JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1373–1378

Received April 24, 2002
Accepted June 14, 2002
ISSN 0022-3573

Quercetin 3,7-dimethyl ether: a vasorelaxant flavonoid isolated from *Croton schiedeanus* Schlecht

M. F. Guerrero, P. Puebla, R. Carrón, M. L. Martín and L. San Román

Abstract

The vasorelaxant profile of quercetin 3,7-dimethyl ether, a flavonoid isolated from *Croton schiedeanus* Schlecht (Euphorbiaceae), was assessed in aortic rings isolated from Wistar rats. To gain insight into its structure–activity relationship, we compared this substance with quercetin 3,4′,7-trimethyl ether (ayanin), another flavonoid isolated from this plant, quercetin 3,3′,4′,7-tetramethyl ether, a flavonoid synthesized by us, and quercetin. In addition we examined the interaction of quercetin 3,7-dimethyl ether with the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway. According to their pEC50 values (concentration producing a 50 % inhibition of the maximal contractile response) to phenylephrine-induced precontraction in rat isolated aorta, the potency order was quercetin 3,7-dimethyl ether > quercetin > quercetin 3,4′,7-trimethyl ether > quercetin 3,3′,4′,7-tetramethyl ether (4.70±0.18; 3.96±0.07; 3.64±0.02; 3.11±0.16). The relaxant effect of quercetin 3,7-dimethyl ether was significantly decreased by the removal of endothelium as well as by methylene blue, an inhibitor of guanylyl cyclase, and by N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), an NO-synthase inhibitor. Therefore, quercetin 3,7-dimethyl ether has a NO/cGMP pathway-related profile, with increased vasorelaxant activity due to hydroxylation at positions 3 and 4 of the B ring. In addition, methylation at positions 3 and 7 with respect to quercetin of the C and A rings, respectively, seems to further enhance the vasorelaxant activity of quercetin 3,7-dimethyl ether.

Laboratorio de Farmacognosia y Farmacología, Facultad de Farmacia, Universidad de Salamanca, E-37007 Salamanca, Spain

M. F. Guerrero, R. Carrón, M. L. Martin, L. San Román

Departamento de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, E-37007 Salamanca, Spain

M. F. Guerrero, P. Puebla

Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, AA 11430 Bogotá, Colombia

M. F. Guerrero

Correspondence: M. L. Martin, Laboratorio de Farmacognosia y Farmacología, Facultad de Farmacia, Universidad de Salamanca, E-37007 Salamanca, Spain. E-mail: marisam@usal.es

Acknowledgment and funding: Project ALFA-RELAPLAMED.2 and Subprogram X, "Química Fina Farmacéutica" of the CYTED supported this work. We thank the School of Pharmacy of the University of Salamanca and the project "Búsqueda de Principios Bioactivos en Plantas Medicinales Colombianas" developed at the Department of Pharmacy, School of Sciences, National University of Columbia.

Introduction

There is increasing interest in the cardioprotective effects of flavonoid compounds in disorders such as hypertension and coronary artery disease (Chan et al 2000; Middleton et al 2000). The antioxidant properties of these compounds seems to be the main reason for their cardiovascular effects since they act as scavengers of reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide, which play a role in the genesis of such diseases (Nakazono et al 1991; Duarte et al 1993; Griendling & Alexander 1997; Givertz & Colucci 1998). In addition, enhanced synthesis of NO may also play an important role (Lemos et al 1999).

In previous work we reported the hypotensive and vasorelaxant effects in rats of *Croton schiedeanus* Schlecht (Guerrero et al 2001), a medicinal plant used in Colombian folk medicine for hypertension. From this plant, we isolated ayanin, a bioflavonoid that, in-vitro, induces relaxation in Wistar rat aorta mediated by endothelium-dependent NO production and subsequent activation of guanylate cyclase (unpublished data) and that, in-vivo, exerts protective cardiovascular effects against the increase in blood pressure and heart rate also through a mechanism that depends on the NO/cyclic guanosine monophosphate (cGMP) pathway (Guerrero et al 2002). Further phytochemical fractionating of this plant led us to the identification of quercetin 3,7-dimethyl ether (QDME).

In searching for structure–activity relationships, we wished to compare the vasorelaxant profile of this flavonoid with those of ayanin (quercetin 3,4′,7-trimethyl ether), quercetin 3,3′,4′,7-tetramethyl ether (QTME), a flavonoid synthesized in our laboratory, and with quercetin, a well-known flavonoid. We also wished to asses the interaction of QDME with the nitric oxide (NO)/cGMP pathway.

Materials and Methods

Drugs and solutions

The following salts and drugs were used: NaCl, $CaCl_2$, $NaHPO_4$, $NaHCO_3$, glucose (Panreac); KCl, $MgCl_2$, KH_2PO_4 and $MgSO_4$ (Probus); ascorbic acid (Prolabo); phenylephrine hydrochloride, methylene blue (Sigma); L-NAME and dimethyl sulfoxide (DMSO; Merck). All flavonoids, methylene blue and N^G-nitro-L-arginine methyl ester (L-NAME) were dissolved in dimethylsulfoxide (DMSO). This vehicle was used at a final concentration in the bath (see Pharmacology, below) of 0.1%.

Chemistry

QDME and ayanin (Figure 1) were isolated from *Croton schiedeanus*. The aerial part of *Croton schiedeanus* Schlecht (Euphorbiaceae) was collected from the Tocaima region, Cundinamarca, Colombia in November 1998. Its identity was confirmed by José Luis Fernández and a voucher specimen has been deposited (No. 432164) in the Herbarium of Natural Sciences Institute of National University of Colombia.

QTME was synthesized according to the method described by Rao & Owoyale (1976).

Melting points were determined on a Büchi 510-K melting point apparatus and are uncorrected. ^1H and ^{13}C NMR were recorded on Bruker WP 200 SY (200 MHz and 50 MHz) and Bruker 400 DRX (400 MHz and 100 MHz) instruments using $CDCl_3$ or DMSO-d_6 and tetramethyl silane (TMS) as internal standard. Sephadex LH-20 (Fluka, 25–100 μm) and silica gel 60 (Merck, 230–400 mesh) were used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis.

Extraction and isolation of ayanin and QDME

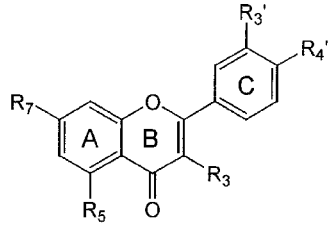
The aerial plant (5 kg) was dried and soaked in 96% EtOH (20 L) at room temperature for 3 days. The EtOH was removed under vacuum to yield a dark residue (80 g), which was partitioned between $CHCl_3$ and H_2O to give a $CHCl_3$-soluble fraction (45 g). The residue of the $CHCl_3$ extract was fractionated with 4% NaOH, yielding an acid part (9 g) and a neutral part (32 g). The acid part was subjected to silica-gel column chromatography; gradient elution with 0–100% EtOAc–*n*-hexane afforded 24 fractions. Fractions 14–21, eluting with 15–20% EtOAc–*n*-hexane, were recrystallized from acetone–MeOH to give ayanin (1.7 g) as a yellow solid.

The neutral part was separated by column chromatography over silica gel; gradient elution with 0–100% EtOAc–*n*-hexane afforded 68 fractions. Fractions 40–43, eluting with 40% EtOAc/*n*-hexane, were obtained as an oil after removal of the solvent; further purification over Sephadex LH-20 using MeOH–CH_2Cl_2–*n*-hexane (1:1:1) as eluent yielded 0.5 g of ayanin as an amorphous solid, which was purified by crystallization from acetone–MeOH.

Fractions 44–45, eluting with 40% EtOAc–*n*-hexane, were obtained as an oil after removal of the solvent. Further purification over Sephadex LH-20 using MeOH–CH_2Cl_2–*n*-hexane (1:1:1) as eluent yielded 0.3 g of QDME, a flavonoid previously isolated from *Notholaena californica* (Wollenweber et al 1981).

Ayanin. m.p., ^1H and ^{13}C NMR spectra are identical to those reported (Malan & Roux 1979; Agrawal 1989).

QDME. Colourless powder from acetone–MeOH, m.p. 220–222°C. ^1H NMR (DMSO, 400 MHz): δ = 3.77 (3H, s, OMe-3), 3,81 (3H, s, OMe-7), 6,29 (1H, d, J = 2.1 Hz, H-6), 6.62 (1H, d, J = 2.1 Hz, H-8), 6.90 (1H, d, J = 8.4 Hz, H-5′), 7.45 (1H, dd, J_1 = 2.1 Hz, J_2 = 8.4 Hz, H-6′), 7.57



Quercetin methyl ethers	R_3	R_5	R_7	R'_3	R'_4
Quercetin	OH	OH	OH	OH	OH
Quercetin 3,7-dimethyl ether (QDME)	OMe	OH	OMe	OH	OH
Quercetin 3,4′,7-trimethyl ether (ayanin)	OMe	OH	OMe	OH	OMe
Quercetin 3,3′,4′,7-tetramethyl ether (QTME)	OMe	OH	OMe	OMe	OMe

Figure 1 The chemical structures of quercetin methyl ethers.

(1H, d, J = 2.1 Hz, H-2′), 9.70 (1H, br s, OH). ^{13}C NMR (DMSO, 100 MHz): δ = 56.0 (OMe-7), 59.5 (OMe-3), 92.1 (C-8), 97.6 (C-6), 105.1 (C-10), 115.5 (C-2′), 115.7 (C-5′), 120.6 (C-6′), 120.7 (C-1′), 137.8 (C-3), 145.2 (C-3′), 148.8 (C-4′), 155.9 (C-2), 156.1 (C-9), 160.9 (C-5), 165.0 (C-7), 178.0 (C-4).

Synthesis of QTME

A solution of quercetin (1 g, Aldrich) in acetone (30 mL) was boiled under reflux with methyl sulfate (0.96 mL, 3 equivalents) and potassium carbonate (1.4 g, 3 equivalents) for approximately 1 h (Rao & Owoyale 1976). After filtration and concentration of the solvent, a solid was obtained and crystallization from acetone–MeOH yielded 0.6 mg of tetra-*O*-methyl quercetin as a yellow powder, 150–152°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 3.84 (6H, s, 2×OMe), 3.94 (6H, s, 2×OMe), 6.30 (1H, d, J = 2.2 Hz, H-6), 6.44 (1H, d, J = 2.2 Hz, H-8), 6.96 (1H, d, J = 8.4 Hz, H-5′), 7.66 (1H, d, J = 2.5 Hz, H-2′), 7.70 (1H, dd, J_1 = 2.5 Hz, J_2 = 8.4 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 55.8 (OMe-7), 56.0 (OMe-3′and 4′), 60.1 (OMe-3), 92.2 (C-8), 97.8 (C-6), 106.0 (C-10), 110.2 (C-2′), 111.2 (C-5′), 122.1 (C-6′), 122.3 (C-1′), 138.9 (C-3), 148.8 (C-3′), 151.4 (C-4′), 155.8 (C-2), 156.7 (C-9), 162.0 (C-5), 165.4 (C-7), 178.7 (C-4).

Pharmacology

Rat aortic ring preparation and experimental procedure

Male Wistar rats (320–470 g) from the Animal House of the University of Salamanca (P.A.E.-SA001) were anaesthetized with ether and sacrificed. The descending thoracic aorta was dissected and placed in an oxygenated Krebs solution with the following composition (in mM): NaCl, 118.0; KCl, 4.75; $CaCl_2$, 1.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11 and ascorbic acid 0.1.

Rings of thoracic aorta (5–6 mm in length) were carefully excised and submerged in Allhin organ chambers containing 5 mL of Krebs solution of bathing medium maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture (pH = 7.4). The rings were mounted by means of two parallel L-shaped stainless-steel holders inserted into the lumen. One holder served as an anchor while the other was connected to a force-displacement transducer (Harvard UF1) to measure isometric contractile force recorded by a MacLab/8-computer system (A.D. Instruments Ltd, London, UK). A basal tension of 2 g was applied. Each preparation was allowed to equilibrate for 60–90 min in Krebs solution before initiating the experimental procedures, and during this period the incubation media were changed every 15 min.

After equilibration, intact aortic rings incubated in Krebs solution were exposed to KCl (80 mM) or phenylephrine (10^{-6} M) until the contractile response reached a steady tension. Then, QDME, ayanin, QTME or quercetin were added cumulatively to the bath (10^{-6} to 10^{-3} M). In some rings, DMSO was added as control.

In another set of experiments, L-NAME (10^{-4} M) or methylene blue (10^{-6} M) were added 15 min before phenylephrine-induced contraction and the cumulative addition of quercetin 3,7-dimethyl ether. The endothelium of some rings was removed by gently rubbing the internal surface with the tip of small forceps. Removal of the endothelium was confirmed by lack of relaxation to 10^{-5} M acetylcholine.

All the provisions concerning the protection of animals used for experiments stipulated by Spanish law and European Community (EEC 1986) specifications were applied.

Data analysis and statistics

The response of aortic rings was expressed as a percentage of the initial contraction to KCl (80 mM) or phenylephrine (10^{-6} M). Concentration–response curves were analysed to give the negative logarithm of flavonoid concentration producing a 50% inhibition of the maximal contractile response (pEC50) by sigmoidal curve-fitting analysis. The maximum relaxation (R_{max}) values corresponded to the percentage of relaxation obtained with the maximum flavonoid concentration dissolved and assayed.

All results are expressed as means±the standard error of the mean (s.e.m.) of n = 8–11. Differences in the concentration–response curves were analysed by one-way analysis of variance, followed by the Dunett post-hoc test with a criterion set for statistical significance at $P < 0.05$. Excel 97 and SPSS 7.5 software was used for data analysis.

Results

Effect of QDME on the sustained contraction induced by phenylephrine or KCl in endothelium intact aorta rings

The maximal contractile responses to phenylephrine (10^{-6} M) and KCl (80 mM) were 3825±45 and 4042±110 mg (n = 99 and 32), respectively.

Figures 2 and 3 show the relaxant responses to the tested flavonoids in KCl- and phenylephrine-precontracted aortic rings, respectively. QDME showed a concentration-

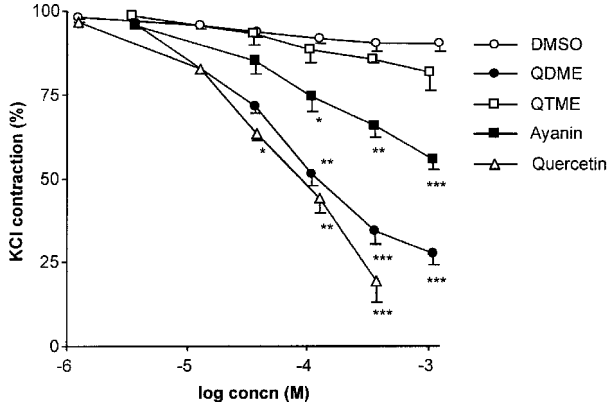


Figure 2 Concentration–response curves to quercetin 3,7-dimethyl ether (QDME), quercetin, quercetin 3,4′,7-trimethyl ether (ayanin) and quercetin 3,3′,4′,7-tetramethyl ether (QTME) in rat endothelium-intact aortic rings precontracted with 80 mM KCl. Each point represents the mean±s.e.m. of n = 8–11 experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control (DMSO) rings.

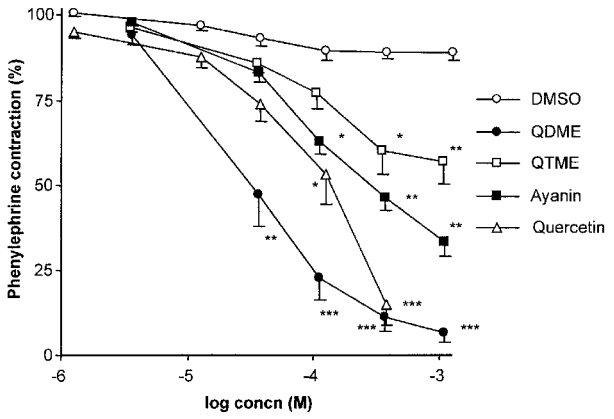


Figure 3 Concentration–response curves to quercetin 3,7-dimethyl ether (QDME), quercetin, quercetin 3,4′,7-trimethyl ether (ayanin) and quercetin 3,3′,4′,7-tetramethyl ether (QTME) in rat endothelium-intact aortic rings precontracted with 10^{-6} M phenylephrine. Each point represents the mean±s.e.m. of n = 8–11 experiments. $*P<0.05$, $**P<0.01$, $***P<0.001$ vs control (DMSO) rings.

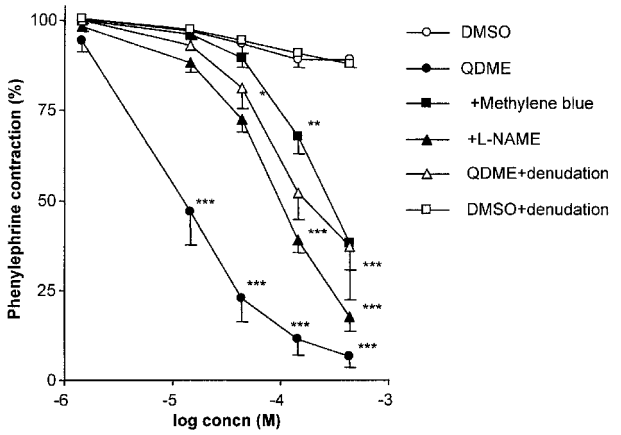


Figure 4 Concentration–response curves to quercetin 3,7-dimethyl ether (QDME) in rat endothelium-denuded and endothelium-intact aortic rings precontracted with 10^{-6} M phenylephrine, in the absence or presence of methylene blue (10^{-6} M) or L-NAME (10^{-4} M). Each point represents the mean±s.e.m. of n = 8–11 experiments. $*P<0.05$, $**P<0.01$, $***P<0.001$ vs control (DMSO) rings.

Table 1 Comparison of the relaxant potency (pEC50) and maximal relaxation (R_{max},%) values obtained with quercetin 3,7-dimethyl ether (QDME), quercetin, quercetin 3,4′,7-trimethyl ether (ayanin) and quercetin 3,3′4′7-tetramethyl ether (QTME) in Wistar-rat endothelium-intact aortic rings precontracted with KCl (80 mM) or phenylephrine (10^{-6} M)

Flavonoids	n	KCl 80 mM		Phenylephrine 10^{-6} M	
		pEC50	R_{max}	pEC50	R_{max}
QDME	16	3.98±0.04	72±3	4.70±0.18	94±3
Ayanin	35	2.76±0.05	45±3	3.64±0.02	67±4
QTME	15	1.81±0.59	18±6	3.11±0.16	43±7
Quercetin	12	4.18±0.01	81±6	3.96±0.07	85±6

dependent relaxation, the maximum response being observed against phenylephrine (>95%). DMSO (0.1%), the vehicle used for the flavonoids, had no effect on vascular tone.

The pEC50 and R_{max} values obtained with the flavonoids are compared in Table 1. QDME had a significantly higher potency in causing vasorelaxation as compared with quercetin, ayanin and QTME (5-, 11- and 38-fold shifts in the curve to the right, respectively, in phenylephrine-precontracted rings). Its effects were more pronounced against phenylephrine- than KCl-precontracted rings. Quercetin showed a slightly greater response in KCl-precontracted rings than QDME (less than a 2-fold shift in the curve).

Effect of endothelium denudation and the NO/cGMP pathway in the relaxation induced by QDME

The relaxation induced by QDME was significantly inhibited by previous removal of the endothelium as well as by L-NAME, 10^{-4} M and methylene blue (10^{-6} M) (Figure 4). Furthermore, the maximum relaxation in methylene blue- and L-NAME-preincubated rings was prevented by only 38% and 17%, respectively.

Discussion

We investigated the structure–activity relationship of two flavonoids isolated from *Croton schiedeanus* – QDME and ayanin – as compared with QTME, a flavonoid obtained by synthesis, and with quercetin, a well-known flavonoid, in causing vasorelaxation in rat isolated aorta. This may be helpful for the synthesis of active compounds for the treatment of hypertension and coronary artery disease. The mechanism of action of QDME was also explored.

Our data show that QDME is able to elicit an important vasorelaxant response, producing close to 100% relaxation in aortic rings precontracted with phenylephrine. According to the pEC50 values for phenylephrine-induced precontraction, the potency order was QDME > quercetin > ayanin > QTME. Since QDME and quercetin elicited the most marked effects it can be assumed that 3′ and 4′ hydroxylation of the B ring improves the relaxant potency. Although QDME and quercetin showed similar responses in KCl-precontracted rings, QDME showed a greater response than quercetin in phenylephrine-precontracted rings. Thus, methylation of positions 3 and 7 of the C and A rings, respectively, could add some degree of greater activity.

However, when methylation occurs at the A, B and C rings at the same time, the effects of these substitutions may offset each other (Ko et al 1999). This point of view is supported by the intermediate and rather slight response obtained with ayanin and QTME, respectively, in which 3′ and 4′ methylations of the B ring seem to decrease the response.

The cardiovascular effects of flavonoid compounds such as reduction of high blood pressure, a decrease in cardiac and renal hypertrophy and direct vasodilatation are associated with the antioxidant properties of these substances (Duarte et al 2001a, b). It is known that the presence of a 4-oxo substitution in the C ring, especially in association with the C2-C3 double bond, increases scavenger activity of flavonoids by delocalizing electrons from the B ring (Middleton et al 2000). Likewise, such a configuration could increase the cardioprotective effects of these compounds. Nevertheless, substitutions of the A and C rings also seem to play an important role in this.

Flavones and flavonols are structurally similar, flavonols having an extra hydroxyl substitution at the carbon 3 position (Figure 1). This hydroxyl substitute seems to be particularly sensitive for flavonoid compounds (Chan et al 2000). The C3 methyl substitution could further increase the relaxant potency. Such could also be the case of QDME. Therefore, hydroxylation at position 3 of the C ring may not be the best combination with the C2-C3 double bond of C, whereas the extra methyl substitution at position C7 increases the vasodilator actions of the flavonoids. Although other authors have found that flavonols with an extra hydroxyl substitution at the carbon 3 position, such as quercetin and kaempferol, can elicit a greater relaxant response (Duarte et al 1993), others have shown that flavones and flavonols have similar effects (Chan et al 2000). In view of our results, we may assume that 3,4 hydroxylation of the B ring must be preserved and that C3 and C7 methyl substitutions of the C and A rings further increase the relaxant activity.

Removal of the endothelium induced an important decrease in the relaxant response by QDME (20-fold shift in the curve to the right). Therefore, the role of endothelium factors in the vasodilator effect of this compound is clear. Indeed, the relaxant response to QDME was inhibited after the inhibition of NO and cGMP synthesis using L-NAME, a nitric oxide synthase inhibitor, and methylene blue, a drug that interferes directly with soluble guanylyl cyclase activated by NO (Gruetter et al 1981; Wang et al 1995) and inhibits vasorelaxation induced by hydrogen peroxide (Burke & Wolin 1987) or organic peroxide (Thomas & Ramwell 1986). However, at higher concentrations QDME relaxation is only affected to a certain extent. Accordingly, the NO/cGMP pathway might be involved in causing the vasorelaxation to QDME but another mechanism of action must also contribute to the response.

It is well known that NO interacts with O_2^- to produce peroxynitrite, resulting in a decreased vasodilator effect (Butler et al 1995) and that polyphenolic flavonoids are powerful antioxidants and exert free radical scavenging properties (Robak & Gryglewski 1988; Rice-Evans et al 1997). One explanation for the relaxant effect of QDME is that it could result from the prevention of NO breakdown through O_2^- removal, as has been proposed for other vasorelaxant flavonoids (Lemos et al 1999).

Furthermore, other endothelial vasorelaxant factors derived from cyclooxygenase and different endothelium-independent mechanisms could be implicated in the vasorelaxation of this compound, as had been proposed for others flavonoids (Ko et al 1991; Chan et al 2000). According to the inhibition of potassium contraction induced by this flavonoid, at least at high concentrations, QDME may interfere with calcium-dependent and -independent contractile mechanisms. Further studies are needed to establish the exact mechanism of action of this quercetin-derived compound.

Conclusions

In our study we observed a correlation between the structure and mechanisms of action of quercetin 3,7-dimethyl ether, quercetin, 3,4′,7-trimethyl ether (ayanin) and quercetin 3,3′,4′,7-tetramethyl ether. Extra hydroxyl substitutions at the C3′ and C4′ positions of the B ring increased the vasorelaxant potency. In addition, methyl substitutions at positions C3 and C7 of the C and A rings can further extend the response. The vasorelaxation induced by QDME is partially NO/cGMP pathway-dependent, although interactions with contractile mechanisms of endothelium-independent origin could also contribute to the increase in relaxation. Such actions may also add to the reported cardioprotective effects of flavonoid compounds.

References

Agrawal, P. K. (ed.) (1989) *Carbon-13 NMR of flavonoids*. Elsevier, Amsterdam, pp 158–168

Burke, T. M., Wolin, M. S. (1987) Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. *Am. J. Physiol.* **252**: H721–H732

Butler, A. R., Flitney, F. W., Williams, D. L. H. (1995) NO, nitrosonium ions, nitroxide ions, nitrosothiols and iron-nitrosyls in biology: a chemist's perspective. *Trends Pharmacol. Sci.* **16**: 18–22

Chan, E., Pannangpetch, P., Woodman, O. (2000) Relaxation to flavonoids and flavonols in rat isolated thoracic aorta: mechanism of action and structure-activity relationships. *J. Cardiovasc. Pharmacol.* **35**: 326–333

Duarte, J., Pérez-Vizcaíno, F., Utrilla, P., Jiménez, J., Tamargo, J., Zarzuelo, A. (1993) Vasodilating effects of flavonoids in rat aortic smooth muscle: structure-activity relationships. *Gen. Pharmacol.* **24**: 857–862

Duarte, J., Jiménez, R., Villar, I. C., Pérez-Vizcaíno, F., Jiménez, J., Tamargo, J. (2001a) Vasorelaxant effects of the bioflavonoid Chrysin in isolated rat aorta. *Planta Med.* **67**: 567–570

Duarte, J., Pérez-Palencia, R., Vargas, F., Ocete, M. A., Pérez-Vizcaíno, F., Zarzuelo, A., Tamargo, J. (2001b) Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br. J. Pharmacol.* **133**: 117–124

Givertz, M. M., Colucci, W. S. (1998) New targets for heart-failure therapy: endothelin, inflammatory cytokines and oxidative stress. *Lancet* **52** (suppl. 1): S134–S138

Griendling, K. K., Alexander, R. W. (1997) Oxidative stress and cardiovascular disease. *Circulation* **96**: 3264–3265

Gruetter, C. A., Kadowitz, P. J., Ignarro, L. J. (1981) Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrite and amyl nitrite. *Can. J. Physiol. Pharmacol.* **59**: 150–156

Guerrero, M. F., Carrón, R., Martín, M. L., San Román, L., Reguero, M. T. (2001) Antihypertensive and vasorelaxant effects of aqueous extract from *Croton schiedeanus* Schlecht in rats. *J. Ethnopharmacol.* **75**: 33–36

Guerrero, M. F., Puebla, P., Martín, M. L., Carrón, R., San Román, L., Reguero, M. T., Arteaga, L. (2002) Inhibitory effect of N(G)-nitro-L-arginine methyl ester on the anti-adrenergic response elicited by ayanin in the pithed rat. *Planta Med.* **68**: 322–325

Ko, F. N., Huang, T. F., Teng, C. M. (1991) Vasodilatory action mechanisms of apigenin isolated from *Apium graveloens* in rat thoracic aorta. *Biochim. Biophys. Acta* **1115**: 69–74

Ko, W. C., Kuo, S. W., Sheu, J. R., Lin, C. H., Tzeng, S. H., Chen, C. M. (1999) Relaxant effects of quercetin methyl ether derivatives in isolated guinea pig trachea and their structure-activity relationships. *Planta Med.* **65**: 273–275

Lemos, V. S., Freitas, M. R., Muller, B., Lino, Y. D., Queiroga, C. E. G., Côrtes, S. F. (1999) Dioclein, a new nitric oxide- and endothelium-dependent vasodilator flavonoid. *Eur. J. Pharmacol.* **386**: 41–46

Malan, E., Roux, D. G. (1979) Flavonoids from *Distemonanathus benthamianus Baillon*. Methoxylated flavones and inter-relationships of benthamianin, [2]Benzopyrano[4,3-*b*][1]benzopyran. *J. Chem. Soc. Perk. T.* **1**: 2696–2703

Middleton, E., Kandaswami, C., Theoharides, T. C. (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **52**: 673–751

Nakazono, K., Watanabe, N., Natsuno, K., Sasaki, J., Sato, T., Inque, M. (1991) Does superoxide underlie the pathogenesis of hypertension. *Proc. Natl Acad. Sci. USA* **88**: 10045–10048

Rao, K. V., Owoyale, J. A. (1976) Partial methylation of quercetin: direct synthesis of tamarixetin, ombuin and ayanin. *J. Heterocyclic Chem.* **13**: 1293–1295

Rice-Evans, C., Miller, N. J., Paganga, G. (1997) Antioxidant properties of phenolic compounds. *Trends Pharmacol Sci.* **2**: 152–159

Robak, J., Gryglewski, R. J. (1988) Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **37**: 837–841

Thomas, G., Ramwell, P. (1986) Induction of vascular relaxation by hydroperoxides. *Biochem. Biophys. Res. Commun.* **139**: 102–108

Wang, Y. X., Cheng, X., Pang, C. C. Y. (1995) Vascular pharmacology of methylene blue in vitro and in vivo: a comparison with N(g)-nitro-L-arginine and diphenyleneiodonium. *Br. J. Pharmacol.* **114**: 194–202

Wollenweber, E., Smith, D. M., Reeves, T. (1981) Flavonoid patterns and chemical races in the California Cloak-Fern, *Notholaena california*. *Stud. Org. Chem.* **11**: 221