Configuration of Guibourtacacidin, and Synthesis of Isomeric Racemates

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1. Diacetates of the four possible racemates of 4',7-dimethoxyflavan-3,4-diol have been synthesized. 2. Comparison of their nuclear-magnetic-resonance spectra and the ionophoretic mobilities of the diols in borate buffer with those of the corresponding derivatives of guibourtacacidin shows that the natural 4',7-dihydroxyflavan-3,4-diol has a 2,3-*cis*-3,4-*trans* configuration, but is accompanied by 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis* isomers. These occur in the approximate proportions 5:1:1. 3. The occurrence of guibourtacacidins in *Guibourtia coleosperma* appears to be of taxonomic significance. Their association with a large excess of related tannins in the heartwood suggests that flavan-3,4-diols with these configurations are suitable precursors in tannin biosynthesis.

Rhodesian copalwood (*Guibourtia coleosperma*) contains traces (0.004%) of a 4',7-dihydroxyflavan-3,4-diol (guibourtacacidin) in association with high concentrations of related polymers (Roux, 1959). The compound did not crystallize although pure by chromatography, but synthetic ($\pm$)-4',7-dihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol crystallized readily (Roux & de Bruyn, 1963). Guibourtacacidin formed an amorphous dimethyl ether and dimethyl ether diacetate, and also gave an ethyl ether with ease on handling in ethanolic solution. In all these respects the compound was reminiscent of the free phenolic forms of natural isomelacacidin, isoteracacidin (Clark-Lewis & Mortimer, 1960; Clark-Lewis, Katekar & Mortimer, 1961) and synthetic 2,3-*cis*-flavan-3,4-*trans*-diols (Drewes & Roux, 1964*b*, 1965).

Guibourtacacidin is shown to consist of an isomeric mixture of 4',7-dihydroxyflavan-3,4-diols in which this configuration is predominant.

EXPERIMENTAL AND RESULTS

Nuclear-magnetic-resonance spectra were recorded by Dr K. Pachler, Chemical Physics Group, C.S.I.R., Pretoria, South Africa, and by Dr A. Melera, Varian Associates, Zurich, Switzerland, on Varian A-60 and HR-100 spectrometers with deuteriochloroform as solvent and tetramethylsilane as internal standard. The sample concentration was 50–100 mg./0.5 ml. of solution. Band positions are expressed on the τ -scale. Coupling constants are measured with an accuracy of 0.02 cyc./sec. C, H, Br, methoxyl and acetyl determinations were by K. Jones, Micro-Analytical Laboratory, C.S.I.R., Pretoria. All melting points are uncorrected.

*Synthesis of ($\pm$)-4',7-dimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol (III; R = CH₃)*

Peonol (2-hydroxy-4-methoxyacetophenone). This was prepared by a modification of the method of Adams (1919). Resacetophenone (20 g.), dissolved in a minimum of cold aq. 10% (w/v) NaOH, was treated with dimethyl sulphate (16 ml.). The mixture was heated for 1.5 hr. at 90° with frequent shaking, and the solution kept alkaline (litmus) by the addition of more 10% NaOH solution when required. The mixture was cooled, acidified with dil. HCl and extracted with ether. The ethereal extract was concentrated to about 100 ml., and unchanged resacetophenone was removed by extraction with aq. Na₂CO₃ solution. The ether fraction, when concentrated under vacuum, gave a red-brown oil that crystallized with difficulty from ethanol after treatment with charcoal. Recrystallization from ethanol gave colourless needles (15 g.), m.p. 50° (cf. Tahara, 1891).

2'-Hydroxy-4,4'-dimethoxychalcone. This was prepared by the methods used by Kostanecki & Osius (1899) and Crabtree & Robinson (1922). To a warm solution (80°) of peonol (15 g.) and anisaldehyde (14 g.) in ethanol (200 ml.) under nitrogen was added aq. 50% (w/v) KOH solution (25 g.). The mixture was maintained at 60–70° for 4 hr. with shaking while the nitrogen atmosphere was maintained. After cooling it was diluted with water and acidified with dil. HCl, when the crude chalcone separated. This crystallized from ethanol in bright-yellow needles (7.5 g.), m.p. 113–114° (Found: C, 72.0; H, 5.9; OCH₃, 21.9. Calc. for C₁₇H₁₆O₄: C, 71.8; H, 5.7; OCH₃, 21.8%). Kostanecki & Osius (1899) recorded m.p. 113–114°.

4',7-Dimethoxyflavanol. This was synthesized by methods similar to those used by Juppen & Kostanecki (1904) and King & Bottomley (1954). 2'-Hydroxy-4,4'-dimethoxychalcone (7.5 g.) in aq. 50% (v/v) ethanol (300 ml.) was treated with NaOH (15 g.) and warmed to 80° to obtain solution. The solution was cooled to 70° and 28% (v/v)

(I)* 2,3-*trans*-3,4-*trans* (II)* 2,3-*trans*-3,4-*cis* (III)* 2,3-*cis*-3,4-*cis* (IV)* 2,3-*cis*-3,4-*trans*

* For racemic compounds only the 2*R* enantiomers are shown.

H₂O₂ (50 ml.) was added slowly with shaking. The reaction was exothermic, and after initial cooling was allowed to come to room temperature slowly. The solution was acidified with conc. HCl, when the flavonol separated as pale-yellow needles (2.25 g.), m.p. 195–196° (Found: C, 68.4; H, 4.7; OCH₃, 21.8. Calc. for C₁₇H₁₄O₅: C, 68.5; H, 4.7; OCH₃, 20.8%). Juppen & Kostanecki (1904) recorded m.p. 196–197°.

4',7-Dimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol. 4',7-Dimethoxyflavonol (200 mg.) in ethanol-methanol (3:1, v/v) (40 ml.) was hydrogenated for 2 hr. at 125° and 120 kg./cm.² by using copper chromite catalyst (300 mg.) (Adkins, 1937; see Vogel, 1954). The residual flavonol was filtered off and the diol crystallized from aq. ethanol as white needles (67.5 mg.), m.p. 86–88° (with water of crystallization), and m.p. 154–155° after drying under vacuum over CaCl₂ (Found: C, 68.1; H, 6.2; OCH₃, 21.3. C₁₇H₁₈O₅ requires C, 67.5; H, 6.0; OCH₃, 20.5%).

The use of Raney nickel catalyst gave the same product but in much lower yield (2.5% compared with 33% above) under optimum conditions.

3,4-*cis*-Diacetoxy-4',7-dimethoxy-2,3-*cis*-flavan. 4',7-Dimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol (108 mg.) was acetylated overnight at room temperature with pyridine (0.4 ml.) and acetic anhydride (0.4 ml.). The product was recovered from water, and recrystallized from ethanol in white needles (117 mg.), m.p. 143–144° (Found: C, 65.2; H, 5.7; OCH₃, 16.1; CO·CH₃, 22.6. C₂₁H₂₂O₇ requires C, 65.3; H, 5.7; OCH₃, 16.1; CO·CH₃, 22.3%).

The diacetate was shown to have the 2,3-*cis*-3,4-*cis* configuration by examination of the chemical shifts and spin-spin splitting of 2-, 3- and 4-protons on the heterocyclic ring from its nuclear-magnetic-resonance (n.m.r.) spectrum (Table 1).

Synthesis of 4',7-dimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol (I; R = CH₃)

2'-Acetoxy-4,4'-dimethoxychalcone (cf. Kostanecki & Osius, 1899). 2'-Hydroxy-4,4'-dimethoxychalcone (19 g.), dissolved in a minimum of pyridine (30 ml.), was acetylated

with acetic anhydride (60 ml.) at room temperature. The thick yellow oil obtained on pouring into water was triturated with CCl₄, when the acetate crystallized as light-yellow plates. These crystallized from ethanol as light-yellow needles (20 g.), m.p. 107° (Found: C, 69.9; H, 5.8; OCH₃, 19.4; CO·CH₃, 13.5. Calc. for C₁₉H₁₈O₅: C, 69.9; H, 5.6; OCH₃, 19.0; CO·CH₃, 13.2%). Kostanecki & Osius (1899) recorded m.p. 103–104°.

2'-Acetoxy-4,4'-dimethoxychalcone dibromide. 2'-Acetoxy-4,4'-dimethoxychalcone (20 g.) was dissolved in CCl₄ (350 ml.), and bromine (10 g.) in CCl₄ (40 ml.) was added cautiously. The solution was left standing overnight at room temperature and evaporated at 50° under reduced pressure to small volume. On cooling the crude chalcone dibromide precipitated as orange crystals. These were recrystallized from benzene-light petroleum (b.p. 40–60°) (1:1, v/v) to give clusters of colourless needles (23 g.), m.p. 147–148° (decomp. and reddening) (Found: C, 47.0; H, 3.9; Br, 33.4; OCH₃, 12.9; CO·CH₃, 8.9. C₁₉H₁₈Br₂O₅ requires C, 46.9; H, 3.7; Br, 32.9; OCH₃, 12.8; CO·CH₃, 8.9%).

4',7-Dimethoxy-2,3-*trans*-dihydroflavonol. 2'-Acetoxy-4,4'-dimethoxychalcone dibromide (5 g.) was dissolved in aq. 80% (v/v) acetone (100 ml.), boiled for 15 min., and then for a further 3 min. with aq. 10% Na₂CO₃ (50 ml.). The mixture was cooled and diluted with water, when an oil settled out. This hardened on standing to form light-yellow needles. These were recrystallized from ethanol to give pale-yellow needles (1.5 g.), m.p. 135° (Found: C, 67.8; H, 5.4; OCH₃, 20.6. Calc. for C₁₇H₁₆O₅: C, 68.0; H, 5.4; OCH₃, 20.7%). Kulkarni & Joshi (1957) recorded m.p. 133°.

3-Acetoxy-4',7-dimethoxy-2,3-*trans*-dihydroflavonol. 4',7-Dimethoxydihydroflavonol (123 mg.) was dissolved in the minimum of pyridine (0.4 ml.), and acetic anhydride (0.65 ml.) was added. The product was worked up as before, and crystallized from ethanol in large shiny plates (60 mg.), m.p. 129–130° (Found: C, 66.4; H, 5.4; OCH₃, 18.0; CO·CH₃, 12.4. Calc. for C₁₉H₁₈O₆: C, 66.7; H, 5.3; OCH₃, 18.1; CO·CH₃, 12.6%). Kulkarni & Joshi (1957) found a different m.p. (140–142°) for the same compound.

From analysis of the n.m.r. spectrum of the compound a 2,3-*trans* arrangement of substituents was shown, as

indicated by the large coupling constants ($J_{2,3}=12.0-12.1$ c./sec.) of the 2- and 3-protons. The following τ values (p.p.m., in parentheses) of the protons were recorded: 3-acetyl protons (8.0) (singlet); 4'- and 7-methoxyl protons (6.19) (singlet); 2-H (4.68) (doublet); 3-H (4.21) (doublet); 8-H (3.55) (doublet); 6-H (3.38) (quartet); 3'-H+4'-H (3.07) (doublet); 2'-H+6'-H (2.58) (doublet); 5-H (2.16) (doublet).

4',7-Dimethoxy-2,3-trans-flavan-3,4-trans-diol. 4',7-Dimethoxy-2,3-trans-dihydroflavonol (800 mg.) in methanol (40 ml.) was hydrogenated with Adams catalyst (79.86% PtO₂; W. C. Hereaus, Hanau, Germany) (550 mg.) for 4 hr., under conditions originally used by Freudenberg & Roux (1954). The product crystallized from aqueous ethanol in colourless needles (452 mg.), m.p. 119–120° (Found: C, 67.1; H, 6.3; OCH₃, 20.8. C₁₇H₁₈O₅ requires C, 67.5; H, 6.0; OCH₃, 20.5%).

Phatak & Kulkarni (1959) obtained a similar compound, m.p. 114–115° (diacetate, m.p. 136°), which was then regarded as 2,3-trans-3,4-cis, from reduction of the dihydroflavonol with LiAlH₄.

3,4-trans-Diacetoxy-4',7-dimethoxy-2,3-trans-flavan. 4',7-Dimethoxy-2,3-trans-flavan-3,4-trans-diol (105 mg.) was acetylated with pyridine (0.4 ml.) and acetic anhydride (0.6 ml.) as above. The product crystallized from ethanol as colourless needles (100 mg.), m.p. 135–136° with sintering at 107–108° (Found: C, 65.2; H, 5.6; OCH₃, 16.0; CO·CH₃, 22.0. C₂₁H₂₂O₇ requires C, 65.3; H, 5.7; OCH₃, 16.1; CO·CH₃, 22.3%).

The n.m.r. spectrum of the diacetate (Table 1) confirmed its 2,3-trans-3,4-trans arrangement of substituents, and the purity of the compound.

Synthesis of 4',7-dimethoxy-2,3-trans-flavan-3,4-cis-diol (II; R=CH₃)

4',7-Dimethoxy-2,3-trans-flavan-3,4-trans-diol was epimerized at C-4 by using NaBH₄-BF₃ in diglyme [bis-(2-methoxyethyl) ether] to give the 2,3-trans-3,4-cis isomer (cf. Kashikar & Kulkarni, 1961).

To a suspension of NaBH₄ (280 mg.) in dry diglyme (20 ml.) was added ethereal BF₃ (45%) (1.5 ml.). A solution of 4',7-dimethoxy-2,3-trans-flavan-3,4-trans-diol (300 mg.) in dry diglyme (15 ml.) was added to the above suspension with stirring. The mixture was kept at room temperature for 2 hr., heated at 70° for 2 hr. and finally left to stand overnight at room temperature. The excess of reagent was decomposed with acetic acid (5 ml.), the mixture diluted with water and the product extracted with ether. The ethereal extract was washed with water, dried over Na₂SO₄ and evaporated to dryness under reduced pressure at 40°. The product crystallized from ethanol as colourless needles (130 mg.), m.p. 138–140° (Found: C, 67.5; H, 6.2; OCH₃, 20.7. C₁₇H₁₈O₅ requires C, 67.5; H, 6.0; OCH₃, 20.5%).

Phatak & Kulkarni (1959) obtained a similar compound, m.p. 136°, which was then regarded as having the 2,3-trans-3,4-trans arrangement. However, n.m.r. spectrometry of their diacetate showed that it was a mixture of 3,4-trans and 3,4-cis isomers.

3,4-cis-Diacetoxy-4',7-dimethoxy-2,3-trans-flavan. The diol (100 mg.) was acetylated with acetic anhydride (0.4 ml.) and pyridine (0.4 ml.) as before, and the product recovered from water. The diacetate did not crystallize, and was recovered by pouring its solution in acetic acid (0.5 ml.) into an excess of water (114 mg.), m.p. 80–83° (Found: C, 65.4; H, 5.9; OCH₃, 16.4; CO·CH₃, 22.0. C₂₁H₂₂O₇ requires C, 65.3; H, 5.7; OCH₃, 16.1; CO·CH₃, 22.3%).

The n.m.r. spectrum of the diacetate (Table 1) confirmed its 2,3-trans-3,4-cis arrangement of substituents, as well as its purity.

Synthesis of 4',7-dimethoxy-2,3-cis-flavan-3,4-trans-diol (IV; R=CH₃)

Attempted epimerization of 4',7-dimethoxy-2,3-cis-flavan-3,4-cis-diol. The conditions were identical with those used by Kashikar & Kulkarni (1961) and successfully employed above for the conversion of the 2,3-trans-3,4-trans into the 2,3-trans-3,4-cis isomer. The product from 300 mg. of starting material was dissolved in a minimum of ethanol and

Table 1. Nuclear-magnetic-resonance spectra of 3,4-diacetoxy-4',7-dimethoxyflavan racemates

	(a) Chemical shifts: τ values (p.p.m.)							
	Me (acetyl)		Me (methoxyl)		H			
	3-	4-	7-	4'	2-	3-	4-	5-
2,3-trans-3,4-trans	8.15	8.00	6.24	6.21	4.95	4.49	3.78	2.91
2,3-trans-3,4-cis	8.16	7.87	6.25	6.20	4.82	4.53	3.84	2.80
2,3-cis-3,4-trans	8.11	7.90	6.23	6.19	4.70	4.77	4.15	2.65
2,3-cis-3,4-cis	8.08	7.92	6.22	6.20	4.73	4.40	3.73	2.89
Guibourtacadin	8.12	7.89	6.23	6.19	~4.70	~4.70	4.13	2.65

(b) Spin-spin coupling constants for 2-, 3- and 4-protons (cyc./sec.)

	$J_{2,3}$	$J_{3,4}$
2,3-trans-3,4-trans	9.0	6.9
2,3-trans-3,4-cis	10.3	3.1
2,3-cis-3,4-trans	0.9*	2.8
2,3-cis-3,4-cis	1.2*	4.2
Guibourtacadin	<1.0	2.7

* Values obtained by analysis of 3-proton quartet.

Table 2. *Thin-layer chromatography and paper ionophoresis of 4',7-dimethoxyflavan-3,4-diol racemates*

Chromatography was carried out on Kieselgel G (Merck) 0.25 mm. thick in chloroform-ethyl acetate (2:1, v/v) with 10 cm. migration. The chromatograms were sprayed with toluene-*p*-sulphonic acid (Roux, 1957) and conc. H₂SO₄-40% formaldehyde (20:1, v/v).

Racemate	Thin-layer chromatography <i>R_F</i>	Ionophoretic mobility (cm.)
2,3- <i>trans</i> -3,4- <i>trans</i>	0.28	-2.1
2,3- <i>trans</i> -3,4- <i>cis</i>	0.31	+3.0
2,3- <i>cis</i> -3,4- <i>cis</i>	0.43	+1.7
Product of attempted inversion of 2,3- <i>cis</i> -3,4- <i>cis</i> racemate		
(i) crystals, m.p. 155-156°	0.43	+1.7
(ii) mother liquor	0.46*	+1.9*
	0.94†	-1.7†

* Residual 2,3-*cis*-3,4-*cis* isomer showed different mobilities due to impurities.

† Possibly 4-ethyl ether of desired 2,3-*cis*-3,4-*trans* racemate.

Table 3. *Thin-layer chromatography of 3,4-diacetoxy-4',7-dimethoxyflavans*

Chromatography was carried out on Kieselgel G (Merck) 0.25 mm. thick in benzene-acetone (19:1, v/v) with 10 cm. migration. The chromatograms were sprayed with conc. H₂SO₄-40% formaldehyde (20:1, v/v) to give the colours indicated.

	<i>R_F</i>	Colour with spray
2,3- <i>trans</i> -3,4- <i>trans</i>	0.65	Brick red
2,3- <i>trans</i> -3,4- <i>cis</i>	0.60	Brick red
2,3- <i>cis</i> -3,4- <i>cis</i>	0.64	Pink
2,3- <i>cis</i> -3,4- <i>trans</i>	0.51	Brick red
Acetylation products of 2,3- <i>cis</i> -3,4- <i>cis</i> isomer		
	0.51	Brick red
	0.75	Pink

water added (2 drops), when colourless needles (106 mg.) formed, m.p. and mixed m.p. with the 2,3-*cis*-3,4-*cis* isomer, 155-156°. The mother liquors were examined by paper electrophoresis (Drewes & Roux, 1964a) and by thin-layer chromatography on Kieselgel G (E. Merck A.-G., Darmstadt, Germany) (Table 2). These indicated that the desired 2,3-*cis*-3,4-*trans* isomer had not formed, although traces of a high-*R_F* (0.94) component might represent its 4-ethyl ether.

Repetition of the reaction under milder conditions (50° for 1 hr.) gave similar results, but with a higher (84%, compared with 32% above) recovery of starting material.

Attempted total synthesis of (±)-4',7-dimethoxy-2,3-cis-flavan-3,4-trans-diol. With 2'-acetoxy-4,4'-dimethoxychalcone dibromide as starting material, attempts were made to synthesize the above compound via the 3-bromoflavanone and 3-bromoflavan-4β-ol by the methods of Kulkarni (1962) and Joshi & Kulkarni (1957) (cf. Clark-Lewis, Jackman & Williams, 1962). Difficulties were encountered in the conversion of the 3-bromoflavan-4β-ol into the desired (±)-4',7-dimethoxy-2,3-*cis*-flavan-3,4-*trans*-diol.

(±)-3,4-*trans*-Diacetoxy-4',7-dimethoxy-2,3-*cis*-flavan. Acetylation of the 2,3-*cis*-3,4-*cis* isomer was performed according to the method of Fujise, Adachi & Hishida (1962a), Fujise, Onuma & Hishida (1962b) and L. R. Williams (unpublished work) (cf. Clark-Lewis, Jackman & Spotswood, 1964).

(±)-4',7-Dimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol (400 mg.) and anhydrous sodium acetate (110 mg.) were added to acetic anhydride (4 ml.) and acetic acid (1 ml.). The mixture was heated under reflux for 4 hr., poured into ice-water and allowed to harden. Thin-layer chromatograms of the product in benzene-acetone (19:1, v/v) (10 cm. migration) on Kieselgel G (Merck) (0.25 mm. thickness), when sprayed with conc. H₂SO₄-40% formaldehyde (20:1, v/v), showed two main red bands (Table 3), in addition to four minor impurities at *R_F* 0.25-0.42. The main bands were separated by the preparative method with six 20 cm. × 20 cm. chromatoplates and a layer 1 mm. thick under identical conditions, except that chromatograms were developed for 1.5-2.0 hr. to the top of the plates (18.0 cm. migration). The two main bands were located, and the silica gel was stripped off the plate and extracted with benzene-ethanol (9:1, v/v). The product with lower *R_F* crystallized from ethanol as colourless prisms (213 mg.), m.p. 109-110° after sintering at 104°. Repeated crystallization from ethanol gave colourless prisms (120 mg.), m.p. 109-110° without sintering at 104° (Found: C, 65.3; H, 5.9; OCH₃, 17.0; CO·CH₃, 22.6. C₂₁H₂₂O₇ requires C, 65.3; H, 5.7; OCH₃, 16.1; CO·CH₃, 22.3%). The n.m.r. spectrum of the diacetate (cf. Table 1 and the Discussion section) showed that the compound was mainly the (±)-3,4-*trans*-diacetoxy-4',7-dimethoxy-2,3-*cis*-flavan, together with a low admixture of the 2,3-*cis*-3,4-*cis* isomer (starting material). Continued treatment of the product with the

acetylating mixture for a further 3 hr. did not alter the m.p. (108–109°) appreciably, or eliminate the 2,3-*cis*-3,4-*cis* isomer.

The higher-*R_F* product gave long colourless needles (40 mg.) from ethanol, m.p. 131–132°.

Paper ionophoresis of the dimethyl ether of guibourtacacidin. Paper ionophoresis of methylated guibourtacacidin was run in sodium borate under the conditions used by Drewes & Roux (1964a). Spraying with toluene-*p*-sulphonic acid showed the main band at –2.10 cm., accompanied by a weak band at ±3.0 cm. These correspond to 2,3-*cis*-3,4-*trans* and/or 2,3-*trans*-3,4-*trans* (–2.10 cm.), and 2,3-*trans*-3,4-*cis* (+3.20 cm.) isomers when compared with previous work (Drewes & Roux, 1964a, 1965). A band corresponding to the 2,3-*cis*-3,4-*cis* isomer (+1.70 cm.) was absent.

Nuclear-magnetic-resonance spectrum of the diacetyl-dimethoxy derivative of guibourtacacidin.

The n.m.r. spectrum of the amorphous diacetyl dimethyl ether showed (Table 1) that this derivative of the flavan-3,4-diol has a predominantly 2,3-*cis*-3,4-*trans* arrangement of substituents.

However, when the spectrum was run at high amplitude, weak signals of the 2- and 4-protons of the 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis* arrangements are also evident. These could be recognized from the signals of the corresponding protons of the pure racemates (cf. Table 1) and from their mixture (see below).

Signals from the 2- and 4-protons of the 2,3-*cis*-3,4-*cis* isomer were completely absent.

Examination of the integral curve of the signals from the 4-protons, which are well separated (cf. Table 1), indicates that the isomers are present in the proportions 2,3-*cis*-3,4-*trans* (5), 2,3-*trans*-3,4-*trans* (1) and 2,3-*trans*-3,4-*cis* (1).

Nuclear-magnetic-resonance spectrum of a lithium aluminium hydride reduction product of (±)-4',7-dimethoxydihydroflavonol

Phatak & Kulkarni (1959) obtained a mixed diol, m.p. 113–121°, from reduction of the above dihydroflavonol with LiAlH₄. This they resolved by acetylation and fractional crystallization of the diacetates. The respective diacetates (m.p. 132°) (and residue from mother liquors) were hydrolysed to afford two diols, m.p. 114–115° (also available by reduction with NaBH₄) and 136°.

The latter, kindly made available by Dr A. B. Kulkarni, and examined for its infrared spectrum by Roux & de Bruyn (1963), was acetylated with acetic anhydride-pyridine at room temperature as above. The product was recovered from water, dried and its n.m.r. spectrum examined. Comparison with the spectra of the 2,3-*trans*-3,4-*trans* and 2,3-*trans*-3,4-*cis* diacetates (Table 1) showed, particularly from the signals of the well-separated 4-acetyl protons, that their product consists of these two racemates in the ratio 11:9.

DISCUSSION

The relative configuration of guibourtacacidin, an optically active 4',7-dihydroxyflavan-3,4-diol from *G. coleosperma*, is determined by complete

synthesis of the 3,4-diacetates of the methyl ethers of four possible isomeric racemates, thus enabling comparison of the n.m.r. spectra of the natural and synthetic compounds to be made.

Previous attempts at synthesis of guibourtacacidin isomers were made by Phatak & Kulkarni (1959), who reduced (±)-4',7-dimethoxydihydroflavonol with lithium aluminium hydride to form a mixed 3,4-diol. This was resolved into 2,3-*trans*-3,4-*cis*- and 2,3-*trans*-3,4-*trans*-diols by fractional crystallization of their acetates. However, from the present work, these products do not appear to be pure geometrical isomers, as evidenced by the n.m.r. spectrum of the amorphous diacetate of their diol, m.p. 136° (presumed 3,4-*trans*), and the low melting point (114–115°) of a diol (presumed 3,4-*cis*). These early structural assignments are no longer accurate, and must be reversed.

The racemate of 2,3-*trans*-3,4-*trans* configuration (I; R=CH₃), m.p. 119–120°, was obtained by synthesis of (±)-4',7-dimethoxy-2,3-*trans*-dihydroflavonol via the chalcone dibromide (Joshi & Kulkarni, 1957) followed by catalytic hydrogenation of the former with Adams catalyst (Freudenberg & Roux, 1954). The 2,3-*trans*-3,4-*cis* racemate (II; R=CH₃), m.p. 138–140°, was formed from the 2,3-*trans*-3,4-*trans* isomer by epimerization at C-4 (Saayman & Roux, 1964) with boron trifluoride and sodium borohydride in diglyme (cf. Kashikar & Kulkarni, 1961).

The 2,3-*cis*-3,4-*cis* racemate (III; R=CH₃), m.p. m.p. 154–155°, was obtained from synthetic 4',7-dimethoxyflavonol in good yield (33%) by hydrogenation with copper chromite (cf. Fujise, Fujise & Hishida, 1963) under pressure. On the other hand, synthesis by the conventional route of the 2,3-*cis*-3,4-*trans* isomer (IV; R=CH₃) gave difficulties similar to those experienced by Kulkarni & Joshi (1957) in their attempted preparation of stereochemically related 5-methoxyflavan-3,4-diols. Since only 6-methyl derivatives of corresponding configuration have been prepared hitherto (Kulkarni & Joshi, 1957; Joshi & Kulkarni, 1957; Kulkarni, 1962; Clark-Lewis *et al.* 1962), complete synthesis by their method of analogues that contain either 5- or 7-methoxyl substituents apparently presents serious difficulties. Similarly, epimerization of 2,3-*cis*-3,4-*cis* to the 2,3-*cis*-3,4-*trans* isomer with sodium borohydride-boron trifluoride was unsuccessful, although involving similar inversion of a 4(*eq*)- to a 4(*ax*)-hydroxyl as in the successful epimerization of the 2,3-*trans*-3,4-*trans* to the 2,3-*trans*-3,4-*cis* isomer. However, the desired 2,3-*cis*-3,4-*trans*-diacetate, m.p. 109–110°, was obtained by prolonged acetylation (cf. Fujise *et al.* 1962a,b; Clark-Lewis *et al.* 1964) of the 2,3-*cis*-3,4-*cis*-diol, followed by preparative thin-layer chromatography of the reaction products on silica gel.

Thus selective epimerization at C-4 by the acetylation technique converts flavan-3,4-*cis*-diols into flavan-3,4-*trans*-diacetates (Clark-Lewis *et al.* 1964), the reaction probably proceeding via a cyclic intermediate, whereas sodium borohydride-boron trifluoride apparently effects the reverse, the sodium borate formed probably stabilizing the 3,4-*cis*-diols through complex-formation (cf. Drewes & Roux, 1964a). In the latter epimerization the presence of both boron trifluoride and sodium borohydride is required (Kashikar & Kulkarni, 1961), and the reaction mechanism must therefore be analogous to the lithium aluminium hydride-aluminium chloride epimerizations of alcohols (Elie & Rerick, 1960).

The methyl ethers and their diacetates show significant R_F differences by thin-layer chromatography on silica gel by using the irrigants chloroform-ethyl acetate for the methyl ethers, and benzene-acetone for their diacetates. These chromatographic methods, coupled with paper ionophoresis in sodium borate (Drewes & Roux, 1964a), provide simple techniques for establishing the identity of the methyl ethers of geometrical isomers of flavan-3,4-diols.

The relative configurations of all four racemates (I, II, III and IV; R = CH₃, only 2*R* enantiomers indicated) were established by n.m.r. spectroscopy and paper ionophoresis by comparison with previous work (cf. Clark-Lewis *et al.* 1962; Drewes & Roux, 1964a,b, 1965). Guibourtacacidin dimethyl ether diacetate had an n.m.r. spectrum similar to that of the corresponding 2,3-*cis*-3,4-*trans* racemate (IV; R = H) (cf. Table 1). These spectra are characterized by the uniquely high upfield position ($\tau = 4.14$ p.p.m.) of the 4-proton ($J_{3,4} = 2.7-2.8$ cyc./sec.), which permits unequivocal differentiation from other spectra (cf. Table 1). This assignment is supported by the non-crystallinity of guibourtacacidin, and evidence of the ease of formation of its 4-ethyl ether (Roux & de Bruyn, 1963), properties that are characteristic of natural and synthetic flavan-3,4-diols of this configuration (cf. Clark-Lewis & Mortimer, 1960; Clark-Lewis *et al.* 1961; Drewes & Roux, 1964a,b). Paper ionophoresis of the methyl ether in borate buffer (Table 2), and the inability of the methyl ether to form a complex with sodium borate (Roux & de Bruyn, 1963), also support the predominantly 3,4-*trans* assignment for guibourtacacidin.

Evidence of the presence of low proportions of 2,3-*trans*-3,4-*trans* (I; R = H) and 2,3-*trans*-3,4-*cis* (II; R = H) isomers from n.m.r. spectra of guibourtacacidin dimethyl ether diacetate, when run at high amplitude, is not surprising, since epimerization of (–)-leuco-fisetinidin [(–)-3',4',7-trihydroxyflavan-3,4-diol] by the method of Drewes & Roux (1964b, 1965) affords a mixture of four diastereoisomers that is resolved into only two spots by paper

chromatography. The major area consists of a similar and complete overlap of 2,3-*trans*-3,4-*trans*, 2,3-*trans*-3,4-*cis* and 2,3-*cis*-3,4-*trans* isomers, which could not be readily resolved (S. E. Drewes & D. G. Roux, unpublished work). Also, synthetic ($\pm$)-4',7-dihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol and guibourtacacidin were shown to have R_F differences in 2% (v/v) acetic acid (0.52 and 0.55 respectively) and in butan-1-ol-acetic acid-water (6:1:2, by vol.) (0.82 and 0.83) that were very small (Roux & de Bruyn, 1963). Collateral evidence of the presence of 2,3-*trans*-3,4-*trans*, 2,3-*trans*-3,4-*cis* and 2,3-*cis*-3,4-*trans*-4',7-dihydroxyflavan-3,4-diols in *G. coleosperma* in the approximate proportions 1:1:5 is available from the ionophoretic behaviour of methylated guibourtacacidin (cf. Table 2 and Drewes & Roux, 1964a, 1965).

The complete absence of the 2,3-*cis*-3,4-*cis* isomer (III; R = H), as shown by both methods, is surprising considering the hitherto associated occurrence of natural 2,3-*cis*-3,4-*cis* and 2,3-*cis*-3,4-*trans* isomers (cf. Clark-Lewis & Mortimer, 1960; Clark-Lewis *et al.* 1961; Drewes & Roux, 1964c).

The predominant guibourtacacidin (2,3-*cis*-3,4-*trans* isomer) may have the absolute configuration 2*R*:3*S*:4*S* (IV; R = H) or 2*S*:3*R*:4*R* (enantiomer), but the former is favoured purely on biological grounds on account of its association in *G. coleosperma* heartwood with a 3',4',7-trihydroxyflavan-3,4-diol of corresponding absolute configuration (Drewes & Roux, 1964c), and from the observation that flavan-3,4-diols of this absolute configuration (isomelacacidin, isoteracacidin) have been identified amongst the Leguminosae.

The isolated occurrence of guibourtacacids and their related polymers in *G. coleosperma* is possibly of taxonomic significance, serving to distinguish it from closely related *G. tessmannii* and *G. demersii* (Roux & de Bruyn, 1963). The association of a monomeric flavan-3,4-diol of predominantly 2,3-*cis*-3,4-*trans* configuration with a large excess of a polymer, both of which yield the same anthocyanidin, suggests that monomeric forms of this configuration also serve as biogenetic precursors of leuco-anthocyanidin 'condensed tannins'.

The development of redness in guibourtacacidin on pyrolysis (150°) (Roux & de Bruyn, 1963) may now be correlated with its predominantly 2,3-*cis*-3,4-*trans* configuration and therefore 4(*ax*)-hydroxyl arrangement (cf. Drewes & Roux, 1964d).

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