6·0 (9·4)	
5 (heartwood edge)	6·8	41·5	7·6 (10·3)	
6–10 (sapwood)	2·9	53·5	1·7 (2·5)	841

* Central sample. † Value for combined heartwood samples.

Table 4. *Molecular-weight gradation in black-wattle heartwood tannins separated on cellulose sheets with* 2 % *acetic acid as irrigant*

The fisetinidin was generated in 3N-hydrochloric acid–propan-2-ol (1:4, v/v).

R_F of fraction	Conversion into fisetinidin (%)	Average mol. wt. of fraction
0·0	4·2	1657
0·1	5·1	1428
0·2	6·2	1016
0·3	5·9	780
0·4	8·6	584
0·5–0·6	15·6*	290*

* Values for (+)-mollisacacidin, forming the major portion of this fraction.

Pigman, Anderson, Fischer, Buchanan & Browning (1953).

The methanol-soluble extracts were examined for total solids, leucoanthocyanidin content and average molecular weight of the tannins (Table 3). The remainder of the cross-section was cut by vertical sectioning along the very marked and widely-spaced annual rings, in order to determine the percentages of non-tannins in various sapwood and heartwood areas corresponding to the different years of growth (Table 3).

Molecular-weight distribution of black-wattle heartwood tannins. The combined heartwood extractives of the same section of the tree (Table 3), of average molecular weight 1123, were fractionated by separation on paper sheets as for wattle bark and quebracho (Roux & Evelyn, 1958*a*), and the molecular weights of the tannins estimated after methylation with diazomethane (Table 4). Fraction R_F 0·5–0·6 contained (+)-7:3′:4′-trihydroxyflavan-3:4-diol (Keppler, 1957) and was recrystallized from water. The yields of fisetinidin generated from each fraction were estimated (Table 4).

Vertical sampling of central heartwood of Acacia mollissima. A tree (10–11-year-old and 66 ft. high) was felled and sections were cut from the trunk at average heights indicated in Table 5. A core $\frac{3}{4}$ in. in diameter was drilled from the centre of each section and the average molecular weights of the extractives were determined as before (Table 5). At 14 ft. height the cross-section of the wood was abnormally light-coloured and no clear differentiation into heartwood and sapwood areas was possible. The concen-

Table 5. *Vertical and radial examination of heartwood of* Acacia mollissima

Height of sample (ft.)*	Sample no.	Conversion into fisetinidin (%)	Average mol.wt. of tannins
	Vertical samples from centres of heartwoods		
51·5	1	6·5	915†
46·5	2	6·8	825
25·0	3	6·7	742
19·5	4	5·0	736
14·0	5	2·3	774
2·0	6	5·5	756
	Radial samples of heartwood from base		
2·0	04	—	686
	03	—	693
	02	—	686
	6·1	—	756
	2	—	732
	3	—	713
	4	—	729

* Average height of cross-section.
† Single estimation only.

tration of total extractives and of leucofisetinidins in this section was below average (see Table 5). Also, the heartwood from each section of this tree appeared lighter than that from a 10-year-old sample previously studied (Tables 3 and 4), and those obtained from black-wattle trees in the Grahamstown area. The average molecular weight of the tannins present were also lower (compare Tables 3 and 5), and the cross-section of sample 6 was therefore sampled radially by drilling to confirm these lower average values.

Chromatographic study of some wattle heartwood components. Examination of wattle-heartwood extract on two-dimensional paper chromatograms showed that two components predominate in the higher-R_F region. These, (*a*) R_F 0·75, 0·38 and (*b*) 0·61, 0·53 in water-saturated butan-2-ol and 2% acetic acid respectively, both give green colorations with ferric salts, yellow with bisdiazotized benzidine and grey–black with ammoniacal silver nitrate. Toluene-*p*-sulphonic acid reagent (Roux, 1957) differentiates between them, (*a*) giving a greenish-yellow fluorescence under ultraviolet light, and (*b*) giving a scarlet coloration. The former runs in the same position as (±)-fustin (see Roux & Evelyn, 1958*c*), derived from the heartwood of *Rhus succedanea* or *R. glabra* (Roux & Freudenberg, 1958). When the appropriate region of the chromatogram was treated with boiling 3N-hydrochloric acid, compound (*a*) was readily converted into fisetin. The fisetin was extracted with amyl alcohol and identified after chromatography: (1) by its greenish yellow fluorescence under ultraviolet light; R_F 0·72 in both butan-1-ol–acetic acid–water (4:1:5, by vol.) and water-saturated butan-2-ol (cf. Bate-Smith & Westall, 1950; Roux & Evelyn, 1958*c*); (2) by light-absorption [$\lambda_{max.}$ 250, 320 and 365 mμ (cf. Kirby & White, 1955)]. Fisetin is formed from (±)-fustin under identical conditions. Substance (*a*) and fustin afford similar colorations with 1% ferric alum (green), bisdiazotized benzidine (pale yellow) and vanillin–toluene-*p*-sulphonic acid (yellow fluorescent) spray reagents, and (*a*) is therefore tentatively identified as fustin. The second substance (*b*) is readily converted into fisetinidin chloride with hot HCl, and was previously isolated by Keppler (1957) and later by Clark-Lewis & Roux (1958, 1959) as a (+)-7:3′:4′-trihydroxyflavan-3:4-diol (2:3-*trans*-3:4-*cis* substituents). Fisetin is also present in almost all heartwood extracts and also in the freshly felled tree. Complex leucofisetinidins are present in the medium to low R_F regions (R_F 0·0–0·4 in direction 2), corresponding to the molecular-weight gradation 584–1657 (Table 4). (+)-Mollisacacidin, fustin and fisetin and complex leucofisetinidins are not present in the sapwood but originate at or near the sapwood/heartwood junction, and are present throughout the heartwood.

DISCUSSION

(−)-7:3′:4′-Trihydroxyflavan-3:4-diol, present in high concentration at the sapwood/heartwood interface and also in the sapwood and portions of the heartwood of *S. quebracho-colorado* (Roux, 1958*a*; Roux & Evelyn, 1958*b*; Freudenberg & Weinges, 1958), has been shown to be enantiomorphous (Clark-Lewis & Roux, 1958, 1959) with (+)-7:3′:4′-trihydroxyflavan-3:4-diol (mollisacacidin), previously found in the heartwood of *A. mollissima* by Keppler (1957). Both monomeric forms were shown to be associated with, and apparently converted into, polymeric leucofisetinidins or tannins in each tree (Roux, 1958*b*; Roux & Evelyn, 1958*a*, *b*), but comparison of the heartwoods is of interest in view of some marked differences between them. In *A. mollissima* the concentration of extractable tannins is low (2–3%) and (+)-leucofisetinidin is present throughout the heartwood, whereas in *Schinopsis* spp. the tannin content of the heartwood is high (16–25%). The concentration of (−)-leucofisetinidin declines rapidly from the heartwood edge; it is absent from the central heartwood of mature (120–140-year-old) specimens.

The isolation of (+)-catechin and gallic acid from the peripheral heartwood of *Schinopsis* spp. is in accordance with the isolation of gallic acid from commercial quebracho extract by Perkin & Gunnell (1896) and confirms the chromatographic identification of (+)-catechin and gallic acid in the wood extractives by King & White (1957*a*, *b*).

Vertical sampling of the heartwood of *S. quebracho-colorado* shows a vertical 'coning' effect present in the distribution of the average molecular weight of the tannins and concentration of non-tannins in the upper portion of the tree (Table 1, samples 37, 104 and 206·5 in. above ground). The regularity of these distributions may be disturbed by the proximity of heartwood 'branches' proceeding into side limbs, and, although all samples were spaced as far away from branches as possible, the distribution of the molecular weight of the tannins is markedly asymmetrical at 37 in. above ground, and the deposition of tannins relative to the annual rings is similarly asymmetrical at 8 in. Such variation must be considered when vertical samples are compared (Table 1). The radial samples from the peripheral to the central heartwood at each level show an increase in average molecular weight of the tannins, confirming the previous findings by Roux & Evelyn (1958*b*). Where the vertical samples are widely spaced in the upper portion of the tree, the central heartwood sample of each level shows an expected gradational increase in molecular weight from the uppermost sample, which contains recently deposited tannins, to those towards the base, which contain 'older' heartwood components. The vertical and radial gradations in molecular weight are accompanied by parallel variations in the tan/non-tan ratio with increasing age of the heartwood (Tables 1 and 2; Roux & Evelyn, 1958*b*). The central heartwood tannins in the relatively young (42-year-old) tree have a much lower degree of condensation (mol.wt. 726–1203) than those from the mature tree (mol.wt. 1748), as shown in Tables 1 and 2. Allowing for the variation which exists in most plant material, the molecular-weight increase appears to run parallel

to the increasing age of the heartwood. In the 'mature' commercial log examined (Table 2) the average molecular weight of the outer and central heartwood tannins (910 and 1784 respectively) falls on either side of the value 1327 obtained for the commercial extract by Roux & Evelyn (1958*a*). The commercial extract is obtained by aqueous extraction of sawdust derived from the logs.

The presence of sapwood enclosed by heartwood in an old quebracho log is of significance. By analogy with the studies of Erdtman (1952, 1955) on conifers, the enclosed sapwood probably resulted from injury to the cambium opposite the enclosed sapwood area. The enclosed sapwood island contains polyphenolic constituents typical of 'normal' sapwood, and it appears that condensed tannin components once deposited in the surrounding heartwood do not migrate into these enclosed sapwood areas. This evidence, in conjunction with the apparent progressive vertical and radial increase in molecular weight with the age of the heartwood, suggests that 'flavonoid' compounds predominating only on the outer periphery of the heartwood, namely (−)-leucofisetinidin and possibly (+)-catechin, condense in the cells in which they are initially deposited, to form condensed tannins. The degree of condensation increases with the aging of the tannins, and apparently no radial translocation of components as suspected for sapwood (Erdtman, 1952, 1955) occurs in quebracho heartwood.

Black-wattle heartwood contains low and very variable percentages (4·4–8·1%) of methanol-soluble extractives. This is evident from radial samples (Table 3) and from vertical samples (Table 5), where sample 5 at 14 ft. height is low in leucoanthocyanidin content and the wood so abnormally light-coloured as to permit no differentiation into heartwood and sapwood. Extraction of the wood is known to be incomplete, owing to the exceptionally low solubility of the residual tannins. These factors limit interpretation of the distribution of the average molecular weight of tannins in the heartwood. The soluble heartwood tannins contain an even molecular-weight gradation over the range 300–1650, having an average molecular weight of 1123 (Tables 3 and 4). In some instances the average value must be much lower (Table 5) and wide differences may exist between trees (cf. Tables 3 and 5). Apparently such wide variation in average molecular weight of the extractable tannins does not exist within vertical and radial samples of particular specimens of black-wattle heartwood examined (Tables 3 and 5).

Sapwoods and heartwoods of *A. mollissima* and *Schinopsis* spp. show variation in the concentration and distribution of flavonoid compounds within individual species and also 'abnormal' variation within individual specimens. Examination of specimens from different localities is therefore necessary. (+)-7:3′:4′-Trihydroxyflavan-3:4-diol appears to be the main precursor of wattle-heartwood tannins, while fustin and fisetin probably originate from simple dehydrogenation of the parent flavan-3:4-diol. For quebracho tannins not only (−)-7:3′:4′-trihydroxyflavan-3:4-diol, but also (+)-catechin, as previously indicated by King & White (1957*a*, *b*), appear to be intermediates in tannin formation, but no direct evidence has been obtained of the presence of the (+)-catechin moiety in condensed quebracho tannins. Hillis (1958) and Hillis & Carle (1958) previously considered that heartwood tannins of *Eucalyptus* spp. could originate from both leucoanthocyanins and catechins, and Hathway (1958) has supported a similar view for the formation of oak-bark tannins.

For wattle and quebracho trees these flavan precursors are not present in the leaves as in *Eucalyptus* spp. (Hillis, 1955) or as in oaks (Hathway, 1959), but originate in the sapwood (King & White, 1957*a*, *b*; Roux, 1958*b*) in which they may be translocated by means of medullary rays and deposited in the periphery of the heartwood. The present work shows that, after deposition, condensation of the flavans into tannins or their conversion into related substances (as in *A. mollissima*) probably occurs in the cells, as also concluded by Hillis (1958) from microscopic examination of the cells of *Eucalyptus* spp.

The different tannins present in the heartwoods and the barks of both *Acacia* spp. and *Schinopsis* spp. (Roux & Evelyn, 1958*b*) may be due to the formation of bark and wood from different cambia, as suggested by Erdtman (1959) for a similar phenomenon in *Pinus* spp.

SUMMARY

1. The isolation of (+)-catechin and gallic acid from the young heartwood of *Schinopsis* spp. and the tentative identification of fustin and fisetin in the heartwood of *A. mollissima* are described.

2. These heartwoods contain enantiomorphous (−)- and (+)-leucofisetinidins respectively, and the vertical and radial distribution of their tannins in relation to average molecular weight, leucoanthocyanidin content and concentration of associated 'non-tannins' are examined and contrasted.

3. There is evidence that typical condensed tannins are formed in the cells in which their flavan precursors are initially deposited in the heartwood periphery.

4. A vertical 'coning' effect is present in the heartwood of the upper portion of the trunk of *Schinopsis quebracho-colorado* with regard to molecular weight and tannin/non-tannin ratio.

5. The heartwood tannins of *A. mollissima* consist of a gradation over the molecular-weight range 290–1650 in which the yield of anthocyanidins decreases with increasing average molecular weight.

Thanks are due to Mr S. P. Sherry, Wattle Research Institute, Pietermaritzburg, Natal, for sections of *A. mollissima*, and to the Director, Union Department of Forestry, Pretoria, for the complete trunk of *S. quebracho-colorado*. This work was financed by the annual grant of the South African Wattle Growers' Union to the Leather Industries Research Institute.

REFERENCES

Bate-Smith, E. C. & Westall, R. G. (1950). *Biochim. biophys. Acta*, **4**, 427.
Clark-Lewis, J. W. & Roux, D. G. (1958). *Chem. & Ind.* p. 1475.
Clark-Lewis, J. W. & Roux, D. G. (1959). *J. chem. Soc.* p. 1402.
Erdtman, H. (1952). *Progr. org. Chem.* **1**, 22.
Erdtman, H. (1955). *Experientia*, Suppl. **2**, p. 156.
Erdtman, H. (1959). *Biochemistry of Wood*, vol. 2, p. 1. London: Pergamon Press Ltd.
Freudenberg, K. & Weinges, K. (1958). *Liebigs Ann.* **613**, 61.
Hathway, D. E. (1958). *Biochem. J.* **70**, 34.
Hathway, D. E. (1959). *Biochem. J.* **71**, 533.
Hillis, W. E. (1955). *Aust. J. biol. Sci.* **9**, 263.
Hillis, W. E. (1958). *Nature, Lond.*, **182**, 1371.
Hillis, W. E. & Carle, A. (1958). *Holzforschung*, **12**, 136.
Keppler, H. H. (1957). *J. chem. Soc.* p. 2721.
King, H. G. C. & White, T. (1957*a*). *J. Soc. Leath. Tr. Chem.* **41**, 368.
King, H. G. C. & White, T. (1957*b*). *Proc. chem. Soc., Lond.*, p. 341.
Kirby, K. S. & White, T. (1955). *Biochem. J.* **60**, 582.
Perkin, A. G. & Gunnell, O. (1896). *J. chem. Soc.* **69**, 1303.
Pigman, W., Anderson, E., Fischer, R., Buchanan, M. A. & Browning, B. L. (1953). *T.A.P.P.I.* **36**, 4.
Roux, D. G. (1957). *Nature, Lond.*, **180**, 793.
Roux, D. G. (1958*a*). *Chem. & Ind.* p. 161.
Roux, D. G. (1958*b*). *Nature, Lond.*, **181**, 1454.
Roux, D. G. & Bill, M. C. (1959). *Nature, Lond.*, **183**, 42.
Roux, D. G. & Evelyn, S. R. (1958*a*). *Biochem. J.* **69**, 530.
Roux, D. G. & Evelyn, S. R. (1958*b*). *Biochem. J.* **70**, 344.
Roux, D. G. & Evelyn, S. R. (1958*c*). *J. Chromat.* **1**, 537.
Roux, D. G. & Freudenberg, K. (1958). *Liebigs Ann.* **613**, 56.
Roux, D. G. & Maihs, E. A. (1960). *Biochem. J.* **74**, 44.
White, T. (1958). *Chemistry and Technology of Leather*, vol. 1, pp. 133–5. New York: Reinhold Publishing Corporation.

Biochem. J. (1960) **76**, 23

Condensed Tannins

5. THE OXIDATIVE CONDENSATION OF (+)-CATECHIN

BY S. R. EVELYN, E. A. MAIHS AND D. G. ROUX
Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(*Received* 15 *September* 1959)

Post-mortem oxidation, whether atmospheric or enzymic, affects the composition of polyphenols present in vegetable extracts and is invariably associated with the well-known 'browning' phenomenon. (+)-Catechin and (−)-*epi*catechin from the leaves of *Uncaria gambir* and heartwood of *Acacia catechu* respectively are known to be associated with dark-coloured condensed tannins in gambier and Burma-cutch extracts. Some of the tannins appear to be present in the original plant material (Hathway & Seakins, 1957*b*), but others may originate during manufacture of the extracts (Howes, 1953). Atmospheric oxidation or autoxidation of (+)-catechin, for example, results in a product which possesses marked tanning properties, as shown by its reaction with collagen monolayers (Ellis & Pankhurst, 1954).

Hathway & Seakins (1955) and Hathway (1958) recognized two different types of autoxidation polymers, namely those obtained at low pH (< 2), which are of the Freudenberg & Maitland (1934) type, and those obtained within a higher pH range (2–8), which occur by polymerization through quinones (Hathway & Seakins, 1957*b*).

In previous work the tacit assumption was made that polymerization does, in fact, occur under the above conditions. This aspect is examined in the present study.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Acetyl values are by Miss E. Paulus of this Laboratory. Infrared-absorption curves are by Dr J. R. Nunn, National Chemical Research Laboratory, C.S.I.R., Pretoria.

Conditions of oxidation of (+)*-catechin.* (+)-Catechin was isolated from cube gambier (obtained from the leaves of *Uncaria gambir*) by the method of Perkin & Yoshitake (1902), m.p. 175°. Atmospheric oxidation of (+)-catechin