Racemic Diastereoisomers of 1-Amino-2-hydroxycyclopentanecarboxylic Acid

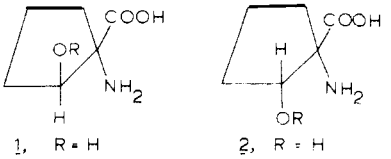
Dimitri E. Gaitanopoulos, James Nash, Danny L. Dunn, and Charles G. Skinner*

Department of Chemistry, North Texas State University, Denton, Texas 76203, and The Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401. *Received January 10, 1975*

The synthesis and characterization of the two diastereoisomeric forms of 1-amino-2-hydroxycyclopentanecarboxylic acid have been accomplished. A previously reported synthesis produced a racemic mixture of the threonine analog *trans*-2-hydroxy-1-aminocyclopentanecarboxylic acid (trans with respect to the hydroxy and carboxyl group). The alternate allothreonine analog was produced by conversion of cyclopentene oxide to *trans*-2-methoxycyclopentanol, followed by oxidation to 2-methoxycyclopentanone and conversion to a hydantoin. Fractional crystallization of the hydantoin sample, followed by hydrolysis, produced *cis*-2-hydroxy-1-aminocyclopentanecarboxylic acid (cis with respect to the hydroxy and carboxyl group) in high purity. Neither of the isomeric forms significantly inhibited the growth of the bacterial strains examined nor were they effective in inhibiting Jensen sarcoma cells in tissue culture.

Cycloleucine (1-aminocyclopentanecarboxylic acid, NSC-1026) has been reported to be a metabolic antagonist of valine and is active against selected experimental tumors.[1] 1-Amino-2-hydroxycyclopentanecarboxylic acid was recently synthesized as a potential antagonist of threonine (or serine), but the product isolated proved to be ineffective as a cytotoxic agent.[2] This previously reported analog was produced via the acetoxymercurimethoxy adduct of 1-cyclopentenecarboxylic acid and appeared to be a relatively pure diastereoisomer. It was subsequently identified as the threonine analog *trans*-2-hydroxy-1-aminocyclopentanecarboxylic acid, **2** (trans with respect to the hydroxy and carboxyl groups). In an effort to improve the yield as well as to prepare the alternate isomeric form, different synthetic routes were examined. Using a general procedure which has been reported for the synthesis of serine,[3] cyclopentene oxide was converted to *trans*-2-methoxycyclopentanol in 89% yield[4] and subsequently oxidized to 2-methoxycyclopentanone.[5] The ketone was condensed with KCN and $(NH_4)_2CO_3$ to produce a hydantoin in 69% yield, which, upon alkaline hydrolysis, produced the diastereoisomeric 1-amino-2-methoxycyclopentanecarboxylic acids in 95% yield. Acid hydrolysis of the methoxy derivative produced the corresponding hydroxyamino acids in 90% yield.

A preliminary examination of the 1-amino-2-hydroxycyclopentanecarboxylic acid sample recovered indicated that it consisted of a mixture of isomeric forms, as evidenced by ion-exchange chromatography (Beckman/Spinco amino acid analyzer), in an 84–16% ratio. The minor component was identical with the previously reported **2** and the major component was identified as the allothreonine analog *cis*-2-hydroxy-1-aminocyclopentanecarboxylic acid, **1** (cis with respect to the hydroxyl and carboxyl groups).



1, R = H 2, R = H
1a, R = CH_3 2a, R = CH_3

As described in the Experimental Section, an effort to separate the diastereoisomeric forms through fractional precipitation at the hydantoin stage proved to be fruitful. The hydantoin reaction mixture yielded two major fractions of about equal proportion; one sample (A) was obtained by concentrating the mother liquor, and the second sample (B) was obtained by neutralizing the filtrate. Alkaline hydrolysis of each of these products, followed by fractional crystallization, yielded a mixture of the isomeric forms of 1-amino-2-methoxycyclopentanecarboxylic acid as indicated by amino acid analysis. The ir spectrum for **2a** was essentially identical with that of the previously reported compound.[2] The compounds were also distinguished through paper chromatography using a previously reported solvent which separated threonine and allothreonine[6] giving R_f values of 0.42 and 0.48 for isomers **2a** and **1a**, respectively. NMR spectra of isomers **2a** and **1a** were determined in D_2O (HCl) using Me_4Si (sealed capillary tube) as the standard and possessed the same general features.

Selected samples of 1-amino-2-methoxycyclopentanecarboxylic acids were subsequently cleaved in the presence of HBr to produce the corresponding free amino acids, **2** and **1**. Both fractions melted with decomposition, **1** at 276–277° and **2** at 286–287°. **2** had an ir spectrum identical with that of the previously reported analog.[2] Both amino acids possessed major absorption bands at 6.20, 7.15, and 9.15 μ; however, **2** had a strong band at 7.60 μ which was absent in **1**. Retention times for the two isomers of the amino acid analyzer were 103 (pH 3.25) and 135 min (pH 4.25) for **1** and **2**, respectively. Using the most highly purified samples from this synthesis route, the integrated spectra calculated for 99.0% purity of **1** and 79.9% purity of **2** (the alternate isomer being the contaminant in both cases). Paper chromatographic separation using cyclohexylamine–water–methyl ethyl ketone–1-butanol (1:2.5:5:5)[6] produced R_f values of 0.38 and 0.45 for **1** and **2**, respectively, which correspond with R_f values of 0.41 and 0.54 for allothreonine and threonine, respectively.

Evidence that these are the more probable structural assignments for **1** and **2** is obtained by comparing the NMR chemical shifts for the β-methine protons of the corresponding 1-amino-2-methoxycyclopentanecarboxylic acid fractions. A study of the NMR spectra of L-hydroxyproline in acid solutions showed the two β-hydrogens to have different chemical shifts in both *trans*- and *allo*-L-hydroxyproline.[7] In all cases the β-hydrogen trans to the carboxyl group had a higher chemical shift than did the β-hydrogen cis to the carboxyl group. Compound **1a** had a triplet due to the β-methine proton centered at δ 4.89, whereas compound **2a** has a triplet β-methine centered at δ 4.20.[2] By analogy with the study of Pogliani and Ellenberger,[7] these data confirm the structure of **2** and **1**.

The homologization of threonine and allothreonine through insertion of an ethylene group between the 2 and 4 carbons is illustrated in Figure 1. For example, based on the absolute configuration of threonine,[14] the only conformation which would permit the formation of a cyclopentane structure must produce the trans form (trans

* Address correspondence to this author at North Texas State University

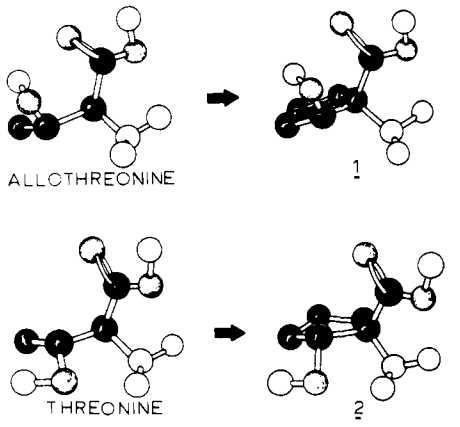


Figure 1. Cyclopentane analogs produced by homologization of threonine and allothreonine.

with respect to the hydroxyl and carboxyl groups). The conformer of allothreonine required to produce the cyclopentane analog would yield the corresponding cis isomer.

Since the major rationale for this study was to separate and define the relative biological activities of the two isomeric forms, a series of microbiological and tissue culture assays was undertaken. Using typical microbial procedures, neither isomer demonstrated any significant toxiciy to growth of *Streptococcus faecalis, Escherichia coli, Leuconostoc mesenteroides*, or *Lactobacillus arabinosus* at concentrations up to 300 μg/ml. In retrospect this is not surprising since the originally tested analog which was inactive[2] now appears to have been a threonine analog. Using Jensen sarcoma cells in tissue culture some inhibition of growth was observed for both isomeric forms; however, the biological activities are disappointing as indicated in Table I. Under comparable assay conditions, cycloleucine was an active growth inhibitor in the tissue culture system.

Table I. Inhibitory Properties of Cyclic Analogs in Jensen Sarcoma Culture[a]

Analog concn, μg/ml	Threonine concn,[b] μg/ml	No. of new cells,[c] 72 hr	% control
Control (+)	18 (normal media)	3.85×10^6	100
Control (−)	None	0.43×10^6	11
Control	50	2.84×10^6	74
trans-2-Hydroxy-1-aminocyclopentanecarboxylic acid (2)			
50	None	0.46×10^6	12
50	18	2.62×10^6	68
50	50	2.61×10^6	68
cis-2-Hydroxy-1-aminocyclopentanecarboxylic acid (1)			
50	None	0.45×10^6	12
50	18	2.67×10^6	69
50	50	2.63×10^6	68
Cycloleucine			
50	None		0
50	25	0.18×10^6	5

[a] Medium McCoy 7a + FBs, ref 13. [b] Serine concentration in medium = 26 μg/ml. [c] Original inoculum = 0.45×10^6 cells.

Experimental Section

6-Methoxy-1,3-diazaspiro[4.4]nonane-2,4-dione. *trans*-2-Methoxycyclopentanol, bp 176–178°, prepared from cyclopentane oxide,[4] was oxidized with chromium trioxide to produce 2-methoxycyclopentanone,[5] bp 60–62° (16 mm). Using the general procedure of Rogers and Henze,[8] a mixture of 17.27 g (0.151 mol) of 2-methoxycyclopentanone, 19.7 g (0.302 mol) of KCN, and 60 g (0.72 mol) of $(NH_4)_2CO_3$ was allowed to react in aqueous EtOH. The reaction mixture was reduced to dryness in vacuo, dissolved in 75 ml of H_2O, and cooled for 24 hr to yield a crude product which was recrystallized from 50% EtOH–H_2O to give 7.88 g of material, mp 151–154° (A). The original aqueous filtrate from A was reduced in vacuo to a small volume, cooled in an ice bath, and neutralized with concentrated HCl to pH 7. A solid product precipitated which was filtered and recrystallized from EtOH–H_2O to yield 8.37 g of product, mp 144–148° (B). Anal. ($C_8H_{12}O_3N_2$, fraction A) C, H, N; ($C_8H_{12}O_3N_2$, fraction B) C, H, N.

1-Amino-2-methoxycyclopentanecarboxylic Acids. Both fractions of the hydantoins, A and B, individually were hydrolyzed by the same general procedure,[3,9] whereby a 7-g (0.038 mol) sample of the corresponding hydantoin, 19 g (0.06 mol) of $Ba(OH)_2 \cdot 8H_2O$, and 110 ml of H_2O were sealed in a Pyrex vessel and heated 2 hr at 160°. After cooling, CO_2 was passed through the reaction mixture to remove Ba ions, and the clear filtrate was taken to dryness in vacuo.

The resulting residue from hydrolysis of A was dissolved in hot EtOH and placed in a deep freeze to yield two fractions which crystallized in sequence and were characterized by amino acid analysis: A_1, 3.83 g (2.4% **2a**, 97.6% **1a**); A_2, 1.21 g (0.9% **2a**, 99.1% **1a**). A third fraction was recovered from the mother liquor by the addition of ether: A_3, 0.81 g (4.5% **2a**, 95.5% **1a**).

The analogous residue from the alkaline hydrolysis and work-up of fraction B was taken up in EtOH–H_2O and cooled to yield sequentially two fractions [B_1, 2.09 g (75.7% **2a**, 24.3% **1a**); B_2, 2.36 g (27.9% **2a**, 72.1% **1a**)], and a third fraction was obtained upon the addition of acetone [B_3, 1.12 g (9.0% **2a**, 91.0% **1a**)].

A chromatographic analysis of these fractions using a Beckman/Spinco amino acid analyzer indicated a mixture of two ninhydrin active components one with a retention time of 91 min (pH 3.25) (**1a**) and the other 141 min (pH 4.25) (**2a**).

Column Conditions. Approximately 1 mg of sample (**2**, **2a**, **1**, **1a**) was dissolved in 2 ml of 0.2 *N* sodium citrate buffer (pH 2.2) containing 15% polyethylene glycol, and 50 μl of the solution was added to the surface of the resin and washed in with an additional 50 μl of buffer under nitrogen. The resin column was Beckman AA-15 and was 55–56 cm high. Using the general procedure for analysis of protein hydrolysate samples,[10,11] the column was held at 55° and sequentially eluted with 0.2 *M* sodium citrate buffer solutions. The initial buffer eluent (pH 3.25) was maintained until the first component was eluted (90–110 min) and was immediately followed by the second buffer (pH 4.25) using a flow rate of 70 ml/hr. The eluent fractions were continuously monitored by ninhydrin reagent containing titanium chloride, and the absorption at 570 mm was plotted on a linear recorder. The area under the absorption peaks was calculated by HXW triangulation.[12] Isoleucine was initially used as a marker; however, due to the consistent elution pattern of the two isomeric forms this practice was discontinued. The elution properties of fraction **2a** proved to be identical with the previously reported sample of 1-amino-2-methoxycyclopentanecarboxylic acid,[2] and characterization of the compound was also confirmed by a comparison of ir and NMR spectra. Subsequent hydrolysis of the methoxy derivative yielded a hydroxyamino acid sample which was identical with the isomer previously reported:[2] NMR of fraction A_2 (D_2O, H^+) δ 2.00–3.00 (m, 6 H, $CH_2CH_2CH_2$), 4.01 (s, 3 H, CH_3O), 4.89 (t, 1 H, CH). The ir spectrum (KBr pellet using a Perkin-Elmer 237 spectrophotometer) of **1a** was similar to **2a**, the major difference being two strong peaks at 8.90 and 9.11 μ as compared to a single major peak in this region at 8.85 μ for **2a**. Anal. ($C_7H_{13}NO_3$, **1a**) C, H, N.

DL-1-Amino-2-hydroxycyclopentanecarboxylic Acid. Sample A_1 (1.21 g) of 1-amino-2-methoxycyclopentanecarboxylic acid was heated under reflux in the presence of 10 ml of 48% HBr for 2.5 hr. The solvent was removed in vacuo, and the residue was repeatedly treated with EtOH and taken to dryness to remove excess HBr. The residue was finally dissolved in 95% EtOH, adjusted to pH 8 with concentrated NH_4OH, and cooled to yield 998 mg of product which was recrystallized from EtOH–H_2O to yield 487 mg of material (1): mp 277–278° dec; NMR (D_2O, H^+)

δ 1.64–3.00 (m, 6 H, $CH_2CH_2CH_2$), 4.92 (t, 1 H, CH); ir (KBr) 3.40, 6.10, 6.53, 7.16, 9.20 μ. Anal. ($C_6H_{11}NO_3$) C, H, N.

In an effort to validate the integrity of the hydrolytic procedure, sample B_2 from the fractional precipitation of 1-amino-2-methoxycyclopentanecarboxylic acid (2.09 g) was cleaved in the presence of 16 ml of 48% HBr in the same manner. There was recovered 1.58 g of crude product which was recrystallized from EtOH–H_2O to produce 771 mg of material, mp 286–287 dec. An amino acid analysis of this material indicated it to be 79.9% isomer 1 and 20.3% isomer **2**.

Microbiological Assays. Samples of 1 and **2** (99 and 80% pure by analysis on an amino acid analyzer, respectively) were examined for their inhibition to growth of *Escherichia coli* 9723 and *E. coli* W, *Streptococcus faecalis* 8043, *Leuconostoc mesenteroide* 10830, and *Lactobacillus arabinosus* 8014 using previously reported assay conditions.[2]

Tissue Culture Studies. Jensen sarcoma cells (ATCC-CCL-45) were seeded in replicate T-25 Falcon flasks using an initial inoculum of 400000 cells/flask in McCoy's 7a medium supplemented with 10% fetal calf serum. The flasks were gassed with 8% CO_2–92% air and incubated at 37° for 24 hr. After incubation, cell numbers were determined for zero time. The medium in the remaining flasks was replaced with a fresh solution containing the analog to be tested and incubation was continued. At the end of 48 hr, cell numbers were measured in replicate flasks. In the remaining flasks, the medium was again replaced with freshly prepared medium containing the analog and incubation was continued to a total of 72 hr when a final cell count was made. Cells were harvested and counted in a Coulter counter, Model B.[13] Highly purified samples of 1 and **2** (greater than 98% purity by analysis on an amino acid analyzer) were used for this study.

Acknowledgment. This study was supported in part by The Robert A. Welch Foundation (B-342) and the North Texas State University Faculty Research Fund. D.L.D. holds the Noble Foundation Fellowship, 1974–1975.

References and Notes

(1) S. K. Carter, *Chemother. Fact Sheet*, Program Analysis Branch, Chemotherapy, National Cancer Institute (July 1970).
(2) J. D. Huddle and C. G. Skinner, *J. Med. Chem.*, 14, 545 (1971).
(3) P. G. Nadeau and R. Gaudry, *Can. J. Res., Sect. B*, **27**, 421 (1949).
(4) S. Winstein and R. B. Henderson, *J. Am. Chem. Soc.*, **65**, 2196 (1943).
(5) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. Weedon, *J. Chem. Soc.*, 39 (1946).
(6) T. L. Hardy and D. O. Holland, *Chem. Ind. (London)*, 517 (1954).
(7) L. Pogliani and M. Ellenberger, *J. Am. Chem. Soc.*, **96**, 1621 (1974).
(8) B. G. Rogers and H. R. Henze, *J. Am. Chem. Soc.*, **63**, 2190 (1941).
(9) P. Tailleur and L. Berlinguet, *Can. J. Chem.*, **39**, 1309 (1961).
(10) S. Moore and W. H. Stein, *Methods Enzymol.*, **6**, 821 (1963).
(11) D. H. Spackman, S. Moore, and W. H. Stein, *Anal. Chem.*, **30**, 1190–1206 (1958).
(12) Beckman Instruments Amino Acid Analyzer Instruction Manual A-TB-049A, Spinco Division, Beckman Instruments, Palo Alto, Calif., 1969.
(13) M. K. Patterson, Jr., in "Tissue Culture: Methods and Applications", P. F. Kruse, Jr., and M. K. Patterson, Jr., Ed., Academic Press, New York, N.Y., 1973, pp 192–194.
(14) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. 1, Wiley, New York, N.Y., 1961, pp 176–183.

Synthesis of 5-Substituted Aminomethyluracils via the Mannich Reaction

Thomas J. Delia,* John P. Scovill, William D. Munslow,

Malcolm H. Filson Laboratories, Department of Chemistry, Central Michigan University, Mt. Pleasant, Michigan 48859

and Joseph H. Burckhalter[1]

Interdepartmental Program in Medicinal Chemistry, College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48104. *Received June 20, 1975*

An extension of the Mannich reaction, in which aminomethylation of the five position of uracil, is reported. Thus, primary and secondary alkylamines and primary aromatic amines containing ring-activating groups led to the title compounds **3–10**. Compound **11** in which the aromatic ring contains the ring-deactivating nitro group was synthesized in an alternative way. All compounds were characterized by their elemental and spectral properties.

Over the years there has been a continuing interest in analogs of thymine which might have cytotoxic activity. Thus, compounds such as 5-fluorouracil,[2] 5-trifluoromethyluracil,[3] and 5-mercaptomethyluracil[4] are effective as inhibitors of cell growth. In view of the biological significance of these 5-substituted uracils we became interested in extending the derivatization at the 5 position of uracil by the use of the Mannich reaction. There exists a close parallel between in vivo thymidine 5-phosphate synthesis and the Mannich reaction. The biological requirements for thymidine 5-phosphate synthesis are formaldehyde, tetrahydrofolic acid, and deoxyuridine monophosphate[5] while those for the Mannich reaction are formaldehyde, an amine, and a compound containing a reactive hydrogen.

During the past decade, the Mannich reaction has been applied to 6-methyluracil,[6–8] 2-thiouracil,[6–8] 6-aminouracil derivatives,[7] and 6-chlorouracil derivatives[9] in addition to uracil.[10,11] Accordingly, we have synthesized a series of 5-substituted aminomethyluracil derivatives (Scheme I, Table I) in order to ascertain the scope of the reaction as well as any differences the side chain might have on biological activity. Of particular emphasis during this investigation was the use of aromatic amines.

Uracil was treated with 2 equiv each of paraformaldehyde and the corresponding amine in aqueous ethanol. The products derived from the aliphatic amines were quite hygroscopic and consequently they were isolated as salts. Products derived from aromatic amines generally precipitated from the reaction mixture as nearly insoluble solids. These were conveniently purified by successive washings with aqueous ethanol, water, and acetone. In all cases the structural assignments were based on elemental analyses and ^1H NMR spectral data.

The ^1H NMR spectra of compounds **3–5**, as salts, and **6**, as free base, were obtained in D_2O and the spectra of compounds **7–10** were obtained in trifluoroacetic acid. In all cases, the C-6 proton (δ 7.86–7.60), the protons of the