# Syntheses and Antimicrobial Properties of Isothiocyanate Derivatives

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**Abstract** □ Nine reaction products of isothiocyanates with cysteine, *N*-(*N*-substituted-thiocarbamoyl)cysteines, and eight reaction products of isothiocyanates with 2,3-dimercapto-1-propanol, 2,3-bis(substituted-thiocarbamoylthio)-1-propanols, were synthesized. The antimicrobial activity of these compounds was investigated.

**Keyphrases** □ Isothiocyanate derivatives—synthesized and screened for antimicrobial activity □ Cysteine, reaction products with isothiocyanates—synthesized and screened for antimicrobial activity □ 2,3-Dimercapto-1-propanol, reaction products with isothiocyanates—synthesized and screened for antimicrobial activity □ Antimicrobial activity—isothiocyanate derivatives synthesized and screened

Some naturally occurring isothiocyanates with antimicrobial activity are known (1–4), and their biological activity is based on the isothiocyanate group (—N=C=S) which reacts with the functional group of the peptide (5). However, little has been reported on the antimicrobial activity of the derivatives of isothiocyanates (6).

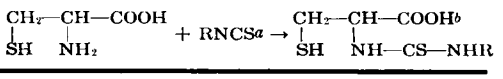
This paper is concerned with the antimicrobial properties of chemically related compounds in which the isothiocyanate group is bound to aliphatic and aromatic radicals.

## DISCUSSION

The experimental compounds were synthesized in this laboratory. Tables I and II show the characteristics of these new compounds.

All of the following strains of microorganisms, except *Lactobacillus hiochi*, were grown on glucose–bouillon liquid medium (pH

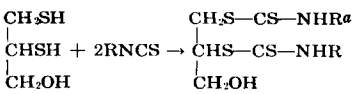
**Table I—Characteristics of *N*-(*N*-Substituted-thiocarbamoyl)cysteines**




| Compound | R | Melting[c] Point | Formula | Analysis, % Calc. | Analysis, % Found | Solubility in Water, mg/ml |
|---|---|---|---|---|---|---|
| 1 | $CH_3CH_2$ | 185° dec. | $C_6H_{12}N_2O_2S_2$ | C 34.62<br>H 5.81<br>N 13.46<br>S 30.75 | 34.67<br>5.99<br>13.56<br>30.97 | 1.0 |
| 2 | $CH_3(CH_2)_2$ | 180° dec. | $C_7H_{14}N_2O_2S_2$ | C 37.84<br>H 6.35<br>N 12.61<br>S 28.80 | 38.18<br>6.32<br>12.83<br>28.05 | 0.5 |
| 3 | $(CH_3)_2CH$ | 205° dec. | $C_7H_{14}N_2O_2S_2$ | C 37.84<br>H 6.35<br>N 12.61<br>S 28.80 | 37.52<br>6.23<br>12.66<br>28.71 | 0.5 |
| 4 | $CH_3(CH_2)_3$ | 200° dec. | $C_8H_{16}N_2O_2S_2$ | C 40.67<br>H 6.83<br>N 11.86<br>S 27.10 | 40.43<br>6.80<br>11.81<br>26.62 | 0.25 |
| 5 | $(CH_3)_2CHCH_2$ | 190° dec. | $C_8H_{16}N_2O_2S_2$ | C 40.67<br>H 6.83<br>N 11.86<br>S 27.10 | 40.60<br>6.83<br>11.86<br>27.09 | 1.0 |
| 6 | $C_6H_5$ | 160° dec. | $C_{10}H_{12}N_2O_2S_2$ | C 46.88<br>H 4.72<br>N 10.93<br>S 25.00 | 46.79<br>4.42<br>10.72<br>24.97 | 0.5 |
| 7 | $C_6H_5CH_2$ | 190° dec. | $C_{11}H_{14}N_2O_2S_2$ | C 48.89<br>H 5.22<br>N 10.37<br>S 23.02 | 48.61<br>5.27<br>10.34<br>23.55 | 0.25 |
| 8 | $C_6H_5(CH_2)_2$ | 191° dec. | $C_{12}H_{16}N_2O_2S_2$ | C 50.70<br>H 5.67<br>N 9.86<br>S 22.76 | 50.17<br>5.67<br>9.95<br>22.41 | 0.5 |
| 9 | $p$-$ClC_6H_4$ | 166–167.5° | $C_{10}H_{11}ClN_2O_2S_2$ | C 41.32<br>H 3.78<br>N 9.63<br>S 22.06 | 41.10<br>3.80<br>9.74<br>22.16 | 0.1 |

[a] Obtained commercially or synthesized from corresponding primary amines by the method of Mumm and Richter (8). [b] *N*-(*N*-Allylthiocarbamoyl)cysteine (7) is the sole known compound of this type. These compounds gave a red color with the nitroprusside test, demonstrating the free sulfhydryl group. In the IR spectrum of these compounds, a weak band near 2600 $cm^{-1}$ indicated the presence of a sulfhydryl group. [c] Melting points are uncorrected.

**Table II**—Characteristics of 2,3-Bis[alkyl (alkenyl, aryl, and aralkyl)-thiocarbamoylthio]-1-propanols



| Compound | R | Melting[b] Point | Formula | Analysis, % | | Solubility in Water, mg/ml |
|---|---|---|---|---|---|---|
| | | | | Calc. | Found | |
| 10 | $CH_3$ | 125° dec. | $C_7H_{14}N_2OS_4$ | C 31.11<br>H 5.19<br>N 10.39<br>S 47.40 | 31.09<br>5.30<br>9.97<br>47.47 | 1.0 |
| 11 | $CH_3(CH_2)_2$ | 113–114° | $C_{11}H_{22}N_2OS_4$ | C 40.45<br>H 6.79<br>N 8.58<br>S 39.27 | 40.47<br>6.70<br>8.32<br>38.94 | 0.1 |
| 12 | $(CH_3)_2CHCH_2$ | 102–103° | $C_{13}H_{26}N_2OS_4$ | C 44.07<br>H 7.34<br>N 7.90<br>S 36.72 | 43.88<br>7.34<br>7.36<br>36.44 | 0.1 |
| 13 | $(CH_3)_2CH(CH_2)_2$ | 131–132° | $C_{15}H_{30}N_2OS_4$ | C 47.08<br>H 7.91<br>N 7.33<br>S 33.51 | 46.92<br>7.95<br>7.42<br>33.06 | 0.1 |
| 14 | $CH_2CHCH_2$ | 119.5–120° | $C_{11}H_{18}N_2OS_4$ | C 40.99<br>H 5.63<br>N 8.69<br>S 39.72 | 41.03<br>5.31<br>8.61<br>39.50 | 0.03 |
| 15 | $C_6H_5CH_2$ | 140.5–141° | $C_{19}H_{22}N_2OS_4$ | C 54.01<br>H 5.20<br>N 6.63<br>S 30.35 | 53.83<br>5.20<br>6.71<br>30.66 | 0.1 |
| 16 | $C_6H_5(CH_2)_2$ | 139–139.5° | $C_{21}H_{26}N_2OS_4$ | C 55.99<br>H 5.77<br>N 6.22<br>S 28.46 | 55.91<br>5.63<br>6.24<br>28.86 | 0.1 |
| 17 | $p$-$ClC_6H_4$ | 137–138° | $C_{17}H_{16}Cl_2N_2OS_4$ | C 44.06<br>H 3.45<br>N 6.04<br>S 27.67 | 44.10<br>3.39<br>6.31<br>27.61 | 0.03 |

[a] These compounds were negative to the nitroprusside test. In the IR spectrum, no peak occurred near 2600 $cm^{-1}$, indicating the absence of a sulfhydryl group. [b] Melting points are uncorrected.

**Table III**—Culture Conditions for Tested Microorganisms

| Strain | Temperature | Days |
|---|---|---|
| *Lactobacillus hiochi* No. 2[a] | 30° | 10 |
| *Streptococcus aureus* FDA 209P | 37° | 2 |
| *Pseudomonas aeruginosa* IFO 3080 | 37° | 2 |
| *Saccharomyces cerevisiae* IFO 0599 | 25° | 3 |
| *Aspergillus niger* ATCC 9642 | 25° | 3 |

[a] Isolated in the Research Laboratory, Takeda Pharmaceutical Industries Ltd. of Japan.

6.0). *L. hiochi* No. 2 was grown on a liquid medium (pH 4.5) which contained 1% glucose, 1% yeast extract, 1% polypeptone, and 13% ethanol.

Table III shows tested microorganisms and culture conditions. The antimicrobial activity of a solution or suspension of these compounds is shown as the minimum inhibitory concentration (MIC) (micrograms per milliliter) in Table IV.

Compounds 7, 8, 9, 14, and 17 exhibited the highest antimicrobial activity, especially against yeast and mold, while Compounds 7 and 8 showed the highest potency for all tested microorganisms. However, these results were unsatisfactory (Table IV), because of the low solubilities of the compounds in water. Therefore, nonionic

**Table IV**—Effects of Derivatives of Isothiocyanates on Microorganism Growth

| Compound | MIC | | | | |
|---|---|---|---|---|---|
| | *Lactobacillus hiochi* | *Streptococcus aureus* | *Pseudomonas aeruginosa* | *Saccharomyces cerevisiae* | *Aspergillus niger* |
| 1 | 1000 | 1000 | 500 | 500 | 500 |
| 2 | 1000 | 1000 | 1000 | >1000 | 1000 |
| 3 | 1000 | >1000 | >1000 | >1000 | 1000 |
| 4 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 5 | 1000 | >1000 | >1000 | >1000 | >1000 |
| 6 | 1000 | 1000 | 1000 | 500 | 1000 |
| 7 | 250 | 250 | 250 | 30 | 7.8 |
| 8 | >1000 | 250 | 1000 | 30 | 15.6 |
| 9 | >1000 | >1000 | >1000 | >1000 | 1000 |
| 10 | 1000 | >1000 | >1000 | >1000 | 130 |
| 11 | 1000 | >1000 | >1000 | >1000 | >1000 |
| 12 | 1000 | >1000 | >1000 | >1000 | >1000 |
| 13 | 1000 | >1000 | >1000 | >1000 | >1000 |
| 14 | 1000 | >1000 | 250 | 130 | 60 |
| 15 | >1000 | >1000 | >1000 | 500 | 500 |
| 16 | 1000 | 1000 | 1000 | 1000 | 1000 |
| 17 | 1000 | 500 | 1000 | 62.5 | 31.3 |

Table V—Effects of Compounds 7 and 8, in which Sorbitan Fatty Acid Esters were Added, on Microorganism Growth

| Sorbitan Ester | MIC, μg/ml | | | |
|---|---|---|---|---|
| | *Streptococcus aureus* | | *Aspergillus niger* | |
| | Compound 7 | Compound 8 | Compound 7 | Compound 8 |
| None (control) | 250 | 250 | 7.8 | 15.6 |
| Monolaurate | <7.8 | <7.8 | 7.8 | 15.6 |
| Monopalmitate | 125 | 125 | 7.8 | 15.6 |
| Monostearate | 250 | 125 | 7.8 | 15.6 |
| Tristearate | 62.5 | 125 | 15.6 | 15.6 |
| Monooleate | 125 | 250 | 7.8 | 15.6 |
| Trioleate | 62.5 | 125 | 7.8 | 31.3 |

surfactants were employed as solubilizing agents.

Table V shows the MIC of surfactants plus Compounds 7 and 8. The surfactants used had no antimicrobial activity. Only for *Streptococcus aureus* were the compounds with added surfactants more effective than those compounds alone. The sorbitan monolaurate suspensions of the compounds had good MIC values, below 7.8 μg/ml. Since the MIC's of Compounds 7 and 8 alone were 250 μg/ml, the compounds with the sorbitan fatty acid esters may have enhanced activities.

## EXPERIMENTAL

***N*-(*N*-Substituted-thiocarbamoyl)cysteines**—These compounds were obtained by the method of Todrick and Walker (7).

**2,3-Bis(substituted-thiocarbamoylthio)-1-propanols**—2,3-Dimercapto-1-propanol was dissolved in about 30 parts of water. Two equimolar quantities of the corresponding isothiocyanate were added and gentle shaking was maintained for 3 hr, whereupon a yellow granular solid started to separate. The mixture was stored overnight and filtered, and the solid was recrystallized from ethanol.

In the preparation of 2,3-bis[aryl(aralkyl)-thiocarbamoylthio]-1-propanol, the same procedure was employed except that dimethyl sulfoxide was used instead of water.

**Antimicrobial Activity**—The antimicrobial activity was determined by determining the MIC (micrograms per milliliter) macroscopically or microscopically using the normal dilution method.

The concentration of each surfactant was 1 mg/ml of liquid medium (pH 6.0).

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# Transport Mechanisms of β-Lactam Antibiotics across Everted Rat Gut


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**Abstract** □ Penicillins and cephalosporins, both monobasic and amphoteric, were tested for active transfer by measurement of the ratio of drug between serosal and mucosal solutions in a standard everted rat gut preparation. In no instance did any of these β-lactam antibiotics demonstrate active transport. Parallel experiments with glucose, a compound known to be actively transported in this preparation, always resulted in a positive ratio. A linear relationship between rate of transfer and concentration for two compounds, sodium ampicillin and sodium nafcillin, provided further evidence for passive transport. Although sodium nafcillin showed an apparent saturable process between 20 and 40 mg/ml, surface tension measurements showed that the CMC for sodium nafcillin lies within this range.



**Keyphrases** □ Antibiotics, β-lactam—transport mechanisms across everted rat gut □ β-Lactam antibiotics—transport mechanisms across everted rat gut □ Cephalosporins—transport mechanisms across everted rat gut □ Transport mechanisms—β-lactam antibiotics across everted rat gut


Since penicillins are known to be actively secreted into the renal tubules (1–3) and actively transported from the cerebrospinal fluid to the blood (4), the possibility that there may also be a specialized process in the intestinal absorption of β-lactam antibiotics must be considered. Earlier *in vitro* studies with an everted gut preparation indicated that the transport of phenoxymethyl penicillin and phenoxybenzyl penicillin (phenbenicillin) is a saturable process (5, 6), although additional studies did not produce evidence of active transport (6).

In the present work, the everted rat intestinal sac was employed to investigate the transport mechanism of several penicillins and cephalosporins, in-