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Condensed Tannins

11. ISOLATION OF A CONDENSED TANNIN FROM BLACK-WATTLE HEARTWOOD, AND SYNTHESIS OF $(\pm)$ -7:3':4'-TRIHYROXYFLAVAN-4-OL*

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Bate-Smith & Swain (1953) suggested that leuco-anthocyanins, which are closely related to the catechins, should be regarded as prototypes of condensed tannins. In this connexion, Hillis (1954, 1956, 1958) has investigated the extent to which leuco-anthocyanins might be precursors of condensed tannins, and might be responsible for the red colour which develops when leather that has been tanned with mangrove and various *Eucalyptus* extracts is exposed to sunlight.

Robinson & Robinson (1935) isolated the first crystalline compound related to the flavan-3:4-diols, peltogynol, and thereafter King & Bottomley (1954) isolated the first member of the structurally simpler and more general series of crystalline flavan-3:4-diols. The progress of condensation of such compounds in the wood was examined (Roux & Evelyn, 1958*a, b*, 1960) by anthocyanidin formation, by number-average molecular weights and by tannin analyses on radial and vertical drillings. These methods give some evidence for the presence

of intact flavan-3:4-diol units in polyleuco-anthocyanins, which may be regarded as one class of condensed tannins. The association of authenticated monomeric flavan-3:4-diols and the corresponding polyleuco-anthocyanin tannins, which Roux (1958*a, b*) demonstrated for the heartwoods of certain *Acacia* and *Schinopsis* spp., is similar to the association of leuco-anthocyanins and complex leuco-anthocyanins in cacao beans (Forsyth, 1952, 1955). On account of the isolation and chemical degradation of a complex leuco-cyanidin from cacao (Forsyth, 1953; Forsyth & Roberts, 1960), the isolation of other polyleuco-anthocyanins that may have tanning properties is of scientific importance. The isolation of one of these polymeric tannins from *A. mearnsii* is described, and certain of its properties are compared with those of synthetic $(\pm)$ -7:3':4'-trihydroxyflavan-4-ol.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Analysis of C, H, acetyl groups, methoxyl groups and ash are by Weiler and Strauss, Oxford, and by K. Jones, Microanalytical Section,

* Part 10: Roux & Paulus (1961*c*).

National Chemical Research Laboratory, C.S.I.R., Pretoria. Infrared comparisons are by Dr J. R. Nunn of the same laboratories. Molecular weights are by A. E. Maihs of these laboratories. A 2 dm. tube was used for optical rotations. Two-way chromatograms were developed in water-saturated butan-1-ol followed by 2% (v/v) acetic acid (solvent combination 1), or in water-saturated butan-2-ol followed by 2% (v/v) acetic acid (solvent combination 2).

Isolation of a (-)-leuco-fisetinidin tannin from the heartwood of Acacia mearnsii

Separation of the tannin. In addition to flavonoid compounds possessing high R_F values (Roux & Paulus, 1960, 1961*a, c*), the heartwoods of freshly felled trees contain a prominent component in the middle R_F range (0.47 and 0.25 in water-saturated butan-1-ol and 2%, v/v, acetic acid respectively). This compound showed tanning properties. The dried, wax-free ethyl acetate extract (75 g.) of black-wattle heartwood obtained as described by Roux & Paulus (1960) was added slowly with shaking to 400 ml. of the lower phase of a water-butan-2-ol-petroleum (b.p. 45–60°) (5:3:2) mixture. The solution was warmed to 60°, then cooled to room temperature and filtered. The filtrate was introduced into the first eight tubes of a 160 tube, 50 ml. underphase, automatic Craig countercurrent machine, and the mixture separated by 160 transfers as described by Roux & Paulus (1960). The tannin component was distributed in tubes 31–61, but in tubes 31–40 tannin of lower R_F value was present. The contents of tubes 41–61 were combined, the organic phase was separated, and the aqueous phase extracted with ethyl acetate. The combined organic phase and ethyl acetate extracts were reduced to small volume (200 ml.) under vacuum, and applied as bands to 40 sheets of Whatman no. 3 chromatographic paper (22½ in. × 18½ in.). Chromatograms were developed by upward migration of 2% (v/v) acetic acid. The tannin band was located with toluene-*p*-sulphonic acid (Roux, 1957*b*) and ammoniacal silver nitrate spray reagents on test strips. Bands were removed and eluted with 70% (v/v) ethanol. The solid (0.7 g.) obtained on evaporation was pale brown.

Two-way paper chromatography showed traces of impurities accompanying the tannin. These were separated on paper chromatograms as above with butan-1-ol-acetic acid-water (6:1:2) (downward) followed by 2% (v/v) acetic acid (upward) for successive separations on different sheets. The buff-coloured product (0.4 g.) appeared free of associated compounds by two-way paper chromatography with solvent combinations 1 and 2.

The tannin settled from aqueous solution as a white sludge and did not crystallize from dilute ethanol, methanol or acetic acid. The tannin migrated as a discrete spot in 2% (v/v) acetic acid and in water-saturated butan-2-ol. In water-saturated butan-1-ol and butan-1-ol-acetic acid-water (4:1:5, upper phase) the tannin moved as an elongated oval spot, although it had been twice separated on paper sheets in butan-1-ol-acetic acid-water (6:1:2). The elongated oval spot was coloured uniformly when sprayed with ammoniacal silver nitrate (grey), bisdiazotized benzidine (yellow), toluene-*p*-sulphonic acid (pink) and ferric alum (green) reagents.

The tannin gave an immediate precipitate with the gelatin-salt reagent (Atkin & Thompson, 1939; Thomas &

Frieden, 1923), indicating strong tanning properties. The monomeric (+)-7:3':4'-trihydroxyflavan-3:4-diol from wattle heartwood gives no precipitate.

The tannin, dried at 110° for 2 hr., had $[\alpha]_D^{20} -32.8 \pm 0.4^\circ$ in methanol (*c*, 0.8), and $-33.3 \pm 0.5^\circ$ in acetone-water (1:1) (*c*, 0.6); λ_{max} , 205 and 280.5 m μ in water and 283 m μ in ethanol; $E_{1\text{cm}}^{1\%}$, 190.8, 193.1 in ethanol [$E_{1\text{cm}}^{1\%}$ for (+)-mollisacacin, 223.7, 222.9] (Found: C, 61.3, 61.2, 62.2; H, 5.4, 5.4, 5.8; ash, 0.0. $\text{C}_{15}\text{H}_{14}\text{O}_6$ requires C, 62.1; H, 4.9%). The infrared-absorption curve over the range 2.5–15 μ was similar to that of (+)-7:3':4'-trihydroxyflavan-3:4-diol, and showed the absence of significant carbonyl-group absorption.

The tannin gave resorcinol, β -resorcylic acid and protocatechuic acid on microfusion with KOH under anhydrous conditions (Roux, 1958*c*). Traces of phloroglucinol (purple with bisdiazotized benzidine) were also detected, but other resorcinol-containing flavans, e.g. (-)-robinetinidol and (+)-7:3':4'-trihydroxyflavan-3:4-diol also yield traces of phloroglucinol. The colour reactions of the tannin with the spray reagents was consistent with the presence of resorcinol and catechol nuclei only, and the pink with toluene-*p*-sulphonic acid suggested a 3:4-diol grouping (cf. Roux & Maihs, 1960). A test for *p*-hydroxybenzyl alcohol groups with quinoxinemonochloroimide (Gierer, 1954) was inconclusive.

A deep-pink colour was produced when the tannin (1 mg.) was heated in 3*N*-HCl (1 ml.) and propan-2-ol (4 ml.) solution under pressure at 100° (Pigman, Anderson, Fischer, Buchanan & Browning, 1953). The pink was shown to be due entirely to fisetinidin chloride, by paper-chromatographic comparison with synthetic material (Leon & Robinson, 1931; Freudenberg & Maitland, 1934); R_F 0.43 in 90% formic acid-3*N*-HCl (1:1) and 0.73 in water-acetic acid-10*N*-HCl (10:30:3); λ_{max} , 523 m μ shifted to 540 m μ (blue) after forming a complex with $\text{Al}_2(\text{SO}_4)_3$ (cf. Roux, 1957*a*). Both the tannin and (+)-7:3':4'-trihydroxyflavan-3:4-diol gave increasing yields of fisetinidin up to 90 min. (maximum yield) under the above conditions, the concentration of fisetinidin declining gradually if the tubes were heated for longer periods. From ϵ_{max} values of fisetinidin chloride calculated from data of Roux & Evelyn (1958*a*) the yield from the tannin was 6.9–7.3%, compared with 22.9–24.0% from (+)-7:3':4'-trihydroxyflavan-3:4-diol under the same conditions.

The tannin reddened rapidly in solution or in the solid form when exposed to sunlight. It was readily soluble in methanol and acetone, and moderately in ethanol and water.

Methoxyl derivative of (-)-leuco-fisetinidin tannin. Tannin (500 mg.) in methanol (100 ml.) was methylated with excess of ethereal diazomethane at -10° for 24 hr. The solution was reduced to small volume (2–3 ml.), under vacuum, treated with charcoal and filtered. The filtrate poured into water gave a white amorphous powder that did not crystallize from ethanol or methanol. The product (400 mg.) dried at 110° for 2 hr. had $[\alpha]_D^{21} -30.2 \pm 0.2^\circ$ in acetone (*c*, 0.9) (Found: C, 66.9; H, 6.2; OMe, 28.5. $\text{C}_{15}\text{H}_{11}\text{O}_3(\text{OMe})_3$ requires C, 65.1; H, 6.1; OMe, 28.0%).

Heating the methylated tannin in 3*N*-HCl-propan-2-ol (1:4) under pressure gave a low yield of a red anthocyanidin (blue with ammonia), R_F 0.68 in 3*N*-HCl-90% formic acid (2:1) and 0.95 in the (1:1) mixture. An anthocyanidin with similar R_F values is formed from (+)-7:3':4'-trimethoxyflavan-3:4-diol.

A separate sample was methylated under identical conditions (Found: C, 66.5; H, 6.3; OMe, 29.4%), and the molecular weight, determined in acetone (three estimations) by the Ray (1952) ebulliometric method as modified by Evelyn (1954) and Evelyn, Cooper & van Berge (1954), was 1087 ± 23 ; $[C_{15}H_{11}O_3(OMe)_3]_3$ requires 996.

The infrared-absorption curve of the methylated tannin was similar to that of (+)-7:3':4'-trimethoxyflavan-3:4-diol over the range 2.5–15 μ and reaffirmed the absence of carbonyl groups in the tannin.

Acetyl derivative of (-)-leuco-fisetinidin tannin. Tannin (400 mg.) was acetylated with acetic anhydride (2.0 ml.) and pyridine (1.6 ml.) for 6 hr. at room temperature and poured into water. The white amorphous product (450 mg.) was redissolved in acetic acid and filtered into water. The flocculent product dried at 50° for 8 hr. over P_2O_5 had $[\alpha]_D^{21} + 8.3 \pm 0.2^\circ$ in tetrachloroethane (c, 1.2) (Found: C, 60.5; H, 4.6; $CO \cdot CH_3$, by acid hydrolysis, 37.8, 37.0%; mol.wt., 1539 ± 16 . $[C_{15}H_{10}O_2(CO \cdot CH_3)_4]_3$ requires C, 60.2; H, 4.8; $CO \cdot CH_3$, 37.6%; mol.wt. 1374). The infrared-absorption curve (KBr) over the range 2.5–15 μ was similar to that of penta-acetyl-(+)-mollisacacidin.

Acetylation of methylated (-)-leuco-fisetinidin tannin. Tannin (100 mg.) previously methylated with diazomethane (28.5% of methoxyl groups) was treated with pyridine (0.4 ml.) and acetic anhydride (0.5 ml.) at room temperature for 6 hr. The product (90 mg.) was isolated by the method used for acetylated (-)-leuco-fisetinidin tannin, dried at 110° for 2 hr. and had $[\alpha]_D^{21} + 12.2 \pm 0.8$ in acetone (c, 0.8) (Found: C, 65.0, 65.4; H, 6.0, 6.2; $CO \cdot CH_3$, 6.1; OMe, 20.6. $C_{30}H_{22}O_7$ requires C, 64.2; H, 5.9; $CO \cdot CH_3$, 11.5; OMe, 24.9%). Acetylation under the same conditions for only 2 hr. gave a product of lower acetyl-group but correspondingly higher methoxyl-group content (Found: $CO \cdot CH_3$, 2.3; OMe, 27.7%). The infrared-absorption curve (6.1% of acetyl groups) was almost identical with that of the methylated tannin except for additional peaks at 1725, 1360 and 1230 cm^{-1} associated with carbonyl and acetate groups.

Hydrogenation of (-)-leuco-fisetinidin tannin. The tannin was unchanged when treated with hydrogen in the presence of palladium catalyst under conditions (Weinges, 1958) used by Roux & Paulus (1961a) to convert (+)-7:3':4'-trihydroxyflavan-3:4-diol into (-)-7:3':4'-trihydroxyflavan-3-ol [(+)-fisetinidol].

Distribution of (-)-leuco-fisetinidin tannin in wattle heartwood

Examination of the heartwoods of a 50- to 60-year old freshly felled specimen of *A. mearnsii* by radial sampling and two-way paper chromatography (solvent combination 1) showed that the tannin was formed in the peripheral heartwood and was present throughout the heartwood. The tannin disappears slowly during aging of the wood after felling, and is absent from wood kept for 2–3 years, probably owing to atmospheric oxidation.

Synthesis of (±)-7:3':4'-trihydroxyflavan-4-ol

(±)-Butin (500 mg.), synthesized from resacetophenone and protocatechuic aldehyde via the cyclization of butein (Saiyad, Nadkarni & Wheeler, 1937), was hydrogenated with platinum oxide (300 mg.) in methanol for 6 hr.; 105.5 ml. of H_2 was absorbed. A paper chromatogram of

the methanolic solution showed the presence of a single substance of higher R_F (0.30) than (±)-butin (0.22) in 2% (v/v) acetic acid, and of lower R_F (0.83) than (±)-butin (0.92) in water-saturated butan-2-ol.

The methanol was removed under vacuum in a rotary evaporator on a water bath at 40–50°, and the viscous residue dissolved in 2–3 ml. of cold water and filtered. Crystals (200 mg.) deposited slowly from the filtrate when kept at 0° for 2–3 days. After drying over P_2O_5 under vacuum these melted at 94–96° with reddening, and the red melt decomposed sharply at 118–120°. Recrystallization from water does not alter either the m.p. or decomposition point (Found: C, 59.2; H, 6.2; loss at 80°, 8.5. $C_{15}H_{14}O_6$, 1.5 H_2O requires C, 59.8; H, 5.7; H_2O , 9.0%). After drying at 80° over P_2O_5 under vacuum (Found: C, 65.2; H, 5.5. $C_{15}H_{14}O_5$ requires C, 65.7; H, 5.2%).

The colours given with spray reagents were: bisdiazotized benzidine, yellow; ferric alum, olive green; silver nitrate, grey-black; toluene-*p*-sulphonic acid, deep blue fading to pale pink. The flavan-4-ol reddened on heating, on exposure to sunlight in the solid state or in solution, and on the addition of warm dilute HCl.

An intense blue-purple was produced after brief heating of the flavan-4-ol with 3*N*-HCl-propan-2-ol (1:4) under pressure at 100°. The colour persisted if the mixture was cooled immediately, but faded rapidly on continued heating.

(±)-7:3':4'-Trimethoxyflavan-4-ol. The hydrogenation product of (±)-butin (500 mg.) in methanol (100 ml.) was methylated overnight with excess of diazomethane in ether at -10°, and the solvents were subsequently removed in a rotary evaporator on a warm (40–50°) water bath. The product crystallized readily from ethanol (4 ml.) at room temperature in clusters of white needles (275 mg.), m.p. 135–137°. Recrystallizing from ethanol increased the m.p. to 139–140° (Found: C, 68.0; H, 6.6; OMe, 29.8. $C_{18}H_{20}O_5$ requires C, 68.3; H, 6.4; OMe, 29.4%). The infrared-absorption curve of (±)-7:3':4'-trimethoxyflavan-4-ol showed strong OH absorption at 3475 cm^{-1} , but no absorption at 1665 and 1345 cm^{-1} frequencies characteristic of the C=O group in trimethyl-(±)-butin. A spot of 7:3':4'-trimethoxyflavan-4-ol placed on paper from methanol solution and sprayed with ethanolic toluene-*p*-sulphonic acid reagent turned deep blue-violet at 60° and red on more prolonged heating at higher temperatures, or on aging at room temperature.

(±)-4-Acetoxy-7:3':4'-trimethoxyflavan. (±)-7:3':4'-Trimethoxyflavan-4-ol (100 mg.) was treated with 0.4 ml. of pyridine and 0.4 ml. of acetic anhydride at room temperature for 5 min. and poured into water. The oily product solidified rapidly and crystallized slowly from ethanol or ethanol-water in fine needles, m.p. 115–118° with sintering at 85–90° (Found: C, 67.0; H, 6.2; $CO \cdot CH_3$, 11.2; OMe, 26.9. $C_{19}H_{22}O_6$ requires 67.0; H, 6.2; $CO \cdot CH_3$, 12.0; OMe, 26.0%).

Comparison of colour and condensation reactions

Spraying chromatograms of the flavan-4-ol, flavan-3:4-diol and tannin with toluene-*p*-sulphonic acid with subsequent heating at 90° caused all the compounds to assume a final pink. For the flavan-4-ol and flavan-3:4-diol, the pink was preceded by a blue, which was very intense with the flavan-4-ol and less pronounced with the flavan-3:4-diol. The flavan-3-ols [(+)- and (-)-fisetinidols] gave no reaction. Toluene-*p*-sulphonic acid reagent gave

Table 1. *Condensation and colour reactions of flavans related to 7:3':4'-trihydroxyflavan-4-ol*

Compound	Period before first appearance of condensation* product	Colour of condensation product	Colour of soln.
(±)-7:3':4'-Trihydroxyflavan-4-ol	Instantaneous	Red-brown	Deep mauve→red
(+)-7:3':4'-Trihydroxyflavan-3:4-diol	30 sec.	Light pink	Deep pink
(-)-7:3':4'-Trihydroxyflavan-3-ol	10 min.	Buff	Colourless
(+)-7:3':4'-Trihydroxyflavan-4-one-3-ol	No product	†	Pale yellow†
(-)-7:3':4'-Trihydroxyflavan-4-one	No product	†	Pale orange-yellow§
(+)-Catechin	5 min.	Buff	Colourless
(+)-7:3':4':5'-Tetrahydroxyflavan-3:4-diol	5-10 sec.	Light pink	Pink

* Where condensation was not immediate the elevated temperature (60°) was maintained to promote the reaction.

† White crystals consisting of unchanged material separate on cooling.

‡ Colour due to conversion into fisetin.

§ Colour due to conversion into butein.

|| Previously shown by Bate-Smith & Swain (1953).

similar colours with the trimethyl ethers of the flavan-4-ol (blue turning to pink) and of flavan-3:4-diol (pink). A positive test is obtained even in the absence of a *p*-hydroxyl group in the 7-position, and therefore the reagent appears specific for the secondary benzyl alcohol (4-hydroxyl) grouping in flavonoids, and indicates that (-)-leuco-fisetinidin tannin contains this grouping.

Solid (±)-7:3':4'-trihydroxyflavan-4-ol turns red rapidly when exposed to sunlight. Such reddening is also shown by (+)- and (-)-7:3':4'-trihydroxyflavan-3:4-diol and by the leuco-fisetinidin tannin, but not by (+)- or (-)-7:3':4'-trihydroxyflavan-3-ol, (+)-fustin or (-)-butin.

The analogues of (±)-7:3':4'-trihydroxyflavan-4-ol when dissolved (3 mg.) in 1 ml. of boiling water and treated immediately with 1 ml. of cold 10N-HCl gave the condensation and colour reactions shown in Table 1. Similar reactions of (+)-catechin and (+)-7:3':4':5'-tetrahydroxyflavan-3:4-diol are included for comparison.

DISCUSSION

The heartwood of *A. mearnsii* contains a number of polymeric tannins of the condensed type. These are already present at the heartwood periphery where the concentration of the monomeric (+)-7:3':4'-trihydroxyflavan-3:4-diol is at a maximum (Roux & Paulus, 1960), but those of very low R_f and high molecular weight increase towards the heartwood centre, corresponding with a decline of the flavan-3:4-diol (Roux & Paulus, 1960; Roux & Evelyn, 1960). This suggests a progressive transformation of the flavan-3:4-diol into condensed tannins of various degrees of condensation. The concentration of other flavonoid compounds present, for example (+)-fustin and (-)-fisetinidol, do not undergo such marked changes (cf. Roux & Paulus, 1960, 1961a). The presence of leuco-fisetinidin tannins in the peripheral heartwood is also found in *Schinopsis* spp. (Roux, 1958b; Roux & Evelyn, 1958b; Roux & Paulus, 1961b), indicating that some of the associated flavan-3:4-diols undergo relatively rapid transformation into tannins [cf. Freudenberg (1960)].

The leuco-fisetinidin tannin isolated in the present study exists in a colourless reduced form in the wood, and shows absorption maxima at 205 and 280 m μ typical of the flavans and their constituent phenols. This differentiates it immediately from the dark oxidation polymers (λ_{max} 280 and 420 m μ) containing repeating quinonoid groups, which were obtained by Hathway & Seakins (1957) and shown to be present in the commercial extracts prepared from *Acacia catechu* heartwood and *Uncaria gambir* leaves.

The tannin isolated shows similarity to (+)-7:3':4'-trihydroxyflavan-3:4-diol in colour reactions, degradation products and ultraviolet- and infrared-absorption curves, but also marked differences. For example, the tannin appears to be trimeric and the yield of fisetinidin chloride obtainable from it approximates to a third of the yield of anthocyanidin from the flavan-3:4-diol. Calculated to a C_{15} basis, the tannin reacts with diazomethane to the equivalent of three methoxyl groups, corresponding to the methylation of all the phenolic hydroxyl groups in 7:3':4'-trihydroxyflavan-3:4-diol. Direct acetylation affords an acetyl derivative containing the exact equivalent of four acetyl groups per C_{15} unit, and presumably represents the substitution of the equivalent of three phenolic and one aliphatic hydroxyl groupings. The tannin differs in this respect from the leuco-cyanidin isolated by Forsyth & Roberts (1960) from cocoa beans, where acetylation and methylation procedures both resulted in the substitution of an equal number of hydroxyl groups. Acetylation of the methylated derivative of the tannin causes demethylation, probably by direct substitution of methoxyl by acetyl groups, but without increase in the total number of hydroxyl groups substituted. This suggests that after methylation the substitution of the total equivalent of four hydroxyl groups, as obtained by direct acetylation, may be hindered owing to steric hindrance of the remaining hydroxyl grouping.

On the assumption that the tannin is a polymer of 7:3':4'-trihydroxyflavan-3:4-diol, it is apparent that the equivalent of at least one aliphatic hydroxyl group per C_{15} unit is involved in direct condensation, or has undergone conversion into a tertiary alcohol as a result of condensation, or alternatively has become sterically hindered following condensation. The high negative rotation ($[\alpha]_D - 33^\circ$) of the tannin in relation to the positive rotation ($[\alpha]_D + 12^\circ$ to $+33^\circ$) of the (+)-7:3':4'-trihydroxyflavan-3:4-diol (cf. Clark-Lewis & Roux, 1959) is not understood at present, but the stereochemistry of the tannin holds future interest in view of the known interrelation between (-)-fisetinidol, (+)-fustin, (-)-butin and (+)-7:3':4'-trihydroxyflavan-3:4-diol (Roux & Paulus, 1960, 1961a, c).

The reactivity of the 4-hydroxyl group in the flavan-3:4-diols has been predicted (Roux, 1959; Freudenberg, 1960) from a knowledge of lignin and lignin model substances, and is confirmed by a study of the properties of polymerization of ($\pm$)-7:3':4'-trihydroxyflavan-4-ol in direct comparison with those of its analogues (Table 1). The ease of polymerization of flavan-4-ols and flavan-3:4-diols does not necessarily implicate only the 4-hydroxy substituent, since flavan-3-ols are shown to condense with relative ease and closely related flavans apparently condense at different rates [compare (+)-7:3':4'-trihydroxyflavan-3:4-diol with (+)-7:3':4':5'-tetrahydroxyflavan-3:4-diol, and (-)-fisetinidol with (+)-catechin]. Nevertheless, the abnormally high reactivity of the flavan-4-ol and flavan-3:4-diol compared with the flavan-3-ol and other analogues (Table 1) emphasizes the high reactivity of the *sec*-(*p*-hydroxybenzyl) alcohol group. This, together with the ease of hydrolysis of the tannin to the anthocyanidin with mineral acid, suggests that the 4-position is at least partly implicated in linking of flavonoid units in the tannin.

The flavan-4-ol also reddens when exposed to direct or indirect sunlight. Such reddening is typical of flavan-3:4-diols and of condensed tannins present in wattle wood and bark (*A. mearnsii* and related spp.), blackwood (*Acacia melanoxydon*), quebracho wood and bark (*Schinopsis* spp.), mangrove bark (*Rhizophora* spp.) and *Eucalyptus* spp. but does not occur with catechins (flavan-3-ols) or with condensed tannins from *A. catechu* heartwood and *U. gambir* leaves. This behaviour suggests that the hydroxyl group in the 4-position is responsible for redness (as distinct from brownness developed on oxidation) inherent in condensed tannins of the leuco-anthocyanidin type.

The synthesis of ($\pm$)-7:3':4'-trihydroxyflavan-4-ol from the corresponding flavan-4-one affords an easy method of obtaining hydroxylated flavan-4-ols. The reduction will, however, be inhibited,

where present, by a 5-hydroxyl group, owing to hydrogen bondage between this and the 4-carbonyl group (cf. Freudenberg & Weinges, 1958). The reduction also has stereochemical aspects and must be considered in conjunction with catalytic reduction of natural ($\pm$)-fustin to ($\pm$)-7:3':4'-trihydroxyflavan-3:4-diol under identical conditions (Roux & Freudenberg, 1958). The 2-phenyl and 3-hydroxyl groups of ($\pm$)-fustin (2:3-*trans*-configuration) may be assigned the 2(*eq*), 3(*eq*) conformation suggested for natural flavanols by Mahesh & Seshardi (1955). The *O*-trimethyl derivative of the reduction product of ($\pm$)-fustin easily forms an *isopropylidene* derivative and the parent flavan-3:4-diol was therefore assigned a 2:3-*trans*-3:4-*cis*-configuration (Clark-Lewis & Roux, 1959). This suggests a 2(*eq*), 3(*eq*), 4(*ax*) conformation for the ($\pm$)-7:3':4'-trihydroxyflavan-3:4-diol (cf. King & Clark-Lewis, 1955; Chandorkar & Kulkarni, 1957), although a 4-hydroxyl group in the equatorial position would be expected from slow reduction under neutral conditions (cf. Barton, 1953) unless it assumed that the 4-carbonyl group is strongly hindered by the α -hydroxyl group in natural fustin. A study of molecular models shows that this hindrance may at most be described as partial.

($\pm$)-Butin [2(*eq*)-phenyl conformation] may similarly afford a ($\pm$)-7:3':4'-trihydroxyflavan-4-ol of 2(*eq*)-phenyl-4(*ax*)-hydroxyl conformation and 2:4-*trans*-configuration from catalytic reduction with platinum oxide in neutral solution, although the alternative of a 4(*eq*)-hydroxyl group cannot be excluded. The former would be related stereochemically to the natural (+)- and (-)-7:3':4'-trihydroxyflavan-3:4-diols, their artificial racemate being identical with the reduction product of ($\pm$)-fustin (Clark-Lewis & Roux, 1959). This is believed to be the first synthesis of a flavan-4-ol containing a number of free phenolic hydroxyl groups, although Freudenberg (1956) cites a 7:4-dihydroxyflavan of which details have not been described.

SUMMARY

1. An optically active condensed tannin has been isolated from the heartwood of black wattle. The tannin resembles the accompanying (+)-7:3':4'-trihydroxyflavan-3:4-diol in most chemical and physical properties, but is trimeric on a C_{15} basis.

2. Evidence available suggests that the tannin is a polymer of 7:3':4'-trihydroxyflavan-3:4-diol condensed possibly through the heterocyclic ring system.

3. ($\pm$)-7:3':4'-Trihydroxyflavan-4-ol has been synthesized from ($\pm$)-butin by catalytic hydrogenation. The stereochemical significance of the reduction is discussed.

4. The properties of the flavan-4-ol in comparison with those of the flavan-3-ol suggest that the hydroxyl group in position 4 might be involved in the biochemical condensation of flavan-3:4-diols, and also in the redness developed in these compounds and in associated or related condensed tannins.

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Diphosphopyridine Nucleotide Pyrophosphorylase in the Nuclei Isolated from Poisoned and Regenerating Rat Liver

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The stimulus for this work was a paper by Morton (1958) in which it was suggested that, since diphosphopyridine nucleotide is required for more enzymic reactions than any other known coenzyme, alteration of its rate of synthesis could modify cell division and differentiation and perhaps lead to

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malignant growth. An important enzyme in its synthesis is diphosphopyridine nucleotide pyrophosphorylase, which is located exclusively in the nuclei (Hogeboom & Schneider, 1952). Low activities of this enzyme were obtained in the nuclei from spontaneous mouse-mammary-gland carcinoma, hepatomas after feeding aminoazobenzene, Ehrlich ascites cells, post-operative human tumours and foetal mouse liver (Morton, 1958; Branster & Morton, 1956). These findings were correlated with

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