Synthesis and Biological Activity of 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic Acid

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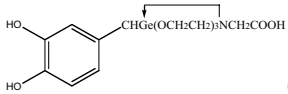


Abstract: The new germanium compound of caffeic acid, (**1**) , has been obtained to compare anti-tumor activities with 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0]undecane-1-yl)-hydroxycinnamic acids which have been researched previously. Compound was prepared which mainly used caffeic acid, germanium dioxide, sodium hypophosphite, triethanolamine as materials by reducing reaction, Micheal addition reaction and transesterification. The structure is comfirmed by ^{1}H-NMR and MS. Biological investigation has demonstrated that the compound is stronger anti-tumor activity than 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0]undecane-1-yl)-hydroxylcinnamic acids with lower toxicity.

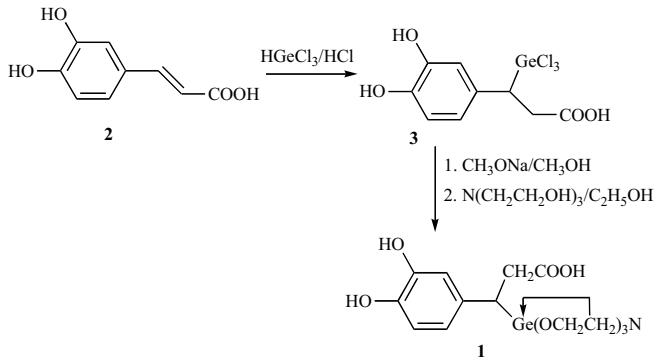
Key Words: Germanium, caffeic acid, germatrane, 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0]undecane-1-yl)-hydroxycinnamic acids, 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0] undecane-1-yl)-caffeic acid, U14 tumor-cell, anti-tumor activity, tumor inhibitory.

1. INTRODUCTION

Atrane compounds of the Group 14 elements are attractive compounds because of their specific biological activity [1-5]. Detailed investigation of the biological activity of germanium organic compounds has showen that they posses low toxicity and salutary act on manifold alive organisms [6], especially germatranes (CASname: 5-aza-2, 8, 9-trioxa-1-germatricyclo [3. 3. 3. 0] undecane) act strong biological properties. It has been found those compounds that germatranes in β-position of Carboxylic acid or carboxylate and phosphonic acid or phophonate have activity of anti-tumer, Moreover , the introduction of one methylene group between heterocycle and germatranyl group dramatically lowers the acute toxicity of the compound, while the activity remains high [7-13]. Now the aim of this work is to introduce the germatrane structure to caffie acid which has two hydroxyl substitutions in benzene ring and strong anti-tumor activities to prepare the desired compound 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid [14,15], to test its biological activity and compare with the activities of 3-(2, 8, 9-Trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-hydroxylcinnamic acids which have one hydroxyl substitution in benzene ring reported in reference [16].

2. RESULTS AND DISCUSSION

3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid was obtained as result of the following conversions: Michael addition of trichlorogermane and caffeic acid in refluxing hydrochloric acid, trichlorogermanic bodies (**3**) into trimethoxy derivatives by methanolizing, and transesterification with triethanolamine to compound (**1**). (yields 63%) (Scheme **1**).The melting point is 215-217 °C.



Scheme 1. Synthesis of 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid.

The analytical data for 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid can be seen from Experimental and show that the characteristic signals of NMR for germatrane are 3.25ppm for methine which is attached to phenyl, 2.96ppm for methylene attached to Carboxy, 3.60ppm for N-CH_2, and 4.01ppm for O-CH_2, 6.46-7.39ppm for C_6H_3; the characteristic MS-fragment ion of GC-MS for germatranes is 220 for M^+-$Ge(OCH_2CH_2)_3N$ and IR data 1633.5cm^{-1} explained the methylene of germantrane ring.

The experimental results for anti-tumor U14 activities of 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid are presented in Table **1**. The results showed that the tumour inhibitory rate of the cyclophosphamide

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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	2.18±1.04		
cyclophosphamide(CPA)	10	1.02±0.42	53. 05	<0.01
3, 4-dihydroxylphenyl	10	1.11±0.54	49.31	<0.01

group was 53.05% 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 caffeic acid germatrane was 49.31%, and the mice's weight increased significantly during administering drug with cells having no abnormal changes. Therefore, 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid has 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 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. Compared with the activities of 3-(2, 8, 9-Trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-hydroxylcinnamic acids seen in Table **2** [16], the tumour inhibitory rate of 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0] undecane-1-yl)-caffeic acid was higher.

3. EXPERIMENTAL

$HGeCl_3$ was prepared according to Ref. [17].

Elementary can be analyzed on Perkin-Elmer/Foss Heraeus CHN-O-Rapid; IR were recorded on Nexus 870 FT-IR; ^1H 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: 30 hybrid mice of Kunming species, female of 16-25g 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 Guangdong Pharmaceutical University. Mice were administered 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic acid for ten days continuously and used the group isotonic sodium chloride as model control and cyclophosphamide as positive control, in which the crystal of 3-(2, 8, 9-trioxa-aza-1-germatricyclo[3. 3. 3. 0] undecane-1-yl)-caffeic acid was 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 [18].

3.1. 3-(2, 8, 9-trioxa-aza-1-germatricyclo [3. 3. 3. 0] undecane-1-yl)-caffeic Acid

Caffeic acid (3.6 g, 0.02mol) and trichlorogermane (3.6 g, 0.02 mol) in 50 ml concentrated hydrochloric acid were

Table 2. Tumor Inhibitory Activity of RCHGe $(OCH_2CH_2)_3NCH_2COOH$(reference [16])

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-OH C_6H_5	10	1.48±0.57	38.00	<0.01
3-OH C_6H_5	10	1.17±0.46	48.76	<0.01
4-OH C_6H_5	10	1.45±0.63	36.25	<0.01

refluxed for 7 h. The resultant white composition was filtered off and dissolved in methanol (15 ml). Then sodium methoxide (5.0 ml) was added to the mixture, and stirred at rt for 5 h, the methanol was evaporated in vacuum, triethanolamine (3.2 ml) in ethanol was added to residue and then refluxed for 6 h 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 5.04 g (Yield63%). Compound: mp215~219°C (ether), Anal. Calcd for $C_{15}H_{21}GeNO_7$: C, 45.05; H, 5.29; N, 3.50. Found: C, 45.06; H, 5.29; N, 3.58. IR(cm^{-1}): 3438, 3074, 2960.8, 2935.2, 2874.2, 1727.2, 1633.5, 1580.9, 1493.7, 1404.01, 1287.2, 873.1, 744.7, 705.1. ^{1}H NMR ($CDCl_3$), δ (ppm): 2.96 (2H, d, -CH), 3.25 (1H, t, $-CH_2$), 3.60 (6H, t, 3×$-NCH_2$), 4.01 (6H, t, 3×$-OCH_2$); 6.46-7.39 (3H, m, $-C_6H_3$). GC-MS (%): 400 [M^+, 5], 220 [Ge $(OCH_2CH_2)_3N$, 100], 160 [30], 146 [15], 130 [7], 116 [5], 100 [3]. HPLC Purity: 98.5%.

4. SUPPLEMENTARY MATERIAL

IR, ^{1}H NMR spectra and GC-MS spectra for the structure reported in this paper have been deposited at the Nanjing University Analytical and Guangdong Pharmaceutical University Center.

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