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19. CONFIGURATION OF DIOL GROUPS IN FLAVAN-3,4-DIOLS BY PAPER CHROMATOGRAPHY AND PAPER IONOPHORESIS*

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The significance attached to the formation of isopropylidene derivatives in high yield from methylated flavan-3,4-*trans*-diol precursors of wattle tannins results in unreliable conclusions regarding the stereochemistry of the diol group, owing to inversion of the 4-hydroxyl group under acid conditions (Drewes & Roux, 1964). Accurate conclusions are, however, possible from comparative oxidation rates of 3,4-*cis*- and 3,4-*trans*-diols with lead tetra-acetate (Bokadia *et al.* 1961) or periodate (Drewes & Roux, 1964), or from the spin-spin coupling constants of the 2-, 3- and 4-protons obtained from their nuclear-magnetic-resonance spectra (Corey, Philbin & Wheeler, 1961; Clark-Lewis & Jackman, 1961; Lillya, Drewes & Roux, 1963). Moreover, formation of isopropylidene derivatives by ketal exchange offers a possible alternative for deciding between alternative arrangements (Brown & MacBride, 1963).

The present work shows that correlation of both paper-chromatographic and paper-ionophoretic behaviour of methylated flavan-3,4-diols with their known stereochemistry offers simple but apparently reliable criteria for differentiating between *cis*- and *trans*-glycol arrangements.

EXPERIMENTAL AND RESULTS

Nuclear-magnetic-resonance spectra were recorded on a Varian A-60 spectrometer with deuteriochloroform as solvent and tetramethylsilane as internal standard. Band positions are expressed as p.p.m. downfield with the standard as origin (Table 1). Coupling constants are measured with an accuracy of ± 0.2 cye./sec. C, H,

methoxyl and acetyl estimations are by K. Jones, Micro-analytical Laboratory, C.S.I.R., Pretoria. All melting points are uncorrected.

Synthesis of (±)-3',4',5',7-tetramethoxy- and (±)-3',4',7-trimethoxy-2,3-cis-flavan-3,4-cis-diols

(±)-3',4',5',7-Tetramethoxy-2,3-*cis*-flavan-3,4-*cis*-diol. (±)-Dihydrorobinetin from *Robinia pseudacacia* was methylated with diazomethane to give (±)-3',4',5',7-tetramethoxyflavan-3-ol-4-one, m.p. 165°. The methylated dihydroflavonol (500 mg.) in ethanolic $\text{N-H}_2\text{SO}_4$ (20 ml.) was refluxed for 6 hr. while O_2 was bubbled through the solution. 3',4',5',7-Tetramethoxyrobinetin (3',4',5',7-tetramethoxyflavonol), m.p. 194–195° (300 mg.), crystallized from the solution (Found: C, 63.7; H, 5.1. Calc. for $\text{C}_{19}\text{H}_{18}\text{O}_7$: C, 63.7; H, 5.0%). Dean & Nierenstein (1925) and Shah & Kulkarni (1958) record m.p. 193° and 194–195° respectively for the same compound.

Raney nickel, freshly prepared from Raney alloy (Hopkin and Williams Ltd.: Ni-Al; 42, 58), was used for reduction of the flavonol. To tetramethoxyrobinetin (192 mg.) dissolved in ethanol-methanol (3:1, v/v) (50 ml.) was added freshly prepared Raney nickel (0.4 g.) and the mixture heated with shaking under H_2 for 4.75 hr. at 90–95° and 38.5 kg./cm.². After removal of the catalyst the solution was concentrated to small volume (2 ml.). White crystals (92.4 mg.), m.p. 160°, separated at room temperature (48% yield). Recrystallization from ethanol raised the m.p. to 165° (Found: C, 63.5; H, 6.3; OCH_3 , 33.5. $\text{C}_{19}\text{H}_{22}\text{O}_7$ requires: C, 63.0; H, 6.1; OCH_3 , 34.2%).

(±)-3,4-*cis*-Diacetoxy-3',4',5',7-tetramethoxy-2,3-*cis*-flavan. Acetylation of the diol (100 mg.) with acetic anhydride (0.4 ml.) and pyridine (0.4 ml.) gave the diacetate (90 mg.), which crystallizes from ethanol, m.p. 169° (Found: C, 61.9; H, 5.8; OCH_3 , 27.6; $\text{CO}\cdot\text{CH}_3$, 19.6. $\text{C}_{23}\text{H}_{26}\text{O}_9$ requires: C, 61.9; H, 5.8; OCH_3 , 27.8; $\text{CO}\cdot\text{CH}_3$, 19.3%). Details of the nuclear-magnetic-resonance spectrum of this compound are listed in Table 1.

* Part 18: Drewes & Roux (1964).

$J_{2,3}$	$J_{3,4}$	
(±)-3,4- <i>cis</i> -Diacetoxy-3',4',5',7-tetramethoxy-2,3- <i>cis</i> -flavan	4.1 (4.0)	
~ 1.0		
(±)-3,4- <i>cis</i> -Diacetoxy-3',4',7-trimethoxy-2,3- <i>cis</i> -flavan	3.8 (4.2)	
~ 1.0		
(±)-3-Acetoxy-3',4',5',7-tetramethoxy-2,3- <i>cis</i> -flavan	—	
~ 1.0		

Table 2. Paper chromatography of isomeric methylated flavan-3,4-diols on 0.1 M-sodium borate-impregnated paper

	R_F			
	Borate paper		Normal paper	
	Butan-1-ol	Ethyl acetate	Butan-1-ol	Ethyl acetate
3',4',5',7-Tetramethoxyflavan-3,4-diols				
2,3- <i>cis</i> -3,4- <i>cis</i> (m.p. 165°)	0.75	0.90	0.84	0.90
2,3- <i>trans</i> -3,4- <i>trans</i> (m.p. 229°)	0.87	0.95	0.85	0.90
2,3- <i>trans</i> -3,4- <i>cis</i> (m.p. 167°)	0.68	0.86	0.86	0.90
3',4',7-Trimethoxyflavan-3,4-diols				
2,3- <i>cis</i> -3,4- <i>cis</i> (m.p. 149°)	0.73	0.89	0.78	0.90
2,3- <i>trans</i> -3,4- <i>trans</i> (m.p. 151°)	0.86	0.95	0.81	0.90
2,3- <i>trans</i> -3,4- <i>cis</i> (m.p. 185°)	0.62	0.82	0.82	0.90
3',4',7,8-Tetramethoxyflavan-3,4-diols				
2,3- <i>cis</i> -3,4- <i>cis</i> (m.p. 145°) (melacacidin)	0.64	—	0.79	—
2,3- <i>cis</i> -3,4- <i>trans</i> (amorphous) (isomelacacidin)	0.87	—	0.80	—

Table 3. Paper ionophoresis of isomeric methylated flavan-3,4-diols in 0.1 M-sodium borate

	Mobility (cm.)	M^*
3',4',5',7-Tetramethoxyflavan-3,4-diols		
2,3- <i>cis</i> -3,4- <i>cis</i>	+1.80	+0.57
2,3- <i>trans</i> -3,4- <i>trans</i>	0.00	0.00
2,3- <i>trans</i> -3,4- <i>cis</i>	+3.15	+1.00
3',4',7-Trimethoxyflavan-3,4-diols		
2,3- <i>cis</i> -3,4- <i>cis</i>	+1.50	+0.48
2,3- <i>trans</i> -3,4- <i>trans</i>	-1.85	-0.49
2,3- <i>trans</i> -3,4- <i>cis</i>	+3.20	+1.02
3',4',7,8-Tetramethoxyflavan-3,4-diols		
2,3- <i>cis</i> -3,4- <i>cis</i>	+1.80	+0.57
2,3- <i>cis</i> -3,4- <i>trans</i>	-2.40	-0.76

* Mobility of compound relative to 3',4',5',7-tetramethoxy-2,3-*trans*-flavan-3,4-*cis*-diol.

Isomeric 2,3-*trans*-3,4-*cis* and 2,3-*trans*-3,4-*trans* ($\pm$)-3',4',5',7-tetramethoxy- and ($\pm$)-3',4',7-trimethoxy-flavan-3,4-diols. The synthesis of these compounds has been described (Drewes & Roux, 1964).

Paper chromatography of methylated flavan-3,4-diols. Whatman no. 1 paper, 11 in. $\times$ 18 in., was impregnated with sodium borate by soaking in 0.1 M-sodium borate and air-drying (cf. Swain, 1953). Chromatograms of all methylated flavan-3,4-diols were run simultaneously on single sheets by upward migration for 10 hr. (10–11 in. migration) with water-satd. butan-1-ol and water-satd. ethyl acetate as irrigants. The same compounds were also run in the same solvents on ordinary Whatman no. 1 paper. The R_F values (Table 2) are an average of three runs. Mixtures of isomeric *trans*-*cis* and *trans*-*trans* methylated flavan-3,4-diols were readily separated on the borate paper.

Paper ionophoresis of methylated flavan-3,4-diols. The apparatus used was the horizontal open strip type (Grassman & Hannig, 1952) in which the paper dips at each end into 0.1 M-sodium borate solution, a saturated atmosphere being maintained by enclosure with a plastic lid. The paper, Schleicher and Schull no. 2043 (4 cm. $\times$ 41 cm.), was dipped into borate solution and, after equilibration (1 hr.), the compound was applied as a streak at the origin. A potential of 170–175 v, giving a current of 5 ma, was applied for

18 hr. with a Shandon Vokam power supply type 2541. The rise in temperature was negligible, making cooling unnecessary.

After drying, the strips were sprayed with ethanolic toluene-*p*-sulphonic acid (Roux, 1957). The sodium borate caused interference, but repeated spraying and heating at 80–100° gave sharply defined pink bands. The ionophoretic mobilities (Table 3) are also expressed in relation to the mobility of ($\pm$)-3',4',5',7-tetramethoxy-2,3-*trans*-flavan-3,4-*cis*-diol as unity (Table 3). The mobilities of the compounds examined were reproducible. Mixtures of isomeric *trans*-*cis* and *trans*-*trans* methylated flavan-3,4-diols were completely resolved by paper ionophoresis run under these conditions.

DISCUSSION

The stereochemistry of synthetic ($\pm$)-3',4',5',7-tetramethoxy- and ($\pm$)-3',4',7-trimethoxy-2,3-*cis*-flavan-3,4-*cis*-diols may be inferred from their method of synthesis, but is confirmed by examination of the spin-spin coupling constants of the 2-, 3- and 4-protons of their 3,4-diacetates (Table 1). Assuming a half-chair conformation (Clark-Lewis & Jackman, 1961; Drewes & Roux, 1964) for the heterocyclic ring and a predominantly equatorial arrangement of the 2-phenyl group, then from the correlation between the dihedral angle of vicinal protons and their coupling constants (Karplus, 1959; Conroy, 1960) the coupling constants of the above ($J_{2,3} \sim 1$ cyc./sec. and $J_{3,4} = 3.8\text{--}4.2$ cyc./sec.) are in agreement with a 2(*ax*),3(*eq*),4(*ax*) arrangement of protons, and therefore a 2(*eq*),-3(*ax*),4(*eq*) or 2,3-*cis*-3,4-*cis* arrangement of substituent groups. The values of these coupling constants correlate with those found by Clark-Lewis, Jackman & Williams (1962) for ($\pm$)-3,4-*cis*-diacetoxy-3',4'-dimethoxy-6-methyl-2,3-*cis*-flavan. Similarly, racemic 3',4',5',7-tetramethoxyflavan-3-ol formed under slightly more drastic conditions than used for the corresponding 2,3-*cis*-flavan-3,4-*cis*-diol has the 2,3-*cis* arrangement ($J_{2,3} \sim 1$ cyc./

sec.), and is therefore the epimeric racemate of the 2,3-*trans*-(−)-robinetinidol previously isolated from wattle wood (Roux & Maihs, 1960).

Paper chromatography of these methylated flavan-3,4-diols, of isomeric 2,3-*trans*-3,4-*cis*- and 2,3-*trans*-3,4-*trans*-flavan-3,4-diols (Drewes & Roux, 1963, 1964; Lillya, Drewes & Roux, 1963), and of the methyl ethers of melacacidin (2,3-*cis*-3,4-*cis* configuration) and isomelacacidin (2,3-*cis*-3,4-*trans*) (Clark-Lewis & Mortimer, 1960), shows little or no differentiation of R_F values in water-satd. butan-1-ol and in water-satd. ethyl acetate (Table 2). However, on paper impregnated with 0.1M-sodium borate, the 2,3-*trans*-3,4-*cis*-diols have appreciably lower R_F values (ΔR_F 0.12–0.14 for butan-1-ol and 0.05–0.09 for ethyl acetate) than the 2,3-*trans*-3,4-*trans*-diols (Table 2). Parallel behaviour is apparent for methylated 2,3-*cis* isomers, naturally occurring melacacidin (2,3-*cis*-3,4-*cis*) and isomelacacidin (2,3-*cis*-3,4-*trans*) having similar R_F values (0.79, 0.80 respectively) in butan-1-ol on ordinary paper, but the former shows a marked reduction in R_F (0.64, 0.87) on borate paper in the same solvent. This suggests that in these compounds the 3,4-*cis*-diol group alone is capable of forming a complex with borate, and the method therefore enables separation of isomeric 3,4-*cis*- and 3,4-*trans*-diols where these are formed simultaneously during reduction of methylated dihydroflavonols with lithium aluminium hydride–aluminium chloride (cf. Drewes & Roux, 1964).

The above evidence of complex-formation of flavan-3,4-*cis*-diols prompted examination of their ionophoretic mobilities in 0.1M-sodium borate (Table 3), since cyclitols (Angyal & McHugh, 1957) and related polyhydroxy compounds show differential mobilities in aqueous sodium borate solution. All methylated flavan-3,4-*cis*-diols showed positive, although variable, mobilities (Table 3), 2,3-*trans*-3,4-*cis*-diols having higher mobilities than isomeric 2,3-*cis*-3,4-*cis*-diols. Methylated 2,3-*trans*-flavan-3,4-*trans*-diols and a 2,3-*cis*-flavan-3,4-*trans*-diol show either no mobility (3',4',5',7-tetramethoxy-2,3-*trans*-flavan-3,4-*trans*-diol) or negative mobility, the latter presumably due to electro-osmotic flow. Owing to the variable behaviour of these 3,4-*trans* compounds, corrections for electro-osmotic flow could not be applied, and the relative mobilities M are accordingly related to that of racemic 3',4',5',7-tetramethoxy-2,3-*trans*-flavan-3,4-*cis*-diol (Table 3).

Comparison of the R_F values in butan-1-ol on paper impregnated with borate of geometrical isomers of two groups of analogues (3',4',5',7-tetramethoxy and 3',4',7-trimethoxy) with their ionophoretic mobilities (Tables 2 and 3) shows that 3,4-*cis*-diols of lowest R_F (2,3-*trans*-3,4-*cis* configuration) also have the highest mobility, whereas

those of intermediate R_F (2,3-*cis*-3,4-*cis*) have intermediate mobilities. This suggests that in the former stronger complex-formation with the borate ion occurs than in the latter group. Ionophoretic mobilities in borate solution therefore apparently offer a method for distinguishing between methylated flavan-3,4-*cis*- and flavan-3,4-*trans*-diols, in which the degree of mobility of 3,4-*cis*-diols might be significant.

The apparent absence of complex-formation of sodium borate with flavan-3,4-*trans*-diols is consistent with the earlier finding by Roux & de Bruyn (1963) that the potentiometric test with borate buffer for *cis*-glycol groups (Boeseken, 1921) gives no depression of pH with (+)-mollisacacidin trimethyl ether [(+)-3',4',7-trimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol]. Similar behaviour of guibourtacacidin dimethyl ether (Roux & de Bruyn, 1963) suggests also a 3,4-*trans* configuration for this natural flavan-3,4-diol.

SUMMARY

1. The chromatographic behaviour on paper impregnated with aqueous M-sodium borate, and the paper-ionophoretic mobility in the same solvent, of geometrical isomers of methylated flavan-3,4-diols of known relative configuration are examined.

2. Isomers of 3,4-*cis*-diol configuration have lower R_F values than those of the 3,4-*trans*-diol arrangement, and only the former group show positive ionophoretic mobility. The degree of positive mobility might be of structural significance.

3. These simple methods, although standardized against a limited range of compounds, appear useful for establishing the purity of geometrical isomers, and for differentiating between 3,4-*cis*- and 3,4-*trans*-diol arrangements.

4. Racemic 3',4',5',7-tetramethoxy-2,3-*cis*-flavan-3,4-*cis*-diol, 3',4',7-trimethoxy-2,3-*cis*-flavan-3,4-*cis*-diol, 3',4',5',7-tetramethoxy-2,3-*cis*-flavan-3-ol and their acetates have been synthesized, and their stereochemistry is confirmed by nuclear-magnetic-resonance spectroscopy.

Nuclear-magnetic-resonance spectra were examined during the tenure of a Charles Bullard Fellowship, 1962–1963, at Harvard University by D. G. R. Thanks are due to Dr D. R. Cooper of our laboratories for advice on experimental conditions for paper ionophoresis. This work is supported by the annual grant of the African Territories Wattle Industry Fund to the Leather Industries Research Institute.

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20. CHROMOPHORIC PROPERTIES OF FLAVAN-4-OLS, FLAVAN-3,4-DIOLS AND CONDENSED TANNINS

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Structural features involved in the commonly observed reddening that develops in certain condensed tannins when these are exposed to sunlight have been examined by comparing the characteristics of the free phenolic forms of flavan, flavan-3-ol, flavan-4-ol and flavan-3,4-diol analogues with 3',4',5',7-, 3',4',7- and 4',7-patterns of phenolic substituents (Roux & Paulus, 1961, 1962; Roux, 1963*a*; Roux & de Bruyn, 1963).

The benzylic 4-hydroxyl position, in conjunction with free phenolic hydroxyl groups in at least the 4'- and 7-positions, has been shown to be contributory to redness induced by indirect sunlight and heat (Roux, 1963*a*; Roux & de Bruyn, 1963). These preliminary conclusions have been summarized (Roux, 1963*b*).

Synthesis of the free phenolic form of two new flavan-4 β -ols now enables the correlation of some of the main structural factors involved in reddening.

EXPERIMENTAL AND RESULTS

C, H, methoxyl and acetyl estimations are by K. Jones, Micro-analytical Laboratory, C.S.I.R., Pretoria. Some of the nuclear-magnetic-resonance spectra are by Dr K. Pachler, Chemical Physics Group, C.S.I.R., Pretoria. All

nuclear-magnetic-resonance spectra were recorded on a Varian A-60 spectrometer with deuterochloroform as solvent and tetramethylsilane as internal standard. Band positions are expressed as p.p.m. downfield with the standard as origin (Table 1). Coupling constants are measured with an accuracy of ± 0.2 cyc./sec. Two-dimensional chromatograms were run with water-satd. butan-1-ol or butan-2-ol for the first direction, and 2% (v/v) acetic acid for the second direction.

Synthesis of flavan-4 β -ols

Synthesis of ($\pm$)-7-hydroxyflavanone and ($\pm$)-7-hydroxyflavan-4 β -ol. 2',4'-Dihydroxychalcone was prepared by the acid condensation method of Goel, Jain & Seshadri (1958) from resacetophenone dibenzoate (m.p. 81°) and freshly distilled benzaldehyde, as yellow needles, m.p. 147°, in 55% yield. Direct condensation of resacetophenone with benzaldehyde under alkaline conditions (Saiyad, Nadkarni & Wheeler, 1937) gave a lower yield (20%), m.p. 146–147°.

($\pm$)-7-Hydroxyflavanone. The flavanone was prepared by the following modified method. 2',4'-Dihydroxychalcone (600 mg.) dissolved in aqueous 1% sodium acetate (43 ml.) and ethanol (20 ml.) was refluxed for 7.5 hr. and left overnight. Buff crystals, m.p. 176–185° (453 mg., 75% yield), separated. These were recrystallized from ethanol in slightly buff plates, m.p. 190–191° (300 mg.). Saiyad *et al.* (1937) cite m.p. 189–190° and a 60% yield from the alkaline method of isomerization.