Volume 9, number 2 FEBS LETTERS July 1970

AFFINITY OF ANTAMANIDE FOR SODIUM IONS

Th.WIELAND, H.FAULSTICH, W.BURGERMEISTER, W.OTTING and W.MÖHLE
Max-Planck-Institute for Medical Research,
Department of Chemistry, Heidelberg, Germany

and

M.M.SHEMYAKIN, Yu.A.OVCHINNIKOV, V.T.IVANOV and G.G.MALENKOV
Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow, USSR

Received 27 May 1970

Antamanide (I) (AA) is a cyclodecapeptide isolated from the green mushroom *Amanita phalloides* [1] which counteracts the toxic effects of the liver poison phalloidine [2]. To further the understanding of its mechanism of action observations have been made in both the laboratories given above. The contributions from Heidelberg are distinguished by (Hd), those from Moscow by (Mo).

$$\begin{array}{lcr} \text{Pro–Phe–Phe–Val–Pro} & & \\ \uparrow \qquad\qquad\qquad\qquad \downarrow & & \text{(I)} \\ \text{Pro–Phe–Phe–Ala–Pro} & & \end{array}$$

In a mass-spectroscopic analysis of a sample of AA which had been in contact with Na^+ ions during isolation, A. Prox found a peak of $m/e = 1169$ (= 23 + mass peak 1146 of the cyclopeptide). This peak was more pronounced when a sample of AA, which had been obtained by adding a 5% NaCl solution to an alcoholic solution of the cyclopeptide, was examined. Two additional peaks at 1169 and 1168 ($1169–H^+$) could also be observed (Hd).

An interaction of AA with Na^+ ions could also be seen in the IR spectra. In the absence of Na^+ the spectrum (in KI) shows two bands of equal height (at 1630 and 1660 cm^{-1}) in the carbonyl region, whereas in the Na^+-containing sample the 1630 cm^{-1} band was about 10% more intense (Hd). IR spectra in solutions (fig. 1) are even more informative, revealing more non-hydrogen bonded NH-groups in the Na-complex (NH stretching band at 3412 cm^{-1}) than in the free AA; changes in CO stretching (amide I) bands are also well developed (Mo).

A quantitative confirmation of a specific affinity of AA for Na^+ ions was given by vapour pressure determinations. The vapour pressures of ethanolic 10^{-2} M solutions of AA and of NaBr (or KBr) composed as shown in figs. 2 and 3, were measured osmometrically using either a Mechrolab (Mountain View) or Knauer (Berlin-West) instrument. Without complex formation the straight diagonals of figs. 2 and 3 would have been expected as a result of a linear decrease of vapour pressure with increasing salt concentration. Assuming various values of the formation constant, K, of the complex between AA and Na^+, the theoretical curves of fig. 2 were constructed. It appears that the experimental data best satisfy a value of $K = 1000–2000$ for Na^+ whereas with K^+ ions (fig. 3) K has a value near 50 (Hd).

The most reliable values of K were obtained by the three following methods.

1. *Potential measurements with ion-specific glass electrodes (Hd)*

Ion activities were determined with glass electrodes, Ingold 401 p Na and 401 p K respectively, in stirred ethanolic solutions at 25° connected with a pH-meter (Knick). At first the equilibration curves of the electrodes in the range of $10^{-4}–10^{-2}$ M were set up for both Na^+ and K^+; then the electrochemical potentials

North-Holland Publishing Company – Amsterdam

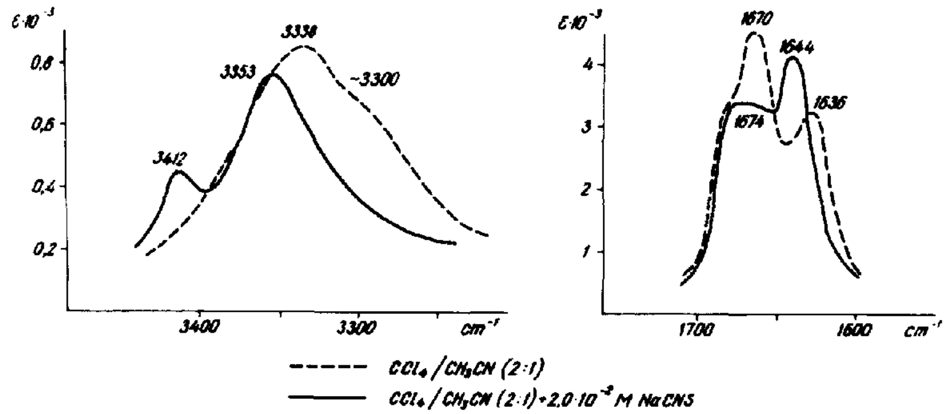

Fig. 1. IR-spectra of antamanide and its Na^+-complex in solution.

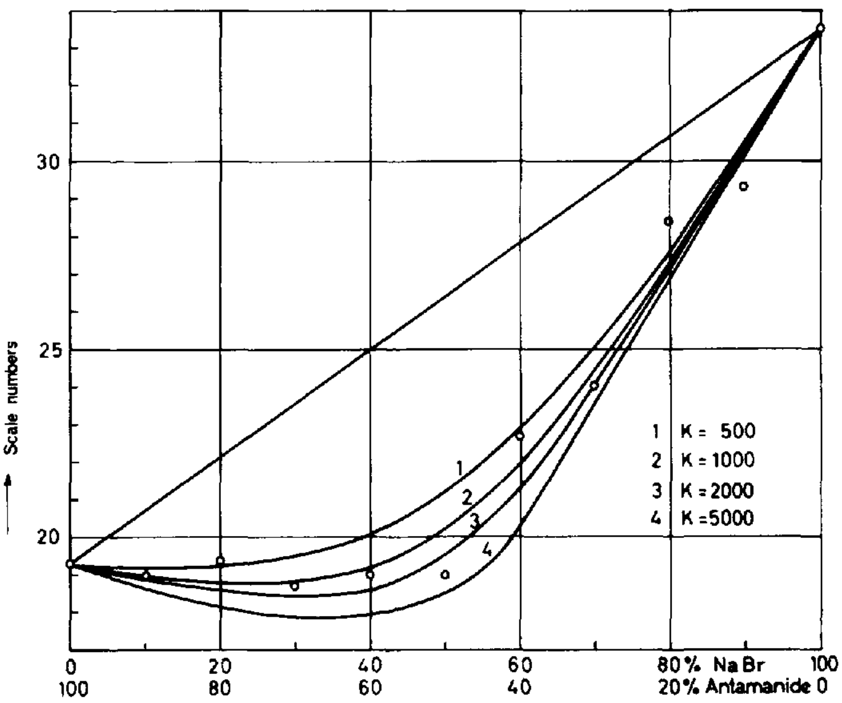

Fig. 2. Vapour pressure values (decreasing with increasing scale numbers on ordinate) of mixtures of ethanolic solutions of 10^{-2} M NaBr with 10^{-2} antamanide in different ratios. Straight line: increasing NaBr concentration without antamanide; curves 1–4 with antamanide, calculated for complex constants K of 500, 1000, 2000 and 5000. Circles are experimental data from vapour pressure osmometry.

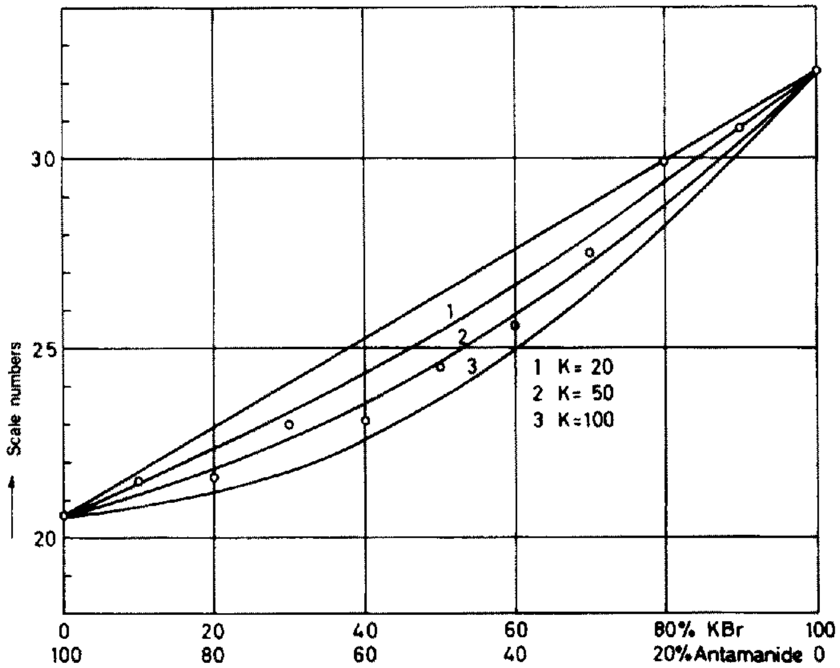

Fig. 3. As fig. 2, but with KBr instead of NaBr.

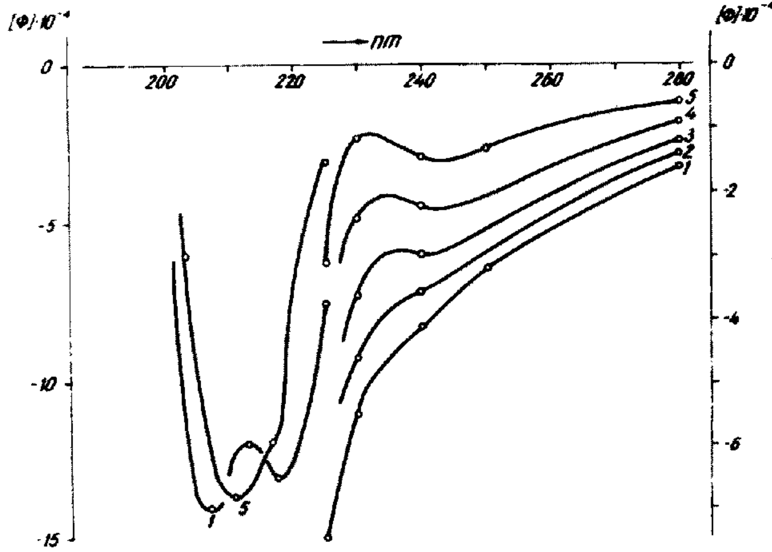

Fig. 4. ORD curves of antamanide and its Na^+-complex in 95% ethanol. $c_{AA} = 0.5 \times 10^{-4}$. 1) $c_{NaCl} = 0$; 2) $c_{NaCl} = 0.2 \times 10^{-3}$ M, degree of complexing (α) = 0.20; 3) $c_{NaCl} = 0.6 \times 10^{-3}$ M, $\alpha = 0.43$; 4) $c_{NaCl} = 2.0 \times 10^{-3}$ M, $\alpha = 0.72$; 5) $c_{NaCl} = 2.0 \times 10^{-2}$, $\alpha = 0.97$.

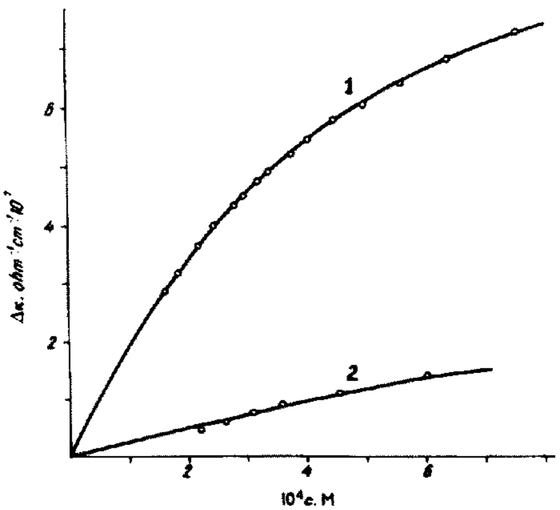

Fig. 5. Decrease of electrical conductivity of NaCl and KCl ethanolic solutions ($c = 1 \times 10^{-4}$ M) in the presence of antamanide. 1) NaCl; 2) KCl. $\Delta\kappa$ = decrease of conductivity, c = concentration of antamanide.

Table 1

Complex	c_{AA}	c_{Alkali}	K	$K_{average}$
AA–Na^+	1×10^{-4}	1.2×10^{-4}	1880	2020
	1×10^{-3}	1.7×10^{-3}	2200	
AA–K^+	3×10^{-4}	1.0×10^{-3}	160	185
	1×10^{-3}	6.6×10^{-4}	210	

of different ratios of AA and alkali-metal ions were measured. Assuming that activities are equal to concentrations (c) in 10^{-4}–10^{-3} M solutions, the complexing constants were calculated from the law of mass action. The values of table 1 were obtained.

2. *Optical rotatory dispersion (ORD) measurements (Mo)*

The ORD curves of AA and its Na^+-complex in 95% ethanol differ essentially (fig. 4), from which a value of K = 1300 can be calculated (for details of the method see [3]). Li^+, 5.6×10^{-2} M, does not cause any changes in the ORD curve of AA and the effects of K^+, Rb^+ and Cs^+ are too small to permit quantitative studies.

3. *Conductometric measurements (Mo)*

Electrical conductivity of NaCl and KCl solutions in absolute ethanol decreases after addition of AA, for the mobility of the complexed cations is less than that of free Na^+ and K^+ (fig. 5). The dependence of conductivity of given salt solution on the AA concentration permits the calculation of the stability constant of the complex (for the details see [3]). K for Na^+ and K^+ was found to be 2800 and 270 respectively. The conductivity method can be used for the estimation of the effective Stokes' radii of the complex cations. They are 5.95 Å for Na-AA^+ and 5.5 Å for K-AA^+.

It is necessary to remember that the ORD measurements were made in aqueous ethanol solutions, while the conductometric measurements were made in absolute ethanol. The presence of water usually decreases the stability constants of macrocyclic complex-forming compounds [3]. Comparison of the K values of Na-AA^+ complex obtained by different methods shows that this substance also follows this rule.

From all these experiments it follows that a distinct complex forms between AA and Na^+ ions which is more stable than the complex with K^+ ions.

References

[1] Th.Wieland, G.Lüben, H.Ottenheyn, J.Faesel, J.X.de Vries, W.Konz, A.Prox and J.Schmid, Angew. Chem. 80 (1968) 209; Angew. Chem. Internat. Edit. 7 (1968) 204.

[2] Th.Wieland, Fortschr. Chem. Org. Naturstoffe 25 (1967) 216; Science 159 (1968) 946.

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