Cyclic Tetrapeptides of Glycine or Alanine Combined with Sarcosine; the Co-existence of Neutral and Protonated Species

By JOHANNES DALE* and KIRSTEN TITLESTAD
(*Kjemisk Institutt, Universitetet i Oslo, Oslo 3, Norway*)

Summary All combinations between glycine and sarcosine, and between alanine and sarcosine, in a cyclic tetrapeptide have been synthesized; when n.m.r. spectroscopy can be applied, the same *cis,trans,cis,trans*-conformation, neutral or protonated, is indicated, as long as α-methyl groups do not give rise to steric hindrance.

WE have recently shown[1] that the unique centrosymmetric *cis,trans,cis,trans*-amide conformation established for cyclotetrasarcosyl both by n.m.r. spectroscopy of solutions[2] and by *X*-ray diffraction of the solid[3] (Figure 1), is also adopted by other cyclic tetrapeptides having one sarcosine residue replaced by glycine or alanine, or two diametric residues replaced by glycine.

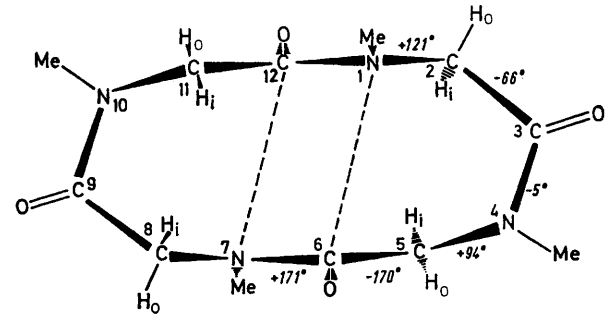

FIGURE 1. *The crystal conformation of cyclotetrasarcosyl.*[3]

In these cases the *trans*-preferred NH-amide groups are able to fit into one or both *trans*-positions, and the α-methyl group of alanine can select an extra-annular position. It was of obvious interest to examine whether this ring conformation would still be adopted when one NH-amide group must end up in *cis*-position, or when two alanine residues have a relative configuration such that one α-methyl group must become intra-annular.

We now report a series of such hitherto unknown cyclic tetrapeptides intermediate in composition between cyclotetrasarcosyl and cyclotetraglycyl or cyclotetra-alanyl, obtained by cyclization of tetrapeptide trichlorophenyl esters in refluxing pyridine.† The yields of the various alanine-containing rings are highest when the amide-conformation substituent-configuration sequence 1,12-*trans*, 2-L, 3,4-*cis*, 5-L, 6,7-*trans*, 8-D, 9,10-*cis*, 11-D required by the ring conformation (Figure 1) can be satisfied. Thus, cyclo-(Ala-Sar-Ala-Sar) is obtained in 30% yield when the configuration is D,L, whereas less than 10% of a mixture of the D,L- and L,L-isomers is obtained from L,L-tetrapeptides. Similarly, since the obtained main isomer of cyclo-(Ala_3-Sar) most likely (see below) has the required configuration L,L,D, (or D,D,L) it is understandable that the yield is only 5% from L,L,L- and D,L,L-tetrapeptides. Configurational change at one amino-acid residue might well have occurred to this extent in refluxing pyridine.

The i.r. spectra do not allow clear conclusions as to the presence of *cis*-NH-amide groups, but precise information can be obtained from their n.m.r. spectra. Unfortunately, the solubility properties of cyclic tetrapeptides containing more than one NH-amide group are such that trifluoroacetic acid (TFA) or solvent mixtures containing it had to be used, and the question arises whether new lines are due to new conformers or just protonated conformers of the same kind.[4,5]

Cyclo-(Sar_4) has exactly the same simple type of spectrum in TFA as in a series of non-acidic solvents.[1] However, since a crystalline monoperchlorate can be precipitated, it seems unlikely that no molecules are protonated in TFA. One proton may either be switching rapidly between amide groups of the same basicity (for example both *trans*) so as to exert an average effect and maintain the apparent symmetry, or it may be exchanging rapidly between molecules.

Cyclo-(Gly-Sar_3) has the expected simple spectrum of one conformer in $CDCl_3/Me_2SO$,[1] but in TFA additional lines appear, corresponding to about 30% of a second species, and less in mixtures with other solvents. Both old and new NH lines have coupling constants of 10 Hz, and the new CH_2 quartets resemble the original ones. All new NMe lines appear unresolved in the *cis*-NMe region, but become resolved when benzene is added; one of these moves faster to higher field with more benzene (Figure 2). This suggests preferred protonation of the only *trans*-NMe-amide group, which is thereby shifted to lower field. Benzene solvates preferentially this positively charged group and shifts the signal back again to the *trans*-NMe region. Since two coexistent species are observed, the proton must either be in one fixed position or shifting rapidly within the same molecule. Exactly the same behaviour is found for cyclo-(L-Ala-Sar_3), but the protonated species in TFA is present in only about 20%.

† K. Titlestad, *Chem. Comm.*, in the press.

Cyclo-(Gly_2-Sar_2) shows a similar phenomenon; the main (presumably non-protonated) conformer has one *cis*- and one *trans*-NMe group, while additional absorption (less than 50%) is found in the *cis*-region and half of it is shifted to the *trans*-region on addition of benzene. Again, preferred or specific protonation of the only *trans*-NMe-amide group is suggested, but the spectrum is more complex with broad lines, perhaps due to the possibility of having two conformers of the same type (Figure 1) in this case (NH,-

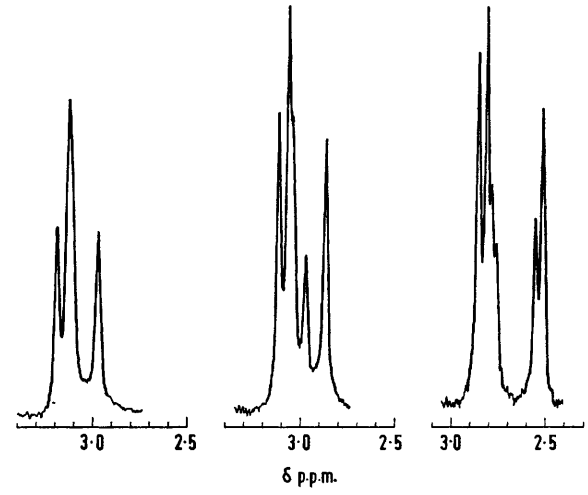

FIGURE 2. 100 MHz *N.m.r. spectra of cyclo-(Gly-Sar_3) in the* NMe-*region. The solvent is TFA alone (left) and with increasing amounts of benzene (middle and right).*

NH,NMe,NMe in either 1,12-*trans*, 3,4-*cis*, 6,7-*trans*, 9,10-*cis* or in 3,4-*cis*, 6,7-*trans*, 9,10-*cis*, 1,12-*trans*). Only the former is allowed for cyclo-(L-Ala_2-Sar_2), and its spectrum is very simple (Figure 3) with two NH, *cis* and *trans*, (of which the *cis* NH is exchanged more rapidly with deuterium), two NMe-signals (*cis* and *trans*), and two CH_2-quartets. This fits exactly the cyclo-(Sar_4)-type conformation, but whether it is neutral or rapidly protonated and deprotonated cannot be decided. Both these cyclic tetrapeptides thus do indeed accept one *cis*-NH-amide group, but were obtained in only 10% yield.

Cyclo-(Gly-Sar-Gly-Sar) has extra lines with intensity up to 50% depending on TFA concentration. The new NMe lines are again shifted upfield with benzene, but so little that it still may be the *trans*-amide groups which are preferentially protonated. The close transannular interaction between the *trans*-amide groups may stabilize the charge, since otherwise N-alkyl amide groups are more basic than those of NH-type.[6] The corresponding cyclo-(L-Ala-Sar-D-Ala-Sar) has a simple spectrum (Figure 3) betraying the symmetry of the cyclo-(Sar_4) conformation but lines are somewhat broadened, and this molecule may perhaps be present exclusively in an unsymmetrically mono-protonated form. Both NH protons are here exchanged very slowly with deuterium. The second isomer (L,L) has a similar spectrum at high temperature

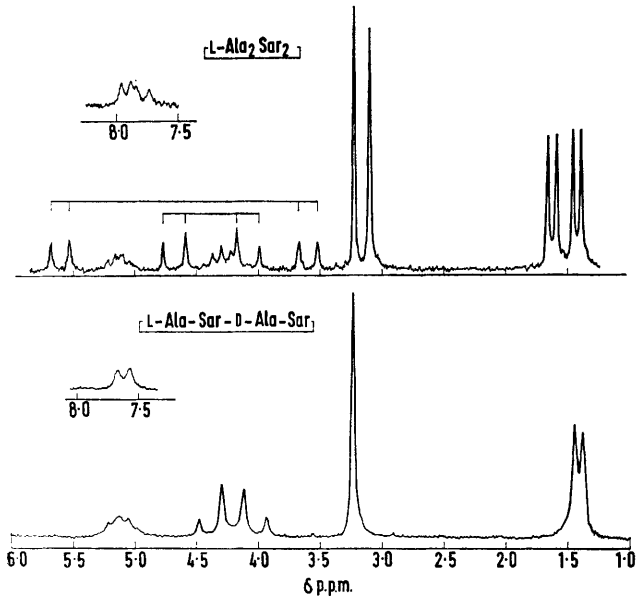

FIGURE 3. *The* 100 MHz *n.m.r. spectra in TFA solution of cyclo-*(L-*Ala*$_2$-*Sar*$_2$) (*top*) *and cyclo-*(L-*Ala-Sar*-D-*Ala-Sar*) (*bottom*).

which on cooling splits (coalescence at *ca.* 30°) into a 1:2 mixture of two species. It is not clear, however, whether these represent different ring conformations, or whether one or both are protonated.

No satisfactory spectrum was obtained for cyclo-(Gly_3-Sar) but cyclo-(Gly_4) shows in TFA two methylene doublets and two overlapping NH-triplets at room temperature, and these become broad at −20°. This can only fit the amide sequence *cis,trans,cis,trans*, and not all-*trans*,[7] Most likely, it is again the familiar ring conformation, although the cyclotetradepsipeptide conformation[8] cannot be ruled out on this basis.

Cyclo-(Ala_3-Sar) has in TFA a simple spectrum with three α-Me-doublets, one NMe line, and one CH_2 quartet with geminal coupling constant of 18Hz, all features fitting the cyclo-(Sar_4) conformation with NMe-amide in *cis*. This is the reason for assigning to this product the L,L,D-configuration.

For cyclo-(Ala_4), obtained in 10% yield, more than eight α-Me doublets are observed, corresponding to at least two totally unsymmetrical or several symmetrical conformers (or protonated conformers). An all-*trans* conformation would have been sterically possible for the expected L,L,L,L-isomer, but should have given a single α-Me doublet.

(*Received*, 20*th October* 1971; *Com.* 1831.)

[1] J. Dale and K. Titlestad, *Chem. Comm.*, 1970, 1403.
[2] J. Dale and K. Titlestad, *Chem. Comm.*, 1969, 656.
[3] P. Groth, *Acta Chem. Scand.*, 1970, **24**, 780.
[4] I. M. Klotz, S. F. Russo, S. Hanlon, and M. A. Stake, *J. Amer. Chem. Soc.*, 1964, **86**, 4774.
[5] J. W. O. Tam and I. M. Klotz, *J. Amer. Chem Soc.*, 1971, **93**, 1313.
[6] A. Veis and C. F. Nawrot, *J. Amer. Chem. Soc.*, 1970, **92**, 3910.
[7] R. Schwyzer, B. Iselin, W. Rittel, and P. Sieber, *Helv. Chim. Acta*, 1956, **39**, 872.
[8] J. Konnert and I. L. Karle, *J. Amer. Chem. Soc.*, 1969, **91**, 4888.