Synthesis and physico-chemical properties of nitrocaffeic acids

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ABSTRACT: The synthesis and spectroscopic properties of the three isomers of nitrocaffeic acid are described. The three pK_a s of each isomer were measured by UV–visible spectroscopy. The comparison of the UV–visible spectra of nitrocaffeic acids and those obtained from the reaction of caffeic acid with reactive nitrogen species led to the conclusion that the nitration of caffeic acid with acidic nitrite does not significantly occur and confirmed the absence of nitration when caffeic acid reacts with peroxynitrite. Attempts to obtain free radical species from nitrocaffeic acids by classical methods showed a different reactivity to that of nitroaromatics and catechols. Nitrocaffeic acids do not autoxidize under aqueous basic conditions and are insensitive to t-BuOK or $O_2^-\cdot$ (two reactants known for their capabilities to oxidize catechols and reduce nitroaromatics). Nitroaromatic anion radicals may be obtained using sodium borohydride as reductant and are particularly stable under an uncontrolled atmosphere. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: nitrocaffeic acids; synthesis; physico-chemical properties

INTRODUCTION

Nonvitamin phenolic compounds are bioactive substances widely occurring in food plants and therefore potentially present in human plasma in a diet-dependent concentration. Among these compounds, caffeic acid is the most abundant of the hydroxycinnamic acids and is found naturally in various agricultural products such fruits, vegetables, wine, olive oil and coffee beans.[1,2] It is now well established that caffeic acid inhibits LDL oxidation induced by different systems and scavenges reactive oxygen species.[3–6] However, there have been few studies on the reactions of caffeic acid and related compounds with reactive nitrogen species. Caffeic acid and hydroxycinnamic acids inhibit the peroxynitrite-dependent tyrosine nitration.[7] Whereas monohydroxycinnamic acids (ferulic and coumaric acids) react with peroxynitrite in a nitration reaction, caffeic acid was readily oxidized by peroxinitrite.[7–9] Kono *et al.*[5] reported the formation of nitro compounds when caffeic or chlorogenic acids (an ester formed between caffeic acid and quinic acid) react with acidic nitrite.[10,11]

These last assertions, based on spectral considerations (presence or absence of significant modification in the visible region of the new product) prompted us to report

the full experimental procedure of the synthesis and the spectroscopic data (UV, acid–base properties) of the three nitrocaffeic acids. Attempts to characterize free radicals resulting from the one-electron reduction or oxidation of these nitrocaffeic acids give some interesting information concerning their oxido-reductive behaviour. We report here the ESR data of these compounds.

RESULTS

Synthesis

Nitrocaffeic acids were prepared according to Scheme 1, as briefly reported previously.[6] 2-Nitrocaffeic acid (2-NCA) and 5-nitrocaffeic acid (5-NCA) were obtained from vanillin in 40 and 59% overall yield, respectively. Vanillin was treated with acetic anhydride to afford acetylvanillin. Vanillin and acetylvanillin were nitrated and converted into the 5-nitro and 2-nitroferulic acids, respectively, by a Doebner modification of the Knoevenagel reaction. 5-Nitro- and 2-nitroferulic acids were treated with boron tribromide to give 5-NCA and 2-NCA. 6-Nitrocaffeic acid (6-NCA) was obtained by demethylation of the commercially available 6-nitro-3,4-dimethoxycinnamic acid using boron tribromide. In each case, the demethylation requires 5 equiv. of boron tribromide and a prolonged reflux in order to achieve total demethylation.

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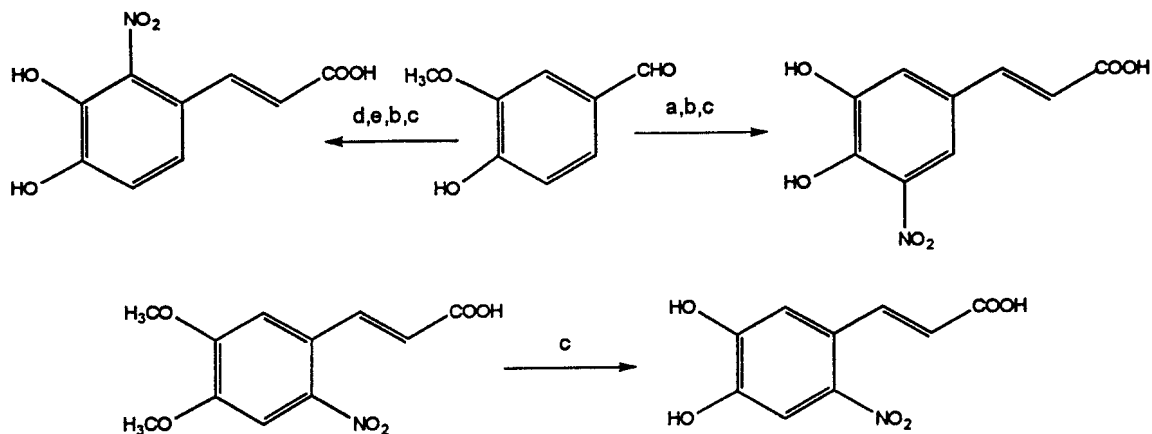
Scheme 1. (a) HNO$_3$, AcOH, $-10\,^\circ$C, 98%; (b) malonic acid, anhydrous pyridine, piperidine, 60 $^\circ$C, 6 days, 75–77%; (c) BBr$_3$, CH$_2$Cl$_2$, reflux, 12 h, 80–85%; (d) NaOH, Ac$_2$O, r.t., 1 h, 85%; (e) HNO$_3$, $-20\,^\circ$C then NaOH, 5%, then 4 M HCl, 75%

UV spectroscopy and acid–base properties

The pK_a s of the three nitrocaffeic acids were measured spectrophotometrically using the Henderson–Hasselbach procedure.[12] They are reported in Table 1.

The UV spectra at pH between 4 and 7 of NCAs showed the presence of an absorbance maximum in the visible region (430 nm) only for 6-NCA (Fig. 1). Moreover, there was no significant change in this visible region when the pH was modified except under strongly basic conditions (pH >12).

Free radical formation

As shown previously[13] in the cases of nitrohydroquinone, 3-nitrocatechol and 4-nitrocatechol, the nitrocaffeic acids did not autoxidize in aerated aqueous basic solutions whatever the pH, concentration and temperature conditions. This behaviour is strongly different to that of the other polyphenolic compounds which generally auto-xidize very easily under these conditions.[14]

Potassium *tert*-butoxide (*t*-BuOK) in dry dimethyl sulfoxide (DMSO) is known to afford semiquinonic radicals from hydroquinone, catechol or caffeic acid,[14,15] but leads to the formation of nitroaromatic anion radicals from nitroaromatics.[16,17] No ESR signals were detected during the addition of *t*-BuOK in *x*-NCA solutions. Neither nitroaromatic anion nor semiquinonic radicals were obtained. Chemically the studied compounds are unaffected by *t*-BuOK: the starting material was totally recovered after acidification of the solution. As a positive control, *t*-BuOK was reacted with caffeic acid and nitrobenzene. The expected radicals were easily obtained in the two cases.

Superoxide anion can reduce nitroaromatic compounds[18] and oxidize catechols.[19] The reactivity of the three nitrocaffeic acids towards superoxide anion (generated using potassium peroxide in DMSO in the presence of 18-C-6 crown ether) was evaluated. The ESR spectrum of superoxide anion was recorded at 77 K. In the presence of our compounds, the initially yellowish solution turned orange–red and the superoxide anion signal disappeared but no other signal was observed even at room temperature. Finally, the reduction by sodium borohydride in aqueous solution was the only efficient

Table 1. pK_as and UV data for CA (caffeic acid), 2-NCA, 5-NCA and 6-NCA

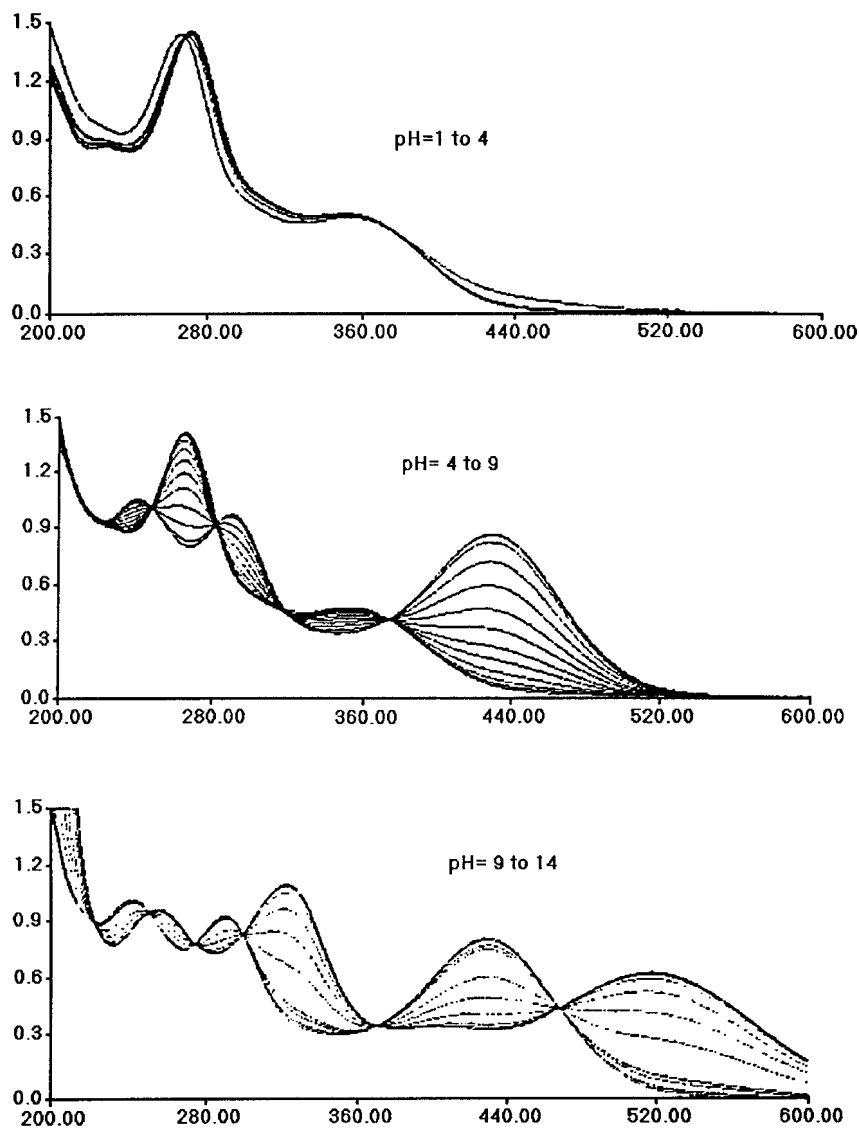| Compound	Measured pK_a	Wavelength (nm)	Isobestic points (nm)	Absorption maxima (nm)
CA	3.89	312	—	212; 282; 312
	8.37	339	233; 259; 317	339
	12.25	339	282	339
2-NCA	1.53	209	—	209; 296
	6.72	235	221; 261; 313	208; 235; 285; 325
	11.97	255	216; 254; 280; 329	255; 361
5-NCA	2.30	217	—	217; 291
	5.52	315	325	315
	13.15	315	266; 325	315; 334
6-NCA	2.58	266	—	266
	7.03	266	249; 285; 322; 374	266; 430
	12.24	430	251; 275; 299; 369; 468	322; 430; 517

Figure 1. UV–visible spectra of 6-NCA at various pH

method to produce nitroaromatic anion radicals (Fig. 2). The radicals are very stable under an uncontrolled atmosphere, but the $[NaBH_4]/[ArNO_2]$ concentration ratio must be fixed in the range 100–200 and the $[ArNO_2]$ concentration must not be higher than 0.01 M. The resulting pH was 8.8. The nitroaromatic anion radicals might be observed in the pH range 8.8–10. Otherwise, the solution was ESR silent. As a control, the *o*-nitrophenol radical anion was generated by this procedure. The hyperfine splitting constants are very close to those reported previously by Neta and Meisel.[20] The hyperfine coupling constants of the nitroaromatic anion radicals were attributed on the basis of the constants of the nitrobenzene anion radical[20] and by calculations. MacLachlan calculations using the parameters of Rieger and Franenkel[21] and Campredon *et al.*[16] give the spin density. For the nitrogen atom we used the parameters $h_N = 2.2$, $k_{C—N} = 1.2$ and $k_{N—O} = 1.67$ and for the oxygen atom

$h_{O(NO_2)} = 1.4$, $h_{O(OH/O-)} = 1.47$, $h_{O(C=O)} = 1.5$, $k_{C—O(OH/O-)} = 1.37$ and $k_{C—O(C=O)} = 1.6$. The nitrogen coupling constants can be calculated with the relation $a_N = 99\rho_{(N\pi)} - 2[35.89\rho_{(O\pi)}]$.

The hydrogen coupling constants were calculated using the MacConnell relation, $a_H = Q_{CH} \rho(C)$, where Q_{CH} is the MacConnell constant ($Q_{CH} = 25$ G) and $\rho(C)$ is the spin density of the carbon linked to the given hydrogen. The propenoic acid moiety presents a diedral angle of 20° versus the aromatic ring,[22] but we used an angle of 30° as previously used by Dixon *et al.*[14] Calculations were performed for each molecule with the nitro group rotated out of the plane of the ring, in steps of 10° for the deformed geometry (i.e. a twisted angle between the plane of the ring and the nitro group) and are depicted in Fig. 3. The calculations predict an angle of twist of ca 35–45° for 5-NCA and 6-NCA and ca 65° for 2-NCA.

Table 2. Experimental and calculated (in parentheses) hyperfine constants (in gauss) of nitroaromatic anion radicals obtained from 2-NCA, 5-NCA and 6-NCA

Parameter	2-NCA	5-NCA	6-NCA
a_1	—	—	—
a_2	19.62	2.75 (1.87)	1.60 (0.62)
a_3	nd	nd	nd
a_4	nd	nd	nd
a_5	1.10 (0.75)	15.25	2.25 (1.96)
a_6	0.40 (0.47)	2.75 (3.15)	15.15
a_α	nd (0.15)	nd (0.02)	0.85 (0.18)
a_β	0.55 (0.67)	nd (0.17)	1.65 (0.97)

DISCUSSION

The three nitrocaffeic acids were easily obtained from commercially available materials and were fully and unambiguously characterized. Using UV spectroscopy, the pK_as were measured showing a strong first acidity (particularly for 2-NCA) and a remarkable second acidity for 5-NCA. The electron-withdrawing properties of the nitro group (inductive and mesomeric) do not account alone for the observed pK_as. *Ortho* interactions (hydrogen bond, twisting phenomenon) have an increased importance in tetrasubstituted aromatic compounds. The comparison of the UV spectra of NCAs with the reported spectra[9] obtained from the reaction of acidic nitrite with caffeic acid shows that the absorbance peak at 472 nm was absent of the UV spectra of NCAs. Nevertheless, the absorbance peak at 440 nm observed in the UV spectra recorded during the reaction of chlorogenic acid with acidic nitrite may probably be attributed to the formation of a nitroaromatic compound. The calculations of the hyperfine hydrogen coupling constants of the nitroaromatic anion radicals show the necessity to consider a twisted angle between the propenoic acid moiety and the aromatic ring and revealed a pronounced twisted angle between the aromatic ring and the nitro group. Nevertheless, these calculations were made on the anion radical, which probably possesses a slightly different conformation from that of the neutral molecule. Attempts to obtain semiquinonic radicals by several known methods (aqueous basic conditions, *t*-BuOK, $O_2^{-\cdot}$) were unsuccessful and the nitroaromatic radical anion obtained under the previously reported conditions[13] was the unique detectable radical. Since NCAs seemed to be difficult to oxidize (into a semiquinonic radical), we felt that they were poor antioxidant agents. As expected, their $OH^\cdot$ scavenging activities are modest (500–800-fold less active than CA) but the $O_2^{-\cdot}$ scavenging properties were comparable to those of CA, 2-NCA being the best $O_2^{-\cdot}$ scavenger of the series.[6] We assume that the scavenging of $O_2^{-\cdot}$ by NCAs is probably due to their strong acidity.
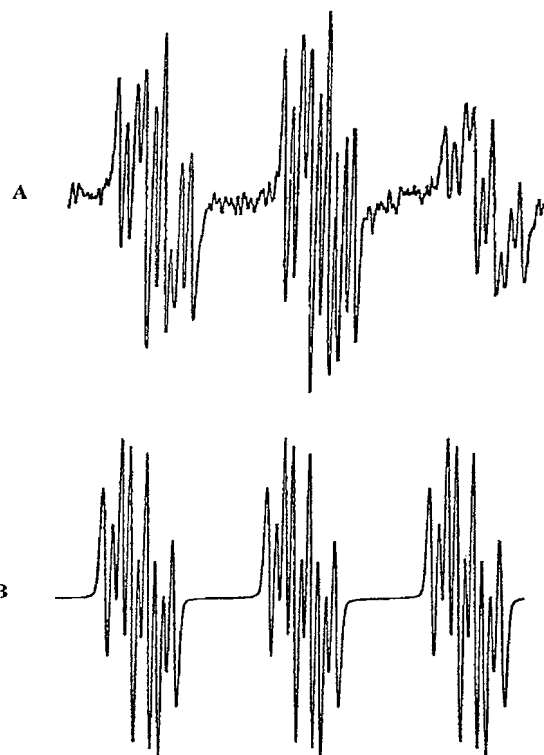

Figure 2. (A) ESR spectrum of the radical obtained from 6-NCA and sodium borohydride with its computer simulation (B) using a linewidth of 0.2 G

CONCLUSION

In this paper, the detailed synthesis of the three isomers of nitrocaffeic acids have been reported. Their spectroscopic data have been recorded and compared with previously reported results[5,7–9] on the reactivity of caffeic acid versus nitrogen reactive species such as peroxynitrite and nitrite. We conclude that the nitration of caffeic acid with acidic nitrite does not occur significantly whereas chlorogenic acid probably leads to nitroaromatic compounds. In the absence of isolated products, the nitration of caffeic acid by nitrite[9] seem to us erroneous. We are currently attempting to isolate and characterize the products of the reactions of caffeic acid and its esters with acidic nitrite. On the other hand, peroxynitrite undoubtedly oxidizes caffeic acid (and probably other catechols or hydroquinones) but the real nature of the isolated compound is questionable since the UV spectrum and the stability of caffeoyquinone are very different to those previously reported by Davies.[23] We are currently attempting to synthesize the different possible products of oxidation of caffeic acid in order to clarify the exact nature of the compound obtained from the reaction of caffeic acid with peroxynitrite. The present results show a clear protective role of the nitro group in the nitrocatechol moiety versus the oxidation leading to modest radical scavenging activities. In
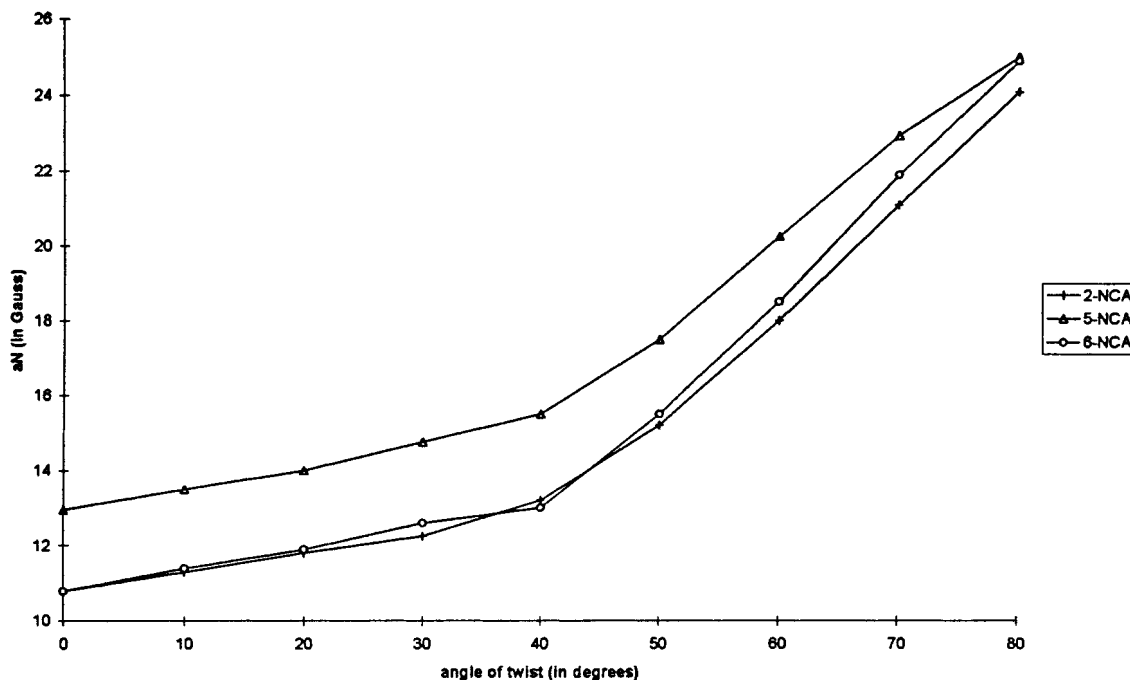
Figure 3. Dependence of calculated nitrogen hyperfine coupling constants for 2-NCA (+), 5-NCA (△) and 6-NCA (○) on the angle of twist of the nitro group out of the plane of the aromatic ring

contrast, the reduction of the nitro group by the classical method is ineffective. The reduction requires the use of sodium borohydride, as shown previously[13] in the case of simple nitrocatechols and nitrohydroquinone. The nitroaromatic anion radicals are fairly stable under an uncontrolled atmosphere even when pure oxygen is bubbled into the solution. The relative absence of reactivity of the nitrocatechol moiety (compared with catechol) may explain the low toxicity of nitecapone or entacapone[24] (two new COMT inhibitors which possess cardioprotective[25] and antioxidant[26] properties).

EXPERIMENTAL

Syntheses. TLC analyses were performed on a 3×10 cm plastic sheet precoated with silica gel $60F_{254}$ (Merck) [solvent system: ethyl acetate–hexane (1:4)]. SiO_2, 200–400 mesh (Merck), was used for column chromatography. Melting points were obtained on a Reichert Thermopan melting point apparatus equipped with a microscope and are uncorrected. NMR spectra were obtained on a Bruker AC 200 spectrometer in the appropriate solvent with TMS as internal reference. Mass spectra were recorded on a Ribermag R 10-10 spectrometer (electron ionization, 60 eV). Elemental analyses were performed by CNRS Laboratories (Vernaison) and were within 0.4% of the theoretical values.

Acetylvanillin. To a vigorously stirred solution of 40 g of vanillin and 10.8 g of NaOH in 200 ml of water was added dropwise a solution of 27.2 ml of acetic anhydride in 240 ml of diethyl ether. After 1 h of stirring, the organic layer was washed with water (5×100 ml) in order to eliminate the excess of acetic anhydride, dried over MgSO$_4$ and evaporated to give 43.4 g of acetylvanillin (85% yield): m.p. 77 °C (lit.[27] 77 °C); ^{1}H NMR (CDCl$_3$), δ2.31 (s, 3H, COCH$_3$), 3.87 (s, 3H, OCH$_3$), 7.19 (d, 1H, ^{3}J = 7.8 Hz, H5), 7.45 (m, 2H, H2 and H6), 9.92 (s, 1H, CHO); EIMS (60 eV) *m/z* 194 (11%, M$^{+\cdot}$), 152 (100%, [M-COCH$_3$]$^{+\cdot}$).

2-Nitrovanillin. Acetylvanillin (25 g) was slowly added to a solution of 120 ml of nitric acid ($d = 1.5$) at −20 °C and the mixture was poured into 500 g of crushed ice. The precipitate was filtered and washed with water. The resulting solid was dissolved in a solution of 10 g of NaOH in 200 ml of water. The solution was stirred for 15 min and then acidified with 4 M HCl. The precipitate was filtered, washed with water and dried to give 19 g of 2-nitrovanillin (75% yield) (this compound is very sensitive to light): m.p. 137 °C (lit.[27] 137 °C); ^{1}H NMR (DMSO-d_6), δ3.83 (s, 3H, OCH$_3$), 7.22 (d, 1H, ^{3}J = 8.5 Hz, H5), 7.45 (d, 1H, ^{3}J = 8.5 Hz, H6), 9.72 (s, 1H, CHO); EIMS (60 eV), *m/z* 196 (100%, M − H$^{+\cdot}$).

5-Nitrovanillin. To a solution of 25 g of vanillin in 50 ml of acetic acid maintained at −10 °C were added dropwise 4 ml of fuming nitric acid. The precipitate was filtered, washed with cold methanol (5×20 ml) and dried to give 32 g of 5-nitrovanillin (98% yield): m.p. 176 °C (lit.[28] 176 °C); ^{1}H NMR (CDCl$_3$), δ3.95 (s, 3H, OCH$_3$), 7.61 (d,

1H, ^4J = 1.7 Hz, H2), 8.09 (d, 1H, ^4J = 1.7 Hz, H6), 9.85 (s, 1H, CHO); EIMS (60 eV), *m/z* 197 (100%, M$^{+\cdot}$), 151 (38%, [M-NO$_2$]$^{+\cdot}$).

2- and 5-nitroferulic acids. Nitrovanillin (3 g) was dissolved in 15 ml of pyridine and 10 drops of piperidine, then 3.14 g of malonic acid were added. After complete dissolution, the solution was kept for 6 days at room temperature and then heated at 100 °C for 4 h. The solution was cooled and poured into a mixture of crushed ice (200 g) and concentrated HCl (100 ml). The precipitate was filtered, washed with ice-cold water and dried in presence of P$_2$O$_5$ to give 2- or 5-nitroferulic acid. 2-Nitroferulic acid (77% yield): m.p. 200–205 °C (decomp.); ^{1}H NMR (DMSO-d_6), δ3.82 (s, 3H, OCH$_3$), 6.48 (d, 1H, ^3J = 15.7 Hz, Hβ), 7.12 (d, 1H, ^3J = 15.7 Hz, Hα), 7.14 (d, 1H, ^3J = 8.7 Hz, H6), 7.64 (d, 1H, ^3J = 8.7 Hz, H5); EIMS (60 eV), *m/z* 239 (31%, M$^{+\cdot}$), 193 (90%, [M-NO$_2$]$^{+\cdot}$), 65 (100%). 5-Nitroferulic acid (75% yield): m.p. 245 °C (decomp.) (lit.[25] 149 °C); ^{1}H NMR (DMSO-d_6), δ3.92 (s, 3H, OCH$_3$), 6.64 (d, 1H, ^3J = 16.0 Hz, Hβ), 7.54 (d, 1H, ^3J = 16.0 Hz, Hα), 7.62 (d, 1H, ^4J = 1.7 Hz, H2), 7.75 (d, 1H, ^4J = 1.7 Hz, H6); EIMS (60 eV), *m/z* 239 (96%, M$^{+\cdot}$), 51 (100%).

Nitrocaffeic acids. To a suspension of 1 g of 2- or 5-nitroferulic acid or 1.055 g of 3,4-dimethoxy-6-nitrocinnamic acid in 50 ml of dichloromethane were added dropwise at room temperature under a nitrogen atmosphere 20 ml of BBr$_3$ (20 mmol) in dichloromethane. The mixture was refluxed overnight. To the cooled solution were added 200 ml of water. After 1 h of hydrolysis, the organic layer was separated and the aqueous layer extracted with ethyl acetate (5 × 50 ml). The organic layers were collected and refluxed with charcoal for 0.5 h. The suspension was filtered over Celite and dried over MgSO$_4$. After evaporation of the solvent the residue was precipitated in a mixture of acetone and water to give the nitrocaffeic acid. 2-Nitrocaffeic acid (80% yield): elemental analyses for C$_9$H$_7$NO$_6$, calculated C 48.01, H 3.13, N 6.22, O 42.64; found C 48.14, H 3.21, N 6.34, O 42.31%; m.p. 220 °C (decomp.); ^{1}H NMR (DMSO-d_6), δ6.41 (d, ^3J = 15.5 Hz, Hβ), 6.97 (d, ^3J = 8.0 Hz, H6), 7.15 (d, ^3J = 15.5 Hz, Hα), 7.33 (d, ^3J = 8.0 Hz, H5); ^{13}C NMR, δ167.3 (COOH), 146.5 (C4), 144.2 (C3), 141.6 (Cα), 138.1 (C2), 122.8 (C1), 120.0 (Cβ), 117.2 (C5), 116.3 (C6); EIMS (60 eV), *m/z* 225 (69%), 179 (56%), 51 (100%). 5-Nitrocaffeic acid (80% yield): elemental analyses for C$_9$H$_7$NO$_6$, calculated C 48.01, H 3.13, N 6.22, O 42.64; found C 48.08, H 3.15, N 6.07, O 42.70%; m.p. 210 °C (decomp.); ^{1}H NMR (DMSO-d_6), δ 6.41 (d, ^3J = 16.0 Hz, Hβ), 7.34 (d, ^4J = 2.0 Hz, H6), 7.48 (d, ^3J = 16.0 Hz, Hα), 7.66 (d, ^4J = 2.0 Hz, H2); ^{13}C NMR, δ 167.0 (COOH), 147.0 (C3), 143.8 (C4), 142.0 (Cα), 137.0 (C5), 124.9 (C1), 118.0 (Cβ), 117.0 (C2), 115.0 (C6); EIMS (60 V), *m/z* 225 (90%), 179 (17%), 51 (100%). 6-Nitrocaffeic acid (85% yield); elemental analyses for

C$_9$H$_7$NO$_6$, calculate C 48.01, H 3.13, N 6.22, O 42.64; found, C 48.00, H 3.18, N 6.41, O 42.41%; m.p. 240 °C (decomp); ^{1}H NMR (DMSO-d_6), 6.22 (d, ^3J = 15.7 Hz, Hβ), 7.09 (s, H2), 7.51 (s, H5), 7.93 (d, ^3J = 15.7 Hz, Hα); ^{13}C NMR, δ 167.1 (COOH), 151.3 (C3), 146.9 (C4), 140.2 (Cα), 139.6 (C6), 123.0 (C1), 121.0 (Cβ), 114.5 (C5), 111.0 (C2); EIMS (60 eV), 225 (11%), 208 (6%), 179 (100%).

pK_a measurement. The pK_a s of the nitro compounds were measured in water at 25 °C spectrophotometrically on a Perkin-Elmer Lambda 15 UV–visible spectrometer. The initial concentration of the solution of NCA was 100 μM. Starting from pH = 1, the pH was adjusted by adding 50 μl of 1 M NaOH (generally the total volume of NaOH did not exceed 2 ml for an initial volume of 100 ml). All the manipulations were carried out under an N$_2$ atmosphere in order to minimize the possible autoxidation of NCA under basic conditions. pK_a s were measured using the Henderson–Hasselbach relationship[12] $\log(A_{BH} - A)/(A - A_{B^-})$ = pH $-$pK_a, where A_{BH} and A_{B^-} represent the absorbances of the species at acidic and at basic pH, respectively, and A is the measured absorbance. pK_as are reported in Table 1.

ESR spectroscopy. ESR spectra were recorded using a Varian E-109 spectrometer operating at 9.5 GHz with a 100 kHz high-frequency modulation amplitude ranging from 0.1 to 0.4 G. The sample solutions were examined in a flat quartz cell inserted in an E-238 cavity operating in the TM$_{110}$ mode for the ESR spectra recorded at room temperature and in a quartz tube inserted in an E-232 cavity operating in the TE$_{104}$ mode for the ESR spectra recorded at 77 K.

Nitroaromatic anion radicals were obtained spontaneously from a mixture of sodium borohydride (30 mg) and the nitro compound (1 mg) in distilled water (0.5 ml). The resulting pH was 8.8 unless indicated otherwise. The initially yellowish solution turned orange–red after a few seconds and the ESR spectra were immediately recorded. Spectral simulations were carried out using a computer program.[29]

Superoxide anions were produce from potassium peroxide in DMSO (0.5 mM KO$_2$ was dissolved in DMSO in the presence of 5 mM 18-C-6). The nitro compound was then added and the temperature was immediately lowered to 77 K. The ESR spectra were immediately recorded. Attempts to characterize semi-quinonic or nitroaromatic radicals were made using the same solution at room temperature.

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