Acta Chemica Scandinavica B 29 (1975) 353—361

Conformational Processes in Simple Cyclic Peptides

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The elementary steps in conformational interconversion paths for cyclic tri-, tetra-, and penta-peptides are discussed. When *trans*-amide groups are present, a full exchange requires its rotation as such "through the ring" or an "outward" rotation about the CN partial double bond *via* the *cis*-amide configuration.

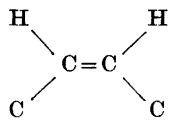
The conversion of the externally hydrogen-bonded crystal conformer of certain NH-containing cyclic pentapeptides to more stable conformers in solution, presumably internally hydrogen-bonded, can be followed at low temperatures by NMR spectroscopy.

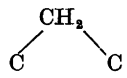
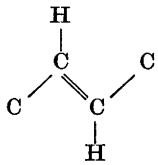
Most known cyclic peptides built up of "natural" amino acids are not amenable to studies of conformational flexibility by dynamic NMR spectroscopy, since all or most of the amino acids carry α-substituents, so that "ring inversion" will not generally lead to an identical conformer, and since the amino acids as a rule are different, so that also "pseudorotation" will lead to non-identical conformers. Cyclic peptides of glycine alone, such as the pentamer and hexamer, are the simplest examples of compounds suitable for the study of site exchange of the α-hydrogens as well as of the amino acid residues. However, the interconversion mechanism would be expected to consist of a series of several steps and lead, as in the case of cycloalkanes,[1] to both types of exchange simultaneously, since the amide bonds are here of NH-type and therefore most likely all *trans* in rings of this size. Also, the low solubility makes NMR studies difficult.

Cyclic peptides of sarcosine [2] and its combinations with glycine [3,4] are much more suitable for such studies. They are more soluble, and a cyclic tripeptide and a variety of cyclic tetrapeptides are also available. Furthermore, amide bonds of NMe-type can equally well be *cis* as *trans*, and the site exchange for the amino-acid residues in homodetic rings, and quite generally the interconversion of different conformers, must therefore involve *cis,trans*-isomerization which in open chains is found to have a barrier of around 19 kcal/mol.[5] This process can be studied separately when the site exchange for the α-hydrogens is more rapid than *cis,trans* exchange. Certain NMR-spectroscopic observations on cyclic peptides, which we have in part already reported, and some new experimental results will be interpreted in the following by analysis of the probable processes involved.

APPROACH TO THE ANALYSIS OF THE CONFORMATIONAL PROCESSES

The analysis of conformational interconversion paths, composed of a sequence of elementary processes, is simple in cycloalkanes as they consist of constitutionally identical ring elements and bonds. The situation in cycloalkenes is also relatively simple despite the more complex constitutional situation, because low-energy conformational processes leading to CH_2-hydrogen site exchange and involving barriers of the order of 5 – 15 kcal/mol, can safely be considered independent of the high-energy *cis,trans* configurational isomerization of the double bonds involving barriers of the order of 50 – 60 kcal/mol.[6]

Thus, a *cis*-olefin  is a con-

formationally rigid C_4-unit comparable to the saturated C_3-unit  and a *trans*-olefin  is a stiff conformational C_4-unit comparable to the saturated C_2-unit C–C provided it can be rotated about its long axis. On the other hand, schemes of *cis,trans*-configuration exchange can be considered separately, neglecting the detailed conformational situation in the saturated parts of the molecule during the processes.

In cyclic peptides, even though the amide groups have partial double-bond character, the energy barriers for the two types of processes are shown in the following to be much more similar, but the cycloalkene situation applies in many cases as an approximation.

CYCLIC TRIPEPTIDES

Cyclotrisarcosyl [8] is the only example of a cyclic tripeptide which can undergo a conformational process. Since in its single observable conformation all three amide groups are *cis*, only the exchange of the inner with the outer hydrogen within each of the three CH_2-groups can be observed. The corresponding AB quartet coalesces at 135 °C (100 MHz), giving a calculated free energy of activation of 20.1 kcal/mol.[7] This barrier is higher than the normal barrier for *cis,trans* isomerization of amides, so that a multi-step interconversion path through less stable conformers having one or two *trans* amide groups cannot *a priori* be excluded. This possibility seems, however, very unlikely considering that the geometrically closely similar olefinic compound, *cis,cis,cis*-cyclonona-1,4,7-triene, where the energy required to convert a *cis*- to a *trans*-double bond is expected to be of the order of 50 – 60 kcal/mol,[6] shows an activation free energy to CH_2 hydrogen site exchange [8,9] of only 14.5 kcal/mol.* A one-step synchronous ring-flattening inversion of the whole molecule is entirely out of question, although this mechanism has been favoured [8] for cyclonona-1,4,7-triene on the basis of an apparent ** very large negative entropy of activation.

It remains to consider a multi-step path with intact *cis*-amide groups. The basic elementary step in composite interconversion mechanisms for cycloalkanes smaller than cyclohexadecane is generally the local "outward" flattening at a "corner" whereby four carbon atoms are brought into coplanarity.[1] In cyclic peptides not less than five ring atoms must be in a common plane at or near the critical point of the barrier if a *cis*-amide group is to preserve its planarity. In reality there may be some temporary departure from planarity of the amide group in question, as well as of others in the molecule. It is also unclear whether in cyclotrisarcosyl it is the coplanarity of the $C_\alpha CNC_\alpha C$ system or the adjacent $NC_\alpha CNC_\alpha$ system which is reached most easily or early in a sequence of two close steps. For these reasons the passage from the initial stable conformation A (Fig. 1) to an intermediate less stable conformation B can most simply be described as the flipping of one CH_2 group from one side of the ring to the other, involving both adjacent *cis*-amide groups and hence the rough coplanarity of seven ring atoms (Fig. 1).

The intermediate conformation B has got changed dihedral angle sign in two bonds (and temporary near-eclipsing in two others) and can, by a further pseudorotation-like movement over presumably very low barriers pass to an equivalent mirror image conformation B′ whereby the sign in two further bonds is changed. Halfway between B and B′ a conformation C with a pseudo-twofold axis of symmetry is attained, which is possibly lower in energy. The final reversion to the mirror image A′ of the initial conformation goes over a barrier equivalent to the first.

This three-step cycle is analogous to, but more detailed than, the exchange mechanism proposed [9] for the "inversion" of one crown-form of cyclonona-1,4,7-triene to an identical

* The even lower reported value of 11 kcal/mol [10] is due to an error of calculation.

** "Observed" negative or extremely large activation entropies for monomolecular processes are now considered with much suspicion due to systematic errors in their determination by NMR.[11]

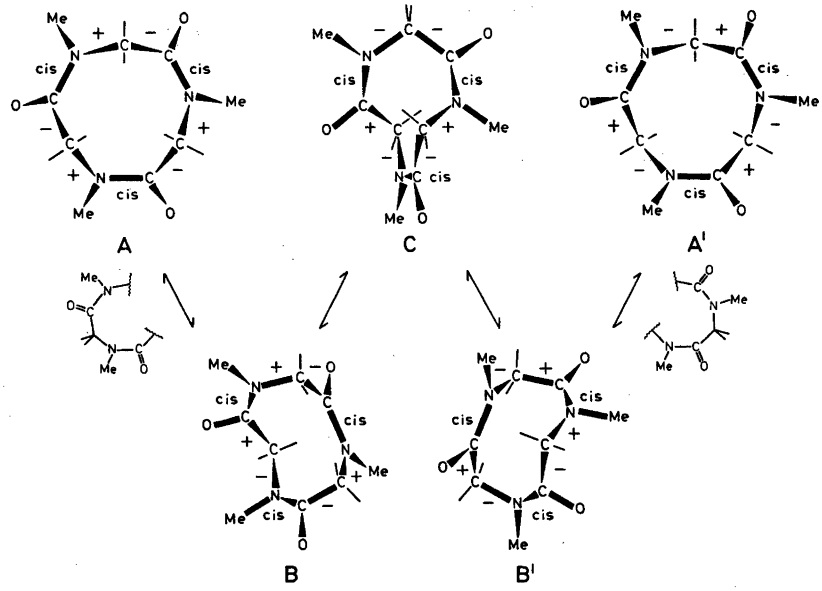


Fig. 1. Conformational interconversion scheme for c-Sar_3. Dihedral angle signs are defined with respect to the ring skeleton.

crown (note that the triolefin is not chiral). Since torsional strain in the $C_\alpha C$- and $C_\alpha N$-bonds is probably small, it is possible to get a qualitative feeling of strain on the basis of valency-angle strain in mechanical models. On this basis the first (and last) barrier is higher than any separating B and B′ (Fig. 1), hence the one which is measured by dynamic NMR spectroscopy. That the observed barrier is higher in the tripeptide than in the triolefin, can be explained by a greater transannular repulsion between the "inner" CH_2-group and the opposite

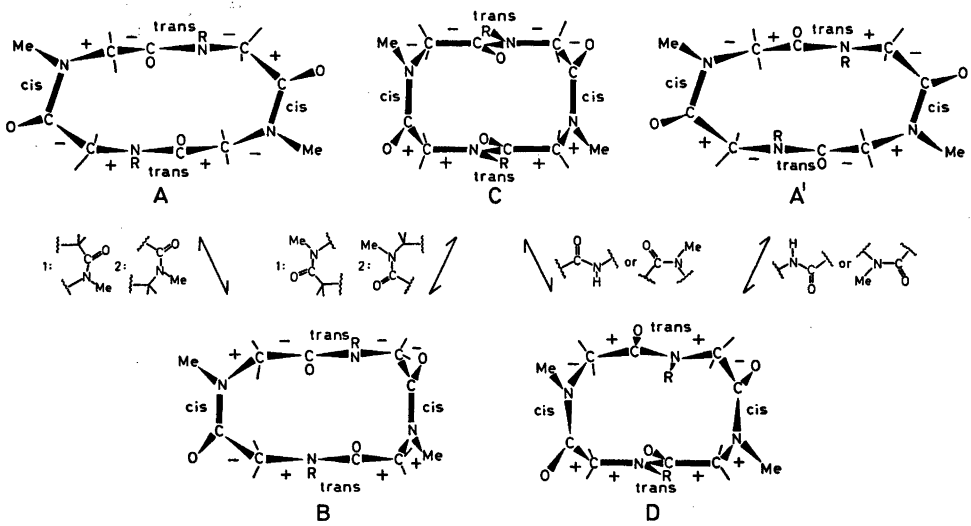


Fig. 2. Conformational interconversion scheme for c-Sar_4 (R=Me), c-$GlySar_3$ (R=Me and H), and c-GlySarGlySar (R=H).

amide group in the intermediate conformation B, and the barrier towards it, due to the presence of the carbonyl and *N*-methyl groups in the amide as compared with only hydrogen in the olefin. This is in accord with the extremely high barrier for the aromatic analogue cyclotriveratrylene; no NMR coalescence is observed [12] up to 200 °C and a conformationally chiral derivative [13] has been resolved. (See note added in proof on p. 360).

CYCLIC TETRAPEPTIDES

The uniquely preferred conformation of a large number of simple cyclic tetrapeptides has been shown both for solutions by NMR spectroscopy [2,3] and subsequently for the solid state by X-ray methods [14,15] to have a *cis,trans,cis,-trans* sequence of amide groups (A in Fig. 2). Of particular interest here are cyclotetrasarcosyl (c-Sar_4), cycloglycyltrisarcosyl (c-$GlySar_3$) and cycloglycylsarcosylglycylsarcosyl (c-GlySarGlySar). These have one, respectively both, *trans*-amide positions occupied by the *trans*-preferred NH-type amide group. The NMR-spectrum of c-Sar_4 itself [2] shows no sign of coalescence of its two AB quartets until the temperature reaches 155 °C, and by further heating the two single lines expected for the CH_2 groups if the amide groups retain their configuration do not develop. Instead, a single line is formed at the same time as also the two NMe lines coalesce to one line at ~180 °C. Thus, full exchange is obtained over a critical barrier as high as ~23 kcal/mol, and it may be concluded that α-hydrogen exchange requires either a mechanism by which also *cis-trans*-configuration is exchanged, or the passage of barriers as high as those leading to *cis,trans* exchange.*

The NMR-spectrum of c-$GlySar_3$ reveals a similar situation for this compound, with a slightly lowered coalescence temperature now observed for the CH_2-quartets only. Exchange of *cis,trans* configuration can in this case of course not be observed, since there is not a sufficient concentration of conformers with *cis* amide groups of NH-type. The NMR spectrum of c-GlySarGlySar, on the other hand, shows coalescence of the two CH_2 quartets to two single lines already at room temperature and again no change in the NMe and NH signals. The following analysis in terms of a multi-step interconversion path can rationalize these observations.

The first step in converting A to A′ (Fig. 2) may be the flattening of either the $N_tC_\alpha C_cN_cC_\alpha$ or the $C_\alpha C_cN_cC_\alpha C_t$ five-ring-atom systems at one of the *cis*-amide groups; this is essentially equivalent to flipping a CH_2 group to the other side of the ring. Whichever of the two types of CH_2 is flipping first, the other has to follow immediately to relieve strain, and therefore the two steps have been considered together in Fig. 2. Two dihedral angle signs are thereby changed to give the intermediate conformation B. The analogous two-step flipping over similar barriers of the two remaining CH_2 groups produces a second intermediate conformation C which has two further dihedral angles of changed sign. Conformation C can not be changed further by using partial-ring-flattening steps, but only be brought back to the initial situation.

In order to change the sign of the remaining four dihedral angles and obtain the inverted conformation A′, which has exchanged all outer with inner hydrogens, but is otherwise identical with the initial conformation A, it is necessary to rotate both *trans*-amide groups as such "through the ring", one after the other. Strictly, these two rotational steps might as well occur alternately with the CH_2-flipping steps according to several different schemes and probably over very similar barriers. The scheme presented in Fig. 2 has been chosen to demonstrate first those steps which involve only CH_2 flipping and can occur irrespective of whether the *trans*-amide groups are of NMe- or NH-type. The steps involving rotation of the *trans*-amide groups can occur only when the *N*-substituent is hydrogen and not the larger methyl group. It is sufficient that only one of these two steps is blocked for the whole mechanistic path to become blocked. This explains why only c-GlySarGlySar can undergo easy CH_2-exchange.

When at least one *trans*-amide group is of NMe type, it is therefore necessary to perform

* The high barrier was initially proposed [2] to be due to transannular interactions between the *trans*-amide groups, but this had to be abandoned when much lower barriers were observed for c-GlySarGlySar,[3] and when the X-ray structures [14,15] revealed normal van der Waals contacts.

a rotation about the partial double bond of the amide group through the *cis*-configuration, with both carbonyl and *N*-methyl pointing out, and further to the other *trans*-configuration (Fig. 2). This in itself requires about 19 kcal/mol,[5] and with the added accompanying ring strain, the observed value of ~ 23 kcal/mol seems reasonable. However, at the temperature necessary for this to occur rapidly, *cis,trans*-isomerization of any amide group will occur rapidly, and full *cis,trans* exchange must be observed in cyclotetrasarcosyl. Assuming that this process occurs stepwise through intermediate conformations, several interconversion paths may be followed. It can only be stated that at least four steps are required, for example the itinerary *ctct*→*ccct*→*cctt*→*cctc*→*tctc* or *ctct*→*ccct*→*cccc*→*cctc*→*tctc*. It is then assumed that increasing temporarily the number of *cis*-amide groups is more advantageous than increasing the number of *trans*-amide groups in this medium-sized ring.

Recently published data [16] on the behaviour of three proline-containing cyclic tetrapeptides also fit well with the interconversion scheme in Fig. 2. Thus, c-Gly-L-ProGly-D-Pro can take the centrosymmetric conformation A with each proline ring linking an external α-position and a *cis*-amide nitrogen and shows no NMR-spectral change with temperature, since inverted or pseudorotated conformers are not populated. On the other hand, c-Gly-L-ProGly-L-Pro does show a low-energy process (13 – 15 kcal/mol) in dynamic NMR-spectroscopy, and since conformation A would give steric problems, we propose that its lowest-energy conformation is the unsymmetrical one B (Fig. 2) which can be converted directly to an equivalent pseudorotated conformation by two successive *trans*-amide rotation steps. The NH-signal is thereby averaged between the two non-equivalent positions, but geminal site exchange in the vicinal α-CH_2 group can of course not take place. Finally, the corresponding *N*-methylated compound, c-Sar-L-Pro-Sar-L-Pro, which must also take the unsymmetrical conformation B, shows no coalescence below 70 °C. This may be explained by the much higher barrier expected for rotation of the *N*-methyl *trans*-amide groups, and we predict that coalescence should become observable in the 150 – 200 °C temperature range.

EXCHANGE PROCESSES IN HIGHER CYCLIC PEPTIDES

Cyclopentasarcosyl, which has been shown to have one unique conformation with a *cis*,-*cis,cis,trans,trans* amide sequence in the solid.[4,17] and predominantly the same in solution (see below), undergoes two separate processes in dynamic NMR spectroscopy. At about 50 °C the five CH_2 quartets coalesce to an unresolved group of presumably five lines ($\Delta G^{\ddagger} \approx 16$ kcal/mol) and by further heating this group narrows directly to a single line, at the same time as the five NMe lines coalesce to one line ($\Delta G^{\ddagger} \approx 19$ kcal/mol). The higher sarcosine homologues behave similarly; thus, cyclooctasarcosyl, which has a single conformation

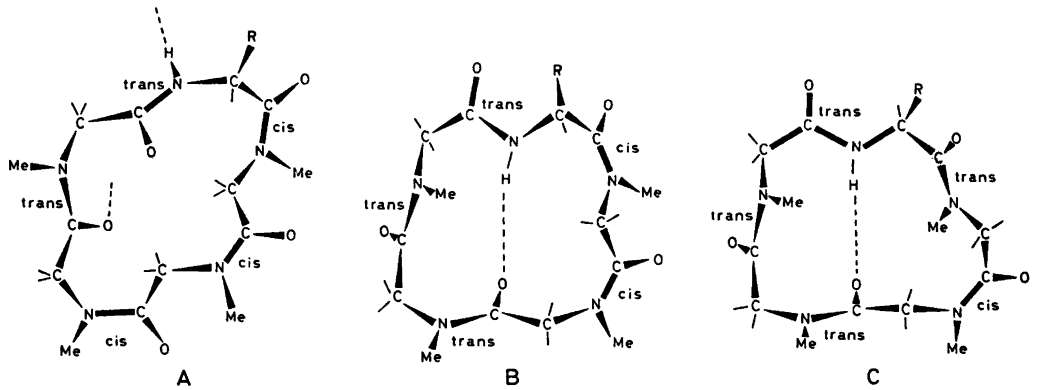


Fig. 3. Crystal conformation (A) and proposed conformations (B and C) for species formed in solution from c-AlaSar$_4$ (R = Me) and c-GlySar$_4$ (R = H).

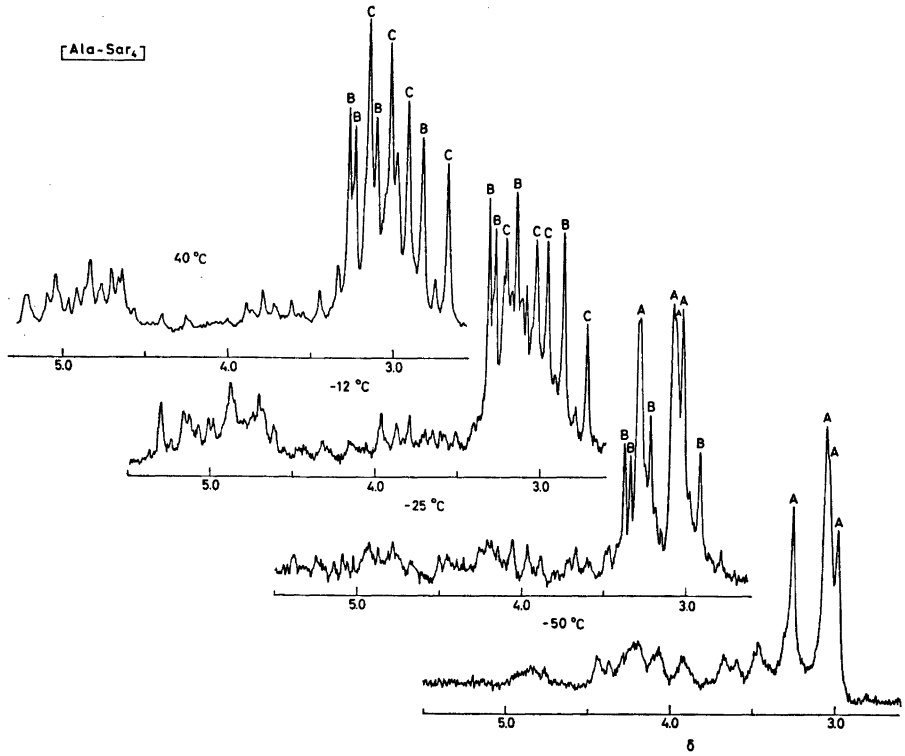


Fig. 4. ^{1}H NMR spectra at 100 MHz of c-AlaSar$_4$ (CH_2- and NMe-regions only). Crystals were dissolved in $CHFCl_2$ at -50 °C (lower spectrum) and the solution allowed to warm up in the probe. $CDCl_3$ was used as solvent at the highest temperature (40 °C).

with the *cis,cis,trans,trans,cis,cis,trans,trans* amide sequence [18] shows CH_2 quartet coalescence at 40 °C ($\Delta G^{\ddagger} \approx 15$ kcal/mol) and coalescence to single CH_2 and NMe lines at 100 °C (≈ 19 kcal/mol).

It is thus clear that the 15-membered and higher rings permit rotation of the *trans*-amide groups "through the ring" even when these are *N*-methyl substituted, the carbonyl oxygen now being the smaller substituent, thereby providing a low-barrier mechanism for geminal CH_2-hydrogen exchange. The higher barrier represents the activation energy for *cis,trans* isomerization of the amide groups leading to full exchange.

CONFORMATIONAL TRANSFORMATIONS OF SOME NH-CONTAINING CYCLIC PENTAPEPTIDES

Finally, a few examples of the conversion of one conformation to others more stable in solution will be given. The experiments were based on the following reasoning:

When, as here, the conformational interconversion barriers are so high (~ 19 kcal/mol) that temperatures of the order of 100 °C are needed to obtain life times short enough (~ 0.01 s) to observe coalescence phenomena in dynamic NMR spectroscopy, it becomes possible at temperatures below -20 °C to slow down the process sufficiently to follow by static NMR spectroscopy the interconversion between different conformers. This means that if the crystalline material is dissolved in $CDCl_3$ at -50 °C, when life-times are of the order of hours even for barriers as low as 17 kcal/mol, the NMR spectrum of the crystal conformer can be observed and identified. By heating slowly to room temperature, when life-times are of the order of seconds, the gradual appearance of other conformers may be observed. Strictly, a given *cis,trans*-sequence may

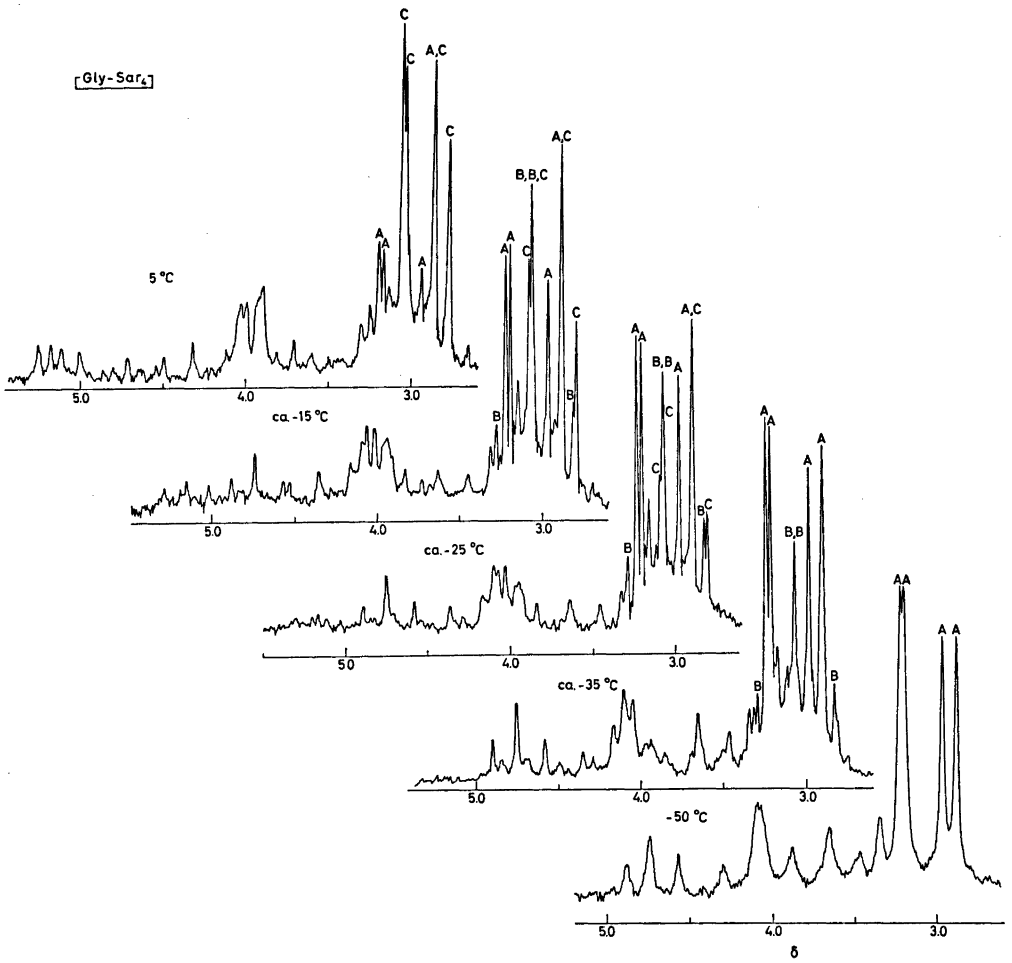


Fig. 5. ^{1}H NMR spectra at 100 MHz of c-GlySar$_4$ (CH_2- and NMe-regions only). Crystals were dissolved in $CHFCl_2$ at -50 °C (lower spectrum) and the solution allowed to warm up in the probe.

give rise to a mixture of ring conformations differing in torsional angles of the single bonds, and even when only one is observed after dissolution at low temperature, this may still be different from that found in the crystal. However, in the present type of cyclic peptides of moderate ring size a given *cis,trans*-sequence will effectively define the whole ring conformation, since the CC_α torsion angle has consistently been found close to 180 or 60° and the NC_α torsion angle roughly 90°.[14,15,17–19]

When as a first experiment crystals of c-Sar$_4$ are dissolved at -50 °C and allowed to varm up, no spectral change is observed, thus proving that this peptide is homogeneous with the same conformer in solution as in the crystal. This had already been concluded independently for c-Sar$_4$, [2,14] as well as for c-GlySar$_3$,[3,15] c-GlySarGlySar, [3,15] and c-AlaSar$_3$,[3,15] by using symmetry arguments and geminal coupling constants for the solutions, and X-ray methods for the crystals.

In the case of c-Sar$_5$ the identity of the dominant solution conformation could not be deduced from its room-temperature NMR-spectrum by symmetry arguments.[2] When now the crystals are dissolved at -50 °C, the spectrum contains just the same five CH_2 quartets

and the five strong NMe lines, and heating produces no change except for the appearance of the very weak extra NMe lines observed earlier. This shows that the crystal conformer, known to be *cis,cis,cis,trans,trans*, is identical with the one which is strongly predominant in solution.

In contrast, all other cyclic pentapeptides containing one or more glycine or alanine residues give dramatic changes on heating, whereby the crystal conformer is transformed stepwise to other conformers. Often the final mixtures contain no more of the crystal conformer, and some conformers are only of transient existence. The best example is c-Ala-Sar$_4$, since its crystal conformer (A, Fig. 3) has been determined by X-ray methods [19] and found to be exactly the same as found [17] for c-Sar$_5$ [*cis,cis,cis,trans,-trans*(NH)]. The amide group of NH-type occupies one of the *trans*-positions and makes an external hydrogen bond with the oxygen of the other *trans*-amide group of a neighbouring (identical) molecule (Fig. 3). After dissolution at -50 °C, the NMR spectrum shows four NMe lines and four CH_2 quartets, as well as the expected lines for the alanine residue;* this set of lines must belong to conformer A. At -30 °C a new set of lines due to a second conformer B appears, and its concentration grows rapidly to become equal to that of A, whereafter, on further heating, a third set appears. The corresponding conformer C dominates the final equilibrium at room temperature (~ 60 %), with the rest made up of mainly conformer B and only traces of A (Fig. 4).

We draw the conclusion that C is the most stable conformer in solution and that it must be produced through B as an intermediate. Since B accumulates temporarily, this must also be more stable than A, and the critical barrier between A and B must be lower than the critical barrier between B and C. Strictly, one cannot know whether the process goes through additional intermediate conformers, but in view of the fact that c-Sar$_5$ with no NH present does not undergo a change, the only conceivable driving force is the establishment of an internal hydrogen bond in solution to replace the external hydrogen bond of the solid. Already conformer B needs therefore a sequence of three *trans* amide groups for steric reasons to obtain the necessary internal hydrogen bond, and we propose the sequence *cis,cis,trans,trans,-trans* (NH) (Fig. 3) in analogy with the crystal conformation of c-Gly$_6$ [20] and c-Gly$_4$Ala$_2$.[21]*

The only simple way of further improving the situation is the direct change of a second *cis*-amide group to *trans*, and we prefer the sequence *trans,cis, trans,trans,trans* (NH) for C (Fig. 3). Here the sequence of the same three *trans*-amide groups resemble even more closely the situation in c-Gly$_4$Ala$_2$, and the remaining *trans,cis* sequence resembles the situation in each half of a cyclic tetrapeptide.

A very similar behaviour is observed for c-GlySar$_4$, except that B never approaches A in concentration and that the final equilibrium is more completely dominated by conformer C (80 %) with the remainder now made up of the crystal conformation A and only traces of B (Fig. 5).

Also c-Gly$_2$Sar$_3$ and c-D-Ala-L-AlaSar$_3$, which have the correct sequence of *cis*- and *trans*-preferred amide bonds, may be expected to start out from the same initial crystal conformation *cis,cis,cis,trans*(NH),*trans*(NH), and do in fact show analogous transformations upon dissolution and subsequent heating.

Added in proof. An alternative to the CH_2-flipping mechanism A⇌B⇌C⇌B′⇌A′ for c-Sar$_3$ shown in Fig. 1 is the amide-group flipping mechanism A⇌B′⇌C⇌B⇌A′. During the flipping of one amide group (A⇌B′) the other two amide groups will approach each other momentarily across the ring, and so the higher barrier for the more bulky amide group as compared to the unsubstituted olefin receives a more convincing explanation.

* Actually, the α-Me group gives rise to two doublets instead of one, but since hardly any splitting is noticeable in the NMe-lines, it is very likely that we have about equal populations of the crystal conformer and its inverted conformer with an identical ring skeleton but exchanged α-H and α-Me positions. For the present discussion these are equivalent.

* These cyclic hexapeptides have only *trans*-amide bonds of NH-type and can therefore form both external and internal hydrogen bonds in the crystals.

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Received October 7, 1974.