of crude compound, m.p. 135–140°, was 8.8 g. One recrystallization from ethyl alcohol gave analytically pure substance.

Condensation of II with Primary Amines. 2-(N-Methylaminomethyl)pyrazolo[1,5-*c*]quinazoline.—One hundred milliliters of ethyl alcohol containing methylamine (0.17 g. per ml.) was added dropwise over a period of 15 min. to 2.0 g. of II dissolved in 100 ml. of ethyl alcohol. After stirring the reaction mixture at room temperature for 20 hr. the solution was concentrated to a viscous residue at the water pump. The residue was taken up in a minimum amount of ethyl alcohol and the solution was treated with 5 ml. of ethyl alcohol saturated with hydrogen chloride gas. Addition of this solution to 1 l. of dry ether resulted in the formation of a white precipitate which was collected and recrystallized from ethyl alcohol–ether. The desired compound was isolated as the monohydrochloride salt.

Preparation of 1-(Pyrazolo[1,5-*c*]quinazolin-2-ylomethyl)pyridinium Chloride. Method A.—A mixture of 1.6 g. (0.01 mole) I, 1.13 g. (0.01) mole of chloroacetyl chloride and 10 ml. of pyridine was heated on the steam bath for 3 hr. On chilling the solution, a white crystalline mass was obtained which was collected on a filter and then recrystallized from ethyl alcohol–ether.

Method B.—A mixture of 2.17 g. (0.01 mole) of 2-chloromethylpyrazolo[1,5-*c*]quinazoline and 10 ml. of pyridine was heated in a sealed tube at 100° for 4 hr. The crystalline mass that was formed was collected on a Büchner funnel and recrystallized from ethyl alcohol–ether. Compound 13 in Table I was prepared in similar manner.

1,2-Dihydropyrazolo[1,5-*c*]quinazoline.—5(*o*-Aminophenyl)-pyrazole (1.6 g.; 0.01 mole) and a molar equivalent of 37% formalin were added to 25 ml. of ethyl alcohol containing one pellet of sodium hydroxide. The solution was heated at reflux on the steam bath for 15 min. After filtering off some amorphous material, the filtrate was neutralized and evaporated to viscous residue *in vacuo*. This residue was extracted with a small amount of hot alcohol. On standing at room temperature, a crystalline substance was obtained which was recrystallized from ethyl alcohol to give an analytically pure product.

General Method for the Preparation of 2-Aryl Substituted 1,2-Dihydropyrazolo[1,5-*c*]quinazolines.—One-tenth molar equivalents of I and of an aromatic aldehyde were dissolved in 50 ml. of ethyl alcohol and the resulting solution was refluxed on the steam bath for 3 hr. After removal of the solvent *in vacuo* the remaining semisolid residue was triturated well with water. The precipitate was collected and recrystallized from a suitable solvent for analysis.

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Synthesis of D- and L-2-Aminobutylisothiourea Dihydrobromide Isomers and Their Conversion to Guanidothiols, Disulfides, and Thiazolines

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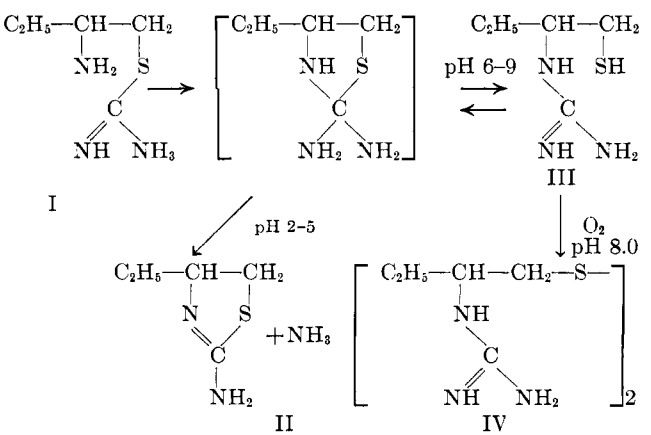
Optically active D- and L-2-aminobutyl bromide was prepared from the enzymatically resolved 2-aminobutyric acids, establishing their configuration. Condensation with thiourea yielded the D- and L-2-aminobutylisothioureas, which readily underwent a pH-dependent intramolecular rearrangement to give the D- and L-2-guanidobutane thiols or D- and L-4-ethyl-2-aminothiazolines. Oxidation of the thiols yielded the optically active 2-guanidobutyl disulfides.

Among the many aminoalkylisothioureas that are capable of protecting mice against a single lethal dose of X radiation, one compound, DL-S, 2-aminobutylisothiourea·HBr (2-ABT), seemed of particular interest. Treatment with this compound at a dose level of 4–5 μmoles per mouse (as compared with 16 μmoles per mouse for a parent compound, S,2-aminoethylisothiourea·di·HBr, AET[3]) prior to 900 r. whole-body X-irradiation enables 95–100% of the mice to survive more than 30 days. Ion exchange analysis[4] confirms that 2-ABT(I) participates in the same intramolecular rearrangements as the parent compound, AET, forming 2-amino-4-ethylthiazoline, II, at a pH of 2.5–5.0, and 2-guanidobutane thiol, III, at a pH of 6.0–9.0. Oxidation of the thiol with air or oxygen at an alkaline pH yields the corresponding 2-guanidobutyldisulfide, IV.



I II III IV

In view of these findings, especially the increased activity on a molar dose level, it seemed desirable to prepare the optically active isomers of 2-ABT and the corresponding thiazolines and examine them for protective activity in mice. If a difference in protective activity exists between the optical isomers, then these compounds, isotopically labeled, might provide some insight to the sensitive cellular and biochemical processes affected by radiation. Indeed, when prepared by the methods described herein, the D-2-ABT is twice as active as the L isomer in protecting mice against 900 r. X-radiation. Intracellular distribution studies using S^{35}- and C^{14}-labeled compounds[5] reveal significant differences in binding in the cellular fractions between the two compounds. In addition, the 2-ABT isomers were found to have interesting pharmacological proper-

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(3) R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., *Radiation Res.*, **7**, 22 (1957).

(4) J. X. Khym, D. G. Doherty, and R. Shapira, *J. Am. Chem. Soc.*, **80**, 3342 (1958).

(5) R. H. Bradford, R. Shapira, and D. G. Doherty, *Intern. J. Radiation Biol.*, **3**, 595 (1961).

ties when compared to the other radioprotective isothioureas.[6]

A direct method for the preparation of the necessary starting materials, D- and L-2-aminobutanols, in quantity, is the enzymic resolution of DL-2-aminobutyric acid followed by the reduction of the stereoisomers with lithium aluminum hydride to the corresponding amino alcohols. This procedure offers the additional advantage of correlating the configuration of the alcohol with that of the α-amino acid. Previous experience with the papain-catalyzed asymmetric anilide synthesis[7] had indicated that its specificity is probably broad enough to include 2-aminobutyric acid and prompted us to use this method rather than the acylase resolution of Birnbaum, *et al.*[8] After the initial preparation of D- and L-2-aminobutanol by this procedure, the resolution of DL-2-aminobutanol *via* salt formation by Radke, *et al.*,[9] came to our attention, and was used for subsequent batches. The [−]-2-aminobutanol was isolated in satisfactory yield from the D-tartrate salt and proved to be equivalent to the D-2-aminobutanol prepared from D-2-aminobutyric acid. However, contrary to the statement by Radke, *et al.*,[9] that seeding with [−]-2-aminobutanol-L-glutamate precipitates the [+]-2-aminobutanol-L-glutamate, we found it essential to seed with the L-2-aminobutanol-L-glutamate to achieve the desired resolution. The corresponding 2-aminoalkyl bromides, not previously reported, were readily prepared by treatment of the amino alcohols with concentrated hydrobromic acid.[10] It was essential, in order to avoid the formation of thiazoline, to prepare the isothiourea in a relatively anhydrous medium. Both optically active isothioureas exhibit polymorphism in their crystallization, the lower melting form being the first one obtained. The polymorphs were identical in their analyses and properties in solution. They also yielded the same derivatives and possessed the same biological activity. In addition, the melting point of an analytical sample of D-2-ABT, stored two years at room temperature in a desiccator, had risen from its initial value of 157–158° to 177–178° and, on re-analysis, yielded results identical with those obtained initially.

The optical properties of the guanidothiols and disulfides are similar to those of cysteine–cystine. Conversion of the L-thiol to the disulfide changes $[\alpha]^{21}D$ from −7.3 to +200°. The disulfides exhibit a large temperature coefficient—1.2°/degree—as well as a marked solvent effect, the specific rotation of the L-disulfide at 0° being +225° in water and at 21° being +200° in water, +175° in *N* HCl, +92° in *N* NaOH, and +175° in 8 *M* urea (*c*, 1%). The disulfides also have a weak absorption band in the ultraviolet, $\epsilon_{max}^{247} = 335$ (water), which is absent in the thiol. The alkaline solution of the L-disulfide disproportionates on standing to the equilibrium mixture of thiol–disulfide as indicated by the decrease in rotation, loss of the ultraviolet peak, and confirmed by the nitroprusside reaction, the equilibrium value being approximately 60% —S—S—/40% —SH. Inclusion of the asymmetric carbon atom in the ring, L-2-amino-4-ethylthiazoline, increases the negative rotation from +7.3° of the thiol to −25.8° for the cyclic compound. The conversion of the isothiourea to the thiazoline at an acid pH can readily be followed by the change in rotation, and was in good agreement with the results obtained with the DL isomer utilizing ion exchange analysis.[4]

The high anomalous rotation observed when an optically active thiol is oxidized to the disulfide has been the subject of speculation since it was noted by Van't Hoff for the conversion of cysteine $[\alpha]^{20}D = +20.5°$ to cystine $[\alpha]^{20}D = -223°$ (*c*, 1%, 1 *N* HCl). Kauzmann and Eyreing[11a] suggested that it was due to forces restricting the freedom and orientation of the groups in the molecule. Fieser[11b] extended this, proposing that cystine formed a symmetrical, intramolecular hydrogen bonded three-ring system at pH's where the rotatory power was maximum. In homocystine, $[\alpha]^{25}D = +75.5°$ (*c*, 1%, *N* HCl) the lack of high rotation was attributed to the presumably less stable nonsymmetrical hydrogen bonded ring system. Fregda[11c] compared the rotations of a series of carboxylic acid disulfides and concluded that ring formation played a negligible role, the high optical activity arising from a disulfide bond in the molecule in proximity to an asymmetric center. Additional support for this was provided by Balenović, *et al.*,[11d] who prepared β-homocystine and found that, contrary to Fiesers' proposition, it had a rotation, $[\alpha]^{14}D = -262°$ (*c*, 1%, *N* HCl), higher than cystine. They also observed that disulfides, in contrast to the thiols, have weak absorption bands in the ultraviolet, a region associated with specific rotation in the visible spectrum. The important consequences of the nonplanarity of the —S—S— bond to the steric properties of the disulfides was pointed out by Calvin,[11e] and Foss[11f] noted the intrinsic asymmetry of the disulfide bond. Taking these facts into consideration, Strem, *et al.*,[11g] in a discussion of the rotatory dispersion of cystine, postulated that the 90° dihedral angle gave rise to a screw sense in all disulfides. With no asymmetric center, right and left senses could occur with equal frequency, but when asymmetric R groups are present, such as in cystine or the basic disulfides we report, a preferred configuration would give rise to an intrinsic rotation associated with the disulfide bond. This last explanation, with the additional corollary that the asymmetric atom be separated by not more than one methylene group from the disulfide bond, seems to fit best the present experimental observations.

Configurationally, the optically active compounds in this series are given the designation D and L since they arise from the corresponding amino acids by reactions not involving the asymmetric carbon atom. Alternatively, the absolute notation of Cahn, Ingold, and Prelog[12] may be used, the D series corresponding to the (R) and the L series to the (S). It is interesting to note than in L-cysteine, the exception in this system, the

(6) V. DiStefano and D. E. Leary, *Pharmacol. Exptl. Therap.*, **126**, 304 (1959).

(7) D. G. Doherty and E. A. Popenoe, Jr., *J. Biol. Chem.*, **189**, 447 (1951).

(8) S. M. Birnbaum, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *ibid.*, **194**, 455 (1952).

(9) F. H. Radke, R. B. Fearing, and S. W. Fox, *J. Am. Chem. Soc.*, **76**, 2801 (1954).

(10) F. Cortese, *Org. Syn.*, **2**, 91 (1943).

(11) (a) W. Kauzmann and H. Eyreing, *J. Chem. Phys.*, **9**, 49 (1941); (b) L. Fieser, *Rec. trav. chim.*, **69**, 410 (1950); (c) A. Fregda, *Acta Chem. Scand.*, **4**, 1307 (1950); (d) K. Balenović, I. Jambrešić, B. Gašpert, and D. Cerar, *Rec. trav. chim.*, **75**, 1252 (1956); (e) M. Calvin, in "Glutathione, A Symposium," Academic Press, New York, N. Y., 1954, p. 7; (f) O. Foss, in "Organic Sulfur Compounds," N. Kharasch, ed., Pergamon Press, New York, N. Y., 1961, p. 78; (g) J. Strem, Y. S. R. Krishna-Prasad, and J. A. Schellman, *Tetrahedron*, **13**, 180 (1961).

(12) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).

naturally occurring amino acid has the (R) configuration, as does the more radioprotective (D-2-ABT) of the two isothioureas.

Experimental[13]

N-Isocaproyl-DL-2-aminobutyric Acid.—A solution of 103 g. (1 mole) of DL-2-aminobutyric acid in 250 ml. of 4 *N* sodium hydroxide was cooled in ice and 148 g. (1.1 moles) of isocaproyl chloride and 275 ml. of 4 *N* sodium hydroxide added in portions with vigorous shaking over 0.5 hr. Acidification with concd. hydrochloric acid yielded a gum, which crystallized on scratching. The precipitate was filtered, washed with water, and dried. Yield: 182 g. (90%) m.p. 106–110°. Recrystallization from ethyl acetate–petroleum ether raised the m.p. to 111–113°.

Anal. Calcd. for $C_{10}H_{19}O_3N$ (201.3): C, 59.68; H, 9.51; N, 6.97. Found: C, 59.69; H, 9.60; N, 7.11.

N-Isocaproyl-L-2-aminobutyryl Anilide.—The acyl amino acid was resolved essentially by the procedure of Doherty and Popenoe.[7] Finely ground papain was mixed with 100 ml. of 0.1 *M* citrate buffer (pH 5), 150 ml. of water, 2.5 g. potassium cyanide, and adjusted to pH 5 with acetic acid. The mixture was stirred 1 hr. and filtered with suction through a pad of Hyflo filter aid. Isocaproyl-DL-aminobutyric acid, 100.5 g. (0.5 mole) was dissolved in a mixture of 150 ml. of *N* sodium hydroxide, 150 ml. of 0.1 *M* citrate buffer, 270 ml. of 2 *N* sodium acetate, and 45.5 ml. of redistilled aniline, and warmed to 45°. The enzyme solution was added, the mixture made up to 1 l. with water, and incubated at 37°. The anilide, which began crystallizing after 10 min. was filtered off after 24 hr., washed with water, and dried. The filtrate was reincubated and reserved for the isolation of isocaproyl-D-aminobutyric acid. Yield: 68 g. (99%), m.p. 166–168°, $[\alpha]^{23}D$ −67.5°, (*c*, 2%, CH_3CO_2H). The anilide was dissolved in 300 ml. of hot absolute ethanol, treated with Norite, and filtered to remove a small amount of protein, diluted to 1 l. with water, and allowed to crystallize overnight at 0°. Yield: 57 g., m.p. 171–172.5°, $[\alpha]^{23}D$ −70.0° (*c*, 2%, AcOH).

Anal. Calcd. for $C_{16}H_{24}N_2O_2$ (275.37): C, 69.53; H, 8.75; N, 10.10. Found: C, 69.43; H, 8.86; N, 9.91.

L-2-Aminobutyric Acid.—The L-anilide, 56 g., was refluxed 4 hr. in 300 ml. of 20% hydrochloric acid, concentrated *in vacuo* to dryness three times with additional amounts of water to remove excess hydrochloric acid, taken up in 500 ml. of water, and treated with Norite. The colorless filtrate was neutralized by passing through an IR-4B column in the OH^- form,[7] and the eluate wash water concentrated *in vacuo* to 100 ml. The pH was adjusted to 6.6 with acetic acid, 500 ml. of ethanol was added, and the mixture allowed to crystallize overnight at −5°. Yield: 17 g. (81%), $[\alpha]^{23}D$ + 21.0° (*c*, 5%, *N* HCl).[8] Alternatively, the filtrate, after hydrolysis, could be neutralized by Dowex-3 OH^- in a batch process and a similar yield obtained.

Anal. Calcd. for $C_4H_9O_2N$ (103.12): C, 46.59; H, 8.79; N, 13.58. Found: C, 46.70; H, 8.92; N, 13.33.

D-2-Aminobutyric Acid.—The small precipitate that formed in the reincubated anilide filtrate was filtered off after 3 days and the filtrate concentrated *in vacuo* to *ca.* 100 ml. Acidification with cold 2 *N* hydrochloric acid to pH 1.5 yielded 57 g. of crystalline N-isocaproyl-D-2-aminobutyric acid mixed with some denatured protein. Recrystallization from ethyl acetate–petroleum ether gave 35 g. of pure compound. Yield: 70%, m.p. 102–103°, $[\alpha]^{23}D$ + 16.4° (*c*, 5% EtOH). The acyl D-amino acid was hydrolyzed and the amino acid isolated as previously described for the L isomer. Yield: 16 g. (90%), $[\alpha]^{23}D$ −21.0° (*c*, 5%, *N* HCl).

Anal. Calcd. for N-Isocaproyl-D-2-aminobutyric acid, $C_{10}H_{19}O_3N$ (201.25): C, 59.68; H, 9.51; N, 6.97. Found: C, 59.74; H, 9.49; N, 6.98. Calcd. for D-2-aminobutyric acid, $C_4H_9O_2N$ (103.12): C, 46.59; H, 8.79; N, 13.58. Found: C, 46.50; H, 8.95; N, 13.45.

D- and L- 2-Aminobutanols.—The isomeric aminobutyric acids were reduced to the corresponding alcohols by lithium aluminum hydride in tetrahydrofuran according to the method of Vogel and Pöhm[14] and isolated by a modified procedure. Lithium aluminum hydride, 4.2 g. (0.11 mole), was added to 100 ml. of dry tetrahydrofuran (dried over sodium hydride and distilled from lithium aluminum hydride) in a 200-ml., three-necked flask fitted with a stirrer and condenser. The mixture was cooled to 0°, 10.3 g. (0.1 mole) of 2-aminobutyric acid was added in 1-g. portions over 1 hr. and, following the last addition, the mixture was refluxed for 6 hr. The mixture was cooled in ice, diluted with an equal volume of ether, and the excess hydride decomposed by the cautious addition of a few drops of water. The precipitate was filtered, dried, and added to 20 ml. ice-cold 40% sodium hydroxide. The resulting solution was extracted with three 50-ml. portions of ether, and the combined extracts dried over sodium hydroxide pellets and distilled through a 50-cm. vacuum-jacketed, Vigreux column. Yield: 6.2 g. (69%), b.p. 178° (740 mm.),[9] D $[\alpha]^{21}D$ −10.0°, L $[\alpha]^{21}D$ +10.2° (neat).

Resolution of DL-2-Aminobutanol.—The procedure of Radke *et al.*,[9] gave a good yield of the D-2-aminobutanol L-tartrate, which was readily converted to the free D-2-aminobutanol. Resolution with L-glutamic acid, however, did not follow the exact course reported by these authors. Copious seeding of three separate batches of the resolution mixture with pure D-2-aminobutanol L-glutamate, as recommended, over a 3-week period failed to yield any precipitate. However, seeding with pure L-2-aminobutanol L-glutamate obtained *via* the 2-aminobutyric acid route gave an immediate and nearly quantitative precipitation of the L-2-aminobutanol L-glutamate.

DL-, D-, and L-2-Aminobutylbromide·HBr.—Two methods were used for the preparation of the aminoalkyl bromides.

Method A.—A mixture of 34 g. (0.2 mole) of 2-aminobutanol and 250 ml. of dry chloroform was cooled in an ice bath in a 500-ml. round bottom flask and 46 g. (0.22 mole) of thionyl bromide added dropwise with stirring. After it was allowed to stand overnight at room temperature, the mixture was evaporated *in vacuo* to dryness, taken up in absolute ethanol, and precipitated with ethyl acetate. Yield: 39.6 g. (85%).

Method B.—2-Aminobutanol, 34 g. (0.2 mole), was added dropwise to 160 ml. of 48% hydrobromic acid in a 300-ml. three-necked round-bottom flask fitted with a stirrer and short Vigreux column and cooled in an ice bath. Upon completion, the solution was refluxed and slowly distilled, 80 ml. being collected in 3 hr. This was replaced with 80 ml. of 48% hydrobromic acid and the distillation continued for an additional 3 hr. The resultant solution was evaporated *in vacuo* to dryness and the product recrystallized from an absolute ethanol–ethyl acetate mixture. Yield: 37 g. (79%), DL- m.p. 188–189°.

Anal. Calcd. for $C_{10}H_{11}NBr_2$ (232.97): C, 20.63; H, 4.76; N, 6.01. Found: C, 20.69; H, 4.58; N, 6.18. D-, M.p. 185–187°, $[\alpha]^{22}D$ −5.08° (*c*, 2% abs. EtOH). Found: C, 20.61; H, 4.59; N, 6.05. L-, M.p. 181–183°, $[\alpha]^{22}D$ +5.06° (*c*, 2% abs. EtOH). Found: C, 20.60; H, 4.82; N, 6.15.

DL-, D-, and L-2-Aminobutylisothiourea·Di·HBr.—Two procedures were used to prepare these compounds. For the DL isomer only one crystalline form was obtained by either method while the D and L isomers both gave polymorphic crystal forms by either method. Initially only the lower melting form was obtained, subsequent preparations yielded the higher melting form.

Method A.—Thiourea, 7.6 g. (0.1 mole), was mixed with 12 ml. of absolute ethanol, and 50 ml. of ethyl acetate and heated on a steam bath to boiling. DL-2-Aminobutylbromide·HBr, 23.3 g. (0.1 mole), was added and the mixture refluxed 1 hr. Complete solution occurred, followed by precipitation of an oil in 10–20 min. Crystallization was induced by scratching with a glass rod and, after short cooling in an ice bath, the precipitate was filtered off, washed with ethyl acetate, and dried *in vacuo*. Yield: 25 g. (81%), 170–172°. It was recrystallized by solution in a minimum amount of hot absolute ethanol, dilution with four volumes of isopropyl alcohol, and cooling to 0°. Yield: 21.6 g., m.p. 177–179°. The same procedure with the optically active halides using half quantities of all components yielded 9–11 g. of crude product.

Method B.—Thiourea, 7.6 g. (0.1 mole), was dissolved in 60 ml. of hot isopropyl alcohol, 23.3 g. (0.1 mole) of DL-2-aminobutylbromide·HBr added, and the solution refluxed 1 hr. on the steam bath. Ethyl acetate, 30 ml., was added to the hot solution, the mixture seeded, cooled to 0°, and the precipitate treated as in A. Yield: 22 g., m.p. 170–173°. Recrystallization gave 19.5 g., m.p. 177–179°. On a one-half scale using the optically active halides, this procedure yielded 9–11 g. of crude product.

Anal. Calcd. for $C_5H_{15}N_3SBr_2$ (309.11): C, 19.43: H, 4.89; N, 13.59; S, 10.37. DL-, Found: C, 19.59; H, 4.84; N, 13.52; S, 10.50. Low melting form D-crude, m.p. 152–154°; recrystal-

(13) All melting points in capillary tube were uncorrected. Analyses by Galbraith Laboratories, Knoxville, Tenn.

(14) O. Vogel and M. Pöhm, *Monatsh. Chem.*, **83**, 541 (1952).

lized, 157–158°. Found: C, 19.49; H, 4.94; N, 13.33; S, 10.39. L-crude, m.p. 148–150°; recrystallized, m.p. 155–157°. Found: C, 19.30; H, 4.70; N, 13.43; S, 10.32. High melting form D-crude, m.p. 177–179°; recrystallized, m.p. 183–185°. Found: C, 19.34; H, 5.00; N, 13.40; S, 10.58. L-crude, m.p. 176–179°; recrystallized, m.p. 184–186°. Found: C, 19.35; H, 4.80; N, 13.50; S, 10.45.

The optical rotations of the polymorphic forms of the L and D isomers are identical: D- $[\alpha]^{22}D$ −12.5° (*c*, 5% 0.2 *M* HCl). Converted to the mercaptoguanidine by the addition of sodium hydroxide to pH 8.0, the rotation is $[\alpha]^{22}D$ +7.50° (*c*, 2%, 0.4 *M* phosphate buffer pH 8.0). Correspondingly, the L isomer in acid gives a rotation of $[\alpha]^{22}D$ +12.0° (*c*, 5% 0.2 *M* HCl), and as the mercaptoguanidine $[\alpha]^{22}D$ −7.3° (*c*, 2% 0.4 *M* phosphate buffer pH 8.0).

D- and L-2-Guanidobutanethiol Flavianate.—D-2-Aminobutylisothiourea (1.1 g.) was dissolved in 5 ml. of water, and the pH brought to 8.0 by the addition of 3.4 ml. of sodium hydroxide. The addition of 3.5 ml. of 1 *M* flavianic acid precipitated a yellow gum, which crystallized upon scratching with a glass rod. The product was filtered, dried (1.3 g.), and recrystallized from 10 ml. of hot 50% ethanol; 1.1 g., m.p. sinter 115°; melts 128–30°. The L-flavianate was prepared in a similar fashion, and had the same melting point.

Anal. Calcd. for $C_{15}H_{19}N_5O_8S_2$ (461.48); C, 39.04; H, 4.15; N, 15.18; S, 13.89. Found: D-, C, 39.16; H, 3.97; N, 15.09; S, 13.61. L-, C, 38.90; H, 4.08; N, 15.06; S, 13.70. D-, $[\alpha]^{22}D$ +6.6°; L-, −6.2° (*c*, 0.5% water).

Bis-DL-, D-, and L-(2-Guanidobutyl) Disulfide Dihydrobromide.—A solution of DL-2-aminobutylisothiourea dihydrobromide, 15.5 g. in 50 ml. of water, was immediately converted to 2-guanidobutanethiol by the addition of 50 ml. of 1 *N* sodium hydroxide, the pH adjusted to 9.0, a few milligrams of cupric chloride added and oxygen bubbled through until the nitroprusside test was negative (*ca.* 3–4 hr.). The solution was acidified to pH 4 with hydrobromic acid, evaporated to dryness *in vacuo*, and the solid extracted with absolute ethanol. Ether was added to the ethanol extract to turbidity, and the mixture allowed to crystallize at −5°. Yield: 8.2 g. (72%), m.p. 173–175°. Recrystallization from absolute ethanol-acetone raised the melting point to 180–182°. The D and L disulfides were obtained in a similar manner.

Anal. Calcd. for $C_{10}H_{26}N_6S_2Br_2$ (454.34): C, 26.43; H, 5.77; N, 18.50; S, 14.11. Found: DL-, C, 26.22; H, 5.74; N, 18.23; S, 13.95. D-, M.p. 183–184°, $[\alpha]^{22}D$ −198°. Found: C, 26.66; H, 5.75; N, 18.21; S, 14.24. L-, M.p. 183–185°, $[\alpha]^{22}D$ +200°. Found: C, 26.71; H, 5.90; N, 18.30; S, 13.90.

DL-, D-, and L-2-Amino-4-ethylthiazoline Hydrobromide.—The thiazolines were prepared by the method of Gabriel.[15] For the corresponding aminoalkyl bromide hydrobromide, 11 g. (0.05 mole) was mixed with 5.0 g. (0.05 mole) potassium thiocyanate and 25 ml. water in an evaporating dish and heated on the steam bath overnight. The residue was extracted with 100 ml. hot isopropanol, potassium bromide filtered off and the filtrate evaporated *in vacuo* to dryness. The crystalline product was removed with ethyl acetate, filtered, and dried. Yield: 8 g. (75%), DL-, m.p. 102–104°.

Anal. Calcd. for $C_5H_{11}N_2S$ Br (211.14): C, 28.44; H, 5.25; N, 13.27; S, 15.19. Found: C, 28.46; H, 5.06; N, 13.12; S, 15.39. D-, M.p. 121–22°, $[\alpha]^{21}D$ +26° (*c*, 2% H_2O). L-, M.p. 121–122.5°, $[\alpha]^{21}D$ −25.8° (*c*, 2%, H_2O). Found: C, 28.54; H, 5.19; N, 13.10; S, 15.35.

The DL thiazoline yielded a flavianic acid salt identical with that obtained by Khym, *et al.*,[4] from the rearrangement of the isothiourea in acid solution.

(15) S. Gabriel, *Ber.*, **22**, 1141 (1889); G. W. Raiziss and W. C. Le Roy, *J. Am. Chem. Soc.*, **63**, 3124 (1941); A. Schoberl, R. Hamm, and M. Kawohl, *Chem. Ber.*, **84**, 571 (1951).

Aroyldiazoacetic Esters. II. Synthesis with Anhydrous Methyl Diazoacetate. Hydrolysis of Aroyl Halides in 96% Methyl Diazoacetate[1-4]

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Preparation of aroyldiazoacetic esters by direct interaction of aroyl halides and methyl diazoacetate (I) is a general procedure if I is anhydrous. There is described a procedure for drying I azeotropically with *n*-pentane, which permits quantitative estimation of water present. Interaction of aroyl chlorides with 96% methyl diazoacetate resulted in hydrolysis products, either alone or together with the aroyldiazoacetic ester. A bimolecular hydrolysis of aroyl chlorides in 96% methyl diazoacetate is proposed. A new mechanism for aroyldiazoacetic ester formation is postulated.

The reaction of acyl halides with diazoacetic esters to give acyldiazoacetic esters is well known. However, benzoyl bromide appears to be the sole example of an *aroyl* halide undergoing this reaction.[6] The heterocycles, furoyl bromide and chloride, react with methyl diazoacetate (I) to give methyl (α-furoyl)diazoacetate in 80% and unstated yields, respectively.[7] Our attempts to extend this reaction led to carboxylic anhydrides and an O-aroylglycollate as products.[8] The present paper describes a procedure for drying I azeotropically, reaction of anhydrous I with several aroyl halides to give crystalline aroyldiazoacetic esters, and reaction of I containing known amounts of water with aroyl halides.

The products obtained from interaction of methyl diazoacetate with aroyl chlorides in our previous study indicated water to be present in I. In the preparation of I by the procedure of Womack and Nelson,[9] the effect of certain operations on the water content of the product diazoacetic ester had not been previously determined. We report that I can be dried azeotropically with *n*-pentane, and the azeotrope collected in a modification of the distilling head used in the Dean–Stark procedure[10] to afford a direct measure of water present. The modification substitutes a capillary tube of known cross section for the usual graduated test tube of the

(1) Paper I: J. H. Looker and D. N. Thatcher, *J. Org. Chem.*, **22**, 1233 (1957).

(2) From the Ph.D. thesis of Charles H. Hayes, University of Nebraska, 1959.

(3) Presented before the 138th National Meeting of the American Chemical Society, New York, N. Y., September, 1960.

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(5) Dow Chemical Company Fellow, 1955–1956.

(6) H. Staudinger, J. Becker, and H. Hirzel, *Ber.*, **49**, 1978 (1916).

(7) T. Reichstein and H. J. Morsman, *Helv. Chim. Acta*, **17**, 1119 (1934).

(8) J. H. Looker and D. N. Thatcher, *J. Org. Chem.*, **23**, 403 (1958). This paper contains references to extensive review articles on aliphatic diazo chemistry.

(9) E. B. Womack and A. B. Nelson, *Org. Syn.*, **24**, 56 (1944).

(10) The unmodified Dean–Stark distilling receiver is available from E. H. Sargent & Co., and is listed as a water trap for determination of water in petroleum products in accordance with A.S.T.M. method D-95.