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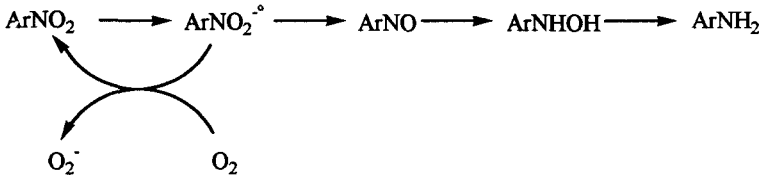
ANTIOXIDANT PROPERTIES OF NITROCAFFEIC ACIDS

Jean-Luc Grenier, Nicole Cotelle, Philippe Cotelle* and Jean-Pierre Catteau

Laboratoire de Chimie Organique physique, URA CNRS 351,
Université des Sciences et Techniques de Lille, 59655 Villeneuve d'Acsq, France

Abstract: A series of nitrodihydroxybenzenes and nitrocaffeic acids were prepared and their hydroxyl radical (OH°) and superoxide anion ($O_2^{\cdot-}$) scavenging activities and xanthine oxidase inhibition activities were evaluated. 2-Nitrocaffeic acid is the more potent $O_2^{\cdot-}$ 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 anion[3-5] (Scheme 1).



Scheme 1

However, we have found that nitrocatechols afford very stable nitroaromatic anion radicals even in the presence of large amounts of oxygen[6].

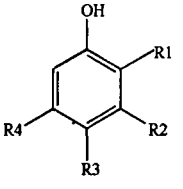
On the other hand, we have described new synthetic polyhydroxyflavones[7,8] and benzoic acids[9] with scavenger and antioxidant properties. In these works, it has also been found that 7-hydroxyflavones are competitive inhibitors of xanthine oxidase. Such compounds would be well adapted to the pathogenesis 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.

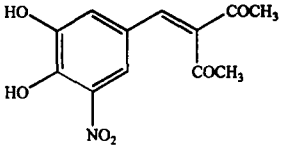
The superoxide anion and hydroxyl radical scavenging activities were investigated by Electron Spin Resonance (ESR) spectroscopy as previously described by Kitagawa[10] and us[11] respectively. The inhibition of xanthine oxidase was studied by UV spectroscopy[8]. The results are reported in table 1.

Table 1. OH° and O_2^- scavenging activities and inhibition of xanthine oxidase by nitrodihydroxyphenyl derivatives



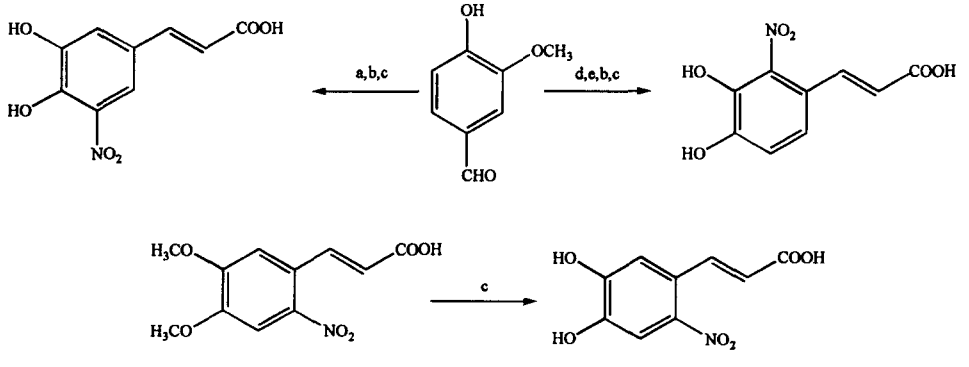
	COMPOUND R1	R2	R3	R4	OH° scavenging activity[11] (IC_{50}; µM)	O_2^- scavenging activity[10] (IC_{50}; µM)	Xanthine oxidase inhibition[8] (% of inhibition)
1	OH	H	H	H	.01	30	5
2	OH	NO_2	H	H	180	250	5
3	OH	H	H	NO_2	130	250	9
4	NO_2	H	OH	H	85	1000	12
5	OH	H	CHCHCOOH	H	.5	95	18
6	OH	NO_2	CHCHCOOH	H	425	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 position[13] (**6**) or at the 5 position[13] (**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° scavenging activities. These results are in accordance with those previously reported on the antioxidant properties of nitecapone[12] (scheme 2).



Scheme 2

Compounds **2** and **4** were obtained by known methods[14,15]. Compounds **6,7** and **8**[16] 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°C, 98%; (b) malonic acid, anhydrous pyridine, piperidine, 60°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°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° 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

1. Grunberg, E.; Titsworth, E.H. *Ann. Rev. Microbiol.* **1973**, *27*, 317
2. Biaglow, J.E.; Varnes, M.E.; Koch, C.H.; Sridhar, R. *Free Radicals and Cancer* (Ed. R.A. Floyd) M. Dekker, New York, **1982**
3. Mason, R.P.; Holtzman, J.L. *Biochemistry* **1975**, *14*, 1626
4. Mason, R.P.; Holtzman, J.L. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 1267
5. Berson, A.; Wolf, C.; Chachaty, C.; Fau, D.; Pessayre, D. *J. Chim. Phys.* **1994**, *91*, 1809
6. Grenier, J.L.; Cotelle, N.; Cotelle, P.; Catteau, J.P. *J. Chim. Phys.* **1995**, *92*, 97
7. Cotelle, N.; Bernier, J.L.; Hénichart, J.P.; Catteau, J.P.; Gaydou, E.; Wallet, J.C. *Free Radic. Biol. Med.* **1992**, *13*, 211
8. Cotelle, N.; Bernier, J.L.; Catteau, J.P.; Pommery, J.; Wallet, J.C.; Gaydou, E. *Free Radic. Biol. Med.* in press
9. Capelle, S.; Cotelle, N.; Cotelle, P.; Bernier, J.L.; Catteau, J.P. *Redox Report* **1994**, *1*, 131
10. Kitagawa, S.; Fujisawa, H.; Sakurai, H. *Chem. Pharm. Bull.* **1992**, *40*, 304
11. Capelle, S.; Planckaert, B.; Cotelle, P.; Catteau, J.P. *J. Chim. Phys.* **1992**, *89*, 561
12. Suzuki, Y.J.; Tsuchiya, M.; Safadi, A.; Kagan, V.E.; Packer, L. *Free Radic. Biol. Med.* **1992**, *13*, 517
13. The IUPAC nomenclature for **6**, 7 and **8** is: (E)-3,4-dihydroxy-(2,5 or 6)-nitro cinnamic acid respectively.
14. Rosenblatt, D.H.; Epstein, J.; Levitch, M. *J. Am. Chem. Soc.* **1953**, *75*, 3277
15. Kehrmann, F.; Sandoz, M.; Monnier, R. *Helv. Chim. Acta*, **1921**, *4*, 948
16. *Compound* **6**: Elemental analyses for $C_9H_7NO_6$ 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; ^{1}H nmr (DMSO-d6): δ 7.33 (d, ^{3}J= 8 Hz, H5), 7.15 (d, ^{3}J= 15.5 Hz, Hα), 6.97 (d, ^{3}J= 8 Hz, H6), 6.41 (d, ^{3}J= 15.5 Hz, Hβ); ^{13}C nmr: δ 167.3 (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); IEMS (60eV): 225 (69%), 179 (56%), 51 (100%).
Compound **7**: Elemental analyses for $C_9H_7NO_6$ 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; ^{1}H nmr (DMSO-d6): δ 7.66 (d, ^{4}J= 2 Hz, H2), 7.48 (d, ^{3}J= 16 Hz, Hα), 7.34 (d, ^{4}J= 2 Hz, H6), 6.41 (d, ^{3}J= 16 Hz, Hβ); ^{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); IEMS (60eV): 225 (90%), 179 (17%), 51 (100%).
Compound **8**: Elemental analyses for $C_9H_7NO_6$ 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; ^{1}H nmr (DMSO-d6): δ 7.93 (d, ^{3}J= 15.7 Hz, Hα), 7.51 (s, H5), 7.09 (s, H2), 6.22 (d, ^{3}J= 15.7 Hz, Hβ); ^{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); IEMS (60eV): 225 (11%), 208 (6%), 179 (100%).
17. De La Bretèche, M.L.; Servy, C.; Lenfant, M.; Ducrocq, C. *Tetrahedron Lett.* **1994**, *39*, 7231
18. D'Ischia, M.; Costantini, C. *Bioorg. Med. Chem.* **1995**, *3*, 923

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