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O-DEMETHYLATION OF *MESO*-DIMETHYL DIHYDROGUAIARETIC ACID IN *SPODOPTERA LITURA*

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Key Word Index—*Spodoptera litura*; Noctuidae; *meso*-dimethyl dihydroguaiaretic acid; *meso*-monomethyl dihydroguaiaretic acid; diarylbutadiene; *O*-demethyldiarylbutadiene.

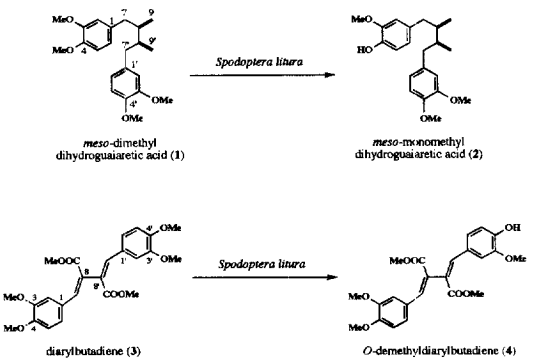
Abstract—Biotransformation of *meso*-dimethyl dihydroguaiaretic acid and diarylbutadiene in *Spodoptera litura* larvae has been investigated. These acyclic lignans were demethylated at the *para*-position of the veratryl group and transformed to *meso*-monomethyl dihydroguaiaretic acid and *O*-demethyldiarylbutadiene by larvae, respectively. © 1997 Elsevier Science Ltd

INTRODUCTION

We previously showed that 7,9′,7′,9-bisepoxylignan, (+)-eudesmin, was first oxidatively demethylated at the *para*-position of its veratryl (3,4-dimethoxyphenyl) group and then conjugated with glucose by the larvae of *Spodoptera litura* [1]. The yield of lignan glucoside was estimated to be 84.8%. A 7,9′-epoxylignan, (−)-magnofargesin was also transformed, but in contrast, *O*-demethylation occurred at the *meta*-position of the 3,4,5-trimethoxyphenyl group, to (−)-3-*O*-demethylmagnofargesin [2]. In order to clarify the molecular recognition of enzymes for the oxidative cleavage of methoxy groups on the aromatic rings, *meso*-dimethyl dihydroguaiaretic acid (**1**), a diarylbutane type lignan in which terminal groups are fully reduced, was fed to larvae of *S. litura*. As a result, a sole *O*-demethylated metabolite **2** was obtained. Diarylbutadiene (**3**), a derivative of diarylbutyrolactone type lignan [3], was also fed [4], because it has a α,β-unsaturated veratryl group which in (−)-magnofargesin was not transformed by larvae. Consequently, it was also transformed to *O*-demethylated metabolite **4**.

RESULTS AND DISCUSSION

Meso-dimethyl dihydroguaiaretic acid (**1**) was incorporated into the artificial diet using cellulose



powder as an inert carrier [1]. Artificial diet containing 1% of **1** was fed to the larvae for 2 days and then artificial diet without **1** was fed for an additional 2 days. Faeces were collected, extracted with dichloromethane and the extract was subjected to silica gel column chromatography to give metabolite **2** and unreacted **1**. No other metabolites, like a glucoside, could be identified by TLC, GC and GC-mass spectrometry. The EI-mass spectrum of metabolite **2** showed a $[M]^+$ at *m/z* 344, which was one CH_2 mass unit less than **1**. It also showed a significant mass fragments at *m/z* 151 (base peak) and at *m/z* 137. Those peaks indicated the existence of veratryl and guaiacyl (4-hydroxy-3-methoxyphenyl) group of diarylbutane type lignans. Infrared spectrum of **2** contained a hydroxyl band at 3441 cm^{-1}. The ^1H NMR spectrum of **2** showed the existence of a hydroxyl (δ 5.49) and three methoxyl proton signals (δ 3.85–3.86). NMR and DEPT spectra showed that **2** has a guaiacyl group. The GC of **2** coincided completely with that of authentic *meso*-monomethyl dihydroguaiaretic acid.

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All our results led us to conclude that **2** is *meso*-monomethyl dihydroguaiaretic acid.

Diarylbutadiene (**3**) was administered to the larvae by the same method. Faeces were collected, extracted with dichloromethane and the extract was subjected to silica gel column chromatography to give **4** and unreacted **3**. From GC-mass spectral analysis, two other metabolites were detected but they could not be identified due to the small quantities present. The structure of the metabolite **4** was deduced from mass-spectral and NMR data. The metabolite **4** had a molecular formula $C_{23}H_{24}O_8$ which was one CH_2 mass unit less than **3**. Infrared spectrum of **4** contained a hydroxyl adsorption band at 3441 cm^{-1}. The mass spectrum showed a $[M]^+$ at *m/z* 428. It also showed significant mass fragments at *m/z* 151 and at *m/z* 137. Those peaks indicated the existence of veratryl and guaiacyl groups. The ^1H NMR spectrum of **4** contained signals for three methoxyl groups (δ 3.70–3.75) and a hydroxyl group (δ 5.80). NMR and DEPT spectra of **4** supported the existence of a guaiacyl group. As a result, the structure of **3** was firmly deduced as *O*-demethyldiarylbutadiene (**4**).

We already revealed that *S. litura* metabolizes 7,9′,7′,9-bisepoxylignans and 7,9′-epoxylignan. In this report, we attempted biotransformation of a diarylbutane type and a diarylbutyrolactone type lignan by this insect. As a result, both acyclic lignans were oxidatively demethylated at the *para*-methoxy group on the veratryl group and aryl group, respectively. No lignan glucosides were obtained. The conversion rate of **1** to metabolite **2** was 5.2%; this was lower than those of **3** and **4** (11.5%) and (+)-eudesmin to (+)-de-*O*-methyleudesmin-4′-*O*-β-D-glucoside (84.8%). This insect can cleave the α,β-unsaturated veratryl group of **3**, while that of (−)-magnofargesin was not attacked. With regard to biotransformation of some lignans in mammals, it has been demonstrated that *O*-demethylation on aromatic rings can be catalysed by intestinal bacteria [5, 6]. Since it was also revealed that this insect's faecal flora can transform some terpenoids [7], we have to clarify whether the faecal flora or endogenous enzymes of this insect are responsible for *O*-demethylation of these lignans.

We also attempted biotransformation of these two lignans by *A. niger*, a fungus which can metabolize some lignans, but they were not transformed.

EXPERIMENTAL

Preparation of lignans. A diarylbutane **1** was prepd by the methylation of *meso*-dihydroguaiaretic acid by methyl iodide and K_2CO_3 in Me_2CO. *Meso*-dihydroguaiaretic acid was isolated from the bark of *Machilus thunbergii* [8]. A diarylbutyrolactone **3** was prepd by Pelter's method [3].

Insect and cultivation conditions. 50 larvae of *S. litura* were reared at 25°, and fed a commercial artificial diet (Insecta, LF, Nihon Nosan Kogyo) until larvae had reached the third instar. Thereafter, larvae were fed artificial diet consisting of: kidney bean (wet) 100 g, agar 4.5 g, H_2O 180 ml.

Administration of **1** *and isolation of metabolite* **2** *from faeces.* 50 larvae (fourth to fifth instar) were starved for 2 days before administration of **1** (100 mg). *Meso*-dimethyl dihydroguaiaretic acid (**1**) was incorporated into the artificial diet (9.9 g) using cellulose powder as an inert carrier and fed to larvae. After eating all of the artificial diet containing **1** (2 days), the larvae were fed untreated artificial diet. Faeces were collected for 4 days, extracted by CH_2Cl_2 (100 ml × 3) and then EtOAc (100 ml × 2), and evapd under red. pres. The combined extract was sepd into acidic, phenolic and neutral portions in the usual way. The phenolic portion was subjected to silica gel CC repeatedly to give a metabolite **2** (5 mg) and unreacted **1** (51 mg). No metabolic products were detected from acidic and neutral portions by TLC and GC-MS.

Administration of **3** *and isolation of metabolite* **4** *from faeces.* 305 mg of diarylbutadiene (**3**) was administered to the larvae of *S. litura via* the artificial diet at the same concn as **1**, and the larvae were reared under the same conditions. Faeces were collected, extracted with CH_2Cl_2 (100 ml × 3) and then EtOAc (100 ml × 2), and the solvent was evapd under red. pres. to give the extract. The extract was subjected to silica gel CC to give **4** (35 mg) and unreacted **3** (113 mg).

GC MS analysis. Analysis was carried out using a capillary column: HP-5MS (Cross linked 5% Ph Me Silicone 0.25 mm i.d. × 30 m). Programming from 180 to 290° at 4° min^{-1} and held at 290°. Injection and detection ports were held at 300°. The flow rate of carrier gas (He) was 1 ml min^{-1}. *Meso*-dimethyl dihydroguaiaretic acid (**1**) was detected at R_t 18.60 min, and *meso*-monomethyl dihydroguaiaretic acid (**2**) was at R_t 18.43 min. Diarylbutadiene (**3**) was detected at R_t 25.48 min, and *O*-demethyldiarylbutadiene (**4**) was at R_t 25.01 min.

Meso-*dimethyl dihydroguaiaretic acid* (**1**). Crystals. $C_{22}H_{30}O_4$, EIMS *m/z* (rel. int.): 358 ($[M]^+$, 25), 152 (15), 151 (100), 107 (5). IR ν_{max} cm^{-1}: 2955, 1590, 1516, 1417, 1261, 1237, 1157, 1030. NMR see Table 1.

Meso-*monomethyl dihydroguaiaretic acid* (**2**). Colourless oil. $C_{21}H_{28}O_4$. EIMS *m/z* (rel. int.): 344 ($[M]^+$, 50), 152 (15), 151 (100), 137 (60), 107 (5). IR ν_{max} cm^{-1}: 3441, 2957, 1607, 1591, 1516, 1418, 1265, 1237, 1155, 1031. NMR see Table 1.

Diarylbutadiene (**3**). Yellow oil. $C_{24}H_{26}O_8$, EIMS *m/z* (rel. int.): 442 ($[M]^+$, 80), 351 (100), 273 (75), 181 (20), 151 (70). IR ν_{max} cm^{-1}: 3021, 2954, 2838, 1708, 1631, 1597, 1515, 1250, 1143, 1024. ^1H NMR (500.0 MHz, $CDCl_3$) δ 3.70 (*s*, 4,4′-OMe), 4.75 (*s*, 3,3′-OMe), 3.86 (*s*, —COOMe), 6.80 (*d*, *J* = 8.5 Hz, H-5,5′), 7.12 (*dd*, *J* = 8.5 Hz, 2, H-6′,6″), 7.15 (*d*, *J* = 2 Hz, H-2,2″), 7.89 (*s*, H-7,7′). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 52.4

[a-f] Values with the same letter are interchangeable.

Table 1. NMR spectral data for diarylbutane **1** and **2**

posit.	^{1}H NMR data* 1	2	^{13}C NMR data† 1	2
1			134.5	133.7
2	6.65 (*d*, *J* = 2)	6.62 (*d*, *J* = 2)[a]	111.0	111.4[f]
3			148.7	146.3
4			147.1	143.5
5	6.79 (*d*, *J* = 8)	6.82 (*d*, *J* = 8)[b]	112.3	113.9
6	6.70 (*dd*, *J* = 8, 2)	6.66 (*dd*, *J* = 8, 2)[c]	120.9	121.6
7	2.30 (*dd*, *J* = 13.4, 9.3)	2.30 (*dd*, *J* = 13.4, 9.3)	39.2	39.0[g]
	2.76 (*dd*, *J* = 13.4, 4.8)	2.76 (*dd*, *J* = 13.4, 4.8)		
8	1.77 (*m*)	1.77 (*m*)	38.8	38.9[h]
9	0.85 (*d*, *J* = 6.7)	0.84 (*d*, *J* = 6.7)[d]	16.2	16.1[i]
1′			134.5	134.4
2′	6.65 (*d*, *J* = 2)	6.64 (*d*, *J* = 2)[a]	111.0	111.0[f]
3′			148.7	148.6
4′			147.1	147.0
5′	6.79 (*d*, *J* = 8)	6.78 (*d*, *J* = 8)[b]	112.3	112.2
6′	6.70 (*dd*, *J* = 8, 2)	6.69 (*dd*, *J* = 8, 2)[c]	120.9	120.9
7′	2.30 (*dd*, *J* = 13.4, 9.3)	2.30 (*dd*, *J* = 13.4, 9.3)	39.2	39.2[g]
	2.76 (*dd*, *J* = 13.4, 4.8)	2.76 (*dd*, *J* = 13.4, 4.8)		
8′	1.77 (*m*)	1.77 (*m*)	38.8	38.7[h]
9′	0.85 (*d*, *J* = 6.7)	0.86 (*d*, *J* = 6.7)[d]	16.2	16.2i
OMe	3.85 (*s*, 6H)	3.84 (*s*, 6H)	55.8 (×2)	55.8 (×2)
	3.86 (*s*, 6H)	3.85 (*s*, 3H)	55.9 (×2)	55.7
OH	—	5.49 (*s*)		

* ^{1}H NMR recorded at 500.0 MHz in $CDCl_3$, *J* in hertz, and TMS as internal standard.
† ^{13}C NMR at 125.7 MHz in $CDCl_3$, TMS as internal standard.
[a–i] Interchangeable.

(9,9′-OMe), 55.6 (4,4′-OMe), 55.8 (3,3′-OMe), 110.8 (C-5,5′), 111.8 (C-2,2′), 124.4 (C-6,6′),[a] 124.6 (C-7,7′)[a] 127.5 (C-8,8′), 142.3 (C-1,1′), 148.7 (C-4,4′), 150.5 (C-3,3′), 167.7 (C-9,9′).

O-*Demethyl diarylbutadiene* (**4**). Yellow oil. $C_{23}H_{24}O_8$, 428 ([M]$^+$, 90), 337 (100), 273 (45), 260 (45), 181 (20), 151 (45), 137 (20). IRν_{max} cm^{-1}: 3014, 2947, 2850, 1707, 1630, 1597, 1515, 1252, 1143 1026. ^{1}H NMR (500.0 MHz, $CDCl_3$) δ 3.70 (*s*, 4-OMe), 4.75 (*s*, 3,3′-OMe), 3.86 (*s*, —COOMe), 6.80 (*d*, *J* = 8.5 Hz, H-5), 6.84 (*d*, *J* = 8.5 Hz, H-5′), 7.06 (*dd*, *J* = 8.5, 2 Hz, H-6′), 7.11 (*dd*, *J* = 8.5, 2 Hz, H-6), 7.13 (*d*, *J* = 2 Hz, H-2),[a] 7.14 (*d*, *J* = 2, H-2′),[a] 7.87 (*s*, H-7′),[b] 7.88 (*s*, H-7).[b] ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 52.4 (C-9,9′), 55.7 (4,3′-OMe),[a] 55.8 (3-OMe),[a] 110.9 (C-5), 111.4 (C-2′),[b] 111.9 (C-2),[b] 114.6 (C-5′), 124.1 (C-7), 124.5 (C-6),[c] 124.6 (C-7′),[c] 125.3 (C-6′), 127.1 (C-8′),[d] 127.5 (C-8),[d] 142.3 (C-1′),[e] 142.5 (C-1),[e] 146.4 (C-4′), 147.4 (C-3′), 148.7 (C-4), 150.5 (C-3), 167.7 (C-9′),[f] 167.8 (C-9).[f]

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