ELSEVIER

Journal of Chromatography A, 883 (2000) 27–37

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

New stationary phase for liquid chromatography with chemically bonded pinane ligand: synthesis and characterization by nuclear magnetic resonance and high-performance liquid chromatographic investigations

Jürgen Wegmann[a], Stefan Bachmann[a], Heidi Händel[a], Christof Tröltzsch[b], Klaus Albert[a,*]

[a]*Institut für Organische Chemie der Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany*

[b]*Abteilung für Analytische Chemie, Institut für Physikalische Chemie, Soldtmann Strasse 16, D-17489 Greifswald, Germany*

Received 24 January 2000; received in revised form 23 March 2000; accepted 27 March 2000

Abstract

A new bicyclic phase for liquid chromatography was prepared by solution polymerization approaches. To introduce a C_4 spacer the starting molecule 3-formylpinane was reduced to the alcohol followed by a substitution of the hydroxy group through a bromide. The obtained halide reacted with magnesium and allyl bromide to the 3-(but-3′-enyl)pinane which was hydrosilylated with trichlorosilane and finally immobilized to silica gels with different pore sizes using the technique of solution polymerization. To elucidate the structure of 3-(but-3′-enyl)pinane high-resolution two-dimensional nuclear magnetic resonance (NMR) spectra were carried out. The new phases were characterized, on the one hand by employing ^{13}C and ^{29}Si solid-state NMR spectroscopy and on the other hand, by separating a standard test mixture consisting of mainly monosubstituted aromatic compounds. The results achieved in chromatography were correlated with the information gained from ^{29}Si CP/MAS NMR measurements.

Keywords: Stationary phases, LC; Nuclear magnetic resonance spectrometry; Pinanes; Basic aromatic compounds

1. Introduction

Challenging analytical problems are often the reason for progress in the fields of chemistry, biology, pharmacy and medical science. High-performance liquid chromatography (HPLC) is currently the most commonly applied technique to separate and analyze multi-component mixtures and therefore often the matter of choice for unresolved analytical problems. For this reason the development of new packing materials for reversed-phase HPLC is a subject of considerable interest and importance.

C_{18}-phases are mostly used in reversed-phase liquid chromatography [1]. However there is still a need for tailored stationary phases for sophisticated applications in environmental as well as in pharmaceutical and medical science. Beside the variation of the alkyl-chain length [2,3] new packing materials were introduced by immobilizing a large number of functional groups onto silica. Due to the properties of these groups different interactions take place during the separation process. Stationary phases

*Corresponding author. Tel.: +49-7071-2975-335; fax: +49-7071-295-875.

E-mail address: klaus.albert@uni-tuebingen.de (K. Albert)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00407-6

containing aromatic selectors such as benzene, pyrene [4] fluorene [5–7], acridine [8], were described in a couple of studies. Other reversed-phase materials possess large molecules, for example, cyclophanes [9], crown ethers [10] and fullerenes [11]. To combine different interactions during the separation process so called mixed phases were introduced by Buszewski et al. [12] recently. Beside from the above mentioned selectors alicyclic compounds were used as organic ligands to synthesize reversed-phase materials, namely adamantane [13], menthol [14] and norpinane [15]. According to these alicyclic phases we attached pinane as selector by a C_4 spacer.

There are different possibilities to bind a selector onto the silica surface. On one hand it is possible to modify the silica with a spacer containing a functional group, for example an aminoalkyl spacer, and connect the organic selector to silica by an amido coupling. The amido coupling allows polar interactions between the solutes and the stationary phase. Our intention was the synthesis of a totally non-polar phase containing an alicyclic organic compound. Therefore we decided to use the silanization reaction scheme which has been demonstrated to be a very useful approach for binding organic moieties to silica. Using this technique it was necessary to bind a linker with a terminal double bond to the pinane molecule. After the hydrosilylation of the double bond the organic selector was immobilized onto silica [16].

According to the outlined reaction scheme 3-(but-3′-enyl)pinane was synthesized and characterized employing high-resolution two-dimensional nuclear magnetic resonance (NMR) techniques. In a further step 3-(but-3′-enyl)pinane was functionalized using trichlorosilane and finally bonded to silica with two different pore sizes. The structure of the new reversed-phase packing materials was investigated by solid-state NMR spectroscopy. ^{29}Si CP/MAS (cross polarization magic angle spinning) NMR spectroscopy led to detailed information about the surface of the chemically modified silica [17,18], whereas information about the structure of the organic ligand was obtained by ^{13}C CP/MAS NMR spectroscopy. Finally the applicability of the new stationary phases in HPLC was investigated by separating a test mixture, which was introduced by Engelhardt and co-workers in 1990 [19,20]. The test mainly consists of monofunctional benzene derivatives and evaluates the column properties. It will be shown that the information obtained by ^{29}Si CP/MAS NMR spectroscopy is consistent to the results for liquid chromatography.

2. Experimental

2.1. Materials and general procedures

Formylpinane was obtained from BASF (Ludwigshafen, Germany) and purified as described elsewhere [21]. Lithium aluminium hydride, phosphorus tribromide and allyl bromide were purchased from Aldrich. Trichlorosilane was obtained from Wacker (Burghausen, Germany). Methanol (gradient grade for HPLC), ammonium dihydrogenphosphate and diammonium hydrogenphosphate were purchased from Merck (Darmstadt, Germany). Two silica gels were used for the synthesis of the stationary phases: ProntoSil-200-5-SI (surface area 200 m^2/g) and ProntoSil-120-5-SI (surface area 350 m^2/g), both obtained from Bischoff Chromatography (Leonberg, Germany).

The syntheses were performed under moistureless conditions using anhydrous solvents. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl and were stored under argon as well as trichlorosilane. The elemental analyses were carried out on a Carlo Erba analyzer, Model 1106.

2.2. NMR spectroscopy

The COSY and the HMQC spectra were measured on a Bruker AMX 400 NMR spectrometer [Bruker, Rheinstetten, Germany ^{1}H NMR: 400.13 MHz, internal standard tetramethylsilane (TMS), 300 K, $^{13}C\{^1H\}$NMR: 100.6 MHz] using an inverse probe. The two-dimensional COSY experiment was recorded with a COSY45 pulse sequence. A total of 512 increments with 16 transients and 2K data points were acquired in simultaneous mode with a spectral width in both dimensions of 3200 Hz. A zero filling up to 1K data points in the F1 dimension and 512 in the F2 dimension, respectively, and multiplication

with a pure sine wave was applied prior to Fourier transformation.

The HMQC spectrum was measured using a bird sequence [22]. Five hundred and twelve increments with 16 transients and 2K data points were acquired. A spectral width of 3200 Hz was used as spectral width in the F1 dimension and 14 700 Hz in the F2 dimension. Processing was performed with a zero filling up to 1K in F2 and 512 data points in the F1 dimension and a multiplication with a qsine wave in F2 and a pure sine wave in F1 prior to Fourier transformation.

^{29}Si CP/MAS NMR spectra were obtained on a Bruker DSX 200 spectrometer at 4.7 T with samples of 200–300 mg in double bearing 7 mm rotors of ZrO_2. Magic angle spinning (MAS) was carried out at 3500 Hz. The spectra were recorded with a proton pulse of 6.5 μs, a recycle delay of 1 s and a contact time of 5 ms. ^{13}C CP/MAS NMR spectra were performed by use of a Bruker ASX 300 instrument. For ^{13}C NMR spectra, sample spinning was executed at 4000 Hz, the proton 90° pulse length was 7 μs, and the contact time and delay time were 1.2 ms and 1 s, respectively. All chemical shifts were referenced externally to TMS.

To process the CP/MAS NMR spectra, 1D WINNMR software (Bruker, Rheinstetten, Germany) was used. Zero filling up to 2K data points and an exponential multiplication of the free induction decay (FID) system with a line broadening of 20 Hz for ^{29}Si CP/MAS NMR spectra and 10 Hz for the ^{13}C CP/MAS NMR spectra, respectively, were performed before Fourier transformation.

2.3. Chromatography

The chromatographic runs were performed on a Merck–Hitachi L 6200 instrument (Merck, Darmstadt, Germany). The stationary phases were packed into 250×4 mm stainless steel columns (Bischoff Chromatography) by a high-pressure slurry packing procedure on a Knauer Pneumatic HPLC pump (Knauer, Berlin, Germany). The standard test mixture was obtained from Bischoff Chromatography. Separations were carried out using HPLC-grade solvents at a flow-rate of 1 ml/min. A mixture of methanol–water (51:49, v/v) was used as mobile phase. Columns were temperature regulated at 25°C with a water jacket. Detection for all separations was at 256 nm.

2.4. 3-(Hydroxymethyl)pinane

To a suspension of 4 g (0.1 mol) lithium aluminium hydride in 200 ml anhydrous tetrahydrofuran 48 g (0.28 mol) 3-formylpinane was added dropwise and the mixture was stirred for 3 h at room temperature. After refluxing for another hour, the suspension was carefully hydrolyzed with ice water. Sulfuric acid (10%) was added until all the precipitated aluminium hydroxide was dissolved. The two resulting phases were separated, the aqueous phase was washed three times with 50 ml diethyl ether, the combined organic phases were purified twice with 100 ml of a saturated sodium chloride solution. After drying the organic phase over sodium sulfate, diethyl ether was evaporated and the residual colorless liquid was distilled (yield 43.6 g, 90.7%).

2.5. 3-(Bromomethyl)pinane [23]

A 42 g (0.25 mol) amount of 3-(hydroxymethyl)pinane was dissolved in 150 ml dry pentane. After the addition of 10.3 ml pyridine, 15.6 ml (0.16 mol) phosphorus tribromide was added dropwise to the solution at 0°C. The mixture was stirred for 30 min at 0°C then refluxed for 3 h while a white precipitate was settling down. The precipitate was dissolved with a 5% sodium hydroxide solution and the resulting two phases were separated. The organic phase was treated with 10% sulfuric acid, followed by concentrated sulfuric acid. Then the organic phase was washed with a saturated sodium carbonate solution, until the wash solution was neutral. The residual liquid was dried over magnesium sulfate and finally fractionally distilled (yield 23.5 g 40.7%).

2.6. 3-(But-3′-enyl)pinane

A 23.1-g (0.1 mol) amount of (bromomethyl)pinane was added dropwise to a suspension of 2.5 g magnesium (0.1 mol) in 100 ml anhydrous mildly boiling diethyl ether. After the addition of 3-(bromomethyl)pinane the mixture was refluxed for 1 h before an excess of allyl bromide (11 ml, 0.13 mol) was added at room temperature. The mixture

was refluxed for 8 h and then treated with ice water and hydrogen chloride. The organic phase was washed with sodium hydrogencarbonate solution, water and finally dried over calcium chloride. The ether was evaporated and the colorless liquid was fractionally distilled (yield: 11.9 g, 62%).

2.7. 3-(4′-Trichlorosilylbutanyl)pinane

A 7-g (36 mmol) amount of 3-(but-3′-enyl)pinane was dissolved in a suspension of 24 mg hexachloroplatinic acid in 25 ml anhydrous tetrahydrofuran. After stirring the mixture for 15 min 7 g trichlorosilane was added and the suspension was stirred for 48 h at room temperature. A dark brown solution was formed and the solvent was evaporated in vacuum (yield 11.4 g, 98%).

2.8. Preparation of the Pinane200 and Pinane120

A 5-g amount of ProntoSil-200-5-SI (about 8.4 $\mu mol/m^2$ surface hydroxyl groups [1]) was dried under vacuum conditions at 180°C for 4 h. After cooling to 70°C, the flask was aerated and the silica was suspended in 20 ml of toluene. An excess of 3-(4-trichlorosilylbutanyl)pinane (3.9 g, 12 mmol) was added and the suspension was stirred for 15 min. Finally 1 ml of water was added to induce the silanization and the mixture was refluxed for 20 h. The modified silica was filtered and after washing twice with 50 ml each of toluene, methanol and light petroleum, respectively, and then was dried at 60°C for 4 h.

For the synthesis of Pinane120 5 g of ProntoSil-120-5-SI was used and 6.8 g 3-(4-trichlorosilylbutanyl)pinane (0.21 mmol) was added after the activation of the silica. The reaction conditions were the same as those described above.

3. Results and discussion

To immobilize the pinane as bicyclic selector onto the silica surface a spacer had to be introduced to the pinane molecule. Therefore the starting molecule 3-formylpinane was reduced to the primary alcohol with lithium aluminium hydride. After that the hydroxy group was substituted with phosphorus tribromide to the 3-bromomethoxypinane [23]. The following Grignard reaction with allyl bromide allows one to attach the spacer to the pinane residue.

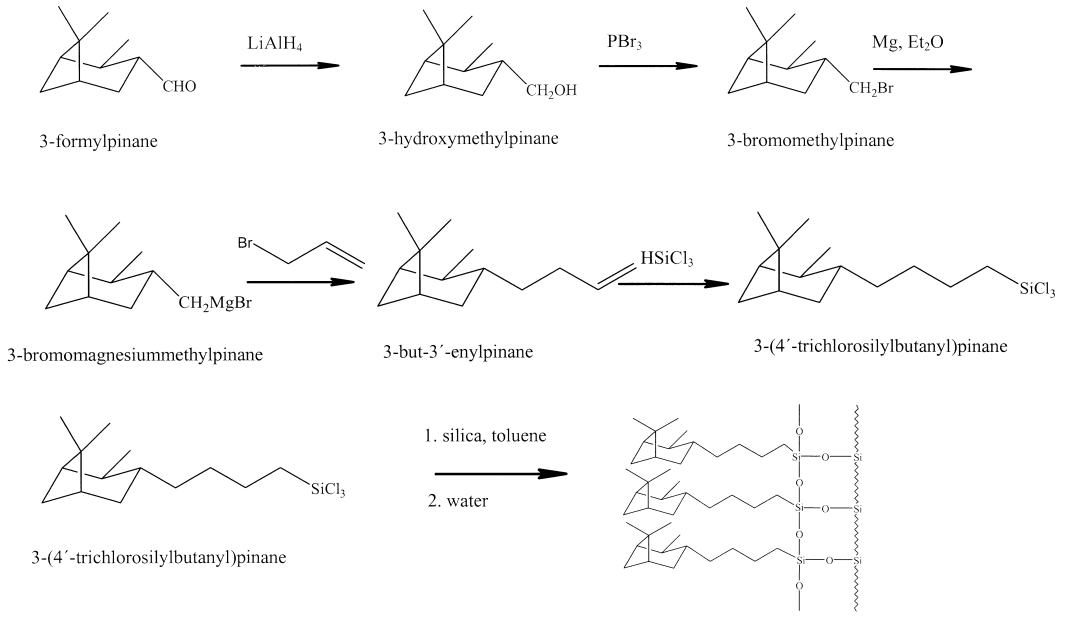

Fig. 1. Reaction scheme for the synthesis of the new stationary pinane phases.

Table 1
Properties of the pinane phases

	Silica	Particle size (μm)	Pore size (Å)	Carbon content (%)	Coverage (μmol/m^2)
Pinane120	ProntoSil-5-120Si	5	120	18.1	5.4
Pinane200	ProntoSil-5-200Si	5	200	10.6	4.4

The silanization of the terminal double bond with trichlorsilane was done using hexachloroplatinic acid as a catalyst. The yield of this reaction is close to 100%. In comparison to the less moisture-sensitive triethoxysilane, trichlorosilane is more reactive and leads to higher coverage and cross linking on the silica surface [6]. Finally the technique of solution polymerization was used to modify two different silica. Hereby the silica was activated under vacuum at 180°C before it was suspended in toluene. The silane was added and at least a little amount of water was necessary to start the polymerization. Fig. 1

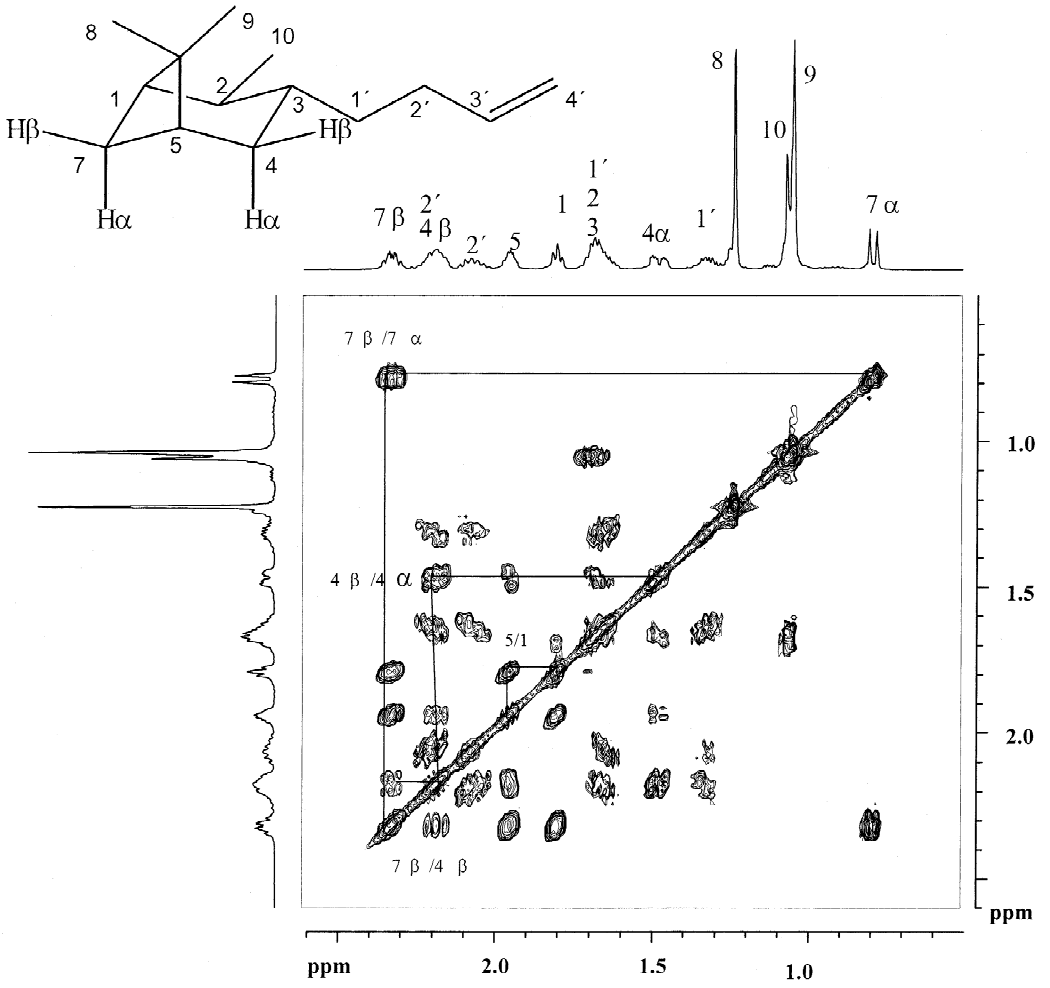

Fig. 2. COSY45 spectrum of 3-(but-3′-enyl)pinane (aliphatic part).

shows the reaction scheme and the material properties are summarized in Table 1.

Using solid-state NMR spectroscopy, it is possible to monitor the synthetic progress of new stationary phases and to prove that the organic ligand is chemically bonded to the silica surface as was expected. However due to the broader linewidth caused by homo- and heteronuclear dipole–dipole interactions in the solid-state the precise assignment of the signals in the ^{13}C CP/MAS NMR spectrum for a bicyclic system like pinane is very difficult. For this reason a detailed structure elucidation of the organic ligand 3-(but-3′-enyl)pinane obtained by high-resolution two-dimensional NMR techniques was needed to assign exactly the determined signals in the ^{13}C CP/MAS NMR spectrum.

At first a H,H COSY spectrum of 3-(but-3′-enyl)pinane (Fig. 2 shows the aliphatic part) had to be performed to assign the signals of the protons. The bicyclic system leads to a complicated coupling system between the protons. Due to their chiral neighborhood the protons H-4α and H-4β as well as the protons H-7α and H-7β possess different chemical shifts. Proton H-7α is high field shifted (0.8 ppm) and shows only a geminal coupling to proton 7β (2.3 ppm) in the COSY spectrum, whereas the coupling constant for the expected vicinal ^{3}J coupling to the protons H-5 and H-1 is zero due to a dihedral angle

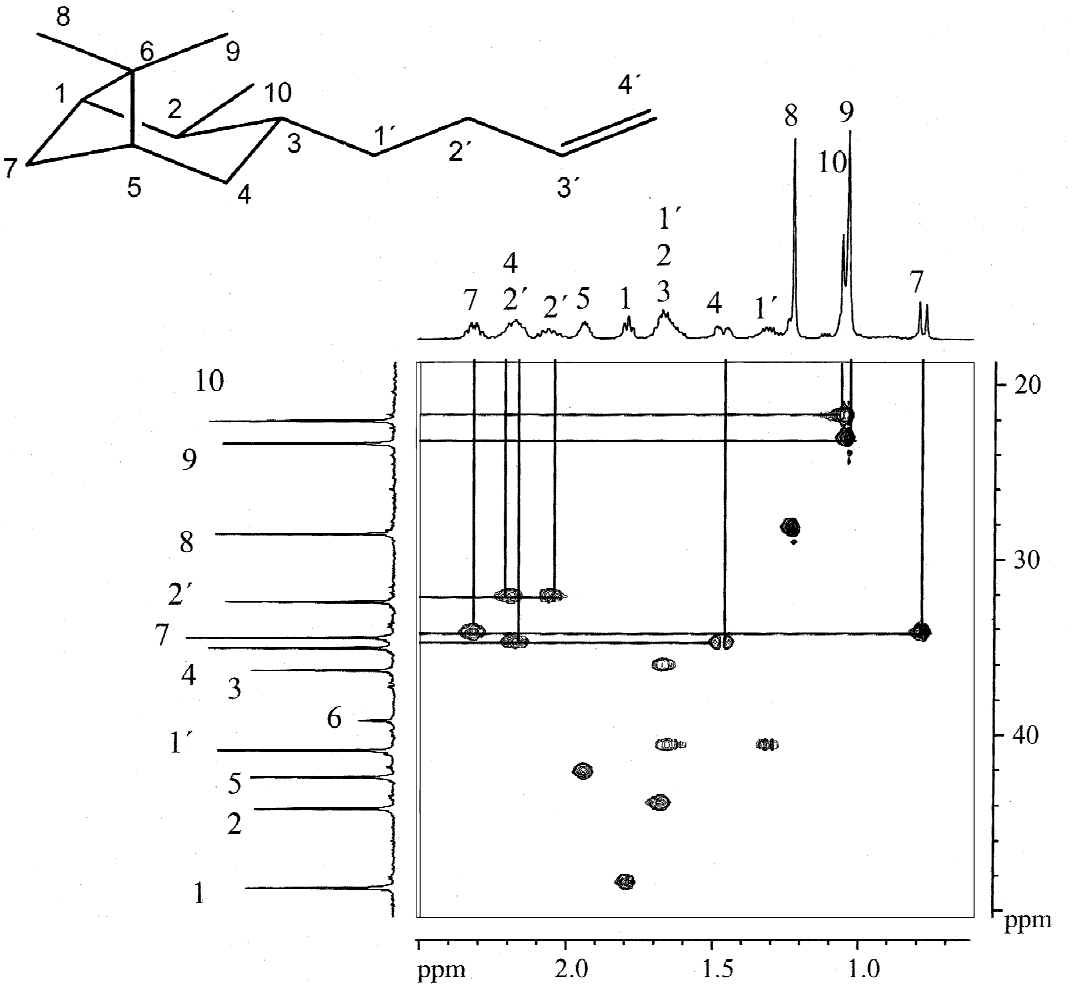

Fig. 3. HMQC spectrum of 3-(but-3′-enyl)pinane (aliphatic part).

of 270° between these protons. Therefore proton H-7α has to be located trans to the bridge atom C-6. Beside the ^{3}J-coupling to proton H-5 and H-1 and the geminal coupling to H-7α proton H-7β shows a long range coupling to proton H-4β, caused by the taut bicyclic system. For the same reason the proton H-1 possesses a long range coupling to proton H-5. The coupling constants are 6.3 Hz for H-7 and H-4 and 5.8 Hz for H-1 and H-5, respectively. Due to steric effects the methyl protons H-8 show a low field shift in comparison to the protons H-9. The methyl protons H-10 show the expected coupling to proton H-2.

The signals of the ^{13}C NMR spectra were assigned using the HMQC spectra (Fig. 3). The above mentioned duplet of the protons H-10 the signal at 22.1 ppm belongs to C-10 whereas C-9 shows a resonance at 23.5 ppm. The assignments of the protons H-7, H-2′ and H-4 allows one to distinguish between C-2′ (32.4 ppm), C-7 (34.5 ppm) and C-4 (35.0 ppm). The signals of the methine carbon atoms were assigned with a HMBC spectrum.

Using the knowledge obtained from the high-resolution NMR investigation of 3-(but-3′-enyl)pinane it was finally possible to assign the signals in the ^{13}C CP/MAS NMR spectra in suspended and solid-state as mentioned above. Fig. 4 shows the spectra of Pinane120 in solid and suspended states. Chloroform was added dropwise to the rotor to obtain a higher resolution in the NMR spectra due to the higher mobility of the carbon atoms. The differences between the spectra in suspended and in solid states are significant in the range of 34–38 ppm. The addition of the solvent allows one to distinguish between the signals of the carbons C-3, C-4, C-7 and C-2′. Furthermore, at 42 ppm a

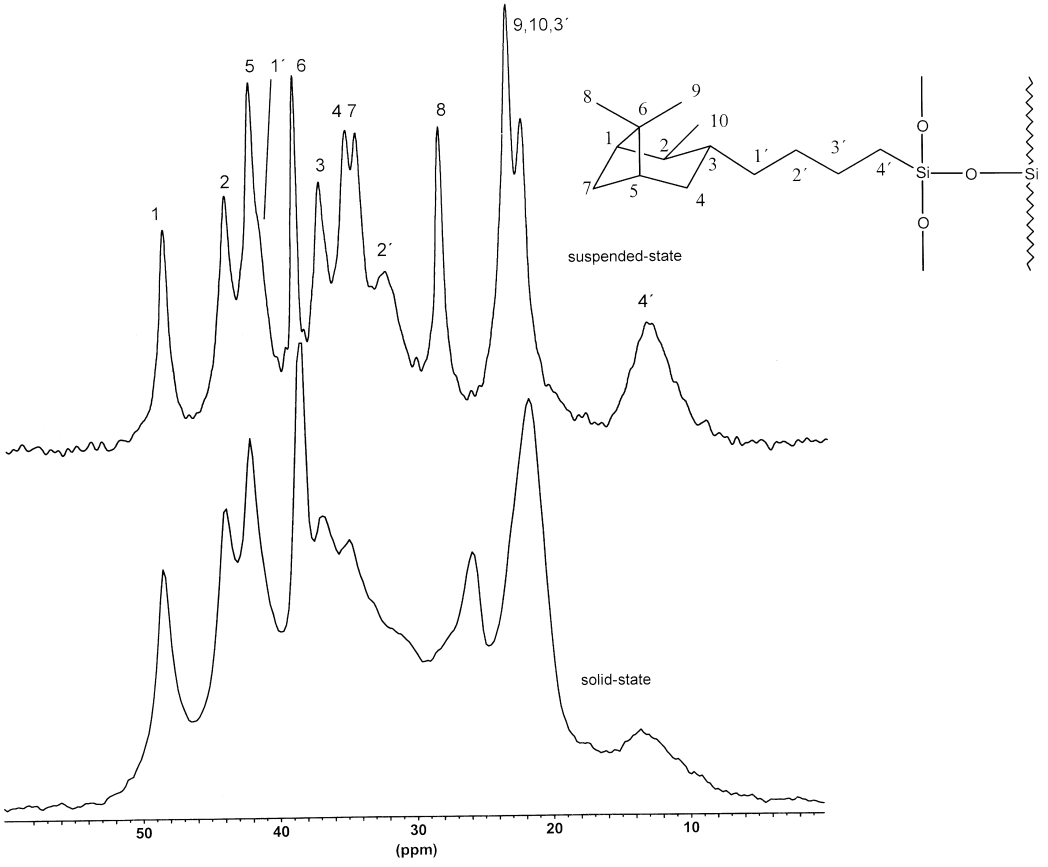

Fig. 4. ^{13}C CP/MAS spectra of Pinane120 in suspended-state (above) and solid-state (below).

shoulder is clearly visible, which might belong to C-1′ of the spacer. Both phases possess the same ^{13}C CP/MAS spectra, therefore these measurements allow only to elucidate the structure of the organic ligand on the surface.

To gain information about the cross linking and silane functionality ^{29}Si CP/MAS spectra measurements were carried out. The signal assignment of silyl species can be summarized briefly [17,18]: a higher degree of cross-linking of silicon species and/or an increase of oxygen in the neighborhood leads to an upfield shift in NMR spectra. The signals of trifunctional species (T^n) appear in the range of −49 to −66 ppm and signals from the native silica (Q^n) from −91 to −110 ppm. The ^{29}Si CP/MAS NMR spectra of the two different pinane phases are shown in Fig. 5. Obviously both phases possess a high cross linking according to the signals at −56 ppm (T^2) and at −65 ppm (T^3), while there is no signal for T^1 species visible in the spectra. However the ratio Q^3/Q^4 is quite different. The reduced signal intensity of the Q^3 species for Pinane120 shows the lower amount of free OH-groups on the surface and the higher density of the organic ligand. Therefore, the phase Pinane120 should show lower silanophilic interactions in HPLC.

To prove this hypothesis a mixture of different monosubstituted aromatic compounds was separated by HPLC using a methanol–water (49:51, w/w) mixture. The used mixture consists of uracil, aniline, phenol, *m*-toluidine, *p*-toluidine, ethylbenzene, *N*,*N*-diethylaniline, toluene and ethyl benzoate. Especially the basic solutes aniline, *N*,*N*-dimethylaniline, *m*- and *p*-toluidine are sensitive to silanophilic interactions [19]. A so called good reversed-phase column should not possess free silanol groups on the surface, which are able to interact with basic solutes. These interactions lead to a peak tailing which is bothersome for the separation of basic pharmaceutical compounds. The elution order of aniline and phenol also depends on these interactions. Usually phenol elutes after aniline on a reversed phase. Furthermore the isomeric toluidines should not be separated on a reversed-phase material, which does not have any polar groups to interact with, because *p*- and *m*-toluidine differ only in their basic strengths due to

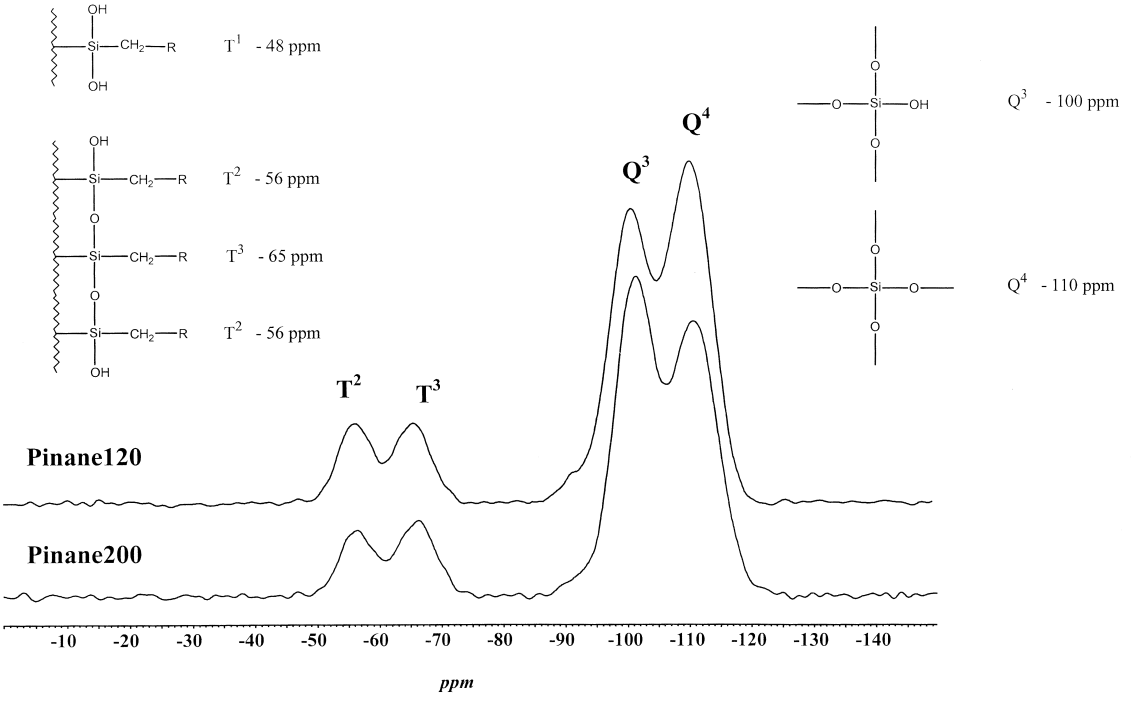

Fig. 5. ^{29}Si CP/MAS spectra of Pinane120 (above) and Pinane200 (below).

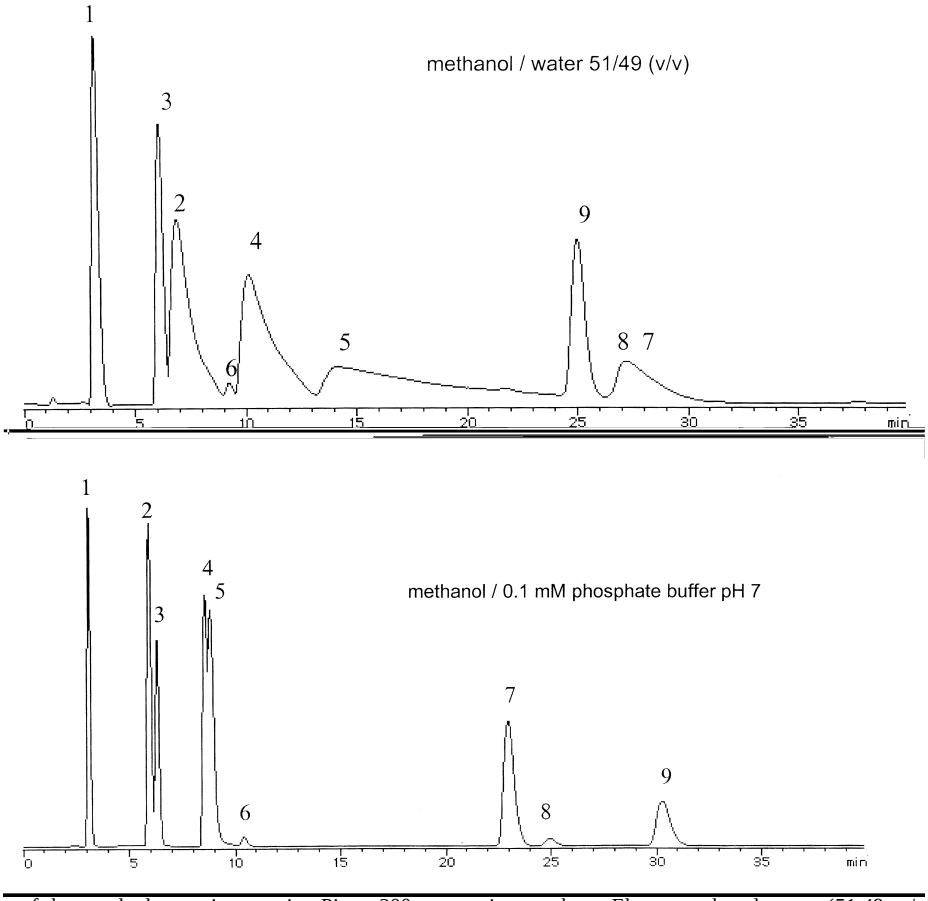

Fig. 6. Separation of the standard test mixture using Pinane200 as a stationary phase. Eluent: methanol–water (51:49, v/v) with a 0.1 m*M* phosphate buffer (below) and without buffer (above). Peaks: 1=uracil, 2=aniline, 3=phenol, 4=*m*-toluidine, 5=*p*-toluidine, 6=ethylbenzene, 7=*N*,*N*-dimethylaniline, 8=toluene, 9=ethyl benzoate.

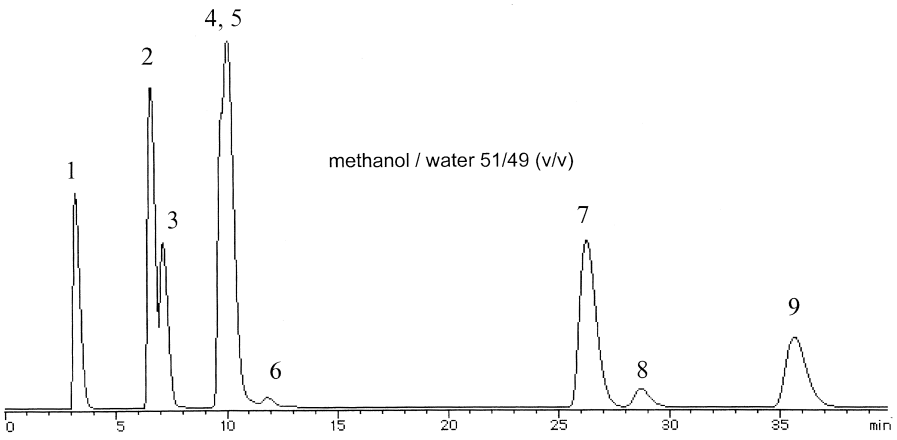

Fig. 7. Separation of the standard test mixture using Pinane120 as a stationary phase. Eluent: methanol–water (51:49, v/v). Peaks: 1=uracil, 2=aniline, 3=phenol, 4=*m*-toluidine, 5=*p*-toluidine, 6=ethylbenzene, 7=*N*,*N*-dimethylaniline, 8=toluene, 9=ethyl benzoate.

the higher inductive effect of the methyl group in the *para* position.

The chromatographic runs with Pinane200 are shown in Fig. 6. As expected from the ^{29}Si CP/MAS spectrum the separation of the test mixture leads to a very pronounced peak tailing. Moreover, phenol is eluted before aniline and the toluidines are separated. Only the use of a 1 m*M* phosphate buffer diminishes the high silanophilic properties of this phase. The elution order of aniline and phenol changes and the peak tailing of the aromatic compounds disappears.

As mentioned above, Pinane120 has a lower amount of free hydroxyl groups on the surface and a higher density of the organic ligand. Without using a buffer in the mobile phase, the separation of the test mixture leads to a very good peak shape, as shown in Fig. 7. There is not any peak tailing for the basic organic solutes and the elution order for phenol and aniline is the expected one of a good reversed-phase column. Furthermore, in comparison to the run with Pinane200 the isomeric toluidines are not separated anymore.

According to Engelhardt and Jungheim, C_8-phases show a different elution order in comparison to C_{18}-phases as far as ethyl benzoate and toluene are concerned [19]. Using a C_{18}-phase toluene is eluting after ethyl benzoate whereas with a C_8-phase the ethyl benzoate is eluting after toluene. Interestingly the retention behavior of both compounds using the pinane phases is similar as achieved with C_8-phases.

4. Conclusion

A new stationary phase was introduced in which pinane is attached to silica with different pore sizes by a butyl spacer. The structure elucidation of the new phase was obtained by employing on the one hand high-resolution two-dimensional NMR measurements of 3-(but-3′enyl)pinane and on the other hand suspended and solid-state NMR investigations of the new reversed-phase materials. The difference in the two synthesized stationary phases was determined by employing ^{29}Si CP/MAS NMR spectroscopy. The results obtained in spectroscopy are confirmed by separating a standard test mixture of aromatic compounds with the new materials. Both phases were able to separate the different aromatic solutes. To obtain a sufficient peak symmetry with Pinane200 as stationary phase, a phosphate buffer was added to the mobile phase. The stationary phase Pinane120 did not show the bothersome silanophilic properties and a sufficient peak shape was achieved without using buffer in the mobile phase.

Acknowledgements

We acknowledge Bischoff Chromatography (Leonberg, Germany) for donation of the silica. Furthermore, we thank BASF (Ludwigshafen, Germany) for providing the 3-formylpinane. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References

[1] K.K. Unger (Ed.), Packings and Stationary Phases in Chromatographic Techniques, Chromatogr. Sci, Vol. 47, Marcel Dekker, New York, Basel, 1990.
[2] L.C. Sander, K.E. Sharpless, N.E. Craft, S.A. Wise, Anal. Chem. 66 (1994) 1667.
[3] M. Pursch, L.C. Sander, H.-J. Egelhaaf, M. Raitza, S.A. Wise, D. Oelkrug, K. Albert, J. Am. Soc. 121 (1999) 3203.
[4] K. Kimata, K. Hosoya, N. Tanaka, T. Araki, D.G. Patterson, J. Chromatogr. 595 (1992) 77.
[5] R. Brindle, K. Albert, J. Chromatogr. A 757 (1997) 39.
[6] W. Wielandt, A. Ellwanger, K. Albert, E. Lindner, J. Chromatogr. A 805 (1998) 71.
[7] A. Ellwanger, R. Brindle, M. Kaiser, W. Wielandt, E. Lindner, K. Albert, J. Chromatogr. A 805 (1999) 133.
[8] A. Ellwanger, R. Brindle, K. Albert, J. High Resolut. Chromatogr. 20 (1997) 39.
[9] T. Shinbo, Y. Sudo, Y. Shimabukuro, T. Kanamori, T. Masuoka, T. Iwatsubo, A. Yamasaki, K. Ogasawara, K. Mizoguchi, J. Chromatogr. 8093 (1998) 95.
[10] G.D.Y. Sogah, D.J. Cram, J. Am. Soc. 101 (1979) 303.
[11] K. Jinno, in: B. Buszewski (Ed.), New Materials and Technologies Used in Environmental Chemistry, Top Kurier Publ. Torun, 1996.
[12] B. Buszewski, R. Gadzala-Kopciuch, R. Kaliszan, M. Markuszewski, M.T. Matyska, J.J. Pesek, Chromatographia 48 (1998) 615.
[13] S.S. Yang, R.K. Gilpin, Anal. Chem. 59 (1987) 2750.
[14] B. Lynch, J.D. Glennon, C. Tröltzsch, U. Menyes, M. Pursch, K. Albert, Anal. Chem. 69 (1997) 1756.
[15] A.M. Stalcup, D.E. Martire, L.C. Sander, S.A. Wise, Chromatographia 27 (1989) 405.
[16] L.C. Sander, S.A. Wise, Anal. Chem. 56 (1984) 504.
[17] K. Albert, E. Bayer, J. Chromatogr. 544 (1991) 345.

[18] D.W. Sindorf, G.E. Maciel, J. Am. Chem. Soc. 105 (1983) 1487.
[19] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[20] H. Engelhardt, M. Arangio, T. Lobert, LC·GC Int. 12 (1997) 803.
[21] W. Himmele, H. Siegel, Tetrahedron Lett. 12 (1976) 911.
[22] A. Bax, R.H. Griffin, B.L. Hawkins, J. Magn. Reson. 55 (1983) 301.
[23] H. Brunner, H. Leyerer, J. Organometal. 334 (1987) 369.