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Beneficial Effects of Different Flavonoids, on Functional Recovery after Ischemia and Reperfusion in Isolated Rat Heart

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Abstract—Three newly synthesised lipid peroxidation inhibitors (**7**, **11**, **14**) were evaluated for their effects on myocardial functional recovery during reperfusion after 30 min global ischemia in isolated rat hearts. The flavonoid compounds (**7**, **11**, **14**, rutin) reduce ischemia/reperfusion-induced cardiac dysfunction.

Introduction

Flavonoids are polyphenolic compounds which constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets.[1] Recent interest has increased greatly owing to their antioxidant capacity with free radical scavenging properties attributed to the catechol or pyrogallol group. The redox properties of polyphenols allow them to act as enzyme inhibitors,[2,3] reducing agents, hydrogen donating antioxidants and in some cases they also chelate transition metal ions.[4,5] These properties play an important part in reducing the risk of free radical-related oxidative damage associated with degenerative disease such as in the treatment and prevention of cancer[4,6,7] and cardiovascular disease.[8] However, the compounds with catechol moiety present a potent prooxidant activity in particular conditions.[9–11] Therefore, our research interest in this field has also been focused in the synthesis of modified flavonoids. In this report, we reveal the chemical synthesis of three new flavonoids [3′,5′-di-*tert*-butyl-7,4′-dihydroxyflavone (flavone **7**), 3′,5′-di-*tert*-butyl-2,4,4′-trihydroxychalcone (chalcone **11**), 3-(4″-hydroxy-3″,5″-di-*tert*-butylbenzylidene)-7,4′-dihydroxy-3′,5′-di-*tert*-butylflavanone (arylidene, **14**)] (Scheme 1) where the 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety was associated to the flavonoid backbone and evaluate the possible role of these flavonoids compared with a xanthine oxidase inhibitor (allopurinol),[12] a flavonol known for its antioxidant properties (rutin: rhamnoside quercetin),[13] and a food additive antioxidant (BHT[14]) in protecting the myocardium from the damaging effects of ischemia/reperfusion injury using the isolated perfuse working rat heart to generate potent cardioprotective therapy agents.

Chemistry

The flavones were prepared according to the Baker–Vankataraman procedure[15,16] (Scheme 2). 2-Hydroxy-4-methoxyacetophenone **1** was condensed with 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride in the presence of DMAP in dry pyridine at 60 °C during 2 h to give the diester **2** in 57% yield. This result was not surprising since 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride reacted in dry pyridine at 60 °C to give 4-(3,5-di-*tert*-butyl-4-hydroxybenzoyloxy)-3,5-di-*tert*-butylbenzoic acid in 60% yield. Nevertheless, the diester **2** was treated with sodium hydroxide in dry DMSO to give the diarylpropane-1,3-dione **3** in 48% yield. The cyclisation of the diarylpropane-1,3-dione **3** was carried out in refluxing acetic acid in the presence of sulfuric acid during 1 h leading to the flavone **4** in 76% yield, which was deprotected using boron tribromide in refluxing dichloromethane to give **5** in 77% yield. The flavone **5** was saponified with sodium hydroxide in dry DMSO for 4 h at 120 °C to give the target flavone **7** in 45% yield. Alternatively **4** was saponified to give the flavone **6** in 54% yield, which was deprotected using boron tribromide to give **7**[18] in 84% yield. Finally, the target

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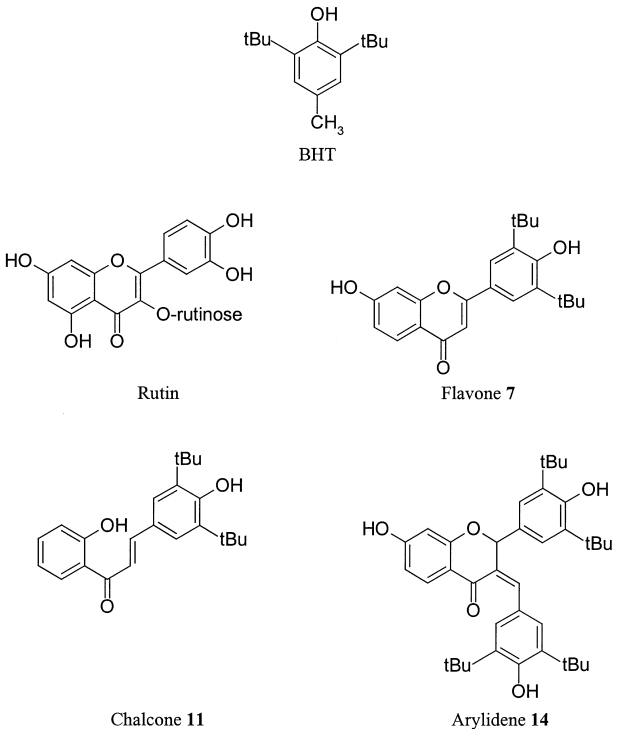
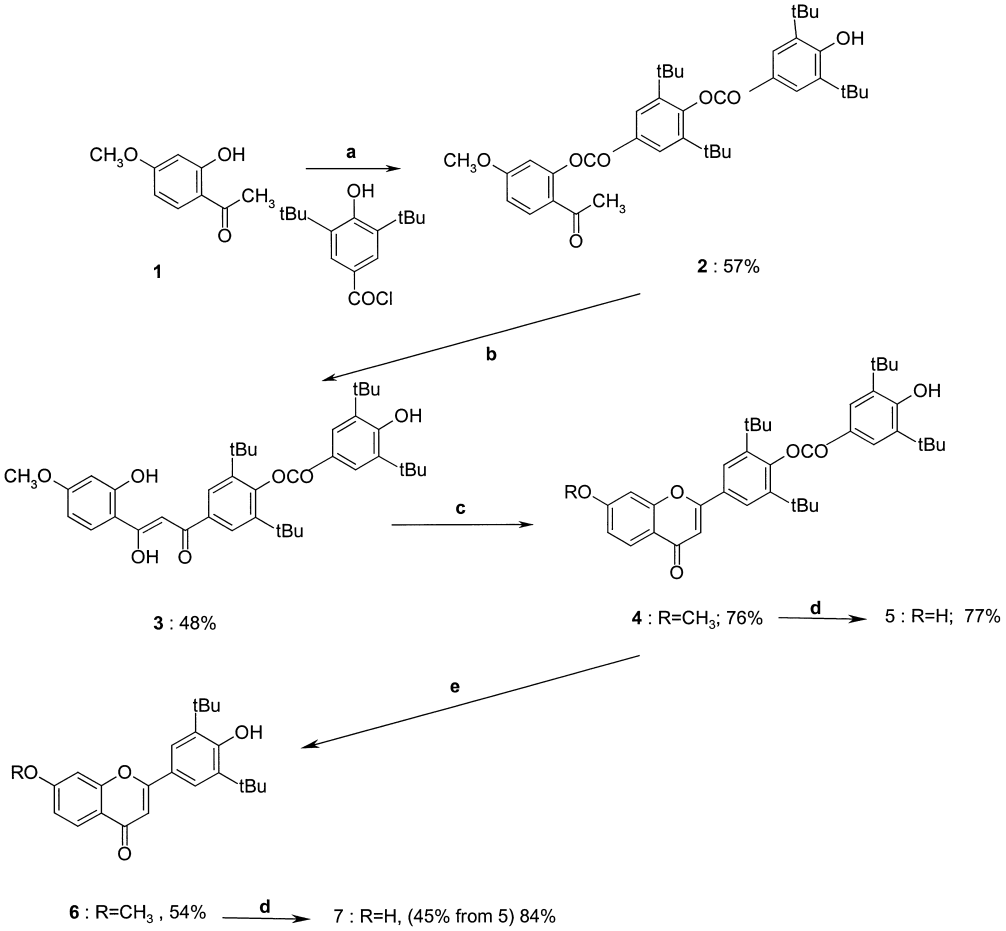


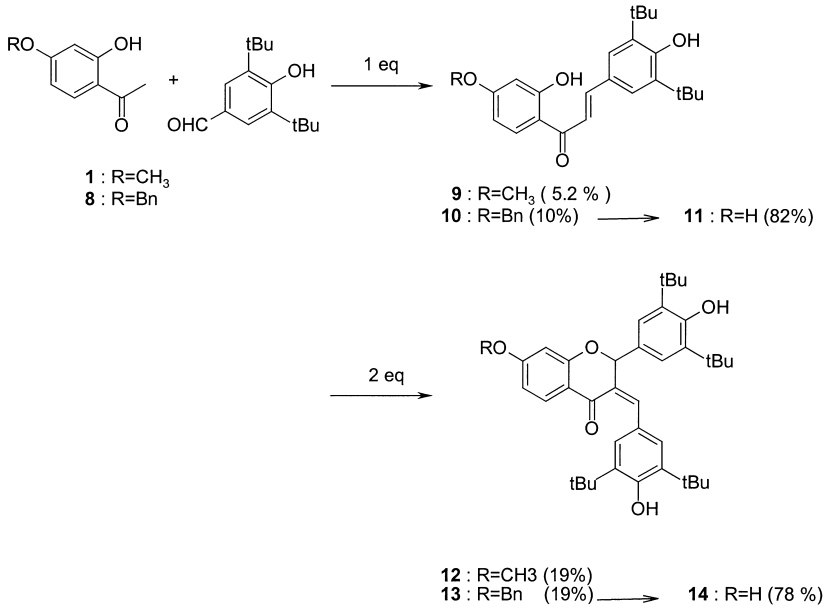
Scheme 1. Chemical structures of BHT, rutin, **7**, **11**, **14**.

flavone **7** was obtained in 6.22% overall yield (calculated from 2,4-dihydroxyacetophenone) in a six-step procedure (versus 4.75% yield when the deprotection was realised before saponification procedure).

The chalcones **9**–**10** and arylidenes **12**–**13** were obtained from the reaction of **1** or **8** with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (Scheme 3). Best results in chalcones were obtained when the acetophenone was treated with one equivalent of the benzaldehyde and the arylidene with two equivalents of the benzaldehyde. Nevertheless, in all the cases, chalcone, arylidene and starting material coexist in the final mixture. The reaction of **1** and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde led to the chalcone **9** and arylidene **12**, which were isolated and treated with boron tribromide. Unfortunately, at −78 °C the demethylation did not occur, whereas at room temperature the target chalcone and arylidene were totally destroyed. These deceptive results led us to prepare the 4-benzyloxy-2-hydroxyacetophenone **8** and reacted it with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde. The resulting chalcone **10** and arylidene **13** were separated and readily deprotected using boron tribromide at −78 °C. The target chalcone **11**[19] and arylidene **14**[20] were obtained in 8 and 15% overall yield (calculated from **1** and **8**), respectively.



Scheme 2. Reagents and conditions: (a) pyridine, DMAP, 2 h, 60 °C; (b) DMSO, NaOH, 2 h, 60 °C; (c) AcOH, H_2SO_4. (d) BBr_3, CH_2Cl_2, 24 h, reflux; (e) DMSO, NaOH, 4 h, 120 °C.



Scheme 3. Synthesis of chalcones and arylidenes.

Biological Activities

Male Sprague–Dawley (300–350 g) were studied according to the Langendorff method.[17] Briefly, the heart was rapidly excised, placed in a cold (4 °C) perfusion buffer then immediately cannulated at the aorta root and perfused with a modified Krebs–Henseleit bicarbonate buffer (37 °C) gassed with 95% oxygen and 5% carbon dioxide (10 mL/min). DMSO solutions of compound were prepared at 10^{-2} M and diluted in Krebs to give final concentration of 5–10 μM in a total DMSO concentration of 0.01% (v/v). The same amounts of pure DMSO were added to the control. A compliant water-filled latex balloon was inserted into the left ventricle through the mitral valve and connected to a pressure transducer. The filling pressure was then adjusted to 5 mmHg. Left ventricular developed pressure (LVDP, mmHg) was recorded continuously.

Five different drug regimes were studied according to the following protocol. After a 10 min initial period, isolated hearts were perfused with either Krebs alone, Krebs + BHT (10 μM), Krebs + rutin (10 μM), Krebs + allopurinol (10 μM), flavone **7** (5 μM), chalcone **11** (5 μM), arylidene **14** (5 μM) in a recirculating manner for 15 min. Hearts were then subjected to a 30 min ischemic period followed by 30–40 min reperfusion using Krebs and the assigned drug regime at the same dose.

Biochemical analysis

The levels of lipid peroxides and enzymatic antioxidant systems in isolated rat heart muscle subjected to global ischemia followed by 30 min of reperfusion were measured.

After each experimental ischemia/reperfusion sequence, the homogenate heart was frozen in liquid nitrogen. Lipid peroxides (TBARS) were assayed in the cardiac homogenate and enzymatic antioxidant systems[22] (GSH peroxidase, superoxide dismutase and catalase) were determined in the centrifuged supernatant. In the experimental protocol studied in this work, there was no significant increase in the activity of enzymatic antioxidant systems (Table 1) or in the level of peroxidation compared to control hearts.

The process of lipid peroxidation, assessed by assaying thiobarbituric acid reactants, is not a predominant phenomenon of reperfusion-induced injury, at least in the experimental model used here. However, enzymatic antioxidant systems investigated in this study do not seem modified. This could mean that the small quantity of oxygen free radicals produced does not overwhelm the enzymatic antioxidant systems of myocardium that is in agreement with lipid peroxidation results.

Table 1. Preparation and measurement of antioxidant enzymes in the heart were performed according to the method described by Brown et al.[22 a]

	SOD (U/mg proteins)	GPX (U/mg proteins)	CAT (U/mg proteins)
Controls	8035±767	1834±229	20±5
BHT	9636±327	1803±112	20±3
Allopurinol	9800±450	1912±234	21±1
Rutin	9077±667	1829±233	21±6
7	8433±1061	1982±525	21±3
11	8740±1056	1879±678	20±7
14	8935±989	1906±634	21±6

[a]Inhibition of the rate of reduction of cytochrome C by superoxide radicals was used as an indirect measurement of SOD activity (1 U = SOD activity required to inhibit cytochrome C reduction by 50%). Disappearance of hydrogen peroxide was monitored at 240 nm as a measure of catalase activity (1 U = 1 μmol of H_2O_2 degraded/min per mg of protein). Glutathione peroxidase activity was determined by the disappearance of NADPH is monitored at 340 nm (1 U = 1 μmol of NADPH converted to NADP/min per mg of protein). All biochemical analyses were standardised per gram of protein content, as determined by Bio-Rad protein assay (Richmond, CA, USA). Values are mean + S.D. not significantly different from control; $*p > 0.05$

Functional recovery

The results demonstrated that flavonoids at the concentration of 5 μM and more especially chalcone **11** exhibited significant myocardial protection. This was evidenced by improved recovery of post-ischemic left ventricular function (left ventricular developed pressure; LVDP: Fig. 1) and diastolic function (left ventricular end-diastolic pressure, LVEDP: Fig. 2) as compared to the control and BHT groups. Values for developed pressure in the flavonoid-treated groups were significantly higher than those in the control and BHT group throughout the reperfusion period (rutin: 81.3±3.8 mmHg, **7**: 87.12±9.21, **11**: 102.25±1.17, **14**: 87.12±9.2 versus control: 38±6, BHT: 29.79±4.25). In contrast to the control group and the BHT treated group, the flavonoid groups and allopurinol group displayed significant reduction in diastolic developed pressure (allopurinol: 5±2, rutin: 9.33±3.78, **7**: 10.5±1.9, **11**: 9.5±1.73, **14**: 14.5±2 versus control: 30±5, BHT: 21.5±0.5).

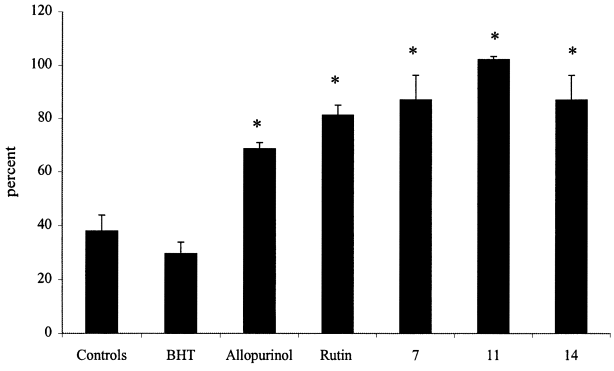


Figure 1. Effects of flavonoids on post-ischemic recovery of left ventricular developed pressure (LVDP) in the isolated heart. Post-ischemic LV recovery was expressed as the ratio 100×post-ischemic LVDP/pre-ischemic LVDP. Results are expressed as mean±SEM of six rats per group. *$p <$ 0.01 compared with controls.

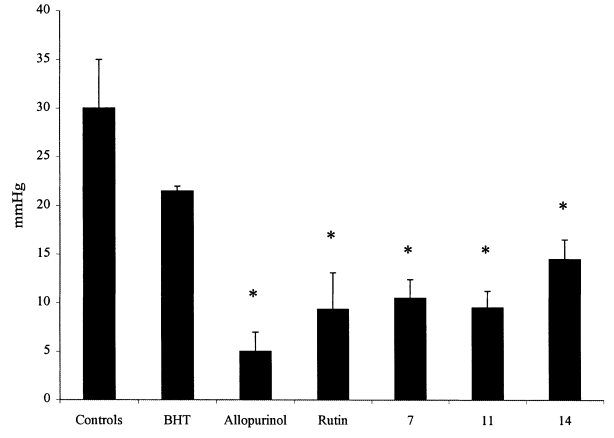


Figure 2. Effects of flavonoids on left ventricular post-ischemic LV end-diastolic developed pressure (LVEDP, mmHg) in the isolated heart. Results are expressed as mean±SEM of six rats per group. *$p < 0.001$ compared with controls.

Conclusion

In this paper, we provide evidence that flavone **7**, chalcone **11**, arylidene **14**, where the BHT moiety and the flavonoid backbone were associated, possess potent cardioprotective properties more important than rutin and allopurinol. Flavonoid-treated hearts and more especially chalcone-treated hearts were resistant to ischemia reperfusion injury, as shown by the improvement of the post-ischemic ventricular function and myocardial stunning. Previous in vitro study[21] has revealed that new flavonoid compounds (**7**, **11**, **14**) are powerful lipid peroxidation inhibitors and poor xanthine oxidase inhibitors. These results suggest that the same flavonoids possess cardioprotective effects that may be attributed to their peroxyl radical scavenging activities although no difference has been observed in homogenate heart TBARS measurements. This may provide a potential therapeutic approach for the prevention of post-ischemic myocardial dysfunction.

References and Notes

1. Bravo, L. *Nutrition Reviews* **1998**, *56*, 317.
2. Ferte, J.; Kuhnel, J. M.; Chapuis, G.; Roland, Y.; Lewin, G.; Schwaller, M. A. *J. Med. Chem.* **1999**, *42*, 478.
3. Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. M. *Free Radical Biol. Med.* **1996**, *20*, 35.
4. Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1990**, *186*, 343.
5. Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. *Free Radical Res.* **1995**, *22*, 375.
6. Sui, Z.; Nguyen, V. N.; Altom, J.; Fernandez, J.; Hilliard, J.; Bernstein, J. I.; Barrett, J. F.; Ohemeng, K. A. *Eur. J. Med. Chem.* **1999**, *34*, 381.
7. Miranda, C. L.; Stevens, J. F.; Helmrich, A.; Henderson, M. C.; Rodriguez, R. J.; Yang, Y. H.; Deinzer, M. L.; Barnes, D. W.; Buhler, D. R. *Food Chem. Toxicol.* **1999**, *37*, 271.
8. Hertog, M. G.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. *Lancet* **1993**, *342*, 1007.
9. Rhaman, A.; Hadi, S. M.; Parish, J. H.; Ainley, K. *Carcinogenesis* **1989**, *10*, 1833.
10. Cao, G.; Sofic, E.; Prior, R. L. *Free Radical Biol. Med.* **1997**, *22*, 749.
11. Sugihara, N.; Arakawa, T.; Ohnishi, M.; Furuno, K. *Free Radical Biol. Med.* **1999**, *27*, 1313.
12. Reimer, K. A.; Jenning, M. D. *Circulation* **1985**, *71*, 1069.
13. Manach, C.; Morand, C.; Texier, O.; Favier, M.-L.; Agullo, G.; Demigné, C.; Régérat, F.; Rémésy, C. *J. Nutr.* **1995**, *125*, 1911.
14. Babich, H. *Environ. Res.* **1982**, *29*, 1.
15. Baker, W. *J. Chem. Soc.* **1933**, 1381.
16. Mahal, H. S.; Venkataraman, K. *J. Chem. Soc.* **1934**, 1767.
17. Neviere, R.; Cepinskas, G.; Madorin, W. S.; Hoque, H.; Karmazyn, M.; Sibbald, W. J.; Kvietys, P. R. *Am. J. Physiol.* **1999**, *277*, H885.
18. **7**, ^{1}H NMR (DMSO-d_6, 200 MHz, δ in ppm): 1.41 (18H, s, di-*tert*-butyl), 6.77 (1H, s, H_3), 6.92 (1H, dd, $^3J = 8.60$ Hz, $^4J = 2.20$ Hz, H_6), 6.99 (1H, d, $^4J = 2.20$ Hz, H_8), 7.70 (1H, s, OH), 7.76 (2H, s, H cycle B), 7.88 (1H, d, $^3J = 8.60$ Hz, H_5), 10.72 (1H, s, OH). ^{13}C NMR ($CDCl_3$, 50 MHz, δ in ppm):

30.05 (C–$\underline{C}H_3$), 34.67 (C–$\underline{C}H_3$), 102.50 (C_8), 105.04 (C_3), 114.76 (C_6), 116.11 (C_{4a}), 122.26 (C_1'), 122.95 (C_2'), 126.47 (C_5), 139.21 (C_3'), 157.33 (C_4'), 157.40 (C_{8a}), 162.52 (C_7), 163.32 (C_2), 176.26 (C_4).

19. **11**, ^{1}H NMR ($CDCl_3$, 300 MHz, δ in ppm): 1.50 (18H, s, di-*tert*-butyl), 5.64 (1H, s, OH in *ortho* position of di-*tert*-butyl), 7.00 (2H, m, H_5), 7.45 (1H, d, H in α of CO, $^3J = 15.3$ Hz), 7.55 (2H, s, H aromatic of B ring), 7.85 (1H, d, H_3, $^3J = 9.4$ Hz), 7.90 (1H, s, OH in *ortho* position of CO). ^{13}C NMR ($CDCl_3$, 50 MHz, δ in ppm): 30.18 (C-(CH_3)), 34.39 (C–(CH_3)), 116.78 (C_6), 120.24 (C_4), 126.11 (CH=), 156.95 (C_1), 193.75 (C=O).

20. **14**, ^{1}H NMR ($CDCl_3$, 200 MHz, δ in ppm): 1.32 (18H, s, di-*tert*-butyl), 1.40 (18H, s, di-*tert*-butyl), 5.24 (1H, s, OH), 5.50 (1H, s, OH), 6.38 (1H, d, H_8), 6.56 (2H, m, H_6 and H_2), 6.53 (1H, s, H_2), 7.11 (2H, s, H_2'), 7.30 (2H, s, H_2''), 8.09 (1H, s, H_β), 10.80 (1H, s, OH).

21. Lebeau, J.; Furman, C.; Duriez, P.; Bernier, J. L., Teissier, E.; Cotelle, N. *Free Radical Biol. Med.* **2000**, *29*, in press.

22. Brown, J. M.; Grosso, M. A.; Terada, L. S.; Whitman, G.; Banerjee, A.; White, C. W.; Harken, A. H.; Repine, J. E. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2516.