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REACTION OF AZIRIDINECARBOXYLIC ACIDS WITH THIOLS IN AQUEOUS SOLUTION. THE FORMATION OF β-AMINO ACID

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Abstract: Enantiomers of 3-methyl-2-aziridinecarboxylic acids (1-d and 1-l) and 2-aziridinecarboxylic acids (2-d and 2-l) reacted easily with thiophenol, cysteine and glutathione in aqueous solution or in sodium phosphate buffer solution at room temperature and gave predominantly β-amino acid derivatives with sulfur substituents at their α-position.

From 1-d and thiophenol, (2*S*, 3*R*)-3-amino-2-phenylthiobutanoic acid (3-d) was produced predominantly. In order to confirm the structure, 3-d was converted to (3*S*, 4*R*)-3-phenylthio-4-methylazetidin-2-one (5) using the Ohno reaction. The configurations of 3-d and 5 were determined by X-ray diffraction and ^{13}C NMR spectrum analysis, respectively. We concluded that the ring-opening reaction of unactivated aziridinecarboxylic acids with thiols in aqueous solution occurred predominantly on C-2 of the aziridine ring with inversion of the configuration at this position. The reaction offers a good route for stereoselective synthesis of peptides or β-lactam derivatives.

The ring-opening reaction of alkyl aziridinecarboxylates, which are activated by an electronegative substituent on the ring nitrogen,[1] with nucleophilic reagents in organic solvents was studied extensively. Usually, α-amino acid derivatives formed as the predominant product.[2]

Recently, in an effort to elucidate the mechanism of detoxification of aziridines in living cells, we studied the metabolic reaction of aziridinecarboxylic acid in living hepatic cells isolated from rats and observed that the aziridine ring easily reacted with the intracellular SH compound glutathione to give α-glutathio-β-amino acid derivatives.[3] Thus, we envisaged that the aziridinecarboxylic acid, although an unactivated compound,[1] may react with SH-nucleophile in aqueous solution at room temperature to give stereospecifically unusual β-amino acids. Here we report that thiophenol, cysteine and glutathione act as suitable nucleophiles capable of opening of aziridine rings to give optically pure β-amino acid derivatives in aqueous or sodium phosphate buffer solution.[4] The starting compounds[5] were (2*R*, 3*R*)- and (2*S*, 3*S*)-3-methyl-2-aziridinecarboxylic acid (1-d and 1-l), which were obtained from d- or l-threonine, and (2*R*)- and (2*S*)-2-aziridinecarboxylic acid (2-d and 2-l), which were obtained from d- or l-serine, according to the literature procedure.[6] Their absolute configurations are shown in Scheme 1.

R : CH_3 1-d 1-l
R : H 2-d 2-l

Scheme 1

RESULTS

Reaction of 3-methyl-2-aziridinecarboxylic acid (1-d or 1-l) with thiophenol. --- The reaction of 1-d and thiophenol in 0.2 M sodium phosphate buffer solution (pH 8.0) at room temperature gave 57% of 3-amino-2-phenylthiobutanoic acid (3-d) and 25% of 2-amino-3-phenylthiobutanoic acid (4-d). The total yield of both amino acids exceeded about 80%. Although the 3-d / 4-d ratio was almost independent of the acidity of the reaction medium as shown in Table 1, it depended greatly on the solvent used in the reaction and β-amino acid formation increased in going from aprotic to protic solent as shown in Tables 2 and 3.

Table 1. Effect of pH on Product Ratio of 3-d and 4-d in Reaction of 3-Methyl-2-aziridinecarboxylic Acid (1-l or 1-d) with Thiophenol

Phosphate buffer[a] pH	Total yield[b] 3-d + 4-d (%)	Product ratio 3-d / 4-d
6.0	75	2.1
8.0	82	2.3
9.0	83	2.2
10.0	77	2.1

[a] 0.2 M Sodium phosphate buffer was used. [b] The reaction was carried out at room temperature for 20 h and the yields of the products were determined by the direct HPLC analyses of the reaction mixture.

Table 2. Solvent Effect on Product Ratio of 3-d and 4-d Formed from Reaction of 1-d with Thiophenol

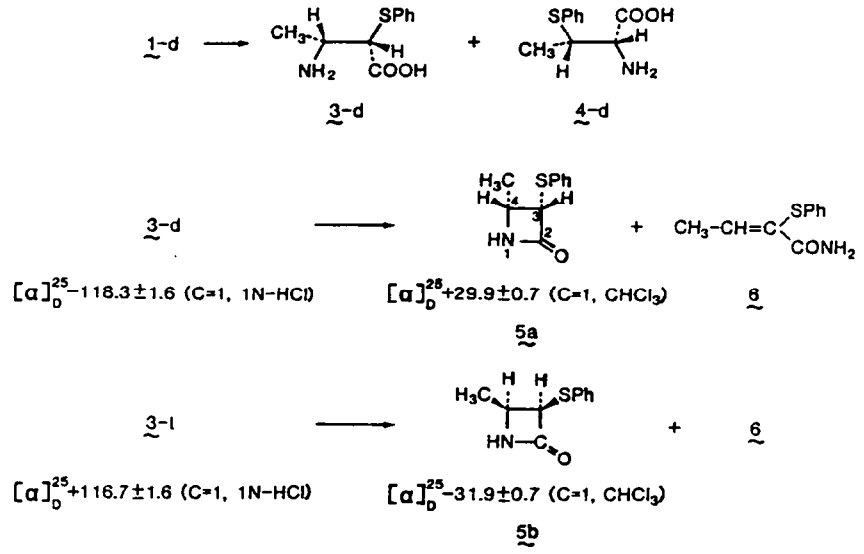
Solvent (v/v)	Total yield 3-d + 4-d (%)	Product ratio 3-d / 4-d
Phosphate buffer (pH 8)	90.1	2.3
H_2O	91.4	2.35
95% EtOH-H_2O	94.2	1.65
95% DMF-H_2O	81.3	1.05
95% CH_3CN-H_2O	90.4	0.95

Table 3. Effect of Water in DMF on Product Ratio 3-d / 4-d Observed in Reaction of 3-Methyl-2-aziridinecarboxylic Acid (1-d) with Thiophenol

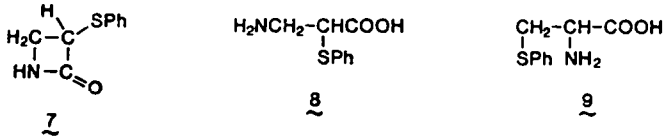
Solvent[a] (v/v)	Total yield 3-d + 4-d (%)	Product ratio 3-d / 4-d
H_2O	91.4	2.35
50% DMF-H_2O	77.6	2.18
70% DMF-H_2O	89.3	1.89
80% DMF-H_2O	92.1	1.71
90% DMF-H_2O	88.5	1.60
95% DMF-H_2O	81.3	1.05
99% DMF-H_2O	80.0	0.50

[a] Water (no buffer) was used.

The absolute configuration of 3-d was determined as 2-*S*, 3-*R* by X-ray diffraction analysis,[7] and 3-d was converted to (3*S*, 4*R*)-3-phenylthio-4-methylazetidin-2-one (**5a**) using the Ohno reaction.[8] The NMR study of the β-lactam **5a** showed a cis relationship between the 3-phenylthio and 4-methyl substituents. On the other hand, the β-lactam **5b** which was derived from 3-l by the above procedure has the absolute value of the optical rotation coincided with that of **5a**. In preparing **5a** or **5b**, we observed the formation of 2-phenylthio-2-butenamide (**6**) as the by-product.[9] The yield of **6** increased when the reaction was carried out at lower temperature.



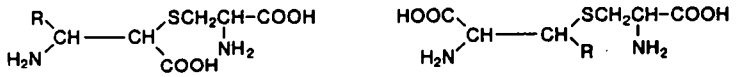
Reaction of 2-aziridinecarboxylic acid (2-d and 2-l) with thiophenol. --- Each enantiomer of **2** and thiophenol gave an equimolar mixture of 3-amino-2-phenylthiopropanoic acid (**8**) and 2-amino-3-phenylthiopropanoic acid (**9**) in 80% yield under reaction conditions similar to those described above. The β-lactam **7** formation reaction for the compound **8** proceeded in a manner similar to that of **3**-d to give the same results.



Reaction of aziridinecarboxylic acids (1 and 2) with cysteine. --- d- or l-Cysteine reacted easily with each enantiomer of **1** or **2** under mild reaction conditions and formed predominantly β-amino acid with a cysteinyl group at the α-position.

From 1-d and l-cysteine, 2-amino-3-[(1*S*,2*R*)-(2-amino-1-carboxypropyl)thio]propanoic acid (one stereoisomer of **10**) and methyllanthionine (one stereoisomer of **11**) were produced in 63% and 17% yield, respectively. The absolute configuration of the former was deduced from ^{13}C nmr and the reaction mode of 1-d and thiophenol. The structure of the later was determined by comparison with an authentic sample of methyllanthionine which was supplied by Osaka Univ.

Under similar reaction conditions, 1-l or each optical isomer of **2** gave **10** and **11** or **12** and **13** respectively. The yields are shown in Table 4.



10 (R : CH_3)
12 (R : H)

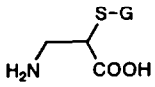
11 (R : CH_3)
13 (R : H)

Table 4. Yield and Product Ratio of Reaction of 3-Methyl-2-aziridinecarboxylic Acid (1-d or 1-l) or 2-Aziridinecarboxylic Acid (2-d or 2-l) with d- or l-Cysteine[a]

Configuration of 1 or 2	Configuration of cysteine	Total yield (%)	Product ratio 10 / 11 from 1 or 12 / 13 from 2
1-l	l	94.3	4.3
1-l	d	76.4	2.6
1-d	l	80.4	3.6
1-d	d	88.0	4.1
2-l	l	96.8	3.0
2-l	d	97.5	2.6
2-d	l	95.3	2.5
2-d	d	93.7	4.2

[a] Reaction was carried out in 0.2 M phosphate buffer (pH 8.0) at 37°C for 20 h and the yields were determined directly based on HPLC of the reaction solution.

Reaction of aziridinecarboxylic acid (1 and 2) with glutathione. --- In buffer solution, the reaction proceeded smoothly at room temperature and β-amino acid formed predominantly. Compound 2 gave 80% or more of *S*-(2-amino-1-carboxyethyl)glutathione (14) with small amount of *S*-(2-amino-2-carboxyethyl)glutathione (15), while 1 formed only S-(2-amino-1-carboxypropyl)glutathione (16). The structures of these products were determined by NMR and by their conversion to cysteine derivatives 10, 12 or 13 by hydrolysis.



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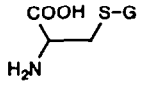

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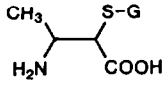

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Table 5. Products and Yield for the Reaction of Aziridinecarboxylic Acid (1-l, d or 2-l, d) and Glutathione

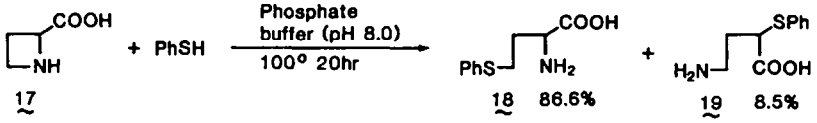
Aziridinecarboxylic acid, configuration	Total yield[a] (%)	Product ratio
1-l	86.3	only 16
1-d	85.0	only 16
2-l	79.2	14/15 5.5
2-d	55.6	14/15 4.9

[a] The yields of the glutathione conjugates were determined by HPLC after their conversion to cysteine derivatives (10, 12 or 13) by 6 N HCl at 110°C.

Reaction of ethylenimine with l-cysteine or glutathione. --- In sodium phosphate buffer solution, cysteine or glutathione reacted very rapidly with ethylenimine and the product was 2-amino-3-[(2-aminoethyl)thio]-propanoic acid from cysteine, or S-(2-aminoethyl)glutathione from glutathione in 92% or 91% yield, respectively. The authentic sample of the former was commercially available[10] and the structure of the latter was determined by its conversion to the former by acid hydrolysis.

Reaction of 2-azetidinecarboxylic acid (17) with thiophenol. --- In order to compare the reactivity of aziridine derivatives with that of four-membered analogues, we studied the reaction of azetidinecarboxylic acid (17)[11] with thiophenol.

The reaction was carried out under ordinary conditions but the temperature was modified to 100°C.[12] The product was mainly 2-amino-4-phenylthiobutanoic acid (18) and the minor product coincided with an authentic sample of 4-amino-2-thiophenylbutanoic acid (19), which was prepared independently from 2-pyrrolidinone according to the method of Zoretic et al.[13]



17 18 86.6% 19 8.5%

DISCUSSION

Reported here is the first observation of the reaction of unactivated[1] aziridinecarboxylic acids with thiols in aqueous solution[14] and β-amino acid formation, revealing a new characteristic of aziridine derivatives.

The reactivity of the aziridine ring with nucleophiles could be determined mainly by two factors. The first is the effect of the strong electronegativity of the nitrogen atom reinforced by the large ring strain of the three-membered ring.[15] Azetizine, which has a less strained four-membered ring system, is much less active with thiols than aziridines, and strain-free pyrrolidine or proline does not react with thiophenol under ordinary reaction conditions. The second factor is that, when the compound reacts with a nucleophile, the reactivity is also enhanced by a ring nitrogen substituent capable of stabilizing a negative charge developing on the aziridine nitrogen in the transition state. Therefore, most ring-opening reactions of aziridines by nucleophiles have been done using such activated aziridine derivatives.[2] Here, the positive charge that developed in the transition state of C-N bond rupture of the aziridine ring should occur more predominantly on the β-carbon atom than at the α-position which is adjacent to the electronegative substituent. Therefore, the reaction of activated aziridinecarboxylic acids and nucleophilic reagents in organic solvents takes place at the β-carbon, giving α-amino acid derivative.[2] In our experiments, the reaction of thiophenol with 1-d in CH_3CN or DMF also gave predominantly the α-amino acid derivative 4-d as shown in Table 2 or 3. The acidity of the SH group of the reagents should be enough for the participation on the nitrogen to form the activated aziridine.[16] The four-membered cyclic amine displayed a similar tendency.

In aqueous solution however, the reaction of thiols with aziridinecarboxylic acids predominantly occurred at the C-2 position of the aziridine ring to form β-amino acid derivatives, as shown in Tables 2 and 3. And this tendency was always observed in the series from thiophenyl, cysteine to glutathione, regardless of the bulkiness of the molecules, as shown in Tables 4 and 5.

In aqueous solution, solvation by water molecule would strongly prevent the participation of thiols on the weakly basic nitrogen of the aziridine ring.[17] Consequently, the reaction of the nucleophilic reagents would be forced to proceed at the C-2 position of the aziridine ring due to the I-effect of the COOH group. Simple calculation of the electronic states of (LU + 1) MO of aziridinecarboxylic acid by the MNDO method of Dewar[18] indicated little advantage for C-2 attack by the nucleophilic reagent.

In conclusion, we found the new method for the stereospecific conversion of serine and threonine into the unusual β-amino acids.

EXPERIMENTAL

All melting points were taken with a Yanagimoto melting point apparatus and are uncorrected. H-NMR were recorded on a Varian Associates EM-390 or XL-200 spectrometer and are reported in ppm from internal or external tetramethylsilane on a delta scale. Column chromatography was performed over Mitsubishi Kasei Diaion HP-20 (50-100 mesh) or Merck silica gel (70-230 mesh). HPLC was performed over Toyo Soda Co. TSK-GEL SP-2SW (0.4 x 25 cm) or TSK-GEL DEAE-2SW (0.4 x 25 cm).

All chiral aziridinecarboxylic acids were obtained as described in literature.[6] The Li salts of aziridinecarboxylic acids were used.

Reaction of 3-methyl-2-aziridinecarboxylic acid (1-d or 1-l) with thiophenol. To a vigorously stirred solution of 600 mg of 1-d in 20 ml of 0.2 M sodium phosphate buffer (pH 8.0) was added 900 mg of

thiophenol at room temperature for 20 h. The solvent was removed in vacuo until a white precipitate appeared. The resulting slurry was desalted with Dowex 50W × 8, giving 980 mg (80%) of solid which contained 64% of (2*S*, 3*R*)-3-amino-2-phenylthiobutanic acid (3-d) and 36% of 2-amino-3-phenylthiobutanoic acid (4-d) by HPLC. Both were separated by column chromatography of Diaion HP-20 with 50% aqueous methanol. As the first fraction, we obtained 443 mg of 3-d as prisms from aqueous ethanol. Mp (dec) 210°C; ^{1}H-NMR (D_2O + DCl) δ 1.90 (d, 3H, CH_3), 4.22 (m, 1H, NCHC), 4.45 (d, 1H, COCHS), 8.00 (m, 5H, Ph); ^{13}C-NMR (D_2O + DCl) δ 173.2, 134.8, 130.9, 130.6, 130.5, 55.0, 48.4, 16.8. Anal. Calcd for $C_{10}H_{13}NO_2S$: C, 56.85; H, 6.20; N, 6.63. Found: C, 56.68; H, 6.16; N, 6.61. $[\alpha]^{25}_D$ −118.3 ± 1.6 (c = 1.1 N HCl). The second fraction was recrystallized from H_2O, giving 250 mg of 4-d as white crystals: Mp (dec) 210°C; ^{1}H-NMR (D_2O + DCl) δ 1.97 (d, 3H, CH_3), 4.50 (m, 1H, SCHC), 4.76 (d, 1H, COCHN), 7.97 (m, 5H, Ph); ^{13}C-NMR (D_2O + DCl) δ 170.4, 134.3, 132.2, 130.2, 129.7, 57.9, 44.9, 19.0. Anal. Calcd for $C_{10}H_{13}NO_2S$: C, 56.85; H, 6.20; N, 6.63. Found: C, 56.90; H, 6.17; N, 6.46.

The reaction of l-l with thiophenol was carried out by the same procedure described above. The yield of the mixture of 3-l and 4-l was 81.1% and the ratio was 2:1. 3-l was separated by HPLC, mp (dec) 210°C; ^{1}H-NMR was the same 3-d. Anal. Found: C, 56.90; H, 6.17; N, 6.46. $[\alpha]^{25}_D$ +116.7 ± 1.6 (c = 1, 1 N HCl). 3-l was considered as antipode of 3-d.

Reaction of d- or l-2-aziridinecarboxylic acid (2-d or 2-l) with thiophenol. The reaction procedure was the same as above. From 2-d, the mixture of (2*S*)-3-amino-2-phenylthiopropanoic acid (8-d) and 2-amino-3-phenylthiopropanoic acid (9-d) was obtained. The absolute configuration of 8-d was estimated from the similarity to 3-d formation. The product ratio of 8-d and 9-d was 1:1 and the total yield was 80.0%. 8-d was purified using HPLC followed by freezedrying. ^{1}H-NMR (D_2O + DCl) δ 3.85 (m, 2H, CH_2N), 4.65 (t, H, SCHCO), 8.05 (m, 5H, Ph). Anal. Calcd for $C_9H_{11}NO_2S$: C, 54.80; H, 5.62; N, 7.10. Found: C, 54.81; H, 5.55; N, 7.02. For 9-d, ^{1}H-NMR (D_2O + DCl) δ 4.05 (m, 2H, CH_2S), 4.85 (m, H, NCHCO), 8.00 (m, 5H, Ph). Anal. Found: C, 54.71; H, 5.53; N, 6.93.

From 2-l, the yield of the mixture of 8-l and 9-l was 76.6% (8/9 = 1/1). For 8-l, ^{1}H-NMR was the same as that of 8-d; ^{13}C-NMR (D_2O + DCl) δ 173.1, 135.7, 130.9, 130.5, 129.5, 48.0, 39.9. Anal. Calcd was the same as that of 8-d. Found: C, 54.50; H, 5.57; N, 7.04. For 9-l, ^{1}H-NMR was the same as that of 9-d; ^{13}C-NMR (D_2O + DCl) δ 170.7, 132.9, 132.3, 129.0, 52.8, 35.0. Anal. Found: C, 54.61; H, 5.59; N, 7.02.

Preparation of (3*S*, 4*R*)-3-phenylthio-4-methylazetidin-2-one (5-a). To a suspended solution of 200 mg of 3-d in 80 ml of acetonitrile was added 640 mg of triphenylphosphine and 520 mg of dipyridyldisulfide and the mixture was heated in a sealed tube at 120°C for 30 min. The product was purified by column chromatography using silica gel with dichloromethane containing 5% of ether and recrystallized from cyclohexane to afford 80 mg of 5-a as colorless crystals. The yield was 86.6%, mp 109-110°C; ^{1}H-NMR ($CDCl_3$) δ 1.40 (d, 3H, CH_3), 4.11 (m, j = 5, 6.2 Hz, 1H, CHN), 4.61 (dd, j = 5, 1.7 Hz, 1H, CHS), 6.3 (brs, 1H, NH), 7.22-7.44 (m, 5H, ArH); ^{13}C-NMR ($CDCl_3$) δ 166.8, 134.9, 129.7, 129.1, 126.8, 57.6, 49.6, 17.1; IR ($CHCl_3$) 1760 cm^{-1}; $[\alpha]^{25}_D$ +29.9 ± 0.7 (c = 1, $CHCl_3$). Anal. Calcd for 5, $C_{10}H_{11}NOS$: C, 62.15; H, 5.74; N, 7.25. Found: C, 62.06; H, 5.74; N, 7.25.

When we used 3-l as starting material, optical isomer 5-b was obtained. All of the physical data were essentially the same as those for 5a except $[\alpha]^{25}_D$ −31.9 ± 0.7 (c = 1, $CHCl_3$) for 5b. Under the same procedure, each optical isomer of 3-phenylthio-azetidine-2-one (7) were obtained from 8.

The formation of 2-phenylthio-2-butenamide (6)[6] was also observed. Mp 108-109°C; ^{1}H-NMR ($CDCl_3$) δ 2.07 (d, 3H, CH_3), 7.22 (m, 5H, Ph), 7.75 (q, H, HC=C); IR ($CHCl_3$) 1675 cm^{-1}. Anal. Calcd for 6, $C_{10}H_{11}NOS$: C, 62.15; H, 5.74; N, 7.25. Found: C, 61.91; H, 5.71; N, 7.25.

Reaction of 2-aziridinecarboxylic acid (2 and l) with cysteine. In the solution of 1 mM of 2-aziridinecarboxylic acid (2-d) in 0.2 M phosphate buffer (pH 8.0), 40 mM of l-cysteine was added at 37°C for 20 h. The products, (2*S*)-2-amino-3-[(2-amino-1-carboxyethyl)-thio]propanoic acid (12) and lanthionine (13), were purified using preparative HPLC followed by freezedrying and the structures were studied using mass, ^{1}H-NMR and ^{13}C-NMR spectra. For 12, MH+ 209, ^{1}H-NMR (D_2O) δ 2.5 (m, 2H, CH_2S), 2.7 (m, 2H, CH_2N), 2.96 (t, 1H, CH), 3.3 (m, 1H, CH), ^{13}C-NMR (D_2O) δ 176.5, 173.5, 54.8, 48.3, 41.4, 33.6. Lanthionine (13) matched an authentic sample which was supplied by Osaka Univ. The yield of 12 was 72.6% and 13 was 24.2%, which were analyzed by HPLC.

The reaction of 3-methyl-2-aziridinecarboxylic acid (1-d) with cysteine was carried out under similar conditions. The products obtained from 1-l and l-cysteine were a 81.1/18.9 mixture of 2-amino-3-[(1*S*,2*R*)-(2-amino-l-carboxypropyl)thio]propanoic acid (**10**) and methyllanthionine (**11**). The yield was 94.3%. For **10**, ^{1}H-NMR (D_2O) δ 1.86 (d, 3H, CH_3), 3.7 (m, 2H, SCH_2) 3.95 (d, H, SCHCO), 4.10 (q, H, NCH), 4.45 (m, H, NCHCO).

Reaction of 2-aziridinecarboxylic acid with glutathione (GSH). The solution of 1 mM of 2-d and 10 mM of GSH in 0.2 M phosphate buffer (pH 8.0) was allowed to react at 30°C for 20 h. The products, a mixture of S-(2-amino-1-carboxyethyl)glutathione (**14**-d) and [(2*R*)-2-amino-2-carboxyethyl]glutathione (**15**-d) were hydrolyzed using 6 N HCl for 24 h at 110°C to 67% of **12** and 12.2% of **13** and they were analyzed by HPLC. The products were purified using preparative HPLC and freezedrying and the structures were studied using mass-, ^{1}H-, ^{13}C-NMR spectra. For **14**-d, MH+ 395, ^{1}H-NMR (D_2O) δ 1.60 (m, 2H, CH_2), 1.93 (m, 2H, CH_2), 2.5 (m, 2H, CH_2S), 2.75 (m, 2H, CH_2N), 3.17 (m, 1H, CH), 3.4 (m, 1H, CH), 3.4 (s, 2H, CH_2), 4.0 (m, 1H, CH), ^{13}C-NMR (D_2O) δ 175.2, 173.8, 173.7, 173.0, 172.5, 53.7, 53.2, 45.2, 42.0, 40.2, 33.2, 31.8, 26.3.

The reaction of 3-methyl-2-aziridinecarboxylic acid 1-d or 1-l with glutathione was carried out under the same condition with aziridinecarboxylic acid **2** described above and the product was isolated and purified using HPLC. The structure of the product was determined as *S*-(2-amino-1-carboxypropyl)glutathione (**16**) by ^{1}H-NMR and by conversion it to cysteine derivative **10** by hydrolysis. In the reaction mixture, the formation of *S*-[2-amino-2-carboxypropyl]glutathione which are methyl analogue of **15** was not observed. The exclusive formation of **16** indicated well that the reaction of glutathione occurred only on the C-2 position of the aziridine ring of **1**.

Reaction of ethylenimine with l-cysteine. To a solution of 355 mg of ethylenimine in 0.2 M phosphate buffer (pH 8.0) was added 1.0 g of l-cysteine at 0°C. The reaction was exothermic. The product was purified by column chromatography on Dowex 50W × 8 with aqueous ammonia and coincided with an authentic sample of 2-amino-3-[(2-aminoethyl)thio]-propanic acid purchased commercially.

Reaction of 2-azetidinecarboxylic acid (17) with thiophenol. The reaction of **17** and thiophenol was carried out under ordinary conditions at 100°C for 20 h. The product, 2-amino-4-phenylthiobutanoic acid (**18**), was recrystallized from H_2O: mp (dec) 240°C: M+ 211: ^{1}H-NMR (D_2O + DCl) δ 2.70 (m, 2H, CCH_2C), 3.61 (t, 2H, SCH_2), 4.70 (t, H, NCHCO), 7.90 (m, 5H, Ph). ^{13}C-NMR (D_2O + DCl) δ 172.2, 134.5, 130.8, 130.3, 128.0, 67.4, 52.4, 30.0, 29.4. The minor product **19** was coincided with an authentic sample of 4-amino-2-thiophenylbutanoic acid which prepared independently from 2-pyrrolidinone according to the method of Zoretic et al.[13]

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