[CONTRIBUTION FROM THE BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY[1]]

# Synthesis of Aminoalkylisothiuronium Salts and their Conversion to Mercaptoalkylguanidines and Thiazolines[2]

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A series of aminoalkylisothiuronium salts and N-substituted aminoalkylisothiuronium salts have been prepared from the corresponding aminoalkyl halide and thiourea or an N-substituted thiourea. The 1,2- and 1,3-aminoalkylisothioureas either underwent intratransguanylation at neutral *p*H to yield mercaptoalkylguanidines or were converted at acid *p*H to 2-aminothiazolines and 2-aminopenthiazolines. The formation of the ring compounds was catalyzed by the presence of $CO_2$.

## Introduction

S,2-Aminoethylisothiuronium·Br·HBr (AET) and S,3-aminopropylisothiuronium·Br·HBr (APT) have been reported to significantly increase the survival of mice when administered prior to an otherwise lethal dose of X-radiation to the whole body.[3] Since these compounds were more active, on a molar basis, as well as less toxic than the best previous protective agent, 2-mercaptoethylamine,[4] we considered it worth while to prepare and examine for protective activity a series of aminoalkylisothiuronium compounds to see if any correlation could be obtained between structure, chemical properties and radiation-protective activity. This report is concerned with the preparation and chemical reactions of such compounds. The extensive biological testing of these compounds for radiation-protective activity in mice has been reported elsewhere.[5]

In the course of the biological evaluation, a series of simple tests were performed to exclude the possibility, unlikely in view of the normal stability of isothiuronium salts at neutral *p*H's, that AET and APT were converted in neutral solution to the corresponding 2- and 3-mercaptoalkylamines. We found that both AET and APT, when neutralized to *p*H 7–8, gave strong positive nitroprusside tests, indicative of a free –SH group. The same solutions also gave a strong Sakaguchi test, showing the presence of a guanido group. These color tests demonstrated that mercaptoethyl- and mercaptopropylguanidine (MEG and MPG) had been formed from their respective isothiuronium salts. An investigation of the reaction yielded evidence that the transformation was *p*H dependent. If a solution of AET was refluxed at its own *p*H (3.5), both color tests rapidly became negative in aliquots that were neutralized and tested. A crystalline product was isolated in essentially quantitative yield and was identified as 2-aminothiazoline·HBr (2-AT). These results implicate a common cyclic intermediate (I) that could either open to form the mercaptoguanidine or split off ammonia to form the thiazoline or penthiazoline. Since the conversion of AET to MEG was instantaneous at *p*H 7.0 and independent of the concentration, the reaction can be regarded as an intratransguanylation. The propyl compound, APT, intratransguanylated in a similar fashion through a penthiazoline intermediate although the optimum *p*H for rapid conversion was shifted from *p*H 7.0 to 9.0. That the reaction proceeded through the postulated intermediate seemed likely, in view of the rapid and complete conversion of AET in aqueous solution (*p*H 2.5, 10 min. reflux) to 2-AT. Although aminolysis of an S-substituted thiourea to yield a guanidine derivative is well known, the conditions required are usually more alkaline than in the present case. So far as we are aware there has been no previous mention of an intratransguanylation of this nature to yield a stable compound. Intermediates similar to those postulated here, where the nitrogen has been replaced by an oxygen to give an oxathiazole, have been proposed to explain the formation of ethylene sulfide from ethylene oxide and thiourea.[6,7] In addition, thiazoline ring intermediates have been shown to exist in glutathione[8] and coenzyme A,[9] and the lability of 2-methylthiazoline to ammonium ion has been demonstrated by Linderstrøm-Lang and Jacobsen.[10]

We prepared a series of related compounds to examine the necessity for the reaction to proceed through the cyclic intermediate. When the carbon chain was extended to four, *i.e.*, S,4-aminobutylisothiuronium·Br·HBr, no intratransguanylation occurred since an unlikely seven-membered ring intermediate would have to be formed, and the compound was stable at neutral *p*H. Substitution of the amine nitrogen atom of AET or APT with one alkyl group did not affect the reaction. However, replacement of both hydrogens of the amine nitrogen with either alkyl groups or a ring system yielded stable isothiuronium salts that could be hydrolyzed only by base to the corresponding thiols.

The extent and direction of these reactions at various *p*H's has been extensively studied by ion-exchange techniques.[11] It was observed that the products obtained depended on the base used for

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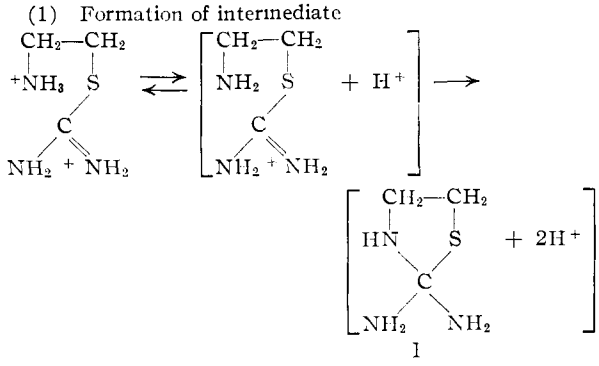
neutralization. Thus, when AET was dissolved in one equivalent of NaOH or an excess of 0.4 *M* phosphate buffer *p*H 7.0, 2-mercaptoethylguanidine was obtained exclusively, whereas AET dissolved in one equivalent of $NaHCO_3$ yielded a solution at *p*H 7.0 composed of 60% MEG and 40% 2-AT.[12] To determine whether this was a bicarbonate ion effect or the formation of a carbonate intermediate by the addition of $CO_2$ to a basic group, we performed an experiment in which an AET solution, *p*H 4.5, was shaken in a pressure bottle at room temperature under one atmosphere of $CO_2$. The conversion to 2-AT was complete in one hour, in contrast to the mixture of MEG and 2-AT obtained after four days at room temperature at this *p*H.[11] An effect of bicarbonate ion on the hydrolysis of S-alkylisothiuronium salts has been reported by Horak,[13] who showed that bicarbonate salts prepared from the halides at low temperatures decomposed quantitatively on heating to yield the thiols.

The mechanism of the rearrangement of AET to MEG and 2-AT may be best explained by the formation of an unstable intermediate I that decomposes to the products in the following series of continuous steps. Similar transformations may be postulated for APT.

Step 1, which is probably rate limiting, involves the formation of an unstable intermediate I, common to all reactions, by the competition between protons and the adjacent carbonium ion for the primary amino group, with the resultant attack of the carbonium ion on the amino group (or *vice versa*) and the liberation of a proton from the amino group. The formation of I is strongly *p*H dependent and is slow at very high proton concentrations. Step 2, the acid-catalyzed formation of 2-AT$^+$, proceeds by the attack of a proton on a guanylamino group of I, attracting electrons and liberating $NH_4^+$ and 2-AT$^+$, a fast step at high proton concentrations. In step 3, the rearrangement of I to MEG occurs rapidly at neutral *p*H since, at low proton concentrations, the uncharged amino groups of I cause a migration of the adjacent C–S bonding electrons to the sulfur atom, thus forming guanidiniumethylmercaptide ion. Step 4, in the *p*H range of 4 to 7, probably involves the attack by $CO_2$ or $H_2CO_3$ on the uncharged guanylamino groups of I, attracting electrons and resulting in the liberation of ammonium bicarbonate and 2-AT. Thus, when AET is neutralized by the addition of one equivalent of bicarbonate, steps 3 and 4 compete and a mixture of MEG and 2-AT is obtained.

The preparation of isothioureas has been reviewed by Schroeder[14] and several of the modifications mentioned are applicable to the syntheses of aminoalkylisothiuronium salts. Since both aminoalkyl and alkylaminoalkylisothiuronium salts of two- and three-carbon chain length between the nitrogen and sulfur are capable of these rearrangements, care must be taken during the synthesis to avoid conditions favorable to the formation of thiazolines, whose presence in the resultant mixtures makes them difficult to crystallize and purify. We found it better to use solvent mixtures containing only trace amounts of water, in which the starting materials were soluble but the product was insoluble. Reaction times were kept short by the

(1) Formation of intermediate



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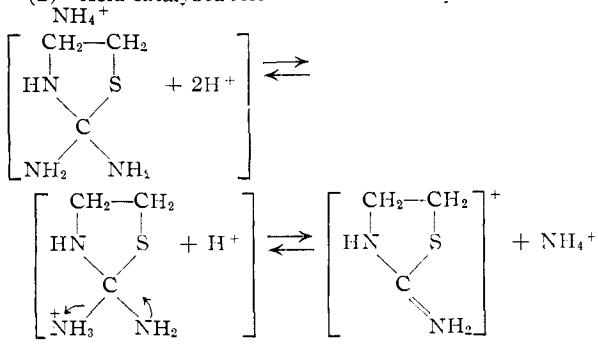
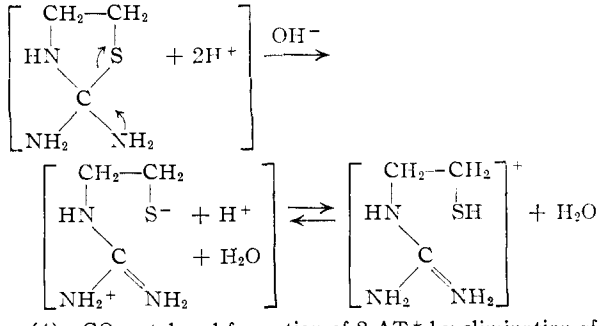
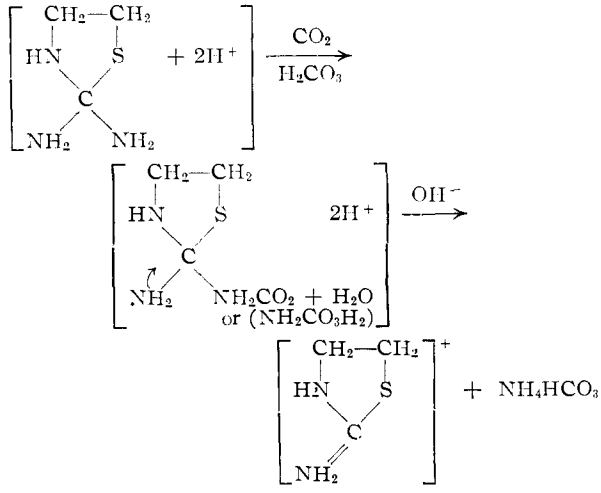
(2) Acid-catalyzed formation of 2-AT$^+$ by elimination of $NH_4^+$



(3) Rearrangement to MEG at neutral *p*H



(4) $CO_2$-catalyzed formation of 2-AT$^+$ by elimination of $NH_4HCO_3$



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use of the more reactive bromides rather than the chlorides, since long reflux times and high reflux temperatures accelerated the decomposition of the products. Absolute isopropyl alcohol or mixtures of absolute ethanol and ethyl acetate were the two most useful solvent systems. The only isothiourea of this type previously reported was AET, which had been prepared by Clinton, *et al.*,[15] by refluxing a mixture of 2-bromoethylamine·HBr and thiourea in ethanol for six hours. In our hands, this procedure yielded a product containing appreciable amounts of 2-AT, a more toxic by-product than AET. In absolute isopropyl alcohol, the aminoalkyl bromides reacted completely in 10–30 minutes, whereas the corresponding chlorides required 1–3 hours and gave increased amounts of thiazolines.

Inasmuch as those amino and alkylaminoalkylisothiuronium salts capable of intratransguanylation at neutral *p*H can also, under more acid conditions, be converted readily to thiazolines and penthiazolines, they are excellent intermediates for the synthesis of a wide variety of these compounds as well as nitrogen- and sulfur-containing spirononanes and spirodecanes. The transformations of several such substituted isothiuronium salts have been studied in detail by ion-exchange techniques and will be reported subsequently.[16]

## Experimental

Aminoalkyl halides used for the preparation of the aminoalkylisothiuronium compounds listed in Table II were synthesized by six general methods (listed A–F in Table II).

**Method A.**—The Gabriel synthesis of amines[17] was used for preparation of the halide precursors of compounds 1 and 2.

**Method B.**—Pyrrolidine, piperidine and morpholine were alkylated with ethylene chlorohydrin, propylene chlorohydrin and butylene chlorohydrin to produce the amino alcohols used in the preparation of compounds 17–25.[18] The amino alcohols were converted to the alkyl chlorides in Table I by reaction with thionyl chloride in chloroform.[19] The method is illustrated by the following two preparations.

**3-(1-Pyrrolidinyl)-propylchloride·HCl.**—To a mixture of 47.0 g. (0.36 mole) of 3-(1-pyrrolidinyl)-propyl alcohol and 200 ml. of dry chloroform was added dropwise 47.6 g. (0.44 mole) of fresh thionyl chloride. The solution was held at room temperature during the addition, stirred overnight, evaporated to dryness, and recrystallized from absolute alcohol and ethyl acetate. The purified product weighed 58.0 g. (61.5%), m.p. 142–143°.

**4-(4-Morpholinyl)-butylchloride·HCl.**—By the preceding method, reaction of 4-(4-morpholinyl)-butyl alcohol with thionyl chloride gave a purified product (68%), m.p. 151–152°.

**Method C.**—Lithium aluminum hydride reduction of γ-phenoxybutyronitrile by the procedure of Nystrom and Brown[20] for the reduction of nitriles gave 4-phenoxybutylamine and 4-phenoxybutylamine·HBr in good yields. Acid hydrolysis (concd. HBr) of either compound led to the formation of 4-bromobutylamine·HBr, the halide precursor of compound 3, Table II.

**4-Phenoxybutylamine·HBr.**—In a 1-liter flask was mixed 7.6 g. (0.2 mole) of crushed lithium aluminum hydride and 300 ml. of dry ether. To the stirred solution was added 32.2 g. (0.2 mole) of γ-phenoxybutyronitrile in 200 ml. of ether at a rate to produce gentle reflux. Water was then added dropwise to the ice-cooled flask, and then 500 ml. of a 20% solution of sodium-potassium tartrate (Rochelle Salt). The ether layer was separated and the aqueous layer extracted twice with 100-ml. portions of ether. After drying over calcium sulfate, the ether solution was evaporated at reduced pressure to an oil. The oil could be distilled to give 4-phenoxybutylamine,[21] 146–148° (17 mm.), or dissolved in 200 ml. of *n*-propyl alcohol and the solution saturated with dry HBr. The white precipitated product was filtered, washed with cold *n*-propyl alcohol, and dried to give 42.5 g. (86.5%), m.p. 159–160°.

**4-Bromobutylamine·HBr.**—A mixture of 37.0 g. (0.15 mole) of 4-phenoxybutylamine·HBr and 100 ml. of HBr (48%) (0.625 mole) was refluxed for 6 hours and then evaporated under reduced pressure to dryness. Recrystallization from absolute ethyl alcohol–ethyl acetate gave 30.0 g. of product (85.5%), m.p. 146–146.5°.

**Method D.**—Hydroxyalkylamine hydrochlorides were prepared by treating hydroxyalkylamines with dry HCl in *n*-propyl alcohol.[22] The salt was then converted to the aminoalkylchloride hydrochlorides with thionyl chloride in dry benzene or chloroform.[23] This method was used in the preparation of 2-aminoethylchloride hydrochloride,[23] 3-aminopropylchloride hydrochloride,[24] N-methylaminoethylchloride hydrochloride,[25] N-dimethylaminoethylchloride hydrochloride[26] and N-diethylaminoethylchloride·HCl[18] and these compounds were used in the preparation of compounds 4, 5, 6 and 7[10] in Table II.

**Method E.**—Hydroxyalkylamines used in the preparation of compounds 1, 2, 12, 13, 14 and 15 (Table II) were refluxed in a large excess of concentrated HBr and the water formed was removed from the higher boiling HBr–$H_2O$ azeotrope, as described in the preparation of 2-bromoethylamine·HBr.[27] N-Phenylaminoethylbromide·HBr was prepared by the modified procedure of Pearlman.[28]

**N-Isopropylaminoethylbromide·HBr.**—2-Isopropylaminoethanol prepared from acetone and aminoethanol by the

TABLE I

AMINOALKYLCHLORIDE HYDROCHLORIDES

| | Amino alcohol B.p., °C., 20 mm. | Yield, % | Chloride·HCl M.p., °C. | Yield, % |
|---|---|---|---|---|
| 2-(1-Pyrrolidinyl)-1-ethanol[a] | 85 | 83 | 173–174[j] | 75 |
| 3-(1-Pyrrolidinyl)-1-propanol[b] | 110 | 73 | 142–143 | 62 |
| 4-(1-Pyrrolidinyl)-1-butanol[c] | 122 | 67 | 111–113[k] | 60 |
| 2-(1-Piperidinyl)-1-ethanol[d] | 95 | 86 | 231–232[l] | 77 |
| 3-(1-Piperidinyl)-1-propanol[e] | 115 | 74 | 208–209[m] | 71 |
| 4-(1-Piperidinyl)-1-butanol[f] | 126 | 68 | 162–163[n] | 70 |
| 2-(4-Morpholinyl)-1-ethanol[g] | .. | .. | 182–183[o] | 80 |
| 3-(4-Morpholinyl)-1-propanol[h] | 130 | 76 | 168–169[p] | 76 |
| 4-(4-Morpholinyl)-1-butanol[i] | 140 | 61 | 151–152 | 68 |

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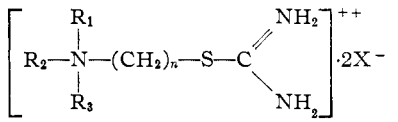
TABLE II

AMINOALKYLISOTHIURONIUM SALTS

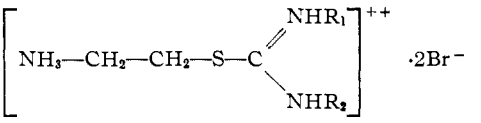


| $R_1$ | $R_2$ | $R_3$ | $n$ | X | Method | M.p., °C. | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found | Sulfur, % Calcd. | Sulfur, % Found, |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | H | H | 2 | Br | A, E | 193–194[a] | $C_3H_{11}N_3SBr_2$ | ... | ... | .. | .. | ... | ... | ... | ... |
| H | H | H | 3 | Br | A, E | 144–145 | $C_4H_{13}N_3SBr_2$ | 16.28 | 16.47 | 4.44 | 4.64 | 14.24 | 14.33 | 10.87 | 10.86 |
| H | H | H | 4 | Br | C | 187–188 | $C_5H_{15}N_3SBr_2$ | 19.43 | 19.43 | 4.89 | 4.90 | 13.59 | 13.67 | 10.37 | 10.10 |
| H | H | H | 2 | Cl | D | ..... | $C_3H_{11}N_3SCl_2$ | 18.76 | 18.82 | 5.77 | 5.89 | 21.87 | 22.08 | 16.69 | 16.49 |
| H | H | H | 3 | Cl | D | ..... | $C_4H_{13}N_3SCl_2$ | 23.30 | 23.24 | 6.35 | 6.57 | 20.38 | 20.11 | 15.55 | 15.31 |
| $CH_3$ | H | H | 2 | Cl | D | 164–166 | $C_4H_{13}N_3SCl_2$ | 23.30 | 22.70 | 6.35 | 7.67 | 20.38 | 19.78 | 15.55 | 14.78 |
| $CH_3$ | $CH_3$ | H | 2 | Cl | D | 181–183[b] | $C_5H_{15}N_3SCl_2$ | 27.28 | 27.34 | 6.87 | 6.93 | 19.09 | 19.06 | 14.57 | 14.40 |
| $CH_3$ | $CH_3$ | $CH_3$ | 2 | Br | F | 120–122 | $C_6H_{17}N_3SBr_2$ | 22.30 | 22.71 | 5.30 | 5.46 | 13.00 | 12.43 | 9.92 | 9.30 |
| $C_2H_5$ | $C_2H_5$ | $C_2H_5$ | 3 | Br | F | 218–220 | $C_{10}H_{25}N_3SBr_2$ | 31.67 | 31.42 | 6.65 | 6.40 | 11.08 | 11.24 | 8.45 | 8.49 |
| $C_2H_5$ | $C_2H_5$ | H | 2 | Cl | D | 196–197[c] | $C_7H_{19}N_3SCl_2$ | 33.87 | 33.72 | 7.71 | 7.52 | 16.93 | 16.91 | 12.92 | 13.02 |
| $CH_3$ | $CH_3$ | $CH_3$ | 3 | Br | F | 194–195 | $C_7H_{19}N_3SBr_2$ | 24.92 | 24.93 | 5.68 | 5.57 | 12.46 | 12.38 | 9.51 | 9.37 |
| $C_2H_5$ | $C_2H_5$ | H | 3 | Br | E | 195.5–197.5 | $C_8H_{21}N_3SBr_2$ | 27.36 | 27.51 | 6.03 | 5.87 | 11.97 | 11.78 | 9.13 | 9.04 |
| $C_3H_7$ | H | H | 2 | Br | E | 103–106 | $C_6H_{17}N_3SBr_2$ | 22.30 | 22.29 | 5.30 | 5.26 | 13.00 | 12.98 | 9.92 | 10.03 |
| $C_3H_7$ | H | H | 3 | Br | E | 134–135 | $C_7H_{19}N_3SBr_2$ | 24.94 | 25.06 | 5.68 | 5.69 | 12.46 | 12.51 | 9.51 | 9.94 |
| $C_6H_5$ | H | H | 2 | Br | E | 217–218 | $C_9H_{15}N_3SBr_2$ | 30.27 | 30.47 | 4.23 | 4.32 | 11.77 | 11.78 | 8.98 | 8.97 |
| $CH_3CO$ | H | .. | 2 | Cl | B | 193–194 | $C_6H_{21}N_3SOCl$ | 30.38 | 30.29 | 6.12 | 5.99 | 21.26 | 21.05 | 16.22 | 16.09 |
| $-CH_2CH_2CH_2CH_2-$ | | H | 2 | Cl | B | 179–181[d] | $C_7H_{17}N_3SCl_2$ | .... | ... | .. | .. | ... | ... | ... | ... |
| $-CH_2CH_2CH_2CH_2-$ | | H | 3 | Cl | B | 223–224 | $C_8H_{19}N_3SCl_2$ | 36.92 | 37.06 | 7.36 | 7.10 | 16.15 | 16.35 | 12.32 | 12.70 |
| $-CH_2CH_2CH_2CH_2-$ | | H | 4 | Cl | B | 206–207[e] | $C_9H_{21}N_3SCl_2$ | ... | ... | .. | .. | ... | ... | ... | ... |
| $-CH_2CH_2CH_2CH_2CH_2-$ | | H | 2 | Cl | B | 226–227[f] | $C_8H_{19}N_3SCl_2$ | ... | ... | .. | .. | ... | ... | ... | ... |
| $-CH_2CH_2CH_2CH_2CH_2-$ | | H | 3 | Cl | B | 202–204 | $C_9H_{21}N_3SCl_2$ | 39.41 | 39.40 | 7.72 | 7.70 | 15.32 | 15.32 | 11.69 | 11.62 |
| $-CH_2CH_2CH_2CH_2CH_2-$ | | H | 4 | Cl | B | 171–173 | $C_{10}H_{23}N_3SCl_2$ | 41.66 | 41.73 | 8.04 | 8.20 | 14.58 | 14.52 | 11.12 | 11.29 |
| $-CH_2CH_2-O-CH_2CH_2-$ | | H | 2 | Cl | B | 239–240[g] | $C_7H_{17}N_3SOCl_2$ | ... | ... | .. | .. | ... | ... | ... | ... |
| $-CH_2CH_2-O-CH_2CH_2-$ | | H | 3 | Cl | B | 221–223 | $C_8H_{19}N_3SOCl_2$ | 34.78 | 35.02 | 6.93 | 6.78 | 15.21 | 15.40 | 11.61 | 11.50 |
| $-CH_2CH_2-O-CH_2CH_2-$ | | H | 4 | Cl | B | 192–194 | $C_9H_{21}N_3SOCl_2$ | 37.24 | 37.03 | 7.29 | 7.30 | 14.48 | 14.41 | 11.05 | 11.18 |

[a] R. O. Clinton, *et al.*, THIS JOURNAL, **70**, 950 (1948); m.p. 194–195°. [b] *Ibid.*, m.p. 182–183°; and R. R. Renshaw, *ibid.*, **60**, 1765 (1938); m.p. 181–182°. [c] N. F. Albertson and R. O. Clinton, *ibid.*, **67**, 1222 (1945); m.p. 194–195°. [d] H. G. Kolloff, *et al.*, *ibid.*, **71**, 3988 (1949); m.p. 174–175°. [e] *Ibid.*, m.p. 168–169.5°. [f] Ref. *a*; m.p. 225–225.5°. [g] Ref. *a*; m.p. 233–235°.

TABLE III

AMINOALKYLISOTHIURONIUM SALTS



| $R_1$ | $R_2$ | M.p., °C. | Formula | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found | Sulfur, % Calcd. | Sulfur, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $CH_3$ | H | 214–215 | $C_4H_{13}N_3SBr_2$[a] | 16.28 | 16.15 | 4.44 | 4.42 | 14.24 | 14.27 | 10.87 | 10.92 |
| $C_2H_5$ | H | 141–144 | $C_5H_{15}N_3SBr_2$[b] | 19.43 | 19.62 | 4.89 | 5.03 | 13.59 | 13.79 | 10.37 | 10.49 |
| $CH_2CHCH_2$ | H | 102–105 | $C_6H_{15}N_3SBr_2$[c] | 22.44 | 22.49 | 4.71 | 4.59 | 13.09 | 13.17 | 9.98 | 9.71 |
| $CH_2$ | $CH_2$ | 121–125 | $C_5H_{14}N_3SBr_2$[d] | 19.56 | 19.33 | 4.27 | 4.45 | 13.68 | 13.46 | 10.44 | 10.49 |
| $C_6H_5$ | H | 221–222 | $C_9H_{15}N_3SBr_2$[e] | 30.27 | 30.45 | 4.23 | 4.18 | 11.77 | 11.82 | 8.98 | 8.81 |
| $C_2H_5$ | $C_2H_5$ | 152–154 | $C_7H_{19}N_3SBr_2$[f] | 24.94 | 24.93 | 5.68 | 5.83 | 12.46 | 12.24 | 9.51 | 9.47 |
| $C_4H_9$ | $C_4H_9$ | 135–137 | $C_{11}H_{27}N_3SBr_2$[g] | 33.59 | 33.61 | 6.92 | 6.95 | 10.69 | 10.70 | 8.15 | 8.27 |
| $C_6H_5CH_2$ | $C_6H_5CH_2$ | 118–119 | $C_{17}H_{23}N_3SBr_2$[h] | 44.26 | 44.35 | 5.03 | 5.00 | 9.11 | 9.09 | 6.95 | 7.01 |

[a] Prepared from 1-methyl-2-thiourea ("Organic Syntheses," Coll. Vol. III, John Wiley & Sons, Inc., New York, N. Y. 1955, p. 617). [b] Prepared from 1-ethyl-2-thiourea, procedure from ref. *a*. [c] Prepared from 1-allyl-2-thiourea, EK #12. [d] Prepared from ethylenethiourea (ref. *a*, p. 394). [e] Prepared from 1-phenyl-2-thiourea, EK #1569. [f] Prepared from 1,3-diethyl-2-thiourea, EK #1803. [g] Prepared from 1,3-dibutyl-2-thiourea, EK #2138. [h] Prepared from 1,3-dibenzyl-2-thiourea, EK #6858.

procedure of Hancock and Cope[29] was brominated by the procedure described in reference 27. The product obtained (80%) had a m.p. of 188–189°. *Anal.* Calcd. for $C_5H_{13}NBr_2$: N, 5.66. Found: N, 5.60.

**N-Isopropylaminopropylbromide·HBr.**—3-Isopropylaminopropanol[30] prepared from acetone and 3-aminopropanol by the procedure above[29] was brominated also as above. The product obtained (82%) had a m.p. of 190–191°. *Anal.* Calcd. for $C_6H_{15}NBr_2$: N, 5.36. Found: N, 5.18.

**Method F.**—Bromoalkyltrialkylammonium bromides used in the synthesis of compounds 8, 9 and 11 in Table II were prepared from the anhydrous trialkylamines and corresponding alkyldibromide by the procedure of Krüger and Bergell.[31] The compounds prepared were 2-bromoethyltrimethylammonium bromide,[32] 3-bromopropyltriethylammonium bromide[33] and 3-bromopropyltrimethylammonium bromide.[33]

**3-Bromopropyltrimethylammonium Bromide.**—In a 100-ml. pressure bottle, 40.2 g. (0.2 mole) of 1,3-dibromopropane and 20 ml. of dry toluene was cooled in an ice-bath and 12 g. (0.2 mole) of anhydrous trimethylamine was added. After being sealed, the reaction mixture was incubated at 40° overnight. The solid product was chopped out, washed with ligroin, ether and recrystallized, decolorized from ethanol, yielding 37 g. of purified product, m.p. 208° (70.5%).

Aminoalkylisothiuronium compounds listed in Tables II and III were prepared from the corresponding aminoalkyl halide salt and thiourea (Table II) or from 2-bromoethylamine hydrobromide and a substituted thiourea (Table III). In our hands, aminoalkylbromide salts were more reactive than the corresponding chlorides, and isopropyl alcohol was

(29) "Organic Syntheses," Coll. Vol. III, John Wiley & Sons, Inc., New York, N. Y., 1955, p. 501.

(30) R. C. Elderfield, *et al.*, THIS JOURNAL, **68**, 1579 (1946).

(31) M. Krüger and P. Bergell, *Ber.*, **36**, 2901 (1903).

(32) Reference 31 and A. Bode, *Ann.*, **267**, 268 (1892).

(33) R. Lucius, *Arch. Pharm.*, **245**, 254 (1907).

the best solvent for the reaction. Compounds 1–6 and 13–15 in Table II and compounds in Table III were all found subject to further reaction, *i.e.*, thiazoline formation and/or rearrangement (see text). The method designed to limit this secondary reaction in the sensitive isothiuronium compounds is given below for the synthesis of compound 1 (Table II).

**S,2-Aminoethylisothiuronium·Br·HBr.**—Thiourea (alcohol-soluble, recrystallized, 76.1 g., 1.0 mole) was added to 400 ml. of hot isopropyl alcohol (reagent ACS containing 0.5% water) in a 1-liter flask fitted with an efficient condenser, and was refluxed gently for 5 minutes to effect almost complete solution. Recrystallized 2-bromoethylamine·HBr (205 g., 1.0 mole) was added, and the refluxing continued. All was in solution within a few minutes and after about 10 minutes, crystallization of the product began, accompanied by vigorous boiling of the solvent. The reaction mixture was heated for an additional 20 minutes; the precipitated AET was filtered, washed with isopropyl alcohol and then ethyl acetate, and dried *in vacuo;* yield 230 g., *i.e.*, 82% of theoretical; m.p. 193–194° (ref. 34; m.p. 193–194°). A pure product requiring no recrystallization was obtained by this method if the starting materials were of high quality. The dry compound was stable under normal conditions of temperature and humidity (*i.e.*, a shelf life of at least a year in tightly closed bottles). AET is hygroscopic, however, under continued exposure to high humidity; it absorbs water, cakes together, and is converted in significant amounts to 2-aminothiazoline—a transformation that can be detected by a drop in melting point by as much as 30°.

Isothiuronium compounds also have been prepared by refluxing the reactants for many hours in ethanol,[34] propanol,[35] butanol[36] and toluene.[37] In our experiments, isopropyl alcohol, acetonitrile, absolute ethanol, and mixtures of absolute ethanol and ethyl acetate were the best solvents in terms of speed of reaction, yield and purity of products obtained.

**2-Aminothiazoline·HBr.**—AET·Br·HBr (28.1 g., 0.1 mole) was dissolved in 100 ml. of water, refluxed one-half hour and evaporated *in vacuo* to dryness. The residue was recrystallized twice from an isopropyl alcohol–ethyl acetate mixture; yield 15.5 g., 85%, m.p. 175–176°. A mixed melting point with an authentic sample of 2-aminothiazoline·HBr was unchanged. This compound was also obtained if AET was prepared in solvents containing an appreciable amount of water and refluxed for a long time; *e.g.*, 16 hours reflux in ethanol gave a 70% yield of the thiazoline.

(34) R. O. Clinton, *et al.*, THIS JOURNAL, **70**, 950 (1948).
(35) R. R. Renshaw, *et al.*, *ibid.*, **60**, 1765 (1938).
(36) C. H. Grogan, *et al.*, *J. Org. Chem.*, **18**, 728 (1953).
(37) F. J. Bandelin and J. V. Tuschhoff, THIS JOURNAL, **74**, 4271 (1952).

OAK RIDGE, TENN.

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[CONTRIBUTION FROM THE KERCKHOFF LABORATORY OF BIOLOGY AND THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

# Electrophoresis and Ultracentrifuge Studies of Milk Proteins. I. $\beta_1$- and $\beta_2$-Lactoglobulin[1,2]

BY HENRY KLOSTERGAARD AND R. A. PASTERNAK

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$\beta_1$- and $\beta_2$-lactoglobulin have been isolated from milk of individual cows. The properties of these two distinct protein species have been studied by electrophoresis and ultracentrifuge methods. The electrophoretic patterns indicate that at *p*H 4.8 both $\beta_1$- and $\beta_2$-lactoglobulin are inhomogeneous. Under similar conditions in the ultracentrifuge only $\beta_1$-lactoglobulin showed evidence of inhomogeneity.

## Introduction

The whey protein, $\beta$-lactoglobulin, has been studied extensively since its isolation by Palmer[3] in 1934. It was considered to be a homogeneous protein, largely because of its easy crystallizability. The homogeneity of $\beta$-lactoglobulin was, however, made doubtful by the discovery of Li[4] that the protein showed composite electrophoretic boundaries near its isoelectric point.

Since then, many observations have indicated that the usual $\beta$-lactoglobulin preparations are composed of two species. Polis, *et al.*,[5] succeeded by repeated fractional crystallizations in getting in small yield a fraction which appeared to be electrophoretically homogeneous. This fraction was assumed to consist of one protein species only; it was called $\beta_1$-lactoglobulin. Polis, *et al.*, were unsuccessful in isolating the second species in pure form. Fractions were obtained which according to the electrophoretic pattern were not homogeneous but only enriched in the second component, $\beta_2$, and it was assumed that some residual $\beta_1$-lactoglobulin was still present.

The existence of two species of $\beta$-lactoglobulin also was suggested by the observation of Block and Zweig[6] that two bands appeared in paper electrophoresis of the protein in barbitol buffer at *p*H 8.6.

Recently $\beta$-lactoglobulin was reinvestigated extensively by Ogston and Tilley.[7] They concluded that $\beta$-lactoglobulin was composed of two very similar species, one of which dimerized reversibly.[8]

Aschaffenburg and Drewry[9] recently discovered by the use of paper electrophoresis as a test procedure that the whey of individual cows differed in its protein composition; some samples showed the two $\beta$-lactoglobulin bands, others only one or the other. These authors isolated the two proteins in crystalline form.

In the present study, two cows were selected such that the milk of one contained $\beta_1$-lactoglobulin alone and the milk of the other $\beta_2$-lactoglobulin alone. From these two sources the two $\beta$-lactoglobulins were isolated in crystalline form. Each

(1) Contribution No. 2156 from the Gates and Crellin Laboratories of Chemistry.
(2) Supported by the Carnation Company and by a grant from the National Science Foundation.
(3) A. M. Palmer, *J. Biol. Chem.*, **104**, 359 (1934).
(4) C. H. Li, THIS JOURNAL, **68**, 2746 (1946).
(5) B. D. Polis, H. W. Schmuckler, J. H. Custer and T. L. McMeekin, *ibid.*, **72**, 4965 (1950).
(6) R. J. Block and G. Zweig, *Arch. Biochem. Biophys.*, **48**, 386 (1954).
(7) A. G. Ogston and J. M. A. Tilley, *Biochem. J.*, **59**, 644 (1955).
(8) For a more complete review of the lactoglobulin problem see ref. 7.
(9) R. Aschaffenburg and J. Drewry, *Nature*, **176**, 218 (1955).