ELSEVIER

Journal of Chromatography A, 685 (1994) 166–171

JOURNAL OF CHROMATOGRAPHY A

Short communication

Immobilization of peralkylated β-cyclodextrin on silica gel for high-performance liquid chromatography

Ionel Ciucanu*, Wilfried A. König

Institute for Organic Chemistry, Hamburg University, Martin Luther King Platz 6, D-20146 Hamburg, Germany

First received 3 March 1994; revised manuscript received 25 May 1994

Abstract

The synthesis of mono-O-5-pent-1-enyl-β-cyclodextrin was studied in order to obtain the best yield of mono-O-substitution. Permethylated mono-O-5-pent-1-enyl-β-cyclodextrin, purified by liquid chromatography, was characterized by fast atom bombardment MS and ^{1}H NMR spectrometry. GC–MS analysis of partially methylated alditol acetates obtained from mono-O-pent-1-enyl-β-cyclodextrin showed that the 5-pent-1-enyl ether group was in the α-D-glucopyranoside residue in the 2-OH position in a proportion of 95.9% for the O-substitution reaction in dimethyl sulfoxide and in the presence of sodium hydroxide. Silica gel with chemically bonded peralkylated(methyl or propyl)-β-cyclodextrin proved to be an efficient stationary phase for the separation of enantiomers.

1. Introduction

Cyclodextrins (CDs) are extensively used in high-performance liquid chromatography (HPLC) as stationary phases bonded to a solid support or as additives in mobile phases for the separation of various types of organic compounds and enantiomers.

CDs can be bonded to silica beads via several spacer arms [1–7] and these methods can be generally classified in three main ways. First, a spacer arm is grafted on silica gel and the CD is reacted with the reactive terminal group of the spacer arm [5]. Second, the reactive group of the spacer arm coupled to a CD molecular is reacted with silanol groups on the surface of silica gel [6]. Third, part of the spacer arm is coupled to silica gel and another part to the CD and immobilization consists in reaction of the reactive groups of these two parts [1–4,7]. In the first and third approaches, the presence of unreacted groups of spacers exhibit complex retention properties. Moreover, thermodynamic and theoretical parameters of the inclusion complex cannot be correctly evaluated when the silica gel surface and the position of the spacer arm on the CD are not well characterized.

* Corresponding author. Present address: University of Timisoara, Department of Chemistry, str. Pestalozzi 16, RO-1900 Timisoara, Romania.

0021-9673/94/$07.00
SSDI 0021-9673(94)00564-P

This paper described a procedure to obtain silica gel with well characterized chemically bonded peralkylated β-CDs, using the second approach for immobilization.

2. Experimental

2.1. Reagents and materials

β-Cyclodextrin, dimethoxymethylhydrosilane (DMMHS), dichloromethylhydrosilane (DCMHS), 5-bromo-1-pentene and hexachloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$) were obtained from Fluka (Buchs, Switzerland). Microporous spherical silica gel (LiChrosorb Si 100) with a medium particle diameter of 5 μm, a mean pore size of 110 nm and a specific surface area of 320 m^2/g, dimethyl sulfoxide (DMSO), dimethylacetamide (DMAA), dimethylformamide (DMFA), dioxane, iodomethane, 1-iodopropane and thin-layer chromatography (TLC) aluminium roll pre-coated silica gel 60 F_{254} were purchased from Merck (Darmstadt, Germany). Water was purified by means of a Milli-Q water-purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus

HPLC was performed with a Spectra-Physics (Santa Clara, CA, USA) Model 8500 chromatograph equipped with a UV detector operated at 254 and 280 nm. The HPLC columns (250 mm × 4.6 mm I.D.) were packed by the usual slurry method.

Gas chromatography (GC) was carried out on a Fractovap 2101 gas chromatograph (Carlo Erba, Milan, Italy) equipped with split injection, a flame ionization detector and a Hewlett Packard Model 3390A integrator.

Electron impact mass spectrometry (EI-MS) was performed with a Finnigan MAT Model 311 A mass spectrometer (MAT, Bremen, Germany) coupled to a Hewlett-Packard HP-5840 gas chromatograph.

Fast atom bombardment mass spectrometry (FAB-MS) was carried out on a VG mass spectrometer (VG Analytical, Manchester, UK) using nitrobenzyl alcohol as matrix.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM 400 spectrometer operating at 400.13 MHz for proton (^1H). All measurements were carried out on solutions in deuteriochloroform and tetramethylsilane was used as the internal standard.

2.3. Preparation of stationary phases

To a solution of 1 mol of β-CD (dried in vacuum for 24 h) in dipolar aprotic solvents was added finely powdered sodium hydroxide and then 5-bromo-1-pentene under a nitrogen atmosphere. The suspension was rapidly stirred at room temperature and after 24 h the solution was evaporated under reduced pressure. For peralkylation, the reaction products dissolved in fresh dipolar aprotic solvent were stirred at room temperature for 12 h with 3 equiv. of sodium hydroxide and 3 equiv. of alkyl halide (iodomethane, 1-iodopropane) per replaceable hydrogen [8]. The reaction mixture was carefully added to 50 ml of cold water and excess of unreacted sodium hydroxide was neutralized with 1 *M* hydrochloric acid. The peralkylated product was extracted three times with chloroform. The combined extracts were washed with water and dried over anhydrous magnesium sulfate. The filtered chloroform solution was concentrated under reduced pressure and the residue was purified by liquid chromatography over silica gel using light petroleum and a light petroleum–acetone (1:1) as eluents.

For the hydrosilylation reaction, to 1 mol of the appropriate peralkylated mono-O-pent-1-enyl-β-CD dissolved in 150 ml of anhydrous toluene was added 1.2 mol of DMMHS or DCMHS in a 250-ml three-necked, round-bottomed flask equipped with a nitrogen inlet and reflux condenser. A few drops of a 4% solution of hexachloroplatinic acid hexahydrate in anhydrous tetrahydrofuran were added at intervals of 2 h. The reaction mixture was heated at 50–90°C with stirring. The progress of the reaction was monitored by IR spectrometry, measuring the decrease in the absorption band at 2060–

2080 cm^{-1} (Si–H), usually requiring more than 36 h. The excess of hydrosilane was removed under reduced pressure. The products were purified by liquid chromatography on silica gel using hexane–toluene (5:1) as eluent. When hydrosilylation was performed with DCMHS, the platinum catalyst was removed by stirring overnight with a drop of mercury.

2.4. Preparation of O-alkylated alditol acetates

Permethylated mono-O-pent-1-enyl-β-CD (10 mg) was hydrolysed in 1 ml of trifluoroacetic acid–80% formic acid (1:1) at 80°C for 20 h. The solution containing the hydrolysate was evaporated to dryness under reduced pressure. The reduction was carried out in water (10 ml) with sodium borohydride (100 mg) for 6 h at room temperature. After acidification with few drops of 80% formic acid and concentration to dryness in the presence of methanol, the product, dried using a high-vacuum pump, was acetylated with acetic anhydride (0.2 ml) and pyridine (0.2 ml) at 100°C for 2 h.

2.5. Immobilization of stationary phase

A 6-g amount of silica gel (dried in vacuum at 150°C for 10 h) was added to 150 ml of a 10% solution of peralkylated mono-O-5-(dimethoxymethylsilyl)pentyl-β-CD in dry toluene with gentle shaking and then the suspension was refluxed for 30 h under nitrogen without stirring. After cooling, the resulting silica gel was filtered through a membrane filter, washed successively with toluene, methanol and diethyl ether, and then dried under vacuum at 40°C for 10 h. The amounts of bonded peralkylated-β-CD were calculated from the results of elemental analysis [9]. The values were 187 μmol/g for permethylated-β-CD and 175 μmol/g for perpropylated-β-CD.

3. Results and discussion

The first step of the coupling technique is to obtain a monofunctionalized derivative of β-CD. Normally this functionalization of CD is possible in one position (2-, 3- or 6-OH) or more positions as the β-CDs have 21 hydroxyl groups. Discrimination by selective reaction between the primary and secondary hydroxyl groups of a β-CD is complicated because of statistical and steric problems. It is known that 2- and 6-OH are more acidic than 3-OH in α-D-glucose [10] and for this reason the basicity of the reaction medium could affect the regioselectivity.

The reaction of β-CDs in dipolar aprotic solvents with various amounts of 5-bromo-1-pentene and powdered sodium hydroxide [8,11] was studied in order to obtain a high yield of mono-O-pent-1-enyl-β-CD. The best results were obtained with DMSO as solvent, 1.2 mol of 5-bromo-1-pentene and 2.5 mol of sodium hydroxide for 1 mol of β-CD. The reaction products showed a high yield of mono-O-pent-1-enyl-β-CD and small amounts of di- and tri-substituted derivatives (Table 1). The first control of the reaction was made by TLC using 25%

Table 1
Yields of permethylated mono-O-pent-1-enyl-β-CDs and substitution position of the 1-pentenyl group as a function of solvent (1.2 mol of 5-bromo-1-pentene per mole of β-CD and 2.5 mol of sodium hydroxide per mole of β-CD)

Solvent	Yield (%)	Relative proportion (%)		
		2-Substitution	3-Substitution	6-Substitution
DMSO	63 ± 2.1	95.9	0.6	3.5
DMAA	50 ± 2.6	73.9	1.2	24.7
DMFA	31 ± 1.8	56.8	1.7	41.5
DMSO–dioxane (1:1, v/v)	53 ± 1.9	60.7	1.7	37.6

ammonia solution–ethanol–2-propanol (2:1:1) as eluent, which separated very well monofunctionalized β-CDs with a 1-pentenyl group in the 2-OH ($R_F = 0.46$), 3-OH ($R_F = 0.42$) or 6-OH position ($R_F = 0.33$). Identification of the spots was made with standard compounds. The optimum reaction temperature was 22°C because at higher temperatures the amounts of di- and trisubstituted β-CD derivatives were larger. After 1 day at room temperature, the reaction mixture was permethylated [8] and then fractionated by liquid chromatography on silica gel.

The purity of permethylated mono-O-pent-1-enyl-β-CD was determined by FAB-MS and ^{1}H NMR spectrometry. Permethylated mono-O-pent-1-enyl-β-CD was analysed in a variety of FAB matrices to determine the relative sensitivity of the molecular ion. The best result was obtained with nitrobenzyl alcohol, which generated $[M + H]^+$ ion over $[M + Na]^+$ and $[M + K]^+$ ions. The loss of the methanol molecule gave an intense fragment ion $[M + H - 32]^+$ (Fig. 1). The presence of the O-pent-1-enyl group in permethylated-β-CD gave a slight modification of the proton chemical shift by comparison with the ^{1}H NMR spectrum of permethylated-β-CD [12]. The proton absorption at $\delta = 5.68–5.83$ gave a multiplet that may be ascribed to vinyl CH from the 1-pentenyl group.

In order to determine the position of the O-pent-1-enyl group in the α-D-glucopyranosyl residue, the fraction of permethylated mono-O-pent-1-enyl-β-CD was successively hydrolysed, reduced and acetylated, and O-methylated alditol acetates were analysed by GC (Fig. 2). The peaks were identified by EI-MS and the intensities and the characteristic fragment ions are listed in Table 2. The interpretation of the mass

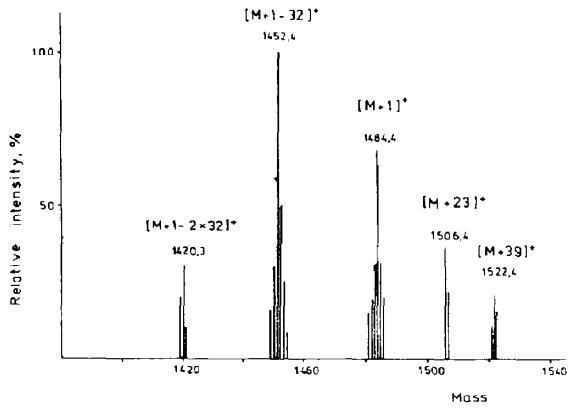

Fig. 1. Positive-ion FAB-MS of permethylated mono-O-pent-1-enyl-β-CD.

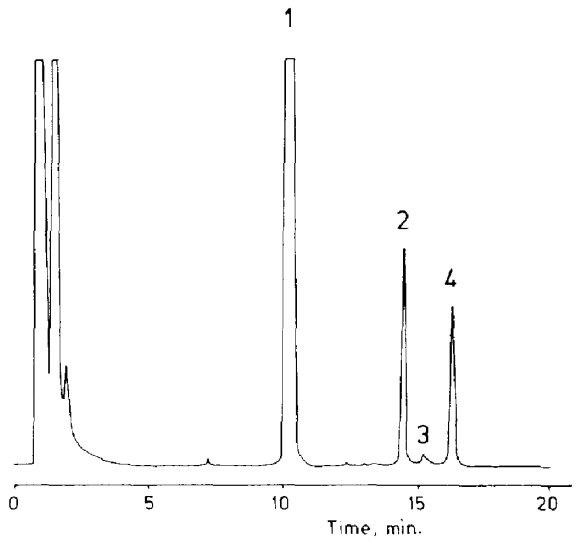

Fig. 2. Gas chromatogram of partially methylated alditol acetates from mono-O-pent-1-enyl-β-CD synthesized in DMFA. Capillary column, SE-30; column temperature increased from 140 to 200°C at 4°C/min; carrier gas, hydrogen. Peaks: 1 = 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methylglucitol; 2 = 1,4,5-tri-O-acetyl-3,6-di-O-methyl-2-O-pent-1-enylglucitol; 3 = 1, 4, 5-tri-O-acetyl-2, 5-di-O-methyl-3-O-pent-1-enylglucitol; 4 = 1, 4, 5-tri-O-acetyl-2, 3-di-O-methyl-6-O-pent-1-enylglucitol.

Table 2
Partial EI mass spectra of derivatives obtained from permethylated mono-O-pent-1-enyl-β-CD after hydrolysis, reduction and acetylation

Peak No.[a]	*m/z* (% of base peak)
1	43(100), 101(20), 117(61), 129(10), 161(6), 173(5), 189(1), 233(20)
2	43(90), 69(100), 113(30), 117(5), 129(63), 171(9), 173(8), 215(3), 233(32), 319(3)
3	43(100), 69(87), 117(55), 167(13), 215(2), 227(6), 287(23), 319(3)
4	43(100), 69(59), 101(26), 117(81), 129(43), 161(11), 171(2), 233(3), 243(3), 287(3), 319(5)

[a] See Fig. 2.

spectra was based on the known fragmentations of partially methylated alditol acetates [13,14]. All compounds with a 1-pentenyl group gave a characteristic fragment ion at m/z 69 $[CH_2CH_2CH_2CH=CH_2]^+$ and m/z 319 $[M-85]^+$. The high abundance of primary fragment ions results from fission between two methoxylated carbons in the chain and decreases when methyl is replaced with acetyl. The compounds in peak 1 gave similar mass spectrum to those reported [13,15] and was consistent with 1,4,5-tri-O-acetyl-2,3,4-tri-O-methylglucitol. The compound in peak 2 was identified as 1,4,5-tri-O-acetyl-3,6-di-O-methyl-2-O-pent-1-enylglucitol. The presence of a 1-pentenyl group on 2-OH was indicated by the absence of fragment ions at m/z 287 and 117 and by the presence of a primary intense fragment ion at m/z 233 and a secondary fragment ion at m/z 129 formed from m/z 171, due to the loss of $CH_3CH=CH_2$. The compound in peak 3 was identified as 1,4,5-tri-O-acetyl-2,6-di-O-methyl-3-O-pent-1-enylglucitol owing to the presence of intense fragment ions at m/z 287 and 117. The compound in peak 4 gave an intense fragment ion at m/z 117 and characteristic fragment ions at m/z 161 and 243. These data and the absence of fragment ions at m/z 233 and 171 indicate the compound to be 1,4,5-tri-O-acetyl-2,3-di-O-methyl-6-O-pent-1-enylglucitol.

Table 1 gives the quantitative results for the distribution of the 1-pentenyl group in the α-D-glucopyranosyl residue of mono-O-pent-1-enyl-β-CD. As expected for such base-catalysed, kinetically controlled alkylation reaction, the 2-OH position was preponderantly substituted. The formation of anions is the rate-determining step. The best yield of 2-OH substitution was obtained using DMSO as solvent. These results are not in accord with those obtained by Schurig et al. [16], which indicated the 6-OH position for substitution in this solvent. We observed that the selectivity of mono-O-substitution decreases with decreasing polarity of the dipolar aprotic solvent (DMSO > DMAA > DMFA) [17]. Similar results were obtained by adding dioxane to DMSO. The very low yield at the 3-OH position can be explained by hydrogen bonding and steric hindrance due to their direction in the cavity of the CD [18].

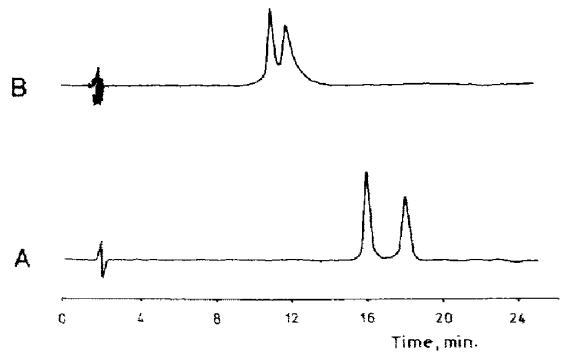

Fig. 3. Separation of enantiomers of hexobarbital on (A) permethylated β-CD and (B) perpropylated β-CD bonded stationary phases. Mobile phase, 0.5% triethylammonium acetate buffer (pH 4.2)–methanol (70:30, v/v); flow-rate, 0.8 ml/min; detection, UV at 254 nm.

Preliminary chromatographic evaluation of peralkylated (methyl or propyl) chemically bonded β-CDs showed the successful separation of racemates of some drugs. Fig. 3 shows the separation of racemic hexobarbital. Comparison between columns A and B indicates a change in the retention time, selectivity and shape of the peaks. These modifications can be only attributed to the replacement of methyl with propyl groups in the β-CD. Clearly, more studies need to be done with these columns in order to give a good explanation for the mechanism of chiral recognition.

Acknowledgements

A research fellowship from Alexander von Humboldt Foundation (Bonn, Germany) to I.C. is gratefully acknowledged. We thank Annegret Meiners and Manfred Preusse for recording the spectra.

References

[1] K. Fujimura, T. Ueda and T. Ando, *Anal. Chem.*, 55 (1983) 446–450.

[2] Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funazo and T. Shono, *Anal. Chem.*, 55 (1983) 1852–1857.
[3] M. Tanaka, Y. Kawaguchi, M. Nakae, Y. Mizobuchi and T. Shono, *J. Chromatogr.*, 299 (1984) 341–350.
[4] M. Tanaka, J. Okazaki, H. Ikeda and T. Shono, *J. Chromatogr.*, 370 (1986) 293–301.
[5] D.W. Armstrong, *US Pat.*, 4539399 (1985).
[6] K. Fujimura, S. Suzuki, K. Hagashi and S. Masuda, *Anal. Chem.*, 62 (1990) 2198–2205.
[7] Y. Kuroda, T. Koto and H. Ogoshi, *Bull. Chem. Soc. Jpn.*, 66 (1993) 1116–1120.
[8] I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[9] G.E. Brendsen and L. de Galan, *J. Liq. Chromatogr.*, 1 (1978) 561–586.
[10] R.I. Gelb, M. Schwartz and D.A. Laufer, *Bioorg. Chem.*, 11 (1982) 274–280.
[11] I. Ciucanu, S. Negura, W. Hirschvogel and C.-t. Luca, *Rev. Roum. Chim.*, 37 (1992) 843–847.
[12] J.R. Johnson and N. Shankland, *Tetrahedron*, 41 (1985) 3147–3152.
[13] H. Bjoerndal, C.G. Hellerqvist, B. Lindberg and B. Svensson, *Angew. Chem.*, 16 (1970) 643–652.
[14] J. Loenngren and S. Svensson, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.
[15] P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg and J. Loenngren, *Chem. Commun.*, (1976) 1–74.
[16] V. Schurig, D. Schmalzig, U. Muehleck, M. Jung, M. Schleimer, P. Mussche, C. Duvekot and J.C. Buyten, *J. High Resolut. Chromatogr.*, 13 (1990) 713–717.
[17] D.W. Meek, in J.J. Langowski (Editor), *The Chemistry of Non-Aqueous Solvents*, Academic Press, New York, 1960, p. 23.
[18] W. Saenger, *Angew. Chem.*, 92 (1980) 343–361.