New Insights into the Acid-Promoted Reaction of Caffeic Acid and Its Esters with Nitrite: Decarboxylation Drives Chain Nitrosation Pathways toward Novel Oxime Derivatives and Oxidation/Fragmentation Products Thereof

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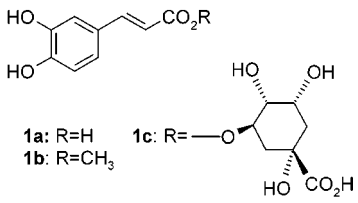
In 0.05 M acetate buffer, pH 4, containing 1% methanol, caffeic acid (**1a**) (2×10^{-3} M) reacted smoothly with nitrite (NO_2^-) (4×10^{-3} M) to afford as main products the novel 2-hydroxy- and 2-methoxyaldoximes **7a**,**b**, the 2-oxoaldoxime **9a**, 3,4-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, and the known furoxan **3c** and benzoxazinone **4b** in smaller amounts. At lower **1a** concentration (e.g., 1×10^{-4} M), **7a** was the main product, whereas with 0.1 M **1a** and 0.5 M NO_2^- **3c** and **9a** were prevailing. At pH 2, **7a** was still the most abundant product, together with 3,4-dihydroxybenzaldehyde and some **9a**, whereas at pH 1 **9a** and 3,4-dihydroxybenzaldehyde were formed in higher yields. No evidence for ring nitration products, including the previously reported 4,5-dihydroxy-2-nitrobenzaldehyde, was obtained. At 2×10^{-3} M concentration and at pH 4, caffeic acid methyl ester (**1b**) reacted with NO_2^- chiefly via ring nitration and/or dimerization to give **5a**, the novel nitrated neolignan derivative **10**, and the parent **6**. Chlorogenic acid (**1c**) afforded only the ring nitrated derivative **5b**. A unifying mechanism for the reaction of **1a** and its esters with NO_2^- is proposed involving reversible formation of nitroso intermediates via chain nitrosation at the 2-position of the (*E*)-3-(3,4-dihydroxyphenyl)propenoic system. In the case of **1a**, decarboxylation would drive the nitroso intermediates toward the formation of oximes **7a**,**b** and **3c**, reflecting nucleophilic addition of water, methanol, and NO_2^-, and their oxidation or breakdown products, viz. **9a**, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, and the benzoxazinone **4b**. In the case of esters **1b**,**c**, to which decarboxylation is precluded, ring nitration or dimerization become the favored routes, triggered by preliminary oxidation at the catechol moiety.

Introduction

Epidemiological[1] and biomedical data[2,3] that accrued over the past decades consistently supported a chemopreventive role of caffeic acid ((*E*)-3-(3,4-dihydroxyphenyl)propenoic acid, **1a**) and other related polyphenolic dietary constituents (e.g., chlorogenic acid, **1c**) widely found in vegetables, fruits, cereals, coffee, and honeybee propolis,[4] in the development of gastric and colorectal tumors.



There is general consensus that the antimutagenic and anticancer effects of **1a** and its congeners stem at least in part from their potent nitrite (NO_2^-) scavenging properties, suggesting an inhibitory role in acid-dependent mutagenic *N*-nitrosamine formation in the stomach. In simulated gastric juice, **1a** was reported to react rapidly and completely with an equimolar quantity of NO_2^-, blocking the elevation of serum *N*-nitrosodimethylamine in rats receiving aminopyrine and NO_2^-.[5] Comparative studies indicated that **1a** was much more effective than its esters, e.g., **1c**,[6] and other related compounds, such as ferulic acid and *p*-coumaric acid[7] as scavenger of NO_2^- and inhibitor of nitrosamine formation, yet the causes for such differences were not addressed at the chemical level.

Although the emphasis in all those studies was on the beneficial consequences of the interaction of **1a** with

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NO_2^-, scattered reports in the literature indicated that **1a** and its derivatives may cause genotoxicity upon nitrosation in the Ames tester strain TA100[8] and may enhance nitrosation and nitrosamine formation in the gastric juice.[9,10] In the latter studies, the balance of stimulating versus suppressive effects on nitrosation processes was found to be critically dependent on the concentration of **1a** and individual features of the gastric juice.

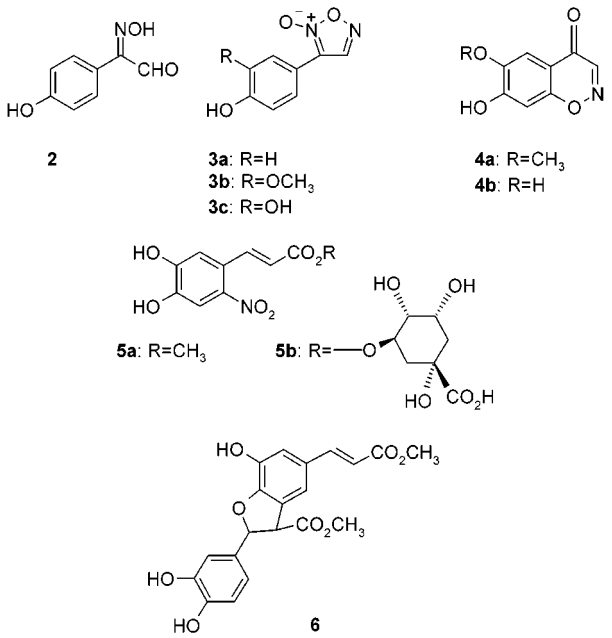
Despite the unabated interest raised by the interaction of **1a** with NO_2^-, elucidation of the underlying chemistry has been hurdled by the intrinsic complexity of the reaction pathways and the elusive character of the products. Comparative investigations[11] of the reaction of **1a** and **1c** with NO_2^- in acetate buffer at pH 4 revealed markedly different spectrophotometric courses, although no explanation was offered. Indirect insights derived from two related studies on the reactions of *p*-coumaric acid and ferulic acid with NO_2.[12−14] These indicated substantial conversion to decarboxylation and chain breakdown products, such as benzaldehydes, 2-(4-hydroxyphenyl)-oximinoacetaldehyde (**2**), furoxans (**3a**,**b**), 2-methoxy-4,6-dinitrophenol, and the unusual 7-hydroxy-6-methoxy-1,2-(4*H*)benzoxazin-4-one (**4a**).

With a view to filling this longlasting gap in the chemistry of caffeic acid derivatives, we undertook a detailed investigation into the main products formed by reaction of **1a** with NO_2^-, in comparison with the methyl ester **1b** and **1c**, under mildly acidic conditions mimicking those occurring in the stomach. When the study was being completed, a paper appeared reporting preliminary data on the reactions of **1a**−**c** with acidic NO_2^-.[15] At pH 2, **1a** was shown to react at the propenoic acid chain to give the furoxan **3c**, the 1,2-(4*H*)-benzoxazin-4-one **4b,** and 4,5-dihydroxy-2-nitrobenzaldehyde, the latter denoting ring nitration, whereas **1b** and **1c** were converted into their nitro derivatives **5a** and **5b**, respectively, as the sole products. Under more acidic conditions (pH 1), both **1a** and **1b** were reported to give dark polymeric precipitates; in the case of **1b**, ethyl acetate extraction of the reaction mixture led to the isolation of the dihydrobenzofuran lignan **6**, an antimitotic agent inhibiting tubulin polymerization.[16]

Because of certain inconsistencies and discrepancies between these and our results, we were then prompted to extend the study to a reexamination of the same reactions under the conditions described by the previous authors.[15]

Results and Discussion

Acid-Promoted Reaction of 1a with NO_2^-. In a first series of experiments **1a** (2×10^{-3} M) was reacted with



NO_2^- (2 molar equiv) in acetate buffer containing 1% methanol at pH 4. Under such conditions, reversed-phase HPLC analysis indicated a complex mixture of products, most of which eluted faster than **1a**,while only two displayed higher retention times. These latter proved to be the furoxan **3c** and the benzoxazinone **4b** by straightforward ^{1}H and ^{13}C NMR analysis in comparison with literature data.[15] Of the faster moving products, two were readily identified as 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzoic acid. The other two products, one of which was the main component of the mixture, were isolated by PTLC fractionation of the ethyl acetate extractable fraction and were subjected to extensive spectral analysis. The ^{1}H NMR spectrum of the most abundant product featured the resonances of a 3,4-dihydroxyphenyl moiety and two additional doublets for coupled protons at δ 7.36 and 5.06, exhibiting one-bond connectivities in the HMQC spectrum with ^{13}C NMR signals at δ 153.9 and 73.2, respectively. A similar pattern of resonances was displayed by the companion product, with the additional feature of a 3H singlet at δ 3.29 for a methoxyl group.

On this basis, the products were formulated as the novel oximes **7a**,**b**. In product **7b**, the methoxyl group derived evidently from the methanol added as cosolvent, as confirmed in separate experiments carried out with acetone in the place of methanol. The *E* configuration of the aldoxime functionalities was deduced on the basis of the chemical shifts of the relevant protons and carbons.[17] Attempts to obtain satisfactory mass spectra were defeated under a variety of conditions. Accordingly, **7a**,**b** were acetylated with Ac_2O/pyridine to afford similar sets of two related products each. Both ^{1}H NMR analysis and EI-MS spectra consistently indicated for the products the structures of the peracetylated derivatives **7c**,**d** and of the nitriles **8a**,**b**, the latter derived by the Ac_2O-promoted dehydration of the aldoxime functionalities.

Another product, yellow in color, could be obtained along with **3c** in much higher yields by similar reaction

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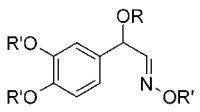
of **1a** at 0.1 M concentration with 0.5 M NO_2^- in acetate buffer, pH 4/methanol 1:1 v/v. The compound shared with **7a**,**b** the proton spin system for a 4-substituted catechol ring, although the H-2 and H-6 protons resonated relatively downfield. It also exhibited a singlet at δ 7.91 correlating with a carbon signal at δ 147.8 and displaying cross-peaks in the HMBC spectrum with carbon signals at δ 187.1 (denoting a carbonyl group) and 129.2. On this basis, the product was formulated as the novel 2-oxoaldoxime **9a**. This conclusion was corroborated by the EI-MS spectrum of the trimethyl derivative **9b**, obtained by reaction with diazomethane, which displayed a discernible molecular ion peak at *m*/*z* 223 and a well-defined pattern of fragmentation peaks at *m*/*z* 165 (M − CH_3O − HCN) and 137 (M − CH_3O − HCN − CO) indicating sequential losses of the elements of the *O*-methyl 2-ketoaldoxime chain.

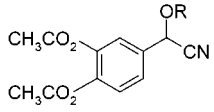
To assess whether the material balance could be improved by detection of putative aromatic nitration products of **1a** escaping HPLC and TLC analysis, the synthesis of 6-nitrocaffeic acid[18] was pursued by nitration of **1a** with tetranitromethane in aqueous bicarbonate at pH 8.0. Careful scrutiny of the reaction mixtures failed however to reveal detectable formation of this product by reaction of **1a** with acidic NO_2^-.

Under the typical conditions used for **1a**, both 3,4-dihydroxybenzoic acid and 3,4-dihydroxybenzaldehyde reacted slowly with NO_2^- to afford complex patterns of products which did not figure among those obtained from **1a**. Notably, no detectable formation of 4,5-dihydroxy-2-nitrobenzaldehyde[19] from 3,4-dihydroxybenzaldehyde was observed under such conditions.

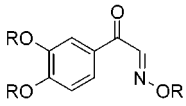
To provide a more complete inventory of the products formed from 2×10^{-3} M **1a** and 4×10^{-3} M NO_2^- at pH 4, and to rule out the possibility that the isolated products could be artifactually generated during work up or chromatographic separation, a typical reaction was continued until most of the starting material was consumed (about 4 h), then the mixture was carefully neutralized and extracted with ethyl acetate. The ^{1}H NMR spectrum of the whole extractable fraction displayed the characteristic resonances of **7a** as the main constituent, as well as those of **7b** (ca. 50% of **7a**), **3c** (ca. 15% of **7a**), 3,4-dihydroxybenzaldehyde (ca. 10% of **7a**) and of the benzoxazinone **4b** (ca. 10% of **7a**). Apparently, no dimerization product of **1a** related, e.g., to the neolignan **6**, was detected.



7a: R=H, R'=H
7b: R=CH_3, R'=H
7c: R=R'=$COCH_3$
7d: R=CH_3, R'=$COCH_3$



8a: R= $COCH_3$
8b: R=CH_3



9a: R=H
9b: R=CH_3

In a subsequent series of experiments, the concentration of **1a** was varied systematically maintaining NO_2^- at 2 molar equiv. At 100 μM concentration, **1a** reacted rapidly to give mainly **7a**, all other products being below detection limits. At 0.1 M concentration, on the other hand, **1a** afforded **3c** as the chief product, with some **9a**. When NO_2^- concentration was varied in the range $1-10 \times 10^{-3}$ M keeping **1a** at 2×10^{-3} M concentration, HPLC analysis revealed consistent changes in substrate consumption but only barely appreciable variations in product distribution. Moreover, in all cases examined, product distribution from **1a** remained virtually unchanged during the reaction course, for at least 4 h.

Changing the nature of the buffer (e.g., phosphate in the place of acetate)[11] or excluding oxygen from the medium (purging with Ar) reduced only slightly the extent of substrate consumption without, however, affecting product distribution.

The reaction of **1a** (2×10^{-3} M) with NO_2^- (4×10^{-3} M) was also carried out at pH 1 and 2, as reported.[15] Under both conditions, 3,4-dihydroxybenzaldehyde and **9a** were among the main products. At pH 2, oximes **7a**,**b** were also formed. Neither 4,5-dihydroxy-2-nitrobenzaldehyde nor oximinoaldehyde products related to those previously reported from ferulic acid[12] were detected in the reaction mixtures from **1a**.

Acid-Promoted Reaction of 1b and 1c with NO_2^-. Reaction of 2 mM **1b** with NO_2^- (2 molar equiv) in acetate buffer at pH 4 resulted in the formation of two main products, the most abundant of which (43% yield) proved to be **5a**. The other product (10% yield) was apparently a dimer sharing several spectral features (^{1}H,^{13}C HMQC, HMBC) in common with those of the dihydrobenzofuran neolignan **6**,[16] along with some telltale differences. These included the presence of four 1H singlets in the aromatic region of the ^{1}H NMR spectrum, one of which appearing relatively downfield (δ 7.67), and a pronounced downfield shift of the H-2 proton on the dihydrobenzofuran moiety (δ 6.65 vs. 5.97 for **6**).[16] Both H-2 and H-3 proton resonances correlated in the HMBC spectrum with quaternary carbon signals at δ 151.5 and 132.9, the latter giving a cross-peak also with the proton signal at δ 7.67. The typical pH-dependent nitrocatechol chromophore, and the conversion to a triacetyl derivative by acetylation, provided definitive evidence for the structure of the nitrated dihydrobenzofuran lignan **10**. Although two different stereoisomers were possible, spectral analysis suggested a single isomer, which was regarded as having a 2,3-trans configuration, in accord with literature data[16] and with preliminary molecular mechanics calculations (MM^+). Morever, no detectable nOe was observed between the protons on the 2- and 3-positions of the benzofuran ring. Although the value for the vicinal coupling constant for the relevant protons (J = 4.4 Hz) was smaller than that of the parent dihydrobenzofuran neolignan **6** (J = 8.3 Hz),[16] it is possible that the nitro group on the adjacent aromatic ring causes a significant distortion of the dihydrofuran moiety. This influence of the nitro group is apparent from the dramatic downfield shift of the H-2 proton of the dihydrobenzofuran moiety.

Despite careful analysis, no apparent chain nitrosation/breakdown product of **1b** could be detected.

When **1b** (2×10^{-3} M) was allowed to react with NO_2^- (2 molar equiv) at pH 1, formation of **5a** was decreased (ca. 21% yield) but not suppressed, at variance with the

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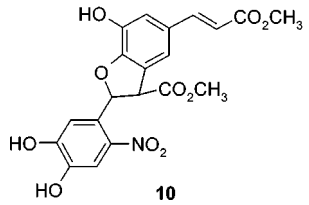

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previous report.[15] Compound **10** was likewise present in detectable amounts (5%), along with **6** (8%). In fact, **6** was also detected by HPLC as a minor component in the early stages of the reaction of **1b** with NO_2^- at pH 4, but its levels gradually decreased as **10** began to accumulate.

Reaction of 2×10^{-3} M **1c** with NO_2^- (2 molar equiv) proceeded virtually to completion to give mainly **5b**, in accord with the previous report.[15]

Mechanistic Issues. The present survey confirms the radically divergent reaction pathways of **1a** and its esters **1b/c** with acidic NO_2^-. The structures of main products from **1a** support previous hypotheses[15] suggesting regioselective nitrosation at the 2-position of the propenoic acid moiety as the initial reaction step. This would be brought about by NO^+ or a related nitrosating species produced by acid promoted decomposition of NO_2^- via HNO_2.

Brief semiempirical (AM1/PM3) calculations predicted for both **1a** and **1b** the 2-position of the propenoic acid sector as the most reactive site, as apparent from the relatively high HOMO coefficient and the highest total charge density (Table 1).

This result conforms to expectations based on mere inspection of canonical resonance forms for **1a** and **1b** (not shown) which underscore the primary role of the *p*-hydroxyl group on the catechol ring in pushing π electron density toward the 2-position of the propenoic acid chain. In accord with the chain activating effect of the aromatic *p*-hydroxyl group are the similar patterns of reactivity with acidic NO_2^- displayed by *p*-coumaric and ferulic acids[12−14] and the complete lack of reactivity of cinnamic acid (as observed in separate experiments).

On this basis, it can be safely concluded that the different behaviors exhibited by **1a** and its esters toward acidic NO_2^- do not reflect an intrinsically different π electron makeup, but are determined by the availability of a decarboxylation route, paving the way to otherwise hardly accessible evolution pathways of the first formed nitroso intermediate(s) (Scheme 1).

The facile decarboxylation of the putative nitroso intermediates from **1a** would be warranted by concomitant isomerization of the nitroso group to a stable aldoxime functionality. This step would be preceded by competing attacks of the various nucleophiles to the resonance-stabilized nitroso cation, or a related species, wherebyproduct distribution would mirror the relative nucleophilicities and concentrations of all species in the medium. Consistent with this view, under conditions of low methanol and NO_2^- concentration, H_2O is the most effective nucleophile causing **7a** to prevail over **7b** and **3c**, whereas with relatively high NO_2^- concentrations, formation of a 1,2-nitronitroso compound, precursor to **3c**,[20] would become the main route. The preferential formation of *E* oximes would be in accord with their greater stability compared to the *Z* isomers,[21] as deduced from brief molecular mechanics calculations (MM+) on structures **7a** and **9a**, but may also stand in witness of a concerted decarboxylation mechanism.[15]

Table 1. Total Charge Densities (TCD) and HOMO Coefficients of 1a/b

		HOMO		TCD	
compd	carbon	AM1	PM3	AM1	PM3
1a	2	0.328	0.315	−0.203	−0.200
	3	0.139	0.137	−0.021	0.012
	2′	0.019	0.018	−0.161	−0.140
	5′	0.169	0.170	−0.195	−0.171
	6′	0.362	0.362	−0.092	−0.074
1b	2	0.331	0.318	−0.199	−0.202
	3	0.146	0.144	−0.026	−0.005
	2′	0.013	0.012	−0.162	−0.142
	5′	0.169	0.171	−0.194	−0.170
	6′	0.363	0.363	−0.093	−0.075

In Scheme 1, oxime **9a** is shown to arise from oxidation of **7a**. This was confirmed in separate experiments in which **7a** was reacted with NO_2^- at pH 4 to give **9a** along with 3,4-dihydroxybenzaldehyde. Formation of the latter may follow from *O*-nitrosation of the oxime OH group in **7a**, which would set the scene for sequential or concerted losses of HNO_2 and HCN by a process reminiscent of the Wohl degradation of aldoses.[22] The importance of the unsubstituted OH group α to the oxime group in providing the driving force for chain breakdown was apparent from the resilience of **7b** to give rise to 3,4-dihydroxybenzaldehyde under the reaction conditions.

The formation of a benzoic acid derivative is apparently unprecedented in the chemistry of caffeic acid analogues and derivatives with acidic NO_2^-. In principle, this acid could arise by oxidation of 3,4-dihydroxybenzaldehyde; however, this possibility was ruled out by the lack of appreciable conversion of the latter compound to 3,4-dihydroxybenzoic acid under the reaction conditions. Thus, the only plausible route to 3,4-dihydroxybenzoic acid was oxidative breakdown of the oxime **9a** or of a related species by a mechanism akin to that operative in the case of 3,4-dihydroxybenzaldehyde formation. Unfortunately, this mechanism could not be convincingly validated by direct experimental proof, since neither **9a** nor its precursor **7a** afforded significant amounts of 3,4-dihydroxybenzoic acid upon reacting with NO_2^- under various acidic conditions. A possible explanation is that 3,4-dihydroxybenzoic acid is formed mainly via a 2-oxonitrile intermediate produced specifically from Z oximes, because of the favorable anti arrangement of the breaking bonds, and that *E*−*Z* isomerization does not occur to a sufficient extent with isolated **7a** or **9a** under the conditions of the mechanistic experiments. Alternatively, *Z* oximes may be directly generated by decarboxylation of the nitroso intermediates, which cannot be ruled out on the basis of available evidence. Indirect proof of the actual formation in the pathway of *Z* oximes would be provided by the generation of benzoxazinone **4b**, which purportedly involves intramolecular cyclization of an *o*-quinone *Z* oxime intermediate.

Many of the mechanistic scenarios proposed in this study show noticeable similarities with those reported

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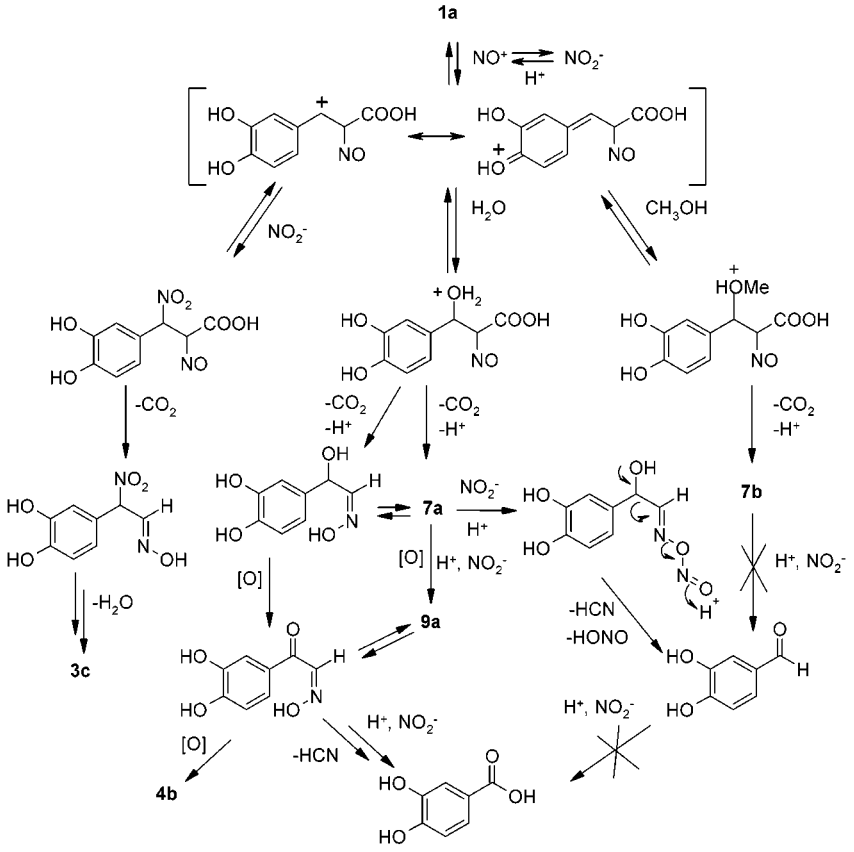
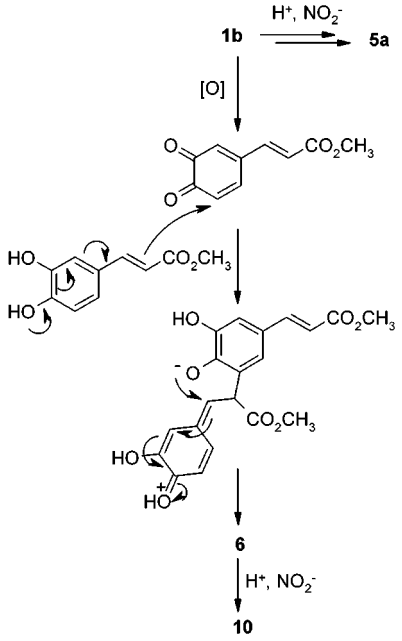
Scheme 1



in a recent paper dealing with the reaction of *p*-coumaric acid with nitrite.[14] In that paper the authors provided a detailed spectral characterization of hydroxy- and oxo-aldoxime derivatives, as well as of benzaldehyde, benzoxazinone and furoxan products, in excellent agreement with our data. By elegant experiments using ^{18}O -labeled water, they conclusively demonstrated that the hydroxy and oxo group in the aldoxime products were derived from the water of the medium. However, in contrast with the mechanistic pathways in Scheme 1, in the previous paper[14] decarboxylation was suggested to precede the addition of water, but not of nitrite, whereby chain hydroxylation would be the result of the conjugated addition of water to a *β*-nitrososstyrene derivative. Although the proposed routes are not mutually exclusive, and may well concur to product distribution, we would favor the one indicated in Scheme 1, in which water or methanol would add to a highly electrophilic nitroso cation or a related species (e.g. a quinone methide).

In the case of esters **1b**,**c**, the unfavorable equilibrium with the putative nitroso intermediate(s) cannot be driven by decarboxylation or other irreversible routes, whereby aromatic nitration and dimerization become the only viable pathways (Scheme 2). Three possible options for catechol ring nitration leading to **5a** and **5b** could be envisaged, namely NO_2^- induced oxidation of the catechol to semiquinone followed by radical coupling with NO_2; nucleophilic attack of NO_2^- to the *o*-quinone; or electrophilic nitrosation of the catechol ring followed by oxidation. The latter route seems untenable in view of previous experience gained in catechol nitration[23] with acidic

Scheme 2



NO_2^-, indicating that the free *o*-dihydroxy functionality is an essential requisite for nitration. This point was definitively confirmed in separate experiments in which the *O*,*O*-dimethyl derivative of **1b** was prepared and shown to remain virtually unaffected upon prolonged exposure to acidic NO_2^-.

Whether nitration occurs via the semiquinone or the quinone route cannot be assessed on the basis of available data. Two points, however, are worthy of note. First, nitration of **1b** is significant at pH 4 and is drastically

(23) (a) Napolitano, A.; d'Ischia, M.; Costantini, C.; Prota, G. *Tetrahedron* **1992**, *48*, 8515−8522. (b) d'Ischia, M.; Costantini, C. *Bioorg. Med. Chem.* **1995**, *3*, 923−927.

decreased at pH 1, as noted by the previous authors,[15] at variance with simple catechols, which are conveniently converted to 6-nitrocatechols with NO_2^- under strongly acidic conditions (e.g., 1% sulfuric acid).[23,24] Second, formation of **6** from **1b** at acidic pH is convincing evidence for the generation of **1b** quinone which would suffer nucleophilic attack by **1b** through the 2-position (a mechanistic point that might be construed as confirmatory proof of the high reactivity of this site predicted also for **1b**).

On these bases, and considering that NO_2^- concentration may be still high at pH 4 but very low at pH 1 (pK_a $HNO_2 = 3.25$),[25] the NO_2^--quinone route seems a plausible, though not exclusive option.

Formation of **10** from **1b** raised a relevant issue concerning the temporal sequence of the catechol nitration and dimerization steps. The finding that **6** was smoothly converted to **10** by reaction with NO_2^- at pH 4, and that **1b** and **5a** did not exhibit mutual reactivity at the same pH, was taken as definitive evidence of the sequence depicted in Scheme 2.

In product **10**, the selective nitration of the unconjugated catechol ring argues further in favor of an oxidative mechanism, which would be hindered in the hydroxydihydrobenzofuran moiety.

Conclusions

The study of the reactions of **1a** and related antioxidant and antinitrosating agents with NO_2^- is an area of great promise for elucidating mechanisms of colorectal carcinogenesis and for the design of novel antioxidants and anticancer agents for rational chemopreventive strategies. In this perspective, the results of the present investigation (a) substantially modify and expand the current knowledge of the reaction behavior of **1a** and related compounds with acidic NO_2^-; (b) throw light on a range of reaction products and mechanisms that apparently eluded the attention of previous workers; (c) provide an improved background to understand at chemical level the biological properties of these important dietary constituents.

The remarkable facility to decarboxylation may be the underlying cause of the greater efficiency of **1a** as NO_2^- scavenger compared to its esters. Loss of CO_2 would provide the driving force for an irreversible sequence of reaction steps that outcompete otherwise facile nitrosation/nitration at the catechol moiety. This latter moiety, in fact, directs reactivity toward the side chain of **1a** through resonance effects and comes into play as target site only when decarboxylation is precluded, as in **1b**,**c**.

Elucidation of the reaction sequences of **1a** delineated a mechanism by which the nature and concentration of nucleophilic constituents in the medium gain importance in the early stages, governing the balance of competing routes. This variability may have several biological implications, currently under assessment in our laboratory. As a relevant example, it may offer a plausible explanation to the reported concentration-dependent change of effect, from stimulating to suppressive, of **1a** on gastric nitrosamine formation: whereas at high concentration **1a** can efficiently scavenge NO_2^-, at low concentrations, viz. with high NO_2^-/**1a** ratios, it may be converted to products with potential nitrosative properties, e.g. the furoxan **3b**.

Experimental Section

General Methods. Caffeic acid (**1a**), chlorogenic acid (**1c**), cinnamic acid, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, and tetranitromethane were used as obtained. Caffeic acid methyl ester (**1b**) was prepared from caffeic acid by Fischer esterification (HCl (g)/dry methanol). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluensulfonamide in ethanolic KOH. CAUTION! Tetranitromethane is highly toxic and is no more commercially available. Diazomethane is explosive and must be collected in peroxide-free ether in a dry ice/acetone bath and kept at −20 °C.

UV spectra were performed with a diode array spectrophotometer. ^1H (^{13}C) NMR spectra were recorded at 400.1 (100.6) MHz. $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, and $^1H-^{13}C$ HMBC NMR experiments were run at 400.1 MHz using standard pulse programs from the Bruker library. For EI/MS spectra samples were ionized with a 70 eV beam, and the source was taken at 230 °C. Main fragmentation peaks are reported with their relative intensities (percent values are in parentheses).

Analytical and preparative TLC analyses were performed on F254 0.25 and 0.5 mm silica gel plates or high performance TLC (HPTLC) using chloroform-methanol 95:5 (eluant A), 90:10 (eluant B), 85:15 (eluant C), chloroform-methanol 90:10 containing 1% or 0.2% acetic acid (eluant D or E), dichloromethane-ethyl acetate 90:10 (eluant F) or benzene−ethyl acetate 70:30 (eluant G). Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid),[26] 10% ferric chloride in ethanol and 2 M sodium hydroxide were used for product detection on TLC plates. Column chromatography was performed on silica gel 60, 0.040−0.063 mm, 230−400 mesh.

Analytical and preparative HPLC was performed with an instrument equipped with a UV detector set at 280 nm. Octadecylsilane coated columns, 4.6 × 250 mm or 22 × 250 mm, 5 μm particle size were used for analytical or preparative runs, respectively. Flow rates of 1 or 12 mL/min were used. Different isocratic and gradient elution conditions were used as follows: 0.1 M formic acid−methanol 97:3 (solvent A), 0.1 M formic acid-methanol 10:90 (solvent B), 0−5 min solvent A, 5−35 min from 0 to 40% solvent B, 35−45 40% solvent B (eluant system I); 0.1 M formic acid /methanol 70:30 v/v (eluant II) or 80:20 (eluant III); 0.1 M formic acid/acetonitrile 65:35 v/v (eluant IV).

Molecular mechanics (MM+) and semiempirical AM1/PM3 calculations were carried out with the Hyperchem 5.0 package produced by Hypercube Inc. (Waterloo, Ontario, Canada) 1997.

Methyl (*E*)-3-(3,4-Dimethoxyphenyl)propenoate. Compound **1a** (200 mg, 1.1 mmol) in DMF (2 mL) was treated with methyl iodide (1 mL) and sodium carbonate (0.5 g), and the mixture was stirred at 60 °C for 48 h. The mixture was then diluted with water (10 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic layers were dried over sodium sulfate and taken to dryness to give a residue which, after purification by silica gel chromatography (30 × 1.5 cm column) using chloroform as the eluant, afforded the title compound (40 mg, 16% yield). ^1H NMR (acetone-d_6) δ (ppm): 3.73 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 6.43 (d, J = 15.8 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 8.4, 1.8 Hz, 1H), 7.30 (d, J = 1.8 Hz, 1H), 7.60 (d, J = 15.8 Hz, 1H). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.60; H, 6.37.

(*E*)-3-(4,5-Dihydroxy-2-nitrophenyl)propenoic Acid. To **1a** (500 mg, 2.8 mmol) dissolved in methanol (3 mL) 0.1 M sodium carbonate (20 mL) was added under vigorous stirring followed by a solution of tetranitromethane (335 μL, 2.8 mmol) in ethyl acetate (6 mL) in three aliquots over 30 min. The

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(25) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 75th ed.; CRC Press: Boca Raton, FL, 1995.

(26) Klebanoff, S. J. *Free Rad Biol. Med.* **1993**, *14*, 351−360.

mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were dried over sodium sulfate and taken to dryness to give the title compound[19] as an oily residue (410 mg, 65% yield).

General Procedure for Reaction of 1a/b and Other Catecholic Compounds with Nitrite. To **1a/b** (1 mmol) dissolved in methanol (5 mL) were added sequentially 0.05 M acetate buffer pH 4 (500 mL) and sodium nitrite (2 mmol), and the mixture was kept under stirring at room temperature. The reaction course was followed by HPLC (eluant system I for **1a** and eluant III for **1b**). After 4 h or after complete consumption of the substrate, the pH of the reaction mixture was raised to 7.0 by addition of sodium hydrogencarbonate. The mixture was extracted with ethyl acetate (3 × 150 mL), and the combined organic layers were dried over sodium sulfate and taken to dryness.

In other experiments, the reaction of **1a** was run as follows: (i) as above but without addition of methanol; (ii) purging with argon the solution of **1a** in the acetate buffer prior to addition of sodium nitrite; (iii) using substrate concentrations in the range 0.1−100 mM and 2 molar equiv of nitrite; (iv) with nitrite varying in the range 0.5−5 molar equiv with respect to the substrate at 2 mM concentration; (v) as in the general procedure but in 0.1 M phosphoric acid, pH 1 or 2.

Reactions of 3,4-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, cinnamic acid, or methyl (*E*)-3-(3,4-dimethoxyphenyl)propenoate (1 mmol) dissolved in methanol (5 mL) with nitrite (2 mmol) was carried out under the general conditions. The reaction course was followed by HPLC (eluant system IV in the case of 3,4-dihydroxybenzoic acid or I in the case of 3,4-dihydroxybenzaldehyde) or by TLC (eluant B for cinnamic acid or eluant F for methyl (*E*)-3-(3,4-dimethoxyphenyl)propenoate).

Isolation of 3,4-Dihydroxybenzaldehyde, 3,4-Dihydroxybenzoic acid, 4b, 7a−d, and 8a/b. For preparative purposes, the reaction of **1a** with nitrite was carried out in 0.05 M acetate buffer, pH 4.0 using 200 mg of the starting material. After workup of the reaction mixture, the residue (60 mg) was fractionated by PTLC (eluant D) to give four main bands. The less polar (R_f 0.33, 13 mg, 9% yield) was identified as 3,4-dihydroxybenzaldehyde by comparison of the spectral (UV, ^{1}H NMR) and chromatographic properties with those of an authentic sample. Another fraction (R_f 0.30, 4 mg, 2% yield) consisted of **4b** identified by comparison of the spectral properties (^{1}H NMR) with those reported.[15] The other two bands were found to consist of pure **7b** (R_f 0.19, 10 mg, 5% yield) and **7a** (R_f 0.07, 20 mg, 10% yield). Purification of the aqueous layer by preparative HPLC (eluant II) afforded 3,4-dihydroxybenzoic acid (t_R 16 min, 10 mg, 6% yield) identified by spectral analysis (^{1}H and ^{13}C NMR) and comparison with an authentic sample.

Compound **7a** or **7b** (15 mg) was treated with acetic anhydride (1 mL) and pyridine (40 μL) overnight at room temperature. After removal of the volatile components, the residues were fractionated by HPTLC (eluant G) to afford in each case two main fractions. The less polar bands were found to consist of **8a** (R_f 0.64, 11 mg, 46% yield) or **8b** (R_f 0.64, 9 mg, 45% yield), while the others contained pure **7c** (R_f 0.49, 12 mg, 42% yield) or **7d** (R_f 0.50, 7 mg, 28% yield).

(*E*)-2-Hydroxy-2-(3,4-dihydroxyphenyl)ethanaloxime (7a). UV: λ_{max} (CH_3OH) 280 nm. ^{1}H NMR (CD_3OD) δ (ppm): 5.06 (d, J = 7.0 Hz, 1H), 6.71 (d, J = 2.0 Hz, 1H), 6.72 (d, J = 7.6 Hz, 1H), 6.80 (dd, J = 7.6, 2.0 Hz, 1H), 7.36 (d, J = 7.0 Hz, 1H). ^{13}C NMR (CD_3OD) δ (ppm): 73.2 (CH), 115.4 (CH), 117.2 (CH), 119.7 (CH), 134.7 (C), 147.0 (C), 147.3 (C), 153.9 (CH). Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.50; H, 4.84; N, 7.47.

(*E*)-2-(3,4-Dihydroxyphenyl)-2-methoxyethanaloxime (7b). UV: λ_{max} (CH_3OH) 280 nm; ^{1}H NMR (CD_3OD) δ (ppm): 3.29 (s, 3H), 4.61 (d, 1H, J = 7.4 Hz), 6.65 (dd, 1H, J = 8.0, 2.0 Hz), 6.73 (d, 1H, J = 8.0 Hz), 6.77 (d, 1H, J = 2.0 Hz), 7.30 (d, 1H, J = 7.4 Hz). ^{13}C NMR (CD_3OD) δ (ppm): 57.3 (CH_3), 82.6 (CH), 115.7 (CH), 117.2 (CH), 120.4 (CH), 132.1 (C), 147.3 (C), 152.4 (CH). Anal. Calcd for $C_9H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.10. Found: C, 54.93; H, 5.45, N, 7.01.

(*E*)-*N*-Acetoxy-2-acetoxy-2-(3,4-diacetoxyphenyl)ethanaloxime (7c). ^{1}H NMR (CD_3OD) δ (ppm): 2.15 (s, 6H), 2.26 (s, 6H), 6.42 (d, 1H, J = 6.0 Hz), 7.27 (d, 1H, J = 8.0 Hz), 7.34 (d, 1H, J = 2.0 Hz), 7.37 (dd, 1H, J = 8.0, 2.0 Hz), 8.00 (d, 1H, J = 6.0 Hz). EI/MS m/z 351 (M^+, 1), 309 (7), 291 (3), 267 (12), 249 (13), 207 (50), 165. (95), 147 (50), 138 (40), 137 (100). HRMS for $C_{16}H_{17}NO_8$: calcd 351.0954, found 351.0932.

(*E*)-*N*-Acetoxy-2-(3,4-diacetoxyphenyl)-2-methoxyethanaloxime (7d). ^{1}H NMR (CD_3OD) δ (ppm): 2.12 (s, 3H), 2.26 (s, 6H), 3.41 (s, 3H), 4.98 (d, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 8.4 Hz), 7.29 (d, 1H, J = 2.0 Hz), 7.32 (dd, 1H, J = 8.4, 2.0 Hz), 7.78 (d, 1H, J = 7.6 Hz). EI/MS m/z 323 (M^+, 1), 281 (11), 239 (13), 221 (29), 179 (100), 148 (100). HRMS for $C_{15}H_{17}NO_7$: calcd 323.1005, found 323.1011.

2-Acetoxy-2-(3,4-diacetoxyphenyl)acetonitrile (8a). UV: λ_{max} (CH_3OH) 300 nm. ^{1}H NMR (CD_3OD) δ (ppm): 2.15 (s, 3H), 2.27 (s, 6H), 6.55 (s, 1H), 7.34 (d, 1H, J = 8.4 Hz), 7.45 (d, 1H, J = 2.0 Hz), 7.49 (dd, 1H, J = 8.4, 2.0 Hz). EI/MS m/z 291 (M^+, 6), 249 (84), 207 (100), 165 (100), 147 (100). HRMS for $C_{14}H_{13}NO_6$: calcd 291.0743, found 291.0735.

2-(3,4-Diacetoxyphenyl)-2-methoxyacetonitrile (8b). UV: λ_{max} (CH_3OH) 300 nm. ^{1}H NMR (CD_3OD) δ (ppm): 2.27 (s, 6H), 3.52 (s, 3H), 5.45 (s, 1H), 7.31 (d, 1H, J = 8.4 Hz), 7.37 (d, 1H, J = 2.0 Hz), 7.42 (dd, 1H, J = 8.4, 2.0 Hz). EI/MS m/z 263 (M^+, 12), 221 (100), 179 (100), 148 (100). HRMS $C_{13}H_{13}NO_5$: calcd 263.0794, found 263.0772.

Isolation of 3c and 9a. The reaction of **1a** (500 mg, 2.78 mmol) dissolved in methanol (12 mL) with nitrite (1.0 g) was carried out as above in 0.05 M, acetate buffer, pH 4.0 (12 mL). After workup of the reaction mixture, the residue (376 mg) was fractionated by chromatography on silica gel (30 × 1.5 cm column) using chloroform/ethyl acetate (9:1 to 1:1 gradient mixtures) to afford two main fractions. The fraction collected with chloroform/ethyl acetate from 9:1 to 7:3 proved to consist of **3c** [15] (63 mg, 12% yield), while the fraction eluted with chloroform/ethyl acetate 1:1 (67 mg) was further purified by PTLC (eluant C) to give pure **9a** (R_f 0.51, 38 mg, 8% yield). The latter compound was subjected to methylation by treatment with diazomethane to afford, after PTLC fractionation (eluant A), **9b**.

2-(3,4-Dihydroxyphenyl)-2-oxoethanaloxime (9a). UV: λ_{max} (CH_3OH) 234 nm, 273 nm, 322 nm (CH_3OH, 0.1 M NaOH) 267 nm, 379 nm. ^{1}H NMR (acetone-d_6) δ (ppm): 6.91 (d, 1 H, J = 8.8 Hz), 7.61 (dd, 1H, J = 8.8, 2.0 Hz), 7.62 (d, 1H, J = 2.0 Hz), 7.91 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 115.2 (CH), 117.1 (CH), 124.5 (CH), 129.2 (C), 141.7 (C), 145.3 (C), 148.7 (CH), 187.1 (C). Anal. Calcd for $C_8H_7NO_4$: C, 53.04; H, 3.89; N, 7.73. Found: C, 53.22; H, 3.92; N, 7.80.

***N*-Methoxy-2-(3,4-dimethoxyphenyl)-2-oxoethanaloxime (9b).** EI/MS: m/z 223 (M^+, 32), 165 (100), 137 (13), 122 (11), 107 (15). HRMS for $C_{11}H_{13}NO_4$: calcd 223.0844, found 223.0865.

2-Oxy-3-(3,4-dihydroxyphenyl)-1,2,5-oxadiazole (3c). UV: λ_{max} (CH_3OH) 247, 316. ^{1}H NMR (CD_3OD) δ (ppm): 6.89 (d, J = 8.4 Hz, 1H), 7.32 (dd, J = 8.4, 2.0 Hz), 7.54 (d, J = 2.0 Hz, 1H), 8.92 (s, 1H). ^{13}C NMR δ (ppm): 113.9 (CH), 115.4 (C), 116.2 (C), 117.3 (CH), 120.1 (CH), 146.4 (CH), 147.5 (C), 149.7 (C). EI/MS: m/z 194 (M^+, 33), 134 (100).

Isolation of 5a, 6, and 10. For preparative purposes, reaction of **1b** with nitrite was carried out in 0.05 M acetate buffer pH 4.0 as described above, using 200 mg (1.0 mmol) of the starting material. After workup of the reaction mixture, the residue (192 mg) was fractionated by PTLC (eluant E) to afford two main fractions consisting of **5a** (R_f 0.44, 107 mg, 45% yield) identified by comparison of the spectral properties (^{1}H NMR) with those previously reported [15] and of **10** (R_f 0.35, 46 mg, 10% yield). Compound **10** was acetylated with acetic anhydride/pyridine as described above for **7a/b** to afford a main product purified by PTLC (eluant F). The reaction of **1b** (200 mg) with nitrite (2 molar equiv) was also carried out in 0.1 M phosphate buffer, pH 1.0. Fractionation of the residue (296 mg) obtained from work up of the reaction mixture on PTLC (eluant D) afforded **5a** (R_f 0.38, 51 mg, 21% yield), **6** (R_f 0.35, 30 mg, 8% yield) identified by comparison of the spectral

properties with those of an authentic sample,[16] and **10** (R_f 0.30, 22 mg, 5% yield).

Methyl (*E*)-3-(4,5-Dihydroxy-2-nitrophenyl)propenoate (5a). UV: λ_{max} (CH_3OH) 274 nm, (CH_3OH, 0.1M NaOH) 258, 306, 322, 432 nm. ^1H NMR (CD_3OD) δ (ppm): 3.78 (s, 3H), 6.26 (d, J = 16.0 Hz, 1H), 7.03 (s, 1H), 7.52 (s, 1H), 8.08 (d, J = 16 Hz, 1H). ^{13}C NMR (CD_3OD) δ (ppm): 113.8 (CH), 115.4 (CH), 121.6 (CH), 125.6 (C), 142.7 (C), 144.3 (CH), 149.3 (C), 153.2 (C), 169.7 (C).

Methyl (*E*)-3-[7-Hydroxy-2-(4,5-dihydroxy-2-nitrophenyl)-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propenoate (10). UV: λ_{max} (CH_3OH) 303, 325 nm (CH_3OH, NaOH 0.01 M) 275, 319, 367, 483 nm;^1H NMR (CD_3OD) δ (ppm): 3.75 (s, 3H), 3.81 (s, 3H), 4.13 (d, 1H, J = 4.4 Hz), 6.30 (d, 1H, J = 16.0 Hz), 6.65 (d, 1H, J = 4.4 Hz), 6.96 (s, 1H), 7.03 (s, 1H), 7.06 (s, 1H), 7.54 (d, 1H, J = 16.0 Hz), 7.67 (s, 1H). ^{13}C NMR (CD_3OD) δ (ppm): 53.0 (CH_3), 54.2 (CH_3), 58.7 (CH), 85.9 (CH), 114.3 (CH), 114.6 (CH), 117.1 (CH), 118.5 (CH), 118.5 (CH), 128.1 (C), 131.4 (C), 132.9 (C), 139.5 (C), 144.2 (C), 147.1 (CH), 147.4 (C), 151.5 (C), 154.3 (C), 170.3 (C), 174.5 (C). Anal. Calcd for $C_{21}H_{17}NO_{10}$: C, 55.69; H, 3.97; N, 3.25. Found: C, 55.47; H, 3.85; N, 3.32. **Acetyl Derivative.** ^1H NMR ($CDCl_3$) δ (ppm): 2.30 (s, 3H), 2.32 (s, 3H), 2.34 (s, 3H), 3.79 (s, 3H), 3.86 (s, 3H), 4.26 (d, 1H, J = 4.4 Hz), 6.29 (d, 1H, J = 16.0 Hz), 6.78 (d, 1H, J = 4.4 Hz), 7.19 (d, 1H, J = 1.6 Hz), 7.35 (d, 1H, J = 1.6 Hz), 7.57 (d, 1H, J = 16.0 Hz), 7.67 (s, 1H), 8.10 (s, 1H). EI/MS: *m*/*z* 470 (97), 428 (24), 386 (66), 326 (47), 267 (29), 194 (100), 134 (21).

Reaction of 1c with Nitrite. Isolation of 5b. To **1c** (200 mg, 0.56 mmol) dissolved in methanol (3 mL) were added sequentially 0.05 M acetate buffer pH 4 (3 mL) and sodium nitrite (204 mg) under vigorous stirring at room temperature. The reaction course was followed by HPLC (eluant III). After 1 h or after complete consumption of the substrate, the mixture was taken to a small volume and fractionated by preparative HPLC (eluant II) to give **5b** (t_R 23 min, 90 mg, 40% yield) identified by comparison of the spectral properties (^1H NMR) with those reported.[15] UV: λ_{max} (MeOH) 276, 339, 430 nm; λ_{max} (CH_3OH, NaOH 0.01 M) 262, 341, 439 nm. ^1H NMR (CD_3OD) δ (ppm): 2.12 (m, 4H), 3.74 (dd, J = 10.0, 3.2 Hz, 1H), 4.20 (m, 1H), 5.42 (m, 1H), 6.31 (d, J = 15.6 Hz, 1H), 7.05 (s, 1H), 7.57 (s, 1H), 8.15 (d, J = 15.6 Hz, 1H). ^{13}C NMR (CD_3OD) δ (ppm): 39.5 (CH_2), 41.3 (CH_2), 73.2 (CH), 73.3 (CH), 75.3 (C), 78.2 (CH), 113.2 (CH), 116.0 (CH), 121.2 (CH), 125.6 (C), 140.7 (C), 144.0 (CH), 150.2 (C), 156.2 (C), 169.1 (C), 181.8 (C).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **7a/7b** and **9a**. ^1H NMR, ^1H-^1H COSY, ^1H–^{13}C HMQC, and HMBC spectra of compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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