Inhibition of Angiotensin-I-Converting Enzyme by Tetrahydroxyxanthones Isolated from Tripterospermum lanceolatum

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INHIBITION OF ANGIOTENSIN-I-CONVERTING ENZYME BY TETRAHYDROXYXANTHONES ISOLATED FROM *TRIPTEROSPERMUM LANCEOLATUM*

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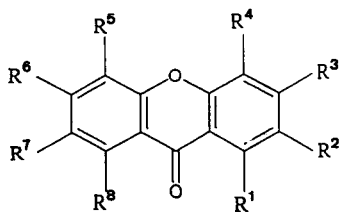
ABSTRACT.—Five tetrahydroxyxanthones, 3,4,6,7-tetrahydroxyxanthone [**1**], 1,3,5,6-tetrahydroxyxanthone [**2**], 3,4,5,6-tetrahydroxyxanthone [**3**], 1,3,6,7-tetrahydroxyxanthone [**4**], and 2,3,6,7-tetrahydroxyxanthone [**5**] isolated from *Tripterospermum lanceolatum* inhibited angiotensin-I-converting-enzyme activity in a dose-dependent manner.

The mode of inhibition of the tetrahydroxyxanthones (THXs) was found to be competitive inhibition. When the tetrahydroxy groups of THXs were blocked with acetyl groups, the angiotensin-I-converting-enzyme inhibitory activity was abolished, suggesting that the tetrahydroxy groups are indispensible for the inhibitory activity.

The angiotensin-I-converting enzyme (ACE) is of pharmaceutical interest due to the use of some of its inhibitors as antihypertension agents. Many inhibitors were found to be chelating agents (1) and peptides analogous to the substrate, and following extensive clinical studies, some ACE inhibitors have been developed as novel antihypertension drugs (2–4).

Tripterospermum lanceolatum Hayata (Gentianaceae) has been used traditionally as an antihypertensive herb in China. It was reported previously that norathyriol, a 1,3,6,7-tetrahydroxyxanthone, possessed a vaso-relaxing action in rat thoracic aorta (5) as well as antiplatelet activity (6). In the present investigation, we studied the inhibitory effects of active principles, tetrahydroxyxanthones



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
1	H	H	OH	OH	H	OH	OH	H
2	OH	H	OH	H	OH	OH	H	H
3	H	H	OH	OH	OH	OH	H	H
4	OH	H	OH	H	H	OH	OH	H
5	H	OH	OH	H	H	OH	OH	H
1 tetraacetate	H	H	OAc	OAc	H	OAc	OAc	H
2 tetraacetate	OAc	H	OAc	H	OAc	OAc	H	H
3 tetraacetate	H	H	OAc	OAc	OAc	OAc	H	H
4 tetraacetate	OAc	H	OAc	H	H	OAc	OAc	H
5 tetraacetate	H	OAc	OAc	H	H	OAc	OAc	H

(THXs) (7), isolated from *T. lanceolatum*, on ACE activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—THXs were isolated from *T. lanceolatum* (7). The sources of other materials used in these experiments were as follows: ACE from Sigma Co., St. Louis, Mo.; 2,4,6-trichloro-s-triazine (TT) from E. Merck, München, Germany; hippuryl-L-histidyl-L-leucine (HHL) and hippuric acid from Serva Co., Heidelberg, Germany.

ASSAY OF ANGIOTENSIN-I-CONVERTING ENZYME (ACE).—The activity of ACE was determined according to the method of Shihabi and Scaro (8) with some modifications. The reaction mixture contained the following components: 3.0 mM HHL, 1.8 μg ACE, and various amounts of compounds to be tested for their anti-ACE activities. The reaction mixture (250 μl) was started by adding 50 μl ACE, and the mixture was incubated at 37° for 10 min. To 100 μl of reaction mixture, 10 μl of 1 N HCl was added to stop the reaction, and then 0.4 ml of 1% TT reagent and 0.5 ml of 80 mM phosphate buffer, pH 8.3, were added to the reaction mixture to develop the reaction product which absorbed at 382 nm.

IN VIVO STUDY OF THXs.—Male spontaneously hypertensive rats of 300–350 g body wt with blood pressure 160–180 mm Hg were used for these experiments. Rats were deprived of food for 12 h but had H_2O on demand. The rats were studied without anesthesia.

The tail veins were cannulated for intravenous injection of angiotensin I or ACE inhibitors, while the tail arteries were cannulated for recording arterial pressure via a pressure transducer connected to a polygraph (Gould, model RS3400) (9).

RESULTS AND DISCUSSION

INHIBITION OF ACE BY THXs AND THEIR DERIVATIVES.—The effect of THXs on ACE activity was examined under the assay conditions described in the Experimental section, and the results are shown in Figure 1. 3,4,6,7-Tetrahydroxyxanthone [**1**] showed the highest inhibitory activity against ACE among the THXs tested, followed by 1,3,5,6-THX [**2**], 3,4,5,6-THX [**3**], 1,3,6,7-THX [**4**], and 2,3,6,7-THX [**5**]. IC_{50}'s of these THXs were determined to be 35.4 μM, 69.2 μM , 238.5 μM, and 530.8 μM for **1**, **2**, **3**, and **4**, respectively, while that of **5** is higher than 769 μM.

The inhibition of ACE activity by THXs was analyzed by changing the concentration of HHL substrate in the presence of various concentrations of THXs. As shown in the double-reciprocal plots in Figure 2, all four THXs studied inhibited the activity of ACE competitively with respect to HHL substrate. K_i values of ACE for THXs were determined to be 14.2 μM, 34.2 μM,

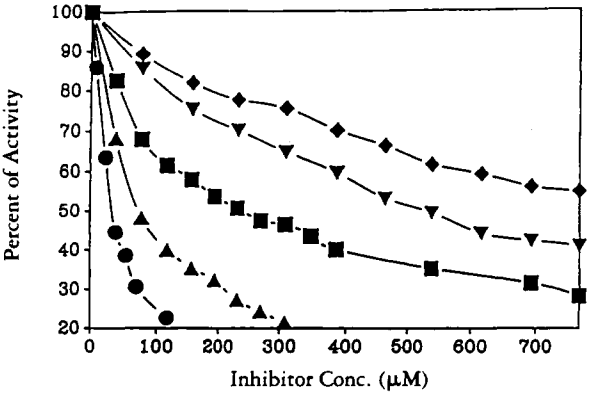


FIGURE 1. Inhibitory effects of tetrahydroxyxanthones (THXs) on angiotension-I-converting enzyme: 3,4,6,7-THX [**1**] (-●-●-), 1,3,5,6-THX [**2**] (-▲-▲-), 3,4,5,6-THX [**3**] (-■-■-), 1,3,6,7-THX [**4**] (-▼-▼-), 2,3,6,7-THX [**5**] (-◆-◆-).

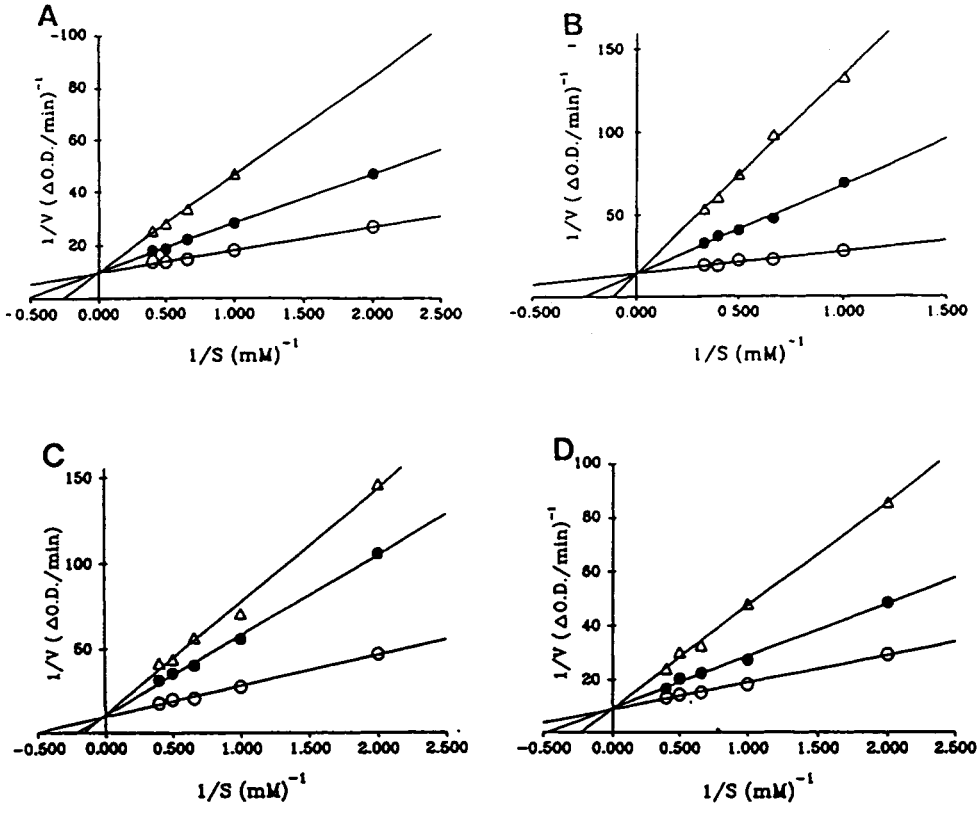


FIGURE 2. Lineweaver-Burk plot for the inhibition of angiotension-I-converting enzyme by tetrahydroxyxanthones (THXs): without inhibitor (-○-○-); panel A 3,4,6,7-THX [**1**] 19.2 μM (-●-●-), 38.5 μM (-Δ-Δ-); panel B 1,3,5,6-THX [**2**] 115.3 μM (-●-●-), 230 μM (-Δ-Δ-); panel C 3,4,5,6-THX [**3**] 192.3 μM (-●-●-), 384.6 μM (-Δ-Δ-); panel D 1,3,6,7-THX [**4**] 230.7 μM (-●-●-), 461.5 μM (-Δ-Δ-).

126.0 μM, and 250.2 μM for **1**, **2**, **3**, and **4**, respectively.

The tetraacetate derivatives of the above mentioned THXs were prepared for studying their effects on ACE activity. The results show that very little inhibitory activity was found for the tetraacetate derivatives of THXs (Table 1).

TABLE 1. Effect of Compounds **1–10** on Angiotensin-I-Converting-Enzyme Activity.

Compound	Inhibition of Activity (%)		
	100 μM	200 μM	400 μM
1	83.13	90.00	95.00
2	56.40	67.66	75.32
3	34.28	46.17	55.47
4	15.48	23.51	40.27
5	11.68	17.23	26.76
1 tetraacetate	9.82	[a]	[a]
2 tetraacetate	<1.00	[a]	[a]
3 tetraacetate	7.00	[a]	[a]
4 tetraacetate	<1.00	[a]	[a]
5 tetraacetate	<1.00	[a]	[a]

[a]The solubility of the test compound is low.

IN VIVO STUDY OF THXs.—Typical examples of the vasopressive response to angiotensin I before and after iv injection of **1** at doses of 40 mg/kg are given in Figure 3. After iv injection of angiotensin I at a concentration of 200 ng/kg, the blood pressure of the rats increased. The vasopressive response to angiotensin I was inhibited 33.8, 47.8, and 52.7% with 20, 40, and 80 mg/kg of **1**, respectively, and the inhibition persisted but level of inhibition decreased after a further series of injection of angiotensin I (Table 2).

THXs that were isolated from *T. lanceolatum* were found to be very effective inhibitors against the ACE activity as demonstrated by in vitro and in vivo experiments. When the hydroxy groups of these active principles were blocked with acetyl groups, the ACE-inhibitory activity of the modified compounds was greatly reduced. This suggests that the free hydroxy groups are essential for the inhibitory activity of THXs on ACE activity.

ACE is a zinc-containing peptidyl dipeptide hydrolase (10), and its complete amino acid sequence has been determined from its cDNA (11,12). ACE

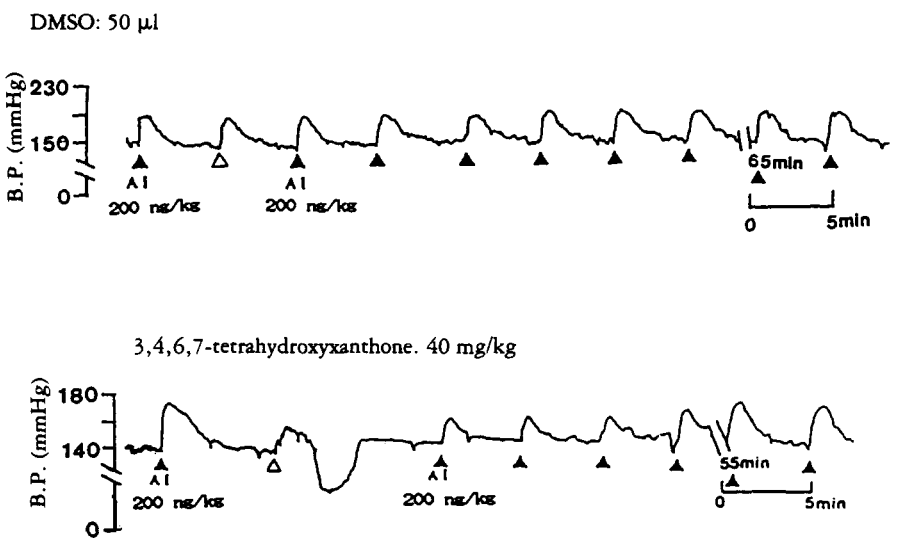


FIGURE 3. Effects of iv administration of angiotensin I (200 ng/kg) with DMSO (50 µl) or with 3,4,6,7-THX [**1**] (40 mg/kg).

TABLE 2. Inhibitory Effects of 3,4,6,7-Tetrahydroxyxanthone [**1**] on the Hypertensive Response to Angiotensin I in Spontaneously Hypertensive Rats.

Drug (dose)	Percent of inhibition[a]		
	10 min	20 min	30 min
1 (20 mg/kg)	33.8 + 1.2%	5.9 + 2.1%	0%
1 (40 mg/kg)	47.8 + 3.1%	30.8 + 3.2%	10.0 + 5.0%
1 (80 mg/kg)	52.7 + 4.7%	32.5 + 5.2%	13.0 + 5.3%
Captopril (0.04 mg/kg)	10.0%	0%	0%
Captopril (0.4 mg/kg)	78.5%	66.6%	45.5%

[a]Mean + SE: 3,4,6,7-tetrahydroxyxanthone [**1**] ($n = 4$), Captopril ($n = 2$). Time intervals are time after administration of the drug.

contains 1277 amino acid residues consisting of two homologous, repeated domains each of which has a potential catalytic site. Two highly conserved segments (residues 361–365 and 959–963), each with sequence HEMGH analogous to that observed in other zinc metalloproteases (13, 14), were predicted to constitute the zinc binding site.

The present investigation demonstrated that four hydroxy groups of THXs were required for their strong inhibition on ACE, and that each of two hydroxyl groups was probably involved in the binding of zinc ion. Blocking of tetrahydroxy groups caused a significant reduction of inhibitory activity, which indicates that the free hydroxyl groups of THXs are probably important for chelating the zinc ions to inactivate the ACE activity.

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