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Microbial transformation of parthenolide

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Abstract

Microbial transformation of the germacranolide parthenolide using *Rhizopus nigricans*, *Streptomyces fulvissimus* and *Rhodotorula rubra* yielded three new compounds: 11αH-dihydroparthenolide, 9β-hydroxy-11βH-dihydroparthenolide and 14-hydroxy-11βH-dihydroparthenolide. 11βH-dihydroparthenolide was the only common metabolite produced by all microorganisms.

Keywords: Parthenolide; Hydroxylation; Feverfew; *Tanacetum parthenium*; Asteraceae; Microbial; Transformation; NMR

1. Introduction

Parthenolide (**1**), a germacranolide sesquiterpene lactone, is the major constituent of the European feverfew (*Tanacetum parthenium*) and several other members of the Asteraceae and Magnoliaceae (Fischer, Vargas, & Menelorou, 1991). In recent years, parthenolide (**1**) has attracted considerable attention (Castaneda-Acosta, Fischer, & Vargas, 1993), because of its wide spectrum of biological effects, which include cytotoxic (Ogura, Cordell, & Farnsworth, 1978; Picman, 1986; Abdel Sattar, Galal, & Mossa, 1996), antibacterial and antifungal activities (Picman, 1986; Fischer, 1991). Parthenolide (**1**) was also found to be the most active germacranolide against *Mycobacterium tuberculosis* and *M. avium* (Fischer et al., 1998). Reports on its anti-inflammatory (Heptinstall, Williamson, White, & Mitchell, 1985), antirheumatic action (Patrick, Heptinstall, & Doherty, 1989) and its action as the active principle in European feverfew, used in treatment of migraine, have renewed interest in this compound. Thus, many studies on the mechanism of its action in the treatment of migraine have been documented (Bejar, 1996; Weber et al., 1997).

Despite the diverse pharmacological actions and the potential of using parthenolide (**1**) for different indications, there are no studies concerning the mammalian or microbial metabolism of this compound. This investigation utilized microorganisms as models to prospectively mimic and predict mammalian biotransformation of parthenolide (**1**). Furthermore, the microbial metabolites obtained can be used as reference standards for monitoring mammalian metabolic studies (Lin & Rosazza, 1998). The use of microorganisms as models for xenobiotic metabolism (Rosazza & Smith, 1979) has been successfully utilized in mammalian metabolism studies of many biologically active natural and synthetic compounds (Davis, 1988).

2. Results and discussion

Screening-scale experiments have shown that most microorganisms, used in this study, were capable of converting parthenolide (**1**) into compound **2**. Thus, preparative-scale fermentation of **1** with *Streptomyces fulvissimus* NRRL 1453B or *Rhizopus nigricans* NRRL 1477 gave **2** in 20–30% yield. The EIMS of compound **2** showed a parent ion peak at m/z 250. Examination of the ^{1}H NMR spectrum (Table 1) did not show the two downfield doublets at δ 6.34 and 5.62 due to the exocyclic methylene in **1**. However, a new methyl signal at δ 1.25 (d, $J=6.8$ Hz), which was attributed to

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Table 1
^{1}H NMR assignments of compounds **1**–**6**

Proton	**1**[a]	**2**	**3**	**4**	**5**	**6**
H-1	5.21 (dd, br, 4.0, 12.2)	5.15 (dd, 2.3, 11.9)	5.18 (br, d, 10.1)	5.37 (br, dd,12.3, 1.2)	5.29 (dd, 12.4, 3.9)	5.61 (dd, 7.7,7.7)
H-2α	2.09–2.24 (m)	2.11 (dddd, 2.3, 6.0, 13.0, 13.0)	1.87 (br, dd, 15, 6.7)	2.14 (m)	2.18 (br, s)	1.89 (m)
H-2β	2.46 (ddd, 13.8, 12.2, 12.5)	2.37 (dddd, 5.0, 11.9, 13.3, 13.0)	2.36 (m)	2.46 (dddd, 4.5, 5.4, 12.2, 13.4)	2.44 (dddd, 13.2, 12.8, 5.7, 5.4)	2.27 (m)
H-3α	1.25 (m)	1.21 (ddd, 5.5, 5.6, 13.0)	1.21 (m)	1.12 (ddd, 5.5, 5.6, 13.0)	1.18 (br,d, 6.1)	1.06 masked (dd)
H-3β	2.09–2.24 (m)	2.16 (m)	2.10 (m)	2.14 (m)	2.11 (dddd, 1.5. 2.0, 7.5, 5.4)	2.13 (m)
H-5	2.79 (d, 8.9)	2.69 (d, 9, 10)	2.73 (d, 9.0)	2.60 (d, 8.9)	2.68 (d, 9.1)	2.75 (d, 9.4)
H-6	3.86 (dd, 8.9, 8.3)	3.80 (dd, 8.4, 9, 10)	3.96 (t, 9.3)	3.8 (t, 8.6)	3.79 (t, 9.1)	3.82 (t, 9.6)
H-7	2.78 (m)	2.28 (m)	2.36 (m)	1.96 (m)	1.87 (m)	1.89 (m)
H-8α	2.09–2.24 (m)	1.80 (m)	1.55 (m)	1.96 (m)	1.87 (m)	2.4 (dd, 13.7, 5.4)
H-8β	1.73 (m)	2.28 (m)	2.10 (m)	1.86 (m)	1.73 (m)	2.27 (m)
H-9α	2.09–2.24 (m)	1.80 (m)	2.10 (m)	4.16 (m)	1.87 (m)	1.56 (m)
H-9β	2.38 (m)	2.25 (m)	2.36 (m)	–	2.72 (dd, 6.2, 7.2)	2.27 (m)
H-11α	–	–	2.71 (m)	–	–	–
H-11β	–	2.27 (dq, 6.8, 10.3)	–	2.29 (m)	2.27 (dq, 7.0, 5.2)	2.27 (m)
H-13α	6.34 (d, 3.6)	1.25 (d, 6.8)	–	1.30 (d, 7.0)	1, 24 (d, 7.0)	1.24 (d, 7.0)
H-13β	5.62 (d, 3.1)	–	1.18 (d, 10.1)	–	–	–
H-14	1.72 (s)	1.68 (s)	1.65 (s)	1.73 (s)	4.04 (d, 11.8) and 4.38 (d, 11.8)	4.10 (d, 12.7) and 4.03 (d, 12.7)
H-15	1.31 (s)	1.27 (s)	1.29 (s)	1.31 (s)	1.20 (s)	1.53 (s)

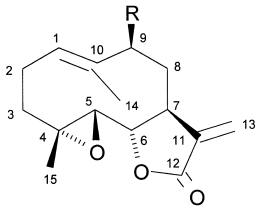
[a] Taken from the reference by Ruangrungsi et al. (1987) and included for the purpose of comparison.

H-13 was observed. The physical and spectral properties (see Section 3) of compound **2** were indistinguishable from those reported for 11βH-dihydroparthenolide (Ruangrungsi et al., 1987; Ruangrungsi, Rivepiboon, Lange, & Decicco, 1988; Fischer et al., 1991; Castaneda-Acosta et al., 1993).

Metabolite **3** could only be formed by two organisms of those tried, namely, *Rhizopus nigricans* NRRL1477 and *Rhodotorula rubra* NRRL y1592. Preparative-scale fermentation, of parthenolide (**1**) with the first organism gave **3** in 13% yield. The MS and ^{1}H and ^{13}C NMR spectral data for **3** were generally similar to those of **2**, except for some minor differences. Most notably, the C-13 ^{13}C NMR signal in **3** resonated at δ 11.2, versus 13.2 in **2**. Furthermore, H-7, which occupies a pseudoaxial position in **2**, was more deshielded in **3**, while, H-13 was more shielded in **2** than **3** (see Table 1). This observation strongly suggested that **3** was epimeric with **2** at C-11. Thus compound **3** is characterized as 11αH-dihydroparthenolide and is hitherto unreported from any other source.

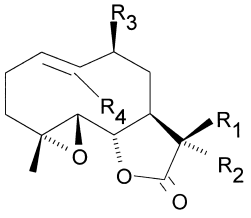
Metabolism of parthenolide (**1**) by *Streptomyces fulvissimus* NRRL 1453B gave, in addition to compound **2**, a highly polar minor metabolite, compound **4**, in a 3% yield. This new metabolite possessed gross NMR spectral data that were generally similar to those of **2**, except for the presence in the ^{13}C NMR spectrum of an oxygenated methine at δ 80.0. The presence in the IR spectrum of a broad band at ν_{max} 3500 cm^{-1} suggested the presence of a hydroxylated methine. The ^{13}C NMR spectra were most informative in determining the position of the hydroxyl group in **4**. A comparison of ^{13}C NMR spectra of **4** and **2** indicated major difference in the region around C-9 (Table 2). In the ^{13}C NMR of **4**, C-8 is shifted downfield by 8.5 ppm, while C-7 is shifted upfield by about 3 ppm. These shifts established that the hydroxyl must be placed at C-9 (Breitmair & Voelter, 1974). The physical and spectral data of **4** were found to be indistinguishable from those obtained for 9β-hydroxydihydroparthenolide. The latter was prepared by reduction of 9β-hydroxyparthenolide (**7**), which was obtained by isolation from *Anvillea garcinii* (Abdel Sattar et al., 1996). Compound **4** was also obtained by incubation of **2** with *S. fulvissimus* NRRL 1453B. Microbial hydroxylation of C-9 of germacranolides might prove to be particularly useful, because naturally occurring germacranolides with oxygenation at this position are quite rare.

Compound **5** was obtained as a minor metabolite by *Rhizopus nigricans* NRRL 1477 (4% yield). It exhibited ^{1}H NMR and ^{13}C NMR signals that were similar, but not identical, to those of **2**, with the C-13 methyl group resonating at δ 13.6 and thus, maintaining its α disposition. This was further confirmed by the pro-



1 R=H

7 R= OH



2 $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$

3 $R_2 = R_3 = H$, $R_1 = R_4 = CH_3$

4 $R_1 = H$, $R_3 = OH$, $R_2 = R_4 = CH_3$

5 $R_1 = R_3 = H$, $R_2 = CH_3$, $R_4 = CH_2OH$

6 same as 5 but R_4 *cis*

duction of **5** by fermentation of **2**, using the same organism. The ^{13}C NMR spectrum of **5**, however, lacked the C-14 methyl signal and instead, it exhibited a hydroxymethyl group at δ 59.8. The broad band at ν_{max} 3500 cm^{-1} in the IR spectrum confirmed the presence of this group. The presence of the less deshielded methyl signal at δ 1.20, due to H-15, established that hydroxylation, indeed, involved C-14, rather than C-15, of **1**.

Compound **5** was found to be different from **6**, obtained by chemical hydroxylation of the allylic C-14 hydroxyl group of **2**, with inversion of configuration from *trans* to *cis* (Haruna & Ito, 1981). It was then possible to conclude that the microbial reaction has proceeded with the retention of the *trans* configuration of the double bond. It is interesting to add that both metabolites **4** and **5** are products of allylic oxidation reactions, which are considered common microbial bio-conversions of many unsaturated steroids and terpenoids (Fonken & Johnson, 1972).

3. Experimental

(−)-Parthenolide (**1**) was isolated and purified from the aerial parts of the local plant *Tarchonanthus camphoratus* (Al-Sheddi, private communication). M.p.'s were determined in open capillary tubes using an Electrothermal 9100 capillary melting-point apparatus and are uncorr. IR spectra were recorded in KBr using a PYE Unicam infrared spectrophotometer and specific rotations were obtained at amb. temperature on a Perkin-Elmer digital polarimeter model 241 MC. The ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ on a Bruker DRX-500 NMR spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as ppm using tetramethylsilane (TMS) as internal standard and coupling constants are expressed in Hz. Electron ionization (EI) mass spectra were taken on a Shimadzu QP500 OGC/mass spectrometer. Thin layer chromatographic analyses were carried out on pre-coated silica gel 60 F254 (Merck) using *n*-hexane–EtOAc mixtures as solvent systems and spots were visualized under short wavelength UV light or by spraying with *p*-anisaldehyde spray reagent. The adsorbent used for column chromatography was silica gel 60/230–400 mesh (EM Science).

Table 2
^{13}C NMR assignments of compounds **1**–**6**

Carbon	**1**[a]	**2**	**3**	**4**	**5**	**6**
1	125.2 d	125.1 d	125.1 d	126.6 d	128.8 d	127.3 d
2	24.1 t	24.0 t	24.0 t	24.2 t	23.9 t	24.0 t
3	36.3 t	36.3 t	36.8 t	36.8 t	37.0 t	37.0 t
4	61.5 s	61.4 s	61.4 s	61.8 s	61.7 s	60.4 s
5	66.3 d	66.3 d	66.6 d	66.5 d	66.7 d	63.9 d
6	82.4 d	82.1 d	82.2 d	81.7 d	82.6 d	81.7 d
7	47.6 d	51.9 d	46.8 d	48.9 d	52.0 d	46.8 d
8	30.6 t	29.7 t	26.2 t	38.2 t	30.6 t	24.6 t
9	41.1 t	41.1 t	41.0 t	80.0 d	36.5 t	27.0 t
10	134.6 s	134.4 s	134.3 s	136.9 s	138.3 s	140.0 s
11	139.2 s	42.4 d	41.2 d	42.5 d	43.0 d	41.9 d
12	169.2 s	179.6 s	178.6 s	177.4 s	178.1 s	178.4 s
13	121.1 t	13.2 q	11.2 q	13.6 q	13.6 q	13.4 q
14	16.5 q	16.8 q	16.8 q	11.3 q	59.8 t	65.9 t
15	17.2 q	17.1 q	17.1 q	17.7 q	17.2 q	18.3 q

[a] Taken from the reference by Castaneda-Acosta et al. (1993) and included for the purpose of comparison.

3.1. Microorganisms

Microorganisms were obtained from either American Type Culture Collection (ATCC) or Northern Regional Research Laboratories (NRRL); organisms were maintained on Sabourad dextrose agar (Oxoid) slants at 4°C and were used for the preliminary screening. The twenty microorganisms, used in this study were: *Aspergillus alliaceous* NRRL 315, *Aspergillus flavipes* ATCC 11013, *Aspergillus niger* NRRL 599, *Aspergillus niger* NRRL 2295, *Aspergillus ochraceous* NRRL 398, *Aspergillus ochraceous* NRRL

405, *Candida albicans*, laboratory isolate, *Cunninghamella blackesleeana* MR 198, *C. echinulata* NRRL 1382 (ATCC 42616), *C. elegans* NRRL 1392 (ATCC 10028a), *Gymnascella citrina* NRRL 6050, *Lindera pinnespora* NRRL 2237, *Penicillium chrysogenum* ATCC 10002, *P. chrysogenum* ATCC 10002 k, *P. purpureus* UI 193, *P. vermiculatum* NRRL 10009, *Rhizopus nigricans* NRRL 1477, *Rhodotorula rubra* NRRL y1592, *Saccharomyces cerevisae* (Baker's yeast) and *Streptomyces fulvissimus* NRRL 1453 B.

3.2. Media (Ibrahim, Galal, Mossa, & El-Feraly, 1997)

All fermentation experiments were carried out in a medium of the following composition: 10 g dextrose, 10 ml glycerol, 5 g yeast extract, 5 g peptone, 5 g K_2HPO_4, 5 g NaCl and 1000 ml distilled water. The pH was adjusted to 6.0 before autoclaving at 121°C for 15 min.

3.3. Fermentation procedures (Ibrahim et al., 1997)

Cells of microorganisms were transferred from two-week old slants into sterile culture media and kept on gyratory shaker for 72 h to give stage I culture. Stage I cultures (5 ml) were used as inocula for stage II cultures (50 ml/250 ml flask). After 24 h incubation of stage II cultures, parthenolide (**1**) was added as a soln in Me_2CO (10 mg 0.25 ml^{-1}). Both substrate and organism controls were made. Each fermentation was sampled by extracting 5 ml of the culture medium with $CHCl_3$ (5 ml). After evapn of the solvent, the residue was chromatographed on silica gel G plates using EtOAc–hexane (1:1) as the mobile phase.

3.4. Fermentation of parthenolide (*1*) with Streptomyces fulvissimus NRRL 1453B

Parthenolide (**1**, 650 mg), dissolved in 16 ml of Me_2CO, was evenly distributed among 65 flasks containing stage II cultures. Fermentation was stopped after 6 days. The mixture was filtered and fermentation broth was extracted three times with equal vols of $CHCl_3$. The combined extracts were dried over anhydrous Na_2SO_4 and solvent evaporated to give 1.3 g of a brownish residue. The crude residue was subjected to preliminary purification by passing its EtOAc solution through a short column to give 0.78 g of residue, which was then loaded on a silica gel column. Elution with *n*-hexane–EtOAc (2:1) gave 200 mg of compound **2**. Further elution of the column with hexane–EtOAc (1:1) yielded **4**, which crystallized from ether to give 20 mg of cubic crystals; R_f 0.26 (*n*-hexane–EtOAc, 1:2); m.p. 164°C; $[\alpha]_D$ −26° (*c* 0.03; $CHCl_3$); IR (ν_{max}) (KBr) cm^{-1}: 3460 (OH) and 1750 (lactone CO); EIMS *m/z* 266 (>1) $[M]^+$; ^1H and ^{13}C NMR ($CDCl_3$) δ: see Tables 1 and 2, respectively.

3.5. Fermentation of parthenolide (*1*) with Rhizopus nigricans NRRL 1477

Parthenolide (**1**, 350 mg), dissolved in 8.75 ml of Me_2CO, was evenly distributed among 35 flasks each containing 50 ml of stage II cultures. Fermentation was stopped after 6 days and broth extracted three times with equal vols of $CHCl_3$. Evapn of the combined extracts gave 490 mg of a semisolid residue. Fractionation of the crude extract on silica gel column using 25% EtOAc in hexane gave compounds **2** (30 mg) and **3** (22 mg), respectively.

Compound **2**, R_f 0.44 (*n*-hexane–EtOAc, 7:3); m.p. 156–157°C; $[\alpha]_D$ −74.0° (*c* 0.021; $CHCl_3$) (−62°) (Ruangrungsi et al., 1987); EIMS (*m/z*) (% relative intensity): 250 (M^+) (>1).

Compound **3**, R_f 0.39 (30% EtOAc in *n*-hexane); m.p. 151–152°C; $[\alpha]_D$ +27.7° (*c* 0.084; MeOH); IR (ν_{max}) (KBr) cm^{-1}: 1765 (lactone CO); EIMS (*m/z*) (% relative intensity): 250 (M^+) (>1); for the ^1H and ^{13}C NMR for **2** and **3** see Tables 1 and 2, respectively.

Compound **5** (8 mg) was obtained by further elution with 60% EtOAc in *n*-hexane; colorless gum; R_f 0.36 (40% Me_2CO in CH_2Cl_2); $[\alpha]_D$ −280.0° (*c* 0.04; $CHCl_3$); IR (ν_{max}) (KBr) cm^{-1}: 3610 (OH) and 1760 (lactone CO); EIMS (*m/z*) (% relative intensity): 266 (M^+) (>1); for the ^1H- and ^{13}C NMR assignments see Tables 1 and 2, respectively.

3.6. Preparation of 9β-hydroxydihydroparthenolide (*4*) from 7

9β-Hydroxy- parthenolide (**7**) was isolated and purified from *Anvillea garcinii* as reported in the literature (Abdel Sattar et al., 1996). To compound **7** (55 mg), dissolved in 4 ml absolute EtOH, sodium borohydride (35 mg) was added and the mixture was stirred for 1 h at room temperature. The reaction was quenched by addition of 0.3 ml of 25% acetic acid, the solvent was distilled off, residue dissolved in EtOAc, washed with 10% $NaHCO_3$ and dried over anhydrous Na_2SO_4. After evapn of the solvent, the residue was chromatographed on a silica gel column. Elution of the column using *n*-hexane–EtOAc (1:1) gave 20 mg of colorless cubes that were indistinguishable from those of compound **4**.

3.7. Preparation of *6* from *2*

Compound **2** (67 mg) was dissolved in CH_2Cl_2 (5 ml) and treated with selenium dioxide (16 mg) and *tert*-butylhydroperoxide (0.14 ml). The mixture was stirred at room temperature for 19 h, then CH_2Cl_2 (50

ml) was added. The reaction mixture was washed with a 2% soln of sodium bisulfite and the solvent was distilled off to leave an oily residue that was crystallized from a mixture of *n*-hexane and EtOAc to give 27 mg of **6** as cubic crystals, m.p. 195–196°C; R_f 0.28 (20% MeCN in $CHCl_3$); $[\alpha]_D$ −99.7° (*c* 0.06; $CHCl_3$); IR (ν_{max}) (KBr) cm^{-1}: 3450 (OH), 1780 (CO); EIMS (*m*/*z*) (% relative intensity): 266 (M^+) (>1); for the ^1H and ^{13}C NMR assignments see Tables 1 and 2, respectively.

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