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SYNTHESIS AND PROPERTIES OF 2-CARBOXYETHYLGERMANIUM SESQUIOXIDE (GE-132) AND RELATED COMPOUNDS AS BIOACTIVE ORGANOGERMANIUM COMPOUND

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ABSTRACT

There has been considerable interest in recent years in the synthesis of bioactive organogermanium compounds. Recently, we observed that 2-carboxyethylgermanium sesquioxide (Ge-132) has a unique chemical structure and various physiological activities. Therefore, we synthesized the number of Ge-132 related compounds (germanium sesquioxides and sesquisulfides, germatranes and thiatranes, germalactones, and amino acids containing germanium), which were found to exhibit more or less activity than the lead compound Ge-132.

Among the various physiological activities, the preventing effect on the formation of advanced glycation end products (AGE) *in vitro* and *in vivo* will be present.

INTRODUCTION

Several organogermanium compounds were reported as physiologically active substances since Winkler synthesized the first organogermanium compound in 1887. In 1962, Kaars [1] demonstrated that trialkylgermanium acetate had antifungal activities, the first description concerning physiological activities of organogermanium compound. Then in 1968, carboxyethyl germanium sesquioxide[2], Ge-132, was synthesized by Asai and Kakimoto, who were interested in the philosophical significance of the existence of germanium in coal, residues of living organisms. In 1974, spirogermanium [3] was synthesized and is now under development as a cytotoxic anticancer agent. Recently, some antitumor agents[4] were synthesized as shown in Fig. 1. However, these activities were not so strong. Animal experiment revealed that Ge-132 is a unique physiologically active compound of extremely low toxicity, with biological responce modifying activity. Ge-132 is currently in phase III clinical study as an agent for adjuvant immunochemotherapy in immunocompromised patients with cancer. More recently, we observed that Ge-132 and related compounds prevent the formation of advanced glycation end products (AGE) *in vitro* and *in vivo*, which may contribute to the development of diabetic complications. In this paper, the synthesis, properties and biological activities of Ge-132 and related compound will be present.

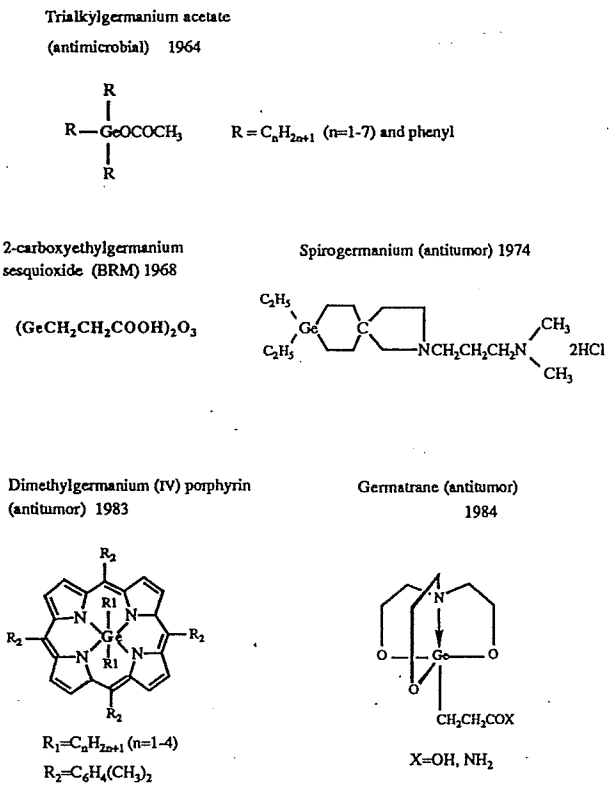


Fig. 1. Reported Germanium Compounds Which Showed the Biological Activity

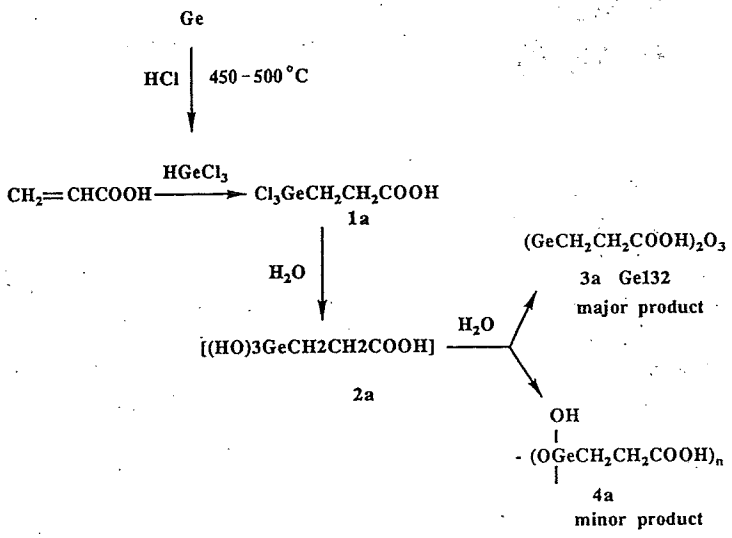
2-CARBOXYETHYLGERMANIUM SESQUIOXIDE (GE-132)

This novel organogermanium compound was synthesized in 1967 at Asai Germanium Institute headed by Asai. His goal of synthesizing water-solble organogermanium compounds originated from his conception that germanium should have some important significance for life, since this element is distributed universally in coal and contained particularly in valued chinese herbs such as ginseng.

Synthesis

The synthetic procedure is shown in Scheme 1. Acrylic acid is treated with trichlorogermane-ether complex or trichlorogermane in conc. hydrochloric acid to form trichlorogermylpropanoic acid of reasonably high purity (**1a**) in a hight yield. **1a** is hydrolyzed to afford Ge-132 (**3a**) as a major product and poly[1-hydroxy-1-(2'-carboxyethyl)germoxane (**4a**) as a minor product *via* the trihydroxygermyl adduct (**2a**).

The trichlorogermylation in conc.hydrochloric acid followed by hydrolysis is preferred for the large-scale preparation and this method made possible the routine preparation of Ge-132 in a one-pot operation.



scheme 1

Chemical structure and physicochemical properties

In the crystalline state X-ray crystallography [5] showed that Ge-132 is a polymer with a three-dimensional network structure formed from the parent skeleton of twelve-size ring in which three O atoms are attached to one Ge atom. On the other hand, the structure of isomer **4a** of Ge-132 was confirmed by X-ray crystallographic analysis to be a linear polymer which has an infinite fibrous structure consisted of *trans-cis-trans-cis* linkage dissimilar to that of polystyrene as shown in Fig. 3. The thermal analysis of compound **3a** and **4a** showed that the latter undergoes selfdehydration to afford the former. Therefore, the linear polymer seems to be a precursor of Ge-132.

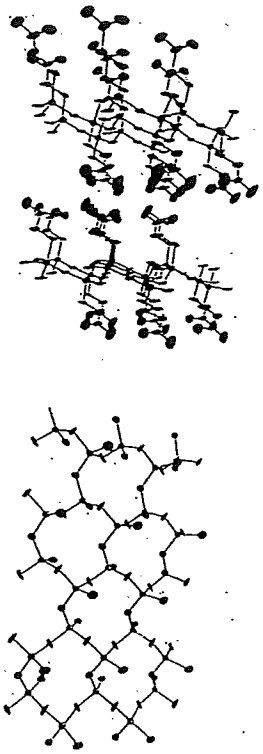


Fig. 2. Crystal Structure of Ge-132 (The carboxylate chain has been omitted for clarify)

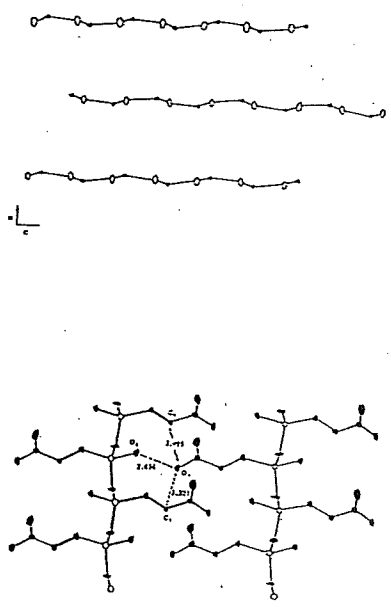
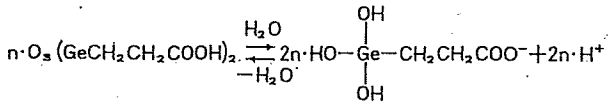


Fig. 3. Crystal Structure of poly[1-hydroxy-1-(2'-carboxyethyl)germoxane]

Ge-132 is soluble in water to about 0.98%, and in solution this compound is hydrolyzed and dissociated to the monomer, trihydroxygermylpropanoic acid. This fact was clarified from ^{17}O-NMR spectrum of [^{17}O]-labeled Ge-132 and ^{13}C-NMR spectrum of Ge-132 in the solid state and in solution.



I II

^{13}C-NMR spectra

δ ppm.	Ge-CH_2	CH_2	COOH
I (Solid)	15.5	28.2	178.0
II (in D_2O)	13.7	28.3	178.9

Pharmacological activities

General pharmacological studies by means of multidimentional observation by Irwin's method in mice receiving 1000 and 4000 mg/kg of Ge-132 (i.p.) revealed that Ge-132 has no significant effect in normal animals. Therefore, it may have no significant action on specific pharmacological receptors.

However, Ge-132 exerted favorable effects in certain desease model in animals, abnormal cells or abnormal methabolism. Ge-132 showed the following pharmacological activities : 1) antitumor activities [6] against certain tumor bearing animals, 2) antiviral activities [7] in virus-infected animals. These two activities were considered to be based on BRM activities [8] such as activation of macrophase increase of NK activity and increase of cell-mediated and humoral immunity. Further it has been revealed that these BRM activities are based by interferon induction, because the formation of interferon γ mRNA in the spleen cell incubated with Ge-132 under the existence of mitogen are confirmed by means of PCR method, 3) regulation of Ca^{2+} metabolism [9], and 4) alleviation of cancerous pain and osteocope [10], 5) a protective effect [11] against fee radicals, 6) Finally, inhibitory activity [12] of the formation of advanced glycation end products (AGE) in tissue, which is accelerated in diabetes mellitus.

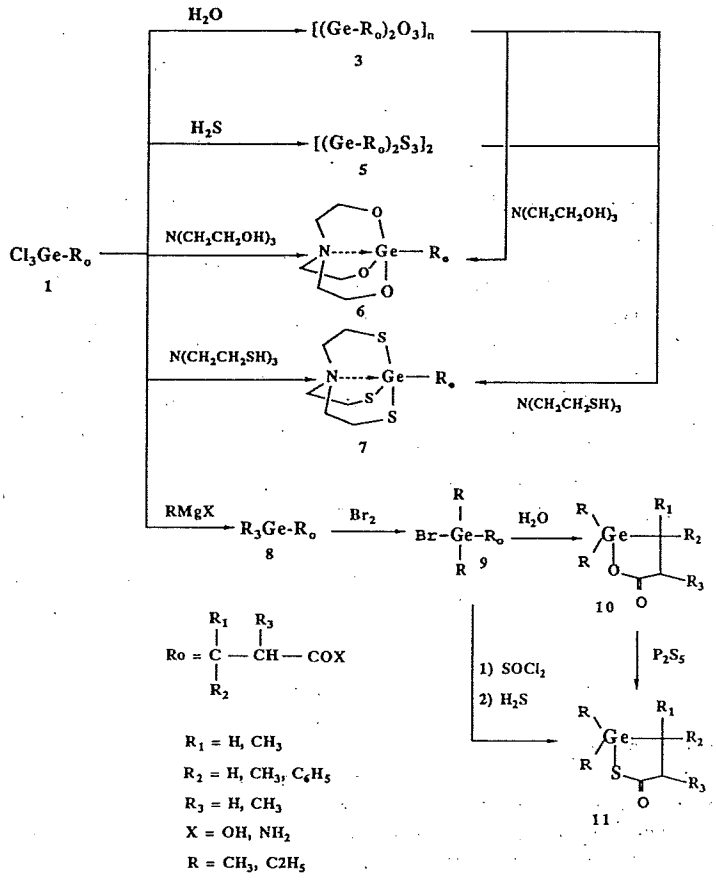
Thus, Ge-132 is a unique drug having pharmacological activities related to an augmenting effect on biophlactic mechanisms.

Toxicity [13]

By all routes of administration, the acute LD_{50} values of Ge-132 for mice and rats were over a few grams per kg body weight. Oral administration of 3g/kg to rats for 6 weeks and/or 6 months, and intravenous administration of 125, 250, and 500 mg/kg to beagle dogs for 6 months gave no signs of toxicity. In reproduction tests through three generations of rats neither teratogenicity nor significant effect on reproductive functions was demonstrated. Therefore, GE-132 seems to have neither acute nor chronic toxity.

Synthesis [14-19]

As shown in Scheme 2, the synthesis of Ge-132 analogs is composed of the formation of trichlorogermyl adducts (1) by the reaction of trichlorogermane with α,β -unsaturated compounds and hydrolysis of the adducts with H_2O to produce a series of sesquioxide analogs (3), or sulfhydrylation with H_2S to produce sesquisulfide series analogs (5). It is worthy to note the difference in the structure between sesquioxide and sesquisulfide, i.e. in the former germanium sesquioxide units bind each other infinitely to form a planar network, and in the latter three units bind to form thiogermaadamantane skeleton.



scheme 2

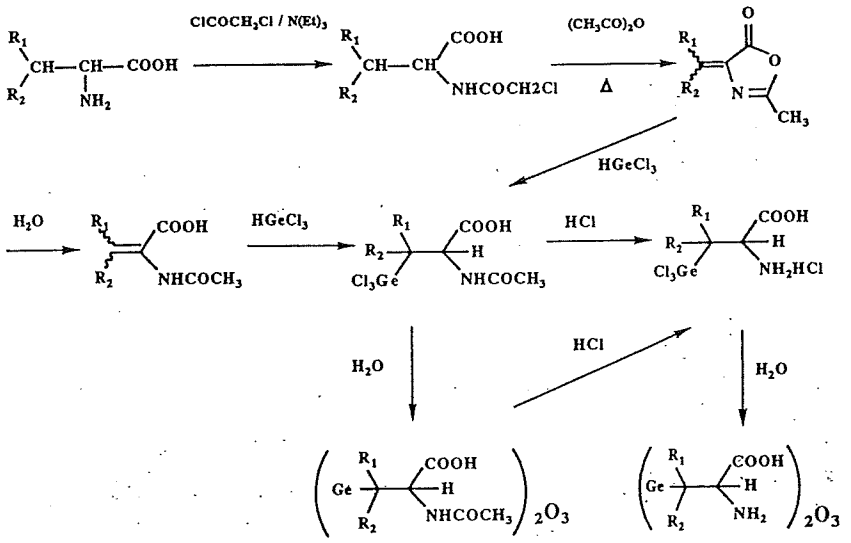
Furthermore, treatment of trichlorogermyl adducts or trialkoxygermyl adducts with trihydroxyethanolamine gives germatrane derivatives (**6**). Using tris(2-mercptoethyl)amine instead of trihydroxyethanolamine in this reaction, trithiagermatranes (**7**), in which the O of the germatrane skeleton is replaced with S, are produced. X-ray structural analysis showed that the Ge-N distance (2.63 Å) is longer than that in the carbamoylethyl germatrane (2.23 Å) investigated earlier because of decreasing electron supply to germanium due to the low electronegativity of sulfur compared with oxygen. Although **6** is generally hydrolyzed to afford triethanolamine and trihydroxygermyl products, **7** is very stable to water.

Secondly, we synthesized the germa- γ-lactones (**10**) by bromination of trialkylgermylpropanica cid (**8**) followed by hydrolysis in good yields. Treatment with P_2S_5, the compounds **10** were converted into the corresponding germa-γ-thiolactones (**11**).

Thirdly, we focused on the synthesis of amino acids and peptides containg germanium in order to dissolve in water easily. These amino acids were prepared from the trichlorogermylation of α,β-unsaturated amino acids and azulactones followed by hydrolysis as shown in Scheme 3. The synthesis of peptides is now being carried out.

Physiological activities

Base on the physiological activities of Ge-132, such as host-mediated antitumor activity, enkephalinase inhibitory activity, bone formation activity, and preventing activity of the AGE formation , these derivatives were evaluated and were found to exhibit more or less activity than the lead compound Ge-132.



scheme 3

Among these various physiological activities, we would like to focus on preventing effect the Maillard reaction, the amino-carbonyl reaction in the human body by Ge-132 and 2-amino-2-carboxylethylgermanium sesquioxide (Ge-385) *in vitro* and *in vivo*.The formation of AGE by the Maillard reaction may possibly be the major factor in ethiology of diabetic complications, mutations, and final aging.

The effects of Ge-132 on the formation of AGE from Nα-t-Boc-lysine and glucose under physiological condition were investigated. The optical density (O.D.) due to Maillard reaction products were decreased dose-dependently to Ge-132, as shown in Fig 4. 10mM Ge-132 was found to completely prevent the progress of glycation and in this reacion mixture residual lysine was found to be remained intact over 90% by HPLC analysis (Fig.5). These results suggest that Ge-132 may be concerned with the inhibition of an early stage in the Maillard reaction.

The effect of Ge-132 on the Amadori products prepared from Nα-t-Boc-lysine and glucose was also examined. As shown in Fig.6 and Fig.7, Ge-132 was also found to prevent the formation of AGE from the Amadori products to a certain extent.

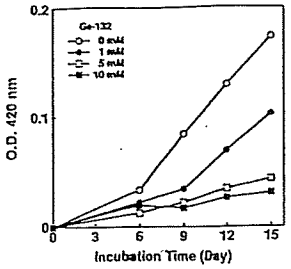


Fig. 4. Effects of Ge-132 on the Formation of AGE from Nα-t-Boc-Lysine and Glucose under Physiological Conditions (pH 7.4, 40 • C)

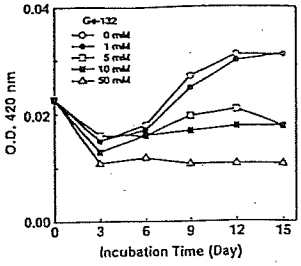


Fig. 6. Effects of Ge-132 on the Formation of AGE from Nα-t-Boc-Nε-Fructose lysine (Amadori Compound) under Physiological Conditions

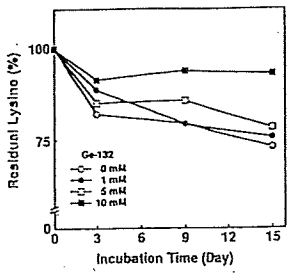


Fig. 5. Effects of Ge-132 on the Reaction of Nα-t-Boc-Lysine and Glucose under Physiological Conditions

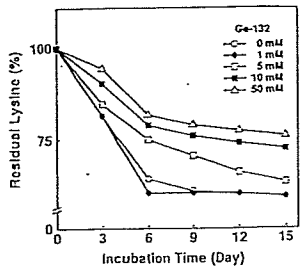


Fig. 7. Effects of Ge-132 on the Decomposition of Nα-t-Boc-Nε-Fructose lysine (Amadori Compound) under Physiological Conditions

Glycation of bovine serum albumine (BSA) and Ge-compound

The effects of Ge-132 and Ge-385 on the glycation of BSA are summarized in Fig.8. Ge-132 seems to have the weak effect for preventing glycation at its low concentration. The characteristic effects of Ge-385 were comparatively examined with Ge-132. Even though Ge-385 was present in the racemate, glycation continued up to day 10. Therefore, according to the concentration of Ge-385, fluorescence intensity decreased, but this was not observed in the case of Ge-132.

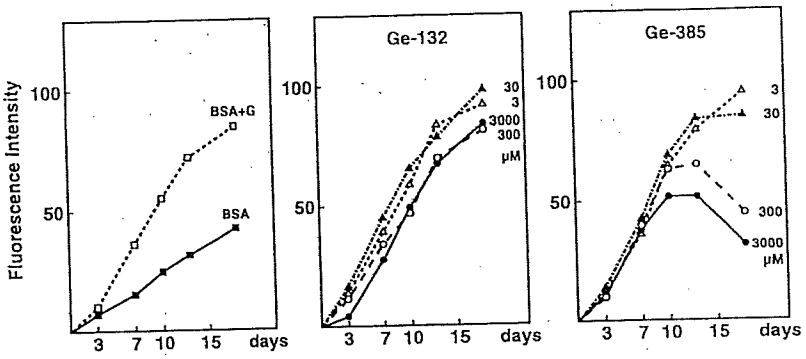


Fig. 8. Effects of Germanium Compounds on the Formation of Advanced Glycation End Products (AGE) from Bovine Serum Albumine (BSA) and Glucose *in vitro*

$(GeCH_2\underset{\underset{R}{|}}{C}HCOOH)_2O_3$,

R=H : Ge-132

$R=NH_2$: Ge-385

Effects of Ge-compound on diabetic rats

As discribed above, these organogermanium compounds seem to prevent the formation of AGE *in vitro*. Therefore, the effects of administration of these two compounds on glycation of collagen in tissues of diabetic rats were examined. Rats with diabetes caused by streptozocin were put into three groups: untreated rats, rat treated with Ge-132, and rats treatead with Ge-385. The compound was added to the drinking water and given at the dose of 100mg/kg body weight for 14 weeks. Levels of plasma glucose and glycated hemoglobin were measured every 4 weeks, and the differences in the three groups were not observed. After the treatment period, collagen was removed from abdominal skin and tail tendons, and collagen-linked fluorescence was measured (Fig.9 and Fig.10).

The rat given Ge-132 had significantly less collagen-linked fluorescence in both the skin and the tail tendons than the untreated rats. Treatment with Ge-385 had less effect on collagen fluorescence in either tissue than the rats treated with Ge-132. These results suggest that Ge-132 inhibited the formation of AGE in tissue which is accelarated in diabeties mellitus.

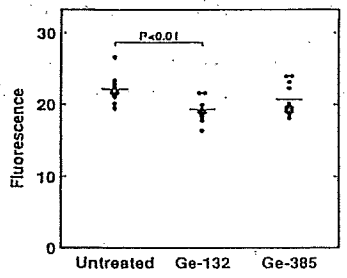


Fig. 9. Collagen-Linked Fluorescence in Skin from Diabetic Rats without Treatment, Rats Treated with Ge-132, and Rats Treated with Ge-385.

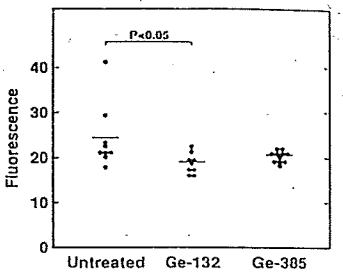


Fig. 10. Collagen-Linked Fluorescence Tail Tendons from Diabetic Ra without Treatment, Rats Treat with Ge-132, and Rats Treated Ge-385

The mechanism of the inhibition of glycation by these germanium compounds has not clarified yet. Therefore, we focus on the reaction of sugar with Ge-132 under physiological conditions and the results will be presented in a future paper.

CONCLUSION

As described above, Ge-132 is a unique organogermanium with high safety and various physiological activities which are associated not only with biological response modification, but also with homeostasis augmentation. These findings suggested that derivatives of Ge-132 may also show analogous physiological profiles, and these compounds were found to exhibit more or less activity than the lead compound Ge-132.

The origin of such unique physiological activities may be related an action on cell membrane. Although more sophisticated molecular biological studies remain to be conducted, a new type of therapeutic agents may be derived from these organogermanium derivatives.

Acknowledgment

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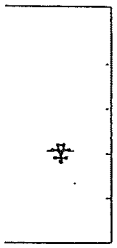

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