SYNTHESIS OF

1-AMINO-2-HYDROXYCYCLOPENTANECARBOXYLIC ACID

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This investigation involved the synthesis of 1-amino-2-hydroxycyclopentanecarboxylic acid, a potential structural analog of the natural amino acids, serine and threonine. The title compound also includes the structural features present in an established antitumor agent, cycloleucine.

1-Cyclopentenecarboxylic acid was prepared by a series of reactions involving formation of the cyanohydrin from cyclopentanone, dehydration of the cyanohydrin to 1-cyano-cyclopentene; and finally, hydrolysis of the nitrile. This intermediate was used for all the further synthesis transformations in this study.

The desired amino acid analog was prepared through a series of reactions involving methoxymercuration of 1-cyclopentenecarboxylic acid, replacement of the mercury by bromine, ammonolysis of the 1-bromo-2-methoxycyclopentanecarboxylic acid; and finally, cleavage of the methoxy group with hydroiodic acid to yield 1-amino-2-hydroxycyclopentanecarboxylic acid.

During the course of this study, the bromination of the mercuric acetate-methanol adduct of 1-cyclopentene-

carboxylic acid was also examined. Using an excess of bromine at 50-55°, 1-bromo-2-hydroxycyclopentanecarboxylic acid is the major reaction product; however, with lower molar ratios of bromine in the reaction, the intermediate, 1-bromomercuri-2-methoxycyclopentanecarboxylic acid, is produced. In contrast, bromination of the adduct at 10-15° leads mainly to 1-bromo-2-methoxycyclopentanecarboxylic acid.

Attempted ammonolysis of the chlorohydrin adduct, prepared from 1-cyclopentenecarboxylic acid and a variety of hypochlorinating reagents, resulted in the synthesis of the isomeric 2-amino-1-hydroxycyclopentanecarboxylic acid.

The structures of 1-amino-2-hydroxycyclopentanecarboxylic acid and 2-amino-1-hydroxycyclopentanecarboxylic acid were characterized and confirmed by elemental analysis, infrared, and nuclear magnetic resonance spectroscopy. These compounds are currently under study in a variety of biological systems.

SYNTHESIS OF
1-AMINO-2-HYDROXYCYCLOPENTANECARBOXYLIC ACID

THESIS

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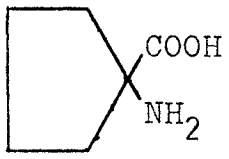
CHAPTER I

INTRODUCTION

In the search for effective chemotheraputic agents, many amino acid analogs have been synthesized in recent years. Some of these antimetabolites have been found to possess antitumor properties, and others inhibit the growth of certain bacteria and viruses. The subject of amino acid analogs has been thoroughly reviewed by Shive and Skinner (5).

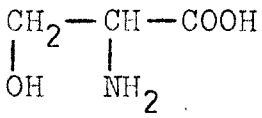
One amino acid analog that has received considerable attention is 1-aminocyclopentanecarboxylic acid. This



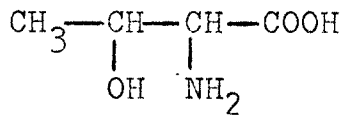
1-Aminocyclopentanecarboxylic acid

substance, and several related derivatives have been studied as potential chemotheraputic agents (3). 1-Aminocyclopentanecarboxylic acid, called cycloleucine, has shown promise as a chemotheraputic agent in the treatment of such diverse problems as acne (2) and leukemia (4).

Among the aliphatic amino acids found in proteins, there are two that also contain an hydroxyl group; namely, serine and threonine.



Serine

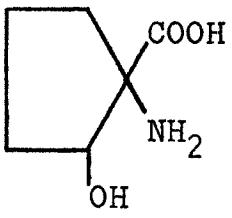


Threonine

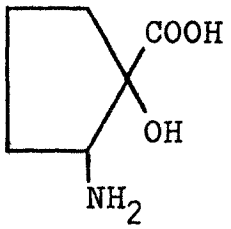
Both of these amino acids are important metabolically. Threonine is required in the diet of both man and rats, but apparently can be synthesized by many microorganisms. The catabolic paths of threonine give rise to such diverse products as glycine and glucose. Although serine is readily synthesized by the body, and thus is not required in the diet, it is nevertheless very important. Various metabolic products of serine are found in lecithins and cephalins. In addition, certain molds form serine derivatives which are antibiotics; namely O-diazoacetyl serine and cycloserine.

Few effective analogs of serine and threonine are known (5). An analog of serine, α-methylserine, is reported to inhibit the growth of Leuconostoc mesenteroides P-60, but other bacteria are unaffected by this compound. The higher homologs of threonine, 2-amino-3-hydroxypentanoic acid and 2-amino-3-hydroxyhexanoic acid, are reported to inhibit the growth of certain bacteria. Thus, it was of interest to prepare a new amino acid analog, 1-amino-2-hydroxycyclopentanecarboxylic acid. This structure contains

the cyclopentane ring found in cycloleucine and the combination of α-amino and β-hydroxyl groups found in serine and threonine. It is also an α-substituted analog, similar to α-methylserine. Thus, it may be anticipated that this cyclopentane derivative could be an effective analog of serine and/or threonine. This synthesis was accomplished through a series of reactions similar to the procedure which has been used for the preparation of threonine (1). Also, in the course of this study, the isomeric compound, 2-amino-1-hydroxycyclopentanecarboxylic acid was prepared.



1-Amino-2-hydroxy-
cyclopentanecarboxylic acid



2-Amino-1-hydroxy
cyclopentanecarboxylic acid

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CHAPTER II

EXPERIMENTAL

Organic Syntheses

All melting points were determined with a Thomas-Hoover capillary melting point apparatus, and are uncorrected. Ascending paper chromatography techniques were used with Whatman No. 1 paper. The solvent systems included: n-butanol-acetic acid-water (3:1:1) (system 1); 65% pyridine (system 2) and cyclohexylamine-water-methylethylketone-n-butanol (1:2.5:5:5) (system 3). Nuclear magnetic resonance (nmr) spectra were obtained on deuterium oxide solutions containing a small amount of HCl. The spectra were determined using a Jeolco JNM-PS-100 instrument. Infrared (ir) spectra were obtained on KBr pellets with a Perkin-Elmer 237 grating spectrophotometer. The gas chromatographic data were obtained on a Loenco Model 2300 Series Graphimatic gas chromatograph.

Cyclopentanone cyanohydrin. A mixture of cyclopentanone (100 g), potassium cyanide (60.6 g), and water (200 ml), was cooled in an ice bath, and 40% sulfuric acid (200 ml) was added dropwise with stirring over a 3-hour period. Stirring was continued overnight with cooling in an ice bath. The resulting mixture was extracted several times with ether, and the ether solution was dried ($MgSO_4$).

The solvent was removed in vacuo, and the residual oil was distilled under reduced pressure. After removal of the excess cyclopentanone, the cyanohydrin distilled at 118-9°/17 mm to yield 89.7 g of product (86% of theory based on KCN). The reported bp is 118-9°/14 mm (1).

1-Cyanocyclopentene. Thionyl chloride (143 g) was slowly added during a 2-hour period to a cold well-stirred mixture of cyclopentanone cyanohydrin (89.7 g) and absolute ether (250 ml). The mixture was heated under reflux for 3.5 hours, then cooled, and poured into a beaker of crushed ice. The ether layer was separated and washed successively with 2N HCl, 2N NaOH, and finally, water. After drying over $MgSO_4$, the ether solution was concentrated in vacuo, and the residual oil distilled to yield 1-cyanocyclopentene (64.5 g, 86% of theory), bp 58-9°/13 mm. The reported bp is 69°/15 mm (6).

1-Cyclopentenecarboxylic acid. A mixture of 1-cyanocyclopentene (32.2 g), potassium hydroxide (43 g), and water (430 ml) was heated under reflux for 48 hours. The mixture was then cooled and acidified with concentrated hydrochloric acid. The resulting precipitate was collected by filtration and recrystallized from ethanol-water to yield 1-cyclopentenecarboxylic acid (19.3 g, 50% of theory), mp 120-1°. The reported mp is 121° (3).

Anal. Calcd. for $C_6H_8O_2$: C, 64.27; H, 7.19. Found: C, 64.47: H, 7.28.

2-Amino-1-hydroxycyclopentanecarboxylic acid.

A solution of monochlorourea (4) was prepared by passing chlorine (51.9 g) through a cold mixture of urea (75 g), calcium carbonate (62.5 g), and water (75 ml). The mixture was filtered and the filter cake washed with water to give 375 ml of solution. A 40-ml portion of this solution was added to a mixture of 1-cyclopentenecarboxylic acid (11.2 g), acetic acid (5 ml), and crushed ice (25 g), and the resulting mixture was stirred overnight with cooling in an ice bath. After all attempts to isolate the chlorohydrin were unsuccessful, the impure material was mixed with ammonium hydroxide (350 ml) and allowed to stand at room temperature for a 7-day period. The resulting solution was concentrated *in vacuo* and desalted by a modification of the procedure reported by Piez *et. al.* (5). A portion of the residue, in 70% ethanol, was poured onto a column of Dowex 1-8X (hydroxide form). After the column was thoroughly washed with 70% ethanol, the amino acid was eluted with 1N HCl in 70% ethanol. The amino acid (185 mg) appeared just ahead of the HCl and easily crystallized from water-ethanol; mp 295-8° dec.; R_f 0.44 (system 1), 0.36 (system 2), 0.44 (system 3); ir (KBr) 3.08, 3.37, 6.15, 9.0, and 12.6 microns; nmr δ 3.80 (triplet, 1 H, methine hydrogen), 1.60-2.64 (multiplet, 6 H, $-CH_2-CH_2-CH_2-$).

Anal. Calcd. for $C_6H_{11}NO_3$: N, 9.64. Found, N, 9.65.

1-Bromo-2-methoxycyclopentanecarboxylic acid. The following series of reactions is a modification of the procedure, developed by Carter and West (2), for the synthesis of threonine. A mixture of 1-cyclopentenecarboxylic acid (56.2 g, 0.5 mole), mercuric acetate (159.3 g, 0.5 mole), and methanol (750 ml) was stirred at room temperature for 7 days. The precipitate which formed was filtered and washed twice with 200 ml of methanol, to yield 149.2 g of adduct, mp 195-200° (presumably 1-acetoxymercuri-2-methoxycyclopentanecarboxylic acid). Subsequently, an additional 6.7 g of product precipitated from the filtrate after standing several more days (total yield 77% of theory). The powdered adduct was added in small portions to a solution of potassium bromide (90 g, 0.75 mole) in water (500 ml); after which, the mixture was cooled to 10° and exposed to direct sunlight. To this solution, bromine (80 g, 0.5 mole) and potassium bromide (90 g, 0.75 mole) in 150 ml of water was added slowly with stirring. Near the end of the addition period, the adduct dissolved producing a clear solution. This solution was then filtered to remove a small amount of insoluble material, and finally extracted with 75 ml of ether. Upon acidification of the aqueous phase with 47% hydrobromic acid, a dense oil separated. This aqueous-organic mixture was extracted six times with 200-ml portions of ether: the oil dissolved in the first portion of ether. The ether phase was dried ($MgSO_4$) and evaporated under

reduced pressure to yield an amber oil mixed with a little solid. The mixture was again dissolved in ether, filtered and concentrated to yield crude 1-bromo-2-methoxycyclopentanecarboxylic acid (73.6 g, 66% of theory based on weight of 1-cyclopentenecarboxylic acid). Repeated attempts to obtain a satisfactory analysis on this material were unsuccessful.

1-Amino-2-methoxycyclopentanecarboxylic acid.

A partially purified sample of 1-bromo-2-methoxycyclopentanecarboxylic acid (44.6 g) was added to 1350 ml of concentrated ammonium hydroxide which had been cooled to 10-15°, and the mixture was stirred occasionally at room temperature for 13 days. The resulting dark reaction mixture was filtered, and then concentrated almost to dryness under reduced pressure. The residue was covered with acetone, and shaken occasionally for 4 days to induce solidification. The solid residue was recovered, and washed with acetone to yield 18.5 g of the crude product which was contaminated with ammonium bromide. An ascending paper chromatograph of this material indicated the presence of a small amount of 1-amino-2-hydroxycyclopentanecarboxylic acid· however, the major product was a ninhydrin-active compound which had R_f values of 0.68 (system 1), 0.86 (system 2), and 0.43 (system 3). The reaction product was dissolved in water, treated with decolorizing charcoal,

and recrystallized twice from water-ethanol to yield white crystals that sublime above 285°.

Anal. Calcd. for $C_7H_{13}NO_3 \cdot H_2O$: C, 47.44; H, 8.53; N, 7.91. Found: C, 47.66; H, 8.53; N, 7.93.

An anhydrous sample of 1-amino-2-methoxycyclopentanecarboxylic acid was obtained from the monohydrate by vacuum sublimation at 124-140°/0.15 mm. This material chromatographed identically with the monohydrate in all three systems: ir (KBr) 3.38, 6.12, 7.20, 7.50, and 8.85 microns; nmr δ 4.20 (triplet, 1 H, methine hydrogen), 3.52 (singlet, 3 H, $-O-CH_3$), 1.68-2.80 (multiplet, 6 H, $-CH_2-CH_2-CH_2-$).

Anal. Calcd. for $C_7H_{13}NO_3$: C, 52.81; H, 8.13; N, 8.80. Found: C, 52.65; H, 8.43; N, 8.83.

1-Bromomercuri-2-methoxycyclopentanecarboxylic acid. Using a lower ratio of bromine to mercuric acetate-methanol adduct than that previously described, a different product is isolated. For example, the adduct (75 g), prepared from 28.03 g (0.25 mole) of 1-cyclopentenecarboxylic acid, was suspended in 350 ml of water which contained 35.7 g (0.3 mole) of potassium bromide. A solution of 35.7 g (0.3 mole) of potassium bromide and 31.9 g (0.2 mole) of bromine dissolved in 100 ml of water was slowly added, while the reaction mixture was strongly illuminated with two incandescent lamps, and the temperature maintained below 35°, using an ice bath. As the last portion of the $KBr-Br_2$ solution was added, a precipitate formed. The precipitated

1-bromomercuri-2-methoxycyclopentanecarboxylic acid (9.57 g, 12 5% of theory) was recrystallized from ethanol-water, mp 156-7°

Anal. Calcd. for $C_7H_{11}BrHgO_3$: C, 19.85; H, 2.62. Found: C, 19.24; H, 2.81.

1-Amino-2-hydroxycyclopentanecarboxylic acid. A mixture of 49% hydroiodic acid (10.3 g) and 1-amino-2-methoxycyclopentanecarboxylic monohydrate (0.70 g) was heated under reflux for 2 hours. The reaction mixture was then concentrated to dryness in vacuo, and the resulting residue was dissolved in absolute ethanol and reduced to dryness twice to remove excess HI. The clear, viscous residue was finally dissolved in a small volume of ethanol, adjusted to pH 8-9 with concentrated ammonium hydroxide, and placed in the refrigerator. The precipitated amino acid was filtered, and recrystallized from water-ethanol to yield 0.26 g (46% of theory) of 1-amino-2-hydroxycyclopentanecarboxylic acid; mp 2[illegible]-90°dec ; mp of mixture with 2-amino-1-hydroxycyclopentanecarboxylic acid, 275-82°; R_f, 0.45 (system 1); 0 .2 (system 2), and 0 45 (system 3); ir (KBr) 3.35, 6.05-6 40, 7.20, 7.55, 9.15, 11.7, and 12.7 microns; nmr δ 4 54 (triplet, 1 H, methine hydrogen), 1.60-2.80 (multiplet, 6 H, $-CH_2-CH_2-CH_2-$).

Anal. Calcd. for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65 Found: C. 49.42; H, 7.57; N, 9.97.

In a separate experiment, the mercuric acetate-methanol adduct of 1-cyclopentenecarboxylic acid was brominated at a higher temperature (50-55°), and the impure bromo acid ammonolyzed at 90-100° for 24 hours in a sealed bomb. The resulting mixture, after many recrystallizations, and, finally, treatment on a column of Dowex 1, as previously described, yielded 2-amino-1-hydroxycyclopentanecarboxylic acid as the only recognizable product. The identity of this compound was confirmed by ir and melting point data.

Gas Chromatographic Analysis

The trimethylsilyl- (TMS-) derivatives of DL-threonine, DL-allothreonine, 1-amino-2-methoxycyclopentanecarboxylic acid, and 1-amino-2-hydroxycyclopentanecarboxylic acid were prepared in the following manner: A 10-15 mg sample of the amino acid was heated with 1-1.5 ml of trimethylsilyl-diethylamine (TMSDEA) and about 1 mg of trichloroacetic acid at 70-80° for about 30 minutes with adequate protection from atmospheric moisture. Most of the excess TMSDEA and the byproduct, diethylamine, were then removed under reduced pressure, leaving the TMS-derivative mixed with TMSDEA. Gas chromatographic analysis on an SE-30 column of the TMS-derivatives of both threonine and allothreonine indicated the presence of two components in each: however, no separation of the TMS-derivatives of these amino acids was observed. Similarly, analysis of the TMS-derivatives of

1-amino-2-methoxycyclopentanecarboxylic acid and 1-amino-2-hydroxycyclopentanecarboxylic acid indicated the presence of two components. The shapes of these two chromatographs were essentially the same, with similar retention times for the TMS-derivatives of both compounds.

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CHAPTER III

RESULTS AND DISCUSSION

An intermediate product which was required in all of the reaction sequences described in this study is 1-cyclopentenecarboxylic acid. This compound was synthesized, as shown in Figure 1, through a series of reactions involving formation of the cyanohydrin from cyclopentanone, dehydration of the cyanohydrin to 1-cyanocyclopentene, and finally, hydrolysis of the nitrile to yield 1-cyclopentenecarboxylic acid.

Several routes for the synthesis of 1-amino-2-hydroxycyclopentanecarboxylic acid were explored. Since many α-amino acids can be conveniently prepared by ammonolysis of the corresponding α-halo acid, it was hoped that the desired compound could be prepared by ammonolysis of the halohydrin prepared from 1-cyclopentenecarboxylic acid. Thus an attempt was made to prepare the desired compound by a reaction sequence similar to one used by Hedgecoth and Skinner in the preparation of β-hydroxyaspartic acid (15). In this method, the unsaturated acid was treated with commercial bleach solution in an attempt to produce the desired chlorohydrin. However, these attempts produced no isolable product

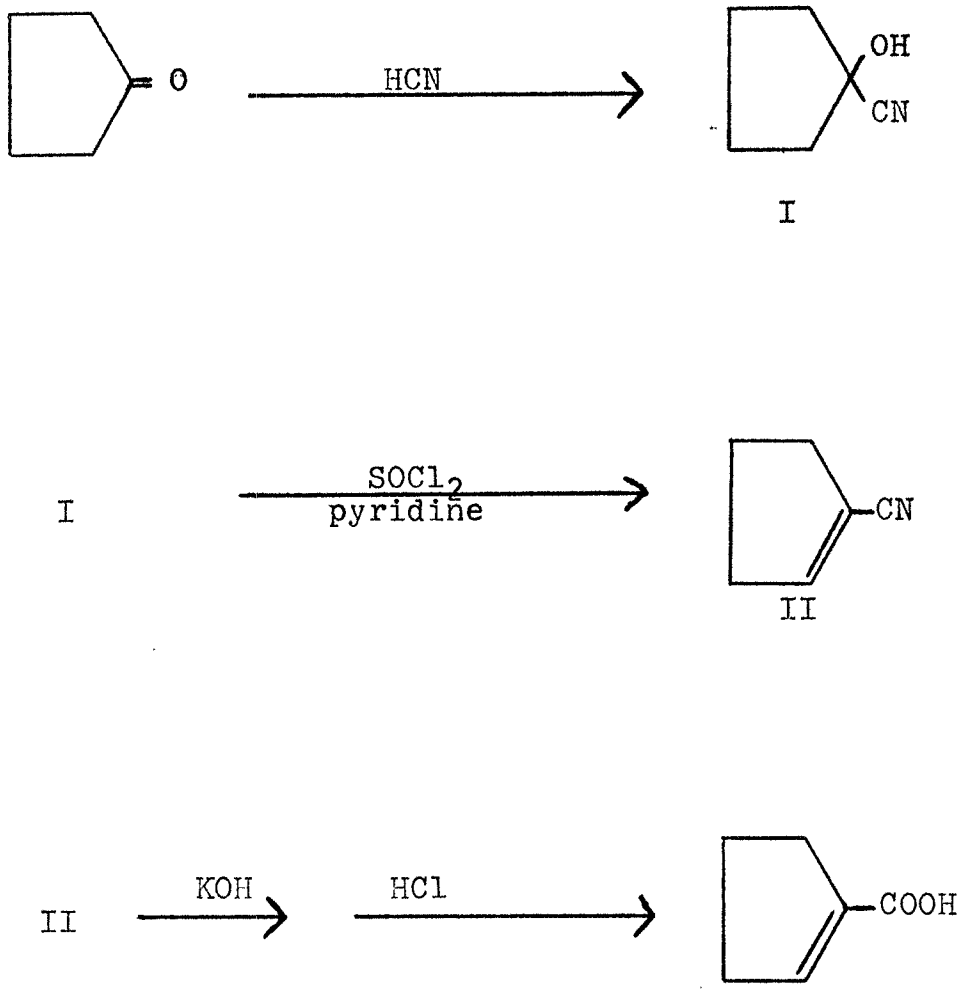

Fig 1--Synthesis of 1-cyclopentenecarboxylic acid

Another approach to the synthesis of the chlorohydrin was patterned after the procedure of Braun (2). In this latter work, chlorine was bubbled through an aqueous solution of crotonic acid, and the resulting chlorohydrin was isolated as its potassium salt. However, when this route was applied to 1-cyclopentenecarboxylic acid, the only material isolated contained no chlorine and this reaction sequence was therefore discarded.

An alternate series of reactions involving the chlorohydrin intermediate did lead to an aminohydroxy acid. However, the product isolated was not the desired 1-amino-2-hydroxycyclopentanecarboxylic acid; but rather, the isomeric 2-amino-1-hydroxycyclopentanecarboxylic acid was obtained. The chlorohydrin intermediate was prepared by the action of monochlorourea (in acetic acid solution) on 1-cyclopentenecarboxylic acid, utilizing a procedure which had been reported for the synthesis of trans-2-chlorocyclopentanol (?) In this method, the monochlorourea is used as a source of hypochlorous acid, which adds to the double bond to yield the chlorohydrin. It is well established that hypochlorous acid adds to α, β-unsaturated acids to yield the α-chloro-β-hydroxy derivative (2); thus, the action of monochlorourea on 1-cyclopentenecarboxylic acid would be anticipated to form 1-chloro-2-hydroxycyclopentanecarboxylic acid. Upon ammonolysis of this chlorohydrin,

the only amino acid isolated was found to be 2-amino-1-hydroxycyclopentanecarboxylic acid. These results are understandable in the light of a series of studies by Erlenmeyer (11), Melikoff (17), and Bruch (3). In all of these investigations, it was found that 2-chloro-3-hydroxy-substituted acids produced the corresponding 2-hydroxy-3-amino derivatives on ammonolysis. The formation of these anomolous products is explained on the basis of the formation of an epoxy intermediate, which reacts further with ammonia to yield a product in which the hydroxyl group forms at the α-rather than the β-position. The series of reactions which could lead to the formation of 2-amino-1-hydroxy-cyclopentanecarboxylic acid by this mechanism is shown in Figure 2. As demonstrated in this sequence of reactions, the aminohydroxy acid produced is probably the isomer in which the amine and hydroxyl groups are trans. This would be anticipated, since it is well known that epoxide rings open to produce trans substituted products under these conditions (22).

The synthesis of 2-amino-1-hydroxycyclopentanecarboxylic acid has been previously described by Burrows and Turner (4). They reported that the compound did not melt below 310°; however, in this investigation, the product isolated and found to be 2-amino-1-hydroxycyclopentanecarboxylic acid melted at 295-8°. This difference in melting points may be explained by assuming that the material obtained in this

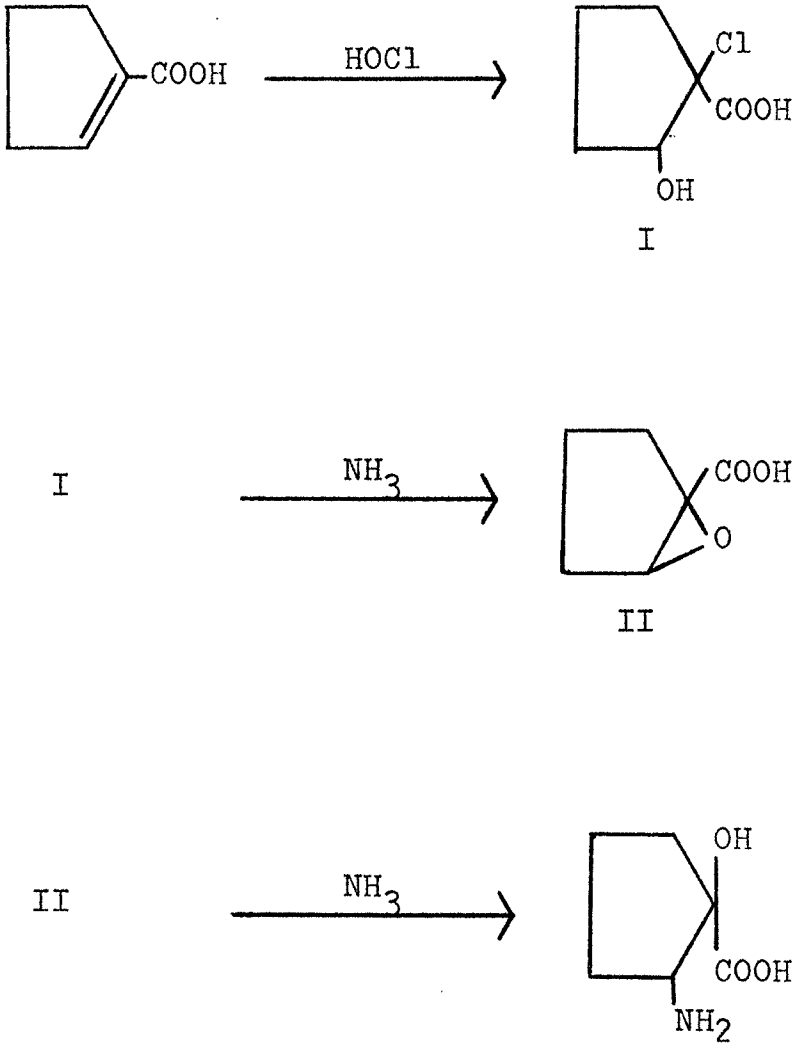

Fig. 2--Synthesis of 2-amino-1-hydroxycyclopentane-carboxylic acid.

investigation is the trans-aminohydroxy acid; whereas, the product reported by Burrows and Turner is either the cis isomer, or more probably, a mixture of cis and trans isomers. Their report gave no information on the stereochemistry of the compound.

The actual synthesis of the desired analog, 1-amino-2-hydroxycyclopentanecarboxylic acid, was accomplished by a route similar to the methods by Carter and West for the syntheses of serine and threonine (5,6). In their procedures, the aminohydroxy acids were prepared by ammonolysis of the appropriate bromomethoxy acids; followed by the hydrolysis of the methyl ether with hydrobromic acid. In both of these syntheses, the bromomethoxy acids were prepared by methoxymercuration and subsequent bromination of the appropriate unsaturated acid or ester.

The adduct obtained from the methoxymercuration of 1-cyclopentenecarboxylic acid, shown in Figure 3, failed to give a satisfactory elemental analysis; however, a similar observation was made in the case of the methoxymercuration of crotonic acid (20). In this latter study, it was postulated that the adduct was a mixture of acetoxymercuri- and hydroxymercuri-compounds. All attempts to purify the adduct obtained from 1-cyclopentenecarboxylic acid, so that a satisfactory analysis could be obtained, failed. The product proved to be insoluble in all of the solvent systems examined, and decomposed in the presence of dilute acids.

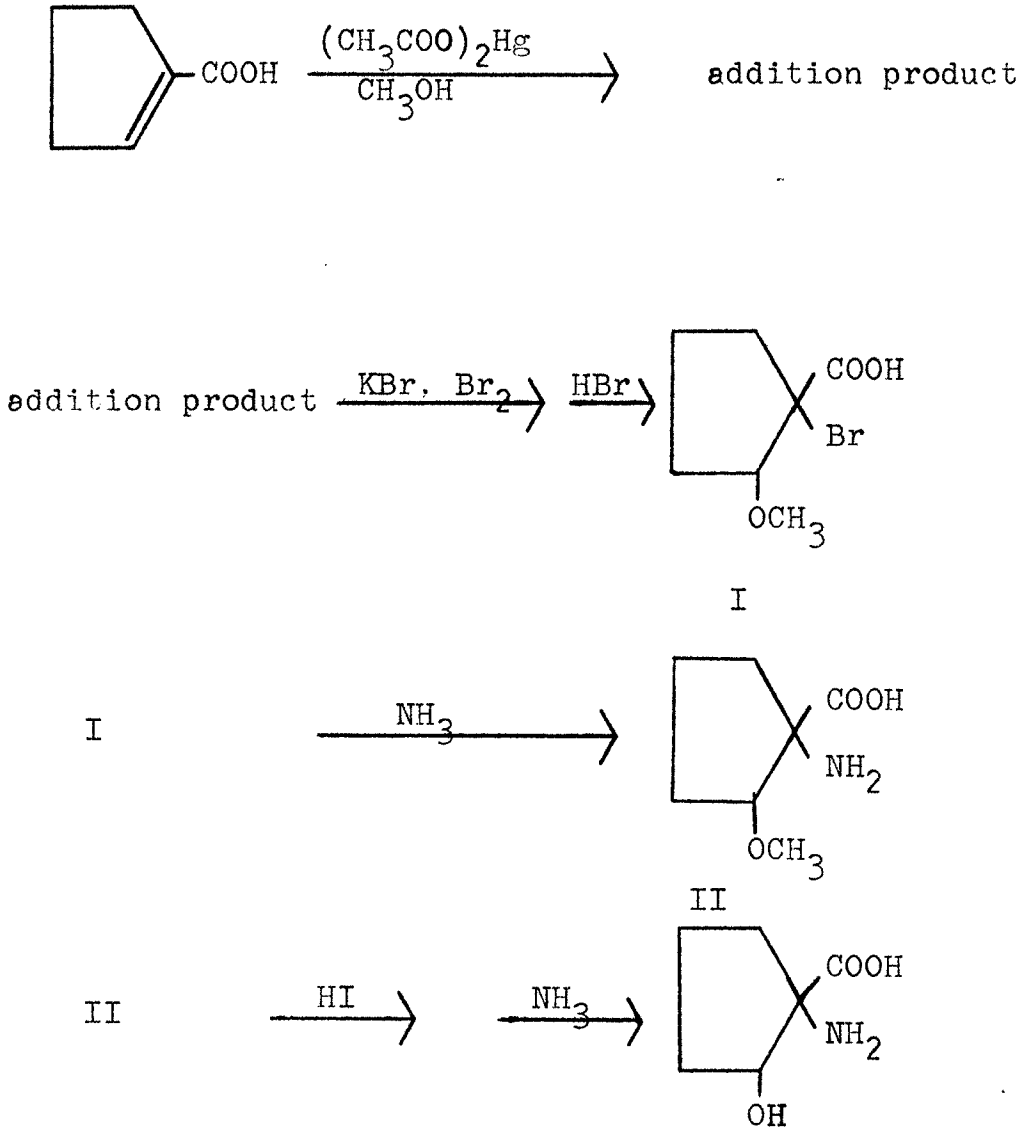

Fig. 3--Synthesis of 1-amino-2-hydroxycyclopentane-carboxylic acid.

The methoxymercuration adduct obtained from 1-cyclopentenecarboxylic acid produced a variety of products upon bromination, depending on the conditions employed. When the sample was reacted with an equimolar quantity of bromine in potassium bromide solution (assuming the adduct to be primarily 1-acetoxymercuri-2-methoxycyclopentanecarboxylic acid) the product isolated proved to be 1-bromomercuri-2-methoxycyclopentanecarboxylic acid, which resulted simply by replacement of the acetate anion with a bromide ion. When the bromination reaction was carried out with an excess of bromine at 50-55° under strong illumination, the major product was apparently 1-bromo-2-hydroxycyclopentanecarboxylic acid. The presence of the hydroxy-, rather than the methoxy-acid derivative, is suggested by the fact that the characterized product obtained on ammonolysis did not contain the methoxy function. It would appear to be unlikely that ammonolysis of a bromomethoxy acid would cleave an ether function, since ethers do not usually undergo hydrolysis even in the presence of strong base. The methyl ether was probably hydrolyzed during the bromination stage by the hydrobromic acid present as a result of hydrolysis:

$$Br_2 + H_2O \rightleftarrows HBr + HOBr.$$

In addition, it was observed that a sample of the impure bromohydroxy acid originally obtained in this reaction mixture produced 2-amino-1-hydroxycyclopentanecarboxylic

acid, probably by the same epoxide intermediate discussed earlier in connection with the analogous chlorohydrin.

In an alternate series of experiments, the bromination of the adduct at a temperature of 10° proceeded smoothly to yield 1-bromo-2-methoxycyclopentanecarboxylic acid. On ammonolysis, this substance produced 1-amino-2-methoxycyclopentanecarboxylic acid. The aminomethoxy acid was first isolated as a crystalline monohydrate; however, an anhydrous sample of this derivative was subsequently obtained by vacuum sublimation of the monohydrate. Structural integrity should have been maintained since it has been demonstrated that many amino acids sublime without loss of optical activity under these conditions (12).

The final step in the preparation of 1-amino-2-hydroxycyclopentanecarboxylic acid involved the hydroiodic acid cleavage of the methoxy compound. The hydroiodide salt initially produced was converted directly to the free amino acid by treatment with ammonium hydroxide.

The two isomeric aminohydroxy acids prepared, 1-amino-2-hydroxy- and 2-amino-1-hydroxycyclopentanecarboxylic acid, were characterized by different melting points, solubilities, ir spectra, and nmr spectra. The ir spectra of both derivatives are presented in Figures 4 and 5.

In the nmr spectrum of 1-amino-2-hydroxycyclopentanecarboxylic acid, the absorption of the methine hydrogen (adjacent to the hydroxyl group) was found at 4.54 δ.

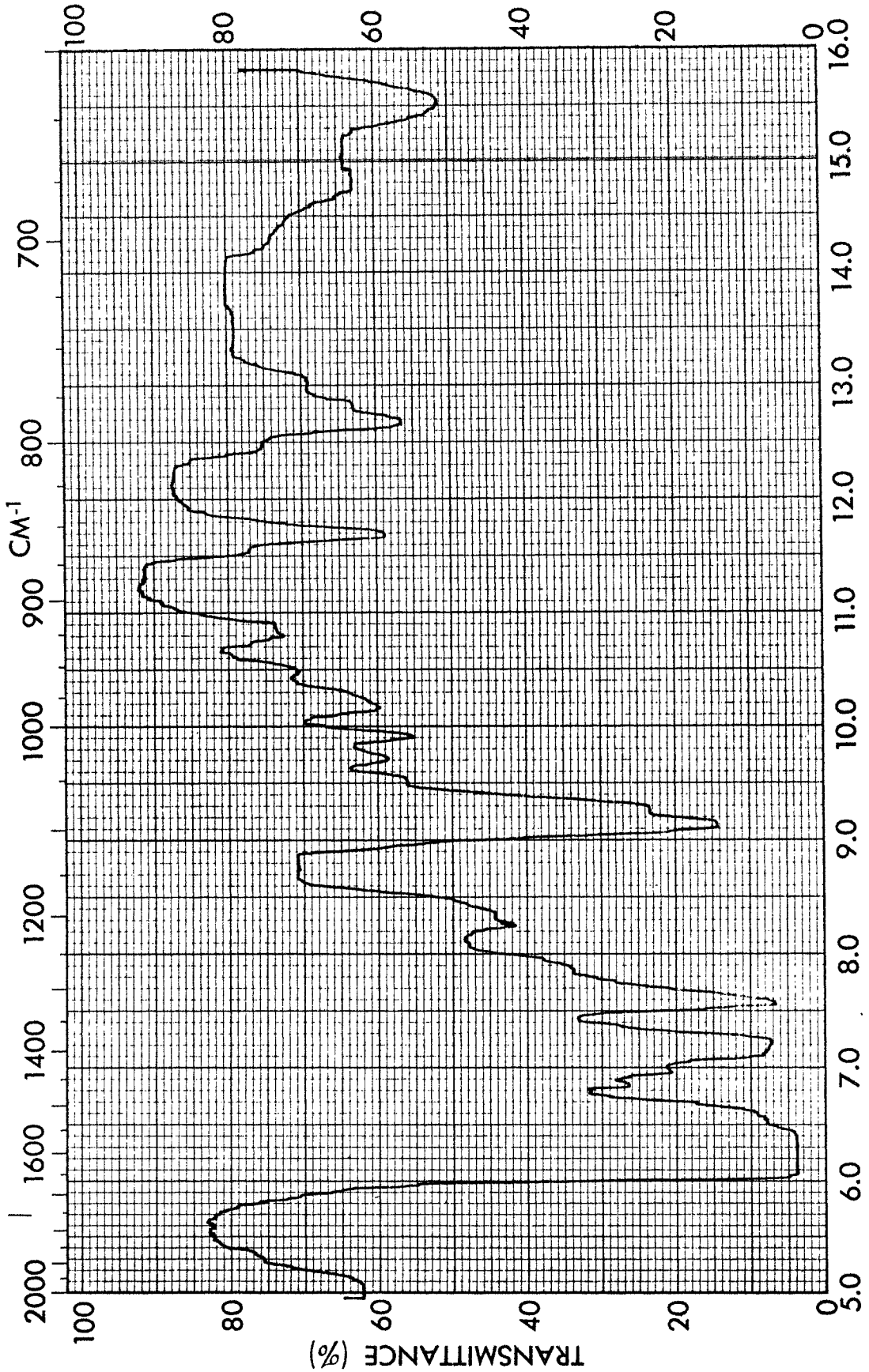

Fig. 4--Infrared spectrum of 1-amino-2-hydroxycyclopentanecarboxylic acid

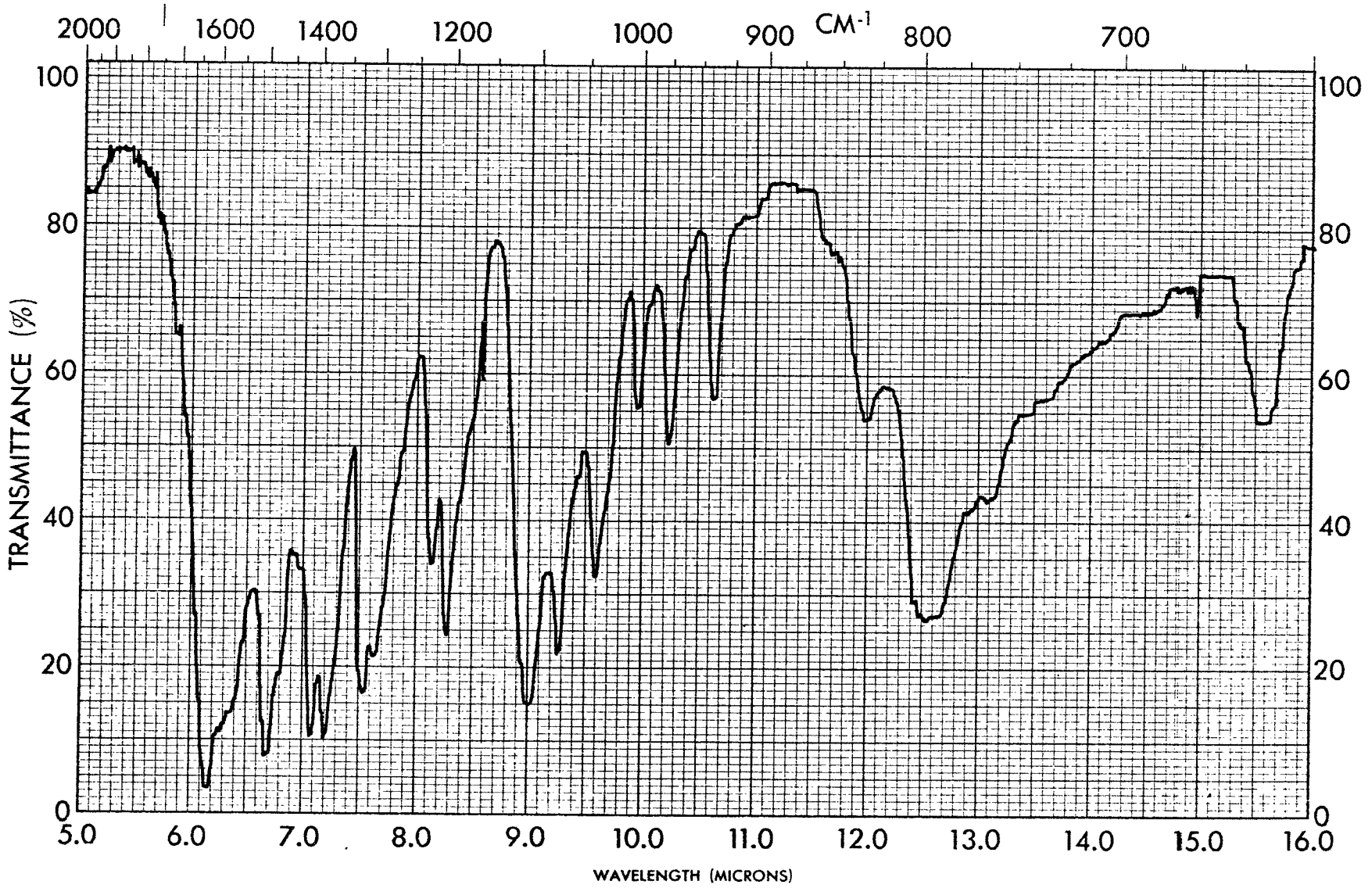

Fig. 5--Infrared spectrum of 2-amino-1-hydroxycyclopentanecarboxylic acid

However, in the spectrum of 2-amino-1-hydroxycyclopentanecarboxylic acid, the methine hydrogen (adjacent to the amine group) absorbs at 3.80 δ. This data is consistent with that observed with model compounds, serine and isoserine. In the spectrum of serine, the β-hydrogens absorb at 4.18 δ, and in the spectrum of isoserine, the β-hydrogens absorb at 3.48 δ. Thus, in both pairs of isomers, protons adjacent to hydroxyl groups are found further downfield than protons adjacent to amine groups. This is consistent with the greater deshielding effect of the more electronegative hydroxyl group. In addition, the methine hydrogen of 1-amino-2-hydroxycyclopentanecarboxylic acid was found to absorb at 4.20 δ.

An investigation of the stereochemistry of 1-amino-2-hydroxycyclopentanecarboxylic acid was of interest in this study. Meyer and Rose (18) proved the stereochemistry of threonine by converting it into the corresponding dihydroxy acid of known configuration This conversion was accomplished by diazotization of the hydroxyamino acid with nitrous acid; however, when a similar attempt at diazotization was carried out with 1-amino-2-hydroxycyclopentanecarboxylic acid, the resulting oily product decomposed within a few minutes. In some related experiments, additional attempts were made to prepare the cis- and trans-1,2-dihydroxycyclopentanecarboxylic acid by stereoselective hydroxylation of 1-cyclopentenecarboxylic acid.

These hydroxylation attempts included the use of permanganate (16, 21), halogens and silver carboxylates (9, 14, 23), and performic acid (10); methods which have been extensively reviewed by Gunstone (13). The only product isolated and characterized from any of these experiments was glutaric acid. This compound was isolated from the reaction of performic acid with 1-cyclopentenecarboxylic acid. Under the conditions used, the cyclopentene ring cleaved with the loss of one carbon, presumable as CO_2. The identity of the product as glutaric acid was established by ir, nmr, and elemental analysis.

There is a single report in the literature (19) on the preparation of 1,2-dihydroxycyclopentanecarboxylic acid; however, the structure was not definitely established and no conclusions were drawn concerning the stereochemistry of the molecule. Since this product was prepared from the epoxide of 1-cyanocyclopentene through acid hydrolysis, it may be assumed to have the trans-diol arrangement.

Gas chromatographic analysis of the trimethylsilyl derivatives of 1-amino-2-hydroxycyclopentanecarboxylic acid and 1-amino-2-methoxycyclopentanecarboxylic acid suggests that these compounds are mixtures of cis and trans isomers. This is to be expected since the replacement of the bromomercuri group with bromine is known to proceed with racemization, even though the initial addition product is trans (7) Thus the bromomethoxy acid and the aminomethoxy

acid obtained *via* ammonolysis would be expected to be mixtures of *cis* and *trans* forms. The trimethylsilyl derivatives of analogous model compounds, threonine and *allo*threonine, were not separated under the conditions used; however, it appears that the *cis* and *trans* cyclopentane analogs are more easily separable. It remains possible that one of the peaks obtained from each of the cyclopentane derivatives may be due to incomplete silylation (or perhaps, double silylation of the amine group) since it has been reported that some amino acids produce more than one silylated derivative (1).

In conclusion, *cis*-2-amino-1-hydroxycyclopentanecarboxylic acid and a mixture of *cis*- and *trans*-1-amino 2-hydroxycyclopentanecarboxylic acid have been synthesized in an attempt to produce a new chemotheraputic agent bearing a structural relation to the natural amino acids, serine and threonine. No microbiological activity has yet been established; however, both of these compounds have been submitted for biological assay in tissue culture systems, to Dr. P. F. Kruse, Jr. of the Samuel R. Noble Foundation in Ardmore, Oklahoma.

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This investigation involved the synthesis of 1-amino-2-hydroxycyclopentanecarboxylic acid, a potential structural analog of the natural amino acids, serine and threonine. The title compound also includes the structural features present in an established antitumor agent, cycloleucine.

1-Cyclopentenecarboxylic acid was prepared by a series of reactions involving formation of the cyanohydrin from cyclopentanone, dehydration of the cyanohydrin to 1-cyanocyclopentene; and finally, hydrolysis of the nitrile. This intermediate was used for all the further synthesis transformations in this study.

The desired amino acid analog was prepared through a series of reactions involving methoxymercuration of 1-cyclopentenecarboxylic acid, replacement of the mercury by bromine, ammonolysis of the 1-bromo-2-methoxycyclopentanecarboxylic acid; and finally, cleavage of the methoxy group with hydroiodic acid to yield 1-amino-2-hydroxycyclopentanecarboxylic acid.

During the course of this study, the bromination of the mercuric acetate-methanol adduct of 1-cyclopentene-

carboxylic acid was also examined. Using an excess of bromine at 50-55°, 1-bromo-2-hydroxycyclopentanecarboxylic acid is the major reaction product; however, with lower molar ratios of bromine in the reaction, the intermediate, 1-bromomercuri-2-methoxycyclopentanecarboxylic acid, is produced. In contrast, bromination of the adduct at 10-15° leads mainly to 1-bromo-2-methoxycyclopentanecarboxylic acid.

Attempted ammonolysis of the chlorohydrin adduct, prepared from 1-cyclopentenecarboxylic acid and a variety of hypochlorinating reagents, resulted in the synthesis of the isomeric 2-amino-1-hydroxycyclopentanecarboxylic acid.

The structures of 1-amino-2-hydroxycyclopentanecarboxylic acid and 2-amino-1-hydroxycyclopentanecarboxylic acid were characterized and confirmed by elemental analysis, infrared, and nuclear magnetic resonance spectroscopy. These compounds are currently under study in a variety of biological systems.