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Improved chiral stationary phase based on cellulose triacetate supported on non-macroporous silica gel diol for the high-performance liquid chromatographic separation of racemic flavanones and diastereomeric flavanone glycosides[a]

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SUMMARY

Microcrystalline cellulose triacetate (MCCTA) and depolymerized MCCTA were dissolved and coated on non-macroporous silica gel diol. The chiral stationary phases obtained were found to be superior to a commercially available column based on cellulose triacetate for the enantiomeric separation of polyhydroxylated flavanones. Diastereomeric flavanone glycosides could also be resolved, together with the aglycones in a mixture. As an example of the analysis of a complex matrix, the separation of naringenin enantiomers in a tomato skin extract is presented.

INTRODUCTION

Since the work of Hesse and Hagel[1], many papers have dealt with the enantiomeric separation of racemic compounds on microcrystalline cellulose triacetate (MCCTA) as a chiral stationary phase (CSP) (*e.g.*, refs. 2–7). With the preparation of 10-μm particles, MCCTA became suitable as a packing material for high-performance liquid chromatography (HPLC), and columns packed with MCCTA are commercially available (Merck, Daicel, Macherey & Nagel). Although MCCTA shows excellent enantioselectivity towards many enantiomers, the efficiency (number of theoretical plates) of such columns for analytical purposes is poor. Further, the choice of eluents and the flow-rate (compressibility) are limited. Consequently, many papers describe separations of single racemates and not of complex mixtures.

Okamoto and co-workers[8–12], Shibata and co-workers[13,14] and Ichida *et al.*[15] supported cellulose triacetate (CTA) and other polysaccharide derivatives on macroporous silica gel and obtained CSPs for HPLC. Coated CTA has different enantio-

[a] Dedicated to Prof. Dr. H. Thaler on the occasion of his 85th birthday.

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selective properties towards racemic compounds owing to an altered structure. MCCTA has a crystalline form which is referred to as CTA I[a] and which is lost on dissolution. Reprecipitation usually yields the modification CTA II[a,16]. The elution order of enantiomers is often reversed on CTA II, a phenomenon that has been investigated by several research groups[15,17,18]. It is interesting that the coating solvents play an important role in chiral recognition[14,15]. Recently, it was reported that CTA I can also be obtained from solution[19,20].

Optical resolution of flavanones has been achieved on various cellulose-based and other polymeric CSPs. Recently, we reported the enantiomeric HPLC separation of several hydroxylated flavanones on MCCTA as a chiral stationary phase[21]. Flavanones occur naturally in immense variety[22] and many others have been synthesized. Various monomethyoxylated flavanones were successfully separated by our group on a poly-N-acryloyl-S-phenylalanine ethyl ester CSP (ChiraSpher, Merck)[23,24] and on a poly(diphenyl-2-pyridylmethyl methacrylate) CSP [Chiralpak OP(+), Daicel][24].

Many cellulose and amylose phenylcarbamate derivatives have shown high separation factors for the parent compound flavanone[8,9,12]. With Cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD, Daicel), flavanone, methoxylated and some hydroxylated flavanones were resolved, but the group of the polyhydroxylated flavanones could not be separated without derivatization[23,24].

Some racemic 3-hydroxyflavanones, which are also classified as flavanonols or dihydroflavonols[25], were resolved on a poly(triphenylmethyl methacrylate) stationary phase [Chiralpak OT(+), Daicel] by HPLC[26]. It should be mentioned additionally that on the same type of column the separation of a racemic biflavanone has been reported[27].

Although the enantioselectivity for hydroxylated flavanones using MCCTA (CTA I) as a CSP is very good (separation factors up to 2)[21], the efficiency of such columns is low, which makes it difficult to determine racemic flavanones in complex matrices such as plant extracts.

CTA II has also been evaluated as a CSP for flavanones, but with a commercially available column packed with CTA II coated on macroporous silica gel (Chiralcel OA, Daicel) hydroxylated flavanones such as naringenin were only partially resolved. However, the column efficiency for CTA coated on silica gel was usually much better compared with MCCTA[13,18]. As far as we can ascertain, no efficient CPS for the direct enantiomeric separation of polyhydroxylated flavanones is commercially available. Therefore, we tried to improve this type of CSP for the analysis of racemic flavanones. We found that CTA coated on non-macroporous silica gel diol (pore size 10 nm) resulted in improved and cheap HPLC columns.

EXPERIMENTAL

Materials

Homoeriodictyol was synthesized by Wagner (Munich, F.R.G.) and pinocembrin was a gift from Wollenweber (Darmstadt, F.R.G.). Taxifolin was obtained from

[a] In a previous paper[21], the abbreviations CTA I, CTA II, CTA III and CTA IV were not related to a modification as here, but were used for the consecutive numbering of the CSPs used in that study.

Sigma (Deisenhofen, F.R.G.); all other flavanones (Table I) were from Roth (Karlsruhe, F.R.G.).

Methanol and 2-propanol were of HPLC grade and dichloromethane, 2-propanol, isooctane, *n*-hexane and ethoxyethanol were of analytical-reagent grade, all purchased from Baker (Gross-Gerau, F.R.G.).

LiChrosorb diol (7 μm) (pore size 10 nm) and microcrystalline CTA were obtained from Merck (Darmstadt, F.R.G.) and Nucleosil diol (7 μm) (pore size 10 nm) from Macherey & Nagel (Düren, F.R.G.).

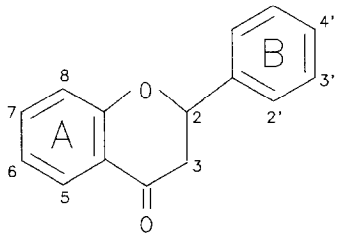
High-performance liquid chromatography

HPLC was performed using a gradient system from Beckman (Munich, F.R.G.) with two 114 M pumps and a high-pressure mixing chamber, an Altex 210 A sampling valve (Beckman) equipped with a 20-μl sample loop and a Pye Unicam variable-wavelength UV detector (Philips, Kassel, F.R.G.) set at either 254 or 280 nm. The results were recorded with Model 3390 A integrator (Hewlett-Packard, Waldbronn, F.R.G.) and a Pye Unicam PU 4850 video chromatography control centre, respectively.

Enantiomeric separation was verified by co-chromatography of the (−)- and (+)-enantiomers collected by chromatography on MCCTA[21].

TABLE I

SUBSTITUTION PATTERN OF FLAVANONES



Compound	*Name*
1	5,7-Dihydroxyflavanone (pinocembrin)
2	5,7-Dihydroxy-4′-methoxyflavanone (isosakuranetin)
3	5,7,4′-Trihydroxyflavanone (naringenin)
4	5,7,4′,3′-Tetrahydroxyflavanone (eriodictyol)
5	5,7,4′-Trihydroxy-3′-methoxyflavanone (homoeriodictyol)
6	5,7,3′-Trihydroxy-4′-methoxyflavanone (hesperetin)
7	5,4′-Dihydroxy-7-methoxyflavanone (sakuranetin)
8	5-Hydroxy-7-methoxyflavanone (pinostobin)
9	Flavanone
10	5-Methoxyflavanone
11	6-Hydroxyflavanone
12	6-Methoxyflavanone
13	4′-Hydroxyflavanone
14	4′-Methoxyflavanone
15	2′-Hydroxyflavanone
16	3,5,7,3′,4′-Pentahydroxyflavanone (dihydroquercetin, taxifolin)
17	Naringenin-7-O-glucoside (prunin)
18	Naringenin-7-O-neohesperidoside (naringin)

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.).

The following gradients were applied using solvent A [*n*-hexane–2-propanol (9:1, v/v)] and solvent B [methanol–2-propanol (2:1, v/v)]: (I) 10 min 10% B, 15 min 10–15% B, 7 min 15–25% B (Fig. 2); (II) 1 min 5% B, 22 min 5–15% B, 30 min 15–100% B (Fig. 3); (III) 1 min 20% B, 20 min 20–60% B (Fig. 5).

Preparation of the CTA–diol CSPs

CSP I is the commercially available CSP.

CSP II (method 1). MCCTA (0.8 g) was dissolved in 15 ml of dichloromethane, 3 ml of *n*-hexane were added and the solution was stirred until precipitated CTA was redissolved. A 3-g amount of the silica gel diol was added and the suspension was cooled to −70°C while stirring, then 25 ml of *n*-hexane were added while still stirring. The stationary phase was separated with a glas filter, washed three times with 20 ml of *n*-hexane and dried.

CSP III (method 2). MCCTA was depolymerized with a mixture of acetic acid, anhydrous acetic acid and sulphuric acid as described[28] for 30 min. The resulting material was dried and reprecipitated from dichloromethane with 2-propanol. A 0.8-g amount of depolymerized CTA was dissolved in 20 ml of dichloromethane, 9 ml of isooctane were added and the solution was stirred until precipitated CTA was redissolved. Then 3 g of the silica gel diol were added and the solvents were slowly evaporated under vacuum.

Columns

The commercially available column (CSP I) was made of stainless-steel (250 × 4.6 mm I.D.), packed with CTA supported on macroporous silica gel (10 μm) [Chiralcel OA; Baker (Daicel)].

Other columns were made of stainless-steel, packed with the stationary phase by a conventional slurry method (2-propanol as slurry solvent and methanol as packing solvent) at a pressure of 44 MPa (440 bar). The column dimensions were 250 × 4.0 mm I.D. for CSP II and 125 × 4.6 mm I.D. for CSP III.

Sample clean-up for qualitative analysis

Dried tomato skins (0.5 g) were extracted with 30 ml of methanol–water (70:30, v/v) at 70°C for 1 h and the filtered extracts were evaporated under vacuum to about 4–5 ml. This solution was added to a polyamide cartridge (Chromabond PA; Macherey & Nagel), the cartridge was washed with 8 ml of water and the flavonoids were eluted with 6 ml of methanol. The methanolic solution was evaporated to dryness and the residue was dissolved in 1 ml of methanol–2-propanol (2:1, v/v). *n*-Hexane (1 ml) was added and the solution was filtered.

RESULTS AND DISCUSSION

Fig. 1A shows the enantiomeric separation of naringenin (compound 3) on a commercially available column (CSP I) packed with CTA coated on macroporous silica gel (Chiralcel OA, Daicel). The peak shape is not symmetrical (asymmetry factor

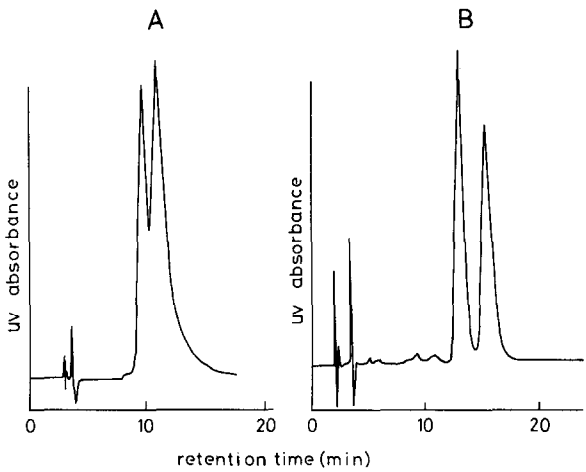


Fig. 1. Enantiomeric separation of naringenin (*cf.*, Table I) on two CSPs based on cellulose triacetate. (A) CTA coated on 10-μm macroporous silica gel (CSP I; Chiralcel OA, Daicel), *n*-hexane–methanol–2-propanol (72:20:8, v/v/v), 1 ml/min; (B) dissolved MCCTA coated on 7-μm Nucleosil diol (CSP II), *n*-hexane–methanol–2-propanol (75:16.3:8.7, v/v/v), 1 ml/min.

2.5–3) and the resolution (R_s = 0.6) is incomplete. The reason for the tailing in the chromatogram may be non-specific adsorption on the silica gel support, which as far as we know consists of macroporous silica gel treated with diphenyldimethoxysilane and N,N-bistrimethylsilylacetamide[28]. Rimböck *et al.*[18] suggested in their studies of CTA coated on silica gel that there are interactions with uncoated parts of this type of stationary phase, which contribute to the overall chromatographic properties of such columns.

There have been several publications describing the coating of cellulose derivatives on a silica gel support. In the first reports on this topic, the coating solvents were slowly evaporated under reduced pressure[10,14,18], whereas in subsequent work the solvents were partially evaporated and the wet material was added to *n*-hexane[11]. The support was usually a silica gel derivative of pore size 100 or 400 nm (macroporous silica gel).

During our investigations, we found that the use of a phase material consisting of a silica gel diol supported with CTA resulted in peak shapes (asymmetry factors 1.4–1.8) superior to those obtained with the commercial column, as illustrated, *e.g.*, in Figs. 1B and 5.

The silica gel diol was supported with (1) dissolved MCCTA (CSP II) and (2) dissolved depolymerized MCCTA (CSP III).

CSP II

Initial attempts to coat silica gel diol by dissolving MCCTA and evaporating the solvents failed, because no powdery material was obtained. The coating of MCCTA on silica gel has been described[10,18], but no details about the quality of the prepared stationary phase were given. Perhaps the molecular weight of MCCTA was different.

We therefore developed a new method in which dissolved MCCTA (in an almost

TABLE II

ENANTIOMERIC SEPARATION OF NARINGENIN: COMPARISON OF CSP I, II AND III

CSP	k'_1[a]	α[b]	R_s[c]	*HETP*[d]	*Mobile phase*[e]
I	2.4	1.20	0.60	0.59	A
II	6.0	1.21	1.16	0.21	B
III	8.2	1.21	1.41	0.13	B

[a] k'_1 = capacity factor of first-eluted enantiomer.

[b] $\alpha = k'_2/k'_1$.

[c] $R_s = 1.198 \cdot \frac{t_2 - t_1}{w_{1/2\,(1)} + w_{1/2\,(2)}}$, where t = retention time and $w_{1/2}$ = peak width at half-height.

[d] HETP = height equivalent to a theoretical plate (mm).

[e] A = *n*-hexane–methanol–2-propanol (72:20:8), 1 ml/min; B = *n*-hexane–methanol–2-propanol (75:16:9), 1 ml/min.

saturated solution) was precipitated at low temperatures (about −70°C) with *n*-hexane. The obtained stationary phases were powdery, but many particles were still agglomerated. This might be the reason why not all of the columns that we prepared where equally good. However, all of them had a better resolution than the commercial column (CSP I), and the separation factor, α, was in the same range (*e.g.*, Table II).

CSP III

Using depolymerized MCCTA (see Experimental), no agglomeration of particles occurred when the solvents (dichloromethane–isooctane, 10:4) were evaporated in vacuum. With these stationary phases the separation factor, α, for naringenin enantiomers was almost the same as with CSP II, but the height equivalent to a theoretical plate (HETP) was lower (Table II) and a better resolution was attained. The reproducibility was satisfactory. Because of the relative high back-pressure of the CTA–diol columns, CSP III was packed in short columns (125 × 4.6 mm I.D.).

Despite the relatively high back-pressure of the HPLC columns [4–6 MPa (40–60 bar) at a flow-rate of 1 ml/min and a mobile phase composed of *n*-hexane–methanol–2-propanol (75:17:8)] we found that non-macroporous silica gel diol is suitable as a support for CTA.

Because we have insufficient information about the commercially available column (CSP I), it is difficult to interpret the differences in resolution and peak symmetry. The decrease in HETP for CTA–diol may be also due to the smaller particle size of 7 μm. From the viewpoint of practical use, however, we can state that CTA–diol columns are better for the separation of polyhydroxylated flavanones.

We also tried to increase the efficiency further by using 5-μm particles. Although it was possible to pack columns with CTA-coated 5-μm silica gel diol, the reproducibility was not good and for most of the columns the HETP was not better than that for the 7-μm columns. The main problem may be the packing procedure itself, because using very short columns (60 × 4.6 mm I.D.) resulted for several columns in a decrease in HETP (about 0.06 mm). Fig. 2 demonstrates that a separation of naringenin enantiomers is possible on this very short column with 5-μm particles.

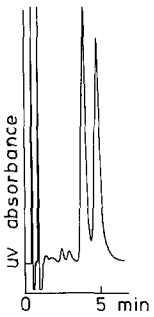


Fig. 2. Optical resolution of naringenin enantiomers on CTA–diol (CSP III type) with 5-μm particles (column 60 × 4.6 mm I.D.). Mobile phase, *n*-hexane–methanol–2-propanol (75:17:8); flow-rate, 1 ml/min; $\alpha = 1.27$; $R_s = 1.4$; pressure = 8 bar.

There are still many problems to be solved, but these results may encourage further investigations in this direction in order to produce efficient cellulose-derived HPLC columns.

The chromatographic data for flavanones with different substitution patterns on a CTA–diol column (CSP III) with an apolar mobile phase are listed in Table III. With

TABLE III

CHROMATOGRAPHIC DATA OF FLAVANONES ON A CTA-COATED DIOL STATIONARY PHASE (CSP III, SHORT COLUMN)

Compound	*Normal-phase mode*			*Reversed-phase mode*[d]	
	k'_1[a]	α[b]	*Mobile phase*[c]	k'_1[a]	α[b]
1	3.75 (−)	1.19	A	2.13 (−)	1.15
2	5.44 (−)	1.13	A	2.55 (−)	1.10
3	11.18 (−)	1.17	A	1.75 (−)	1.22
4	20.84 (−)	1.14	A	1.48 (−)	1.18
5	14.97 (−)	1.15	A	1.67 (−)	1.17
6	13.60 (−)	1.14	A	1.68 (−)	1.18
7	7.22	1.00	A	3.26	1.00
8	4.56	1.00	B	4.12	1.00
9	2.20	1.00	B	1.62	1.00
10	5.87	1.00	B	1.24	1.00
11	3.24	1.00	A	1.31	1.00
12	2.41	1.00	B	2.05	1.00
13	4.54	1.00	B	1.38	1.00
14	3.59	1.00	B	2.07	1.00
15	9.03	1.00	B	1.23	1.00
16	12.10	1.00	C	0.97	1.00

[a] k'_1 = capacity factor of first-eluted enantiomer; the calculation is based on 1,3,5-tri-*tert.*-butylbenzene as non-retained compound[4].

[b] α = separation factor, k'_2/k'_1.

[c] Mobile phase (1 ml/min): (A) *n*-hexane–methanol–2-propanol (80:13.4:6.6); (B) *n*-hexane–methanol–2-propanol (95:3.3:1.7); (C) *n*-hexane–methanol–2-propanol (70:20:10).

[d] Mobile phase: methanol–water (70:30), 0.5 ml/min.

CTA coated on silica gel diol (CTA–diol), only flavanones with 5,7-dihydroxy substitution (phloroglucinol type at ring A) (compounds 1–6, Table I) were resolved. The (−)-enantiomers were eluted before the (+)-enantiomers. These flavanones were also resolved on an MCCTA column (CTA I)[22]. It is interesting to note that (±)-taxifolin (compound 16), a polyhydroxylated 3-hydroxyflavanone, was not resolved on CTA–diol (CSP III), whereas the analogous racemate without the hydroxyl group in position 3 (eriodictyol, compound 4) was separated.

Several eluents were tested with regard to chromatographic behaviour and chiral recognition of naringenin (Table IV). The best resolution was achieved with *n*-hexane–2-propanol–methanol (R_s = 1.5). On substituting *tert.*-butyl methyl ether for 2-propanol the separation factor, α, and the resolution decreased slightly. With *n*-hexane–methanol–ethoxyethanol and *n*-hexane–2-propanol the resolution (R_s = 0.8) was worse. Although naringenin was eluted very fast with methanol (k'_1 = 0.4), a partial resolution was observed. A methanol–water mobile phase resulted in a high separation factor (α = 1.36) and a resolution of R_s = 1.1. None of these mobile phases damaged the stationary phase and the retention times were constant.

Not recommended are acetonitrile-containing mobile phases such as *n*-hexane–*tert.*-butyl methyl ether–acetonitrile (60:30:10) or isooctane–diethyl ether–acetonitrile (150:80:20). The columns were irreversibly damaged by loss of phase material.

Similar to the situation of MCCTA (CTA I)[21] is the influence of temperature on the separation factor, α: decreasing the temperature increases the α values. For example, for naringenin α = 1.16 at 30°C and 1.37 at 5°C with a mobile phase composition of *n*-hexane–2-propanol–methanol (75:8.5:16.5) at a flow-rate of 1 ml/min (measured with a CSP II-type column).

Regarding the separability of different flavanones, the normal-phase mode (apolar eluents) is better than the reversed-phase mode (polar eluents). Fig. 3 illustrates the separation and optical resolution of a mixture of six racemic flavanones (compounds 1–6, Table I) applying gradient I (see Experimental). Except for hesperetin and homoeriodictyol (positional isomers at 3′ and 4′), where two peaks are overlapping, all other flavanones were at least partially resolved. In contrast, in the reversed-phase mode the flavanones are eluted in a narrow retention zone (Table III).

TABLE IV

OPTICAL RESOLUTION OF NARINGENIN ENANTIOMERS ON CSP III: EVALUATION OF MOBILE PHASES

Mobile phase	k'_1 [a]	α [b]	R_s [c]
n-Hexane–methanol–2-propanol (75:17:8)	5.5	1.23	1.5
n-Hexane–methanol–*tert.*-butyl methyl ether (75:17:8)	5.4	1.19	1.4
n-Hexane–methanol–ethoxyethanol (75:17:8)	4.0	1.14	0.8
n-Hexane–2-propanol (75:25)	8.6	1.19	0.8
Methanol	0.4	1.30	– [d]
Methanol–water (70:30)	1.4	1.36	1.1

[a] k'_1 = capacity factor based on a void volume of 1.15 ml as determined with tri-*tert.*-butylbenzene[4].
[b] $\alpha = k'_2/k'_1$.
[c] See Table II.
[d] Partial resolution, R_s not calculated.

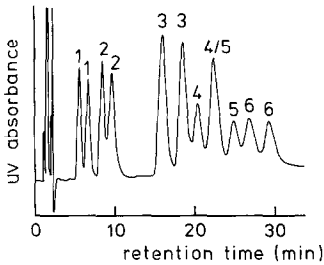
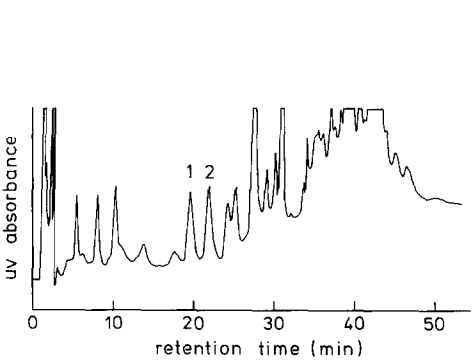


Fig. 3. Gradient elution (I) in the normal-phase mode of a mixture of six flavanones on CSP III (125 × 4.6 mm I.D.). For details, see Experimental. Peaks: 1 = pinocembrin; 2 = isosakuranetin; 3 = naringenin; 4 = hesperetin; 5 = homoeriodictyol; 6 = eriodictyol (*cf.*, Table I). (−)-Enantiomers are eluted before (+)-enantiomers.

Another example of the separation of complex mixtures is the analysis of a tomato skin extract on a short column (125 × 4.6 mm I.D.) applying gradient II in the normal-phase mode (Fig. 4). Naringenin enantiomers are separated from the matrix substances. Peak identification was performed by co-chromatography of naringenin enantiomers and by recording the UV spectra with a diode-array detector. Most naturally occurring flavanones were isolated in an optically active form[29]. The observation that in tomato skins both enantiomers were present may be due to racemization or spontaneous cyclization of naringenin–chalcone during sample preparation. This point is now under investigation.

Flavanone glycosides, which are diastereomers due to the chiral sugar moiety, were separated from the aglycones and were also resolved on CTA–diol (CSP III), as demonstrated in Fig. 5. The elution order with respect to C_2 stereochemistry was confirmed by enzymatic hydrolysis of the enriched fractions of the diastereomers and determination of the enantiomeric aglycone. Flavanone glycosides have been separated as their acetates[30] or benzoates[31] on a silica gel column previously, but direct separation by HPLC has not yet been reported.



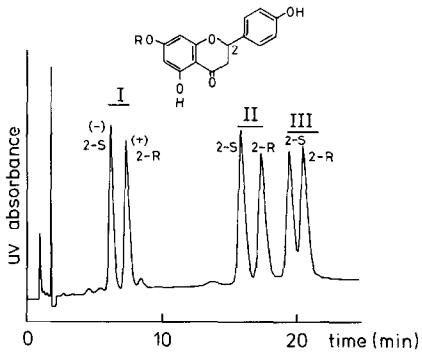


Fig. 4. Naringenin enantiomers in a tomato skin extract on CSP III (CTA–diol) by gradient elution (II) in the normal-phase mode. For details, see Experimental. Peaks: 1 = (−)(2*S*)-naringenin; 2 = (+)(2*R*)-naringenin.

Fig. 5. Separation of naringenin enantiomers and diastereomeric naringenin glycosides in a single chromatographic run on CTA III by gradient elution. For details, see text. Peaks: I = naringenin, R = H; II = naringenin-7-O-glucoside, R = β-D-glucopyranose; III = naringenin-7-O-neohesperidoside, R = α-L-rhamnopyranosyl-β-D-(1,2)-glucopyranose.

CONCLUSION

Although the resolving power (separation factor) of a chiral stationary phase of silica gel diol coated with CTA (CTA–diol) is lower than that of MCCTA[21], the former is superior to MCCTA and to a commercial CTA-coated silica gel column (Chiralcel OA) for the enantiomeric separation of polyhydroxylated flavanones owing to its higher efficiency. With gradient elution the analysis of complex mixtures is possible. The described chiral stationary phase can be easily prepared and may also be an alternative to the very expensive commercially available column for the solution of other problems.

REFERENCES

1 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277.
2 G. Blaschke, *J. Liq. Chromatogr.*, 9 (1986) 341.
3 A. Hussenius, R. Isaksson and O. Matson, *J. Chromatogr.*, 405 (1987) 155.
4 H. Koller, K.-H. Rimböck and A. Mannschreck, *J. Chromatogr.*, 282 (1983) 89.
5 K. R. Lindner and A. Mannschreck, *J. Chromatogr.*, 193 (1980) 308.
6 K.-H. Rimböck, F. Kastner and A. Mannschreck, *J. Chromatogr.*, 329 (1985) 307.
7 A. Mannschreck, H. Koller and R. Wernike, *Kontakte (Darmstadt)*, 1 (1985) 40.
8 Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173.
9 Y. Okamoto, R. Aburatani, T. Fukumoto and K. Hatada, *Chem. Lett.*, (1987) 1857.
10 Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, *Chem. Lett.*, (1984) 739.
11 Y. Okamoto, R. Aburatani and K. Hatada, *J. Chromatogr.*, 389 (1987) 95.
12 Y. Okamoto, H. Sakamoto, K. Hatada and M. Irie, *Chem. Lett.*, (1986) 983.
13 T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
14 T. Shibata, T. Sei, H. Nishimura and K. Deguchi, *Chromatographia*, 24 (1987) 552.
15 A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi and Y. Toga, *Chromatographia*, 19 (1984) 280.
16 R. H. Marchessault and P. R. Sundararjan, in G. O. Aspinall (Editor), *The Polysaccharides*, Vol. 2, Academic Press, London, 1983, pp. 82–89.
17 E. Francotte, R. M. Wolf, D. Lohmann and R. Müller, *J. Chromatogr.*, 347 (1985) 25.
18 K.-H. Rimböck, M. A. Cuyegkeng and A. Mannschreck, *Chromatographia*, 21 (1986) 223.
19 Daicel Chemicals Industries, *Jpn. Pat.*, 63 117 001 (1988); *C.A.*, 109 (1988) 131 104d.
20 E. J. Roche, J. P. O'Brien and S. R. Allen, *Polym. Commun.*, 27 (1986) 138.
21 M. Krause and R. Galensa, *J. Chromatogr.*, 441 (1988) 417.
22 B. A. Bohm, in J. B. Harborne (Editor), *The Flavonoids, Advances in Research*, Chapman and Hall, London, New York, 1988, pp. 348–372.
23 M. Krause and R. Galensa, *Lebensmittelchem. Gerichtl. Chem.*, 43 (1989) 12.
24 M. Krause and R. Galensa, *J. Chromatogr.*, submitted for publication.
25 B. A. Bohm, in J. B. Harborne (Editor), *The Flavonoids, Advances in Research*, Chapman and Hall, London, New York, 1988, pp. 372–379.
26 H. Takahashi, S. Li, Y. Harigaya and M. Onda, *Heterocycles*, 26 (1987) 3239.
27 M. Niwa, S. Otsuji, H. Tatamatsu, G.-Q. Liu, X.-F. Chen and Y. Hirata, *Chem. Pharm. Bull.*, 34 (1986) 3249.
28 Daicel Chemical Industries, *Eur. Pat. Appl.*, 0 121 776 A1 (1984); *C.A.*, 102 (1985) 26 690z.
29 H. Arakawa and M. Nakazaki, *Justus Liebigs Ann. Chem.*, 636 (1960) 111.
30 R. Galensa and K. Herrmann, *J. Chromatogr.*, 198 (1980) 217.
31 D. Treutter, R. Galensa, W. Feucht and P. P. S. Schmid, *Physiol. Plant.*, 65 (1985) 95.