Biotransformation of Glabratephrin, a Rare Type of Isoprenylated Flavonoids, by *Aspergillus niger*

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Microbial transformation of glabratephrin, the major isolated compound from *Tephrosia purpurea*, afforded pseudosemiglabrin. The formation of the transformed compound seems to be performed via ring opening-closure of a five-membered ring causing transformation from a spiro into a fused system. The structure of the transformed compound was determined by comprehensive NMR studies, including DEPT, COSY, HMQC, NOE and MS.

Key words: Tephrosia purpurea, Microbial Transformation, Pseudosemiglabrin

Introduction

Microbial transformation is known to be a useful tool to obtain more active or less toxic compounds and to achieve selective conversions of compounds to useful derivatives which are difficult to be produced synthetically (Venisetty and Cidii, 2003). This technique has been widely applied for the conversion of natural products into potentially useful substances. Microbial transformations are known for their high stereoselectivity and environmental-friendly nature (Choudhary *et al*., 2005). Additionally, microorganisms are able to transform a huge variety of organic compounds, such as terpenes, steroids and alkaloids (Fraga *et al*., 1998; Lin and Rosazza, 1998; Orden *et al*., 2005). The biotransformation described here was performed by *Aspergillus niger*, a fungus used for biotransformation of several compounds (El-Hassane *et al*., 2000; Galal *et al*., 1999; Haridy *et al*., 2006). The objective of the current study was to determine the ability of *A. niger* to accomplish the conversion of prenylflavonoids. Microbial transformation of glabratephrin (**1**) afforded pseudosemiglabrin (**2**).

Results and Discussion

Compound **2** was obtained by fermentation of compound **1** using *Aspergillus niger* and purified by using Sephadex column chromatography. Compound **2** was isolated as crystalline needles and demonstrated the molecular ion peak $[M]^+$ in the EI mass spectrum at m/z = 392 (43%) – exact mass determination at m/z = 392.1258 – in full agreement with the molecular formula $C_{23}H_{20}O_6$. A base peak appeared at m/z = 332 (100%), corresponding to [M–acetic acid]. The ^{1}H NMR spectrum of compound **2** showed two singlet signals at δ_H 1.10 (3H) and 1.32 (3H) assigned to the *gem*-Me_2 adjacent to an oxygen function, one singlet at δ_H 2.24 (s, 3H) assigned to the methyl moiety of the acetate group, one singlet at δ_H 4.30 assigned to H-5″ (1H), and two doublets at δ_H 6.63 (d, J = 6.6 Hz), 5.53 (d, J = 6.6 Hz) due to H-2″ and H-6″, respectively. The flavone structure was indicated by the following signals: one singlet at δ_H 6.79 for H-3 (1H), two doublets at δ_H 8.18 (d, J = 8.5 Hz) and 6.94 (d, J = 8.5 Hz) for H-5 and H-6, respectively, and two multiplet signals at δ_H 7.52 and 7.91 for the aromatic protons of ring B.

The ^{13}C NMR spectrum of compound **2** revealed the presence of 21 carbon atoms and their multiplicities (by DEPT analysis) confirmed the number of hydrogen atoms of the formula given above. The carbon atoms were assigned to: three methyl carbon signals at δ_C 23.03, 27.42, 20.12, nine methine carbon signals, two of them related to the four aromatic carbon atoms at δ_C 126.02 for C-2′, C-6′ and 128.93 for C-3′, C-5′, and nine quaternary carbon signals, of them two carbonyl carbon signals at δ_C 177.39 and 169.58. All proton and carbon signals were determined by ^{1}H-^{1}H COSY and HMQC (Table I). The stereochemistry of compound **2** was deduced from the chemical shifts and the coupling constants and confirmed by NOE spectra with inspection of Dreiding models. The *cis*-orientation of H-2″ and H-6″ was deduced from the coupling constant (J = 6.6 Hz). Addition-

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Table I. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **2**[a].

Proton	δ_H	Carbon	δ_C
		C-2	162.49
H-3	6.79 s	C-3	107.33
		C-4	177.39
		C-4a	118.16
H-5	8.18 d (8.5)	C-5	128.46
H-6	6.94 d (8.5)	C-6	111.61
		C-7	164.39
		C-8	111.36
		C-8a	153.59
		C-1′	131.13
H-2′,4′,6′	7.91 m	C-2′,6′	126.02
		C-4′	131.57
H-3′,5′	7.52 m	C-3′,5′	128.93
H-2″	6.63 d (6.6)	C-2″	108.74
		C-4″	84.50
H-5″	4.30 s	C-5″	76.65
H-6″	5.53 d (6.6)	C-6″	47.71
gem-Me_2	1.10 s	*gem*-Me_2	23.03
	1.32 s		27.42
OAc	2.24 s	OAc	20.12, 169.58

[a] TMS as internal standard.

ally, irradiation of the signal at δ_H 5.53 (H-6″) enhanced the signal at δ_H 6.63 (H-2″) and the signal at δ_H 4.30 (H-5″), suggesting the same orientation of these three protons. On the basis of these results, the structure of compound **2** was assigned to be the isoprenylflavonoid pseudosemiglabrin (Waterman and Khalid, 1980). ^{13}C NMR data of compound **2** are given in Table I. The formation of **2** seems to proceed via ring opening-closure of the five-membered ring leading to transformation of the spiro into the fused system. First, hydrolysis of the lactone ring resulted in intermediate A. Then A undergoes oxidative elimination to give B, which under decarboxylation achieved the desired transformed compound **2** (Fig. 1).

Experimental

General

^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and 2D spectra were recorded on a JEOL 500 MHz Lambda spectrometer with TMS as internal standard. EIMS was performed on a JEOL SX102A mass spectrometer. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer.

Microorganism

The fungal culture of *Aspergillus niger* was obtained from the culture collection of Botany Department, Faculty of Science, El-Minia University, Egypt. The fungus was maintained on YMPGA (yeast extract–malt extract–peptone–glucose–agar) slants (Lodder, 1970) at 4 °C.

Medium

YMPG (yeast extract–malt extract–peptone–glucose) liquid medium was used for screening experiments (Lodder, 1970).

Fermentation procedures

Screening experiments were performed in 250 mL Erlenmeyer flasks containing 50 mL YMPG medium. The medium was autoclaved at 121 °C for 15 min. Sterile culture medium was inoculated with a spore suspension of *Aspergillus niger* (2-week-old culture) and kept on a rotary shaker (150 rpm) at room temperature (28 °C) for 48 h. After incubation, compound **1** (30 mg) was added as solution in methanol (5 mg/50 mL medium). Six flasks were prepared for the compound. Both substrate and organism controls were also performed. After two weeks of incubation, cultures were filtered and filtrates were extracted with methylene chloride to afford compound **2** (20 mg).

Plant material

T. purpurea was collected at Elba Mountain, Aswan, South of Egypt, in 2003. A voucher specimen is deposited at the Department of Botany, South Valley University, Aswan, Egypt.

Pseudosemiglabrin (**2**): Crystalline needles; $[\alpha]_D^{25} = -126°$ (c = 0.31, $CHCl_3$). – IR (KBr): ν = 1745, 1650 cm^{-1}. – ^{1}H NMR ($CDCl_3$): The ^{1}H assignments were achieved by ^{1}H-^{1}H correlation spectroscopy (COSY), see Table I. – ^{13}C NMR ($CDCl_3$): The ^{13}C assignments were achieved by HMQC and HMBC, see Table I. – EIMS: *m/z* (%) = 392 $[M]^+$, 332 $[M-CH_3COOH]^+$, 317 $[M-CH_3COOH-CH_3]^+$, 289, 263, 230, 187, 160, 102 (calcd. for $C_{23}H_{20}O_6$: 392.12598).

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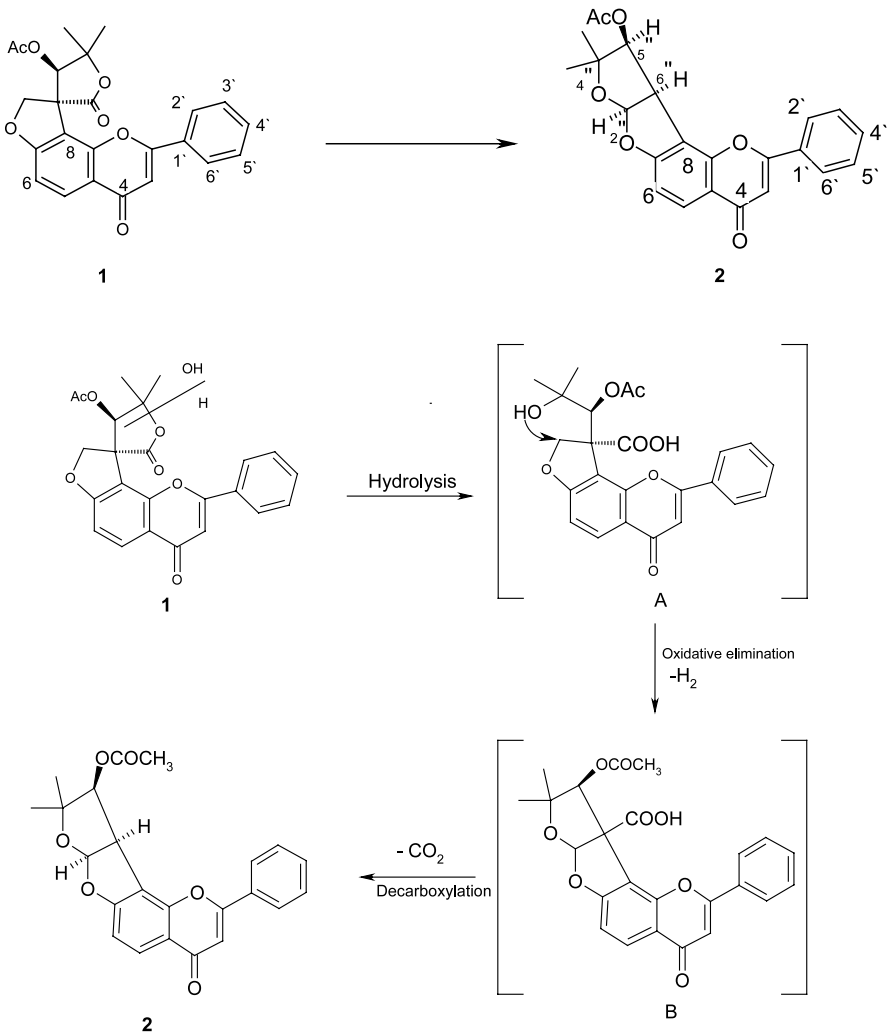

Fig. 1. Plausible mechanism for the formation of compound **2**.

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