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Inhibition of gastric H^+, K^+-ATPase by chalcone derivatives, xanthoangelol and 4-hydroxyderricin, from *Angelica keiskei* Koidzumi

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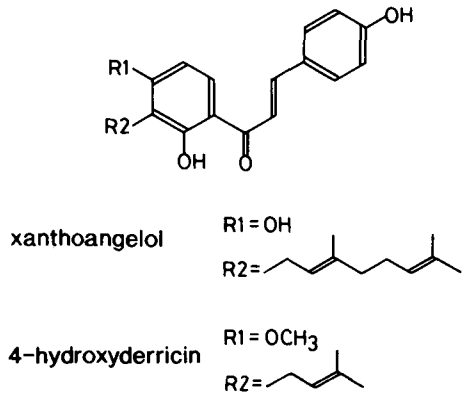
Abstract—Two chalcone derivatives, xanthoangelol (I) and 4-hydroxyderricin (II) isolated from *Angelica keiskei* Koidzumi, inhibited pig gastric H^+, K^+-ATPase with IC50 values of 1·8 and 3·3 μM, respectively. The inhibition by I or II was competitive with respect to ATP and was non-competitive with respect to K^+. I and II also inhibited K^+, stimulated *p*-nitrophenyl phosphatase, with IC50 values of 1·3 and 3·5 μM, respectively. Proton transport in-vitro was inhibited by I or II, in a dose-dependent manner. I at 100 mg kg^{-1}, i.p. significantly inhibited acid secretion and the formation of stress-induced gastric lesions. These results suggest that the antisecretory effect of I is due to the inhibition of gastric H^+, K^+-ATPase.

Angelica keiskei Koidzumi (Ashitaba in Japanese) is a plant, found along the Pacific coast of Japan, that is used as a diuretic, laxative, analeptic and a lactagogue. The main constituents, xanthoangelol (I) and 4-hydroxyderricin (II) have been isolated and identified (Kozawa et al 1978). It was found in a preliminary screening that these chalcone derivatives have a potent inhibitory activity on gastric H^+, K^+-ATPase. The present investigation reports their detailed inhibitory effects on the gastric H^+, K^+-ATPase.



Chemical structures of xanthoangelol (I) and 4-hydroxyderricin (II).

Materials and methods

Preparation of gastric H^+, K^+-ATPase. Gastric microsomal vesicles containing H^+, K^+-ATPase were prepared by density gradient centrifugation from pig fundic mucosa, as described by Saccomani et al (1977). The purified vesicles were collected and lyophilized to be rendered freely permeable to cations and were stored at −80°C. For the proton transport experiments, fresh non-lyophilized vesicles were used.

Preparation of xanthoangelol and 4-hydroxyderricin. These compounds were isolated from the roots of *Angelica keiskei* Koidzumi, as described by Kozawa et al (1978).

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Assay of H^+, K^+-ATPase. The assay medium consisted of 2 mM $MgCl_2$, 2mM Tris-ATP, 40 mM Tris/HCl pH 7·4 and 5 μg membrane protein, with or without 20 mM KCl in a total volume of 1 mL. The medium was incubated for 20 min at 37°C. The reaction was terminated by addition of 1 mL cold trichloroacetic acid (10%). The inorganic phosphate derived from the ATP was measured according to Fiske & Subbarow (1925). Drugs were dissolved in dimethylsulphoxide.

Assay of K^+-stimulated p-nitrophenyl phosphatase (K^+-pNPPase). The assay medium contained 5 mM $MgCl_2$, 5 mM pNPP, 40 mM Tris/HCl pH 7·4 and 5 μg membrane protein, with or without 20 mM KCl, in a total volume of 1 mL. After 20 min of incubation at 37°C, the reaction was terminated with 1 mL M NaOH. The absorbance of the reaction medium was read at 410 nm.

Protein was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

Proton transport experiment. Proton transport was measured by a spectrophotometric method (Lee & Forte 1978). The incubation mixture contained 1 mM $MgCl_2$, 150 mM KCl, 0·5 mM EDTA, 20 mM PIPES/NaOH pH 7·4, 300 μg fresh vesicles from pig gastric microsomes and 10 μM acridine orange, with or without drugs in a final volume of 2 mL. Mg-ATP 0·48 mM was added and the reaction initiated with 10 μM valinomycin. Incubation was at room temperature (20°C). Quenching of fluorescence was monitored in a Shimadzu Spectrophotometer UV-240 at 493 nm (excitation) and 530 nm (emission).

Antisecretory activity. The effect of xanthoangelol on gastric acid secretion was tested in pylorus-ligated rats (Shay et al 1945). The drug was given i.p. at the time of ligation. The volume of gastric juice was collected and measured after 4 h of ligation and sample was used for determination of acid concentration. Samples were titrated against 0·1 M NaOH. Acid output was calculated by multiplying the volume of gastric juice by the acid concentration.

Induction of gastric lesion. Wistar male rats, 200–250 g, were deprived of food, but had free access to tap water for 18 h before the experiment. Gastric lesions were induced by restraint stress (Takagi & Okabe 1968) and the animals killed. Stomachs were fixed with formalin and opened along the greater curvature. The area of each lesion was measured by microscopy and the summed area per stomach used as the lesion index. Drugs were given p.o. 10 min before stress loading.

Results

Xanthoangelol (I) and 4-hydroxyderricin (II) inhibited pig gastric H^+, K^+-ATPase, in a dose-dependent manner (Fig. 1A). The concentrations of each compound required for 50% inhibition (IC50) were 1·8 and 3·3 μM, respectively. Kinetic studies were conducted by varying ATP concentrations from 0·5 to 4 mM. Double reciprocal plots showed that I inhibited H^+, K^+-ATPase, competitively with ATP, without altering the V_{max}

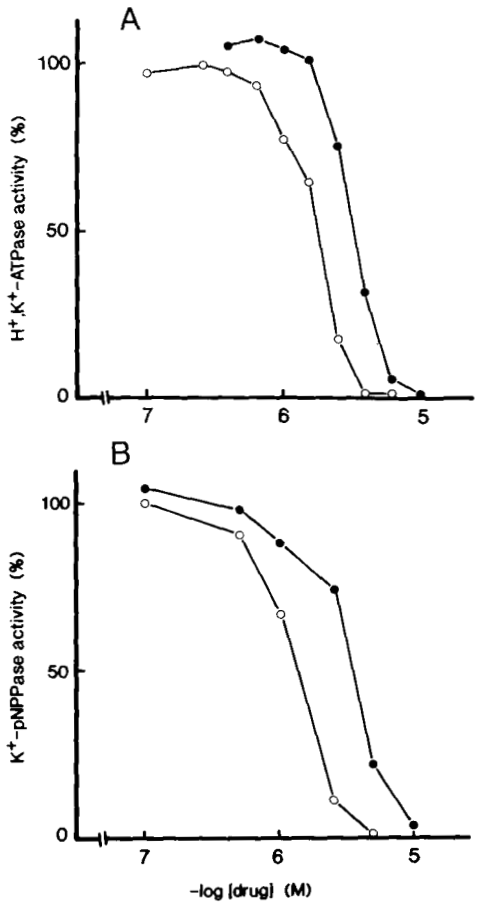

FIG. 1. A. Effects of xanthoangelol (○) and 4-hydroxyderricin (●) on the H^+, K^+-ATPase from pig gastric mucosa. Each value is the average of duplicate experiments. B. Effects of xanthoangelol (○) and 4-hydroxyderricin (●) on K^+-pNPPase from pig gastric mucosa. Each value is the average of duplicate experiments.

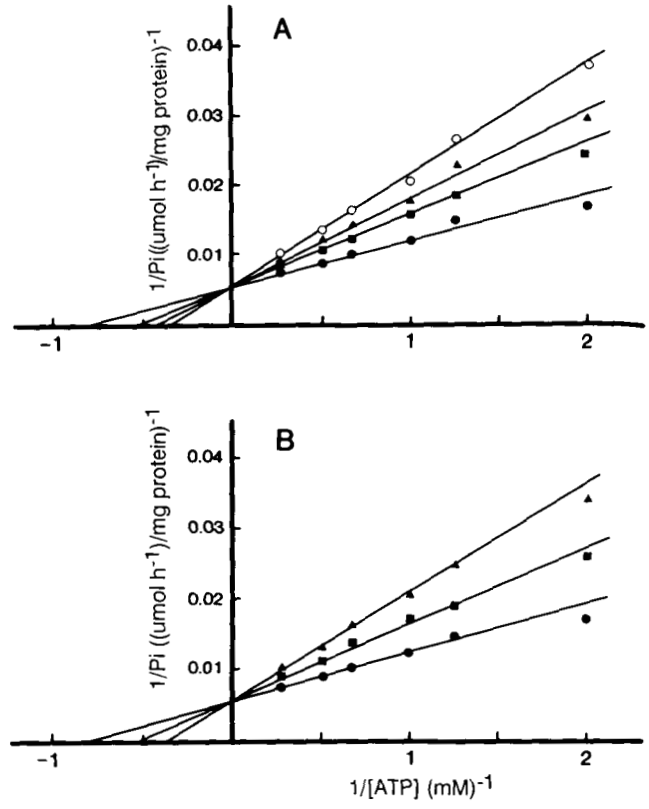

FIG. 2. Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase vs concentrations of ATP in the presence of 0 (●), 1 (■), 1·5 (▲) and 2 μM (○) xanthoangelol (A) or 0 (●), 2 (■) and 4 μM (▲) 4-hydroxyderricin (B). Each value is the average of duplicate experiments.

200 μmol h^{-1} (mg protein) $^{-1}$ while increasing the apparent K_m value from 1·2 to 2·0, 2·5 and 2·9 mM in the presence of 1, 1·5 and 2 μM I, respectively (Fig. 2A). The calculated K_i value was 1·6 μM. The inhibitory effect of II was also competitive with ATP (Fig. 2B), the apparent K_m values changed from 1·1 to 1·8 and 2·6 mM in the presence of 2 and 4 μM II. The calculated K_i value was 2·7 μM. The effect of varying K^+ concentrations on the inhibition of H^+, K^+-ATPase by I and II was also investigated. These two compounds behaved as non-competitive inhibitors with respect to K^+ (Fig. 3). The apparent V_{max} values were changed from 212 to 116 and 76 μmol Pi h^{-1} (mg protein) $^{-1}$ in the presence of 0·8 and 1·6 μM I, while the apparent K_m value of K^+ 0·44 mM was not affected. The calculated K_i was 0·96 μM. For II, the apparent V_{max} value was 236 μmol Pi h^{-1} (mg protein) $^{-1}$ without the compound, 160 and 116 μmol Pi h^{-1} mg^{-1} protein with 2·5 and 5 μM II, respectively. The apparent K_m value of K^+ 0·42 mM remained unchanged. The calculated K_i was 5·1 μM.

Both compounds inhibited K^+-pNPPase in the pig gastric membrane the same extent as seen with H^+, K^+-ATPase (Fig. 1B), with IC50 values of 1·3 and 3·5 μM for I and II, respectively.

Table 1. Effect of xanthoangelol (100 mg kg^{-1}) on acid output in pylorus-ligated rats.

	Volume (mL)	Acidity (m equiv/4h)	Total acid output (μ equiv/4h)
Control (n = 6)	4·0 ± 0·3	96·1 ± 3·5	389·8 ± 39·6
Xanthoangelol (n = 5)	1·8 ± 0·2**	81·1 ± 2·7*	145·1 ± 19·5**

Xanthoangelol was given i.p. at the time of pylorus-ligation.
Each value represents the mean ± s.e. Significant difference from the control; * $P < 0·05$, ** $P < 0·01$.

Table 2. Effects of xanthoangelol and 4-hydroxyderricin on stress induced gastric lesion.

	Dose (mg kg^{-1})	n	Lesion index (mm^2)	Inhibition (%)
Control	—	6	4·20 ± 0·72	
Xanthoangelol	100	5	1·25 ± 0·21*	70·2
Control	—	5	2·48 ± 0·37	
Xanthoangelol	100	6	1·05 ± 0·29*	57·7
	200	5	0·52 ± 0·09**	78·9
Control	—	6	3·04 ± 0·83	
4-Hydroxyderricin	50	6	3·15 ± 0·41	−3·4
Control	—	8	3·86 ± 0·53	
4-Hydroxyderricin	100	6	3·92 ± 0·46	−1·4
Control	—	6	5·17 ± 1·25	
4-Hydroxyderricin	200	6	7·75 ± 1·67	−50·0

Drugs were given p.o. 10 min before stress loading.
Each value represents the mean ± s.e. Significant difference from the control; * $P < 0·05$, ** $P < 0·01$.

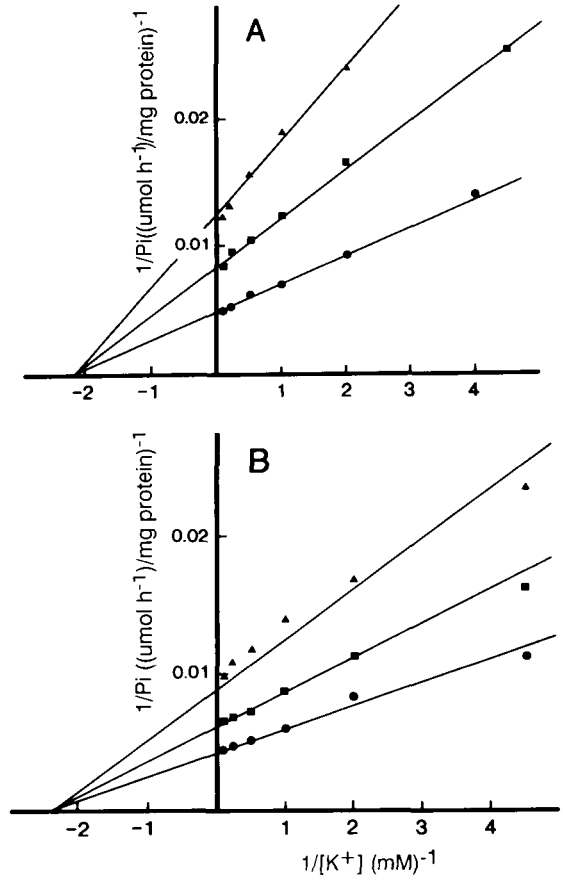

FIG. 3. Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase vs concentrations of KCl in the presence of 0 (●), 0·8 (■) and 1·6 μM (▲) xanthoangelol (A) or 0 (●), 2·5 (■) and 5 μM (▲) 4-hydroxyderricin (B). Each value is the average of duplicate experiments.

Proton transport was measured by observing the quenching of acridine orange fluorescence, in-vitro. Both compounds inhibited proton transport in a dose-dependent manner (Fig. 4).

The effect of I on acid output in-vivo was studied in pylorus-ligated rats. I, 100 mg kg^{-1} i.p., caused significant reductions in the volume, acidity and total acid output (Table 1). I and II were also tested against the stress-induced gastric lesion. I in doses of 100 mg kg^{-1} p.o. significantly inhibited formation of the mucosal lesion (Table 2). II was inactive, even at 200 mg kg^{-1} p.o.

Discussion

The present studies show that xanthoangelol (I), a major constituent of *Angelica keiskei* Koidzumi, is a potent inhibitor of gastric H^+, K^+-ATPase. Chalcones are widely distributed in the plant kingdom and their physiological and biochemical activities have been reported (Kyogoku et al 1979; Wagner et al 1986; Tanaka et al 1987). However, it has not been reported that chalcone derivatives have inhibitory effects on the gastric H^+, K^+-ATPase. The antisecretory effect of I was demonstrated in proton transport experiments in-vitro and in pylorus-ligated rats. I was also effective in inhibiting the formation of gastric lesions. This suggests that the anti-ulcer and antisecretory effects of I may be due to the inhibition of H^+, K^+-ATPase.

It has been shown that the ATP hydrolytic site of the enzyme is located in the cytosolic site and a high affinity K^+ site is on the luminal face across the membrane (Ray & Nandi 1986). The H^+, K^+-ATPase is phosphorylated on the cytosolic site by ATP in the presence of Mg^{2+}. The enzyme-phosphate complex is dephosphorylated by luminal K^+. Kinetic studies revealed that the inhibition of H^+, K^+-ATPase by I or II is competitive with respect to ATP and is non-competitive with respect to K^+. It is, therefore, suggested that these compounds bind competitively to the ATP site and inhibit the formation of phosphoenzyme by ATP. The formation of the complex of these inhibitors and enzyme may result in non-competitive inhibition with respect to K^+.

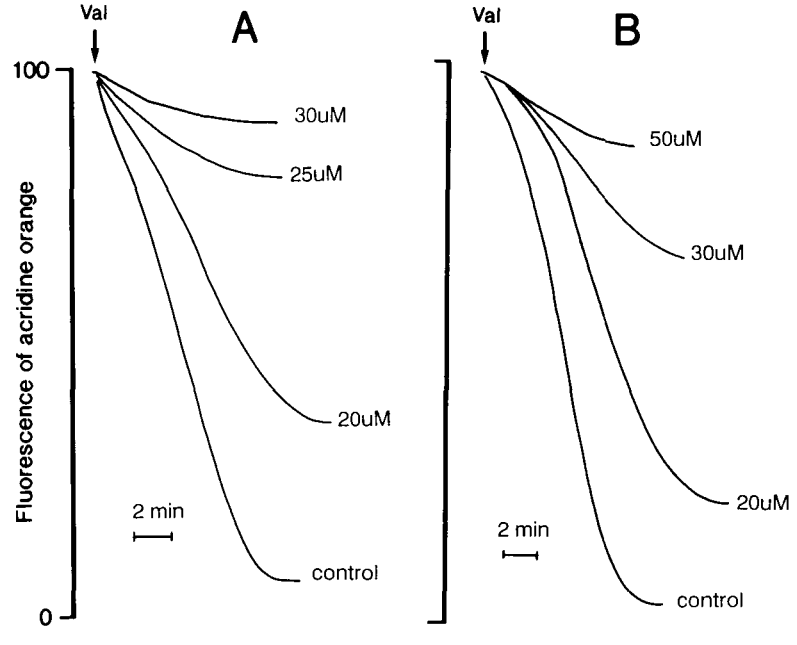

FIG. 4. Effects of xanthoangelol (A) and 4-hydroxyderricin (B) on H^+, K^+-ATPase mediated proton transport. Mg-ATP 0·48 mM was added before the reaction was initiated by 10 μM valinomycin (Val).

The potency of both compounds in the inhibition of enzyme in-vitro is of the same magnitude or greater compared with those of the H^+, K^+-ATPase inhibitors omeprazole (Im et al 1985) and SCH 28080 (Scott & Sundell 1985) which exhibited IC50 values of 6·0 and 2·5 μM in our assay system, respectively. The antisecretory and anti-ulcer activity in-vivo, however, was less effective. II, in particular, was inactive in doses up to 200 mg kg^{-1}, p.o. for inhibiting gastric lesions. One possible explanation for the discrepancy observed in-vitro and in-vivo is the poor hydrophobicity of the compounds. Since these compounds act at the cytosolic site after absorption, a poor hydrophobicity may prevent them from reaching the H^+, K^+-ATPase site. Also, the possibility that these compounds may be readily degraded to inactive metabolites cannot be ruled out.

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Comparison of intestinal and peritoneal dialysis of theophylline and phenobarbitone in rats

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Abstract—Intestinal dialysis of drugs by oral administration of activated charcoal has been compared with peritoneal dialysis in rats. The average amounts of theophylline transported over 120 min into the intestinal lumen and the peritoneal cavity were 15·7 and 16·5% of the intravenous dose (10 mg kg^{-1}), respectively, showing no significant difference, whereas the amount of the same intravenous dose of phenobarbitone transported from the blood into the intestinal lumen (7·8%) was significantly smaller than that entering the peritoneal cavity (12·5%). The net water flux showed that secretion predominated in the peritoneal transport whilst absorption predominated in the intestinal transport for both drugs. However, the net water flux in the intestinal lumen after intravenous theophylline (as aminophylline) was significantly smaller than that following phenobarbitone. The differences in transport across the two membranes could be due to differences in the intrinsic properties of the membranes, such as the surface area, the thickness of the membrane and the distribution of blood vessels. Differences could also be due to differences in the pharmacological effects of the drugs.

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In acute drug overdoses, haemoperfusion, haemodialysis, peritoneal dialysis, and combined haemodialysis-haemoperfusion have been used as methods of haemopurification (Weinberger & Hendeles 1980; Gibson 1981). Oral administration of activated charcoal has also been employed to inhibit absorption of excess drugs from the gastrointestinal (GI) lumen into the blood. The GI mucous membranes have a large surface area when considered as a whole. In particular, the total absorptive or exsorptive area of the small intestine has been calculated to be about 200 m^2 in an adult human, and is far larger than that of the peritoneal membrane (about 2 m^2, Csaky 1984).

We have previously reported that intravenously administered drugs are generally transported into the small intestinal lumen and the bile in rats, and that drugs can be removed by adsorption onto orally administered activated charcoal (Arimori & Nakano 1986a, 1987, 1988a, b). However, GI dialysis by oral administration of activated charcoal has not been clearly established as an effective means of removing drugs which have been parenterally administered or have already been absorbed into the systemic circulation from the GI tract. The present study compared GI