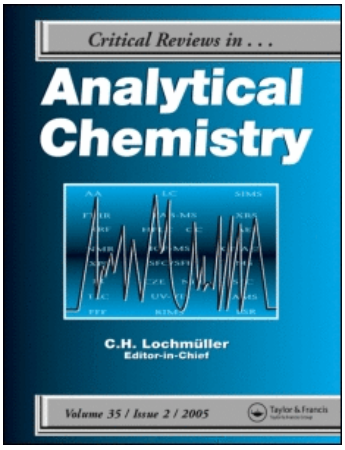

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Some Aspects of the Analysis of Biologically Active Organogermanium Substances

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Some Aspects of the Analysis of Biologically Active Organogermanium Substances

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An attempt of synthesis of information concerning organogermanium compounds has been accomplished. Organic germanium derivatives exhibit biological activity. This fact is very important for the whole ecosystem. There are some organogermanes which are characterized by confirmed neurotropic activity. Others have positive influence on the blood circulation. Some others are used in anti-cancer therapy. Germanium derivatives, because of their properties that are similar to but not identical to, silica properties, are still in the sphere of immense interest. Numerous hopes are set on their applications, first of all with possibilities of pharmacological use against different diseases, including malignant tumors and AIDS. Generally organogermanium preparations are less toxic than their silicon analogs.

Keywords benzyl germanium derivatives: di-, tri-, tetra-, hexa-, properties, determination, separation, furylgermanium

INTRODUCTION

Germanium was isolated from silver ore, argyrodite, by German chemist Winkler in 1886 (1). One year later, Winkler succeeded elaborating the first synthesis of organogermanium compound—tetraethylgermanium. The next several years did not show further progress in organogermanium chemistry. Almost forty years later, in 1925, investigations of organogermanium compounds were started.

Inter-war years were characterized by intensive exploration of germanium compounds. After a decrease of interest in 1940–1950, the situation changed in the 1960s. In that time, derivatives of elements of the 14th group were investigated in different extents. Organosilicons and organotin seemed to be more interesting, and organogermanes were less interesting. This fact confirms the data in Table 1, which shows a correlation between a number of performed analyses and a number of published papers. Between 1966 and 1969, one can observe a significant increase of interest concerning tin and germanium compounds (1).

The last several years brought new analyses and, connected with them, discoveries in the area of organogermanium chemistry. The number of papers and scientists researching organogermanium compounds had significantly increased (2–7).

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PHYSICAL GERMANIUM PROPERTIES

Germanium is a grey-white substance, with metallic sheen, that shows some metallic properties. Its melting point is 949°C, density is 5.35 g/cm^3, and atomic mass is 72.59. The germanium atom has four valence electrons, which can form four covalent bonds with other atoms. In such a case a tetrahedric structure of diamond type is formed. As an effect of supplying of energy some bonds can be cleaved and electrons are moved from their normal positions. As a result, so-called spontaneous conductivity can be obtained. Melting with other metals can modify electric properties of germanium. Electric conductivity of germanium initially decreases with an increase of temperature, the increases. Germanium is a typical electric semi-conductor. Under high pressure it changes its properties and behaves as a metal. Some contaminants can cause that electric current to go only in one direction. This property found wide application in electronics (8).

CHEMICAL PROPERTIES OF ORGANOGERMANIUM COMPOUNDS

Investigation of chemical and biological properties of germanium started after the discovery of its semi-conductor properties. In 1925–32, aromatic and alicyclic derivatives of germanium were synthesized by the use of Grignard reagents. In 1925, Dennis and Hance obtained tetraalkylgermanes R_4Ge (where R –methyl, ethyl, propyl, butyl) (9). In the same time tetraphenylgermanium tetraphenylgermanium was synthesized from $GeCl_4$ and phenylmagnesium bromide (PhMgBr). The

TABLE 1
Number of published papers concerning organic Si, Ge, Sn, Pb derivatives in years 1966 and 1969 (1)

Compound	Number of papers		Proportional increase [%]
	1966	1969	
Si	615	823	34
Ge	148	208	40
Sn	207	537	159
Pb	71	82	15

synthesis of tetra-alkyl and tetraphenyl germanes was difficult and complicated; the only way was the use of the Grignard reaction. First organohalogermanes were synthesized by Morgan and Drews in 1925. They obtained bromophenylgermane and tetraphenylgermane from $GeBr_4$ and PhMgBr (10). But because of by-products, which were difficult to separate, this reaction was not propagated. In the same year, organic compounds containing Ge-O bond were obtained, namely Ph_3GeOH, $Ph_3GeOGePh_3$ and $(Ph_2GeO)_4$ (1). Ten years later it was shown that the reaction of $GeCl_4$ with magnesiumorganic reagents led to hexaorganodigermane R_3GeGeR_3 (where R – 4-MeC_6H_4, $PhCH_2$). In the middle of the 20th century it was observed that in the reaction of $GeCl_4$ with an excess of PhMgBr, hexaphenyldigermane was formed (1). (Earlier, in 1931–50, organomagnesium reagents were used for the synthesis of tetraorganogermanium derivatives.) For the synthesis of organogermanium compounds containing Ge-Ge bond, e.g. R_3GeGeR_3, the Wurtz-Fittig reaction was applied. In 1932, hexa-ethyldigermane was obtained by heating Et_3GeBr with sodium at 210–270°C, without solvent and by the reaction of Et_3GeBr with sodium in liquid ammonia. In 1933, Simmons obtained hexaryldigermoxane (where Ar–p-MeC_6H_4, m- MeC_6H_4 (11). One year later, $(PhCH_2)_3GeOGe(CH_2Ph)_3$ was synthesized. The first heterocyclic derivative, tetra-2-thienylgermanium, was synthesized in 1937, by the use of Grignard reagent. In the same year, by the same method, the first cycloalkylgermanium was synthesized (1). In 1962, Kaars showed that tri-organogermanium acetates have fungistatic activity (12). However, in 1968 Asai synthesized oxide $(GeCH_2CH_2COOH)_2O_3$ (Ge-132) (13).

IMPORTANCE OF GERMANIUM DERIVATIVES FOR THE ECOSYSTEM

Biological Properties

The investigations of Asai were based on his conviction that germanium can play an important role in living organisms' processes (13). His theory was supported by the presence of germanium in highly valuated Chinese herbs and in numerous medicinal plants, e.g. ginseng, garlic, soya. In the 1970s, dietetic supplements of germanium became popular, however their supposed therapeutic properties were not entirely confirmed (3). Germanium exists in all living organisms in trace amounts — 0.07–1.5 ppm. Medical investigations confirmed its specific properties, which can be used in overcoming of various diseases. Generally germanium derivatives are less toxic than their silicon analogs. M.G. Voronkov confirmed it in 1968. The investigations of E. Lukevics and L. Ignatovich (1, 3) showed that although biological activity of organogermanium and organosilicon derivatives were similar, they could exhibit different extents of activities and in some cases these activities had opposite directions (14). Organogermanium derivatives, as well as organosilicon derivatives, are more active than carbon analogs.

Analyses of organic germanium c ompounds were preceded by long-term investigations of inorganic derivatives. They started in 1922, when tests showed that germanium dioxide stimulated red blood particle production. In the same year it turned out to be very toxic. Next years brought increasing interest in germanium chemistry, especially in the middle of the 20th century. Numerous investigations concerning biological properties of inorganic germanium derivatives were carried out. In 1936, M. Rothermundt and K. Burschkies decided to prove usefulness of organogermanium derivatives in chemotherapy (15). They determined toxicity of many types of substances, e.g. R_4Ge, R_3GeX, R_3GeGeR_3, where R alkyl, cyclohexyl, aryl or benzyl. They stated that organogermanium compounds had low toxicity and their usefulness was moderate. Toxicity of tetralkylgermanium derivatives was tested by Italian pharmacologists in years 1963–66. According to their results, tested derivatives were practically not toxic (1).

Neurotropic Activity

In spite of intensive research concerning chemistry of organogermanium, numerous compounds are unsatisfactorily examined, such as benzyl and furyl germanium derivatives. Furylgermanium derivatives show similar properties as furylsilanes. Furylsilanes substituted in the position 2 have been known for a few dozen years. They show significant biological activity (16). On the other hand, their analogs substituted in the position 3 are still insufficiently examined. They probably also exhibit biological activity. Investigations of silylo- and germylosubstituents of trifluoroacetylfuran showed their biological activity (16). Medical tests of 5-triethylgermyl-2-trifluoroacetylfuran confirmed its strong anesthetic and analgesic activity. It can also influence an action of muscles and coordination of motion. It is interesting that 5-trimethylsilyl-2-trifluoroacetylfuran and its germanium analogue have comparable toxicity, but methyl group substitution by the ethyl group changes this relation, and the germanium derivative is significantly more toxic (16).

Neurotropic properties of trialkylgermyl-2-trifluoroacetylfuran depend on alkyl substituent by the germanium atom. The highest efficiency during the test with the use of hexobarbital (a drug which causes short-lived anesthesia) showed triethylgermyl derivative. It extends hexobarbital action by 37%. Substitution of ethyl groups by methyl causes protection against loss of memory (100% efficiency). Silicon-containing derivatives have lower efficiency (16.7%) (16).

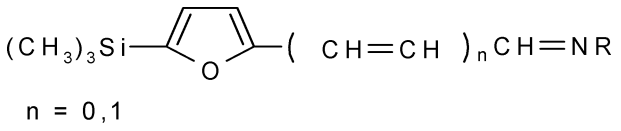


FIG. 1. 5-Substituted of (2-trimethylsilyl)furan.

Particularly well examined in respect of psychotropic activity are aminoalkyl(2-furyl)- and aminoalkyl(2-thienyl)silanes (17). This type of compounds, according to tests carried out on white mice and rats, had sedative action. Increase of furyl groups decreases toxicity of 3-aminopropyl(2-furyl)silane, but also decreases its effectiveness. In respect of lethal doses 1-(2′-furyl)silatrane can be compared with phenylsilatrane. On the other hand 1-(2′-thienyl)silatrane is at about 400 times less toxic than phenylsilatrane. Its neurotropic activity is lower, too. It is interesting that 1-(3′-furyl)- and 1-(3′-thienyl)silatrane have an opposite action than 1-(2′-furyl)- and 1-(2′-thienyl)silatrane. The former have sedative activity; they decrease mobility of organisms. The latter, on the contrary, stimulate muscles. Substitution in the furan ring in position 5 in 1-(2-furyl)silatrane significantly decreases its toxicity. Analogously, toxicity of 1-(thienyl)silatrane can be decreased (17).

5-Substituted derivatives of (trimethylsilyl)furan (Fig. 1) exhibit distinct influence on inhibition of tumor development (lung cancer of mice and melanosis). In the case of melanosis cancer development was in stopped 60% (17).

Close relations between organosilicon and organogermanium compounds and their similar properties justify this wide list of organosilicon potential applications. Investigations concerning siloxy- and germoxytranes $RnMOGe(OCH_2CH_2)_3N$ (where M = Si, Ge) showed their moderate influence on motion muscle activity (18). 1-Hydroxygermatrane inhibited decease of mice caused by anoxemia. When it was used, life of rodents was twice prolonged during so-called hypoxia (a defficiency of oxygen in the tissues of the body). Furyl- and phenylgermatranes are more active inhibitors of anoxemia than thienylgermatranes. 2-Furylgermanium derivatives protect more efficiently against hypoxia than 3-furylgermatranes. An introduction of a methylene group between heteroaromatic ring and germatrane group decreases the activity. On the other hand, a substitution of the thiophene ring in the position 5 by the methyl group increases the activity over 2.5 times (17). Activity of bromobenzylgermatranes depends on the position of bromine atom in the aromatic ring. Orto- and parabromobenzylgermatranes are significantly more active (3 times) than meta derivatives (3, 4).

Furylgermatranes are able to increase activity of some anesthetics. In tests of anesthesia by ethanol (used among others for general anesthesia) 2-thienylgermatranes exhibited unexpected activity. They stimulated and induced the central nerve system, whereas 3-thienylgermatranes, on the contrary, caused sedative effects. An introduction of the methyl group increases the activity of both compounds. On the other hand, an introduction of the ethyl group in the position 5 changes the activity of 2-thienylgermatrane from stimulating into sedative. Among all germatranes, p-tolylgermatrane is the most efficient antidepressive. Aryl- and heteroarylgermatranes prolong the anesthesia caused by hexobarbital. Particular action of this kind show 2-furfuryl derivatives (prolongation is twice as long). All furylgermatranes derivatives extend the time of hexobarbital prolongation, but they reduce anesthetic properties of ethanol (3).

Arylgermatranes are characterized by extraordinary properties connected with an improvement of memory and concentration. Benzylgermanium derivatives are an important group of biologically active compounds. The most biologically active are benzylgermatranes (19). They are weakly toxic. They show anesthetic and anti-convulsive properties. Some of these compounds efficiently inhibit amnesia of animals caused by the electric shock, e.g. benzylgermatrane and o- and p-bromobenzylgermatrane (4).

Influence on the Blood Circulation System

Some of organogermanes positively influence the blood circulation system of organisms. Ge-132 can serve as an example (20). Investigation showed that this compound decreased the blood arterial pressure and the pulse rate. Adrenaline derivative 3,4-bis(trimethylsiloxy)-4-α-(trimethylsiloxy)-β-(methyl)(triethylgermylamine)ethylbenzene is twice as efficient in increasing blood pressure as adrenaline itself and seven times less toxic (3). Then 4-(5-trimethylgermyl-2-furyl) substituted during tests carried out on cats increased the stream blood in the neck arteria, expanded the coronary vessels and decreased the blood arterial pressure. The next aspect of organogermanium derivatives is their influence on the blood clotting. 3-(5′-Triethylgermyl-3′-isoxazolinyl)pyridine, among others, belongs, to the most effective agents which decrease the blood clotting (3). Analogous silicon compounds are significantly less active in this area of activity.

Anti-Cancer Activity

The development of wide spectrum of Ge-132 biological activity has stimulated further research, especially in the pharmacology concerning anti-cancer therapy (1). Ge-132 was treated as a strong analgetic and immunological system supporting remedy (21). Further studies of this compound led to the conclusion that its derivatives can penetrate DNA structures. It is very important for the activity of anti-cancer drugs. The investigation results suggest that Ge-132 not only overcomes cancer development but also causes the increase of interferon production (the protein synthesis, including viral proteins in cells, inhibiting substance). At the same time any negative influence on the cells is observed. These properties caused an idea to insert germanium into pharmaceutical preparation in order to profit from its anti-cancer activity and its low toxicity. This can lead to less noxious preparations used in cancer treating. Ge-132 derivatives show inhibiting activity towards various types of cancer cells (2, 8, 22–24). It is necessary to remark that the anti-cancer mechanism of organogermanium compounds is not

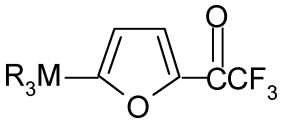


FIG. 2. General scheme of 2-trifluoroacetylfuran derivatives, which cytotoxicity is shown in Table 2.

completely known. Presumably they can be an important element of chemotherapy (one of the most often used methods of cancer treating), which supports surgical cancer treating. Therefore the main aim of contemporary chemotherapy is to elaborate medicines operating with more versatility and more selectivity on degenerated groups of cells. In the anti-cancer chemotherapy basic roles are still played by metal-organic preparations, usually platinum and ruthenium complexes. Unfortunately, some of them are not selective, and therefore significantly toxic (25). It causes numerous undesirable side effects during the therapy.

Cytotoxicity of some germanium and silicon derivatives has been accurately tested in vitro (Fig. 2, Table 2). Tests were carried out on three different cancer cells: mice liver cancer (HT-22A), fibromatous human sarcoma (HT-1080) and mice embryo neuroma (Neuro 2A). Preparations 1–5 have only limited influence in the investigation cancer cells (16).

The evaluation of cell vitality was carried out by the transformation salt tetrazoline (MTT) test and by the use of crystal violet. The MTT test is a colorimetric test for toxicity; it is based on the transformation of yellow tetrazolium salts into violet formazan, insoluble in water, formazan. This process takes place in mitochondria of living cells. If the cells were injured or destroyed earlier injured or destroyed by a toxin (mycotoxin), the reaction is less intensive or not observed. These changes can be photometrically measured. Crystal violet, a triphenylmetane dye, is also used in processes of cell dying. The evaluation of the results was done by the comparison of lethal dosis (LD_{50}) results obtained for individual virus (cancer cell), i.e. comparison of medicine (the analyzed compound) concentrations causing inhibition in 50% (set-back of the virus' growth) (16).

The most effective preparation inhibiting cancer turned out to be 5-triethylgermyl-2-trifluoroacetylfuran (LD_{50} for Neuro 2A 3.3 ml). This compound is a stronger inhibitor of the cancer development than analogous silicon derivative. It was shown that the presence of silyl and germyl groups significantly increases the absorptivity of grease of the substance. It improves its ability to penetrate by the cell membranes and in consequence biological activity of the preparation is higher than the activity of the carbon analogs. The biological activity, besides the presence of silicon or germanium atoms, is influenced by the presence of heterocyclic rings. A type and efficiency of this activity depends on a nature and structure of the heterocyclic compound and its substituents. Latvian authors reported that 2-acetyl-trisilylthiophene is four times more toxic than analogous furan derivative. Numerous accesible medicines contain furan, e.g. ranitidine (used in treating of gastric and duodenal ulcer), furosemide (for acute and chronic kidney insufficiency), and prazosin (used for reducing of arterial blood pressure) (26).

Other Reported Biological Activity of Organogermanes

Protective properties of organogermanes against harmful radiation were confirmed by many tests. Such activity exhibited, among others: germanothiazolydil, germanodithioacetal and germatrane sulphide (3, 27). They effectively protected mice during gamma radiation emitted by so-called cobalt bomb. Besides, they are characterized by low toxicity.

Tests carried out with Ge-132 showed that it helps in the inhibition of osteoporosis (a disease characterized by progressive decrease in bone mass) (28). Germanium compounds were shown to be useful also in treating of diabetes and malaria (3).

TABLE 2
Cytotoxicity of chosen 2-trifluoroacetylfuran derivatives (Fig. 2) (16)

Kind of cancer cells	Substituent	R_3M (in Fig. 2)				
		Me_3C (1)	Me_3Si (2)	Et_3Si (3)	Me_3Ge (4)	Et_3Ge (5)
HT-1080	CV	Lack of activity	Lack of activity	Lack of activity	Lack of activity	72*
	MTT	Lack of activity	Lack of activity	Lack of activity	Lack of activity	72
	NO %	4	6	5	18	250
MG-22A	CV	Lack of activity	Lack of activity	Lack of activity	Lack of activity	6
	MTT	Lack of activity	Lack of activity	Lack of activity	Lack of activity	10
	NO %	5	7	15	14	250
Neuro 2A	CV	Lack of activity	—	—	Lack of activity	3.3
	MTT	Lack of activity	—	—	Lack of activity	4
	NO %	5	—	—	18	300

*-LD_{50} (μg.mL^{-1}): MTT and CV.
- not analyzed.
NO—amount of nitrogen oxide formed by the immunological system: CV.

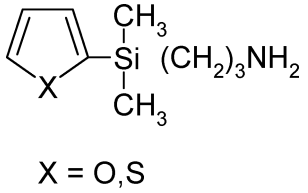


FIG. 3. Methyl(2-furyl)-, methyl(2-thienyl)(3-aminopropyl) silane (17).

Organogermanes were applied also in agriculture, in the cultivating of cereals. Germanium o-chlorobenzamide derivative shows fungistatic activity, e.g. fights against Gaecemannomyces graminis (a fungus causing gangrene of corn stalk base) (29). Methyl(2-furyl)- and methyl(2-thienyl)(3-aminopropyl)silane (Fig. 3) can be used in fighting against rust of wheat, decay of tomatoes caused by the pathogenetic fungus Phytophora and cucumber mold (17). Because of the distinct similarity of silicon and germanium derivatives, analogous activity of germanium compounds can be expected. At the end of the 1960s, diphenyl(iminodiacetoxy)germanium was used as an insecticide (18). In 1962, for the first time, fungistatic activity of trialkyl(acetoxy)germanium was investigated by Kaars (12).

TOXICITY

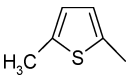
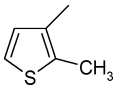
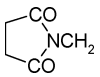
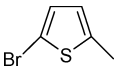
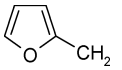
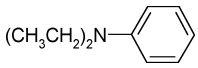
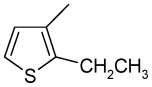
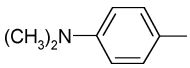
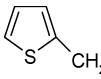
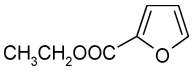
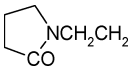
Toxicological investigation showed that the majority of tested germano-organic derivatives are less toxic than their tin or silicon analogs (14). However their too long action can cause kidney injures. A range of toxicity mainly depends on the germanium substituents. Therefore, tetraalkylgermanium compounds, germoxanes, and germanols (LD_{50} = 3000–5000 mg/kg), among others, belong to the less toxic germanium derivatives whereas to thienylgermatrane (LD_{50} = 15–20 mg/kg) belongs the most toxic.

Tetraorganylgermanes R_4Ge (where R = Et, Pr, Bu, $PhCH_2$, Ph) exhibited low toxicity to mice (3). A lethal dose of n-alkyl derivatives is between 2000 and 10000 mg/kg^{-1}. Tetraisopropylgermanium is more toxic (620 mg/kg) than tetra(n-propyl) germanium (5690 mg/kg). On the other hand tricyclohexylgermanol and hexaphenyldigermoxane are practically not toxic to mice (3). Toxicity of hexaorganodigermoxane strongly depends on a kind of substituent by the germanium atom, e.g. hexaethyldigermoxane is more toxic (LD_{50} = 240 mg/kg) than hexabutyldigermoxane (LD_{50} = 6000 mg/kg).

Germatrane derivatives $RGe(OCH_2CH_2)_3N$ (Table 3) are significantly less toxic than their silicon analogs (30). The most toxic tested germatranes (2-thienylgermatrane) is 55 times less toxic than 2-thienylsilatrane. Then 2-furylgermatrane are characterized by very low toxicity (LD_{50} > 1000 mg/kg). Generally, thienylgermatrane derivatives belong to highly toxic substances (LD_{50} = 16–89 mg/kg) (30). Only 5-ethyl-2-thienylgermatrane is an exception (LD_{50} is over 1000 mg/kg).

A comparison of toxicity of 5-methyl, 5 ethyl and 5-bromo-2-thienylgermatrane led to the conclusion that exchange of the ethyl group by the methyl decreased the toxicity. However 5-methyl- and 5-bromoderivative showed similar harming (30).

TABLE 3
Toxicity of chosen derivatives of $RGe(OCH_2CH_2)_3N$ (30)

R	LD_{50} (mg × kg^{-1})	R	LD_{50} (mg × kg^{-1})
	16.5		1690
	20.5		2050
	20.5		2500
	20.5		2960
	89		3250
	89		3680
H	320	H_2C=CH	5600
	325	OH	6500
	1090		10000

Thienyl derivatives of germatranes substituted in the position 2 are the most toxic. On the other hand, furyl derivatives substituted in the position 2 are slightly less toxic than their analogs substituted in the position 3. Insertion of the methylene group between furan ring and germanium atom decreases toxicity. Tests exhibited small toxicity of tricyclohexyl-, tribenzylchloro-, tribenzylbromo and tribenzyliodogermanium to mice (LD_{50} = 1250–5000 mg/kg) (30).

PREPARATION OF SAMPLES TO THE CHROMATOGRAPHIC ANALYSIS

Separation and determination of germanium organic derivatives is more difficult than similar operations on their silicon analogs. The difference of properties causes standard columns applied to silicon compounds to be useless during attempts of isolation, separation or determination of germanium derivatives. Some organic germanium derivatives exhibit the ability to modify column packing (31, 32). However, because of immense development of specific packing, the most often used method of isolation, even from the most complicated matrices, is still an extraction liquid-solid (scale micro and macro). On the other hand, the most popular technique in processes of separation is high performance liquid chromatography (HPLC). This method was also utilized for elaboration of optimum chromatographic

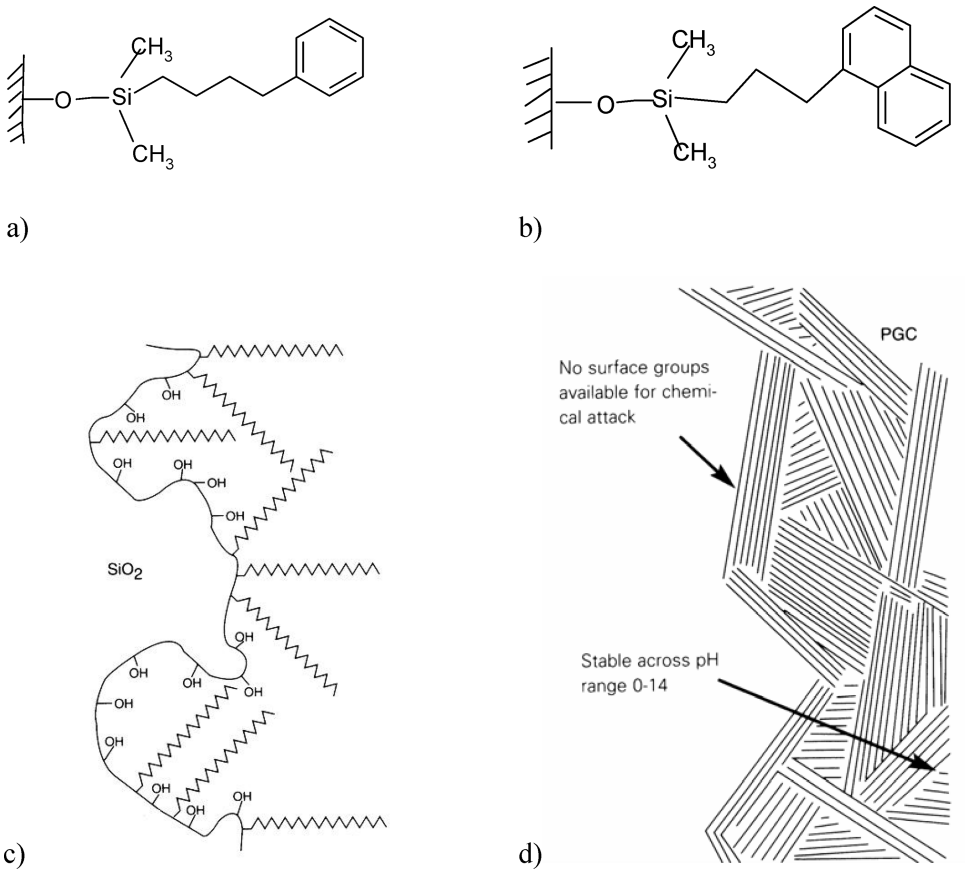


FIG. 4. Scheme of chemically bonded stationary phases: a) phenylbutyl (RP Si–PB) b) naphthylpropyl (RP Si–NAF), c)octadecyl RP Si–C_{18}, d) hypercarb (RP Si–PGC).

conditions for separation of numerous organogermanium compounds (5–7, 31).

Biological activity of compounds can be determined by different methods, e.g. by the analysis of a mechanism and its interaction with the constitutional transport proteins (albumin, transferrin). Characterization of these mechanisms is carried out by the use of various spectroscopic methods. In every method it is necessary to isolate the investigated compound from a complex mixture. The most convenient technique is solid phase extraction (SPE), then separation and determination by HPLC technique. At present these methods are the most often used analytical procedures. An application of these methods to investigations of benzylgermanium derivatives was hindered by their properties (8, 14). Alcohols and esters of low molecular weight could not be used in the extraction of benzylgermanium compounds because they react one with the other. The most optimum solvents were anhydrous hexane and anhydrous diethyl ether.

In the pharmacy and biomedicine particular attention is given to the analysis of biologically active substances. For isolation and determination of complex mixtures, the use of a selective column is often necessary (33, 34). Stability and reproducibility of columns, extracting as well as chromatographic, is a condition of proper analytic procedure. In chromatography and related techniques the most important factor is selection of a mobile phase and a stationary phase in respect of the analyzed substance.

In the SPE extraction column packings of various polarity are used. The most popular packing is silica gel. It is used in the so-called normal phase system. Despite many attitudes, in the medical and pharmaceutical analysis, more often its modifications are obtained by condensation with hydrophobic hydrocarbon chains of various lengths (C_2, C_8, C_{18}, C_{22} or C_{30}). This chain can be ended by one or two phenyl groups (Fig. 4a, b) (35). Stationary phases based on silica are widespread in extraction as well as in liquid chromatography. First of all, they are used in reversed phase (RP) systems. The most important in the laboratory practice are these sorbents, which have 2, 8 or 18 carbon atoms in the molecule (Fig 4c). These materials should exhibit great reproducibility of the surface, i.e. proportional share of coal in the elemental analysis, porosity parameters, concentration of metallic contamination, etc. (34).

A convenience of the RPs is connected with a fact of strong adsorption of water contained in solvents by adsorbents used in the normal phase system. It causes a decrease of adsorbent activity and as a consequence loss of separation ability of the

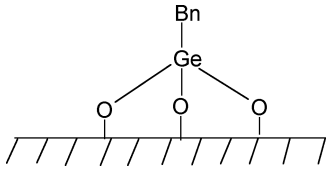


FIG. 5. Scheme of the stationary phase modified by benzyltrichlorogermanium (Bn = $PhCH_2$) (32)

column. Hydrophobic packing used in the RP HPLC are free of this disadvantage, and therefore are more livel (33). These phases can be used for the separation of substances that are hardly soluble or even insoluble in water, e.g. aromatic hydrocarbons. More and more often porous graphitized coal is used, e.g. hypercarb (Fig. 4d) (7, 31, 32).

The retention process in the RP chromatography is based on specific and unspecific interactions between the stationary phase, the mobile phase and the separated substance. Optimization of the chromatographic process is carried out by changes of type, composition and character of the mobile phase or type, properties and topography of the stationary phase. Modification of these agents enables the steering of the chromatographic process (33).

A composition of a mobile phase is created in order to obtain retention factors (k') higher than 1. The best separated peaks are obtained when k' are between 2 and 10. The final effect of the stationary and mobile phase interaction (a mixture separation) depends on thermodynamic properties of the chromatographic system.

SEPARATION AND DETERMINATION BY HPLC TECHNIQUE

Initially, attempts of separation and determination of benzylhalogenogermanium derivatives were made with the use of columns filled by standard adsorbents (SiO_2, Al_2O_3). These attempts led to a conclusion that these germanium compounds were able to modify the adsorbent surface. This ability decreased with an increase of benzyl (also furyl) groups connected with germanium atom (6, 7).

Separation of monobenzyl germanium derivatives was not possible, because extremly strong combinations with the stationary phase were formed (Fig. 5). A recovery of germanium compounds was possible after their transformation into inorganic germoxanes by the use of concentrated inorganic acids such as hydrochloric or sulfuric (VI) (32).

Dibenzyldichlorogermanium derivatives do not form such strong combinations with the surface of the stationary phase, but strongly polar solvents are needed (Fig. 6) (32).

Contrary to the two above-mentioned groups, tribenzylchlorogermanium derivatives relatively weakly interact with the surface of the stationary phase (Fig. 7) because of their sterically spacious group. Due to this fact, mixtures of organic solvents with water can elute them, however, in a form of hexabenzyldigermoxane. The use to separate relatively polar and anhydrous solvent (e.g. dichloromethane) generates elution of the compounds in unchanged state (32).

In the case of tetra- and hexabenzylgermanium derivatives modification of the stationary phase surface was not observed (6, 7, 32).

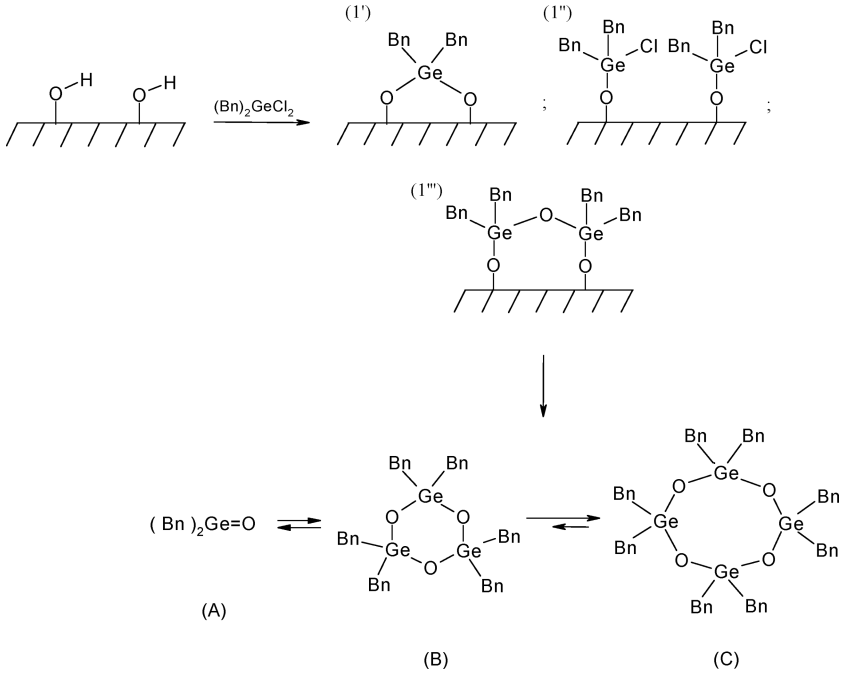


FIG. 6. Scheme of the stationary phase modified by dibenzyldichlorogermanium (Bn = $PhCH_2$) (32).

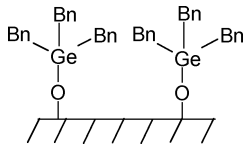


FIG. 7. Scheme of the stationary phase modified by tribenzylchlorogermanium (Bn = $PhCH_2$) (32)

Also furylgermanes cause numerous difficulties during chromatographic separation and determination. Practically, there is a lack of information concerning 3-furylgermanium derivatives. Slightly better is a situation of information concerning furylsilanes. Furylsilanes as well as furylgermanes belong to the compounds that should be separated and determinated by the use of so-called dedicated stationary phases, because columns commonly considered as standard are not effective in their case.

No doubt that all investigations concerning optimization of isolation from various matrices, chromatographic separation and determination are very important, because of the high degree of difficulty. The references recommend for these purposes the use of dedicated phases and anhydrous organic solvents of high purity (5–7, 31–35). Aryl stationary phases, recommended especially for π-electron containing compounds, belong to the dedicated phases Aryl phases are especially useful in separation and determination of benzene ring containing substances, for example phenylbutyl phase (Fig. 4a), naphtylpropyl phase (Fig. 4b) and Hypercarb (Fig. 4d). The lack of free silanol groups on the silica surface and the presence of π-electrons cause an increase of interactions between analyzed substances and the stationary phase. In these conditions, excellent results could be achieved in numerous processes.

The first dedicated phases appeared in 1989 prepared by Pidgeon et al. (36). In the last decade of the 20th century Buszewski with his coworkers obtained phases suitable for separation of proteins, amines, basic drugs and polycyclic aromatic hydrocarbons of differentiated geometry (35, 37–40).

Cyclodextrine phases are also often used, especially for the separation of drugs containing a chiral carbon atom. According to common opinion, Armstrong had the priority in this area. They were used in the middle of the 20th century, but gained bigger popularity in the 1980s (41). As many important biologically active compounds exist in forms of mixtures of chiral isomers (e.g. drugs), various phases designed for their separation were prepared, e.g. so-called Pirkle's phases [phases containing chiral active centers (π-donor, π-acceptor) (42, 43).

In the future, besides further development of dedicated phases, more attention will be paid to chiral germanium compounds. Another expected direction that can be applied in the area of these compounds is so-called chemometry. It is a relatively new method based on computer modeling, which can optimize the retention process. On the other hand this method has some disadvantages, because in respect to separation selectivity, universal packing does not exist. It causes that sometimes an efficient separation of all homologues of one series is not possible. Therefore the results can be incorrect. Computer modeling is especially troublesome during testing newly received compounds. Then it is completely useless when some troubles appear, e.g. with separation of low yield reaction products and when problems with elution appear. At present the solution of analytical needs requires the use of various chromatographic and related methods.

SUMMARY

When Mendelejew, in his table, predicted such an interesting element like germanium (for Mendeleev "eco-silicium") (1), he could not understand, how important it would be 100 years later. Initially, germanium and its compounds were not popular. Recently, especially organic germanium derivatives aroused distinct interest. Numerous profits of their application were reported in the industry, medicine and related sciences.

Increase of germanium compounds' popularity had an influence on a number of investigations. This number is still not immense, but it has the tendency to increase. Every paper increases our knowledge.

This paper is an attempt of a synthesis of information concerning germanium and its compounds in order to help the research in the area of organogermanium chemistry.

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