CORRELATIONS OF ELECTROPHORETIC MOBILITIES IN BORATE BUFFER WITH STRUCTURAL FACTORS OF SOME FLAVONOID COMPOUNDS

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INTRODUCTION

The versatility of two-dimensional paper chromatography for studying the distribution and structure of flavonoid compounds, and also the elegant use of thin-layer chromatography for resolving mixtures of their derivatives has been widely demonstrated. By comparison, no systematic data exist on their electrophoretic mobilities (*cf.* refs. 1, 2). The reason for this must perhaps be sought in the observation that chromatographic effects on borate-impregnated paper closely parallel many of the coordination effects commonly achieved in similar systems by paper electrophoresis. Electrophoresis, nevertheless, is capable of revealing many small structural differences between compounds, as is shown by the present systematic attempts at correlating paper electrophoretic behaviour with flavonoid structure.

EXPERIMENTAL AND RESULTS

Origin of compounds

Most substances used are natural compounds or their synthetic conversion products. The majority were previously described by ROUX[3] and ROUX, MAIHS AND PAULUS[4]. Eriodictyol was kindly supplied by Dr. B. H. KOEPPEN, Stellenbosch University, Stellenbosch, Cape. Similarly, pinocembrin, pinobanksin, chrysin and tectochrysin, originating from various *Pinus* spp. (*cf.* ref. 5) were supplied by Prof. H. ERDTMAN, Royal Technical University, Stockholm. Kaempferol, (+)-aromadendrin and (+)-afzelechin from *Eucalyptus calophylla*[6,7] were kindly supplied by Dr. W. E. HILLIS, C.S.I.R.O., Melbourne. (—)-Melacacidin and isomelacacidin were isolated by preparative paper chromatography from the heartwood of *Acacia melanoxylon*[8]. 4′,7-Dihydroxy-, 7-hydroxy- and 4′-hydroxyflavanones were prepared by synthesis[9,10].

Electrophoretic method

Mobilities were measured by horizontal paper electrophoresis using a Vokam power supply (Shandon Scientific Co.) and Schleicher and Schüll 2043 (4 × 41 cm) paper. A constant current of 0.31 mA/cm width of paper was applied (4 × 4 cm papers requiring a total of 5 mA) for 6 h using borate buffer pH 8.8 (12.6 g/l sodium borate, 3.1 g/l boric acid). The distance of anodic migration was measured from the origin to the centre of the band. Bands were located under U.V. light or by spraying with

ammoniacal silver nitrate, *bis*-diazotized benzidine, or toluene-*p*-sulphonic acid, depending on the structure of the flavonoid (*cf.* ROUX *et al.*[3,4,11,12]). An average of two runs was used to calculate the migration distance. (+)-Catechin was used as reference compound with each run, and relative mobilities were expressed with (+)-catechin as unity.

Flavonoid compounds were made up in buffer just prior to the start of each run. In some cases (flavonols) a trace of ethanol was added to promote solubility.

Where ammoniacal silver nitrate was used for spraying, colour development did not occur until the strips were washed with distilled water, probably due to masking of the reducing (*ortho*-hydroxyphenolic) groups by borate ions.

Results

The relative mobilities (M) of the flavonoid compounds are given in Table I. Their stereochemistry[13] is as shown, but where they are racemates, only one enantiomer corresponding to the 2 *R* form of 2,3-*trans*-flavan-3-ols [(+)-catechin] is shown.

DISCUSSION

Consideration of structures I–XXXVI suggests that co-ordination of the borate ion might occur (a) at vicinal hydroxyls which may either be phenolic (3′,4′- or 7,8-positions) or aliphatic (3,4-position); (b) with the 5-hydroxyl and 4-carbonyl positions as in certain flavones, flavanones, flavonols and dihydroflavonols; (c) with the 3-hydroxyl and 4-carbonyl as in dihydroflavonols (flavanonols) or flavonols.

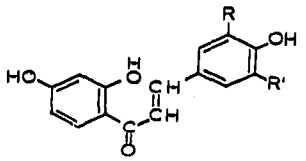
The effects of such co-ordination on electrophoretic mobility, where operative, are considered below, and further consideration of stereochemically related compounds show that other structural factors affect mobility to a variable degree.

Effects of possible co-ordination sites

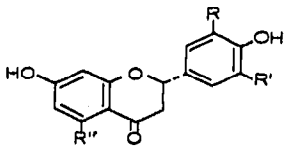
(*a*) *Vicinal hydroxyl groups.* Comparison of the flavonols, kaempferol (IX) (0.06) and quercetin (X) (0.25); of the dihydroflavonols, aromadendrin (XIV) (0.99) and taxifolin (XV) (1.64); of the chalcones, dahlia chalcone (I) (0.16) and butein (II) (0.33); and of the flavan-3-ols, (+)-afzelechin (XXI) (0.33) and (+)-catechin (XXII) (1.00), shows that provision of a phenolic *ortho*-hydroxyl system through introduction of a 3′-hydroxyl produces enhanced relative mobility ($\Delta M = +0.17$ to $+0.67$) irrespective of the type of compound under consideration. Similarly comparison of (+)-leucofisetinidin (XXIV) (1.05), with one (3′,4′) phenolic co-ordinating position, and isomelacacidin (XXXV) (1.64), with two (3′,4′ and 7,8) such positions, illustrates a similar ($\Delta M = +0.59$) effect on the introduction of additional vicinal phenolic hydroxyls, both compounds having a 3,4-*trans*-glycol grouping which exerts no effect (see below).

Examination of the mobilities of isomelacacidin (XXXV) (1.64) and (—)-melacacidin (XXXIV) (1.71) shows a small but significant difference in mobility which may be ascribed to additional co-ordination with the 3,4-*cis*-glycol grouping of the latter compared with the absence of co-ordination with borate at the 3,4-*trans*-glycol group of the former (see below). These compounds are identical in all other respects, both having the 2,3-*cis*-configuration of substituents.

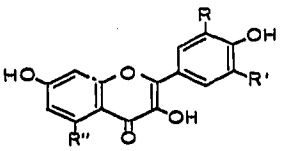
(*b*) *5-Hydroxyl and 4-carbonyl position.* Correlation of the mobilities of pairs



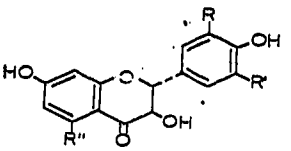
I Dahlia chalcone (R=R′=H)
II Butein (R=OH, R′=H)
III Robtein (R=R′=OH)



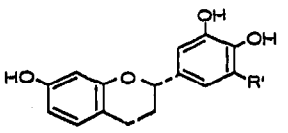
IV Butin (R=OH, R′=R″=H)
V Robtin (R=R′=OH, R″=H)
VI Eriodictyol (R=R″=OH, R′=H)



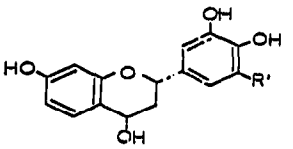
VII Fisetin (R=OH, R′=R″=H)
VIII Robinetin (R=R′=OH, R″=H)
IX Kaempferol (R=R′=H, R″=OH)
X Quercetin (R=R″=OH, R′=H)
XI Myricetin (R=R′=R″=OH)



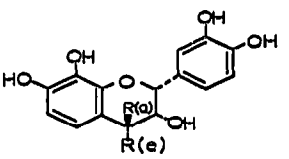
XII Fustin (R=OH, R′=R″=H)
XIII Dihydrorobinetin (R=R′=OH, R″=H)
XIV Aromadendrin (R=R′=H, R″=OH)
XV Taxifolin (R=R″=OH, R′=H)
XVI Ampeloptin (R=R′=R″=OH)



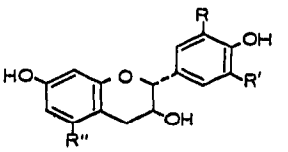
XVII



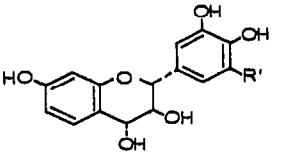
XVIII



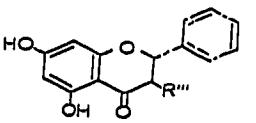
XXXIV Melacacidin ($R_{(a)}$=H, $R_{(e)}$=OH)
XXXV Isomelacacidin ($R_{(a)}$=OH, $R_{(e)}$=H)



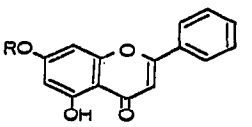
XIX Fisetinidol (R=OH, R′=R″=H)
XX Robinetinidol (R=R′=OH, R″=H)
XXI Afzelechin (R=R′=H, R″=OH)
XXII Catechin (R=R″=OH, R′=H)
XXIII Gallocatechin (R=R′=R″=OH)



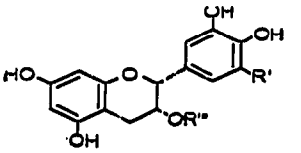
XXIV Leucofisetinidin (R′=H)
XXV Leucorobinetinidin (R′=OH)



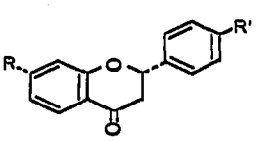
XXVI Pinocembrin (R‴=H)
XXVII Pinobanksin (R‴=OH)



XXVIII Chrysin (R=H)
XXIX Tectochrysin ($R=CH_3$)



XXX Epicatechin (R′=R‴=H)
XXXI Epigallocatechin (R′=OH, R‴=H)
XXXII Epicatechin gallate (R′=H, R‴=galloyl)
XXXIII Epigallocatechin gallate (R′=OH, R‴=galloyl)



XXXVI

TABLE I

RELATIVE MOBILITIES (M)* OF FLAVONOID COMPOUNDS IN A SODIUM BORATE–BORIC ACID BUFFER

Compound	*M**	*Compound*	*M**
Chalcones		*2,3-trans-Flavan-3-ols*	
Dahlia chalcone (I)	0.16	Fisetinidol (XIX)	1.04
Butein (II)	0.33	Robinetinidol (XX)	0.96
Robtein (III)	0.17	Afzelechin (XXI)	0.33
		Catechin (XXII)	1.00
		Gallocatechin (XXIII)	0.93
Flavanones		*2,3-trans-Flavan-3,4-trans-diols*	
Butin (IV)	1.72	Leucofisetinidin (XXIV)	1.05
Robtin (V)	1.49	Leucorobinetinidin (XXV)	0.96
Eriodictyol (VI)	1.54		
Flavonols		*Flavonoids with unsubstituted B-ring*	
Fisetin (VII)	0.38	Pinocembrin (XXVI)	0.85
Robinetin (VIII)	0.24	Pinobanksin (XXVII)	0.93
Kaempferol (IX)	0.06	Chrysin (XXVIII)	0.11
Quercetin (X)	0.25	Tectochrysin (XXIX)	0.00
Myricetin (XI)	0.12		
Dihydroflavonols		*2,3-cis-Flavan-3-ols*	
Fustin (XII)	1.85	Epicatechin (XXX)	0.92
Dihydrorobinetin (XIII)	1.82	Epigallocatechin (XXXI)	0.80
Aromadendrin (XIV)	0.99	Epicatechin gallate (XXXII)	1.46
Taxifolin (XV)	1.64	Epigallocatechin gallate (XXXIII)	1.38
Ampeloptin (XVI)	1.48		
Flavans		*2,3-cis-Flavan-3,4-diols*	
3′,4′,5′,7-Tetrahydroxy (XVII, R′ = OH)	0.84	Melacacidin (XXXIV)	1.71
3′,4′,7-Trihydroxy (XVII, R′ = H)	0.97	Isomelacacidin (XXXV)	1.64
Flavan-4β-ols		*Flavanones with low degree of substitution*	
3′,4′,5′,7-Tetrahydroxy (XVIII, R′ = OH)	0.88	4′,7-Dihydroxy (XXXVI, R = R′ = OH)	0.84
3′,4′,7-Trihydroxy (XVIII, R′ = H)	1.00	7-Hydroxy (XXXVI, R = OH, R′ = H)	0.95
		4′-Hydroxy (XXXVI, R = H, R′ = OH)	0.00

* Mobilities relative to that of (+)-catechin.

of flavanonols, fustin (XII) (1.85) and taxifolin (XV) (1.64), dihydrorobinetin (XIII) (1.82) and ampeloptin (XVI) (1.48); of the flavonols, fisetin (VII) (0.38) and quercetin (X) (0.25), robinetin (VIII) (0.24) and myricetin (XI) (0.12); and of the flavanones, butin (IV) (1.72) and eriodictyol (VI) (1.54), shows that in each instance where a 5-hydroxyl is introduced (latter compound) a reduction ($\Delta M = -0.12$ to -0.34) instead of an anticipated increase in mobility is obtained. This effect is discussed below.

(*c*) *3-Hydroxyl and 4-carbonyl position.* Mobilities of compounds containing the 4-carbonyl but having the 3-hydroxyl either absent (flavanone) or present (flavanonol) *e.g.* butin (IV) (1.72) and fustin (XII) (1.85), robtin (V) (1.49) and dihydrorobinetin (XIII) (1.82), eriodictyol (VI) (1.54) and taxifolin (XV) (1.64), pinocembrin (XXVI) (0.85) and pinobanksin (XXVII) (0.93), show an increase ($\Delta M = +0.08$ to $+0.33$) with the availability of this possible co-ordination position. However, the effect is more likely to be related to an "affinity factor" (see below).

Lack of co-ordination with borate in both the latter positions is perhaps surprising considering that aluminium salts form very stable complexes with 5-hydroxy- and 3-hydroxy-flavanones[14], although in acid medium.

Hydroxylation at the 4'-position

The relative mobilities of pinobanksin (XXVII) (0.93) and (+)-aromadendrin (XIV) (0.99) show that introduction of a hydroxyl in the 4'-position in flavanonols causes a small increase ($\Delta M = +0.06$). This might be due to partial dissociation of the 4'-hydroxyl (compare effect of strong dissociation of the 7-hydroxyl due to its *para*-position to the 4-carbonyl in flavanones and flavanonols). However, for the flavanones, 7-hydroxyflavanone (XXXVI, R = OH, R' = H) (0.95) and 4',7-dihydroxyflavanone (XXXVI, R = R' = OH) (0.84), introduction of a 4'-hydroxyl has the opposite effect ($\Delta M = -0.11$) on mobility.

Hydroxylation at the 5'-position in the presence of 3',4'-dihydroxyls

Comparison of the stereochemically related (where applicable) pairs of flavan-3-ols, (+)-catechin (XXII) (1.00) and (+)-gallocatechin (XXIII) (0.93), (—)-epicatechin (XXX) (0.92) and (—)-epigallocatechin (XXXI) (0.80), (—)-epicatechin gallate (XXXII) (1.46) and (—)-epigallocatechin gallate (XXXIII) (1.38), (—)-fisetinidol (XIX) (1.04) and (—)-robinetinidol (XX) (0.96); of flavan-4β-ols, 3',4',7-trihydroxy (XVIII, R' = H) (1.00) and 3',4',5',7-tetrahydroxy (XVIII, R' = OH) (0.88); of flavans, 3',4',7-trihydroxy (XVII, R' = H) (0.97) and 3',4',5',7-tetrahydroxy (XVII, R' = OH) (0.84); of flavan-3,4-diols, (+)-leucofisetinidin (XXIV) (1.05) and (+)-leucorobinetinidin (XXV) (0.96); of dihydroflavonols, ($\pm$)-fustin (XII) (1.85) and ($\pm$)-dihydrorobinetin (XIII) (1.82), ($\pm$)-taxifolin (XV) (1.64) and ($\pm$)-ampeloptin (XVI) (1.48); of flavonols, fisetin (VII) (0.38) and robinetin (VIII) (0.24), quercetin (X) (0.25) and myricetin (XI) (0.12); of flavanones, ($\pm$)-butin (IV) (1.72) and ($\pm$)-robtin (V) (1.49); and of the chalcones, butein (II) (0.33) and robtein (III) (0.17), shows that introduction of a 5'-hydroxyl in the presence of 3',4'-dihydroxyls causes a variable ($\Delta M = -0.03$ to -0.22) reduction in mobility.

Hydroxylation at the 3'-position in the presence of a 4'-hydroxyl

Comparison of the stereochemically related (where applicable) pairs of flavan-3-

ols, (+)-afzelechin (XXI) (0.33) and (+)-catechin (XXII) (1.00); of dihydroflavonols, (+)-aromadendrin (XIV) (0.99) and (+)-taxifolin (XV) (1.64); of flavonols, kaempferol (IX) (0.06) and quercetin (X) (0.25); and of the chalcones, dahlia chalcone (I) (0.16) and butein (II) (0.33) show a large positive effect for the non-planar ($\Delta M = +0.65, +0.77$) and a lesser effect ($\Delta M = +0.17, +0.19$) for planar flavonoids on introduction of the 3'-hydroxyl in the presence of the 4'-hydroxyl.

These positive effects on mobilities of the 3'-hydroxyl in the presence of the 4'-hydroxyl contrast with the negative effect on mobility with the introduction of the equivalent 5'-hydroxyl in the presence of 3'- and 4'-hydroxyls, and must be due almost exclusively to the co-ordination with the borate ion to form a negatively charged complex.

Simultaneous hydroxylation at 3',4'-positions

The above theory is supported by the observation that where two hydroxyls are introduced simultaneously in the *ortho*-position, as in the comparison (+)-robanksin XXVII (0.93) and (±)-taxifolin (XV) (1.64); (—)-pinocembrin (XXVI) (0.85) and (±)-eriodictyol (VI) (1.54), the increase in mobility ($\Delta M = +0.74, +0.68$) is of the same order as for the non-planar flavonoids above where the 3'-hydroxyl is introduced.

Hydroxylation at the 8-position in the presence of a 7-hydroxyl

An effect, almost of the same magnitude, is observed when introducing a second *ortho*-hydroxyl group through 8-hydroxylation, in the comparison of (+)-leucofisetinidin (XXIV) (1.05) with isomelacacidin (XXXV) (1.64) ($\Delta M = +0.59$), and the increased mobility must again be due to co-ordination with an additional borate ion only.

Hydroxylation at the 5-position

In order to study this effect independently of others, comparison must be made between mobilities of stereochemically related flavan-3-ols, (—)-robinetinidol (XX) (0.96) and (+)-gallocatechin (XXIII) (0.93); (—)-fisetinidol (XIX) (1.04) and (+)-catechin (XXII) (1.00). Introduction of a 5-hydroxyl into flavan-3-ols thus has a negative effect on mobility ($\Delta M = -0.03, -0.04$) similar to those evident from previous considerations of co-ordination sites where introduction of a 5-hydroxyl into a flavanone has a somewhat larger effect ($\Delta M = -0.18$). Also introduction of the 5-hydroxyl in the presence of a 4-carbonyl plus 3-hydroxyl has an effect of similar magnitude in dihydroflavonols ($\Delta M = -0.21, -0.34$) and in flavonols ($\Delta M = -0.13, -0.12$).

The 5-hydroxyl therefore has a retarding effect on mobility which appears to be accentuated by the presence of a 4-carbonyl or a 4-carbonyl plus 3-hydroxyl. From this it may be concluded that no complexing with borate ions occurs, and the effect is rather similar to the introduction of a 5'-hydroxyl in the presence of the 3',4'-dihydroxyl arrangement where no further complexing is possible as in the flavan-3-ols ($\Delta M = -0.07, -0.08$), dihydroflavonols (—0.16, —0.03), flavonols (—0.14, —0.12) and flavanones (—0.23).

The higher relative mobility of flavanones, flavonols and dihydroflavonols of the "resorcinol" series, when compared with the equivalent compounds of the

"phloroglucinol" series (*cf.* Table I) might be due in part also to a reduction in the acidic properties of the 7-hydroxyl (flavanones and dihydroflavonols) or 7- plus 4-hydroxyls (flavonols) due to strong hydrogen bonding between the 5-hydroxyl and 4-carbonyl groups in these flavonoids of the "phloroglucinol" series[15] (*cf.* discussion of the effects on mobility of ionization induced by the 4-carbonyl group).

Hydroxylation at the 3-position

From the mobility data in Table I, the introduction of a 3-hydroxyl into flavans as in the pairs, (±)-3',4',5',7-tetrahydroxyflavan (XVII, R' = OH) (0.84) and (—)-robinetinidol (XX) (0.96); (±)-3',4',7-trihydroxyflavan (XVII, R' = H) (0.97) and (—)-fisetinidol (XIX) (1.04), or into flavan-4β-ols as in the pairs, 3',4',5',7-tetrahydroxyflavan-4β-ol (XVIII, R' = OH) (0.88) and (+)-leucorobinetinidin (XXV) (0.96); 3',4',7-trihydroxyflavan-4β-ol (XVIII, R' = H) (1.00) and (+)-leucofisetinidin (XXIV) (1.05), may be shown to produce a positive mobility (ΔM = +0.05 to +0.11).

Similar effects are evident when comparing similar flavanone pairs, (+)-eriodictyol (VI) (1.54) and (±)-taxifolin (XV) (1.64); (±)-robtin (V) (1.49) and (±)-dihydrorobinetin (XIII) (1.82); (±)-butin (IV) (1.72) and (±)-fustin (XII) (1.85); and pinocembrin (XXVI) (0.85) and pinobanksin (XXVII) (0.93), the degree of increase of mobility being exceedingly variable (+0.08 to +0.33). Notable is the exceptionally large increase, ΔM = +0.33, with introduction of the 3-hydroxyl into robtin.

The variable degree of increase of mobility, and the relatively small increases (ΔM = +0.08 to +0.13) in most cases whether in the presence or absence of the 4-carbonyl, suggests that this effect is entirely due to a reduction of affinity for cellulose as demonstrated by paper chromatography in 2 % acetic acid[3,4].

Hydroxylation at the 4β-position

Comparison of the mobilities of the pairs, (±)-3',4',5',7-tetrahydroxyflavan (XVII, R' = OH) (0.84) and (±)-3',4',5',7-tetrahydroxyflavan-4β-ol (XVIII, R' = OH) (0.88), (±)-3',4',7-trihydroxyflavan (XVII, R' = H) (0.97) and (±)-3',4',7-trihydroxyflavan-4β-ol (XVIII, R' = H) (1.00) would indicate that the 4β-hydroxyl increases electrophoretic mobility, an effect again similar to that found on paper chromatography in aqueous medium[3,4].

This effect does not operate in all instances as in the stereochemically related pairs (—)-fisetinidol (XIX) (1.04) and (+)-leucofisetinidin (XXIV) (1.05); (—)-robinetinidol (XX) (0.96) and (+)-leucorobinetinidin (XXV) (0.96) no appreciable increase is observed for the same factor.

Carbonyl group at the 4-position

Comparison of 2,3-dihydroflavonols with flavan-3-ol analogues shows that introduction of a 4-carbonyl group greatly increases electrophoretic mobility. The magnitude of the increase may be judged from the pairs (+)-afzelechin (XXI) (0.33) and (+)-aromadendrin (XIV) (0.99); (+)-catechin (XXII) (1.00) and (±)-taxifolin (XV) (1.64); (+)-gallocatechin (XXIII) (0.93) and (±)-ampeloptin (XVI) (1.48); (—)-fisetinidol (XIX) (1.04) and (±)-fustin (XII) (1.85); and (—)-robinetinidol (XX) (0.96) and (±)-dihydrorobinetin (XIII) (1.82), where ΔM = +0.55 to +0.86.

That these differences are due exclusively to the introduction of the 4-carbonyl group is shown by the comparisons (±)-3′,4′,5′,7-tetrahydroxyflavan (XVII, R′ = OH) (0.84) and (±)-robtin (V) (1.49); (±)-3′,4′,7-trihydroxyflavan (XVII, R′ = H) (0.97) and (±)-butin (IV) (1.72), where the increases $\Delta M = +0.65, +0.75$ are of the same magnitude as above.

The induction of high mobility by the 4-carbonyl in flavanones and flavanonols is presumably due to the far stronger ionization in borate buffer of the 7-hydroxyl in these than in their flavan and flavan-3-ol analogues, due to its location *para* to the carbonyl group. The higher acidity of the 7-hydroxyl is, for example, shown in the selective alkylation of the appropriate polyhydroxyisoflavones in the synthesis of prunetin and santal[16].

Unequivocal proof of the above is afforded by comparison of 4′-hydroxyflavanone (XXXVI, R = H, R′ = OH) (0.00) with 4′,7-dihydroxyflavanone (XXXVI, R = R′ = OH) (0.84), and with 7-hydroxyflavanone (XXXVI, R = OH, R′ = H) (0.95), where the relatively high mobility in flavanones correlates with the simultaneous presence of the 7-hydroxyl group.

This large positive mobility effect is the opposite of the strong affinity effect (reduction of R_F) on paper chromatograms in aqueous medium, when the same pairs of compounds are compared[3,4].

Galloyl group at the 3-position

Comparison of the mobilities of the pairs, (—)-epicatechin (XXX) (0.92) and (—)-epicatechin gallate (XXXII) (1.46); (—)-epigallocatechin (XXXI) (0.80) and (—)-epigallocatechin gallate (XXXIII) (1.38), shows a large increase in mobility ($\Delta M = +0.54, +0.54$) due to galloylation of the 3-hydroxyl group.

The increase in mobility must be due to the introduction of an additional phenolic *ortho*-hydroxyl system (co-ordination position for borate ion), and is of the same order as when a second phenolic *ortho*-hydroxyl system is introduced into the flavan nucleus; compare (+)-leucofisetinidin (XXIV) (1.05) and isomelacacidin (XXXV) (1.64) ($\Delta M = +0.59$). The positive mobility on galloylation is the opposite of the chromatographic affinity effect in 2 % acetic acid where the R_F is reduced.

Stereochemical effects

(*a*) *2,3-cis and 2,3-trans configurations*. Comparison of the electrophoretic mobility of pairs of the above configurations, respectively: (—)-epicatechin (XXX) (0.92) and (+)-catechin (XXII) (1.00); (—)-epigallocatechin (XXXI) (0.80) and (+)-gallocatechin (XXIII) (0.93), shows that when the 3-hydroxyl occupies an *axial* position as in 2,3-*cis*-(epi)-flavan-3-ols the mobility is lower ($\Delta M = -0.08, -0.13$) than when it is *equatorial* as in the 2,3-*trans*-flavan-3-ols.

A parallel affinity effect is shown on chromatography in water or 2 % acetic acid[3,4].

(*b*) *3,4-cis- and 3,4-trans-configurations*. The effect of 3,4-*trans*-glycol groups on mobility in borate buffer may be examined by comparison of 2,3-*trans*-flavan-3-ol and 2,3-*trans*-flavan-3,4-*trans*-diol pairs, (—)-fisetinidol (XIX) (1.04) and (+)-leucofisetinidin (XXIV) (1.05); (—)-robinetinidol (XX) (0.96) and (+)-leucorobinetinidin (XXV) (0.96) where the 3,4-*trans*-glycol group does not apparently contribute to mobility.

However, comparison of the 2,3-*cis*-flavan-3,4-*trans*-diol and 2,3-*cis*-flavan-3,4-*cis*-diol pair, isomelacacidin (XXXV) (1.64) and (—)-melacacidin (XXXIV) (1.71) suggests that the 3,4-*cis*-diol grouping contributes to the mobility ($\Delta M = +0.07$), which is probably reduced by the greater affinity of (—)-melacadicin for cellulose[4]. These findings have been confirmed by previous electrophoretic work on the fully methylated ethers of 3,4-*cis*- and 3,4-*trans*-flavandiols, where only the 3,4-*cis*-forms have been shown to have anodic mobility in sodium borate (*cf.* DREWES AND ROUX[17]).

Correlation between mobility and planarity

The relatively low electrophoretic mobilities of the flavonols and chalcones compared with high mobilities of dihydroflavonols, flavanones and also flavan-3-ols, flavan-4-ols and flavan-3,4-diols (Table I) is probably due to the high affinity, resulting from planar structure, of the former compared with the latter group. A similar relationship has been shown in paper chromatography in aqueous medium[3,4,11,12].

Mobility of compounds lacking the ortho-dihydroxy system

Six non-planar compounds (Table I) which do not contain the complex-forming *ortho*-dihydroxy system, namely aromadendrin (XIV) (0.99), pinocembrin (XXVI) (0.85), pinobanksin (XXVII) (0.93), 4',7-dihydroxyflavanone (XXXVI, R = R' = OH) (0.84), 7-hydroxyflavanone (XXXVI, R = OH, R' = H) (0.95) and afzelechin (XXI) (0.33), show relatively high mobility in spite of the proved absence of other co-ordination sites. Amongst these, the high mobility of the flavanones (XIV) (XXVI) (XXVII) (XXXVI, R = R' = OH and R = OH, R' = H) must be ascribed to the strong ionization of the 7-hydroxyl induced by the 4-carbonyl in the boric acid–sodium borate buffer. In the absence of the carbonyl group as in afzelechin (XXI), the greatly reduced but still significant mobility must be due to the far weaker dissociation of 7- and/or 4'-hydroxyls.

The flavanone, chrysin (XXVIII), has mobility (0.11) in spite of the reduced degree of hydroxyl substitution, absence of co-ordinating positions, and high affinity for cellulose due to planarity. Mobility must, therefore, be due to the strong ionization of the 7-hydroxyl as above. In tectochrysin (XXIX), where this position is blocked by methoxylation, mobility drops to zero. Parallel conclusions have been drawn by JURD AND HOROWITZ[18] from examination of the spectral shifts of flavones in sodium acetate buffer.

CONCLUSIONS

Paper ionophoresis of 40 flavonoid compounds in boric acid–sodium borate buffer has enabled tentative correlations between certain structural factors and mobility. Vicinal phenolic hydroxyls and 3,4-*cis*-glycol systems enhance mobility due to complex formation with borate, but no complexing occurs at 3-hydroxy-4-carbonyl and 5-hydroxy-4-carbonyl sites. Strongly enhanced mobility is induced by the introduction of a 4-carbonyl due to strong ionization of the 7-hydroxyl group. Mobility is also enhanced by hydroxylation in the 3- and 4β-positions, and by galloylation of the 3-hydroxyl, but is retarded by hydroxylation in 5'- and 5-positions, 2,3-*cis*- as compared with 2,3-*trans*-arrangements of substituents, and strongly reduced by the overall planarity of the flavonoid unit. Mobility effects produced by hydroxylation in the

5-, 5′-, 4β- and 3-positions and by molecular planarity may be correlated with affinity phenomena for cellulose, but galloylation and introduction of a 4-carbonyl produce effects opposite to those found on paper chromatograms due to factors cited above. In general, hydroxylation in positions where they are capable of ionizing (7 and in some cases 4′) or when of aliphatic character (3,4β), apparently enhances mobility, whereas introduction of undissociated hydroxyls (5′,3 and in flavanones, 4′) retards ionophoretic mobility, provided no simultaneous complexing with borate occurs in these positions.

The relative mobilities of flavonoids are of diagnostic value in establishing their identity, while many of the specific mobility effects in borate buffer might assist in resolving components in complex mixtures or in establishing their homogeneity.

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SUMMARY

Comparison of the electrophoretic mobilities of flavonoid compounds in sodium borate–boric acid buffer has shown that co-ordination of borate with phenolic *ortho*-hydroxyls and ionization of the 7-hydroxyl correlate with large increases in mobility, whereas overall planarity strongly reduces mobility. The effects of hydroxyl, carbonyl and galloyl substituents, and of *cis*- and *trans*-arrangements in the 2,3- and 4-positions are related to smaller differences in mobility. These differences in mobility are of diagnostic value.

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