SciMedCentral

JSM Chemistry

Short Communication

The Importance of Correct Tautomeric Structures for Biological Molecules

Poul Erik Hansen[1]*, John Mortensen[2] and Fadhil S. Kamounah[3]

[1]*Poul Erik Hansen, Department of Science, Roskilde University, Denmark*
[2]*John Mortensen, Department of Science, Roskilde University, Denmark*
[3]*Fadhil S. Kamounah, Department of Science, Roskilde University, Denmark*

***Corresponding author**
Poul Erik Hansen, Department of Science, Systems and Models, Roskilde University, Universitetsvej 1, DK-4000 Roskilde, Denmark, Tel: 4546742432; Fax: 4546743011; E-mail: poulerik@ruc.dk

Submitted: 04 December 2014

Accepted: 12 January 2015

Published: 14 January 2015

ISSN: 2333-6633

Keywords
- Usnic acid
- Isotope effects on chemical shifts
- Tetracycline
- Structure-function relationship
- pK_a value

Abstract

The structures of usnic acid and tetracycline are determined using deuterium isotope effects on 13C chemical shifts in a water environment. In case of usnic acid this is achieved by synthesizing a more water soluble usnic acid with a PEG linker. In the usnic acid case an enolic β-triketone (C-1, C-14 and C-3) tautomeric equilibrium is at hand below pH 5. At pH 7.4 it exists as a mono anion. In case of tetracycline equilibrium between a zwitter ion and a neutral form is found together with an amide functional group and a hydrogen bonded enolic β-diketone system shifted strongly towards one tautomer.

ABBREVIATIONS

DFT: Density Functional Theory

INTRODUCTION

A number of biological compounds turn out to be tautomeric. However, tautomerism is often difficult to establish and structures are often just referred to as one form as seen in a number of cases for usnic acid in (Figure 1). Furthermore, some molecules turn out to be rather strong acids, which of course will influence their properties at physiological pH and can lead to internal proton transfer. A much debated example is that of rifampicin [1]. Tautomerism can be of different kinds. In the present study the tautomeric structures may involve hydrogen's at carbon or hydrogen's at electronegative elements as illustrated for acetyl acetone. For tautomerism involving hydrogen at carbon the equilibrium is usually slow and the amount of a given tautomer is usually depending on the polarity of the solvent. Tautomerism between enolic forms is usually fast and with a very low conversion barrier. An illustrative case is that of usnic acid. Usnic acid is known to have an array of important biological effects [2].The biological effects are believed to be related to ring C [3] (See Figure 1). The structure of ring C is therefore important. In the literature many different forms are given and often as single structures not as a tautomeric equilibrium as that between B and C [1,4] as shown in (Figure 1). One of the difficulties of studying biological effects of usnic acid is its low solubility in water. A more water soluble pegylated derivative is therefore synthesized. Acidity clearly plays a role for the two molecules investigated. The pKa value for usnic acid is for the first ionization 4.4 [5] meaning that at pH 7.4 only 1 per mile is protonated. For tetracycline internal proton transfer is a possibility.

Tetracycline is a very useful antibiotic. The effects are well established but this cannot be said for the structure. Often structures are determined of the hydrochloride as tetracycline itself is sparingly soluble at pH=7. The structure of tetracycline has been investigated and different structures have been proposed based on X-ray structures (solid) [6] and DFT calculations [7]. The structure of tetracycline is usually given as shown in (Figure 2A). One effective way of studying tautomeric systems in solution, is the use of deuterium isotope effects on chemical shifts [8,9]. This can be supplemented by DFT calculations. The goal of the present paper is to introduce techniques to study tautomerism in solution and to turn the attention to the determination of correct structures before making corrections between structure and function.

MATERIALS AND METHODS

2-(2-(2-chloroethoxy) ethoxy) ethanol was purchased from Sigma-Aldrich, Weinheim Germany.

The pegylated usnic acid is synthesized as follows: To a solution of usnic acid (0.64 g, 1.87 mmol) and 2-(2-(2-chloroethoxy) ethoxy) ethanol (0.63 g, 3.74 mmol) in anhydrous DMSO (8.0 ml) was added at room temperature anhydrous potassium carbonate (1.55 g, 11.22 mmol). The mixture was stirred under nitrogen atmosphere at 100 °C for 60 hrs. The mixture was cooled and poured into distilled water (60.0 ml) and stirred for 10 min and then neutralized by 1.0 M HCl to pH 6.8, and then extracted with dichloromethane (4x50 ml). The combined extracts were washed with water and brine and finally dried over anhydrous magnesium sulfate. Evaporation of solvent affords a light brown viscous

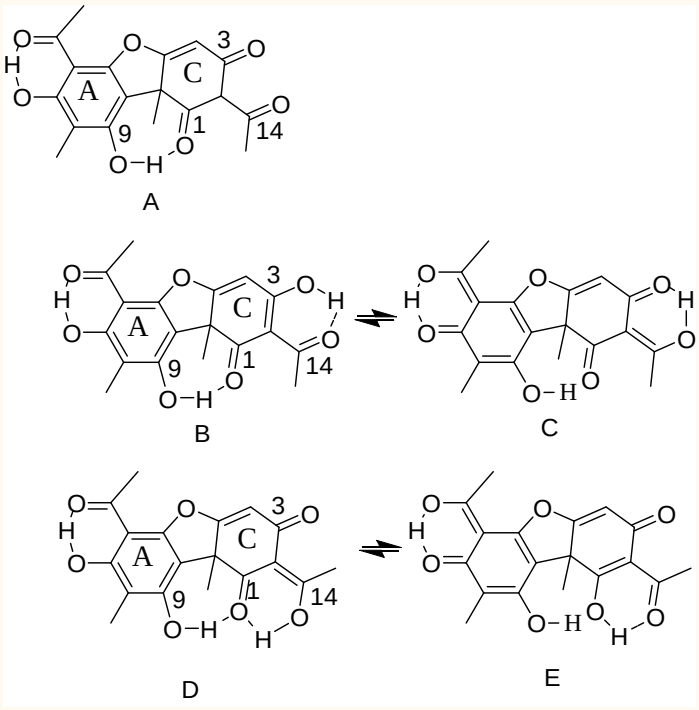

Figure 1 Tautomeric scheme of usnic acid.

residue. The product was purified by column chromatography on silica gel, eluating first with dichloromethane/ethyl acetate (1:1) and then with ethyl acetate to obtain the pegylated usnic acid as a highly viscous amber material. *m/z* 476.44, ^{1}H NMR ($CDCl_3$): 18.81 (OH-3); 10.81 (OH-9); 5.94 (H-4); 4.02, 3.78, 3.74 3.62, CH_2O); 2.65 (CH_3-12); 2.63 (CH_3-14); 2.20 (CH_3-15) and 1.77 (CH_3-10). ^{13}C NMR (DMSO-d_6) in ppm: 201.1 (C-11); 197.8 (C-13); 196.0 (C-1); 191.4 (C-3); 180.0 (C-4a); 157.2 (C-7); 153.5 (C-9); 152.0 (C-5a); 115.2 (C-8); 112.5 (C-6); 108.8 (C-9a); 105.1 (C-2); 97.5 (C-4); 74.1, 72.3, 69.8, 69.7, 62.3, and 60.2 (OCH_2); 58.9 (C-9b); 31.8 (C-10); 31.8 (C-14); 27.5 (C-12) and 8.7 (C-15).

NMR spectra are recorded on a Varian Mercury instrument at 300 MHz or 75 MHz for ^{1}H and ^{13}C NMR.

The isotope effects are determined by subtraction of data for spectra recorded in a 50:50 mixture of D_2O and DMSO-d_6 and H_2O and DMSO-d_6 and multiplied by a factor 2. The calculations were done in the Gaussian program package [10] using the B3LYP/6-3G (d,p) functional [11,12].

RESULTS AND DISCUSSION

The structure of usnic in $CDCl_3$ was shown to be a tautomeric equilibrium between the B and C forms (Figure 1) with almost equal amounts of each tautomer [8]. In (Figure 3) deuterium isotope effects are determined for a pegylated form of usnic acid at pH 3.4 in a D_2O: DMSO-d_6 (1:2). The isotope effects observed for carbons 1 and 3 are almost the same as those in $CDCl_3$ showing that the same forms and the same equilibrium also is at hand in a mixture of DMSO and D_2O at this pH.

The pK_a value is determined in a mixture of water, methanol and chloroform as 4.4 [4]. In the present study the pK_a value of the pegylated usnic acid is determined in water: DMSO mixtures as (1.5:1). 4.3; (1:1) 4.3; (0.5:1) 4.84 and pure DMSO 5.76 leading to a pK_a value of 4.3. At pH 7.4 only about one thousand of the molecules will be fully protonated and an anion with the charge distribution as seen in (Figure 5) will be present. Considering the low solubility dimerization is clearly also a possibility. As derivatives of usnic acid with an amine are shown to have biological effects [13] and not having the very acidic proton. It is interesting that the anion of usnic acid has a strong hydrogen bond between OH-9 and O-1 (Figure 5). A similar picture is not seen for the amine derivative. In this case the C-1=O bond is clearly more double bond like. It will be interesting to see how the biological effects of usnic acids substituted at O-9 compare to usnic acid.

Tetracycline is a very interesting molecule as it contains a dike to (C-11, C-11a and C-12 and a "triketone" moiety (C-1, C-2, C-13 and C-3) and the possibility for zwitter ion formation. The pK_a values have been determined as 3.3, 7.8 and 9.5 [14] suggesting that a zwitter ionic structure could be present. Establishing the correct equilibrium is clearly very important. Structures are shown in (Figure 2). The structure of tetracycline has been determined at pH=1, the hydrochloride, using isotope effects on ^{13}C chemical shifts. In that case similar results were obtained in a 50:50 mixture of D_2O and DMSO-d_6 and in D_2O [15]. In the present study tetracycline is investigated at pH=6.3 in a 50:50 mixture of D_2O and DMSO-d_6. The deuterium isotope effects at ^{13}C chemical shifts are given in (Figure 4). The isotope effects are total isotope effects as all XH protons are exchangeable [15]. The very large deuterium isotope effects at C-12 show that the equilibrium is shifted so that C-12 is primarily on the OH form. This was also predicted based on model compounds [15] as well as theoretical calculations [6]. The large negative isotope effect at C-11 is due to the equilibrium isotope effect contribution. The isotope effect at C-10 clearly shows that the OH group is hydrogen bonded. The

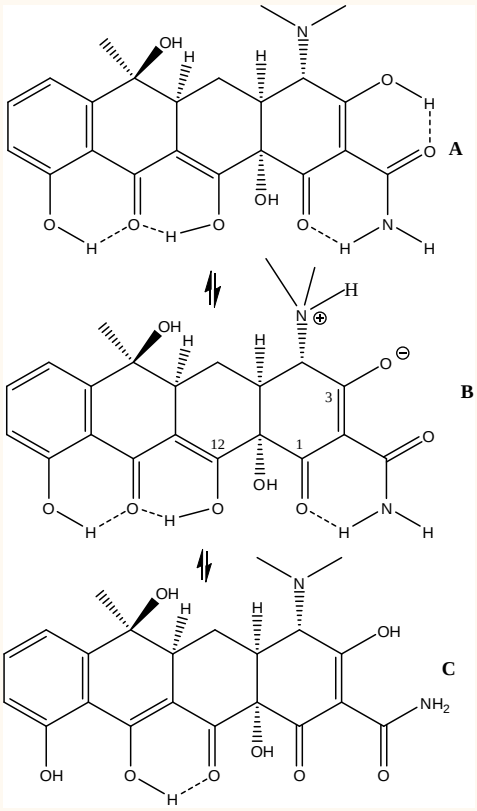

Figure 2 Tautomeric and rotameric forms of tetracycline. Only a few forms are shown.

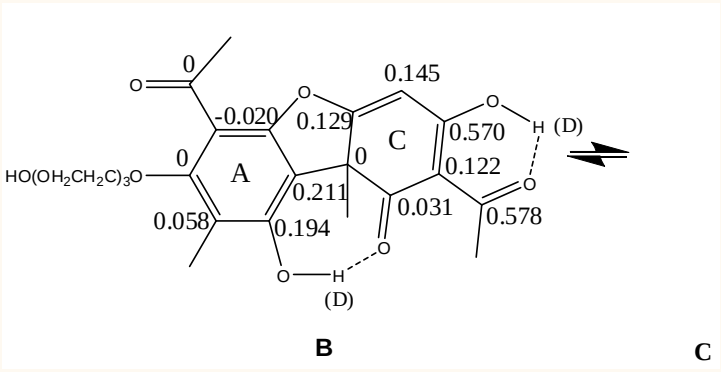

Figure 3 Deuterium isotope effects on ^{13}C chemical shifts of usnic acid in DMSO-d_6:D_2O (2:1). For structure of C see (Figure 1).

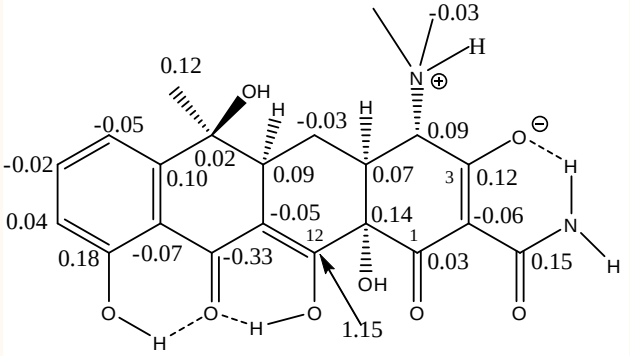

Figure 4 Deuterium isotope effects on ^{13}C chemical shifts of tetracycline.

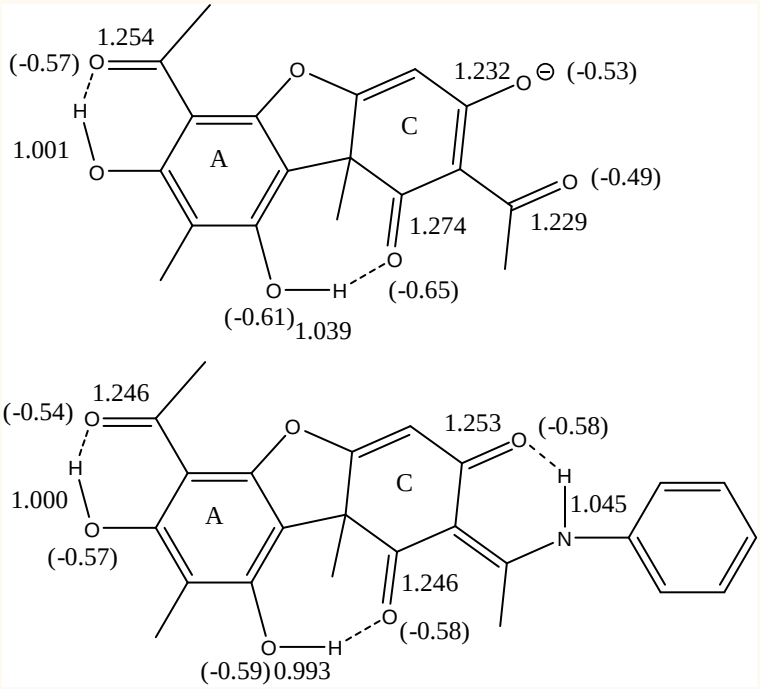

Figure 5 Calculated structure of usnic acid anion and an amine derivative. Numbers in brackets are Mulliken charges and bond lengths are in Å.

deuterium isotope effect at the amide carbon is in agreement with an amide group is which both NH protons are exchanged [16]. The deuterium isotope effect at C-3 and the N $(CH_3)_2$ methyl carbons are 0.15 and -0.03 ppm, respectively. The finding of a relatively large positive isotope effect at C-3 indicates that C-3 is protonated part of the time. For C-3 to be protonated fully one would expect an isotope effect of ~0.9ppm, the value found for 2-carbamoyl-5, 5-dimethyl-1, 3-cyclohexanedione [17]. The effect of deuteriation at the NH group at the CH_3 carbon can be judged from $DMANH^+$ [18]. In that compound the effect is -0.12 ppm (2*-0.06 ppm, assuming no long-range effect is seen). Deuteriation may shift the equilibrium causing an equilibrium isotope effect [8]. Deuteriation will always prefer the stiffer bond (the bond with the highest XH stretching frequency). From the calculations of Othersen et al [7] the NH stretching frequency is higher than that of the OH bond. As the ^{13}C chemical shifts of the CH_3 group is shifted to lower frequency upon deuteriation and that of C-3 is likewise shifted to lower frequency, both will gain a positive contribution due to the equilibrium. However, this effect will be much larger at C-3 than at CH_3 as the chemical shift is ~2.6 ppm for CH_3 but ~8 ppm for C-3(calculated [6]). We may there for conclude that the equilibrium is shifted towards the zwitter ionic form. The mole fraction of the neutral form is maximally 0.15/0.99 ~0.15. The equilibrium contribution will decrease this number, whereas hydrogen bonding to the ND proton will increase this [17].

CONCLUSION

The pegylated form of usnic acid is a tautomeric equilibrium between the two enolic forms B and C (Figure 1) in a water environment at pH 4.3. At pH 7.4 it is fully on a mono anionic form. Calculations show a strong hydrogen bond between OH-9 and C-1=O. The fact that it is pegylated at OH-7 should have no effect on its biological function. It is of course important to notice that the triketo form for the C-ring does not exist at all and that the form E of (Figure 1) is not present either.

Tetracycline exists as equilibrium between a zwitter ionic form and a neutral form both with an amide functional group and in the former case with a hydrogen bond from the NH proton. In addition, a hydrogen-bonded enolicβ-diketone system shifted strongly towards one tautomer.

ACKNOWLEDGEMENTS

The authors would like to thank Andrias Højgaard and Mette Nielsen for their valuable contribution to the tetracycline work.

REFERENCES

1. Pyta K, Przybylski P, Wicher B, Gdaniec M, Stefańska J. Intramolecular proton transfer impact on antibacterial properties of ansamycin antibiotic rifampicin and its new amino analogues. Org Biomol Chem. 2012; 10: 2385-2388.
2. Cocchietto M, Skert N, Nimis PL, Sava G. A review on usnic acid, an interesting natural compound. Naturwissenschaften. 2002; 89: 137-146.
3. Takai M, Uehara Y, Beisler JA. Usnic acid derivatives as potential antineoplastic agents. J Med Chem. 1979; 22: 1380-1384.
4. Livertox. Clinical and Research Information on Drug-Induced Liver Injury.
5. Sharma RK, Jahnke PJ. Acidity of usnic acid. Indian J Chem.1966; 4: 16-18.
6. Stezowski JJ. Chemical-structural properties of tetracycline derivatives. 1. Molecular structure and conformation of the free base derivatives. J Am Chem Soc. 1976; 98: 6012-6018.
7. Othersen OG, Beierlein G, Lanig H, Clark T. Conformations and tautomers of tetracycline, J Phys Chem B. 2003; 107:13743-13749.

8. Hansen PE. Deuterium Isotope Effects on 13C Nuclear Shiel¬ding of Intra molecular Hydrogen bonded Systems. Magn Reson Chem. 1986; 2: 903-910.

9. Bolvig S, Hansen PE. Isotope Effects on chemical shifts as an analytical tool in structural studies of intra molecularly hydrogen bonded compounds. Curr Org Chem. 2000; 4: 19-54.

10. Gaussian 09, Revision A.1, Frisch M J, Trucks GW, Schlegel HB, Scuseria G, Robb MA. Cheese man J R, Fox D J et al. Gaussian, Inc., Wallingford CT. 2009.

11. Becke AD. Density Functional thermo chemistry. III. The role of exact exchange. J Chem Phys. 1993; 98: 5648-5652.

12. Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B Condens Matter. 1988; 37: 785-789.

13. Bazin MA, Le Lamer AC, Delcros JG, Rouaud I, Uriac P, Boustie J, et al. Synthesis and cytotoxic activities of usnic acid derivatives. Bioorg Med Chem. 2008; 16: 6860-6866.

14. Stephens CR, Murai K, Brunigs KJ, Woodward RB. Acidity constants of the tetracycline antibiotics, J Am Chem Soc. 1956; 78: 4155-4158.

15. Hansen PE. Hydrogen-bonding and Tautomerism studied by Isotope Effects on Chemical Shifts. J Mol Struct. 1994; 321: 79-87.

16. Reuben J. Intra molecular Hydrogen Bonding as Reflected in the Deuterium Isotope Effects on Carbon-13 Chemical Shifts. Correlation with Hydrogen Bond Energies. J Am Chem Soc. 1986; 108: 1735-1738.

17. Hansen PE, Duus F, Bolvig S, Jagodzinski TS. Intra-molecular Hydrogen Bonding of the Enol forms of? -Ketoamides and?-Ketothioamides. Deuterium Isotope Effects on 13C Chemical Shifts. J Mol Struct.1996; 378: 45 - 59.

18. Grech E, Klimkiewich J, Nowicka-Scheibe J, Piertzak M, Schilff W, Pozharski AF, et al. Deuterium Isotope Effects on 15N, 13C and 1H Chemical Shifts of Proton Sponges. J.Mol.Struct. 2002; 615: 121-140.

Cite this article

Hansen PE, Mortensen J, Kamounah FS (2015) The Importance of Correct Tautomeric Structures for Biological Molecules. JSM Chem 3(1): 1014.