

Antiallergic Activity of Aqueous Extracts and Constituents of *Taxus yunnanensis*

Junko KOYAMA,*^a Izumi MORITA,^a Norihiro KOBAYASHI,^a Keiichi HIRAI,^b Eriko SIMAMURA,^b Takahiro NOBUKAWA,^c and Shigetoshi KADOTA^d

^aKobe Pharmaceutical University; Higashinada-ku, Kobe 658–8558, Japan; ^bDepartment of Molecular and Cell Structural Science, Kanazawa Medical University; ^cMedical Research Institute, Kanazawa Medical University; Uchinada, Ishikawa 920–0293, Japan; and ^dInstitute of Natural Medicine, University of Toyama; Sugitani, Toyama 930–0194, Japan. Received May 26, 2006; accepted August 21, 2006

The H₂O, H₂O/MeOH (1 : 1) extracts from the wood of *Taxus yunnanensis* showed a remarkable inhibitory effect on induced histamine release from the human basophilic cell line, KU812. The eleven constituents purified from the wood extracts of *Taxus yunnanensis* were tested by an *in vitro* histamine release inhibition assay. Among them, secoisolariciresinol and taxiresinol were found to show inhibitory activities. A new neolignan, 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol, was isolated from the wood of *Taxus yunnanensis*.

Key words *Taxus yunnanensis*; lignan; antiallergy; neolignan; taxane-type diterpene

Allergies have become big problem around the world. Two of the most common types are pollen allergies and atopic dermatitis. In the past fifty years, these problems have dramatically increased. Though traditional Chinese medicines lack the definition of immunology, patients suffering from allergic diseases are treated with orally administered traditional medicinal drugs. This indicates that medicinal plants used for the allergy treatment may contain antiallergic compounds. The lignans and flavonoids of polyphenol are widely distributed in the plant kingdom, and have been recognized to have various biological activities including antiallergic actions, and various *in vitro* and *in vivo* studies of the antiallergic effect have been reported.^{1–4}

Taxus yunnanensis CHENG *et al.* L. K. FU, which belongs to the plant family Taxaceae is widely distributed in the Yunnan Province of the People's Republic of China and commonly known as "Yunnan Hongdoushan". The wood of *Taxus yunnanensis* has been used in traditional Chinese medicine by several ethnic societies in the Yunnan Province for the treatment of kidney problems and diabetic conditions.⁵ During the course of our characterization studies on the bioactive constituents of Chinese natural medicines, the H₂O extract and H₂O/MeOH extract from the wood of *Taxus yunnanensis* were found to show a remarkable inhibitory effect on induced histamine release from the human basophilic cell line, KU812.⁶ In the present study, one new neolignan, 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol, was isolated together with ten known compounds (three phenols, five lignans, two taxane-type diterpenes) from the wood of *Taxus yunnanensis*. In addition, we examined the inhibitory effects of these isolates using an *in vitro* histamine release test.

MATERIAL AND METHODS

General Experimental Procedures Five hundred megahertz for the ¹H-NMR and 125 MHz for the ¹³C-NMR, CDCl₃, TMS as the int. standard; CC: silica gel (Mallinckrodt, AR, U.S.A.) in amounts equivalent to 50 times the extracts; TLC: silica gel (Merck, 60F₂₅₄; thickness, 0.5 mm).

The optical rotation was measured using a spectrophotometer in CHCl₃.

Plant Material The bark and wood of *Taxus yunnanensis* CHENG *et al.* L. K. FU was collected from Mt. Laojunshan at an altitude of 3800 m, 100 km west of Lijiang City in the Yunnan Province of the People's Republic of China in 2000, and brought to Japan with the permission of the State Pharmaceutical Administration of the People's Republic of China. The identification was made by one of the authors (T. Nobukawa). A voucher sample (TMPW 21495) is preserved in the Museum for Material and Medica, University of Toyama, Japan.

Extraction and Isolation The wood of *Taxus yunnanensis* was chopped into small pieces and crushed into a powder. The dried wood powder (750 g) was extracted with H₂O (31×3) under reflux for 45 min to yield a H₂O extract (51.0 g). The residue was further extracted with H₂O/MeOH (1 : 1) (31×3) and MeOH (31×3) to give the H₂O/MeOH extract (35.1 g) and the MeOH extract (7.5 g), respectively. The extraction of the bark powder (20 g) was worked up in a similar manner as described above. The H₂O extract was 2.2 g, the H₂O/MeOH extract 1.4 g, and the MeOH extract 0.41 g, respectively. Compounds **1–10** were isolated in the same manner as previously reported.⁷ Compound **11** had the same *R_f* value as secoisolariciresinol (*R_f*=0.36 in 10% MeOH/CHCl₃, 3.5 mg).

2-[2-Hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**11**): White amorphous solid. IR (CHCl₃) cm⁻¹: 3620, 3540, 1606, 1516. ¹H-NMR (CDCl₃) δ: 1.91 (2H, m, 8'-CH₂), 2.66 (2H, t, *J*=7.5 Hz, 7'-CH₂), 3.59 (1H, m, 8-H), 3.68 (2H, t, *J*=6.5 Hz, 9'-CH₂), 3.85 (3H, s, 3-OCH₃), 3.87 (3H, s, 3'-OCH₃), 3.89 (1H, dd, *J*=11, 5 Hz, 9-CH), 3.95 (1H, dd, *J*=11, 6 Hz, 9-CH), 5.53 (1H, d, *J*=7.5 Hz, 7-H), 6.66 (1H, s, 4'-H), 6.67 (1H, s, 6'-H), 6.86 (1H, d, *J*=8.5 Hz, 5-H), 6.90 (1H, dd, *J*=8.5, 2 Hz, 6-H), 6.93 (1H, d, *J*=2 Hz, 2-H). ¹³C-NMR (CDCl₃) δ: 31.96, 34.55, 53.77, 55.96, 55.99, 62.24, 63.88, 87.84, 108.83, 112.42, 114.29, 115.98, 119.37, 127.77, 133.10, 135.35, 144.14, 145.58, 146.50, 146.64. MS *m/z*: 360.1572 (Calcd for C₂₀H₂₄O₆: 360.1571, M⁺-H₂O).

* To whom correspondence should be addressed. e-mail: j-koyama@kobepharma-u.ac.jp

$[\alpha]_D + 12.1^\circ$ ($c=0.215$, CHCl_3).

Cell Culture and Stimulation The KU812 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan) and maintained in RPMI-1640 (Gibco, NY, U.S.A.) supplemented with 10% fetal bovine serum (MP Biomedicals, Eshwege, Germany), 100 units/ml penicillin G, 100 $\mu\text{g/ml}$ streptomycin, and 10 mM HEPES buffer. The KU812 cells were cultured at 37 °C in a humidified 5% CO_2 incubator. IL-4 was added to the culture medium to a final concentration of 1 ng/ml. The cells were inoculated and maintained for 20 d. The medium changes were done every 3–4 d.

Histamine Release Measurement The antiallergic effect of the test samples on the induced histamine release from the human basophilic cell line, KU812 was evaluated by the method of Tachibana *et al.* Briefly, KU812 cells (1.5×10^6 cells) were washed and resuspended in a Tyrode buffer. Five millimolar calcium ionophore A23187 (Wako, Osaka, Japan) was incubated with the test compounds (1.5 $\mu\text{g/ml}$) and then added to the cell suspension. The mixture was incubated at 37 °C for 20 min, and the reaction was terminated by cooling at 4 °C for 15 min. The cell suspension was then centrifuged and the amount of histamine in the supernatant was measured by the HPLC peak area using Cosmosil 5C₁₈-PAQ (see below). The percentage histamine release was calculated as follows: histamine release (%) = (test – negative control) / (positive control – negative control) \times 100. The supernatant from the unstimulated cells was used as the negative control, and the supernatant from the stimulated cells only with A23187 was the positive control.

IC₅₀, the concentration that inhibited histamine release from the KU812 cells by 50% relative to control, was interpolated from graphed dose–response results (1.5, 30, 75, 105 $\mu\text{g/ml}$). IC₅₀ values were determined graphically.

HPLC Analysis The HPLC separations were carried out on a Waters alliance 2690 system (Waters, MA, U.S.A.) equipped with an auto sampler, a column thermostat and a UV–vis diode array detector working at 220 nm. The histamine was separated on a Cosmosil 5C₁₈-PAQ column (4.6 mm i.d. \times 150 mm, Nacalai Tesque, Kyoto, Japan). The mobile phase was 25 mM potassium dihydrogenphosphate containing 10 mM sodium hexanesulfonate (pH 2.5)–acetonitrile (99.7:0.3), and the flow-rate was 1.0 ml/min. Among the peaks of the supernatant, histamine was identified by spiking with standards. Histamine eluted at 20.5 min.

Statistics Values are expressed as mean \pm S.D. One-way ANOVA test was used for statistics analysis.

RESULTS

The dried powder of the wood was extracted with H_2O , $\text{H}_2\text{O/MeOH}$ (1:1), and MeOH under reflux for 45 min. The H_2O extract and $\text{H}_2\text{O/MeOH}$ extract contained vanillin (1), vanillic acid (2), coniferyl aldehyde (3), isotaxiresinol (4), secoisolariciresinol (5), α -conidendrin (6), taxiresinol (7), (7'*R*)-7'-hydroxyariciresinol (8), taxusin (9), 9-dehydro-13-acetylbaccatin III (10), and one new neolignan (11). The structures of the phenols, lignans, and taxane-type diterpenes of *Taxus yunnanensis* in this study are shown in Chart 1. The lignans, isotaxiresinol (4) and secoisolariciresinol (5), were found to be the major components of the wood of *Taxus yunnanensis*.

Compound 11, $\text{C}_{20}\text{H}_{26}\text{O}_7$, was isolated as a white amorphous solid with $[\alpha]_D + 12.1^\circ$ ($c=0.215$, CHCl_3). The IR spectrum of 11 showed an absorption at 3400 cm^{-1} corresponding to the hydroxyl group. The $^1\text{H-NMR}$ spectrum suggested the presence of two aromatic ortho coupled protons at δ 6.86 (1H, d, $J=8.5$ Hz) and δ 6.90 (1H, dd, $J=8.5, 2$ Hz), a meta coupled aromatic proton at δ 6.93 (1H, d, $J=2$ Hz), and two aromatic protons at δ 6.66 (1H, s) and δ 6.67 (1H, s). Two methoxy signals were observed at δ 3.85 and δ 3.87, along with two oxymethylenes (δ 3.68, 3.95), one oxymethine (δ 5.53), two methylenes (δ 1.91, 2.66), and one methine (δ 3.59). The $^{13}\text{C-NMR}$ spectrum showed twenty carbon signals consisting with two methoxy carbons (δ 55.96, 55.99), four methylene carbons (δ 31.96, 34.55, 62.24, 63.88), seven methine carbons, and seven quaternary carbons. The structure of 11 was determined by analysis of the ^1H -, ^{13}C -NMR, heteronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC) (Fig. 1), COSY and IR spectroscopic data as 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol. Though neolignan (11) is a novel compound, the lacton of two units of 11 was a known

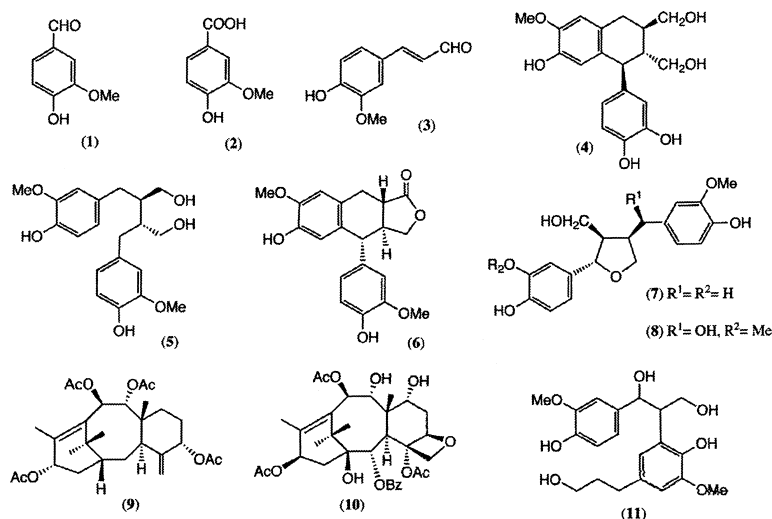
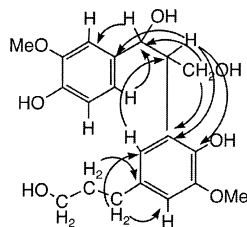
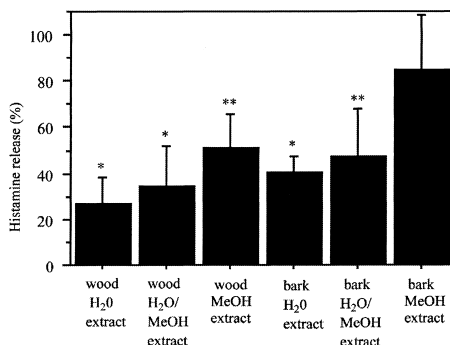


Chart 1. Structures of Compounds 1–11

Fig. 1. Selected HMBC Correlations of **11**Fig. 2. Histamine Release (%) of the Extracts from *T. yunnanensis*

All samples were tested at a concentration of 1.5 $\mu\text{g/ml}$. Histamine releases were calculated from the mean of four data. Each value represents the mean \pm S.D. * $p < 0.01$ and ** $p < 0.05$, significant differences from the positive control using a one-way ANOVA test.

compound called lappaol H which was the dineolignan with four units of coniferyl alcohol, and isolated from the seeds of *Arctium lappa* L.⁸⁾

The inhibitory effects on induced histamine release from the human basophilic cell line, KU812, of the H₂O, H₂O/MeOH, and MeOH extracts of the wood and the bark from *Taxus yunnanensis* were examined. As shown in Fig. 2, all the extracts except for the MeOH extract of the bark significantly inhibited the histamine release from cell stimulated with A23187. The H₂O extract of the wood showed the strongest inhibitory effect. All of the isolated compounds from *Taxus yunnanensis* were tested for their inhibitory effects on the histamine release (Table 1). As the result, secoisolariciresinol (**5**: 89.0%, IC₅₀=68.3 $\mu\text{g/ml}$) and taxiresinol (**7**: 86.1%, IC₅₀=59.5 $\mu\text{g/ml}$) showed inhibitory effects, and these effects were alike compared to the first line antiallergic drug, sodium cromoglicate (93.5%, IC₅₀=56.3 $\mu\text{g/ml}$), that inhibits the degranulation of the mast cell. Although due to the meager amount obtained, the IC₅₀ values of **6**, **8**—**11** could not be determined. The inhibitory effect on the histamine release of the wood extracts of *Taxus yunnanensis* might be mainly due to lignans, isotaxiresinol (**4**), secoisolariciresinol (**5**), and taxiresinol (**7**), that play a vital role because of their high content in the wood. It is interesting that lignans and coniferyl aldehyde (**3**) having a C₆C₃ unit had the inhibitory effect of histamine release, but neolignan (**11**) did not show

Table 1. Inhibitory Effects of Constituents from *T. yunnanensis* on the Release of Histamine from KU812 Cells

Compound	Histamine release (%)	
	1.5 $\mu\text{g/ml}$	IC ₅₀ ($\mu\text{g/ml}$)
1	95.7 \pm 5.4	>100
2	94.5 \pm 3.9	>100
3	93.8 \pm 3.7	94.3
4	94.3 \pm 5.7	70.0
5	89.0 \pm 4.7**	68.3
6	96.4 \pm 3.7	—
7	86.1 \pm 2.9*	59.5
8	93.3 \pm 8.4	—
9	98.3 \pm 8.1	—
10	99.5 \pm 3.0	—
11	108.3 \pm 12.6	—
Sodium cromoglicate	93.5 \pm 6.3	56.3

Each value represents the mean \pm S.D. of three—seven experiments. IC₅₀ was interpolated from graphed dose—response results (1.5, 30, 75, 105 $\mu\text{g/ml}$). * $p < 0.01$ and ** $p < 0.05$, significant differences from the positive control using a one-way ANOVA test. —, not tested due to insufficient amounts.

any antiallergic activity at a 1.5 $\mu\text{g/ml}$ concentration. It is postulated that the compounds that show the antiallergic activity of the bark are different from lignans as a very small amount of lignans is found in the bark. Though two taxane-type diterpenes did not show an antiallergic activity at this test, the investigation of the activity of other taxane-type diterpenes in the bark are now in progress.

In conclusion, the H₂O, H₂O/MeOH (1 : 1) extracts of the wood of *Taxus yunnanensis* showed a remarkable inhibitory effect on the induced histamine release from the human basophilic cell line, KU812. Secoisolariciresinol (**5**) and taxiresinol (**7**) inhibited the histamine release. One new neolignan, 2-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol, was also isolated from the wood of *Taxus yunnanensis*.

REFERENCES

- 1) Homma M., Minami M., Taniguchi C., Oka K., Morita S., Niitsuma T., Hayashi T., *Planta Med.*, **66**, 88—91 (2000).
- 2) Taniguchi C., Homma M., Takano O., Hirano T., Oka K., Aoyagi Y., Niitsuma T., Hayashi T., *Planta Med.*, **66**, 607—611 (2000).
- 3) Morikawa T., Sun B., Matsuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
- 4) Serra M. F., Diaz B. L., Barreto E. O., Pereira A. N. B., Lima M. C. R., Barbosa-Filho J. M., Cordeiro R. S. B., Martins M. A., De Silva P. M. R., *Planta Med.*, **63**, 207—212 (1997).
- 5) Chiang Su New Medical College (ed.), "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technologic Publisher, Shanghai, 1977, p. 2342.
- 6) Tachibana H., Sunada Y., Miyase T., Sano M., Maeda-Yamamoto M., Yamada K., *Biosci. Biotechnol. Biochem.*, **64**, 452—454 (2000).
- 7) Banskota A. H., Tezuka Y., Nguyen N. T., Awale S., Nobukawa T., Kadota S., *Planta Med.*, **69**, 500—505 (2003).
- 8) Ichihara A., Kanai S., Nakamura Y., Sakamura S., *Tetrahedron Lett.*, **33**, 3035—3038 (1978).