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Equilibrium molecular structure of orotic acid from gas-phase electron diffraction data and quantum-chemical calculations

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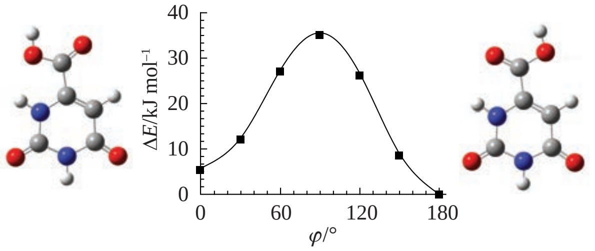
The accurate equilibrium structures of planar *anti* and *syn* orotic acid conformers with different positions of the carboxyl group with respect to the C=C bond were determined from gas-phase electron diffraction data using the dynamic model and taking into account anharmonic vibrational corrections calculated with *ab initio* force field. The high accuracy of coupled-cluster computations was exploited in the analysis of structural effects due to electron-acceptor substituents.



Keywords: equilibrium molecular structures, *syn* and *anti* conformers, pseudo-conformer model, orotic acid, gas-phase electron diffraction, coupled-cluster computations.

Orotic acid (6-carboxyuracil, vitamin B13) is a key precursor in the biosynthesis of pyrimidine nucleobases.[1] However, its molecular structure has not been accurately determined experimentally.[2] Two orotic acid conformers with a *synperiplanar* conformation of the carboxyl group and the *anti* and *syn* positions of a carbonyl group with respect to the C=C bond of the uracil ring were detected in the matrix isolation IR spectra. Their amounts were estimated at 85 and 15%, respectively, from the experimental intensities of bands due to ν(O–H) and ν(N–H).[3] The presence of a negligibly small amount of the further *anti* conformer with the *anticlinal* conformation of the carboxyl group was predicted by quantum-chemical calculations (for instance, 1.8% at 480 K, M06/aug-cc-pVTZ).[3] The aim of this work was to determine the accurate equilibrium structures of the lowest energy orotic acid conformers by gas-phase electron diffraction (GED) and high-level *ab initio* computations.

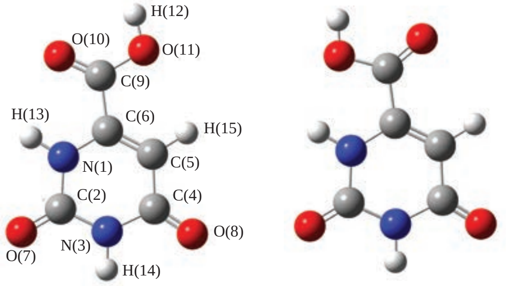
The GED experiment was carried out on an EG-100M apparatus with an accelerating voltage of ~60 kV for the long (LD = 362.28 mm) and short (SD = 193.94 mm) nozzle-to-film distances at a nozzle temperature of 520(3) K.[†] A commercial sample (purity, 98%) was used without further purification. The total electron scattering intensities $I(s)$[†] were obtained by the initial processing of electron diffraction patterns using a standard algorithm.[4]

The quantum-chemical calculations (MP2[5] and B3LYP[6,7]) were carried out using the Gaussian program package,[8] except for the CCSD(T)[9,10] structure optimization performed with the Molpro program.[11,12] According to the MP2 and B3LYP calculations (with the cc-pVTZ basis set[13]), the lowest energy *anti* and *syn* conformers of orotic acid are characterized by the s-*trans* and s-*cis* conformations of the O(10)=C(9)–C(6)=C(5) fragment, respectively; they have the *synperiplanar* carboxyl group and the planar overall structures of C_s symmetry (Figure 1). These conformers are separated by a very high barrier to internal rotation of the carboxyl group around the C(6)–C(9) bond (~ 35.6 kJ mol⁻¹, MP2/cc-pVTZ).[†] The planar structures were also optimized at the CCSD(T) level of theory with all electrons being correlated (ae) in conjunction with the cc-pwCVTZ basis set.[14] The final structure of the CCSD(T)_ae/cc-pwCVQZ quality (Table 1) was estimated using the additivity approximation

$$r_e^{BO} = r_e(\text{CCSD(T)\_ae/cc-pwCVTZ}) + \Delta r_{T\to Q}, \qquad (1)$$

where structural changes due to the enlargement of the basis set from triple- to quadruple-ζ size $\Delta r_{T\to Q} = r(\text{cc-pwCVQZ}) - r(\text{cc-pwCVTZ})$ were estimated at the MP2_ae level.

The harmonic and anharmonic (cubic) force fields, which are required for the calculations of vibrational corrections to the experimental internuclear distances $r_{a,ij}$ in order to derive the semiexperimental equilibrium structure, r_e^{se}, were computed at the MP2/cc-pVTZ level. The total corrections $\Delta r_{ij} = r_{a,ij} - r_{e,ij}^{se}$ including harmonic shrinkages, kinematic and dynamic anharmonicity effects and centrifugal distortions[†] were calculated according to Sipachev.[15–17] An attempt to describe the experimental data with a mixture of two conformers did not lead to satisfactory



anti　　*syn*

Figure 1 Molecular models of two lowest-energy conformers of orotic acid.

[†] See details in Online Supplementary Materials.

Table 1 Equilibrium structures of the *anti* and *syn* conformers of orotic acid from the GED data and *ab initio* calculations (bond lengths in Å and angles in °).

Distances and angles	r_eGED ^{a,b}	r_aGED ^{a}	$r_e^{BO\ c}$	r_eGED ^{a,b}	r_aGED ^{a}	$r_e^{BO\ c}$
		anti			*syn*	
C(2)–N(1)	1.377(5)^3	1.389(5)	1.377	1.379(5)^3	1.391(5)	1.379
N(3)–C(2)	1.380(5)^3	1.390(5)	1.381	1.378(5)^3	1.388(5)	1.378
C(4)–N(3)	1.394(5)^3	1.404(5)	1.394	1.395(5)^3	1.406(5)	1.395
C(5)–C(4)	1.463(3)^4	1.473(3)	1.463	1.462(3)^4	1.471(3)	1.461
C(6)–N(1)	1.371(5)^3	1.382(5)	1.372	1.375(5)^3	1.386(5)	1.375
C(6)=C(5)	1.348(13)^6	1.355(13)	1.347	1.348(13)^6	1.355(13)	1.346
O(7)=C(2)	1.209(1)^5	1.213(1)	1.208	1.209(1)^5	1.213(1)	1.209
O(8)=C(4)	1.212(1)^5	1.215(1)	1.211	1.211(1)^5	1.215(1)	1.211
C(9)–C(6)	1.490(3)^4	1.500(3)	1.490	1.493(3)^4	1.503(3)	1.492
O(10)=C(9)	1.205(1)^5	1.209(1)	1.204	1.199(1)^5	1.203(1)	1.198
O(11)–C(9)	1.337(13)^6	1.347(13)	1.335	1.349(13)^6	1.358(13)	1.347
H(12)–O(11)	0.971(9)^7	0.986(9)	0.966	0.971(9)^7	0.986(9)	0.966
H(13)–N(1)	1.013(9)^7	1.029(9)	1.008	1.011(9)^7	1.027(9)	1.006
H(14)–N(3)	1.014(9)^7	1.030(9)	1.009	1.014(9)^7	1.030(9)	1.009
H(15)–C(5)	1.081(9)^7	1.096(9)	1.076	1.082(9)^7	1.097(9)	1.076
N(3)–C(2)–N(1)	114.0(8)^8		113.4	114.2(8)^8		113.6
C(4)–N(3)–C(2)	127.7(4)^9		128.5	127.7(4)^9		128.4
C(5)–C(4)–N(3)	113.7(6)^{10}		113.3	113.6(6)^{10}		113.3
O(7)=C(2)–N(1)	123.4(11)^{11}		123.3	123.0(11)^{11}		123.0
O(8)=C(4)–C(5)	125.4(11)^{11}		125.4	125.6(11)^{11}		125.6
C(9)–C(6)–N(1)	110.8(7)^{12}		113.1	115.1(7)^{12}		117.4
O(10)=C(9)–C(6)	122.5(4)^{13}		122.4	124.5(4)^{13}		124.5
O(11)–C(9)–C(6)	113.1(4)^{13}		113.0	111.4(4)^{13}		111.4
H(12)–O(11)–C(9)	106.7^d		106.7	106.9^d		106.9
H(13)–N(1)–C(2)	118.1^d		118.1	116.7^d		116.7
H(14)–N(3)–C(2)	115.1^d		115.1	115.2^d		115.2
H(15)–C(5)–C(4)	119.0^d		119.0	119.6^d		119.6

^{a} Values in parentheses are estimated total errors (2.5σ). ^{b} Parameters with equal superscripts were refined in one group; differences between parameters in each group were assumed at the values of the r_e^{BO} structure. ^{c} Computed structure of CCSD(T)_ae/cc-pwCVQZ quality [see equation (1)]. ^{d} Assumed at the computed value.

results (R_f = 4.2%). Alternatively, the model of pseudo-conformers was applied in the GED analysis. The lowest frequency vibration [~67 cm^{-1} (*anti*) and ~57 cm^{-1} (*syn*), MP2/cc-pVTZ] is a pure mode describing the carboxyl group rotation around the C(6)–C(9) bond (a potential energy distribution for torsion in this mode is about 90%). Therefore, it could be separated from other vibrations. The weighting of pseudo-conformers was carried out according to a potential energy function from MP2/cc-pVTZ calculations.† Table 1 summarizes the structural analysis data and the determined structures of *anti* and *syn* conformers. The application of the model of pseudo-conformers reduced the disagreement factor R_f by 0.9% (to 3.3%) due to a strong anharmonicity of the dynamic mode.[18] Figure 2 shows the radial distribution functions obtained by Fourier transformation of the final molecular electron scattering intensities $sM(s)$.†

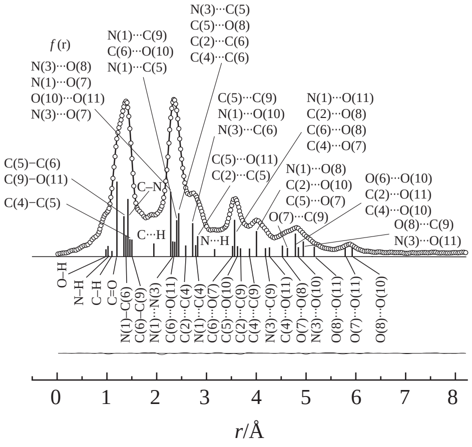


Figure 2 Experimental radial distribution curve $f(r)_{exp}$ (open circles) derived with a damping factor of exp(0.002 s^2) and its theoretical counterpart $f(r)_{theor}$ (solid line) for the dynamic model of orotic acid with vertical bars of molecular terms. Difference curve, $\Delta f(r) = f(r)_{exp} - f(r)_{theor}$.

For both conformers (see Table 1), the computed structures of CCSD(T)_ae/cc-pwCVQZ quality, r_e^{BO}, are consistent with the r_e^{se} ones to within a few thousandths of Å and a few tenths of degree for the bond lengths and angles, respectively. The high accuracy of this quality r_e^{BO} structure was confirmed in many studies.[19–22] The high-accuracy computed structure was exploited in the analysis of fine structural effects due to electron-acceptor substituents. The bond angle in the ring at the carboxyl group $\angle_e^{BO}$ [C(5)=C(6)–N(1)] = 122.6° (*anti* conformer) increased by 0.9° in comparison to this angle in uracil ($\angle_e^{BO}$ [C(5)=C(6)–N(1)] = = 121.7°).[20] Note that the above effect was observed in 5-fluorouracil due to the fluorine substituent. The angle $\angle_e^{BO}$ [C(4)–C(5)–C(6)] in 5-fluorouracil[21] also increased (by 1.5°) with respect to this angle in uracil.[20]

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.01.025.

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