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CYCLIC OLIGOMERS OF 4-AMINOPENTANOIC ACID[1]

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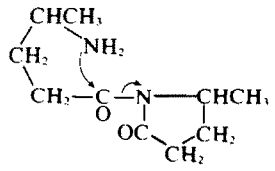
Abstract—Among the neutral products obtained on heating N-(4-amino-pentanoyl)-4-aminopentanoic acid *p*-nitrophenyl ester in dilute pyridine solution are the lactam of 4-aminopentanoic acid and the cyclic tetramers of the amino acid. The 10-membered ring product of direct cyclization was not detected.

INTRODUCTION

For a conformational study, we desired to obtain the isomers of 5,10-dimethyl-1,6-diazacyclodecan-2,7-dione, the cyclic dimer of 4-aminopentanoic acid. To this end we prepared by standard peptide procedures the hydrobromide of N-(4-aminopentanoyl)-4-aminopentanoic acid p-nitrophenyl ester, starting with racemic 4-aminopentanoic acid derived from the commercially obtainable 5-methyl-2-pyrrolidinone. Cyclization was carried out by prolonged storage of the dimer active ester at 0.025 M in boiling pyridine. Cyclic amides were separated from other products by treatment with methanol and a mixed-bed ion exchange resin.

The principal neutral product of the cyclization process was the original lactam, 5-methyl-2-pyrrolidinone; it accounted for at least 40% of the amino acid residues in the starting material. Other neutral products accounted for a further 20% of the 4-aminopentanoic acid units. These turned out to be chiefly cyclic tetramers of 4-aminopentanoic acid, products of cyclodimerization.

To yield the pyrrolidinone instead of the 10-membered ring, the nitrophenyl ester probably underwent base-catalyzed intramolecular cyclization to the acyl lactam; this, as indicated below, can lead directly to fragmentation into two molecules of pyrrolidinone.



The remaining neutral products were subjected to a variety of fractionation processes: extraction, fractional crystallization, sublimation, partition chromatography and gel filtration chromatography. Fractions were examined by mass spectrometry, using a direct probe at varying inlet temperatures up to 260°. No indication of a product of mass 198, the desired result of cyclization, was obtained, nor was there any indication of its most likely fragmentation products (M^+–CH_3, M^+–CO). The principal components were of mass 396, corresponding to the cyclic tetramer. Judging from gel filtration experiments, the tetramer comprised about 80% of the non-lactam neutral product; 20% was of higher molecular weight.

Three of the isomeric cyclic tetramers were isolated in small amounts. The N–H resonances of these products in trifluoroacetic acid are shown in Fig. 1. One product, m.p. 376–380°, has a spectrum indicating that all of the amide protons and all of the methyl groups are identical in the NMR time average; it is therefore either the diastereomer

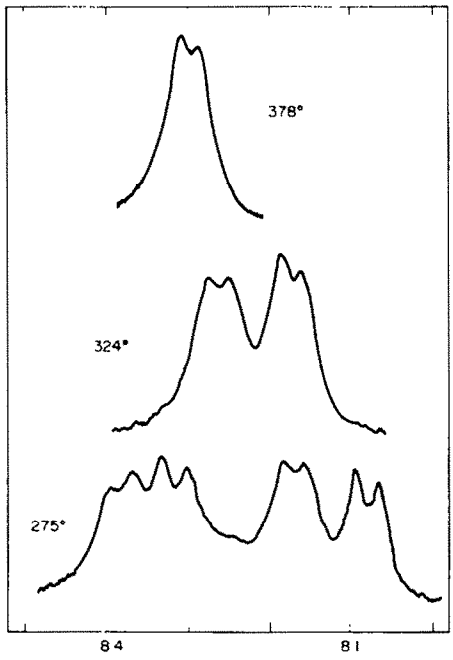


Fig. 1. Amide proton resonances of isolated cyclic tetramers of 4-aminopentanoic acid, about 40 mg/ml in trifluoroacetic acid, 30°. The reference is internal TMS. The isomers are identified by melting point. The 4(γ)-proton resonances (not shown) of the m.p. 276° isomer were split, 3H at 4.30 and 1H at 4.19. The 4.19 ppm proton is coupled to the 8.33 ppm N–H proton. The 4-proton resonances of the other two isomers were not separately resolved.

in which all residues are of the same configuration or that in which the configurations alternate around the ring, R–S–R–S. A product of m.p. 324° exhibits only two kinds of amide proton and is thereby identified as the R–R–S–S isomer. The third isolated product, m.p. 274–6°, shows four different N-H units and is thus the asymmetric isomer, R–R–S–R (S–S–R–S).

Thus it appears that rather than cyclize by intramolecular reaction of amino group with nitrophenyl ester carbonyl, the dimer active ester undergoes fragmentation via intramolecular attack of amide, a less reactive nucleophile, to form 5-membered rings. Competitive with fragmentation is intermolecular amide formation, even though intermolecular reaction is inhibited by dilution under the conditions used. It is clear that formation of the 10-membered 1,6-diamide is not at all favored, presumably for steric reasons. This may be contrasted with the facile formation of the cyclic dimer of ε-aminocaproic acid, a 14-menbered ring, from linear dimer active ester[2] or azide.[3]

The stereochemical properties of the 10-membered 1,6-diamide ring must be closely similar to those described for the 1,6-cyclodecadienes.[4-7] Models of the diamide or the diene with two *trans* amide or double bonds can be constructed without bond angle or torsional strain. There is, however, considerable crowding of the π systems of the parallel planar unsaturated groups; they are about 2.5 A apart, but have a van der Waals sum of about 3.4 A. The cyclic dimer of ϵ-aminocaproic acid does have a structure with parallel planar *trans* amide groups[8] but because the saturated chains bridging the amide groups, are two atoms longer, there is no $\pi-\pi$ interference. Models with one *cis* and one *trans* unit in the 10-menbered series have torsional strain. Models with two *cis* units can relieve the $\pi-\pi$ crowding and retain favorable torsional angles;[4,6] for cyclodecadiene the *cis–cis* is in fact the most stable form.[5,6] However for monosubstituted amides *cis* configurations are generally less stable than *trans* by 2 kcal or more.[9]

The considerations above, applied to the intermediate stages in the cyclization process, could rationalize the absence of 10-membered ring among the products from N-(4-aminopentanoyl)-4-aminopentanoic nitrophenyl ester. However, cyclizations of di-α-t-butyl L-(γ)-glutamyl-L-glutamate pentachlorophenyl ester, an analog in which methyl is replaced by t-butoxycarbonyl, does yield 36% of the 10-membered ring after 1 day at 20–40°.[10] Consistent with the presence of strain, the product is reported to show unusual susceptibility to ring-opening hydrolysis. In the present case no reaction is detectable after a day at room temperature. After a day at 115° only lactam, tetramers and starting materials are found.

The bulkier side groups of the (γ)-glutamyl derivative do not reduce strain in the 1,6-diazacyclodecane-2,7-dione ring. The difference in the two results may be kinetic rather than thermodynamic. Formation of the 5-membered acyl lactam is apparently the favored intramolecular process in the aminopentanoic system, but this reaction may be sufficiently inhibited by the electron-withdrawing nature of a t-butoxycarbonyl group so that cyclization to the 10-membered ring becomes the dominant intramolecular process in the glutamic acid system.

EXPERIMENTAL

M.ps were determined in a Mel-Temp apparatus and are corrected. A Perkin–Elmer Model 257 grating IR spectrophotometer was used to obtain the IR spectra. Mass spectra were obtained with a Varian MAT Model CH-7 mass spectrometer operated with a resolution (10% valley) of 1000. Proton NMR spectra were obtained using either a Varian T-60 instrument or the 250 MHz instrument of the NMR Facility for Biomedical Studies, Carnegie-Mellon University. Analyses were performed by Micro-Tech Laboratories, Stokie, Illinois. Thin-layer chromatographic plates were Quantum Industries type Q-6.

dl-t-*Butyl 4-aminopentanoate hydrochloride* (**1**) was prepared by the usual H_2SO_4 catalyzed esterification using isobutylene in dioxane.[11] A 50% yield of chromatographically homogeneous ester hydrochloride, m.p. 149–151°, ν(KBr): 1730 (ester), was obtained and used without further purification.

dl-N-*Carbobenzyloxy-4-aminopentanoic acid* (**2**) was obtained in 70% yield by Schotten-Baumann acylation of *dl*-4-aminopentanoic acid,[12] itself obtained by barium hydroxide hydrolysis[13] of *dl*-5-methyl-2-pyrrolidinone (Aldrich Chemical Co., Milwaukee). The product was crystallized from EtOAc-hexane, m.p. 105-109°, chromatographically homogeneous (Found: C, 62.28; H, 6.87; N, 5.54. $C_{13}H_{17}NO_4$ requires: C, 62.14; H, 6.82; N, 5.57%).

Resolution of N-*carbobenzyloxy-4-aminopentanoic acid.* Resolution was accomplished via multiple recrystallization of the quinine salt from EtOH–ether. The *d*-enantiomer was obtained from the more soluble salt: m.p. 75.5–77°, $[\alpha]_D^{20}$ 17.4° (24.5 mg/ml in EtOAc). The *l*-form had m.p. 77–78° (Found: C, 62.15; H, 6.80; N, 5.45. $C_{13}H_{17}NO_4$ requires: C, 62.14; H, 6.82; N, 5.57%).

A sample of *l*-4-aminopentanoic acid was obtained on cleavage of the *l*-carbobenzyloxy derivative by HBr in trifluoroacetic acid and recovery by ammonia neutralization of a 2-propanol soln of the hydrobromide: m.p. 196–202°, $[\alpha]_D^{20}$ – 11.1° (27.4 mg/ml in water), reported, – 12°.[14]

Diastereomeric mixture of N - *carbobenzyloxy* - 4 - *aminopentanoyl* - 4 - *aminopentanoic* t - *butyl esters* (**3**). The acid **1** (10.2 g, 0.076 mole), the ester hydrochloride **2** (16 g, 0.077 mole) and 7.7 g (0.076 mole) of N-methyl-morpholine were combined in 150 ml of dimethylformamide vacuum distilled from copoly(ethylene, maleic anhydride), Monsanto EMA-11. The solution was cooled to 0° in an ice bath and 16 g (0.083 mole) of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Story Chemical Co., Muskegon, Michigan) dissolved in a minimum amount of dimethylformamide was added. After 2–3 hr at 0°, the soln was stirred at room temp overnight before the solvent was removed in vacuum. The residual oil was dissolved in chloroform and washed thoroughly with 5% $NaHCO_3$ aq, 5% citric acid and saturated salt. The chloroform soln was dried over Na_2SO_4, evaporated, and the residue was crystallized from chloroform–hexane. 13 g (42%), m.p. 96.5–99.5°, was obtained: *m/e*: 406(M^+), 301 (metastable, $M^+ \rightarrow M-C_4H_9$); ν(KBr): 1730 (ester), 1686 (carbamate), 1642 amide; $\delta(CDCl_3)$: 1.5 (s, 9, t-Bu), 1.2 (d, 6, 5-Me), 5.33 (s, 2, benzyl CH_2), 7.73 (s, 5, aromatic) (Found: C, 65.44; H, 8.72; N, 6.72. $C_{22}H_{34}N_2O_5$ requires: C, 65.00; H, 8.43; N, 6.89%).

The single isomer derived from the dextrorotatory enantiomer only of 4-aminopentanoic acid, by the same route, had m.p. 112.5–113° and the same spectral observables (Found: C, 64.78; H, 8.60; N, 6.88. $C_{22}H_{34}N_2O_5$ requires: C, 65.00; H, 8.43; N, 6.89%).

Mixed N - *carbobenzyloxy* - 4 - *aminopentanoyl* - 4 - *aminopentanoic acids* (**4**). The ester **3** was stirred 1 hr with trifluoroacetic acid (15 g in 35 ml). Trifluoroacetic acid was removed in vacuum and the residue was mixed with 1 l of ice-water. The precipitate was crystallized from water. Chromatographically homogeneous product, m.p. 154–158°, was obtained in at least 90% yield (Found: C, 61.62; H, 7.46; N, 7.88. $C_{18}H_{26}N_2O_5$ requires: C, 61.70; H, 7.48; N, 7.99%); *m/e*: 350 (M^+); ν(KBr): 1692 (carboxyl, carbamate), 1642 (amide).

Mixed N - *carbobenzyloxy* - 4 - *aminopentanoyl* - 4 - *aminopentanoic* p - *nitrophenyl esters* (**5**). The acid **4** was coupled to *p*-nitrophenol by dicyclohexylcarbodiimide in pyridine.[15] The mixed esters, running as a single component on thin layer chromatograms, was obtained in 78% yield, m.p. 120–125°, after recrystallization from EtOAc–hexane: *m/e*: 333 (M^+–$O_2NC_6H_4O$); ν(KBr): 1762 (ester), 1693 (carbamate), 1640 (amide) (Found: C, 61.31; H, 6.30: N, 9.00. $C_{24}H_{29}N_3O_7$ requires: C, 61.13; H, 6.20; N, 8.91%).

Cyclization of 4 - *aminopentanoyl* - 4 - *aminopentanoic* - p - *nitrophenyl esters.* The blocked dimer nitrophenyl ester **5** (11.6 g, 0.025 mole) was dissolved in 60 ml trifluoroacetic acid, and HBr was bubbled through the soln 1 hr. The solvent was evaporated and the residue triturated with anhyd ether until it solidified. This product was dissolved in 1 l. of dried pyridine (distilled from KOH and stored over 5A molecular sieve). The soln was heated to reflux, with care taken to exclude moisture, one week. The pyridine was then removed by evaporation.

When the residue was taken up in MeOH (100 ml) a small quantity of insoluble solid remained. This solid was recrystallized from MeOH, to yield 78 mg, m.p. 376–380° (sealed tube under nitrogen) (Found: C, 60.32; H, 9.05; N, 14.27. $(C_{10}H_{18}N_2O_2)_n$ requires: C, 60.58; H, 9.15; N, 14.13%); *m/e*, direct inlet, 260°: 396 (M^+), 368 (M^+–CO); ν(KBr): 1648, 1558 (amide I, II); NMR, see Fig. 1.

The MeOH soln of the original product was treated by stirring overnight with several equivalents (*ca.* 30 g) of mixed bed ion exchange resin, BioRad Ag 501 × 8, previously washed with MeOH, then decolorizing with charcoal. The solvent was evaporated and the residue triturated with ether to produce 1.03 g of solid. The ether, on evaporation, yielded about 2 g of 5-methyl-2-pyrrolidinone, identified by TLC, IR and mass spectrometry.

Mass spectra of the ether extract revealed no products of *m/e* 198. At sufficiently high inlet temperatures some ions of *m/e* 396 were present. Fractional crystallization of the ether-insoluble product from MeOH–ether yielded 36 mg of a second chromatographically homogeneous tetramer, m.p. 324° d (sublimes > 300°) (Found: C, 59.95; H, 9.01; N, 14.16. $(C_{10}H_{18}N_2O_2)_n$ requires: C, 60.58; H, 9.15; N, 14.13%); *m/e*, direct inlet, 260°: 396 (M^+), 368 (M^+–CO); ν(KBr): 1655, 1545 (amide I, II); NMR, see Fig. 1. All other fractions were combined and partition chromatographed on a silica gel column using a butanol–acetic acid–water (3/1/1) solvent system. One additional homogeneous substance was thereby obtained, 46 mg, m.p. 274–6° d. (Found: C, 60.12; H, 9.07; N, 14.21. $(C_{10}H_{18}N_2O_2)_n$ requires: C 60.58; H, 9.15; N, 14.13%); *m/e*, direct inlet 220°: 396 (M^+), 368 (M^+–CO); ν(KBr): 1655, 1545 (amide I, II) NMR, see Fig. 1.

The bulk of the product, which was not separated into components, was identified as a mixture of polymers of 4-aminopentanoic acid by elemental analysis and NMR spectrum of a reprecipitated (MeOH–ether) and dried sample. The proton spectrum (trifluoracetic acid) showed resonances characteristic of α(2.70), β(2.03), γ(4.18, 4.3), σ(1.4) and NH (*ca.* 8.25) protons in the required 2:2:1:3:1 ratio, with no indication of additional end group resonances.

The N–H and 4-proton regions of the spectrum are consistent with a composition including about 50% of the m.p. 275° component and perhaps 20% of the m.p. 324° component, but additional N–H absorptions, not exhibited by any of the isolated cyclic tetramers, are present as well. A sample of this material, subjected to gel filtration chromatography on Sephadex LH-20 in EtOH, separated into two fractions, neither homogeneous to TLC. The two fractions had identical IR spectra (KBr) with amides I and II band at 1645 and 1545, closely similar in the fingerprint region to the spectra of the isolated cyclic tetramers, although with lines less sharp. The mass spectrum of the larger, slower running fraction was characterized by the *m/e* 396 and 368 peaks of the isolated tetramers; no larger species were detected, and the distribution of fragments was similar. The mass spectrum of the smaller, faster running fraction, which comprised 0.2 of the total, also showed the *m/e* 396 peak as the only evidence of volatile (260°, direct inlet) material. If this fraction contained higher oligomers, they were not volatile.

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