Note

Glycosides of a Phenylpropanoid and Neolignans: Oviposition Stimulants in Pine Inner Bark for Cerambycid Beetle, *Monochamus alternatus*

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INTRODUCTION

The cerambycid beetle, *Monochamus alternatus*, is a vector of the pathogenic pine wood nematode, *Bursaphelenchus xylophilus*.^{1,2)} After landing on a dying pine, the female beetle moves to a site favorable for oviposition, cuts a characteristic slit in the outer bark, inserts her ovipositor into the inner bark through the slit and lays eggs. Irrespectively of pine volatiles, the methanol and water extracts of the inner bark of *Pinus densiflora* stimulate the female beetle to oviposit in the presence of free moisture.³⁾ In order to explore oviposition stimulants for the female beetle, a 70% aqueous acetone extract of pine inner bark, from which ether solubles and ethanol insolubles had been removed,⁴⁾ was divided into fractions I, II and III with a Sephadex LH-20 column.⁵⁾ A combination of fractions I and II⁴⁾ and that of fractions I and III⁵⁾ were found to be active. The flavan-3-ol (+)-catechin⁴⁾ and the flavanonol glucoside (–)-2,3-*trans*-dihydroquercetin-3'-*O*- β -D-glucopyranoside⁵⁾ were characterized as oviposition stimulants involved in fraction II for the female beetle. Polymeric proanthocyanidins and two dimeric proanthocyanidins, *i.e.*, procyanidins B-1 [epicatechin-(4 β → 8')-catechin] and B-3 [catechin-(4 α → 8')-catechin], were also characterized as stimulants involved in fraction III.⁵⁾ We describe here the isolation and identification of glycosides of a phenylpropanoid and neolignans as oviposition stimulants from fraction I.

MATERIALS AND METHODS

1. Instruments

Negative FAB-MS spectra were taken with the mass spectrometer described previously.⁵⁾ Measurements of ¹H and ¹³C NMR, ¹³C-¹H and ¹H-¹H COSYs and HMBC spectra were performed with the same spectrometer as before.⁵⁾ Optical rotations were also measured with the polarimeter

used previously.^{4,5)}

2. Isolation of Compounds 1 to 5

Fractions I (11.5% of oven-dried inner bark), II and III (15.4%) were prepared according to the previous method.⁵⁾ Fraction I (1.25 g) was applied to an Amberlite CG-50 (37–75 μ m, Rohm & Haas) column (2.5 × 30 cm) and eluted with water (600 ml) and then 20% aqueous 2-propanol (400 ml). Eluates (10 ml each) were collected. Subfractions I-1 (665 mg), I-2 (277 mg) and I-3 (92 mg) were obtained from eluate nos. 9–19, 20–60 and 61–100, respectively. Adsorbed materials were recovered as subfraction I-4 (41 mg) by elution with ethanol. This fractionation was repeated. Yields of subfractions I-1 to I-4 were estimated to be 6.5% of oven-dried inner bark; 2.7%; 0.9%; and 0.4%, respectively. Subfraction I-2 (300 mg) was further chromatographed over MCI GEL CHP 20P (2.5 × 41 cm, 75–150 μ m, Mitsubishi Chem. Corp.) by elution with water containing the amount of methanol increased by 10% [water → 90% aqueous methanol]. Eluates (10 ml each) were collected. Eluate nos. 337–371, 389–398, 404–425, 446–454 and 528–536 afforded brownish compounds **1** (89 mg), **2** (44 mg), **5** (56 mg), **3** (23 mg) and **4** (11 mg), respectively. Individual compounds **1**, **2** and **5** were applied to each of DEAE-cellulose (1.5 × 30 cm, Wako) columns [water → 10 mM aqueous formic acid], and compounds **3** and **4** were separately applied to a HPLC Zorbax ODS (9.4 × 250 mm, 25–100 μ m, Rockland Tech.) column [methanol/5% aqueous acetic acid (2:3)]. Finally, all the compounds were passed through MCI GEL CHP 20P columns (1.5 × 24 cm) [water → 40% aqueous methanol]. Yields of the white amorphous solids **1** to **5** thus isolated were 0.8% of oven-dried inner bark; 0.4%; 0.2%; 0.1%; and 0.5%, respectively.

3. Hydrolysis of Compounds 1 to 5

Compounds **1**, **2** and **5** (30–60 mg) in water (5–10 ml) were treated with β -glucosidase (12–24 mg, from sweet almonds, Funakoshi) at 28°C for 3–8 hr. Compounds **3** and **4** (20–50 mg) in 0.1M H₂SO₄ (4–10 ml) were heated at 100°C for 15 min and cooled. The reaction mixtures were extracted with ethyl acetate, and the aglycones in the organic layers were purified by silica gel TLC with water-saturated ethyl acetate. The aqueous layers were neutralized to pH 6.5 with an aqueous barium hydroxide solution, and the barium sulfate formed was removed. The sugars in the filtrates were purified by paper chromatography.⁵⁾ The aglycones and the sugars thus purified were obtained in yields of 85%–93% and 75%–84%, respectively.

4. Bioassay

The assay with the female *M. alternatus* adults⁶⁾ was performed in 1997–1998 according to the same procedure as

described in the previous papers.⁴⁾ All samples were tested with fraction III. Statistical comparisons of oviposition responses (%) were also made in the fashion employed in the preceding paper.⁵⁾

RESULTS

1. Identification of Compounds 1 to 5

1.1 Compound 1 A deprotonated molecular ion ($[M-H]^-$) was observed at m/z 343 in the negative FAB-MS spectrum of **1**. The hydrolysis of **1** with β -glucosidase gave D-glucose⁵⁾ and an aglycone. The 1H and the ^{13}C NMR spectra of the aglycone coincided with those⁷⁾ of dihydroconiferyl alcohol, respectively. In the ^{13}C NMR spectrum of **1**, the C-9 carbon signal moved downfield by 7.8 ppm due to glucosylation⁸⁾ in comparison with that of the aglycone. The HMBC spectrum of the pentaacetate of **1** revealed the presence of a linkage between C-9 and the anomeric carbon of the sugar moiety through the oxygen concerned. The configuration at C-1' was confirmed to be β -form, because the carbon signal of the acetate was observed at 101.9 ppm. The $[\alpha]_D^{28}$ of **1** was -21.8° (MeOH, $c=1.4$). The results are listed in Table 1. Thus, **1** was identified as dihydroconiferyl alcohol-9- O - β -D-glucopyranoside.

1.2 Compound 2 The hydrolysis of **2** with β -glucosidase yielded the dihydrobenzofuran type neolignan cedrusin. [2, 3-dihydro-7-hydroxy-2-(4'-hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol]^{9,10)} and D-glucose. From Table 1 as well as the results, **2** was found to be cedrusin-4'- O - β -D-glucopyranoside.

1.3 Compound 3 Cedrusin and a sugar were produced by the hydrolysis of **3** with 0.1M H_2SO_4 . The sugar was confirmed to be L-rhamnose by measuring its 1H and ^{13}C NMR (D_2O) spectra and those of its specimen. On the basis of the results and Table 1, **3** was elucidated to be cedrusin-4'-

O - α -L-rhamnopyranoside.

1.4 Compound 4 The hydrolysis of **4** with 0.1M H_2SO_4 resulted in the formation of 7- O -methyl cedrusin [dihydrodehydroconiferyl alcohol]¹¹⁾ and L-rhamnose. From these

1 : R = β -D-glucopyranosyl

2 : $R_1 = H$, $R_2 = \beta$ -D-glucopyranosyl

3 : $R_1 = H$, $R_2 = \alpha$ -L-rhamnopyranosyl

4 : $R_1 = CH_3$, $R_2 = \alpha$ -L-rhamnopyranosyl

5 : R = β -D-xylopyranosyl

Table 1 Analytical data of compounds **1** to **5**.

	1	2	3	4	5
FAB-MS spectra					
$[M-H]^-$ m/z	343	507	491	505	509
NMR spectra ^{a)}					
$\Delta\delta$ (ppm)	C-9 +7.8				
in carbon	C-1'	+3.6	+3.9	+4.0	+3.9
signals ^{b)}	C-4'	+3.5	+4.7	+4.6	+2.3
	C-5'	+2.0	+3.0	+3.0	+2.6
Connectivity ^{c)}	C-9	C-4'	C-4'	C-4'	C-4'
δ (ppm) ^{d)}	101.9	101.5	98.9	98.9	101.4
Specific rotation					
$[\alpha]_D^{28}$	-21.8°	-47.3°	-69.1°	-62.1°	
	(MeOH, $c=1.4$)	(MeOH, $c=0.8$)	(EtOH, $c=0.6$)	(MeOH, $c=0.6$)	

^{a)} Measured in methanol- d_4 . ^{b)} Observed downfield shifts of the carbon signals in the ^{13}C NMR spectra of **1-5** in comparison with those of the aglycones.

^{c)} Carbons which showed connectivities with the anomeric carbons through the oxygens in the HMBC spectra of the peracetates of **1-5**. ^{d)} Observed chemical shifts of the anomeric carbons in the ^{13}C NMR spectra of the peracetates of **1-5**.

results and Table 1, **4** was identified as 7-*O*-methyl cedrusin-4'-*O*- α -L-rhamnopyranoside.

1.5 Compound 5 The hydrolysis of **5** with β -glucosidase furnished the 2-*O*-1'' type neolignan 1-(4'-hydroxy-3'-methoxyphenyl)-2-[4''-(3-hydroxypropyl)-2''-hydroxyphenoxy]-1,3-propanediol^{12,13} and a sugar. This sugar was found to be D-xylose by measuring its ¹H and ¹³C NMR (D₂O) spectra and those of its specimen. From Table 1 as well as the results, **5** was confirmed to be 4'-*O*- β -D-xyloside of the neolignan. The 1-H signal (1/3H, d, *J* = 5.9 Hz) due to *threo* isomer¹² of **5** and another one (2/3H, d, *J* = 5.4 Hz) due to its *erythro* isomer¹² were observed at 4.95 ppm and 4.90 ppm, respectively.

2. Oviposition Stimulant Activity of Compounds 1 to 5 in the Presence of Subfraction I-1 and Fraction III

When subfractions I-1 and I-2 were assayed separately with fraction III, no activity was found (Table 2). However, a combination of subfractions I-1 and I-2 induced an oviposition response of 58%, accounting for the oviposition stimulant activity of fraction I. Addition of subfractions I-3 and/or I-4 to the combination of subfractions I-1 and I-2 were ineffective in enhancing the activity. Combinations without either subfraction I-1 or I-2 failed to induce oviposition, and only subfraction I-1 with subfraction I-4 induced a response of 20% or 25%.

Compounds **1** to **5** isolated from subfraction I-2 exhibited no activity when assayed individually with subfraction I-1 and fraction III (Table 3). On the other hand, a combination of **1** and **2** and another one of **3**, **4** and **5** elicited responses of 20% and 38%, respectively. Elimination of any one of **3**, **4** and **5** led to reduction in the activity. Addition of either **1** or **2** to the combination of **3**, **4** and **5** failed to increase activity. However, **1** to **5** induced a response of 53% when tested all together, accounting entirely for the activity of subfraction I-2.

DISCUSSION

Glucoside **1** of dihydroconiferyl alcohol was first isolated from *P. contorta*.⁷⁾ Glucoside **2** of cedrusin and its rhamnoside **3** have been isolated from *P. sylvestris*.¹⁴⁾ Rhamnoside **4** of 7-*O*-methyl cedrusin has been found in *P. massoniana*.¹⁵⁾ The xyloside **5** of the 2-*O*-1'' type neolignan isolated here was a 1:2 mixture of *threo* and *erythro* isomers, as was previously¹²⁾ found in *P. sylvestris*. Fraction III involves proanthocyanidins, condensed tannins.⁵⁾

Both fractions I-1 and I-2 were indispensable to induce

Table 2 Oviposition responses of female *Monochamus alternatus* to subfractions I-1 to I-4 of fraction I with fraction III.

Substrate ^{a)}						Oviposition response(%) ^{b)}	
I	+	III				68 a	
I-1	+	III				8 b	
I-2	+	III				8 b	
I-3	+	III				0 b	
I-4	+	III				5 b	
I-1	+	I-2	+	III		58 a	
I-1	+	I-2	+	I-3	+	III	55 a
I-1	+	I-2	+	I-4	+	III	53 a
I-1	+	I-2	+	I-3	+	I-4 + III	65 a
I-1	+	I-3	+	III			8 b
I-2	+	I-3	+	III			0 b
I-2	+	I-4	+	III			5 b
I-2	+	I-3	+	I-4	+	III	0 b
I-3	+	I-4	+	III			0 b
I-1	+	I-4	+	III			20 c
I-1	+	I-3	+	I-4	+	III	25 c

^{a)} with 25–35% moisture on a wet basis. ^{b)} Responses with different letters differed significantly ($p < 0.05$, a one-way ANOVA followed by a TURKEY-KRAMER multiple comparison test). $N = 40$.

Table 3 Oviposition responses of female *Monochamus alternatus* to compounds **1** to **5** in the presence of subfraction I-1 and fraction III.

Substrate ^{a)}											Oviposition response(%) ^{b)}			
I-1	+	I-2	+	III							58	a		
I-1	+	1	+	III							3	b		
I-1	+	2	+	III							0	b		
I-1	+	3	+	III							8	b		
I-1	+	4	+	III							0	b		
I-1	+	5	+	III							5	b		
I-1	+	1	+	2	+	III					20	c		
I-1	+	3	+	4	+	5	+	III			38	d		
I-1	+	3	+	4	+	III					20	c		
I-1	+	3	+	5	+	III					18	c		
I-1	+	4	+	5	+	III					15	c		
I-1	+	1	+	3	+	4	+	5	+	III	38	d		
I-1	+	2	+	3	+	4	+	5	+	III	35	d		
I-1	+	1	+	2	+	3	+	4	+	5	+	III	53	a

^{a),b)} See Table 2.

oviposition (Table 2). The glycosides **1** to **5** isolated from subfraction I-2 were characterized as oviposition stimulants for *M. alternatus*. These five compounds exhibited an additive rather than a synergistic effect between them (Table 3).

A few monomeric phenylpropanoids have been characterized as an oviposition stimulant (*trans*-asarone for the carrot fly *Psila rosae*¹⁶⁾), an antifeedant (isoasarone for the common cutworm *Spodoptera litura*¹⁷⁾) and a growth inhibitor (myristicin for the silkworm *Bombyx mori*¹⁸⁾). On the other hand, some of neolignans have been known as antifeedants (asatone for the papilionid butterfly *Luehdorfia puziloi*¹⁹⁾ and magnolol for the fall webworm *Hyphantria cunea*²⁰⁾), an insecticide (conocarpan for the European corn borer *Ostrinia nubilalis*²¹⁾) and a growth inhibitor (licarin A for *S. litura*²²⁾). However, the activity of glycosides of phenylpropanoids and neolignans towards phytophagous insects has not been known. This is the first report on their oviposition stimulant activity towards the insects.

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要 約

フェニルプロパノイドならびにネオリグナンの配糖体：マツ内樹皮に含まれるマツノマダラカミキリ産卵刺激物質

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アカマツ内樹皮の含水アセトン抽出物からエーテル可溶部を除き、残渣のエタノール可溶部を Sephadex LH-20 カラムで画分 I, II 及び III に分画した。画分 II と III に含まれるマツノマダラカミキリ産卵刺激物質は既に同定されているので、画分 I を Amberlite CG-50 カラムで画分 I-1, I-2, I-3 及び I-4 に分画し、画分 I-2 から産卵刺激物質として、フェニルプロパノイド配糖体 dihydroconiferyl alcohol-9-*O*- β -D-glucopyranoside (**1**) と 4 種類のネオリグナン配糖体、即ち, cedrusin-4'-*O*- β -D-glucopyranoside (**2**), cedrusin-4'-*O*- α -L-rhamnopyranoside (**3**), 7-*O*-methyl cedrusin-4'-*O*- α -L-rhamnopyranoside (**4**) 及び 1-(4'-hydroxy-3'-methoxyphenyl)-2-[4''-(3-hydroxypropyl)-2''-hydroxyphenoxy]-1,3-propanediol-4'-*O*- β -D-xylopyranoside (**5**) を単離・同定した。画分 I-1 と画分 III の存在下に 2 種以上の配糖体の混合物は産卵を刺激したが、両画分が存在しても個々の配糖体は産卵を刺激しなかった。