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Nitroxygenation of quercetin by HNO

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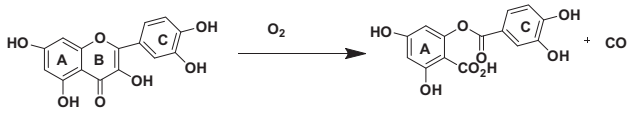
ABSTRACT

The flavonol quercetin undergoes both enzymatic and non-enzymatic reactions with nitroxyl (HNO/NO^-), similar to analogous reactions with dioxygen, but in which N is regioselectively found in the ring-cleaved product. Here we report on kinetic and thermodynamic analysis of the non-enzymatic nitroxygenation reaction in water, which is orders of magnitude faster than the comparable dioxygenation. The second order rate constants were determined from variable temperature reactions, which allowed determination of the reaction activation enthalpy ($\Delta H^{\neq}$ = 9.4 kcal/mol), entropy ($\Delta S^{\neq}$ = −8.3 cal/mol K), and free energy ($\Delta G^{\neq}$ = 11.8 kcal/mol). The determined standard state energy (ΔG^{o}) and activation free energy, as well as the low entropic energy of reaction, are consistent with a proposed single electron transfer (SET) rate determining step.

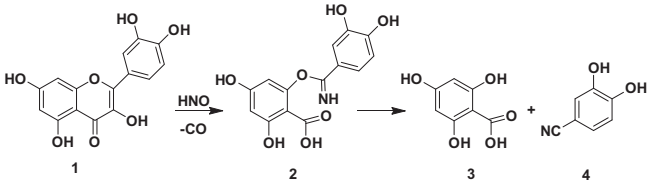
Quercetin and other flavonoids are antioxidants found in fresh fruits and vegetables that have been shown to play an important preventative role in cardiovascular diseases and aging.[1–3] A number of enzymes have been found which decompose flavonoids by reaction with dioxygen, Scheme 1.[4,5] The same reaction occurs non-enzymatically, though typically at higher pH.[4]

In a series of mechanistic studies, the Speier group proposed that in organic solvents the non-enzymatic reaction proceeds by a way of a rate-limiting single electron transfer (SET) between the deprotonated flavonol and oxygen, but in protic solvents a second slower bimolecular reaction competes.[5] Metal complex models for the enzymatic reactions have also been examined by Speier[6] and others.[7,8] In both the free or metal-bound non-enzymatic oxygenations, the reactions are typically observed at temperatures above 70 °C, with reported activation free energies of greater than 24.1 kcal/mol.[5a,6f]

Nitroxyl, HNO, is the reduced and protonated congener of NO, which is isoelectronic with singlet O_2. HNO displays biological effects distinct from that of NO, for example, as an enzyme inhibitor[9] and ionotropic agent that may be used in the treatment of heart failure.[10] Recently, we reported the unprecedented substitution of HNO for dioxygen in the activity of Mn-substituted Quercetin Dioxygenase, Mn-QDO, resulting in the incorporation of both heteroatoms of HNO regioselectively into the product, Scheme 2.[9] In these reactions, HNO is generated in situ from a precursor, and in the presence of enzyme and substrate, and like dioxygenation, cleaves the central *O*-heterocyclic ring to release CO. The reaction likely proceeds through an analogous depsidic product **2**, which



Scheme 1.



Scheme 2.

decomposes to give the observed 2,4,6-trihydroxybenzoic acid and 3,4-dihydroxybenzonitrile products. Importantly, like dioxygenation, a non-enzymatic nitroxygenation of quercetin with HNO proceeds at high pH yielding the same regioselective products, again suggesting the deprotonated quercetinate anion is the dominant reactant.

The coupling of HNO to an enolic carbon center is similar to the so-called nitroso aldol reactions, NA, in which nitroso compounds couple with activated ketones and aldehydes yielding both O- and N-bound adducts, Scheme 3.[11] The early examples of these aldol condensations utilized nitrosobenzene and strongly activated enolates or silyl enol ethers, typically yielding N-bound

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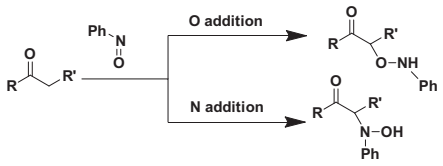

Scheme 3.

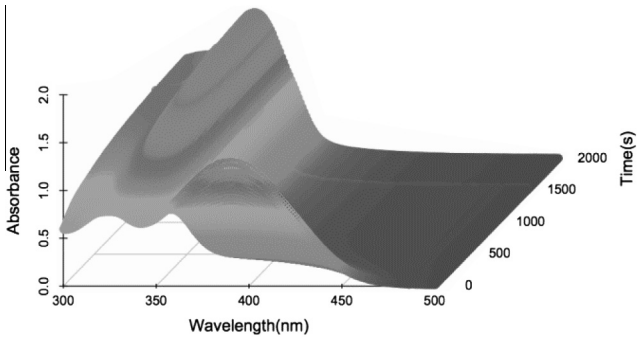

Figure 1. Absorbance spectra obtained over the course of reaction of quercetin (0.04 mM) with HNO donor Angeli's salt (1.00 mM) in pH 8.0 sodium phosphate buffer at 298 K.

Table 1
Temperature dependence of AS decomposition

T (K)	k_1 (s^{-1})[a]	
	HNO	DNO
288	5.0×10^{-4}	4.0×10^{-4}
293	7.0×10^{-4}	6.0×10^{-4}
298	1.8×10^{-3}	1.5×10^{-3}
303	2.6×10^{-3}	2.3×10^{-3}
308	3.4×10^{-3}	2.8×10^{-3}

[a] Experimental variance within 5%.

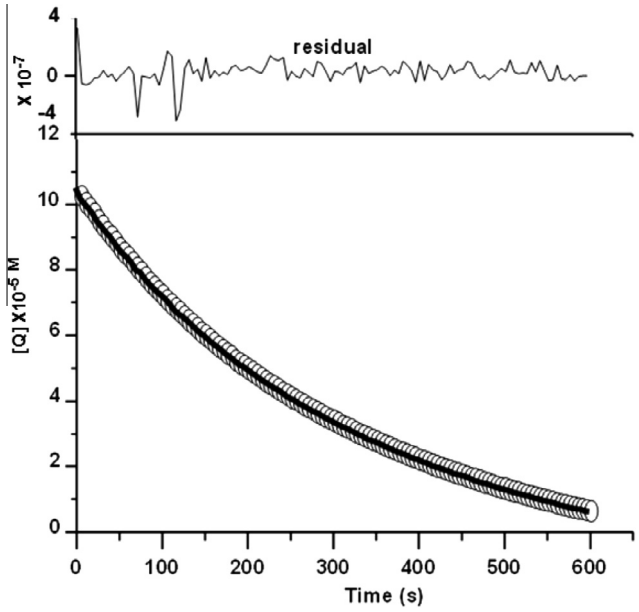

Figure 2. Overlay of modeled (dot) and experimentally-derived (dash) concentrations for a typical reaction, with residual plot above.

Table 2
Temperature dependence of k_3

T (K)	k_3 (M^{-1} s^{-1})[a] × 10^4	
	HNO	DNO
288	0.782 (±0.15)	0.418 (±0.13)
293	1.10 (±0.05)	0.573 (±0.08)
298	1.34 (±0.09)	0.852 (±0.07)
303	1.85 (±0.07)	1.32 (±0.05)
308	2.49 (±0.12)	2.06 (±0.09)

[a] Experimental variance is within 5%, average of three trials (error in parenthesis).

Table 3
Determined activation parameters at 20 °C for Eq. 3

	$\Delta H^{\neq}$ (kcal/mol)	$\Delta S^{\neq}$ (cal/mol K)[a]	$-T\Delta S^{\neq}$ (kcal/mol)	$\Delta G^{\neq}$ (kcal/mol[b])
HNO	9.38 (±0.04)	−8.27 (±0.03)	2.42	11.80
DNO	13.61 (±0.02)	5.07 (±0.04)	−1.48	12.13

[a] From intercepts of the Eyring plots.
[b] From the equation $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$.

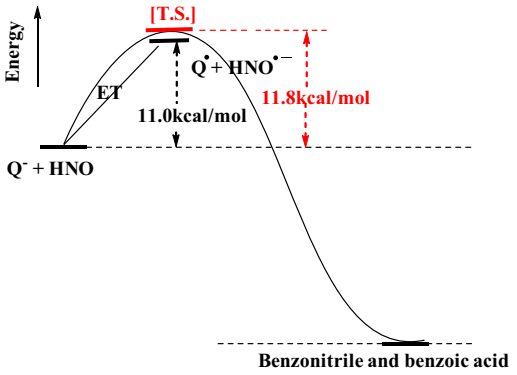

Figure 3. Reaction coordinate diagram for Eq. 3 showing determined activation energy in red, and calculated value for initial outer-sphere electron transfer mechanism.

hydroxyamino products.[12–14] More recent work by Yamamoto and coworkers have shown a much wider scope of NA reactivity,[15] with Lewis-acid catalysis yielding O-bound aminooxy adducts.[16,17] These reactions are proposed to occur through bimolecular nucleophilic attack, rather than outersphere SET.

In this Letter, we investigate the kinetics and thermodynamics of the non-enzymatic reaction to address questions regarding the mechanism of nitroxygenation. Reactions between quercetin and HNO were monitored by the decay of the quercetin absorption band with λ_{max} of 400 nm, as shown in Figure 1. The majority of experiments were run at pH 8.0 in phosphate buffer, conditions at which quercetin ($pK_a = 7.1$)[18] is almost 90% deprotonated and compatible with the use of Angeli's Salt (AS) as a stable source of HNO. Under analogous conditions, no side reaction of quercetin with the byproduct NO_2^-, was seen on the timescale of the measured reactivity.

$$HN_2O_3^- \rightarrow HNO + NO_2^- \quad (k_1) \tag{1}$$

$$HNO + HNO \rightarrow N_2O + H_2O \quad (k_2) \tag{2}$$

$$Q^- + HNO \rightarrow \text{Product} \quad (k_3) \tag{3}$$