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Dipodazine, a diketopiperazine from *Penicillium dipodomyis*

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Abstract

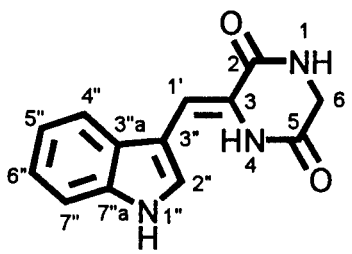
Dipodazine, (*Z*)-1′,3-didehydro-3-(3″-indolylmethylene)-piperazine-2,5-dione (**1**), has been isolated from *Penicillium dipodomyis* and is also present in *P. nalgiovense*. The structure was established by spectroscopical methods.

Keywords: *Penicillium dipodomyis*; *Penicillium nalgiovense*; Diketopiperazine; Dipodazine; Chemotaxonomy

1. Introduction

Penicillium dipodomyis has been isolated from underground seed caches and cheek pouches of banner-tailed kangaroo rats (*Dipodomys spectabilis*) (Frisvad, Filtenborg, & Wicklow, 1987). *P. nalgiovense* is one of the most dominant fungi associated with cheese and mould-fermented sausages (Andersen, 1995; Berwal & Dincho, 1995; Lund, Filtenborg, & Frisvad, 1995). Together with *P. flavigenum*, they are penicillin producers belonging in the *P. chrysogenum* complex of taxonomically related xerophilic penicillia (Raistrick & Ziffer, 1951; Andersen & Frisvad, 1994; Samson, van Reenen-Hoekstra, Frisvad, & Filtenborg, 1995; Banke, Frisvad, & Rosendahl, 1997). Although *P. nalgiovense* is regarded as a domesticated form of *P. chrysogenum* (Stolk, Samson, Frisvad, & Filtenborg, 1990), it was concluded from isozyme analysis that *P. nalgiovense* is closer related to *P. dipodomyis* than to *P. chysogenum* and *P. flavigenum* (Banke et al., 1997).

In support of this, during chemotaxonomical HPLC investigations of terverticillate penicillia (Svendsen & Frisvad, 1994), it was discovered that *P. dipodomyis* and meat associated *P. nalgiovense* share the ability to produce unknown blue-flourescent metabolites. In the present study the major component, dipodazine (**1**), has been isolated and characterized.



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2. Results and discussion

Following extraction of a *P. dipodomyis* culture with EtOAc, **1** was isolated by liquid partitioning and silica gel VLC as a grey solid. The purity was confirmed by ^{1}H and ^{13}C NMR. The HREIMS showed a molecular ion at m/z 241.0854, corresponding to the composition $C_{13}H_{11}O_2N_3$ (Δ +0.3 mmu). The conjugated indole moeity was implied by the pattern of ^{1}H NMR signals

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(δ 7.93, 7.63, 7.42, 7.17, 7.10 and 7.00) identical to the corresponding signals from *N*-acetyl-(*E*)-α,β-didehydrotryptophan ethyl ester (Genet, Bénetti, Hammadi, & Ménez, 1995). The D_2O exchangeable signal at δ 11.62 was attributed to an indole NH. Furthermore the D_2O exchangeable signals at δ 9.45 and δ 8.10, together with carbonyl ^{13}C NMR signals at δ 164.5 and δ 160.7, indicated the presence of two secondary amide groups. Considering the formula and indole moeity, this suggested a Trp–Gly derived cyclic dipeptide. The sequence of H-4″, H-5″, H-6″ and H-7″ was deduced from COSY. All proton signals were assigned to their respective carbon signals by HMQC. HMBC results established the positions of C-3″a and C-7″a atoms by the cross peaks of $^3J_{C\text{-}3''a,H\text{-}2''}$, $^3J_{C\text{-}3''a,H\text{-}1'}$ and $^3J_{C\text{-}7''a,H\text{-}2''}$. The cross peaks originating from $^3J_{C\text{-}1',H\text{-}2''}$ and $^3J_{C\text{-}2,H\text{-}1'}$ connected the CH-1′ group to the indole 3″-position and the diketopiperazine. Moreover, $^2J_{C\text{-}5,H\text{-}6}$ and $^3J_{C\text{-}2,H\text{-}6}$ confirmed the diketopiperazine ring structure. The assignments of C-3 and C-3″ remains interchangeable, due to lack of definite HMBC correlations. NOESY exhibited a cross peak between H-4″ and the vinyllic H-1′ of the conjugated side chain. NOE difference spectroscopy with decoupling of indole H-1″ gave signal enhancement of H-2″ (9%) and H-7″ (3%), establishing the presence of the 3″-substituted indole ring. Decoupling of H-1 resulted in enhancement of H-6 (4%). Similar decoupling of H-4 resulted in enhancement of H-2″ (10%), thus assuring the (*Z*)-configuration of **1**.

The structure of **1** has been reported at a conference as an intermediate in the synthetic pursuit of the echinulines (Oikawa, Yoshioka, & Yonemitsu, 1978). However, without any characterizing data. The fact that **1** is produced by both *P. dipodomyis* and *P. nalgiovense* supports the close relationship between the two species suggested by Banke et al. (1997). Our results show that both *P. dipodomyis* and meat associated *P. nalgiovense* isolates, but not the type culture and the cheese associated *P. nalgiovense* isolates, produce **1**. This character difference can possibly be attributed to domestication, as the *P. nalgiovense* isolates represents two different functional lineages of industrial strains.

3. Experimental

The following isolates were retrieved from the IBT Culture Collection at the Department of Biotechnology (IBT), Technical University of Denmark: *P. dipodomyis*: IBT 3353 (ex soil, Walnut Crater, AZ), IBT 3792 (ex cheek pouches of *Dipodomys spectabilis*, Arizona, USA), IBT 17759 (ex barley, Wyoming, USA); *P. nalgiovense*: IBT 3798 (ex salami, Germany), IBT 3800 (type culture, ex cheese, former Czechoslovakia), IBT 4090 (ex salami, Italy), IBT 12044 (ex salami, Denmark), IBT 12679 (ex cheese, Denmark) and IBT 13039 (ex cheese, Crete). All isolates were screened for production of **1**, with the results discussed above (Smedsgaard, 1997). IBT 17759 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 12.64 g crude product. 12 g of this was partitioned with $H_2O/CHCl_3$. An insoluble residue was isolated by filtering through kieselguhr, which was subsequently washed with acetone. This acetone extract (836 mg) was subjected to silica gel VLC using 50 ml step-gradient elution from EtOAc to MeOH, concentrating **1** in a residue (64.1 mg). Further washing 5× with 1 ml $CHCl_3$–MeOH (90:10) left **1** insoluble as a grey solid (14.5 mg). UV: λ_{max} nm (rel. abs. in CH_3CN–H_2O): 226 (100), 280 (34), 340 (77). ^1H NMR (399.938 MHz, DMSO-d_6): δ 11.62 (1H, br s exc D_2O, H-1″), 9.45 (1H, br s exc D_2O, H-4), 8.10 (1H, br s exc D_2O, H-1), 7.93 (1H, d, $J=2.8$ Hz, H-2″), 7.63 (1H, d, $J=7.7$ Hz, H-4″), 7.42 (1H, d, $J=8.1$ Hz, H-7″), 7.17 (1H, t, $J=7.1$ Hz, H-6″), 7.10 (1H, t, $J=7.1$ Hz, H-5″), 7.00 (1H, s, H-1′), 4.00 (2H, d, $J=1.8$ Hz, H-6) ^{13}C NMR (100.573 MHz, DMSO-d_6): δ 164,5 (C-5), 160.7 (C-2), 135.7 (C-7″a), 127.0 (C-3″a), 126.2 (C-2″), 122.6 (C-3 or C-3″), 122.1 (C-6″), 119.9 (C-5″), 118.1 (C-4″), 111.8 (C-7″), 107.9 (C-3″ or C-3), 107.6 (C-1′), 45.0 (C-6). EIMS (probe) 70 eV, *m/z* (rel. int): 241 $[M]^+$ (100), 156 (47), 155 (41). HREIMS (probe) 70 eV, *m/z*: 241.0854 $[M]^+$ $C_{13}H_{11}O_2N_3$ (Δ +0.3 mmu).

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