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Preparation of a cyclophane-bonded stationary phase and its application to separation of naphthalene derivatives

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Abstract

A cyclophane (CP44)-bonded silica gel stationary phase was prepared and elution behaviour of hydrophobic solutes was investigated in the reversed-phase mode. Aromatic compounds were retained on the stationary phases more strongly than the corresponding alicyclic compounds, as was expected by the complex-forming ability of the cyclophane. The stationary phases also showed isomer-selective separation for monomethyl- and dimethylnaphthalenes. The isomers having methyl groups at the α-position were eluted prior to those having methyl groups at the β-position, i.e., the retention order for methylnaphthalene was, α<β and that for dimethylnaphthalene, α,α<α,β<β,β. Moreover, some dimethylnaphthalene isomers which cannot be separated on ordinary reversed-phase stationary phases were separated finely on this stationary phase. The separation mechanism is discussed on the basis of the structure of the cyclophane-involved complex. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, LC; Cyclophane-bonded stationary phases; Naphthalenes

1. Introduction

Current attention is being paid to the application of highly-selective organic host compounds to chromatography. Most extensively studied host compounds are cyclodextrins, naturally-occurring host compounds having hydrophobic cavities and being able to form inclusion complexes with organic compounds [1]. A number of cyclodextrin-bonded stationary phases have been prepared, and used successfully for separation of a wide variety of organic compounds [2,3].

Cyclophanes are another group of promising host compounds bearing hydrophobic cavities [4–6]. They are fully synthetic host compounds, and therefore able to be designed as required for given guest molecules. Although many excellent cyclophanes have been synthesized, few reports have been published on their application to chromatographic stationary phases.

Odashima et al. recently reported the preparation of a cyclophane-immobilized Sepharose gel and its application to the separation of water-soluble aromatic guests [7]. Although they gave interesting results, the gel has the disadvantage that it is neither usable in an organic mobile phase and under a high

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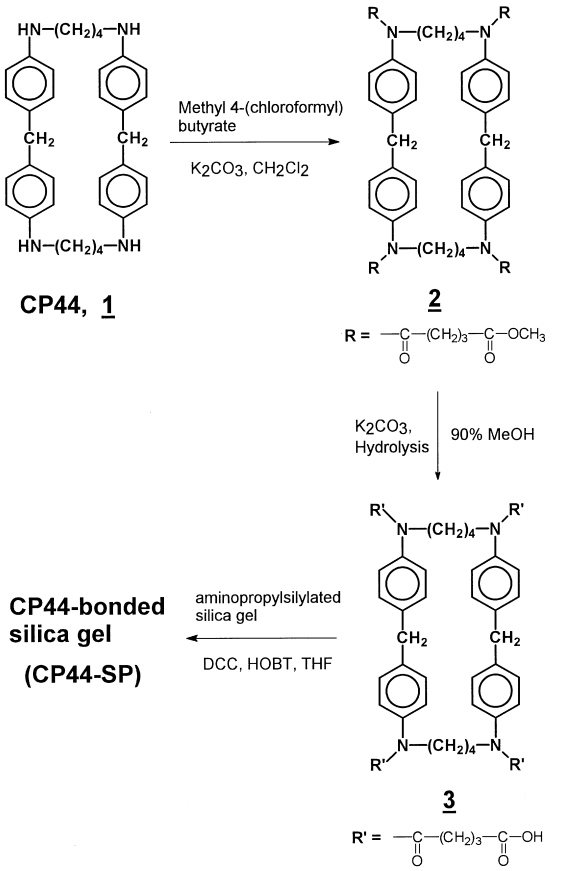

Fig. 1. Synthetic routes to CP44-bonded silica gel.

pressure (ordinary HPLC conditions) nor applicable to separation of multi-ring aromatic compounds.

This paper deals with a new cyclophane-bonded stationary phase for HPLC. A cyclophane-bonded stationary phase was prepared by binding a cyclophane, named CP44 (Fig. 1), covalently to silica gel, and the retention behaviour of 2- or 3-ring aromatic compounds on the stationary phase was examined.

2. Experimental

2.1. Reagents and materials

Naphthalene, 1- and 2-methylnaphthalene, 1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,6- and 2,7- dimethylnaphthalenes, *cis*- and *trans*-decahydronaphthalenes, biphenyl, dicyclohexyl, phenylcyclohexane, anthracene, phenanthrene and 1-hydroxybenzotriazole (HOBT) were purchased from Wako (Osaka, Japan). 2,3-Dimethylnaphthalene was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Methyl 4-(chloroformyl)butyrate and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from Aldrich (Milwaukee, USA). Silica gel (M.S. GEL SIL: particle size 5 μm, pore size 120 Å and surface area 350 m^2/g) was obtained from Dohkai (Fukuoka, Japan). All other reagents were of guaranteed reagent grade.

Octadecylsilylated silica gel-prepacked column (L-Column ODS (polymeric); 150 mm×4.6 mm I.D.) and phenylethylsilylated silica gel-prepacked column (ULTRON N-Phenyl; 150 mm×4.6 mm I.D. were obtained from Chemicals Inspection and Testing Institute (Tokyo, Japan) and Shinwa (Kyoto, Japan), respectively.

2.2. Preparation of CP44-bonded stationary phase

1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**1**,CP-44) was synthesized as described by Odashima et al. [8], and bound covalently to silica gel according to the scheme in Fig. 1.

*2.3. Synthesis of 1,6,20,25-tetra(4′-carboxylbutanoyl)-1,6,20,25-tetraaza [6.1.6.1] paracyclophane (**3**)*

To a mixture of **1** (5.0 g, 10 mmol) and anhydrous K_2CO_3 (5.5 g, 40 mmol) in dry CH_2Cl_2 (350 ml) was added dropwise a solution of methyl 4-(chloroformyl)-butyrate (9.9 g, 60 mmol) in dry CH_2Cl_2 (80 ml) over a period of 1 h at 0°C. The reaction mixture was gradually warmed to room temperature and stirred for 3 days. The solution was washed with saturated $NaHCO_3$, water, and brine, and dried. After removal of the solvent the residue was purified by chromatography over silica gel eluted with benzene–ethyl acetate (1:3, v/v) to give 8.6 g of **2** (yield 84%).

To a solution of K_2CO_3 (6.9 g, 50 mmol) in 90% aqueous solution of methanol (500 ml) was added **2** (5.0 g, 4.9 mmol), and the solution was refluxed for 2 h. After cooling, the solution was acidified with

concentrated HCl. The precipitated solid, **3**, was collected, and recrystallized from methanol–water (4:1, v/v) (4.3 g, yield 91%).

2.4. Bonding of CP44 to silica gel

Silica gel was aminopropylsilylated and end-capped according to the procedure described in another paper [9].

To a suspension of **3** (1.7 g, 1.8 mmol), DCC (1.8 g, 8.6 mmol) and HOBT (1.2 g, 8.6 mmol) in terahydrofuran (50 ml) was added amino-propylsilylated silica gel (3.0 g, 1.2 mmol NH_2/g), and the mixture was stirred for 24 h. The reaction mixture was then treated with a mixture of acetic acid (0.27 ml, 4.7 mmol), DCC (0.89 g, 4.3 mmol) and HOBT (0.59 g, 4.3 mmol) for 24 h to acetylate residual amino groups. The particles were filtered, washed sequentially with terahydrofuran, ethanol and hot ethanol, and dried at 60°C under vacuum for 4 h. The quantity of bound CP44 estimated from the element analysis was 0.17 mmol/g of stationary phase.

2.5. Chromatographic measurements

The packing of the CP44-bonded stationary phase (CP44-SP) into a stainless-steel column (150 mm× 4.6 mm I.D.) was carried out by the conventional high-pressure slurry-packing procedure.

Chromatography was carried out using a standard apparatus: a pump (Model 576, GL Sciences, Tokyo, Japan), a UV detector (SPD-10A, Shimadzu, Kyoto, Japan), a refractive index detector (SE-51, Syowa Denko, Tokyo, Japan), a recorder (Chromatocorder 12, System Instruments, Tokyo, Japan) and an injector (model 7125, Rheodyne, CA, USA). The column temperature was controlled by dipping the column in a thermostated water bath. The standard chromatographic conditions were as follows. The eluent was a mixture of acetonitrile and water (for the ratio, see the footnote of table). The flow-rate was 1 ml/min. A 1-μl volume of 10 m*M* solute solutions in methanol was injected into the Rheodyne valve.

3. Results and discussion

Table 1 shows the retention of various aromatic compounds and their corresponding alicyclic compounds on the CP44-SP, in comparison with those on octadecylsilylated- and phenylethylsilylated-silica gel stationary phases (ODS-SP and phenyl-SP). k' is the capacity factor, and α is the ratio of the capacity factor of each alicyclic compound to that of the corresponding aromatic compound. The α values of dicyclohexyl and decahydronaphthalene on the CP44-SP were below 1, indicating that aromatic compounds were retained more strongly than alicyclic compounds. This is in marked contrast to the results obtained with the ordinary reversed-phase stationary phases (ODS-SP and phenyl-SP), where more hydrophobic alicyclic compounds were eluted

Table 1
Comparison of the retention between aromatics and the corresponding alicyclics

Compound	CP44-SP[a]		ODS-SP[b]		Phenyl-SP[a]	
	k'	α	k'	α	k'	α
Dicyclohexyl	2.25	0.97	14.71	12.71	5.18	2.63
Phenylcyclohexane	1.58	0.68	2.86	2.47	2.91	1.48
Biphenyl	2.31	1.00	1.16	1.00	1.97	1.00
trans-Decahydronaphthalene	1.21	0.88	7.39	7.91	3.52	2.36
cis-Decahydronaphthalene	1.33	0.96	6.92	7.42	3.39	2.28
Naphthalene	1.38	1.00	0.93	1.00	1.49	1.00
Cyclohexane	0.73	1.26	2.18	3.97	1.68	1.77
Benzene	0.58	1.00	0.55	1.00	0.95	1.00

[a] Acetonitrile–water (60:40, v/v).
[b] Acetonitrile–water (85:15, v/v).
Temperature; 25°C.

after the corresponding aromatic compounds. In the case of one-ring compounds (benzene and cyclohexane), no reversal of elution order between the CP44-SP and the reversed-phase stationary phases was observed, i.e., benzene was eluted prior to cyclohexane.

The retention of solutes on the CP44-SP mainly stemmed from two kinds of interaction; one is CP44-involved complex formation and the other, hydrophobic bonding. According to Odashima et al. [10], CP44 has a hydrophobic cavity whose rectangular opening is about 3.5 Å wide and 7.9 Å long. Since this width is about the same size as the thickness of aromatic compounds and too narrow for aliphatic compounds, CP44 can form an inclusion complex easily with aromatic compounds, but not with alicyclic compounds. Therefore, the retention due to the CP44-involved complex formation occurs only for aromatic compounds. On the other hand, the hydrophobic interaction works more strongly on alicyclic compounds than on aromatic compounds. The balance of these two interactions determines the elution order. The retention order of naphthalene> decahydronaphthalene and biphenyl>dicyclohexyl on the CP44-SP reflects that the CP44-involved interaction for aromatic compounds surpasses the hydrophobic interaction for alicyclic compounds. The reason why the retention of phenylcyclohexane is less than that of dicyclohexyl can be given as follows: as shown by Odashima et al. [11], the complex formation constants of CP44 with benzene derivatives are much smaller than those with naphthalene derivatives, indicating that a multi-aromatic ring is necessary for stable complex formation. Phenylcyclohexane consists of one phenyl and one cyclohexyl ring. The replacement of the cyclohexyl ring by a phenyl one in dicyclohexyl brings about a large decrease in the retention by hydrophobic interaction because of the loss of one cyclohexyl ring, whereas an added phenyl ring gives rise to a small increase in the retention by the CP44-involved complex formation. Eventually, the balance of these two effects would make the retention of phenylcyclohexane weaker than that of dicyclohexyl. Similarly, no reversal of elution order between benzene and cyclohexane can be explained in terms of the low complex formation constant for benzene.

Table 2 shows the retention (k') and the retention ratio (α) of 2- and 3-ring aromatic compounds, where α stands for the ratio of the capacity factor of each aromatic compound to that of naphthalene. Of interest is that the elution order of naphthalene and 1-methylnaphthalene was different between the CP44-SP and reversed-phase stationary phases, i.e., the retention order on the CP44-SP was naphthalene>1-methylnaphthalene, whereas that on ODS and phenyl-SPs was naphthalene<1-methylnaphthalene. Since the retention order on the ODS stationary phase can be regarded as the order of hydrophobicity, the reversal of the order on the CP44-SP indicates that the CP44-involved retention worked more strongly for naphthalene than for 1-methylnaphthalene. Taking into consideration that 2-methylnaphthalene was retained strongly on the CP44-SP, the position of the methyl group on the naphthalene ring plays an important role for the CP44-involved retention. The difference in retention between other isomer pairs of 1,5-dimethyl-

Table 2
Retention (k') and retention ratio (α) of some aromatic compounds on CP44-SP, ODS-SP and phenyl-SP

Aromatics	CP44-SP		ODS-SP		Phenyl-SP	
	k'	α	k'	α	k'	α
Naphthalene	1.46	1.00	4.21	1.00	1.78	1.00
1-Methylnaphthalene	1.37	0.94	6.27	1.49	2.14	1.20
2-Methylnaphthalene	1.74	1.19	6.61	1.57	2.16	1.21
1,5-Dimethylnaphthalene	1.51	1.03	9.51	2.26	2.52	1.41
2,6-Dimethylnaphthalene	2.10	1.44	10.71	2.54	2.63	1.48
Anthracene	4.40	3.01	10.25	2.43	2.81	1.57
Phenanthrene	3.18	2.18	9.14	2.17	2.69	1.51

Eluent; acetonitrile–water (60:40, v/v).
Temperature; 20°C.

naphthalene–2,6-dimethylnaphthalene and anthracene–phenanthrene also supports that the CP44-SP has an isomer-selectivity for aromatic compounds.

In order to examine this isomer-selectivity of the CP44-SP in more detail, the elution behaviour of ten geometrical isomers of dimethylnaphthalenes was measured. The results are shown in Table 3, where α is defined as the ratio of the capacity factor of each dimethylnaphthalene to that of 1,8-dimethylnaphthalene. The retention order on the CP44-SP was: 1,8-<1,4-<1,7-<1,5-<1,2-<1,3-<1,6-< 2,3-<2,7-<2,6-, whereas that on the ODS-SP was: 1,8-<1,2-<1,4-<1,5-<2,3-<1,7-<1,6-<1,3-< 2,7-<2,6-. The retention order on the CP44-SP had the following tendency; 1-methylnaphthalene<α,α-dimethylnaphthalene<α,β-dimethylnaphthalene <2-methylnaphthalene<β,β-dimethylnaphthalene, where α and β stand for 1-, 4-, 5-, 8- and 2-, 3-, 6-, 7-positions on naphthalene ring, respectively. This order is quite different from that observed on the ordinary reversed-phase stationary phases (ODS- and phenyl-SPs), where 1-methylnaphthalene<2-methylnaphthalene < α,α-dimethylnaphthalenef ≤ α,β-dimethylnaphthalenef ≤ β,β-dimethylnaphthalene. This difference is chiefly ascribed to the contribution due to the CP44-involved complex formation. Odashima et al. proposed on the basis of NMR and structure-modification studies that the complex between CP44 and naphthalene has the structure where the naphthalene ring is included in the cyclophane cavity with its long axis tilted about 30° ('pseudo axial' form) [11]. According to this 'pseudo axial' complex form, the distance between a methyl moiety of methyl-naphthalene and the cyclophane ring is greater for the β-position than for the α-position. The balance of this steric hindrance in the complex formation and the overall hydrophobic interaction can explain the above retention order for the naphthalene derivatives: Addition of methyl group in α-position of 2-methylnaphthalene gives rise to a large steric hindrance in the complex formation which surpasses the increase in the hydrophobic interaction, and hence decreases the retention (α,β-<

Table 4
Effect of temperature on the retention of various aromatics on CP44-SP

Aromatics	k' (capacity factor)			
	0°C	10°C	20°C	30°C
Naphthalene	1.83	1.64	1.46	1.28
1-Methylnaphthalene	1.61	1.49	1.37	1.22
2-Methylnaphthalene	2.14	1.94	1.74	1.51
Biphenyl	3.08	2.80	2.48	2.09
Anthracene	5.73	5.12	4.40	3.60
Phenanthrene	3.92	3.59	3.18	2.70
1,2-Dimethylnaphthalene	1.81	1.69	1.56	1.38
1,3-Dimethylnaphthalene	1.81	1.72	1.58	1.40
1,4-Dimethylnaphthalene	1.63	1.56	1.45	1.30
1,5-Dimethylnaphthalene	1.73	1.63	1.51	1.33
1,6-Dimethylnaphthalene	1.82	1.73	1.60	1.41
1,7-Dimethylnaphthalene	1.64	1.57	1.46	1.30
1,8-Dimethylnaphthalene	1.54	1.48	1.39	1.25
2,3-Dimethylnaphthalene	2.38	2.20	1.98	1.71
2,6-Dimethylnaphthalene	2.53	2.34	2.10	1.80
2,7-Dimethylnaphthalene	2.35	2.19	1.99	1.72

Eluent; acetonitrile–water (60:40, v/v).

Table 3
Retention (k') and retention ratio (α) of dimethylnaphthalene isomers on CP44-SP, ODS-SP and phenyl-SP

Dimethylnaphthalene	CP44-SP		ODS-SP		Phenyl-SP	
	k'	α	k'	α	k'	α
1,4-Dimethylnaphthalene	1.45	1.04	9.49	1.10	2.54	1.03
1,5-Dimethylnaphthalene	1.51	1.08	9.51	1.10	2.52	1.02
1,8-Dimethylnaphthalene	1.39	1.00	8.65	1.00	2.47	1.00
1,2-Dimethylnaphthalene	1.56	1.12	9.06	1.05	2.51	1.02
1,3-Dimethylnaphthalene	1.58	1.14	9.93	1.15	2.60	1.05
1,6-Dimethylnaphthalene	1.60	1.15	9.88	1.14	2.58	1.04
1,7-Dimethylnaphthalene	1.46	1.05	9.59	1.11	2.57	1.04
2,3-Dimethylnaphthalene	1.98	1.42	9.59	1.11	2.56	1.04
2,6-Dimethylnaphthalene	2.10	1.51	10.71	1.24	2.63	1.07
2,7-Dimethylnaphthalene	1.99	1.43	10.59	1.22	2.62	1.06

Eluent; acetonitrile–water (60:40, v/v).
Temperature; 20°C.

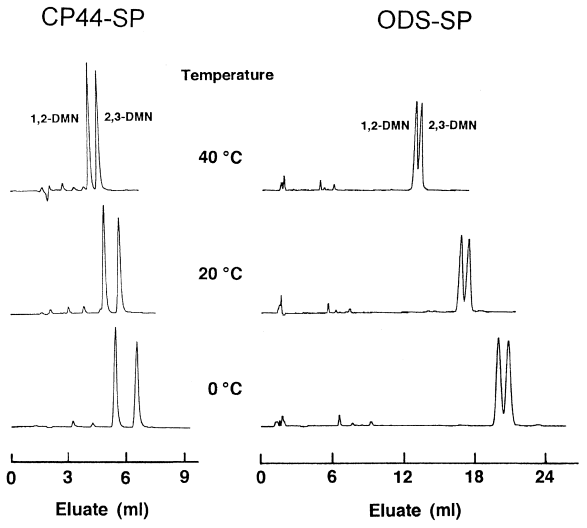

Fig. 2. Elution profiles of a mixture of 1,2- and 2,3-dimethylnaphthalenes (1,2-DMN and 2,3-DMN) at varying temperature.

2-). Contrary to this, addition of methyl group in α-position of 1-methylnaphthalene gives a little effect in the complex formation because 1-methylnaphthalene itself has a low complex formation ability, and therefore, increase the retention due to the increase in hydrophobicity (1-<α,α-). Similarly, the orders of α,α-<α,β- and 2-<β,β- can be explained. The difference in retention between anthracene and phenanthrene shown in Table 2 can also be explained in terms of the above complex structure model, because anthracene and phenanthrene can be regarded as 2,3- and 1,2-substituted naphthalenes, respectively.

Table 4 shows the effect of column temperature on the retention of aromatic compounds. The retention of all the aromatic compounds examined was increased with the decrease of the temperature. It is noteworthy that the difference in retention of several isomer pairs become greater with the decrease of the temperature, as is usually observed in host-involved separation systems [12]. Fig. 2 shows one example where the temperature change of chromatograms of 1,2- and 2,3-dimethylnaphthalenes on the present CP44-SP is shown in comparison with that on the ODS-SP. The separation of 1,2- and 2,3- dimethylnaphthalenes became better on the CP44-SP as the temperature decreased, but little change was observed on the ODS-SP. Another example is shown in Fig. 3. Fine separation of 2,6-dimethylnaphthalene from 2,7-dimethylnaphthalene was observed on the CP44-SP at low temperature, whereas no separation

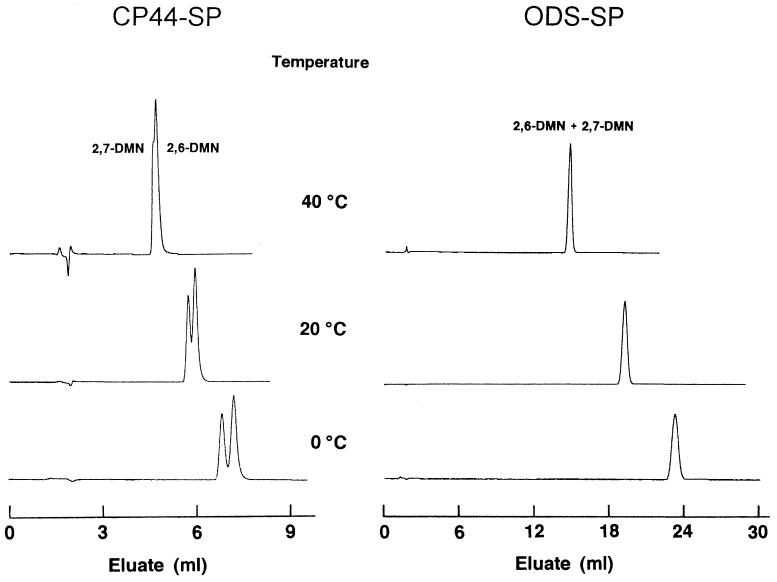

Fig. 3. Temperature dependence of the separation of a mixture of 2,6- and 2,7-dimethylnaphthalenes (2,6-DMN and 2,7-DMN) on CP44-SP and ODS-SP.

was obtained on the ODS-SP at any temperature. Since their mutual separation is very difficult because of the close resemblance of their physicochemical properties, this result is very attractive from the practical point of view.

References

[1] M.L. Bender, M. Komiyama, Cyclodextrin Chemistry, Springer-Verlag, Heidelberg, 1978.
[2] Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funazo, T. Shono, Anal. Chem. 55 (1983) 1852.
[3] D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner Jr., S.-C. Chang, Anal. Chem. 62 (1990) 1610.
[4] I. Tabushi, Acc. Chem. Res. 15 (1982) 66.
[5] F. Diederich, Angew. Chem. Int. Ed. Engl. 27 (1988) 362.
[6] J.E. Forman, R.E. Barrans Jr., D.A. Dougherty, J. Am. Chem. Soc. 117 (1995) 9213.
[7] K. Manabe, K. Odashima, K. Koga, Chem. Pharm. Bull. 40 (1992) 580.
[8] K. Odashima, A. Itai, Y. Iitaka, K. Koga, J. Org. Chem. 50 (1985) 4478.
[9] Y. Sudo, T. Yamaguchi, T. Shinbo, J. Chromatogr. A 736 (1996) 39.
[10] K. Odashima, A. Itai, Y. Iitaka, K. Koga, J. Am. Chem. Soc. 102 (1980) 2504.
[11] K. Odashima, T. Soga, K. Koga, Tetrahedron Lett. 52 (1981) 5311.
[12] T. Shinbo, T. Yamaguchi, H. Yanagishita, D. Kitamoto, K. Sakaki, M. Sugiura, J. Chromatogr. 625 (1992) 101.