Peptides of Azaserine

*Koppaka V. Rao (1) and B. Ravindranath*

College of Pharmacy, University of Florida, Gainesville, Florida 32610

The synthesis of the *cis* and *trans* forms of the cyclic dipeptide of O-diazoacetyl-DL-serine is described.

The discovery of azaserine (O-diazoacetyl-L-serine, **1**) as the metabolite of a *Streptomyces* sp. marked an important step in the chemotherapy of cancer (2,3). It was the first of a family of amino acids and peptides such as 6-diazo-5-oxo-L-norleucine (**2**, DON, **4**), alazopeptin (**3**) (5) and azotomycin (**4**) (6.7) with the unique diazo group to be isolated from *Streptomycete* cultures. Azaserine has found some limited application as a chemotherapeutic agent in certain forms of leukemia (8).

$$\bar{N} = N = CH\text{-}CO\text{-}O\text{-}CH_2\text{-}CH\text{-}COO^-$$
$$\underset{NH_3{}^+}{\mid}$$
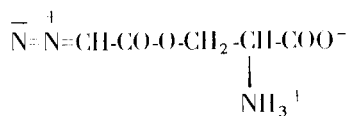
1

Since the synthesis of azaserine was reported (9a,b), a number of analogs, as for example, the 2- and 3-methyl derivatives were synthesized and subjected to screening for biological activity (10). None showed significant activity. This suggested that the role played by azaserine has specific requirements with regard to the size and stereochemistry. To some extent, this may be rationalized on the basis of its well-known function as a "glutamine-antagonist" as elucidated by Buchanan and associates (11).
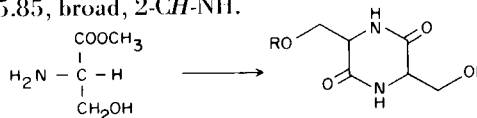
A comparison 6-diazo-5-oxo-L-norleucine and one of its peptide forms, azotomycin showed that the latter was more active and was tolerated by patients better (12). In a microbial system such as *Bacillus subtilis*, the peptide forms **3** and **4** show greater activity than DON. It was postulated that the increased activity of **3** might be because it could be a preferred transport form of **2** (13). In the analogous case of azaserine, no such peptide derivatives have been available for comparison either through isolation or synthesis. As a first step in this direction, the synthesis of a cyclic dipeptide form was considered. It is expected that such a cyclic dipeptide structure would confer greater stability to the molecule and be potentially capable of generating azaserine in the body.

DL-Serine methyl ester was converted to a mixture of the *cis* and *trans* forms of 3,6-bis-hydroxymethylpiperazine-2,5-dione (**5**) (14,15). The mixture was separated by fractional crystallization with the *cis* form being the predominant isomer (60-80%).

Acylation of the *cis* isomer of **5** with chloroacetyl chloride in chloroacetic acid gave the bis-O-chloroacetyl derivative (**6**). Treatment of **6** with sodium azide in aqueous dioxane afforded the 3,6-bis-O-azidoacetyl derivative (**7**). Catalytic hydrogenation of **7** in a neutral solvent (aqueous ethanol with either Pd-C or Pt-catalyst gave a mixture of products but in the presence of two equivalents of hydrochloric acid, the bis-O-glycyl derivative (**8**) was obtained as the crystalline dihydrochloride. On diazotization at $0\text{-}5°$ with sodium nitrite and acid, **8** gave the bis-O-diazoacetate **9** which was obtained as a faint yellow crystalline solid.

In an alternative scheme, base-catalyzed reaction of **5** (*cis*) with the *p*-nitrophenyl ester of Z-glycine in dimethylformamide gave the bis-O-Z-glycyl ester (**10**) which underwent hydrogenation in the presence of two equivalents of hydrochloric acid to form **8**. This approach was found to be superior to the scheme described above, especially for the synthesis of the *cis* **8**.

The *cis* 3,6-diazoacetoxymethylpiperazine-2,5-dione showed the expected spectral properties- uv: max 250 nm; log E, 4.53 which was completely lost on treatment with acid; ir: 3100, 3050 (NH), 2060 ($\bar{N}=N=CH$) and 1680 cm^{-1} (ester) and nmr (DMSO-d$_6$): τ 1.60, broad, 2-NH; τ 4.62, singlet 2-CHN$_2$; τ 5.70, broad, 2-CH-CH_2-O and τ 5.85, broad, 2-CH-NH.

$$\begin{array}{c} COOCH_3 \\ \mid \\ H_2N - \overset{\mid}{C} - H \\ \mid \\ CH_2OH \end{array} \longrightarrow$$

5, R = H (*cis* and *trans*)
6, R = ClCH$_2$CO- (*cis* and *trans*)
6a, R = Br CH$_2$CO- (*trans*)
7, R = N$_3$CH$_2$CO- (*cis* and *trans*)
8, R = HN$_2$CH$_2$CO- (*cis* and *trans*)
9, R = $\bar{N}$=N=CH-CO- (*cis* and *trans*)
10, R = C$_6$H$_5$CH$_2$O-CO-NH - CH$_2$CO- (*cis*)

The first sequence of reactions was repeated with the trans isomer 5 with some modifications which were necessitated by its lower solubility. The bis-O-bromoacetyl derivative (6a) was prepared to achieve a more facile displacement by the azide. However, the yield of 6a was not as high as that of 6 (trans) and the latter was therefore used in the scheme. The trans 3,6-bisdiazoacetoxymethylpiperazine-2,5-dione (9) separated as a crystalline solid during the diazotization step. Its spectral properties were very similar to those of the cis isomer and in agreement with the assigned structure.

The two cyclic peptides are being subjected to antitumor evaluation.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and were uncorrected. Spectra were obtained using the following instruments: Beckman DB (uv), Beckman Acculab 3 (ir), Varian A60A with TMS as internal standard (nmr) and Hitachi-Perkin Elmer SMU-6E (mass spectra).

3,6-Bis-hydroxymethylpiperazine-2,5-dione (5) (14,15).

The following is an improved procedure for 5. A solution of DL-serine methyl ester hydrochloride (5 g.) in methanol (50 ml.) was passed through a column (2.5 x 25 cm) of weak base type ion exchange resin (such as Amberlite IR-45,50-100 mesh) which has been previously washed with 5% aqueous sodium bicarbonate (250 ml.) followed by water (300 ml.) and methanol (200 ml.). The effluent and the methanol wash (100 ml.) were concentrated to an oil which was kept at room temperature for 3-5 days. The semi-solid mass was triturated with a mixture of methanol and ether (1:1) and filtered. The solid (2.1 g., 75%) was recrystallized from hot water to give the trans isomer of 5 as a colorless crystalline solid, m.p. 284-285°; (reported m.p. 285°, 15); Yield, 0.67 g. (24%). The mother liquors on evaporation gave the crude cis isomer which was recrystallized from methanol; m.p. 228-230° (reported m.p. 228-230°, 15), yield, 0.8 g. (32%).

3,6-Bis-chloroacetoxymethylpiperazine-2,5-dione (6 cis).

A mixture of cis 5 (1 g.), chloroacetic acid (4 g.) and chloroacetyl chloride (2 ml.) was stirred at room temperature for 4 hours. The mixture was diluted with water, the solid filtered and crystallized from aqueous methanol; m.p. 210-211°, yield, 1.4 g. (75%); nmr (trifluoroacetic acid): τ 1.6, broad, 2-NH; τ 5.2, broad, 2-CH-CH$_2$; τ 5.7, s, 2-CH$_2$Cl.

Anal. Calcd. for C$_{10}$H$_{12}$Cl$_2$N$_2$O$_6$: C, 36.76; H, 3.98; Cl, 21.67; N, 8.53. Found: C, 36.44; H, 3.75; Cl, 21.95; H, 3.75.

A mixture of trans 5 (1 g.), chloroacetic acid (4 g.), chloroacetyl chloride (2 ml.) and 8-10 drops of sulfuric acid was stirred for 2 days at room temperature with occasional warming to maintain a clear solution. The product (6 trans) was worked up and crystallized as above; m.p. 214-215°, yield, 1.4 g. (75%).

Anal. Calcd. for C$_{10}$H$_{12}$Cl$_2$N$_2$O$_6$: C, 36.76; H, 3.98; Cl, 21.67; N, 8.53. Found: C, 36.48; H, 3.73; Cl, 21.41; N, 8.35.

3,6-Bis-bromacetoxymethylpiperazine-2,5-dione (6a).

Trans 5 (0.5 g.) was stirred with bromoacetic acid (3 g.), bromoacetyl bromide (1.5 ml.) and sulfuric acid (2 drops) at room temperature for two days. The mixture was diluted with methanol and filtered; m.p. 188-190°, yield, 0.72 g. (60%).

Anal. Calcd. for C$_{10}$H$_{12}$Br$_2$N$_2$O$_6$: C, 28.35; H, 2.84; Br, 38.22; N, 6.76. Found: C, 28.55; H, 3.08; Br, 37.92; N, 6.93.

3,6-Bis-azidoacetoxymehtylpiperazine-2,5-dione (7 cis).

A solution of 6 (cis, 1 g.) in 50% aqueous dioxane (25 ml.) was stirred with sodium azide (2 g.) for two days. After concentration to dryness and trituration with water, the solid was filtered and crystallized from aqueous methanol, m.p. 157-158°, yield, 0.9 g. (86%).

Anal. Calcd. for C$_{10}$H$_{12}$N$_8$O$_6$: C, 35.30; H, 3.55; N, 32.98. Found: C, 35.25; H, 3.57; N, 32.78.

The trans isomer 7 was prepared in an analogous manner except that the solvent was dimethylsulfoxide-water (7:3, 10 ml.). The product crystallized from methanol as colorless needles, m.p. 175-177°, yield, 0.65 g. (60%) from 1 g. of 6 (trans).

Anal. Calcd. for C$_{10}$H$_{12}$N$_8$O$_6$: C, 35.30; H, 3.55; N, 32.93. Found: C, 34.97; H, 3.44; N, 32.70.

3,6-Bis aminoacetoxymethylpiperazine-2,5-dione (8 cis).

The cis 7 (1 g.) was hydrogenated in 50% aqueous dioxane (100 ml.) and 1N-hydrochloric acid (6 ml., 2 eq.) in the presence of Adam's catalyst (0.2 g.) for 30 minutes. The mixture was filtered and the filtrate concentrated to near dryness. Addition of ethanol gave a crystalline solid which was recrystallized from ethanol, m.p. 204-206°, yield, 0.6 g. (57%).

Anal. Calcd. for C$_{10}$H$_{18}$Cl$_2$N$_4$O$_6$: C, 33.25; H, 4.98; Cl, 19.63; N, 14.51. Found: C, 32.98; H, 5.11; Cl, 19.48; N, 15.51.

The trans isomer of 8 was prepared and crystallized in a similar manner; m.p. 203-205°, yield, 0.6 g. (60% from 1 g. of 7).

Anal. Calcd. for C$_{10}$H$_{18}$Cl$_2$N$_4$O$_6$: C, 33.25; H, 4.98; Cl, 19.63; N, 15.51. Found: C, 33.42; H, 5.05; Cl, 19.33; N, 15.32.

3,6-Bis-diazoacetoxymethylpiperazine-2,5-dione (9 cis).

A solution of 8 cis (1 g.) in water (20 ml.) was cooled to 0° and treated with 1N hydrochloric acid (0.4 ml.) and sodium nitrite (0.4 g.). During one hour, the absorbance (250 nm) rose to 50,000 units (unit = volume x dilution factor x absorbance reading). The solution was neutralized with sodium bicarbonate and extracted twice with a mixture of phenol and chloroform (3:1). The combined organic layer was shaken with water (10 ml.) and a mixture of ether and hexane (1:1, 100 ml.). The aqueous layer was freed from organic solvents and freeze-dried. The solid was taken up in a mixture of methanol and chloroform (1:2, 20 ml.) and applied to a column of Florisil (25 g.) prepared in a solvent mixture comprised of chloroform, 9 and methanol, 1. Elution of the column with the same solvent mixture gave the major band. Concentration of the fractions gave a crystalline solid which was recrystallized from ethanol, m.p. 213-215°, yield, 0.24 g. (30%).

Anal. Calcd. for C$_{10}$H$_{10}$N$_6$O$_6$: C, 38.71; H, 3.25; N, 27.09. Found: C, 38.52; H, 3.53; N, 26.93.

For the preparation of 9 (trans), a solution of 8 (trans, 0.25 g.) in water (5 ml.) was cooled to 0° and treated with 1N hydrochloric acid (0.2 ml.) and sodium nitrite (0.2 g.). After 20 minutes, the crystalline solid was filtered and recrystallized from water; m.p. 233-235°, yield, 75 mg. (35%); λ max, 250 nm; Log E, 4.52.

Anal. Calcd. for C$_{10}$H$_{10}$N$_6$O$_6$: C, 38.71; H, 3.25; N, 27.09. Found: C, 39.01; H, 3.11; N, 27.24.

3,6-Bis-Z-glycyloxymethylpiperazine-2,5-dione (10).

To 5 (1 g.) in warm dimethylformamide (25 ml.) was added Z-glycine p-nitrophenyl ester (4 g.) and triethylamine (0.3 ml.). After 24 hours, the solvent was removed by concentration under reduced pressure and the residue crystallized from ether and then from methanol to give the desired product, m.p. 181-183°, yield,

2.2 g. (70%); nmr (DMSO-d$_6$): τ 2.50, broad, 2-NH: τ 2.65, singlet, 2-C$_6$H$_5$; τ 4.90, singlet, 2-O-CH_2-C$_6$H$_5$; τ 5.70, broad, 2-CH-CH_2; τ 6.10, doublet, 2-CH_2-NH.

Anal. Calcd. for C$_{26}$H$_{28}$N$_4$O$_{10}$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.25; H, 4.89; N, 10.30.

Catalytic hydrogenation of **10** (1 g.) was carried out in aqueous ethanol (30 ml. (1:1) containing 1N hydrochloric acid (4 ml.) in presence of Adam's Catalyst (0.2 g.) for 2 hours. The mixture was filtered, the filtrate concentrated to dryness and the solid crystallized from ethanol, yield, 0.4 g. (60%). The product was identical with **8** (cis) in all respects.

Acknowledgement.

REFERENCES

(1) To whom correspondence is to be addressed.

(2) S. A. Fusari, R. P. Frohardt, A. Ryder, T. H. Haskell, D. W. Johannessen, C. C. Elder and Q. R. Bartz, *J. Am. Chem. Soc.*, **76**, 2878 (1954).

(3) S. A. Fusari, T. H. Haskell, R. P. Frohardt and Q. R. Bartz, *ibid.*, **76**, 2881 (1954).

(4) H. W. Dion, S. A. Fusari, Z. L. Jakubowski, J. L. Zora and A. R. Bartz, *ibid.*, **78**, 3075 (1956).

(5) S. E. Devoe, N. E. Rigler, A. J. Shay, J. H. Martin, T. C. Boyd, E. J. Backus, J. H. Mowat and N. Bohonos, "Antibiotics Annual," Medical Encyclopedia Inc., 730 (1957).

(6) K. V. Rao, S. C. Brooks, M. Kugelman and A. A. Romano, *ibid.*, 943 (1960).

(7) K. V. Rao, "Antimicrobial Agents Annual," 179 (1962).

(8) J. H. Burchenal, M. L. Murphy and C. T. C. Tan, *Pediatrics*, **18**, 643 (1956).

(9a) J. A. Moore, J. R. Dice, E. D. Nicolaides, R. D. Westland and E. L. Wittle, *J. Am. Chem. Soc.*, **76**, 2884 (1954). (b) E. D. Nicolaides, R. D. Westland and E. L. Wittle, *ibid.*, **76**, 2887 (1954).

(10) H. A. DeWald, D. C. Behn and A. M. Moore, *ibid.*, **81**, 4364 (1959).

(11) S. C. Hartman, B. Levenberg and J. M. Buchanan, *ibid.*, **77**, 501 (1955).

(12) A. J. Weiss, G. Ramierez, T. Grage, J. Strawitz, L. Goldman and V. Downing, *Cancer Cehmotherapy Reports*, **52**, 611 (1968).

(13) R. F. Pitillo, C. Woolley, R. W. Brockman and D. H. Ho, *ibid.*, **55**, 47 (1971).

(14) H. Brockmann and H. Musso, *Chem. Ber.*, **87**, 581 (1954).

(15) A. Martin Municio, *Annales real. Soc. espàn. fis. y. quim.*, (Madrid) 51B, 565 (1955); *Chem. Abstr.*, **50**, 14775f (1956).