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On the detection of both carbonyl and hydroxyl oxygens in amino acid derivatives: a ^{17}O NMR reinvestigation

Vassiliki Theodorou,[a] Anastassios N. Troganis[b] and Ioannis P. Gerothanassis[a,*]

[a]*Department of Chemistry, University of Ioannina, GR-45 110 Ioannina, Greece*
[b]*Department of Biological Applications and Technologies, University of Ioannina, GR-45 110 Ioannina, Greece*

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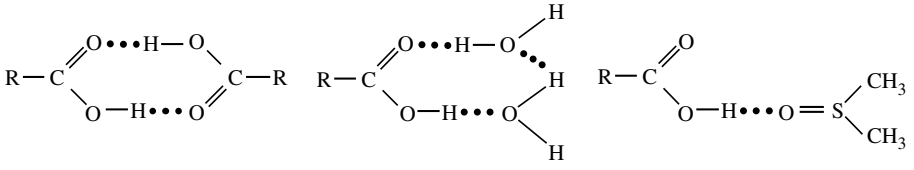
Abstract—The hypothesis and the conclusions of previous ^{17}O NMR studies on the detection of both oxygens of the carboxylic group of Boc–[^{17}O]Tyr(2,6-diClBzl)–OH in DMSO-d_6 solution (V. Tsikaris et al., *Tetrahedron Lett.* **2000**, *41*, 8651) are reconsidered. The appearance of two discrete resonances at 340 and 175 ppm of this protected amino acid is not now attributed: (a) to the reduction of the intramolecular conformational exchange rate, due to the effect of intramolecular hydrogen bonding of the hydroxy part of the carboxyl with the carbonyl oxygen of the Boc-group, and (b) to the effect of solvent viscosity, suggested in the mentioned study. The cause of this phenomenon is now attributed to a strong hydrogen bonding of the polar proton acceptor solvent DMSO with the carboxy group, which effectively reduces the proton exchange rate, thus becoming slow on the ^{17}O NMR time scale.

^{17}O NMR spectroscopy provides a powerful and sensitive tool for studying intra- and intermolecular hydrogen bonding effects both in solution and in the solid state.[1–4] Since the oxygen atom is one of the most important atoms constituting hydrogen-bonding structures, ^{17}O NMR might provide novel and complementary information not readily available from other methods.

In carboxylic acids, only one resonance is observed in the ^{17}O NMR spectra for both C=O and OH oxygens,[1–9] due to the intermolecular proton transfer through a cyclic dimeric form (Scheme 1A) in the solid state,[10,11] a cyclic dimer form or higher linear polymeric forms in apolar media,[11,12] or within hydrogen-bonded complexes consisting of one carboxylic acid molecule and two solvent molecules[13–16] (Scheme 1B) in protic solvents, such as H_2O and MeOH. These phenomena are considered to be very fast processes on the NMR time scale and an average ^{17}O resonance was usually observed at 250–260 ppm.

Recently, Tsikaris et al.[17] claimed that the two resonances at 340.3 and 175 ppm observed for the carboxylic oxygens of the labelled amino acid Boc–[^{17}O]Tyr(2,6-diClBzl)–OH (Scheme 2Aa) in DMSO-d_6 solution, should be attributed to two phenomena, which strongly reduce the intramolecular conformational exchange rate: firstly, to a strong intramolecular hydrogen bond interaction between the carboxy group (COOH) and the



(A) (B) (C)

Scheme 1.

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* Corresponding author. Tel.: +30-2-6510-98389; fax: +30-2-6510-98682; e-mail: igeroth@cc.uoi.gr

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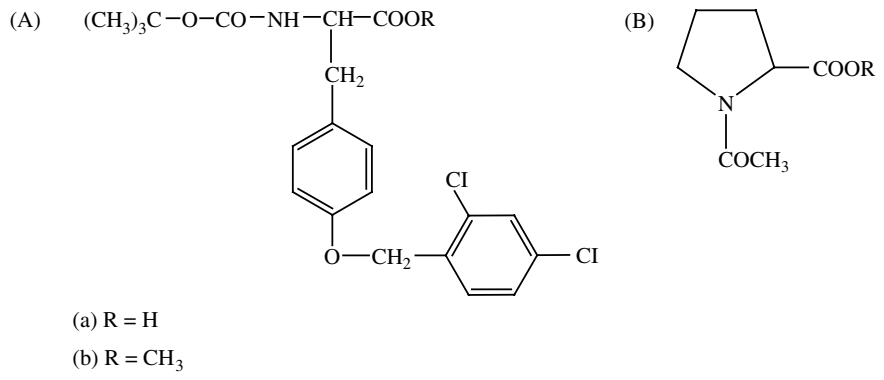

Scheme 2.

carbonyl part (C=O) of the Boc group, stabilising a γ-turn like structure and, secondly, to the effect of DMSO viscosity. The purpose of this communication is to critically evaluate the conclusions of Tsikaris et al.[17] and to provide a coherent comment on the observation of both carbonyl and hydroxyl oxygens in amino acid derivatives.

In their report,[17] Tsikaris and co-workers presented a series of experiments in order to support their hypothesis:

(i) The ^{17}O NMR spectrum of HCl[^{17}O]Tyr(2,6-diClBzl)–OH in DMSO-d_6 has only one resonance at 251.8 ppm, which was interpreted with the hypothesis that a γ-turn structure cannot exist. However, no details were provided on the preparation of the salt. In any case either with aqueous HCl or with HCl gas, fast proton transfer of the carboxylic group would be facilitated and one resonance would be observed.

(ii) The ^{17}O NMR spectrum of Boc–[^{17}O]Tyr(2,6-diClBzl)–OH in $CDCl_3$, revealed a single resonance at 247.5 ppm. It was claimed that the viscosity of the solvent strongly affects the intramolecular conformational exchange rate, being rapid in the case of chloroform. This hypothesis is erroneous for two reasons. Firstly, the differences in viscosity between DMSO (1.788 mPa s at 30 °C)[18] and $CHCl_3$ (0.512 mPa s at 30 °C)[19] solutions would not be expected to reduce an intramolecular conformational exchange of the ns time scale to an ms time scale or ever longer. Secondly, in nonpolar solvents, such as chloroform, a very fast intermolecular proton transfer occurs through the cyclic dimeric or linear polymeric forms of a carboxylic acid.

In order to further support our hypothesis, we performed some complementary experiments with Ac–[^{17}O]Pro–OH, which is one of the best characterised models of γ-turn structures,[9,20] Ac–[^{17}O]Pro–OMe[21], Boc–[^{17}O]Tyr(2,6-diClBzl)–OMe[21] (Scheme 2) and [^{17}O]PhCOOH.[22] The samples were prepared by dissolving the correct amount of each compound in 600 μL commercial DMSO-d_6, which, according to proton NMR spectra, contained 0.4 M of H_2O.

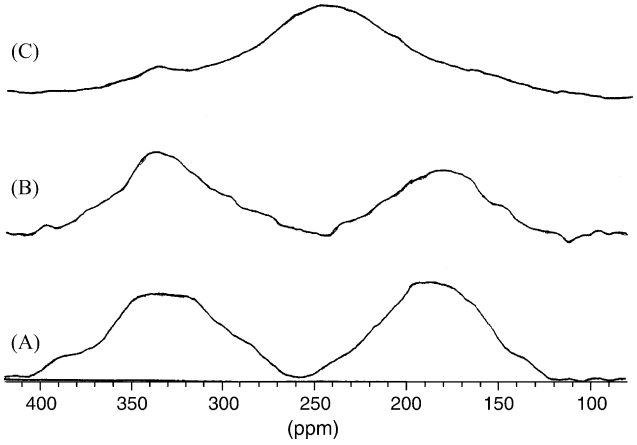

Figure 1. ^{17}O NMR spectra at 54.4 MHz of Ac-[^{17}O]ProOH ($C = 40$ mM) (A), [^{17}O]PhCOOH ($C = 30$ mM) (B), and [^{17}O]PhCOOH ($C = 100$ mM) (C) in DMSO-d_6, containing 0.4 M of water. NMR spectra were recorded on a Bruker AMX-400 spectrometer at 40 °C. 800 000 scans were accumulated with a repetition rate of 20 s^{-1} and an exponential line broadening of 200 Hz was imposed on the accumulated data before Fourier transformation.

Two resonances at 320 and 180 ppm were observed for the two oxygens (C=O, OH) of Ac–[^{17}O]Pro–OH in DMSO-d_6 (Fig. 1A). DMSO is an important dipolar aprotic solvent with a high dielectric constant, which behaves as a strong hydrogen bond acceptor with proton donor substrates, such as COOH and OH groups.[23,24] Dimethyl sulfoxide also interacts with other acids and electrophiles[25] enabling ionisation or other processes. As the concentration of the acceptor is increased, the cyclic dimer of the carboxylic acid (Scheme 1A) is suppressed effectively, in favour of the complex with solvent molecules (Scheme 1C). DMSO, therefore, disrupts the potential hydrogen bonds of the cyclic dimers, the S=O oxygen interacting strongly with the COOH, and reduces the intermolecular proton transfer, resulting in a clear differentiation of the two oxygens. The chemical shifts of the two resonances are in good agreement with those observed for the two oxygens (C=O, –OCH_3) of the two methyl esters Ac–[^{17}O]Pro–OMe (335 and 130 ppm) and Boc–[^{17}O]Tyr-(2,6-diClBzl)–OMe (342 and 135 ppm) in $CDCl_3$ (40 °C). On the contrary, the ^{17}O NMR of Ac–[^{17}O]Pro–OH in $CDCl_3$ indicates only one resonance at 255 ppm due to

the formation of cyclic dimers or linear polymeric forms, as revealed by IR spectroscopy, despite the presence of a major amount of the γ-turn structure.

The ^{17}O NMR spectrum of [^{17}O] benzoic acid, has two broad resonances for the two oxygens of the carboxyl in DMSO-d_6 at 40 °C (Fig. 1B). Since this carboxylic acid cannot form a γ-turn structure, the presence of two resonances must be attributed to the formation of new intermolecular hydrogen bonds with solvent molecules, the PhCOOH·DMSO complex (Scheme 1C), with disruption of the cyclic dimers (Scheme 1A). Further confirmation that the two resonances are really connected by proton exchange can be obtained from variable concentration experiments. According to Meschede and Limbach[26] the pseudo-first order proton exchange rate constant k_{obs} observed by NMR (in the slow exchange regime) is given by $k_{obs} = K_2kC$, where K_2 is the dimerisation constant, C the concentration of the acid, and k the true exchange rate in the dimer. Therefore, increasing C should increase k_{obs}. Indeed, increasing the concentration from 30 mM (Fig. 1B) to 100 mM (Fig. 1C) results in an averaged single resonance absorption at 250 ppm. As expected, in $CDCl_3$ solution a single resonance is observed due to the formation of the dimers (Scheme 1A). IR spectra of PhCOOH, both in DMSO and chloroform, are consistent with the ^{17}O NMR results.

In conclusion, the detection of both carbonyl and hydroxyl oxygens in ^{17}O NMR of amino acid derivatives in DMSO solution, contrary to the case in $CDCl_3$ solution, should not be attributed to a reduction of the intramolecular conformational exchange rate due to the formation of an intramolecular γ-turn like hydrogen bond interaction and to the effect of solvent viscosity. This phenomenon should be attributed to the strong hydrogen bond interaction of DMSO-d_6 with the COOH group, which suppresses almost completely the formation of cyclic dimers and, thus, reduces effectively the exchange rate for proton transfer.

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