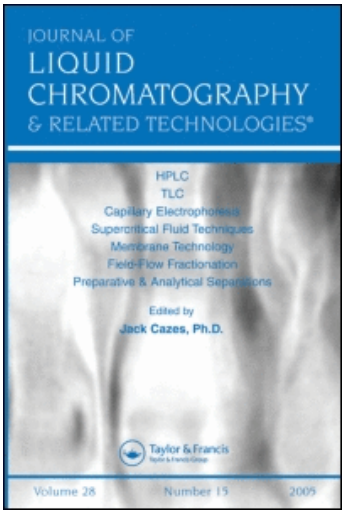

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Separation of Tribenzylhydrogermanium Nitrile Derivatives by Means of HPLC with Participation of Π-Π Interactions

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Abstract: This paper is a continuation of an investigation concerning optimization of chromatography of tribenzylhydrogermanium nitrile derivatives. Organic derivatives of germanium compounds cause numerous difficulties during determinations, because of easy precipitation on chromatographic columns. Optimal conditions of separation and determination of 3-tribenzylgermanylbutyronitrile and 4-tribenzylgermanylbutyronitrile have been elaborated, taking advantage of π-π interactions between the stationary phase and analyzed compounds. In the investigation three aryl stationary phases and various compositions and contents of the mobile phase were considered. Obtained results showed, that the best selectivity yielded a column containing porous graphitized coal. The highest separation factor (2.85) has been obtained using such a column and dichloromethane, as a mobile phase and flow 0.3 mL $\cdot$ min^{-1}. A significantly lower separation factor (1.30) for the same mobile phase gave the naphthylpropyl stationary phase. Obtained results confirm a predominating influence of π-π interactions.

Keywords: Aryl stationary phases, Isomers, Tribenzylgermanes, Chromatography

INTRODUCTION

Germanium compounds are a new group of chemical compounds of increasing practical application in various branches of industry. They exhibit some specific properties, in many points different than properties of silicon compounds. For these reasons they cause many synthetic and analytic problems.

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Germanium compounds show high biological activity, low toxicity, and positive influence on living organisms. They are significantly less toxic, than silicon compounds. Therefore, in spite of the high price of starting materials (e.g., GeO_2), interest in these compounds is increasing. Germanium is an element, which occurs in all living organisms. Bacteria, fungi, and seaweed adsorb many germanium compounds. The highest concentration of germanium was found in plants, e.g., in aloe and garlic. Also, tomato juice, soya, and fish exhibited the presence of germanium compounds. Organic germanium compounds stimulate healing of injuries and show antibacterial activity.

Some organic germanium compounds show anticancer activity.[1–3] In spite of initiation of various special programs guided to overcome tumour diseases, the number of new patients and deaths is still increasing.[4] For example, world wide, in 2002 of 1,023,152 new cases of large intestine cancer, i.e., only one type of this disease, was reported. Large intestine cancer, the world over, cause 9.5% illness of men and 9.3% of women. It is third, among the most often tumour diseases. In 2002 more than half a million (528,978) people have died because of large intestine cancer. In Europe, this illness is the second among the most common reason of the death of men and women.

Finding the proper drug for this illness is an imperative aim, justified by the increasing number of illness and death. As a consequence, new more and more complicated structures are needed. This concerns analogues of natural compounds and other pharmacologically active compounds. It creates a need for the synthesis and analysis of new compounds.[5–8] More and more, new specialized stationary phases are needed for separation of these compounds. Such properties possess chemically bonded aryl phases, intended for π electron containing compounds.

The main aim of this work was optimization of the chromatographic separation of two isomers: 3-tribenzylgermanylbutyronitrile and 4-tribenzylgermanylbutyronitrile (Fig. 1) using aryl stationary phases.

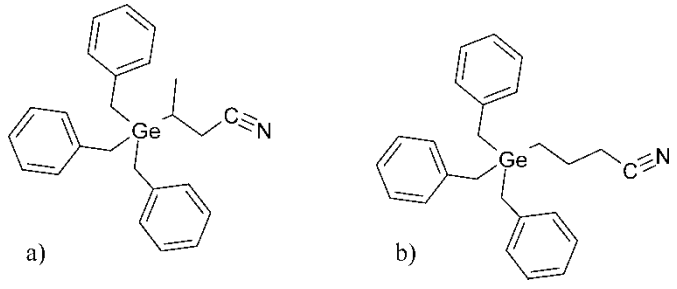

Figure 1. Structures of: a) 3-tribenzylgermanyl-butyronitrile b) 4-tribenzylgermanyl-butyronitrile.

EXPERIMENTAL

Materials and Methods

As reported in the chemical literature, germanium derivatives[9] were subjected to separation by means of aryl stationary phases. Obtained germanium compounds, i.e., 3-tribenzylgermanylbutyronitrile (**germ. 1**) and 4-tribenzylgermanylbutyronitrile (**germ. 2**) (Fig. 1a and 1b) were dissolved in dichloromethane (HPLC purity, Fluka AG, Buchs, Switzerland), obtaining concentration at about 10 μ/mL. Prepared samples were analyzed by the high performance liquid chromatography at 19°C, applying wavelength detection of 268 nm. During the investigation three stationary phases were used: commercial hypercarb column, packed by porous graphitized coal (RP Si-PGC, Fig. 2a, Thermo Electron Corporation, UK), phenylbutyl (RP Si-PB, Fig. 2b[8]) and naphthylpropyl (RP Si-NAF, Fig. 2c[8]).

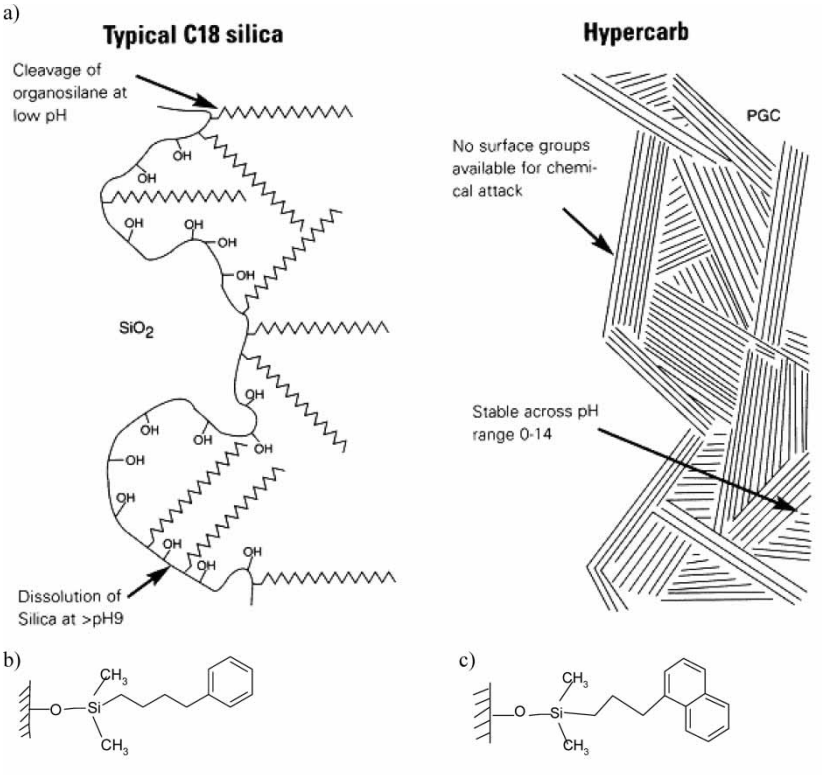

Figure 2. Scheme of chemically bonded stationary phases: a) typical C_{18} silica and hypercarb (RP Si-PGC) b) phenylbutyl (RP Si-PB) c) naphthylpropyl (RP Si-NAF).

The organogermanium compounds were prepared by the method described in the literature:[10]

3-tribenzylgermanyl-butyronitrile: ^{1}H NMR ($CDCl_3$): $\delta = 1.14$ (d, 3H, CH_3), 1.27–1.42 (m, 1H, Ge-CH), 2.10 (t, 2H, CH_2-CN), 2.33 (s, 6H, CH_2Ph), 6.78–7.28 (m, 15H, H-Ph). UV ($CHCl_3$): $\lambda_{max} = 254$, 261, 268, 276 nm.

MS (EI), m/z (% rel. int.): [M^+] 415 (3), 373 (3), 347 (8), 324 (25), 280 (3), 257 (7), 179 (3), 165 (83), 91 (100), 65 (20).

4-tribenzylgermanyl-butyronitrile: ^{1}H NMR ($CDCl_3$), δ (ppm), 0.69–0.78 (m, 2H, Ge-CH_2-C), 1.27–1.42 (m, 2H, C-CH_2-C), 2.10 (t, 2H, CH_2-CN), 2.27 (s, 6H, CH_2Ph), 6.78–7.28 (m, 15H, H-Ph). UV ($CHCl_3$): $\lambda_{max} = 254$, 261, 268, 276 nm.

MS (EI), m/z (% rel. int.): [M^+] 415 (1), 324 (44), 296 (9), 255 (11), 165 (32), 139 (4), 115 (6), 91 (100), 65 (16).

Apparatus

Chromatographic measurements were performed on a: liquid chromatograph (model SPD-6A, Shimadzu, Kyoto, Japan), equipped with a gradient pump (Shimadzu, model LC-6A, Kyoto, Japan), UV detector, a sampling valve (Rheodyne, model 7125; Berkeley, CA, USA), with a 20 μL sample loop, and Shimadzu model C-R6A data recorder.

^{1}H NMR spectra were recorded on a Bruker-200 in $CDCl_3$, with HMDS as internal standard. MS-spectra were performed with a Shimadzu Mass-Spectrometer GC/MS-QP5050, column Phenomenex BPX-5 30 m × 0.25 mm ID × 0.25 μm FT, total flow 52.7 mL · min^{-1}.

RESULTS AND DISCUSSION

In this work, optimal conditions of chromatographic separation of two isomers have been reported: 3-tribenzylgermanylbutyronitrile and 4-tribenzylgermanylbutyronitrile using aryl stationary phases Table 1. In Table 2 are shown

Table 1. Characteristics of aryl bonded phase

Column name	Manufacturer of column	Reversed phase code	Carbon (%)	Column dimensions (mm)
Phenylbutyl	Home made	Si-PB	14.9	125 × 4.6
Naphthylpropyl	Home made	Si-NAF	16.1	125 × 4.6
Hypercarb	Thermo electron corporation	Si-PGC	100	100 × 4.6

Table 2. Chosen dependence ln k for **germ. 1** and **germ. 2** from on type of stationary and mobile phase. Chromatographic conditions: – flow – 0.5 or 0.3 mL · min^{-1}, wavelength – 268 nm, temperature – 20°C

Type of stationary phase	Mobile phase[a]	Flow rate (mL · min^{-1})	k_1	k_2	$\alpha = k_2/k_1$
RP Si-PB	Dichloromethane	0.5	4.89	5.65	1.16
		0.3	10.41	11.87	1.14
	Methanol	0.5	6.20	6.95	1.12
		0.3	13.55	16.07	1.19
RP Si-NAF	Dichloromethane	0.5	2.86	3.85	1.35
		0.3	6.6	8.61	1.30
	Methanol	0.5	4.32	4.35	1.00
		0.3	10.04	10.39	1.04
RP Si-PGC	Dichloromethane	0.5	0.71	1.30	1.83
		0.3	0.87	2.48	2.85
	Methanol	0.5	1.01	2.16	2.14
		0.3	2.99	4.81	1.61

[a]In the Table only optimal data of chromatographic separation and determination were reported (for retention time less than 25 min).

the optimal results of the isomer separation process. Studies concerning a capacity factor k′ dependence on a number of methylene groups in alkylbenzene contained in individual stationary phase, exhibited the best separation properties for the column RP Si–PGC.

During the optimization, various compositions of the mobile phase were tested: dichloromethane/water (100/0, 90/10, and 80/20), methanol/water (100/0, 85/15, and 70/30), using flow 0.5 and 0.3 mL · min^{-1} and three

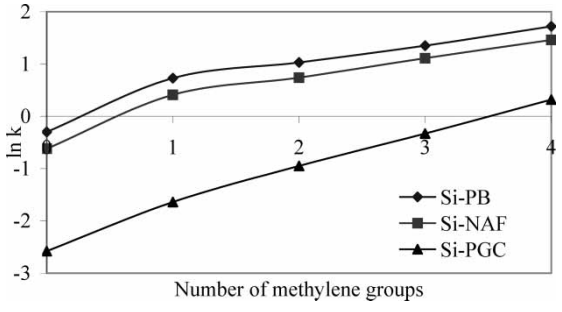

Figure 3. Dependence ln k on the number of carbon atoms in alkyl chain of alkylbenzenes for phenylbutyl, naphthylpropyl and hypercarb packing. Chromatographic conditions: mobile phase 75/25 vol.% acetonitrile/water, flow – 0.5 mL × min^{-1}, wavelength – 254 nm, temperature − 20°C.

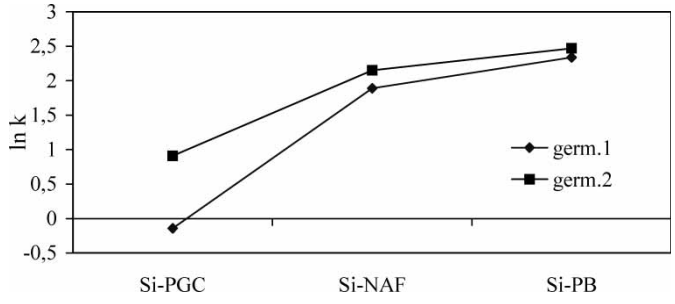

Figure 4. Effect of the separation of **(germ. 1)** and **(germ. 2)** with the use of aryl stationary phases RP Si-PB, RP Si-NAF, and RP Si-PGC. Mobile phase: dichloromethane (100 vol.%), flow rate: 0.3 mL · min^{-1}, detection –268 nm (see Table 2).

aryl stationary phases: phenyl butyl, naphthylpropyl, and porous graphitized coal (hypercarb column).

An application of columns commonly considered as standard, containing octadecyl and octyl packing did not yield satisfactory results of investigated isomers separation.[9] The use of pure methanol or methanol/water mixture in different ratio caused band spreading and peak tailing. Similar results were obtained with the use of pure or aqueous acetonitrile. Therefore, because columns commonly accessible and most often used, i.e., C_{18} and C_8 were not useful, studies concerning aryl stationary phases (including hypercarb) chromatography were undertaken.

Aryl packing with various numbers of π electrons were applied. The literature data confirm that aryl stationary phases are dedicated to determination of compounds containing aromatic rings.[8] Then, predominating interactions during the chromatographic process between a stationary phase and analyzed compounds are interactions of π-π types (Fig. 3). In this way, in numerous determinations, isomer separation could be achieved, as well as significant shortening of the time of analysis. The same has happened in the herein reported case (Table 2 and Figs. 4–6): interactions between the

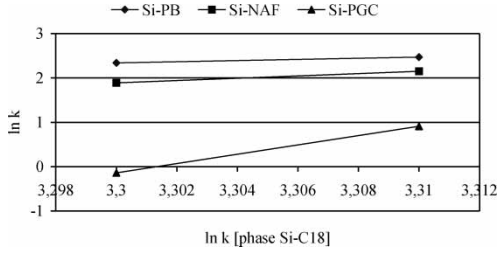

Figure 5. Dependence of ln k of the RP Si-PB and RP Si-NAF and RP Si-PGC phases on ln k obtained for the octadecyl phase for **germ. 1** and **germ. 2**.

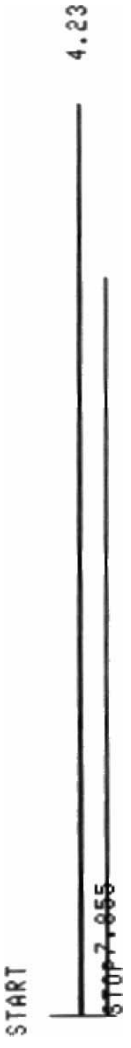

Figure 6. A chromatogram of separation of the **germ. 1** (4.23 min) and **germ. 2** (7.855 min) on the stationary RP Si-PGC phase. Mobile phase: dichloromethane (100 vol.%); flow – 0.3 mL · min^{-1}, wavelength – 268 nm, temperature −20°C.

stationary phase and the chromatographer compound increased with a number of π electrons, yielding better separation and shorter retention time.

The application of aryl phases made possible the separation and determination of 3-tribenzylgermanylbutyronitrile and 4-tribenzylgermanyl-butyronitrile in a very short time (under 6 min). From among the used stationary phases, the highest selectivity showed the phase containing porous graphitized coal (RP Si-PGC). It enabled achieving the highest

separation factor 2.85, when pure dichloromethane with flow 0.3 mL.min^{-1} was applied (Table 2).

A significantly lower separation factor for pure dichloromethane and flow 0.5 mL $\cdot$ min^{-1} yielded the naphthylpropyl stationary phase. Satisfactory separation could be obtained by also using phenylbutyl stationary phase; however retention time was longer (less than 20 min). The presence of methanol or water containing mixtures caused disturbances of the elution process, precipitation of chromatographer compounds on the column and difficulties of determination.

To recapitulate, application of octadecyl stationary phase to the analysis of above mentioned compounds[9] did not allow for elaboration of optimal conditions of their chromatographic separation and determination. During the analysis peak spreading and tailing of peaks appeared, independently of flow and mobile phase composition. Only application of aryl stationary phases dedicated to separation of π electron-containing compounds enabled elaboration of optimal conditions of chromatography separation and analysis of investigated isomers. This effect is exactly shown in Figures 4–6. Obtained results showed the predominant influence of π-π interactions on the chromatographic process.

CONCLUSIONS

3-tribenzylgermanylbutyronitrile and its isomer: 4-tribenzylgermanyl-butyronitrile can be conveniently separated and determined by high performance liquid chromatography using aryl stationary phases. All tested aryl phases gave satisfactory results. The highest selectivity and the best separation could be achieved with the phase containing porous graphitized coal (hypercarb). Obtained results confirmed the predominant influence of $\pi-\pi$ interactions on the chromatographic process.

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