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Solistatin, an aromatic compactin analogue from *Penicillium solitum*

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Abstract

Solistatin, (+)-(3*R*,5*R*)-7-(2′-methyl-1′-naphthyl)-3-hydroxyheptan-5-olide (**1**), has been isolated from *Penicillium solitum*. The structure and relative stereochemistry were established by NMR spectroscopy and mass spectrometry. The absolute stereochemistry was determined by chemical degradation and comparison of CD data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Penicillium solitum; β-Hydroxy-δ-lactone; Solistatin; Compactin; Chemotaxonomy

1. Introduction

Penicillium solitum occasionally appears as a contaminant on different food items like hard and semi-hard cheeses (Lund, Filtenborg, & Frisvad, 1995), Danablu cheese and rye bread (Lund, 1995a) and on fruits and cereals (Frisvad & Filtenborg, 1989). The species is very closely related to *P. commune* and *P. palitans* and the three species can hardly be differentiated by the morphology of their conidiogenous structures (Lund, 1995b). However, only cultures of *P. solitum* grown on YES agar display an orange reverse. Moreover, all three may be distinguished by the typical HPLC profile of secondary metabolites (Frisvad & Thrane, 1987). *P. solitum* produces cyclopenin, cyclopenol, viridicatin and a number of compactins (Frisvad & Filtenborg, 1989). During our work with isolates from cheese associated *P. solitum*, we became aware of the presence of solistatin. Solistatin (**1**) is an aromatic analogue of compactin, indicating a biosynthetical relationship.

2. Results and discussion

Solistatin, (+)-(3*R*,5*R*)-7-(2′-methyl-1′-naphthyl)-3-hydroxyheptan-5-olide (**1**), was isolated from *P. solitum* by column chromatography as a semisolid colorless residue. The purity of **1** was determined by HPLC and ^{1}H NMR analysis. The molecular ion appeared in HREIMS at *m*/*z* 284.1405, corresponding to the composition $C_{18}H_{20}O_3$ (Δ −0.7 mmu). The presence of the 2′-methyl-1′-naphthylmethyl moiety was suggested by the EIMS base-peak at *m*/*z* 155 $[CH_3–C_{10}H_6–CH_2]^+$ and UV absorption in the region 250–330 nm. This was substantiated by the ^{1}H NMR and ^{13}C NMR aromatic signals and methyl group singlet (δ 2.51). COSY revealed the sequence of H-5′, H-6′, H-7′ and H-8′ and the correlation between H-3′ and H-4′. The corresponding carbon signals were assigned using HMQC. NOESY served to connect H-4′ with H-5′, H-3′ with Me and H-8′ with H-7. The remaining aromatic carbon signals were assigned from the HMBC cross-peaks. The results includes $^3J_{\text{H–Me,C-3′}}$ and $^3J_{\text{H-3′,C–Me}}$, which together with $^2J_{\text{H–Me,C-2′}}$, $^2J_{\text{H-7,C-1′}}$, $^3J_{\text{H-3′,C-1′}}$ and an unusual homoallylic $^4J_{\text{H-6,C-2′}}$ coupling identified the relative position of C-1′ and C-2′ atoms. The

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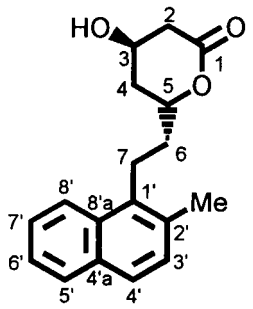

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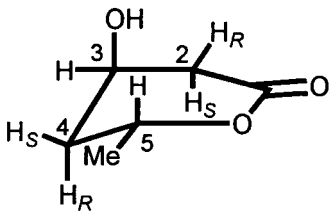

2

positions of C-4′a and C-8′a atoms are established by the cross-peaks of $^3J_{\text{H-6}',\text{C-4}'\text{a}}$ and $^3J_{\text{H-7}',\text{C-8}'\text{a}}$, respectively.

The assignment of the lactone moiety is in accordance with data from (+)-(3*R*,5*R*)-3-hydroxytetradecan-5-olide and compactin (Brown, Smale, King, Hasenkamp, & Thompson, 1976; Yada, Sato, Kaneko, & Ichihara, 1993). COSY confirmed correlation between H-3 and H-2. Furthermore, the coinciding multiplets of H-4 and H-6 showed correlation to H-3, H-5 and H-7. Simultaneous decoupling of H-3 and H-5 reduced the H-4 coupling pattern to a pair of germinal doublets ($J=14.3$ Hz) of which H-4*S* was partly obscured by overlap of H-6a. At the same time H-6b was simplified, revealing the germinal coupling and the coupling constants to H-7 (ddd, $J=14.3$, 10.8 and 6.1 Hz). Saturation of H-7 simplified the pattern from H-6b and gave the coupling constant to H-5 ($J=3.7$ Hz). It proved impossible to characterize H-4*S* and H-6a individually due to overlap. The value of the chemical shift (δ 170.1), indicated C-1 to arise from an ester group. C-1 was correlated to H-2 and H-3 through HMBC $^2J_{\text{H-2,C-1}}$ and $^3J_{\text{H-3,C-1}}$. The remaining carbon signals were readily assigned by HMQC.

The relative stereochemistry was established by the coupling constants of the lactone ring, confirming the conformation suggested by an energy minimized (AM1) molecular model (**2**). A W-coupling ($^4J_{\text{H-2}R,\text{ H-4}S}=1.7$ Hz) indicates the relative orientation of H-2*R* and H-4*S* atoms. The couplings of H-3 to H-2 and H-4 are all in the range of $J=3.5$–5.0 Hz. This indicates the equatorial orientation of the H-3 atom in accordance with the Karplus equation. Accordingly, the hydroxyl group must be axially oriented. H-4*R* has a large coupling to H-5 ($^3J_{\text{H-4}R,\text{H-5}}=11.4$ Hz), indicating the H-5 atom to be axially oriented.

In order to assign absolute stereochemistry, solistatin was dehydrated with TsOH in benzene to yield (5*R*)-7-(2′-methyl-1′-naphthyl)-2-hepten-5-olide. The wavelengths, signs and relative intensity of the CD curve of the product agree with the corresponding data of (−)-(5*R*)-2-tetradecen-5-olide (Yada et al., 1993). Since the absolute configuration of the lactone ring is identical to that found in compactin (Brown et al., 1976; Keck & Kachensky, 1986), this result strongly supports the proposed biosynthetic relation. The synthesis of racemic 7-(2′-methyl-1′-naphthyl)-3-hydroxyheptan-5-olide, being an effective inhibitor of cholesterol biosynthesis, is claimed in a patent (Oka, Terahara, & Endo, 1979). However, the description totally lacks any characterization of the compound.

3. Experimental

Two isolates of cheese associated *P. solitum*, from the IBT Culture Collection at the Department of Biotechnology (IBT), Technical University of Denmark, were found to be producers of **1**, as indicated by HPLC (Frisvad & Thane, 1987; Smedsgaard, 1997): IBT 14859 (ex Danablu cheese, Denmark) and IBT 15170 (ex Manchego cheese, Spain). IBT 15170 on YES was selected for the present investigation due to good production of **1** and relative low production of other metabolites in the chromatographic window. IBT 15170 was cultivated for 3 weeks in the dark at 25°C on 200 YES agar plates (three point mass inoculation) and extracted repeatedly with EtOAc to give 5.859 g crude product. The extract was partitioned using a modified Kupchan-scheme: Partitioning with $H_2O/CHCl_3$ followed by *n*-heptane/90% $MeOH_{aq}$ and 60% $MeOH_{aq}/CHCl_3$ gave 3.022 g $CHCl_3$ extract. Silica gel VLC using 50 ml step-gradient elution from *n*-heptane to EtOAc, then EtOAc/MeOH followed by silica gel LC with *n*-heptane–EtOAc yielded 161.9 mg purified product. Repeated separation on RP-18 HPLC using H_2O–CH_3CN (50:50) gave 10.4 mg semi-solid colorless solistatin. UV: $\lambda_{\max}^{\text{EtOH}}$ nm (log ε): 227 (4.09), 275 (2.84), 283 (2.87), 292 (2.75), 306 (2.13), 312 (1.95), 321 (2.09). $[\alpha]_D^{20}$ +32.0° (EtOH; *c* 0.251). CD: $\Delta\varepsilon_{243}$ −0.076, $\Delta\varepsilon_{287}$ +0.041 (EtOH; c 0.0015). ^{1}H NMR (399.938 MHz, $CDCl_3$): δ 8.03 (1H, br d, $J=8.8$ Hz, H-8′), 7.80 (1H, br d, $J=8.2$ Hz, H-5′), 7.64 (1H, d, $J=8.2$ Hz, H-4′), 7.50 (1H, ddd, $J=8.8$, 6.8 and 1.5 Hz, H-7′), 7.41 (1H, ddd, $J=8.2$, 6.8 and 1.1 Hz, H-6′), 7.30 (1H, d, $J=8.4$ Hz, H-3′), 4.85 (1H, dddd, $J=11.4$, 8.2, 3.7 and 3.5 Hz, H-5), 4.41 (1H, dddd,

$J = 5.0$, 3.7, 3.6 and 3.5 Hz, H-3), 3.40 (1H, ddd, $J = 13.9$, 11.0 and 5.1 Hz, H-7a), 3.18 (1H, ddd, $J = 13.9$, 10.8 and 6.0 Hz, H-7b), 2.79 (1H, dd, $J = 17.6$ and 5.0 Hz, H-2*S*), 2.67 (1H, ddd, $J = 17.6$, 3.7 and 1.7 Hz, H-2*R*), 2.51 (3H, s, Me), 2.00 (2H, m, H-4*S* and H-6a), 1.91 (1H, dddd, $J = 14.3$, 10.8, 6.1 and 3.7 Hz, H-6b), 1.84 (1H, ddd, $J = 14.3$, 11.4 and 3.29 Hz, H-4*R*). ^{13}C NMR (100.573 MHz, $CDCl_3$): δ 170.1 (C-1), 134.1 (C-2′), 133.0 (C-1′), 132.5 (C-4′a), 131.8 (C-8′a), 129.1 (C-3′), 128.5 (C-5′), 126.3 (C-4′), 126.0 (C-7′), 124.5 (C-6′), 123.2 (C-8′), 75.4 (C-5), 62.8 (C-3), 38.6 (C-2), 36.0 (C-4 or C-6), 35.6 (C-4 or C-6), 23.9 (C-7), 20.0 (Me). EIMS (probe) 70 eV, *m*/*z* (rel. int): 284 $[M]^+$ (52), 266 $[M\text{-}H_2O]^+$ (20), 180 (47), 155 (100), 154 (66). HREIMS (probe) 70 eV, *m*/*z*: 284.1405 $[M]^+$ $C_{18}H_{20}O_3$ (Δ −0.7 mmu). Solistatin (7 mg) was dehydrated with TsOH in benzene under reflux for 1 h. The product was purified using silica gel VLC eluted with 5 ml step gradient from *n*-heptane to EtOAc to give 2.5 mg of (5*R*)-7-(2′-methyl-1′-naphthyl)-2-hepten-5-olide with NMR data as previously described (Brown et al., 1976). UV: λ_{max}^{EtOH} nm (log ε): 227 (4.45), 274 (2.24), 283 (2.28), 291 (2.16), 306 (151), 312 (1.33), 321 (1.42). $[\alpha]_D^{20}$ −35.4° (EtOH; *c* 0.153). CD: $\Delta\varepsilon_{226}$ −5.094, $\Delta\varepsilon_{240}$ −0.049, $\Delta\varepsilon_{256}$ −0.080 (EtOH; *c* 0.0057). CD data for (−)-(5*R*)-2-tetradecen-5-olide are: $\Delta\varepsilon_{236}$ −1.3, $\Delta\varepsilon_{255}$ −2.4 (MeOH) (Yada et al., 1993).

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