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NIGRIFORTINE, A DIKETOPIPERAZINE METABOLITE OF *PENICILLIUM NIGRICANS*

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Key Word Index—*Penicillium nigricans*; nigrifortine; diketopiperazine; biosynthesis.

Abstract—The structure of a novel diketopiperazine metabolite, named nigrifortine, isolated from cultures of *Penicillium nigricans* is deduced from its ^{1}H NMR, mass and UV spectra together with biosynthetic reasoning.

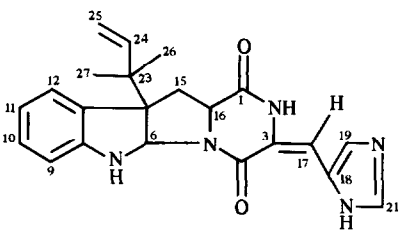
INTRODUCTION

In recent years an increasing number of simple and substituted diketopiperazine alkaloids, derived from two amino acids, have been isolated from fungi. Over 40 such substances are listed [1, 2] as fungal metabolites, the biosynthesis of which frequently involves one or more aromatic amino acid precursors. Although a strain of *Penicillium nigricans* has been shown to elaborate the simple symmetrical dimer L-phenylalanine anhydride [3], neither the equivalent dimer of tryptophan nor a derivative of it has yet been reported.

RESULTS AND DISCUSSION

In the course of studies on the biosynthesis of the indolic penitrem mycotoxins an isolate of *P. nigricans* in our laboratory [4] became the focus of attention since it could be induced by calcium chloride to sporulate in submerged fermentation and, concomitantly, produce penitrem mycotoxins together with the antibiotic griseofulvin [5]. Mycelial extracts, made from shaken flask cultures given [*benzene ring-U*-^{14}C]tryptophan during the phase in which penitrems are biosynthesized, were found also to contain a less polar substance which, from TLC autoradiography, was evidently derived from tryptophan. The yield of the compound, 6 mg from the mycelium grown submerged in 100 ml of the medium, was *ca* twice that of penitrems and thus it was at least a principal secondary metabolite produced under these conditions. The specific activity of the metabolite derived from [^{14}C]tryptophan was 4.27×10^4 dpm/mg, an incorporation of 4%. Cultures given [2-^{14}C]mevalonic acid also incorporated the radiolabel into the metabolites with similar efficiency (specific activity of metabolite, 3.64×10^4 dpm/mg).

Fast atom bombardment (FAB) and electron impact (EI) mass spectrometry gave the molecular formula $C_{32}H_{36}N_4O_2$ and fragmentation losses equivalent to two isoprenes. The loss of the first isoprene is analogous to the loss (69 mass units = C_5H_9) evident in the fragmentation of roquefortine (**1**) [6], a substituted indolic diketo-



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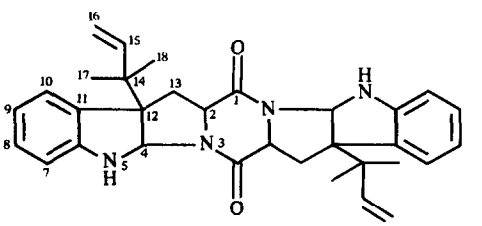

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Table 1. 250 MHz ^{1}H NMR spectra of nigrifortine (2) and roquefortine (1) (δ-values in 5 mg/ml deuterochloroform)

Nigrifortine																		
Proton	2	4	5	7	8	9	10	13a	13e	15	16c	16t	17	18				
Chemical shift (δ)	3.85	5.42	4.9	6.45	7.0	6.68	7.18	2.40	2.50	5.98	5.10	5.05	1.00	1.10				
Signal type	*dd*	*s*	*s*	*dd*	*dt*	*dt*	*dd*	*dd*	*dd*	*dd*	*dd*	*dd*	*s*	*s*				
Coupling	2, 13a	2, 13e	7, 8	7, 9	8, 9	8, 10	9, 10	13a, 13e	15, 16e	15, 16t	16c, 16t	—	—	—	—			
Constant (Hz)	6.75	10.5	7.5	1.0	7.5	1.0	7.5	12.75	10.5	17	1.0	—	—	—	—			
Roquefortine																		
Proton	2	6	7	9	10	11	12	15a	15e	16a	17	19	21	24	25c	25t	26	27
Chemical shift (δ)	12.9	5.64	4.95	6.6	7.1	6.75	7.15	2.60	2.45	4.05	6.20	7.68	7.7	6.00	5.15	5.10	1.00	1.15
Signal type	*s*	*s*	*s*	*dd*	*dt*	*dt*	*dd*	*dd*	*dd*	*dd*	*s*	*s*	*s*	*dd*	*dd*	*dd*	*s*	*s*
Coupling	9, 10	9, 11	10, 11	10, 12	11, 12	15a, 15e	15e, 16	15a, 16	24, 25c	24, 25t	25c, 25t	—	—	—	—	—	—	—
Constant (Hz)	7.5	1.0	7.5	1.0	7.5	11.25	11.25	6.25	11.0	17	1.0	—	—	—	—	—	—	—

piperazine produced concurrently with the penitrem toxins by *P. crustosum* [7].

The ^{1}H NMR spectrum integrated for only 18 protons, instead of the expected 36, indicating a symmetrical dimer. Interpretation of the spectrum (Table 1), by comparison with that of roquefortine, indicated a structure (**2**), which is consistent with the other spectral data and is in accord with evidence concerning incorporation of indolic and isoprene precursors. The trivial name, nigrifortine, is proposed on account of the producing organism and analogies with roquefortine. Nigrifortine is, therefore, the first example of a prenylated diketopiperazine fungal metabolite in the form of an indolic symmetrical dimer. The compound had no apparent effect when given intraperitoneally to mice at up to 40 mg/kg.

EXPERIMENTAL

Penicillium nigricans Bainer ex. Thom. (IMI 228669), recently included within the description of *P. janczewskii* Zaleski [8], was grown in submerged culture in 500 ml Erlenmeyer flasks containing 100 ml Czepek Dox broth supplemented with yeast extract (0.5%) and $CaCl_2$ (2%). DL-[*benzene-ring-U*-^{14}C]Tryptophan (10 μCi) was distributed equally between two flasks at 3 days. DL-[2-^{14}C]mevalonic acid was given similarly to other cultures and all mycelia were harvested 2 days later. Freeze-dried mycelia were extracted with Me_2CO (200 ml/g dry wt) and a portion of the extract chromatographed over silica gel using $CHCl_3$–Me_2CO (93:7).

Chromatograms were autoradiographed using X-ray film for 3 days at −80°. [^{14}C]Nigrifortine was isolated from the remaining extract by prep. TLC using $CHCl_3$–Me_2CO (19:1) followed by reversed-phase HPLC through an ODS column (250 × 4.6 mm), using MeOH–H_2O (5:1), flow rate 2 ml/min and UV detection at 242 nm. [^{14}C]Nigrifortine had a retention vol. of 13 ml in this system and was isolated as a white solid.

The nigrifortine thus isolated was dissolved in MeOH (1 ml), a portion of which (3 × 100 μl) was assayed for ^{14}C by liquid scintillation counting. The nigrifortine concn of the remaining MeOH soln was determined by use of a calibrated HPLC assay, based on the conditions described above, which allowed calculation of the sp. act. of the nigrifortine isolated. Dried mycelium grown similarly in a 60 l. fermenter [5] was the source of nigrifortine, purified by prep. TLC and HPLC, used to calibrate the assay and to obtain the physical data from which the structure is deduced.

FAB spectra showed an M_r of 508. EI spectra (including HRMS) showed the following important ions for nigrifortine *m/z* (rel. int.): 508 $[M]^+$ (44), 439 $[M-69]^+$ (100), 371 $[M-137]^+$ (19). HRMS $[M]^+$ 508.2856, $C_{32}H_{36}N_4O_2$ (calc. 508.2838). UV λ_{max}^{MeOH} nm (ε): 208 (31 300), 242 (6280), 298 (2360). NMR: 250 MHz (Table 1).

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