ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2015, **51**, 14175

Received 5th April 2015,
Accepted 9th June 2015

DOI: 10.1039/c5cc02831k

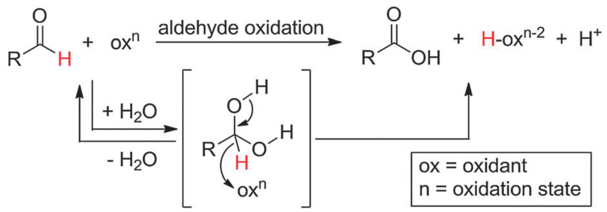
www.rsc.org/chemcomm

Mimicking the active site of aldehyde dehydrogenases: stabilization of carbonyl hydrates through hydrogen bonds†

A. J. K. Roth, M. Tretbar and C. B. W. Stark*

Aldehyde hydrates are important but highly unstable, transient intermediates in biological and synthetic oxidations to carboxylic acids. We here report *N*-oxides as the first class of chemical reagents capable of stabilizing such water adducts. This stabilizing effect (studied in solution and in the solid state) seems to be based on the formation of hydrogen bonds.

Aldehyde dehydrogenases (ALDH's) are highly important enzymes involved in oxidative detoxification processes.[1] They convert toxic endogenous and exogenous aldehydes into less hazardous and more easily excretable (or biosynthetically reusable) carboxylic acids. The molecular mode of action of ALDH's is quite well understood and there are two distinct pathways: a covalent[2] and a non-covalent mechanism.[3–5] Before the oxidation can occur in the non-covalent mechanism, water has to add to the carbonyl group to form the reactive tetrahedral hydrate intermediate (Scheme 1). This pre-equilibrium is crucial for the efficiency of the overall reaction.[3] Most synthetic aldehyde oxidations are believed to proceed through the same hydrate intermediate or an analogue (RC(OH)X) thereof.[6,7] Generally, the addition of water to carbonyl compounds is highly ineffective as the hydrate intermediate is both enthalpically and entropically disfavored.[8] Thus, for most aldehydes even when water is used in large excess or as a solvent, only trace amounts of the aldehyde hydrate can be detected. In fact, for most carbonyl compounds the related hydrate has never been observed. At the active site of ALDH's a three-dimensional array of Lewis-acids and Lewis-bases stabilizes the water adduct.[4] Artificial ligands or reagents to mimic this stabilization have until to date never been reported. Such a hydrate-stabilizing reagent would be of great interest (i) to investigate the properties of as yet unknown carbonyl hydrates and (ii) to mimic the high efficiency of the biological oxidation process in synthetic organic chemistry.



Scheme 1 Common mechanism of aldehyde oxidation through hydrate intermediates.

We recently presented a simple method for a TPAP[9]-catalyzed direct conversion of primary alcohols to carboxylic acids with a large excess of *N*-methylmorpholine *N*-oxide (NMO), where NMO plays a dual role, acting as stoichiometric co-oxidant as well as reagent for the stabilization of the intermediate aldehyde hydrate.[7] We initially suggested that Lewis-basic (but non-Brønsted-basic) reagents such as phosphine oxides, sulfoxides, *N*-oxides and the like should be able to shift the carbonyl/hydrate equilibrium even without the three-dimensional arrangement of an enzyme.[7] *N*-Oxides such as NMO proved to be particularly promising[10] and a stabilization through hydrogen bonds[11] was suggested (Fig. 1). Preliminary support came from simple ^{1}H-NMR studies[7] but the real nature of the interaction remained ambiguous. Different models with different geometries and stoichiometry between the aldehyde hydrate and NMO have been postulated (Fig. 1).[7]

In the search for experimental evidence for the molecular mode of hydrate stabilization, we decided to first investigate the interaction between an aldehyde hydrate and an *N*-oxide in

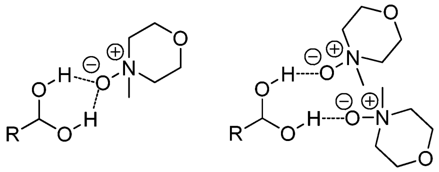


Fig. 1 Previously postulated modes of carbonyl hydrate stabilization *via* hydrogen bonds to *N*-oxides.

Fachbereich Chemie, Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany. E-mail: stark@chemie.uni-hamburg.de

† Electronic supplementary information (ESI) available: Experimental details and crystallographic data. CCDC 1019891 and 1019897. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5cc02831k

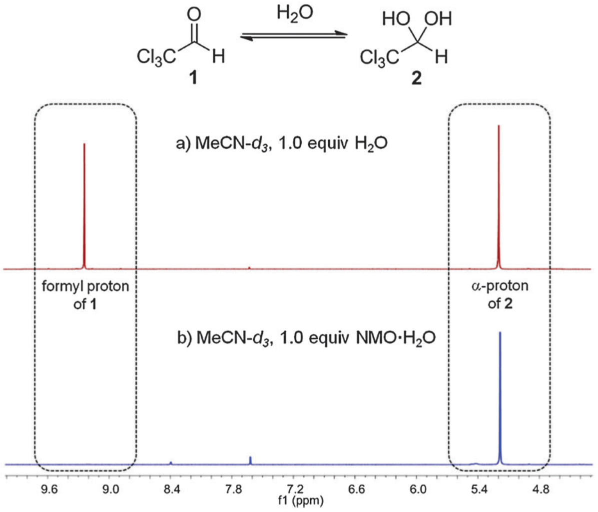


Fig. 2 ^{1}H-NMR spectra of **1** (c = 0.25 M) in MeCN-d_3 with (a) 1.0 equiv. of H_2O; (b) 1.0 equiv. of NMO·H_2O.

solution. Chloral **1** was selected as a model compound as it forms a reasonably stable hydrate that can be studied in solution and also be used for co-crystallization experiments. NMR studies in MeCN-d_3 showed that in presence of 1.0 equiv. of water the equilibrium between chloral **1** and its hydrate **2** lies at 63% hydrate (Fig. 2). On addition of 1.0 equiv. of NMO·H_2O to a solution of chloral **1** in MeCN-d_3 the equilibrium is completely shifted towards the hydrate and not even a trace of the carbonyl compound is detectible (Fig. 2).

To elucidate the stoichiometry of this hydrate stabilizing interaction, we carried out Job Plot-experiments.[12] For this purpose, the variation of the chemical shift of the *N*-methyl group of NMO was monitored by ^{1}H-NMR depending on the composition of a solution of NMO and chloral hydrate at constant overall concentration (Fig. 3). In these experiments the maximum chemical shift is determined at a 1 : 1 ratio of the two components which clearly indicates a 1 : 1 interaction in solution.

Next the situation in the solid state was studied. After some experimentation, investigations to obtain co-crystals[13] from chloral hydrate and NMO were successful. Co-crystals readily formed in large amounts and high quality when cooling a concentrated solution containing both compounds in stoichiometric amounts. The ease of formation of these co-crystals suggests a fairly attractive intermolecular interaction.[14] Moreover, this effect is not limited to NMO as *N*-oxide but is also observed when pyridine *N*-oxide is used. In both cases high quality crystals suitable for X-ray analysis were obtained. Hydrogen bond interactions between the respective two components were determined in the crystal structure resulting in 1 : 1 complexes. These findings are in accordance with the stoichiometry observed in solution for the chloral hydrate–NMO system. The two structures, however, differ quite significantly: with NMO, ring-shaped structures consisting of two NMO and two hydrate molecules are formed (Fig. 4). With the aromatic *N*-oxide on the other hand, linear structures of alternating *N*-oxide and hydrate molecules are detected (Fig. 5). In both cases there are no hydrogen bond interactions between different OH-groups (neither intra- nor intermolecular)[15] but only contacts between the added hydrogen bond acceptor and the two protons of the *gem*-diol. Ortep-plots of the crystal structures are shown in Fig. 4 and 5, respectively. In addition schematic representations of both structures are shown in Fig. 6.

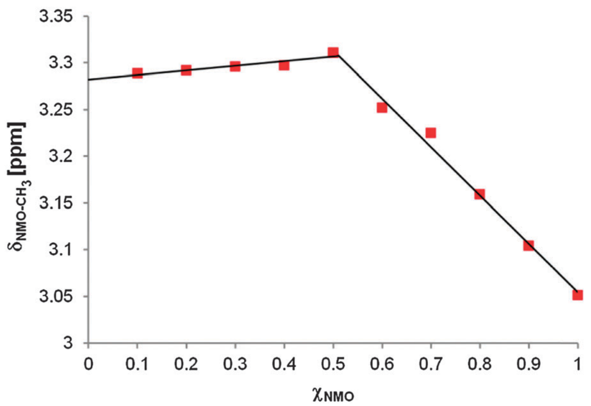
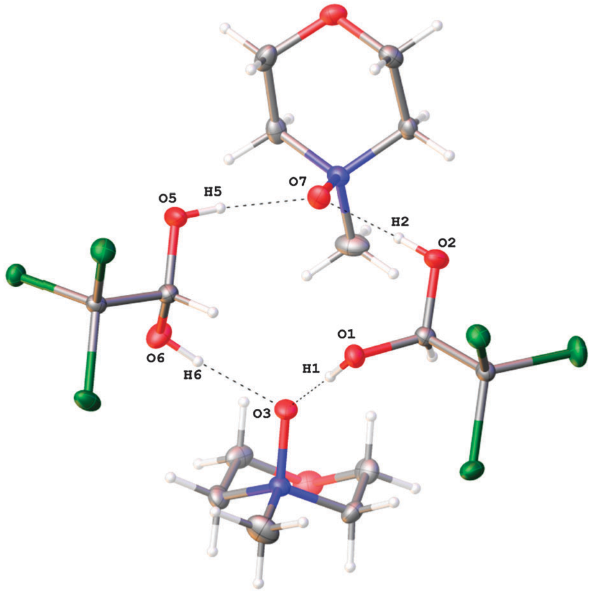


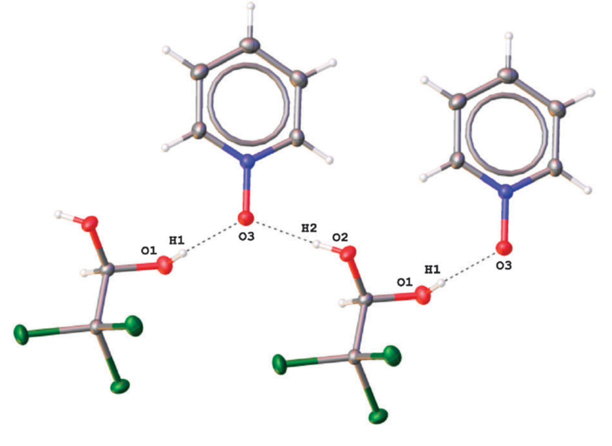
Fig. 3 Job Plot of the system NMO–chloral hydrate in DMSO-d_6 (c = 0.3 M).

According to the classification by Jeffrey,[11] hydrogen bonds can be characterized as strong, moderate or weak depending on the distance between the proton and acceptor (d(H···A), A = acceptor) and the bond angle between donor (D), proton and acceptor (bond angle D–H–A). In the present case, the hydrogen bond interactions found in both complexes are moderate hydrogen bonds concerning their bond lengths and – in most cases – even strong hydrogen bonds with regard to the bond angles (>170°). Selected data from the X-ray analysis of co-crystals are summarized in Table 1 (for details see the ESI†).



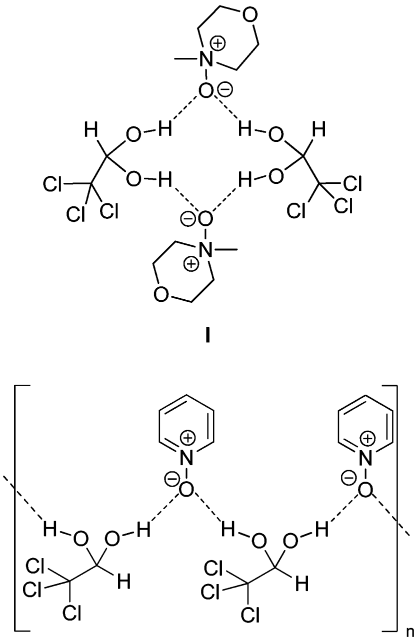
I

Fig. 4 Crystal structure of the NMO–chloral hydrate complex **I**; ellipsoids are drawn at 50% probability.



II

Fig. 5 Crystal structure of the pyridine-*N*-oxide–chloral hydrate complex **II**; ellipsoids are drawn at 50% probability.



I

II

Fig. 6 Schematic representation of the complexes between NMO and chloral hydrate (**I**), pyridine-*N*-oxide and chloral hydrate (**II**).

Table 1 Hydrogen bonds in the NMO–chloral hydrate complex (**I**) and pyridine-*N*-oxide–chloral hydrate complex (**II**)

	H-bond	$d(\mathrm{H}\cdots\mathrm{A})$ (Å)	Angle (D–H–A) (°)
I	O1–H1–O3	1.80	171
	O6–H6–O3	1.80	172
	O5–H5–O7	1.82	174
	O2–H2–O7	1.79	177
II	O1–H1–O3	1.93	159
	O2–H2–O3	1.85	177

In summary we were able to provide experimental evidence for the stabilization of aldehyde hydrates by *N*-oxides *via* hydrogen bonds. A 1 : 1 hydrogen bond interaction between model substrate chloral hydrate and NMO was observed in solution using Job Plot-experiments. The mode of interaction found in the solution phase could be confirmed for the solid state using crystal structures with two different *N*-oxides. These findings uncover for the first time a class of chemical reagents for the stabilization of aldehyde hydrate intermediates and mimic the action of ALDH's (non-covalent mechanism) in this respect. Moreover, these results explain the effect of NMO·H_2O in the TPAP-catalyzed oxidation of primary alcohols to directly produce carboxylic acids.[7,16] We believe that these findings will be relevant in the study of as yet unknown aldehyde hydrates and the development of synthetic protocols for the oxidation of primary alcohols or aldehydes to acids mimicking the efficiency of biological oxidation and detoxification processes. Investigations on structure–activity relationships of the use of different *N*-oxides for aldehyde hydrate stabilization are currently underway.

Financial support of this research by the Fonds der Chemischen Industrie (FCI) is gratefully acknowledged.

Notes and references

1 S. A. Marchitti, C. Brocker, D. Stagos and V. Vasiliou, *Expert Opin. Drug Metab. Toxicol.*, 2008, **4**, 697.

2 For studies on the covalent mechanism involving the formation of a thiohemiacetal with a cystein residue, see: (*a*) S. Sheikh, T. D. Hurley and H. Weiner, *J. Biol. Chem.*, 1997, **272**, 18817; (*b*) S. J. Perez-Miller and T. D. Hurley, *Biochemistry*, 2003, **42**, 7100; (*c*) T. Wymore, D. W. Deerfield and J. Hempel, *Biochemistry*, 2007, **46**, 9495.

3 For studies on the non-covalent mechanism involving an aldehyde hydrate, see: (*a*) K. T. Eisses, *Bioorg. Chem.*, 1989, **17**, 268; (*b*) C. Corbier, F. Della Seta and G. Branlant, *Biochemistry*, 1992, **31**, 12532; (*c*) H. Teng, E. Segura and C. Grubmeyer, *J. Biol. Chem.*, 1993, **268**, 14182; (*d*) L. P. Olson, J. Luo, Ö. Almarsson and T. C. Bruice, *Biochemistry*, 1996, **35**, 9782; (*e*) B. DeLaBarre, P. R. Thompson, G. D. Wright and A. M. Berghuis, *Nat. Struct. Biol.*, 2000, **7**, 238; (*f*) A. Gruez, V. Roig-Zamboni, S. Grisel, A. Salomoni, C. Valencia, V. Campanacci, M. Tegoni and C. Cambillau, *J. Mol. Biol.*, 2004, **343**, 29; (*g*) F. Fan, M. W. Germann and G. Gadda, *Biochemistry*, 2006, **45**, 1979; (*h*) G. Gadda, *Biochemistry*, 2008, **47**, 13745; (*i*) P. Ferreira, A. Hernández-Ortega, B. Herguedas, J. Rencoret, A. Gutiérrez, M. J. Martínez, J. Jiménez-Barbero, M. Medina and Á. T. Martínez, *Biochem. J.*, 2010, **425**, 585.

4 For crystal structures of ALDH's in complex with an aldehyde hydrate at the active site, see: (*a*) J. A. R. G. Barbosa, J. Sivaraman, Y. Li, R. Larocque, A. Matte, J. D. Schrag and M. Cygler, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 1859; (*b*) S. Mukherjee, D. Dutta, B. Saha and A. K. Das, *J. Mol. Biol.*, 2010, **401**, 949; (*c*) F. Salvi, Y.-F. Wang, I. T. Weber and G. Gadda, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2014, **70**, 405.

5 For the role of hydrogen bonds and proton transfer in such enzymatic reactions, see: K. B. Schowen, H.-H. Limbach, G. S. Denisov and R. L. Schowen, *Biochim. Biophys. Acta*, 2000, **1458**, 43. See also ref. 11.

6 In synthetic aldehyde oxidations the X-group in RC(OH)X may be a hydroxide, a hydroxylate or a substituted oxygen atom. For an instructive overview, see: G. Tojo and M. Fernàndez, in *Basic Reactions in Organic Synthesis*, ed. G. Tojo, Springer, New York, 2007. For leading references on mechanistic investigations and practical procedures for the oxidation to carboxylic acids, see: (*a*) F. H. Westheimer and N. Nicolaides, *J. Am. Chem. Soc.*, 1949, **71**, 25; (*b*) K. Heyns and H. Paulsen, *Angew. Chem.*, 1957, **69**, 600; (*c*) J. Roček and C.-S. Ng, *J. Org. Chem.*, 1973, **38**, 3348; (*d*) B. O. Lindgren and T. Nilsson, *Acta Chem. Scand.*, 1973, **27**, 888; (*e*) E. J. Corey and G. Schmidt, *Tetrahedron Lett.*, 1979, **20**, 399; (*f*) P. L. Anelli, C. Biffi, F. Montanari and S. Quici, *J. Org. Chem.*, 1987, **52**, 2559; (*g*) A. E. J. de Nooy, A. C. Besemer and H. van Bekkum, *Tetrahedron*, 1995, **51**, 8023; (*h*) M. Jáky and J. Szammer, *J. Phys. Org. Chem.*, 1997, **10**, 420; (*i*) M. Zhao, J. Li, E. Mano, Z. Song,

D. M. Tschaen, E. J. J. Grabowski and P. J. Reider, *J. Org. Chem.*, 1999, **64**, 2564; (*j*) K. B. Wiberg and F. Freeman, *J. Org. Chem.*, 2000, **65**, 573; (*k*) R. Mazitschek, M. Mülbaier and A. Giannis, *Angew. Chem.*, 2002, **114**, 4216 (*Angew. Chem., Int. Ed.*, 2002, **41**, 4059); (*l*) J. C. Qiu, P. P. Pradhan, N. B. Blanck, J. M. Bobbitt and W. F. Bailey, *Org. Lett.*, 2012, **14**, 350; (*m*) D. Könning, T. Olbrisch, F. D. Sypaseuth, C. C. Tzschucke and M. Christmann, *Chem. Commun.*, 2014, **50**, 5014. See also ref. 7.

7 A.-K. C. Schmidt and C. B. W. Stark, *Org. Lett.*, 2011, **13**, 4164. See also ref. 16.

8 For studies on the formation of aldehyde hydrates and their stabilization, see: (*a*) I. H. Williams, D. Spangler, D. A. Femec, G. M. Maggiora and R. L. Schowen, *J. Am. Chem. Soc.*, 1983, **105**, 31; (*b*) E. J. Meijer and M. Sprik, *J. Am. Chem. Soc.*, 1998, **120**, 6345; (*c*) Y.-H. Hsieh, N. Weinberg, K. Yang, C.-K. Kim, Z. Shi and S. Wolfe, *Can. J. Chem.*, 2005, **83**, 769; (*d*) S. H. Hilal, L. L. Bornander and L. A. Carreira, *QSAR Comb. Sci.*, 2005, **24**, 631; (*e*) S. T. Arroyo, J. A. S. Martín and A. H. García, *J. Phys. Chem. A*, 2007, **111**, 339; (*f*) A. S. Medvedeva, I. V. Mitroshina, A. V. Afonin, K. A. Chernyshev, D. A. Bulanov and A. V. Mareev, *Russ. J. Org. Chem.*, 2013, **49**, 828; (*g*) D. Kaur, R. Kaur and S. Khanna, *Struct. Chem.*, 2014, **25**, 437.

9 W. P. Griffith, S. V. Ley, G. P. Whitcombe and A. D. White, *J. Chem. Soc., Chem. Commun.*, 1987, 1625. For reviews see: (*a*) S. V. Ley, J. Norman, W. P. Griffith and S. P. Marsden, *Synthesis*, 1994, 639; (*b*) P. Langer, *J. Prakt. Chem.*, 2000, **342**, 728.

10 (*a*) E. Maia, A. Peguy and S. Pérez, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1981, **37**, 1858; (*b*) H. Chanzy, E. Maia and S. Pérez, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1982, **38**, 852; (*c*) V. Kabrelian, W. Berger, M. Keck and B. Philipp, *Acta Polym.*, 1988, **39**, 710; (*d*) H.-P. Fink, P. Weigel, H. Purz and J. Ganster, *Prog. Polym. Sci.*, 2001, **26**, 1473, for a review on *N*-oxides, see: (*e*) D. Bernier, U. K. Wefelscheid and S. Woodward, *Org. Prep. Proced. Int.*, 2009, **41**, 173.

11 (*a*) G. A. Jeffrey and W. Saenger, *Hydrogen bonding in biological structures*, Springer, Berlin, 1991; (*b*) G. A. Jeffrey, *An introduction to hydrogen bonding*, Oxford University Press, New York, 1997; (*c*) T. Steiner, *Angew. Chem.*, 2002, **114**, 50 (*Angew. Chem., Int. Ed.*, 2002, **41**, 48); (*d*) G. R. Desiraju, *Angew. Chem.*, 2011, **123**, 52 (*Angew. Chem., Int. Ed.*, 2011, **50**, 52).

12 (*a*) V. M. S. Gil and N. C. Oliveira, *J. Chem. Educ.*, 1990, **67**, 473; (*b*) K. Hirose, *J. Inclusion Phenom. Macrocyclic Chem.*, 2001, **39**, 193; (*c*) E. J. Olson and P. Bühlmann, *J. Org. Chem.*, 2011, **76**, 8406.

13 For investigations on the formation of co-crystals with *N*-oxides, see: (*a*) L. Huang, H. Liang and K.-B. Yu, *J. Chem. Crystallogr.*, 2007, **37**, 523; (*b*) B. Hatano, A. Aikawa, H. Katagiri, H. Tagaya and H. Takahashi, *Chem. Lett.*, 2007, **36**, 1272; (*c*) N. R. Goud, N. J. Babu and A. Nangia, *Cryst. Growth Des.*, 2011, **11**, 1930.

14 J. H. ter Horst, M. A. Deij and P. W. Cains, *Cryst. Growth Des.*, 2009, **9**, 1531.

15 G. M. Brown and H. A. Levy, *Cryst. Struct. Commun.*, 1973, **2**, 107.

16 (*a*) A.-K. C. Schmidt and C. B. W. Stark, *Org. Lett.*, 2011, **13**, 5788, see also: (*b*) A.-K. C. Schmidt and C. B. W. Stark, *Synthesis*, 2014, 3283.