Journal of Molecular Structure 920 (2009) 323–326

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

# A theoretical and experimental NMR study of (+)-biotin methyl ester

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ARTICLE INFO

*Article history:*
Received 22 October 2008
Received in revised form 7 November 2008
Accepted 10 November 2008
Available online 24 November 2008

This paper is dedicated to Professor Gloria Inés Yranzo from the University of Córdoba (Argentina) who untimely passed away in September 2008

*Keywords:*
Biotin
NMR
CPMAS
Hydrogen bonds
GIAO

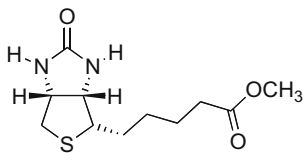
ABSTRACT

The structure in solution and in the solid state of (+)-biotin methyl ester is discussed based on NMR chemical shifts measured in $CDCl_3$ solution and in the solid state together with GIAO calculations at the B3LYP/6-311++G** theoretical level. In the solid state, only the extended conformation exists while in $CDCl_3$ solution a small amount of a IMHB conformation is present in a fast dynamic equilibrium with the preceding one. The HB involves the C=O oxygen atom of the ester.

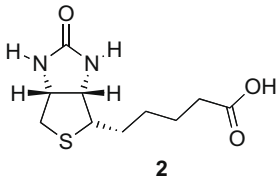


## 1. Introduction

We have been reporting the characterization by NMR of some relevant pharmaceutical and biological compounds, for instance, Omeprazole [1,2] and curcumin [3]. In particular, we have been interested in biotin methyl ester **1** (3aS,4S,6aR)-hexahydro-2-oxo-1*H*-thieno[3,4-d]imidazole-4-pentanoic acid methyl ester [4–8], a derivative of biotin **2**, in molecular recognition studies trying to improve the binding of **2** with synthetic receptors.



**1**



**2**

Biotin, also known as vitamin H or B7, is a water soluble B-complex vitamin. Biotin is a cofactor in the metabolism of fatty acids and leucine, and in gluconeogenesis. Biotin is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids. It plays a role in the citric acid cycle, which is the process by which biochemical energy is generated during aerobic respiration. Biotin not only assists in various metabolic reactions, but also helps to transfer carbon dioxide being also helpful in maintaining a steady blood sugar level [9].

The attachment of biotin to various chemical sites, called biotinylation, can be used as an important laboratory technique to study various processes including protein localization, protein interactions, DNA transcription and replication [10]. Biotin binds very tightly to the tetrameric protein avidin (also streptavidin and neutravidin), with a dissociation constant $K_d$ in the order of $10^{-15}$ mol/L [11,12]. This is often used in different biotechnological applications. Biotinylated antibodies are used to capture avidin or streptavidin in the ELISA techniques. Until 2005, very harsh conditions were required to break the biotin-streptavidin bond [13].

Due to its importance many structural, both experimental and theoretical, studies have been devoted to biotin: the X-ray structure was determined by Jerry Donohue [14], data concerning UV [15], IR [16] and NMR spectra [17] were reported several times as well as theoretical [18] and conformational studies [19].

We decided to determine the NMR parameters of **1**, a simple derivative of biotin, useful as a model compound due to its much higher solubility in organic solvents. Contrary to the much studied **2** its derivative **1** has not been properly studied by NMR.

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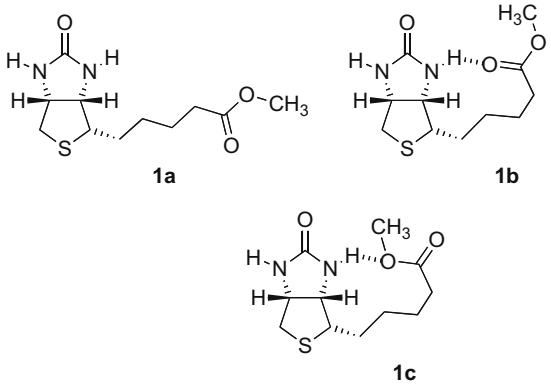
doi:10.1016/j.molstruc.2008.11.027

## 2. Results and discussion

The X-ray structure of **1** is known (BIOTME10 [20], Fig. 1):

The structure represented in Fig. 1 present three important characteristics: (i) the valeric acid tail is extended; (ii) the fusion of 5,5 tetrahydroimidazalone and tetrahydrothiophene rings is *cis*; (iii) the hydrogen bonds (HBs) are intermolecular involving N—H and C=O bonds and forming a catemer.




We have calculated, at the B3LYP/6-311++G** theoretical level, three conformations of (+)-biotin: extended **1a** (like in Fig. 1), folded with the N—H towards the C=O group **1b** and folded with the N—H towards to $OCH_3$ group **1c**. The relative energies are 0.0, 9.3 and 10.7 kJ $mol^{-1}$, respectively. The fact that **1b** is more stable than **1c** corresponds to the better HB acceptor ability of the C=O compared with the OR in ester groups, that is, HBs are stronger with the carbonyl than with the ether group [21,22] but in any case, the extended conformation is much more stable in the gas phase. Solvent effects could involve intermolecular HBs not present in the gas phase, therefore, it is expected that **1a** will be further favored in solution.

### 2.1. NMR results

We have reported in Table 1 the results we have obtained.

We have used the following equations used to transform σ into δ (including $^1$H TMS $\sigma$ = 31.97 ppm, $^{13}$C TMS $\sigma$ = 184.75 ppm, $^{15}$N $MeNO_2$ $\sigma$ = −154.43 calculated at the same level):

$^{15}$N NMR: $\delta^{15}N = -148 - 0.95\ \sigma^{15}N$, this work.
$^{13}$C NMR: $\delta^{13}C = 175.7 - 0.963\ \sigma^{13}C$, from Ref. [23].
$^1$H NMR: $\delta^1H = 31.0 - 0.97\ \sigma^1H$, from Ref. [24].

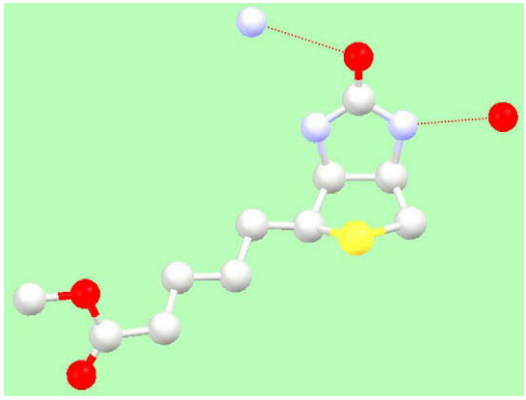


**Fig. 1.** X-ray structure of biotin methyl ester showing the hydrogen bonds (HBs).

We have compared the experimental results with the three calculations and gathered the regression equations in Table 2.

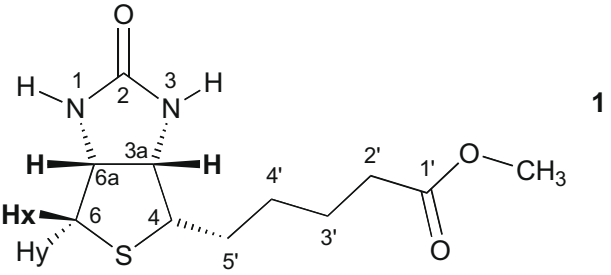
We must remember that in the solid state, CPMAS measurements, the (+)-biotin methyl ester has the structure **1a**. Therefore, the $^{15}$N NMR results are useless because there are too similar. On the other hand, the small differences in the $^{13}$C spectra are significant and shows that conformer **1a** is that present in CPMAS (Eqn. 10) and in $CDCl_3$ (Eqn. 7). But the most significant differences are found in $^1$H NMR (Eqns. 13–15, see Fig. 2).

A slightly better correlation ($r^2$ = 0.99838 vs. 0.99810) is obtained with a mixture of 88% of **1a** and 12% of **1b** (inclusion of some amount of **1c** does not improve the correlation). A dynamic situation between **1a** and **1b** involving a wagging of the tail could explain the NOESY results. This conclusion is consistent with those of Crisp and Jiang [25] using ROESY experiments, although we think that conformer **1b** is present in small amounts.

## 3. Experimental

Solution NMR spectra were recorded on a Bruker DRX 400 (9.4 Tesla, 400.13 MHz for $^1$H, 100.62 MHz for $^{13}$C and 40.56 MHz for $^{15}$N) spectrometer with a 5-mm inverse-detection H-X probe equipped with a *z*-gradient coil. Chemical shifts (δ in ppm) are given from internal solvent, $CDCl_3$ 7.26 for $^1$H and 77.0 for $^{13}$C and for $^{15}$N NMR nitromethane (0.00) was used as external standard.

**Table 1**
Chemical shifts (δ, ppm) and coupling constants (*J*, Hz).




| Atom | Exp. $CDCl_3$ | Exp. CPMAS | Calc. **1a** | Calc. **1b** | Calc. **1c** |
|---|---|---|---|---|---|
| N1 | −291.7[a] | −284.5 | −292.5 | −289.6 | −291.7 |
| C2 | 163.4 | 165.8 | 157.6 | 157.0 | 157.3 |
| N3 | −300.5[b] | −294.5 | −300.3 | −299.9 | −299.7 |
| C3a | 61.9 | 64.7 | 65.1 | 63.3 | 62.5 |
| C4 | 55.3 | 59.2 | 63.7 | 67.3 | 66.4 |
| C6 | 40.5 | 41.3 | 47.0 | 46.6 | 46.5 |
| C6a | 60.1 | 60.9 | 62.6 | 63.6 | 63.9 |
| C1' | 174.1 | 173.5 | 172.7 | 175.5 | 171.7 |
| C2' | 33.6 | 36.0 | 34.9 | 36.9 | 38.8 |
| C3' | 24.8 | 27.6 | 27.6 | 23.5 | 25.5 |
| C4' | 28.3 | 33.3 | 32.8 | 34.3 | 31.6 |
| C5' | 28.2 | 30.3 | 32.5 | 25.9 | 25.4 |
| $CH_3$ | 51.6 | 53.4 | 50.6 | 52.0 | 51.7 |
| NH1 | 5.22 | – | – | – | – |
| NH3 | 5.64 | – | – | – | – |
| H3a | 4.31[c] | – | 4.01 | 4.34 | 4.19 |
| H4 | 3.16[d] | – | 2.94 | 2.95 | 2.94 |
| H6 y | 2.73[e] | – | 2.56 | 2.12 | 2.25 |
| H6 x | 2.92[f] | – | 2.81 | 2.85 | 2.84 |
| H6a | 4.51[g] | – | 4.28 | 4.16 | 4.28 |
| H2' | 2.34[h] | – | 2.21 | 2.34 | 2.27 |
| H3' | 1.69 | – | 1.50 | 1.70 | 1.65 |
| H4' | 1.45 | – | 1.24 | 1.54 | 1.58 |
| H5' | 1.69 | – | 1.58 | 1.63 | 1.50 |
| $CH_3$ | 3.67 | – | 3.53 | 3.69 | 3.98 |

$^{15}$N—$^1$H coupling constants: [a]$^1J$ = 93.9 (N1H); [b]$^1J$ = 94.2 (N3H).
$^1$H—$^1$H coupling constants: [c]$J$ = 1.5 (NH3); [d]$J$ = 8.2, 6.6 (H5′) and 4.6 (H3a); [e]$J_{gem}$ = −12.9; [f]$J_{gem}$ = −12.9, $J$ = 5.0 (H6a); [g]$J$ = 7.7 (H3a), 5.0 (H6 x), 1.1 (H6 and NH1); [h]$J$ = 7.5 (H3′).