160—161°. These values agreed with those in the literature[4-6] and also with those of the isomer synthesized through the Nefkens' method.[6] The yield was 20% of the theoretical. Optical purity of this phthalyl-L-glutamic acid (I) was confirmed by the fact that the hydrolyzing solution of the end-product, cholestanyl hydrogen-L-glutamate, with 6N HCl gives a value of $[\alpha]_D^{22}$ +23.6° ($c=6$, H_2O), which is identical to that of L-glutamic acid hydrochloride.

Phthalyl-DL-glutamic Acid (II)——A mixture (2.34 g, $[\alpha]_D^{22}$ −15.28°, $c=3$, dioxane) of the phthalyl-L-glutamic acid (I) and the DL-isomer (II) was dissolved in dioxane (60 ml) at 60° and to the resulting solution was gradually added 4 times its volume of water (60°) with stirring. The solution was kept at the same temperature for 2 hr to precipitate white crystals. By repeating this procedure, 0.9 g of crystals ($[\alpha]_D^{22}$ 0°, $c=3$, dioxane; mp 190—193°) was obtained. These values agreed with those of the DL-isomer (II) being prepared by a known method[7] *via* DL-α-pyrrolidonecarboxylic acid from the L-glutamic acid.

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Radiation Protective Agents. VI.[1] Synthesis of L-2-Pyrrolidinylmethyl Derivatives

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The radiation protective effects of various organic chemicals have been extensively investigated[3-6] and it has been generally recognized that mercaptoethlamine (MEA) and its isothiuronium salt (AET) are the most effective to the radioprotection. Based on this fact, we have examined the syntheses[7] and the radioprotective effects[8] of compounds related to AET.

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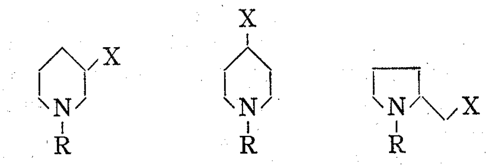
The structure-effect relationship is still ambiguous, but in considering past studies, it may be said that structures having C_2 and/or C_3 units between N and S atoms are essentially required in chain systems.[9] This paper concerns the reactions of cyclic amino halides with nucleophiles such as thiourea, sodium thiosulfate,[10] and sodium phosphorothioate.[11] The amino halides (**3Aa, b, 3Ba, b**) were treated with an equimolar amount of the nucleophiles in appropriate solvents (with thiourea: **3Ab**; in isopropanol under reflux, **3Bb**; in *n*-butanol at 100°, with sodium thiosulfate: **3Ab**; in methanol under reflux, with sodium phosphorothioate: **3Ab, 3Ba**; in water at room temperature) to give the corresponding salts in the following yields: **3Ac**; 80%, **3Bc**; 67%, **3Ad**; 81%, **3Ae**; 82%, **3Be**; 77%. In contrast, the other amino halides (**1Ab, 1Ba, b, 2Ab, 2Ba, b**), except the reaction of **1Bb** with thiourea, did not give unequivocal results; **1Bb** yielded **3Bc** in 48% yield. Prolonged heating caused the decomposition of products or the reagents and the change of solvent did not improve the reactions.



1A: R=H **2A**: R=H **3A**: R=H
1B: R=Me **2B**: R=Me **3B**: R=Me

a: X=Cl
b: X=Br
c: $X=S\overset{+}{C}(NH_2)NH_2$
d: $X=SSO_3H$
e: $X=SPO_3HNa$

Chart 1

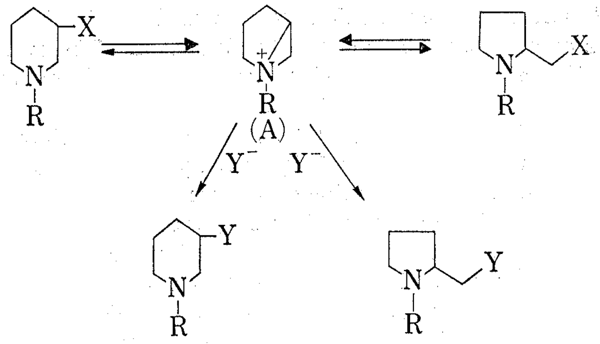


Chart 2

As previously reported,[12] the *β*-amino halides underwent the solvolysis at fast rates attributed to strong *β*-N-participation (Chart 2). Usually, the reactive intermediate (A) reacts with the nucleophile (Y^-) to yield the corresponding products. If the reaction does not proceed *via* a S_N1 process, the ion (A) would not be formed, therefore the displacement would take place *via* a S_N2 process. On the basis of these facts, it is of interest to know the reaction of amino halides with nucleophiles because the presence of the participation by nitrogen is ambiguous in these reactions.

The facts that only the primary halides yielded the expected products would suggest that the reactions involve a S_N2 mechanism. The reactions of the secondary halides may be overwhelmed by a S_N1 process or difficult to proceed under the conditions used. Unrecovery of a starting material may indicate that the reactions proceed through undesired processes. Anyway, these facts show difficulty in the preparation of these types of secondary salts.

The compounds prepared here have satisfactorily the radioprotective effects by assays in mice.[13] The effects correspond to those of MEA and AET.

Experimental

All melting points are corrected. Infrared (IR) spectra were recorded on a Hitachi-Perkin-Elmer Model 225 Spectrometer. Nuclear magnetic resonance (NMR) spectra were measured in D_2O using TMS

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as an external standard by a Varian 100 Mc (HA-100) Spectrometer and peaks are represented in ppm from TMS. Optical rotation were measured by a JASCO Automatic polarimeter (DIP-SL).

L-2-Pyrrolidinylmethyl Isothiuronium Bromide Hydrobromide (3Ac)——A solution of 311 mg (4 mmole) of thiourea and 985 mg (4 mmole) of L-2-pyrrolidinylmethyl bromide hydrobromide (**3Ab**) dissolved in 4 ml of iso-PrOH was refluxed for 45 min. After cooling, a precipitate was assembled by filtration and was recrystallized from iso-PrOH to give colorless needles, 1.0 g (80% yield), mp 164—165°; NMR: 2.0—3.0 (4H, br), 3.9 (2H, t), 4.06 (2H, d), 4.1—4.5 (1H, br). *Anal.* Calcd. for $C_6H_{15}N_3SBr_2$: C, 22.44; H, 4.71; N, 13.09. Found: C, 22.23; H, 4.73; N, 13.10. Dipicrate (recrystallized from H_2O) mp 184—185° (decomp.). *Anal.* Calcd. for $C_{18}H_{19}O_{14}N_9S$: C, 35.01; H, 3.10; N, 20.42; S, 5.19. Found: C, 35.18; H, 3.22; N, 20.86; S, 5.15.

N-Methyl-L-2-pyrrolidinylmethyl Isothiuronium Bromide Hydrobromide (3Bc)——A solution of 150 mg (2 mmole) of thiourea and 563 mg (2 mmole) of N-methyl-L-2-pyrrolidinylmethyl bromide hydrobromide (**3Bc**) dissolved in 2 ml of *n*-BuOH was heated at *ca.* 100° for 16 min. White crystals appeared were collected and purified recrystallization from EtOH. There was obtained 450 mg of colorless needles (67% yield), mp 187—188.5°. NMR: 2.4—3.0 (4H, br), 3.48 (3H, s), 3.6—4.0 (2H, br), 4.13 (2H, d), 4.1—4.4 (1H, br). *Anal.* Calcd. for $C_7H_{17}N_3SBr_2$: C, 25.09; H, 5.11; N, 12.54. Found: C, 24.80; H, 5.07; N, 12.32.

L-2-Pyrrolidinylmethyl Thiosulfuric Acid (**3Ad**)——To a aqueous solution of sodium thiosulfate (1.8 g (7.5 mmole) in 7.5 ml of H_2O) was added 1.8 g (7.5 mmole) of L-2-pyrrolidinylmethyl bromide hydrobromide (**3Ab**) and then the solution was refluxed for 30 min. To the cold solution was added EtOH and white crystals come out were collected by filtration. The crystals were recrystallized from MeOH to give colorless needles of 1.2 g (81% yield), mp 154—156° (decomp.). $[\alpha]_D^{20}$ +40.2 (H_2O). IR: ν_{max}^{Nujol} cm^{-1}: 1245, 1170, 1020, ($RS \cdot SO_2 \cdot OH$). NMR: 2.0—3.0 (4H, m), 3.6—4.0 (4H, q), 4.4—4.6 (1H, br). *Anal.* Calcd. for $C_5H_{11}O_3NS_2$: C, 30.44; H, 5.62; N, 7.10; S, 32.51. Found: C, 30.61; H, 5.63; N, 7.00; S, 32.44.

Sodium Hydrogen L-2-Pyrrolidinylmethyl Phosphorothioate (3Ae)——To a aqueous solution of L-2-pyrrolidinylmethyl bromide hydrobromide (**3Ab**) (1.96 g (8 mmole) in 8 ml of H_2O) was added 1.4 g (8 mmole) of anhyd. trisodium monothiophosphate[14] at portionwise. The solution was stirred for 4 hr at room temperature. Under this condition, trisodium monothiophosphate disappeared. When 20 ml of EtOH and 40 ml of acetone were added, white solid come out was collected by filtration. The solid was purified from H_2O–EtOH to give colorless powder of 1.4 g (82% yield). $[\alpha]_D^{24}$ +2.8 (H_2O). NMR: 2.13—2.7 (4H, m), 3.3—3.9 (4H, m), 4.0—4.5 (1H, m). The presence of a C–S bond was characterized by nitroprussid color test.

Sodium Hydrogen N-Methyl-L-2-pyrrolidinylmethyl Phosphorothioate (3Be)——To a aqueous solution of N-methyl-L-2-pyrrolidinylmethyl bromide hydrobromide (**3Bb**) (2.6 g (10 mmole) in 5 ml of H_2O) was added 1.8 g (10 mmole) of anhyd. trisodium monothiophosphate[14] at portionwise. When the solution was stirred for 7 hr at room temperature, trisodium monothiophosphate disappared. Then 50 ml of acetone and 20 ml of EtOH were added and white precipitate obtained were collected by filtration. The solid was redeposited from H_2O–EtOH–acetone to give colorless powder of 1.8 g (77% yield). NMR: 2.3—2.7 (4H, m), 3.36 (3H, s), 3.5—3.7 (4H, m), 4.0—4.3 (1H, m). The presence of a C–S bond was characterized by nitroprussid color test.

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