Synthesis and Biological Activity of 3-(2, 8, 9-Trioxa-aza-1-germatricyclo [3. 3. 3. 0]undecane-1-yl)-hydroxycinnamic Acids

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Abstract: The new germanium compounds of cinnamic acid, $RC_6H_4CHGe(OCH_2CH_2)_3NCH_2COOH$ R=H (**I**), 2-OH (**II**), 3-OH (**III**), 4-OH (**IV**), have been obtained to study anti-tumor activities. Compounds (**I-IV**) were prepared by the electrophilic addition reaction and nucleophilic substitution reaction. Biological investigation has demonstrated that all compounds (**I-IV)** are lower toxicity with strongly anti-tumor activity than positive control.

Key Words: Germanium, cinnamic acid, hydroxyl-cinnamic acid germanium, synthesis, trichlorogermane, germatrane, u14 tumor-cell, antitumor activity, tumor inhibitory.

1. INTRODUCTION

Germatranes (CASname: 5-aza-2, 8, 9-trioxa-1-germatricyclo [3. 3. 3. 0] undecane) contain hypervalent germanium atom transannular bond to nitrogen, which is responsible for their chemical stability [1, 2, 7], and the nature of substituent at germanium atom influences the strength of the transannular N → Ge bond the values of bond angles and biological properties [3-11]. It has been found that compounds which germatrane cycle is in β-position of Carboxylic acid and phosphonic acid have activities of anti-tumor and the introduction of one methylene group between heterocycle and germatranyl group dramatically lowers the acute toxicity of the compound, while the activity remains high [4-10]. One can expect that in cinnamate germatranes series, it will lead to the low toxic compounds and thus to the increase of their therapeutic index. Therefore, in this investigation we focused our attention to the cinnamategermatranes.

2. RESULTS AND DISCUSSION

Cinnamic acid germatranes (**I-IV**) were obtained as result of the following conversions: Michael addition of trichlorogermane and cinnamic acid in refluxing concentrated hydrochloric acid, trichlorogermanic bodies into trimethoxy derivatives by methanolysis, and transalkoxylation with triethanolamine to germatranes **I-IV**, (yields 40.2, 57.8, 63.0, and 70.1%, respectively. Scheme **1**). The melting point was about 190°C.

The analytical data for cinnamategermatranes (**I-IV**) can be seen from Experimental and show that the characteristic signals of NMR for germatranes are 2.11-2.42ppm for methine which is attached to phenil, 2.34-2.52ppm for methylene attached to Carboxy, 2.60-2.66ppm for N-CH_2, and 3.58-3.62ppm for O-CH_2, 6.60-7.30ppm for C_6H_4 and the characteristic MS-fragment ion of GC-MS for germatranes is 220 for M^+-$Ge(OCH_2CH_2)_3N$.

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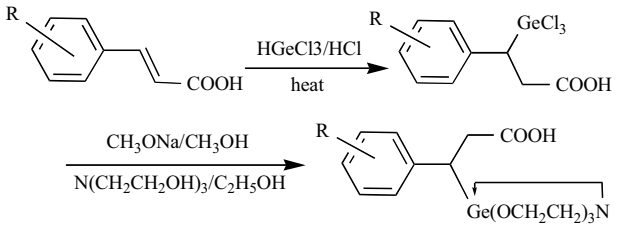

Compound (I) R=H
Compound (II) R=2-OH
Compound (III) R=3-OH
Compound (IV) R=4-OH

Scheme (1). The synthesis route of 3-(2, 8, 9-Trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-hydroxycinnamic acids.

The experimental results for anti-tumor U14 activities of cinnamategermatranes are presented in Table **1**. The results showed that the tumour inhibitory rate of the cyclophosphamide group was 53.01% and the mice's body weight decreased evidently during administering drug, from which the substantial damage of cell could be observed. And the tumour inhibitory rate of cinnamic acid germatranes (I-IV) were 33.33% for compound (I), 38.0% for compound (II), 48.76% for compound (III) and 36.25% for compound (IV), and the mice's weight increased significantly during administering drug with cells having no abnormal changes. Therefore, the cinnamategermatranes have inhibitory effect and lower toxicity than positive control. The results of electron microscope were as follows: in blank control, the tumor cell was growing strongly, and obvious hyperemia, hydroncus; inflammatory cell infiltration was observed around the connective tissue. Liver, spleen, kidney, adrenal gland, and stomach were normal essentially, heart and lung with hyperemia were observed but the substantial damage of cell could not be observed. Methyl green-pyronin staining indicated that the apoptosis of tumor cells was less observed. However, in group administered, it was appeared that the tumor's hyperemia, necroses were obvious; the staining tissue was under the staining mirror the cells of apoptosis of the chromatin in compact structure or grain stained seriously were observed beside dense necrosis; when stained with

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Table 1. Tumor Inhibitory Activity of RCHGe $(OCH_2CH_2)_3NCH_2COOH$

Group	Number of Animal	Weight of Tumor	Inhibitory (%)	P value
isotonic sodium chloride	10	2.27±1.24		
cyclophosphamide(CPA)	10	1.07±0.54	53.07	<0.01
C_6H_5	10	1.52±0.74	33.33	<0.01
2-OHC_6H_5	10	1.48±0.57	38.0	<0.01
3-OHC_6H_5	10	1.17±0.46	48.76	<0.01
4-OHC_6H_5	10	1.45±0.63	36.25	<0.01

methyl green-pyronin the nuclei were green or green-blue; cytoplasm was red-purple, and heart, kidney, liver, lung, adrenal gland and stomach had no abnormal changes, there were a number of megakaryocytic in spleen.

3. EXPERIMENTAL

$HGeCl_3$ was prepared according to Ref [12].

^{1}H NMR spectra were conventionally recorded on Varian 200 Mercury instrument using D_2O as a solvent. Mass spectra were recorded on Hp6890 (70ev).

Anti-tumor U14 activity was studied by the following methods: 60 hybrid mice of Kunming species, female of 25±1.0 g in weight, were provided by the Center of Experimental Animal in our college. The oncocyte of mice's cervix cancer U14 which was introduced from Shanghai Institute of Pharmacy, regularly handed from generation to generation, were reserved by the Pathology Group of Jiangxi traditional of Chinese Medicine. Mice were administered cinnamic acid germatranes (**I-IV**) for ten days continuously and used the group isotonic sodium chloride as model control and cyclophosphamide as positive control, in which the crystal of cinnamic acid germatranes (**I-IV**) were made up to a concentration of 100μg /ml with doubly distilled water and fed to a mouse of 200μg / (10g.d) *via* stomach lavage in every test group, and 10 mice were fed *via* stomach lavage with normal isotonic sodium chloride of 0.3mL/d in model control, and the cyclophosphamide dissolved in isotonic sodium chloride and obtained 10mg/ml then administered by 75mg/kg and injected into abdonmen of by 0.2ml, once a day in positive control.

Calculate the tumour inhibitory rate according to the following equation:

Tumour inhibitory rate = (Average tumour weight of model contrast group- Average tumour weight of administered group) / Average tumour weight of model contrast group×100%

Tests were indicated and determined according to Ref [13].

3.1. Cinnamic Acid Germatranes

Cinnamic acid (6.37 g, 0.043 mol) and trichlorogermane (7.7 g, 0.043 mol) in 30 ml concentrated hydrochloric acid were refluxed for 5 h. The resultant white composition was filtered off and dissolved in methanol (30 ml). Then sodium methoxide (10 ml) was added to the mixture, and stirred at rt for 4 h, the methanol was evaporated in vacuum, triethanolamine (6.4 ml) in ethanol was added to residue and then refluxed for 6h and filtered off to obtain white solid. The water solution of white solid was dealt with acidification and filtered to obtain a solid substance, which was crystallized with ether. Eventually get a pure compound (I). Compound (I): mp183~185°C(ether), Anal. Calcd for $C_{15}H_{21}GeNO_5$: C, 48.97; H, 5.75; N, 3.81. Found: C, 48.81; H, 5.73; N, 3.69. ^{1}H NMR (D_2O) d: 2.96 (2H, d, $-CH_2$), 3.25 (1H, t, -CH), 3.59 (6H, t, $-NCH_2$), 4.01 (6H, t, $-OCH_2$); 6.46-7.39 (5H, m, $-C_6H_5$). GC-MS (%): 368[M^+, 5], 220[Ge $(OCH_2CH_2)_3N$, 100], 160(35), 46(18), 130(7), 116(5), 100(5), 91(50).

Compounds (II-IV) were prepared analogously, recrystallized from ether.

Compound (II), mp 193~195°C(ether), Anal. Calcd for $C_{15}H_{21}GeNO_6$: C, 47.42; H, 5.57; N, 3.69. Found: C, 47.31; H, 5.55; N, 3.73. ^{1}H NMR (D_2O) d: 2.63 (2H, d, $-CH_2$); 3.11 (1H, t, -CH), 3.61 (6H, t, $-NCH_2$), 3.93 (6H, t, $-OCH_2$), 6.53-7.64 (4H, m, $-C_6H_4$). GC-MS (%): 380[M^+, 3], 220[Ge $(OCH_2CH_2)_3N$, 100], 160(30), 146(15), 130(7), 116(5), 100(3).

Compound (III), mp 189~191°C(ether), Anal. Calcd for $C_{15}H_{21}GeNO$: C, 47.72; H, 5.57; N, 3.69. Found: C, 47.50; H, 5.56; N, 3.55. ^{1}H NMR (D_2O) d, 3.12 (2H, d, $-CH_2$), 3.27 (1H, t, -CH), 3.59 (6H, t, $-NH_2$), 3.93 (6H, t, $-CH_2$), 6.45-7.51 (4H, m, C_6H_4). GC-MS (%):380[M^+, 3], 220[Ge $(OCH_2CH_2)_3N$, 100], 160(34), 146(13), 130(5), 116(3), 100(5).

Compound (IV), mp 203~206°C(ether), Anal. Calcd for $C_{15}H_{21}GeNO_6$: C, 47.42; H, 5.57; N, 3.69. Found: C, 47.43; H, 5.49; N, 3.71. ^{1}H NMR (D_2O) d, 3.15 (2H, d, $-CH_2$), 3.31 (1H, t, -CH), 3.48(6H, t, $-NH_2$), 4.13(6H, t, $-OCH_2$), 6.33-7.29 (4H, m, $-C_6H_4$). GC-MS (%):380[M^+, 3], 220[Ge $(OCH_2CH_2)_3N$, 100], 160(30), 146(13), 130(7), 116(4), 100(7).

4. SUPPLEMENTARY MATERIAL

^{1}H NMR spectra and GC-MS spectra for the structures **I-IV** reported in this paper have been deposited at the Nanjing University Analytical Center.

5. ACKNOWLEDGEMENTS

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