[Chem. Pharm. Bull. 36(1) 244—248 (1988)]

Inhibitors of Xanthine Oxidase from *Alpinia galanga*[1)]

TADATAKA NORO,* TAKESHI SEKIYA, MASAKO KATOH (née ABE), YASUSHI ODA, TOSHIO MIYASE, MASANORI KUROYANAGI, AKIRA UENO and SEIGO FUKUSHIMA

School of Pharmaceutical Science, University of Shizuoka, 2–2–1 Oshika, Shizuoka 422, Japan

(Received June 29, 1987)

Xanthine oxidase (XO) inhibitors were isolated from the rhizomes of *Alpinia galanga* (Zingiberaceae), and were identified as *trans-p*-coumaryl diacetate (**1**), *trans*-coniferyl diacetate (**2**), [1′*S*]-1′-acetoxychavicol acetate (**3**), [1′*S*]-1′-acetoxyeugenol acetate (**4**) and 4-hydroxybenzaldehyde (**5**). The type of inhibition by either **1** or **3** with respect to xanthine as a substrate was uncompetitive.

Keywords——xanthine oxidase; inhibitor; uncompetitive inhibition; *Alpinia galanga*; Zingiberaceae; xanthine; *p*-coumaryl diacetate; 1′-acetoxychavicol diacetate; phenylpropenol

Introduction

In the course of our investigations on inhibitors of xanthine oxidase (XO, EC 1.2.3.2), we have reported the inhibitory activities of flavonoids,[2)] xanthones[3)] and anthraquinones.[1)] They may be candidates as drugs for the treatment of gout.[4)] A preliminary screening test *in vitro*, aiming to find XO inhibitors among many oriental crude drugs and plant materials, suggested that the rhizomes of *Alpinia galanga* SWARTZ[5,6)] (=*Languas galanga* STUNTZ.,[7)] Zingiberaceae) show an inhibitory effect on the enzyme. The rhizomes of *A. galanga* have been used for flavoring food[5)] and as a stomachic.[6)] Other biological activities of *A. galanga* are as follows: antitumor,[8)] pungency,[8,9)] antibacterial,[10)] anti-ulcer,[11)] and insecticidal.[12)] In present paper, we describe the isolation and activity of XO inhibitors from the rhizomes of *A. galanga*.

Results and Discussion

The activity of XO *in vitro* towards xanthine as a substrate was assayed spectrophotometrically at 290 nm by the method described in the previous paper.[1)] The rhizomes of *A. galanga* were extracted with chloroform, methanol and water under reflux as described in Experimental, where the activities and yields of the extracts are also given. The most active chloroform extract was fractionated repeatedly by silica gel column chromatography, and the fractions were monitored by thin layer chromatography (TLC, silica gel) and also by measurement of inhibitory activity against XO. Five strong inhibitors were isolated, *trans-p*-coumaryl diacetate (**1**), *trans*-coniferyl diacetate (**2**), [1′*S*]-1′-acetoxychavicol acetate (**3**),[10,11)] [1′*S*]-1′-acetoxyeugenol acetate (**4**),[10,11)] and 4-hydroxybenzaldehyde (**5**) (Chart 1). The spectral data were consistent with those of the synthetic compounds[13,14)] and the literature values[10,11)] (Tables I and II). The activities of the inhibitors **1**—**5** against XO are shown in Table III. The most active compound is **1**. The concentration of **1** in the assay mixture required to give 50% inhibition (IC_{50}) was 3.5×10^{-6} M. Synthetic *dl*-**3** or *dl*-**4** showed similar

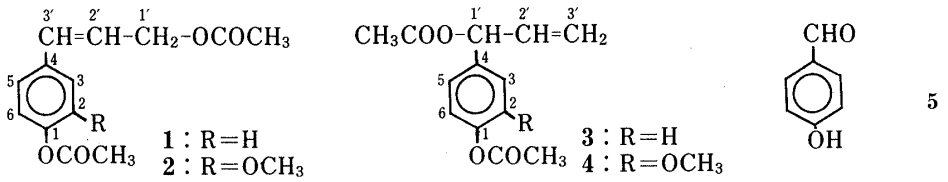

Chart 1

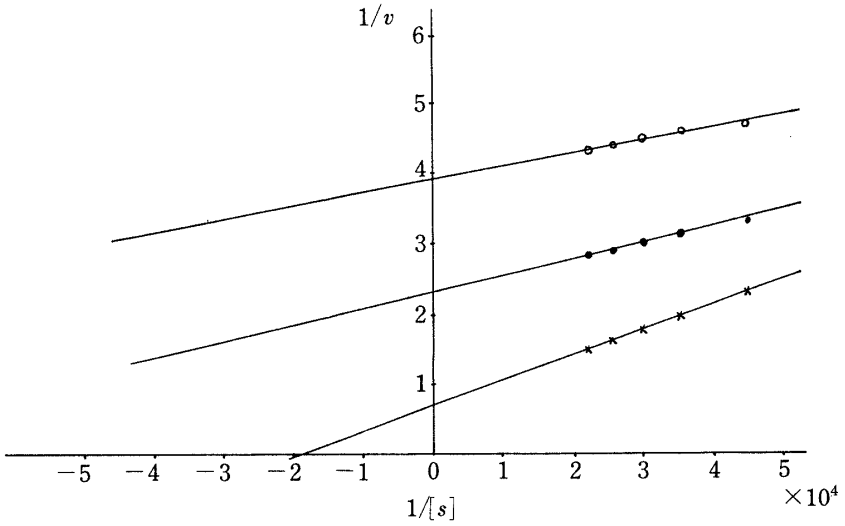

Fig. 1. Inhibitory Effects of **1** and **3** on Xanthine Oxidase

Lineweaver–Burk plots in the absence (0 M, ×—×) and in the presence of **1** (4.27×10^{-6} M, ○—○) or **3** (4.27×10^{-6} M, ●—●) with xanthine as the substrate. v, μM substrate metabolized/mg enzyme/min; s, substrate.

inhibitory activity to naturally occurring optically active **3** or **4**. These data show that phenylpropenol acetate derivatives have strong inhibitory activity against XO, and 3-phenyl-2-propen-1-ol derivatives are stronger inhibitors than 1-phenyl-2-propen-1-ol derivatives. Kinetic studies were done on the effect of **1** or **3** on the oxidation of xanthine by XO under the standard assay conditions. The results are shown as Lineweaver–Burk plots[15] in Fig. 1. The type of inhibition by **1** and **3** was uncompetitive.

In *A. galanga*, **3** was found to be the main constituent in this experiment. The biological activities of **3** and **4** were reported to be as follows; antitumor,[8] pungency,[9] antibacterial[10] and anti-ulcer.[11] Monoterpenes,[16] sesquiterpenes,[11,16] diterpenes[17] and flavonoids[18] have been reported as constituents of *A. galanga*, but these constituents were not XO inhibitors, as examined by this assay method. This is the first report of the isolation of **1**, **2** and **5** from the rhizomes. We have identified strong XO inhibitors, and this represents another biological activity of the rhizomes of *A. galanga*.

Experimental

The following instruments were used to obtain physical data. Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a JASCO IRA-202 infrared spectrophotometer. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were recorded on a JEOL FX-90Q spectrometer (89.55 and 22.50 MHz, respectively). Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Mass spectra (MS) were recorded on a JEOL JMS-01SG-2 mass spectrometer. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. The spectrophotometric measurements were carried out with a Hitachi model 101 spectrophotometer. Silica gel $60GF_{254}$ (Merck) was used for TLC, and detection was achieved by

illumination with an ultraviolet lamp or by spraying 20% H_2SO_4 aqueous solution followed by heating. For column chromatography, Silica gel 60 (Merck) was used.

Enzyme and Chemicals——Xanthine oxidase (EC 1.2.3.2), Tween 80, sodium phosphate dibasic 12 hydrate and potassium phosphate monobasic were obtained from previously described sources.[2] The buffer, the substrate solution and the enzyme solution were prepared as described previously.[2]

Test Solution——The test solution was prepared as described previously.[2]

Assay of Xanthine Oxidase Activity——For the assay of XO activity the method described previously[1] was employed.

Estimation of Xanthine Oxidase Inhibitory Activity——The same method as described previously[2] was used for the estimation of XO inhibitory activity. (Table III)

Lineweaver–Burk Plots[15]——The Lineweaver–Burk plots for XO with xanthine as a substrate under our assay conditions in the absence and in the presence of **1** or **3** are shown in Fig. 1.

Extraction and Isolation——The dried rhizomes (1 kg, commercial products) of *Alpinia galanga* SWARTZ were extracted three times with 3 l of $CHCl_3$ under reflux for 3 h, then with MeOH and H_2O in the same way successively, and the extracts were concentrated *in vacuo*. These extracts were assayed for inhibitory effects against XO with 10 μg of test sample per 1 ml of assay solution. The activities and yields of the extracts were followed; $CHCl_3$ ext. (62.6%, 10.1 g), MeOH ext. (25.9%, 56.0 g) and H_2O ext. (0%, not measured). The most active $CHCl_3$ extract was fractionated repeatedly on a silica gel column with a benzene–$CHCl_3$–MeOH, hexane–acetone or hexane–AcOEt gradient system as the eluent. The each fraction was monitored by TLC (hexane : AcOEt = 7 : 1 or hexane : acetone = 4 : 1), and by measurement of inhibitory activity against XO. The yields of active compounds were as follows: **1** (150 mg), **2** (14 mg), **3** (770 mg), **4** (130 mg) and **5** (30 mg).

***p*-Coumaryl Diacetate (1)**——Colorless prisms from hexane–benzene. mp 48 °C. *Anal.* Calcd for $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found: C, 66.61; H, 5.99. MS *m/z* (rel. int%): 234 (M^+, 11), 192 (50), 150 (33), 149 (40), 133 (34), 132 (18), 131 (23), 121 (27), 107 (17), 105 (12), 103 (13), 94 (16), 77 (16), 42 (100). IR (KBr) cm^{-1}: 1755, 1730. NMR (Tables I and II). **1** was identical with synthetic *p*-coumaryl diacetate on the basis of melting point, spectral comparisons and elemental analysis.

Coniferyl Diacetate (2)——Colorless oil. *Anal.* Calcd for $C_{14}H_{16}O_5$: C, 63.62; H, 6.10. Found: C, 63.65; H, 6.07. MS *m/z* (rel. int%): 264 (M^+, 8), 223 (10), 222 (66), 180 (21), 179 (32), 163 (14), 151 (15), 147 (13), 137 (12), 131 (41), 124 (15), 119 (21), 103 (17), 91 (20), 77 (12), 65 (11), 42 (100). IR (liq. film) cm^{-1}: 1755, 1730. NMR (Tables I and II). **2** was identical with synthetic coniferyl diacetate on the basis of spectral comparisons and elemental analysis.

[1′*S*]-1′-Acetoxychavicol Acetate (3)[10,11]——Colorless oil. *Anal.* Calcd for $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found: C, 66.63; H, 6.00. $[\alpha]_D^{20}$ −53° (*c* = 0.95, EtOH, lit. −80°).[11] NMR (Tables I and II). **3** was identical with synthetic *dl*-1′-acetoxychavicol acetate on the basis of spectral comparisons and elemental analysis, and literature values[10,11] were also in agreement.

[1′*S*]-1′-Acetoxyeugenol Acetate (4)[10,11]——Colorless oil. *Anal.* Calcd for $C_{14}H_{16}O_5$: C, 63.62; H, 6.10. Found: C, 63.59; H, 6.08. $[\alpha]_D^{20}$ −17.8° (*c* = 0.44, EtOH) NMR (Tables I and II). **4** was identical with synthetic *dl*-1′-acetoxyeugenol acetate on the basis of spectral comparisons and elemental analysis, and literature values[10,11] were also in agreement.

4-Hydroxybenzaldehyde (5)——Colorless needles from hexane–benzene. mp 115.5 °C. *Anal.* Calcd for $C_7H_6O_2$: C, 68.84; H, 4.95. Found: C, 68.65; H, 5.02. **5** was confirmed by direct comparison of the melting point and spectral

TABLE I. ^{1}H-NMR Chemical Shifts[a]

Proton No.	1	2	3	4
2	7.07 (1H, d, *J* = 8.5 Hz)	—	7.06 (1H, d, *J* = 8.6 Hz)	—
3	7.38 (1H, d, *J* = 8.5 Hz)	6.97 (1H, br s)	7.36 (1H, d, *J* = 8.6 Hz)	6.96 (1H, br s)
5	7.38 (1H, d, *J* = 8.5 Hz)	6.97 (1H, br s)	7.36 (1H, d, *J* = 8.6 Hz)	7.26 (1H, br s)
6	7.07 (1H, d, *J* = 8.5 Hz)	6.97 (1H, br s)	7.06 (1H, d, *J* = 8.6 Hz)	6.98 (1H, br s)
1′	4.71 (2H, d, *J* = 5.2 Hz)	4.71 (2H, d, *J* = 5.9 Hz)	6.26 (1H, d, *J* = 5.9 Hz)	6.25 (1H, d, *J* = 5.7 Hz)
2′	6.21 (1H, dt, *J* = 15.9, 5.2 Hz)	6.21 (1H, dt, *J* = 15.9, 7.1 Hz)	5.99 (1H, m)	6.00 (1H, m)
3′a	6.64 (1H, d, *J* = 15.9 Hz)	6.62 (1H, d, *J* = 15.9 Hz)	5.27 (1H, d, *J* = 16.3 Hz)	5.30 (1H, d, *J* = 17.9 Hz)
3′b	—	—	5.23 (1H, d, *J* = 9.6 Hz)	5.25 (1H, d, *J* = 10.1 Hz)
OCH_3	—	3.83 (3H, s)	—	3.84 (3H, s)
$OCOCH_3$	2.09 (3H, s)	2.09 (3H, s)	2.09 (3H, s)	2.30 (3H, s)
	2.28 (3H, s)	2.30 (3H, s)	2.28 (3H, s)	2.11 (3H, s)

a) Measured at 89.55 MHz in $CDCl_3$ solution.

TABLE II. ^{13}C-NMR Chemical Shifts[a]

Carbon No.	1	2	3	4
1	150.8 (s)	139.9 (s)	150.6 (s)	139.8 (s)
2	121.8 (d)	151.3 (s)	121.6 (d)	151.3 (s)
3	127.7 (d)	110.6 (d)	128.4 (d)	111.8 (d)
4	134.3 (s)	135.3 (s)	136.5 (s)	137.8 (s)
5	127.7 (d)	119.6 (d)	128.4 (d)	119.7 (d)
6	121.8 (d)	122.8 (d)	121.6 (d)	122.8 (d)
1′	64.9 (t)	64.8 (t)	75.7 (d)	75.7 (d)
2′	123.8 (d)	123.7 (d)	136.2 (d)	136.2 (d)
3′	133.3 (d)	133.6 (d)	117.0 (t)	117.0 (t)
OCH_3	—	56.0 (q)	—	56.0 (q)
$OCOCH_3$	20.9 (q)	20.5 (q)	21.1 (q)	20.6 (q)
	21.0 (q)	20.9 (q)	×2	21.1 (q)
$OCOCH_3$	169.0 (s)	168.7 (s)	169.1 (s)	168.8 (s)
	170.6 (s)	170.6 (s)	169.7 (s)	169.7 (s)

a) Measured at 22.5 MHz in $CDCl_3$ solution.

TABLE III. Inhibitory Activity of Constituents of the Rhizomes of *Alpinia galanga* on Xanthine Oxidase

Compounds	IC_{50} ($\times 10^{-6}$ M)[a]
p-Coumaryl diacetate (**1**)	3.5
Coniferyl diacetate (**2**)	6.1
(1′*S*)-1′-Acetoxychavicol acetate (**3**)	10.7
(1′*S*)-1′-Acetoxyeugenol acetate (**4**)	9.8
4-Hydroxybenzaldehyde (**5**)	19.6

a) IC_{50} of the reference inhibitor, norathyriol,[3] 0.9×10^{-6} M, each value of IC_{50} is the average of four measurements.

data with those of an authentic sample.

Synthesis of *p*-Coumaryl Diacetate (1) and Coniferyl Diacetate (2)——*p*-Coumaryl alcohol[13] and coniferyl alcohol[14] were acetylated by the usual method.

Synthesis of *dl*-1′-Acetoxychavicol Acetate and *dl*-1′-Acetoxyeugenol Acetate——**3** and **4** were prepared according to Mitsui *et al.*[11] The synthetic **3** and **4** were in *dl*-form.

Acknowledgement The authors thank Dr. Mitsuo Uchida and Mrs. Hisayo Kitamura, Analytical Center of School of Pharmaceutical Science, University of Shizuoka, for the MS measurements and elemental analysis.

References and Notes

1) This forms Part V of "Studies of Enzyme Inhibitors," Part IV: T. Noro, K. Noro, T. Miyase, M. Kuroyanagi, K. Umehara, A. Ueno and S. Fukushima, *Chem. Pharm. Bull.*, **35**, 4314 (1987). A part of this work was presented previously: Abstracts of Papers, the 104th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, March 1984, p. 138.
2) T. Noro, Y. Oda, T. Miyase, A. Ueno and S. Fukushima, *Chem. Pharm. Bull.*, **31**, 3984 (1983); S. Nishibe, A. Sakushima, T. Noro and S. Fukushima, *Shoyakugaku Zasshi*, **41**, 116 (1987).
3) T. Noro, A. Ueno, M. Mizutani, T. Hashimoto, T. Miyase, M. Kuroyanagi and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 4455 (1984).
4) R. W. Rundles, *Trans. Assoc. Am. Physicians*, **76**, 126 (1963); M. Isa, G. Hayashi and Y. Kowa, *Nippon Yakurigaku Zasshi*, **64**, 108 (1968).
5) M. Konoshima, G. Honda and T. Ono, *Shoyakugaku Zasshi*, **30**, 18 (1976).
6) Chiang Su New Medicinal College, "Dictionary of Chinese Crude Drugs, 中葯大辞典," Shanghai Scientific Technologic Publisher, 1977, p. 125.

7) Research Institute for Drugs of Chinese Medical and Technological College *et al.*, "Zhōng-yào-zhì, 中葯志," Vol. 2, Rénmínwèishēng Pub. Co., Peking, 1961, p. 126.
8) H. Itokawa, H. Morita, T. Sumitomo, N. Totsuka and K. Takeya, *Planta Medica*, **1987**, 32.
9) H. Itokawa, H. Morita, T. Sumitomo, C. Hata and T. Shinohara, Abstracts of Papers, the 32nd Annual Meeting of the Japanese Society of Pharmacognosy, Okayama, October 1985, p. 39.
10) A. M. Janssen and J. J. C. Scheffer, *Planta Medica*, **1985**, 507; I. C. Chopra, B. N. Khajuria and C. L. Chopra, *Antibiotics and Chemotherapy*, **7**, 378 (1957) [*Chem. Abstr.*, **52**, 13014*a* (1958)].
11) S. Mitsui, S. Kobayashi, H. Nagahori and A. Ogiso, *Chem. Pharm. Bull.*, **24**, 2377 (1976).
12) R. S. Dixit and S. L. Perti, *Bull. Reg. Res. Lab. Jammu. India*, **1**, 169 (1963) [*Chem. Abstr.*, **60**, 11312*b* (1964)].
13) K. Freudenberg and G. Gehrke, *Chem. Ber.*, **84**, 443 (1951).
14) K. Freudenberg and H. H. Hubner, *Chem. Ber.*, **85**, 1181 (1952).
15) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934); M. Dixon and E. C. Weff, "Enzymes," 3rd ed., Longman Group Ltd., London, 1979, p. 341.
16) H. L. De Pooter, M. N. Omar, B. A. Coolsaet and N. M. Schamp, *Phytochemistry*, **24**, 93 (1985).
17) H. Morita and H. Itokawa, *Chem. Lett.*, **1986**, 1205.
18) A. G. R. Nair, R. Gunasegaran and B. S. Joshi, *Indian J. Chem.*, **21B**, 979 (1982).