PERGAMON

Phytochemistry 52 (1999) 1275–1277

PHYTOCHEMISTRY

Microbial transformation of $\Delta^{9(15)}$-africanene

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Received 11 May 1999

Abstract

Incubation of $\Delta^{9(15)}$-africanene with fungi *Aspergillus niger* and *Rhizopus oryzae* for 8 days yielded two oxidized derivatives, 10α-hydroxy-$\Delta^{9(15)}$-africanene and 9α,15-epoxyafricanane. The structure of the two products were assigned by interpretation of their spectral data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial transformation; *Aspergillus niger*; *Rhizopus oryzae*; $\Delta^{9(15)}$-africanene

1. Introduction

Biotransformations are useful methods for producing medicinal and agricultural chemicals from both active or inactive natural products and also biotransformations are important tools for introducing chemical functions into inaccessible sites of molecules, and then by enhance the biological activity (Charney & Herzog, 1967; Kieslich, 1975). Bacteria and fungi have provided hydroxylation, decarboxylation, oxidation and hydrolysis products (Brannon, Boaz, Wiley, Mabe & Horton, 1968; Biellmann, Branlant, Gero-Robert & Poiret, 1973; Biellmann, Wennig, Daste & Raynand, 1968). The tricyclic sesquiterpenes of the class africanenes are very rare in nature. To study the different derivatives of africanene class, we examined the biotransformation of $\Delta^{9(15)}$-africanene to elucidate their metabolic pathways and to generate their derivatives.

$\Delta^{9(15)}$-africanene (**1**) is a tricyclic sesquiterpene isolated from various marine soft corals of *Sinularia* sp. (Kashman, Bodner, Finer-Moore & Clardy, 1980; Braekman et al., 1980) and some of its derivatives are also isolated from terrestrial sources (Toyoto, Nagashima, Shima & Asakawa, 1992; Catalan, De Fenik, De Arriazu & Kokke, 1992); the absolute configuration of **1** was assigned by X-ray diffraction study of its derivatives (Kashman, Bodner, Finer-Moore & Clardy, 1980; Braekman et al., 1980). The present paper deals with the biotransformation of $\Delta^{9(15)}$-africanene (**1**) to 10α-hydroxy-$\Delta^{9(15)}$-africanene (**2**) and 9α,15-epoxyafricanane (**3**) by the fungi, *Aspergillus niger* and *Rhizopus oryzae*. *A. niger* is widely used in biotransformations (Kasahara, Miyazawa & Kameoka, 1996; Asakawa, Takahashi, Toyota & Noma, 1991), whereas few reports are their on *R. oryzae* (Lisker, Zhang & Chu Fun, 1990; Petzoldt, Dahl & Vorbrueggen, 1986).

2. Results and discussion

To investigate the course of biotransformation of **1** by *A. niger* and *R. oryzae*, compound **1** was incubated with *A. niger* and *R. oryzae* separately for 8 days. Two main products (**2** and **3**) and many minor products were detected by TLC analysis of the ethyl acetate extract of the culture medium after biotransformation. These products were not detected by TLC analysis of the culture of *A. niger* and *R. oryzae* to which substrate was not fed. From the above results, it was demonstrated that *A. niger* and *R. oryzae* transformed

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PII: S0031-9422(99)00379-9

Table 1
^{13}C-NMR Data of Compounds **1**–**3** in $CDCl_3$

Carbon	**1**	**2**	**3**
1	52.7 *d*	48.9 *d*	49.8 *d*
2	18.9 *s*	18.9 *s*	19.7 *s*
3	23.4 *t*	23.5 *t*	23.6 *t*
4	22.1 *d*	22.1 *d*	21.8 *d*
5	43.3 *t*	43.3 *t*	43.2 *t*
6	33.7 *s*	33.9 *s*	33.3 *s*
7	51.3 *t*	51.6 *t*	44.4 *t*
8	42.2 *d*	41.3 *d*	44.4 *d*
9	158.3 *s*	161.0 *s*	82.9 *s*
10	33.7 *t*	75.5 *d*	29.6 *t*
11	27.7 *t*	36.3 *t*	37.3 *t*
12	24.1 *q*	24.2 *q*	24.2 *q*
13	20.5 *q*	20.4 *q*	19.7 *q*
14	33.9 *q*	33.9 *q*	33.9 *q*
15	104.5 *t*	108.6 *t*	68.9 *t*

1 into more polar compounds **2** and **3**, and many minor products separately. After 8 days ca. 95% of the starting substrate **1** was transformed and the culture was extracted with EtOAc followed by column chromatography yielded **2** (5 mg, ca. 5%) and **3** (10 mg, ca. 10%) respectively. The structures of **2** and **3** were determined by spectral data.

Compound **2** was obtained as a solid. The ^{1}H-NMR spectrum of compound **2** showed signals for the presence of an exocyclic methylene at δ 5.15 (1H, *d*, J = 1.5 Hz) and 4.90 (1H, *d*, J = 1.5 Hz), an allylic methine-bearing hydroxyl at δ 4.45 (1H, *br t*, $W_{1/2} = 6$ Hz), three tertiary methyls at δ 1.05 (3H, *s*), 0.95 (3H, *s*), and 0.90 (3H, *s*), and a tri-substituted cyclopropane ring at δ 0.54 (2H, *m*) and 0.25 (1H, *m*). The spectral data were similar to those of $\Delta^{9(15)}$-africanene (**1**), except for the signal at δ 4.45 and down field chemical shift of exocyclic protons at δ 4.65 (1H, *br s*) and 4.82 (1H, *br s*) to δ 4.90 and 5.15, respectively. This suggesting the allylic oxidation of **1** at C-10 and was also supported by ^{13}C-NMR spectral signals at δ 161.0 (*s*), 108.6 (*t*), and 75.5 (*d*) (Table 1). The foregoing spectral data are reminiscent to those of 10α-hydroxy-$\Delta^{9(15)}$-africanene, which was earlier isolated from the soft coral *Sinularia dissecta* (Ramesh, Srinivasa Reddy, Rao & Venkateswarlu, 1999). Thus, the structure of compound **2** was established as 10α-hydroxy-$\Delta^{9(15)}$-africanene.

Compound **3** was obtained as a solid. The ^{1}H-NMR spectrum of compound **3** showed signals for the presence of methylene group at δ 3.57 (1H, *d*, J = 13.5 Hz) and 3.46 (1H, *d*, J = 13.5 Hz), three tertiary methyls at δ 1.0 (3H, *s*), 0.95 (3H, *s*), and 0.92 (3H, *s*), and a tri-substituted cyclopropane ring at δ 0.55 (2H, *m*), 0.21 (1H, *m*), respectively. The spectral data were similar to those of **1** except for the presence of signals at δ 3.57 and 3.46 and was devoid of exocyclic methylene protons at δ 4.65 and 4.82 as observed for **1**. This suggested that the epoxidation of **1** at C_9–C_{15}. Further, the presence of epoxide was also inferred from the ^{13}C-NMR signals at δ 82.9 (*s*) and 68.9 (*t*). The foregoing spectral data were reminiscent to those of 9α,15-epoxyafricanane, which was earlier isolated from the soft coral *Sinularia dissecta* (Ramesh, Srinivasa Reddy, Rao & Venkateswarlu, 1999). Thus, the structure of compound **3** was established as 9α,15-epoxyafricanane. The above results explain that both the fungi *A. niger* and *R. oryzae* were stereoselectively hydroxylating the allylic position of the double bond, and epoxidising the double bond of $\Delta^{9(15)}$-africanene (**1**).

3. Experimental

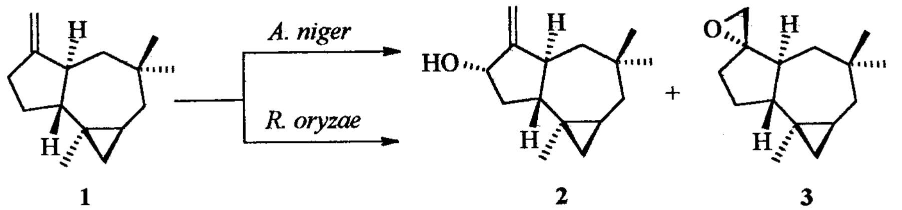
Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded on Perkin-Elmer 240C instruments. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Unity 400 MHz and Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in Hertz. Mass spectra were recorded on VG Auto Spec-M instrument.

3.1. Preculture of Aspergillus niger (ATCC # 9642)

Spores of *A. niger*, which had been preserved at low temperature, were inoculated into sterilized culture medium [sucrose (30 g/l) + sodium nitrate (3 g/l) + dipotassium phosphate (1 g/l) + magnesium sulphate (0.5 g/l) + potassium chloride (0.5 g/l) + ferrous sulphate (0.01 g/l) + p^{H} 7.3 ± 0.2] in a flask which was shaken at 37° for 3 days.

3.2. Biotransformation of $\Delta^{9(15)}$-africanene (1)

Precultured *A. niger* transferred into four 200 ml Erlenmeyer flasks each containing 100 ml of medium,



1 **2** **3**

and stirred for 3 days. After the growth of *A. niger*, compound **1** (25 mg) was added separately to the each medium and the organism cultivated for eight more days.

3.3. Isolation of metabolites 2 and 3

After the fermentation the culture medium and mycelium were separated by filtration. The medium was extracted with EtOAc (3 × 50 ml). The combined EtOAc extract was dried over anhydrous Na_2SO_4 and the solvent was evaporated to yield a crude extract (120 mg). The ethyl acetate extract was subjected to column chromatography on silica gel with Hexane-EtOAc gradient, to give the pure metabolites **1** (5 mg), **2** (5 mg), and **3** (10 mg).

The same experimental procedure was repeated for biotransformation of $\Delta^{9(15)}$-africanene (**1**) with *Rhizopus oryzae* (ATCC # 24563), which lead to isolation of compounds **1** (3 mg), **2** (7 mg), and **3** (13 mg).

3.4. 10α-Hydroxy-$\Delta^{9(15)}$-africanene (2)

Colourless needles, mp 98°C, $[\alpha]^{25D}$ + 19.1° (*c* 1.2, $CHCl_3$), IR (KBr) ν_{max} 3500, 1620, 1375 and 890 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz)δ 5.15 (1H, *d*, J = 1.5 Hz, H-15_a), 4.90 (1H, *d*, J = 1.5 Hz, H-15_b), 4.45 (1H, *br t*, $W_{1/2} = 6$ Hz, H-10_β), 2.46 (1H, *m*, H-8_α), 1.88 (1H, *m*, H-1_β), 1.80 (4H, *m*, H-5_β, H-7_β, H-11_α, H-11_β), 1.22 (1H, *m*, H-5_α), 1.18 (1H, *m*, H-7_α), 1.05 (3H, *s*), 0.95 (3H, *s*), 0.90 (3H, *s*), 0.54 (2H, *m*, H-3_β, H-4), 0.25 (1H, *m*, H-3_α), ^{13}C-NMR ($CDCl_3$, 50 MHz), see Table 1; EIMS *m/z* 220 $[M]^+$ (8), 205 (16), 187 (12), 123 (30), 105 (43).

3.5. 9α,15-Epoxyafricanane (3)

Solid, mp 125°C. $[\alpha]_D^{25}$ +16.2° (*c* 1.1, $CHCl_3$). IR (KBr) γ_{max} 2950, 1375, 1080 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ 3.57 (1H, *d*, J = 13.5 Hz, H-15), 3.46 (1H, *d*, J = 13.5 Hz, H-15), 1.86–1.76 (2H, *m*, H-10_α, H-5_α), 1.76–1.66 (2H, *m*, H-10_β, H-1_β), 1.66–1.58 (2H, *m*, H-8_α, H-11_β), 1.44–1.38 (1H, *m*, H-7_β), 1.25 (1H, *m*, H-11_α), 1.21–1.02 (1H, *m*, H-7_α, H-5_β), 1.00 (3H, *s*), 0.95 (3H, *s*), 0.92 (3H, *s*), 0.55 (2H, *m*, H-3_α, H-4), 0.21 (1H, *m*, H-3_β). ^{13}C-NMR ($CDCl_3$, 50 MHz), see Table 1. EIMS *m/z* 189 $[M^+$-31] (20), 123 (25), 95 (45).

Acknowledgements

We are thankful to the Department of Ocean Development for financial assistance, the Director IICT, and Dr. J.S. Yadav for their encouragement, and UGC for providing fellowship to P. Ramesh.

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