the principal products. Ammonia is formed by the hydrolysis of amide groups in the casein, but no deamination of amino acids occurs.

3. Negligible conversion of casein or casein hydrolysate into cellular material takes place during incubation of these substances with *Entodinium caudatum*.

4. Urea plays no part, either as substrate or end product, in the nitrogen metabolism of *Entodinium caudatum*.

5. Cell-free extracts of the entodinia contain a proteinase which, in addition to hydrolysing casein, also hydrolyses the amide and ethyl ester of *N*^α-benzoyl-L-arginine. Activity is greatest at pH 6.5-7.0, and is increased in the presence of cysteine or calcium ions.

6. A peptidase, which hydrolyses a wide range of peptides is also found in the cell-free extracts. Its activity against L-leucylglycine is greatest at pH 5.8-6.0, and is increased by cobalt and to a lesser extent by zinc ions.

7. The proteinase and peptidase can be separated by electrophoresis on paper at pH 8.6.

We thank Dr P. N. Hobson for his advice during the course of this work, and Mr T. H. Blackburn for the gift of casein. We are also grateful to Dr R. L. M. Syngé, Dr F. Bealing, Dr G. Jones and Dr E. I. McDougall for their advice, gifts of chemicals and the use of their apparatus. Mr W. Shand and Mr R. Summers gave valued assistance in these experiments.

REFERENCES

- Abou Akkada, A. R. & Howard, B. H. (1960). *Biochem. J.* **76**, 445.
 Abou Akkada, A. R. & Howard, B. H. (1961). *Biochem. J.* **78**, 512.
 Bergmann, M., Fruton, J. S. & Pollok, H. (1939). *J. biol. Chem.* **127**, 643.
 Bier, M. & Nord, F. F. (1951a). *Arch. Biochem. Biophys.* **31**, 335.
 Bier, M. & Nord, F. F. (1951b). *Arch. Biochem. Biophys.* **33**, 320.
 Blackburn, T. H. & Hobson, P. N. (1960a). *J. gen. Microbiol.* **22**, 272.
 Blackburn, T. H. & Hobson, P. N. (1960b). *J. gen. Microbiol.* **22**, 290.
 Conway, E. J. (1957). *Microdiffusion Analysis and Volumetric Error*, 4th ed. London: Crosby Lockwood and Son Ltd.
 Duke, J. A., Bier, M. & Nord, F. F. (1952). *Arch. Biochem. Biophys.* **40**, 424.
 Gordon, W. G., Semmet, W. F., Cable, R. S. & Morris, M. (1949). *J. Amer. chem. Soc.* **71**, 3293.
 Heald, P. J. & Oxford, A. E. (1953). *Biochem. J.* **53**, 506.
 Hestrin, S. (1949). *J. biol. Chem.* **180**, 249.
 Howard, B. H. (1959). *Biochem. J.* **71**, 671.
 Hull, M. E. (1947). *J. Dairy Sci.* **30**, 881.
 Kunkel, H. G. & Tiselius, A. (1951). *J. gen. Physiol.* **35**, 89.
 Lampson, G. P. & Singher, H. O. (1960). *Proc. Soc. exp. Biol., N.Y.*, **103**, 368.
 Lewis, D. (1955). *Brit. J. Nutr.* **9**, 215.
 McDonald, I. W. (1952). *Biochem. J.* **51**, 86.
 Mellvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
 Mandl, L., Ferguson, L. T. & Zaffuto, S. F. (1957). *Arch. Biochem. Biophys.* **69**, 565.
 Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
 Nomoto, M. & Narahashi, Y. (1959). *J. Biochem., Tokyo*, **46**, 653.
 Ogle, J. D. & Tytell, A. A. (1953). *Arch. Biochem. Biophys.* **42**, 327.
 Porter, W. L. & Hoban, N. (1954). *Analyt. Chem.* **26**, 1846.
 Schlottke, E. (1936). *S.B. naturf. Ges. Rostock*, 3rd Series, **6**, 59.
 Schwert, G. W., Neurath, H., Kaufman, S. & Snoke, J. E. (1948). *J. biol. Chem.* **172**, 221.
 Sym, E. A. (1938). *Acta Biol. exp., Varsovie*, **12**, 192.
 Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 627.
 Viswanatha, T. & Liener, I. E. (1956). *J. biol. Chem.* **221**, 961.
 Warner, A. C. I. (1956a). *J. gen. Microbiol.* **14**, 733.
 Warner, A. C. I. (1956b). *J. gen. Microbiol.* **14**, 749.
 Weil, L. & Kochohaty, W. (1937). *Biochem. J.* **31**, 1255.

Biochem. J. (1962) **82**, 320

Condensed Tannins

12. POLYMERIC LEUCO-FISETINIDIN TANNINS FROM THE HEARTWOOD OF *ACACIA MEARNSII**

By D. G. ROUX AND E. PAULUS

Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(Received 26 June 1961)

The heartwood of the black-wattle tree (*Acacia mearnsii*) contains, amongst others, a 'leuco-fisetinidin' tannin which apparently corresponds to

the trimeric form of the accompanying (+)-7,3',4'-trihydroxyflavan-3,4-diol (Roux & Paulus, 1961c). The isolation, from the same source, of related tannins of progressively higher molecular weight is described in the present study.

* Part 11: Roux & Paulus (1961c).

EXPERIMENTAL AND RESULTS

Analyses of C, H and methoxyl and acetyl groups are by K. Jones, Microanalytical Laboratory, National Chemical Research Laboratory, C.S.I.R., Pretoria, and by Weiler and Strauss, Oxford. Infrared comparisons are by Dr J. R. Nunn of the same Laboratories, Pretoria. Molecular-weight estimations are by E. A. Maihs, of this Institute, using a modified Ray ebulliometer (Evelyn, 1954; Evelyn, Cooper & van Berge, 1958). Two-dimensional paper chromatograms were on Whatman no. 1 paper with water-saturated butan-1-ol and then 2% (v/v) acetic acid.

Preliminary fractionation of wattle-heartwood tannins

The powdered solids (80 g.), obtained by extraction of the heartwood (drillings) of *A. mearnsii* with methanol, were extracted with light petroleum (b.p. 60–80°) in a Soxhlet apparatus to remove waxes. A proportion of the wax appeared to be occluded by tannins and could not be removed by single extraction with light petroleum. The solids were therefore redissolved in methanol, the solvent was removed in a rotary evaporator and the dry solids were re-extracted with light petroleum as before. The process was repeated three or four times to ensure complete removal of waxes, and thus to avoid interference in the subsequent Craig partition separation caused by emulsions in the front tubes containing upper phase. The wax-free product was dissolved in 500 ml. of the lower phase of water–butan-2-ol–light petroleum (b.p. 40–150°) (5:3:2, by vol.) with warming, and the warm solution was filtered rapidly through Whatman no. 541 paper. The solution was introduced into the first 10 tubes of a 160-tube, 50 ml. underphase, automatic Craig machine. The upper and lower phases of the above-mentioned mixture were used for countercurrent separation, and after 160 transfers the contents of the tubes were examined by two-dimensional paper chromatography.

The 'leuco-fisetinidins' present in the extract (cf. Fig. 1) were distributed as follows: 1, (+)-7,3',4'-trihydroxyflavan-3,4-diol (36–71); 2, unstable leuco-fisetinidin (72–114); 3, leuco-fisetinidin tannin (41–66); 4, 'trimeric tannin' (41–61); 5, 11–40; 6, 1–15.

Specific rotations of the unstable leuco-fisetinidin and of the leuco-fisetinidin tannins 5 and 6 could not be measured as the solutions were too coloured for polarimetry.

'Leuco-fisetinidin 2'. The component with R_F 0.79 in water-saturated butan-1-ol and 0.56 in 2% (v/v) acetic acid was isolated from tubes 72–114, where it was accompanied by (+)-mollisacacidin in tubes 72–90, and by (+)-fustin and (–)-fisetinidinol in tubes 91–114. The solids from tubes 91–114 were streaked on Whatman no. 3 paper (200 mg./sheet) and the chromatogram was developed in 2% (v/v) acetic acid by upward migration. The band was located with toluene-*p*-sulphonic acid spray (Roux & Maihs, 1960) (pink) on a test strip. The band was cut and eluted with 70% (v/v) ethanol. The solids from tubes 72–90 were separated by similar methods but on pre-washed (water, 24 hr.) sheets of the same paper with butan-1-ol–acetic acid–water (6:1:2, by vol.). The product obtained from the eluates (0.1 g.) could not be crystallized from water, but darkened rapidly when kept at 0° to yield fisetin [identified by paper chromatography, and by its ultraviolet and infrared spectra (cf. Roux & Paulus, 1960)].

'Leuco-fisetinidin 2' had higher R_F (0.79) than (+)-7,3',4'-trihydroxyflavan-3,4-diol (0.59) in water-saturated butan-1-ol, but their R_F values were similar (0.56 and 0.52 respectively) in 2% (v/v) acetic acid. The substance gave fisetinidin chloride when heated with 3*N*-HCl–propan-2-ol (1:4, v/v) under pressure (cf. Roux, 1957). The leuco-fisetinidin gave the same colour reactions as (+)-7,3',4'-trihydroxyflavan-3,4-diol with selective spray reagents (cf. Roux & Maihs, 1960), namely ferric alum (green), bis-diazotized benzidine (yellow), ammoniacal AgNO₃ (brown-black) and toluene-*p*-sulphonic acid (purple-red, changing to pink).

'Leuco-fisetinidin 3'. This leuco-fisetinidin from tubes 41–66 of the Craig machine possessed R_F values (0.54, 0.39) between those of (+)-7,3',4'-trihydroxyflavan-3,4-diol (0.59, 0.52) and of the 'trimeric' leuco-fisetinidin (0.42, 0.22) in both water-saturated butan-1-ol and 2% (v/v) acetic acid respectively. The substance (100 mg.) was separated from these by preparative chromatography with 2% (v/v) acetic acid when isolating the trimeric tannin (Roux & Paulus, 1961c), but was contaminated with (+)-fustin and with minor components. Interfering components were separated with butan-1-ol–acetic acid–water (6:1:2, by vol.) on cellulose sheets as described above. The product (21 mg.) gave fisetinidin chloride with the 3*N*-HCl–propan-2-ol (1:4, v/v) solution, and gave the same colour reactions with the above-mentioned spray reagents as (+)-7,3',4'-trihydroxyflavan-3,4-diol. Slight differences were, however, noticed with ammoniacal AgNO₃ (brown only) and with toluene-*p*-sulphonic acid (pink only). Micro-degradation under anhydrous conditions (Roux, 1958) gave mainly resorcinol, β -resorecylic acid and protocatechuic acid. The substance gave a precipitate with the gelatin–salt reagent, indicating its tanning properties (Atkin & Thompson, 1937).

Fig. 1. Chromatographic distribution of components in the methanolic extractives of the heartwood of *Acacia mearnsii*. Solvents: direction 1, water-saturated butan-1-ol; direction 2, 2% (v/v) acetic acid. 1, (+)-7,3',4'-Trihydroxyflavan-3,4-diol; 2, 'leuco-fisetinidin 2'; 3, 'leuco-fisetinidin 3' tannin; 4, trimeric leuco-fisetinidin tannin; 5, pentameric leuco-fisetinidin tannin; 6, 'decameric' leuco-fisetinidin tannin; 7, fisetin; 8, butein; 9, (–)-butein; 10, (+)-fustin; 11, (–)-fisetinidinol; 12 and 13, components of unknown structure.

Table 1. Comparison of ultraviolet-absorption spectra of (+)-mollisacacidin and polymeric leuco-fisetinidin tannins

Compound	$\lambda_{\min.}$ (m μ)	$\epsilon_{\min.}^*$	$\lambda_{\max.}$ (m μ)	$\epsilon_{\max.}^*$
(+)-7,3',4'-Trihydroxyflavan-3,4-diol	252	1253	282	6638
Trimeric tannin	256	1404	282	5660
Pentameric tannin	258	2112	281	5422
'Decameric' tannin	260	2928	281	5025

* Molar-extinction coefficients for the tannins and the flavan-3,4-diol are calculated to the same C_{15} basis (mol.wt., 290.29).

Trimeric (-)-leuco-fisetinidin tannin. This component from tubes 41-61 was previously isolated and examined by Roux & Paulus (1961c).

The pure tannin (10 mg.), freed of all (+)-7,3',4'-trihydroxyflavan-3,4-diol, in 0.1N-acetic acid (4 ml.), was placed in a small pressure tube, which was heated at 97° for 2 hr., and, after cooling, the contents were examined by two-dimensional paper chromatography. In addition to the unchanged tannin spot (R_f 0.42 and 0.22) a weak spot was present on the chromatogram in the exact position occupied by (+)-7,3',4'-trihydroxyflavan-3,4-diol (R_f 0.59 and 0.52) in water-saturated butan-1-ol and 2% (v/v) acetic acid. The new spot gave a pink colour with toluene-*p*-sulphonic acid spray, yellow with bisdiazotized benzidine, grey-black with ammoniacal $AgNO_3$ and green with ferriol alum. Attempts to improve the low yield of the conversion product by altering the conditions were unsuccessful.

'Pentameric' leuco-fisetinidin tannin. The component was the main constituent in tubes 11-30, but was associated with the trimeric tannin in tubes 31-40. When examined on Whatman no. 3 sheets the contents of tubes 11-30 contained traces of other known components of the mixture. The tannin was purified by the preparative method with both solvent systems as for 'leuco-fisetinidin 3'. The product (1.5 g.) was light brown, sparingly soluble in water, but soluble in absolute methanol and ethanol. It did not crystallize from water or ethanol-water but settled out as a white sludge. The product was dried, dissolved in absolute methanol and filtered. After recovery it was dried at 110° for 2 hr. (Found: C, 61.1; H, 5.0; ash, 0. $C_{15}H_{14}O_6$ requires C, 62.1; H, 4.9%). The substance had the same ultraviolet-absorption spectrum as the trimeric tannin (Table 1) and also the same infrared absorption over the range 2.5-15 μ . The yield of fisetinidin chloride under the conditions of Pigman, Anderson, Fischer, Buchanan & Browning (1953) was lower than that from the trimer (Table 2). The R_f values of the tannin (0.20 and 0.16) were lower than those of the trimer in both solvents. No hydrolysis product could be detected after treatment with 0.1N-acetic acid as described above. The compound precipitated gelatin in aqueous medium, indicating its tanning properties.

Methylated 'pentameric' tannin. The tannin (300 mg.) in methanol (100 ml.) was methylated with excess of ethereal diazomethane (cf. Roux & Paulus, 1961c). The white product (280 mg.) was dissolved in methanol and filtered after boiling with charcoal. The solid recovered from the filtrate was dried at 110° for 2 hr. (Found: C, 65.2; H, 6.4; OMe, 28.2; mol.wt., 1774 $\pm$ 66. $[C_{15}H_{11}O_6(OMe)_4]_5$ requires C, 65.1; H, 6.1; OMe, 28.0%; mol.wt., 1660).

Acetylated 'pentameric' tannin. The tannin (300 mg.) was acetylated with acetic anhydride-pyridine (cf. Roux &

Table 2. Estimated yields of fisetinidin chloride from monomeric and polymeric leuco-fisetinidins

Compound	Estimated percentage yield of fisetinidin chloride	Average of four determinations
(+)-7,3',4'-Trihydroxyflavan-3,4-diol	22.9-24.0	23.52
Trimeric tannin	6.5-7.1	6.75
'Pentameric' tannin	4.5-5.1	4.78
'Decameric' tannin	4.8-5.2	4.95

Paulus, 1961c) and the mixture was poured into water. The buff precipitate was dissolved in acetic acid, filtered and poured into water. The product was dried at 110° for 2 hr. (Found: C, 60.7; H, 4.6; $CO \cdot CH_3$, 37.5; mol.wt., 2484 $\pm$ 48. $[C_{15}H_{10}O_6(CO \cdot CH_3)_4]_5$ requires C, 60.2; H, 4.8; $CO \cdot CH_3$, 37.6%; mol.wt., 2290).

Oxidation of methylated derivative. The methylated tannin (1 g.) in acetone-water (1:1, v/v) (20 ml.) was oxidized with $KMnO_4$ (cf. Roux, 1952). The MnO_2 formed was removed and the clear solution acidified slightly after concentration under vacuum. The acid solution was treated with sodium hydrogen carbonate and extracted with ether to remove non-acidic material. The acids were extracted with ether after reacidification of the solution. The extractives were examined by paper chromatography on Whatman no. 1 paper with butan-1-ol-morpholine-water (5:1:4, by vol.) and with Rhodamine B as spray reagent (Freudenberg & Chen, 1960). Veratric acid (R_f 0.43) was the only acid detected in significant amounts, and *m*-hemipinic acid (R_f 0.18) was absent. The solid extractives were dissolved in water (charcoal), from which white globules of veratric acid (28 mg.) separated, m.p. and mixed m.p. with authentic veratric acid 179-181° with sublimation commencing at 175°. The odour of aliphatic acids was noticeable after initial acidification.

'Decameric' leuco-fisetinidin tannin. The contents of tubes 1-15 (lower phase) were separated by chromatography as for the 'leuco-fisetinidin 3'. Material remaining on the starting-line was retained in each separation and was dissolved in methanol and filtered. The solids recovered (2.8 g.) were dried at 110° for 2 hr. (Found: C, 62.3; 61.9; H, 5.2, 5.2; ash, 0; $C_{15}H_{14}O_6$ requires C, 62.1; H, 4.9%). The ultraviolet-absorption spectrum (Table 1) was similar to, and the infrared absorption curve over the range 2.5-15 μ was identical with, those of the pentameric and trimeric tannins present. The compound gave the same colour reactions as the other leuco-fisetinidins, and the yield of fisetinidin chloride (Table 2) was the same as for

the pentamer. The substance was soluble in methanol and acetone-water (1:1, v/v), slightly soluble in ethanol and sparingly soluble in water, the soluble portion showing tanning properties. Alkali-fusion under anhydrous conditions (Roux, 1958) gave resorcinol, β -resorcylic acid and protocatechuic acid as the main degradation products. Traces of phloroglucinol were also formed. Treatment with 0.1 N-acetic acid produced no change, as with the pentamer.

Derivatives were formed as for the pentameric tannin.

Methylated 'decameric' tannin. Yield, 240 mg. from 300 mg. of tannin. The amorphous white product did not crystallize from ethanol-water or aqueous acetic acid, but settled out as a white sludge. The compound was dried at 110° for 2 hr. (Found: C, 64.8; H, 6.1; OMe, 30.9, 30.6; mol.wt., 3618 ± 81 . $[\text{C}_{15}\text{H}_{11}\text{O}_3(\text{OCH}_3)_2]_{10}$ requires C, 65.1; H, 6.1; OMe, 28.0%; mol.wt., 3320). $[(\text{C}_{15}\text{H}_{11}\text{O}_3(\text{OMe})_2)_{11}]$ requires mol.wt., 3652.) The infrared-absorption curve over the range 2.5–15 μ showed the absence of significant carbonyl absorption, and was identical with those of the methylated trimeric and pentameric derivatives. Degradation of the methylated product gave veratric acid only as for the pentamer.

Acetylated 'decameric' tannin. Yield, 320 mg. of pale-buff amorphous product from 300 mg. of tannin. The compound did not crystallize from ethanol, methanol or acetic acid-water mixtures. The substance was dried at 110° for 2 hr. (Found: C, 60.6; H, 4.9; $\text{CO} \cdot \text{CH}_3$, 36.0; mol.wt., 4366 $\pm$ 128. $[\text{C}_{15}\text{H}_{14}\text{O}_6(\text{CO} \cdot \text{CH}_3)_4]_{10}$ requires C, 60.2; H, 4.8; $\text{CO} \cdot \text{CH}_3$, 37.6%; mol.wt. 4580). $[(\text{C}_{15}\text{H}_{14}\text{O}_6(\text{CO} \cdot \text{CH}_3)_4)_9]$ requires mol.wt., 4122.)

Infrared-absorption curves of acetates

The acetates of (+)-7,3',4'-trihydroxyflavan-3,4-diol (penta-acetate), trimeric, pentameric and 'decameric' tannins (tetra-acetates per C_{15} unit) were dried at 110° for 2 hr. and disks made with dried anhydrous KBr (0.6 mg./250 mg. of KBr) for infrared examination. All the curves showed low peaks of the same absorption density at 3500 cm^{-1} , where absorption due to valence-stretching vibration of an unbonded hydroxyl group is expected.

DISCUSSION

Heartwood extracts from the black-wattle tree contain a number of leuco-fisetinidins in addition to the flavonoid compounds already described (Roux & Paulus, 1960, 1961*a, b, c*). Two of these, the unstable 'leuco-fisetinidin 2' and (+)-7,3',4'-trihydroxyflavan-3,4-diol, have no tanning properties, but the remainder (leuco-fisetinidin tannins 3–6, Fig. 1) give positive reactions with the gelatin-salt reagent (cf. Atkin & Thompson, 1937).

The trimeric leuco-fisetinidin tannin is optically active and has been examined previously (Roux & Paulus, 1961*c*). Two tannins of lower R_f in both solvent systems (5 and 6, Fig. 1) have progressively higher molecular weights and fall in a range (M , 1000–3000) where accurate estimations of molecular weight are difficult. Roux (1953) recommended the use of tannin derivatives in non-polar solvents to avoid deviations from ideality, and

Evelyn (1954) showed the ebulliometric method, with a modified Ray apparatus, to be the most satisfactory. With methylated and acetylated derivatives, the tannin designated as 'pentameric' has been found to approximate more closely to a pentameric than a hexameric tannin. The acetylated derivative of the 'decameric' tannin has a molecular weight slightly less than required, and the methylated derivative shows a value close to an undecamer. The limitations of the method prevent greater accuracy for the 'decameric' tannin, and this substance, represented as a spot at the origin (Fig. 1), may not necessarily be a homogeneous polymer.

The monomer, trimer, 'pentamer' and 'decamer' show a progressive decrease in R_f with increase of molecular weight in both solvent systems on two-dimensional paper chromatograms (Fig. 1). They all have similar absorption maxima at 281–282 $m\mu$, but the molar extinction coefficient, when calculated to a $\text{C}_{15}\text{H}_{14}\text{O}_6$ basis, decreases progressively with increasing molecular weight (Table 1). The minimum at 252 $m\mu$ shifts progressively to 260 $m\mu$ with increasing molecular weight, and also becomes less pronounced (Table 1). The yield of fisetinidin chloride which may be generated from these tannins decreases progressively with increase of molecular weight over the low range (monomer to 'pentamer') but reaches a minimum value at the 'pentamer' (Table 2).

The infrared-absorption curves over the range 2.5–15 μ of the trimeric, 'pentameric' and 'decameric' tannins and of their acetyl and methoxyl derivatives are almost identical with and similar to those of the flavan-3,4-diol. The 'pentameric' and 'decameric' tannins have the same analysis as the trimer (Roux & Paulus, 1961*c*) and as the flavan-3,4-diol. On a C_{15} basis all form trimethoxyl derivatives, but tannins show the substitution of one hydroxyl group less (four acetyl groups) than the flavan-3,4-diol (five acetyl groups) after acetylation. Infrared-absorption curves of these acetates, although not conclusive, indicate that free hydroxyl groups may be absent from the acetates of the polymeric tannins.

The absence of *m*-hemipinic acid from the oxidation products of the methylated 'pentameric' and 'decameric' tannins suggests that these tannins of biochemical origin differ from the oxidation polymers of (+)-catechin (compare Hathway, 1958). Furthermore, the single absorption maximum of the leuco-fisetinidin tannins at 281–282 $m\mu$ differentiates them from the oxidation polymers of catechins, which exhibit two maxima at 280 and 410–430 $m\mu$ (Hathway & Seakins, 1957). The mode of condensation of the flavonoid units in the polymeric leuco-fisetinidin tannins therefore does not apparently involve C·C links of the type suggested

by Hathway (1958) for the oxidation polymers of catechins, but the partial hydrolysis of the trimer to a flavan-3,4-diol, and the apparent elimination of one aliphatic hydroxyl group in the polymers compared with the monomer, indicate the possibility of some 4-ether linkages. More than one type of linkage appears possible in these condensed leuco-fisetinidin tannins of biological origin, a proportion of the links being labile under acid conditions when the compounds are highly dispersed in 3*N*-hydrochloric acid-propan-2-ol (1:4) mixtures under conditions developed by Pigman *et al.* (1953).

SUMMARY

1. Tannins approximating in molecular weight to pentameric and decameric leuco-fisetinidins have been isolated from the heartwood extractives of *A. mearnsii*. Their properties are compared with trimeric (–)-leuco-fisetinidin tannin and (+)-7,3',4'-trihydroxyflavan-3,4-diol, which accompany them in the heartwood.

2. The trimeric tannin undergoes partial hydrolysis to the flavan-3,4-diol (chromatographic evidence).

3. These natural tannins are distinct from the oxidation polymers of flavan-3-ols (catechins), and

available evidence points to their being polymers of the flavan-3,4-diol.

This work was financed by the annual grant of the South African Wattle Growers' Union to the Leather Industries Research Institute.

REFERENCES

- Atkin, W. R. & Thompson, F. C. (1937). *Procter's Leather Chemists' Pocket Book*, p. 136. London: E. and F. N. Spon Ltd.
- Evelyn, S. R. (1954). *J. Soc. Leath. Tr. Chem.* **38**, 142.
- Evelyn, S. R., Cooper, D. R. & van Berge, P. C. (1958). *J. Polym. Sci.* **33**, 53.
- Freudenberg, K. & Chen, C.-L. (1960). *Ber. dtsch. chem. Ges.* **93**, 2533.
- Hathway, D. E. (1958). *J. chem. Soc.* p. 520.
- Hathway, D. E. & Seakins, J. W. T. (1957). *J. chem. Soc.* p. 1562.
- Pigman, W., Anderson, E., Fischer, R., Buchanan, M. A. & Browning, B. L. (1953). *T.A.P.P.I.* **36**, 4.
- Roux, D. G. (1952). *J. Soc. Leath. Tr. Chem.* **36**, 393.
- Roux, D. G. (1953). *J. Soc. Leath. Tr. Chem.* **37**, 259.
- Roux, D. G. (1957). *Nature, Lond.*, **179**, 305.
- Roux, D. G. (1958). *J. Amer. Leath. Chem. Ass.* **53**, 384.
- Roux, D. G. & Maihs, A. E. (1960). *J. Chromat.* **4**, 65.
- Roux, D. G. & Paulus, E. (1960). *Biochem. J.* **77**, 315.
- Roux, D. G. & Paulus, E. (1961*a*). *Biochem. J.* **78**, 120.
- Roux, D. G. & Paulus, E. (1961*b*). *Biochem. J.* **80**, 62.
- Roux, D. G. & Paulus, E. (1961*c*). *Biochem. J.* **80**, 476.

Biochem. J. (1962) **82**, 324

Condensed Tannins

13. INTERRELATIONSHIPS OF FLAVONOID COMPONENTS FROM THE HEARTWOOD OF *ROBINIA PSEUDACACIA*

BY D. G. ROUX AND E. PAULUS

Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(Received 26 June 1961)

The wood of the locust tree (*Robinia pseudacacia* L.) consists mainly of yellow-brown heartwood with a narrow margin of white sapwood, the heartwood being well known for its durability and resistance to insects. The heartwood was shown to contain 7,3',4',5'-tetrahydroxyflavonol (robinetin), by Schmid & Pietsch (1931), Brass & Kranz (1932) and Schmid & Tadros (1932), and also the corresponding flavanonol, (+)-dihydorobinetin, by Freudenberg & Hartmann (1954). (+)-Dihydorobinetin apparently occurs mainly in partially racemized form, and Freudenberg & Roux (1954) converted it by catalytic hydrogenation into (±)-7,3',4',5'-tetrahydroxyflavan-3,4-diol.

Weinges (1958) briefly noted the presence of (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol in *R. pseudacacia* heartwood but cited no details other than its optical rotation. He demonstrated, however, the stereochemical identity of (+)-dihydorobinetin and (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol at C-2 and C-3 by means of the same interconversion. The flavan-3,4-diol was converted into robinetinidin chloride (Freudenberg & Roux, 1954; Roux & Freudenberg, 1958), and the flavan-3,4-diol may accordingly be regarded as a monomeric leuco-robinetinidin.

The heartwood of *R. pseudacacia* is examined in detail in the present work as in the bark tannins of