The Structure-Activity Relationships of Antibacterial L-5-Alkylthiomethylhydantoin S-Oxides and Related Compounds*

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The structure-activity relationships of antibacterial L-5-alkylthiomethylhydantoin S-oxides and related compounds prepared were examined. S-Propyl-L-cysteine-S-oxide, its *N*-carbamoyl derivative and *dl*-3-alkylthio-2-methylpropionamide S-oxides which do not have hydantoin ring exhibited far less activities. 5-Alkyl- and alkenylthiomethyl-5-methylhydantoin S-oxides and 5-alkylthioethylhydantoin S-oxides bearing hydantoin structure did not have either any appreciable activity. From these results, it has strongly been suggested that the structure required for antibacterial activity is summarized as follows: (1) Hydantoin ring and (2) sulfoxide function should be necessary, (3) the hydrogen atom on the 5-C of the hydantoin ring is essential, and (4) the methylene-unit between the sulfur atom and the hydantoin ring is not two (or more), but one.

INTRODUCTION

In the previous paper¹⁾, the authors reported that L-5-alkyl- and alkenylthiomethylhydantoin S-oxides have remarkable antimicrobial activity, in particular, potent antibacterial activity, and both hydantoin ring and sulfoxide function in the structure might be essential for emergence of the activity. This paper describes further study on the structure-activity relationships.

MATERIALS AND METHODS

1. Instrumental Analyses

Instrumental analyses employed were described in the previous paper.¹⁾

2. Compounds

Preparations of L-5-alkylthiomethylhydantoin S-oxides and several related compounds were described in the previous paper.¹⁾

L-5-Methylthioethylhydantoin S-oxide was synthesized similarly from L-methionine. DL-5-Pentylthioethylhydantoin S-oxide was synthesized from acrolein and 1-pentanethiol as starting materials by the applications of the methods of Pfister *et al.*,²⁾ and Bucherer and Lieb³⁾ followed by oxidation of the hydantoin derivative obtained.

DL-5-Methyl-5-propylthiomethylhydantoin S-oxide and DL-5-allythiomethyl-5-methylhydantoin S-oxide have been prepared previously.⁴⁾

dl-3-Alkylthio-2-methylpropionamide S-oxide (alkyl : ethyl and propyl) were synthesized from methacrylamide by addition of alkanethiol⁵⁾ followed by hydrogen peroxide oxidation.

dl-3-Alkylthio-2-methylpropionamide S-oxide (alkyl : ethyl and propyl) were synthesized from methacrylamide by addition of alkanethiol⁵⁾ followed by hydrogen peroxide oxidation.

3. Bioassay

Antibacterial activities were assayed by measuring the inhibitory effects on the growth of *Escherichia coli* AHU 1041. Cultivation medium and assay were described in the previous paper.¹⁾

* Hydantoin Derivatives of S-Alkyl- and Alkenyl-L-cysteine-S-oxides (Part II)

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ous paper.¹⁾

RESULTS

1. Compounds Prepared

1.1 L-Methylthioethylhydantoin S-oxide

The sulfoxide was prepared from L-methionine in a similar manner as described in the preparation of L-5-propylthiomethylhydantoin S-oxide (L-(±)-PHSO);¹⁾ mp 132°C(dec.), $[\alpha]_D^{19} -48^\circ$ ($c=1.0$, H₂O). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: around 3200 (νNH 's of hydantoin), 1770 & 1720 (νCO 's of hydantoin), and 1020 (νSO). MS $m/e(\%)$: 190(M^+ , 0.8), 126($\text{M}^+ - \text{CH}_3\text{SOH}$, 100), 99(7.8), 64($\text{CH}_3\text{SOH}^{1+}$, 8.4), 63($\text{CH}_3\text{SOH}^{1+}$, 3.3), 56(8.2), 41(6.1). This compound gave one spot on *tlc*.

1.2 DL-5-Pentylthioethylhydantoin S-oxide.^{2,3)}

Addition of 1-pentanethiol to acrolein in the presence of triethylamine gave 3-pentylthiopropional in 64% yield; bp 105–106°C (12 mmHg). 3-Pentylthiopropional was then converted into DL-5-pentylthioethylhydantoin by the treatment of potassium cyanide and ammonium carbonate in aqueous ethanol followed by neutralization with hydrochloric acid in 84% yield; mp 98–99°C. The hydantoin was oxidized to form the sulfoxide in acetic acid solution by the addition of 30% hydrogen peroxide in 66% yield; mp 138°C(dec.). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: around 3200 (νNH 's of hydantoin), 1765 & 1725 (νCO 's of hydantoin), 1000 & 980 (νSO). MS $m/e(\%)$: 246(M^+ , 2.5), 127(18.4), 126($\text{M}^+ - \text{C}_5\text{H}_{11}\text{SOH}$, 74.0), 125(43.5), 100(32.2), 99(9.5), 56(31.8), 55(9.9), 43(C_5H_7^+ , 100), 41(23.6). This compound gave one spot on *tlc*.

1.3 dl-3-Alkylthio-2-methylpropionamide S-oxides (alkyl: ethyl and propyl)

dl-3-Alkylthio-2-methylpropionamides (alkyl: ethyl and propyl) were prepared from methacrylamide by the treatment with ethanethiol or 1-propanethiol in the presence of sodium methoxide in methanol. dl-3-Ethylthio-2-methylpropionamide: yield, about 50%; mp 43–45°C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3360 & 3180 (νNH of CONH₂), 1670 (νCO of CONH₂), 1640(shoulder), 1463, 1430, 1370. dl-2-Methyl-3-propylthiopropionamide: yield, about 50%; mp 39–41°C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3360 & 3190 (νNH of CONH₂), 1668 (νCO of CONH₂), 1640 (shoulder), 1460, 1428, 1370.

dl-3-Alkylthio-2-methylpropionamides were

then oxidized to their sulfoxides by 30% hydrogen peroxide in more than 80% yield. dl-3-Ethylthio-2-methylpropionamide S-oxide: mp 105–107°C(dec.). Anal. Found: C, 44.36; H, 7.99. Calcd. for C₆H₁₃NO₂S: C, 44.15, H, 8.03%. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3340 & 3190 (νNH of CONH), 1690 (νCO of CONH₂), 1640(shoulder), 1465, 1420, 1370, 1298, around 1020 (νSO). MS $m/e(\%)$: 164($\text{M}^+ + 1$, 1.7), 163(M^+ , 2.7), 134($\text{M}^+ - \text{C}_2\text{H}_5$, 83.3), 117(25.6), 87(25.4), 86 ($\text{M}^+ - \text{C}_2\text{H}_5\text{SO}$, 25.6), 78($\text{C}_2\text{H}_5\text{SOH}^{1+}$, 44.0), 73(23.7), 69(27.5), 63(26.8), 44(CONH_2^{1+} , 100), 43(45.8), 41(69.0), 39(24.3) 29(38.9), 27(33.7). dl-2-Methyl-3-propylthiopropionamide S-oxide: mp 88–90°C(dec.). Anal. Found: C, 47.51; H, 8.51. Calcd. for C₇H₁₅NO₂S: C, 47.33; H, 8.53%. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3344 & 3190 (νNH of CONH₂), 1680 (νCO of CONH₂), 1625, 1458, 1408, 1295, 1003 & 995 (νSO). MS $m/e(\%)$: 178($\text{M}^+ + 1$, 0.9), 177(M^+ , 1.1), 161(2.4), 160 (2.2), 134($\text{M}^+ - \text{C}_3\text{H}_7$, 47.6), 118(24.5), 117(22.3), 92($\text{C}_3\text{H}_7\text{SOH}^{1+}$, 23.4), 86($\text{M}^+ - \text{C}_3\text{H}_7\text{SO}$, 35.6), 74(29.6), 73(26.5), 69(32.9), 63(27.5), 44 (CONH_2^{1+} , 77.4), 43(C_3H_7^+ , 100), 39(39.5), 27(36.4).

2. Effects of L-5-Alkylthiomethylhydantoin S-oxides and Related Compounds on the Growth of E. coli

Effects of several sulfoxide derivatives on the growth of *E. coli* are shown in Table 1. dl-3-Alkylthio-2-methylpropionamide S-oxides (alkyl: ethyl and propyl) did not have any activity.

Further, structure-activity relationships among L-(±)-PHSO and its analogues are shown in Table 2.

DISCUSSION

It was initially considered that oxidizing ability of sulfoxide function might have the inhibitory action against *E. coli*, because the activity disappeared by addition of thiol compounds into culture medium. However, the sulfoxide compounds which do not have a hydantoin structure exhibited far less activities as shown in Table 1 and in the previous paper.¹⁾

It was therefore suggested that both sulfoxide function and hydantoin ring were required for the activity. The results shown

Table 1 Effects of several sulfoxide derivatives on the growth of *Escherichia coli* AHU 1041.

Compounds tested	Concn. mM	Growth* OD _{610nm}
dl-3-Ethylthio-2-methylpropionamide S-oxide	0.4896	0.806
dl-2-Methyl-3-propylthiopropionamide S-oxide	0.4896	0.837
L-5-Propylthiomethylhydantoin S-oxide	0.0306	0.000
	0.0153	0.121
L-5-Butylthiomethylhydantoin S-oxide	0.0306	0.032
	0.0153	0.243
Control		0.735

* The bacterium was cultured in semi-synthetic medium, pH 6.8, at 37°C for 17 hr with shaking (110 rpm). Inoculation: *ca.* 8×10^6 cells/6 ml medium.

Table 2 Structure-activity relationships among L-5-propylthiomethylhydantoin S-oxide (L-(±)-PHSO) and its analogues.

Compounds tested	Concn, µg/ml	<i>E. coli</i> growth* OD _{610nm}
L-(+)-PHSO	12.5	0
DL-5-Methyl-5-propylthiomethylhydantoin S-oxide	100	1.867
L-5-Allylthiomethylhydantoin S-oxide	12.5	0
DL-5-Allylthiomethyl-5-methylhydantoin S-oxide	100	1.726
L-5-Methylthiomethylhydantoin S-oxide	12.5	0.315
L-5-Methylthioethylhydantoin S-oxide	100	1.868
L-5-Pentylthiomethylhydantoin S-oxide	12.5	0.029
DL-5-Pentylthioethylhydantoin S-oxide	100	1.809
Control		1.914

* The bacterium was cultured in nutrient broth, pH 6.7, at 37°C for 18.5 hr with shaking (110 rpm). Inoculation: *ca.* 8×10^6 cells/6 ml medium.

in Table 2 give informations on the structure-activity relationships. DL-5-Propyl- and allylthiomethyl-5-methylhydantoin S-oxides had no comparable activity with L-(±)-PHSO. L-5-Methylthioethylhydantoin S-oxide (hydantoin derivative of L-methionine sulfoxide) and DL-5-pentylthioethylhydantoin S-oxide did not have any appreciable activity, while L-5-alkylthiomethylhydantoin S-oxides (alkyl: methyl and pentyl) did have comparable activity with L-(±)-PHSO.

The chemical structures of 5-alkyl- and alkenylthiomethylhydantoin S-oxides and far less active sulfoxide compounds are shown in Fig. 1. From these results, it was further suggested that the hydrogen atom on the 5-C of the sulfur atom and the hydantoin ring was not two (or more), but one for the exhibition of antibacterial activity. Therefore, the structure required for antibacterial activity is sum-

marized in Fig. 2.

Although the previous paper¹⁾ described that there was no significant difference in antibacterial activity between the two stereoisomers, *i.e.*, L-(+)-PHSO and L-(-)-PHSO, other stereoisomers, D-(+)-PHSO and D-(-)-PHSO, have not been prepared to determine their antibacterial activities.

It was observed that L-(±)-PHSO decomposed slowly at pH around 6 but quickly at pH around 7 in the M/10 phosphate buffer solution and generated onion-like flavor.⁶⁾ The authors presumed that the decomposition of L-(±)-PHSO might be responsible for the antibacterial activity. In the buffer solution (pH 7.0) L-(±)-PHSO decomposed completely after one hour incubation at 37°C to give 5-methylenehydantoin and propyl propanethiosulfinate. The resulting propyl propanethiosulfinate exhibited potent antibacterial activity.

(active)

5-Alkyl- and alkenylthiomethylhydantoin S-oxides

R: alkyl- and alkenyl

(far less active)

R: $\text{CH}_3(\text{CH}_2)_2$ R': H & CONH_2

(far less active)

R: $\text{CH}_3(\text{CH}_2)_2$ & $\text{CH}_2=\text{CHCH}_2$

(inactive)

R: CH_3CH_2 & $\text{CH}_3(\text{CH}_2)_2$

(far less active)

R: CH_3 & $\text{CH}_3(\text{CH}_2)_4$

Fig. 1 Chemical structures of antibacterial 5-alkyl- and alkenylthiomethylhydantoin S-oxides and far less active sulfoxide compounds.

- 1) Hydantoin ring
- 2) S-Oxide function
- 3) R'=H
- 4) n=1

Fig. 2 Structure required for antibacterial activity.

The detailed decomposition reactions of L-(±)-PHSO and its analogues in neutral aqueous solutions and the studies on mode of action will be presented in subsequent papers.

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要 約

抗菌性 L-5-Alkylthiomethylhydantoin S-oxides および関連化合物の構造と活性の関係

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抗菌(とくに抗細菌)活性を有する L-5-alkylthiomethylhydantoin S-oxides および調製した関連化合物を用いて構造-活性の関係を調べた。

ヒダントイン環をもたない S-propyl-L-cysteine-S-oxide, その N-carbamoyl 誘導体および dl-3-alkylthio-2-methylpropionamide S-oxides はほとんど活性を示さなかった。ヒダントイン環をもつものでも 5-alkyl- および alkenylthiomethyl-5-methyl 誘導体や 5-alkylthioethyl 誘導体はほとんど不活性であった。

これらの結果を総合すると, 抗細菌活性を示すために必要な構造は, (1) ヒダントイン環を有すること。 (2) スルホキシド基を有すること。 (3) ヒダントイン環の 5-C に水素を有すること。 および (4) S とヒダントイン環の間に 1 個の CH_2 基を有することである。