# Optical resolution of flavanones by high-performance liquid chromatography on various chiral stationary phases

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ABSTRACT

Six commercially available chiral stationary phases [Chiralcel OD, Chiralpak OP (+), ChiraSpher, Cyclobond I, acetylated Cyclobond I and Cyclobond II) were evaluated for the high-performance liquid chromatographic separation of flavanone, six monosubstituted flavanones and one disubstituted flavanone. At least some of the flavanones were resolved on each column, except on Cyclobond II. For the β-cyclodextrin stationary phases possible inclusion phenomena are discussed.

INTRODUCTION

The flavanones naringenin (5,7,4'-trihydroxyflavanone) and eriodictyol (5,7,3',4'-tetrahydroxyflavanone) are central intermediates in the biosynthesis of flavonoids[1]. A large number of flavanones have been isolated as secondary plant metabolites, many of them in an optically active form[2]. However, there are many reports that do not comment on the stereochemistry of flavanones.

Of primary interest to us was the development of a high-performance liquid chromatographic (HPLC) method to determine flavanone enantiomers in plant extracts, *i.e.*, to gain information about the optical purity of flavanones without isolating them. It has been demonstrated for a flavanoneglycoside (naringin) by NMR that racemization of the aglycone occurred during maturation[3].

Chalcones, which also have been reported as natural products[4], are isomeric forms of the flavanones. Some chalcones cyclize spontaneously in aqueous medium in a pH-dependent reaction to racemic flavanones[5]. Stereochemical investigations therefore may be helpful in deciding whether chalcones or the corresponding flavanones were originally accumulated in plant cells.

During our search for a suitable HPLC column we tested many chiral stationary phases (CSP) with a series of different flavanones. We did not find a CSP for the resolution of all flavanones. Polyhydroxylated flavanones were separated on microcrystalline cellulose triacetate[6] or cellulose triacetate supported on silica gel diol[7]. In this paper we report the enantiomeric separation of flavanones with a low degree of

substitution (hydroxyl or/and methoxyl groups) by HPLC on five CSPs. The parent compound flavanone also has been resolved on various cellulose- and amylose-carbamate CSPs by Okamoto and co-workers[8-11] and several 3-hydroxyflavanones[12] and a biflavanone[13] on Chiralpak OT (+).

EXPERIMENTAL

*Materials*
    Flavanones (Fig. 1) were purchased from Roth (Karlsruhe, F.R.G.) and methanol, 2-propanol (HPLC grade), *n*-hexane, dioxane and *tert.*-butyl methyl ether (analytical-reagent grade) from Baker (Gross-Gerau, F.R.G.).
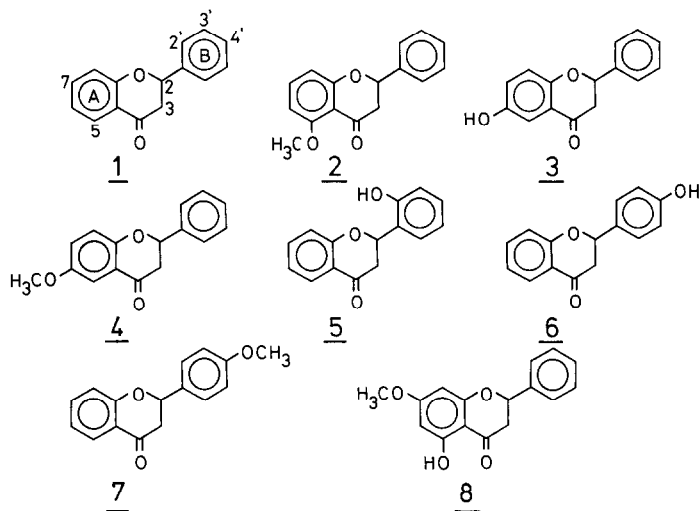


Fig. 1. Structures of flavanones tested. 1 = Flavanone; 2 = 5-methoxyflavanone; 3 = 6-hydroxyflavanone; 4 = 6-methoxyflavanone; 5 = 2'-hydroxyflavanone; 6 = 4'-hydroxyflavanone; 7 = 4'-methoxyflavanone; 8 = pinostrobin (5-hydroxy-7-methoxyflavanone).

*HPLC*
    The HPLC apparatus used consisted of a gradient system from Beckman (Munich, F.R.G.) with two pumps (114 M) and a high-pressure mixing chamber, a sampling valve (Altex 210; Beckman) equipped with a 20-$\mu$l sample loop and a Pye Unicam variable-wavelength UV detector set at 254 nm (Philips, Kassel, F.R.G.). The results were recorded with an integrator (3390 A from Hewlett-Packard, Waldbronn, F.R.G. or C-R6A from Shimadzu, Duisburg, F.R.G.) or a video chromatographic control center (Pye Unicam 4850; Philips).
    Chromatography was performed at ambient temperature unless specified otherwise. The column temperatures were adjusted, if necessary, with a column oven from Techlab (Erkerode, F.R.G.).
    Some of the results were monitored with a polarimetric detector (Chiramonitor 750/25) from ACS (Macclesfield, U.K.), supplied by Zinsser (Frankfurt, F.R.G.).

*Columns*

The following columns were used:

Chiralcel OD (250 × 4.6 mm I.D.), 10 μm (Daicel, Baker);

Chiralpak OP (+) (250 × 4.6 mm I.D.), 10 μm (Daicel, Baker);

ChiraSpher (250 × 4.0 mm I.D.), 5 μm (Merck, Darmstadt, F.R.G.);

Cyclobond I (β-cyclodextrin) (250 × 4.6 mm I.D.), 5 μm (ASTEC; ICT, Frankfurt, F.R.G.);

Acetylated Cyclobond I (250 × 4.6 mm I.D.), 5 μm (ASTEC; ICT);

Cyclobond II (γ-cyclodextrin) (250 × 4.6 mm I.D.), 5 μm (ASTEC, ICT).

RESULTS AND DISCUSSION

*Chiralcel OD*

This chiral stationary phase consists of macroporous silica gel coated with cellulose tris(3,5-dimethylphenylcarbamate) (Fig. 2). Okamoto and co-workers[8–11] reported only the separation of flavanone on various cellulose- and amylose-derived CSPs. Table I shows that Chiralcel OD is capable of resolving many other substituted flavanones. Of the 8 flavanones studied (Fig. 1), only 4'-hydroxyflavanone is not separated, not even partially, as monitored with a polarimetric detector. The first-eluted enantiomer of the other seven flavanones is always leavorotatory.
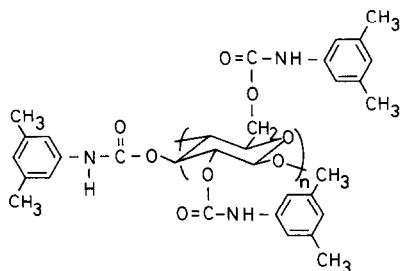


Fig. 2. Structure of Chiralcel OD [cellulose tris(3,5-dimethylphenylcarbamate)].

TABLE I

ENANTIOMERIC SEPARATION OF FLAVANONES ON CHIRALCEL OD

Mobile phase, *n*-hexane–2-propanol (90:10, v/v); flow-rate, 1 ml/min. $k'_1$ = Capacity factor of the first-eluted enantiomer as indicated; α = separation factor.

| Compound | $k'_1$ | α |
|---|---|---|
| Flavanone | 1.34 (−) | 1.49 |
| 5-Methoxy- | 2.90 (−) | 1.37 |
| 6-Hydroxy- | 2.56 (−) | 1.16 |
| 6-Methoxy- | 1.62 (−) | 1.29 |
| 2'-Hydroxy- | 1.76 (−) | 1.23 |
| 4'-Hydroxy- | 4.99 | 1.00 |
| 4'-Methoxy- | 1.85 (−) | 1.25 |
| Pinostrobin | 1.96 (−) | 1.60 |

Substituted flavanones have larger $k'$ values than flavanone. The influence of a hydroxyl group on the retention behaviour is different depending on its position; 6- and 4'-hydroxyflavanones ($k' = 2.56$ and $4.99$, respectively) are more retained than 2'-hydroxyflavanone ($k' = 1.76$) or pinostrobin (5-hydroxy-7-methoxy-) ($k' = 1.96$). The capacity factors of the hydroxy-substituted flavanones are larger in the pairs 6-hydroxy–6-methoxyflavanone and 4'-hydroxy–4'-methoxyflavanone, but the enantioselectivity decreases for 6-hydroxy- and is lost for 4'-hydroxyflavanone. It seems that hydrogen bonding between the hydroxyl groups of the flavanones and the carbamate function of the CSP hinders chiral recognition in these instances. Pinostrobin (with a hydroxyl group in the 5-position, $\alpha = 1.60$), on the other hand, is better resolved than flavanone ($\alpha = 1.49$). With pinostrobin intramolecular hydrogen bonding between the 5-hydroxy and the carbonyl groups is also possible. Other polyhydroxylated flavanones such as naringenin (5,7,4'-trihydroxyflavanone) were not resolved on this column without derivatization. However, the separation of naringenin tribenzoate was possible, indicating that hydroxyl groups of the solutes hinder chiral recognition.

In conclusion, Chiralcel OD is a CSP with relatively high separation factors for many flavanones. Fig. 3 demonstrates the resolution of pinostrobin with $n$-hexane–2-propanol (90:10) as the mobile phase.
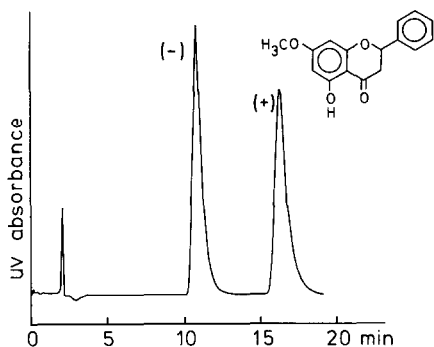


Fig. 3. Enantiomeric separation of pinostrobin on Chiralcel OD with $n$-hexane–2-propanol (90:10) at 1 ml/min as the mobile phase.

*Chiralpak OP (+)*

Chiralpak OP (+) is based on macroporous silica gel coated with poly(diphenyl 2-pyridylmethylmethacrylate). This type of CSP is chiral only due to its helicity[14], as shown in Fig. 4. As demonstrated in Table II, the chromatographic behaviour of four flavanones was studied. The polar modifier plays an important role in the separation process. $n$-Hexane–2-propanol (95:5) resulted in separation factors between 1.09 and 1.22. Small amounts of methanol in the mobile phase (eluent B) decrease the capacity factors and enantioselectivity is observed only for 5-methoxyflavanone. On the other hand, increasing the concentration of 2-propanol to 50% (eluent C) decreases the capacity factors even more but, except for 4'-methoxyflavanone, a separation is achieved. It seems that with apolar eluents polar interactions between the chiral
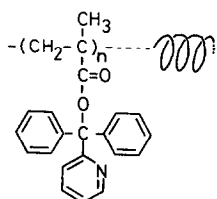
Fig. 4. Structure of Chiralpak OP (+) [poly(diphenyl 2-pyridylmethylmethacrylate)].

TABLE II

SEPARATION OF RACEMIC FLAVANONES ON CHIRALPAK OP (+)

Mobile phases: A = $n$-hexane–2-propanol (95:5, v/v); B = $n$-hexane–methanol–2-propanol (95:5:2.5, v/v/v); C = $n$-hexane–2-propanol (50:50, v/v); D = methanol. Flow-rate, 1 ml/min; temperature, 5°C.

| Compound | Mobile phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | B | | C | | D | |
| | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ | $k'_1$ | $\alpha$ |
| Flavanone | 4.19 | 1.14 | 3.56 | 1.00 | 2.48 | 1.07 | 3.45 | 1.14 |
| 5-Methoxy- | 8.46 | 1.22 | 4.99 | 1.07 | 2.23 | 1.20 | 1.28 | 1.00 |
| 6-Methoxy- | 4.87 | 1.11 | 3.56 | 1.00 | 2.55 | 1.12 | 2.09 | 1.00 |
| 4'-Methoxy- | 5.66 | 1.09 | 3.39 | 1.00 | 2.57 | 1.00 | 1.16 | 1.00 |

polymer and the solutes are important for the recognition process, although a general rule cannot be derived from the results obtained. With methanol as eluent flavanone is most retained (probably owing to a non-polar interaction) and a separation ($\alpha = 1.14$) is also possible.

In general, the resolving power and efficiency of this CSP is not as good as those of Chiralcel OD. The elution order has only been determined for flavanone, as shown in Fig. 5.
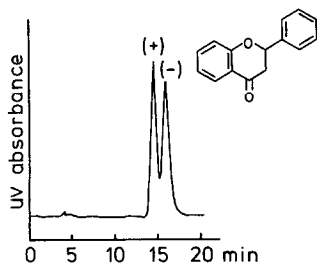


Fig. 5. Optical resolution of flavanone on Chiralpak OP (+) with $n$-hexane–2-propanol (95:5) at 1 ml/min as the mobile phase at 5°C.

*ChiraSpher*

In contrast to the above two CSPs, ChiraSpher is based on small-pore silica gel (pore size 10 nm). The chiral polymer [poly-N-acryloyl-(S)-phenylalanine ethyl ester] (Fig. 6) is fixed on the silica gel by an *in situ* polymerization procedure[15]. Table III illustrates that all flavanones were resolved (at least partially) on this CSP. The hydroxylated flavanones are much more retained than the methoxylated type (except pinostrobin) and therefore the eluents were not the same. Small amounts of dioxane in *n*-hexane (eluent A) as polar modifier resulted in a separation of flavanone and 6-methoxy- and 4'-methoxyflavanone (5-methoxyflavanone was not eluted within 30 min). The same separation factor as with eluent A was observed for flavanone and 6-methoxyflavanone with *n*-hexane–2-propanol (98:2) (eluent B), whereas α decreased slightly for 4'-methoxyflavanone. The capacity factor for 5-methoxyflavanone ($k'$ = 5.33) was much higher than those for the other methoxyflavanones.
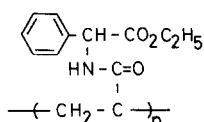


Fig. 6. Structure of ChiraSpher [poly-N-acryloyl-(S)-phenylalanine ethyl ester].

The hydroxylated flavanones were analysed with a mobile phase containing a larger amount of 2-propanol [*n*-hexane–2-propanol (90:10) (eluent C)]. Pinostrobin was eluted relatively fast ($k'$ = 1.65) compard with 6-hydroxy- ($k'$ = 5.37), 2'-hydroxy- ($k'$ = 6.06) and 4'-hydroxyflavanone ($k'$ = 13.09). Hydrogen bonding between the

TABLE III

OPTICAL RESOLUTION OF FLAVANONES ON CHIRASPHER

Mobile phases: A = *n*-hexane–dioxane (96:4, v/v); B = *n*-hexane-2-propanol (98:2, v/v); C = *n*-hexane-2-propanol (90:10, v/v). Flow-rate, 0.5 ml/min.

| Compound | $k'_1$ | α | Mobile phase |
|---|---|---|---|
| Flavanone | 2.46 (+) | 1.07 | A |
|  | 1.48 | 1.07 | B |
| 5-Methoxy- | 5.33 | 1.03 | B |
| 6-Hydroxy- | 5.37 (+) | 1.03 | C |
| 6-Methoxy- | 4.12 (+) | 1.07 | A |
|  | 1.83 | 1.07 | B |
| 2'-Hydroxy- | 6.06 (+) | 1.04 | C |
| 4'-Hydroxy- | 13.09 (+) | 1.04 | C[a] |
| 4'-Methoxy- | 3.13 (+) | 1.07 | A |
|  | 2.53 | 1.05 | B |
| Pinostrobin | 1.65 (+) | 1.03 | C |

[a] Flow-rate 1 ml/min.

hydroxyl group of the flavanones and the amide and/or ester group of the stationary phase seems to be responsible for the strong retention of these compounds, with the exception of pinostrobin. The latter deviation may be due to intramolecular hydrogen bonding of the hydroxyl group to the keto group of pinostrobin.

The reason for the observed large differences in retention behaviour between the positional isomers 5-methoxy- and 6-methoxyflavanone and 2'-hydroxy- and 4'-hydroxyflavanone may be attributed to different tight fits in the cavities of the polymer of the CSP.

Although this type of CSP was regarded as most suitable for the resolution of polar racemates[15], no separation of polyhydroxylated flavanones such as naringenin (5,7,4'-trihydroxyflavanone) or naringenin tribenzoate was achieved.

A good resolution can be achieved for several flavanones in a single chromatographic run with *n*-hexane-dioxane (100:4) as the mobile phase owing to the high efficiency of the column, as shown in Fig. 7. Other polar modifiers such as *tert.*-butyl methyl ether were also tested with good results.
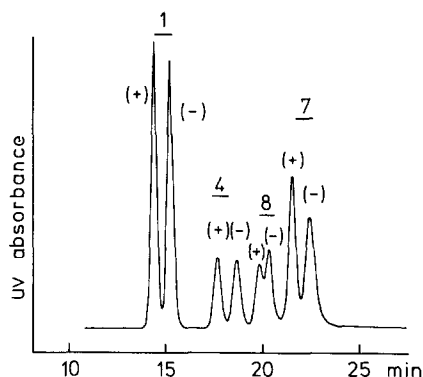


Fig. 7. Enantiomeric separation of flavanones on ChiraSpher with *n*-hexane-dioxane (100:4) at 1 ml/min as the mobile phase. For peak identification, see Fig. 1.

*Cyclobond I*

Cyclodextrins are cyclic glucoamyloses which have the form of a truncated cone. In the presence of water the cavity is relatively hydrophobic and lipophilic molecules or parts of a molecule can form inclusion complexes[16]. When enantiomers form inclusion complexes and there are interactions between the chiral centre or substituents near the chiral centre with the mouth of the cyclodextrin cavity, chiral recognition may occur[17]. Most optical resolutions reported in the literature were performed with β-cyclodextrins either as mobile phase additives or bonded to silicagel as stationary phase[17–19]. Only a few enantiomeric separations with α- or γ-cyclodextrins have been described[20,21].

A molecule of the size of benzene, naphthalene or biphenyl fits in the cavity of the β-cyclodextrin. Better enantioselectivity has been observed for molecules with naphthalene or biphenyl moieties than for smaller molecules, because of the requirement for a "tight fit" between the hydrophobic part of a compound and the
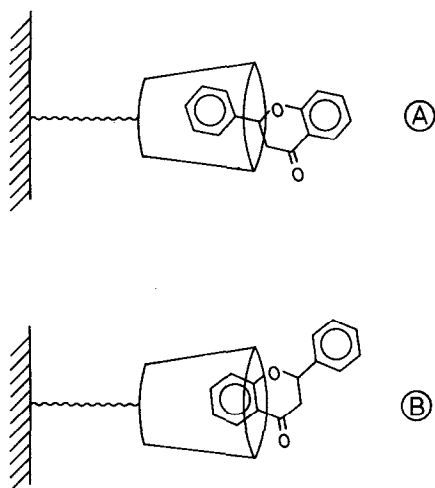
Fig. 8. Proposed inclusion orientations of flavanone in the $\beta$-cyclodextrin cavity: (A) inclusion of the phenyl group (ring A); (B) inclusion of ring B (see Fig. 1).

cyclodextrin cavity[17]. However, it was shown recently that single-ring compounds were resolved on $\beta$-cyclodextrin CSP when certain structural features are present[22].

Regarding the structure of the flavanones, an inclusion with the phenyl group (ring B) (Fig. 8A) or parts of the bicycle (ring A) (Fig. 8B) is possible. Recently the complexation of $\beta$-cyclodextrin with catechin and epicatechin, which have some structural similiarities with the flavanones, has been reported[23].

Table IV shows that inclusion with the $\beta$-cyclodextrin CSP also results in chiral discrimination for several flavanones. Compared with flavanone ($k'_1$ = 2.93) the introduction of a hydroxy group in 6- or 4'-position decreases the capacity factors (1.81; 1.93), which might be attributed to the higher polarity. 2'-Hydroxyflavanone ($k'_1$ = 2.54) is more strongly retained than 4'-hydroxyflavanone and the difference in

TABLE IV

ENANTIOMERIC SEPARATION OF FLAVANONES ON CYCLOBOND I ($\beta$-CYCLODEXTRIN)

Mobile phase, methanol–water (50:50, v/v); flow-rate, 1 ml/min.

| Compound | $k'_1$ | $\alpha$ | $R_s$ |
|----------|--------|----------|-------|
| Flavanone | 2.93 (−) | 1.11 | 1.3 |
| 5-Methoxy- | 1.78 | 1.00 | — |
| 6-Hydroxy- | 1.81 (+) | 1.11 | 1.2 |
| 6-Methoxy- | 2.85 (−) | 1.11 | 1.1 |
| 2'-Hydroxy- | 2.54 (−) | 1.33 | 3.5 |
| 4'-Hydroxy- | 1.93 | 1.00 | — |
| 4'-Methoxy- | 2.98 (−) | 1.06 | 0.7 |
| Pinostrobin | 3.35[a] | [a] | [a] |

[a] $k'$ of the second peak; first peak eluted as a shoulder.

retention behaviour between 5-methoxyflavanone ($k'_1 = 1.78$) and 6-methoxyflava-none ($k'_1 = 2.85$) is also very large.

Regarding the chiral separations it is remarkable that 2'-hydroxyflavanone is best resolved ($\alpha = 1.33$) and 4'-hydroxyflavanone not at all (not even with a larger amount of water in the mobile phase). 4'-Methoxyflavanone, on the other hand, is partially resolved. The reason for this behaviour is not clear. Maguire[24], in a study of hydantoin derivatives, observed that the introduction of a $p$-hydroxy group increased retention and separation factors. An inclusion of the $p$-hydroxyphenyl moiety of 4'-hydroxyflavanone with hydrogen bonding to the primary hydroxyl groups of the cyclodextrin molecule (as observed for the hydantoins) seems not to occur, because then the retention should also increase. Hydrogen bonding of the 4'-hydroxy group, however, may be one reason for the loss of enantioselectivity, because racemic 4'-methoxyflavanone is differentiated. Perhaps with 4'-hydroxyflavanone a different inclusion complex is formed.

It is further of interest that the elution order for 6-hydroxyflavanone [(+)-before (−)-enantiomer] is opposite to that for the other flavanones. 5-Methoxy-flavanone is not separated, although a partial resolution was monitored with a polarimetric detector, also with the opposite elution order [(+)- before (−)-enantiomer].

The chiral recognition mechanism involving inclusion phenomena with Cyclo-bond I has been described in detail for several compounds[25]. One requirement is that inclusion occurs and that interaction with the rim of the cyclodextrin cavity can occur to result in chiral differentiation.

Both proposed inclusion orientations for the flavanones will probably contrib-ute to the overall chromatographic behaviour. A definite recognition model cannot be derived from the results obtained. With the flavanones without B-ring oxygenation inclusion complex A (Fig. 8) seems more probable to be responsible for enantio-selectivity because in complex B (Fig. 8) no polar groups are present at the phenyl group opposite the chiral centre to interact with the (polar) rim of the cyclodextrin cavity. Additionally, substituents in 5- and 6-positions may preclude full penetration of the A-ring of the flavanones into the cyclodextrin cavity. This may also limit the extent to wich complex B is involved in enantioselectivity. However, the difference in retention between 5-methoxyflavanone ($k' = 1.78$) and 6-methoxyflavanone ($k' = 2.85$) could also be explained by steric hindrance of 5-methoxyflavanone in complex B. The extent to which one of the complexes contributes to retention (achiral interaction) and enantioselectivity may be different for each flavanone.

Fig. 9 shows the influence of methanol concentration on the capacity factors for 2'-hydroxyflavanone. Up to a concentration of 80% methanol resolution is attained. The high separation factors may be due to rigid fixation of the B-ring with additional hydrogen bonding via the 2'hydroxy group in the $\beta$-cyclodextrin molecule (as in Fig. 8A), although the other orientation (as in Fig. 8B) may also explain the enantio-selectivity. Neither interaction seems to be possible with the hydroxy group in the 4'-position.

Although the situation with the flavanones might be different, it should be noted that there is a carbonyl group opposite the chiral centre (on the other side of the phenyl group), a structural requirement which was thought to be important in resolving singel-ring compounds[22].
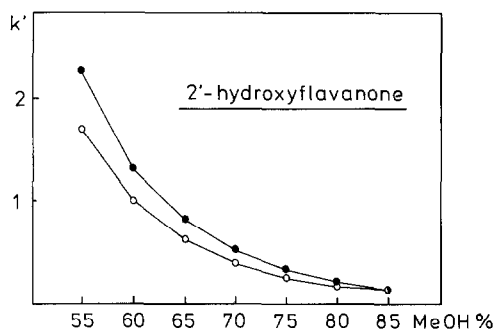
Fig. 9. Plot of methanol concentration in water against capacity factors ($k'$) of 2'-hydroxyflavanone on Cyclobond I. Flow-rate, 1 ml/min. ○ = ( − )-Enantiomer; ● = ( + )-enantiomer.

## Acetylated Cyclobond I

The mouth of the cyclodextrin molecule is extended by acetylating the 2-hydroxyl groups[19] and further binding sites are introduced. On bonded acetylated Cyclodextrin CSP (acetylated Cyclobond I) there have been only a few reports of enantiomeric separations[19,26,27]. Table V demonstrates that several flavanones were successfully resolved on this CSP, although the separation factors are smaller than with Cyclobond I. With the same eluent [methanol–water (50:50)] all capacity factors are higher than on Cyclobond I, which may be due to the increase in hydrophobicity. The most striking difference from Cyclobond I is that 5-methoxyflavanone is resolved, whereas 4'-methoxyflavanone is not. 5-Methoxy- and 6-hydroxyflavanone are eluted with the ( + )-enantiomer first and flavanone and 6-hydroxy- and 2'-hydroxyflavanone are eluted with the opposite elution order [( − )- before ( + )-enantiomer]. This behaviour is comparable to the situation on Cyclobond I (Table IV). Fig. 10 demonstrates that (except that the capacity factors are generally higher) the elution behaviour of 2'-hydroxyflavanone with respect to methanol concentration is similar to that with $\beta$-cyclodextrin (Fig. 9). Fig. 11 shows a chromatogram of 2'-hydroxy-

TABLE V

ENANTIOMERIC SEPARATION OF FLAVANONES ON ACETYLATED CYCLOBOND I (ACE-TYLATED $\beta$-CYCLODEXTRIN)

Mobile phase, methanol–water (50:50, v/v); flow-rate, 1 ml/min.

| Compound | $k'_1$ | $\alpha$ | $R_s$ |
|---|---|---|---|
| Flavanone | 4.40 ( − ) | 1.07 | 0.7 |
| 5-Methoxy- | 3.05 ( + ) | 1.07 | 0.6 |
| 6-Hydroxy- | 3.55 ( + ) | 1.08 | 0.8 |
| 6-Methoxy- | 5.00 ( − ) | 1.06 | 0.7 |
| 2'-Hydroxy- | 3.61 ( − ) | 1.25 | 2.3 |
| 4'-Hydroxy- | 3.22 | 1.00 | — |
| 4'-Methoxy- | 5.11 | 1.00 | — |
| Pinostrobin | 7.06[a] | [a] | [a] |

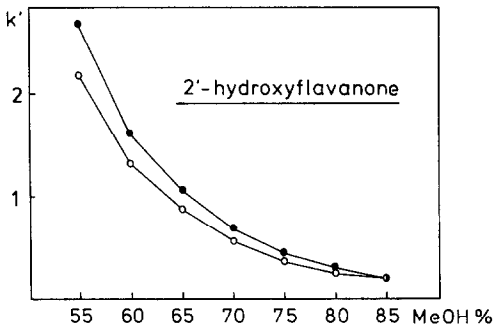[a] $k'$ of the second peak; first peak eluted as a shoulder.

Fig. 10. Plot of methanol concentration in water against capacity factors ($k'$) of 2'-hydroxyflavanone on acetylated Cyclobond I. Flow-rate, 1 ml/min. $\bigcirc$ = (−)-Enantiomer; $\bullet$ = (+)-enatiomer.

flavanone separated on acetylated Cyclobond I with (a) UV and (b) polarimetric detection.

The inclusion phenomena are thought to be similar to those discussed for native $\beta$-cyclodextrin. Although the flavanones are more retained than on Cyclobond I, a decrease in the separation factors of the enantiomers (with the exception of 5-methoxyflavanone) is observed. The increase in retention may be due mainly to the increase in hydrophobicity of the CSP and not to a tighter fit of the solutes in the acetylated cyclodextrin cavity. The reduction of the hydrogen bonding sites of the cyclodextrin or steric reasons (acetoxy groups) may be responsible for the decrease in enantioselectivity. It has also been reported that acetylation of $\beta$-cyclodextrin enhanced (with one exception) the separation of dansylamino acid enantiomers[27].

Except for 5-methoxyflavanone, it can be concluded that the acetylated form of $\beta$-cyclodextrin results in a poorer resolution than $\beta$-cyclodextrin.

$\gamma$-Cyclodextrin has eight glucose units and the cavities are larger than those in $\beta$-cyclodextrin. With $\Gamma$-cyclodextrin bonded CSP (Cyclobond II) no enantiomeric separation was observed with UV detection, as demonstrated in Table VI. Although
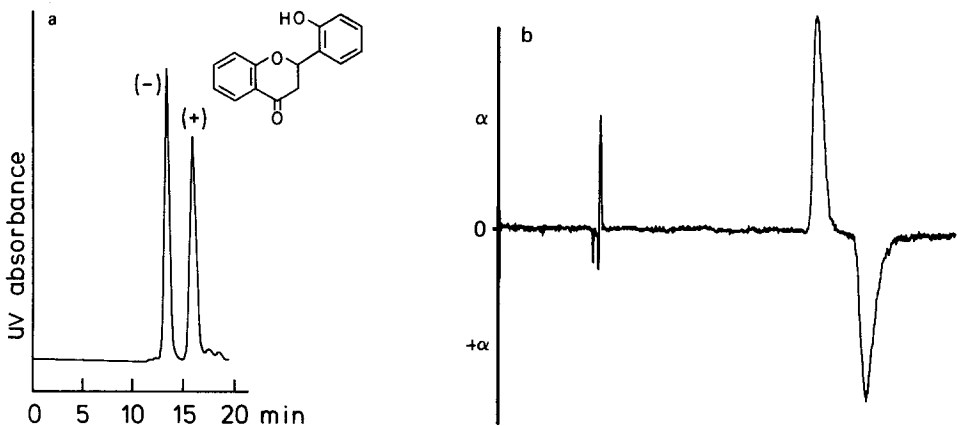


Fig. 11. Enantiomeric separation of 2'-hydroxyflavanone on acetylated Cyclobond I with methanol–water (50:50) at 1 ml/min as the mobile phase. (a) UV detection; (b) polarimetric detection.

TABLE VI

RETENTION BEHAVIOUR OF FLAVANONES ON CYCLOBOND II (γ-CYCLODEXTRIN)

Mobile phase, methanol–water (40:60, v/v); flow-rate, 1 ml/min.

| Compound | $k'$ | Compound | $k'$ |
|----------|------|----------|------|
| Flavanone | 1.70 | 2-Hydroxy- | 1.56 |
| 5-Methoxy- | 1.55 | 4'-Hydroxy- | 1.05 |
| 6-Hydroxy- | 1.19 | 4'-Methoxy- | 1.68 |
| 6-Methoxy- | 1.65 | Pinostrobin | 2.28 |

complexation is also possible with γ-cyclodextrin, the large cavity probably includes the whole molecule, not differentiating between the enantiomers. Capacity factors between Cyclobond I and II cannot be compared directly because the amounts of cyclodextrin bonded to the silica gel are different[28]. It is interesting, however, that the difference in the capacity factors of 5- and 6-methoxyflavanone is very small compared with the situation on β-cyclodextrin.

In conclusion, various chiral stationary phases can be used to resolve flavanones with a low degree of substitution. Except for ChiraSpher, not all of the tested flavanones were separated on each column. Minor structural changes can greatly affect enantioselectivity and resolution.

ACKNOWLEDGEMENTS

REFERENCES

1  W. Heller and G. Forkman, in J. B. Harborne (Editor), *The Flavonoids, Advances in Research*, Chapman & Hall, London and New York, 1988, pp. 399–422.
2  H. Arakawa and M. Nakazaki, *Justus Liebigs Ann. Chem.*, 636 (1960) 111.
3  W. Gaffield, R. E. Lundin, B. Gentili and R. M. Horowitz, *Bioorg. Chem.*, 4 (1975) 259.
4  B. A. Bohm, in J. B. Harborne (Editor), *The Flavonoids, Advances in Research*, Chapman & Hall, London and New York, 1988, pp. 329–340.
5  J. N. M. Mol, M. P. Robbins, R. A. Dixon and E. Veltkamp, *Phytochemistry*, 24 (1985) 2267.
6  M. Krause and R. Galensa, *J. Chromatogr.*, 441 (1988) 417.
7  M. Krause and R. Galensa, *J. Chromatogr.*, 502 (1990) 287.
8  Y. Okamoto, R. Aburatani, S.-I. Miura and K. Hatada, *J. Liq. Chromatogr.*, 10 (1987) 1613.
9  Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
10  Y. Okamoto, R. Aburatani, T. Fukumoto and K. Hatada, *Chem. Lett.*, (1987) 1857.
11  Y. Okamoto, H. Sakamoto, K. Hatada and M. Irie, *Chem. Lett.*, (1986) 983.
12  H. Takahashi, S. Li, Y. Harigaya and M. Onda, *Heterocycles*, 26 (1987) 3239.
13  M. Niwa, S. Otsuji, H. Tatematsu, G.-Q. Liu, X-F. Chen and Y. Hirata, *Chem. Pharm. Bull.*, 34 (1986) 3249.
14  Y. Okamoto and K. Hatada, *J. Liq. Chromatogr.*, 9 (1986) 369.
15  G. Blaschke, *J. Chromatogr. Sci.*, 40 (1988) 179.
16  J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest, 1982.
17  T. J. Ward and D. W. Armstrong, *Chromatogr. Sci.*, 40 (1988) 131.
18  J. Szejtli, *Starch*, 39 (1987) 357.
19  T. J. Ward and D. W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407.

20  D. W. Armstrong, X. Yang, S. M. Han and R. A. Menges, *Anal. Chem.*, 59 (1987) 2594.
21  M. Gazdag, G. Szepesi and L. Huszar, *J. Chromatogr.*, 436 (1988) 31.
22  D. W. Armstrong, Y. I. Han and S. M. Han, *Anal. Chim. Acta*, 208 (1988) 275.
23  E. Haslam, T. H. Lilley, Y. Cai, R. Martin and D. Magnolato, *Planta Med.*, 55 (1989) 1.
24  J. H. Maguire, *J. Chromatogr.*, 387 (1987) 453.
25  D. W. Armstrong, T. J. Ward, R. D. Armstrong and T. E. Beesley, *Science (Washington, D.C.)*, 232 (1986) 1132.
26  P. Macaudiere, M. Claude, R. Rosset and A. Tambute, *J. Chromatogr.*, 450 (1988) 255.
27  M. Tanaka, T. Shono, D. Q. Zhu and Y. Kawaguchi, *J. Chromatogr.*, 469 (1989) 429.
28  D. W. Armstrong and W. Li, *Chromatography*, March (1987) 43.