HETEROCYCLES, Vol. 106, No. 2, 2023, pp. 271 - 276. © 2023 The Japan Institute of Heterocyclic Chemistry
Received, 12th December, 2022, Accepted, 28th December, 2022, Published online, 10th January, 2023
DOI: 10.3987/COM-22-14797

ON THE REACTIVITY OF 2-METHYLENE-3-QUINUCLIDINONE IN WATER

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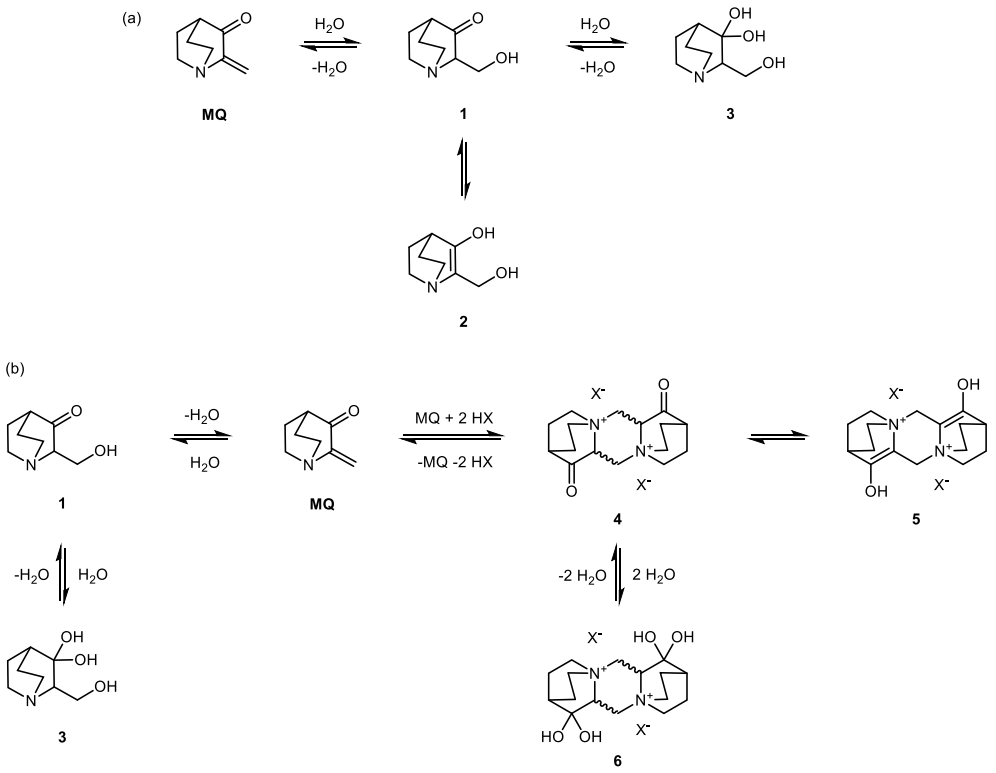
Abstract – The biologically active Michael acceptor 2-methylene-3-quinuclidinone (**MQ**) has a unique chemical reactivity and forms several products upon dissolution in water. With the use of X-ray, NMR and LCMS data the structures of two previously incorrectly characterised compounds are rectified. A complex equilibrium in water, containing novel dimeric species of **MQ**, and its dependence on temperature, pH and concentration is presented.

The unique reactivity of 2-methylene-3-quinuclidinone (**MQ**) has attracted both synthetic[1] and medicinal chemists.[2] As a result of a rigid bicyclic ring system the nitrogen lone pair is forced into a position which nullifies its mesomeric effect on the double bond, leading to a net increase in reactivity by the inductive effect. Upon protonation of the nitrogen the activation of the double bond is even more pronounced. **MQ** is a very reactive Michael acceptor which reacts preferentially with soft nucleophiles and its biological target selectivity stems from selectivity of reactivity rather than through non-covalent interactions. In polar solvents this process is reversible and eventually an equilibrium between reactants and products is formed. Three interconvertible water adducts of **MQ** have been isolated and characterized, suggesting a four-compound equilibrium (Scheme 1a).[1a-c] The hydrochloride salt of compound **3** is offered by several vendors and has been reported to have very low water solubility. With this communication we present results showing that the mixture is more complex (Scheme 1b). In addition, we can correct the structures for two of the previously isolated compounds.

Water adds readily over the double bond to give the water adduct **1**, which can further react with water to form the hydrated ketone **3** if the carbonyl is activated by protonation of the nitrogen. In addition, **MQ** dimerises via two aza-Michael additions forming various dimeric species. These processes are reversible, and the nature of the equilibrium depends on pH, concentration, and temperature.



Scheme 1. (a) Equilibrium based on previously reported **MQ** products in water. (b) Corrected and updated equilibrium of **MQ** in water.

According to literature[1a-c] dissolution of **MQ** in hydrochloric acid (aq.) leads to precipitation of compound **3** as the hydrochloride salt. However, single crystal X-ray diffraction of this material revealed a racemic mixture of compound **6** as the dichloride salt with two crystal waters (Figure 1).[3]

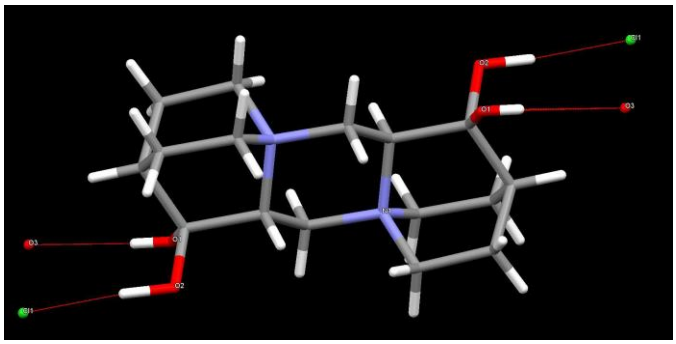
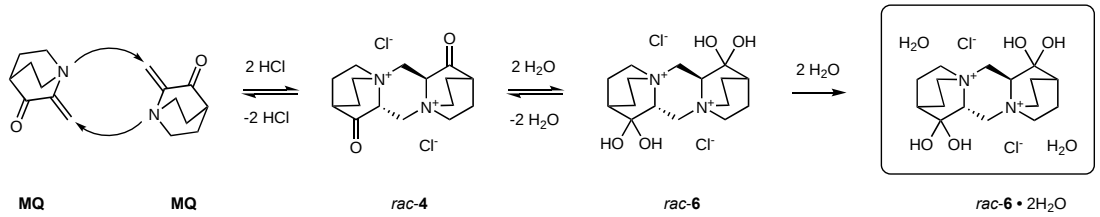


Figure 1. Single crystal X-ray structure of compound **6** including chloride ions (green) and crystal waters (red)

The structure is a symmetric dimer of **MQ** resulting from two aza-Michael additions and subsequent hydration of the ketones (Scheme 2).



Scheme 2. Formation of *rac*-**6** by dimerization of **MQ**, ketone hydration and precipitation of the dichloride dihydrate

LCMS of compound **6** using a pH 4 buffer produced several ions of dimeric structures (Figure 2a). These results prompted further investigation into the nature of the equilibrium of **MQ** in water.

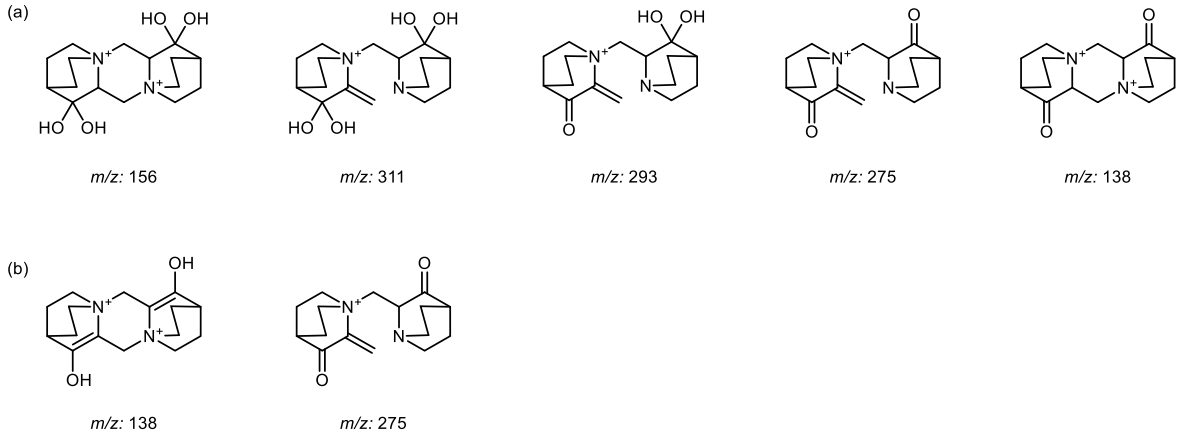


Figure 2. (a) Proposed structures for the major ions detected by LCMS of compound **6** at pH 4. (b) Proposed structures for the major ions of detected by LCMS of compound **5** at pH 9.

Dissolution of **MQ** in water yields a slightly basic solution and addition of acetone or acetonitrile produces a white precipitate that has been reported as compound **2**.[1b] However, NMR and mass spectrometry analyses of this material are consistent with compound **5** rather than compound **2**. Because of the mirror plane of compound **2** carbon 5 and 8, as well as carbon 6 and 7, would be chemically equivalent and have identical shifts (Figure 3).

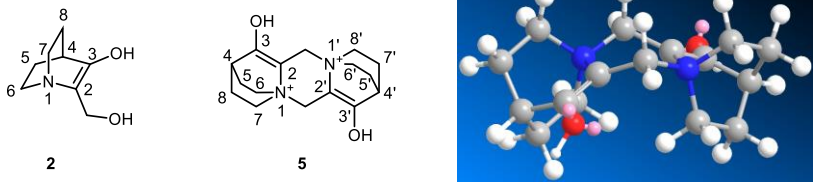


Figure 3. The mirror plane of compound **2** makes carbon 6 and 7, as well as 5 and 8, chemically equivalent. According to the energy minimized structure of compound **5**, carbon 6 and 7, as well as 5 and 8, are chemically non-equivalent.

In contrast, different shifts were seen for carbon 5 and 8, as well as for carbon 6 and 7, although they were not individually assigned (Supporting Information). Energy minimisation of compound **5** produced a non-planar *C*2-symmetric structure with the piperazine ring in a boat conformation where carbons at 5 and 8, as well as 6 and 7, are chemically non-equivalent. The rotational symmetry makes the two carbons in each pair 5/5', 6/6', 7/7' and 8/8' chemically equivalent, explaining the two sets two of signals for all the carbons 5/5', 6/6', 7/7' and 8/8'. Analysis by LCMS using a pH 9 buffer is in line with a dimeric structure (Figure 2b). The same cyclic system has been reported previously.[4]

Compound **1** was prepared by stirring **MQ** in a dilute acetic acid solution followed by basification and recrystallization (Supporting Information).

Attempts to isolate compound **3** were unsuccessful but it was detected by LCMS in an acidic solution. **MQ** was dissolved in pH 4 buffer at room temperature and analysed after 60 minutes by LCMS using a pH 4 buffer as eluent. Two peaks were detected by TIC (Supporting Information). The peak eluting at 3 minutes contained the *m/z* 174 corresponding to $Mw+H^+$ of compound **3**. The peak shape and the presence of *m/z* 156 suggests a rapid equilibrium between compounds **3** and **1**. Compound **6** eluted as the second peak at 6.3 minutes.

NMR studies were performed to further understand the influence of pH, concentration, and temperature on the equilibrium, and to identify additional components. The effect of pH was studied on **MQ** solutions (42 mg/mL) in D_2O in the presence of pH buffers (4, 7.5 and 9). The spectra (^1H, ^{13}C, TOCSY, HSQC and HMBC) were recorded 1 week after mixing to ensure that equilibrium had been reached. Three components were detected at pH 9 and as pH was lowered the equilibrium became more complex due to keto/enol isomerization and hydration of carbonyls (Table 1). For compounds **4** and **6** two isomers were detected, presumably diastereomers with opposite configuration at one of the stereogenic carbons in the piperazine ring. Low levels of additional components were seen at pH 4 and 7.5 but due to small peaks and the complexity of the spectra it was not possible to determine the structures of these low-level components.

Table 1. Component ratios at equilibrium at different temperatures, concentrations, and pH

pH (temp.)	MQ	1	3	4	5	6
9 (25 °C)[a]	1	1.3	-	-	2.2	-
7.5 (25 °C)[a]	1	1.2	-	0.3[c]	4	-
4 (25 °C)[a]	1	1.4	-	1[c]	2	3[c]
4 (5 °C)[b]	-	1	0.78	-	-	0.25[c]

[a] 42 mg/mL MQ. [b] 1.5 mg/mL MQ. [c] Two isomers detected.

MQ (1.5 mg/mL) was dissolved in D_2O containing a pH 4 buffer at 5 °C and spectra were recorded at 5 °C. This led to the disappearance of the signals from **MQ**, **4** and **5** and the appearance of measurable signals of **3**. The protons at carbon 2 and 2' of compounds **1**, **3**, **4** and **6** were never detected by ^{1}H NMR and carbons 2 and 2' appeared as triplets in the ^{13}C NMR spectra because of a very rapid H/D exchange of the α-protons with D_2O.

An updated equilibrium of aqueous solutions of **MQ** is presented and the structures of two previously isolated compounds have been corrected. The complexity of the equilibrium increases as pH is lowered from basic to neutral to acidic due to keto/enol isomerization and hydration of carbonyls. At a lower temperature the addition products are favoured over elimination products as seen by the disappearance of **MQ** and **4** at 5 °C. Moreover, a lower concentration favours monomeric over dimeric **MQ** species.

Compounds **1**, **5** and **6** have been isolated in pure form and compounds **3** and **4** were characterized from mixtures with the other components. Compounds **5** and **6** are insoluble in most solvents but can be dissolved in water and DMSO, however, solution phase analysis on pure material was challenging due to rapid equilibration into the other components. The low water solubility of a polar compound such as **6** suggests very efficient crystal packing.

SUPPORTING INFORMATION

Supplementary (synthesis of the target molecule, ^{1}H spectra, ^{13}C spectra, etc.) data associated with this article can be found, in the online version, at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/27841/106/2

ACKNOWLEDGEMENTS

This work was funded by Aprea Therapeutics.

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3. CCDC 2205553 contains the supplementary crystallographic data for compound **6**. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures.
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