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NOTE

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Manufacture of wood-cement boards VIII: cement-hardening inhibitory compounds of keyaki (Japanese zelkova, *Zelkova serrata*)

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Abstract To isolate and identify the cement-hardening inhibitory components of keyaki (Japanese zelkova, *Zelkova serrata* Makino), methanol extractives containing inhibitory components were fractionated by successive organic solvent extraction and column chromatography, and the inhibition of these fractions was determined. Spectroscopic analysis of one isolated compound identified it to be keyakinin with the C-glucoside structure of a flavonol. According to the inhibitory indices, the compound was found to be the main cement-hardening inhibitory component of keyaki. Extraction of keyaki with hot water or a blend of keyaki with up to 30% hinoki as a suitable species diminished the cement-hardening inhibition of wood-cement board to a great extent, suggesting that such treatments are economical countermeasures to this inhibition.

Key words Wood-cement board · *Zelkova serrata* · Cement-hardening inhibitory compound · Keyakinin

Introduction

Keyaki (Japanese zelkova, *Zelkova serrata* Makino)[1] is native to Japan, Korea, and the southern part of China. It is used in architecture, construction, furniture-making, shipbuilding, and the like in Japan. This wood is rarely utilized by itself for the production of wood–cement board, as it is known to be extremely incompatible with cement.[2] During the manufacture of wood-cement boards, refuse and waste woods are utilized as raw materials in addition to logs. The present study attempts not only to isolate and identify cement-hardening inhibitory components of keyaki as a point of academic interest, but also to provide basic information to the wood industry on the inhibition of cement hardening as a point of practical interest.

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Material and methods

Material

About 70-year-old keyaki (Japanese zelkova, *Zelkova serrata* Makino) wood was used.

Extraction and fractionation

About 3 kg of wood meal was extracted three times with methanol (MeOH) followed by extraction with water (H_2O) three times at room temperature. The combined MeOH and H_2O solutions were evaporated individually under reduced pressure to give 337 g (11.0% on wood) of MeOH extractives and 81 g (2.6%) of H_2O extractives. The MeOH extractives were successively extracted with *n*-hexane, benzene, ethyl acetate (AcOEt), and MeOH to obtain their soluble fractions. The MeOH-soluble fraction was separated by silica-gel column chromatography with solvents of acetone/*n*-hexane (3:7 v/v), acetone/*n*-hexane (7:3), acetone, and MeOH to give four fractions. The third fraction was further chromatographed on silica gel using mixed solvents of H_2O-saturated AcOEt and ethanol (EtOH) to obtain five fractions. From the third and fourth fractions after evaporation of the solvent, a total of 31 g (1.0% on wood) of a pale yellow compound (compound **I**) crystallized directly on standing. The compound was recrystallized from MeOH.

I: mp 212–214°C (ref. 213°C).[3] Anal. calculated for $C_{22}H_{22}O_{11}$: C, 57.14; H, 4.80; found: C, 57.11; H, 4.83.

Acetylation of compound I

A solution of 46 mg **I** in 0.7 ml pyridine and 0.6 ml acetic anhydride was allowed to stand at room temperature. After

standing overnight, the solution was diluted with cold H_2O and then extracted with AcOEt. The AcOEt solution was extracted with 0.5N NaOH and then 0.5N HCl to remove acetic acid and pyridine, respectively, washed with H_2O, dried over sodium sulfate, and finally evaporated to yield the crude acetylation product. The product was purified by silica-gel column chromatography with a mixed solvent of benzene/AcOEt (7:3) to isolate 72mg (91.7%) of **II** as a colorless compound.

II: MS *m/z*: 714 ($M^+ - 42$), 672; ^{1}H-NMR: δ[3]: 1.81 [3H, s, $OCOCH_3$(OAc)], 2.02 (3H, s, OAc), 2.07 (6H, s, 2 × OAc), 2.31 (3H, s, OAc), 2.36 (3H, s, OAc), 2.49 (3H, s, OAc), 3.95 (1H, m, H-5″), 4.03 (3H, s, OCH_3), 4.10–4.46 (2H, m, H-6″), 4.49 (1H, d, J = 10Hz, H-1″), 5.17 (1H, t, J = 9Hz, H-2″), 5.31 (1H, t, J = 9Hz, H-4″), 5.91 (1H, t, J = 9Hz, H-3″), 6.85 (1H, s, H-8), 7.25 (2H, d, J = 9Hz, H-3′, 5′), 7.84 (2H, d, J = 9Hz, H-2′, 6′). ^{13}C-NMR: δ: 20.6, 20.7, 20.8, 21.2, 21.3, 56.7, 62.6, 68.8, 71.0, 72.3, 74.6, 76.3, 98.2, 110.4, 115.7, 122.2 (strong), 127.3, 129.7 (s), 134.0, 149.5, 153.0, 154.2, 158.4, 161.7, 163.9, 168.1, 168.9, 169.1, 170.0, 170.1, 170.5, 170.9. Anal. calculated for $C_{36}H_{36}O_{18}$: C, 57.13; H, 4.79; found: C, 57.58; H, 4.77.

Methylation and subsequent acetylation of **I**

To a solution of 0.42mg **I** in 5ml MeOH was added an excess amount of diazomethane in 10ml diethyl ether at room temperature. After 48h the solution was evaporated under reduced pressure to yield the crude methylation product. The product without purification was acetylated with acetic anhydride and pyridine in the same way as described above. Isolation of the product by silica-gel column chromatography with a mixed solvent of benzene/AcOEt (7:3) gave 45mg (73.7%) of a colorless compound (**III**).

III: MS *m/z*: 672 (M^+), 613; ^{1}H-NMR: δ: 1.78 (3H, s, OAc), 2.03 (3H, s, OAc), 2.05 (3H, s, OAc), 2.08 (3H, s, OAc), 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.94 (1H, m, H-5″), 4.00 (3H, s, OCH_3), 4.04–4.38 (2H, m, H-6″), 5.12 (1H, d, J = 10Hz, H-1″), 5.20 (1H, t, J = 9Hz, H-2″), 5.34 (1H, t, J = 9Hz, H-4″), 6.06 (1H, t, J = 9Hz, H-3″), 6.73 (1H, s, H-8), 7.03 (2H, d, J = 9Hz, H-3′, 5′), 8.08 (2H, d, J = 9Hz, H-2′, 6′). Anal. calculated for $C_{33}H_{36}O_{15}$: C, 58.92; H, 5.40; found: C, 58.87; H, 5.41.

Hydration reaction

The hydration temperature from a wood meal/portland cement/water mixture was measured in the usual procedure.[4]

Inhibitory index and compatibility factor

The inhibitory index[5] and compatibility factor[6] were calculated from the hydration temperature curve for the wood/cement/water mixtures. The inhibitory index of hot water-extracted hinoki wood meal (control) was 1.5.

Spectrometry

The ^{1}H- and ^{13}C-NMR spectra of compounds in deuterochloroform ($CDCl_3$) were recorded with trimethylsilane (TMS) as an internal standard on a Jeol JNM-EX 270 FT NMR spectrometer. The mass spectrometric (MS) spectrum was recorded on a Jeol JMD D-300 mass spectrometer.

Results and discussion

Isolation of a cement-hardening inhibitory component

As can be seen from the inhibitory index and compatibility factor in Table 1, keyaki wood strongly inhibited the hardening of portland cement in each case with calcium chloride, magnesium chloride, and aluminum chloride as accelerators. Furthermore, the methanol extractives of the wood contained cement-hardening inhibitory components, indicating that the components can be eluted from the wood by extraction with a polar solvent of methanol. The water ex-

Table 1. Yield, inhibitory index, and compatibility factor of keyaki wood meal and MeOH and H_2O extractives

Sample[a]	Yield (% on wood)	Accelerator	Time (h)[b]	Inhibitory index	Compatibility factor[c]
Keyaki wood meal	–	$CaCl_2$	>50.0	∞	45.2
		$MgCl_2$	>50.0	∞	42.9
		$AlCl_3$	>50.0	∞	64.2
MeOH extractives[d]	11.0	$CaCl_2$	>50.0	∞	48.8
		$MgCl_2$	>50.0	∞	70.0
		$AlCl_3$	15.0	31.0	80.5
H_2O extractives[d]	2.6	$CaCl_2$	6.0	0.3	90.0
		$MgCl_2$	7.6	3.3	86.4
		$AlCl_3$	5.9	0.5	93.1

[a] Hot water-extracted hinoki (*Chamaecyparis obtsusa* Endl.) was used as a control
[b] Time to reach maximum temperature (hinoki: 5.6h)
[c] Area under the hydration heating curve for 24h
[d] Inhibitory index and compatibility factor were calculated from the hydration temperature curves of hot water-extracted hinoki impregnated with the extractives

tractives obtained by extraction of the methanol extractive-free wood with a solvent of water with greater polarity had only small inhibitory indices, suggesting that almost all of the inhibitory components are present in the methanol extractives.

Because the methanol extractives contain a large number of components of different polarities, they were successively fractionated in the usual way with *n*-hexane, benzene, AcOEt, and MeOH, increasing the polarity to obtain their soluble fractions, as shown in Fig. 1. The yields, inhibitory indexes, and compatibility factors of their soluble fractions are summarized in Table 2. Because cement-hardening inhibitory components are generally polar and the appearance of cement-hardening inhibition required an inhibitory component content[7] of more than 1%, the *n*-hexane-soluble fraction[4] containing nonpolar compounds and the low-yield benzene-soluble fraction were excluded from the hydration reaction tests. As can be seen from Table 2, the methanol-soluble fraction contained a large amount of the inhibitory components.

To separate the inhibitory components, the methanol-soluble fraction was further fractionated by silica-gel column chromatography to give four fractions (F1–F4). The yields, inhibitory indexes, and compatibility factors of the fractions are summarized in Table 3. The main inhibitor was contained in the third fraction (F3) eluted with acetone. The F3 fraction was further separated by silica-gel column chromatography with various solvents to give five fractions. Identical pale yellow crystals (compound **I**) were isolated from the third and fourth fractions. The infrared (IR) spectrum (not shown) of **I** was similar to that of F3, indicating that the net content of **I** in wood is in the range of 1.0% (total isolation yield) to 2.9% (yield of F3).

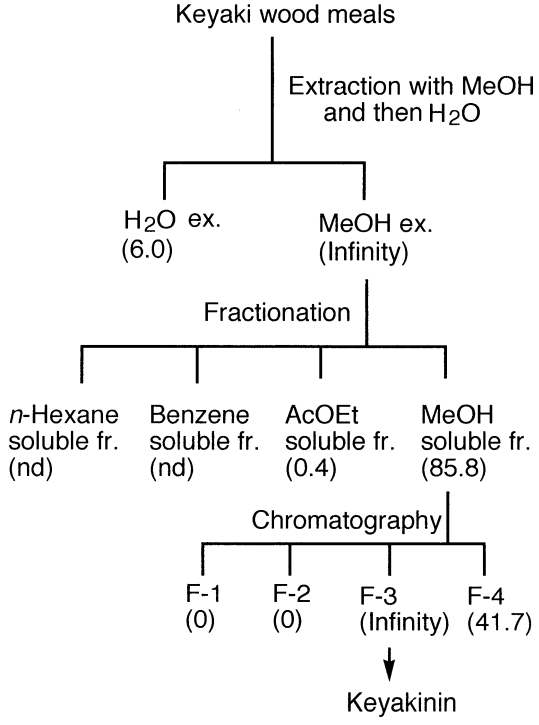

Fig. 1. Separation of inhibitory components. Inhibitory indexes are in parentheses

Identification and inhibitory index of compound **I**

As the IR spectrum of **I** did not show the presence of any acetyl group, **I** was acetylated with acetic anhydride and pyridine to count the number of hydroxyl groups in the molecule. The ^{1}H-NMR spectrum of the acetylation product (**II**) showed peaks due to seven acetyl groups, a methoxyl group, and a C-glucoside unit in the aliphatic region. In the aromatic region, the spectrum showed peaks due to a *p*-hydroxyphenyl nucleus and a five-substituted aromatic nucleus. The striking resemblance of the ^{1}H-NMR spectrum of **II** to that[3] of acetylated keyakinin suggests that **I** is keyakinin (Fig. 2).

To confirm the structure, **I** was methylated with diazomethane and subsequently acetylated to yield compound **III**. The ^{1}H-NMR spectrum of **III** showed peaks due to four aliphatic acetyl groups in the C-glucoside unit and three methoxyl groups in addition to an original methoxyl group. Apparently, three acidic hydroxyl groups on A, B, and the heterocyclic rings contribute to the methylation.

Table 2. Yield, inhibitory index, and compatibility factor of the *n*-hexane-, benzene-, AcOEt-, and MeOH-soluble fractions

Fraction	Yield (% on wood)	Time (h)	Inhibitory index[a]	Compatibility factor
n-Hexane	1.1	ND	ND	ND
Benzene	0.6	ND	ND	ND
AcOEt	1.0	5.8	0.4	92.1
MeOH	8.3	22.6	85.8	55.9

ND, Not determined
[a] $CaCl_2$ was used as an accelerator

Table 3. Yield, inhibitory index, and compatibility factor of fractions 1–4

Fraction	Yield (% on wood)	Time (h)	Inhibitory index[a]	Compatibility factor
1	0.7	6.1	0	90.8
2	0.5	6.3	0	95.5
3	2.9	>50.0	∞	24.8
4	4.1	16.5	41.8	68.5

[a] $CaCl_2$ was used as an accelerator

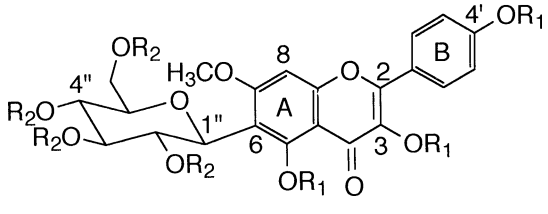

Fig. 2. Chemical structures of compounds **I**, **II**, **III**. Compound **I**, $R_1 = R_2 = H$ (keyakinin); compound **II**, $R_1 = R_2 = COCH_3$; compound **III**, $R_1 = CH_3$, $R_2 = COCH_3$

Table 4. Inhibitory index and compatibility factor of compound **I** (keyakinin)

Accelerator	Time (h)	Inhibitory index	Compatibility factor
$CaCl_2$	18.7	31.1	51.7
$MgCl_2$	11.6	7.0	72.8
$AlCl_3$	14.6	13.0	68.3

Compound **I**: 0.15 g (1% on wood) was impregnated into 15 g of hot water-extracted hinoki wood meal

Table 5. Inhibitory index and compatibility factor of a blend of keyaki and hinoki

Sample	Time (h)	Inhibitory index	Compatibility factor
50% keyaki	11.5	15.1	68.2
30% keyaki	7.3	0.5	98.2
15% keyaki	6.3	0	98.8

$CaCl_2$ was used as the accelerator in all instances

Spectroscopic support for the structure was obtained with a molecular ion peak at *m/z* 672 in the mass spectrum.

The yield of inhibitor **I** is in the range of 1.0%–2.9%, and an inhibitory index with a content of 1% was calculated. As summarized in Table 4, **I** showed greater or lesser inhibition effects in all experiments with $CaCl_2$, $MgCl_2$, and $AlCl_3$ as accelerators. In general, a phenolic compound with a catechol unit such as teracacidin[8,9] has a high inhibitory index even in the presence of $CaCl_2$, and a polyhydric alcohol such as inositol[4] shows strong inhibition even in the presence of $AlCl_3$. Therefore, **I** with both groups in a molecule is a cement-hardening inhibitory compound of wood that belongs to a new category. The chemical structure of **I** suggests that both a C-glucoside unit with four hydroxyl groups and an α-hydroxyl ketone unit, which may form a chelating structure with inorganic ions, contribute to cement-hardening inhibition.

Countermeasures

The most economical countermeasure for this inhibition is to reduce the content of **I** in raw materials for wood-cement boards by mixing it with raw material from some other suitable species. As shown in Table 5, a blend of keyaki with hinoki greatly diminished the cement-hardening inhibition. Of course, the inhibitor can also be removed by extraction of the keyaki flakes with hot water.

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