ANTAMANIDE. EVIDENCE FOR A CONFORMATIONAL CHANGE AND NON PLANAR AMIDE GROUPS *).

H. Faulstich, W. Burgermeister and Th. Wieland

Max-Planck-Institut für medizinische Forschung, Abteilung Chemie, Heidelberg.

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Summary: Detailed CD- and UV-studies indicate a conformational change in antamanide by interaction with cations or polar solvents. The conformation formed on addition of water is similar to that of the complex. A model for antamanide already suggested by Ovchinnikov's (1) group is confirmed by CD-data and by substitutions in position 1 and 4 (2). The unusually high negative dichroism at about 230 nm is ascribed to $n \rightarrow \pi^*$ transitions of transoid tertiary amide groups distorted out of plane. Non planar amides probably also determine the dichroic properties of enniatin B (3) and of some acetylproline derivatives (4).

Antamanide (AA), a cyclic decapeptide was isolated from extracts of the poisonous mushroom Amanita phalloides by Wieland et al. (5), and found to counteract the action of phalloidin. Further investigations by Wieland et al. and Shemyakin et al. (6) revealed that AA formed alkali metal complexes with a high selectivity for sodium over potassium. In a following study the conformation of the sodium complex was described by the Russian group (1) by joint use of ORD- NMR- and IR-data. Recently, minimum energy calculations based on NMR- and CD-data by Tonelli et al. (7) led to a proposal for the conformation of uncomplexed AA in nonpolar solvents.

In early ORD-studies, and recently more detailed CD-studies of AA, intense variations in the $n \rightarrow \pi^*$ region were detected when sodium ions were added e.g. in dioxan.

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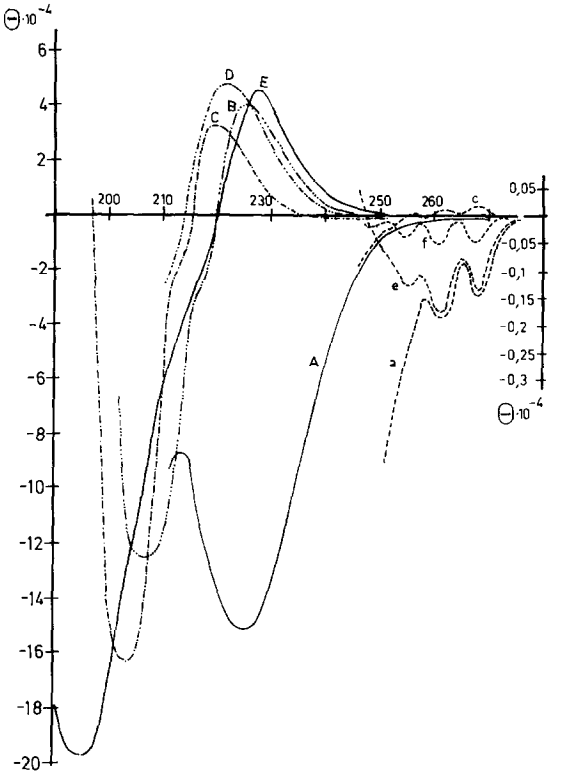

Fig. 1. CD spectra of AA in dioxan (a, A) and methanol-water (1:1) (e, E) and of AA-sodium (1:15) in dioxan (B), in methanol (c, C) and acetonitril (D). AA-sodium (1:2) in acetonitril (f).

By complexation a highly negative molar ellipticity at 224 nm changes to positive value. The CD-spectrum of the complex is almost independent of solvent, as already observed by Ivanov et al. (1).

When the CD of AA was studied in solvents of different polarity (7), changes in both the $\pi \rightarrow \pi^*$ and the $n \rightarrow \pi^*$ region were observed. A more detailed study of the latter revealed that CD curves of the AA sodium complex and of AA in polar solutions, especially those containing water, were similar in shape. For example, the positive dichroic absorption band at about 230 nm, a characteristic of the complex, could be produced from any non polar solution of AA by stepwise addition of water.

in the 250 - 280 nm region the negative dichroic absorption bands of the phenyl residues diminished on complexation, or even grew positive as in

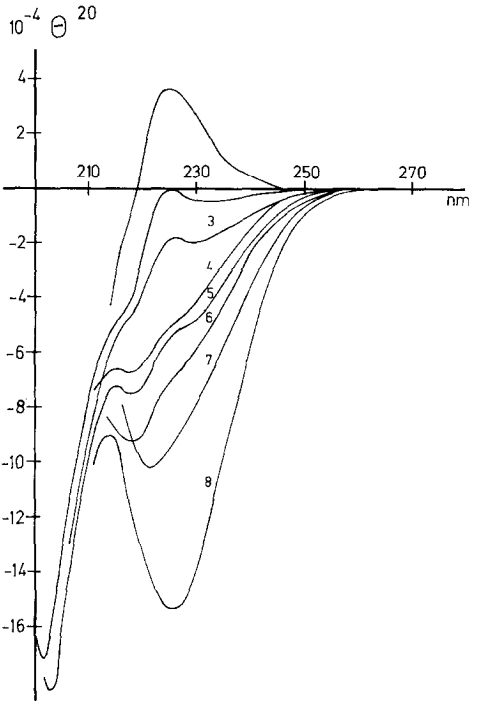

Fig. 2. CD spectra of AA in different solvents: (1) methanol-water (50:50), (2) tetrahydrofurane-water (97:3), (3) methanol, (4) ethanol, (5) tetrahydrofurane (99:1), (6) n-propanol, (7) methylenchlorid, (8) 1,4-dioxane.

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methanol (fig. 1a, 1f, 1c); addition of water on the other hand did not cause any changes (fig. 1e). Therefore it seems reasonable to attribute the positive dichroic absorption bands of these $\pi \rightarrow \pi^*$ transitions to the cationic environment of the phenyl residues in the complex. This corresponds to some extent to observations of Brady et al.(8), who studied the dichroism of L-phenylalanine derivatives. These authors found negative bands, similar to fig. 1a for acylated L-phenylalanines; positive bands as in fig.1c appeared when the amino group was free. For phenylalanine in neutral or acidic medium the presence of the ammonium group could well explain the positive dichroic bands. This explanation, however, would not be valid at pH 13, where the ammonium group is deprotonated.

Parallel to these changes in the CD the complexation reaction or the addition of water could be followed by the UV spectrum. Here again sodium and water caused similar spectral changes, which were useful for calculation of complex constants (6).

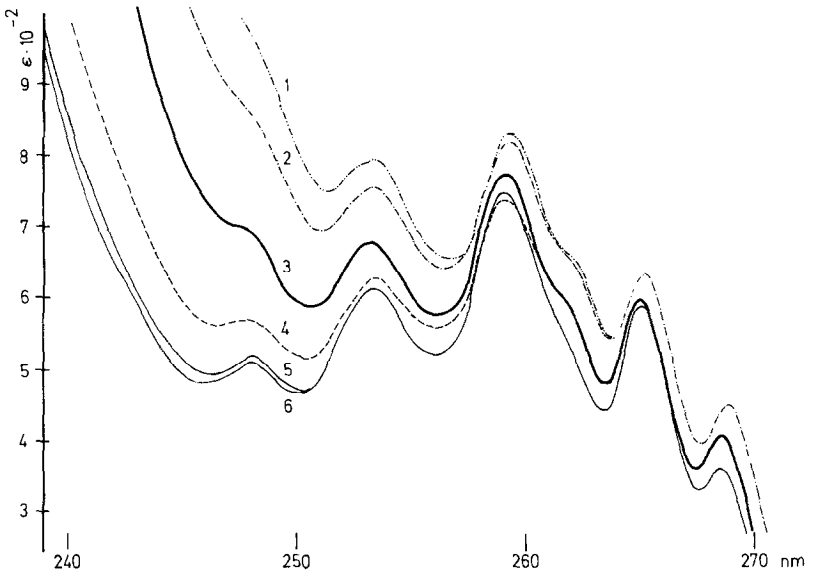

Fig. 3. UV spectra of AA [1x10^{-3}M] in 1,4-dioxan (1), methylenchlorid (2) ethanol (3) and 1,4-dioxan-water (3:1) (4).
AA in ethanol on addition of sodiumperchlorate [1x10^{-2}M] (5) and [1x10^{-1}M] (6)

The reason for all these spectral changes seen in AA solution on the addition of sodium ions or of water is a conformational transition rather than solvent effects as suggested by Tonelli et al.(7). Perhydro-AA which had been prepared simultaneously in our and Ovchinnikov's laboratories, and which will be described elsewhere, displayed a similar dichroism with respect to polarity of solvent or on addition of sodium ions as AA itself (fig. 4). Consequently dichroism of AA down to 210 nm does not depend on the participation of $\pi \rightarrow \pi^*$ transitions of the aromatic side chains. In the UV spectrum of perhydro-AA the molar extinction decreased in the 230 nm region, when the solvent was changed from 1,4-dioxane to 1,4-dioxane-water (fig. 5). This is in agreement with the CD spectrum, but may also be caused by solvent effects.

A similar dichroic absorption band for $n \rightarrow \pi^*$ transitions shifted towards the red was observed by Madison and Schellman in their extensive study of proline derivatives (4). They found a deep trough at 230 nm for acetyl--L-prolineamide and -methylamide, but not for the dimethylamide and the ester, all dissolved in a hydrocarbon solution. The effect was explained by intramolecular hydrogen bonding. Likewise, from the inflection point in the

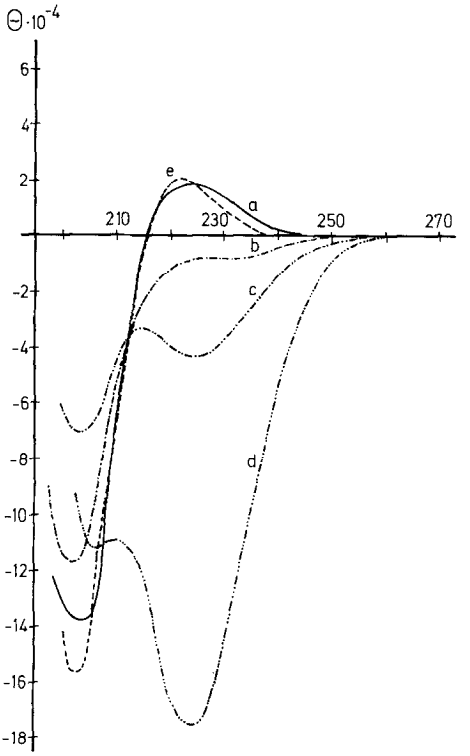

Fig. 4. CD spectra of perhydro-AA in dioxan (d), methanol (c), methanol-water (10:1) (b) and acetonitril-water (96:4) (a). CD spectrum of perhydro-AA-sodium (1:2,in methanol)(e).

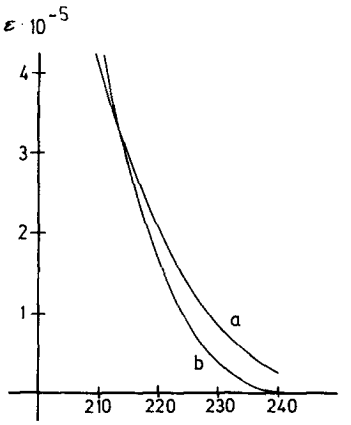

Fig. 5. UV spectra of perhydro-AA [4×10^{-4} M] in 1,4 dioxane-water (1:1) (a) and in 1,4 dioxane (l = 0.1 cm).

ORD spectrum of enniatin B in heptane at 230 nm as published by Shemyakin et al., (3) it can be seen that also for this depsipeptide in non polar solvent a negative Cotton effect is located at about 230 nm. In the latter molecule no intramolecular hydrogen bonds can be involved in the conformational state, so a more comprehensive explanation is necessary for the 230 nm trough.

By studies with space filling models we found that non planar amide groups could provide a more general explanation for this dichroic band. Recently Winkler and Dunitz (10) described distorted secondary amides caused merely by steric hindrance in medium sized rings (C_8 - C_{10}) and suggested that out-of-plane deformations in an actual molecule could be made at a very modest energy cost. If this is true, non-planar amide groups should be more wide-spread than is commonly assumed.

Indeed, in the model of the P_2-conformation of enniatin B (3), slightly distorted tertiary amides are rigidly fixed in an extremely compact molecule. From the models of acetylproline-amide or -methylamide it can be deduced that the intramolecular hydrogen bridge is built only if one of the amides is out of plane. Here the deformation energy may be compensated for by the energy of the hydrogen bond in a completely non polar medium. Madison and Schellman themselves suggested a non-planar amide group for acetylproline-diisopropylamide, but could not explain the fact that the spectrum of the latter resembled the spectra of the hydrogen bridged analoges much more closely. This may be explained by the fact that the amide, methylamide, and diisopropylamide are distorted equally, but that the steric hindrance causes a smaller distortion than does the hydrogen bridge. The shallower trough in the diisopropylamide spectrum, and its location at only 220 nm would be in good agreement with the fact that the more the amide group is distorted out of plane, the less is the energy required for $n \rightarrow \pi^*$ transitions, corresponding to higher wavelengths of the trough.

The striking similarities in the optical properties of AA and enniatin B are not restricted to the 230 nm band in non-polar solvents. From ORD studies we know (1, 3) that, e.g. the ORD curves of AA and of enniatin B in polar solvents such as CF_3CH_2OH-H_2O and CF_3CH_2OH, respectively, resemble each other very much in their trough at about 215 nm. It is in good

agreement with this that the mechanism of conformational change in AA resembles that of enniatin B fairly closely. It may be true that in the case of AA, formation of hydrogen bridges is involved; but in both molecules the planes of amide groups turn about 180°, being distorted out of plane after that process. In AA, the amide bonds in question are between pro^3-ala^4 and pro^8-phe^9. Two intramolecular hydrogen bridges are formed between NH of ala^4 and the CO of val^1, and between NH of phe^9 and CO of phe^6, at the cost of out-of-plane-deformations of 20-30° for the two carbonyls concerned. Again, the space filling model for that conformation, which had essentially already been suggested by Ivanov et al. (1) on the base of IR data, revealed the two distorted tertiary amide bonds rigidly fixed in a completely inflexible molecule. A Dreiding model showed that at a distance of 8 Å between ala^4 (C_β) and phe^9 (C_β) the resulting dihedral angles listed below fitted in well with the values of Tonelli et al. (7) (in parentheses). The latter were derived from NMR data by the Karplus equation: Val^1: 30° (22°); phe^6: 30° (18°); ala^4: 160° (157°); phe^9: 130° (138°); phe^5: 10° (0°); phe^{10}: 10° (0°). The model showed further that 4 lipophilic side chains (val^1, ala^4, phe^6, phe^9) are localized quite near to each other and form a hydrophobic region. This is in good agreement with the observations on analogs of AA synthesized in our laboratories (11, 12). Here, complexation and corresponding biological activity decreased to 10% of the AA values when val^1 was substituted by less lipophilic aminoacids, especially those without a γ-carbon atom in the side chain. To a less extent the ß-methylgroup in the val^1 or ile^1-side chains and the methyl group of ala^4 play their part in complexation and biological behaviour. It should be mentioned here that non planar amides are only in conformity with a rigid, probably fully hydrogenbonded molecule, and therefore render the flexible, non hydrogenbonded conformation of AA suggested by Tonelli et al. (7) unlikely.

In the NMR spectra, the conformational transition caused changes too (1, 7), but no double signals for single protons were found. This does not contradict a conformational change but indicates a low energy barrier for this transition. Also, exchange studies with AA and CD_3OD in chloroform solutions indicated a rather fast exchange, thus suggesting that no intramolecular hydrogen bridges were present (7). These two facts are in agreement with the kinetic data, which suggest that the conformational transition should

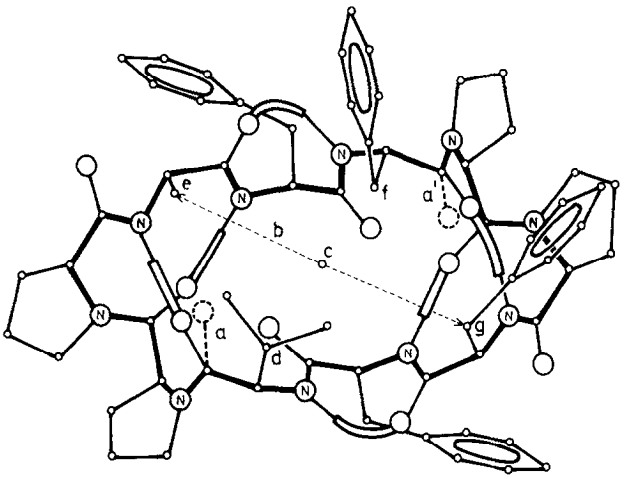

Fig. 6. Model of the AA conformation in non polar solvents. Insight almost parallel to the pseudo-C_2-axis (c), with a distance of 8 Å (b), the distorted amides (a, a') and the four lipophilic side chains (d, e, f, g).

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be a very fast process. By ultrasonic absorption of AA in an organic solvent in the absence of sodium ions, the equilibrium reaction proved to be in the range of microseconds while the complexation reaction, as determined by temperature jump, was in the range of milliseconds. Together with the fact that only small amounts of water or methanol cause a shift in equilibrium (12), the quick to-and-fro-motion of the molecule can explain the high exchange rate of some protons and the sharp average signals actually found in the NMR spectrum (7). The kinetic data will be presented in a forthcoming paper in cooperation with Prof. Eigen's laboratories in Göttingen.

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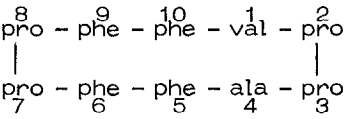

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