Stereochemical studies on ascaulitoxin: a *J*-based NMR configurational analysis of a nitrogen substituted system

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Abstract—The *J*-based NMR configurational analysis was applied to the determination of relative stereochemistry of the ascaulitoxin molecule (**1**), a phytotoxic metabolite with herbicidal activity against *Chenopodium album*. This method is particularly suitable for acyclic structures containing hydroxy(alkoxy) groups, because in this kind of system, besides $^3J_{H-H}$ and $^3J_{C-H}$, $^2J_{C-H}$ values can also be used to extract additional angular information. Ascaulitoxin (**1**), with its bis-amino acid side chain containing four stereocenters, two of which with nitrogen substituents, required a more careful analysis of heteronuclear *J*-couplings, also in comparison with theoretical values obtained by ab initio methods. © 2001 Elsevier Science Ltd. All rights reserved.
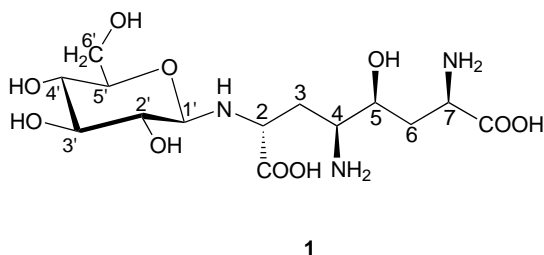
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In the last decades many efforts were made to develop methods for the biological control of the infestant plants by using their natural antagonists. Among these, fungi appear to be the most appropriate agents as mycoherbicides and some of them have also been commercialised.[1] The phytotoxins produced by pathogenic weed fungi can also be used as pure compounds to develop new natural and safe herbicides.[2] These include the phytotoxic metabolites produced in vitro by *Ascochyta caulina* (P. Karst) v.d. Aa and v. Kest, which has been proposed as a mycoherbicide against *Chenopodium album*, a common world-wide weed of many arable crops such as sugar beet and maize.[3] From the in vitro fungal culture filtrates, three new phytotoxins were recently isolated and characterised as non-proteinogenic amino acids.[4–6] The main toxin was

named ascaulitoxin (**1**) and characterised as the β-D-glucopyranoside of the unusual bis-amino acid 2,4,7-triamino-5-hydroxyoctandioic acid. As the configuration of the naturally occurring toxin was still undetermined, the relative configurational analysis of its four stereocenters (C-2, C-4, C-5 and C-7) appears to be of interest and a necessary step for establishing its absolute configuration and to realise its stereoselective synthesis.

Herein, we describe a stereochemical and conformational study on ascaulitoxin (**1**) based on extensive use of heteronuclear $^{2,3}J_{CH}$ values (in combination with ROESY responses), a methodology recently described as *J*-based NMR approach.[7–11] The method has proved particularly useful for the configurational analysis of polyoxygenated/polymethylated frameworks, typically found in polyketides of natural origin. Conversely, the method (which relies also on $^2J_{CH}$ couplings) has not been extended so far to the stereochemical analysis of nitrogen-substituted chains, such as the ascaulitoxin molecule (**1**). However, we envisaged that the *J*-based approach was likely to display more breadth of application, since the need of oxygen (hydroxy, methoxy, etc.) substituents stemmed just on the need of suitable correlations between the values $^2J_{CH}$ couplings and the dihedral angles between the proton and the heteroatom attached to the carbon coupled to the proton. Indeed, in oxygenated (and also dioxygenated) C_2-fragments small $^2J_{CH}$ couplings (in absolute value, e.g. $+2 \rightarrow -1$ Hz) are commonly observed for *anti*-like arrangements

between the proton and the oxygen, whereas large (in absolute value, e.g. $-4 \rightarrow -6$ Hz) are found for *gauche*-like arrangements. For the case of molecular fragments containing nitrogen substituents, although there is still need of experimental measurements of $^2J_{CH}$ couplings in systems with known or predictable structural features, reliable curves describing their angular dependence have been obtained by ab initio methods. In fact, a recently published review covering the angular dependence of a variety of heteronuclear couplings[12] has drawn our attention on the comparative influence of oxygen and nitrogen substituents on $^2J_{CH}$ couplings and suggested that an extension of the *J*-based approach to nitrogen-containing molecules was indeed possible. In fact, our experimentally derived data on the bis-amino acid ascaulitoxin (**1**) would indicate a good correlation between calculated and measured couplings of the latter

type (see Table 1). In this respect, $^2J_{CH}$ couplings at nitrogen-bearing carbons display a pattern of values qualitatively similar (although within a somewhat tighter range) to that of oxygenated fragments, ranging between ca. -1 Hz (*anti*-like arrangements) to ca. -4 Hz (*gauche*-like arrangements).

The dominant rotamers of all C_2-fragments of the ascaulitoxin side chain along with their relative configurations and the NMR data (*J*-couplings and ROEs) used for their assignment are reported in Table 1. NMR data (600 MHz) were measured at 290 and 300 K and in two solvent systems DMSO-d_6 and DMSO-d_6–D_2O (8:2). We also measured all *J*-couplings of the sugar (β-glucopyranoside) portion as an internal control on the accuracy of NMR data (see Table 1). The most intriguing part of the molecule (from a spectro-

Table 1. Dominant rotamers of ascaulitoxin (**1**) side chain[a] C_2-fragments along with their relative configurations. ROESY contacts are classified into strong (s), medium (m) and weak (w) effects

Framment	Segment	$^3J_{H-H}$ (Hz)	$^{2,3}J_{C-H}$ (Hz)	ROE
C2-C3 *anti*		3a 8.2, 8.1[b]	$^2J_{H3a-C2}$ -4.2 (HL)[c]	H2-H3b very s
		3b 9.3, 9.3[b]	$^2J_{H3b-C2}$ -2.7 (HL)	
			$^3J_{H3a-C1} \geq 5$ Hz (HB)[c]	
			$^3J_{H3b-C1}$ 1.9 Hz (HB)	
			$^3J_{H2-C4}$ 0.6 Hz (HL)	
C3-C4 *anti*		3a 7.4, 7.5[b]	$^2J_{H3a-C4}$ -4.8 (HL)	NH4b-H2 s
		3b 7.8, 7.9[b]	$^2J_{H3b-C4}$ -2.0 (HL)	H3b-H5 m
			$^3J_{H4-C2}$ 1.3 Hz (HL)	
			$^3J_{H3a-C5} \geq 5$ Hz (HB)	
			$^3J_{H3b-C5}$ 1.3 Hz (HB)	
C4-C5 *syn*		2.5	$^2J_{H4-C5}$ 1.1 (HL)	
			$^3J_{H4-C6} < 3$ Hz (HB)	
			$^3J_{H5-C3}$ 2.9 Hz (HB)	
			$^2J_{H5-C4}$ -1.9 (HL)	
C5-C6 *anti*		6a 8.0, 9.2[b]	$^2J_{H6a-C5}$ -5.9 (HL)	
		6b 3.0, 3.3[b]	$^2J_{H6b-C5}$ -2.1 (HL)	
			$^3J_{H5-C7}$ 2.1 (HL)	
			$^3J_{H6a-C4}$ 1.8 Hz (HB)	
C6-C7 *anti*		6a 7.2, 8.6[b]	$^2J_{H6a-C7}$ -5.4 (HL), -4.7 (HL)[b]	
		6b 7.6, 5.1[b]	$^2J_{H6b-C7}$ -4.3 (HL), -2.8 (HL)[b]	
			$^3J_{H7-C5}$ 2.4 (HL), 2.7 (HL)[b]	
			$^3J_{H6a-C8}$ 2.8 (HB), 2.2 Hz (HB)[b]	
			$^3J_{H6b-C8} < 3$ Hz (HB), < 3 Hz (HB)[b]	

[a] The coupling constants of the sugar part of the ascaulitoxin (whose relative configuration is, of course, known) have been measured only to judge the accuracy of our experimentally determined heteronuclear *J* values.
C1'-C2': $^3J_{H1'-H2'}$ 9.0 Hz, $^2J_{H2'-C1'}$ -4.6 Hz (HL[c]), $^3J_{H1'-C3'}$ 1.8 Hz (HL), $^2J_{H1'-C2'}$ -2.6 Hz (HL); **C2'-C3'**: $^3J_{H2'-H3'}$ 9.0 Hz, $^2J_{H2'-C3'}$ -4.4 Hz (HL), $^3J_{H2'-C4'}$ 0.4 Hz (HL), $^2J_{H3'-C2'}$ -4.4 Hz (HL), $^3J_{H3'-C1'}$ 1.1 Hz (HL); **C3'-C4'**: $^3J_{H3'-H4'}$ 9.0 Hz, $^2J_{H3'-C4'}$ -3.3 Hz (HL), $^3J_{H3'-C5'}$ 1.2 Hz (HL), $^2J_{H4'-C3'}$ -2.3 Hz (HL), $^3J_{H4'-C2'}$ 0.9 Hz (HL); **C4'-C5'**: $^3J_{H4'-H5'}$ 9.1 Hz, $^2J_{H4'-C5'}$ -4.7 (HL), $^3J_{H4'-C6'}$ 1.7 (HL), $^2J_{H5'-C4'}$ -3.0 (HB), $^3J_{H5'-C3'}$ 2.6 (HL)
[b] spectra recorded at 290 K in DMSO-d_6-D_2O (80:20)
[c] HL=Pulse Field Gradient - Hetero Half-Filtered TOCSY [Total Correlation Spectroscopy] (PFG-HETLOC), HB=Phase-Sensitive - Pulse Field Gradient - Heteronuclear Multiple Bond Correlation (PS-PFG-HMBC)

scopical viewpoint) appears to be the C2–C4 fragment. In fact, in this moiety $^3J_{HH}$ values are all observed in the 7–9 Hz range, leaving open the possibility that such values have to be considered as deriving by weighted averages between fast-interconverting conformers. However, ascaulitoxin (**1**) showed no significant changes of chemical shifts and coupling constants over a wide range of temperatures (260–310 K), a finding in contrast with the conformational averaging hypothesis.[†] In addition, it was not possible to fit the experimental J_{HH} values (at least in the C2–C3 fragment) as weighted averages of the coupling constants calculated (by using a generalised Karplus equation[13]) for differently populated staggered conformations.[‡] Conversely, we found that the same J-values could be explained in terms of a single structural arrangement in which both C2–C3 and C3–C4 fragments exist as staggered conformers, though with a reduced angular separation of 20–30° between substituents (Table 1). Inspection of a Dreiding 3D model of **1** showed that this conformation allowed a hydrogen bond interaction with favorable N–H···N alignment between the nitrogen at C2 (acceptor) and C4-amino group (donor), thus forming a twist-like six-membered ring conformation for this segment. Temperature coefficients measured in DMSO-d_6 for both C4–NH$_2$ exchangeable protons were consistent with this hypothesis, with the two protons displaying very different coefficients ($\Delta\delta/\Delta T = -2.3$ and -6.6 ppb/K). Interestingly, this hypothesis requires a dihedral angle of ca. 100° between H3a and C2-nitrogen, which in turn would give a calculated[12] value around -4 Hz (observed -4.2 Hz, see Table 1) for the corresponding $^2J_{CH}$, while the same coupling for the geminal H3b, with its $-140°$ torsion angle, should be expected around -2.5 Hz (observed -2.7 Hz). Finally, ROESY data also supported this spatial arrangement of the C2–C4 fragment

with the very strong dipolar couplings H2–H3b and H2–NH4b. More straightforward was the analysis of the rest of the molecule, with the only exception of the C6–C7 segment, also characterised by intermediate J_{HH} values (7.2 and 7.6 Hz) at room temperature, this time indeed due to conformational averaging, as indicated by the more definite and interpretable J values obtained at 290 K (8.6 and 5.1 Hz).

Acknowledgements

[†] In principle, a symmetrical double-well potential is also consistent with the observation of little temperature dependence of NMR parameters.

[‡] As a matter of fact, the following system of three equations (and three unknowns) was solved to extract the populations of the three staggered conformers (named, for example, A, B and C) of the C2–C3 fragment:

$$(9.3\pm0.5)\ \text{Hz} = aJ_A + bJ_B + cJ_C$$

$$(8.2\pm0.5)\ \text{Hz} = aJ'_A + bJ'_B + cJ'_C$$

$$1 = a+b+c$$

where J_A, J_B and J_C represent the $^3J_{H2-H3b}$ and J'_A, J'_B and J'_C, the $^3J_{H2-H3a}$ values calculated by the Altona equation for the A, B and C conformers, respectively. The solutions of the above system made no physical sense, as one of them assumed negative values over the whole considered range of experimental J values ($^3J_{H2-H3b} = 9.3\pm0.5$ Hz and $^3J_{H2-H3a} = 8.2\pm0.5$ Hz).

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