



lignan moiety and glucosyl group. Enzymatic hydrolysis of compound (1) and (2) with  $\beta$ -glucosidase gave an aglycone and a glucose. The aglycone was identified as (+)-lariciresinol (3)<sup>1</sup> based on the  $^1\text{H}$ -nmr spectrum and specific rotation. From above results, compound (1) and (2) were to be lariciresinol type glucosides. The  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr assignments and the position of glucosyl linkage were investigated by comparison with nmr spectrum data of lariciresinol-4,4'-bis-*O*- $\alpha$ -L-rhamnoside (4)<sup>2</sup> and made by using nOe and 2D-nmr techniques. Compound (1): The nOe experiments, glucosyl anomeric proton at  $\delta$  4.87 (the residual solvent peak was overlapped), methoxyl groups at  $\delta$  3.82 and  $\delta$  3.85 were irradiated, the signals at  $\delta$  7.13,  $\delta$  6.98 and  $\delta$  6.78 showed nOe enhancement, respectively. The HMBC spectrum, cross peaks were observed between H-8' ( $\delta$  2.35) with C-1', H<sub>2</sub>-7 ( $\delta$  2.50, 2.90) with C-1. Thus, signals at  $\delta$  139.5 and  $\delta$  133.5 were assigned at C-1' and C-1, respectively. And cross peaks were also observed between C-1' with H-5' ( $\delta$  7.13,  $J=8.06$ ) and C-1 with H-5 ( $\delta$  6.70,  $J=8.06$ ). Thus, signals at  $\delta$  7.13 and  $\delta$  6.70 were assigned at H-5' and H-5, respectively. In the same manner, the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were assigned as Table I. On the other hand, from the nOe results,  $\beta$ -D-glucose, OMe ( $\delta$  3.82) and OMe ( $\delta$  3.85) were attached to the position at C-4', C-3 and C-3', respectively. From the above results, compound (1) is lariciresinol-4'-*O*- $\beta$ -D-glucoside.

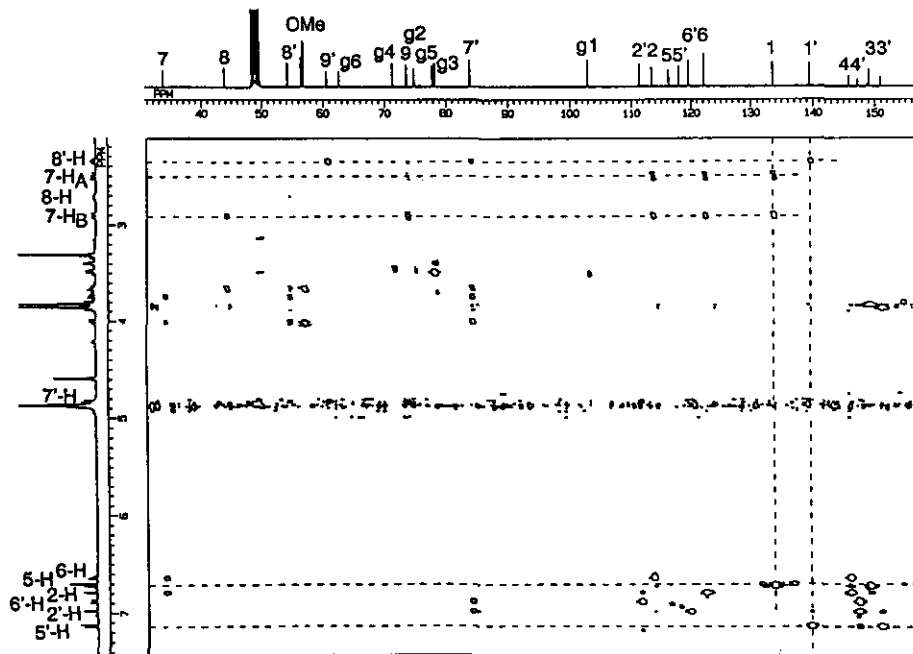


Figure 1. HMBC spectrum of 1.

Jolad *et al.* have reported the isolation of **1** from *Asclepias subulata*,<sup>3</sup> but they purified it after acetylation of the fractions of the ethyl acetate extract. This is for the first time that the glucoside itself has been isolated.

Compound (**2**): In the nOe experiments, when glucosyl anomeric proton at  $\delta$  4.86 (the residual solvent peak was overlapped), methoxyl groups at  $\delta$  3.84 and  $\delta$  3.85 were irradiated, the signals at  $\delta$  7.09,  $\delta$  6.90 and  $\delta$  6.88 showed nOe enhancement, respectively. The HMBC spectrum, cross peaks were observed between H-8' ( $\delta$  2.38) with C-1', H<sub>2</sub>-7 ( $\delta$  2.54, 2.97) with C-1. Thus, signals at  $\delta$  135.7 and  $\delta$  137.2 were assigned at C-1' and C-1, respectively. And cross peaks were also observed between C-1 with H-5 ( $\delta$  7.09,  $J=8.06$ ) C-1' with H-5' ( $\delta$  6.76). Thus, signals at  $\delta$  7.09 and  $\delta$  6.76 were assigned at H-5 and H-5', respectively. In the same manner, the <sup>1</sup>H- and <sup>13</sup>C-nmr spectra were assigned as Table I. On the other hand, from the nOe results,  $\beta$ -D-glucose, OMe ( $\delta$  3.84) and OMe ( $\delta$  3.85) were attached to the position at C-4, C-3' and C-3, respectively. From the above results, compound (**2**) is lariciresinol-4-O- $\beta$ -D-glucoside. Compound (**2**) have not been reported in Nature.

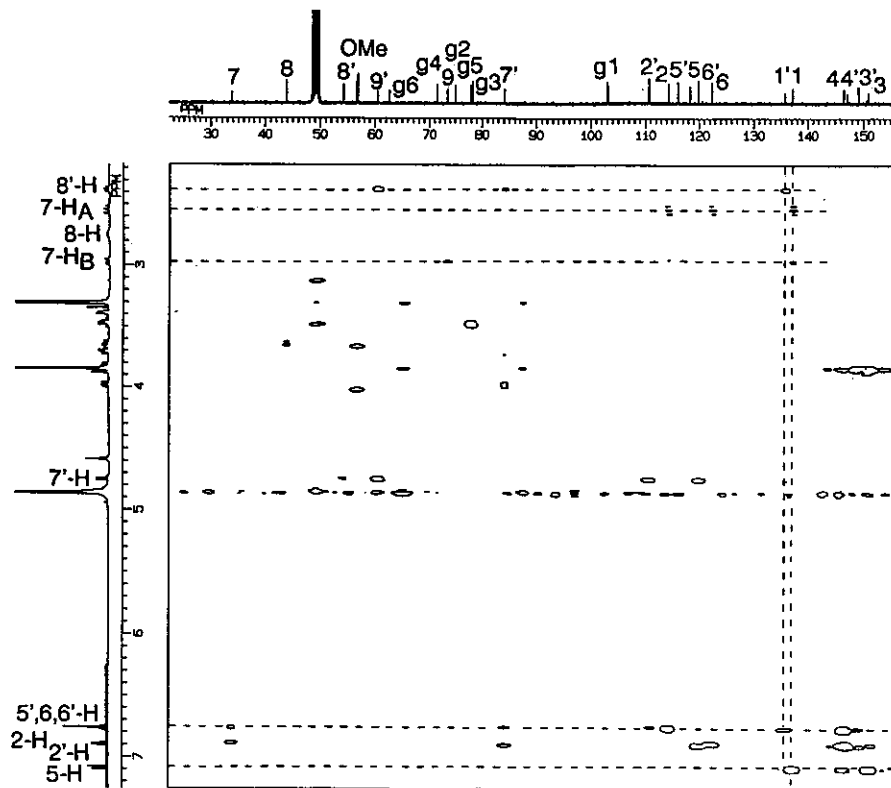


Figure 2. HMBC spectrum of **2**.

Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectrum of 1 and 2 in  $\text{CD}_3\text{OD}$  ( $^1\text{H}$ -, 400 MHz,  $^{13}\text{C}$ -, 100 MHz)

	1		2	
	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$
1		133.5		137.2
2	6.78 (d, $J=1.83$ )	113.4	6.88 (d, $J=1.83$ )	114.4
3		149.1		150.9
4		145.9		146.4
5	6.70 (d, $J=8.06$ )	116.2	7.09 (d, $J=8.06$ )	118.3
6	6.63 (dd, $J=8.06, 1.83$ )	122.2	6.76 (br.s)**	122.3
7	2.50 (dd, $J=13.2, 11.0$ )	33.7	2.54 (dd, $J=13.2, 11.0$ )	33.7
	2.90 (dd, $J=13.2, 4.4$ )		2.97 (dd, $J=13.2, 4.7$ )	
8	2.71 (m)	43.8	2.75 (m)	43.8
9	4.00 (dd, $J=8.06, 6.23$ )	73.7	3.98 (dd, $J=8.06, 6.23$ )	73.5
3-OMe	3.82 (s)	56.4	3.85(s)	56.4
1'		139.5		135.7
2'	6.98 (d, $J=1.83$ )	111.4	6.90 (d, $J=1.83$ )	110.7
3'		150.9		149.1
4'		147.3		147.1
5'	7.13 (d, $J=8.06$ )	117.9	6.76 (br.s)**	116.0
6'	6.88 (dd, $J=8.06, 1.83$ )	119.6	6.76 (br.s)**	119.8
7'	4.82 (d, $J=6.6$ )	83.9	4.75 (d, $J=6.9$ )	84.1
8'	2.35 (m)	54.2	2.38 (m)	54.1
9'		60.5		60.5
3'-OMe	3.85 (s)	56.8	3.84(s)	56.8
Glc-1	4.87*	102.9	4.86*	103.0
Glc-2		75.0		75.0
Glc-3		78.2		78.2
Glc-4		71.4		71.4
Glc-5		77.9		77.9
Glc-6		62.5		62.6

\*The residual solvent peak was overlapped. \*\* Each peaks were overlapped.

## EXPERIMENTAL

$^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectrum were recorded with a JEOL JMX-GSX 400 (400 and 100 MHz, respectively) spectrometer in  $\text{CD}_3\text{OD}$ . Chemical shifts are given on a  $\delta$  (ppm) scale with tetramethylsilane as an internal standard. Optical rotations were determined with a JASCO DIP-360 digital polarimeter. Ir and uv spectra were recorded on a Perkin-Elmer 1725X FT-IR instrument and on a Beckman DU-64 spectrophotometer, respectively. Fabms were measured using a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230-400 mesh) and Sephadex LH-20 (Pharmacia Fine Chemical). Hplc was carried out on Tosoh hplc system (pump, CCPM prep; detector, UV-8010) using a TSK gel ODC 120T (Tosoh) column. Thin layer chromatography was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying 50%  $\text{H}_2\text{SO}_4$  followed by heating.

**Collection and extraction.** Fresh bark of *O. asiaticus* (1.0 kg), collected in June 1991, in Sendai, Japan, were extracted with methanol at room temperature for one month. The MeOH extract was concentrated under

reduced pressure and the residue was suspended in a small excess of H<sub>2</sub>O. This suspension was extracted with CHCl<sub>3</sub>, Et<sub>2</sub>O, EtOAc and *n*-BuOH, successively.

**Isolation** The *n*-BuOH soluble fraction residue (53.0 g) was chromatographed on Sephadex LH-20 column (MeOH-H<sub>2</sub>O=1:1) and on silica gel column (CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O=30:10:1), and subjected to hplc (MeOH-H<sub>2</sub>O=1:3) to give compound **(1)** (6.7 mg) and **(2)** (4.6 mg).

**Lariciresinol-4'-O-β-D-glucoside (1)** An amorphous powder.  $[\alpha]^{23}_D -19.3^\circ$  (*c*=0.6, MeOH). Uv  $\lambda_{max}$ : 277, 226 nm. Ir  $\nu_{max}$ : 3312 (OH), 1598, 1515 cm<sup>-1</sup> (arom. rings). fabms *m/z* 545 [M+Na]<sup>+</sup>. <sup>1</sup>H- and <sup>13</sup>C-nmr; see Table 1.

**Lariciresinol-4-O-β-D-glucoside (2)** An amorphous powder.  $[\alpha]^{23}_D -19.3^\circ$  (*c*=0.4, MeOH). Uv  $\lambda_{max}$ : 278, 225 nm. Ir  $\nu_{max}$ : 3333 (OH), 1595, 1512 cm<sup>-1</sup> (arom. rings). fabms *m/z* 523 [M+H]<sup>+</sup>. <sup>1</sup>H- and <sup>13</sup>C-nmr; see Table 1.

**Enzymatic Hydrolysis of 1 and 2** A solution of **1**, or **2**, (5 mg) in H<sub>2</sub>O (3 ml) was incubated with β-glucosidase (10 mg) at 37°C overnight, then evaporated under reduced pressure, and the residue was extracted with MeOH. The extractive was fractionated by silica gel column chromatography (CHCl<sub>3</sub>-MeOH=19:1 → 7:3) into (+)-lariciresinol (**3**) and glucose. Glucose was identified by pc (BuOH-AcOH-H<sub>2</sub>O=4:1:2), visualizing agent, aniline hydrogen phthalate), *Rf*=0.24.

**(+)-Lariciresinol(3)**  $[\alpha]^{23}_D +19.7^\circ$  (*c*=0.2, Me<sub>2</sub>CO). <sup>1</sup>H-Nmr (CDCl<sub>3</sub>, 400 MHz); δ 2.42 (1H, m, 8'-H), 2.72 (1H, dd, *J*=13.5, 5.0 Hz), 3.81 (3H, s, OMe), 3.88 (3H, s, OMe), 3.90 (3H, s, OMe), 4.79 (1H, d, *J*=6.7 Hz, 7'-H), 6.69-6.86 (6H, m, arom. protons).

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## REFERENCES AND NOTES

1. R. Andersson, T. Popoff, and O. Theander, *Acta Chem. Scand.*, 1975, **B29**, 835.
2. F. Abe and T. Yamauchi, *Phytochemistry*, 1989, **28**, 1737.
3. S. D. Jolad, R. B. Bates, J. R. Cole, J. J. Hoffmann, T. J. Siahann, and B. N. Timmermann, *Phytochemistry*, 1986, **25**, 2581.

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