Pergamon

PII: S0031-9422(96)00222-1

Phytochemistry, Vol. 43, No. 1, pp. 111–113, 1996

0031-9422/96 $15.00 + 0.00

O-DEMETHYLATION OF 7,7′-EPOXYLIGNANS BY *ASPERGILLUS NIGER*

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(*Received in revised form* 27 *February* 1996)

Key Word Index—*Myristica fragrans*; Myristicaceae; 7,7′-epoxylignans; (+)-veraguensin; (+)-galbelgin; galgravin; (+)-verrucosin; (+)-fragransin A_2; nectandrin B; *Aspergillus niger*.

Abstract—Biotransformation of the 7,7′-epoxylignans, (+)-veraguensin, (+)-galbelgin and galgravin by *Aspergillus niger* has been investigated. These lignans were converted to their corresponding 4,4′-*O*-demethyl derivatives, (+)-verrucosin, (+)-fragransin A_2 and nectandrin B. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The recent development of isolation and spectroscopic methods have enabled the determination of elaborate and complicated bioactive natural organic compounds, such as taxol [1] and maitotoxin [2]. However, many bioactive natural organic compounds are known to possess simple structures the value of which is still being evaluated, e.g. polonicumtoxins [3] and sobrerol [4]. Such alkaloids and terpene alcohols are sometimes obtained by metabolic reaction in organisms. Many terpene alcohols have been semisynthesized by biotransformation [5]. This suggests that there are many useful compounds in seeds which have not yet been activated, and the bioactivities of these compounds could be enhanced by biotransformation. From this point of view, we have been studying the biotransformation of lignans and neolignans, which possess simple structure and manifold bioactivities [6–10].

Naturally occurring 7,7′-epoxylignans (2,5-diaryl-3,4-dimethyltetrahydrofurans), (+)-veraguensin (**1**), (+)-galbelgin (**2**) and galgravin (**3**), show platelet-activating factor (PAF) inhibitory activity [11], but the metabolism of these lignans in organisms has not been elucidated. It was discovered that the first metabolic reaction for bisepoxylignans, such as (+)-magnolin, in the rat, an insect (*Spodoptera litura* larvae) and a fungus (*Aspergillus niger*) was the same *O*-demethylation at the *p*-position. In this paper, we report the microbial transformation of three lignans **1**–**3** to (+)-verrucosin (**4**), (+)-fragransin A_2 (**5**) and nectandrin B (**6**), respectively, by *A. niger*. The structures of these metabolic products were determined by a GC–mass spectroscopic method.

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RESULTS AND DISCUSSION

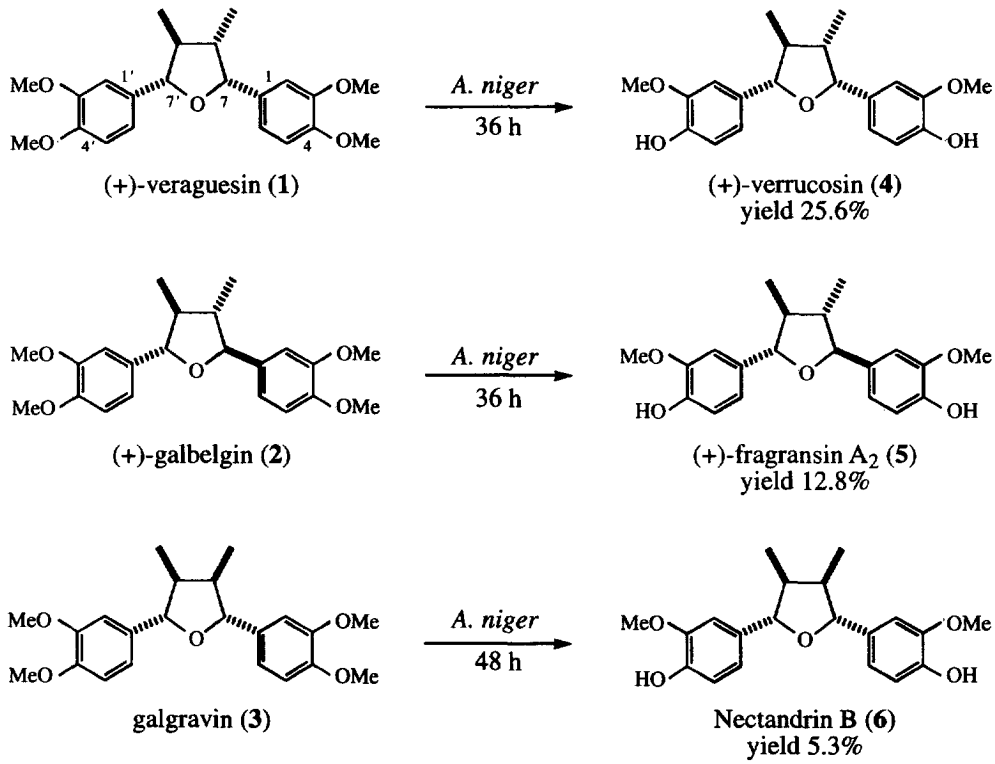
Lignans **1**–**3** were prepared by the methylation of (+)-verrucosin (**4**), (+)-fragransin A_2 (**5**) and nectandrin B (**6**), respectively, isolated from seeds of *Myristica fragrans*. Cultivation media, incubation temperature and other conditions were the same as previously published [7]. Each of the lignans was dropped on the mycelia, cultivated, extracted with ether, then analysed and investigated by GC-mass spectrometry.

(+)-Veraguensin (**1**) was transformed to compound **4** (conversion rate: 25.6% at 36 hr and 11.3% at 48 hr). The GC retention time of compounds **4** (20.73 min) coincided with that of authentic compound **4**. Compound **4** showed a $[M]^+$ at *m/z* 344 (13) and characteristic ions at *m/z* 192 (100), 180 (2), 177 (41), 164 (11), 151 (23) and 145 (62). These ions clearly corresponded with those of (+)-verrucosin (**4**) [12].

(+)-Galbelgin (**2**) was transformed to compound **5** (conversion rate: 12.8% at 36 hr and 11.8% at 48 hr). The GC retention time of compound **5** (20.90 min) coincided with that of authentic compound **5**. Compound **5** also showed a $[M]^+$ at *m/z* 344 (18) and characteristic ions at *m/z* 192 (100), 180 (15), 177 (44), 164 (28), 151 (25) and 145 (62). These spectral data clearly agreed with (+)-fragransin A_2 (**5**) [12].

Galgravin (**3**) was also transformed to compound **6** (conversion rate: 3.8% at 36 hr and 5.3% at 48 hr). GC retention time (21.26 min) coincided with that of authentic compound **6**. GC–mass spectral data showed $[M]^+$ at *m/z* 344 (15) and characteristic ions at *m/z* 192 (100), 180 (3), 177 (43), 164 (15), 151 (25) and 145 (64). All of the spectral data clearly identified compound **6** as nectandrin B [12].

These results suggested that the possible metabolic pathway of lignans **1**–**3** to **4**–**5**, respectively, by *A. niger* is as shown in Scheme 1. The metabolic reaction



Scheme 1. Possible metabolic pathways of 7,7′-epoxylignans **1**–**3** to **4**–**6** by *Aspergillus niger*.

for these 7,7′-epoxylignans by *A. niger* is *O*-demethylation at *p*, the same position as for bisepoxylignans of the magnolin type [6]; however, no accumulation of intermediates was detected, while intermediates accumulated with (+)-magnolin-type lignans were transformed by this fungus.

EXPERIMENTAL

Preparation of neolignans. (+)-Veraguensin (**1**), (+)-galbelgin (**2**) and galgravin (**3**) were prepd by methylation of (+)-verrucosin (**4**), (+)-fragransin A_2 (**5**) and nectandrin B (**6**), respectively, isolated from seeds of *M. fragrans* by modifying previously reported methods [12, 13].

Methylation of lignans. Each lignan (50 mg) was reacted with CH_2N_2 for 3 days in Et_2O at room temp. Products were purified by silica gel CC and identified as **1**–**3** (45–48 mg) by NMR and MS [12].

Fungus and cultivation conditions. Fungus, cultivation media, incubation temp. and other conditions were the same as in ref. [7]. Each lignan (45 mg) was dissolved in 0.3 ml of DMSO and added to the culture medium (corresponding to 5 mg of substrate per Petri dish). The dish was incubated at 28° under static conditions together with two control dishes which contained either mycelia with medium or substrate dissolved in DMSO with medium.

GC–MS analysis. Every 12 hr, each lignan-added dish and control dishes were extracted with Et_2O, then analysed by GC–MS. Analysis was carried out using a caillary column: HP-5MS (cross-linked 5% Ph Me Silicone 0.25 mm i.d. ×30 m). Programming from 180° to 300° at 4° min^{-1} and then held at 300°. The flow rate of carrier gas (He) was 1 ml min^{-1}.

(+)-*Veraguesin* (**11**). R_t: 21.70 min. MS *m/z* (rel. int.): 372 ($[M]^+$, 31), 206 (100), 191 (69), 178 (11), 175 (67), 165 (18), 151 (13), 138 (13).

(+)-*Galbelgin* (**2**). R_t: 21.82 min. MS *m/z* (rel. int.): 372 ($[M]^+$, 26), 206 (100), 191 (77), 178 (41), 175 (74), 165 (25), 151 (16), 138 (20).

Galgravin (**3**). R_t: 22.24 min. MS *m/z* (rel. int.): 372 ($[M]^+$, 33), 206 (100), 191 (74), 178 (15), 175 (69), 165 (18), 151 (15), 138 (15).

Acknowledgements—This work was partially supported by Research Fellowships of the Japan Society for the Promotion of Science (JSPS) and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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