A Common Conformation for Five Cyclic Tetrapeptides

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Summary Cyclo-(Sar$_3$–*N*-MeAla), cyclo-(Sar$_3$–Ala), cyclo-(Sar$_3$–Gly), and cyclo-(Sar–Gly–Sar–Gly) have been synthesized; the similarity of their low-temperature n.m.r. spectra with that of cyclo-(Sar$_4$) suggests that all have the same ring conformation.

THE cyclic tetrapeptide of sarcosine (*N*-methylglycine) was recently[1] synthesized and shown by n.m.r. spectroscopy to have a single and surprisingly rigid, centrosymmetric conformation with the amide-configuration sequence *cis, trans, cis, trans*. A transannular attraction between the two *trans*-amide groups was suggested. This conformation, only slightly modified, has since been confirmed by Groth[2] through *X*-ray crystallography and is shown in Figure 1. The two types of CH_2 group are quite different; those in the 2- and 8-positions are cyclohexane-like, with one outer "equatorial" and one inner "axial" hydrogen, while those in the 5- and 11-positions are of straight-chain type with the inner hydrogen more intra-annular and the outer hydrogen less extra-annular (Figure 1). This difference is also

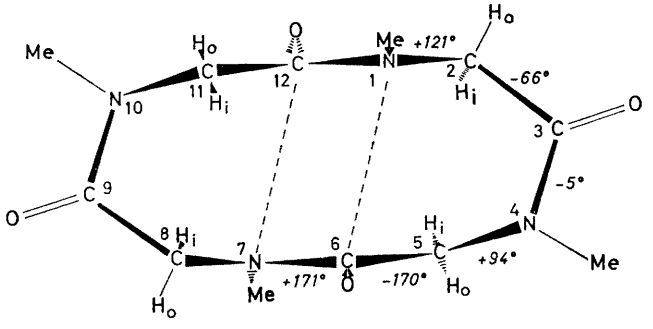

FIGURE 1. *The crystal conformation of cyclotetrasarcosyl.*[2]

reflected in the degree of splitting of n.m.r. chemical shift (Figure 2) between inner and outer hydrogens for the two cases (2·1 p.p.m. against 0·8 p.p.m.), presumably caused by the quite dissimilar orientation of the various hydrogens with respect to the vicinal carbonyl and *N*-methyl groups.

In the similar tetrapeptide we have now synthesized, which has one sarcosine residue replaced by *N*-methyl-L-alanine, the α-methyl group should be best accommodated in the outer 2- (or 8-) position. As the most widely split quartet was the one that was reduced in intensity, and the methine proton occupying the inner position came at very low field, the assignment of the highest-field signal to the outer and the lowest-field signal to the inner 2-CH_2 hydrogen of cyclotetrasarcosyl was straightforward (Table).

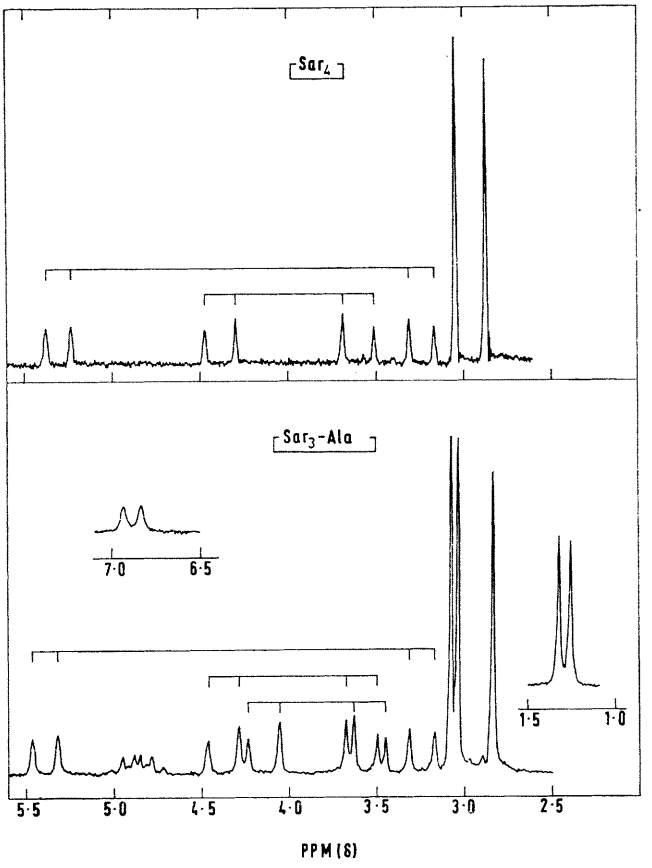

FIGURE 2. *The* 100 *MHz n.m.r. spectra in* $CDCl_3$ *solution of cyclotetrasarcosyl* (top) *and cyclotrisarcosyl-*L*-alanyl* (bottom).

When L-alanine itself was present instead of *N*-methyl-L-alanine, there would be one more reason for the α-methyl group to occupy the 2-position, since thereby NH becomes part of a *trans*-amide group instead of the less favourable *cis*-amide. The i.r. spectrum shows in fact the diagnostic[3] *trans*-CO·NH band at 1550 cm^{-1}, and the disappearance of one higher-field n.m.r. signal (Figure 2) for *N*-methyl links

Properties of cyclic tetrapeptides

Compound	M.p.	I.r. C=O band in KBr(cm.$^{-1}$)	N.m.r. coalescence temp.	Low-temperature n.m.r. spectral data (100 MHz)													
				Chem. shift (p.p.m.) of *N*-Me			Chem. shift (p.p.m.) of CH_2								Gem. coupl. const. (Hz)		
				cis	*trans*		inner		outer		inner		outer				
				N-4 & N-10	N-1	N-7	C-2	C-8	C-2	C-8	C-5 & C-11		C-5 & C-11		C-2	C-8	C-5 & C-11
Cyclo-(Sar–Sar–Sar–Sar)	>350°	1665, 1640	*ca.* 180°	3·05[a]	2·88	2·88	5·3	5·3	3·25	3·25	4·4		3·6		14·5	14·5	18
Cyclo-(Sar–Sar–Sar–*N*-MeAla)	315°	1670, 1640		3·04[a]	2·65	2·87	—	5·3	—	3·25	4·3	4·4	3·55	3·6	—	14·5	18
Cyclo-(Sar–Sar–Sar–Ala)	315° subl.	1690, 1680 1650		3·04 3·08[a]	—	2·84	—	5·35	—	3·25	4·1	4·35	3·55	3·6	—	14·5	18
Cyclo-(Sar–Sar–Sar–Gly)	318°	1685 1650	*ca.* 150°	3·03 3·05[b]	—	2·83	4·85	5·3	3·4	3·25	4·1	4·25	3·55	3·65	15	14·5	18
Cyclo-(Sar–Gly–Sar–Gly)	>350°	1660	*ca.* 20°	3·22[c]	—	—	5·2	5·2	3·85	3·85	4·4		4·15		15	15	19

[a] In $CDCl_3$; [b] in $CDCl_3$–$(CD_3)_2SO$, 8:1; [c] in CF_3CO_2D.

this signal to the *trans*-configuration. This contrasts with the situation in poly-*N*-methyl-L-alanine where the higher-field signal is attributed[4] to the *cis*-configuration. The present assignment is further corroborated by the large observed vicinal NH.CH coupling constant of 10 Hz (Figure 2) corresponding to the *anti*-arrangement.[5] On Groth's crystal structure[2] (Figure 1) this fits best the inner 2-hydrogen (dihedral angle 180°; the others being: outer 2-H, 60°; inner 5-H, 155°; outer 5-H, 35°).

In the case of cyclo-(Sar_3–Gly) the i.r. spectrum again shows the *trans*-amide band, and the same is true when two glycine residues are introduced [cyclo-(Sar–Gly–Sar–Gly)] so as to fit both *trans*-amide positions. All assignments of n.m.r. signals are given in the Table; for the CH_2 group in the 5- (or 11-) position the high-field signal is attributed to the outer hydrogen on the basis of an exceptionally strong down-field displacement observed for this signal when the solvent is changed from chloroform to methanol or water.

The striking similarity between all these n.m.r. spectra with respect to chemical shift for CH_2 and NMe groups, as well as geminal CH_2 coupling constants, strongly suggests that the same ring conformation is adopted by all these cyclic tetrapeptides. No extra lines attributable to other minor conformations were ever observed.

The coalescence temperature (see Table), which is particularly high for cyclotetrasarcosyl, is almost as high for cycloglycyltrisarcosyl, but drops to room temperature for cycloglycylsarcosylglycylsarcosyl.† If the height of the inversion barrier is determined by the strength of the incipient transannular nucleophilic attack by nitrogen on carbonyl carbon,[1,2] it seems as if the basicity of NH were much lower than of NMe. Intramolecular hydrogen bonding, as suggested by Schwyzer for the case of cyclotetraglycyl,[6] must be excluded even for our NH-containing cyclotetrapeptides.

Mechanisms other than a simple inversion for the CH_2 site exchange process cannot be excluded, but seem less likely. Thus, if it were coupled with the *cis,trans*-configuration exchange, the barriers ought not to have decreased on replacing NMe by NH. A rotation of the *trans*-amide group "through the ring" would of course be more easy with NH, but one would then lack an explanation for the high coalescence temperatures of the larger rings of the cyclosarcosyl series.[1]

The ring conformation of Figure 1 requires that if each of the four α-carbon atoms were to carry one substituent in its outer position, the configuration must be the same at C-2 and C-5 and opposite to the configuration at C-8 and C-11. We therefore believe that this is the reason why the cyclotetradepsipeptide cyclo-(D-HyIv–L-*N*-MeIleu–D-HyIv–L-*N*-MeLeu) has had to adopt a different ring conformation, as shown[7] by *X*-ray methods. Only the configuration sequence DDLL could fit the present ring skeleton with the ester groups *trans* and the amide groups *cis*.

The parent linear tetrapeptides were synthesized by conventional methods, the carboxyl group being activated as the 2,4,5-trichlorophenyl ester, and cyclization performed in pyridine. The yields were particularly good for cyclotetrasarcosyl and for cycloglycylsarcosylglycylsarcosyl (40%). Formation of cyclic dipeptides was sometimes noted, both during cyclization of the linear tetrapeptide and during sublimation of impure cyclic products.

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† The rings carrying one α-Me group can of course show no coalescence on heating as long as other conformations are not present.

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