A STUDY OF THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS

IV. NMR SPECTRA OF CYCLOHEXAPEPTIDES CONSTRUCTED OF ALANINE AND GLYCINE RESIDUES: CHEMICAL SHIFTS AND INTRAMOLECULAR HYDROGEN BONDS†

S. L. Portnova, T. A. Balashova,
V. F. Bystrov, V. V. Shilin,
Ya. Bernat,‡ V. T. Ivanov,
and Yu. A. Ovchinnikov

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In preceding papers [1, 2] it was shown that cyclic peptides constructed of L(D)-alanine and glycine residues form in aqueous solutions a dissymmetric (chiral) system with no center or plane of symmetry with respect to the amide chromophores. On passing to less polar solvents, some change in conformation takes place which is accompanied by a change in the intensity of the Cotton effects corresponding to the $n \rightarrow \pi^*$ transition and to a splitting of the $\pi \rightarrow \pi^*$ transition of the amide groups. However, the methods of optical rotatory dispersion and circular dichroism do not permit more definite information to be obtained on the conformational states of the cyclic peptides and the coordinates Φ and Ψ to be evaluated for the individual amino acid residues.

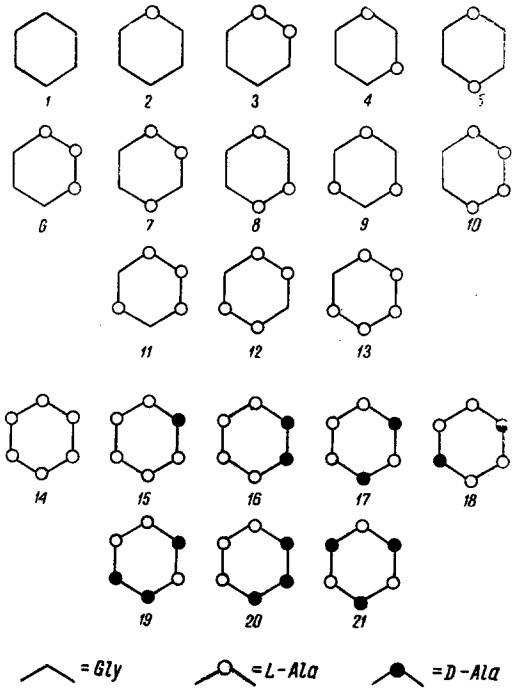

Fig. 1. Cyclic hexapeptides constructed of L- and D-alanine and glycine residues (direction of acylation clockwise).

In this account, which is the subject of two papers (for communication V, see [3]), we describe the results of a study of the conformations of the cyclohexapeptides (1)-(21) (Fig. 1) in polar solvents by the method of nuclear magnetic resonance (NMR). In this, we have made wide use of procedures which we have developed previously in the course of a study of the conformational states of linear peptides [4-8] and also of membrane-active cyclodepsipeptides [9, 10] and gramicidin S [11].

PARAMETERS OF THE SPECTRA

a. Interpretation of the NMR Spectra. Compounds (1)-(21) are sparingly soluble in the majority of ordinary solvents, and only trifluoroacetic acid, dimethyl sulfoxide, and water enable solutions of the cyclopeptides with concentrations sufficient for measuring the NMR spectra under ordinary conditions to be obtained. The spectra of

† For the preceding communication, see [1].

‡Chemical Faculty of the Polytechnic Institute (Gdansk, Poland).

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TABLE 1. Parameters of the ^1H NMR Spectra of the Cyclopeptides (1)-(13)

Cyclopeptide	Solvent	Chemical shifts, ppm					Spin-spin coupling constants, Hz		
		δ_{CH_3}	δ_{CH_2} (Gly)	δ_{CH} (Ala)	δ_{NH} (Gly)	δ_{NH} (Ala)	$^3J_{NH-CH}$ (Gly)	$^3J_{NH-CH}$ (Ala), observed	$^3J_{NH-CH}$ (Ala), with correction
1)	CF_3COOH	—	4,33 (doublet)	—	7,94 (triplet)	—	5,4	—	—
2)	CF_3COOH	1,59	4,06—4,55	4,59	7,93(*k*, *l*) 7,95(*m*) 7,98(*n*, *o*)	7,81	—	5,8	6,3
(3)	$(CD_3)_2SO$	1,27 1,31	3,46—4,00	4,15 4,18	7,88(*k*) 7,92(*l*) 8,23(*m*) 8,41(*n*)	8,05(*a*) 8,14(*b*)	6,5(*k*) 5,5(*m*, *n*)	6,3(*a*) 6,4(*b*)	6,8(*a*) 7,0(*b*)
	CF_3COOH	1,57 1,64	3,98—4,60	4,73	7,84(*k*) 7,92(*l*) 7,99(*m*) 8,08(*n*)	7,64(*a*) 7,79(*b*)	5,0(*n*)	6,4(*a*) 5,9(*b*)	7,0(*a*) 6,4(*b*)
(4)	$(CD_3)_2SO$	1,24 1,26	3,73 (*l*, *n*) 3,77 (*k*, *m*)	4,29(*a*) 4,22(*b*)	7,80(*k*) 7,84(*l*) 8,34(*m*) 8,38(*n*)	8,19(*a*) 8,21(*b*)	4,5(*k*) 5,0(*l*) 5,5(*m*) 4,0(*n*)	8,2(*a*) 5,5(*b*)	8,9(*a*) 6,0(*b*)
	H_2O	1,41	—	—	7,84—8,69	7,84—8,69	—	—	—
	CF_3COOH	1,58 1,61	4,07—4,50	4,30—4,87	8,04(*k*) 7,85—8,69(*l*, *m*, *n*)	7,79(*a*) 7,85—8,69(*b*)	5,0(*k*)	6,4(*a*)	7,0(*a*)
(5)	$(CD_3)_2SO$	1,24	3,35—4,00	4,07(*a*, *b*)	7,55(*k*, *l*) 8,39(*m*, *n*)	8,46(*a*, *b*)	4,7(*k*, *l*) 6,0(*m*, *n*)	5,7(*a*, *b*)	6,2(*a*, *b*)
	H_2O	1,39	~4,0	4,27(*a*, *b*)	7,82(*k*, *l*) 8,53(*m*, *n*)	8,60(*a*, *b*)	5,0(*k*, *l*) 6,0(*m*, *n*)	5,3(*a*, *b*)	5,8(*a*, *b*)
	CF_3COOH	1,56	~4,3	4,66(*a*, *b*)	7,94(*k*, *l*) 8,02(*m*, *n*)	7,79(*a*, *b*)	5,5(*k*, *l*) 6,0(*m*, *n*)	5,9(*a*, *b*)	6,4(*a*, *b*)

TABLE 1 (Continued)

Cyclopeptide	Solvent	Chemical shifts, ppm					Spin–spin coupling constants, Hz		
		δ_{CH_3}	δ_{CH_2} (Gly)	δ_{CH} (Ala)	δ_{NH} (Gly)	δ_{NH} (Ala)	$^3J_{NH-CH}$ (Gly)	$^3J_{NH-CH}$ (Ala), observed	$^3J_{NH-CH}$ (Ala), with correction
(6)	$(CD_3)_2SO$	1,25 1,30 1,32	3,43—3,90	3,90—4,45	8,01(*k*) 8,15(*l*) 8,48(*m*)	7,87(*a*) 8,08(*b*) 8,24(*c*)	6,0(*k*) 5,5(*m*)	7,3(*a*) 5,1(*b*) 7,9(*c*)	7,9(*a*) 5,5(*b*) 8,6(*c*)
	H_2O	1,40 1,45 1,47	—	—	7,85–8,68	7,85—8,68	—	—	—
	CF_3COOH	1,59 1,60 1,62	3,96–4,66	4,50(*a*) 4,77(*b*, *c*)	7,93(*k*) 8,10(*l*) 8,16(*m*)	7,54(*a*) 7,77(*b*) 7,86(*c*)	5,0(*k*) 5,5(*l*) 5,0(*m*)	5,1(*a*) 7,9(*b*) 6,7(*c*)	5,5(*a*) 8,6(*b*) 7,3(*c*)
(7)	$(CD_3)_2SO$	1,22 1,26 1,26	3,42–4,30	4,29(*a*) 3,42–4,25(b, *c*)	7,59(*k*) 7,72(*l*) 8,46(*m*)	8,02(*a*) 8,35(*b*, *c*)	5,0(*k*) 4,0(*l*) 5,5(*m*)	8,0(*a*) 5,6(*b*, *c*)	8,7(*a*) 6,1(*b*, *c*)
	CF_3COOH	1,55 1,57 1,60	3,87–4,60	4,50—4,93	7,85—8,10	7,75(*a*, *b*) 7,85—8,10(*c*)	—	6,6(*a*, *b*)	7,2(*a*, *b*)
(8)	$(CD_3)_2SO$	1,25	3,37—3,95	3,95—4,42	8,12(*k*) 8,21(*l*) 8,28(*m*)	7,97(*a*) 8,10(*b*) 8,25(*c*)	5,0(*h*, *l*, *m*)	6,5(*a*) 6,8(*b*) 6,8(*c*)	7,1(*a*) 7,4(*b*) 7,4(*c*)
(9)	$(CD_3)_2SO$	1,25	~3,7	4,21	8,27	8,00	5,5	6,8	7,4
	H_2O	1,39	3,95 (doublet)	—	8,38	8,22	5,5	6,4	7,0
	CF_3COOH	1,57	δ_A=4,17; δ_B=4,34 J_{AB}=–16,6	4,69	8,01	7,83	5,5 J_{AX}=5,0; J_{BX}=6,1	6,6	7,2
(10)	$(CD_3)_2SO$	1,27	3,35–3,92	3,92—4,40	7,68(*k*) 8,05(*l*)	7,89(*a*) 8,32(*b*, *c*, *d*)	5,0(*k*, *l*)	7,3(*a*) ~6,3(*b*, *c*, *d*)	7,9(*a*) ~6,8(*b*, *c*, *d*)
	CF_3COOH	1,59 1,59 1,62 1,62	3,86—4,90	3,86—4,90	7,99(*k*, *l*)	7,76(*a*, *b*) 7,77(*c*, *d*)	4,0(*k*, *l*)	6,2(*a*, *b*) 5,9(*c*, *d*)	6,7(*a*, *b*) 6,4(*c*, *d*)

TABLE 1 (Continued)

Cyclopeptide	Solvent	Chemical shifts, ppm					Spin–spin coupling constants, Hz		
		δ_{CH_3}	δ_{CH_2}(Gly)	δ_{CH} (Ala)	δ_{NH} (Gly)	δ_{NH} (Ala)	$^3J_{NH-CH}$ (Gly)	$^3J_{NH-CH}$ (Ala), observed	$^3J_{NH-CH}$ (Ala), with correction
(11)	$(CD_3)_2SO$	1,23 1,23 1,27 1,29	3,39 4,02	4,13 4,13 4,20 4,20	8,30(*k*) 8,39(*l*)	7,85(*a*) 7,95(*b*) 8,10(*c*) 8,12(*d*)	~5,5(*k*, *l*)	6,1(*a*) 7,5(*b*) 6,7(*c*) 6,9(*d*)	6,6(*a*) 8,2(*b*) 7,3(*c*) 7,5(*d*)
	H_2O	1,38 1,38 1,44 1,44	—	—	8,44(*k*) 8,50(*l*)	8,05(*a*) 8,15—8,25 (*b*, *c*, *d*)	5,5(*k*) 5,0(*l*)	7,0(*a*) —	7,6(*a*) —
	CF_3COOH	1,53 1,56 1,57 1,60	δ_A=4,00; δ_B=4,21 J_{AB}=−15,2	4,25—4,98	7,96(*k*) 8,03(*l*)	7,60(*a*) 7,68(*b*) 7,9?(*c*) 8,23(*d*)	5,3(*l*) J_{AB}=6,2; J_{BX}=4,3	6,4(*a*) 7,2(*b*) 6,6(*c*) 7,2(*d*)	7,0(*a*) 7,8(*b*) 7,2(*c*) 7,8(*d*)
(12)	$(CD_3)_2SO$	1,26 1,26	3,38—3,98	4,21 4,17	8,12(*k*, *l*)	7,80(*a*, *b*) 8,32(*c*, *d*)	5,0(*k*, *l*)	6,9(*a*, *b*) 6,5(*c*, *d*)	7,5(*a*, *b*) 7,1(*c*, *d*)
	H_2O	1,39 1,40	3,95 (doublet)	4,36 4,36	8,24(*k*, *l*)	8,20(*a*, *b*) 8,33(*c*, *d*)	5,0(*k*, *l*)	6,8(*a*, *b*) 5,8(*c*, *d*)	7,4(*a*, *b*) 6,3(*c*, *d*)
	CF_3COOH	1,55 1,59	δ_A=4,08; δ_B=4,44 J_{AB}=−16,6	4,70 4,77	8,01(*k*, *l*)	7,77(*a*, *b*) 7,93(*c*, *d*)	J_{AX}=4,9; J_{BX}=5,8	6,9(*a*, *b*) 6,4(*c*, *d*)	7,5(*a*, *b*) 7,0(*c*, *d*)
(13)	$(CD_3)_2SO$	1,24 1,24 1,26 1,26	δ_A=3,50; δ_B=3,78 J_{AB}=−15,7	3,95—4,40	8,49	7,78(*a*) 7,81(*b*) 7,90(*c*) 7,97(*d*)	J_{AX}=4,8; J_{BX}=4,5	4,4(*a*) 5,9(*b*) 5,0(*c*) 6,5(*d*)	4,8(*a*) 6,4(*b*) 5,4(*c*) 7,1(*d*)
	CF_3COOH	1,34 1,57 1,59 1,59 1,61 1,65	δ_A=4,06; δ_B=4,45 J_{AB}=16,0	4,49—4,88	8,07	8,29(*e*) 7,67—7,90	J_{AX}=5,1; J_{BX}=6,1	7,7(*e*) —	8,4(*e*) —

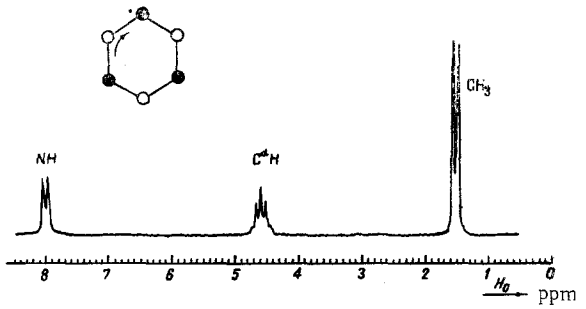

Fig. 2. ^{1}H NMR spectrum of DLDLDL-cyclohexaalanine in CF_3COOH solution at a frequency of 100 MHz.

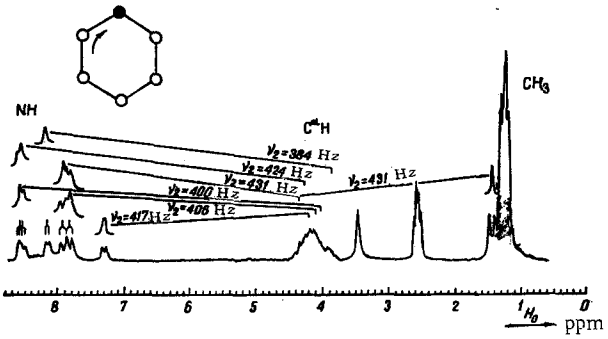

Fig. 3. ^{1}H NMR spectrum of LLLLLD-cyclohexaalanine in $(CD_3)_2SO$ solution at a frequency of 100 MHz (the double-resonance spectra are shown in the top part of the figure).

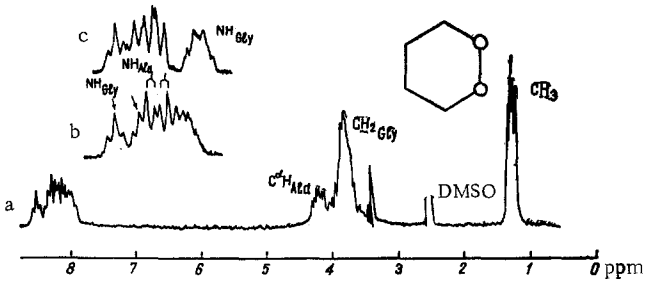

Fig. 4. ^{1}H NMR spectrum of cyclo-(Ala–Ala–Gly–Gly–Gly–Gly) (a) in $(CD_3)_2SO$ solution at a frequency of 100 MHz and the region of the signals of the amide NH protons with an expanded sweep (b). The same region of the spectrum with the addition of 0.02 ml of D_2O (c).

cyclohexaglycyl in the meso form (20) and (21) could be taken only in CF_3COOH solutions. The other compounds were studied in CF_3COOH and $(CD_3)_2SO$. Some cyclodepsipeptides were also studied in aqueous solutions. Only the region of the amide protons was recorded in H_2O, while the chemical shifts of the $C^{\alpha}H$ methine protons of the alanine residues and of the methylene protons of the glycine residues were determined from the spectra of solutions in D_2O. However, as a rule the spectra of aqueous solutions of the cyclopeptides are difficult to interpret because of the broadening of the signals from the NH groups due to the slow exchange of the amide protons with the protons of the solvent. Because of this, it was not always possible to separate the corresponding signals from the alanine and glycine NH protons and to determine the spin–spin coupling constants of the protons in the NH–$C^{\alpha}H$ fragments ($^3J_{NH-CH}$); in those cases where this could be done, the results obtained proved to be extremely close to the values of $^3J_{NH-CH}$ found for solutions in $(CD_3)_2SO$ (Tables 1 and 2).

The parameters of the NMR spectra of compounds (1)–(21) taken at room temperature are given in Tables 1 and 2; the spectra of some of these compounds are shown in Figs. 2-5. The signals of the C–CH_3 group appear in the strong field (δ 1.2-1.6 ppm), the signals from the $C^{\alpha}H$ protons in the 3.8-4.8 ppm region, and the signals from the amide NH protons at 7.3-8.7 ppm. The observed values of the constants have been corrected for the overlapping of the lines and for the electronegativities of the substituents in the peptide fragment [5]. The spin–spin coupling constants $^3J_{NH-CH}$ do not depend on the temperature in the range from 20 to 90°C that was studied.

To interpret the spectra with overlapping NH signals, we used the double-resonance method (see, for example, Fig. 2) and temperature measurements. In view of the fact that in the cyclopeptide series in CF_3COOH solution there is a pronounced overlapping of the lines of the NH protons, Tables 1 and 2 give only those results that relate to well-resolved signals.

On considering the NMR spectra, attention is attracted by their "homogeneity," i.e., the correspondence of the number of signals observed in the spectrum with the structural formula of the cyclopeptide. Thus, in the spectra of compounds (2), (5), (9), (14), and (21), which contain one or several chemically identical alanine residues, in each case there is a doublet corresponding to the alanine NH and CH_3 protons; in compounds (3), (4), and (12) there are two each; and in (6), (7), (8), (18), and (20) three each; and so on. This shows the chemical and optical purity of the compounds studied [12, 13] and also that there is no conformational equilibrium in them which takes place more slowly than, or at a rate comparable with, the frequencies characteristic for the NMR method. From this, and also from the IR spectra (the presence in all the cyclopeptides of a strong amide II band [13]) and x-ray structural analysis, showing that compounds (1) [14] and (3) [15] have only trans amide bonds in the crystalline state, it may be concluded

TABLE 2. Parameters of the ^1H NMR Spectra of the Cyclohexaalanines (14)–(21)

Cyclopeptide	Solvent	Chemical shifts, ppm			Spin–spin coupling constants, Hz	
		δCH_3	$\delta C^{\alpha}H$	δNH	$^3J_{NH-CH}$ observed	$^3J_{NH-CH}$ with corrections
(14)	$(CD_3)_2SO$	1,23	3,86–4,25	8,19	6,9	7,5
	CF_3COOH	1,67	4,50–5,00	7,87	6,8	7,4
(15)	$(CD_3)_2SO$	1,19; 1,19; 1,27; 1,27; 1,27; 1,42(*c*)	3,84(*d*); 4,00(*f*); 4,07(*b*); 4,17(*a*); 4,24(*e*); 4,32(*c*)	4,27(*a*); 7,78(*b*); 7,87(*c*); 8,12(*d*); 8,50(*e*); 8,52(*f*)	4,8(*a*); 7,1(*b*); 8,7(*c*); 4,7(*d*); 8,3(*e*); 4,7(*f*)	5,2(*a*); 7,7(*b*); 9,5(*c*); 5,1(*d*); 9,0(*e*); 5,1(*f*)
	H_2O	1,34–1,47	—	7,58(*a*); 8,02–8,11(*b*, *c*); 8,27(*d*); 8,62(*e*); 8,67(*f*)	5,1(*a*); 5,1(*d*); 4,7(*e*); 7,5(*f*)	5,5(*a*); 5,5(*d*); 5,1(*e*); 8,2(*f*)
	CF_3COOH	1,53; 1,56; 1,58; 1,60; 1,62; 1,63;	4,41; 4,58; 4,65; 4,71; 4,73; 4,75	7,69(*a*); 7,80–8,00(*b*–*f*)	6,5(*a*)	7,1(*a*)
(16)	$(CD_3)_2SO$	1,22; 1,22; 1,27; 1,27; 1,29; 1,39;	3,80; 4,50	7,56(*a*); 7,87(*b*, *c*); 7,96(*d*); 8,06(*e*); 8,36(*f*)	5,7(*a*); 6,8(*b*); 6,3(*c*); 5,4(*d*); 8,3(*e*); 7,4(*f*)	6,2(*a*), 7,4(*b*); 6,8(*c*); 5,9(*d*); 9,0(*e*); 8,0(*f*)
	CF_3COOH	1,55; 1,57; 1,60; 1,61; 1,63; 1,65;	4,32; 4,76; 4,76; 4,76; 4,76; 4,76	7,60(*a*); 7,64(*b*); 7,70–7,95(*c*–*f*)	6,7(*a*); 4,7(*b*)	7,3(*a*); 5,1(*b*)
(17)	$(CD_3)_2SO$	1,20; 1,24; 1,26; 1,26; 1,28; 1,38;	3,94; 4,18; 4,18; 4,18; 4,18; 4,18;	7,59(*a*); 7,84(*b*, *c*); 8,00(*d*); 8,10(*e*); 8,39(*f*)	5,9(*a*); 5,9(*b*); 6,5(*c*); 5,0(*d*); 8,6(*e*); 7,7(*f*)	6,4(*a*); 6,4(*b*); 7,1(*c*); 5,4(*d*); 9,3(*e*); 8,4(*f*)
(18)	$CD_3)_2SO$	1,18; 1,23; 1,26	4,05; 4,17; 4,30	7,38(*a*, *b*); 8,20(*c*, *d*); 8,56(*e*, *f*)	6,8(*a*, *b*); 5,9(*c*, *d*); 7,1(*e*, *f*)	7,4(*a*, *b*); 6,4(*c*, *d*); 7,7(*e*, *f*)
	CF_3COOH	1,53; 1,54; 1,62	4,59; 4,66; 4,86	7,73(*a*, *b*); 7,82(*c*, *d*); 7,90(*e*, *f*)	6,5(*a*, *b*); 6,9(*c*, *d*); 8,6(*e*, *f*)	7,1(*a*, *b*); 7,5(*c*, *d*); 9,3(*e*, *f*)
(19)	$(CD_3)_2SO$	1,15; 1,24; 1,24; 1,24; 1,24; 1,24	3,96; 4,02; 4,09; 4,27; 4,27; 4,27	7,38(*a*); 7,47(*b*); 7,96(*c*); 8,03(*d*); 8,34(*e*); 8,44(*f*)	5,8(*a*); 7,4(*b*); 7,6(*c*); 6,4(*d*); 5,5(*e*); 7,2(*f*)	6,4(*a*); 8,0(*b*); 8,3(*c*); 7,0(*d*); 6,0(*e*); 7,8(*f*)
	CF_3COOH	1,47; 1,48; 1,53; 1,55; 1,64; 1,64	4,59; 4,61; 4,67; 4,76; 4,85; 4,85	7,50(*a*); 7,70(*b*); 7,80(*c*); 7,80–7,96(*d*, *e*, *f*)	4,8(*a*); 6,1(*b*); 7,1(*c*);	5,2(*a*); 6,6(*b*); 7,7(*c*)
(20) (21)	CF_3COOH	1,57; 1,61; 1,62	4,58; 4,87; 4,87	7,50(*a*, *b*); 7,78(*c*, *d*); 7,84(*e*, *f*)	5,6(*a*, *b*); 7,6(*c*, *d*); 5,8(*e*, *f*)	6,1(*a*, *b*); 8,3(*c*, *d*); 6,3(*e*, *f*)
	CF_3COOH	1,53	4,61	8,00	6,9	7,5

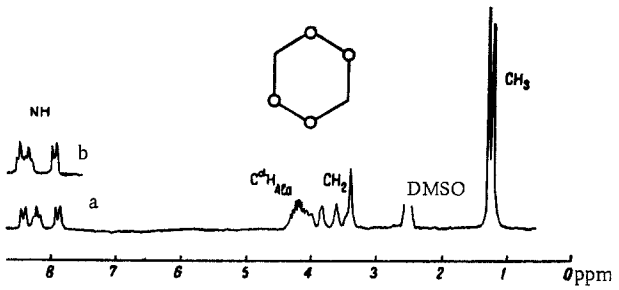

Fig. 5

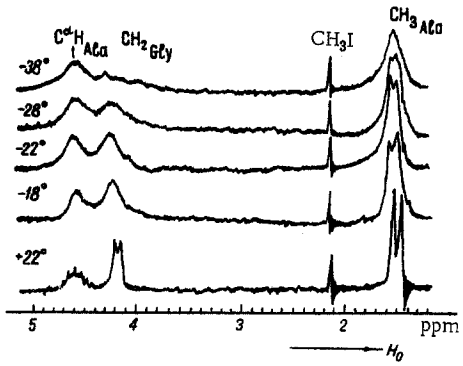

Fig. 6

Fig. 5. ^1H NMR spectrum of cyclo-(Ala–Ala–Gly)$_2$ (a) and the region of the signals of the amide protons in the spectrum of deuterated cyclo-Ala–Ala–Gly–Ala*–Ala–Gly (b) (see text).

Fig. 6. Temperature dependence of the spectrum of cyclo-(Ala–Gly)$_3$ in $CF_3COOH + H_2O$ (7:1) solution.

TABLE 3. Temperature Gradient $(\Delta\delta/\Delta T) \cdot 10^{-3}$ ppm/deg of the Chemical Shifts of the NH Protons in $(CD_3)_2SO$ Solutions*

Compound	Protons studied									
	alanine residues						glycine residues			
	a	b	c	d	e	f	k	l	m	n
(3)	3,9	5,4	—	—	—	—	1,4	3,1	4,0	5,7
(4)	5,0	5,0	—	—	—	—	1,7	2,1	4,4	5,7
(5)	5,9	5,9	—	—	—	—	0,0	0,0	4,2	4,2
(6)	2,9	3,3	5,1	—	—	—	3,3	**	5,8	—
(7)	4,2	6,5	6,5	—	—	—	0,0	1,5	6,3	—
(8)	3,0	5,6	6,4	—	—	—	3,4	4,8	4,8	—
(9)	3,9	3,9	3,9	—		—	4,7	4,7	4,7	—
(11)	2,1	4,2	4,0	4,4	—	—	6,0	5,6	—	—
(12)	2,4	2,4	4,8	4,8	—	—	5,0	5,0	—	—
(13)	1,4	2,8	3,6	3,4	5,1	—	6,4	—	—	—
(14)	3,0	3,0	3,0	3,0	3,0	3,0	—	—	—	—
(16)	5,4	1,7	4,3	3,2	3,4	6,1	—	—	—	—
(18)	0,0	0,0	7,0	7,0	6,4	6,4	—	—	—	—
(19)	2,0	1,7	5,3	6,0	6,3	6,3	—	—	—	—

* The temperature gradient of N-acetylacetamide is $6.1 \cdot 10^{-3}$ ppm/deg [30].

† The signal from the NH(*l*) proton is masked by the other signals.

that in all the compounds considered, regardless of the number and configuration of the alanine residues, there are no appreciable amounts whatever of the cis form of the amide bonds.* This result is not in agreement with the conclusions of Blaha et al. [22, 23] based on IR-spectroscopic investigations that, with the introduction into cyclopeptides of several neighboring amino acid residues with the same configuration, forms with cis amide bonds take a considerable part in the conformational equilibrium.

In addition to this, the "homogeneity" of the NMR spectra yet again shows the equivalence of the alanine or glycine residues in the dominating conformations of the "symmetrical" compounds (1), (5), (9), (12), (14), and (21), since the possibility cannot be excluded of the existence of several equally populated and

*It is known that the energy barriers of cis⇌trans transitions in secondary amides and peptides are extremely high, amounting to 17-24 kcal/mole [8, 17-21]. Consequently, if appreciable amounts of the forms with the cis amide bonds were present in the conformational equilibrium, the observed NMR spectra should represent the result of the superposition of the spectra of each of these forms with intensities proportional to their molar fractions.

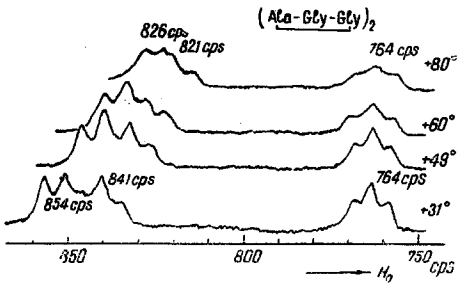

Fig. 7

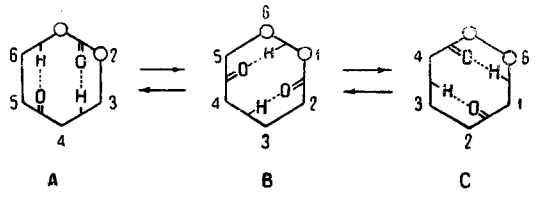

Fig. 8

Fig. 7. Temperature dependence of the position of the NH signals in the spectrum of cyclo-(Ala−Gly−Gly)$_2$ in $(CD_3)_2SO$ solution.

Fig. 8. Possible systems of ten-membered rings with intramolecular H bonds (shown by broken lines) in the molecule of a cyclohexapeptide.

rapidly converting conformations in each of which the alanine or glycine residues are chemically nonequivalent (in a similar manner to the way in which the cyclohexapeptide antibiotic enniatin B, constructed of three chemically equivalent links, assumes a conformation having no elements of symmetry in nonpolar solvents [10, 16]).* In actual fact, the NMR spectrum (Fig. 6) taken of a solution of compound (9) in a mixture of CF_3COOH and H_2O (7 : 1) cooled to −38°C indicates a rapid conformational equilibrium of several forms, since the broadening of the signals reflects the approach to the coalescence temperature.

Further information on the structure of the cyclopeptides studied was obtained by an analysis of the following parameters of the NMR spectra: a) the chemical shifts of the signals from the NH protons at room temperature in CF_3COOH and $(CD_3)_2SO$ solutions; b) the dependence of the chemical shifts of the NH protons on the temperature in $(CD_3)_2SO$ solutions; c) the rate of exchange of the hydrogen of the NH groups for deuterium in $(CD_3)_2SO-D_2O$ or $(CD_3)_2SO-CD_3OD$ solutions; and d) the spin−spin coupling constants $^3J_{NH-CH}$. The first three parameters are considered in the present paper, and the $^3J_{NH-CH}$ constant in the following one.

b. Chemical Shifts and Deuterium Exchange as Indicators of an Intramolecular H Bond. The first three of the parameters mentioned above permit the determination of the positions of the intramolecular H bonds and an evaluation of their stability. Since there are no aromatic groups with their pronounced magnetic anisotropy in the cyclopeptides studied, the interpretation of the chemical shifts of the NH signals is simpler than in the cyclopeptides containing residues of phenylalanine (such as gramicidin S [24-28] tyrosine [29], and histidine [29]). While the signals from the NH groups are located in a narrow range of frequencies for solutions in CF_3COOH (see Tables 1 and 2) (76% of all the signals fall in the range from 7.7 to 8.0 ppm), for solutions in $(CD_3)_2SO$ a considerable differentiation of the NH protons with respect to their chemical shifts is found. As a rule, the NH signals from the two amino acid residues (separately for glycine and for alanine residues) are located in the stronger-field region (7.2-7.8 ppm, see Tables 1 and 2). It may be assumed that these NH groups are shielded from the action of the solvent and form two intramolecular H bonds, while the NH groups that correspond to the signals in the weaker field (8.0-8.6 ppm) are proton-donating for H bonds with the solvent.

This assumption has been confirmed by the dependence of the chemical shifts of the NH protons on the temperature. The method of determining intramolecular H bonds in peptides [28-30] is based on the fact that the positions of the signals from the NH groups participating in intramolecular H bonds are less sensitive to a change in the temperature than the chemical shifts of the solvated NH groups. The temperature gradients of the chemical shifts of the NH groups for the cyclohexapeptides are given in Table 3. In accordance with what has been said above, the smallest temperature gradient corresponds to the signals located in the stronger field [an exception is cyclohexaalanyl (16)]. In order to illustrate the influence of the temperature on the chemical shifts of the signals from the NH groups, Fig. 7 gives the spectra of the cyclopeptide (5) at various temperatures.

* For this reason, there is insufficient basis for the conclusion of the presence of an axis of second-order symmetry in gramicidin S, cyclo-(Val−Orn−Leu−D-Phe−Pro)$_2$ [24], and the cyclohexapeptide cyclo-(Gly−Pro−Gly)$_2$ [25], based on a study of the NMR spectra, which give one group of signals from the amino acid residues of the same type at room temperature.

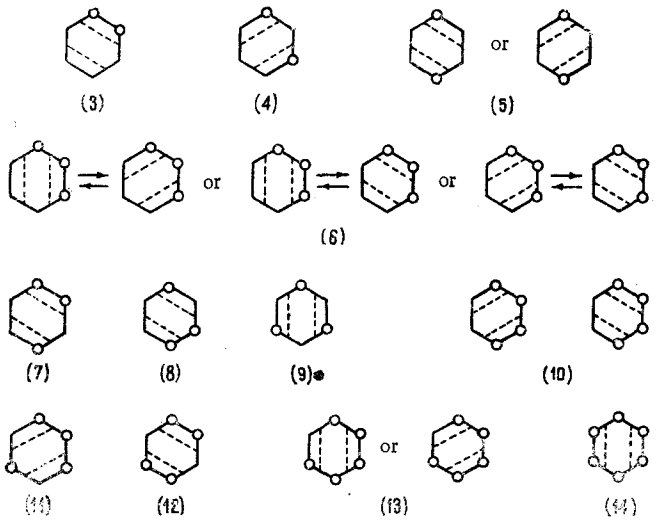

Fig. 9. Positions of the intramolecular H bonds in the dominating structures of the cyclohexapeptides (3)-(14).

In this case, the two glycine NH protons giving a signal in the strong field (7.64 ppm) scarcely change their position, in contrast to the signals of the other NH groups. This shows that the intramolecular H bonds in the cyclopeptide (5) are formed by the NH groups of the two glycine residues.

Thus, the positions of the signals from the NH groups and their dependence on the temperature show that in the cyclohexapeptides studied, the majority of which belong to one and the same conformational type [1], structures with two intramolecular H bonds predominate in polar solvents (dimethyl sulfoxide and water).

The results obtained are in good agreement with the pleated-sheet conformational model proposed for the cyclohexapeptides by Schwyzer [31-33] in order to explain the ease of the occurrence of the reaction of the doubling of linear tripeptides during attempts at their cyclization. Recently, Schwyzer and Ludescher have given a number of NMR results also supporting the pleated-sheet conformation in cyclo-(Gly–Pro–Gly)$_2$ [25]. A characteristic feature of this type of structure is the presence of two trans-annular H bonds closing ten-membered rings. A theoretical analysis of the cyclohexapeptide systems has shown that the pleated-sheet conformation actually corresponds to a minimum in the potential energy on the conformational charts [34]. A similar structure has been found by an x-ray structural analysis of crystalline samples of compounds (1) and (3) [14, 15]. The realization of ten-membered rings stabilized by H bonds has recently been demonstrated for gramicidin S [11], valinomycin, and complexes of valinomycin with potassium in solutions [9] and with potassium tetrachloroaurate in crystals [35], and also for ferrichrome A [36]. Kopple, Ohnishi, and Go [29, 30] also incline to the "pleated-sheet" conformation for cyclo-Tyr–Gly$_5$, cyclo-Leu–Gly$_5$, and cyclo-His–Gly$_2$–Tyr–Gly$_2$ on the basis of NMR results similar to ours. Thus, the results that we have obtained in combination with those published previously permit the "pleated-sheet" conformation to be considered as the most probable for the cyclohexapeptides.

In the general case, three different structures of this type with intramolecular H bonds differing by the positions of the H bonds in the ring are possible for cyclohexapeptides. Thus, for compound (3) these are structures A, B, and C (Fig. 8) in which the H bonds are formed by different pairs of amino acid residues. For some cyclohexapeptides – for example, (9) and (14) – these possibilities are equivalent. The temperature gradients of the chemical shifts of the NH protons for these compounds may be considered as averaged values corresponding to equal lives of the molecules in each of the three states mentioned.* If the value of $\Delta\delta/\Delta T$ is less than this value, it must be assumed that the NH group is participating in intramolecular H bonds.

* Some difference in the temperature gradients is possibly connected with the steric shielding of the lateral methyl groups or with the presence in the conformational equilibrium of a small proportion of structures with a different type of hydrogen bonding – for example, seven-membered rings with a H bond, which exist in linear dipeptides [4, 5] and in the cyclohexapeptides (16)-(19) in nonpolar solvents [37].

TABLE 4. The Deuterium Exchange of the NH Protons of the Cyclohexapeptides

Compound	Medium	Nature of the deuterium exchange*
(3)	$(CD_3)_2SO$+ +12% D_2O	D_2O added in an amt. of 0.02 ml four times $T \simeq 2$ h, rates equal
(5)	$(CD_3)_2SO$+ +8% D_2O	$T \simeq 8$ min, rates equal
(9)	$(CD_3)_2SO$+ +5% CD_3OD	$T \simeq 1$ h 20 min, rates equal
	$(CD_3)_2SO$+ +10% D_2O	$T \simeq 7$ min, rates equal
(11)	$(CD_3)_2SO$+ +10% D_2O	$T \simeq 2$ h, rates equal
(12)	$(CD_3)_2SO$† +5% CD_3OD	NH(*a*, *b*)22% exchange in 1 h 15 min NH(*c*, *d*)andNH(*k*, *l*): $T/2$=1h 15 min
(13)	$(CD_3)_2SO$+ +12% D_2O	$T \simeq 1$ h 20 min, rates equal
(18)	$(CD_3)_2SO$+ +10% D_2O	$T \simeq 7$ min, rates equal

*T is the time during which the intensity of the signal decreases by one half.

†When 5% of D_2O was added to this solution, the exchange accelerated and took place to the extent of 72% for NH (a, b), 93% of NH (k, *l*), and 87% of NH (c, d) in 20 min.

An analysis of the chemical shifts (see Tables 1 and 2) and their temperature gradients (see Table 3) permits the systems of H bonds [in $(CD_3)_2SO$ solution] shown schematically in Fig. 9 to be considered as dominating in compounds (3)-(14).

In order to determine H bonds in the cyclopeptide (12), its deuterated derivative, cyclo-Ala–Ala–Gly–Ala*–Ala–Gly was synthesized. (The asterisk denotes the L-alanine residue replaced by an α-deutero-L-alanine residue.) As follows from its NMR spectrum (top part of Fig. 5), the label proved to be located in the alanine residue ($\delta_{NH} = 8.32$ ppm) not participating in the formation of an intramolecular H bond; i.e., compound (12) has the dominating structure of the H bonds shown in Fig. 9.

A refinement of the dominating system of H bonds for compounds (6), (10), and (13) can be made on the basis of an analysis of the $^3J_{NH-CH}$ constants (see following paper). So far as concerns compounds (5) and (15)-(20), in these cases for the spectral assignments it is necessary to study suitable deuterated or ^{15}N-labeled derivatives.

It is impossible to make any definite conclusions whatever concerning the law of the "selection" of the pairs of H bonds in the series of compounds studied on the basis of the results obtained. Thus, while in compounds (3)-(5), (8), and (10) the H bonds are formed with the minimum participation of the alanine NH groups, in (11)-(13), on the contrary, the glycine NH groups take the less active part in H bonding.

The absence of a clear direction in the formation of an intramolecular H bond in the series of compounds studied (see Fig. 9) is apparently explained by the fact that all three possible types of pleated-sheet structures (A, B, and C; see Fig. 8) differ only slightly in their energies, and the domination of one conformation does not exclude the realization (even if to a smaller extent) of the other two forms.

A consideration of the temperature gradients (Table 3) of the cyclohexaalanyls (18) and (19) shows that there is a definite preference in the formation of a H bond even in those cases where the cyclohexapeptide is constructed of similar amino acid residues of different configurations; i.e., the sequence of D- and L-amino acid residues in the ring determines the dominating position of the H bonds to a substantial extent.

In addition to the temperature dependence of the chemical shifts, the deuterium exchange of the hydrogen of the NH groups (NH → ND) is also being used to study intramolecular H bonds. It is known [38]

that in proteins and polypeptides the NH groups present in an α-helical structure and participating in the formation of H bonds exchange with deuterium far more slowly than the other NH groups. A considerable difference in the rates of deuterium exchange for the free and the bound NH groups has also been found in gramicidin S and its N,N'-diacetyl derivative [11, 24, 39]. Consequently, it may be assumed that in the cyclohexapeptides the NH groups participating in H bonds (the signals of which are located in a stronger field and have a smaller temperature gradient) should exchange with deuterium more slowly than the other NH groups. Deuterium-exchange experiments were performed in $(CD_3)_2SO$ solutions with the addition of a carrier of mobile deuterium (D_2O or CD_3OD) (Table 4). As already mentioned, the cyclohexapeptides are distinguished by the characteristic that a conformational equilibrium accompanied by the "migration" of the H bonds with respect to the ring is possible for them (see Fig. 8). If the "migration" takes place with a rate considerably exceeding the rate of deuterium exchange, all the NH groups become equivalent in their accessibility for D_2O or CD_3OD and, consequently, they should not differ in the rate of deuterium exchange. In actual fact, as follows from Table 4, for the majority of the compounds studied [(3], (5), (9), (11), (13), and (18)] practically identical rates of deuterium exchange are observed. Where it was possible to detect some difference in the rates of deuterium exchange [compound (12)], the result obtained agreed well with the absolute values and temperature gradients of the chemical shifts.

Thus, the study of the temperature dependence of the chemical shifts and of the rate of deuterium exchange of the NH groups has shown that, as a rule, the cyclohexapeptide molecule has a structure of the "pleated-sheet" type with two trans-annular H bonds. This dominating structure is in rapid dynamic equilibrium with two other structures (see Fig. 8).

In the following paper the spin–spin coupling constants of the protons of the NH–CH fragment of the alanine residue of the cyclohexapeptides are considered together with the results of an experimental investigation of the conformational analysis of similar systems.

EXPERIMENTAL

The ^1H NMR spectra were measured on a JNM 4H-100 instrument with a working frequency of 100 MHz and with the stabilization of the resonance conditions with respect to one sample. Tetramethylsilane was used as internal standard ($\delta = 0.00$ ppm) for the CF_3COOH and $(CD_3)_2SO$ solutions, and sodium trimethylsilylpentanesulfonate (DSS) for the aqueous solutions. Before the preparation of the samples, the dimethyl sulfoxide was distilled over CaH_2 to eliminate moisture. The chemical shifts were determined with an accuracy of ± 0.005 ppm and the $^3J_{NH-CH}$ spin–spin coupling constants with an accuracy of about ± 0.1 Hz. The temperature was measured by a copper-constantan thermocouple with an accuracy of ± 1°C with the aid of the thermal attachment to the JNM 4H-100 instrument.

SUMMARY

1. The NMR spectra of cyclohexapeptides constructed of L(D)-alanine and glycine residues have been studied in $(CD_3)_2SO$, CF_3COOH, and H_2O solutions.

2. In all the compounds studied, the amide bonds assume the trans configuration.

3. In polar solvents, the cyclohexapeptides assume the "pleated-sheet" conformation characterized by two trans-annular hydrogen bonds of the 4 → 1 type.

4. The dominating structure of the cyclohexapeptides is in equilibrium with two analogous structures accompanied by the migration of the system of H bonds with respect to the ring.

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