JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 781–786 781
DOI:10.1021/jf901498q

Inhibition of Xanthine Oxidase by *Acacia confusa* Extracts and Their Phytochemicals

Yu-Tang Tung and Shang-Tzen Chang*

School of Forestry and Resource Conservation, National Taiwan University, Taipei 106, Taiwan

Acacia confusa Merr. (Leguminosae) is traditionally used as a medicinal plant in Taiwan. In the present study, the XOD-inhibitory activity of ethanolic extracts from *A. confusa* was investigated for the first time. Results demonstrated that the ethanolic extract of *A. confusa* heartwood had a strong XOD-inhibitory activity. Among all fractions derived from heartwood extracts, the EtOAc fraction exhibited the best inhibitory activity. Following column chromatography and reverse-phase high-performance liquid chromatography, eight specific phytochemicals including melanoxetin, 7,8,3′,4′-tetrahydroxyflavone, transilitin, okanin, 3,7,8,3′-tetrahydroxy-4′-methoxyflavone, 7,8,3′-trihydroxy-3,4′-dimethoxyflavone, 7,3′,4′-trihydroxyflavone, and 7,3′,4′-trihydroxy-3-methoxyflavone were isolated and identified from the EtOAc fraction. In addition, the IC_{50} values indicated that okanin showed the strongest XOD-inhibitory effect (IC_{50} value of 0.076 μM), followed by melanoxetin (0.274 μM) and allopurinol (4.784 μM). The present study revealed that okanin and melanoxetin showed excellent inhibition on XOD in noncompetitive and competitive mode, respectively, and their inhibitory activity is better than that of allopurinol. This is the first study that demonstrates the XOD-inhibitory performance of okanin and melanoxetin.

KEYWORDS: *Acacia confusa*; extracts; xanthine oxidase; okanin; melanoxetin

INTRODUCTION

Xanthine oxidase (XOD) is situated at the end of a catabolic sequence of the purine nucleotide metabolism in humans and a few other uricotelic species. Its major function is to catalyze the oxidation of hypoxanthine to xanthine and that of xanthine to uric acid (*1*). Gout and hyperuricemia are metabolic disorders associated with abnormal amounts of uric acid in the body, resulting in the deposition of urate crystals in joints and kidneys, causing inflammation as well as gouty arthritis and uric acid nephrolithiasis (*2*, *3*). Accordingly, XOD inhibitors are employed to block the final step in uric acid synthesis, reducing the production of uric acid, as well as promoting the production of anti-inflammatory agents to relieve the symptoms of the disease (*4*−*6*).

The use of botanical plants is gaining renewed interest in connection with the treatment of some clinical disorders. Scientists have turned to exploring the potent XOD inhibitor from a wide variety of traditional herbal plants (*7*−*9*). *Acacia* is an evergreen tree; the genus *Acacia* comprises more than 1300 species and occurs in almost all types of habitat (*10*). The *Acacia* species is mainly used as a traditional medicine (*11*−*19*). *Acacia confusa* Merr. (Leguminosae), a native species in Taiwan, is widely distributed on the hills and lowlands of Taiwan and has been traditionally used as a medicine (*20*). Ethanolic extracts of *A. confusa* have been used in Taiwan for wound healing and anti-blood-stasis (*21*) and were reported to contain abundant phenolics (*18*, *20*, *22*−*29*). Furthermore, recent studies have shown that phenolics have been reported to be strong XOD inhibitors (*30*). The putative therapeutic effects of many traditional medicines have been ascribed to flavonoids, in particular, due to their enzyme inhibitory and antioxidant activities (*31*, *32*). Therefore, ethanolic extracts of *A. confusa* may be good candidates for further development as clinically used drugs. However, to the best of our knowledge there is no prior report on the XOD-inhibitory activity of ethanolic extracts from *A. confusa*. In this study, we investigated the XOD-inhibitory activity of ethanolic extracts from *A. confusa* for the first time and detected the active constituents.

*Author to whom correspondence should be addressed (telephone 886-2-33664626; fax 886-2-23654520; e-mail peter@ntu.edu.tw).

MATERIALS AND METHODS

Chemicals. Xanthine oxidase, sodium pyrophosphate, xanthine, and allupurinol were all purchased from Sigma Chemical Co. (St. Louis, MO). The other chemicals and solvents used in this experiment were of analytical grade.

Plant Material. The heartwood, bark, twig, flower, and leaf of *A. confusa* were sampled from the experimental forest of National Taiwan University in Nan-Tou county. The species was identified by Sheng-You Lu of the Taiwan Forestry Research Institute, and a voucher specimen (AC001) was deposited at the School of Forestry and Resource Conservation, National Taiwan University. The materials were air-dried at ambient temperature (25 °C).

Extraction and Isolation. Extraction of heartwood extract from *A. confusa* was carried out according to the method of Wu et al. (*18*). The resulting heartwood extract (125 g) was fractionated successively with EtOAc, *n*-butanol (BuOH), and water to yield soluble fractions of EtOAc (58 g), BuOH (43 g), and H_2O (21 g). The phytochemicals from the EtOAc

Published on Web 01/04/2010

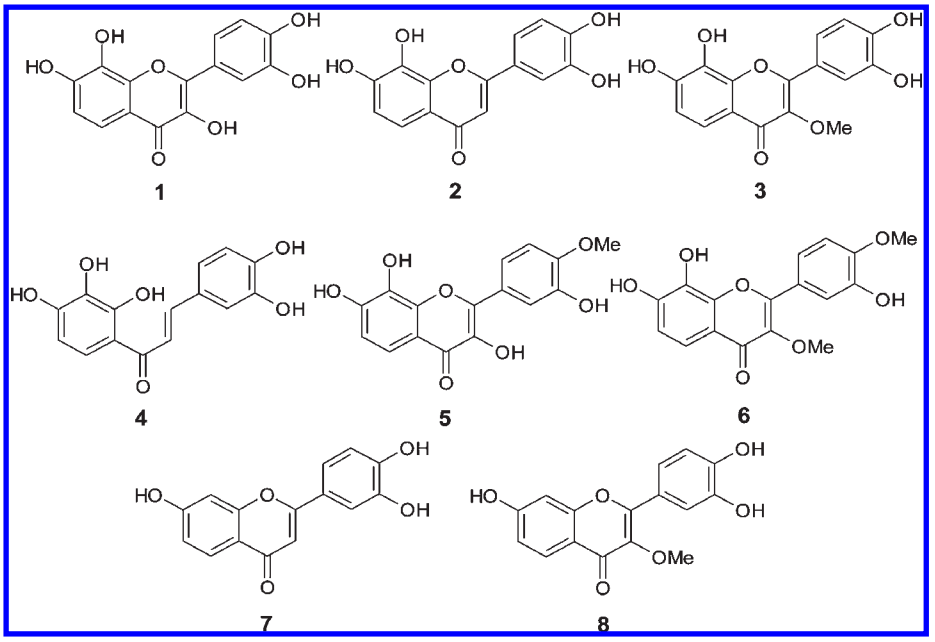

Figure 1. Flavonoids isolated from EA5, EA6, EA7, and EA8 subfractions of *A. confusa* heartwood extract: **1**, melanoxetin; **2**, 7,8,3′,4′-tetrahydroxyflavone; **3**, transilitin; **4**, okanin; **5**, 3,7,8,3′-tetrahydroxy-4′-methoxyflavone; **6**, 7,8,3′-trihydroxy-3,4′-dimethoxyflavone; **7**, 7,3′,4′-trihydroxyflavone; **8**, 7,3′,4′-trihydroxy-3-methoxyflavone.

fraction were separated and purified by semipreparative HPLC on a model PU-980 pump (Jasco) equipped with an MD-910 photodiode array detector (Jasco) and a 250 mm × 10.0 mm i.d., 5 μm Luna RP-18 column (Phenomenex, Torrance, CA). The mobile phase was solvent A, 100% MeOH; and solvent B, ultrapure water. Elution conditions were 0−10 min of 35−45% A to B (linear gradient); 10−25 min of 45−50% A to B (linear gradient); 25−30 min of 50−100% A to B (linear gradient) at a flow rate of 4 mL/min. ESIMS data were collected using a Finnigan MAT-95S mass spectrometer, and NMR spectra were recorded by a Bruker Avance 500 MHz FT-NMR spectrometer. The structures of compounds **1**−**8** (as shown in **Figure 1**) were identified by ESIMS and NMR, and all spectral data were consistent with those reported in the literature (*18*, *20*, *33*−*35*).

Determination of XOD-Inhibitory Activity. Measurement of XOD-inhibitory activity was carried out according to the method of Kong et al. (*8*) with slight modifications. First, 798 μL of 0.1 unit xanthin oxidase (XOD) in buffer (200 mM sodium pyrophosphate/HCl, pH 7.5) and 2 μL of the test extracts or compounds in DMSO were mixed at 37 °C for 5 min. The reaction was started by adding 200 μL of 0.6 mM xanthine in double-distilled water to the mixture. The reaction mixture was incubated at ambient temperature, and the absorbance at 295 nm was determined every 1 min up to 8 min. Allopurinol was used as a positive control. Three replicates were made for each test sample. The percent inhibition ratio (percent) was calculated according to the following equation: % inhibition = [(rate of control reaction − rate of sample reaction)/rate of control reaction] × 100.

Lineweaver−Burk Plots. The type of inhibition involved was determined by the Lineweaver−Burk plot. First, 798 μL of 0.1 unit XOD in buffer (200 mM sodium pyrophosphate/HCl, pH 7.5) and 2 μL of compounds (1.5625, 6.25, 12.5, and 25 μM) in DMSO were mixed in 96-well microplates at 37 °C for 5 min. The reaction was started by adding substrate concentrations of 0.1, 0.2, 0.3, 0.4, and 0.6 mM, respectively in double-distilled water to the mixture. The reaction mixture was incubated at ambient temperature, and the absorbance at 295 nm was determined every 1 min up to 8 min using the ELISA reader. All data obtained from the enzyme assays were plotted using Excel (Microsoft Office 2007, Microsoft, Taiwan).

Statistical Analyses. All results were expressed as mean ± SD (n = 3). The significance of difference was calculated by using the Scheffe test, and values of < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

XOD-Inhibitory Activity of *A. confusa* Extracts. Heartwood, bark, twig, flower, and leaf crude extracts of *A. confusa* were assayed for XOD-inhibitory activity at 100, 50, and 25 μg/mL, and the results are shown in **Figure 2**. The XOD-inhibitory activity of crude extracts from *A. confusa* showed a dose-dependent manner. In the presence of the 100 μg/mL test sample, the XOD inhibition of *A. confusa* decreased in the following order: heartwood extract (80%) > bark extract (59%) > flower extract (46%) = leaf extract (45%) > twig extract (39%). Allopurinol (a positive control), being clinically used as a XOD inhibitory drug, showed 88% inhibition on XOD at a concentration of 10 μg/mL. Among five ethanolic extracts from different plant parts of *A. confusa*, heartwood extract exhibited the best XOD-inhibitory activity. The addition of 100, 50, and 25 μg/mL heartwood extracts resulted in increasing XOD-inhibitory activity, by 80, 75, and 60%, respectively. According to the results reported by Chang et al. (*23*), *A. confusa* heartwood extract dose-dependently inhibited superoxide radicals induced by hypoxanthine−xanthine oxidase, with an IC_{50} value of 1.8 μg/mL. The result indicates that the extract of *A. confusa* heartwood has a good performance of inhibiting superoxide free radicals produced by hypoxanthine and xanthine oxidase.

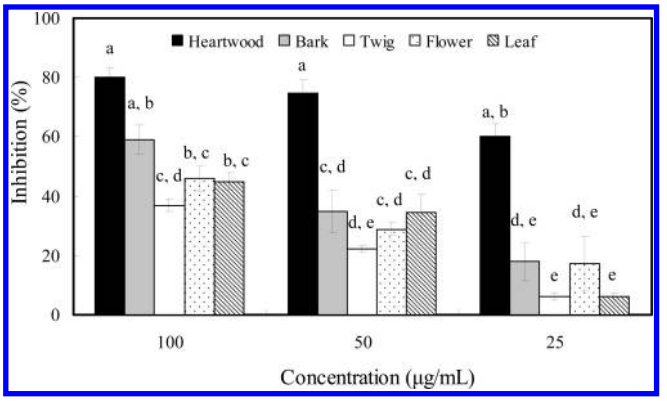

Figure 2. XOD-inhibitory activity of *A. confusa* extracts. The bars marked by different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

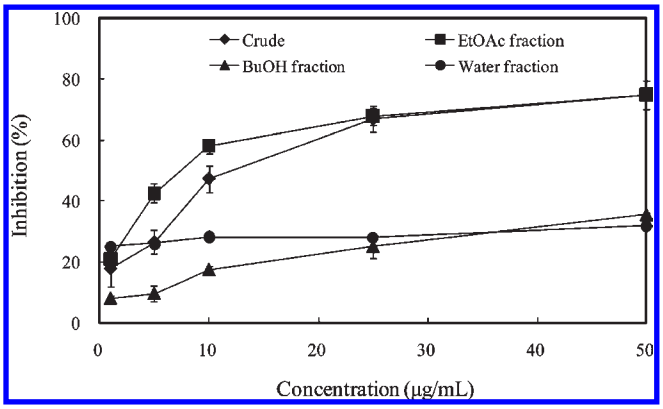

Figure 3. XOD-inhibitory activity of *A. confusa* heartwood extract and its derived fractions.

Moerman (*36*) found that the inner bark of *Populus balsamifera*, being used historically to treat gout, induced 51% XOD inhibition at a concentration of 100 μg/mL. Foster and Duke (*37*) reported that the leaves of *Ledum groenlandicum*, used by the Cree and more broadly to treat gout, showed 50% inhibition at a concentration of 100 μg/mL. Owen and Johns (*7*) also found that the average inhibitory activity of plant extracts from 26 plant species belonging to 18 families traditionally used for the treatment of gout and related symptoms by indigenous people of northeastern America was 25% at 100 μg/mL. Kong et al. (*8*) reported that water extracts of *Cinnamomum cassia* and *Morus alba* barks, Chinese medicinal inhibitors of XOD, showed 31 and 14% inhibition, respectively, at a concentration of 50 μg/mL. Comparisons of the aforesaid results indicated that heartwood and bark extracts of *A. confusa* have an excellent XOD-inhibitory activity, especially the heartwood extract.

XOD-Inhibitory Activity of Ethanolic Extract from *A. confusa* Heartwood. As shown in **Figure 3**, the XOD-inhibitory activity of crude extract and its derived fractions from *A. confusa* heartwood, including the soluble fractions of EtOAc, BuOH, and water, revealed a dose-dependent manner. In the presence of the 50 μg/mL test sample, the XOD inhibition of heartwood extract and its derived fractions decreased in the following order: EtOAc fraction (75%) > crude extract (74%) > BuOH fraction (35%) > water fraction (32%). Of these, the EtOAc fraction showed the strongest activity. The concentration required to inhibit 50% xanthine oxidase (IC_{50}) was determined from the results of a series of concentrations tested. A lower IC_{50} value corresponds to a greater inhibitory activity. The IC_{50} values of the crude extract, EtOAc fraction, BuOH fraction, and water fraction were 11.7, 7.8, >100, and >100 μg/mL, respectively. The IC_{50} value of allopurinol was 1.2 μg/mL. According to the results reported by González et al. (*38*), the methanolic extracts of the twig from *C. cassia*, the flower from *Chrysanthemum indicum*, and the leaf from *Lycopus europaeus* were found to possess significant XOD-inhibitory activity, with IC_{50} values of 18, 22, and 26 μg/mL, respectively. Furthermore, comparison with the results obtained by González et al. (*38*) shows that there are abundant XOD-inhibitory phytochemicals present in the heartwood extract of *A. confusa*, especially in the EtOAc fraction.

Bioassay-Guided Fractionation of *A. confusa* Heartwood Extract. The EtOAc fraction of *A. confusa* heartwood extract showed a stronger XOD-inhibitory activity than the other fractions, indicating that the XOD-inhibitory activity of *A. confusa* heartwood extract had been efficiently enriched in the EtOAc fraction. Moreover, the activity of the EtOAc fraction showed to be about 20-fold more effective than that of BuOH and H_2O fractions. Thus, its constituent characteristics were further investigated in this study. The EtOAc fraction was first subjected to an RP-18 column chromatography with a stepwise gradient of MeOH−H_2O and derived into 10 subfractions. Of these, subfractions EA5, EA6, EA7, and EA8 possessed a high XOD-inhibitory activity as shown in **Table 1**. **Figure 4** shows the HPLC chromatograms of EA5, EA6, EA7, and EA8. These subfractions were finally subjected to preparative HPLC. Consequently, eight major constituents, as shown in **Figure 1**, were isolated and identified as melanoxetin (**1**) (*26*), 7,8,3′,4′-tetrahydroxyflavone (**2**) (*18*), transilitin (**3**) (*34*), okanin (**4**) (*35*), 3,7,8,3′-tetrahydroxy-4′-methoxyflavone (**5**) (*18*), 7,8,3′-trihydroxy-3,4′-dimethoxyflavone (**6**) (*18*), 7,3′,4′-trihydroxyflavone (**7**) (*18*), and 7,3′,4′-trihydroxy-3-methoxyflavone (**8**) (*18*).

Table 1. Elution Solvent, Collected Weight, and XOD-Inhibitory Activity (5 μg/mL) of 10 Subfractions Divided from the EtOAc Fraction

subfraction	mobile phase[a] (%)	weight (g)	inhibition[b] (%)
EA1	10−20	13.9	35.9 ± 1.3d
EA2	30	3.1	39.4 ± 0.5d
EA3	30	1.7	49.4 ± 1.6c
EA4	30−40	2.4	64.1 ± 0.7b
EA5	40−50	6.1	78.7 ± 1.0a
EA6	50−60	3.3	77.8 ± 0.9a
EA7	60	1.1	80.5 ± 1.5a
EA8	70−80	0.9	74.8 ± 2.3a
EA9	80−100	0.5	37.9 ± 1.3d
EA10	THF	6.4	39.3 ± 1.1d
total weight (g)		39.4	
recovery (%)		93.9	

[a] The ratio of MeOH/H_2O (v/v). The inhibition of allopurinol was 78.2%. [b] Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe test.

Comparison of Phytochemicals of *A. confusa* Heartwood Extracts with Well-Known XOD Inhibitor. The XOD-inhibitory effects of phytochemicals isolated from the EtOAc fraction of *A. confusa* heartwood extract were compared with those of allopurinol, which is clinically used as a drug for XOD inhibition. The tested constituents inhibited XOD in a concentration-dependent manner as shown in **Figure 5**. The IC_{50} values indicated that okanin showed the strongest XOD-inhibitory effect (IC_{50} value of 0.076 μM), followed by melanoxetin (0.274 μM), allopurinol (4.784 μM), and 7,8,3′,4′-tetrahydroxyflavone (10.488 μM). Furthermore, the IC_{50} values of the other constituents (3,7,8,3′-tetrahydroxy-4′-methoxyflavone, 7,8,3′-trihydroxy-3,4′-dimethoxyflavone, 7,3′,4′-trihydroxyflavone, and 7,3′,4′-trihydroxy-3-methoxyflavone) were >50 μM. Wu et al. (*18*) reported that okanin and melanoxetin showed the best NO-inhibitory effects. Harrison (*39*) reported that XOD is capable of producing reactive nitrogen species in addition to the reactive oxygen species, and those two can combine to form nitrile. Thus, the fact that okanin and melanoxetin are excellent inhibitors of NO may be due to the XOD-inhibitory effects. Unno et al. (*40*) found that the IC_{50} values of valoneic acid dilactone and ellagic acid, isolated from the aqueous extracts of the *Lagerstroemia speciosa* leaves, were 2.5 and 71.5 μM, respectively. Lin et al. (*41*) reported that esculetin (IC_{50} value of 10.84 μM) showed the best XOD-inhibitory effects among coumarin derivatives, followed by 4-methylesculetin (IC_{50} value of 75.79 μM) and 4-hydroxycoumarins (IC_{50} value of 78.13 μM). Chang et al. (*42*) also reported that caffeic acid, ferulic acid, isoferulic acid, *p*-coumaric acid, and *p*-methoxycinnamic acid were also competitive inhibitors with IC_{50} values of 65.6, 93.9, 143.2, 96.9, and 184.0 μM, respectively. Comparisons of these data revealed that okanin, melanoxetin, and 7,8,3′,4′-tetrahydroxyflavone examined in this study exhibited great XOD-inhibitory activity. The present study revealed that okanin and melanoxetin showed excellent performances in

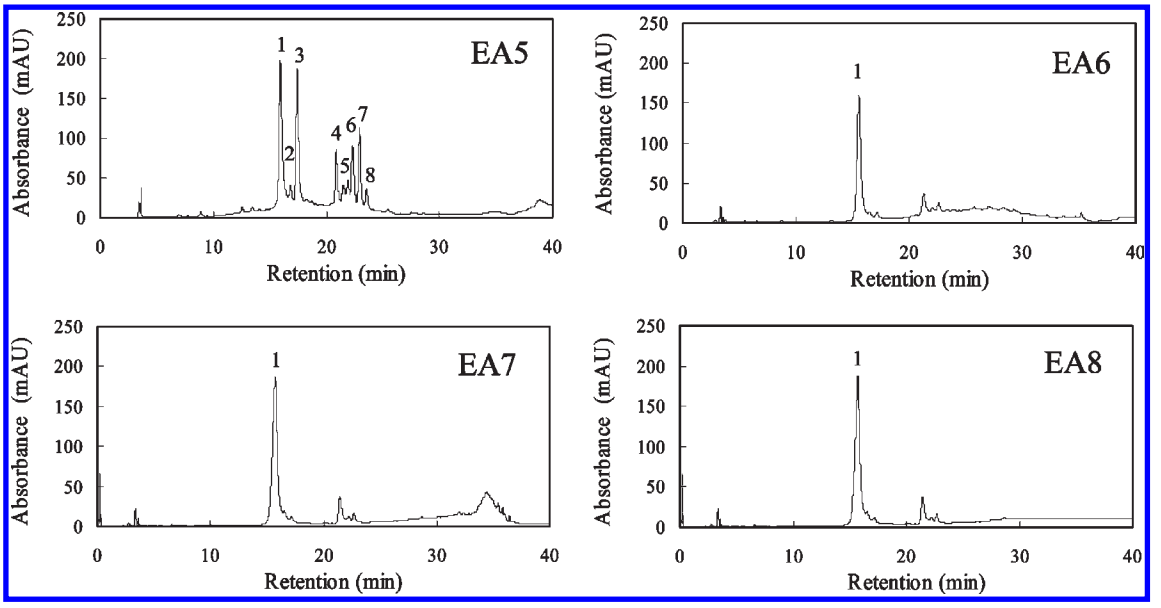

Figure 4. HPLC chromatograms of EA5, EA6, EA7, and EA8 subfractions from the EtOAc fraction of *A. confusa* heartwood extract.

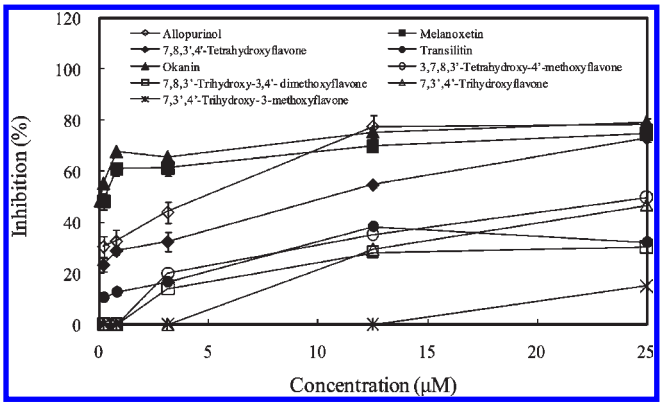

Figure 5. XOD-inhibitory activity of phytochemicals from the EtOAc fraction of *A. confusa* heartwood extract.

XOD inhibition and that their inhibitory activities are better than that of allopurinol.

Structure−Activity Relationships of Flavonoids from *A. confusa* Heartwood. The structure−activity relationships of flavonoids (compounds **1**−**8**) according to their XOD-inhibitory activities were also illustrated in this study. Surprisingly, with the same number of hydroxyl groups in the A- and B-rings, chalcone (okanin) had a greater XOD-inhibitory activity than flavone (7,8,3′,4′-tetrahydroxyflavone). Okanin, which has only two rings connected by a linear chain, displayed a very high affinity to the xanthine oxidase. Nagao et al. (*43*) reported that chalcone (phloretin) had a greater XOD-inhibitory activity than flavone (luteolin). Among the flavones obtained, the decreasing order of XOD-inhibitory activity was as follows: melanoxetin (**1**) > 7,8,3′,4′-tetrahydroxyflavone (**2**) > transilitin (**3**); 3,7,8,3′-tetrahydroxy-4′-methoxyflavone (**5**) > 7,8,3′-trihydroxy-3,4′-dimethoxyflavone (**6**); 7,3′,4′-trihydroxyflavone (**7**) > 7,3′,4′-trihydroxy-3-methoxyflavone (**8**). It revealed that, with the same number of hydroxyl groups in the A- and B-rings, the hydroxyl group at the C3 position in the C-ring had the best XOD-inhibitory activity, followed by the hydrogen and methoxyl groups at the same position. Similarly, Mo et al. (*44*) also showed that the XOD-inhibitory activity of kaempferol (C3−OH) was greater than that of apigenin (C3−H). Thus, these data revealed that the hydroxyl group at the C3 position enhanced the XOD-inhibitory activity. Moreover, for flavonoids with different numbers of hydroxyl groups on the A-ring, the decreasing order of activity found was as follows: 7,8,3′,4′-tetrahydroxyflavone (**2**) > 7,3′,4′-trihydroxyflavone (**7**); transilitin (**3**) > 7,3′,4′-trihydroxy-3-methoxyflavone (**8**). The number of hydroxyl groups on the A-ring was positively related to their inhibitory activities. Similarly, results obtained by Cotelle et al. (*45*) also showed that flavones with a 5,7-dihydroxyl substitution might have better XOD-inhibitory activity than those with only a 5-substitution among 24 flavones with various −OH or $-OCH_3$ substitutions on A- and B-rings tested. In addition, Nagao et al. (*43*) concluded that at least two hydroxyl groups on the A-ring were required for XOD-inhibitory activities of flavonoids. Comparisons of the aforesaid data revealed that an increase in the number of hydroxyl groups on the A-ring led to higher XOD-inhibitory activities. In addition, with the same structure of A- and C-rings, the hydroxyl group at the C4′ position in the B-ring had better effectiveness than the methoxyl group at the same position, such as melanoxetin (**1**) > 3,7,8,3′-tetrahydroxy-4′-methoxyflavone (**5**); transilitin (**3**) > 7,8,3′-trihydroxy-3,4′-dimethoxyflavone (**6**). Furthermore, our findings are also in agreement with the results reported by de Groot and Rauen (*46*), who claimed that flavonols possessing 3′,4′-OH features are effective inhibitors of xanthine oxidase. Selloum et al. (*47*) also reported that the most potent inhibitor of uric acid production was quercetin with a cathechol moiety on the B-ring (3′,4′-OH), followed by the monohydroxylated kaempferol (4′-OH), whereas myricetin with a pyrogallol moiety (3′,4′, 5′-OH) was less active.

Mode of Inhibition of Active Phytochemicals. The kinetics and molecular mechanisms of XOD-inhibitory activity by okanin, melanoxetin, 7,8,3′,4′-tetrahydroxyflavone, and allopurinol are shown in **Figure 6**. Lineweaver−Burk plots led us to support the view that XOD inhibitions of melanoxetin and 7,8,3′,4′-tetrahydroxyflavone are in a competitive mode. As for allopurinol, being clinically used as a XOD-inhibitory drug, it also displays a competitive mode. The competitive inhibition of allopurinol is consistent with the result obtained by Nguyen et al. (*48*). Furthermore, Lineweaver−Burk plots revealed that okanin with high XOD inhibition did so through a noncompetitive inhibition with xanthine as a substrate. This noncompetitive okanin inhibition is consistent with the mechanism of valoneic acid dilactone and ellagic acid reported by Unno et al. (*40*).

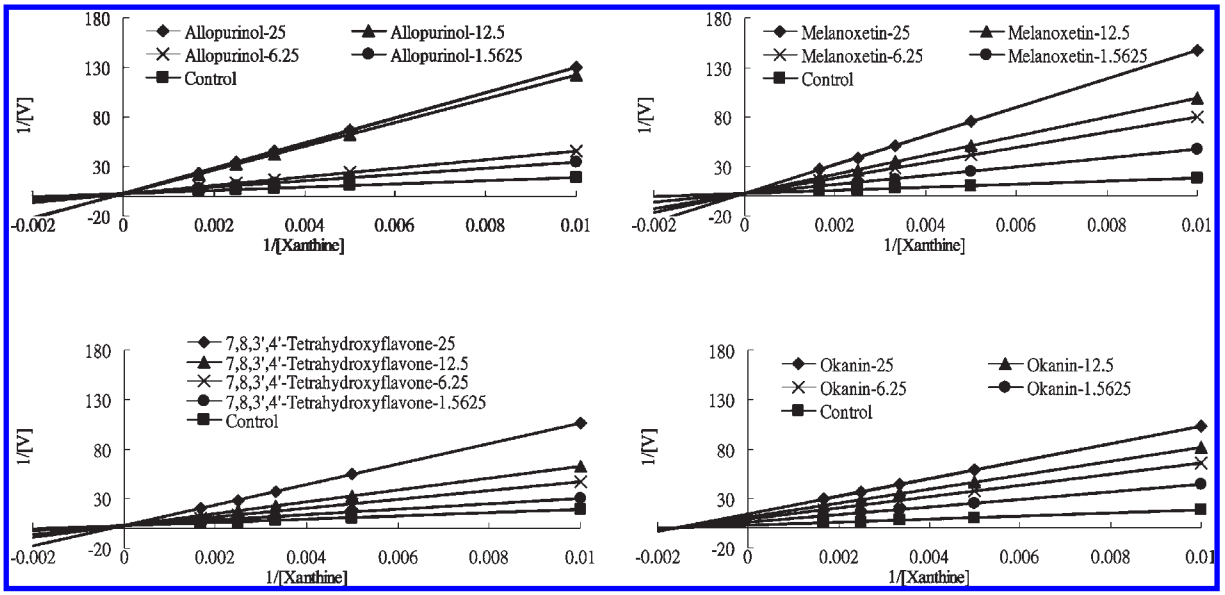

Figure 6. Kinetic assays of xanthine oxidase inhibition by melanoxetin, 7,8,3′,4′-tetrahydroxyflavone, and okanin. A Lineweaver—Burk double-reciprocal plot was constructed for the inhibition of xanthine oxidase by melanoxetin, 7,8,3′,4′-tetrahydroxyflavone, and okanin. The plot is expressed as 1/velocity versus 1/xanthine (mM^{-1}) without or with an inhibitor in the reaction solution.

In conclusion, heartwood extract of *A. confusa* and its EtOAc fraction exhibited a strong XOD-inhibitory action. Among eight specific phytochemicals isolated from the EtOAc fraction of *A. confusa* heartwood extract, it was proven that okanin and melanoxetin showed excellent XOD-inhibitory performance in noncompetitive and competitive modes, respectively, and their inhibitory activity is better than that of allopurinol. It is of great interest that the XOD-inhibitory activity of okanin and melanoxetin could be equal to that of a clinically used drug. Thus, *A. confusa* heartwood extract or its derived phytochemicals have great potential in preventing hyperuricemia caused by xanthine oxidase. However, at this juncture we have no definite information that the ethanolic extract from *A. confusa* heartwood possesses hyperuricemic activity in vivo. Future studies should focus on employing modern medical chemical techniques to modify the structures of certain purified plant ingredients into better agents with high efficacy and activity. In addition, in vivo pharmacological studies should also be made.

ACKNOWLEDGMENT

We thank Shou-Ling Huang (Department of Chemistry, National Taiwan University) for NMR spectral analyses.

LITERATURE CITED

(1) Harris, M. D.; Siegel, L. B.; Alloway, J. A. Gout and hyperuricemia. *Am. Fam. Physician* **1999**, 59, 925–934.

(2) Kramer, H. M.; Curhan, G. The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988−1994. *Am. J. Kidney Dis.* **2002**, 40, 37–42.

(3) Tomita, M.; Mizuno, S.; Yamanaka, H.; Hosoda, Y.; Sakuma, K.; Matuoka, Y.; Odaka, M.; Yamaguchi, M.; Yosida, H.; Morisawa, H.; Murayama, T. Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. *J. Epidemiol.* **2000**, 10, 403–409.

(4) Kelley, W. N. Antihyperuricemic drugs. In *Textbook of Rheumatology*; Kelley, W. N., Harris, E. D., Ruddy, S., Sledge, C. B., Eds.; W. B. Saunders: Philadelphia, PA, 1991; pp 862−877.

(5) Star, V. L.; Hochberg, M. Prevention and management of gout. *Drug* **1993**, 45, 212–222.

(6) Wortmann, R. L. Management of hyperuricemia. In *Arthritis and Allied Conditions*, 12th ed.; McCarthy, D. J., Koopman, W. J., Eds.; Lea and Febiger: Philadelphia, PA, 1993; pp 1807−1818.

(7) Owen, P. L.; Johns, T. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *J. Ethnopharmacol.* **1999**, 64, 149–160.

(8) Kong, L. D.; Cai, Y.; Huang, W. W.; Cheng, C. H. K.; Tan, R. X. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *J. Ethnopharmacol.* **2000**, 73, 199–207.

(9) Sweeney, A. P.; Wyllie, S. G.; Shalliker, R. A.; Markham, J. L. Xanthine oxidase inhibitory activity of selected Australian native plants. *J. Ethnopharmacol.* **2001**, 75, 273–277.

(10) Seigler, D. S. Phytochemistry of *Acacia*—sensu lato. *Biochem. Syst. Ecol.* **2003**, 31, 845–873.

(11) Wadood, A.; Wadood, N.; Wahid, N.; Shah, S. A. Effects of *Acacia arabica* and *Caralluma edulis* on blood glucose levels of normal and alloxan diabetic rabbits. *J. Pak. Med. Assoc.* **1989**, 39, 208–212.

(12) Dafallah, A. A.; Al-Mustafa, Z. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. *Am. J. Chinese Med.* **1996**, 24, 263–269.

(13) Ghosh, N. K.; Babu, S. P.; Sukul, N. C.; Ito, A. Cestocidal activity of *Acacia auriculiformis*. *J. Helminthol.* **1996**, 70, 171–172.

(14) Sotohy, S. A.; Sayed, A. N.; Ahmed, M. M. Effect of tannin-rich plant (*Acacia nilotica*) on some nutritional and bacteriological parameters in goats. *Dtsch. Tierarztl. Woch.* **1997**, 104, 432–435.

(15) Amos, S.; Akah, P. A.; Odukwe, C. J.; Gamaniel, K. S.; Wambede, C. The pharmacological effects of an aqueous extract from *Acacia nilotica* seeds. *Phytother. Res.* **1999**, 13, 683–685.

(16) Gilani, A. H.; Shaheen, F.; Zaman, M.; Anbaz, K. H.; Shah, B. H.; Akhtar, M. S. Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. *Phytother. Res.* **1999**, 13, 665–669.

(17) Hussein, G.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Kakiuchi, N.; Shimotohno, K. Inhibitory effects of sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytother. Res.* **2000**, 14, 510–516.

(18) Wu, J. H.; Tung, Y. T.; Chien, S. C.; Wang, S. Y.; Kuo, Y. H.; Shyur, L. F.; Chang, S. T. Effect of phytochemicals from the heartwood of *Acacia confusa* on inflammatory mediator production. *J. Agric. Food Chem.* **2008**, 56, 1567–1573.

(19) Hammiche, V.; Maiza, K. J. Traditional medicine in Central Sahara: pharmacopoeia of Tassili N'ajjer. *J. Ethnopharmacol.* **2006**, 105, 358–367.

(20) Wu, J. H.; Tung, Y. T.; Wang, S. Y.; Shyur, L. F.; Kuo, Y. H.; Chang, S. T. Phenolic antioxidants from the heartwood of *Acacia confusa*. *J. Agric. Food Chem.* **2005**, 53, 5917–5921.

(21) Kan, W. S. Leguminosae. In *Manual of Medicinal Plants in Taiwan*; Kan, W. S., Ed.; National Research Institute of Chinese Medicine: Taipei, Taiwan, 1978; pp 239−240.
(22) Wu, J. H.; Huang, C. Y.; Tung, Y. T.; Chang, S. T. Online RP-HPLC-DPPH screening method for detection of radical-scavenging phytochemicals from flowers of *Acacia confusa*. *J. Agric. Food Chem*. **2008**, 56, 328–332.
(23) Chang, S. T.; Wu, J. H.; Wang, S. Y.; Kang, P. L.; Yang, N. S.; Shyur, L. F. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *J. Agric. Food Chem*. **2001**, 49, 3420–3424.
(24) Tung, Y. T.; Wu, J. H.; Huang, C. C.; Peng, H. C.; Chen, Y. L.; Yang, S. C.; Chang, S. T. Protective effect of *Acacia confusa* bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats. *Food Chem. Toxicol*. **2009**, 47, 1385–1392.
(25) Tung, Y. T.; Wu, J. H.; Hsieh, C. Y.; Chen, P. S.; Chang, S. T. Free radical-scavenging phytochemicals of hot water extracts of *Acacia confusa* leaves detected by on-line screening method. *Food Chem*. **2009**, 115, 1019–1024.
(26) Tung, Y. T.; Wu, J. H.; Huang, C. Y.; Kuo, Y. H.; Chang, S. T. Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark. *Bioresour. Technol*. **2009**, 100, 509–514.
(27) Tung, Y. T.; Wu, J. H.; Kuo, Y. H.; Chang, S. T. Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. *Bioresour. Technol*. **2007**, 98, 1120–1123.
(28) Lee, T. H.; Qiu, F.; Waller, G. R.; Chou, C. H. Three new flavonol galloylglycosides from leaves of *Acacia confusa*. *J. Nat. Prod*. **2000**, 63, 710–712.
(29) Lee, T. H.; Liu, D. Z.; Hsu, F. L.; Wu, W. C.; Hou, W. C. Structure−activity relationships of five myricetin galloylglycosides from leaves of *Acacia confusa*. *Bot. Stud*. **2006**, 47, 37–43.
(30) Chang, W. S.; Lee, Y. J.; Lu, F. J.; Chiang, H. C. Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Res*. **1993**, 13, 2165–2170.
(31) Havsteen, B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol*. **1983**, 32, 1141–1148.
(32) Brandi, M. L. Flavonoids: biochemical effects and therapeutic applications. *Bone Miner*. **1992**, 19, S3–S14.
(33) Miyazawa, M.; Oshima, T.; Koshio, K.; Itsuzaki, Y.; Anzai, J. Tyrosinase inhibitor from black rice bran. *J. Agric. Food Chem*. **2003**, 51, 6953–6956.
(34) Kim, H. K.; Cheon, B. S.; Kim, Y. H.; Kim, S. Y.; Kim, H. P. Effects of naturally flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure−activity relationships. *Biochem. Pharmacol*. **1999**, 58, 759–765.
(35) Wadsworth, T. L.; Koop, D. R. Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. *Biochem. Pharmacol*. **1999**, 57, 941–949.
(36) Moerman, D. E. *Medicinal Plants of Native America*; University of Michigan Museum of Anthropology Technical Reports; Ann Arbor, MI, 1986; p 910.
(37) Foster, S.; Duke, J. A. *A Field Guide to Medicinal Plants*; Houghton Mifflin: Boston, MA, 1990.
(38) González, A. G.; Bazzocchi, I. L.; Moujir, L.; Ravelo, A. G.; Correa, M. D.; Gupta, M. P. Xanthine oxidase inhibitory activity of some Panamanian plants from Celastraceae and Lamiaceae. *J. Ethnopharmacol*. **1995**, 46, 25–29.
(39) Harrison, R. Physiological roles of xanthine oxidoreductase. *Drug Metab. Rev*. **2004**, 36, 363–375.
(40) Unno, T.; Sugimoto, A.; Kakuda, T. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia speciosa* (L.) Pers. *J. Ethnopharmacol*. **2004**, 93, 391–395.
(41) Lin, H. C.; Tsai, S. H.; Chen, C. S.; Chang, Y. C.; Lee, C. M.; Lai, Z. Y.; Lin, C. M. Structure−activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities. *Biochem. Pharmacol*. **2008**, 75, 1416–1425.
(42) Chang, Y. C.; Lee, F. W.; Chen, C. S.; Huang, S. T.; Tsai, S. H.; Huang, S. H.; Lin, C. M. Structure−activity relationship of C6-C3 phenylpropanoids on xanthine oxidase-inhibiting and free radical-scavenging activities. *Free Radical Biol. Med*. **2007**, 43, 1541–1551.
(43) Nagao, A.; Seki, M.; Kobayashi, H. Inhibition of xanthine oxidase by flavonoids. *Biosci., Biotechnol., Biochem*. **1999**, 63, 1787–1790.
(44) Mo, S.; Zhou, F.; Lv, Y.; Hu, Q.; Zhang, D.; Kong, L. Hypouricemic action of selected flavonoids in mice: structure−activity relationships. *Biol. Pharm. Bull*. **2007**, 30, 1551–1556.
(45) Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. M. Antioxidant properties of hydroxy-flavones. *Free Radical Biol. Med*. **1996**, 20, 35–43.
(46) de Groot, H.; Rauen, U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundam. Clin. Pharmacol*. **1998**, 12, 249–255.
(47) Selloum, L.; Reichl, S.; Müller, M.; Sebihi, L.; Arnhold, J. Effects of flavonols on the generation of superoxide anion radicals by xanthine oxidase and stimulated neutrophils. *Arch. Biochem. Biophys*. **2001**, 395, 49–56.
(48) Nguyen, M. T.; Awale, S.; Tezuka, Y.; Ueda, J. Y.; Tran, Q.; Kadota, S. A. Xanthine oxidase inhibitors from the flowers of *Chrysanthemum sinense*. *Planta Med*. **2006**, 72, 46–51.

Received May 6, 2009. Revised manuscript received September 23, 2009. Accepted September 23, 2009. We thank the Taiwan Forestry Bureau of Council of Agriculture for financial support.