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Synthesis and Antimutagenic Effects of Selena-Morpholine Hydrate

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Abstract: **The synthesis of selenamorpholine hydrate was described and its antimutagenic effects were investigated by the Ames tests. The results indicated that the mutation induced by the indirect mutagen 2-AF was inhibited obviously by selenamorpholine hydrate at the dosage of Se 0.16~100 μg /dish for 35.8%~53.7% (TA_{97}) and the mutation induced by direct mutagen Dexon was inhibited for 6.2%~30.0% (TA_{97}) and 2.3%~34.1% (TA_{100}). The mutagenesis of the indirect mutagen cyclophosphamide was suppressed for 5.4%~16.1% by selenamorpholine hydrate at the dosage of Se 0.16~100 μg /dish.**

Key words: **synthesis; selenamorpholine hydrate; Ames test; antimutation**

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0 Introduction

Since 1949 Clayton and Baumann first reported that dietary selenium reduced liver tumors caused by 3-methyl-4-dimethyl aminoazo benzene[1], numerous epidemiological studies in human subsequently presented the evidence that selenium has an anticarcinogenic effect[2,3]. There is an inverse relationship between human cancer incidence and the selenium content of plants in the local area and between blood selenium levels and cancer deaths. Selenium can prevent or retard the growth of chemically induced tumors in experimental animals. It is effective against mutagenesis induced by a number of chemical mutagens[4~7].

In the system of salmonella TA_{1538}, sodium selenite has been demonstrated to exhibit a restraining effect against the mutagenic action of 2-acetamidofluorene (AAF), N-hydroxy-2-acetamidofluorene (N-OH-AAF) and N-hydroxyaminofluorene (N-OH-AF). In the system of salmonella TA_{100}, selenium significantly decreased the mutagenicity of 7, 12-dimethylbenz [α] anthrancene (DMBA). And it could reduce the rupture of chromosome caused by DMBA in the lymphocyte culture. In 1980, Adams observed that selenium can markedly inhibit the reverse mutation of TA_{100} and TA_{1538} induced by DMBA and liver microsomal S9 activation. Martin reported that selenium could reduce the mutagenicity of cyclophosphamide through inhibition of aromatic hydrocarbon hydroxylase[8]. Sodium selenite can reduce the sister chromatid exchange (SCE) frequency caused by N-OH-AAF. Hu[9] investigated the effect of sodium selenite to the mutagenesis of cell v79 in vitro by carcinogen N-methyl-N-nitro-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU). And it was found that selenite given before the exposure to carcinogens could notably reduced the SCE frequency of cell v79 caused by MNNG and MNU while selenite given simultaneously could not protect the cell from the injury. In 1997, Wang[10] examined the antimutagenic effects of selenium polysaccharide and sodium selenite. Both of the two selenium compounds were effective in inhibiting the mutations induced by the mutagens daunomycin, cyclophosphamide and

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sodium azide.

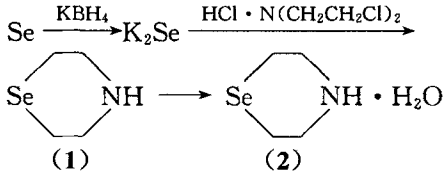
Recently attempts have been made to synthesize organic selenium compounds with lower toxicity than inorganic selenium compounds and apply them in human medication. It was therefore considered worthwhile to synthesize a number of organoselenium compounds to screen them for antimutagenic activities. And our study was initiated to determine the effects of selenamorpholine hydrate to the mutations induced by 2-aminofluorene, Dexon and cyclophosph-amide.

1 Experimental

1.1 Synthesis

General information. Melting point was taken on x-4 microscope melting point apparatus and uncorrected. ^{1}H-NMR spectrum was measured on JEOL FX-90Q spectrometer. Mass spectrum was recorded on VG7070E-HF spectrometer. Element analysis was taken with a CERLO ERBA 1106 instrument.

The structure and synthetic route of the target compound is shown in Scheme 1.



Scheme 1

Selenamorpholine (**1**). To a stirring mixture containing selenium powder 7.9 g(10 mmol) and 6.0g (11 mmol) potassium borohydride, 100 mL ethanol was added under a continuous flow of N_2. The mixture was heated at reflux until it turned to colorless. Then a solution of 4.4 g(11 mmol) sodium hydrate and an ethanol solution of 17.8 g(10 mmol) nitrogen mustard hydrochloride were added dropwise. After being refluxed for 4 h, the mixture was filtered, concentrated and 7.5 g colorless oil (**1**) was obtained by distillation under reduced pressure. (**1**), yield: 50.0%. b.p. 61～63℃, 7mmHg. ^{1}H-NMR, δ_H ($CDCl_3$): 3.23 (4H, m, CH_2NCH_2), 2.63 (4H, m, CH_2SeCH_2), 1.93 (1H, s, NH). MS, m/z: 151 (M^+, 63.0. Se is calculated as ^{80}Se).

Selenamorpholine hydrate (**2**). Compound (**1**) with H_2O at room temperature gives (**2**). m.p. 63～64℃. Anal. calcd. for $C_4H_{11}NOSe$: C, 28.58; H, 6.60; N, 8.33. Found: C, 28.67; H, 6.87; N, 8.16.

1.2 Ames test

1.2.1 Materials

1) Test compound: selenamorpholine hydrate. The selenium content of it is 47.01%.

2) Test strains: Salmonella TA_{97}, TA_{98}, TA_{100} and TA_{102} were supplied by Ames laboratory of America as indicator strains. They were qualified by his-, △uvrB, rfa, R factor, PAQ1 plasmid and spontaneous reversion. The strain was cultured at 37℃, 120 r/min for 10 h and was used when the number of strain reached 1×10^9/mL.

3) Reagents: 2-AF and Dexon were purchased from Wuhan Medicine corporation. Cyclo-phosphamide was obtained from Shanghai 12th pharmaceutical factory.

1.2.2 Methods

1) Solutions: Selenamorpholine hydrate was dissolved and diluted with germ-free distilled water to prepare the solutions with the concentration of Se 1, 0.2, 0.04, 8×10^{-3} g·L^{-1}.

2) The assay for mutagenic activity was performed as described by Ames[11]. The direct mutagen Dexon, indirect mutagen cyclophosph-amide and 2-AF were granted as positive control while germ-free distilled water as negative control. The test was carried out with 3 dishes per treatment and duplicated. Under the condition with or without liver microsomal S9, after 48 hours of incubation at 37℃ the plate was surveyed. Calculate the mean revertants (MR) and the inhibitory rate. Inhibitory rate = (MR of positive control dish − MR of the dish added selenium-containing solution) / MR of positive control dish ×100%.

2 Results and Discussion

2.1 Antimutagenic effect of selenamorpholine hydrate to 2-AF

At the dosage of 0.16～100 μg Se per dish, the mutation induced by 2-AF was inhibited for 35.8%～53.7% (TA_{97}) and 10.2%～18.9% (TA_{98}) by selenamorpholine hydrate (Table 1). It was shown that selenamorpholine hydrate was effective in inhibiting against mutagenesis induced by 2-AF.

Table 1 Inhibitory effect of selenamorpholine hydrate to the mutation induced by 2-AF (+S9)

selenium content /μg·dish^{-1}	2-AF content /μg·dish^{-1}	TA_{97}		TA_{98}		TA_{100}		TA_{102}	
		MR	Inhibitory rate/%	MR	Inhibitory rate/%	MR	Inhibitory rate/%	MR	Inhibitory rate/%
0	20	2.68	—	34.4	—	1.02	—	1.13	—
100	20	1.72	35.8	28.9	16.0	0.95	6.9	1.28	−13.3
20	20	1.49	44.4	30.9	10.2	1.23	−20.6	1.12	0.9
4	20	1.24	53.7	27.9	18.9	1.12	−9.8	1.19	−5.3
0.8	20	1.32	50.7	30.1	12.5	1.13	−10.8	1.34	−18.6
0.16	20	1.24	53.7	30.1	12.5	1.08	−5.9	1.27	−12.4
100	0	1.40	—	1.15	—	0.78	—	0.77	—

2.2 Antimutagenic effect of selenamorpholine hydrate to Dexon

At the dosage of 0.16～100 μg Se per dish, the mutation induced by Dexon was inhibited for 6.2%～30.0% (TA_{97}) and 2.3%～34.1% (TA_{100}) by selenamorpholine hydrate. And the mutation was inhibited for 23.8%(TA_{102}) by selenamorpholine hydrate at the dosage of 100 μg Se per dish. As to TA_{98}, the inhibitory effect was less significant (Table 2).

Table 2 Inhibitory effect of selenamorpholine hydrate to the mutation induced by Dexon (−S9)

selenium content /μg·dish^{-1}	2-AF content /μg·dish^{-1}	TA_{97}		TA_{98}		TA_{100}		TA_{102}	
		MR	Inhibitory rate/%	MR	Inhibitory rate/%	MR	Inhibitory rate/%	MR	Inhibitory rate/%
0	50	4.80	—	3.66	—	6.60	—	2.23	—
100	50	3.36	30.0	3.21	12.3	4.35	34.1	1.70	23.8
20	50	3.75	21.9	3.48	4.9	4.81	27.1	2.39	−7.2
4	50	4.35	9.4	3.49	4.6	6.45	2.3	2.35	−5.4
0.8	50	4.29	10.6	3.54	3.3	6.21	5.9	2.40	−7.6
0.16	50	4.50	6.2	3.61	1.4	6.71	−1.7	2.34	−4.9
100	0	0.94	—	1.03	—	0.92	—	0.86	—

2.3 Antimutagenic effect of selenamorpholine hydrate to cyclophosphamide

At the dosage of 0.16～100 μg Se per dish, the mutation induced by cyclophosphamide was inhibited for 5.4%～16.1% (TA_{100}) by selenamorpholine hydrate (Table 3). It indicated that the antagonism of this organoselenium compound against the mutagenesis induced by cyclophosphamide was not notable.

The results showed that selenamorpholine hydrate had antimutagenic effects against the mutagen 2-AF and Dexon, which is consistent with the conclusion of the previous work[10] that the organic selenium compound has antimutagenic effect. Carcinogenicity is closely connected with mutagenicity, so the antimutagenic effect of selenium compound (inorganic or organic form) may support for its anticarcinogenic properties. Since the mechanism of action of the organic selenium may be different from that of inorganic selenium, the organic selenium has received considerable attention.

Table 3 Inhibitory effect of selenamorpholine hydrate to the mutation induced by cyclophosphamide (TA_{100} as test strain, +S9)

selenium content /μg·dish^{-1}	Cyclophosphamide /μg·dish^{-1}	MR	Inhibitory rate/%
0	200	0.93	—
100	200	0.85	8.6
20	200	0.78	16.1
4	200	1.00	−7.5
0.8	200	0.99	−6.4
0.16	200	0.88	5.4
100	—	0.92	—

In developmental research on antitumor agents, quantities of organic selenium compounds have been synthesized with the purpose of improving antitumor activity. Thus the Ames test would be used as an initial method to screen the antitumor agent and it would provide scientific basis for

the introduction of organic selenium compounds as human medicines.

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Syntheses and Properties Studies on New Dispersed Dyes With Intermediate BIT

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Abstract: The target production were obtained by coupled reaction of 3-amino-5-nitro-2,1-benzoisothiazole, as a diazo component, with some derivatives of anilline or some derivatives of naphthylamine sulfonic acid in proper condition. Their colors are from red to violet. They have excellent tight property. The chemical and physical test of production verified that they were some unique properties.

Key words: BIT; coupled reaction; dispersed dyes; properties test