BIOLOGICALLY ACTIVE ALKALI METAL COMPLEXONES. A ^{13}C-NMR STUDY OF ION–DIPOLE INTERACTION

V.F. BYSTROV*, V.T. IVANOV, S.A. KOZ'MIN, I.I. MIKHALEVA, K.KH. KHALILULINA and YU.A. OVCHINNIKOV

Shemyakin Institute for Chemistry of Natural Products, USSR Academy of Sciences, Ul. Vavilova 32, Moscow V-312, USSR

E.I. FEDIN and P.V. PETROVSKII

Institute of Organoelement Compounds, USSR Academy of Sciences, Moscow, USSR

Received 24 December 1971

1. Introduction

The recently discovered ability of cyclic depsipeptides and peptides to form stable alkali ion complexes (see [1] and ref. therein) has aroused considerable interest. Such compounds are being widely used as tools for the study of processes associated with ion transport through membranes. They are also convenient models for investigating the binding of Na^+ and K^+ ions in biological systems. Among the most popular of the alkali metal complexones are the depsipeptide antibiotics valinomycin and the enniatins, and the cyclic decapeptide antamanide [2, 3]. A characteristic feature of valinomycin is its exceptionally high K^+/Na^+ selectivity; the enniatins are less selective [1, 4], whereas antamanide displays selectivity for the Na^+ ion [4, 5].

Important information on the spatial structure and complexing mechanism of the above compounds in solution has been obtained by proton magnetic resonance spectroscopy [6–12]. It is to be expected that the intensively developing field of ^{13}C-nuclear-magnetic-resonance will open up new possibilities in such studies and that it will become a powerful new method among the physicochemical techniques used for investigating three-dimensional structures. For peptidic compounds data have already accumulated permitting assignment of the ^{13}C-signals to the principal structural elements [13–19]. However, correlations are still lacking that would allow the use of this method in conformational analysis [16].

The present paper compares the ^{13}C-spectra of valinomycin† $\overline{\text{(D-Val–L-Lac–L-Val–D-HyIv)}_3}$ [6, 12], of beauvericin $\overline{\text{(L-MePhe–D-HyIv)}_3}$ [20, 21] an antibiotic belonging to the enniatin series, and a symmetric analog of antamanide (Val^6, Ala^9-antamanide [22–24]

$$\overline{\underset{\substack{1,6\quad 2,7\quad 3,8\quad 4,9\quad 5,10}}{\text{(L-Val–L-Pro–L-Pro–L-Ala–L-Phe)}_2}}$$

with those of their alkali metal (Na^+, K^+) complexes. Beauvericin stands very close in conformational parameters and complexing properties to the thoroughly investigated enniatin antibiotics [9, 21, 23], while the symmetric antamanide analog is very similar to the naturally occurring cyclopeptide [11, 22, 23, 25].

2. Experimental

The ^{13}C-spectra were obtained at 22.63 MHz on a Bruker HX 90/13–18″ spectrometer equipped with a Fabri-Tek 1085 computer (20K core memory). The spectrometer was operated in the Fourier-transform

* To whom to address all correspondence.

† The following abbreviations for residues are used: HyIv: α-hydroxyisovaleric acid; MePhe, *N*-methylphenylalanine; Lac, lactic acid

North-Holland Publishing Company – Amsterdam

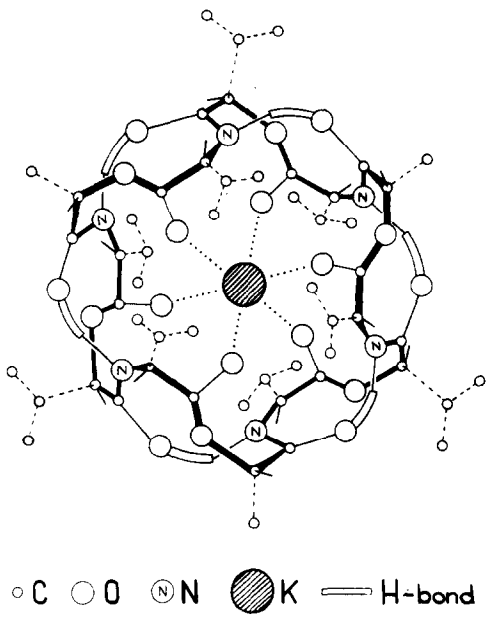

Fig. 1. Spatial structure of the K^+ complex of valinomycin.

mode with multiple scanning (2000–8000 scans at 0.8 sec/scan) and proton noise decoupling at 90 MHz. Solutions of 100–300 mg of substance in 1.4 ml of a 1:1 (v:v) mixture of $CDCl_3$ with CD_3OD were used. The complexes were formed by adding excess KNCS or NaNCS in 1:2.5 mole ratios for valinomycin and 1:5 mole ratios for beauvericin and "symmetric" antamanide. The chemical shifts (± 0.2 ppm) were measured with respect to CS_2 (0.07 ml) as internal reference.

A biosynthetic sample of valinomycin [26] was used whereas beauvericin [21] and "symmetric" antamanide [24] were prepared by total synthesis.

Assignment of the ^{13}C-peaks was made on the basis of data for amino acids and dipeptides [13–19] and also by comparison of the spectra of the compounds among themselves.

3. Results and discussion

It had earlier been shown [1, 6, 9, 11, 12, 22, 23, 27, 28] that the distinctive characteristic of the complexes in question is the location of the ion within the central cavity of the cyclic molecule so that the former is held in place by ion–dipole interaction with those carbonyls that are oriented towards the center of the cavity.

Table 1
^{13}C-chemical shifts of valinomycin and its K^+ complex.

Carbon		Valinomycin	K^+ complex
C=O		20.4	16.8
		20.9	17.3
		21.7	19.8
		22.3	21.2
C^{α}–O	HyIv	113.9	112.7
	Lac	122.1	121.3
C^{α}–N		133.0	130.6
		134.0	130.8
C^{β}–H		162.2	162.3
		163.1	164.2
		163.5	164.2
CH_3		173.6	172.6
		173.6	172.6
		173.8	173.2
		174.0	173.7
		174.0	173.9
		175.8	175.3
		173.1	175.9
Signals from solvents			
$CDCl_3$ [a]		114.9	114.8
CD_3OD [b]		114.3	114.3

[a] Deuterium isotope shift +0.17 ppm, J_{CD} = 32.3 Hz.
[b] J_{CD} (CD_3) = 21.5 Hz.

It was to be expected that with ion–dipole interaction of the type C=O...M^+, where M^+ stands for the monovalent cation (Na^+ or K^+) an additional shift of electron density on the carbonyl bond towards the oxygen will take place. This should decrease the ^{13}C-screening only of those carbonyls that are sufficiently near to the cation.

With valinomycin we have the following: there are 4 ^{13}C=O signals (see fig. 1) of which, owing to symmetry of the chemical and spatial structures of the molecule, each should correspond to the carbonyls of 3 identical amino or hydroxy acid residues. Now, in the spectrum of the K^+ complex only one ^{13}C=O signal falls within the C=O region of the non-complexed valinomycin spectrum (table 1). But since,

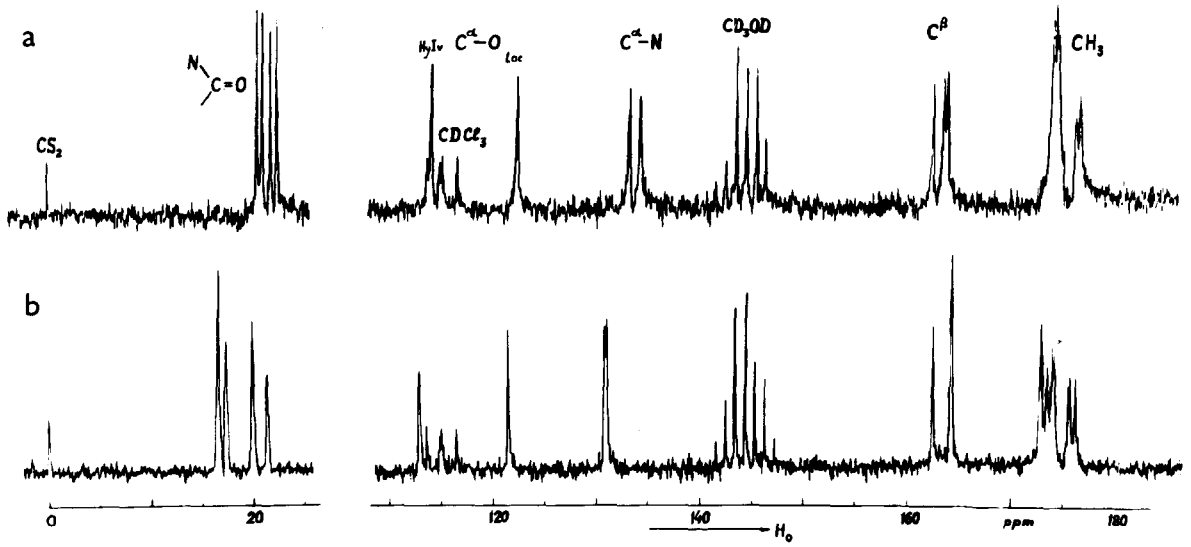

Fig. 2. ^{13}C-NMR spectra of valinomycin (a) and of its K^+ complex (b) in $CDCl_3$–CD_3OD (1:1, v:v) solution. Concentration 300 mg/1.4 ml; 2024 scans; repetition time: 0.8 sec, time of accumulation: 27 min.

from the spatial structures of the complexed and non-complexed molecules [6, 12, 27], it follows that complexing should similarly affect the 2 ester C=O signals and similarly the 2 amide C=O signals, it also follows that all the ^{13}C=O resonances (including the one remaining in the non-complexed valinomycin region) must have undergone a paramagnetic shift as the result of the complexing.

The shifts of the signals belonging to 6 ^{13}C=O groups are quite considerable (3.1–5.5 ppm). These must be assigned to the ester carbonyls which, as had been earlier established [6, 12, 27], are engaged in strong ion–dipole interaction with the K^+ ion located in the molecular cavity. However, the shifts of the other 2 (amide) signals are also quite noticeable (0.5–1.9 ppm downfield). This means that, as well as taking part in hydrogen bonding [6, 12, 27] these carbonyls may also be participating in weak ion–dipole interaction with the K^+ ion (here the O...K^+ distance is around 4.0–4.5 Å).

In conformity with the theory of magnetic screening associated with electrostatic bond polarization [15, 29, 30] it follows that the induced chemical shift should decrease with decrease in the C=O...M^+ angle from 180° to 90°. In enniatin complexes where all 6 carbonyls are symmetrically located around the central cation [1, 9, 28], these angles are much less than 180° (in contrast with valinomycin where these are approx. 180° [6, 12, 27]). Hence, despite the practically identical O...K^+ distance (2.7–2.9 Å) in both types of complexes, the change in ^{13}C=O resonance should be less for the enniatins. In fact, in the K^+ complex of beauvericin the ^{13}C=O signal undergoes less change in position than in the valinomycin complex.

When the K^+ cation is replaced by the smaller Na^+ cation a characteristic change occurs in the enniatin conformation, similar to the closing of a flower [1, 9] This is connected with decrease in the O...M^+ distance and a straightening out of the C=O...M^+ angle causing an increase in the positive charge electrical field component directed along the carbonyl bond. As a result the ^{13}C=O resonance for the Na^+ complex of beauvericin shifts by 1.7 and 2.3 ppm to lower field (table 2).

In the ^{13}C-spectrum of "symmetric" antamanide complex with Na^+ a significant shift to lower field (~ 2.3 ppm) is observed for only one ^{13}C=O line corresponding to 2 symmetrically located identical amino acid residues (table 3). The resonances of the other 4 C=O signals are within the limits of only −1.5 to +0.5 ppm. Hence the carbonyls of only 2 identical amino acid residues (apparently Val^1 and Val^6) of the cyclodepsipeptide residue approach the

Table 2
^{13}C-chemical shifts of beauvericin and its complexes with K^+ and Na^+ ions.

Carbon		Beauvericin	K^+ complex	Na^+ complex
C=O		21.6	20.2	19.9
		22.9	20.9	20.6
$\gtrless$C–(C^β)	MePhe	56.3	56.4	56.5
C– ortho		64.1	63.9	63.9
C– meta		64.1	64.3	64.3
C– para		65.8	65.4	65.5
C^α–O	HyIv	116.8	115.8	*
C^α–N	MePhe	136.2	135.3	135.3
C^β	MePhe	158.2	158.9	159.6
C^β	HyIv	161.3	161.3	161.4
N–CH_3	MePhe	162.8	162.7	163.1
CH_3	HyIv	174.4	175.4	175.4
		176.1	175.5	175.6
Signals from solvants				
$CDCl_3$		115.1	115.0	115.1
CD_3OD		144.3	144.3	144.2

* Hidden by $CDCl_3$ multiplet.

Table 3
^{13}C-chemical shifts of Val^6, Ala^9-antamanide and its Na^+ complex.

Carbon		Val^6, Ala^9-antamanide	Na^+ complex
		19.0	16.7
		20.8	19.3
C=O		21.5	21.0
		21.5	21.7
		21.8	22.1
$\gtrless$C–(C^β)	Phe	54.4	56.2
C–ortho		62.7	63.1
C–meta		64.5	62.3
C–para		66.5	65.0
	Pro	131.9	133.1
	Pro	134.1	133.6
C^α	Val	136.1	136.6
	Ala	136.4	138.4
	Phe	142.5	140.6
C^δ	Pro	145.0	144.6
		146.0	145.4
C^β	Phe	158.5	156.8
	Val	161.2	160.3
		162.7	160.3
		164.6	163.7
C^β, C^γ	Pro	168.2	167.1
		171.0	170.1
	Ala	173.9	173.6
CH_3	Val	175.4	174.2
	Val	175.4	176.0
Signals from solvents			
$CDCl_3$		115.2	114.8
CD_3OD		144.6	144.0

cation located within the internal cavity of the cyclodepsipeptide. This conclusion is in complete agreement with the earlier proposed conformation of the Na^+–antamanide complex [11, 22] of which only the 2 carbonyls of the symmetrically situated Val^1 and Phe^6 residues are in close contact (~ 2.6 Å) with the central ion.

Besides the ion–dipole interaction induced shifts of the ^{13}C=O signal discussed above, marked changes are observed also in the position of a number of other signals. For instance, both signals from the C^α–N atom of valinomycin are shifted by 2.1–3.4 ppm to low field on complex formation, whereas the C^α–O signal is shifted by only –1.2 and –0.8 ppm (table 1). Obviously such selective shifts are due to conformational rearrangement of the molecule on complex formation.

^{13}C-NMR-spectroscopy presents new wide possibilities for studying both the secondary and the tertiary structures of peptide and depsipeptide systems. Particular stress is to be placed on the considerably higher "resolving power" of ^{13}C-magnetic resonance than of proton resonance. For instance, in the ^{13}C-spectra of "symmetric" antamanide and its Na^+ complex individual signals are clearly visible from all of the 25 structurally non-equivalent carbon atoms. Evidently the main difficulty that can be seen so far is assignment of the ^{13}C-signals, in particular those of the carbonyl

groups. This can, of course, be overcome by synthesis of compounds selectively labelled with ^{13}C.

References

[1] M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov, V.K. Antonov, E.I. Vinogradova, A.M. Shkrob, C.C. Malenkov, A.V. Evstratov, I.A. Laine, E.I. Malik and I.D. Ryabova, J. Membrane Biol. 1 (1969) 402.

[2] Th. Wieland, G. Lüben, H. Ottenheym, J. Faesel, J.X. de Vries, W. Konz, A. Prox and J. Schmidt, Angew. Chem. 80 (1968) 209.

[3] Yu.A. Ovchinnikov, V.T. Ivanov, A.I. Miroshnikov, K.Kh. Khalilulina and N.N. Uvarova, Khim. Prirodn. Soedin. (1971) 469.

[4] I.M. Andreev, G.G. Malenkov, A.M. Shkrob and M.M. Shemyakin, Molekul. Biol. 5 (1971) 614.

[5] Th. Wieland, H.Faulstich, W. Burgermeister, W. Otting, W. Möhle, M.M. Shemyakin, Yu.A. Ovchinnikov, V.T. Ivanov and G.G. Malenkov, FEBS Letters 9 (1970) 89.

[6] V.T. Ivanov, I.A. Laine, N.D. Abdullaev, L.B. Senyavina, E.M. Popov, Yu.A. Ovchinnikov and M.M. Shemyakin, Biochem. Biophys. Res. Commun. 34 (1969) 803

[7] D.H. Haynes, A. Kowalsky and B.C. Pressman, J. Biol. Chem. 244 (1969) 502.

[8] M. Ohnishi and D.W. Urry, Biochem. Biophys. Res. Commun. 36 (1969) 4264.

[9] Yu.A. Ovchinnikov, V.T. Ivanov, A.V. Evstratov, V.F. Bystrov, N.D. Abdullaev, E.M. Popov, G.M. Lipkind, S.F. Arkhipova, E.S. Efremov and M.M. Shemyakin, Biochem. Biophys. Res. Commun. 37 (1969) 665.

[10] M. Ohnishi and D.W. Urry, Science 181 (1970) 1091.

[11] V.T. Ivanov, A.I. Miroshnikov, N.D. Abdullaev, L.B. Senyavina, S.F. Arkhipova, N.N. Uvarova, K.Kh. Khalilulina, V.F. Bystrov and Yu.A. Ovchinnikov, Biochem. Biophys. Res. Commun. 42 (1971) 654.

[12] V.T. Ivanov, I.A. Laine, M.D. Abdullaev, V.E. Pletnev, G.M. Lipkind, S.F. Arkhipova, L.B. Senyavina, E.N. Meshcheryakova, E.M. Popov, V.F. Bystrov and Yu.A. Ovchinnikov, Khim. Prirodn. Soedin. (1971) 221.

[13] W.J. Horsley and H.Sternlicht, J. Amer. Chem. Soc. 90 (1968) 3738.

[14] W.J. Horsley, H. Sternlicht and J.S. Cohen, Biochem. Biophys. Res. Commun. 37 (1969) 47.

[15] W.J. Horsley, H. Sternlicht and J. Cohen, J. Amer. Chem. Soc. 92 (1970) 680.

[16] W.A. Gibbons, J.A. Sogn, A. Stern, L.C. Craig and L.F. Johnson, Nature 227 (1970) 640.

[17] A. Allerhand, D.W. Cochran and D. Doddrell, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1093.

[18] M.H. Freedman, J.S. Cohen and I.M. Chaikon, Biochem. Biophys. Res. Commun. 42 (1971) 1148.

[19] H. Sternlicht, C.L. Konyon, S.L. Parker and J. Sinclair, J. Amer. Chem. Soc. 93 (1971) 199.

[20] R.L. Hamill, C.E. Higgins, N.E. Boaz and M. Gorman, Tetrahedron Letters (1969) 4255.

[21] Yu.A. Ovchinnikov, V.T. Ivanov and I.I. Mikhaleva, Tetrahedron Letters (1971) 159.

[22] Yu.A. Ovchinnikov, V.T. Ivanov, V.F. Bystrov, N.D. Abdullaev and A.I. Miroshnikov, Proc. XIth European Peptide Symposium Vienna, 1971, in press.

[23] Yu.A. Ovchinnikov, V.T. Ivanov and A.M. Shkrob, in: Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes (Proc. International Symposium) eds. E. Muñoz, F. Fernandez and D. Vazquez (Springer Verlag, Heidelberg, 1971).

[24] A.I. Miroshnikov, K.Kh. Khalilulina, N.N. Uvarova, V.T. Ivanov and Yu.A. Ovchinnikov, Khim. Prirodn. Soedin., in press.

[25] V.T. Ivanov, S.A. Koz'min, L.B. Senyavina, A.I. Miroshnikov, K.Kh. Khalilulina, N.N. Uvarova, V.F. Bystrov and Yu.A. Ovchinnikov, Khim. Prirodn. Soedin., in press.

[26] G.M. Smirnova, I.M. Blinova, T.A. Kolodnitzkaya and A.S. Khokhlov, Antibiotiki 5 (1971) 387.

[27] M. Pinkerton, L.L. Steinrauf and P.S. Dawkins, Biochem. Biophys. Res. Commun. 35 (1969) 512.

[28] M. Dobler, J.D. Dunitz and F. Krajewski, J. Mol. Biol. 42 (1969) 603.

[29] A.D. Buckingham, Can. J. Chem. 38 (1960) 300.

[30] J.W. Essley, J. Feeney and L.H. Sutcliffe, High Resolution NMR Spectroscopy, Vol. 1 (Pergamon Press, Oxford, 1965) chs. 3.9, 3.10 and 4.4.3.