# 2A06

# 赤外分光法による尿酸−メラミン錯体の多重水素結合構造の決定

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# Multiple hydrogen-bonding structures of uric acid−melamine complex identified by IR spectroscopy

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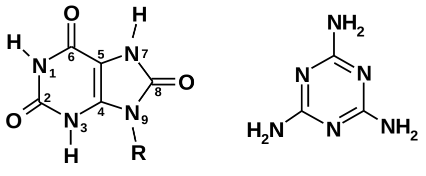
## 1. INTRODUCTION

Melamine (MEL, Chart 1), a nitrogen-rich organic compound, is known to form remarkably stable lattices joined by the network of hydrogen bonds (H-bonds) with cyanuric acid and related compounds. MEL is considered to be safe for its normal uses, but food products that are contaminated with it can be unsafe for consumption. It was illegally used for adulterating foods and feeds to mimic the nitrogen content of protein. Later, this contamination was found to cause a series of food safety incidents. Of these incidents, the most serious was the accumulation of urinary stones in infants ingesting MEL-contaminated formula. Analyses of the stones showed that they are composed of MEL and Uric acid (UA, Chart1) in nearly equimolar amounts, and suggested that 1:1 H-bonded interaction of the two components may be responsible for the stone formation [1,2]. It was also reported that the IR spectra of urinary stones are quite similar to those of mixed crystals of UA and MEL[3].

**Chart 1.**



R=H: UA
R=CH$_3$: 9MUA

MEL

In our preliminary report [4], we employed a laser-desorption (LD)/supersonic jet technique to generate a 1:1 complex of UA and MEL (UA−MEL) in the gas phase and performed its structural analysis using IR spectroscopy. The result suggested that the complex is of non-planar type as stabilized by triple H-bonds. Here, we discuss the nature of these H-bonding interactions in more detail based on IR spectroscopic measurements and theoretical calculations. The result obtained for a binary complex of 9-methyluric acid (9MUA, Chart 1) with MEL is also presented.

## 2. EXPERIMENTAL

The compounds of UA and 9MUA tend to decompose upon heating, and thus vaporized by the LD technique. H-bonded complexes formed by this method were ionized through resonant two-photon ionization using a frequency tunable UV laser, and analyzed by a TOF mass spectrometer. Mass-selected IR spectra were recorded in the region 2500−3700 cm$^{-1}$ by an IR−UV double-resonance scheme. In addition, mixed crystals of UA and MEL were prepared in water and IR measurements were performed using an FT-IR spectrometer.

## 3. RESULTS AND DISCUSSION

Low-energy structures of the UA−MEL complex are shown in Figure 1. The two most stable structures correspond to nonplanar pairs formed by triple H-bonding between UA and MEL. In structure 239, the O2 atom of UA acts as a proton acceptor (A) while the N3H and N9H sites as double donors (D), thus allowing for ADD(UA)−DAA(MEL) type. In previous clinical analyses of urinary stones detected in infants ingesting MEL-contaminated formula, it was speculated that a planar H-bonding structure 678 [1] or 216 [2] is involved in the stone formation.

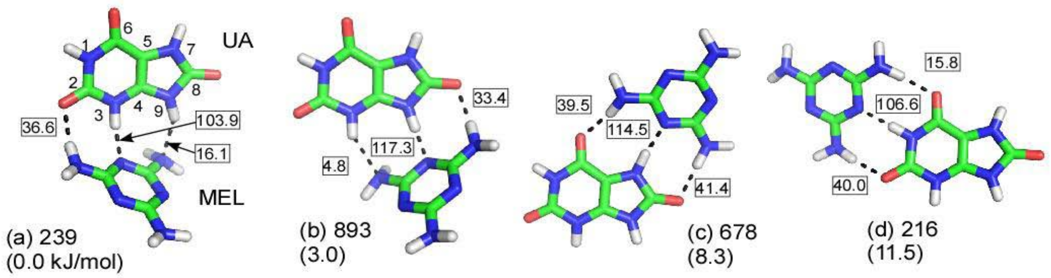


**Figure 1.** Low-energy structures calculated for the UA−MEL complex [M06-2X,SP:CCSD with 6-311++G(d,p)]. Stabilization energy (in kJ/mol) with respect to the most stable structure denoted as 239 is shown in parenthesis. The three digit number indicates the relevant UA sites (either ADD or ADA type), which are H-bonded to MEL. Delocalization energies for H-bonding interactions derived from an NBO analysis are shown in boxes in kJ/mol.

The IR spectrum recorded for the complex of UA−MEL is shown in Figure 2 (top). It is evident that the two bands appearing at 3449 and 3524 cm$^{-1}$ correspond well to the stretching transitions of N1H (orange) and N7H (green) of UA monomer, respectively. The other two free NH stretching transitions of UA, namely N3H and N9H, are absent in the IR spectrum, and thus possible complex geometries are associated with those with these NH sites H-bonded with MEL, namely 239 and 893. By comparing with the calculated IR spectra, the observed complex is assigned to structure 239 [4]. More importantly, a significantly broadened transition appears in the frequency range 2500−2800 cm$^{-1}$, which is a spectral manifestation for strong H-bonding interaction between the two chromophores [5]. This broad band is assigned to the NH stretching transition of the middle H-bond, either N3H in structure 239 or N9H in structure 893.

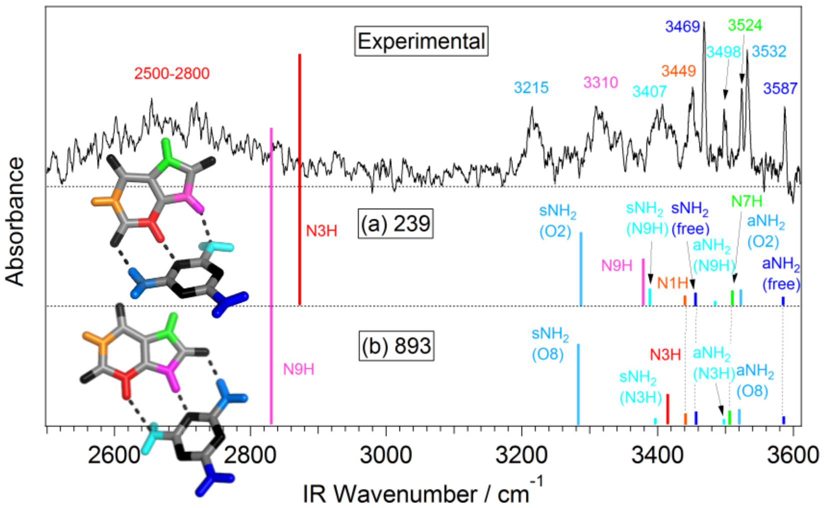


**Figure 2.** (Top) Experimental IR spectrum of the UA−MEL complex. Vibrational frequencies (Scaling factor: 0.955) and IR intensities for nonplanar structures (a) 239 and (b) 893, calculated at the B3LYP/6-311++G(d,p) level.

Such nonplanar structures may be less stable if the N9H site of UA is methylated. Thus, the most stable structures for 9MUA−MEL pairs will be of planar type, analogous to structures 678 and 216 displayed in Figure 1. We will show that the two planar structures are indeed observed for the 9MUA−MEL complex [5].

## REFERENCES

[1] C. G. Skiner *et al*, J. Med. Toxicol. 6, 50 (2010). [2] X. Cong *et al*, J. Clin. Lab. Anal. 27, 59 (2013). [3] F. Grases *et al*, Urology 73, 1262 (2009). [4] H. Asami, S. Urashima and H. Saigusa, The 6th Annual Meeting for Molecular Science (2012, Tokyo) 1B01. [5] H. Asami and H. Saigusa, J. Phys. Chem. B 118, 4851 (2014).