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2D-NMR OF NATURAL PRODUCTS PART III[1]
HOMO- AND HETERONUCLEAR NMR-SPECTROSCOPY OF A CYCLIC TETRAPEPTIDE RELATED TO CHLAMYDOCIN

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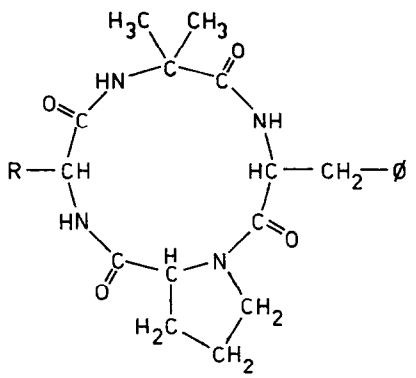
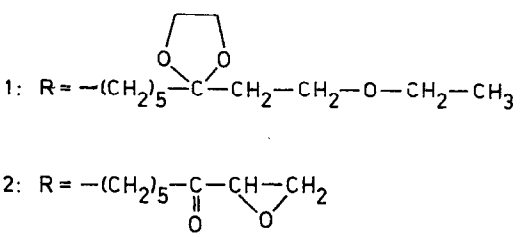
Summary: 2D-^{1}H-^{13}C-shift correlation enables full assignment of the ^{13}C- and proton-spectrum of the Chlamydocin derivate 1. The conformation of 1 in $CDCl_3$ is discussed.

Chlamydocin (cyclo[A^{i}bu-L-Phe-D-Pro-L-(2-amino-8-oxo-9.10-epoxydecanoic acid)], 2), a cyclic tetrapeptide first isolated by Closse and Huguenin[2] from culture filtrates of Diheterospora chlamydosporia, has high cytostatic activity. Studies of the twelve-membered ring conformation of cyclic tetrapeptides have provided interesting insight in unusual peptide bond geometries[3-5]. Conformations with alternating cis-trans planar amide bonds and all trans amide conformations have been discussed[5]. Variation of the solvent often causes conformational interconversions; this has been observed by Rich et al[6] on cyclo(Gly-L-Phe-D-Pro-L-Ala).

We report here the NMR study of 1 which is closely related to Chlamydocin (2).





The ^1H NMR spectrum was analysed by homonuclear double resonance experiments. Partially relaxed ^1H-spectra have been used to unreveal overlapping lines with different T_1-values. An unambiguos assignment of the ^{13}C-resonances has been obtained using J-modulated ^{13}C-spectra and 2D-^1H-^{13}C-shift correlation[10](Fig. 1) which in turn gave additional information for the proton assignment (Tab. 1).

Chemical shifts and coupling constants are invariant with temperature, thus indicating conformational homogeneity of 1. This is supported by rather big chemical shift differences of diastereotopic protons[7].

		δ(^{13}C)	δ(^1H)
A^{i}bu	2	58.94	--
	3$^+$	23.54	1.75
	4$^+$	26.63	1.32
Phe	2	53.61	5.14
	3	35.98	2.92/3.24
	4	137.31	--
	5,9$^{\&}$	129.08	
	6,8$^{\&}$	128.55	7.13-7.30
	7	126.67	
Pro	2	57.92	4.63
	3	24.73	1.75/2.29
	4	25.06	1.75/2.15
	5	47.02	3.21/3.83

		δ(^{13}C)	δ(^1H)
Ada	2	54.57	4.15
	3	29.04	1.56/1.75
	4§	23.50	1.28
	5	29.53	1.28
	6§	25.54	1.28
	7	37.65	1.75
	8	110.76	--
	9	37.20	1.90
	10	66.38	3.47
	11	66.10	3.44
	12	15.13	1.17
	13$^{\%}$	64.84	3.90

Amide protons:

	A^{i}bu	Phe	Ada
δ	5.84	7.50	7.04
^3J(HNCαH)	--	10.3	10.5

Tab. 1: Chemical shifts [ppm] and HNCαH coupling constants [Hz] of 1 in $CDCl_3$
Ada = ethylene ketal of 2-amino-10-ethoxy-8-oxo-decanoic acid (see formula).
+,&,§: chemical shifts may be interchanged.
%: signals of the dioxolane ring.
Carbonyl-carbons have not been assigned.

The chemical shift of Pro-γ-C indicates trans-configuration of the Phe-Pro-peptide bond. Deuterium exchange experiments show that the Phe - amide proton is shielded from the solvent. This information, supported by the torsional angles derived from HNCαH coupling constants[8], indicates a γ-turn hydrogen bond from the amide proton of Phe to the carbonyl oxygen of Ada. The same conclusion can be drawn from the unusual high field shift of Pro-β-C due to the eclipsed Pro - carbonyl oxygen[3]. Based on these evidences molecular model considerations suggest a second γ-turn between the amide proton of Ada and the carbonyl oxygen of Phe, which has been also proposed for cyclo[Gly-L-Phe-D-Pro-

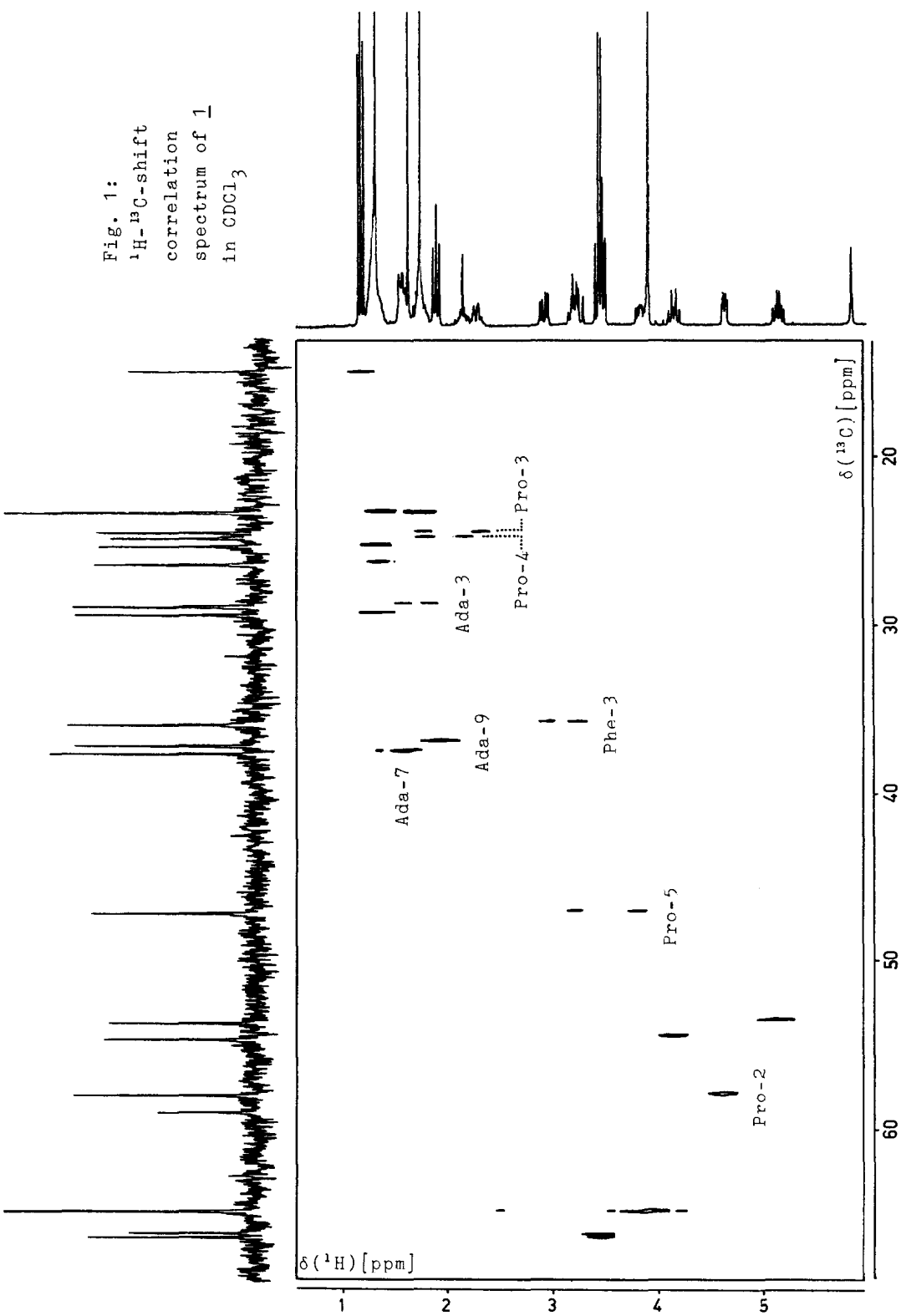


Fig. 1:
^{1}H-^{13}C-shift correlation spectrum of 1 in $CDCl_3$

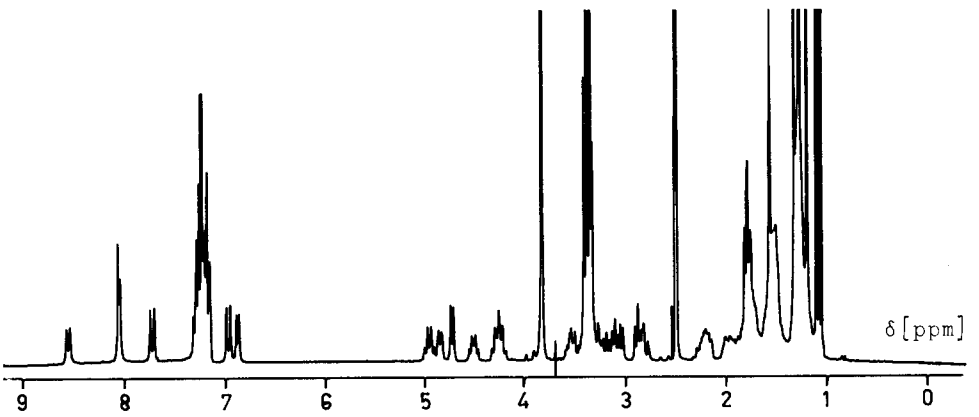


Fig 2: 250 MHz ^{1}H NMR spectrum of 1 in DMSO-d_6

-L-Ala]. Changing the solvent from $CDCl_3$ to DMSO-d_6 results in an extreme downfield shift (~2.2ppm) of the resonance of the amide proton of A^ibu, the only one which is not involved in hydrogen bonding.

In DMSO-d_6 1 exists in two conformations (Fig. 2) which are slowly exchanging on the NMR time scale. Using the Hoffmann-Forsén-method[9] one can get information about the rate of this exchange process. At 24°C the mean lifetime of the two molecular species is in the order of seconds (<5s). Further experiments to gain more information about the nature of these species and about the dynamic properties of this exchange are in progress.

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