XXI Congresso della Società Chimica Italiana

Computational and NMR Analyses for the Elucidation of the Mechanism of Reaction Between Carnosine and 4-Hydroxy-*trans*-2-nonenal

*Giangiacomo Beretta**, Giulio Vistoli, Alessandro Pedretti, Giancarlo Aldini

Istituto Chimico Farmaceutico Tossicologico, Università di Milano,
Viale Abruzzi 42, 20131 Milan, Italy
*jjberetta@ hotmail.com

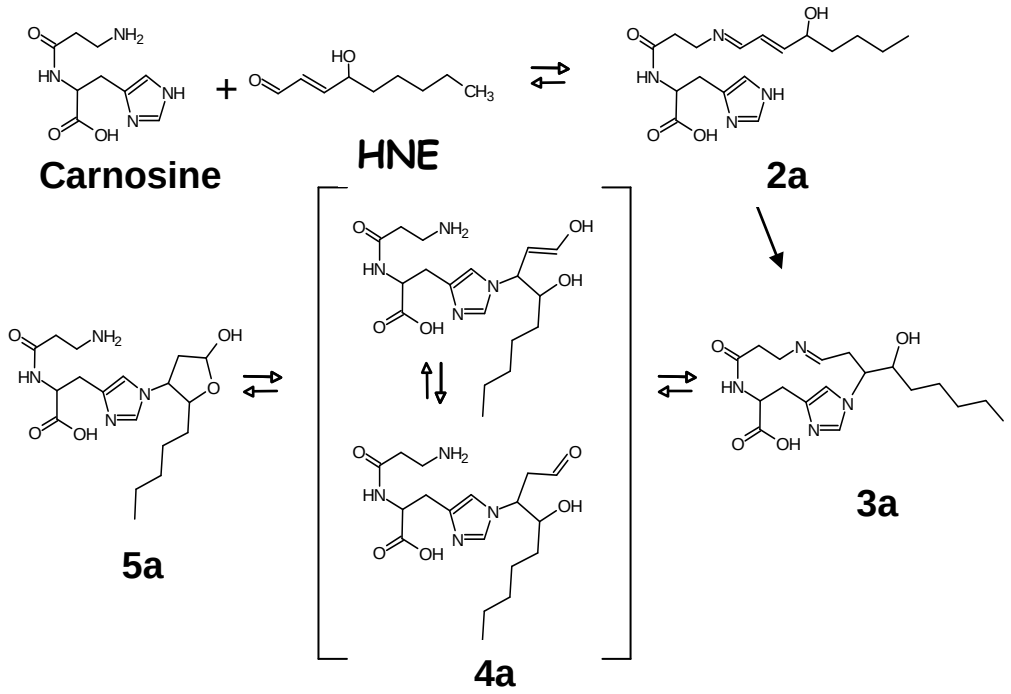
Introduction

♦ Unsaturated aldehydes, generated endogenously during the degradation process of biological molecules, are involved in the onset and progression of many pathologies such as cardiovascular and neurodegenerative diseases [1]. Among these reactive aldehydes, 4-Hydroxy-*trans*-2-nonenal (HNE) is one of the most abundant toxin generated through the β-cleavage of hydroperoxides from ω-6 polyunsaturated fatty acids (arachidonic and linoleic acid) which induces glutathione depletion and protein and DNA modifications [2].

♦ Carnosine (β-alanyl-L-histidine) is a dipeptide found at up to 20 mM in muscle and nerve tissues in humans. Since its discovery at the beginning of the 20th century, carnosine's biological function has not been well clarified. Recently it has been proposed that carnosine could act as a naturally occurring scavenger of harmful reactive aldehydes [3]. In support of this biological role, there is indirect evidence that carnosine suppresses aldehyde-induced cytotoxicity in cultured cells and inhibits aldehyde-induced protein-protein and DNA-protein cross-linking [4].

Background

In previous study [5,6] we have found, using combined analytical techniques (HPLC-UV-DAD, ESI-MS and NMR) the ability of carnosine to quench HNE through the formation of two reaction products which are in a pH-dependent equilibrium:the Michael adduct in hemiacetal form (**5a**) and the 14-membered macrocyclic imine adduct (**3a**). The proposed reaction mechanism is depicted in *scheme 1.*



Scheme 1: **Proposed reaction mechanism of carnosine as HNE quencher**

The reaction starts with the formation of a reversible α,β-unsaturated imine **2a**, followed by the ring closure through an intra-molecular Michael addition to give **3a.** The hydrolysis of the iminic double bond leads to the formation of **4a** that undergoes cyclization to the stable hemiacetalic derivative **5a**

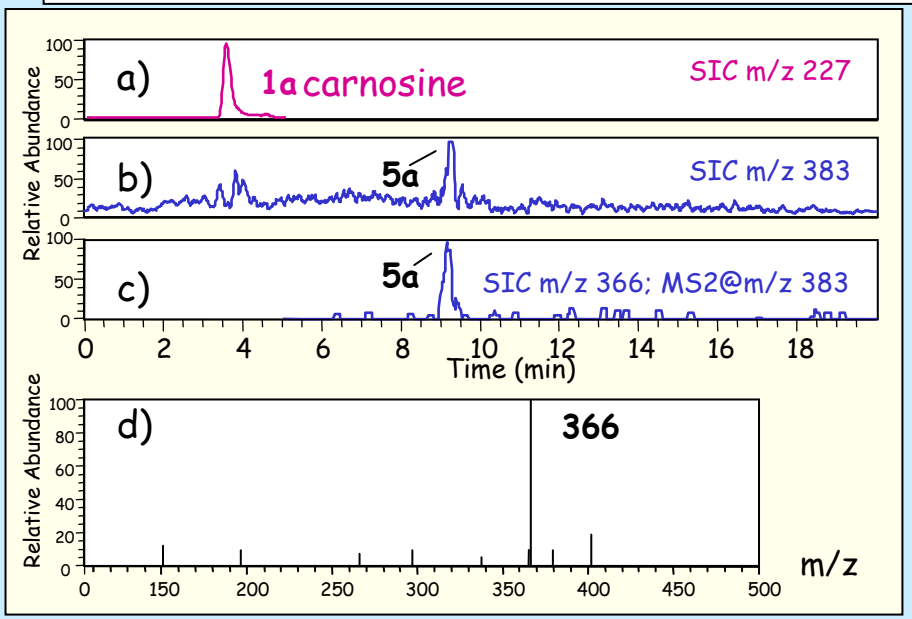
Background

The biological role of carnosine as a quencher of α,β-unsaturated aldehydes has been verified by detecting carnosine-HNE reaction adducts in oxidized rat skeletal muscle homogenate by LC-ESI-MS experiments [5,6]

⇒ to indicate that carnosine acts as a detoxifying agent towards HNE

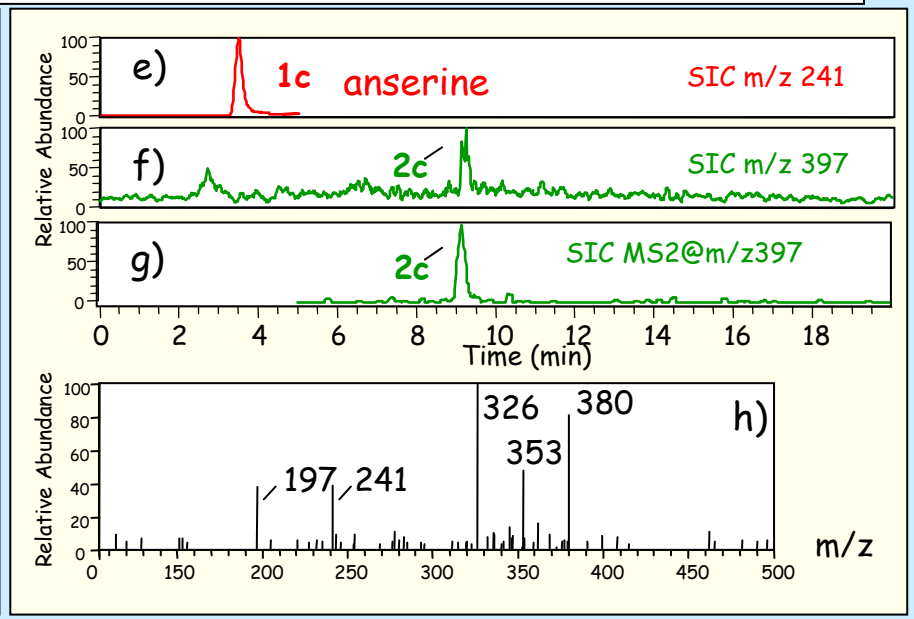
LC-MS and LC-MS/MS analysis (positive ion mode) of rat skeletal muscle homogenate spontaneously oxidized in aerobic conditions for 2 hours at 37 °C

Carnosine – HNE adduct (5a)



MS-MS spectrum of the peak at 9.20 min (parent ion m/z 383)

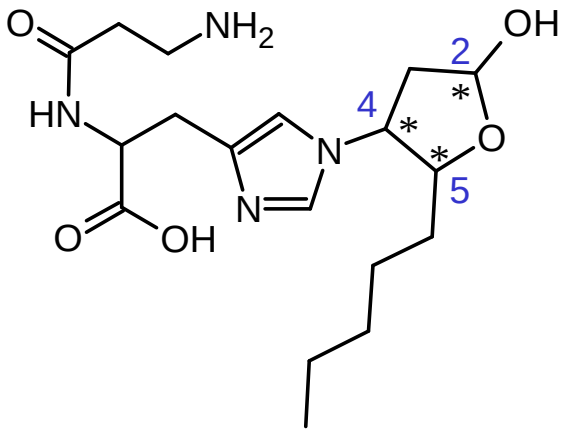
Anserine – HNE adduct (2c)



MS-MS spectrum of the peak at 9.20 min (parent ion m/z 383)

Aim of the study

♣ Because HNE generated in lipid peroxidation is a racemic mixture of 4R- and 4S-enantiomers, the HNE-carnosine Michael adduct, possessing three chiral centers at C-2, C-4 and C-5, in the tetrahydrofuran moiety, is presumed to be composed of at least eight isomers



♣ Aim of the present study was to investigate the configurational isomers of the HNE-carnosine adduct by NMR spectroscopy and by molecular orbital calculations in order to gain a deeper insight into the reaction mechanism of HNE adduction to carnosine

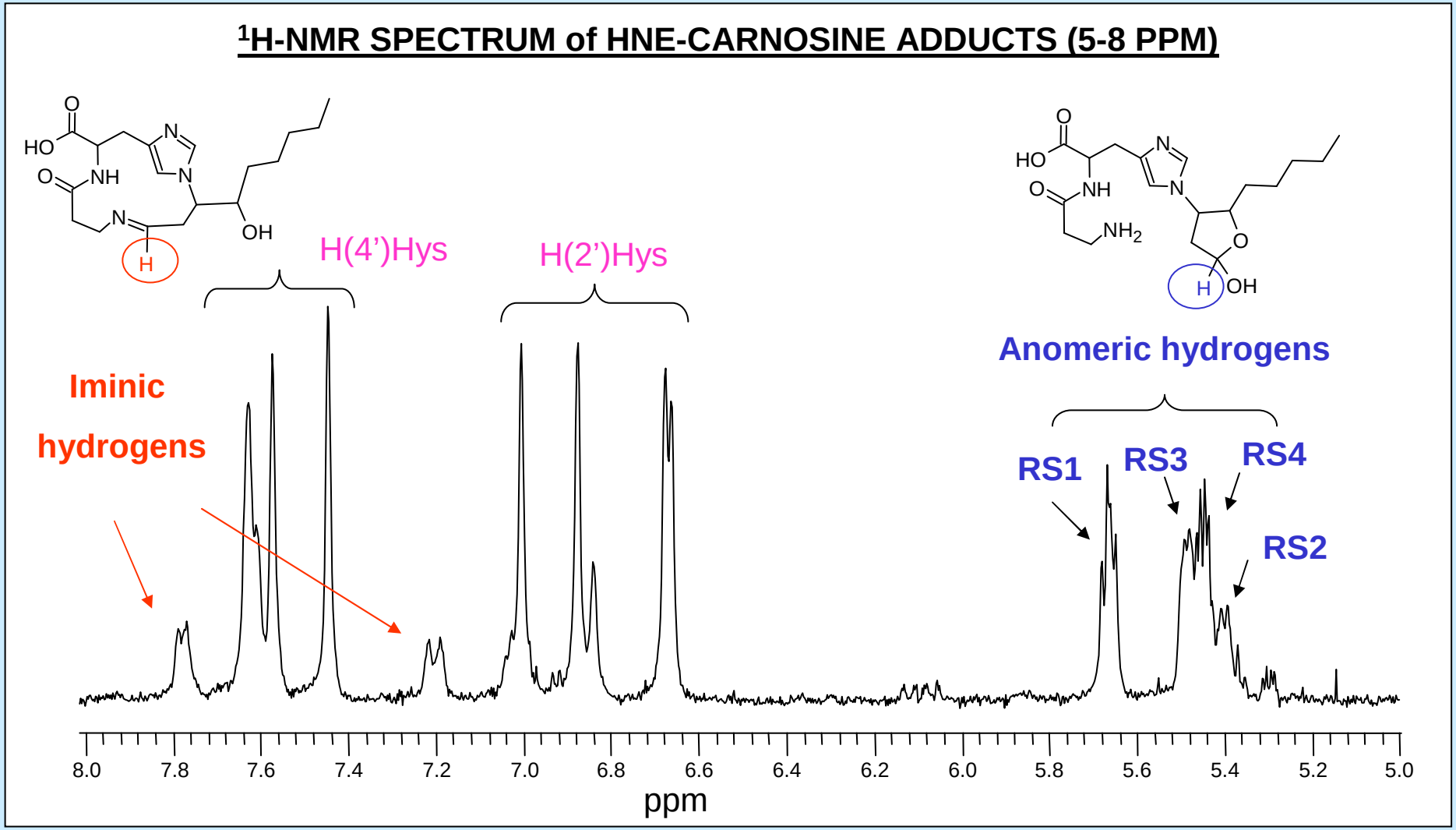
Experimental Procedures

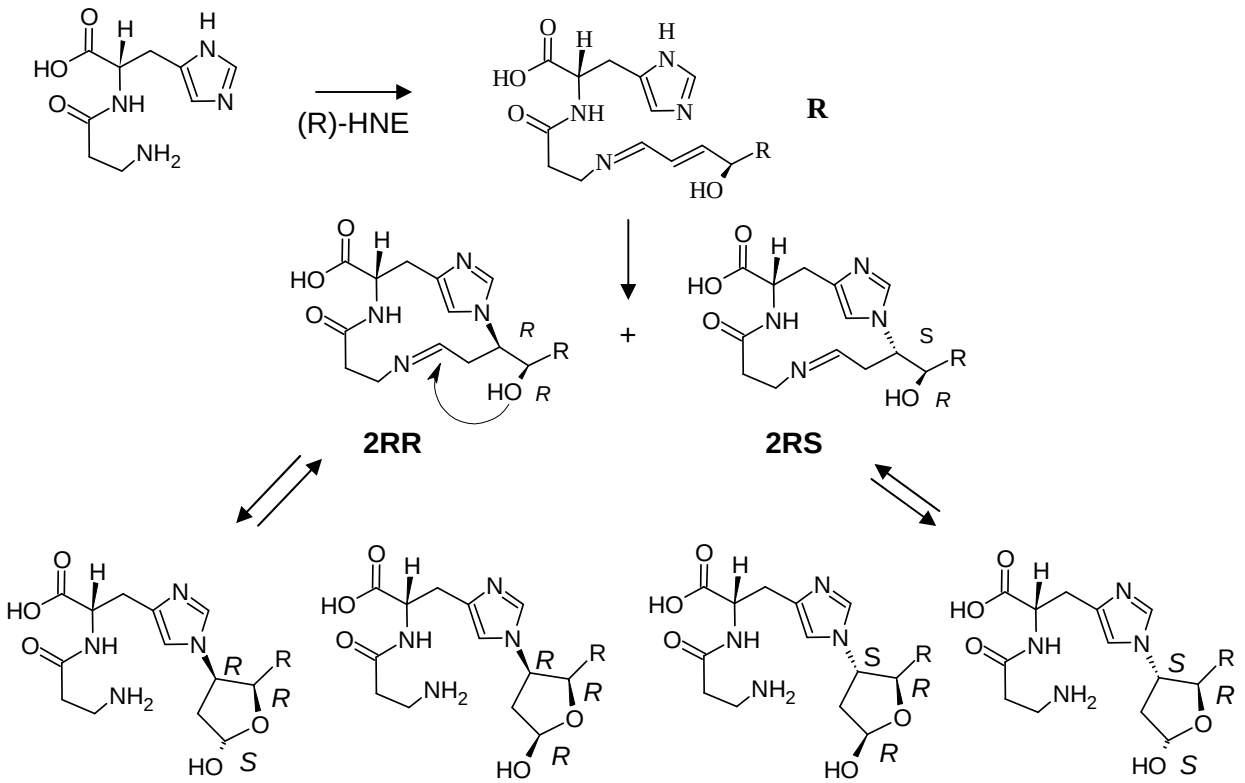
HNE-carnosine reaction: HNE (1.2 mg generated from 1.9 mg of the diethyl-acetal in 500 µl of HCl 1 mM) were mixed with 1.8 mg of carnosine in 1.5 mL of phosphate buffer (10 mM, pH 7.4). After 24 h of incubation at 37°C, 100 µL were analyzed by ESI-MS and the remaining solution dried under vacuum; the residue was dissolved in 0.5 mL of D_2O and analyzed by NMR.

***NMR analyses*:** Standard Varian software was applied for one-dimensional (1D) ^1H NMR experiments; spectra were obtained using a 4.5 kHz spectral window with 30K data points, giving a digital resolution of 0.1 Hz. Water suppression was obtained with the standard Varian software PRESAT

Computational analysis: The conformational profile of all predictable diastereoisomers was explored using molecular dynamic (MD) simulations *in vacuo* (simulation time = 1 ns, T = 300 K) and the preferred conformations were optimized using high-level *ab initio* calculations at B3LYP level of theory in order to calculate the energy properties of the considered isomers.The MD calculations were performed with QUANTA/CHARMm and the *ab initio* ones were performed with GAMESS-US.

Results

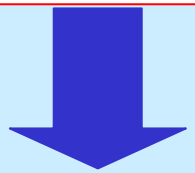

¹H-NMR SPECTRUM of HNE-CARNOSINE ADDUCTS (5-8 PPM)
H(4')Hys
H(2')Hys
Iminic hydrogens
Anomeric hydrogens
RS1
RS3
RS4
RS2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
ppm



	RRS (+SSR) (RS1)	RRR (+SSS) (RS2)	RSR (+SRS) (RS3)	RSS (+SRR) (RS4)
Relative abundance (%)	40	8	20	32
Energy (Kcal/mole)	18.52	30.62	2.65	0

The energy profiles calculated can explain the relative abundance of RS3 and RS4 but not for RS2 and in particular for RS1 ⇒ this suggests that the energy content is not the only driving force regulating the relative abundance of the diasteroisomers.

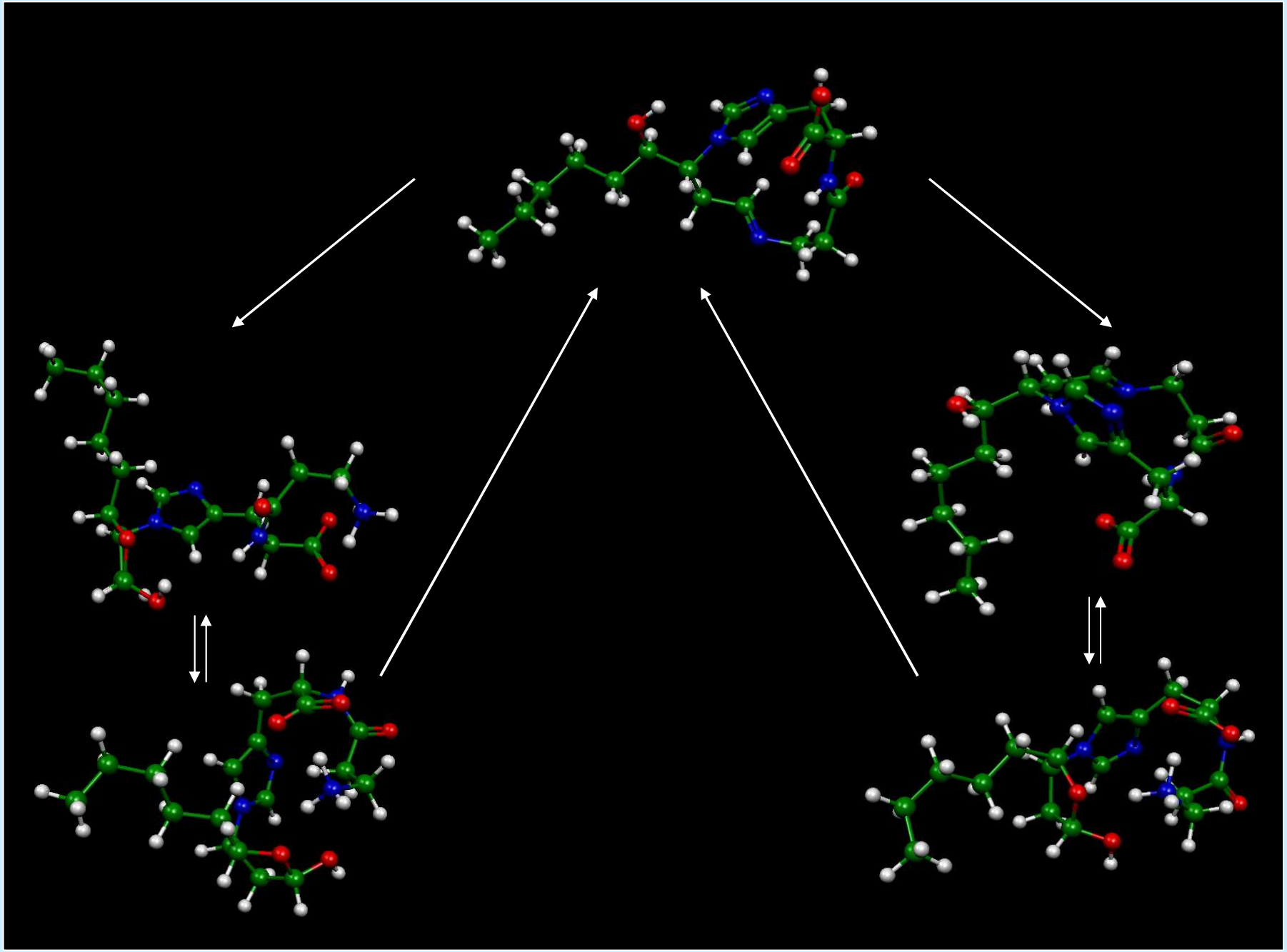
A possible explanation for the different relative abundance could be the interconversion among the diasteroisomers through the intermediate 2RR/2RS, which requires an intramolecular addition between the amino group of the β-alanine residue and the C-2 of the tetrahydrofuran ring.



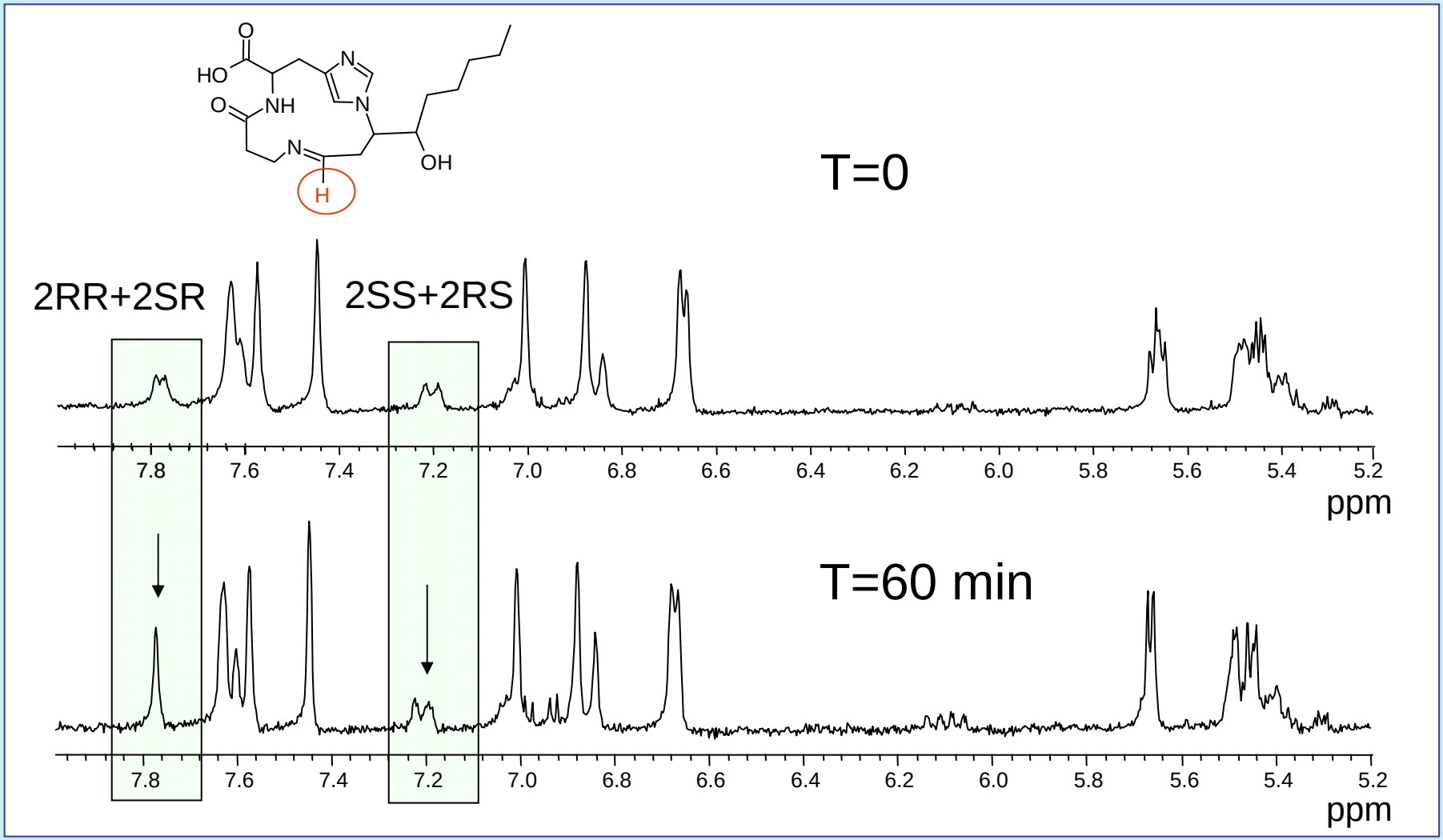
To test this hypothesis ⇒ conformational and energetic profile analyses of the diasteroisomers with the N-term amino group closed to the tetrahydrofuran ring.

	RRS+SSR (RS1)	RRR+SSS (RS2)	RSR+SRS (RS3)	RSS+SRR (RS4)
Relative abundance (%)	40	8	20	32
Energy (Kcal/mole)	18.52	30.62	2.65	0
MD energy (Δ…)	2.83	4.05	8.94	12.47

- The MD simulation indicates that although the proposed conformation is allowed for all the diasteroisomers, it is greatly favored for RS1 and RS2 in respect to RS3 and RS4.
- The possible interconversion between RS1 and RS2 through 2RR and the low energy of RS1 in respect to RS2 well explain the greater abundance of RS1 in respect to RS2.
- The unfavorable interconversion of RS3 and RS4 explains why the relative abundance of these diasteroisomers is mainly driven by their energy content.



The simplfication to singlet of the signal at 7.8 ppm relative to the iminic proton of 2RR is a further confirm of the rapid interconversion of RS1 and RS2 ⇒ this is due to the Deuterium exchange at the adjacent methilene group consequent to RS1 to RS2 interconversion. This was not observed for 2RS, to indicate a slower interconversion of RS3 and RS4.



Conclusions

Computational and NMR analyses shed light on the stereochemistry of the reaction between carnosine and HNE.

The relative abundance of the diasteroisomers RS3 and RS4 is dependent on the energy content while that of RS1 and RS2 to the their interconversion through the intermediate 2RR.

References

[1] K. Uchida, 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res. 2003 Jul;42(4):318-43.
[2] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81-128.
[3] A.R. Hipkiss, Could carnosine be a naturally-occurring scavenger for acrolein and other reactive aldehydes in the brain?, Neurobiol. Aging. 23 (2002) 645-646.
[4] A.R. Hipkiss, et al., Pluripotent protective effects of carnosine, a naturally occurring dipeptide, Ann. N.Y. Acad. Sci. 854 (1998) 37-53.
[5] Aldini G, Carini M, Beretta G, Bradamante S, Facino RM. Carnosine is a quencher of 4-hydroxy-nonenal: through what mechanism of reaction? Biochem Biophys Res Commun. 2002 Nov 15;298(5):699-706
[6] Aldini G, Granata P, Carini M. Detoxification of cytotoxic alpha,beta-unsaturated aldehydes by carnosine: characterization of conjugated adducts by electrospray ionization tandem mass spectrometry and detection by liquid chromatography/mass spectrometry in rat skeletal muscle. J Mass Spectrom. 2002 Dec;37(12):1219-28