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SHORT REPORTS

A DIOXOPIPERAZINE DERIVATIVE FROM *PENICILLIUM MEGASPORUM**

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Key Word Index—*Penicillium megasporum*; dioxopiperazine; megasporizine; physcion; 7-hydroxy-4,6-dimethylphthalide; asperphenamate; phyllostine.

Abstract—Megasporizine, a new dioxopiperazine derivative, has been isolated from *Penicillium megasporum* NHL 2977, together with physcion (parietin), 7-hydroxy-4,6-dimethylphthalide, asperphenamate, and phyllostine. Megasporizine was also isolated from the strains NRRL 2232 and ATCC 48997 along with physcion, asperphenamate, and penicillic acid. The structure of megasporizine has been investigated by spectroscopic means. Megasporizine is a dioxopiperazine which has a methoxy group at the α-carbon of an amino acid residue.

INTRODUCTION

The hyphomycete *Penicillium megasporum* Orpurt et Fennell, which was originally isolated from grassland soil in southern Wisconsin and from heath soil in England [2], has been encountered in the course of a survey of the fungal pollutants of the Nagasaki Prefecture in Japan. This fungus, although one of the rarer *Penicillium* species, nevertheless has a worldwide distribution with most data available for those strains from temperate zones, including the U.S.A., the U.K., Ireland, Canada, India (as *P. giganteum* Roy and Singh), and Lebanon [2–4].

Penicillium megasporum. characterized by large, coarsely spinulose, globose conidia averaging 6.5 μm in diameter, produces predominantly biverticillate penicilli and is assigned to the series Megaspora in the subgenus *Furcatum* [4]. Pitt stated that the species (*P. megasporum* and *P. asperosporum* G. Smith) in the series Megaspora show little affinity with most other *Penicillium* species because of their distinctive conidial size and ornamentation.

Penicillium megasporum NHL 2977 was isolated from marine sludge from Ohmura Bay, Nagasaki Prefecture in April, 1981, by S. Ueda. We have examined our original isolate (NHL 2977) and two typical strains (NRRL 2232 and ATCC 48997) of *P. megasporum* with respect to secondary metabolite production, in an attempt to provide additional means for the taxonomic clarification of this eccentric species.

RESULTS AND DISCUSSION

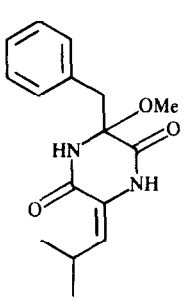
Megasporizine (**1**) was isolated, along with physcion (parietin) (**2**) [5], 7-hydroxy-4,6-dimethylphthalide (**3**) [6], asperphenamate (**4**) [7, 8], and phyllostine (**5**) [9], from the methylene chloride extract of our isolate cultured on rice.

Megasporizine (**1**) gave a molecular ion at *m/z* 288 in EIMS, and elemental analysis confirmed the molecular formula as $C_{16}H_{20}N_2O_3$. The strong ions at *m/z* 197 [M $-C_6H_5CH_2]^+$ and 91 $[C_6H_5CH_2]^+$ in the EIMS suggested the presence of a benzyl group in the molecule. The ^{1}H NMR signals at δ7.32 (5H, *m*), 3.28 (1H, *d*), and 3.17 (1H, *d*), the six ^{13}C NMR signals at δ126.9–134.1, and the one ^{13}C NMR signal at δ43.8 (*Tbr s*) were assigned to one benzyl group. The ^{1}H NMR signals at δ0.89 (3H), 1.00 (3H), and 2.44 (1H), which corresponded to the ^{13}C NMR signals at δ22.1 (2C, *Qm*) and 23.6 (*Dm*), were assigned to an isopropyl residue, which was directly connected to the double bond [the carbon signals at δ125.1 (*Dm*) and 124.2 (*d*) and the proton signal at δ5.76 (1H, *d*)].

The other ^{1}H NMR signals at δ3.31 (3H), 6.80 (1H), and 8.41 (1H) were assigned to a methoxy group, which appeared at δ50.4 (Q, $J = 142$ Hz) in the ^{13}C NMR spectrum, and two NH groups, respectively. The IR absorption at 1685 cm^{-1} and the ^{13}C NMR signals at δ159.7 (*d*) and 163.0 (*br d*) in 1 suggested two amide carbonyls. The carbon signals at δ124.2 (*d*) and 159.7 (*d*), which were assigned to the carbon of the double bond bearing the isopropyl residue and one of the amide carbonyls, respectively, were each changed into a singlet on selective irradiation of the vinylic proton (δ5.76). The above results are consistent with structure **1** for megasporizine.

Gallina and Liberatori discussed the stereochemistry of the double bond in 3-isobutylidenedioxopiperazines in terms of their ^{1}H NMR spectra [10]. The vinylic proton

*Part 25 in the series 'Studies on Fungal Products.' For Part 24 see ref. [1].



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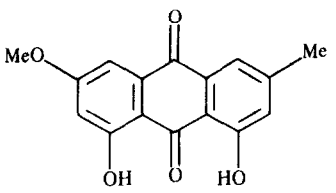

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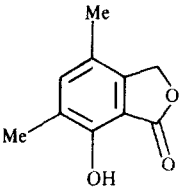

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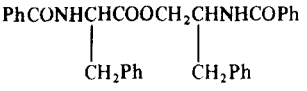

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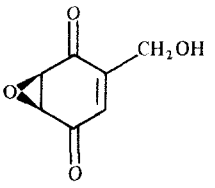

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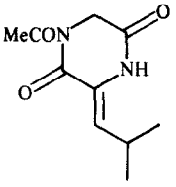

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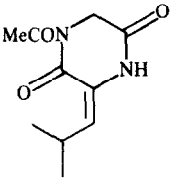

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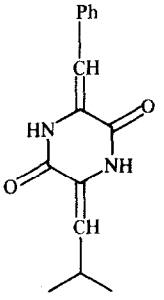

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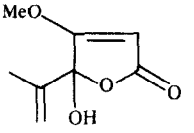

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signal in **6** (*Z*-isomer) appeared at δ6.28, whereas that in **7** (*E*-isomer) at δ5.63, i.e. 0.65 ppm upfield. The vinylic proton of **1** appeared at δ5.76 which corresponded well to that of **7**. This result showed that the configuration of the isobutylidene residue in megasporizine (**1**) is *E*. The absolute configuration of the methoxy group in **1** has not been determined yet.

Megasporizine (**1**) is the first example of a dioxopiperazine which has a methoxy group at the α-carbon of an amino acid residue. It is also a rare example of a dioxopiperazine derived from phenylalanine and leucine. Albonoursin (**8**) [11] and leucylphenylalanine anhydride [12], isolated from *Streptomyces noursei*, are the only other examples.

The metabolites of two typical strains of *P. megasporum* were also investigated on TLC. Megasporizine (**1**), physcion (**2**), and asperphenamate (**4**), along with penicillic acid (**9**) [13], were detected from the strains NRRL 2232 and ATCC 48997.

EXPERIMENTAL

General. Mps: uncorr. Low pressure LC (LPLC) was performed on a Chemco Low-Prep 81-M-2 in a glass column (150 × 10 mm) packed with silica gel CQ-3 (30–50μ; Wako).

Isolation of megasporizine (**1**) *and other metabolites. Penicillium megasporum*, strain NHL 2977, was cultivated at 27° for 3 weeks on rice (450 g). The cultivated rice was extracted with CH_2Cl_2, and the organic layer dried (Na_2SO_4) and evapd. The residue (1.07 g) was chromatographed on silica gel with $CHCl_3$ to give physcion (parietin) (**2**) [5] (mp 210°, 8 mg), with $CHCl_3$–EtOH (50:1) to give 7-hydroxy-4,6-dimethylphthalide (**3**) [6] (mp 160°, 6 mg), and with $CHCl_3$–EtOH (25:1) to give asperphenamate (**4**) [7, 8] [mp 200° (dec.), 69 mg]. The fraction eluted with $CHCl_3$–EtOH (10:1) was purified by LPLC with $CHCl_3$–EtOH (40:1) to give a megasporizine (**1**)-rich fraction and phyllostine (**5**) [9] (mp 55°, 26 mg). The fraction enriched with **1** was further purified by LPLC with C_6H_6–EtOAc (4:1) to obtain pure megasporizine (**1**) (7 mg).

Megasporizine (**1**). Needles (C_6H_6); mp 230–233° (dec.); $[\alpha]_D^{24}$ +55° and $[\alpha]_{365}^{24}$ +270° (MeOH; *c* 0.20). IR ν_{max}^{KBr} cm^{-1}: 3400, 3175 (NH), 1685 (CON); UV λ_{max}^{MeOH} nm (log ε): 225 (sh) (4.19), 250 (4.20); EIMS (probe) 70 eV, *m/z* (rel. int.): 288.1476 $[M]^+$ ($C_{16}H_{20}N_2O_3$ requires 288.1474, 3), 256 $[M-MeOH]^+$ (4), 197 $[M-C_6H_5CH_2]^+$ (100), 169 (13), 91 $[C_6H_5CH_2]^+$ (24); (Found: C, 66.8; H, 7.0; N, 9.6. Calc. for $C_{16}H_{20}N_2O_3$: C, 66.6; H, 7.0; C, 9.7%); ^{1}H NMR (270.17 MHz, $CDCl_3$, TMS as int. std.): δ0.89 [3H, *d*, *J* = 6.7 Hz, CH (Me)$_2$], 1.00 [3H, *d*, *J* = 5.7 Hz, CH (Me)$_2$], 2.44 [1H, *m*, = CHCH(Me)$_2$], 3.17 (1H, *d*, *J* = 13.4 Hz, $\overline{CH_2}$), 3.28 (1H, *d*, *J* = 13.4 Hz, CH_2), 3.31 (3H, *s*, OMe), 5.76 [1H, *d*, *J* = 10.6 Hz, = CH CH(Me)$_2$], 6.80 (1H, *br s*, NH), 7.32 (5H, *m*, aromatic H), 8.41 (1H, *br s*, NH); ^{13}C NMR (100.40 MHz, DMSO-d_6, TMS): δ22.1 (2C, *q*), 23.6 (*d*), 43.8 (*t*), 50.4 (*q*), 87.9 (*s*), 124.2 (*s*), 125.1 (*d*), 126.9 (*d*), 128.0 (2C, *d*), 130.5 (2C, *d*), 134.1 (*s*), 159.7 (*s*), 163.0 (*s*).

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CORRECTED STRUCTURES OF PASSICORIACIN, EPICORIACIN AND EPITETRAPHYLLIN B AND THEIR DISTRIBUTION IN THE FLACOURTIACEAE AND PASSIFLORACEAE

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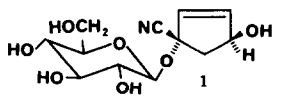
Key Word Index—Flacourtiaceae; Passifloraceae; leaves; cyanogenic glycosides; correction of structures.

Abstract—Three cyanogenic glycosides, passicoriacin, epicoriacin and epitetraphyllin B, have had their structures reassigned as epivalkenin, taraktophyllin and volkenin respectively, based on a reinterpretation of their spectral data.

INTRODUCTION

Recently we reported the presence of tetraphyllin B, epitetraphyllin B, passicoriacin, and epipassicoriacin in vegetative material of *Passiflora coriacea* (Passifloraceae) [1] and that passicoriacin and epipassicoriacin co-occur in *Passiflora suberosa* with two epoxide-containing cyanogenic glycosides [2]. The proposed structures of passicoriacin and epipassicoriacin were based on an earlier structure of epitetraphyllin B [3] that now has been shown to be incorrect [4] and, thus, the proposed structures for passicoriacin, and epipassicoriacin must be changed.

In a subsequent paper, Jaroszewski *et al.* [5] reported cyclopentenoid cyanogenic glycosides from *Taraktogenos heterophylla* and *Hydnocarpus anthelmintica* (Flacourtiaceae). These glycosides have similar spectral properties (some spectra were measured under different conditions)



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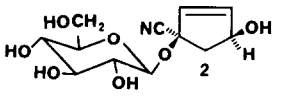

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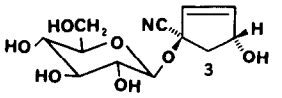

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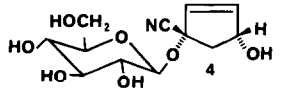

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