sulphur in the presence of Ba^{2+} ions. Choline sulphate and nitrocatechol sulphate were good sulphur sources for the growth of choline sulphate-less fungi both in the absence and the presence of Ba^{2+} ions.

5. When choline sulphate-producing fungi were grown on an adequate sulphur medium a store of choline sulphate accumulated in the mycelia which could be used to maintain further growth when the fungi were transferred to a medium totally deficient in sulphur of any form. In the same circumstances no further growth of choline sulphate-less fungi occurred on the new sulphur-deficient medium.

6. Choline sulphate in fungal mycelia is envisaged as a store of easily assimilated sulphate existing in an activated state. The pathway of utilization is thought to involve transfer of sulphate from choline sulphate to adenosine 3':5'-diphosphate with the formation of adenosine 3'-phosphate 5'-sulphatophosphate and the subsequent reduction of adenosine 3'-phosphate 5'-sulphatophosphate to give SO_3^{2-} ions and eventually cysteine.

The investigation was supported in part by a Royal Society Grant. Dr T. Harada is grateful to the University College of South Wales and Monmouthshire for a fellowship and to the Wellcome Trust for a travel grant.

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Condensed Tannins

6. BIOCHEMICAL AND STEREOCHEMICAL INTERRELATIONSHIPS OF (+)-FUSTIN FROM BLACK-WATTLE HEARTWOOD (*ACACIA MOLLISSIMA*)*

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(Received 21 March 1960)

Condensed tannins from the heartwood of *Acacia mollissima* consist of a molecular gradation (290–1650) of polyphenolic compounds (Roux & Evelyn, 1960) in which (+)-7:3':4'-trihydroxy-flavan-3:4-diol [(+)-mollisacacidin, Keppler (1957)] is the predominant single component. Polymeric

leuco-fisetinidin tannins form the major fraction of the heartwood polyphenols and appear to originate from this monomeric 'leuco-fisetinidin' flavan-3:4-diol (Roux, 1958). Other monomeric flavonoid substances of similar structural pattern are also present. The provisional identification of fustin and fisetin (Roux & Evelyn, 1960) is confirmed in this study, and their probable mechanism of biogenesis

* Part 5: Evelyn, Maihs & Roux (1960).

is discussed. The stereochemical interrelationships of various fustins, and the synthesis of (+)-mollisacacidin are also described.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Mixed melting points were carried out as before (Roux & Maihs, 1960). Analyses of C, H, methoxyl groups and acetyl groups were by Weiler and Strauss, Oxford, and by Pascher, Bonn. Infrared comparisons were made by Dr J. R. Nunn, National Chemical Research Laboratory, C.S.I.R., Pretoria. A 2 dm. tube was used for optical rotations.

Selection of wattle heartwood. Radial examination by two-dimensional paper chromatography (Roux, 1958) of heartwoods of wattle trees, felled in plantations after the normal 8- to 10-year rotation (average wood diameter 7 in.), showed the predominant presence of (+)-mollisacacidin, but relatively low concentrations of fustin and fisetin. In older specimens, estimated to be at least 50 years old (average wood diameter 20 in.) the concentration of (+)-mollisacacidin was similarly high in the peripheral heartwood and persisted with progressively declining concentration into the central heartwood. Both fustin and fisetin, on the other hand, were in low concentration in the peripheral heartwood but increased slightly towards the central heartwood (cf. Table 2). These were observed in each of four 50-year-old specimens of *A. mollissima* examined. The heartwoods of the older trees were selected for the isolation of fustin and fisetin.

Extraction of heartwood and separation of components. The damp heartwood of the freshly cut tree was drilled and the damp drillings (2.6 kg.) were extracted with ethyl acetate (7.5 l.) in two extractions of 48 hr. each. The extracts were dried in a rotary evaporator under vacuum and the powdered residue (37 g.) was extracted with light petroleum (b.p. 40–60°) in a Soxhlet apparatus for 6 hr. The wax-free residue (30 g.) was dissolved in 400 ml. of the lower phase of a water–butan-2-ol–‘benzene’ (5:3:2) mixture and introduced into the first eight tubes of a 160-tube, 50 ml. underphase, fully automatic Craig countercurrent machine (Glasapparatebau Göttingen-Helmut Rettberg). The upper and lower phases of the above mixture were used for countercurrent separation, and after 160 transfers the contents of tubes 120–145 and 146–160 were run off. The organic phase was separated and the aqueous phase extracted with ethyl acetate in each instance. The combined organic phase and ethyl acetate extracts of tubes 120–145 were decreased to 40 ml. as before, and streaked on to eight sheets (5 ml./sheet) of 22½ in. × 18½ in. Whatman no. 3 chromatographic paper. The chromatograms were developed by upward migration in 2% acetic acid. The fustin band (R_F 0.43) was located with reference to the (+)-mollisacacidin band [toluene-*p*-sulphonic acid, Roux (1957b)] on a test strip. The fustin band, which migrated immediately behind this leuco-fisetidin band (R_F 0.57), was cut and stripped in 70% ethanol. Fustin (250–300 mg.) crystallized from a concentrate of the eluents. Fisetin was present, amongst other components, in tubes 146–160 of the Craig machine.

(+)-Fustin. Fustin from the Craig-countercurrent and paper-chromatographic separations, was recrystallized from water as fine needles, m.p. 209–211°. The m.p. in soda

glass was variable and apparently dependent on the rate of heating. In Pyrex glass (cf. Hillis, 1952; Laidlaw & Smith, 1958) the m.p. was elevated to 228–229° and consistent results were obtained. Fustin, dried over CaCl_2 , was analysed as a semi-hydrate (Found: C, 60.2; H, 4.3; loss at 110°, 3.2. $\text{C}_{15}\text{H}_{12}\text{O}_8 \cdot 0.5\text{H}_2\text{O}$ requires C, 60.6; H, 4.4; H_2O , 3.0%). After 2 hr. at 110° the fustin became anhydrous [Found: C, 62.4; H, 4.3. $\text{C}_{15}\text{H}_{12}\text{O}_8$ requires C, 62.5; H, 4.2%. $[\alpha]_D^{25} + 28.3 \pm 1.1^\circ$ in acetone–water 1:1 (c, 0.9)]. (+)-Fustin and (–)-fustin showed identical infrared-absorption curves over the range 2.5–15 μ , and these were almost identical with the infrared curve of ($\pm$)-fustin over the same range.

Tetra-acetyl-(+)-fustin. (+)-Fustin (500 mg.) was acetylated with 2 ml. of pyridine and 3 ml. of acetic anhydride at room temperature for 5 hr. The product was poured into water and, after hardening overnight, was recrystallized twice from ethanol (0.3 g.), m.p. 116–119°. $[\alpha]_D^{25} + 24.4 \pm 0.8^\circ$ in tetrachloroethane (c, 1.3) (Found: C, 60.2; H, 4.50; $\text{CO} \cdot \text{CH}_3$, 38.1. $\text{C}_{23}\text{H}_{20}\text{O}_{10}$ requires C, 60.5; H, 4.4; $\text{CO} \cdot \text{CH}_3$, 37.7%). Tetra-acetyl derivatives of (+)-, (–)- and ($\pm$)-fustin showed identical infrared-absorption curves over the range 2.5–15 μ .

O-Trimethyl-(+)-fustin. (+)-Fustin (500 mg.) in 50 ml. of methanol was methylated with excess of diazomethane at –5° as described for 7:3:4'-trimethoxyflavan-3:4-diol (Roux & Evelyn, 1958). The product (0.3 g.) was recrystallized twice from ethanol, m.p. 138–140°, $[\alpha]_D^{25} - 44.3 \pm 1.6^\circ$ in tetrachloroethane (c, 1.3) (Found: C, 65.5; H, 5.5; OMe, 27.6. $\text{C}_{18}\text{H}_{21}\text{O}_9$ requires C, 65.5; H, 5.6; OMe, 28.2%). The infrared-absorption curves of O-trimethyl-(+)-fustin and (–)-fustin were identical over the range 2.5–15 μ .

Acetyl-O-trimethyl-(+)-fustin. O-Trimethyl-(+)-fustin (90 mg.) was acetylated with 0.4 ml. of pyridine and 0.4 ml. of acetic anhydride for 3 hr. at room temperature. The product was poured into water and recrystallized from ethanol (60 mg.), m.p. 119–121°, $[\alpha]_D^{25} + 2.1 \pm 0.3^\circ$ in tetrachloroethane (c, 0.8) (Found: C, 64.6; H, 5.6; $\text{CO} \cdot \text{CH}_3$, 11.1; OMe, 26.9. $\text{C}_{23}\text{H}_{20}\text{O}_7$ requires C, 64.5; H, 5.4; $\text{CO} \cdot \text{CH}_3$, 11.6; OMe, 25.0%).

($\pm$)-Fustin and derivatives. ($\pm$)-Fustin from *Rhus glabra* was isolated as described by Roux & Freudenberg (1958) and the derivatives were prepared as described above. This racemic mixture showed low negative rotation $[\alpha]_D^{21} - 2.4^\circ$. (Roux & Freudenberg, 1958) and its m.p. 214–216° in soda glass was raised to 226–228° in Pyrex glass. Tetra-acetyl-($\pm$)-fustin, m.p. 145° (Found: C, 60.4; H, 4.3; $\text{CO} \cdot \text{CH}_3$, 36.8. Calc. for $\text{C}_{23}\text{H}_{20}\text{O}_{10}$: C, 60.5; H, 4.4; $\text{CO} \cdot \text{CH}_3$, 37.7%). O-Trimethyl-($\pm$)-fustin, m.p. 140–143°. Acetyl-O-trimethyl-($\pm$)-fustin, m.p. 144–145°.

Tetra-acetyl-(–)-fustin. (–)-Fustin (50 mg.) (Freudenberg & Weinges, 1959; Weinges, 1959) from *Rhus cotinus*, kindly supplied by Professor K. Freudenberg and Dr K. Weinges, was acetylated with 0.2 ml. of pyridine and 0.3 ml. of acetic anhydride at room temperature for 3 hr. The product was poured into 3 ml. of water, allowed to harden overnight and crystallized from ethanol (46 mg.), m.p. 115–116° [m.p. 117–118° found by Weinges (1959)], $[\alpha]_D^{25} - 25.2 \pm 1.1^\circ$ in tetrachloroethane (c, 0.8). The infrared-absorption curves of tetra-acetyl-(–)-fustin and tetra-acetyl-(+)-fustin were superimposable over the range 2.5–15 μ .

Comparison of melting points of (+)-fustin, (–)-fustin, ($\pm$)-fustin and their tetra-acetyl derivatives. In Pyrex glass

the m.p.'s of the above-listed fustins were all elevated to the range 226–230°. An equimolecular mixture of (+) - and (–) - fustin showed no elevation of m.p. A mixed m.p. of a molecular mixture of tetra-acetyl-(+) - fustin (m.p. 116–119°) and tetra-acetyl-(–) - fustin (m.p. 115–116°) showed an elevation to 150–151.5°. The latter agrees approximately with the m.p. (145° and 147–148°) of tetra-acetyl-(±) - fustin from *R. glabra*, and exactly with the m.p. of tetra-acetylfustin (150–151°) from *Rhus succedanea* (Oyamada, 1934, 1939). A mixed m.p. of the artificial racemic mixture and that derived from the natural racemate from *R. glabra* showed no depression (m.p. 147–149°). A comparison of the melting points and optical rotations of the various fustins is shown in Table 1.

Paper chromatography of (+) -, (–) - and (±) - fustin. The naturally occurring fustins of *A. mollissima*, *R. cotinus* and *R. glabra* all have the same R_F values in partitioning mixtures [0.81 in water-saturated butan-2-ol, and 0.80 in butan-1-ol-acetic acid-water (4:1:5)], but show slight but reproducible differences in 2% acetic acid or water when run at identical concentrations on the same sheet of paper. In 2% acetic acid with reference substances (–) - robinetinidol (0.42) and (+) - catechin (0.36) the following R_F values were recorded: (–) - fustin, 0.35; (±) - fustin, 0.36; (+) - fustin, 0.37. As expected, with such small differences in R_F between (+) - and (–) - fustin, the racemic mixture showed no tendency to separate into its optical antipodes.

Hydrogenation of (+) - fustin to (+) - mollisacadin. (+) - Fustin (500 mg.) from *A. mollissima* was hydrogenated in methanol with platinum oxide as catalyst (Freudenberg & Roux, 1954), as described in detail by Roux & Freudenberg (1958) for (±) - fustin. The theoretical volume of hydrogen was absorbed in 12 hr. The product after repeated recrystallization from water (69 mg.) melted at

125–129°, and showed no depression in a mixed m.p. (125–129°) with (+) - mollisacadin from wattle heartwood; $[\alpha]_D^{25} + 36.7^\circ \pm 1.3^\circ$ in acetone-water (1:1) (c, 1.1). The product showed the same R_F as (+) - mollisacadin in 2% acetic acid (0.55), but a distinctly higher R_F than that (0.49) of the enantiomorphous (–) - 7:3':4' - trihydroxyflavan-3:4-diol from *Schinopsis quebracho-colorado* (Clark-Lewis & Roux, 1958, 1959). The hydrogenation product showed the same R_F (0.76) as (+) - and (–) - 7:3':4' - trihydroxyflavan-3:4-diol in water-saturated butan-2-ol (Roux & Evelyn, 1958), and was readily converted into fisetinidin chloride (Roux, 1957a) on treatment with hot hydrochloric acid in propan-2-ol under pressure at 100°.

Isolation and identification of fisetin. The upper phases of tubes 146–160 from the Craig separation were concentrated to dryness as before. The concentrates (1.1 g.) were streaked on to Whatman no. 3 sheets and the chromatograms developed in 2% acetic acid as for (+) - fustin. The bands on the starting line fluoresced a brilliant greenish yellow under ultraviolet light and were eluted as before. The eluents were concentrated to dryness and the solid residue boiled with 500 ml. of *N*-sulphuric acid for 1 hr. The solution was filtered hot and extracted with ether after cooling. The yellow product obtained from evaporation of the ether was recrystallized twice from ethanol-water, yielding 3 mg. of yellow needles. The ultraviolet and visible range spectra (Found: $\lambda_{\max}$, 252, 320, 360 m μ and $\log E_{1\%}^{1\text{cm}}$, 2.62, 2.51, 2.73 respectively) were similar to that of fisetin derived from the refluxing of (±) - fustin in *N*-sulphuric acid in a current of air according to the method of Pew (1948) (Found: $\lambda_{\max}$, 250, 320, 365 m μ and $\log E_{1\%}^{1\text{cm}}$, 2.69, 2.59, 2.84 respectively) (cf. Kirby & White, 1955; Mitsuno & Yoshizaki, 1957). The infrared-absorption curves of the substance and of fisetin were identical over the range 2.5–15 μ .

Table 1. *Melting points and optical rotations of enantiomorphous fustins and their racemates*

Chadenson, Molho-Lacroix, Molho & Mentzer (1955) found m.p. 248° for fustin from *Gleditsia (Gleditschia) triacanthos*. Hasegawa & Shirato (1951) found m.p. 218° for fustin, 158° for tetra-acetylfustin and 143° for *O*-trimethylfustin from *Rhus vernicifera*. Values in parentheses are from the authors listed in the footnotes.

Compound	<i>A. mollissima</i> (+) - Fustin	<i>R. succedanea</i>	<i>R. glabra</i> (±) - Fustin	Racemic mixture of enantiomorphs	<i>Cotinus cogygria</i> (<i>R. cotinus</i>) (–) - Fustin
Phenol					
M.p. (Pyrex glass)	228–229°	—	226–228°	226–228°	228 230°
M.p. (soda glass)	209–211°	(216–218°)*	222° (213–215°)† (223–225°)§	—	(216–218°)§
$[\alpha]_D$	+28.3°	—	(–2.4°)‡	—	(–26°)§
Tetra-acetyl					
M.p.	116–119°	(150–151°)*	145° (147–148°)§	150–151.5°	115–116° (117–118°)§
$[\alpha]_D$	+24.4°	—	—	—	–25.5°
<i>O</i> -Trimethyl					
M.p.	138–140°	(143–144°)*	140–143° (141–142°)†	—	—
$[\alpha]_D$	–44.3°	—	—	—	—
Monoacetyl- <i>O</i> -trimethyl					
M.p.	119–121°	(142–143°)*	144–145°	—	—
$[\alpha]_D$	+2.1°	—	—	—	—

* Oyamada (1939).

† Keppler (1957).

‡ Roux & Freudenberg (1958).

§ Freudenberg & Weinges (1959); Weinges (1959).

Radial distribution of (+)-fustin and (+)-mollisacacidin in black-wattle heartwoods. The cross-section of a freshly felled 50-year-old specimen of *A. mollissima*, of wood diameter 20 in., was sampled radially by drilling at the centre, 4 in. from the centre and 8 in. from the centre (heartwood periphery). Sapwood surrounding the heartwood had an average width of 2 in. The drillings (30 g.) from each position were extracted with methanol (150 ml.) with four extractions of 24 hr. each at room temperature. Residual polyphenols, which could be removed similarly by three successive extractions with acetone-water (1:1), consisted mainly of highly condensed tannins as shown by paper chromatography. Extraction of these tannins was not complete even at this stage. The solid extractives were redissolved in methanol or acetone-water (5% soln.), and spotted on two-dimensional paper chromatographic sheets in 0.5 and 1 mg. concentrations. (+)-Fustin and (+)-7:3':4'-trihydroxyflavan-3:4-diol were dissolved in methanol (0.1% soln.) and applied to two-way paper chromatograms in 10 µg. increments over the range 10–90 µg. The densitometric method of Roux & Maihs (1960) with ammoniacal silver nitrate as spraying reagent was used for the estimation of (+)-fustin and (+)-mollisacacidin in each of the radial samples, and in the ethyl acetate extract of the whole bark (Table 2). The values for (+)-fustin and (+)-mollisacacidin in the 'combined methanol and acetone-water extracts' are lower than anticipated from the values of the 'methanol extract', when also comparing the percentages of extractives in Table 2. A possible explanation for this is that acetone-water extraction removes mainly polymeric condensed tannins, as indicated on paper chromatograms. These tannins are therefore present in much higher concentration at or near the origin on paper chromatograms of the combined extract, and may possibly occlude or retain a proportion of the flavonoid compounds resulting in the relatively low values obtained on estimation.

DISCUSSION

Fustin (2:3-dihydrofisetin), first member of the now relatively large group of 2:3-dihydroflavonols to be found in Nature, was isolated by Oyamada (1934, 1939) from *Rhus succedanea*. Oyamada

failed to record the optical rotation of his fustin, and this omission was repeated in more recent work on fustins by others (Hasegawa & Shirato, 1951; Chadenson, Molho-Lacroix, Molho & Mentzer, 1955; Mitsuno & Yoshizaki, 1957; Keppler, 1957). Freudenberg & Weinges (1959) and Weinges (1959) were the first to record the isolation of a (–)-fustin from *R. cotinus*, and the present isolation of a (+)-fustin of similar but opposite rotation from *A. mollissima* suggested that these substances were pure stereoisomers and possibly also enantiomorphs. This conjecture was proved by comparing the tetra-acetates of (+)- and (–)-fustin. The acetyl derivatives not only had equal and opposite rotations (Table 1) but similar and lower melting points (116–119°) compared with Oyamada's (1939) tetra-acetylfustin (150–151°) and tetra-acetyl-(±)-fustin from *R. glabra* (146–147°). A molecular mixture of tetra-acetyl-(+)-fustin and tetra-acetyl-(–)-fustin gave a melting-point elevation to 150–151.5° and proved the enantiomorphous nature of the parent compounds. *R. glabra* was shown to contain the natural racemate by a mixed melting point of tetra-acetyl-(±)-fustin and the artificial racemate. These interrelationships were confirmed by comparison of the infrared-absorption curves of the enantiomorphous fustins, the natural racemate and their derivatives. Further proof was also provided by the conversion of (+)-fustin into (+)-7:3':4'-trihydroxyflavan-3:4-diol, which is enantiomorphous (Clark-Lewis & Roux, 1958, 1959) with the (–)-leucofisetinidin obtained by Freudenberg & Weinges (1959) by the hydrogenation of (–)-fustin.

From melting-point data in Table 1 it may be concluded that Oyamada's fustin was also a natural racemate similar to that in *Rhus glabra*, and similar racemates apparently also occur in *Rhus vernicifera* (Hasegawa & Shirato, 1951) and in

Table 2. *Estimation of (+)-fustin and (+)-mollisacacidin in radial samples of black-wattle heartwood from a 50-year-old tree*

Location of sample	Extract as % of damp-wood weight	(+)-Fustin (as % of solids in extract)*	(+)-Mollisacacidin (as % of solids in extract)*
Methanol extract			
Heartwood periphery	9.0	2.1	19.6
Middle heartwood	5.3	3.3	11.8
Central heartwood	5.1	2.6	5.9
Combined methanol and acetone-water extracts			
Heartwood periphery	11.6	1.3	13.8
Middle heartwood	8.0	2.3	7.0
Central heartwood	8.3	2.1	2.8
Ethyl acetate extract of whole heartwood	1.2	3.5	5.5

* The radial distribution of (+)-mollisacacidin and (+)-fustin represented is the pattern commonly encountered in the heartwood [compare radial distribution of leucofisetinidins in Table 3 by Roux & Evelyn (1960)]. Heartwoods containing very variable distributions of these compounds and of tannin components are also found.

Gleditsia triacanthos (Chadenson *et al.* 1955). Fustin from *Gleditsia japonica* (Mitsuno & Yoshizaki, 1957) forms a tetra-acetate of similar but slightly lower melting point (110–111°) than the corresponding derivatives of the enantiomorphs (cf. Table 1).

The enantiomorphism of (+)-fustin from *A. mollissima* and (–)-fustin from *Rhus cotinus* constitutes the second example of the presence of enantiomorphous flavonoid compounds in Nature. Previously (+)- and (–)-7:3':4'-trihydroxyflavan-3:4-diols from *A. mollissima* and *Schinopsis quebracho-colorado* respectively were shown to be enantiomorphous by Clark-Lewis & Roux (1958, 1959). From recent work by Weinges (1958, 1959) the absolute configuration of (+)-fustin may be inferred. (+)-Fustin has the same absolute configuration (2:3-*trans*) as (+)-catechin and other naturally occurring dihydroflavonols (Mahesh & Seshardi, 1955; Whalley, 1956; Clark-Lewis & Korytnyk, 1958) and is tentatively represented as (2R:3R)-3:7:3':4'-tetrahydroxyflavan-4-one (cf. Cahn, Ingold & Prelog, 1956; Birch, Clark-Lewis & Robertson, 1957). Similarly, (–)-fustin, shown to have the same configuration as (–)-catechin by Weinges (1959), may be designated as (2S:3S)-3:7:3':4'-tetrahydroxyflavan-4-one. (–)-Fustin from *R. cotinus* has been hydrogenated to (–)-7:3':4'-trihydroxyflavan-3:4-diol (Weinges, 1959), which occurs naturally in *Schinopsis* spp. (Roux, 1958; Freudenberg & Weinges, 1958; Roux & Evelyn, 1958). Thus (–)-fustin and (–)-leucofisetinidin form a group of hitherto unusual naturally occurring (2S)-flavan derivatives and also belong to a natural group of flavonoid compounds ('resorcinol series') unhydroxylated in the 5 position. The 5-deoxyflavanone, (–)-liquiritigenin, from *Glycyrrhiza glabra* although designated as (2S) (Arakawa & Nakazaki, 1960), has the same configuration at the 2 position as (+)-catechin and therefore does not belong to the above-mentioned group.

The conversion of (+)-fustin by hydrogenation with platinum oxide in methanol (method of Freudenberg & Roux, 1954) into (+)-7:3':4'-trihydroxyflavan-3:4-diol, $[\alpha]_D^{25} + 36.7^\circ$, possibly represents the first synthesis of (+)-mollisacacidin [(+)-gleditsin] from fustin, as Keppler (1957) apparently used (±)-fustin from *R. glabra* and therefore obtained (±)-7:3':4'-trihydroxyflavan-3:4-diol (cf. Roux & Freudenberg, 1958; Clark-Lewis & Roux, 1959). (+)-Fustin and (+)-mollisacacidin occurring in the heartwood of *Acacia mollissima* therefore have identical configurations at C-2 and C-3. Freudenberg & Weinges (1959) have drawn attention to a similar relationship between (–)-fustin and (–)-7:3':4'-trihydroxyflavan-3:4-diol found in *R. cotinus*, and between

(+)-dihydrobinetin and (+)-7:3':4':5-tetrahydroxyflavan-3:4-diol occurring in *Robinia pseudacacia*. Mitsuno & Yoshizaki's (1957) undesigned fustin accompanying (+)-gleditsin [(+)-7:3':4'-trihydroxyflavan-3:4-diol] in *Gleditsia japonica* is evidently a pure enantiomer, and therefore probably a (+)-fustin identical with that from *A. mollissima*.

In black-wattle heartwood (+)-mollisacacidin occurs in highest concentration in the peripheral heartwood and decreases in concentration towards the centre (Table 2), whereas (+)-fustin, present in relatively low concentration in the peripheral heartwood, increases in concentration towards the centre. Visual observation of the quantitative chromatograms showed that fisetin, which is almost absent in the peripheral heartwood, increases progressively in concentration towards the centre. These distributions, together with the stereochemical and chemical interrelationships, suggest that (+)-7:3':4'-trihydroxyflavan-3:4-diol is the primary body formed in biosynthesis, and that apart from condensation to polymeric leucofisetinidins, it undergoes interconversion into (+)-fustin and finally into fisetin during aging of the wood. Both condensation and interconversion could result from dehydrogenation mechanisms.

SUMMARY

1. (+)-Fustin and fisetin have been isolated from the heartwood of *Acacia mollissima*.

2. (+)-Fustin has been shown to be enantiomorphous with (–)-fustin from *Rhus cotinus*, whereas *Rhus glabra*, *Rhus succedanea* and *Rhus vernicifera* contain racemic mixtures of these enantiomorphs. The stereochemistry of these fustins is inferred from previous work.

3. (+)-Fustin was converted into (+)-7:3':4'-trihydroxyflavan-3:4-diol [(+)-mollisacacidin] by hydrogenation.

4. The radial distributions of (+)-mollisacacidin, (+)-fustin and fisetin in wattle heartwood were estimated. The findings suggest that (+)-mollisacacidin is the primary precursor from which (+)-fustin and fisetin are formed by dehydrogenation.

Thanks are due to Professor Dr K. Freudenberg and Dr K. Weinges, Chemisches Institut der Universität, Heidelberg for their gift of 50 mg. of (–)-fustin from *R. cotinus*.

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An Inborn Defect in the Metabolism of Tyrosine in Infants on a Normal Diet

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(Received 15 March 1960)

Levine and his coworkers (Levine, Marples & Gordon, 1939, 1941; Levine, Gordon & Marples, 1941; Levine, Dann & Marples, 1943) showed that premature, but not full-term, infants excrete *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenyllactic acid if fed on a high protein diet, but both groups of infants excrete these compounds if given a 'loading' dose of tyrosine or phenylalanine. This was confirmed and extended by Woolf & Edmunds (1950), who showed that, after a 'loading' dose of tyrosine or phenylalanine, both premature and full-term infants exhibit a temporary hydroxyphenyluria followed by a return to normal values though the peak excretion is much higher in the premature group.

Ascorbic acid promptly cured the hydroxyphenyluria in these infants (Levine *et al.* 1939, 1941, 1943; Woolf & Edmunds, 1950) and it rarely appeared in an infant that had ever been fed with ascorbic acid. Adults or infants suffering from scurvy also showed hydroxyphenyluria when fed with large doses of tyrosine (Rogers & Gardner, 1949; Morris, Harpur & Goldbloom, 1950) and here again feeding with ascorbic acid cured the hydroxyphenyluria. Independently of the above

work, Sealock & Silberstein (1939, 1940) had shown that scorbutic guinea pigs developed hydroxyphenyluria if they were fed with tyrosine. The effect of ascorbic acid on tyrosine metabolism by liver slices *etc. in vitro* has been reviewed by Knox (1955) (see also Zannoni & La Du, 1960).

Hydroxyphenyluria also occurs in advanced liver disease (Felix, Leonhardi & Glasenapp, 1951; L. I. Woolf, unpublished work); in this condition ascorbic acid has no effect.

Work *in vitro* on the foetal and neonatal development of enzymes concerned with the metabolism of phenylalanine and tyrosine has been carried out by Kretchmer, Levine, McNamara & Barnett (1956), Kretchmer & McNamara (1956), Reem & Kretchmer (1957), Kenney, Reem & Kretchmer (1958) and Auerbach & Waisman (1959).

During a survey of young infants in Cardiff to detect cases of phenylketonuria (Gibbs & Woolf, 1959), several infants were found whose urine gave a green colour with ferric chloride, but who were not phenylketonurics. As reported below, this has been found to be due to *p*-hydroxyphenylpyruvic acid and these urines have also been found