

INFLUENCE OF HETARYL SUBSTITUENTS ON THE TOXICITY AND NEUROTROPIC ACTIVITY OF GERMATRANES

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The influence of hetaryl substituents on the toxicity and neurotropic activity of germatranes has been investigated. The leading compounds influencing the duration of ethanol and hexobarbital anesthesia, preventing retrogradal amnesia, protecting against hypoxia, and having low toxicity have been found.

In 1994, the first organogermanium pharmaceutical propagermanium was launched in Japan under the trade name Serocion® (Sanwa Kagaku Kenkyusho Co., Ltd.). Its biological activity spectrum includes protection against viruses, immunostimulation, and hepatoprotection. Propagermanium has been introduced into clinics for the treatment of chronic hepatitis. This compound belonging to germsesquioxanes has been shown to possess low toxicity.

This achievement stimulated further investigations of biological activity not only of germsesquioxanes but also of other classes of low toxicity organogermanium compounds.

Earlier we found that many organylgermatranes exhibit very low toxicity (LD_{50} for mice more than $5000 \text{ mg} \cdot \text{kg}^{-1}$). The more detailed investigations allowed us to demonstrate that the acute toxicity and neurotropic activity of germatranes depend strongly on the substituent structure at the germanium atom (see Tables 1-4).

There is a group of low toxicity compounds (Table 1). Their mean lethal dose (LD_{50}) at intraperitoneal administration varies from 10,000 to $3000 \text{ mg} \cdot \text{kg}^{-1}$. This group of compounds includes the derivatives of pyrrolidone (β -pyrrolidinoethyl being less toxic than its α -isomer), adamantane, phthalimide, and *N,N*-dialkylaniline. 1-Hydroxygermatrane ($8400 \text{ mg} \cdot \text{kg}^{-1}$) is a low toxic compound, while the trimethylsilylation of its hydroxyl group increases the toxicity by 2.4 times. Methoxycarbonylpropylgermatrane ($6820 \text{ mg} \cdot \text{kg}^{-1}$), vinylgermatrane ($5600 \text{ mg} \cdot \text{kg}^{-1}$) and *p*-fluorobenzoylaminoethylgermatrane (its chloro derivative being more toxic) also belong to this group of low toxic compounds.

The group of compounds with moderate toxicity (Table 2) includes chloromethylgermatrane ($2960 \text{ mg} \cdot \text{kg}^{-1}$; its bromomethyl derivative appears considerably more toxic — $355 \text{ mg} \cdot \text{kg}^{-1}$), 2- and 4-pyridylgermatranes (2820 and $2580 \text{ mg} \cdot \text{kg}^{-1}$), tris(2-thienyl)siloxy- and triphenylsiloxygermatranes (~ 2500 and $2000 \text{ mg} \cdot \text{kg}^{-1}$), 2- and 3-furyl- and furfurylgermatranes (2050 , 1630 , and $2960 \text{ mg} \cdot \text{kg}^{-1}$, respectively).

RCH_2 -substituted germatranes (Table 3), where $R = 3,5$ -dimethylpyrazolyl-, diethylamino-, bromo-, 2-thienyl, form a group of more toxic compounds ($LD_{50} = 700$ - $325 \text{ mg} \cdot \text{kg}^{-1}$). Hydrogermatrane reveals a similar toxicity ($320 \text{ mg} \cdot \text{kg}^{-1}$).

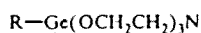
The derivatives of thienylgermatranes are highly toxic compounds with LD_{50} values within the 16 - $89 \text{ mg} \cdot \text{kg}^{-1}$ range (Table 4). 5-Ethyl-2-thienylgermatrane ($> 1000 \text{ mg} \cdot \text{kg}^{-1}$) appears to be an exception in this series of compounds. The introduction of the bromine atom instead of the methyl group does not change the toxicity value.

The most toxic compound among all studied germatranes is 2-thienylgermatrane ($16.5 \text{ mg} \cdot \text{kg}^{-1}$). The introduction of the methyl group in the 5-position of the thienyl ring slightly decreases the toxicity ($20 \text{ mg} \cdot \text{kg}^{-1}$). Phenylgermatrane is two times less toxic than 2-thienylgermatrane but still exhibits high toxicity ($35.5 \text{ mg} \cdot \text{kg}^{-1}$).

High toxicity of phenyl- and thienylgermatranes cannot be explained by the presence of the tricyclic germatrane ring with the pentacoordinated germanium atom or by the presence of π -electron system in the substituent. Alkylgermatranes containing the same germatrane system and vinylgermatrane (π -bond) are nontoxic compounds (Table 5). Hydrolysis of arylgermatranes possible in an animal body cannot explain this phenomenon. Hydrolysis of Ge—C bond decreases the toxicity

TABLE 1

$$LD_{50} = 10000 - 3000 \text{ mg} \cdot \text{kg}^{-1}$$



Acute toxicity of germatranes
(i.p. administration to white mice)

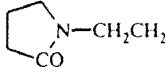
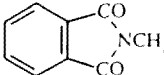
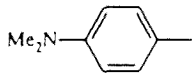
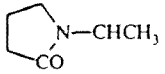
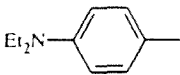
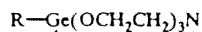
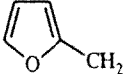
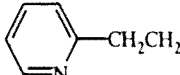

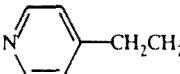
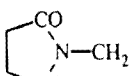
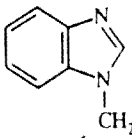
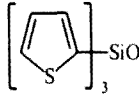

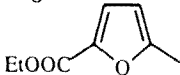
R	LD ₅₀ , mg · kg ⁻¹	R	LD ₅₀ , mg · kg ⁻¹
	10 000	CH ₂ CH ₂ CN	4 300
OH	8 400		4 100
CH ₂ CH(CH ₃)COOMe	6 820		3 680
	6 500	Me ₃ SiO	3 500
CH ₂ =CH	5 600		3 250
1-Ad	> 5 000		
<i>p</i> -FC ₆ H ₄ CONHCH ₂	> 5 000		

TABLE 2

$$LD_{50} = 3000 - 1000 \text{ mg} \cdot \text{kg}^{-1}$$



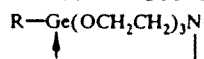
Acute toxicity of germatranes
(i.p. administration to white mice)

R	LD ₅₀ , mg · kg ⁻¹	R	LD ₅₀ , mg · kg ⁻¹
ClCH ₂	2960	CH ₂ CH ₂ COOEt	2400
	2960	<i>p</i> -ClC ₆ H ₄ CONHCH ₂	2050
	2820		2050
	2580	Ph ₃ SiO	~2000
	2500		1780
	~2500		1630
			1090

by more than 500 times. Both products of Ge—O bond hydrolysis — germanic acid and triethanolamine — are also less toxic than the starting 2-thienylgermatrane. As the mechanism of arylgermatrane biological activity is not known yet one can speculate that both parts of the molecule — germatrane system (for binding to the receptor?) and aryl group bond directly to the germanium atom (for toxic bioarylation; thienylmethylgermatrane is less toxic) — are important for the exhibition of high toxicity.

TABLE 3

$$LD_{50} = 1000 - 300 \text{ mg} \cdot \text{kg}^{-1}$$



Acute toxicity of germatranes
(i.p. administration to white mice)

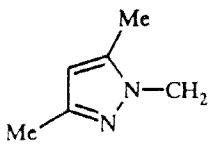
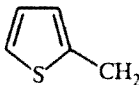
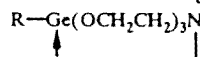
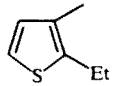
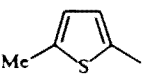
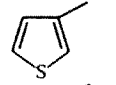
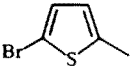
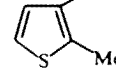

R	LD ₅₀ , mg · kg ⁻¹
	708
Et ₂ NCH ₂	355
BrCH ₂	355
	325
H	320

TABLE 4

$$LD_{50} = 100 - 10 \text{ mg} \cdot \text{kg}^{-1}$$



Acute toxicity of germatranes
(i.p. administration to white mice)

R	LD ₅₀ , mg · kg ⁻¹	R	LD ₅₀ , mg · kg ⁻¹
	89		20,5
	89		20,5
	20,5		16,5

The toxicity of hetarylgermatranes depends strongly on the nature of the heteroatom in hetaryl substituents (Table 6). Thienylgermatranes are much more toxic than the corresponding furan derivatives. The more toxic the thienyl compound, the more pronounced is the difference between thienyl and furyl derivatives: 124 times for 2-isomers, 18 times for 3-isomers, and only 9 times for 2-hetarylmethyl derivatives.


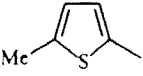
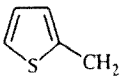
The position of a substituent in the heterocycle also influences the toxicity of hetarylgermatranes (Table 7). 2-Isomers belonging to the thiophene series appear to be the most toxic, while the 2-derivatives in the furan series are less toxic than the 3-isomer. Introduction of the second substituent in the thiophene ring (in position 5 for the 2-isomer and in position 2 for the 3-isomer) changes the toxicity in such a way that the methyl derivatives become similar in their toxicological behavior, while for the ethyl derivatives the 2-isomer becomes less toxic.

Introduction of only one additional methylene group in the molecule of thienylgermatranes dramatically changes their toxicity (Table 8). Insertion of a CH₂ group between the thiophene ring and the germanium atom decreases the toxicity by 20 times. Substitution of the methyl group for the ethyl one reduces noticeably the acute toxicity of the compound (by 4.3 times for the 3-isomer, and by more than 50 times for the 2-isomer).

TABLE 5

Influence of aryl- and hetaryl-
substituents on the toxicity of germatranes

$R-\text{Ge}(\text{OCH}_2\text{CH}_2)_3\text{N}$

R	LD ₅₀ , mg · kg ⁻¹	R	LD ₅₀ , mg · kg ⁻¹
	16,5	CH ₂ =CH	5600
	20,5	1-Ad	>5000
C ₆ H ₅ -	35,5		325

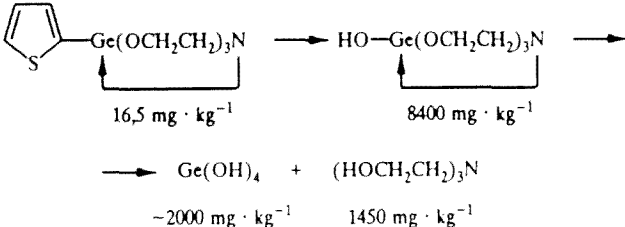
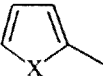
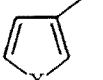
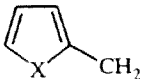


TABLE 6

Influence of the heteroatom in hetarylsubstituents
on the toxicity of germatranes

$R-\text{Ge}(\text{OCH}_2\text{CH}_2)_3\text{N}$

(i.p. administration to white mice)

R	LD ₅₀ , mg · kg ⁻¹	
	O	S
	2050	16,5
	1630	89
	2960	325

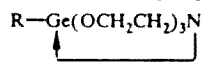
The degree of protection against hypoxia also depends on the structure of substituents at the germanium atom in germatranes.

Some regularities have been observed in the series of 5-membered nitrogen heterocycles (Table 9). 1-Isomer of pyrrolidinoethylgermatrane is more active than the 2-isomer. Introduction of the second carbonyl group in the ring (succinimidomethyl) increases the antihypoxic effect of the compound. Introduction of the double bond (maleinimidomethyl) leads to a further increase in the activity (145.5%). The condensation with benzene ring (phthalimidomethyl) reduces the activity, while the substitution of one carbonyl group for the SO₂ group increases the protection properties.

Furylgermatranes are more active against hypoxia than thienylgermatranes and compounds with nitrogen-containing substituents (Table 10). The potency of protection of 2-furylgermatrane is higher than that of 3-isomer. Insertion of the CH₂

TABLE 7

Influence of the substituent position in the heterocycle on the toxicity of hetaryl germatranes



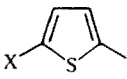
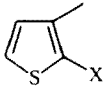


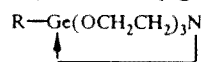

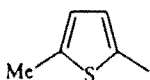
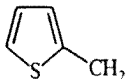
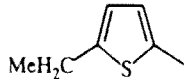

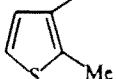
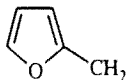
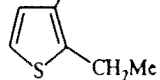
R	LD ₅₀ , mg · kg ⁻¹		
	H	Me	Et
	16,5	20,5	>1000
	89,0	20,5	89,0
	2050	-	-
	1630	-	-

TABLE 8

Effect of one CH₂-group on the toxicity of hetarylgermatranes



R	LD ₅₀ , mg · kg ⁻¹	R	LD ₅₀ , mg · kg ⁻¹
	16,5		20,5
	325		>1000
	2050		20,5
	2960		89,0

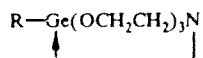
group between the heterocycle and the germanium atom reduces the activity both in the furan and thiophene series, while the introduction of the methyl group in position 5 of the thiophene ring increases the antihypoxic activity by 2.6 times.

Hydroxygermatrane prevents hypoxia-caused death in experimental animals at the same extent as 2-furylgermatrane (Table 11). Silylation and gerylation of its hydroxyl group significantly decreases the antihypoxic properties.

Furylgermatranes exhibit stimulating activity in an ethanol anesthesia test (Table 12). In the thiophene series the 2-isomer is a stimulant as well, while the 3-isomer acts as CNS depressant. Introduction of the methyl group increases the stimulating activity of the 2-isomer (5-CH₃) and the depriving properties of the 3-isomer (2-CH₃). Substitution of the 5-methyl group for the 5-ethyl group changes the action mode of 2-isomer from stimulation of CNS to its depression. Most of the thienylgermatranes prolong the duration of hexobarbital anesthesia. The highest activity is observed for 5-ethyl-2-thienyl derivative (205%). 2-Furfuryl derivative is even more active (236.6%).

TABLE 9

Protection against hypoxia



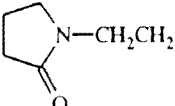
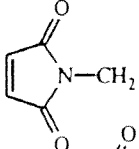
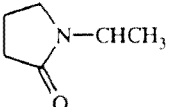
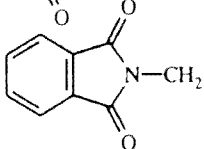
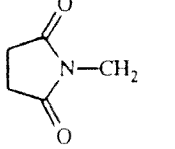
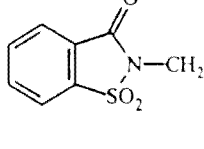
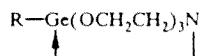



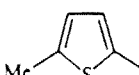



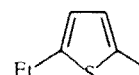
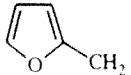

R	% of control	R	% of control
	116,6		145,5
	122,8		114,1
	136,0		139,7

TABLE 10

Protection against hypoxia



R	% of control	R	% of control
	184,8		117,6
	150,5		181,0
	131,8		132,8
	141,3		126,0
	169,5		133,4

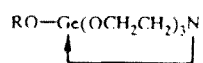
Hydroxygermatrane shortens the duration of ethanol anesthesia (Table 13). Triphenylgermylation of its hydroxyl group increases its stimulating activity, while the silylation in most cases prolongs the ethanol anesthesia. Methyl-di(2-furyl)siloxygermatrane was the most active siloxygermatrane in prolongation of hexobarbital anesthesia.

3-Thienylgermatranes were more active than 2-isomers in memory improvement tests (Table 14). The effectiveness in retrogradal amnesia tests was reduced by the introduction of the methyl group; however, the introduction of the ethyl group did not cause any significant changes.

Summarizing the data obtained, we can conclude that we have found some leading compounds possessing high activity and low toxicity: 2-furfurylgermatrane prolonged the hexobarbital anesthesia by 2.4 times as compared with the control, and

TABLE 11

Protection against hypoxia



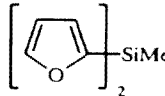
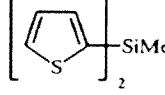
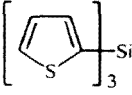
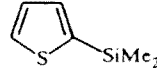
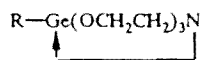
R	% of control	R	% of control
H	186,5		118,8
Me ₃ Si	148,1		114,8
	136,2		111,1
Ph ₃ Si	120,1		
Ph ₃ Ge	116,4		

TABLE 12

Neurotropic activity of germatranes




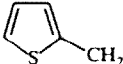

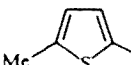

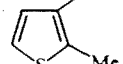

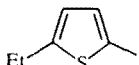
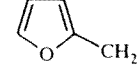
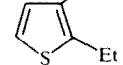
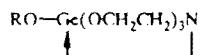
R	% of control		R	% of control	
	ethanol anaesthesia	hexobarbital anaesthesia		ethanol anaesthesia	hexobarbital anaesthesia
	72,9	191,8		117,1	127,0
	61,5	171,4		51,6	150,0
	80,0	58,0		137,8	106,6
	121,4	164,3		161,3	205,0
	79,6	236,6		187,0	126,7

TABLE 13

Neurotropic activity of germatranes



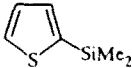
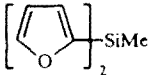
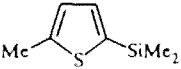
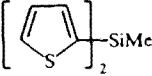
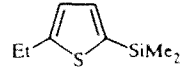
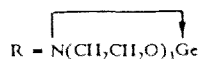
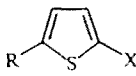
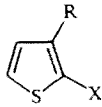
R	% of control		R	% of control	
	ethanol anaesthesia	hexobarbital anaesthesia		ethanol anaesthesia	hexobarbital anaesthesia
H	89,4	132,2			
Me ₃ Si	151,0	95,5		140,9	117,8
Ph ₃ Si	87,4	129,4			
Ph ₃ Ge	46,2	133,3			
	94,5	195,8		110,0	55,5
	156,6	97,8		168,5	74,7

TABLE 14

Neurotropic activity of germatranes



Retrogradal amnesia, (% of control)

X		
H	33	89
Me	20	20,5
Et	40	89

had $\text{LD}_{50} = 2960 \text{ mg} \cdot \text{kg}^{-1}$; triphenylsiloxygermatrane ($\text{LD}_{50} = \sim 2000 \text{ mg} \cdot \text{kg}^{-1}$) shortened the duration of ethanol anaesthesia by 46%; 5-ethyl-2-thienylgermatrane prolonged ethanol and hexobarbital anaesthesias by 1.6 and 2 times, respectively, and also prevented retrogradal amnesia by 89%, while hydroxygermatrane ($\text{LD}_{50} = 8400 \text{ mg} \cdot \text{kg}^{-1}$) was the most active against hypoxia and prevented retrogradal amnesia almost completely.