STRUCTURAL AND CHROMATOGRAPHIC CORRELATIONS OF SOME FLAVONOID COMPOUNDS

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INTRODUCTION

The contributions of correlations between structure, chromatographic behaviour, and reactivity to chromogenic spray reagents towards predicting the structures of those flavonoid compounds which are derived from plant sources, was shown previously by ROUX AND MAIHS[1], and ROUX, MAIHS AND PAULUS[2]. Such work is now extended by similar comparison of wider groups of flavonoid analogues of established absolute configuration.

EXPERIMENTAL AND RESULTS

Origin of substances

(+)-Leuco-fisetinidin [(+)-7,3′,4′-trihydroxyflavan-3,4-diol] from the heartwood of *Acacia mearnsii* (formerly *A. mollissima*[3]) was hydrogenated with palladium catalyst under conditions established by WEINGES[4] to (—)-fisetinidol [(—)-7,3′,4′-trihydroxyflavan-3-ol][5]. (+)-Fustin [(+)-3,7,3′,4′-tetrahydroxyflavan-4-one] also from the heartwood of *A. mearnsii*[6] was converted to (—)-butin [(—)-7,3′,4′-trihydroxyflavan-4-one][7] by hydrogenolysis using conditions similar to those described by PEW[8]. The flavonone was reduced to (+)-7,3′,4′-trihydroxyflavan-4-ol by catalytic hydrogenation with platinum[7], and to (—)-7,3′,4′-trihydroxyflavan by catalytic hydrogenation with palladium[9]. Fisetin was isolated from the heartwood of *Rhus glabra*[6], and butein was synthesised.

(+)-Leuco-robinetinidin [(+)-7,3′,4′,5′-tetrahydroxyflavan-3,4-diol] was isolated from the heartwood of *Robinia pseudacacia*[4,10], and (—)-robinetinidol [(—)-7,3′,4′,5′-tetrahydroxyflavan-3-ol] from the bark of *A. mearnsii*[11]. (+)-Dihydrorobinetin [(+)-3,7,3′,4′,5′-pentahydroxyflavan-4-one] was obtained from *R. pseudacacia* heartwood in partly racemized form[12], and resolved into the optically pure (+)-form by chromatographic methods[7]. This flavanonol was converted successively to (—)-robtin [(—)-7,3′,4′,5′-tetrahydroxyflavanone], (+)-7,3′,4′,5′-tetrahydroxyflavan-4-ol, and (—)-7,3′,4′,5′-tetrahydroxyflavan by the same conversions as outlined for (+)-fustin[7,9]. Robinetin was isolated from *R. pseudacacia*[13], while robtein was synthesised[7].

2,4,4′-Trihydroxychalcone and (±)-liquiritigenin [(±)-7,4′-dihydroxyflavan-4-one] were synthesised[14], and the latter converted to (±)-7,4′-dihydroxyflavan-4-ol by catalytic hydrogenation with platinum[15]. 7,4-Dihydroxyflavonol was also synthesised[15].

Chromatographic methods

The pure substances were applied within the concentration range 10–15 μg on Whatman No. 1 chromatographic paper and the chromatograms mounted on stainless steel frames. Chromatograms were developed with 2 % acetic acid by upward migration to a point 12 in. (30.5 cm) from the starting line over about 6 h. After drying they were sprayed with the reagents described before[1], and also with those described below. R_F values (Table I) were calculated to an average of three values in the presence of (+)-catechin (R_F 0.35) and (+)-gallocatechin (R_F 0.32) as reference compounds.

Other chromatograms were prepared as above but developed in water-saturated butan-1-ol (*n*-butanol) and in butan-1-ol–acetic acid–water (6:1:2, v/v) by upward migration to points about 10 in. (25.4 cm) from the starting lines. R_F values in each solvent systems were calculated as above (Table II).

Spray reagents

(*a*) *Quinonechloroimides.* 2,6-Dichloroquinonechloroimide (2 %) in absolute ethanol was sprayed evenly and after allowing for the evaporation of the alcohol the chromatograms were fumed with ammonia. Blue or mauve colorations were developed by flavans, flavan-3-ols, flavan-4-ols, flavan-3,4-diols, flavanones and flavanonols of the "resorcinol series" with catechol B nuclei. Those with pyrogallol B nuclei gave spots with pale yellow centres and blue edges, changing slowly to brown on aging. In all cases the colorations were developed rapidly with flavan-4-ols and flavan-3,4-diols, and usually, although not in all instances, more slowly by the other types of flavonoids. (+)-Catechin (catechol grouping) (blue) and (+)-gallocatechin (pyrogallol grouping) (blue with yellow centre) also behave consistently.

Those flavonoids examined with a single hydroxyl group on the B nucleus (4'-hydroxyl) gave weaker colours, exceptions being the flavan-4-ol (mauve) and the flavan-3,4-diol (7,4'-dihydroxyflavan-3,4-diol)[15,16] (mauve).

2,6-Dibromoquinonechloroimide and unsubstituted quinonechloroimide give similar colours, but the 2,6-dichloro- and 2,6-dibromo-derivatives are preferred as the colours developed are more intense and the shades of blue and violet more characteristic than with the unsubstituted quinonechloroimide.

(*b*) *Erlich's reagent. p*-Dimethylaminobenzaldehyde (2 %) in 2 *N* hydrochloric acid[17] shows an immediate pink in the cold (15–20°) with (+)-catechin and (+)-gallocatechin, but not with flavonoids containing resorcinol A nuclei. However, amongst the latter group, flavans develop an intense purple after 10–15 min and flavan-3-ols the same colour after 15–20 min. Thereafter flavan-4-ols develop a blue-purple and flavan-3,4-diols a pink, presumably due to the presence of hydrochloric acid in the reagent. These chromatograms were not heated.

(*c*) *p-Toluenesulphonic acid.* With catechol- and phloroglucinol-containing flavan-4-ols this reagent gives an intense blue-purple fading to a light pink at 80°. A stable violet is given by 7,4'-dihydroxyflavan-4-ol.

DISCUSSION

Conclusions which may be drawn from the chromatographic behaviour of the wider range of flavonoid nuclei studied above, reaffirm the earlier deductions by ROUX, MAIHS AND PAULUS[2] regarding the effect of the position of hydroxylation on R_F in

aqueous medium. These flavonoid nuclei contain three types of hydroxyl groups: (a) phenolic, occupying the 5,7,3′,4′ and 5′-positions, (b) benzylic in the 4-position, and (c) aliphatic in the 3-position. In 2 % acetic acid ("adsorptive system") substitution of phenolic hydroxyls at 3′ and 5′ cause small reductions in R_F (compare the three groups of analogues in Table I), whereas substitution in the 5-position has been shown to cause large reduction[2]. The *p*-hydroxybenzyl hydroxyl at C-4 causes small increases in R_F (compare flavans with flavan-4-ols, ΔR_F +0.01 to +0.03, and flavan-3-ols with flavan-3,4-diols, ΔR_F +0.04 to +0.07, in Table I), whereas the truly aliphatic 3-hydroxyl is responsible for large increases (compare flavans with flavan-3-ols, ΔR_F +0.16 and flavan-4-ols with flavan-3,4-diols, ΔR_F +0.17 to +0.22, in Table I). The effect of the 4-hydroxyl groups on R_F in 2 % acetic acid is, therefore,

TABLE I

R_F VALUES OF STEREOCHEMICALLY RELATED FLAVONOID ANALOGUES IN 2 % ACETIC ACID

Flavonoid type	R_F*		
	Pattern of phenolic hydroxylation		
	7,3′,4′,5′ (R = R′ = OH)	7,3′,4′ (R = OH, R′ = H)	7,4′** (R = R′ = H)
(—)-Flavan (I)	0.26	0.32	
(—)-Flavan-3-ol (II)	0.42	0.48	
(+)-Flavan-4-ol (III)	0.27	0.35	0.44
(+)-Flavan-3,4-diol (IV)	0.49	0.52	
(—)-Flavanone (V)	0.19	0.22	0.29
(+)-Flavanonol (VI)	0.35	0.38	

* R_F values are in relation to (+)-catechin (0.35) and (+)-gallocatechin (0.32).
** These compounds were racemates, the R_F of the flavan-3,4-diol indicated, being of the forward running enantiomer.

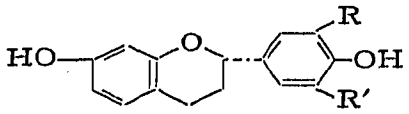
TABLE II

R_F VALUES OF STEREOCHEMICALLY INTERRELATED FLAVONOIDS AND THEIR FLAVONOL AND CHALCONE ANALOGUES IN WATER-SATURATED BUTAN-1-OL AND BUTAN-1-OL–ACETIC ACID–WATER (6:1:2, v/v)

Flavonoid type	R_F*		
	Pattern of phenolic hydroxylation		
	7,3′,4′,5′ (R = R′ = OH)	7,3′,4′ (R = OH, R′ = H)	7,4′** (R = R′ = H)
(—)-Flavan (I)	0.77 (0.80)	0.88 (0.88)	
(—)-Flavan-3-ol (II)	0.59 (0.69)	0.76 (0.79)	
(+)-Flavan-4-ol (III)	0.50 (0.60)	0.72 (0.78)	0.92 (0.91)
(+)-Flavan-3,4-diol (IV)	0.37 (0.47)	0.61 (0.65)	
(—)-Flavanone (V)	0.74 (0.81)	0.84 (0.86)	0.89 (0.91)
(+)-Flavanonol (VI)	0.59 (0.69)	0.76 (0.79)	
Flavonol	0.42 (0.45)	0.70 (0.71)	0.90 (0.88)
Chalcone	0.50 (0.61)	0.82 (0.84)	0.90 (0.91)

* R_F values for water-saturated butan-1-ol are indicated first, and those for butan-1-ol–acetic acid–water are in parentheses. R_F values are in relation to (+)-catechin 0.55 (0.65) and (+)-gallocatechin 0.35 (0.45).
** The flavan-4-ol and flavanone of this group are racemates.

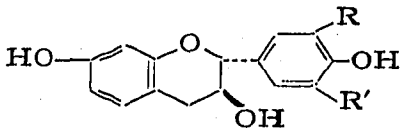
consistent with their properties which are intermediate between the most weakly dissociated phenolic hydroxyls (acidic due to partial dissociation in water) and neutral aliphatic hydroxyls (undissociated). Apart from the nature of the hydroxyl groups, their relative position on the C_{15} skeleton must also exert a large influence on chromatographic behaviour, as shown for the phenolic hydroxyls[2].



(I)

R=OH, R'=H. (—)-7,3',4'-trihydroxyflavan

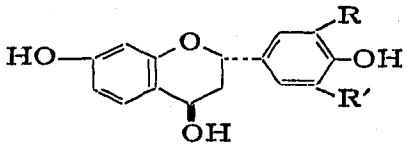
R=R'=OH. (—)-7,3',4',5'-tetrahydroxyflavan



(II)

R=OH, R'=H. (—)-fisetinidol

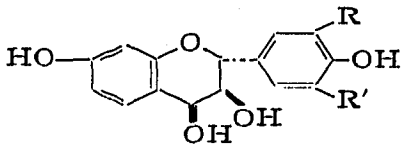
R=R'=OH. (—)-robinetinidol



(III)

R=OH, R'=H. (+)-7,3',4'-trihydroxyflavan-4-ol

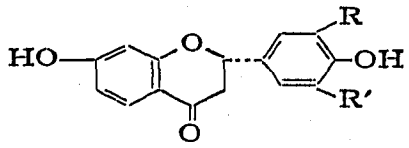
R=R'=OH. (+)-7,3',4',5'-tetrahydroxyflavan-4-ol



(IV)

R=OH, R'=H. (+)-leuco-fisetinidin

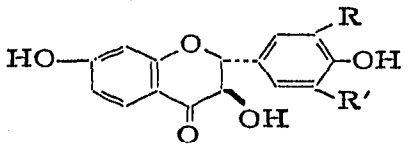
R=R'=OH. (+)-leuco-robinetinidin



(V)

R=OH, R'=H. (—)-butin

R=R'=OH. (—)-robtin



(VI)

R=OH, R'=H. (+)-fustin

R=R'=OH. (+)-dihydrorobinetin

Perhaps the most interesting of the comparisons in Table I are the large differences in R_F between flavans and flavanones of the same (2S) absolute configuration[7,9]. The flavanones in each instance show a lower R_F than the flavan analogue (ΔR_F —0.07 to —0.10) in 2 % acetic acid, and the same conclusion may be reached[2] by comparison of the flavanonols (2R:3R configuration) with the stereochemically related flavan-3-ols (2R:3S configuration) (ΔR_F —0.07 to —0.10). This reduction of R_F due to a carbonyl group at C-4 is difficult to explain, the proton-accepting carbonyl group, capable of forming strong hydrogen bonds with water, should, for example, not reduce the solubility of the flavonoid nucleus appreciably. Furthermore, modern stereochemical concepts suggest that flavans and flavanones have similar conformations of the heterocyclic ring system[18], and differences in their affinity for cellulose, related in this instance also to their solubility in water[2], are therefore unlikely to be attributable to differences in molecular shape.

By comparison, in water-saturated butan-1-ol (Table II), there exist remarkable agreements between the R_F values of flavans and flavanones (ΔR_F 0.03–0.04) and of flavan-3-ols and flavanonols (ΔR_F 0.00) for each group of analogues. However, flavan-3-ols show consistently higher R_F values than flavan-4-ols (ΔR_F 0.04–0.09). Similar comparison of the R_F values of flavans with flavan-3-ols, flavan-4-ols, and flavan-3,4-diols within each group shows that individual reductions of R_F introduced by the 3- and 4-hydroxyls are almost additive in the flavan-3,4-diols. Flavonols have lower R_F values than the corresponding flavanonols (dihydroflavonols) (ΔR_F —0.06 to —0.17) due to affinity effects or low solubility[2] resulting from the planar nature of the flavonol molecule. For the same reason chalcones which are isomeric with flavanones, have lower R_F values, the additional hydroxyl in chalcones (2-position) being strongly bonded with the carbonyl group. Apart from these anomalies, increase of the degree of hydroxylation of the flavan and flavanone groups always leads to reduction in R_F, the interval in water-saturated butan-1-ol being somewhat less regular than in the butan-1-ol–acetic acid–water (6:1:2, v/v) mixture (Table II).

R_F values of the 7,4'-dihydroxy analogues are apparently too high to permit accurate differentiation of similar effects, the comparative values in Table II showing that the magnitude of the above R_F differences decrease with decrease in the degree of phenolic hydroxylation, in both "partitioning" systems.

The spray reagent 2,6-dichloroquinonechloroimide has been used for the chromatographic location of polyhydric phenols[19], and flavan-3,4-diols[20], while unsubstituted quinonechloroimide was used for estimation of benzyl alcohol groups in lignins although the reaction was not specific[21]. For the flavonoids examined, the reaction is similarly not specific, but with those containing *p*-hydroxybenzyl alcohol groups, namely flavan-4-ols and flavan-3,4-diols, the blue develops rapidly and is intense. This reagent and *p*-toluenesulphonic acid are particularly useful for locating flavan-4-ols and flavan-3,4-diols[15] of the resorcinol-phenol group, which possess no phenolic *ortho*-hydroxy groups. These give no reaction with ferric alum, and their reactions with ammoniacal silver nitrate and bisdiazotized benzidine are exceptionally weak.

Erlich's reagent, known to give reactions with free resorcinol nuclei[17,22] may be used to differentiate between flavan-3-ols of the "resorcinol" and "phloroglucinol" series, although this differentiation is achieved almost more effectively with bisdiazotized benzidine and vanillin–*p*-toluenesulphonic acid sprays[1,23]. The slow development of mauves by flavans and flavan-3-ols of the "resorcinol series" with Erlich's reagent may be due to hydrolytic fission of the heterocyclic ring to give "free" resorcinol nuclei.

ACKNOWLEDGEMENT

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SUMMARY

The chromatographic behaviour of flavan, flavan-3-ol, flavan-4-ol, flavan-3,4-diol, flavanone, flavanonol, flavonol and chalcone analogues of robinetinidin and fisetinidin chlorides in 2 % acetic acid, water-saturated butan-1-ol, and in butan-1-ol–acetic

acid–water (6:1:2, v/v), is discussed. Similarly, the diagnostic value of quinonechloroimides, Erlich's reagent, and *p*-toluenesulphonic acid as spray reagents for flavonoids is examined.

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