

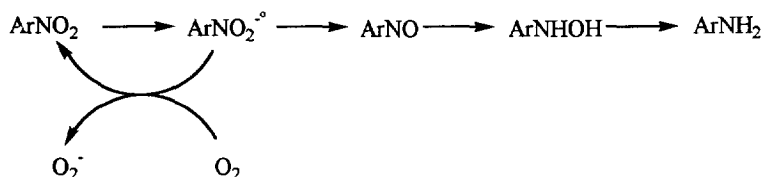
ANTIOXIDANT PROPERTIES OF NITROCAFFEIC ACIDS

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Abstract: A series of nitrodihydroxybenzenes and nitrocaffeic acids were prepared and their hydroxyl radical (OH^\bullet) and superoxide anion (O_2^-) scavenging activities and xanthine oxidase inhibition activities were evaluated. 2-Nitrocaffeic acid is the more potent O_2^- scavenger. 2- (and 5)-Nitrocaffeic acids are the more potent xanthine oxidase inhibitors.

Nitroaromatic drugs comprise a very large group with useful clinical activity as antibacterial, antiprotozoal and anticancer agents^{1,2}. Their activity is solely dependent upon reduction of the nitro group, the products of reduction are responsible for the toxicity of the drug. Their uses depend upon the selectivity of toxicity (differential toxicity towards hypoxic as compared to aerobic cells). The one-electron reduction of nitroaromatics affords the nitroaromatic anion radical which may lead to the nitroso, hydroxyamino and amino derivatives or may react with molecular oxygen to yield superoxide anion³⁻⁵ (Scheme 1).



Scheme 1

However, we have found that nitrocatechols afford very stable nitroaromatic anion radicals even in the presence of large amounts of oxygen⁶.

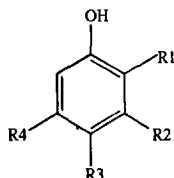
On the other hand, we have described new synthetic polyhydroxyflavones^{7,8} and benzoic acids⁹ with scavenger and antioxidant properties. In these works, it has also been found that 7-hydroxyflavones are competitive inhibitors of xanthine oxidase. Such compounds would be well adapted to the pathogenesis of ischemic injury which is characterized by an overproduction of the superoxide anion due (i) to a leak of electrons in the mitochondria respiratory chain and (ii) to the conversion of xanthine dehydrogenase to xanthine oxidase which

produces superoxide anion when converting hypoxanthine successively to xanthine, then uric acid. Thus, compounds able to both inhibit xanthine oxidase and to scavenge superoxide anion may be useful as protecting agents against cellular injury during reperfusion of ischemic tissues.

In this paper, we report our preliminary results of a new series of nitrocaffeic acids as potent scavengers of superoxide anion and xanthine oxidase inhibitors.

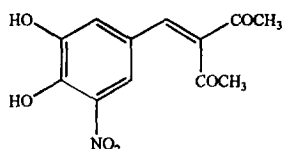
The superoxide anion and hydroxyl radical scavenging activities were investigated by Electron Spin Resonance (ESR) spectroscopy as previously described by Kitagawa¹⁰ and us¹¹ respectively. The inhibition of xanthine oxidase was studied by UV spectroscopy⁸. The results are reported in table 1.

Table 1. OH° and O₂⁻ scavenging activities and inhibition of xanthine oxidase by nitrodihydroxyphenyl derivatives



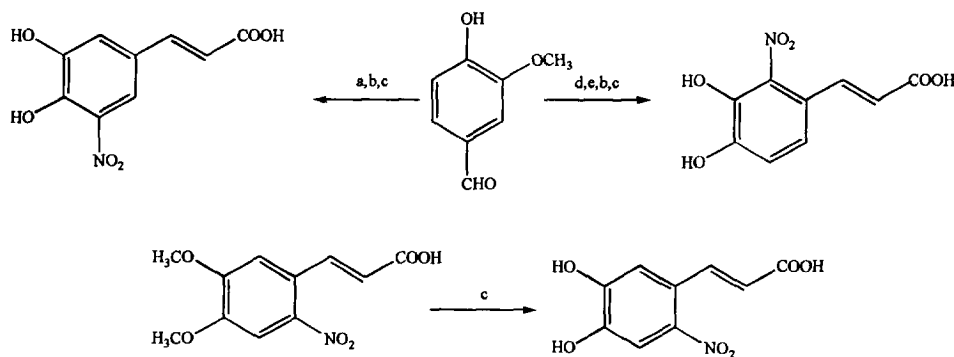
	COMPOUND				OH° scavenging activity ¹¹ (IC ₅₀ ; μM)	O ₂ ⁻ scavenging activity ¹⁰ (IC ₅₀ ; μM)	Xanthine oxidase inhibition ⁸ (% of inhibition)
	R1	R2	R3	R4			
1	OH	H	H	H	.01	30	5
2	OH	NO ₂	H	H	180	250	5
3	OH	H	H	NO ₂	130	250	9
4	NO ₂	H	OH	H	85	1000	12
5	OH	H	CHCHCOOH	H	.5	95	18
6	OH	NO ₂	CHCHCOOH	H	425	35	61
7	OH	NO ₂	H	CHCHCOOH	400	185	70
8	OH	H	NO ₂	CHCHCOOH	250	150	36
9	OH	NO ₂	H	CHC(COCH ₃) ₂	300 ¹²	500 ¹²	---

The inspection of table 1 shows that the xanthine oxidase inhibition requires both the caffeic acid moiety and a nitro group either at the 2 position¹³ (6) or at the 5 position¹³ (7). In the absence of the nitro group or the propenoic acid moiety the xanthine oxidase inhibition decreases (2,3 or 5). The more potent O_2^- scavenger is 6 which is as good O_2^- scavenger as catechol (1). However the presence of a nitro group decreases dramatically the OH^\bullet scavenging activities. These results are in accordance with those previously reported on the antioxidant properties of nitecapone¹² (scheme 2).



Scheme 2

Compounds 2 and 4 were obtained by known methods^{14,15}. Compounds 6,7 and 8¹⁶ were prepared as outlined in scheme 3. Compounds 6 and 7 were obtained from vanillin in 40% and 59% overall yield respectively. Compound 8 was obtained from the demethylation of the commercially available 6-nitro-3,4-dimethoxycinnamic acid by boron tribromide.



Scheme 3: (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_2Cl_2 , reflux, 12h, 80-85%; (d) $NaOH$, Ac_2O , r.t., 1h, 85%; (e) HNO_3 , $-20^\circ C$ then $NaOH$ 5% then HCl 4N, 75%.

Reduction of molecular oxygen to O_2^- by xanthine oxidase, followed by Haber-Weiss reaction (O_2^- -driven Fenton reaction) generating OH^\bullet is an important physiological pathway of reactive oxygen reaction. Compound 6 was found to affect this pathway by exhibiting a variety of its antioxidant abilities: xanthine oxidase inhibitor and O_2^- scavenger. Thus although, the abilities of compound 6 as a xanthine oxidase inhibitor and as O_2^- scavenger independently are not exceptional, its multioxidant capability deserves attention. A further study of this series will be useful in the design of new xanthine oxidase inhibitors. The antioxidant properties of

nitrocatechols are of particular interest since it has been postulated that the neurotoxicity of nitric oxide could be attributed to the formation of nitrocatecholamines^{17,18} (by a reaction of nitric oxide and catecholamines).

References and notes

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16. **Compound 6**: Elemental analyses for C₉H₇NO₆ calc'd 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; ¹H nmr (DMSO-d₆): δ 7.33 (d, ³J= 8 Hz, H₅), 7.15 (d, ³J= 15.5 Hz, H_α), 6.97 (d, ³J= 8 Hz, H₆), 6.41 (d, ³J= 15.5 Hz, H_β); ¹³C nmr: δ 167.3 (COOH), 146.5 (C₄), 144.2 (C₃), 141.6 (C_α), 138.1 (C₂), 122.8 (C₁), 120.0 (C_β), 117.2 (C₅), 116.3 (C₆); IEMS (60eV): 225 (69%), 179 (56%), 51 (100%).
17. **Compound 7**: Elemental analyses for C₉H₇NO₆ calc'd 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. 245°C; ¹H nmr (DMSO-d₆): δ 7.66 (d, ⁴J= 2 Hz, H₂), 7.48 (d, ³J= 16 Hz, H_α), 7.34 (d, ⁴J= 2 Hz, H₆), 6.41 (d, ³J= 16 Hz, H_β); ¹³C nmr: δ 167.0 (COOH), 147.0 (C₃), 143.8 (C₄), 142.0 (C_α), 137.0 (C₅), 124.9 (C₁), 118.0 (C_β), 117.0 (C₂), 115.0 (C₆); IEMS (60eV): 225 (90%), 179 (17%), 51 (100%).
18. **Compound 8**: Elemental analyses for C₉H₇NO₆ calc'd 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; ¹H nmr (DMSO-d₆): δ 7.93 (d, ³J= 15.7 Hz, H_α), 7.51 (s, H₅), 7.09 (s, H₂), 6.22 (d, ³J= 15.7 Hz, H_β); ¹³C nmr: δ 167.1 (COOH), 151.3 (C₃), 146.9 (C₄), 140.2 (C_α), 139.6 (C₆), 123.0 (C₁), 121.0 (C_β), 114.5 (C₅), 111.0 (C₂); IEMS (60eV): 225 (11%), 208 (6%), 179 (100%).
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