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BIOTRANSFORMATION OF LIGNANS: METABOLISM OF (+)-EUDESMIN AND (+)-MAGNOLIN IN *SPODOPTERA LITURA*

MITSUO MIYAZAWA, HIROYUKI KASAHARA, and HIROMU KAMEOKA

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577, Japan

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Key Word Index—Biotransformation; lignan; *Spodoptera litura*; Noctuidae; (+)-eudesmin; (+)-de-4′-*O*-methyleudesmin; (+)-de-4′-*O*-methyleudesmin-4′-*O*-β-D-glucoside; (+)-magnolin; (+)-de-4″-*O*-methylmagnolin; (+)-de-4″-*O*-methylmagnolin-4″-*O*-β-D-glucoside; de-*O*-methylation; glucoside conjugation.

Abstract—Biotransformation of the plant lignans, (+)-eudesmin and (+)-magnolin in *Spodoptera litura* larvae has been investigated. (+)-De-4′-*O*-methyleudesmin and (+)-de-4′-*O*-methyleudesmin-4′-*O*-β-D-glucoside were identified from the (+)-eudesmin-administered larvae faeces, and (+)-de-4″-*O*-methylmagnolin and (+)-de-4″-*O*-methylmagnolin-4″-*O*-β-D-glucoside were from (+)-magnolin-administered, respectively. The metabolic reaction of (+)-eudesmin and (+)-magnolin in *Spodoptera litura* larvae is de-*O*-methylation at *para*-position on veratryl and 3,4,5-trimethoxyl groups followed by glucosylation.

INTRODUCTION

A large number of natural lignans and neolignans which posses biological activities towards insects have been identified in recent decades; e.g. β-peltatin-A-methyl ether, deoxypodophllotoxin and deoxypicropodophyllin and a number of podophyllotoxin analogues inhibit insect larval growth [1] as do (−)-machilusin and its analogue against *Spodoptera litura* larvae [2] and (+)-sesamin and (+)-kobusin against silkworm larvae (*Bombix mori* L.) [3]. There are, however, few reports in which the metabolism of lignans in insects has been investigated. *Spodoptera litura* (Noctuidae) is a well-known rest insect and recently a study of larval growth inhibitory lignans against the insect was reported [2]. We have therefore investigated the metabolism of (+)-eudesmin and (+)-magnolin in larvae of this insect. Previously, we investigated biotransformation of the furofuran lignans, (+)-magnolin and (+)-yangabin in rat [4] and (+)-eudesmin and (+)-magnolin by fungus, *Aspergillus niger* [5], and revealed that the first metabolic reaction in both cases was specific de-*O*-methylation at the *p*-position. Our present results suggest the existence of similar oxidases for the metabolism of magnolin type lignans in mammalia, fungi and insects.

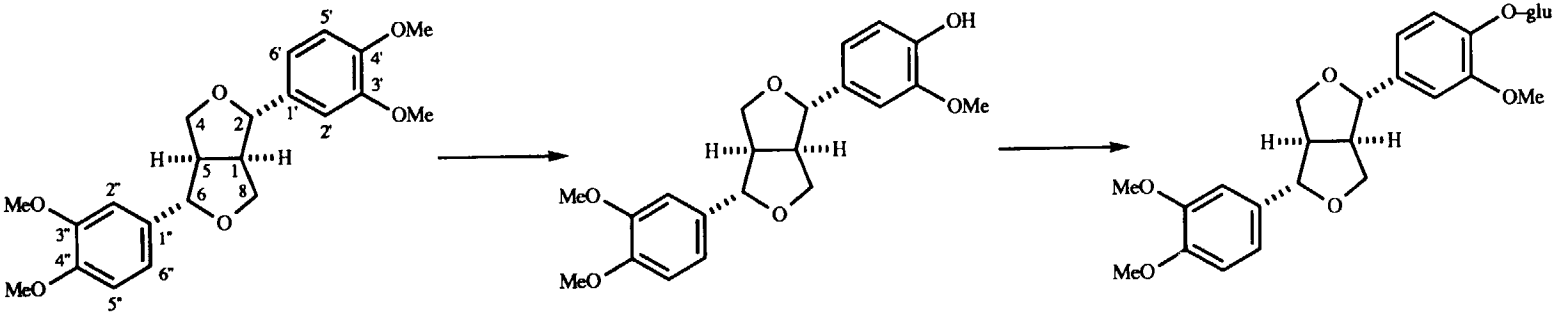
RESULTS AND DISCUSSION

(+)-Eudesmin (**1**) and (+)-magnolin (**2**) were incorporated into the artificial diet using cellulose powder as an inert carrier, and fed to fourth or fifth instar larvae, respectively. Faeces were collected for 4 days, dried, extracted by CH_2Cl_2 (100 ml × 3) and then EtOAc (100 ml × 2), and evaporated under reduced pressure. The CH_2Cl_2 extract of **1**-administered larvae faeces was subjected to silica gel CC to give novel metabolites **3** and **4**. Metabolites **5** and **6** were isolated from the CH_2Cl_2 extract of **2**-administered larvae faeces.

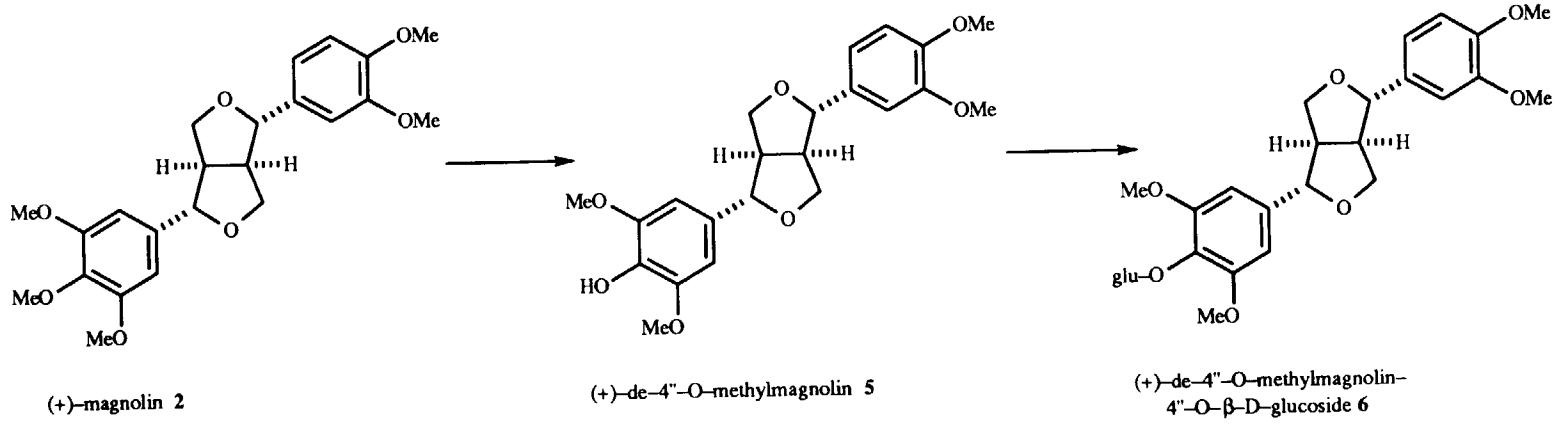
Metabolic product **3** had a molecular formula $C_{21}H_{24}O_6$ as determined by a high resolution mass spectrum and NMR data. The IR spectrum contained a hydroxyl band 3418 cm^{-1}, and the specific rotation showed (+)-form. The ^1H and ^{13}C NMR spectra clearly corresponded with those of (+)-de-4′-*O*-methylmagnolin [5].

The negative FAB-mass spectrum of **4** showed $[M]^+$ at *m/z* 533.2029 $[M - H]^+$, corresponding to the molecular formula $C_{27}H_{33}O_{11}$ (calcd 533.2023). A peak at *m/z* 371 $[M - H - 162]^+$ was due to the loss of a hexose moiety from the $[M]^+$. The ^1H NMR spectrum of **4** showed similar signals to those of **3** except for the lack of signals for 4′-phenolic hydroxyl proton and the existence of that of an anomeric proton at δ4.82 (*d*, *J* = 6 Hz) and those due to the glucosyl group. Acid hydrolysis of **4** gave glucose which was identified by TLC, and an aglycone which was identified as **3** by spectroscopic data. The ^{13}C NMR signal pattern of the sugar showed that **4** contained a β-glucose. The specific optical rotation shows the (+)-form; therefore, **4** is (+)-de-4′-*O*-methyleudesmin-4′-*O*-β-D-glucoside.

Metabolic product **5** had a molecular formula $C_{22}H_{26}O_7$ as determined by a high resolution mass spectrum and NMR data. The IR spectrum contained a hydroxyl band at 3387 cm^{-1}, and the specific rotation



Scheme 1. Metabolic pathway of (+)-eudesmin (**1**) to (+)-de-4′-*O*-methyleudesmin (**3**) and (+)-de-4′-*O*-methyleudesmin-4′-*O*-β-D-glucoside (**4**) in *Spodoptera litura* larvae.



Scheme 2. Metabolic pathway of (+)-magnolin (**2**) to (+)-de-4″-*O*-methylmagnolin (**5**) and (+)-de-4′-*O*-methylmagnolin-4″-*O*-β-D-glucoside (**6**) in *Spodoptera litura* larvae.

showed the (+)-form. The ^{1}H NMR spectrum was similar to that of (+)-de-4′-*O*-methylmagnolin [4, 5]; however, the ^{13}C NMR spectrum of **5** showed signals due to veratryl and syringyl groups [4]. The mass spectrometry confirmed the existence of a veratryl group (*m/z* 151 $[ArCH_2]^+$ and 165 $[ArCO]^+$) and a syringyl group (*m/z* 167 $[ArCH_2]^+$ and 181 $[ArCO]^+$). Therefore, **5** is (+)-de-4′-*O*-methylmagnolin.

The negative FAB-mass spectrum of **6** showed $[M]^+$ at *m/z* 563.2154 $[M - H]^+$, corresponding to the molecular formula $C_{28}H_{35}O_{12}$ (calcd 563.2129). A peak at *m/z* 401 $[M - H - 162]^+$ was due to the loss of a hexose moiety from the $[M]^+$. Acid hydrolysis of **6** gave glucose which was identified by TLC and an aglycone which was identified as **5** by spectroscopic data. The ^{1}H and ^{13}C NMR spectra reconfirmed that **6** was the glucoside of de-4″-*O*-methylmagnolin. The signals for the anomeric proton of **6** shifted at δ4.62 (*d*, $J = 7.5$ Hz) and the anomeric carbon signal at δ105.5 indicated α-configuration for the anomeric proton and hence the presence of a β-glucosidic linkage. The specific optical rotation shows the (+)-form; therefore, **4** is (+)-de-4″-*O*-methylmagnolin-4″-*O*-β-D-glucoside.

The possible metabolic pathways of **1** and **2** are shown in Schemes 1 and 2. Compounds **1** and **2** were completely metabolized to respective metabolites **3** and **5**; furthermore, the large amounts of **3** and **5** were glucoside conjugated and excreted in the faeces. Up to now, there are no reports of the metabolism of these lignans in insect. Although the oxidation of **1** occurred at the *p*-methoxyl of its veratryl group, 3,4,5-trimethoxyl group of **2** was oxidized preferentially to the veratryl group. This result is apparently different from that of the fungus *A. niger*, which preferentially oxidizes the veratryl group [5].

With regard to the conjugation reaction, it is known that insects possess some conjugation systems, e.g. glucuronide, sulphate and glucoside conjugation etc., as secondary metabolic processes of phenols [6]. Lignans **1** and **2** were conjugated as glucosides in *S. litura* larvae. This result differs from the glucuronide conjugation which takes place in rats when **2** and (+)-yangabin are administered [4].

EXPERIMENTAL

Preparation of lignans. (+)-Eudesmin (**1**) and (+)-magnolin (**2**) were isolated from flower buds of *Magnolia fargesii* by previously reported methods [7].

Insect and cultivation conditions. A hundred larvae of *S. litura* were grown at 25°, and fed a commercial artificial diet (Insecta LF, Nihon Nosan Kogyo) until larvae had become third instar. After third instar, larvae were fed artificial diet: kidney bean (wet) 100 g, brewer's dried yeast 11.5 g, L-(+)-ascorbic acid 1 g, formaldehyde soln 3 ml, agar 4.5 g, H_2O 180 ml.

Administration of **1**. Fifty larvae (fourth to fifth instar) were fasted for 2 days before administration of **1** (142 mg). (+)-Eudesmin (**1**) was incorporated into the artificial diet (100 g) using cellulose powder as an inert carrier and fed to larvae. After the artificial diet containing **1** was exhausted (2–3 days), the larvae were fed artificial diet henceforth.

Table 1. ^{1}H NMR spectral data for lignans (**1** and **2**) and metabolic products (**3–6**)

H	1	2	3	4	5	6
1	3.12 *m*	3.11 *m*	3.11 *m*	3.03 *m*	3.10 *m*	3.07 *m*
2	4.76 *d* (4)	4.77 *d* (5)	4.74 *d* (4.5)	4.66 *d* (4.5)	4.77 *d* (5)	4.74 *d* (5)
4ax	3.90 *dd* (4.9)	3.92 *dd* (4.9)	3.89 *dd* (4.9)	3.82 *m*[a]	3.90 *dd* (4.9)	3.90 *m*[b]
4eq	4.26 *dd* (7.9)	4.28 *dd* (7.9)	4.26 *dd* (7.9)	4.20 *dd* (7.9)	4.28 *dd* (7.9)	4.26 *dd* (7.9)
5	3.12 *m*	3.11 *m*	3.11 *m*	3.03 *m*	3.11 *m*	3.07 *m*
6	4.76 *d* (4)	4.75 *d* (5)	4.76 *d* (4.5)	4.70 *d* (4.5)	4.72 *d* (5)	4.71 *d* (5)
8ax	3.90 *dd* (4.9)	3.92 *dd* (4.9)	3.89 *dd* (4.9)	3.82 *m*[a]	3.90 *dd* (4.9)	3.90 *m*[b]
8eq	4.26 dd (7.9)	4.29 *dd* (7.9)	4.26 *dd* (7.9)	4.20 *dd* (7.9)	4.28 *dd* (7.9)	4.26 *dd* (7.9)
2′	6.91 *d* (2)	6.91 *d* (2)	6.90 *d* (2)	6.88 *s*	6.91 *d* (2)	6.89 *d* (2)
5′	6.84 *d* (8)	6.84 *d* (8)	6.89 *d* (8)	6.99 *d* (8)	6.85 *d* (8)	6.83 *d* (8)
6′	6.89 *dd* (2.8)	6.89 *dd* (2.8)	6.82 *dd* (2.8)	6.79 *dd* (8)	6.88 *dd* (2.8)	6.86 *dd* (2.8)
2″	6.91 *d* (2)	6.58 *s*	6.91 *d* (2)	6.86 *d* (2)	6.59 *s*	6.55 *s*
5″	6.84 *d* (8)	—	6.84 *d* (8)	6.80 *d* (8)	—	—
6″	6.89 *dd* (2.8)	6.58 *s*	6.88 *dd* (2.8)	6.84 *dd* (2.8)	6.59 *s*	6.55 *s*
OMe						
3′	3.88 *s*	3.88 *s*	3.90 *s*	3.74 *s*	3.88 *s*	3.86 *s*
4′	3.91 *s*	3.91 *s*	—	—	3.90 *s*	3.88 *s*
3″	3.88 *s*	3.88 *s*	3.87 *s*	3.86 *s*	3.90 *s*	3.81 *s*
4″	3.91 *s*	3.84 *s*	3.90 *s*	3.84 *s*		
5″	—	3.38 *s*	—	—	3.90 *s*	3.81 *s*
OH	—	—	5.58 *s*	—	5.51 *s*	—
Glu-H-	—	—	—	4.82 *d* (6)	—	4.62 *d* (7.5)

Recorded in $CDCl_3$, chemical shift values are reported as δ values from TMS at 270.1 MHz for **1**, **2** and **4**, and 500.0 MHz for **3**, **5** and **6**.

Coupling constant in Hz. [a,b]Values are overlapped with other signals.

Isolation of metabolites **3** *and* **4** *from faeces.* Faeces were collected for 4 days, dried (15.8 g), extracted by CH_2Cl_2 (100 ml × 3) and then EtOAc (100 ml × 2), and evapd under red. pres. The CH_2Cl_2 extract (250 mg) of **1**-administered larvae faeces was subjected to silica gel CC to give novel metabolites **3** (5 mg) and **4** (166 mg).

Administration of **2**. Fifty larvae (fourth to fifth instar) were fasted for 2 days before administration of **2** (348 mg). (+)-Magnolin (**2**) was incorporated into the artificial diet (100 g) using cellulose powder as an inert carrier and fed to larvae.

Isolation of metabolites **5** *and* **6** *from faeces.* Faeces were collected for 4 days, dried (17.6 g), extracted by CH_2Cl_2 (100 ml × 3) and then EtOAc (100 ml × 2), and evapd under red. pres. The CH_2Cl_2 extract (515 mg) and **2**-administered larvae faeces was subjected to silica gel CC to give novel metabolites **5** (10 mg) and **6** (230 mg).

Determination of sugars in compounds **4** *and* **6**. A soln of each glycoside (2 mg) in 8% HCl–dioxane (1:1) (1 ml) was refluxed for 3 hr. Sugars were analysed by TLC on silica gel with EtOAc–H_2O–MeOH–HOAc (13:3:3:4).

(+)-*De*-4′-O-*methyleudesmin* (**3**). Oil. HRMS *m/z*: 372.1602 ($[M]^+$, calcd for $C_{21}H_{24}O_6$: 372.1573). MS *m/z* (rel. int.): 372 ($[M]^+$, 100), 341 (15), 294 (50), 205 (20), 177 (40), 165 (50), 151 (68), 137 (28). $[\alpha]_D$ + 40.6° ($CHCl_3$; *c* 0.75). NMR: see Tables 1 and 2.

(+)-*De*-4′-O-*methyleudesmin*-4′-O-β-D-*glucoside* (**4**). Amorphous powder. FAB-MS *m/z* (rel. int.): 533.2029 $[M - H]^+$ (28) (calcd for $C_{27}H_{33}O_{11}$: 533.2023), 371 $[M - H - 162]^+$ (100). $[\alpha]_D$ + 0.94° ($CHCl_3$; *c* 4.35). NMR: see Tables 1 and 2.

(+)-*De*-4″-O-*methylmagnolin* (**5**). Oil. HRMS *m/z*: 402.1663 ($[M]^+$, calcd for $C_{22}H_{26}O_7$: 402.1648). EIMS *m/z* (rel. int.): 402 ($[M]^+$, 100), 371 (8), 235 (8), 210 (15), 193 (20), 181 (35), 177 (36), 167 (25), 165 (40), 151 (37). $[\alpha]_D$ + 7.59° ($CHCl_3$; *c* 3.0). NMR: see Tables 1 and 2.

(+)-*De*-4″-O-*methylmagnolin*-4″-O-β-D-*glucoside* (**6**). Amorphous powder. FAB-MS *m/z* (rel. int.): 563.2154 $[M - H]^+$ (10) (calcd for $C_{28}H_{35}O_{12}$: 563.2129), 401 $[M - H - 162]^+$ (52). $[\alpha]_D$ + 7.69° ($CHCl_3$; *c* 4.15). NMR: see Tables 1 and 2.

Table 2. ^{13}C NMR spectral data for lignans **1**–**6** (δ, TMS, in $CDCl_3$)

C	1	2	3	4	5	6
1	54.0	54.1	54.1	54.3	54.1	54.0
2	85.6	85.7	85.9	85.7	85.7	85.8
4	71.6	71.7	71.7	71.8	71.7	71.6
5	54.0	54.4	54.1	54.0	54.4	54.5
6	85.6	86.0	85.8	85.6	86.1	85.6
8	71.6	71.9	71.7	71.5	71.9	72.0
1′	133.4	133.5	132.9	136.5	133.5	133.3
2′	109.1	109.2	108.6	111.1	109.3	109.3
3′	149.1	149.0	146.7	149.5	149.2	149.2
4′	148.5	148.7	145.2	145.6	148.7	148.7
5′	110.9	111.1	114.3	117.2	111.1	111.1
6′	118.1	118.2	118.9	118.6	118.2	118.2
1″	133.4	136.8	133.5	133.4	132.2	134.5
2″	109.1	102.8	109.2	109.3	102.8	103.0
3″	149.1	153.4	149.2	149.2	147.2	152.9
4″	148.5	137.5	148.6	148.6	134.3	138.4
5″	110.9	153.4	109.3	110.0	147.2	152.9
6″	118.1	102.8	118.2	118.2	102.8	103.0
Glu						
1	—	—	—	101.8	—	105.5
2	—	—	—	73.2	—	74.1
3	—	—	—	76.0	—	76.3
4	—	—	—	69.4	—	69.8
5	—	—	—	75.9	—	76.0
6	—	—	—	61.4	—	61.9
OMe						
3′	55.8	55.9	55.9	56.1	55.9	55.9
4′	55.8	55.9	—	—	56.0	55.9
3″	55.8	56.2	55.9	56.0	56.4	56.4
4″	55.8	60.9	55.9	56.0	—	—
5″	—	56.2	—	—	56.4	56.4

Recorded at 67.5 MHz for **1**, **2** and **4**, and 125.7 MHz for **3**, **5** and **6**.

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