Cyclo-(L-asparagyl-L-asparagyl) [(3*S*,6*S*)-3,6-Bis(carbamoylmethyl)-piperazine-2,5-dione]: Preparation and Crystal Structure

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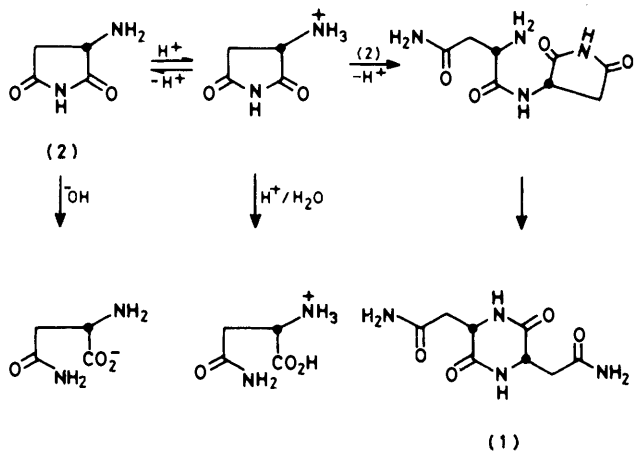
Two methods are described for the preparation of optically pure cyclo-(L-asparagyl-L-asparagyl) [(3*S*,6*S*)-bis(carbamoylmethyl)piperazine-2,5-dione]: (i) by self-condensation of (*S*)-3-aminopyrrolidine-2,5-dione in refluxing acetonitrile, and (ii) by self-condensation of L-asparagine methyl ester at room temperature; the latter method is more efficient. The identity of this previously uncharacterised piperazinedione was confirmed by analytical and spectroscopic data, and by a crystal structure determination, which showed the conformation of the piperazinedione ring to be a bowsprit boat (*i.e.* quasi-equatorial carbamoylmethyl substituents). The angle between the amide units of the piperazinedione ring is 18°. Intramolecular hydrogen bonds are absent from the crystal structure, the packing of which is dominated by intermolecular hydrogen bonds from each piperazinedione CO to the carbamoyl NH_2 (each NH of which is engaged in hydrogen bonding to a different molecule of piperazinedione).

Although piperazine-2,5-diones were found very early in the development of the chemistry of amino acids and peptides,[1] for one of the common natural amino acids, asparagine, there is no clear description of its (3*S*,6*S*)-piperazine-2,5-dione (1) [cyclo-(L-asparagyl-L-asparagyl)] in the literature. A piperazine-2,5-dione derived from L-asparagine was prepared by Emil Fischer,[2] but was not completely characterised. A piperazine-2,5-dione of asparagine has also been obtained by heating diethyl fumarate with ammonia [3,4] but the stereochemical identity of this substance was not established. For a synthetic project we required gram quantities of cyclo-(L-asparagyl-L-asparagyl) (1). We describe here two preparative methods for this compound, the identity of which has been established by analytical and spectroscopic data, and a determination of its crystal structure.

Preparation of Piperazinedione (1) *from* (S)-3-*Aminopyrrolidine*-2,5-*dione*.—Sondheimer and Holley [5] reported that an aqueous solution of (*S*)-3-aminopyrrolidine-2,5-dione (2) deposited crystals of a substance believed to be the piperazine-2,5-dione of asparagine. We expected compound (2) to exhibit pH-dependent behaviour in water. Hydrolysis to asparagine was expected [6] at high and at low pH, whereas at intermediate pH values formation of the piperazinedione (1) might occur from the reaction of the pyrrolidinedione (2) with its conjugate acid (*cf.* Scheme). The optimum pH for the production of the piperazinedione (1) will then be governed by the pK_a of the pyrrolidinedione (2).

Monitoring reactions of the pyrrolidinedione (2) in aqueous phosphate buffers by ^1H n.m.r. spectroscopy showed that formation of the piperazinedione (1) was maximum at *ca.* pH 7. Thus, a solution of the pyrrolidinedione (2) in D_2O–phosphate buffer showed the appearance of signals for the piperazinedione (1) and for asparagine, and the concomitant disappearance of the resonances from the pyrrolidinedione (2). The identity of the piperazinedione (1) was proved by its isolation from the buffered solution and comparison with an authentic sample prepared in another way (see below and Experimental section); the presence of asparagine was confirmed by adding this substance to the n.m.r. tube. Eventually, piperazinedione (1) separated from the solution preventing us from carrying out a kinetic analysis of this system. At pH 6.65 the initial ratio of the piperazinedione (1) : asparagine was *ca.* 1.2 : 1. At other pH's (5.21, 6.03, and 7.55) the proportion of asparagine was greater. In 1M-NaOD in D_2O (pH 13.1) the pyrrolidinedione (2) was rapidly hydrolysed at room temperature to asparagine. The pyrrolidinedione (2) in



Scheme.

1M-DCl in D_2O (pH −0.97) was stable for 5 days at room temperature, but was hydrolysed to aspartic acid on heating the solution at 80 °C for 38 h.

To avoid the formation of asparagine or aspartate from the pyrrolidinedione (2) we heated the dione in anhydrous acetonitrile and obtained a 40% yield of pure piperazinedione (1). This product was identical in all respects with the piperazinedione prepared as described in the next section.

Preparation of the Piperazinedione (1) *from* (L)-*Asparagine Methyl Ester*.—Fischer [2] prepared cyclo-(asparagyl-asparagyl) (1) by heating 3,6-bis(methoxycarbonylmethyl)piperazine-2,5-dione, derived from dimethyl L-aspartate, with ammonia. This process is liable to cause racemisation [7] and an optical rotation was not quoted for the piperazinedione obtained. We repeated Fischer's procedure twice. On the first occasion a 62% yield of once-recrystallised asparagine piperazine-2,5-dione was obtained with $[\alpha]_D^{20}$ −20° (*c* 1.2 in H_2O). On the second occasion a small quantity (4%) of asparagine piperazine-2,5-dione with an optical rotation of 0 was obtained, as well as material (5%) having $[\alpha]_D^{24}$ −5.4°.

We have prepared asparagine piperazine-2,5-dione by self-condensation of L-asparagine methyl ester. Although a small amount of racemisation may occur during this method for preparing piperazine-2,5-diones because of the basic reaction

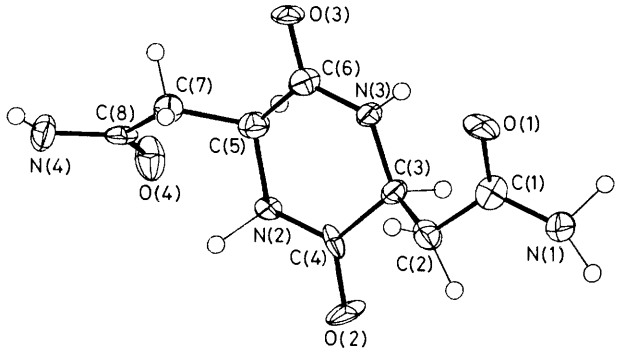


Figure 1. View of the molecule showing atomic numbering

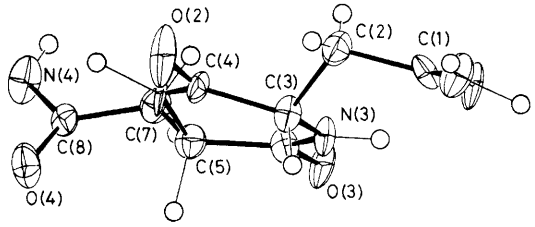


Figure 2. View of the molecule showing the piperazinedione ring conformation

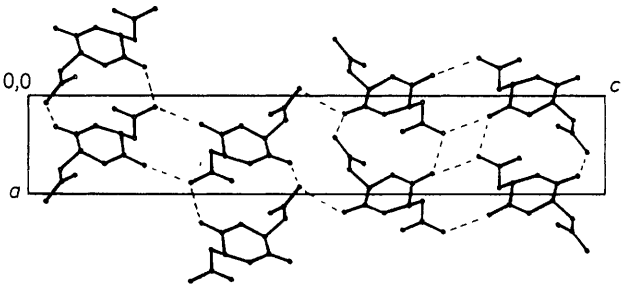


Figure 3. Packing diagram viewed down *b*, with H bonds dashed

conditions, optically pure piperazinediones can be obtained after recrystallisation of the crude product.[8]

We found that N^{α}-benzyloxycarbonyl-L-asparagine can be efficiently converted into its methyl ester by careful control of esterification using 2M-HCl in methanol at −15 °C.[9] The use of higher concentrations of HCl or higher reaction temperatures gave appreciable amounts of dimethyl aspartate. Removal of the benzyloxycarbonyl group from the ester by hydrogenolysis gave L-asparagine methyl ester which, at room temperature during 48 h deposited crystals of the piperazinedione (1). These were recrystallised once to give cyclo-(L-asparagyl-L-asparagyl) (64%). ^1H and ^{13}C N.m.r. spectra (see Experimental section) of the piperazinedione (1) were in accord with the assigned structure. Compound (1) gave a satisfactory combustion analysis. The optical rotation of the piperazinedione (1) prepared by the above method was $[\alpha]_D^{20}$ −26.6° (*c* 1.2 in H_2O), identical with the value obtained for (1) prepared from (*S*)-3-aminopyrrolidine-2,5-dione (see previous section).

To exclude the possibility that samples of the piperazinedione (1) prepared by our methods were contaminated with cyclo-(D-asparagyl-L-asparagyl) we attempted to prepare this substance from diethyl fumarate and ammonia.[4] The asparagine piperazinedione from this reaction could be either the D,L-isomer or the racemate (D,D/L,L) (assuming that preferential crystallisation of one enantiomer from the racemate does not occur). The material obtained was much less soluble in water than cyclo-(L-asparagyl-L-asparagyl) and its ^1H n.m.r spectrum (in $[^2H_6]Me_2SO$) differed from that of the L,L-isomer. We believe therefore that the substance obtained from diethyl fumarate and ammonia is cyclo-(D-asparagyl-L-asparagyl). The ^1H n.m.r. spectrum of cyclo-(L-asparagyl-L-asparagyl) did not show resonances attributable to the D,L-isomer.

Crystal Structure of Cyclo-(L-asparagyl-L-asparagyl).—The crystal structures of several piperazine-2,5-diones have recently been determined [10-21] because these compounds are simple model systems suitable for probing interactions between the side-chains of amino acid residues in proteins (*cf.* refs. 18*a* and 20). The determinations have revealed a variety of conformational possibilities described as planar, chair, bowsprit boat, flagpole boat, and twist-boat. The main factors that determine the conformation are amide resonance (maximum for the planar and boat conformations), steric repulsions between 3,6-substituents (minimised in the bowsprit boat), hydrophobic interactions between the piperazinedione ring and a 3- or 6-substituent (stabilising the flagpole-boat conformation when a substituent contains an aromatic group) and hydrogen bonding (inter- or intra-molecular); some of the crystals contain water molecules engaged in hydrogen bonding. Examination of a molecular model of cyclo-(L-asparagyl-L-asparagyl) shows that the flagpole-boat conformation could be stabilised by intramolecular hydrogen bonding between the carbamoyl groups. The bowsprit-boat conformation could be stabilised by intramolecular hydrogen bonds between each carbamoyl *CO* and the nearest piperazinedione N*H*. However, we find that in the crystal structure of cyclo-(L-asparagyl-L-asparagyl) (Figure 1) the conformation of the piperazinedione ring is an extended bowsprit-boat from which intramolecular hydrogen bonding is absent.

In the ring, N(3), C(4), N(2), and C(6) are coplanar, with a larger deviation from this plane for C(5) (0.31 Å) than C(3) (−0.09 Å) (Table 3); thus, the boat is flattened at the C(3) end. The angle between planes C(3)–C(4)–N(2)–C(5) and C(3)–N(3)–C(6)–C(5) is 18° (Figure 2) [*cf.* 26° in cyclo-(L-alanyl-L-alanyl)].[13] The molecule contains an approximate two-fold axis, ignoring the differing dihedral angles about C(1)–C(2) (58°) and C(7)–C(8) (9°). The C(1)–C(2)–C(3)–N(3) dihedral angle is −54° and that for C(8)–C(7)–C(5)–N(2) is −68°.

The packing is dominated by intermolecular hydrogen bonds between (i) N(1), O(2a), O(2b), and (ii) N(4) and O(3c). There is also a hydrogen bond between N(4) and O(3d), but it is very long (3.22 Å) (Figure 3). The molecular dimensions are normal (Table 2) and are similar to those in other piperazine-2,5-diones [*e.g.* cyclo-(L-alanyl-L-alanyl)].[13] The difference between the C(sp^2)–N (average 1.34$_5$ Å) and C(sp^3)–N (average 1.47 Å) distances is notable.

One unusual property of the crystals of piperazinedione (1) is their high density (1.55 g cm^{-3}). This is probably due to the high proportion of N and O atoms, because the crystals do not show a very tightly bound structure.[22]

Experimental

Solvents were either AnalaR grade or redistilled laboratory reagent. M.p.s are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 257 spectrophotometer. ^1H N.m.r. spectra were recorded at 220 MHz with a Perkin-Elmer R34 spectrometer (internal standard: tetramethylsilane for organic solvents, sodium 3-trimethylsilylpropanesulphonate for D_2O). $^{13}C\{^1H\}$

N.m.r. spectra were recorded at 100.6 MHz with a Bruker WH-400 spectrometer (internal standard: dioxane). Buffers were prepared in D_2O by mixing appropriate volumes of 0.5M-NaOD and 0.5M-KD_2PO_4 in D_2O. The pH values given are direct readings obtained from a Pye-Unicam 405 electrode and Corning PT1-5 meter.

Cyclo-(L-*asparagyl*-L-*asparagyl*) *from* L-*Asparagine Methyl Ester*.—L-N^{α}-Benzyloxycarbonyl-L-asparagine (94%) was prepared from L-asparagine as described by Boissonas *et al.*:[23] m.p. 163—164 °C (lit.,[23] m.p. 163 °C), δ (1 : 1 [$^2H_6$]Me_2CO–D_2O) 2.83 (2 H, d, CH_2), 4.58 (1 H, t, CH), 5.12 (2 H, s, PhCH_2), and 7.4 (5 H, m, phenyl H), $[\alpha]_D^{20}$ +7.5° (*c* 1.5 in AcOH) (lit.,[24] $[\alpha]_D$ +7.6°). A stirred suspension of this compound (5.0 g, 0.018 mol) in dry methanol (50 cm^3) was cooled to −60 °C. Acetyl chloride (7.0 cm^3, 0.1 mol) was added dropwise whilst the reaction temperature was maintained at −60 °C. The temperature was then maintained at −15 °C for 24 h. The solvent was removed at 0 °C under reduced pressure, the residual solid was washed with ethoxyethane and dried to give white crystals of N^{α}-benzyloxycarbonyl-L-asparagine methyl ester (5.18 g, 98%): m.p. 153—153.5 °C (lit.,[23] m.p. 150 °C); t.l.c. (silica gel F_{254}, 5% v/v MeOH in CH_2Cl_2) one spot at R_F 0.6; δ ([$^2H_6$]Me_2CO) 2.7—3.0 (2 H, m, CH_2), 3.67 (3 H, s, OMe), 4.58 (1 H, t, CH), 5.08 (2 H, s, PhCH_2), and 7.3 (5 H, m, phenyl H), $[\alpha]_D^{20}$ −2.9° (*c* 4.0 in AcOH) (lit.,[24] $[\alpha]_D$ −2.0°).

N^{α}-Benzyloxycarbonyl-L-asparagine methyl ester (5.0 g, 0.017 mol) in methanol (30 cm^3) and water (5 cm^3) containing 10% Pd–C catalyst (0.12 g) was hydrogenated (Parr apparatus, initial hydrogen pressure 20 lb in^{-2}) for 4 h. The mixture was filtered through Celite and the solvent was removed from the filtrate to give L-asparagine methyl ester as a yellow oil (2.0 g, 76%), δ (D_2O) 2.72 (2 H, d, CH_2), 3.75 (3 H, s, OMe), and 3.88 (1 H, t, CH); *m/z* [electron impact (E.I.)] 147 (M^+ + 1) (100%), 115 (25), 102 (6), 87 (42), and 43 (15): $\nu_{max.}$ (neat) 3 350s, 3 190s, 1 720s, 1 670s, 1 440m, and 1 210m cm^{-1}. This ester was stored for at least 48 h at room temperature and gave a crystalline mass. This was recrystallised from water to give *cyclo-*(L-*asparagyl*-L-*asparagyl*) as white platelets (1.0 g, 64%) which did not melt <280 °C, δ ([$^2H_6$]Me_2SO) 2.55 (4 H, m, 2 × CH_2), 4.16 (2 H, t, 2 × CH), 6.93 (2 H, br s, 2 × NH), 7.42 (2 H, br s, 2 × NH), and 7.80 (2 H, br s, 2 × ring NH, exchanges the most rapidly on adding D_2O); δ (D_2O) 2.87 (4 H, d) and 4.47 (2 H, t); δ (^{13}C) (D_2O) 38.99 (2 × CH_2), 52.32 (2 × CH), 169.76 (2 × $CONH_2$), and 174.76 p.p.m. (2 × ring CO) [*cf.* 57.68 (2 × CH), 63.63 (2 × CH_2), and 169.11 (2 × CO) for cyclo-(L-seryl-L-seryl); assignments for CH and CH_2 confirmed by off-resonance decoupling]; *m/z* (E.I.) 228 (M^+, 42.1%), 211 (67.1), 183 (52.8), 166 (28.5), 153 (15.7), and 138 (29.2); $\nu_{max.}$ (Nujol) 3 390m, 3 240w, 3 120w, 1 660s, 1 630s, and 1 300m cm^{-1} (Found: C, 42.0; H, 5.25; N, 24.2. $C_8H_{12}N_4O_4$ requires C, 42.1; H, 5.25; N, 24.5%); $[\alpha]_D^{20}$ −26.6° (*c* 1.2 in H_2O).

A crystal from this sample was used for the crystallographic determination.

Cyclo-(L-*asparagyl*-L-*asparagyl*) *from* (S)-3-*Aminopyrrolidine-2,5-dione*.—To a suspension of N^{α}-benzyloxycarbonyl-L-asparagine methyl ester (2.14 g, 7.6 mmol) in water (10 cm^3) was added 0.5 mol dm^{-3} sodium hydroxide (15 cm^3, 7.5 mmol) and the mixture was stirred for 15 min. The solution was filtered and the filtrate was acidified with 1 mol dm^{-3} aqueous hydrochloric acid. After the mixture had been cooled for 30 min the resulting precipitate was filtered off, washed with ice-cold water, and recrystallised from ethyl acetate–light petroleum (b.p. 40—60 °C) to give (*S*)-3-benzyloxycarbonylaminopyrrolidine-2,5-dione (1.40 g, 74%), m.p. 80—81 °C (lit.,[5] m.p. 79—81 °C), δ ($CDCl_3$) 2.6—3.05 (2 H, m, ring CH_2), 4.35 (1 H, dd, CH), 5.05 (2 H, s, PhCH_2), 6.12 (1 H, br, NH), 7.3 (5 H, s, phenyl H), and 9.45 (1 H, br, imide NH), $[\alpha]_D^{20}$ −43° (*c* 3.6 in EtOH) (lit.,[5] $[\alpha]_D$ −43°).

The above imide (0.50 g, 2.0 mmol) in methanol (10 cm^3) containing 10% Pd–C catalyst (0.15 g) was hydrogenated (Parr, initial pressure 10 lb in^{-2}) for 2.5 h. The resulting mixture was filtered and the filtrate was evaporated to give a white solid. This was recrystallised from aqueous methanol to give (*S*)-3-aminopyrrolidine-2,5-dione (0.2 g, 87%), m.p. 143 °C (decomp.) [lit.,[5] m.p. 144 °C (decomp.)]; δ (D_2O) 2.54 (1 H, dd, J_{vic} 5.5 and J_{gem} 18.5 Hz, H-4), 3.09 (1 H, dd, J_{vic} 10 and J_{gem} 18.5 Hz, H-4′), and 3.97 (1 H, dd, J_{vic} 5.5 and 10 Hz, H-3); $[\alpha]_D^{20}$ −77° (*c* 2.5 in MeOH) (lit.,[5] $[\alpha]_D$ −77°).

(*S*)-3-Aminopyrrolidine-2,5-dione (0.15 g, 1.3 mmol) in dry acetonitrile (20 cm^3) was boiled under reflux for 7 days. The precipitated solid was filtered off and recrystallised from water to give cyclo-(L-asparagyl-L-asparagyl) (0.06 g, 40%), δ (D_2O) and $[\alpha]_D^{20}$ in H_2O (*c* 1.1) identical with data for the piperazinedione obtained as described above.

Cyclo-(*asparagyl-asparagyl*) [*Excess of* (3L,6L)-*Isomer*].—This was prepared twice from dimethyl L-aspartate by following the procedure of Fischer and Koenigs.[2] The properties of the two products are described in the text.

Cyclo-(D-*asparagyl*-L-*asparagyl*).—This was prepared as described by Dunn and Fox.[4] The crude product was re-

Table 1. Atomic co-ordinates (× 10^4), with standard deviations in parentheses *

Atom	*x*	*y*	*z*	*U*	Atom	*x*	*y*	*z*	*U*
C(1)	2 816(26)	1 760(21)	8 174(4)	28	O(3)	1 837(17)	6 087(17)	9 529(2)	35
C(2)	661(24)	3 427(25)	8 163(4)	31	O(4)	−3 088(19)	12 796(17)	9 185(3)	42
C(3)	1 139(26)	5 709(23)	8 401(4)	25	H(101)	3 750	845	7 502	63
C(4)	−1 064(24)	7 314(23)	8 347(4)	26	H(102)	5 625	−2	7 871	63
C(5)	−645(25)	8 598(24)	9 093(4)	29	H(401)	−6 387	10 504	9 889	63
C(6)	1 081(26)	6 530(22)	9 167(4)	28	H(21)	395(187)	3 774(185)	7 820(28)	23(32)
C(7)	−2 520(25)	8 925(24)	9 447(4)	29	H(22)	−276(192)	2 789(188)	8 335(31)	78(34)
C(8)	−3 770(24)	11 252(23)	9 427(4)	24	H(31)	2 655(261)	6 384(239)	8 227(38)	73(50)
N(1)	3 834(22)	991(22)	7 810(3)	38	H(201)	−3 825(260)	9 336(246)	8 678(39)	103(50
N(2)	−1 924(20)	8 396(19)	8 683(3)	27	H(301)	2 939(144)	4 123(136)	8 861(20)	23(20)
N(3)	1 901(20)	5 349(18)	8 836(3)	24	H(51)	817(218)	10 122(221)	9 057(36)	79(42)
N(4)	−5 740(22)	11 579(23)	9 693(3)	39	H(71)	−3 145(183)	7 304(187)	9 466(28)	72(29)
O(1)	3 651(18)	1 006(18)	8 517(3)	39	H(72)	−1 751(190)	8 979(181)	9 737(28)	71(29)
O(2)	−2 082(21)	7 515(20)	7 993(2)	49					

* Average isotropic *U* (× 10^3) given for anisotropic atoms.

Table 2. Bond lengths (Å) and angles (°) with standard deviations in parentheses

N(1)–C(1)	1.36(2)	N(1)–C(1)–O(1)	119(4)
C(1)–O(1)	1.26(2)	N(1)–C(1)–C(2)	119(4)
C(1)–C(2)	1.51(2)	O(1)–C(1)–C(2)	121(1)
C(2)–C(3)	1.53(2)	C(1)–C(2)–C(3)	114(1)
C(3)–C(4)	1.51(2)	C(2)–C(3)–C(4)	109(1)
C(3)–N(3)	1.46(1)	C(2)–C(3)–N(3)	113(1)
C(4)–N(2)	1.32(2)	C(4)–C(3)–N(3)	115(1)
C(4)–O(2)	1.26(2)	C(3)–C(4)–N(2)	118(1)
N(2)–C(5)	1.48(2)	C(3)–C(4)–O(2)	120(1)
C(5)–C(6)	1.53(2)	N(2)–C(4)–O(2)	122(1)
C(5)–C(7)	1.53(2)	C(4)–N(2)–C(5)	126(1)
C(6)–N(3)	1.33(2)	N(2)–C(5)–C(6)	111(1)
C(6)–O(3)	1.25(1)	N(2)–C(5)–C(7)	111(1)
C(7)–C(8)	1.50(2)	C(6)–C(5)–C(7)	113(1)
C(8)–N(4)	1.37(2)	C(5)–C(6)–N(3)	118(1)
C(8)–O(4)	1.23(2)	C(5)–C(6)–O(3)	120(1)
		N(3)–C(6)–O(3)	121(1)
		C(3)–N(3)–C(6)	126(1)
		C(5)–C(7)–C(8)	112(1)
		C(7)–C(8)–N(4)	116(1)
		C(7)–C(8)–O(4)	122(1)
		N(4)–C(8)–O(4)	121(1)
N(1)–H(101)	0.90(10)	H(101)–N(1)–H(102)	112(8)
N(1)–H(102)	1.18(11)	O(2a) · · · N(1) · · · O(2b)	127.8(4)
N(4)–H(401)	0.78(10)	O(3c) · · · N(4) · · · O(3d)	138.1(4)
N(1) · · · O(2a)	2.86(1)		
N(1) · · · O(2b)	3.03(2)		
N(4) · · · O(3c)	2.94(2)		
N(4) · · · O(3d)	3.22(1)		

Symmetry related atoms:

a = $x, y-1, z$ b = $-x, y-\frac{1}{2}, 1\frac{1}{2}-z$
c = $x-1, y+1, z$ d = $x-\frac{1}{2}, 1\frac{1}{2}-y, 2-z$

Table 3. Deviation from mean planes (Å) (starred atoms define planes)

Plane	Deviations
1	C(3)* 0.02; C(4)* −0.06; N(2)* 0.06; C(5)* −0.03; N(3) 0.36; C(6) 0.42
2	C(3)* −0.01; N(3)* 0.02; C(6)* −0.02; C(5)* 0.01; N(2) −0.45; C(4) −0.35
3	O(1)* 0.00; N(1)* 0.00; C(1)* 0.01; C(2)* 0.00
4	C(1)*; C(2)*; C(3)*
5	O(4)*; N(4)*; C(8)*; C(7)* (all 0.00)
6	C(8)*; C(7)*; C(5)*
7	N(3)* 0.01; C(4)* −0.01; N(2)* 0.01; C(6)* −0.01; C(5) −0.31; C(3) −0.09

Inter-plane angles (X)

1—2 18.3° 3—4 58.0° 5—6 9.2°

crystallised from water to give the title compound as a white solid (64%) which did not melt <290 °C, δ ([$^2H_6$]Me_2SO) 2.52 (4 H, m, 2 × CH_2), 4.07 (2 H, br t, 2 × CH), and 6.94 (2 H, br s, 2 × NH), 7.41 (2 H br s, 2 × NH), and 7.84 (2H br s, 2 × NH); *m/z* (E.I.) 228 (M^+, 45%), 211 (29), 183 (37), 166 (11), 138 (11), and 125 (18); $\nu_{max.}$ (Nujol) 3 380m, 3 260m, 3 190m, 1 670s, 1 650s, 1 335m, and 1 259w cm^{-1}.

Reactions of (S)-3-*Aminopyrrolidine*-2,5-*dione*.—Samples of (*S*)-3-aminopyrrolidine-2,5-dione (each 0.015 g, 0.13 mmol) were dissolved in 0.5 mol dm^{-3} D_2O–phosphate buffers (0.5 cm^3) with pH values of 5.21, 6.03, 6.65, and 7.55, respectively. These solutions were then incubated at 22 °C and monitored periodically by ^1H n.m.r. spectroscopy. After *ca.* 10 h some precipitation had occurred. This prevented the calculation of percentage composition of the mixture by integration. The reactions were virtually complete after 42 h. The only products observed were asparagine and cyclo-(L-asparagyl-L-asparagyl) (the precipitate). Careful scrutiny of the spectra suggested that the formation of the piperazinedione is maximised in the region of pH 7. A sample of (*S*)-3-aminopyrrolidine-2,5-dione (0.15 g, 1.3 mmol) was dissolved in 0.5 mol dm^{-3} H_2O–phosphate buffer (3.5 cm^3) of pH 7.07 and the solution was maintained at room temperature for *ca.* 2 days. The mixture was concentrated to *ca.* one-third initial volume, filtered, and the solid was washed with cold water (1 cm^3) and dried (P_4O_{10}; *in vacuo*) to give slightly impure 3,6-bis(carbamoylmethyl)piperazine-2,5-dione (1) (0.015 g). This structural assignment was confirmed by ^1H n.m.r. spectroscopy (D_2O) and by addition of authentic piperazinedione (1) to the n.m.r. tube.

The behaviour of (*S*)-3-aminopyrrolidine-2,5-dione (0.015 g, 0.13 mmol) in 1 mol dm^{-3} DCl (0.5 cm^3, pH −0.97) and in 1 mol dm^{-3} NaOD (0.5 cm^3, pH 13.1) was also examined by ^1H n.m.r. spectroscopy. At pH 13.1 hydrolysis to asparagine occurs within 10 min at room temperature. At pH −0.97, the five-membered ring of 3-aminopyrrolidine-2,5-dione (2) survives for 5 days at room temperature. Hydrolysis did proceed at 80 °C and was complete after 38 h at this temperature. The product was shown to be aspartic acid by additions of asparagine and aspartic acid to the n.m.r. tube.

Crystal Data.—$C_8H_{12}N_4O_4$, orthorhombic, space group $P2_12_12_1$, $a = 5.400(3)$, $b = 5.732(3)$, $c = 31.831(18)$ Å, $U = 985.3(9)$ Å^3, $M = 228.2$, $Z = 4$, $D_c = 1.54$ g cm^{-3}, $D_m = 1.55$ g cm^{-3}, Mo-K_α radiation, $\lambda = 0.710\,69$ Å, μ(Mo-K_α) = 1.17 cm^{-1}, $F(000) = 480$.

Data were collected with a Syntex $P2_1$ four circle diffractometer. Maximum 2θ was 50° with scan range ±1.15° (2θ) around the $k_{\alpha1}$—$k_{\alpha2}$ angles, scan speed 0.7—29° min^{-1}, depending on the intensity of a 2-s pre-scan; backgrounds were measured at each end of the scan for 0.25 of the scan time. Three standard reflections were monitored every 100 reflections, and showed only statistical changes during data collection. Unit cell dimensions and standard deviations were obtained by least-squares fit to 15 reflections. 641 Observed reflections [$I/\sigma(I) > 3.0$] (1 083 total) were used in refinement, and corrected for Lorentz and polarisation effects. No absorption correction was made. Systematic absences $h00$, $h \neq 2n$, $0k0$, $k \neq 2n$, $00l$, $l \neq 2n$ indicate space group $P2_12_12_1$.

The structure was solved without difficulty using MULTAN-80.[25] After refinement, hydrogen atoms attached to carbon, N(2), and N(3) were inserted at calculated positions (but not refined). Three of the four hydrogens attached to N(1) and N(4) could be seen on a difference Fourier synthesis and were inserted and refined (with fixed temperature factors); the unlocated hydrogen atom is that involved in the weakest H bond. Final refinement was by full matrix least-squares method, in large blocks with anisotropic temperature factors for all except hydrogen atoms. The absolute configuration of the molecule as refined was shown to be correct by comparison with the known molecular configuration. Unit weights were used and shown to be satisfactory by a weight analysis. The

final R value was 0.075. Computing was with the X-RAY 76 system,[26] on a Burroughs B6700 computer. Scattering factors in the analytical form and anomalous dispersion factors were taken from ref. 27. Final atomic co-ordinates are given in Table 1, and bond lengths and angles in Table 2. Anisotropic temperature factors and observed and calculated structure factors are available as a Supplementary Publication * (SUP No. 23635, 6 pp.).

* For details of the Supplementary Publications Scheme see Instructions to Authors (1983), *J. Chem. Soc., Perkin Trans. 1*, 1983, Issue 1.

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