

Supporting Information

Urocanic acid as a novel scaffold for next-gen nature-inspired sunscreens:

I. Electronic laser spectroscopy under isolated conditions

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Contents

1. Experimental procedures	3
1.1 High-resolution laser spectroscopy	3
1.2 Computational methods	4
2. Synthesis and characterization	5
3. Supplemental Figures and Tables	7
4. Supplemental references	11

1. Experimental procedures

1.1. High-resolution laser spectroscopy

In our studies various Resonance Enhanced MultiPhoton Ionization (REMPI) spectroscopic techniques have been employed to record excitation spectra of supersonically cooled molecules, and to determine their electronically excited-state dynamics. The molecular beam setup and laser systems that have been employed to this purpose have been described in detail before.¹ The major difference with the present experiments is in the seeding of the compounds of interest into the supersonic expansion. While previously compounds have been heated to obtain sufficient vapor pressure, we have now employed laser desorption to bring molecules into the gas phase and subsequently cool them.

In our experiments samples was prepared by mixing a compound of interest with carbon powder at an approximate ratio of 2:1. This mixture was gently pressed and coated on a 55 cm EDM-AF graphite bar. In order to desorb physisorbed molecules from the bar, the bar was irradiated with a 1064 nm IR beam from a Polaris pulsed Nd: YAG laser (New Wave Research) operating at 30 Hz and providing 1.5-2.0 mJ pulses. By placing the sample bar in front of the nozzle of a pulsed valve (Amsterdam Cantilever Piezo valve)² with a 300 μm orifice and a conical-shaped front plate with a conical opening with a diameter of 4 mm and an opening angle of 40° and delivering 34 μs pulses, desorbed molecules were picked up and cooled in a supersonic expansion of 6 bar of Ar. During the experiments, the sample bar was translated as to provide a fresh sample at each laser shot. The resulting molecular beam was skimmed with a 2 mm conical skimmer (Beam Dynamics) after which it entered the ionization chamber where laser excitation and ionization took place. Mass-resolved ion detection took place using a reflectron time-of-flight spectrometer (R.M. Jordan Co.).

REMPI excitation spectra have been recorded using a two-color scheme in which excitation of electronically excited states took place with the frequency-doubled output of a Sirah Precision Scan dye laser operating on DCM or Pyrromethene 597 and pumped by a Spectra-Physics Lab 190 Nd: YAG laser, while for ionization of electronically excited molecules, the output of a counterpropagating Neweks PSX-501 ArF (193 nm, 6.42 eV) was employed. Typically, pulse energies of 1.5-2 mJ and 1 mJ were used for excitation and ionization, respectively. In order to determine whether one or more conformers were contributing to the recorded excitation spectra, UV-UV depletion spectroscopy was employed. In these experiments, a probe signal was generated by the previously mentioned excitation laser fixed at a particular resonant wavelength and the ionization laser. This probe signal was subsequently monitored while the frequency-doubled output of a third laser system consisting of a Cobra-Stretch dye laser operating on DCM or Pyrromethene 597 and pumped by a Spectra-Physics Lab 190 Nd: YAG laser was scanned over a wavelength region of interest. Typically, the depletion laser delivered pulses with an energy of 2-4 mJ and was fired 150 ns prior to the excitation and ionization lasers. A final experiment that was performed was to record the decay of electronically excited states. In

these experiments, the ion yield was monitored as a function of the delay time between the excitation and ionization lasers using a delay generator (Stanford Research Systems DG535).

Urocanic acid was purchased from Sigma Aldrich and used without further purification. Methyl urocanate, N₃-methyl methyl urocanate and N₁-methyl methyl urocanate have been synthesized in-house using synthetic routes as described below.

1.2 Computational methods

Geometry optimization of various conformers of urocanic acid and its derivatives followed by calculation of harmonic force fields was performed for the electronic ground state and the lower two electronically excited singlet states using Time Dependent Density Functional Theory (TD-DFT) at the wB97XD /cc-pVDZ level.³ In order to compare with the experimentally recorded spectra, the equilibrium geometries and force fields obtained from these calculations were employed to predict vibrationally resolved excitation spectra using either the Franck-Condon approximation or taking also Herzberg-Teller coupling into account.⁴ A scaling factor of 0.953⁵ was used for the calculated vibrational frequencies. All calculations have been performed with the Gaussian16, Rev. C.01 suite of programs.⁶

2. Synthesis and characterization

Methyl urocanate

To a solution of 1.0 g (7.24 mmol) urocanic acid in methanol (11 mL) was added to anhydrous sodium sulfate (0.15 g) and concentrated sulfuric acid (0.57 mL), after which the reaction mixture was refluxed for 30 h. After cooling the reaction mixture was filtered through a cotton wool plug, and the filtrate was concentrated in a vacuo. The residue was dissolved in 20 mL water, to which 20 mL of a phosphate buffer (pH 7) was added. Next, saturated aqueous NaHCO₃ solution was added until ca. pH 8. This aqueous mixture was extracted with 3 × 40 mL ethyl acetate, and the combined organic layers were dried (Na₂SO₄) and concentrated in vacuo to afford 0.88 g (yield 80%) of the product as a white solid. No additional purification was necessary. ¹H-NMR (CDCl₃, 400 MHz) δ 9.20 (vbs, 1H), 7.72 (s, 1H), 7.62 (d, J = 15.8 Hz, 1H), 7.29 (s, 1H), 6.47 (d, J = 15.8 Hz, 1H), 3.78 (s, 3H); TLC: R_f = ca. 0.1 (silica, 25% NH₄OH/MeOH/CH₂Cl₂ - 0.5/5/95).

N₃-methyl methyl urocanate

53 mg (1.32 mmol) of a 60% dispersion of NaH in mineral oil was washed twice with pentane. To the residue was added dry N, N-dimethylformamide (3 mL) and 0.1 g (0.66 mmol) of (E)-Methyl 3-(1H-imidazole-4-yl)acrylate. After stirring for 5 minutes, iodomethane (39 mL, 0.089 g, 0.63 mmol) was added. The reaction mixture was stirred for 30 min, and TLC indicated almost complete conversion. The reaction mixture was quenched with water, after which phosphate buffer (pH 7) was added. This was extracted with 2 × 100 mL ethyl acetate. To the combined organic layers diethyl ether (150 mL) was added. The resulting mixture was washed with 2 × 50 mL water and 50 mL brine, dried (Na₂SO₄), and concentrated in vacuo. The crude product was purified by flash chromatography (SiO₂, gradient elution with 25% NH₄OH/MeOH/CH₂Cl₂ - 0.1/1/99 → 0.2/2/98 → 0.3/3/97 → 0.4/4/96) to give 35 mg (yield 32%) of the product as a colorless solid. ¹H-NMR (CDCl₃, 400 MHz) δ 7.54 (d, J = 16 Hz, 1H), 7.44 (s, 1H), 7.07 (d, J = 0.8 Hz, 1H), 6.54 (d, J = 16 Hz, 1H), 3.77 (s, 3H), 3.69 (s, 3H); TLC: R_f = ca. 0.3 (silica, 25% NH₄OH/MeOH/CH₂Cl₂ - 0.5/5/95). Note: recrystallization from toluene is possible, increasing the purity.

N₁-methyl methyl urocanate

To a solution of methyl (E)-3-(1-trityl-1H-imidazol-4-yl)acrylate (9.0 g, 22.8 mmol) in a mixture of CH₂Cl₂ (350 mL) and CH₃CN (350 mL) was added dimethyl sulfate (6.5 mL, 8.6 g, 68.5 mmol). After stirring for two days at RT TLC indicated incomplete conversion. Another 4.3 mL of DMS was added. At t = 7 days TLC indicated incomplete conversion, and 2.2 mL dimethyl sulfate was added. At t = 9 days, not complete, 6.5 mL dimethyl sulfate was added. At t = 10 days, not complete, DIPEA (1.0 mL, 0.74 g, 5.7 mmol) and 6.5 mL dimethyl sulfate were added. At t = 16 days, almost complete, 6.5 mL dimethyl sulfate was added. At t = 45 days, the reaction was considered to be complete. The reaction mixture was concentrated in a vacuo, and the residue was suspended in water (500 mL) and methanol (300 mL), this mixture was stirred for 10 days. The reaction mixture was concentrated in vacuo until most of the methanol was distilled off. The remaining liquid was partitioned in 500 mL DCM and 500 mL saturated NaHCO₃ soln. After shaking and separating the aqueous layer was extracted with 2 x

500 mL DCM. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give 9.01 g. This was flash chromatographed (SiO_2 gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}/28\% \text{NH}_4\text{OH}$ – 99/1/0.1 → 98.5/1.5/0.15 → 98/2/0.2 → 97.5/25.5/0.25 → 97/3/0.3 → 96/4/0.4) to afford 2.12 g of the title product as a white solid. ^1H NMR TLC $R_f = 0.3$ (5 MA/D) note the other isomer runs a bit high.

3. Supplemental Figures and Tables

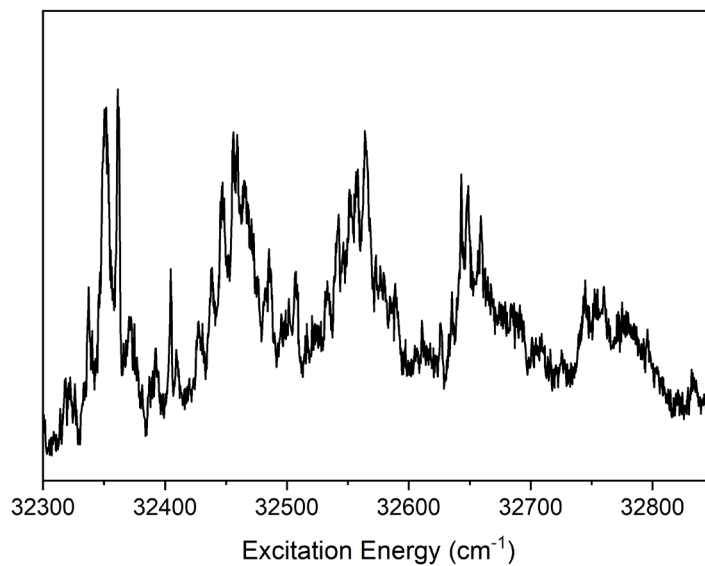


Figure S1. Expanded part of the (1+1') R2PI excitation spectrum of urocanic acid in the region of 32000-32850 cm⁻¹ in the region where both narrow resonances assigned to ${}^1n\pi^* \leftarrow S_0$ transitions, as well as broad resonances assigned to ${}^1\pi\pi^* \leftarrow S_0$ transitions, are observed.

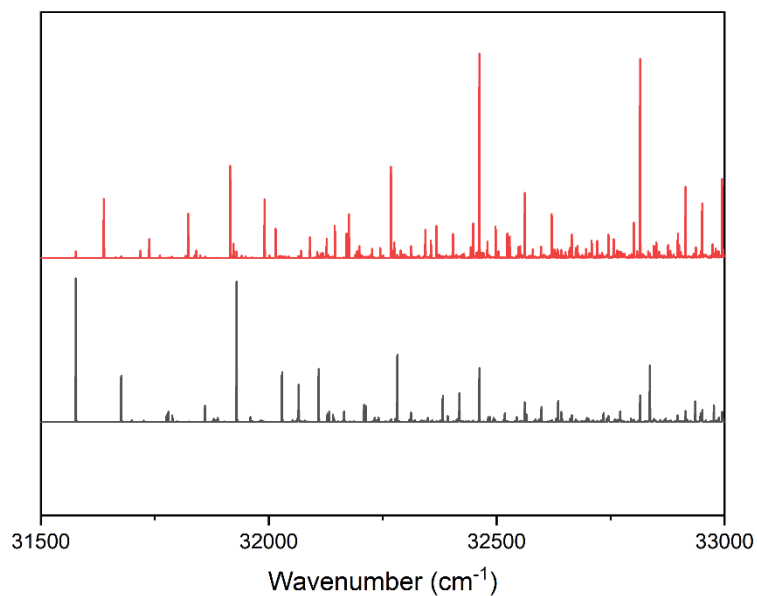


Figure S2. The vibrationally resolved excitation spectrum predicted for the ${}^1n\pi^* \leftarrow S_0$ transition using TD-DFT calculations at the wB97XD/cc-pDVZ level. Red curve: Franck-Condon Herzberg-Teller approximation. Black curve: Franck-Condon approximation. Notice that the black trace has been multiplied by a factor 30.

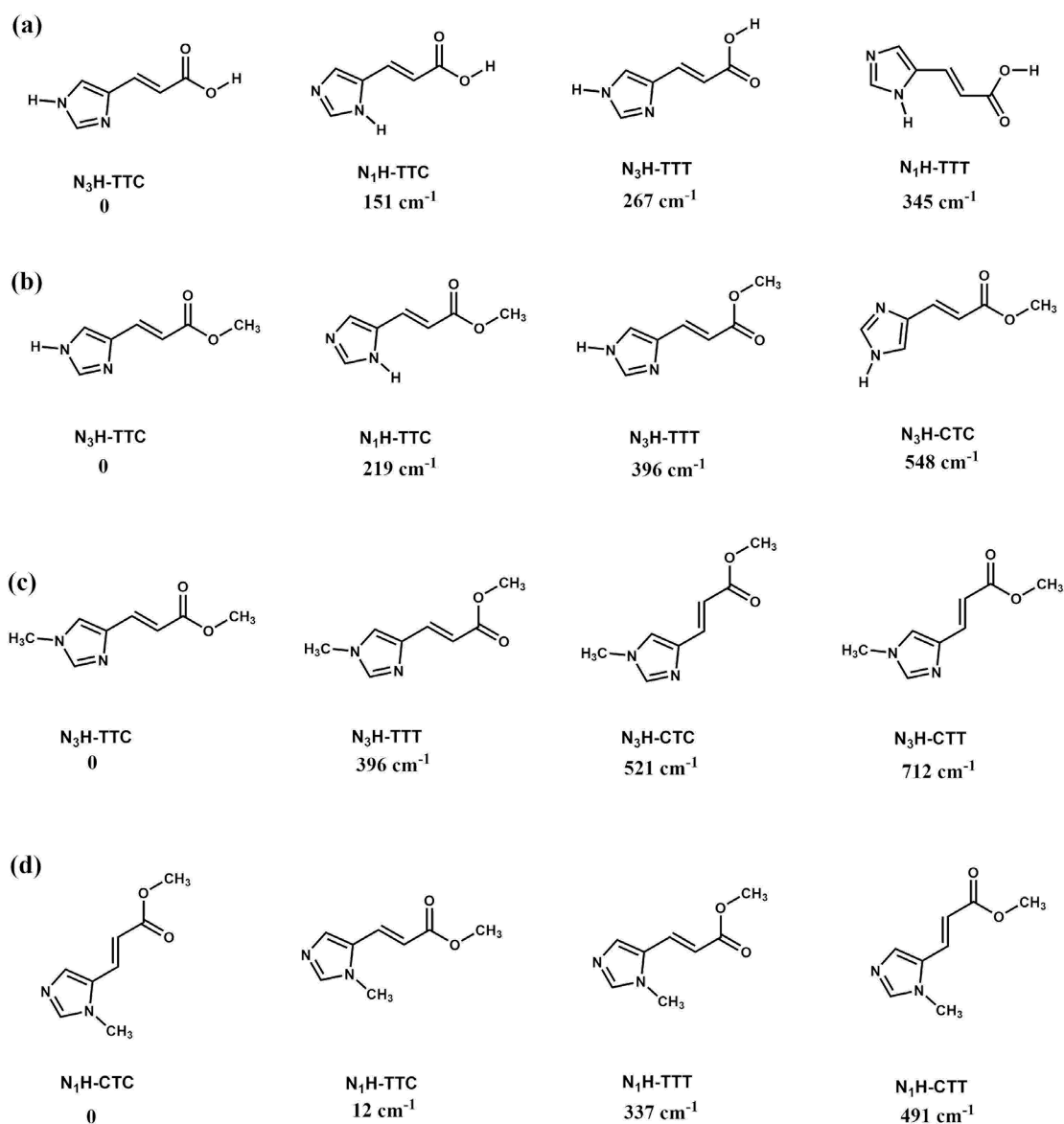


Figure S3. The most stable *trans*- (a) urocanic acid, (b) methyl urocanate, (c) N₃-methyl methyl urocanate, and (d) N₁-methyl methyl urocanate with their relative energies, based on DFT calculations at the wB97XD/cc-pVDZ level.

Table S1. Assignment of higher-intensity bands observed in the ${}^1n\pi^* \leftarrow S_0$ R2PI spectrum of urocanic acid after excitation from the vibrationless level of S_0 for bands up to vibrational excess energies of $\sim 600 \text{ cm}^{-1}$.

Frequency ^a	Intensity (%) ^b	Vibrational assignments	Description
0	100	0^0	
61	400	1^1	Out-of-plane, torsion($O_9=C_8-O_{10}-H_{11}$)
144	150	2^1	$\beta(C_7-C_8-O_{10}-H_{11})$
339	128	3^1	$\beta(C_2-C_6-C_7)$
491	150	1^3	$\beta(C_3-N_4-C_5)$
572	540	$1^2 2^1$	$\beta(C_7-C_8-O_{10})$

^a Frequency with respect to 0-0 band of ${}^1n\pi^* \leftarrow S_0$ transition (31518.6 cm^{-1}).

^b Intensity with respect to intensity of 0-0 band of ${}^1n\pi^* \leftarrow S_0$ transition.

4. Supplemental references

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