Journal of Natural Products
Vol. 51, No. 2, pp. 345-348, Mar-Apr 1988

INHIBITION OF COW'S MILK XANTHINE OXIDASE BY FLAVONOIDS

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Flavonoids, a large group of naturally occurring pigments, are widely distributed in the plant kingdom. They are known to inhibit a number of enzymes such as phosphodiesterase (1–3), Ca^{2+} ATPase (4), aldose reductase (5–7), lipoxygenase (8,9), and cyclooxygenase (10). The inhibition of xanthine oxidase (xanthine: oxygen oxidoreductase, EC 1.2.3.2) by flavonoids has also been reported by several workers (11–15). The major function of this enzyme is to oxidize hypoxanthine and xanthine to uric acid. An inhibitor of xanthine oxidase has been expected to be a therapeutic agent for hyperuricemia that causes gout (16), renal stones (16,17), or ischemic myocardium (18). Allopurinol is a potent inhibitor of xanthine oxidase that has been clinically used (19). The present paper describes inhibition of xanthine oxidase from cow's milk by 103 flavonoids. Some structure-activity relationships are also discussed.

RESULTS AND DISCUSSION

The inhibitory activities of 103 flavonoids against xanthine oxidase at 50 μg/ml were measured. The inhibition percentages of individual flavonoids excluding 19 compounds previously reported (11–15) are presented in Tables 1–5. IC_{50}'s are also listed with flavonoids indicating more than 50% inhibition at 50 μg/ml. Baicalein, robinetin, and 3,6-dimethoxyapigenin were newly found to possess a strong inhibitory effect on xanthine oxidase. Luteolin, diosmetin, quercetin, and naringenin were confirmed to possess strong activity, and 3,6-dimethoxyapigenin showed the highest activity. Generally speaking, flavones and flavonols without a glycosyl group were relatively strong inhibitors. As Iio *et al.* reported (14), the presence of a glycosyl group decreased the inhibitory activity. Methylation of an aromatic hydroxyl group also decreased inhibition except for diosmetin

TABLE 1. Inhibitory Activities of Flavones on Xanthine Oxidase.

Compound[a]	Substituents						Inhibition (% at 50 μg/ml)	IC_{50} (μM)
	5	6	7	8	3′	4′		
cosmosiin	OH		O-R_1			OH	13.9	—
rhoifolin	OH		O-R_2			OH	12.9	—
acacetin	OH		OH			OMe	55.7	14.1
acaciin	OH		O-R_2			OMe	15.1	—
tilianine	OH		O-R_1			OMe	0	—
baicalein	OH	OH	OH				85.5	3.3
baicalein-Me_3	OMe	OMe	OMe				22.0	—
scutellarein	OH	OH	OH			OH	67.3	12.6
scutellarin	OH	OH	O-R_3			OH	16.6	—
cirsimaritin	OH	OMe	OMe			OH	0	—
cirsimarin	OH	OMe	OMe			O-R_1	12.2	—
pectolinarigenin	OH	OMe	OH			OMe	32.1	—
pectolinarin	OH	OMe	O-R_2			OMe	11.5	—

[1]Née Sekino.

TABLE 1. Continued.

Compound[a]	Substituents						Inhibition (% at 50 μg/ml)	IC_{50} (μM)
	5	6	7	8	3′	4′		
scutellarein-Ac_4	OAc	OAc	OAc			OAc	0	—
luteolin-7-R_1	OH		O-R_1		OH	OH	67.2	75.9
lonicerin	OH		O-R_2		OH	OH	20.1	—
chrysoeriol	OH		OH		OMe	OH	61.5	14.0
chrysoeriol-7-R_3	OH		O-R_3		OMe	OH	0.3	—
diosmetin-7-R_4	OH		O-R_4		OH	OMe	30.5	—
diosmetin-7-R_1	OH		O-R_1		OH	OMe	28.9	—
diosmin	OH		O-R_2		OH	OMe	0	—
luteolin-Ac_4	OAc		OAc		OAc	OAc	47.7	—
pedaltin	OH	OMe	OH		OH	OH	49.0	—
cirsiliol	OH	OMe	OMe		OH	OH	0.5	—
cirsiliol-4′-R_1	OH	OMe	OMe		OH	O-R_1	0	—
cirsileneol	OH	OMe	OMe		OMe	OH	1.0	—
cirsileneol-4′-R_1	OH	OMe	OMe		OMe	O-R_1	0	—
wogonin	OH		OH	OMe			50.2	176.1
fukugetin	OH	R	OH		OH	OH	97.2	22.3
flavone							15.4	—

[a]R = -CO-CH-CH-Ph-OH (O), R_1 = glucose, R_2 = rhamnose-glucose, R_3 = glucuronic acid, R_4 = arabinose-glucose.

and 3,6-dimethoxyapigenin. All acetates, isoflavones, and *C*-glycosylflavonoids tested showed less inhibitory activity. Recently, Nishibe *et al.* found 4′-*O*-glycosylated flavonoids with potent inhibitory activity (15). These findings suggested the presence of free hydroxyl groups at C-5 and C-7 to be important for producing inhibitory activity against xanthine oxidase. In the

TABLE 2. Inhibitory Activities of Flavonols on Xanthine Oxidase.

Compound[a]	Substituents								Inhibition (% at 50 μg/ml)	IC_{50} (μM)
	3	5	6	7	3′	4′	5′	2″		
trifolin	O-R_5	OH		OH		OH			16.1	—
kaempferitrin	O-R_6	OH		O-R_6		OH			15.5	—
juglanin	O-R_7	OH		OH		OH			0	—
kaempferol-7-R_1	OH	OH		O-R_1		OH			0	—
kaempferol-3-R_2	O-R_2	OH		OH		OH			14.4	—
hyperin	O-R_5	OH		OH	OH	OH			43.0	—
quercetin-3-R_3	O-R_3	OH		OH	OH	OH			36.5	—
reynoutrin	O-R_8	OH		OH	OH	OH			33.1	—
avicularin	O-R_7	OH		OH	OH	OH			30.8	—
quercetin-3-R_1-2″-R_9	O-R_1	OH		OH	OH	OH		C-R_9	21.0	—
quercimeritrin	OH	OH		O-R_1	OH	OH			0	—
quercetin-3-Me	OMe	OH		OH	OH	OH			98.5	31.6
quercetin-3-Me-4′-R_1	OMe	OH		OH	OH	O-R_1			69.1	35.6
quercetin-3-Me-7-R_1	OMe	OH		O-R_1	OH	OH			39.5	—
isorhamnetin	OH	OH		OH	OMe	OH			49.0	—
persicarin	O-KSO_3	OH		OH	OMe	OH			90.3	34.6
narcissin	O-R_2	OH		OH	OMe	OH			22.2	—
quercetin-Ac_5	OAc	OAc		OAc	OAc	OAc			0	—
myricetin-3-R_{10}	O-R_{10}	OH		OH	OH	OH	OH		88.6	19.1
myricetin-Ac_6	OAc	OAc		OAc	OAc	OAc	OAc		13.6	—
robinetin	OH			OH	OH	OH	OH		82.9	4.3
apigenin-3,6-OMe_2	OMe	OH	OMe	OH		OH			95.0	0.03

[a]R_1 = glucose, R_2 = rhamnose-glucose, R_3 = glucuronic acid, R_5 = galactose, R_6 = rhamnose, R_7 = arabinose, R_8 = xylose, R_9 = gallic acid, R_{10} = rhamnose-galactose.

TABLE 3. Inhibitory Activities of Flavanones, Flavanonols, and Isoflavones on Xanthine Oxidase.

Compound[a]	Substituents[a]								Inhibition (% at 50 μg/ml)	IC_{50} (μM)
	3	5	6	7	8	3′	4′	5′		
Flavanone										
liquiritigenin				OH			OH		100	11.3
liquiritin				OH			$O\text{-}R_1$		18.0	—
prunin		OH		$O\text{-}R_1$			OH		4.2	—
sakuranetin		OH		OMe			OH		0	—
isosakuranetin		OH		OH			OMe		0	—
poncitrin		OH		$O\text{-}R_2$			OMe		0	—
eriodictyol		OH		OH		OH	OH		47.8	—
Flavanonol										
aromadendrin	OH	OH		OH			OH		66.9	93.8
fustin	OH			OH		OH	OH		27.2	—
taxifolin	OH	OH		OH		OH	OH		17.8	—
astilbin	$O\text{-}R_6$	OH		OH		OH	OH		0	—
phellamurin	OH	OH		$O\text{-}R_1$	R_{11}		OH		0	—
Isoflavone										
daidzein				OH			OH		0	—
daidzin				$O\text{-}R_1$			OH		0	—
formononetin				OH			OMe		17.6	—
genistein		OH		OH			OH		0	—
genistin		OH		$O\text{-}R_1$			OH		0.8	—
sophoricoside		OH		OH			$O\text{-}R_1$		15.3	—
tectorigenin		OH	OMe	OH			OH		0	—
tectoridin		OH	OMe	$O\text{-}R_1$			OH		0	—
iristectorigenin A		OH	OMe	OH		OH	OMe		0	—
irigenin		OH	OMe	OH		OH	OMe	OMe	0	—
iridin		OH	OMe	$O\text{-}R_1$		OH	OMe	OMe	0	—
irisolone		OMe	$-O\text{-}CH_2\text{-}O-$				OH		0	—
irisflorentin		OMe	$-O\text{-}CH_2\text{-}O-$			OMe	OMe	OMe	0	—

[a] R_1 = glucose, R_2 = rhamnose-glucose, R_6 = rhamnose, $R_{11} = -(CH_2)_2\text{-}C(OH)(CH_3)_2$.

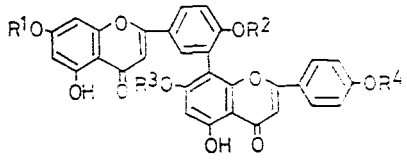
cases of vitexin and orientin possessing free OH at C-5 and C-7, the *C*-glycosyl group might be too bulky and prevent the flavonoids from combining with the enzyme. The lack of activity of isoflavones and 3-*O*-glycosylated flavonols with a free OH at C-5 and C-7 seemed to be due to the steric factor of the bulky group at C-3. The low activity of flavanones and flavanonols suggested the presence of a double bond between C-2 and C-3 to be an important factor. The high activity of diosmetin (IC_{50} 0.07 μM) and 3,6-dimethoxyapigenin (IC_{50}

TABLE 4. Inhibitory Activities of *C*-glycosylflavonoids on Xanthine Oxidase.

Compound[a]	Substituent[a]								Inhibition (% at 50 μg/ml)
	3	5	6	7	8	3′	4′	2″	
vitexin		OH		OH	$C\text{-}R_1$		OH		0
orientin		OH		OH	$C\text{-}R_1$	OH	OH		15.3
isoorientin		OH	$C\text{-}R_1$	OH		OH	OH		25.2
isoorientin-7-R_1		OH	$C\text{-}R_1$	$O\text{-}R_1$		OH	OH		0
swertisin		OH	$C\text{-}R_1$	OMe			OH		24.9
embigenin		OH	$C\text{-}R_1$	OMe			OMe		33.5
embinin		OH	$C\text{-}R_1$	OMe			OMe	$O\text{-}R_6$	22.8
keyakinin	OH	OH	$C\text{-}R_1$	OMe			OH		0

[a] R_1 = glucose, R_6 = rhamnose.

TABLE 5. Inhibitory Activities of Biflavones on Xanthine Oxidase.



Compound	R^1	R^2	R^3	R^4	Inhibition (% at 50 μg/ml)	IC_{50} (μM)
amentoflavone	H	H	H	H	100	35.3
sciadopitysin	Me	Me	H	Me	0	—

0.03 μM) might be attributable to their surface activity, as Iio *et al.* suggested (14).

EXPERIMENTAL

MATERIAL.—We obtained most of the flavonoids tested from plant sources; 3,6-dimethoxyapigenin was obtained through the courtesy of professor T.J. Mabry, University of Texas at Austin. Other chemicals were purchased from commercial sources and used without further purification: xanthine oxidase (Boehringer Mannheim Co. Ltd.), xanthine (ICN Pharmaceuticals Inc.), sodium phosphate dibasic 12 hydrate, potassium phosphate monobasic, and Tween 80 (Wako Pure Chemical Industries, Ltd.).

ASSAY OF XANTHINE OXIDASE ACTIVITY.—Xanthine oxidase activity was measured by the method reported by Noro *et al.* (13). The inhibitory activity (%) was calculated as follows: $(1-B/A)\times 100$, where A is the activity of enzyme without test material, and B is the activity of enzyme with test material.

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Received 28 August 1987