PERGAMON

Phytochemistry 55 (2000) 19–22

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

# Transformation of jervine by *Cunninghamella elegans* ATCC 9245

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Received 26 January 2000; received in revised form 22 May 2000

**Abstract**

Preparative-scale fermentation of the known C-*nor*-D-homosteroidal jerveratrum alkaloid jervine with *Cunninghamella elegans* (ATCC 9245) has resulted in the isolation of (−)-jervinone as the major metabolite. In addition, *C. elegans* ATCC 9245 was able to epimerize C-3 of jervine, producing 3-*epi*-jervine. This epimerization reaction was similar to that reported for tomatidine, the known spirosolane-type *Solanum* alkaloid. The structure elucidation of both metabolites was based primarily on 1D- and 2D-NMR analyses. 

*Keywords:* Microbial metabolism; C-*nor*-D-homosteroidal jerveratrum alkaloid; *Cunninghamella elegans*; ATCC 9245; Jervine; (−)-Jervinone; 3-*epi*-Jervine

## 1. Introduction

*Veratrum* alkaloids are a group of potent hypotensive agents that lower blood pressure by reflex suppression of the cardiovascaular system (Kupchan et al., 1961; Honerjager, 1982). Jervine (**1**) is one of the most extensively studied and widely available C-*nor*-D-homosteroidal jervane alkaloids (Gaffield et al., 1986; Agrawal et al., 1991). Jervine and its 11-deoxo derivative, cyclopamine, are potent teratogens from *Veratrum californicum* that induce cyclopic malformations in sheep (Gaffield and Keeler, 1996). The key structural factor in jervane, solanidane and spirosolane alkaloids that induces the mammalian teratogenicity is the $\Delta^{5,6}$ unsaturated system (Gaffield and Keeler, 1993). Chemical transformation of jervine into testosterone via 14 synthetic steps was also reported (Murai et al., 1977). Jervine and several synthetic jervine derivatives display potent in vivo anti-inflammatory and glucocorticosteroidal activities in rats (Gerashchenko et al., 1974). Antimicrobial activity of jervine and other jerveratrum alkaloids against *Pityrosporum ovale*, *Trichophyton mentagrophytes* and *Saccharomyces cerevisiae*, and against the basidiomycete, *Polystictus versicolor* has been reported (Han and Woo, 1973; Wolters, 1970).

Microbial metabolism studies have been used successfully as model systems to predict metabolic pathways in humans or to increase the efficacy of drugs by metabolic activation. Fungi have enzyme system similar to those of mammalian since they are eukaryotics (Rosazza and Duffel, 1986). Hence, many fungi have recently been used as in vitro models for predicting mammalian drug metabolism (Clark et al., 1985). Steroidal alkaloids have been investigated in metabolism studies because of their interesting biological activities (Vining, 1980). Tomatidine, a spirosolane-type *Solanum* alkaloid with the commonly encountered six-membered C-ring and five-membered D-ring steroidal skeleton, when incubated for 120 h with *Gymnoascus reesii* CBS 39264 suspended in 0.4% NaCl at pH 4.7 resulted in the metabolism of tomatidine to 1,4-tomati-

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0031-9422/00/$ - see front matter 
PII: S0031-9422(00)00202-8

dien-3-one, tomatidone, 4-tomatiden-3-one, 1-tomatiden-3-one, and tomatanin-3α-ol (Rosazza and Duffel, 1986). The explanation of formation of tomatanin-3α-ol was proposed through oxidation followed by stereospecific reduction of tomatidine (Rosazza and Duffel, 1986). Similar metabolites, in addition to 15β-hydroxytomatidone and 4-hydroxy-3,4-secotomatidin-3-oic acid, were also produced by incubation of tomatidine with *Nocardia restrictus* and *Mycobacterium phlei* (Vining, 1980; Rosazza and Duffel, 1986). All organisms failed to induce any *N*-containing side chain modification. Jervine was the subject of a previous microbial metabolism study which enabled the conversion of jervine into an unidentified antibacterial product using *Piricularia oryzae* (Wolters, 1970). *Nocardia* species (ATCC 21145) are able to metabolize rings A and B of veratramine, the related C-*nor*-D-homosteroidal alkaloid, but fail to metabolize rings C and D or its *N*-containing side chain (El Sayed, 1998). In the present study, jervine (**1**) was chosen for a microbial bioconversion study in an attempt to prepare less toxic and more bioactive analogues, to determine whether metabolism in the *N*-containing side chain is possible and to compare its metabolism with that of veratramine and tomatidine.

## 2. Results and discussion

Thirty-five growing cultures were screened for their ability to bioconvert **1**. Few cultures were observed to completely or partially transform **1** into metabolites of lesser or greater polarity. The microbe *Cunninghamella elegans* (ATCC 9245) was chosen for preparative-scale fermentation because it entirely depleted **1** and converted it into less polar and more polar metabolites **2** and **3**, respectively.

The high resolution FT-ICR MS of **2** displayed a peak at $m/z$ 424.2858 $(M + H)^+$, suggesting the molecular formula $C_{27}H_{38}O_3N$ and 10 degrees of unsaturation. The IR, $^{13}C$- and $^{1}H$-NMR spectra of **2** (Table 1) suggested that it is closely similar to jervinone, reported from the rhizomes of *Veratrum album*, with few differences (Atta-ur-Rahman et al., 1992). The FT-IR spectrum of **2** ($CHCl_3$) displayed two strong absorption bands at 1709 and 1665 $cm^{-1}$,

Table 1
$^{13}C$- and $^{1}H$-NMR spectral data of (−)-jervinone (**2**) and 3-*epi*-jervine (**3**)[a]

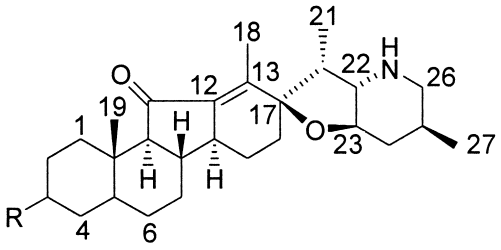
| Position | **2**[b] | | **3**[c] | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 35.2 | 2.36, *ddd* (13.0, 3.5, 3.1) 1.91, *m* | 38.2 | 2.55, *m*; 2.15, *m* |
| 2 | 29.9 | 2.17, 2H, *m* | 28.5 | 1.83, *m*; 1.70, *m* |
| 3 | 199.6 | – | 67.5 | 4.07, *brs* |
| 4 | 125.6 | 5.76, *s* | 30.8 | 2.34, *m*; 1.99, *m* |
| 5 | 168.5 | – | 139.5 | – |
| 6 | 32.8 | 2.34, 2H, *m* | 123.0 | 5.34, *dd* (5.0, 1.0) |
| 7 | 30.8 | 1.91, *m*; 1.50, *m* | 31.5 | 2.36, *m*; 1.60, *m* |
| 8 | 41.4 | 1.64, *m* | 38.0 | 1.62, *m* |
| 9 | 64.3 | 1.65, *m* | 62.8 | 1.80, *d* (13.3) |
| 10 | 38.5 | – | 33.5 | – |
| 11 | 204.5 | – | 207.0 | – |
| 12 | 138.0 | – | 138.5 | – |
| 13 | 146.9 | – | 146.5 | – |
| 14 | 43.8 | 1.95, *m* | 45.2 | 1.98, *m* |
| 15 | 23.9 | 1.96, *m*; 1.32, *m* | 23.9 | 1.92, *m*; 1.36, *m* |
| 16 | 33.6 | 2.35, *m*; 1.92, *m* | 33.5 | 2.36, *m*; 1.60, *m* |
| 17 | 85.4 | – | 86.0 | – |
| 18 | 12.3 | 2.19, 3H, *d* (2.1) | 11.6 | 2.18, 3H, *brs* |
| 19 | 16.9 | 1.16, 3H, *s* | 17.9 | 1.03, 3H, *s* |
| 20 | 40.2 | 2.06, *m* | 39.0 | 2.52, *m* |
| 21 | 10.8 | 0.96, 3H, *d* (7.1) | 10.9 | 0.81, 3H, *d* (7.4) |
| 22 | 66.5 | 2.73, *dd* (9.6, 8.9) | 66.4 | 2.65, *dd* (9.7, 9.0) |
| 23 | 76.3 | 3.31, *ddd* (10.1, 9.6, 3.5) | 76.9 | 3.34, *ddd* (10.3, 9.7, 3.5) |
| 24 | 38.8 | 2.70, *m*; 1.22, *m* | 39.5 | 2.21, *m*; 1.20, *m* |
| 25 | 31.4 | 1.61, *m* | 31.0 | 1.57, *m* |
| 26 | 54.6 | 3.09, *dd* (12.4, 3.5) 2.33, *m* | 54.6 | 3.07, *dd* (12.5, 3.7); 2.32, *m* |
| 27 | 18.8 | 0.95, 3H, *d* (6.7) | 18.7 | 0.80, 3H, *d* (6.6) |

[a] Coupling constants ($J$) are in Hz.
[b] In $CDCl_3$ at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$.
[c] In $CDCl_3$–$CD_3OD$ (9.5:0.5) at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$.

assigned for the α,β-unsaturated cyclopentenone carbonyl at C-11 and the α,β-unsaturated cyclohexenone carbonyl at C-3, respectively. While the reported $[\alpha]_D^{25}$ value for jervinone was +38.5° (*c* 1.0, $CHCl_3$) (Atta-ur-Rahman et al., 1992), metabolite **2** displayed an $[\alpha]_D^{25}$ value of −16.8° (*c* 1.0, $CHCl_3$), which is consistent with the reported value for the parent substrate, jervine (**1**) $[\alpha]_D^{25}$ −147° (*c* 1.0, EtOH), considering the difference in solvent (El Sayed et al., 1996). The $^{13}$C-NMR assignments of **2** were similar to those reported for (+)-jervinone (Atta-ur-Rahman et al., 1992), except for the transposed carbons C-2, C-16 and C-24. The olefinic H-4 proton singlet resonating at δ 5.76 displayed a $^3J$-HMBC coupling with the methylene carbon at δ 29.9 (C-2). Protons $H_2$-2 ($\delta_H$ 2.17) displayed COSY coupling with $H_2$-1 ($\delta_H$ 2.36 and 1.91). Protons $H_2$-16 displayed a $^3J$-HMBC coupling with the quaternary C-13 carbon resonating at δ 146.9. The methyl doublet resonating at δ 0.95 (C-27) displayed a $^3J$-HMBC coupling with C-24 which confirmed the assignment of this carbon. Hence, metabolite **2** was proved to be (−)-jervinone, a different compound from the reported (+)-jervinone (Atta-ur-Rahman et al., 1992).

The high resolution FT-ICR MS of **3** displayed a peak at *m*/*z* 426.3012 $(M + H)^+$, suggesting the same molecular formula of jervine (**1**) ($C_{27}H_{39}O_3N$). The $^{13}$C- and $^1$H-NMR spectra of **3** (Table 1) were similar to those of **1** and suggested that **3** is 3-*epi*-jervine. The broad proton singlet resonating at δ 4.07 which is correlated with the methine carbon at δ 67.5 was assigned H-3 (Table 1). This proton displayed a COSY coupling with $H_2$-2 protons absorbing at δ 1.83 and 1.70. The upfield shifting of C-3 and downfield shifting of H-3 in **3** (−4.1 and +0.57 ppm, respectively) as compared to those of **1** was consistent with **3** being 3-*epi*-jervine (El Sayed et al., 1996). This assumption was supported by the strong NOESY correlations between H-3 and both β-oriented equatorial H-1 (at δ 2.55) and axial H-2 (at δ 1.70) suggesting similar orientation of these protons in metabolite **3**. Metabolite **3** is thus confirmed to be 3-*epi*-jervine, a new natural product. This epimerization reaction was similar to that reported for tomatidine (Rosazza and Duffel, 1986). Hence, these results indicate the parallelism of jervine and tomatidine metabolism patterns.

Only two reported jerveratrum alkaloids were found to possess a C-3 α-hydroxy group: kuroyurinidine (2β,3α,6β-trihydroxy-5α-jervanin-12-ene), reported from the genus *Fritillaria* (Liliaceae) (Sashida et al., 1989) and verdine (1β,3α,6α-trihydroxy-5β-jervanin-12-en-11-one), isolated from *V. album* ssp. *Lobelianum* Bernh. (Tashkhodzhaev et al., 1984).




| | R | Other |
|---|---|---|
| Jervine (**1**) | β-OH | $\Delta^{5,6}$ |
| Jervinone (**2**) | =O | $\Delta^{4,5}$ |
| 3-*epi*-jervine (**3**) | α-OH | $\Delta^{5,6}$ |

## 3. Experimental

### 3.1. General

The $^1$H- and $^{13}$C-NMR spectra were recorded in $CDCl_3$, on a Bruker AMX-500 or 400 NMR spectrometer operating at 500 or 400 for $^1$H-NMR, and 125 or 100 MHz for $^{13}$C-NMR, respectively. The HRMS spectra were measured on a Bioapex FT-ICR MS with electrospray ionization. TLC analyses were carried out on precoated silica gel $G_{254}$ 500 μm, with the following developing system: $CHCl_3$–MeOH–$NH_4OH$ (80:20:0.01). For column chromatography, silica gel 60, 40 μm was used.

### 3.2. Chemicals

Jervine (**1**) was purchased from a commercial source and also isolated from the roots and rhizomes of *Veratrum viride* Aiton (El Sayed et al., 1996).

### 3.3. Organisms

Preliminary microbial metabolism studies were conducted as previously reported (Lee et al., 1990; El Sayed, 1998). Thirty-five microbial cultures obtained from the Department of Pharmacognosy, University of Mississippi culture collection were used for screening. These microbes were selected based on literature precedence for their abilities to catalyze related compounds. The microbes utilized were reported earlier (El Sayed, 1998), in addition to: *Aspergillus niger* ATCC 10581, *Bacillus megaterium* ATCC 9885, *Botrytis allii* ATCC 9435, *Cunninghamella echinulata* NRRL 3655, *Cunninghamella* species NRRL 5695, *Mucor mucedo* UI 4605, *Mucor ramannianus* ATCC 9628, *Nocardia restricta* ATCC14887, *Sporobolomyces pararoseus* ATCC 11386 and *Thamnidium elegans* ATCC 18191. Stock cultures were maintained on agar slants of media recommended by the ATCC and were stored at 4°C.

### 3.4. Microbial metabolism of jervine (*1*) by C. elegans

*C. elegans* (ATCC 9245) was grown in four, 1 l culture flasks, containing 200 ml of compound medium α which consists of (per liter of distilled water): glucose, 20 g; NaCl, 5 g; $K_2HPO_4$, 5 g; yeast extract (BBL, Cockeysville, MD), 5 g; peptone (Difco, Detroit, MI), 5 g. A total of 70 mg of **1** was dissolved in 1 ml EtOH, equally divided between the four flasks and distributed among the 24-h-old stage II cultures. After 14 days, the incubation mixtures were pooled and filtered. The filtrate (0.7 l) was exhaustively extracted with EtOAc ($3 \times 300$ ml), dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue (320 mg) was flash chromatographed over 50 g silica gel 60 starting with (100%) *n*-hexane and gradiently eluted with increasing proportions of EtOAc and finally with (100%) MeOH. Alkaloid-containing fractions were subjected to repeated preparative TLC on silica gel $G_{254}$ using $CHCl_3$–MeOH–$NH_4OH$ (80:20:0.01) as a solvent system to give two metabolites: **2** (28.0 mg, $R_f$ 0.70) and **3** (1.5 mg, $R_f$ 0.32).

### 3.5. (−)-Jervinone (*2*)

Colorless needles from EtOH, mp 243–244°C, $[\alpha]_D^{25}$ −16.8° (*c* 1.0, $CHCl_3$); UV $\lambda_{max}$ (log ε) (MeOH) 222 (4.51), 249 (4.75) nm; IR $\nu_{max}$ ($CHCl_3$): 3547 (NH), 3027–2860, 1709 (C═O), 1665 (C═O), 1630 (C═C), 1485, 1342, 1237, 1118, 1016, 795 $cm^{-1}$; $^{13}C$- and $^{1}H$-NMR, see Table 1; FT-ICR MS *m/z* calculated for $C_{27}H_{38}O_3N$ $[M + H]^+$ 424.2852, found 424.2858.

### 3.6. 3-epi-Jervine (*3*)

Yellow needles from MeOH, mp 248–250°C, $[\alpha]_D^{25}$ +88.7° (*c* 0.042, $CHCl_3$); UV $\lambda_{max}$ (log ε) (MeOH) 221 (4.49), 248 (4.71) nm; IR $\nu_{max}$ ($CHCl_3$): 3698 (OH), 3596 (NH), 3021–2856, 1714 (C═O), 1605 (C═C), 1508, 1458, 1361, 1271, 1158, 1026, 860 $cm^{-1}$; $^{13}C$- and $^{1}H$-NMR, see Table 1; FT-ICR MS *m/z* calculated for $C_{27}H_{40}O_3N$ $[M + H]^+$ 426.3008, found 426.3012.

## Acknowledgements

Dr James O'Neal, National Center for Natural Products Research, University of Mississippi, is acknowledged for the HRMS analysis.

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