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 3N-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.

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Condensed Tannins

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

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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, (+)-dihydrorobinetin, by Freudenberg & Hartmann (1954). (+)-Dihydrorobinetin 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 (+)-dihydrorobinetin 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

Acacia mearnsii (black wattle) polymeric 'leucorobinetinidins' predominate (Roux, 1957*b*; Roux & Evelyn, 1958).

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points are on molecular mixtures of substances (Roux & Maihs, 1960*a*). Analyses of C, H, 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 Laboratory. Unless otherwise stated, two-dimensional chromatograms were run with water-saturated butan-1-ol and then 2% (v/v) acetic acid on Whatman no. 1 paper.

Separation and isolation of components

The trunk of a 28-year-old specimen of *R. pseudacacia* was cut into sections 6 in. thick, and the yellow-brown heartwood contained within the central annual rings 1–22 was removed by drilling. The drillings (4·5 kg.) were extracted with absolute methanol at room temperature, yielding 150 g. (2 days), 43 g. (3 days) and 38·5 g. (14 days) of solids from successive extractions over the periods indicated. The combined solids were extracted three times with light petroleum (b.p. 60–80°) in a Soxhlet apparatus, the solids being redissolved in methanol and finally recovered in a vacuum rotary evaporator between each successive extraction. This ensured the complete removal of waxes, which otherwise interfere with the efficiency of the Craig separation procedure. Examination of the wax-free solid showed the distribution of components as in Fig. 1.

The solids (100 g.) were dissolved in 400 ml. of a water–butan-2-ol–light petroleum (b.p. 40–150°) (5:3:2, by vol.) mixture and introduced into the first eight tubes of a 160-tube, 50 ml. underphase, fully automatic Craig machine (Glasapparatebau Göttingen, Helmut Rettberg). The upper and lower phases of the above-described mixture were used for countercurrent separation, and after 160 transfers the upper and lower layers of every fifth tube were examined by two-dimensional paper chromatography. The tubes contained mixtures of compounds and the paper chromatograms were used as a guide in grouping the tubes into fractions, as follows, to enable facile separation of components on paper. The contents were concentrated under vacuum to give the yields of solids indicated: 1–15 (12·5 g.); 16–35 (15·5 g.); 36–64 (24·5 g.); 65–90 (19·0 g.); 91–107 (3·5 g.); 108–123 (1·5 g.); 124–145 (3 g.); 146–160 (2 g.). The fraction from tubes 1–15 was from the lower phase only, the content of the upper phase being negligible, whereas fractions in the range 16–64 were obtained from both phases. The aqueous lower phases in the range 65–160 were extracted four times with ethyl acetate, and the extracts were added to the corresponding upper phases before recovery of the solids. Brown solids (5 g.) precipitated in tubes 1–35 during the course of the Craig separation. These consisted of a tannin mixed with robinetin and traces of fisetin.

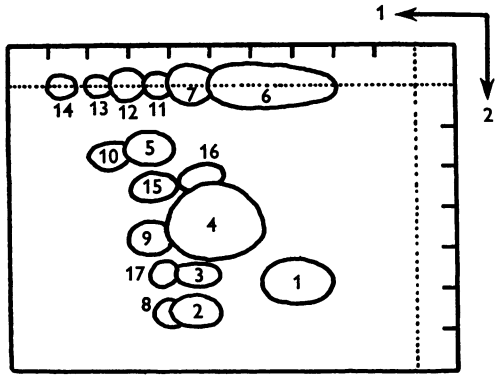


Fig. 1. Chromatographic distribution of the flavonoids present in the methanolic extractives of the heartwood of *Robinia pseudacacia*. Solvents: direction 1, water-saturated butan-1-ol; direction 2, 2% (v/v) acetic acid. 1, (+)-7,3′,4′,5′-Tetrahydroxyflavan-3,4-diol; 2, leuco-robinetinidin; 3, (−)-robinetinidol; 4, (+)-dihydrorobinetin; 5, (−)-robtin; 6, robinetin; 7, robtein; 8, (+)-7,3′,4′-trihydroxyflavan-3,4-diol; 9, (+)-fustin; 10, (−)-butin; 11, fisetin; 12, butein; 13, 7,4′-dihydroxyflavonol (?); 14, 2′,4′,4-trihydroxychalkone; 15, 16 and 17, unknown components.

Flavonoid components present in each group of fractions from the Craig machine were separated on Whatman no. 3 filter-paper sheets (22½ in. × 18¼ in.) with 2% (v/v) acetic acid by the methods and spray reagents described by Roux & Paulus (1960, 1961*a*) and Roux & Maihs (1960*b*). Where the components were not obtained in a sufficiently pure form for crystallization, they were redissolved in methanol and applied to prewashed sheets (with water, 24 hr.) of Whatman no. 3 paper, and separated with butan-1-ol–acetic acid–water (6:1:2, by vol.) by the downward method. Alternatively, separation with 2% (v/v) acetic acid was repeated, depending on the location of impurities present as judged by means of two-dimensional paper chromatography. The following components were isolated.

Flavonoids based on resorcinol and pyrogallol

(+)-7,3′,4′,5′-*Tetrahydroxyflavan*-3,4-*diol and comparison with synthetic racemate.* The compound, distributed in tubes 15–50, was separated from the grouped fractions 16–35 and 36–64. The flavan-3,4-diol (2·3 g.) separated from water (charcoal) in fine needles, which were dried over calcium chloride for 3 days (Found: C, 40·6; H, 6·4; loss at 110°, 30·6. $C_{15}H_{14}O_7,7{\cdot}5H_2O$ requires C, 40·8; H, 6·6; H_2O, 30·6%). After drying at 110° for 2 hr., the crystals redden at 150° and collapse at 172–175° into a viscous red mass (Found: C, 58·7; H, 4·6. Calc. for $C_{15}H_{14}O_7$: C, 58·7; H, 4·6%). $[\alpha]_D^{22}$ +33·9±0·3° [*c* in acetone–water (1:1, v/v) 1·2]. $\lambda_{max.}$ 280·5 mμ (ε 3617) in ethanol. The infrared-absorption curves of the substance and of synthetic (±)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol were identical over the range 2·5–15 μ. When heated with 3N-HCl–propan-2-ol (1:4, v/v) under pressure at 100°, robinetinidin chloride (cf. Roux, 1957*a*) was formed in 24·3, 26·2 and 24·7% yield, calculated from extinction values for the anthocyanidin by Roux & Evelyn (1958).

Derivatives were formed by methods already described (Roux & Paulus, 1960; Roux & Freudenberg, 1958; Clark-Lewis & Roux, 1959).

(+)-7,3′,4′,5′-Tetramethoxyflavan-3,4-diol. Fine needles from ethanol–water (1:1, v/v), m.p. 164–166° (Found: C,

63·0; H, 6·2; OCH_3, 34·1. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1; OCH_3, 34·7%). $[\alpha]_D^{22}$ +19·8±0·1° (*c* in *sym*-tetrachloroethane 1·0).

(+)-3,4-Diacetoxy-7,3′,4′,5-tetramethoxyflavan. Needles from ethanol, m.p. 121–122° (Found: OCH_3, 28·0; $CO{\cdot}CH_3$, 20·1. $C_{23}H_{26}O_9$ requires OCH_3, 27·8; $CO{\cdot}CH_3$, 19·4%).

Isopropylidene derivative of (+)-7,3′,4′,5′-tetramethoxyflavan-3,4-diol. Short fine needles from ethanol, m.p. 138–140°. (Found: C, 66·0; H, 6·7. $C_{22}H_{26}O_7$ requires C, 65·6; H, 6·5%). $[\alpha]_D^{20}$ +8·6±0·3° (*c* in *sym*-tetrachloroethane 0·7).

(+)-3,4,8,3′,4′,5′-Hexa-acetylflavan. The flavan-3,4-diol (0·5 g.) was acetylated with acetic anhydride (7 ml.) and sodium acetate (1·5 g.) by keeping the mixture at boiling for 5 min. The product, after pouring into water and hardening, did not crystallize (Found: C, 58·0; H, 4·9; $CO{\cdot}CH_3$, 45·0. $C_{27}H_{26}O_{13}$ requires C, 58·1; H, 4·7; $CO{\cdot}CH_3$, 46·2%). $[\alpha]_D^{21}$ +21·4±0·1° (*c* in *sym*-tetrachloroethane 1·0).

(±)-7,3′,4′,5′-Tetrahydroxyflavan-3,4-diol (Roux & Freudenberg, 1958) has the same m.p. as the (+)-form, and a mixed m.p. showed no depression. $\lambda_{max.}$ 279·5 mμ (ε 3861) in ethanol. The racemate separates into two enantiomers (R_F 0·40 and 0·46) in 2% (v/v) acetic acid on Whatman no. 1 paper (Roux, Maihs & Paulus, 1961*a*), the (+)-form agreeing with the R_F 0·46 component. The racemate and the (+)-form have identical R_F values (0·41) in water-saturated butan-1-ol.

(+)-*Dihydrorobinetin*. The compound was distributed in tubes 50–95 and was isolated from grouped fractions 36–64, 65–90 and 91–107. Needles (20 g.) from water, m.p. 225–226°. The optical rotation of two different samples from the same trunk varied: $[\alpha]_D^{21}$ +10·7±0·2° [*c*, in acetone–water (1:1, v/v) 1·0] and +16·2±0·1° (*c* 0·9). In the same solvent mixture Freudenberg & Hartmann (1954) recorded $[\alpha]_D$ +13·8°, and Weinges (1958) +29° for the pure optical isomer. The methylated derivative had m.p. 166–167°, $[\alpha]_D^{21}$ −21·1±0·4° (*c* in *sym*-tetrachloroethane 0·8).

(−)-*Robinetinidol*. The component (35 mg.) (R_F 0·55) was separated from tubes 36–64 in the pure form, but in tubes 65–90 (36 mg.) was accompanied by a component, R_F 0·63 in water-saturated butan-1-ol (component 17, Fig. 1), which showed carbonyl absorption on infrared-absorption spectra. Needles from water, m.p. 203–205°. Mixed m.p. with (−)-robinetinidol from the bark of *A. mearnsii* (Roux & Maihs, 1960*a*) showed no depression. $[\alpha]_D^{20}$ −9·7±0·1° [*c* in acetone–water (1:1, v/v) 0·6]. The infrared-absorption curves of the substance and (−)-robinetinidol were identical over the range 2·5–15 μ.

(±)-7,3′,4′,5′-*Tetrahydroxyflavanone* (*robtin*). The compound was distributed in tubes 100–140 and isolated from grouped fractions 91–107, 108–123 and 124–145. Thick rhombs (480 mg.) from ethanol–water, m.p. 276° after losing water of crystallization at 162–164°. The substance was dried over $CaCl_2$ (Found: C, 56·4; H, 4·9; loss at 110° for 2 hr. under vacuum, 8·2. $C_{15}H_{12}O_6{\cdot}1{\cdot}5H_2O$ requires C, 57·2; H, 4·8; H_2O, 8·6%). After drying at 110° for 2 hr. (Found: C, 61·5; H, 4·9. $C_{15}H_{12}O_6$ requires C, 62·5; H, 4·2%). $[\alpha]_D^{20}$ −3·1±0·3° (*c* in methanol 1·1). $\lambda_{max.}$ 234, 276 and 311 mμ in ethanol, and 255, 339 mμ in 0·002M-sodium ethoxide. Infrared absorption over the range 2·5–15 μ is similar to that of dihydrorobinetin, except in the frequency range 1000–1100 cm.$^{-1}$, where differences due to secondary hydroxyl absorption may be expected. Carbonyl absorption for robtin, represented by a shoulder at 1640 cm.$^{-1}$ compared with 1665 cm.$^{-1}$ in butin, merges with the first C=C absorption peak at 1600 cm.$^{-1}$

Robtin gave the following colours with the spray reagents (cf. Roux & Maihs, 1960*b*): ferric alum (blue), bis-diazotized benzidine (deep yellow), ammoniacal silver nitrate (black). Reduction with magnesium in ethanolic 3N-HCl solution gave a blue typical of flavanones, after completion of the reduction. Refluxing (50 mg.) for 0·5 hr. with ethanolic 3N-HCl (5 ml.) gave a yellow colour due to the corresponding chalkone, robtein (see below), R_F 0·63 in butan-1-ol–acetic acid–water (6:1:2, by vol.) on Whatman no. 3 paper, $\lambda_{max.}$ 255, 315 (shoulder) and 387 mμ in ethanol, and 308 (shoulder), 349 mμ in 0·002M-sodium ethoxide. Degradation by micro-fusion with KOH under anhydrous conditions (Roux, 1958*a*) gave resorcinol and gallic acid. These reactions indicate that the compound is a flavanone composed of resorcinol and pyrogallol nuclei.

(±)-7,3′,4′,5′-Tetramethoxyflavanone. Robtin (100 mg.) in methanol (50 ml.) was methylated with diazomethane at −5° for 24 hr., and the solution evaporated to dryness in a rotary evaporator. The product (60 mg.) crystallized from methanol in rhombs, m.p. 147–148° (cf. Dean & Nierenstein, 1925) (Found: C, 66·3; H, 6·0; OCH_3, 36·2. Calc. for $C_{19}H_{20}O_6$ C, 66·3; H, 5·9; OCH_3, 36·1%). $[\alpha]_D^{20}$ 0 (*c* in *sym*-tetrachloroethane 0·7).

(±)-7,3′,4′,5′-Tetra-acetylflavanone. Robtin (100 mg.) was acetylated with acetic anhydride (0·3 ml.) and pyridine (0·2 ml.) for 30 min. and the product poured into water. After hardening, the product was crystallized from ethanol, yielding white needles (60 mg.), m.p. 180–182° (Found: C, 60·0; H, 4·2; $CO{\cdot}CH_3$, 36·2. $C_{23}H_{20}O_{10}$ requires C, 60·5; H, 4·4; $CO{\cdot}CH_3$, 37·7%). $[\alpha]_D$ +0·6±0·1° (*c* in *sym*-tetrachloroethane 0·8).

2′,4′,3,4,5-*Pentahydroxychalkone* (*robtein*). The compound was distributed in tubes 125–155. The contents of the grouped fractions 124–145 and 146–160 were applied to pre-washed sheets of Whatman no. 3 paper and separated by downward migration with butan-1-ol–acetic acid–water (6:1:2, by vol.). Three chalkone bands (R_F 0·63, 0·77, 0·89) appearing yellow in visible, and brown under ultraviolet, light were visible. The predominating deep-yellow chalkone band (R_F 0·63) became deep brick-red on exposure to ammonia. It was eluted with 70% (v/v) ethanol and the separation procedure on filter-paper sheets was repeated to remove traces of robinetin and fisetin. The yellow solid obtained was crystallized from ethanol–water. Bright-yellow crystals (400 mg.) separate slowly (2–3 weeks) when kept at 0°. The compound darkens above 280° but does not melt up to 345° (Found: C, 62·8; H, 4·6. $C_{15}H_{12}O_6$ requires C, 62·5; H, 4·2%). $\lambda_{max.}$ 255, 310 (shoulder) and 387 mμ in ethanol, and 308 (shoulder), 347 mμ in 0·002M-sodium ethoxide. Alkali fusion with KOH under anhydrous conditions gave resorcinol, β-resorcylic acid and gallic acid. Robtein is formed from robtin by isomerization under acidic conditions (see above).

2′,4′,3,4,5-Penta-acetylchalkone. Robtein (100 mg.) was acetylated with acetic anhydride (0·3 ml.) and pyridine (0·2 ml.) for 8 hr. at room temperature. The product was poured into water and recrystallized from ethanol after hardening overnight, m.p. 124–126° (Found: C, 60·2; H, 4·5; $CO{\cdot}CH_3$, 42·6. $C_{25}H_{22}O_{11}$ requires C, 60·2; H, 4·4; $CO{\cdot}CH_3$, 43·2%).

Robinetin. The flavonol was present in tubes 10–145, but in high concentration in tubes 1–35, where it was associated

with the tannin fraction and again in tubes 85–145. From the latter source 1·4 g. of yellow crystals were obtained, darkening above 300°. $\lambda_{max.}$ 254, 317 and 370 mμ in ethanol, and 274, 337 and 420 mμ in 0·002 M-sodium ethoxide. The acetate had m.p. 225–226°.

Robinetin prepared from dihydrorobinetin by the method of Pew (1948) had the same ultraviolet spectrum as described above. The acetate, m.p. 226–228°, showed no depression on mixed m.p. with the corresponding compound above.

A 'leuco-robinetinidin' of unknown structure. This compound (300 mg.), R_F 0·61 and 0·54 in water-saturated butan-1-ol and 2% (v/v) acetic acid, was present mainly in tubes 45–60. Separation on filter-paper sheets gave a product which did not crystallize from water and which darkened and changed into robinetin (80 mg.) (acetate, m.p. 224–225°) when kept at 0°. The substance gave 8·9% yield of robinetinidin chloride (cf. Roux, 1957*a*) with 3 N-HCl–propan-2-ol (1:4, v/v), calculated as for (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol.

Flavonoids based on resorcinol and catechol

The identity of these compounds was established by comparison of derivatives, m.p., ultraviolet- and infrared-absorption spectra with specimens previously obtained by the authors (Roux & Paulus, 1960, 1961*c*).

(+)-7,3′,4′-*Trihydroxyflavan*-3,4-*diol.* The compound was present mainly in tubes 55–80, and obtained from grouped fractions 36–64 and 65–90. It could not be completely separated from the accompanying leuco-robinetinidin of unknown structure, and its identity as (+)-7,3′,4′-trihydroxyflavan-3,4-diol is based on chromatographic evidence.

(+)-*Fustin.* Fustin was distributed in tubes 95–120, and was isolated from the grouped fractions 91–107 and 108–123. Yield, 70 mg. $[\alpha]_D^{20}$ +24·3±0·5° [*c*, in acetone–water (1:1, v/v) 0·9]. The tetra-acetate had $[\alpha]_D^{20}$ +23·1±0·2° in *sym.*-tetrachloroethane. These rotations when compared with those of the corresponding optically pure compounds (cf. Roux & Paulus, 1960) show that the fustin consists almost entirely (about 92%) of the (+)-form.

(±)-*Butin.* The compound was present in tubes 125–155, and accordingly isolated from grouped fractions 124–145 and 146–160. Yield, 165 mg. The butin was of low optical activity, $[\alpha]_D^{20}$ −4·2±0·4° (*c*, in methanol 1·1) compared with the presumably optically pure (−)-form (−15·8°) from *Acacia mearnsii* (Roux & Paulus, 1961*c*).

Butein and fisetin. These compounds were both distributed in tubes 145–160 and isolated from the corresponding grouped fraction by means of the same paper-chromatographic separation used for robtein. Yields: butein (70 mg.) and fisetin (10 mg.).

Flavonoids based on resorcinol and phenol

2′,4′,4-*Trihydroxychalkone.* The sheets from which robtein, butein and fisetin were cut showed the presence of a third chalkone (R_F 0·89) in low concentration. The yellow chalkone bands were eluted and the eluates chromatographed again on fresh sheets with the same solvent to eliminate impurities. The process was repeated and the eluates were concentrated under vacuum. The substance had $\lambda_{max.}$ 295 and 375 mμ in ethanol, and 278 and 430 mμ in 0·002 M-sodium ethoxide. On paper chromatograms the substance was brown under ultraviolet light and gave a deep yellow on exposure to ammonia vapour. The lack of reducing power and the regular interval between robtein and butein (ΔR_F 0·14) and between butein and the chalkone (ΔR_F 0·12) suggested that the chalkone was 2′,4′,4-trihydroxychalkone.

Synthetic 2′,4′,4-trihydroxychalkone (Nadkarni & Wheeler, 1938) had $\lambda_{max.}$ 295 (shoulder) and 375 mμ in ethanol, and 278 and 430 mμ in 0·002 M-sodium ethoxide, R_F 0·89, was brown under ultraviolet light and bright yellow on exposure to ammonia.

Traces of a yellow–green fluorescent compound, R_F 0·81 in the same solvent mixture in Whatman no. 3 as used for the separation of robtein, was also evident. The brilliant yellow–green fluorescence, coupled with the presence of robinetin and fisetin, suggests that the substance was 7,4′-dihydroxyflavonol.

Phenolic components of the sapwood

The white sapwood (83 g.) was ground into a powder in a Wiley mill and extracted with methanol. Two-dimensional chromatograms of the wax-free extract (0·3 g.) showed the presence of (+)-dihydrorobinetin and (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol in about the same relative proportions as in the heartwood (Table 1). Robinetin and the unknown leuco-robinetinidin as well as traces of robtin and fisetin were also present, whereas other components were completely absent.

The white sapwood was separated from the yellow heartwood by a thin (1–2 mm. wide) dark-brown layer. This was separated, ground into a powder and extracted with methanol. Two-dimensional chromatograms showed the presence of fustin, butin, 7,3′,4′-trihydroxyflavan-3,4-diol, fisetin and traces of robinetin.

Table 1. *Estimation of flavonoids in the methanolic extracts of the heartwood and white sapwood of* Robinia pseudacacia

Values in parentheses indicate percentages isolated; —, indicates 'not estimated'.

Flavonoid	Concn. (%) In sapwood	Concn. (%) In heartwood
(+)-Dihydrorobinetin	2·3	17·6 (20·0)
Robinetin	0·8	8·0 (1·4*)
(+)-7,3′,4′,5′-Tetrahydroxyflavan-3,4-diol	0·4	6·2 (2·3)
'Leuco-robinetinidin'	0·2†	1·0† (0·3)
Robtin	0·05	1·5 (0·5)
Robtein	Absent	0·9 (0·4)
(+)-7,3′,4′-Trihydroxyflavan-3,4-diol	Absent	—
Fustin	Absent	0·5 (0·07)
Butin	Absent	0·5 (0·16)
Butein	Absent	0·4 (0·07)
Fisetin	Traces	— (0·01)
2′,4′,4-Trihydroxychalkone	Absent	0·01

* Isolated from tubes 85–145 only.

† Values are with reference to (+)-7,3′,4′,5-tetrahydroxyflavan-3,4-diol.

Concentration of components in heartwood and sapwood extracts of Robinia pseudacacia

The concentration of components in methanolic extracts of the heartwood and sapwood was estimated where possible by the chromatographic and densitometric methods of Roux & Maihs (1960*a*, *b*), ammoniacal $AgNO_3$ being used as spray reagent and the compounds which had been isolated as reference standards (Table 1). In some instances, where R_F differences were small (robinetin, fisetin, robtein, butein and 2′,4′,4-trihydroxychalkone), components were estimated by visual comparison.

DISCUSSION

The main group of flavonoid components in methanolic extract of the fresh heartwood of *R. pseudacacia* consists of a mixture of analogues based on resorcinol and pyrogallol nuclei (Table 1), of which (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol and (−)-robinetinidol, partly racemic (+)-dihydrorobinetin and (−)-robtin (flavanone), robtein (chalkone), robinetin and an unstable leucorobinetinidin have been isolated. This association of related components resembles a similar group, based on a combination of resorcinol and catechol nuclei, in the heartwoods of *Acacia mearnsii* and related wattles (Roux & Paulus, 1960, 1961*a*, *c*, *d*, 1962; Roux, Maihs & Paulus, 1961*b*). Furthermore, tannins in each heartwood correspond in their pattern of hydroxylation to the predominant flavan-3,4-diol present, as previously shown by Roux (1958*b*). Wattle heartwoods contain polymeric 'leuco-fisetinidin' tannins, and *Robinia* heartwood polymeric 'leuco-robinetinidin' tannins. Polymeric 'leuco-robinetinidins', also associated with (−)-robinetinidol, are known to be present in the bark of *A. mearnsii* (Roux, 1957*b*), and at present these represent the only known sources of 'leuco-robinetinidins' in Nature.

Smaller amounts of flavonoids based on resorcinol and catechol, (+)-7,3′,4′-trihydroxyflavan-3,4-diol (chromatographic evidence), (+)-fustin (slightly racemized form), (−)-butin (almost completely racemized), butein, and fisetin and traces of flavonoids based on resorcinol and phenol, 2′,4′,4-trihydroxychalkone, and what is considered to be the corresponding flavonol, were also isolated from *R. pseudacacia*.

The resorcinol–pyrogallol analogues are almost the only phenolic components of the white sapwood and no significant amounts of other flavonoid components or tannins are present (Table 1). The resorcinol–catechol analogues appear to originate separately in the very thin layer of 'dark sapwood' at the sapwood–heartwood interface.

(+)-7,3′,4′,5′-Tetramethoxyflavan-3,4-diol forms an isopropylidene derivative with ease. This suggests that the parent compound, with a 2,3-*trans* arrangement (Weinges, 1958), also has a 3,4-*cis* configuration of substituent groups. The alternative, a 2,3-*trans*–3,4-*trans*-configuration, cannot be excluded entirely as in one instance a *trans*-diol has been bridged by this method (cf. Angyal & Macdonald, 1952).

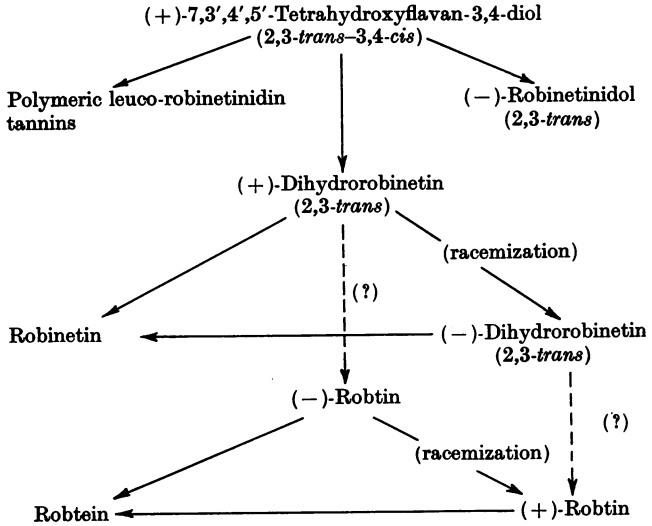
The occurrence of (−)-robinetinidol in low concentation is of interest in view of its stereochemical relationship with the accompanying (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol (Weinges, 1958) as well as its association with polymeric leucorobinetinidin tannins both in the heartwood of *R. pseudacacia* and the bark of *A. mearnsii* (Roux & Maihs, 1960*a*).

The flavanonol (+)-dihydrorobinetin is racemized to varying degrees ($[\alpha]_D + 10{\cdot}7$ and $+16{\cdot}2°$) compared with the optically pure form ($[\alpha]_D + 29°$) isolated by Weinges (1958) presumably from young heartwood. A similar association of optically pure flavan-3,4-diol [(−)-7,3′,4′-trihydroxyflavan-3,4-diol] and partly racemized flavanonol [(−)-fustin] occurs in the heartwood of *Schinopsis* spp. (Roux & Paulus, 1961*b*).

The low rotation ($[\alpha]_D - 3{\cdot}1°$) of the corresponding flavanone, named robtin, suggests that it is partly, if not almost completely, racemized. This conjecture is supported by the similar melting points of the synthetic and therefore racemic 7,3′,4′,5′-tetramethoxyflavanone (Dean & Nierenstein, 1925), and the corresponding derivative of robtin. Robtin is easily isomerized to the corresponding chalkone, robtein, which also occurs naturally in the wood.

An unknown leuco-robinetinidin was isolated. Its similar R_F (0·54) in 2% (v/v) acetic acid compared with (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol (0·46) and other flavan-3,4-diols of this group (cf. Roux *et al.* 1961*a*) suggests that the compound is a flavan-3,4-diol.

The resorcinol–pyrogallol group of flavonoid analogues may originate from a common precursor or from interconversion. The ease of the interconversions: (+)-dihydrorobinetin → (+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol → (−)-robinetinidol (Weinges, 1958), (+)-dihydrorobinetin → robinetin (Freudenberg & Hartmann, 1954), and robtin → robtein, suggests that the complexity of the mixture might at least be due partly to interconversion. On this assumption, the racemic flavanone, robtin, could result from the partially racemized flavanonol, dihydrorobinetin, as the reverse reaction flavanone → flavanonol has been effected *in vitro* by Pacheco (1960). The possible conversion flavanone (robtin) → chalkone (robtein) occurs spontaneously during handling, the facility of the reaction being typical of 5-deoxyflavanones (Seshardi, 1950). Although the partially racemized (+)-dihydrorobinetin is the predominant



Scheme 1. Possible mechanism of transformations of the predominant group of flavonoids in *Robinia pseudacacia* heartwood.

heartwood component, the conversion (+)-dihydrorobinetin → (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol appears unlikely as the natural flavan-3,4-diol is isolated as a pure optical isomer, whereas after racemization of the flavanonol the conversion should result in a racemic flavan-3,4-diol as shown by Freudenberg & Roux (1954). The reverse of this conversion, followed by racemization of the flavanonol, appears more likely. A scheme of interconversion similar to that suggested for the resorcinol–catechol analogues in the heartwood of *A. mearnsii* (Roux & Paulus, 1961*a*) is tentatively proposed (Scheme 1).

SUMMARY

1. Flavonoid components from the heartwood and sapwood of *Robinia pseudacacia* have been isolated and their concentrations have been estimated.

2. The predominant flavonoids are resorcinol–pyrogallol analogues, (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol, (−)-robinetinidol, (+)-dihydrorobinetin (partly racemic), (−)-7,3',4',5'-tetrahydroxyflavanone (robtin) (partly racemic), robinetin, 2',4',3,4,5-pentahydroxychalkone (robtein), an unstable leuco-robinetinidin and polymeric leuco-robinetinidin tannins.

3. (+)-7,3',4',5'-Tetrahydroxyflavan-3,4-diol probably has a 2,3-*trans*–3,4-*cis*-configuration.

4. Resorcinol–catechol analogues, (+)-7,3',4'-trihydroxyflavan-3,4-diol, (+)-fustin (partially racemic), (−)-butin (partly racemic), butein and fisetin, together with traces of 2',4',4-trihydroxychalkone, are also present.

5. A tentative scheme of interconversion of the predominant components is outlined.

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Glucuronide Synthesis in Kidney and Gastrointestinal Tract

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An important site of glucuronide formation is the liver, and in this tissue a major pathway, and the only one so far elucidated, is the enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid to the aglycone (Dutton & Storey, 1954; Storey & Dutton, 1955), which may be one of a wide range of compounds. The enzyme responsible, uridine diphosphate transglucuronylase, has been found in livers of mammals, of birds, of frog and of some fish; it appears to be absent from those of other fish, of tadpoles, aquatic amphibia (Lester & Schmid, 1961), early foetal mammals and, surprisingly, the cat (for review, see Dutton, 1961*a*).

Glucuronide formation has been reported in slices of mammalian kidney (Lipschitz & Bueding, 1939; Karunairatnam, Kerr & Levvy, 1949; Storey, 1950) and of gastrointestinal tract (Zini, 1952; Shirai & Ohkubo, 1954; Hartiala, 1955; Schachter, Kass & Lannon, 1959), and it seemed necessary to investigate this conjugatory mechanism in detail and to determine whether the pathway found in liver functions also in these tissues; evidence of other methods of glucuronide formation, absent or negligible in liver but of possible importance here, might also be forthcoming. Knowledge of the relative significance of such extra-hepatic glucuronide synthesis in the mammalian adult and foetus would be interesting, especially in view of the latter's apparently considerable dependence on maternal tissues for 'detoxication' with glucuronic acid and of recent reports that human foetuses themselves can perform oestrogen glucuronide synthesis (see Diczfalusy, Cassmer, Alonso & de Miquel, 1961*b*); this assessment might throw light on extra-hepatic conjugation in the newborn.

Information on the presence and formation of uridine diphosphate glucuronic acid itself was also desirable; apart from any detoxicatory capacity of the gastrointestinal tract, mucopolysaccharide may be produced by this tissue (Smith & Gallop, 1953; Werner, 1953) and uridine diphosphate glucuronic acid be involved in its synthesis here as it is in other cases (Glaser & Brown, 1955; Markowitz, Cifonelli & Dorfman, 1959).

The present work indicates that, in broken-cell preparations of kidney cortex and gastrointestinal mucosa, glucuronide synthesis can proceed by enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid to various acceptors, and that the nucleotide itself can be formed in these tissues by enzymic oxidation of uridine diphosphate glucose. Development of the system in foetal kidney parallels that in liver, but in foetal stomach maximal activity of uridine diphosphate transglucuronylase appears before birth, falling after. It is probable that bilirubin glucuronide can be formed by gastric mucosa. Preliminary accounts of this work have already appeared (Dutton, 1958, 1959; Dutton & Stevenson, 1959).

EXPERIMENTAL

Materials

Acceptor substrates. These were resublimed *o*-aminophenol, recrystallized *o*-aminobenzoic acid, recrystallized (−)-menthol and bilirubin (from British Drug Houses Ltd., and recrystallized from chloroform). Ascorbic acid (Roche) was added along with *o*-aminophenol, to minimize oxidation.

Uridine diphosphate glucuronic acid. This was 'uridine diphosphoglucuronic acid' (98–100% quoted purity, ammonium salt) from Sigma Chemical Co., St Louis, Mo., U.S.A. A further source was enzymic oxidation of UDP-glucose *in vitro*, under oxygen with DPN (Strominger, Maxwell, Axelrod & Kalckar, 1957).

Other additions. UDP, UTP, UDP-glucose, UDP-*N*-acetylglucosamine, DPN and TPN were also from Sigma; ATP was from C. F. Boehringer und Soehne GmbH., Mannheim, Germany; EDTA was from British Drug

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