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Inhibition of Aldose Reductase by Maesanin and Related *p*-Benzoquinone Derivatives and Effects on Other Enzymes

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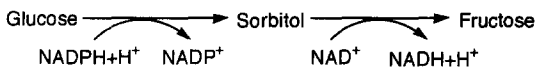
Abstract—A naturally occurring *p*-benzoquinone derivative, maesanin, inhibited porcine lens aldose reductase. Systematic investigation of related *p*-benzoquinone derivatives revealed that 2,5-dihydroxy-*p*-benzoquinone was a potent inhibitor of aldose reductase and aldehyde reductase but had no effect on NADH oxidase. Kinetic analysis showed this *p*-benzoquinone exhibited uncompetitive inhibition against DL-glyceraldehyde and noncompetitive inhibition against NADPH.

Introduction

Aldose reductase (EC 1.1.1.21), a member of the NADPH-dependent aldo-keto reductase family, is a key enzyme in the polyol pathway.[1] The polyol pathway can be found in many tissues such as the lens, retina, nervous and kidney in which diabetic complications appear.[2] This enzyme catalyses the reduction of various aldehydes, including the aldehyde form of glucose, to the corresponding sugar alcohol, sorbitol.



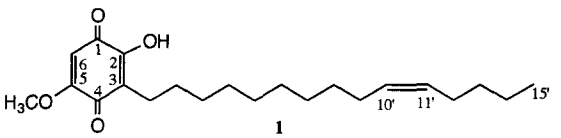
Reduction of glucose to sorbitol provides a common link in the onset of diabetic complications that result in tissue and functional change in the cornea, lens, retina, iris, peripheral nerves and kidney.[3,4] It has been reported that accumulation of sorbitol in the lens contributes to the formation of cataracts in diabetics.[5,6] The intracellular accumulation of sorbitol leads to locally hyperosmotic conditions, which appear to be responsible for the loss of clarity of the lens.[7] Fructose also plays a role in the ingress of H_2O into the cornea.[8] Therefore, the inhibition of aldose reductase would be effective in preventing cataract formation in diabetes.

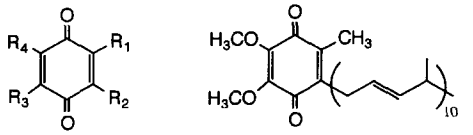
In our continuing search for bioactive substances from tropical plants,[9,10] maesanin, 3-alkyl-2-hydroxy-5-methoxy-1,4-benzoquinone (**1**), was isolated from the fresh fruit of an East African medicinal plant *Maesa lanceolata* (Myrsinaceae).[11,12] This *p*-benzoquinone derivative showed inhibitory activity against porcine lens aldose reductase. In this paper, effects of maesanin and related *p*-benzoquinone derivatives (**2–10**) on aldose reductase and other enzymes were investigated.

Results and Discussion

Developmentally, polyol metabolism is extremely advanced in lens,[13] therefore, aldose reductase activity can be measured in lens homogenate.[14] Maesanin showed the 65% inhibition at 0.1 mM against porcine lens aldose reductase. Figure 2 demonstrates the concentration effect of maesanin on inhibition of porcine lens aldose reductase; the 50% inhibition was observed at 0.07 mM. Rat lens aldose reductase was more sensitive to maesanin; 63.3% inhibition was observed at 10 μM and 54.9% at 1 μM (data not shown).

Maesanin possesses a *p*-benzoquinone moiety as shown in Figure 1. Structurally similar quinone methide antibiotic, citrinin,[15] is also reported to inhibit aldose reductase activity.[16] Therefore, the effects of *p*-benzoquinone derivatives on aldose reductase activity were investigated. As listed in Table 1, *p*-benzoquinone itself





2-9 10

Figure 1. Structure of maesanin and benzoquinone derivatives. **1**, Maesanin; **2–9**, see Tables 1–3; **10**, coenzyme Q_{10}.

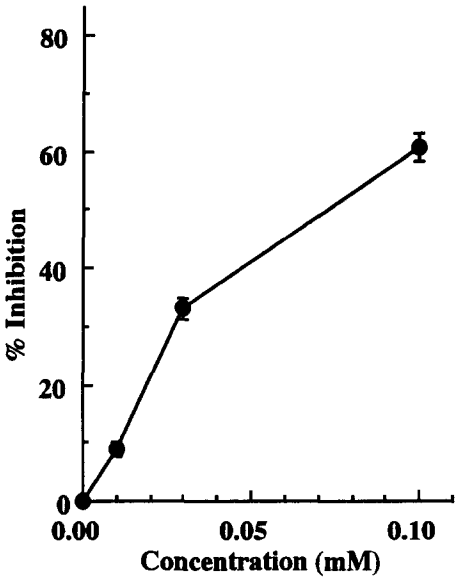


Figure 2. Inhibitory effect of maesanin on porcine lens aldose reductase. Each plot is the mean of triplicate determinations, with the standard deviation indicated by a vertical bar.

(**2**) showed little inhibition against porcine lens aldose reductase. Among the *p*-benzoquinone derivatives tested, 2,5-dihydroxy-*p*-benzoquinone (**3**) was most effective; complete inhibition was observed at 0.1 mM. Tetrahydroxy-*p*-benzoquinone (**4**), *p*-toluquinone (**5**) and coenzyme Q_0 (**7**) also showed slight inhibition. However, duroquinone (**6**) and 2-hydroxy-6-methoxy-3,5-dimethyl-*p*-benzoquinone (**8**) did not inhibit aldose reductase up to 0.1 mM. Since coenzyme Q_0 (**7**) exhibited some inhibition, coenzyme Q_{10} (**10**) which possesses an additional isoprenyl group at 3 position of **7**, was also expected to inhibit aldose reductase. It would be superior if coenzyme Q_{10} exhibits this activity since it exists universally in animal tissues and has been used as a drug for treatment of heart disease.[17] Furthermore, coenzyme Q_{10} possesses antioxidative activity.[18] Lipid peroxidation is also one of the causes of cataracts.[19,20] However, **10** showed no inhibitory activity against porcine lens aldose reductase up to 0.1 mM. Both maesanin (**1**) and coenzyme Q_{10} (**10**) possess a 3-alkyl moiety and a 5-methoxy group, which would not be associated with aldose reductase inhibition. Considering that **3** exhibited potent activity, a hydroxy group adjoined to a quinoid carbonyl would be an important part for aldose reductase inhibition.

Maesanin has a hydroxy group while **3** possesses the two hydroxy groups in *p*-benzoquinone structure. Kinetic analysis for aldose reductase inhibition by **3** using Lineweaver–Burk plots is illustrated in Figure 3. When the concentration of substrate DL-glyceraldehyde was changed, the slopes, obtained from the uninhibited enzyme and from the two different concentrations of **3**, were parallel. On the other hand, the reciprocal plot curves of enzyme activities and concentration of cofactor NADPH intersected at the abscissa. These indicate that **3** exhibited uncompetitive inhibition against DL-glyceraldehyde and noncompetitive inhibition against NADPH.

Table 1. Effect of *p*-benzoquinone derivatives on porcine lens aldose reductase[a]

	R_1	R_2	R_3	R_4	Inhibition (%)[b]
2	H	H	H	H	12.2
3	OH	H	OH	H	100
4	OH	OH	OH	OH	32.5
5	Me	H	H	H	16.6
6	Me	Me	Me	Me	0
7	OMe	OMe	Me	H	28.8
8	OH	Me	Me	OMe	0
9	Phe	H	Phe	H	0

[a]All compounds were tested at final concentration of 0.1 mM.
[b]Inhibitory ratios were calculated as relative inhibition against control reaction.

Many aldose reductase inhibitors have been reported to inhibit aldehyde reductase (EC 1.1.1.20).[21–24] Aldehyde reductase has been suggested to be an important site of action of anticonvulsant drugs. Similarities including the monomeric nature of these enzymes, their overlapping substrate specificities, and similar utilization of NADPH, suggest that both aldose reductase and aldehyde reductase possess certain structural similarities necessary for the binding of inhibitors.[25] Aldose reductase and aldehyde reductase are originated from the same genetic locus, therefore, it has been proposed that both reductases may play a role in the development of chronic diabetic pathologies.[26] In the kidney, aldehyde reductase is expressed preponderantly.[27] Maesanin almost completely inhibited porcine kidney aldehyde reductase at 0.1 mM as shown in Figure 4. Table 2 demonstrates the effect of *p*-benzoquinone derivatives on kidney aldehyde reductase. Among the *p*-benzoquinones examined, **3** and **4** also inhibited aldehyde reductase. Inhibition by **3** was potent, as was aldose reductase.

Aldose reductase is a NADPH-dependent oxidoreductase, therefore, the effects of adenine nucleotide-requiring enzymes would be concentrated.[28] Some adenine nucleotide-linked enzymes, for example lactate dehydrogenase which generates the end product of anoxic glycolysis and glutathione reductase which protects against peroxidation, were not inhibited by some aldose reductase inhibitors.[29,30] NADH oxidase is one of the most important adenine nucleotide-linked oxidation systems by which metabolic energy in mitochondria is produced. Inhibition of this enzyme results in the decrease at ATP level,[31] and the acceleration results in the production of toxic oxygen radicals.[32,33] For example, some flavonoids, known to be potent inhibitors of lens aldose reductase,[34–36] inhibit NADH oxidase in liver mitochondria[37–39] and generate toxic radical species.[38–40] Indeed, *p*-benzoquinones have been reported to affect NADH-linked mitochondrial functions.[41,42] Previously, maesanin was reported to inhibit NADH oxidase and related mitochondrial function in yeast and rat liver mitochondria.[43,44] The activity of NADH oxidase can be measured in prepared mitochondria.[45] Against bovine liver mitochondrial NADH oxidase, it showed almost complete inhibition at 1 μM, as shown in Figure 5. Table 3 shows

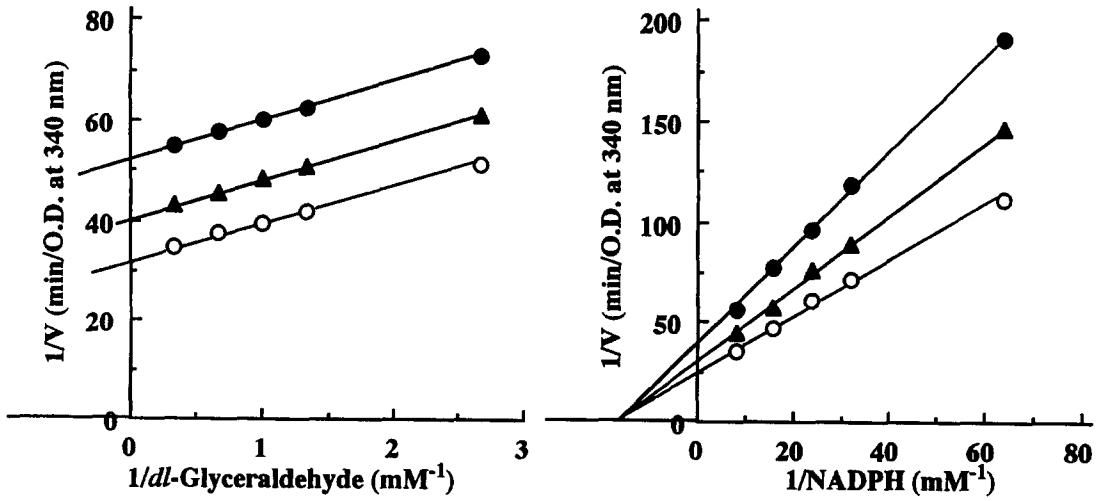


Figure 3. Inhibitory effect of **3** on porcine lens aldose reductase. Lineweaver–Burk plots in the absence (○) and in the presence (●, 0.03 mM; ▲, 0.01 mM) of **3** are shown.

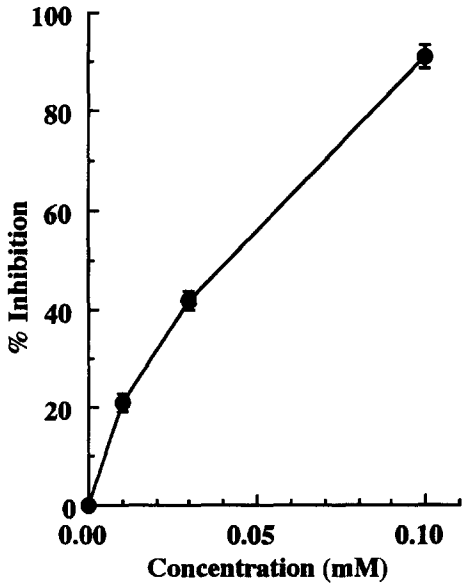


Figure 4. Inhibitory effect of maesanin on porcine kidney aldehyde reductase. Each plot is the mean of triplicate determinations, with the standard deviation indicated by a vertical bar.

the effects of *p*-benzoquinone derivatives on NADH oxidase in mitochondria isolated from bovine liver. Compound **8** has already been reported to inhibit the rat liver mitochondrial respiratory chain.[42] Compounds **2**, **5**, **7** and **9** accelerated the oxidation of NADH, which produces toxic oxygen species. On the other hand, **3** and **4** had no effect on mitochondrial NADH oxidase up to 0.1 mM.

Maesanin exhibited inhibitory activity on lens aldose reductase, but other adenine nucleotide-linked enzymes were also inhibited by this substance. In contrast, 2,5-dihydroxy-*p*-benzoquinone was found to be a potent inhibitor of aldose and aldehyde reductases; but it did not have an effect on mitochondrial NADH oxidase. Hence, 2,5-dihydroxy-*p*-benzoquinone would be expected to be a molecular probe of non-toxic aldose reductase inhibitors.

Experimental

Maesanin was isolated in our previous work.[11,12] 2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone was isolated as an oxidation product of wasabidienone-A, a

Table 2. Effect of *p*-benzoquinone derivatives on porcine kidney aldehyde reductase[a]

	R_1	R_2	R_3	R_4	Inhibition (%)[b]
2	H	H	H	H	0
3	OH	H	OH	H	68.6
4	OH	OH	OH	OH	19.9
5	Me	H	H	H	0
6	Me	Me	Me	Me	0
7	OMe	OMe	Me	H	0
8	OH	Me	Me	OMe	0
9	Phe	H	Phe	H	0

[a]All compounds were tested at final concentration of 0.1 mM.
[b]Inhibitory ratios were calculated as relative inhibition against control reaction.

Table 3. Effect of *p*-benzoquinone derivatives on bovine liver mitochondrial NADH oxidase[a]

	R_1	R_2	R_3	R_4	Inhibition (%)[b]
2	H	H	H	H	−337.3
3	OH	H	OH	H	0
4	OH	OH	OH	OH	0
5	Me	H	H	H	−320.4
6	Me	Me	Me	Me	0
7	OMe	OMe	Me	H	−158.5
8	OH	Me	Me	OMe	25.2
9	Phe	H	Phe	H	−35.5

[a]All compounds were tested at final concentration of 0.1 mM.
[b]Inhibitory ratios were calculated as relative inhibition against control reaction. Minus means the acceleration of the reaction.

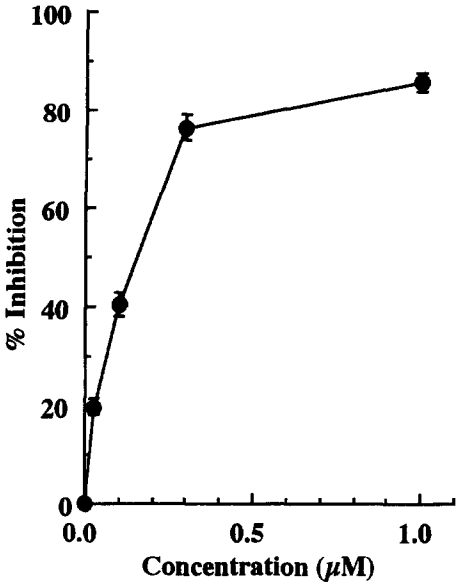


Figure 5. Inhibitory effect of maesanin on bovine liver mitochondrial NADH oxidase. Each plot is the mean of triplicate determinations, with the standard deviation indicated by a vertical bar.

fermentation product of *Phoma wasabiae*.[46,47] *p*-Benzoquinone, tetrahydroxy-*p*-benzoquinone, 2,5-diphenyl-*p*-benzoquinone, duroquinone, coenzyme Q_0 and coenzyme Q_{10} were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2,5-Dihydroxy-*p*-benzoquinone and *p*-toluquinone were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Porcine eyeball and kidney, and bovine liver were obtained from Nippon Ham Co. (Osaka, Japan).

Preparation of lens aldose reductase

Lenses were removed from porcine eyes and homogenized in 3 vol of 135 mM phosphate buffer (pH 7.0) containing 10 mM β-mercaptoethanol.[48] The homogenate was centrifuged at 10,000 *g* for 15 min. The supernatant fluid was saturated with 75% $(NH_4)_2SO_4$. The precipitate obtained by centrifugation was dissolved in the same buffer and used as an enzyme preparation.[49]

Preparation of kidney aldehyde reductase

The porcine kidneys were dissected into cortical and medullary regions, and the cortices were homogenized in 20 mM phosphate buffer (pH 7.5) containing 0.25 M sucrose, 0.5 mM EDTA and 1 mM β-mercaptoethanol. The homogenate was centrifuged at 15,000 *g* for 20 min. The supernatant fluid was saturated with 60% $(NH_4)_2SO_4$. The precipitate obtained by centrifugation was dissolved in 10 mM Tris buffer (pH 7.4) containing 0.5 mM EDTA and 1 mM β-mercaptoethanol, and subjected to a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer. The enzyme eluate was chromatographed on a DEAE-cellulose column (Wako Pure Chemical Ind., Osaka, Japan) equilibrated with the same Tris buffer and developed with 0–0.4 M linear gradient of NaCl. This partially purified aldehyde reductase was free of aldose reductase.[50]

Preparation of liver mitochondria

The bovine liver was homogenized in 0.25 M sucrose containing 0.1 mM EDTA (pH 7.4). The homogenate was centrifuged at 600 *g* for 10 min and the supernatant fluid was decanted and centrifuged at 15,000 *g* for 10 min.[51] The mitochondrial pellet was washed twice with the same solution and finally suspended in 3 mM Tris buffer (pH 7.4) containing 0.07 M sucrose, 0.21 M mannitol and 0.1 mM EDTA.

Assay of reductase activity

Aldose reductase and aldehyde reductase were assayed spectrophotometrically on a Shimadzu MPS-2000 spectrophotometer equipped with a temperature controller. The reaction mixture contained 50 mM Na-phosphate buffer (pH 6.2), 0.125 mM NADPH, 400 mM Li_2SO_4, enzyme solution and 3 mM DL-glyceraldehyde as a substrate.[52] The reaction was initiated by the addition of NADPH. The reaction rate was determined by tracing the decrease in the absorption of NADPH at 340 nm on Shimadzu Graphic Printer PR-3.

Assay of NADH oxidase

Mitochondrial NADH oxidase was assayed by measuring the decrease in absorbance at 340 nm.[53] The reaction mixture consisted of 50 mM phosphate buffer (pH 7.4), 0.1 mM NADH and bovine liver mitochondrial protein.

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