Natural and Synthetic Diastereoisomeric (–)-3',4',7-Trihydroxyflavan-3,4-diols

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1. Rhodesian copalwood (*Guibourtia coleosperma*) contains three diastereoisomeric leuco-fisetinidins. These consist of the (–)-2,3-*cis*-3,4-*cis* (2*R*,3*R*,4*R*) and (–)-2,3-*cis*-3,4-*trans* (2*R*,3*R*,4*S*) 3',4',7-trihydroxyflavan-3,4-diols, and the third was shown to be a 2,3-*trans*-3,4-*cis* isomer by means of paper ionophoresis. 2. There occurrence in similar proportions as tannin precursors also in the tropical hardwoods *G. tessmannii* and *G. demeusii* implies a close taxonomic relationship between these, and with *G. coleosperma*. 3. Epimerization of the natural (–)-3',4',7-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol affords a mixture from which the (–)-2,3-*cis*-3,4-*cis* isomer was separated readily, but the (–)-2,3-*trans*-3,4-*cis* isomer was obtained with difficulty. These were formed by epimerization of the (–)-2,3-*trans*-3,4-*trans* isomer at C-2 and C-4, and at C-4, and at C-4, respectively.

The flavan-3,4-diols possess three asymmetric centres at C-2, C-3 and C-4 and therefore eight stereoisomeric forms are possible. In view of this it is perhaps surprising that so few diastereoisomers of each type of natural leuco-anthocyanidin have been recognized hitherto.

However, a number of flavan-3,4-diols of the 'phloroglucinol series', namely two leuco-pelargonidins (I, R=R'=H) (Ganguly & Seshadri, 1961; Paris & Cubukou, 1962), four leuco-cyanidins (I, R=OH, R'=H) (Ganguly & Seshadri, 1959; Nagarajan & Seshadri, 1961; Laumas & Seshadri, 1958; Chadha & Seshadri, 1962), and two leucodelphinidins (I, R=R'=OH) (Hathway, 1958; Ganguly, Seshadri & Subramanian, 1958) have been isolated, but although these may represent different diastereoisomers, their stereochemistry remains unknown.

On the other hand, those isomeric flavan-3,4-diols that are fully characterized have hitherto been shown to exist in not more than two forms, as in the pairs (–)-teracacidin (II, R=H) and isoteracacidin (III, R=H) (Clark-Lewis, Katekar & Mortimer, 1961), (–)-melacacidin (II, R=OH) and isomelacacidin (III, R=OH) (Clark-Lewis & Mortimer, 1960), (+)-mollisacacidin (IV, R=H) and (–)-leuco-fisetinidin (V) (Drewes & Roux, 1964*a*), whereas (+)-leuco-robinetinidin (IV, R=OH) (Drewes & Roux, 1964*a*) exists in one form only.

Of the above, the leuco-fisetinidins (3',4',7-trihydroxyflavan-3,4-diols) are unique in their existence as enantiomers of 2,3-*trans*-3,4-*trans*

configuration (IV, R=H, and V). The remaining geometrical arrangements have now been shown to occur in diastereoisomers from the heartwood of *Guibourtia coleosperma* (Rhodesian copalwood), bringing the total to five natural diastereoisomeric leuco-fisetinidins. This finding (Drewes & Roux, 1964*d*) links with the occurrence of three guibourt-acacids (4',7-dihydroxyflavan-3,4-diols) in the same wood (Saayman & Roux, 1965).

The epimerization of (–)-leuco-fisetinidin (V) from *Schinopsis lorentzii* has been studied to enable direct comparison with the natural diastereoisomers.

EXPERIMENTAL AND RESULTS

The nuclear-magnetic-resonance spectra were by Dr K. Pachler, Chemical-Physics Group, C.S.I.R., Pretoria, obtained with a Varian A-60 spectrometer with trimethylsilane as internal standard. C and H analyses were by K. Jones, Micro-Analytical Laboratory, National Chemical Research Laboratory, C.S.I.R., Pretoria. All melting points are uncorrected, and mixed melting points were by the method of Roux & Maihs (1960). Two-dimensional chromatograms were run in water-satd. butan-2-ol and then in 2% (v/v) acetic acid on Whatman no. 1 paper, and the *R_F* values of compounds are cited in this sequence.

Isolation of natural leuco-fisetinidins

Drillings (3.9 kg.) from the heartwood of *G. coleosperma* were extracted at room temperature with ethyl acetate (12l.) for 7 days. The solution was concentrated under vacuum, yielding a reddish-brown powder (312g.). The

flavan-3,4-diol content of the extract was enriched as follows. The extract (10g.) was dissolved in ethanol (100ml.), water (350ml.) was added with vigorous stirring, and the resultant precipitate (tannins) was sucked off and discarded. The filtrate was extracted with ethyl acetate and the combined extracts were concentrated to dryness under vacuum, giving a pale-brown powder (5.3g.).

The enriched extract (140g.) from a repetition of the above procedure dissolved partially in the lower phase of water–butan-2-ol–light petroleum (b.p. 60–80°) (5:3:2, by vol.) (800ml.). The undissolved material (15g.) was sucked off, and the filtrate introduced into the first 16 tubes of a Craig countercurrent machine (50ml. underphase). After 160 transfers two fractions were collected from tubes 33–43 and 44–65. Two-dimensional chromatograms (toluene-*p*-sulphonic acid reagent) showed the presence of two leuco-fisetinidins R_F 0.62 and 0.43, and 0.69 and 0.56, respectively in water-satd. butan-2-ol and 2% (v/v) acetic acid in the first fraction, and the second fraction contained these as well as a third leuco-fisetinidin (R_F 0.75 and 0.58 respectively).

(–)-3',4',7-Trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol. The contents of tubes 33–43 were concentrated to small (150ml.) volume, streaked on to 30 sheets of Whatman no. 3 paper (22½ in. × 18½ in.), and the chromatogram was developed in 2% (v/v) acetic acid by upward migration. Reducing bands (ammoniacal AgNO₃) were located at R_F 0.43 and 0.55. The contents of tubes 44–65 were separated as above on 45 sheets.

The low- R_F bands from both fractions were eluted with 70% ethanol, and separated for a second time by chromatography in 2% acetic acid. The eluates from fraction 44–65 gave a light-brown powder (307mg.) on concentration under vacuum. A similar product from fraction 33–43 still contained a proportion of tannin, and further chromatography gave no crystalline material. The brown powder was separated on ten sheets of Whatman no. 3 paper in water-satd. butan-2-ol, and the band at R_F 0.59 was cut out and eluted. The separation was repeated on four sheets with the same solvent. The final eluates were concentrated at 45° under vacuum and the solution (4ml.) was placed in a vacuum desiccator under slight vacuum. Buff needles (25mg.), which sinter at 98–103° and melt slowly with reddening and then blackening from 147°, were obtained, $[\alpha]_D^{18}$ –28.6 ± 3.0° [c 0.15 in acetone–water (1:1, v/v)]. A mixed m.p. with the same compound formed by epimerization of (–)-leuco-fisetinidin (see below) showed the same behaviour, whereas a mixed m.p. with (+)-2,3-*cis*-3,4-*cis* enantiomer melted at 133–135° and darkened from 150° onwards. On paper chromatograms the compound migrated as a discrete spot (R_F 0.62 and 0.43) in the above solvents.

The hydrated (+)-2,3-*cis*-3,4-*cis* enantiomer has m.p. 105–106°, the anhydrous form sintering at 65–67° and melting at 146–106° (decomp.), $[\alpha]_D$ +48.5° (Drewes & Roux, 1964c, 1965), and has R_F 0.62 and 0.39 respectively.

(–)-3',4',7-Trimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol. The (–)-3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol (55mg.) was methylated at 0° with excess of diazomethane. The product failed to crystallize and two-dimensional chromatography showed a trace of impurity migrating slightly ahead of the compound. The contamination gave an orange colour with the toluene-*p*-sulphonic acid spray as opposed to the red of the diol. Paper ionophoresis showed a mobility (+1.85cm.) characteristic of the isomeric 2,3-*cis*-3,4-*cis* racemate (Drewes & Roux, 1964b).

(–)-3,4-*cis*-Diacetoxy-3',4',7-trimethoxy-2,3-*cis*-flavan. The non-crystalline methyl ether (50mg.) was acetylated for 24hr. at room temperature with acetic anhydride (0.2ml.) and pyridine (0.2ml.). A white solid (59mg.), m.p. 65–70°, was recovered from water. The n.m.r. spectrum showed peaks at τ 8.73 and 7.70 p.p.m. due to impurities, but the signals from heterocyclic-ring protons at C-2, C-3 and C-4 were clear and could be interpreted unambiguously by comparison with the racemate (cf. Drewes & Roux, 1964b). The 2-H appears as a singlet (τ 4.70 p.p.m.; $J_{2,3} < 1$ cyc./sec.), 3-H as a doublet (τ 4.36 p.p.m.) and 4-H as a doublet (τ 3.70 p.p.m.; $J_{3,4} \approx 4$ cyc./sec.). Methyl proton signals due to the 3-acetyl (8.06), 4-acetyl (7.89), 7-methoxyl (6.20) and 3',4'-methoxyl groups (6.10) appeared at the τ values indicated.

Purification of the diacetate (55mg.) was effected by preparative thin-layer chromatography on three 20cm. × 20cm. plates with a 1mm. layer of Kieselgel G (E. Merck A.-G., Darmstadt, Germany). Benzene–acetone (9:1, v/v) was employed as solvent and the diacetate was located at R_F 0.62 with the conc. H₂SO₄–40% formaldehyde (20:1, v/v) spray reagent. Needles (5mg.), m.p. 143–145°, were obtained from ethanol, raised to 151–152° on recrystallization. A mixed m.p. with (+)-3,4-*cis*-diacetoxy-3',4',7-trihydroxy-2,3-*cis*-flavan (m.p. 152.5°; Drewes & Roux, 1965) was 143–145° (Found: C, 63.3; H, 6.0. C₂₂H₂₄O₈ requires C, 63.5; H, 5.8%).

(+)-3',4',7-Trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol. The higher- R_F (0.55) bands from the separation of the Craig fractions 33–43 and 44–65 on Whatman no. 3 paper with 2% acetic acid were eluted as above, and a small quantity of the eluate from tubes 44–65 was further separated with water-saturated butan-2-ol. Two-dimensional chromatography showed that the product consisted mainly of one component, with a second of lower concentration underlying it. These had R_F 0.56 and 0.58 (2% acetic acid), and 0.69 and 0.75 (water-satd. butan-2-ol), respectively. After methylation with diazomethane examination of the mixture by paper ionophoresis (cf. Drewes & Roux, 1964b) showed the presence of two compounds. The compound of higher concentration had a migration of –1.80 cm. in sodium borate and was presumed to be the (–)-2,3-*cis*-3,4-*trans* isomer (see below), whereas the compound of low concentration had a mobility of +3.0 cm. corresponding to 2,3-*trans*-3,4-*cis* analogues only (Drewes & Roux, 1964b, 1965). No attempt was made to isolate the latter isomer in view of its low concentration.

Comparison with the (+)-2,3-*trans*-3,4-*cis* enantiomer (Drewes & Roux, 1965) on two-dimensional paper chromatograms showed that the compounds had the same mobilities in both solvent systems (0.75, 0.58). However, comparison with the (–)-2,3-*trans*-3,4-*cis* isomer (see below) showed that the compounds had the same mobility (0.75) in the partitioning mixture but differing mobilities [0.58 and 0.54 for (+) and (–)-forms respectively] in 2% acetic acid (cf. Table 1).

4-Ethyl ether of (–)-3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*trans*-diol. After separation in 2% (v/v) acetic acid and elution of the higher- R_F material from grouped fractions 33–43 and 44–65 as above, the aqueous-alcoholic eluates were left at 0° for 6 weeks. These were evaporated to small volume at 50°, and finally to dryness in a vacuum desiccator. The fractions yielded 450 and 625mg. of brown powder respectively. Examination by two-dimensional chromato-

Table 1. R_F values of diastereoisomeric 3',4',7-tri-hydroxyflavan-3,4-diols

Diastereoisomers	Water-satd. butan-2-ol	2% (v/v) Acetic acid
(+)-Forms		
2,3-trans-3,4-trans	0.79	0.58
2,3-trans-3,4-cis	0.75	0.58
2,3-cis-3,4-cis	0.62	0.39
2,3-cis-3,4-trans	0.69	0.50
(-)-Forms		
2,3-trans-3,4-trans	0.79	0.53
2,3-trans-3,4-cis	0.75	0.54
2,3-cis-3,4-cis	0.62	0.43
2,3-cis-3,4-trans	0.69	0.56

graphy indicated that the mixture of diols had been converted mainly into a compound of higher R_F , 0.80 and 0.61 in water-satd. butan-2-ol and 2% (v/v) acetic acid respectively.

The product from fraction 33-43 (450 mg.) was accordingly separated on 16 sheets of Whatman no. 3 paper, with water-saturated butan-2-ol, and the band at R_F 0.80 was cut out, eluted and concentrated to dryness under vacuum (59 mg.). Although the product was almost pure by two-dimensional chromatography, it failed to crystallize. The above separation was repeated and from the eluates clusters of needles (18 mg.) separated on concentration. Similarly fraction 44-65 gave needles (35 mg.) that blackened beyond 200°. The crude crystals (53 mg.) were recrystallized twice from ethanol-water (1:1, v/v) (3 ml.) to give needles, m.p. 178-184°, that became purple and blackened on further heating, $[\alpha]_D^{21} - 18.3 \pm 1.0^\circ$ (c 0.5 in ethanol) (Found: C, 63.0; H, 6.1; loss at 140° for 2 hr. under vacuum, 3.4. $C_{17}H_{18}O_6 \cdot \frac{1}{2}H_2O$ requires C, 62.4; H, 5.8; H_2O , 2.8%).

A mixed m.p. with the hemihydrate of the 4-ethyl ether of (+)-2,3-cis-3,4-trans-leuco-fisetinidin (m.p. 187°, $[\alpha]_D + 22^\circ$; cf. Drewes & Roux, 1965) gave 178-180° with decomposition.

Conversion of the 4-ethyl ether into the (-)-2,3-cis-3,4-trans-leuco-fisetinidin. Crystalline 4-ethyl ether (5 mg.) (R_F 0.80 and 0.61; cf. above) was heated on the steam bath with 0.1 N-acetic acid (2 ml.) for 20 min. Examination by two-dimensional chromatography showed an almost quantitative conversion into the free diol (R_F 0.69, 0.56). By comparison the (+)-enantiomer (Drewes & Roux, 1965) has the same R_F (0.69) in the partitioning mixture, but a lower R_F (0.50) in 2% acetic acid (cf. Table 1).

Interconversion of the isomeric (-)-2,3-cis-3,4-cis- and 2,3-cis-3,4-trans-leuco-fisetinidins

The crystalline (-)-3',4',7-trihydroxy-2,3-cis-flavan-3,4-cis-diol (3 mg.), m.p. 147°, R_F 0.62 and 0.43 (cf. Table 1), was heated on a steam bath with 0.01 N-HCl (0.5 ml.) for 10 min. Two-dimensional chromatography showed a 40% conversion into the (-)-2,3-cis-3,4-trans isomer (R_F 0.69 and 0.56).

Epimerization of (-)-3',4',7-trihydroxyflavan-2,3-trans-3,4-trans-diol [(-)-leuco-fisetinidin]

Quebracho (*Schinopsis lorentzii*)-heartwood drillings (cf. Roux & Evelyn, 1958) (1.5 kg.) were extracted in the cold

with ethyl acetate (7.5 ml.) over a 7-day period and the extract was evaporated to dryness under vacuum to give a light-brown solid (150 g.). The solid was subjected to countercurrent separation in a Craig machine, the upper and lower phases of water-butan-2-ol-light petroleum (b.p. 60-80°) (5:4:1, by vol.) being used (cf. Roux & Paulus, 1960). After 160 transfers (-)-leuco-fisetinidin was distributed mainly in tubes 81-102. The combined solids from these tubes were streaked on to Whatman no. 3 sheets (18½ in. × 22½ in.) (300 mg./sheet) and developed with 2% (v/v) acetic acid. By cutting out the band R_F at 0.55 and eluting with 70% (v/v) ethanol a total of 6.0 g. of (-)-leuco-fisetinidin, m.p. 126-130°, was obtained.

(-)-Leuco-fisetinidin (500 mg.) was epimerized by autoclaving, as described by Drewes & Roux (1965), in water (50 ml.) containing 1.5-3.0 mg. of $KHCO_3$ at 15 lb./in.² for 3 hr. This gave the best yield of conversion products without side reactions. In addition to the starting material (R_F 0.79 and 0.53), two-dimensional chromatograms on Whatman no. 1 paper showed the presence of a new compound (R_F 0.62 and 0.43). The product was diluted with ethanol and applied to 15 sheets of Whatman no. 3 paper (5 ml./sheet) and the chromatograms were developed in 2% (v/v) acetic acid. Two reducing bands (ammoniacal $AgNO_3$) at R_F 0.45 and 0.55 were cut out and eluted as above.

(-)-3',4',7-Trihydroxy-2,3-cis-flavan-3,4-cis-diol. The eluates from the lower- R_F (0.45) band from two epimerizations (1 g. of starting material) were combined, concentrated to small volume under vacuum and applied to eight pre-washed (with water) Whatman no. 3 sheets. The chromatograms were developed by the downward method with water-satd. butan-2-ol. From a strongly reducing band at R_F 0.63, fine needles (35 mg.) were obtained from ethanol-water (1:1, v/v) by the usual method of elution. The compound sintered strongly at 106° and melted gradually from 140° upwards with reddening and decomposition, $[\alpha]_D^{20} - 47.5 \pm 0.3^\circ$ [c 0.7 in acetone-water (1:1, v/v)] (Found: C, 55.3; H, 5.8; loss at 100° for 2 hr. under vacuum, 12.6. $C_{15}H_{14}O_6 \cdot 2H_2O$ requires: C, 55.2; H, 5.5; H_2O , 11.0%). Mixed m.p. with (+)-3',4',7-trihydroxy-2,3-cis-flavan-3,4-cis-diol ($[\alpha]_D + 48.5^\circ$, m.p. 105-106°, and m.p. of the anhydrous form 146-150° after sintering at 65-67°; Drewes & Roux, 1965) gave sintering at 125-130°, and melting with decomposition from 138°. A mixed m.p. with the natural (-)-2,3-cis-3,4-cis-leuco-fisetinidin from *G. coleosperma* showed no depression, m.p. 140° onwards with reddening and decomposition. The R_F values of the natural and synthetic compounds, R_F 0.62 and 0.43, were also identical (cf. Table 1).

(-)-3',4',7-Trimethoxy-2,3-cis-flavan-3,4-cis-diol. The diol (82 mg.) was methylated with an excess of diazomethane at 0° in methanol-ether for 48 hr. Buff needles (52 mg.), m.p. 95°, were obtained from ethanol-water. These were recrystallized from ethanol to give needles (36 mg.), m.p. 138-140° after drying at 80° for 2 hr., $[\alpha]_D^{20} - 64.2 \pm 1.0^\circ$ [c 0.7 in acetone-water (1:1, v/v)] (Found: C, 65.2; H, 6.1. $C_{18}H_{20}O_6$ requires C, 65.1; H, 6.1%).

(+)-3',4',7-Trimethoxy-2,3-cis-flavan-3,4-cis-diol (Drewes & Roux, 1965) had m.p. 134°, $[\alpha]_D + 58.8^\circ$.

(-)-3,4-cis-Diacetoxy-3',4',7-Trimethoxy-2,3-cis-flavan. The above trimethyl ether (9 mg.) was acetylated with acetic anhydride (0.1 ml.) and pyridine (0.1 ml.) for 24 hr. at room temperature. From water 6.1 mg. of white solid was obtained which crystallized from ethanol in fine needles, m.p. 151-152° (Found: C, 64.0; H, 6.0. $C_{22}H_{24}O_8$ requires C, 63.5;

H, 5.8%). A mixed m.p. with the corresponding (+)-isomer, m.p. 152° (Drewes & Roux, 1965), occurred at 125–127°.

Recovery of (–)-3',4',7-trihydroxy-2,3-trans-flavan-3,4-trans-diol and isolation of associated diastereoisomers

The combined eluates from the higher- R_F (0.55) band in 2% acetic acid, obtained from the epimerization of 500 mg. of starting material, were concentrated to small volume (2 ml.) in a desiccator over CaCl_2 . Needles (65 mg.) separated which were recrystallized three times from water, m.p. 126–130°, $[\alpha]_D^{20} -26.2^\circ$ [c 0.7 in acetone–water (1:1, v/v)]. A mixed m.p. with authentic (–)-leuco-fisetinidin from quebracho heartwood showed no depression.

The above mother liquor was applied to eight pre-washed sheets of Whatman no. 3 paper. The chromatograms were developed with aq. 75% (w/v) phenol (Koeppen, 1961) for 24 hr. Two bands at R_F 0.48 and 0.55 were detected with AgNO_3 spray, the former corresponding in R_F to (–)-leuco-fisetinidin. The band of higher R_F was accordingly cut out and eluted, and the product subjected to two further separations under identical conditions. The brown solid (6 mg.) obtained had almost the same R_F as (–)-leuco-fisetinidin in 2% acetic acid on Whatman no. 1 paper (0.54 and 0.53 respectively), but differing R_F values in water-satd. butan-2-ol (0.75 and 0.79 respectively). On the other hand, the compound had a lower R_F than the (+)-enantiomer in 2% acetic acid (Drewes & Roux, 1965) (0.54 and 0.58 respectively), but both had the same R_F in water-satd. butan-2-ol (0.75) (cf. Table 1).

Methylation of the above diol (6 mg.) with diazomethane at 0° for 48 hr., followed by concentration of the methanolic solution to small volume (1 ml.) under vacuum, and the addition of 2 drops of water, gave needles (5.9 mg.), m.p. 177–180°. Recrystallization from ethanol gave m.p. 186–187°, identical with that of the trimethyl ether below.

(–)-3',4',7-Trimethoxy-2,3-trans-flavan-3,4-cis-diol. Crystals obtained by concentrating the material of higher R_F (2% acetic acid) from an epimerization consisted mainly of unchanged (–)-2,3-trans-3,4-trans-leuco-fisetinidin and melted over the range 110–125°. Such a fraction (55 mg.), m.p. 110–120°, was methylated with diazomethane at 0°. The solution was evaporated to small volume under vacuum, and a few drops of water were added, when clusters of needles (39 mg.), m.p. 94°, formed. From the mother liquor further crystals (9 mg.), m.p. 90–105°, were obtained. Paper ionophoresis (Drewes & Roux, 1964b) in sodium borate showed that the former crystals were almost entirely 3,4-trans isomers, whereas the latter was a 3:2 mixture of 3,4-trans and 2,3-trans-3,4-cis isomers respectively.

The crystals (9 mg.), m.p. 90–105°, were applied to a single sheet (17 cm. wide) of Whatman no. 3 paper, and run under the ionophoretic conditions described by Drewes &

Roux (1964b). The band that had positive migration was cut out and eluted with 0.1 M-sodium borate–ethanol (1:1, v/v). The eluate was acidified with dilute acetic acid, extracted with ethyl acetate, and the combined extracts were washed with dilute NaHCO_3 solution. Concentration under vacuum of the ethyl acetate extract gave a white solid (1.6 mg.), which was recrystallized from ethanol to give needles, m.p. 183–185°.

The above electrophoretic separation was repeated on a larger scale, when from five epimerizations 5.5 mg. of the desired 2,3-trans-3,4-cis isomer was obtained, m.p. 186–187°, $[\alpha]_D^{20} -48 \pm 0.3^\circ$ [c 0.25 in acetone–water (1.8:1.0, v/v)]. The enantiomeric (+)-3',4',7-trimethoxy-2,3-trans-flavan-3,4-cis-diol (Drewes & Roux, 1965) had m.p. 186–187°, and $[\alpha]_D^{20} +42.9^\circ$.

Conversion of (–)-trihydroxy-2,3-cis-flavan-3,4-cis-diol into the (–)-2,3-cis-3,4-trans-isomer

Crystalline (–)-3',4',7-trihydroxy-2,3-cis-flavan-3,4-cis-diol (2 mg.) in 0.01 N-HCl (0.5 ml.) was heated on a steam bath for a total of 20 min. Small portions were withdrawn after 5, 10 and 20 min. and examined by two-dimensional chromatography. The chromatograms showed that after 5 min. the solution consisted of 60% of the 2,3-cis-3,4-cis-diol (R_F 0.62 and 0.43; cf. Table 1) and 40% of the 2,3-cis-3,4-trans isomer (R_F 0.69 and 0.56). After 10 min. a 1:1 mixture was present, and after 20 min. a 60% conversion into the 2,3-cis-3,4-trans isomer had occurred. In the latter case some polymeric material had formed at the origin and a fairly high proportion of a flavonol (fisetin) was present.

Paper-chromatographic examination of heartwood extracts of Guibourtia spp.

The methanolic extracts of each of the heartwoods of *G. coleosperma*, *G. demeusii* and *G. tessmannii* were compared by two-dimensional chromatography. The leuco-fisetinidins were present in approximately the relative proportions shown in Table 2.

Paper-chromatographic comparison of free phenolic diastereoisomeric flavan-3,4-diols

The various natural and synthetic diastereoisomeric leuco-fisetinidins obtained from this and from previous (Drewes & Roux, 1965) work were compared by running them simultaneously on one-dimensional chromatograms in water-satd. butan-2-ol and in 2% (v/v) acetic acid. These relative R_F values were collated with those obtained from two-dimensional chromatograms (cf. Table 1 and Fig. 1).

Table 2. Approximate relative proportions of leuco-fisetinidins in extracts of heartwoods of three *Guibourtia* species

Species	Leuco-fisetinidin.....2,3-cis-3,4-cis	2,3-cis-3,4-trans	2,3-trans-3,4-cis
<i>G. coleosperma</i>	2	5	3
<i>G. demeusii</i>	1	6	3
<i>G. tessmannii</i>	Trace	6	4

Fig. 1. Relative positions of diastereoisomeric leuco-fisetinidins on a two-dimensional chromatogram. Direction 1: water-satd. butan-2-ol; direction 2: 2% (v/v) acetic acid. The isomers are: A, (+)-2,3-*trans*-3,4-*trans*; B, (+)-2,3-*trans*-3,4-*cis*; C, (+)-2,3-*cis*-3,4-*trans*; D, (+)-2,3-*cis*-3,4-*cis*; E, (-)-2,3-*trans*-3,4-*trans*; F, (-)-2,3-*trans*-3,4-*cis*; G, (-)-2,3-*cis*-3,4-*trans*; H, (-)-2,3-*cis*-3,4-*cis*.

DISCUSSION

The heartwood of Rhodesian copalwood (*G. coleosperma*) contains three diastereoisomeric leuco-fisetinidins in very low concentration. Of these, the isomer of lowest R_F and also lowest relative concentration is shown to be a (-)-3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol by means of nuclear-magnetic resonance spectrometry. The absolute configuration is established as 2*R*,3*R*,4*R* (VI) by its identity with a product of epimerization of (-)-leuco-fisetinidin, and by its enantiomorphism with the corresponding compound of known configuration obtained by identical means from (+)-mollisacacidin (Drewes & Roux, 1965).

Similarly, the component of highest relative concentration and intermediate R_F is shown to be a (-)-3',4',7-trihydroxy-2,3-*cis*-flavan-3,4-*trans*-diol by demonstrating the enantiomorphism of its crystalline 4-ethyl ether with the corresponding known ether of the (+)-form (Drewes & Roux, 1965). The above (-)-2,3-*cis*-3,4-*cis*-leuco-fisetinidin is also readily epimerized at C-4 to the (-)-2,3-*cis*-3,4-*trans* diastereoisomer. These factors establish the absolute configuration of the latter as 2*R*,3*R*,4*S* (VII).

The third isomer could not be isolated owing to its close association with the above 2,3-*cis*-3,4-*trans* component, coupled with its low concentration in the wood, but the mobility of its methyl ether in borate buffer is uniquely characteristic of 2,3-*trans*-

3,4-*cis*-flavan-3,4-*trans*-diol analogues (Drewes & Roux, 1964*b*, 1965; Saayman & Roux, 1965). Further, the R_F value of the free phenolic form corresponds to values which are expected from previous work, being in agreement with the (+)-2,3-*trans*-3,4-*cis* isomer (VIII) formed by epimerization from (+)-mollisacacidin (Drewes & Roux, 1965), but migrating in advance of the corresponding (-)-isomer (IX) from (-)-leuco-fisetinidin. This suggests that the natural compound has the 2*R*,3*S*,4*S* (VIII) configuration.

The natural (-)-2,3-*cis*-3,4-*cis* and (-)-2,3-*cis*-3,4-*trans* isomers from *G. coleosperma* have the same absolute configurations as other known pairs of natural flavan-3,4-diols, such as (-)-melacacidin (II, R=OH) and isomelacacidin (III, R=OH), (-)-teracacidin (II, R=H) and isoteracacidin (III, R=H) (cf. Clark-Lewis & Mortimer, 1960; Clark-Lewis & Katekar, 1962; Clark-Lewis *et al.* 1961), which occur in *Acacia* spp. with similar association of these pairs of compounds. However, an exception is the 2,3-*cis*-3,4-*trans*-guibourtacacidin, also from *G. coleosperma* heartwood, which is not accompanied by an isomeric 4',7-dihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol (Saayman & Roux, 1965).

The presence of a 2,3-*trans*-3,4-*cis*-leuco-fisetinidin in *G. coleosperma* heartwood, and previous evidence of the simultaneous occurrence of a guibourtacacidin analogue in this wood (Saayman & Roux, 1965), are the first instances where such natural leuco-anthocyanidins have been recognized.

Natural 3',4',7-trihydroxyflavan-3,4-diols therefore not only constitute the first instance of the natural occurrence of enantiomorphous flavonoids (Clark-Lewis & Roux, 1959) (later fustins were shown to occur similarly; Roux & Paulus, 1960), but now also represent the first instance where the natural existence of all four possible geometrical arrangements for a single flavan-3,4-diol has been recognized. Five of the eight possible diastereoisomeric leuco-fisetinidins occur naturally (IV, R=H, V, VI, VII and VIII), and the remainder (the enantiomers of VI and VII, and also IX) have been formed by epimerization of (+)-mollisacacidin (Drewes & Roux, 1965) and (-)-leuco-fisetinidin.

Since the physical characteristics of all the possible forms of the diastereoisomeric 3',4',7-trihydroxyflavan-3,4-diols are known, those non-crystalline (+)- and (-)- forms that have been isolated from *Pithecolobium dulce* (Rajadurai, 1963) and *Caesalpania coriaria* (Leela & Sastry, 1964) must be either impure or of some modified constitution. Neither of these compounds nor their non-crystalline derivatives, obtained in each instance by precipitation with light petroleum from ethyl acetate solution, show agreement with the diastereoisomeric leuco-fisetinidins or their derivatives.

The heartwood of *G. coleosperma* is hitherto

unique in containing three diastereoisomers of each of two different flavan-3,4-diols, the preponderance of accompanying tannins representing polymeric forms of both of these 4',7-dihydroxy- and 3',4',7-trihydroxy-flavan-3,4-diols. A close taxonomic relationship between *G. demeusii* and *G. tessmannii* and with *G. coleosperma* (cf. Roux & de Bruyn, 1963) is implied by the presence of (–)-2,3-*cis*-3,4-*cis*-, (–)-2,3-*cis*-3,4-*trans*- and 2,3-*trans*-3,4-*cis*-leuco-fisetinidins in approximately the same relative proportions in the heartwoods of each of these species.

Epimerization of (–)-leuco-fisetinidin [(–)-3',4',7-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol] (V) apparently follows the expected course, similar to that proposed for the epimerization of the enantiomeric (+)-mollisacadin (IV, R=H) by Drewes & Roux (1965). However, owing to the closely related R_F values of the conversion products (cf. Table 1) the mixture of (–)-diastereoisomers obtained was resolved into only two 'spots' on two-dimensional chromatograms, the major area consisting of a mixture of (–)-2,3-*trans*-3,4-*trans* (V, starting material), (–)-2,3-*trans*-3,4-*cis* (IX) and (–)-2,3-*cis*-3,4-*trans* (VII) isomers, and the minor area consisting of the (–)-2,3-*cis*-3,4-*cis* form (VI) only. Resolution of the former mixture of three components proved difficult, two of these being amorphous as evidenced by the properties of their (+)-enantiomers (Drewes & Roux, 1964c, 1965). Thus only the predominant (–)-2,3-*trans*-3,4-*trans* (starting material) and (–)-2,3-*trans*-3,4-*cis* isomers could be separated by fractional crystallization and by chromatography respectively, the (–)-2,3-*trans*-3,4-*cis* isomer being obtained in low quantity only. However, after methylation of the mixture the methyl ether of the last-named was separable by paper ionophoresis. Thus the (–)-2,3-*cis*-3,4-*trans* isomer (VII) remains to be isolated from amongst the epimerization products. A similar mixture of diastereoisomeric guibourtacacidins (predominantly 2,3-*cis*-3,4-*trans*-, but with low proportions of 2,3-*trans*-3,4-*trans*- and 2,3-*trans*-3,4-*cis*-4',7-dihydroxyflavan-3,4-diols) from *G. coleosperma* heartwood (Saayman & Roux, 1965), migrate as a single component on two-dimensional chromatograms in the above solvent systems (Roux & de Bruyn, 1963). Such overlap (cf. Fig. 1) emphasizes the possible inaccuracy inherent in the identification of diastereoisomeric flavan-3,4-diols based purely on their concurrence on paper chromatograms.

Examination of the leuco-fisetinidin diastereoisomers (Table 1 and Fig. 1) by paper chromatography shows that in all instances the pairs of (+)- and (–)-enantiomers have the same relative R_F values in water-satd. butan-2-ol but differing R_F values in 2% (v/v) acetic acid. These differences are such that for the 2,3-*trans* forms the (+)-enantiomer always migrates in advance of the

(–)-enantiomer, whereas for the 2,3-*cis* or epi forms the reverse applies. Also in water-satd. butan-2-ol the 2,3-*cis* isomers have lower R_F values than the 2,3-*trans* isomers. These phenomena are parallel to those observed by Roberts & Wood (1953) for catechins, gallocatechins and their epimers.

The presence of a preponderance of leuco-fisetinidin tannins in the heartwoods of *G. coleosperma*, *G. demeusii* and *G. tessmannii* suggests that the associated leuco-fisetinidins of 2,3-*trans*-3,4-*cis*-, 2,3-*cis*-3,4-*cis* and 2,3-*cis*-3,4-*trans* configurations are readily converted into 'condensed' tannins, probably under the partial influence of enzymic systems, as with enantiomers of 2,3-*trans*-3,4-*trans* configuration in other heartwoods (cf. Roux & Evelyn, 1958; Roux & Paulus, 1961, 1962).

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