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BIOTRANSFORMATION OF LIGNANS: *META-O*-DEMETHYLATION OF (−)-MAGNOFARGESIN IN *SPODOPTERA LITURA*

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Key Word Index—*Spodoptera litura*; Noctuidae; 7,9′-epoxylignan; (−)-magnofargesin; (−)-3-*O*-demethylmagnofargesin; biotransformation.

Abstract—Biotransformation of (−)-magnofargesin in *Spodoptera litura* larvae has been investigated. (−)-Magnofargesin was *O*-demethylated, at the *meta*-position of its 3,4,5-trimethoxyphenyl group, to (−)-3-*O*-demethylmagnofargesin. Copyright © 1996 Elsevier Science Ltd

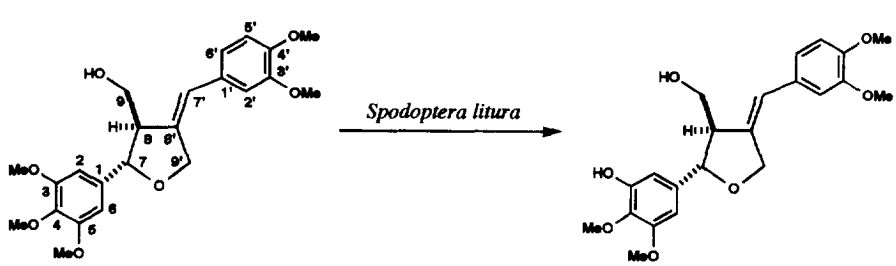
INTRODUCTION

We have been investigating the biotransformation of lignans and neolignans. Thus it was revealed that the first metabolic reaction of 7,9′,7′,9-bisepoxylignans such as (+)-magnolin in mammal (rat) [1], insect (*Spodoptera litura* larvae) [2] and a fungus (*Aspergillus niger*) [3] was the same *O*-demethylation at the *para*-position of their aryl groups. In addition, 7,7′-epoxylignans such as (+)-veraguensin were also *O*-demethylated at *para*-position by *A. niger* [4]. This report deals with biotransformation of (−)-magnofargesin (**1**) [5], a member of the 7,9′-epoxylignans, by *S. litura* larvae.

RESULTS AND DISCUSSION

The faeces of (−)-magnofargesin-administered larvae were collected and extracted with $CHCl_3$ and EtOAc. The sole metabolic product **2** was detected on TLC and GC (conversion rate: 40%). The combined organic layer was chromatographed on a SiO_2 gel column. The metabolic product **2** was recrystallized from ether and 15 mg was isolated.

The metabolic product **2** had a molecular formula $C_{22}H_{26}O_7$([M]$^+$ *m/z* 402.1674 Δ 0.5 mmu), which was one CH_2 mass unit less than **1**, established by HRMS and NMR data. The specific rotation showed that **2** was the (−)-form. The infrared spectrum contained a wide hydroxyl band at 3439 cm^{-1}. The mass spectrum of **2** showed two assignable ions at *m/z* 220 [M − ArCO]$^+$ (Ar = 7-aryl group) and 189 [220 − CH_2OH]$^+$. Those ions were contained in the mass spectrum of **1**. The NMR spectra of **2** were similar to those of **1** except for the existence of a hydroxyl group and the disappearance of a methoxyl group. The previous results for the biotransformation of 7,9′,7′,9-bisepoxylignans and 7,7′-epoxylignans reminded us that the regioselective *O*-demethylation at *para*-position also occurred in this 7,9′-epoxylignan. However, ^{1}H NMR signals for H-2 and 6 shifted at δ 6.58 and 6.62, respectively, while H-2 and 6 of **1** shifted at δ 6.63. Detailed analysis of NMR and DEPT spectra showed that **2** possesses a 4,5-dimethoxy-3-hydroxyphenyl group. All these spec-



(-)-magnofargesin (1)

(-)-3-*O*-demethylmagnofargesin (2)

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tral data show that **2** is (−)-3-*O*-demethylmagnofargesin.

Biotransformation of (−)-magnofargesin (**1**) by *S. litura* larvae afforded the single metabolite, (−)-3-*O*-demethylmagnofargesin (**2**). No glycoside of **2** was obtained while glucosides were obtained when 7,9′,7′,9-bisepoxylignans such as (+)-magnolin were transformed by this insect [9]. Furthermore, **2** is a new lignan differ from the naturally occurring lignan in possessing a phenolic hydroxyl group in the *meta*-position of the aromatic ring [6]. Biological evaluation of **2** is underway.

EXPERIMENTAL

Isolation of (−)-*magnofargesin*. (−)-Magnofargesin (**1**) was prepared from *Magnolia fargesii* by previously reported methods [5].

Table 1. NMR spectral data for (−)-3-*O*-demethylmagnofargesin (**2**)

Position	^{1}H	^{13}C
1		135.0
2	6.54 (*d*, *J* = 2)	105.9
3		149.3
4		137.5
5		152.5
6	6.64 (*d*, *J* = 2)	102.0
7	4.82 (*d*, *J* = 6)	82.0
8	2.97 (*m*)	55.0
9	3.82 (*dd*, *J* = 11, 5)	62.9
	3.95 (*dd*, *J* = 11, 6)	
1′		139.3
2′	6.70 (*d*, *J* = 2)	111.5
3′		148.9
4′		148.2
5′	6.88 (*d*, *J* = 8)	111.2
6′	6.72 (*dd*, *J* = 8, 2)	120.7
7′	6.39 (*dd*, *J* = 2, 2)	121.7
8′		129.9
9′	4.75 (*ddd*, *J* = 14, 2, 2)	70.1
	4.91 (*dd*, *J* = 14, 2)	
OMe		
4	3.86 (*s*)[a]	60.9
5	3.88 (*s*)[a]	55.9
3′	3.90 (*s*)	55.9
4′	3.90 (*s*)	55.9
9-OH	1.66 (*br s*)	
ArOH	5.77 (*s*)	

^{1}H NMR recorded at 270.1 MHz in $CDCl_3$, *J* in hertz, and TMS as internal standard.

^{13}C NMR at 67.8 MHz in $CDCl_3$, TMS as internal standard.

[a]Interchangeable.

Insect and cultivation conditions. Fifty larvae of *S. litura* were grown at 25°, and fed a commercial artificial diet (Insecta LF, Nihon Nosan Kogyo) until larvae had been transformed into the third instar. After the third instar, larvae were fed an artificial diet: kidney bean (wet) 100 g, agar 4.5 g, H_2O 180 ml.

Administration of **1** *to larvae*. Fifty larvae (fourth to fifth instar) were fasted for 2 days before administration of **1** (224 mg). (−)-Magnofargesin (**1**) was incorporated into the artificial diet (100 g) using cellose powder as an inert carrier and fed to larvae. After eating the artificial diet containing **1** (2 days), they were fed the artificial diet furthermore.

Isolation of metabolite **2** *from faeces*. Faeces were collected for 4 days, extracted by $CHCl_3$ (100 ml × 3) and then EtOAc (100 ml × 2), and evaporated under reduced pressure. The combined extract (283 mg) of **1** administered-larvae faeces was separated into acidic (30 mg), phenolic (127 mg) and neutral (116 mg) portions in the usual way. Then the phenolic portion was subjected to SiO_2 CC repeatedly to give a metabolite **2** (15 mg). No metabolic products were detected from acidic and neutral portion by TLC and GC-MS.

GC-MS analysis. The combined extract of **1** administered-larvae faeces was analyzed by GC-MS. 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 150° to 315° at 4° min^{-1} and then held at 315°. The flow rate of carrier gas (He) was 1 ml min^{-1}. (−)-Magnofargesin (**1**) was detected at R_t: 38.78 min.

(−)-3-O-*Demethylmagnofargesin* (**2**). Powder. R_f: 39.36 min. HRMS *m/z*: 402.1674 ($[M]^+$, calcd for $C_{22}H_{26}O_7$: 402.1679). EIMS *m/z* (rel. int.): 402 ($[M]^+$, 60), 220 (60), 202 (20), 190 (21), 189 (100), 181 (10), 161 (25). $[\alpha]_D^{20}$ − 45.77° ($CHCl_3$; *c* 0.2). IR ν_{max} cm^{-1}: 3439, 2935, 1596, 1516, 1464, 1261, 1239, 1026. NMR: Table 1.

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