α-Bromo-β-(5-imidazolyl)propionic Acid and Its Reaction with Cysteine*

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ABSTRACT: D,L-α-Bromo-β-(5-imidazolyl)propionic acid (BIP) was prepared by diazotization of D,L-histidine in 48% HBr. The reagent, isolated as the monohydrate, can be converted to histidine by ammonolysis or to imidazolylpropionic acid by treatment with Raney nickel. The apparent pK_a values of the carboxyl and imidazole groups of BIP are 2.2 and 7.0, respectively. BIP reacts with L-cysteine at 25° ($\bar{k}_2$ at pH 8.0, 3.6×10^{-3} l. mole^{-1} sec^{-1}) to produce a pair of diastereoisomeric new amino acids which are both Pauly and ninhydrin positive. The new amino acids were desulfurized with Raney nickel to produce alanine and imidazolylpropionic acid, demonstrating the new amino acids arise from a simple displacement reaction. The new amino acids (D- and L-α-(*S*-L-cysteinyl)-β-(5-imidazolyl)propionic acids) appear as twin peaks in the region of tyrosine and phenylalanine in a standard amino acid analysis. The chromatographic mobility of the new amino acids like that of cystine is especially sensitive to small changes of pH in the eluting buffer. The study suggests the reagent may be useful for chemical modifications of proteins.

Although the imidazole function of histidine is the most frequently implicated of amino acid side chains in the catalytic action of enzymes, simple alkylating agents for the introduction of imidazoles into proteins are virtually nonexistent. Reagents with this capability have potential as tools for (a) exploration of cooperative functions of catalytic groups, (b) investigation of enzymes of histidine metabolism, and (c) introduction of new or altered metal binding sites into proteins for crystallographic study.

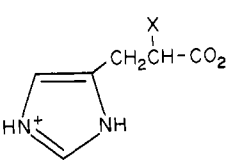
Several simple reagents were designed and prepared to pursue these goals. One of these reagents, α-bromo-β-(5-imidazolyl)propionic acid (BIP)[1] showed particular promise. The purpose of the present paper, therefore, is to characterize this reagent and its reaction with cysteine. The paper which follows (Jolley and Yankeelov, 1972) will describe the use of BIP in specifically introducing a second imidazole function at the active site of papain.

Materials and Methods

D,L-Histidine (free base) was obtained from the Sigma Chemical Co. Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid)) was obtained from Calbiochem. Raney nickel was obtained from W. R. Grace and Co., Raney Catalyst Division, Chattanooga, Tenn., and was used as an aqueous suspension (0.6 g/ml). Hydrobromic acid (Matheson Coleman & Bell) was redistilled before use. Nitrogen (prepurified) was from Matheson. All other reagents were of analytical grade. Distilled, deionized water was used throughout the study.

Paper chromatography (ascending) was performed using Whatman No. 1 paper, while thin-layer chromatography (tank type) employed glass plates coated with cellulose. Butanol–acetic acid–water (12:3:5, v/v) was the developing solvent in both systems. Ninhydrin and Pauly stains were prepared according to Smith and Birchenough (1960). Melting points were taken on a Fisher-Johns hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Infracord, Model 137. Microanalyses were performed by Schwartzkopf Microanalytical Laboratories, Woodside, N. Y., and Galbraith Laboratories, Inc., Knoxville, Tenn. The potentiometric titration was performed under nitrogen using a Radiometer Type 4c meter equipped with a GK-2021 electrode and is corrected for solvent blank. Amino acid analyses were performed with an NC-1 Technicon amino acid analyzer using a 21-hr separation. Sulfhydryl groups were determined by the method of Ellman (1959), but using pH 7.0 instead of pH 8.0 buffer. Measurements on a standard solution of cysteine at pH 7.0 revealed the color yield was within 1% of that given for pH 8.0 (ϵ_{412} 13,600).

D,L-α-*Chloro-β-(5-imidazolyl)propionic Acid* (*Ia*). This compound was prepared from D,L-histidine according to the method of Edlbacher and von Bidder (1942). This compound (mp 190–192°) which was chromatographically homogenous on paper (R_F 0.48) was used as a pilot compound in the study.



I_a, X=Cl
I_b, X=Br

D,L-α-*Bromo-β-(5-imidazolyl)propionic Acid* (*Ib*). D,L-Histidine (free base, 15.2 g, 0.097 mole) was dissolved in 220 ml of 48% HBr. The solution was chilled in an ice-salt bath to −5°. Aqueous sodium nitrite (20 g, 0.29 mole in 40 ml of water)

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[1] Abbreviations used are: DTNB or Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid); CIP, D,L-α-chloro-β-(5-imidazolyl)propionic acid; BIP, D,L-α-bromo-β-(5-imidazolyl)propionic acid.

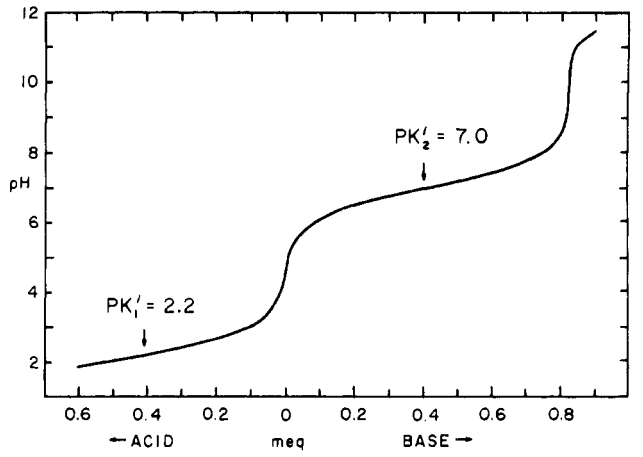


FIGURE 1: Potentiometric titration of α-bromo-β-(5-imidazolyl)-propionic acid monohydrate. The titration was performed under nitrogen on 0.1924-g (0.8118 mequiv) samples of the monohydrate in 0.1 N KCl. The data are corrected for solvent blank.

was added dropwise over a period of 75 min during which time the temperature of the mixture was not permitted to rise above 5°. After the addition was complete, stirring was continued for 60 min at 0°. The dark solution was concentrated by rotary evaporation at reduced pressure without exceeding 58° to produce a yellow oil containing a white precipitate. Higher temperatures resulted in decomposition. The concentrate was extracted with acetone (100 ml in four portions) and the residue of salt was discarded. The acetone extract was concentrated to an oil under a stream of nitrogen at 36°. Additional salt separated and was removed by centrifugation. The oil (crude hydrobromide of Ib) was dissolved in 50 ml of water and again concentrated to ensure removal of excess HBr. Water (80 ml) was added to the concentrate and the pH was adjusted to 4.6 with cold 2 N NH_4OH. The solution was decolorized with Norit and evaporated to dryness at 50°. The residue was dissolved in six parts water (w/v) at 60° and then stored at 4°. The product, which crystallized in the form of igloos, was dried over P_2O_5 at 25° under vacuum at 25 μ: yield, 10.5 g (46%) of Ib as the monohydrate; mp 108–111°; λ_{max}^{KCl} 6.20, 7.22 μ. The product migrated as a single, Pauly-positive component (R_F 0.53) in paper chromatography. *Anal.* Calcd for $C_6H_7BrN_2O_2 \cdot H_2O$: Br, 33.71; C, 30.40; H, 3.83; N, 11.82. Found: Br, 33.74; C, 30.32; H, 3.92; N, 11.69.

α-Bromo-β-(5-imidazolyl)propionic Acid Hydrobromide. This compound was less stable than the monohydrate of Ib and was prepared for characterization purposes only. A sample of analytical quality Ib (mp 108–111°) was dissolved in a minimal amount of 48% HBr. The solution, which was stored at −20°, slowly deposited stout colorless prisms. The crystals were collected on a glass frit and washed quickly with chilled acetone (−80°). The sample was dried without delay at 55° over P_2O_5 under vacuum (20 μ): mp 138–139°, λ_{max}^{KCl} 3.53 μ. *Anal.* Calcd for $C_6H_8Br_2N_2O_2$: Br, 53.28; C, 24.03; H, 2.69; N, 9.34. Found: Br, 51.66; C, 24.36; H, 3.01; N, 9.22.

β-(5-Imidazolyl)propionic Acid. A 1.0-g sample of Ib monohydrate was dissolved in 100 ml of water. A 10-ml portion of an aqueous suspension of Raney nickel was compacted by centrifugation and the supernatant discarded. The nickel was transferred to the solution of BIP and mechanically shaken for 15 min. The suspension was filtered and the light green filtrate was passed through a 3.6 × 3.3 cm column of Dowex A-1 chelating resin. The column was irrigated with deionized water at 4–5 ml/min. The nickel was visibly retained on the first 0.5 cm of the column. The eluate (130 ml) was lyophilized to give 0.67 g of a white powder. The sample was desalted on a column of Dowex 2-X8 resin according to Dréze *et al.* (1954) and then lyophilized several times from water to give 0.298 g (50%) of product: mp 210–212° (lit. (Kraml and Bouthillier, 1955) mp 204–206°). For analysis the sample was allowed to crystallize slowly from ethanol–ether–water (24:11:8, v/v) and dried *in vacuo* over P_2O_5. *Anal.* Calcd for $C_6H_8N_2O_2$: C, 51.41; H, 5.76; N, 19.99. Found: C, 51.54; H, 5.70; N, 19.87.

Results

BIP is a reagent which contains both a powerful nucleophile (imidazole) and a good leaving group (bromide). Successful synthesis of this reagent was projected on its acidic mode of preparation and stability of the neutral reagent anticipated on the basis of its zwitterionic character. It was necessary, however, to test the validity of these assumptions by (a) firmly establishing the structure of the reagent, (b) evaluating the importance of possible polymerization reactions, and (c) determining the behavior of the reagent with free amino acids, before applying the reagent in the study of proteins.

Conversion of D,L-*α-Bromo-β-(5-imidazolyl)propionic Acid to* D,L-*Histidine.* BIP monohydrate was dissolved in 4 ml of 10 N NH_4OH and heated in a water bath for 1 hr at 50°. The solution was evaporated to dryness and the residue dissolved in 2 ml of 10% isopropyl alcohol. Paper chromatography showed a ninhydrin-positive spot which was indistinguishable in mobility from histidine. When examined by automatic amino acid chromatography the product was found to be eluted with authentic histidine[2] and to be formed in 51% yield based on the amount of BIP taken as substrate. Thus, ammonolysis of BIP to produce histidine and hydrogenolysis of BIP to produce β-(5-imidazolyl)propionic acid, both in fair yield, establishes the correctness of structure Ib (Reaction Scheme I).

Question of Stability. BIP was found to suffer only slow decomposition in basic solution. When an aqueous solution of BIP was maintained at pH 10.0 for 4 hr at 21° in the absence of added salt only limited decomposition occurred. Paper Chromatography revealed, in addition to the major spot of the parent compound, three Pauly-positive materials with R_F values of 0.00, 0.26, and 0.39. This surprisingly slow rate of decomposition suggested the feasibility of a potentiometric titration.

Potentiometric Titration of D,L-*α-Bromo-β-(5-imidazolyl)-Propionic Acid Monohydrate.* The titration curve of BIP monohydrate is shown in Figure 1. The titration was performed under nitrogen in 0.1 N KCl on 0.1924-g (0.8118 mequiv) samples of the monohydrate using standardized 0.1 N NaOH and 0.1 N HCl as titrants. The data are corrected for solvent blank. Apparent pK_a values of 2.2 and 7.0 were obtained corresponding to the carboxyl and imidazolyl groups, respectively. These values reflect the inductive effect of the α-bromine in lowering the pK's, since the corresponding values for imidazolylpropionic acid are 4.0 and 7.55 (Pasini and Vercellone, 1955). The equivalent weight derived from the basic leg of the titration curve was 234, which was in good agreement with the theoretical value of 237 for the mono-

[2] A side product, eluted 20 min prior to histidine and 4–5% of its area, was not investigated further.

hydrate of Ib. The latter result would appear to rule out significant polymerization during the course of the titration.

Reactivities of CIP and BIP. The behavior of BIP and CIP with individual amino acids containing functional side chains (cysteine, methionine, lysine, serine, histidine, tyrosine, and tryptophan) was examined in a preliminary way by incubating solutions 0.1 M in each reactant at pH 8.0, 25° for 4 hr. Paper chromatography revealed that cysteine incubated with BIP was converted to a ninhydrin- and Pauly-positive product (R_F 0.16) over the course of 2 hr while CIP failed to react. Subsequent exposures of standard amino acids to BIP at pH 8.0, 25° and pH 6.0, 37° followed by automatic chromatography failed to demonstrate a reaction with other amino acids.

Kinetics of the Reaction of D,L-*α-Bromo-β-(5-imidazolyl)-propionic Acid with* L-*Cysteine.* L-Cysteine·HCl·0.5H_2O (0.668 g, 4 mequiv) was dissolved in 15 ml of nitrogen-purged water. The pH was adjusted to 8.0 with 2 N NaOH. Nitrogen-saturated water was used to bring the volume to 20 ml. BIP monohydrate (0.9482 g, 4 mequiv) was dissolved in 15 ml of water. After pH adjustment to 8.0 with NaOH, the solution was brought to a volume of 20 ml and purged with nitrogen. Care was taken to prepare the BIP solution just prior to reaction. The reaction was performed in a jacketed vessel maintained at 24.8 ± 0.2° in the following way. Ten milliliters of the cysteine solution (0.2 M) was placed in the reaction vessel under a constant stream of nitrogen. To initiate the reaction 10.0 ml of the BIP solution (0.2 M) was added with magnetic stirring. Nitrogen flushing and stirring was continued throughout the duration of the experiment. The pH was maintained at 8.0 by addition of 2 N NaOH by means of polyethylene tubing (Clay-Adams 240) coupled to a buret and pulled to an inverted delivery tip. At specified intervals aliquots (0.1 ml) were removed and diluted with 10 ml of ice-chilled phosphate buffer (pH 7.0) otherwise prepared according to Ellman (1959). An aliquot (0.5 ml) of this primary dilution was further diluted with 4.5 ml of the same buffer at room temperature. Three milliliters of the resulting solution was mixed with 0.02 ml of Ellman reagent and the maximal absorbance at 412 nm was recorded. A plot of the reciprocal of the absorbance at reaction time t less the reciprocal of the absorbance at reaction time zero was made against time. These data are shown in Figure 2. The lower curve shows the plot obtained when the experiment was repeated omitting BIP from the reaction vessel. From the data of Figure 2 and the $\epsilon_{412\ nm}$ of 13,600 for the DTNB (Ellman, 1959) the apparent second-order rate constant for the reaction of BIP with L-cysteine at 25° was found to be 3.6×10^{-3} l. $mole^{-1}$ sec^{-1}. The reaction follows second-order kinetics to about 90 min or two-thirds completion. Curvature detected in the latter phases of reaction may be due to progressive changes in ionic strength or side reactions undetectable in the early phases (see below).

Automatic Chromatography of New Amino Acids and Estimation of Ninhydrin Constant. Amino acid analyses of the reaction mixture of BIP (0.1 M) and L-cysteine (0.1 M) revealed that up to 90-min reaction, the only ninhydrin-positive products present in the chromatogram were cysteine, cystine, and a pair of diastereoisomeric new amino acids. The kinetic behavior of the reaction discussed above is also consistent with this observation. In addition it was found that the reaction could be quenched effectively by the addition of 0.1 N HCl. This was established by the absence of new amino acids from the chromatogram when an identical reaction mixture was quenched at time zero. These facts permitted the estimation of a ninhydrin constant through correlation of cysteine loss (by Ellman determination), with the total area of the doublet due to the new amino acids present in the corresponding chromatographic analysis. The quantity of cysteine converted to product for a given determination was obtained as a difference between the sulfhydryl titer at zero time and that of the quenched aliquot at 60 min.

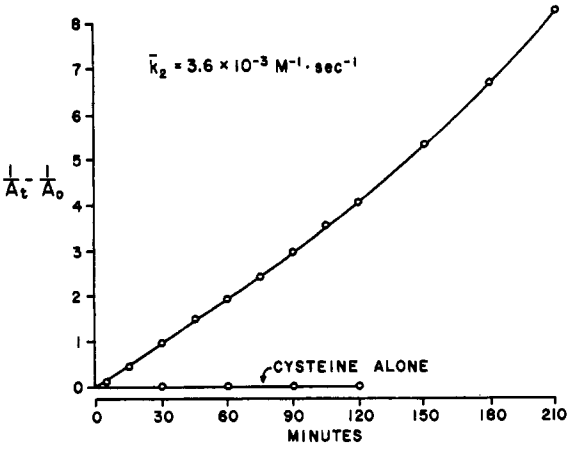


FIGURE 2: Second-order kinetic plot of reaction of 0.1 M BIP with 0.1 M L-cysteine (upper curve). A_0 and A_t represent the absorbance at 412 nm in assay by DTNB. The reaction was performed under nitrogen and the sulfhydryl content of the solution monitored as described in text. The corresponding plot for cysteine behavior under the same conditions but in the absence of BIP is also shown (lower curve).

Conditions for the BIP–L-cysteine reaction were identical with those described above for the rate constant determination. Oxidation of cysteine during the reaction was insignificant (Figure 2). Sulfhydryl groups were determined immediately on the fresh dilutions. Acid-quenched samples were stored at −20° and chromatographed within 24 hr.

At specified times (60–90 min) from initiation of reaction 1.0 ml of the mixture was removed and diluted with 10 ml of cold 0.1 N HCl. For Ellman determination 1.0 ml of this primary dilution was further diluted with 10 ml of the pH 7.0 phosphate buffer described above. An aliquot (0.5 ml) of this secondary solution was further diluted with a mixture of 4.5 ml of pH 7.0 phosphate buffer and 5.0 ml of water. Three milliliters of the final dilution was treated with 0.02 ml of DTNB and maximal absorbance at 412 nm was used to calculate the cysteine content of the quenched samples. For amino acid analysis 0.2 ml of the above primary dilution was treated with 0.2 ml of a 2.5 mM solution of Technicon norleucine and 0.1 ml of 0.1 N HCl. An aliquot of this mixture (0.2 ml) was applied to the 130-cm column. The integrated area (HW) for the doublet was divided by the micromoles of product present as determined by the loss of sulfhydryl titer. Two determinations performed in this manner gave average color values of 0.77 and 0.81 relative to the ninhydrin constant of norleucine (or leucine) for the pair of new amino acids.

The position in the chromatogram for these amino acids relative to norleucine using the recommended standard gradient for the 130-cm column is given in Figure 3. These amino acids, which are structurally related to cystine in having two basic and two acidic groups (structure II), are, like cystine, especially sensitive in their chromatographic mobility to pH changes in the eluting buffers. Thus, while the new amino acids are eluted with tyrosine and phenylalanine in a standard chromatogram, by filling chambers 1–3 of the

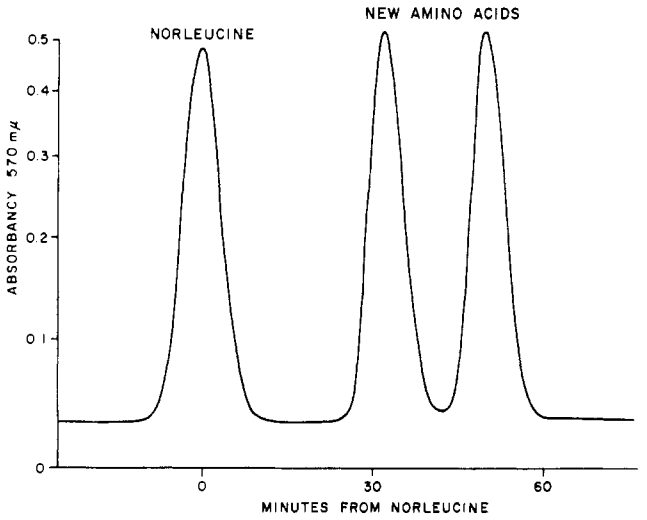


FIGURE 3: Chromatographic behavior of diastereoisomeric new amino acids. Sample was prepared under conditions identical with that of Figure 2. After 60-min reaction a sample was withdrawn and diluted with buffer, HCl, and norleucine as described in text. The sample was applied to the 130-cm column of the amino acid analyzer and eluted under standard conditions.

Autograd with pH 2.88 buffer and chambers 4–9 with pH 5.0 buffer, the new amino acids are eluted in the vacant region just prior to tyrosine.[3]

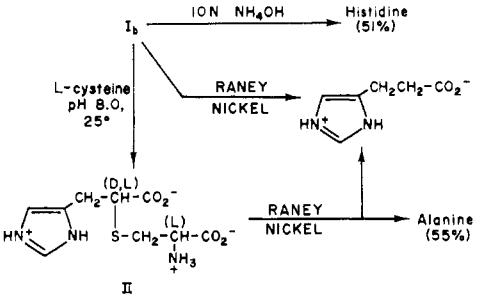
Large-Scale Preparation of D,L-α-(*S*-L-*Cysteinyl*)-β-(*5-imidazolyl*)*propionic Acid.* These diastereoisomers were prepared under conditions identical to those described for the kinetic measurements, but using 10 mmoles of each reactant. The reaction was allowed to proceed for 5 hr while adding 2 N NaOH to maintain pH. Total base uptake was 2.32 ml (4.64 mequiv). The pH of the mixture was lowered to 1.0 with HCl. The volume was brought to 250 ml using 0.1 N HCl as diluent and the solution was stored frozen prior to desalting and chromatography.

A 50-ml portion of the above solution was diluted with an equal volume of water and placed on a 3.2 × 1.8 cm column of Dowex 2-X8 resin and desalted according to Dréze *et al.* (1954). The acetic acid eluate was lyophilized and the product examined by thin-layer chromatography. Two ninhydrin-positive spots were seen: a major component of R_F 0.20 and a minor component of R_F 0.13.

A 148-mg sample of the desalted material in 3.0 ml of water was applied to a 2.9 × 68 cm column of Bio-Gel P-2. The sample was eluted with water at a flow rate of 27 ml/hr and fractions of 3.5 ml were collected. Fractions 68–82 contained the ninhydrin-positive material. Each of these fractions was examined by thin-layer chromatography. Fractions 68–76 contained only a single component corresponding to the new amino acids. In contrast, fractions 77–82 showed the presence of increasing amounts of a second, slower moving component corresponding to the impurity. Fractions 68–74 were combined and lyophilized to give 51 mg of a white powder. The ash content of the sample was found to be 1.1%. Amino acid

[3] The observation that the two diastereoisomers produced are resolvable by ion-exchange chromatography emphasized the desirability of preparing the enantiomeric forms of BIP. Attempts to prepare optically active reagents directly from D- or L-histidine, however, were not successful. This observation is in accord with those of Fargher and Pyman (1921) who obtained D,L-α-chloro-β-(5-imidazolyl)propionic acid from L-histidine.

REACTION SCHEME I



analysis of a weighed sample of this material gave a color yield for the new amino acids of 0.72 times that of norleucine indicating that the sample was approximately 90% pure and therefore quite satisfactory for structural studies.

Both Sephadex G-10 and Bio-Gel P-2 chromatography were used in tandem in an attempt to prepare a sample of analytical quality. The elemental analysis was as follows. *Anal.* Calcd for $C_9H_{13}N_3O_4S$: C, 41.69; H, 5.05; N, 16.21; S, 12.37. Found: C, 40.26; H, 5.28; N, 15.01; S, 11.96; ash, 0.0.

Reductive Cleavage of D,L-α-(*S*-L-*Cysteinyl*)-β-(*5-imidazolyl*)*propionic Acid.* A 0.3-ml aliquot of a Raney nickel suspension (0.6 g/ml) was compacted by centrifugation and the supernatant discarded. To the catalyst was added 1.5 ml of water containing 6.5 mg (25.1 μmoles) of the purified new amino acids. A control mixture was prepared substituting cysteine for the new amino acids. The suspensions were agitated at 25° for 30 min after gas evolution had ceased. The nickel was removed by centrifugation and the supernatants were examined by thin-layer chromatography. Each Raney nickel treated sample produced a strong ninhydrin-positive spot of R_F 0.40 which was indistinguishable from authentic alanine. Automatic amino acid chromatography revealed that the ninhydrin-positive component released from the new amino acids was eluted with authentic alanine and was produced in 55% yield based on the weight of new amino acids subjected to reduction.

To identify the imidazole-containing fragment of the molecule, reduction of the new amino acids was repeated using 30% more Raney nickel and limiting the total time of agitation to 10 min. Thin-layer chromatography of the supernatant revealed a strong Pauly-positive spot of R_F 0.56 which was indistinguishable in mobility and staining characteristics from authentic β-(5-imidazolyl)propionic acid. Some residual starting material with an R_F of 0.16 was observed in the chromatogram under these more limiting conditions.

The results of these desulfurization reactions, as diagramed in Reaction Scheme I, establish the correctness of the skeletal structure assigned to these diastereoisomers, in spite of the somewhat less satisfactory elemental analysis.

Stability of New Amino Acids under Hydrolytic Conditions. A stock solution of 4.02 mM isomeric new amino acids containing 4.19 mM norleucine was prepared. One milliliter of this stock solution and 1.0 ml of 12 N HCl were added to each of a series of hydrolysis tubes. The samples were evacuated, sealed, and maintained at 110° (as for standard protein hydrolysis) for specified intervals. After removal of acid by rotary evaporation, each sample was diluted with 0.1 N HCl and subjected to amino acid analysis at the 0.2-μmole level for norleucine. Since norleucine is completely stable to acid

hydrolysis (Walsh and Brown, 1962), the percentage recovery of the new amino acids after any given interval of exposure was determined by the ratio of the integrated area of the doublet to the area of the norleucine. A semilogarithmic plot of the data (not shown) is approximated by the equation: $\log C_t/C_0 = -1.8 \times 10^{-3}t$, where t is time at 110° in hours. C_t and C_0 are the corrected concentrations of new amino acids at time t and time zero, respectively. The data indicate that under acid hydrolytic conditions, the new amino acids are comparable in their stability to serine.

Discussion

Although some successful preparations of protein reagents derived from basic amino acids have been reported (Liu, 1967; Shaw and Glover, 1970), the design of these reagents is complicated by the presence of nucleophilic and leaving groups within the same molecule. The low pK_a and reactivity of imidazole presents particular challenge in the preparation of imidazole-containing alkylating agents. Polymerization and cyclization reactions are potential deterrents which might thwart the preparation of such agents or seriously complicate their use.

The present study demonstrates that the essential alkylating character of bromoacetic acid is retained when the α-carbon atom bears an imidazolylmethyl substituent. BIP was prepared under strongly acidic conditions to minimize the nucleophilicity of the imidazole function. The reagent is isolated most conveniently as the monohydrate which owes its stability to its zwitterion structure. Although the chloro analog (CIP) is unreactive, BIP reacts selectively[4] with L-cysteine to produce a pair of diastereoisomeric new amino acids: D- and L-α-(*S*-L-cysteinyl)-β-(5-imidazolyl)propionic acids which are identifiable as twin peaks on the amino acid analyzer. The chemical studies presented here can leave little doubt as to the correctness of either the structure of the reagent or the new amino acids derived from its reaction with cysteine.

The use of BIP in synthetically introducing a second imidazole at the active site of papain demonstrates the potential of this reagent in the study of proteins (Jolley and Yankeelov, 1972). BIP and related compounds may have possible uses in medicinal chemistry as inhibitors of histidine decarboxylase (Pages and Burger, 1966).

[4] The possibility of reactions of BIP with other amino acid side chains is discussed elsewhere (Yankeelov and Jolley, 1971).

Acknowledgments

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References

Dreze, A., Moore, S., and Bigwood, E. J. (1954), *Anal. Chim. Acta 11*, 554.

Edlbacher, S., and von Bidder, H. (1942), *Hoppe-Seyler's Z. Physiol. Chem. 276*, 126.

Ellman, G. L. (1959), *Arch. Biochem. Biophys. 82*, 70.

Fargher, R. G., and Pyman, F. L. (1921), *J. Chem. Soc. 119*, 734.

Jolley, C., and Yankeelov, J. A., Jr. (1972), *Biochemistry 11*, 164.

Kraml, M., and Bouthillier, L. P. (1955), *Can. J. Biochem. Physiol. 33*, 590.

Liu, T. Y. (1967), *J. Biol. Chem. 242*, 4029.

Pages, R. A., and Burger, A. (1966), *J. Med. Chem. 9*, 766.

Pasini, C. and Vercellone, A. (1955), *Gazz. Chim. Ital. 85*, 349.

Shaw, E., and Glover, G. (1970), *Arch. Biochem. Biophys. 139*, 298.

Smith, I., and Birchenough, M. (1960), *in* Chromatographic and Electrophoretic Techniques I, Smith, I., Ed., New York, N. Y., Interscience, p 212.

Walsh, K. A., and Brown, J. R. (1962), *Biochim. Biophys. Acta 58*, 596.

Yankeelov, J. A., Jr., and Jolley, C. J. (1971), *Arch. Biochem. Biophys. 147*, 822.