J Wood Sci (2001) 47:493–496

NOTE

Naohiro Takaku · Keigo Mikame · Tomoya Okunishi
Shiro Suzuki · Toshiaki Umezawa · Mikio Shimada

New lignan, isoactifolin, from *Chamaecyparis obtusa* cv. Breviramea

Received: July 25, 2000 / Accepted: November 8, 2000

Abstract A new lignan isoactifolin was isolated from young shoots (with leaves) of *Chamaecyparis obtusa* cv. Breviramea. The structure of the compound was determined based on spectroscopic evidence.

Key words Lignan · Isoactifolin · *Chamaecyparis obtusa* cv. Breviramea · Furan

Introduction

Hinoki cypress (*Chamaecyparis obtusa*) has long been utilized as one of the most important building woods in Japan. Heartwood of this species contains significant amounts of a dibenzylbutyrolactone lignan, hinokinin (**2**).[1,2] A few other lignans were isolated from this and related species. Thus, **2** accounted for about 30% of the resins extracted from *C. obtusa* heartwood.[1] Savinin (**3**) (= hibalactone), a dehydro derivative of **2**, was isolated from heartwood of *Chamaecyparis pisifera*[3] and from young leaves of *C. obtusa*,[4] *C. obtusa* cv. Breviramea,[5,6] and *C. pisifera* cv. Plumosa-aurea.[5,6] (+)-Sesamin (**4**) and deoxypodophyllotoxin (**5**) were obtained from *C. obtusa* cv. Breviramea young leaves[7] and *Chamaecyparis lawsoniana* leaves,[8] respectively. Despite the high content of **2** in *C. obtusa* heartwood and of **3** in *C. obtusa* cv. Breviramea young leaves, a detailed survey of lignans, especially possible biosynthetic precursor lignans of **2** in this species, has not yet been reported. Therefore, we surveyed lignans in *C. obtusa* and *C. obtusa* cv. Breviramea and isolated 11 lignans. One was found to be a new furan lignan, isoactifolin (**1**), and here we report its isolation. Characterization of the other 10 known lignans will be reported elsewhere in relation to stereochemistry.

N. Takaku · K. Mikame[1] · T. Okunishi[2] · S. Suzuki · T. Umezawa (✉) · M. Shimada
Laboratory of Biochemical Control, Wood Research Institute, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan
Tel. +81-774-38-3625; Fax +81-774-38-3682
e-mail: tumezawa@kuwri.kyoto-u.ac.jp

Present address:
[1] Faculty of Bioresources, Mie University, Mie 514-8507, Japan
[2] National Food Research Institute, Ibaraki 305-8642, Japan

Parts of this report were presented at the 44th lignin symposium, Gifu, October 1999

Experimental

Instruments and chromatography

One- and two-dimensional nuclear magnetic resonance (NMR) spectra were obtained with a JNM-LA400MK FT-NMR system (JEOL). Chemical shifts and coupling constants (J) were expressed in δ values and Hz, respectively. Low- and high-resolution electron impact mass spectrometry (EIMS) was performed on a JMS-DX303HF mass spectrometer (JEOL) equipped with a JMA-DA5000 mass data system. High-performance liquid chromatography (HPLC) was conducted with a Shimadzu LC-6A liquid chromatograph, detection being at λ = 280 nm. The reversed-phase column used was a Waters Novapak C_{18} (150 × 3.9 mm), and it was a eluted with CH_3CN-H_2O (37:63) at 1 ml/min. Silica gel column chromatography employed Kieselgel 60 (Merck, 70–230 mesh). Silica gel thin-layer chromatography (TLC) employed Kieselgel 60 F_{254} (Merck, 20 × 20 cm, 0.5 or 0.25 mm). All chemicals used were of reagent grade.

Plant material

Chamaecyparis obtusa cv. Breviramea plants were obtained from a local nursery and were maintained in the experimental forest of Wood Research Institute, Kyoto University, Japan. Young shoots with leaves of the plant were collected in February 1994 and April 1999 and were used for lignan extraction.

Isolation and structural determination of isoactifolin (**1**)

Freeze-dried *C. obtusa* cv. Breviramea young shoots with leaves (163.43 g) were pulverized using a Waring blender and then extracted with hot methanol (400, 150, 150, 150, 150, 150, and 150 ml; total 1300 ml). The combined methanol extracts (43.76 g) were suspended in distilled water (300 ml), which was then extracted with diethyl ether (200 ml × 3). The combined diethyl ether extracts (13.26 g) were submitted to successive purification by repeated column chromatography (solvents: first, EtOAc; second, methanol/dichloromethane = 3:97; third, ethyl acetate/*n*-hexane = 1:1), repeated TLC (solvents: first, acetone/dichloromethane = 3:97; second, ethyl acetate/*n*-hexane = 1:3; third, acetone/dichloromethane = 3:97), and reversed-phase HPLC to afford a new lignan (**1**) (3.6 mg).

Isoactifolin (**1**): data of ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H correlated spectrometry (COSY), and ^{1}H-detected heteronuclear multiple-bond quantum correlation (HMBC) are summarized in Table 1. NOE difference ^{1}H-NMR spectral data are shown in Table 2. MS *m/z* [rel. int. (%)]: 400 (76.4, M^+), 340 (22.1), 219 (27.6), 205 (38.7), 188 (25.1), 173 (23.2), 162 (16.5), 151 (65.1), 137 (30.9), 135 (100.0), 131 (16.9), 77 (19.5). High-resolution MS *m/z* (M^+): calculated for $C_{22}H_{24}O_7$: 400.1522; found: 400.1525.

Results and discussion

Because young leaves of *C. obtusa* cv. Breviramea contain significant amounts of **3**,[4-6] the plant material was subjected to a survey for lignans, especially possible biosynthetic precursors of **2** and **3**. As a result, a new lignan (**1**) (Fig. 1) was isolated from methanol extracts of the young shoots (with leaves) by chromatographic methods. In addition to **1**, several known lignans were isolated, and their characterization including stereochemical properties will be reported elsewhere.

The structure of **1** was determined by mass spectrometry and one- and two-dimensional NMR spectrometry. Compound **1** afforded a molecular ion [M^+] at *m/z* 400.1525 in the high-resolution EIMS, indicating the molecular formula to be $C_{22}H_{24}O_7$ (calculated for $C_{22}H_{24}O_7$: 400.1522). Table 1 shows the ^{1}H-NMR spectral data, which displayed the presence of an aromatic methoxyl group at δ 3.89 and an aromatic methylenedioxy group at δ 5.93. In addition, it revealed six aromatic protons at δ 6.62–6.87, which showed typical patterns of 1,3,4-tri-substituted benzenes. These results suggested that **1** had 3-methoxy-4-hydroxyphenyl (guaiacyl) and 3,4-methylenedioxyphenyl (piperonyl), which are popular aromatic moieties in lignans. It also exhibited a singlet of a phenolic hydroxyl group at δ 5.56 and a singlet of an alcoholic acetyl group at δ 2.02. Other signals in the ^{1}H-NMR spectrum were assigned with ^{1}H-^{1}H COSY (Table 1). It was revealed that a doublet at δ 4.74 (J = 6.6, H-7) was coupled with a multiplet at δ 2.49–2.56 (H-8), which was in turn coupled with two double doublets at δ 4.16 (J = 7.3, J = 11.2, H-9) and 4.31 (J = 7.1, J = 11.2, H-9). The multiplet at δ 2.49–2.56 (H-8) was coupled with a multiplet at δ 2.65–2.74 (H-8′). It was also revealed that the multiplet at δ 2.65–2.74 (H-8′) was coupled with four double doublets at δ 2.51 (J = 10.7, J = 13.4, H-7′), 2.81 (J = 5.0, J = 13.5, H-7′), 3.70 (J = 6.7, J = 8.7, H-9′a), and 4.05 (J = 6.6, J = 8.8, H-9′b). Assignments of H-9′a and H-9′b were done by nuclear Overhauser effect (NOE) difference ^{1}H-NMR spectrometry (Table 2). In the ^{13}C-NMR spectrum, 22 carbon signals were observed, as shown in Table 1. The assignments of the carbon atoms were achieved by a combination of ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrometry and HMBC spectrometry (Table 1). These ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of an acetylated furan lignan, actifolin (**6**), which was isolated from *Actinodaphne longifolia*.[9] However, the HMBC correlations between benzylic protons (H-7 and H-7′) and aromatic carbons (Table 1) indicated that **1** is the regioisomer of **6** in terms of the positions of the aromatic rings. The result was further confirmed by EIMS. Thus, intense fragments at *m/z* 135 and 151 were observed in the EIMS spectrum, which were assigned to 3,4-methylenedioxybenzyl ion and methoxyhydroxybenzyloxy ion, respectively, indicating that guaiacyl substituent and piperonyl substituent attached to C-7 and C-7′, respectively. As for the location of the acetyl group, H-9 (δ 4.16)

Fig. 1. Structures of lignans

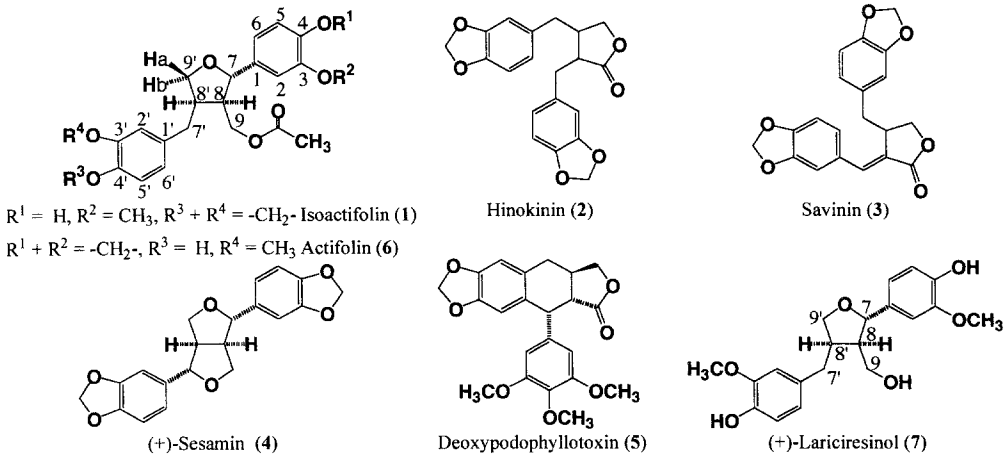

Table 1. NMR data for isoactifolin (**1**) in $CDCl_3$

Carbon no.	^{13}C[a]	^{1}H[a]	^{1}H-^{1}H COSY[b]	HMBC[c]
1	134.26			H-5
2	108.29 or 108.35	6.84 (d, J = 2.0)		
3	146.59			H-5, OMe, OH
4	145.11			H-2, 5, 6, OH
5	114.23	6.87 (d, J = 8.1)	H-6	OH
6	118.86	6.79 (dd, J = 2.0, 8.1)	H-5	H-2, 7
7	83.11	4.74 (d, J = 6.6)	H-8	H-2, 6, 9, 9, 9′b
8	48.96	2.49–2.56 (m)	H-7, 8′, 9, 9	H-9, 9
9	62.67	4.16 (dd, J = 7.3, 11.2)	H-8, 9	H-7, 8
		4.31 (dd, J = 7.1, 11.2)	H-8, 9	
1′	133.78			H-5′, 7′, 7′
2′	108.89	6.66 (d, J = 1.7)		H-6′, 7′, 7′
3′	147.82			H-2′, 5′, OCH_2O
4′	146.04			H-2′, 6′, OCH_2O
5′	108.35 or 108.29	6.73 (d, J = 8.1)	H-6′	
6′	121.48	6.62 (dd, J = 1.7, 8.1)	H-5′	H-2′, 7′, 7′
7′	33.30	2.51 (dd, J = 10.7, 13.4)	H-7′, 8′	H-2′, 6′
		2.81 (dd, J = 5.0, 13.5)	H-7′, 8′	
8′	42.46	2.65–2.74 (m)	H-7′, 7′, 8, 9′a, 9′b	H-7′, 9, 9
9′	72.67	3.70 (dd, J = 6.7, 8.7, H-9′a)	H-8′, H-9′b	H-7′
		4.05 (dd, J = 6.6, 8.8, H-9′b)	H-8′, H-9′a	
OMe	55.97	3.89 (s)		
OCH_2O	100.94	5.93 (s)		
OCOMe	20.92 (Me)	2.02 (s)		
	170.97 (C=O)			H-9, 9, OCOMe
OH		5.56 (s)		

[a] Chemical shifts are δ values; coupling constants (J in parentheses) are given in Hz
[b] Correlations between H-2 and H-6, and between H-2′ and H-6′ were not observed clearly
[c] Protons correlating with carbon resonances

Table 2. Nuclear Overhauser effect difference ^{1}H-NMR spectral data for isoactifolin (**1**)

Irradiation (δ)	Enhanced peaks (δ)
2.70 (H-8′)	2.51, 2.49–2.56, 2.81, 4.05, 6.62, 6.66
3.70 (H-9′a)	2.65–2.74, 4.05, 4.74, 6.62, 6.66
4.05 (H-9′b)	2.49–2.56, 2.65–2.74, 3.70, 6.62, 6.66
4.74 (H-7)	2.49–2.56, 3.70, 4.16, 4.31, 6.62, 6.66, 6.73, 6.79, 6.84

and H-9 (δ 4.31) showed a correlation with the carbonyl carbon (δ 170.97) in the HMBC spectrum (Table 1). In addition, the chemical shift of acetyl protons (δ 2.02) indicated its alcoholic, but not phenolic, acetyl nature, which was further confirmed by the downfield shift of H-9 signals (δ 4.16 and δ 4.31) compared with those of non-acetylated lariciresinol (**7**) (H-9, δ 3.77 and δ 3.92).[10] These results clearly indicated that the acetyl group was attached to the alcoholic hydroxyl group on C-9.

The NOE difference ^{1}H-NMR spectra of **1** (Table 2) indicated that the configurations of **1** were 8,8′-*cis* and 7,8-*trans* (7*R**, 8*S**, 8′*S**) as in **7**.[11] Taken together, the relative structure for **1** was determined as shown in Fig. 1, and we propose isoactifolin as the name of **1**.

Naturally occurring acetylated lignans, such as actifolin (**6**), are not popular, and the isolation of **1** from *C. obtusa* cv. Breviramea was another example. In addition, this was the first isolation of a furan lignan from *Chamaecyparis* plants. Because actifolin (**6**) showed cytotoxicity against a small panel of human tumor cells,[12] it is of interest to examine whether **1** has cytotoxicity.

Acknowledgments This research was partly supported by Grants-in-Aid for Scientific Research (06760160, 07660222, 10660163, 12660150) from the Ministry of Education, Science, Sports, and Culture of Japan and by the Sumitomo Foundation.

References

1. Keimatsu S, Ishiguro T (1936) Untersuchung der bestandteile der harze (VII Mitteilung); über die Konstitution des Hinokinins (in Japanese). Yakugaku Zasshi 56:103–118
2. Ohashi H, Hayashi H, Yamada M, Yasue M (1987) Phenolic heartwood constituents and heartwood color of Japanese cypress (in Japanese). Res Bull Fac Agr Gifu Univ 52:131–139
3. Takashima T, Ogushi M, Kiuchi Y, Akabane F (1968) The occurrence of savinin in the heartwood of *Chamaecyparis pisifera* Endl. (in Japanese). Mokuzai Gakkaishi 14:391–392
4. Kariyone T, Isoi K (1954) Studies on plant waxes. VI. Lignans from the leaves of coniferae (1) (in Japanese). Yakugaku Zasshi 74:1312–1314
5. Masumura M (1955) Studies on the component of young leaves of *Chamaecyparis obtusa* cv. Breviramea. I. Separation of crystallized component (in Japanese). Nippon Kagaku Zasshi 76:423–425
6. Masumura M (1955) Studies on the component of young leaves of *Chamaecyparis obtusa* cv. Breviramea. II. Structure of neutral component, hibalactone (in Japanese). Nippon Kagaku Zasshi 76:425–429
7. Masumura M (1955) Studies on the component of young leaves of *Chamaecyparis obtusa* cv. Breviramea. IV. About hinoki acid and *d*-sesamin (in Japanese). Nippon Kagaku Zasshi 76:1318–1322

8. Fitzgerald DB, Hartwell JL, Leiter J (1957) Distribution of tumor-damaging lignans among conifers. J Natl Cancer Inst 18:83–99
9. Tanaka H, Nakamura T, Ichino K, Ito K (1989) A lignan from *Actinodaphne longifolia*. Phytochemistry 28:952–954
10. Umezawa T, Shimada M (1994) Syntheses of (±)-lariciresinols. Mokuzai Gakkaishi 40:231–235
11. Freudenberg K, Weinges K (1961) Systematik und Nomenklatur der Lignane. Tetrahedron 15:115–128
12. Kwon HC, Choi SU, Lee JO, Bae KH, Zee OP, Lee KR (1999) Two new lignans from *Lindera obtusiloba* Blume. Arch Pharm Res 22:417–422