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N,*N*′-Dimethylformamide-Derived Products from Catalytic Oxidation of 3-Hydroxyflavone

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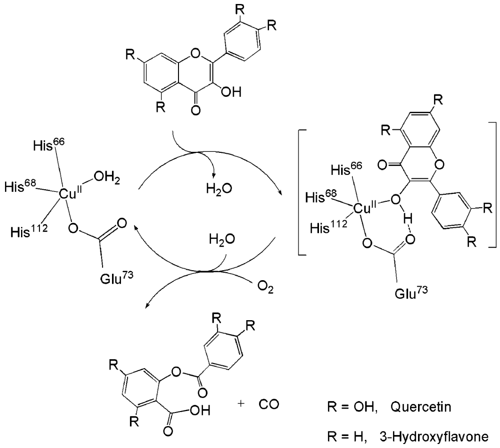
The biomimetic conversion of 3-hydroxyflavone in the presence of a copper(II) catalyst, dioxygen, and *N*,*N*′-dimethylformamide to oxidation products as well as two previously unreported solvent-derived products is seen. The two solvent-derived products were characterized, and their crystal structures were determined.

The development of small molecules for the purpose of biomimetic oxidations has been an area of active study.[1] Such catalysts may be employed for new synthetic transformations as well as bioremediation of aromatic and heteroaromatic waste products.[2] In addition, these compounds may potentially shed light on mechanisms at play for various metalloenzymes.

Quercetin 2,3-dioxygenase (2,3-QD) is a member of the cupin superfamily, which is a group of proteins possessing a common fold.[3] 2,3-QD is responsible for degradation of quercetin (3,4,5,7-tetrahydroxyflavonol) via coordination of the substrates to the copper active site and subsequent oxidative cleavage to yield the corresponding depside (phenolic ester 2-protocatechuoylphloroglucinol carboxylic acid) and carbon monoxide. During the reaction, dioxygen is consumed (Scheme 1).

The copper environment in 2,3-QD from *Aspergillus japonicus* has been investigated by X-ray crystallography[4,5] and electron paramagnetic resonance (EPR).[6] The 1.6 Å resolution crystal structure revealed that the metal is primarily bonded to three histidine nitrogens (His-66, His-68, His-112) and a water molecule in a distorted tetrahedral fashion. A minor mixed trigonal-bipyramidal/square-pyramidal geometry is also present. In the latter, the water is positioned in the equatorial plane and Glu-73 is additionally coordinated. X-ray studies have revealed that anaerobic binding of the flavonol substrate results in ordered copper(II) coordination with displacement of the water molecule by a deprotonated hydroxy group of flavonol and additional coordination by Glu-73.[4] In these studies, it is suggested that the carboxylate group of Glu-73 may serve as a stabilizing group for the bound substrate (Scheme 1). It has also been indicated that deletion of this group results in an inactive enzyme.[6]

Scheme 1



Previously, the dioxygenation of 3-hydroxyflavone using various copper catalysts was reported.[7] In these studies, 3-hydroxyflavone, in the presence of dioxygen and *N*,*N*′-dimethylformamide (DMF) solvent, was converted to the corresponding carboxylic acid ester and carbon monoxide (eq 1). The carboxylic acid ester is further converted under

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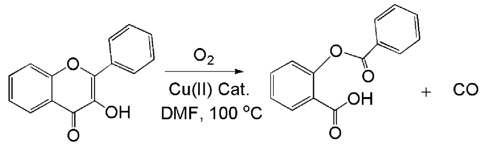
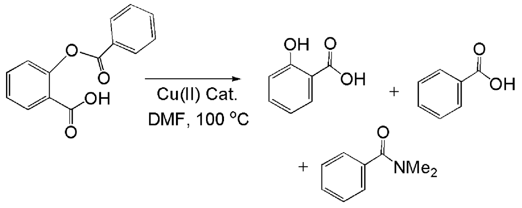


(1)



(2)

reaction conditions to yield benzoic acid, salicylic acid, and *N*,*N*-dimethylbenzamide (eq 2).

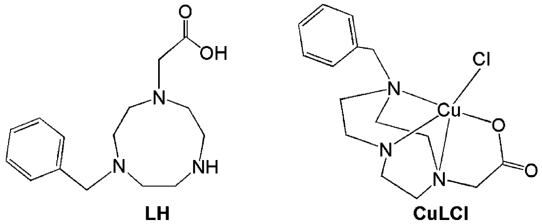


Figure 1. Structure of 1-benzyl-4-acetato-1,4,7-triazacyclononane (LH) and its copper(II) complex (**1**, CuLCl).

In an earlier report, it was determined that the addition of carboxylates to N-coordinated copper(II) catalysts accelerated the oxidation of 3-hydroxyflavone.[8] A copper(II) complex supported by an N-donor chelating ligand possessing a pendent carboxylate group has also been employed as a 2,3-QD model compound.[9] In the present study, we have employed CuLCl (**1**; Figure 1)[10] as a model compound for 2,3-QD. Compound **1** consists of copper(II) bonded to a three nitrogens and one carboxylate ligand (LH; Figure 1) along with a chloride.

Using an assay similar to that employed by Speire et al.,[7b] our studies demonstrated that heating solutions of 3-hydroxyflavone in the presence of **1** also resulted in oxidation of the substrate. The conversion of 3-hydroxyflavone was monitored by the disappearance of the substrate band at 345 nm (Figure S1 in the Supporting Information). Although in previous studies[7e] it was determined that 2,3-QD model studies required temperatures at ~100 °C, we found that **1** was active at lower temperature (Figure S2 in the Supporting Information), with a turnover rate of 6 h^{-1} at 90 °C. Of the solvents tested, conversion of the substrate was only observed with DMF, *N*,*N′*-diethylformamide, and *N*,*N′*-dimethylacetamide, while the substrate was unaffected with dimethyl sulfoxide, CH_3CN, methanol, formamide, or water as the sole solvent. In addition, oxygen served a critical role in this reaction because no oxidation was observed in its absence while the conversion could be accelerated by increasing the oxygen flow rate.

To analyze products generated from the above-mentioned studies, the reactions were monitored by gas chromatography/mass spectrometry (GC/MS) and UV−vis spectroscopy. In a typical reaction, 300 mg (1.26 mmol) of 3-hydroxyflavone was dissolved in 450 mL of freshly distilled DMF and the reaction was heated to 100 °C. The catalyst (0.10 mmol) was dissolved in water (3.0 mL) and added to the reaction mixture. A condenser was attached, and oxygen was bubbled into the reaction mixture at a constant rate (75 mL min^{-1}). Small aliquots were removed periodically and analyzed.

The identity and quantity of species present in the reaction mixture were determined as a function of time (Figure S3 in the Supporting Information). From this information, it is apparent that the substrate is completely oxidized in just over 5 h along with the formation of a new product (**2**, fw = 281), benzoic acid, salicylic acid, *N*,*N′*-dimethylbenzamide, and 2-[bis(dimethylamino)methoxy]benzoic acid. Noticeably, the carboxylic ester product (eq 1) is missing; however, hydrolytic products (salicylic and benzoic acid) as well as *N*,*N′*-dimethylbenzamide (presumably a product of solvent-derived $HN(CH_3)_2$ and the carboxylic acid ester) are observed. The formation of **3** (fw = 253) is closely correlated with the disappearance of product **2** (Figure S3 in the Supporting Information). Using GC/MS (high-resolution, chemical ionization mode), **2** and **3** were identified as having formulas $C_{17}H_{15}NO_3$ and $C_{16}H_{15}NO_2$, respectively. To gain further insights into the identity of **2** and **3**, we monitored large-scale reactions by GC/MS and quenched the reaction (by quickly cooling the reaction mixture to 0 °C) on two separate occasions when **2** and **3** each reached their highest yield (~50% by mass relative to the substrate). Water was then added to the reaction mixture, and the product was extracted with hexanes. Isolation of the crude product followed by crystallization (slow cooling of a warm hexanes solution) afforded X-ray-quality crystals[11] of **2** and **3** in 14% and 32% yield, respectively.

Compound **2** is 3-(dimethylamino)-3-phenyl-3*H*-chromene-2,4-dione, and the structure is shown in Figure 2. Elemental analysis and NMR of the bulk material confirmed the homogeneity of the isolated compound (see the Supporting Information). A substantial rearrangement is observed relative to 3-hydroxyflavone. The phenyl group is shifted to an adjacent carbon, and a dimethylamino group has been incorporated. Overall, this product is oxidized by two electrons relative to the substrate.

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(11) Crystallographic data were collected on a Siemens SMART Platform CCD diffractometer (Mo Kα). **2**: $C_{17}H_{15}NO_3$ (281.30), orthorhombic, space group $P2_12_12_1$, a = 8.7252(13) Å, b = 11.5021(17) Å, c = 13.944(2) Å, V = 1399.4(4) $Å^3$, Z = 4, μ = 0.092 mm^{-1}, D = 1.335 Mg m^{-3}, $F(000)$ = 592. Final R indices (192 parameters) for 1441 observed reflections [$I > 2\sigma(I)$] are R1 = 0.0482 and wR2 = 0.1138, and those for all unique reflections are R1 = 0.0575 and wR2 = 0.1185. **3**: $C_{16}H_{15}NO_2$ (253.29), triclinic, space group $P\bar{1}$, a = 6.9123(6) Å, b = 9.3536(8) Å, c = 11.0002(9) Å, α = 82.5890(10)°, β = 72.4300(10)°, γ = 69.2680(10)°, V = 633.94(9) $Å^3$, Z = 2, μ = 0.088 mm^{-1}, D = 1.327 Mg m^{-3}, $F(000)$ = 268. Final R indices (174 parameters) for 2574 observed reflections [$I > 2\sigma(I)$] are R1 = 0.0345 and wR2 = 0.0820, and those for all unique reflections are R1 = 0.0459 and wR2 = 0.0881.

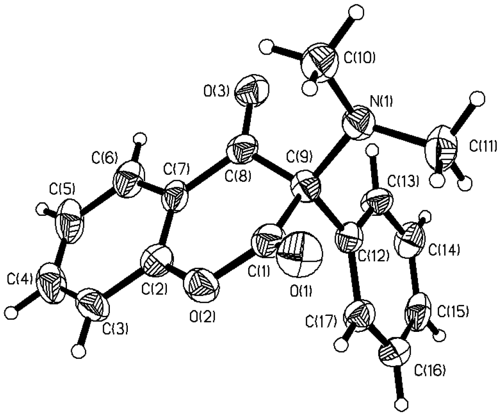


Figure 2. ORTEP representation (50% probability) of **2**. Selected bond lengths (Å) and angles (deg): C(1)−O(1) 1.211(4), C(1)−O(2) 1.366(4), C(1)−C(9) 1.517(5), C(8)−O(3) 1.194(4), C(8)−C(9) 1.516(5), C(9)−N(1) 1.457(5), C(9)−C(12) 1.553(5); O(1)−C(1)−C(9) 123.7(3), C(1)−C(9)−C(8) 111.3(3), O(3)−C(8)−C(9) 122.6(3), C(8)−C(9)−N(1) 108.7(3), C(1)−C(9)−N(1) 114.2(3), C(12)−C(9)−C(1) 108.7(3), C(12)−C(9)−N(1) 110.4(3), C(12)−C(9)−C(8) 102.8(3).

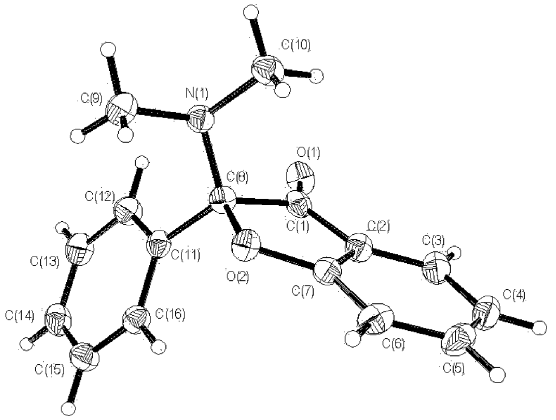
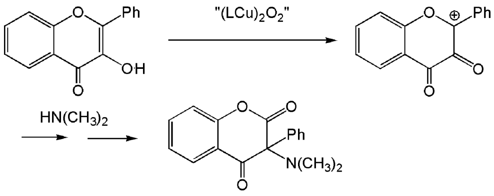


Figure 3. ORTEP representation (50% probability) of **3**. Selected bond lengths (Å) and angles (deg): C(1)−O(1) 1.2136(15), C(1)−C(8) 1.5612(17), C(8)−O(2) 1.4867(14), C(8)−N(1) 1.4312(16), C(8)−C(11) 1.5203(17); O(1)−C(1)−C(8) 123.66(11), C(1)−C(8)−O(2) 103.61(9), C(1)−C(8)−N(1) 111.65(10), C(1)−C(8)−C(11) 107.97(10), O(2)−C(8)−N(1) 111.10(9), O(2)−C(8)−C(11) 108.78(9).

Scheme 2



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Compound **3** is 2-(dimethylamino)-2-phenylbenzofuran-3(2*H*)-one, and the structure is shown in Figure 3. Elemental analysis and NMR also confirmed the homogeneity of this isolated material. Compound **3** differs from **2** by the loss of a carbon monoxide group, giving a five-membered heterocyclic core.

The formation of **2** from 3-hydroxyflavone suggests the involvement of a radical process. Indeed, the addition of a 15-fold excess (relative to **1**) of DMPO to the reaction mixture completely inhibits the oxidation of 3-hydroxyflavone. Aqueous DMF has been reported to serve as a reducing agent for copper(II),[12] silver(I),[13] and gold(III)[14] when heated anaerobically. To test if the reduction of **1** and subsequent oxygenation is responsible for the formation of an oxidizing species, we heated an aqueous solution of **1** at 100 °C in DMF under dinitrogen for 48 h and monitored the solution by EPR before and after heating (Figure S4 in the Supporting Information). The results confirm that copper(I) is quantitatively generated and subsequently reoxidizes upon exposure to air. These experiments immediately suggest a route for generation of an oxidizing species (i.e., "$(LCu)_2O_2$", eqs 3 and 4).[15]

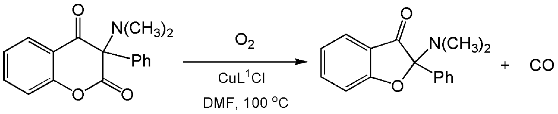
$$2LCu^{II} + HC(O)N(CH_3)_2 + H_2O \rightarrow 2LCu^{I} + CO_2 + (CH_3)_2NH \quad (3)$$

$$2LCu^{I} + O_2 \rightarrow \text{“}(LCu)_2O_2\text{”} \quad (4)$$

The proposed steps for the formation of **2** from 3-hydroxyflavone are shown in Scheme 2 and are based on the observation that no oxygens in **2** are derived from dioxygen or water (from $^{18}O_2$ and $H_2{}^{18}O$ experiments).

In the proposed steps, 3-hydroxyflavone is oxidized to a ketone−carbocation intermediate by "$(LCu)_2O_2$". Subsequent formation of an iminium species followed by phenyl group shift could lead to product **2**.

Using isolated **2**, it was determined that conversion of **2** to **3** proceeds with the liberation of 1 equiv of CO (eq 5)[16] and requires DMF, dioxygen, and the catalyst as well as heat. It is likely that this process is also radically driven.



(5)

The results of this study demonstrate that DMF solvent is an active participant in the biomimetic oxidation of 3-hydroxyflavone by CuLCl. The reaction likely involves a free-radical process for both conversion of the substrate to **2** and conversion of **2** to **3**. Future studies will be aimed at elucidating the steps involved in the formation of **2** in this reaction.

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Supporting Information Available: Oxygenation procedure and characterization data for compounds **2** and **3** (PDF) and crystallographic information (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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