Structure of a New Metabolite from *Aspergillus chevalieri*

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A new metabolite has been isolated from *Aspergillus chevalieri* as colorless needles, mp 294–296°C, [α]_D+46°. It has a dioxopiperazine ring system formed from tryptophan and alanine. Chemical and spectroscopic data indicate that this metabolite is L-alanyl-2-(1,1-dimethylallyl)-L-tryptophan anhydride (I).

During investigation of fungal metabolites, we isolated a new metabolite from a strain of *Aspergillus chevalieri* (Mangin) Thom *et al*. Church IFO 4090. We now report the isolation and structural elucidation of the metabolite.

This mold was grown as a surface culture for 3 weeks at 24°C on malt extract medium. The extract soluble in acetone from culture filtrate was chromatographed on a column of silicic acid. Elution with benzene-acetone (2:1, v/v) gave a new metabolite. This metabolite (I) was obtained as colorless needles, mp 294–296°C, [α]_D+46° (AcOH). Its molecular formula, C_{19}H_{23}O_{2}N_{3}, was determined by elementary analysis and mass spectrometry [m/e: 325 (M+)]. This compound gave a purple color with Ehrlich’s reagent. I showed UV absorption at 223 (ε 36000), 283 (ε 81000) and 292 nm (ε 7100).

The spectrum is close to that of echinulin (II) which is a metabolite of this mold. This suggests the presence of a 2,3-substituted indole system in I. I showed IR absorption at 3380 cm^{-1} due to a secondary amine group and bands at 1670 and 1655 cm^{-1} due to carbonyl absorption of amide groups. Treatment of I with 6N hydrochloric acid afforded alanine and tryptophan. This suggests the presence of a dioxopiperazine ring system in I which is formed from tryptophan and alanine.

The NMR spectrum of I showed a doublet at δ 1.22 (3H, J=7 Hz) due to the alanyl methyl protons which showed coupling with a proton at δ 3.80 (quartet, J=7 Hz). Two doublets of doublets at δ 3.00 (1H, J=10 and 15 Hz) and 3.28 (1H, J=5 and 15 Hz) were assigned to the allylic methylene protons which showed coupling with a proton at δ 4.02 (doublet of doublets, J=5 and 10 Hz). These protons at δ 3.80 and 4.02 were assigned to two hydrogen atoms on carbons adjacent to nitrogen atoms in dioxopiperazine ring system. Three signals at δ 7.13–7.53 (1H), 8.02 (1H), and 10.42 (1H) disappeared on treatment with deuterium oxide and were assigned to the protons of –NH– groups.

A signal at δ 1.48 (6H, singlet) was assigned to two olefinic methyl protons and two doublets of doublets at δ 4.93 (1H, J=2 and 10 Hz) and 4.98 (1H, J=2 and 17 Hz) were assigned to the olefinic protons of a vinyl group. These protons showed coupling with a proton at δ 6.12 (1H, J=10 and 17 Hz). Hydrogenation of I in methanol over palladium-charcoal gave a dihydro-derivative (III), in whose NMR spectrum the olefinic protons of the vinyl group disappeared.

Signals at δ 6.74 (1H, doublet of doublets, J=1.5 and 6 Hz), 6.91 (1H, sextet, J=2, 6 and 10 Hz), and 7.20–7.55 (2H, multiplet) assignable to the aromatic protons were observed, while the spectrum showed no singlet signal assignable to a proton located at position 2 in the indole system. Therefore, location of the γ,γ'-dimethylallyl group in I is assigned at position 2 in the indole system.

This evidence proves that the metabolite is alanyl-2-(1,1-dimethylallyl)-tryptophan anhydride (I). This compound had been synthesized by Houghton and Saxton who isolated two diastereoisomers of L-alanyl-2-(1,1-dimethyl-
allyl)-tryptophan anhydride by chromatography on Kiesel gel G. One has mp 264-272°C, \([\alpha]_D^{22} +22.3^\circ\) (AcOH) and L-alanyl-L-‘tryptophanyl’ configuration. The other has mp 228-235°C, \([\alpha]_D^{22} -68^\circ\) (AcOH) and L-alanyl-D-‘tryptophanyl’ configuration. We therefore conclude that dextrorotatory metabolite has L,L-configuration.

Moreover, Allen has reported in the biosynthetic study of echinulin (II) using y,y-dimethylallyl pyrophosphate, L-alanyl-L-tryptophan anhydride, and cell free extracts from Aspergillus amstelodami that only single isoprene substitution occurred on the tryptophanyl moiety and the reaction product was probably I.3)

**EXPERIMENTAL**

Melting points are uncorrected. UV spectra were measured on a Hitachi 124 spectrometer. IR spectra were recorded on a Hitachi 215 spectrometer and 'H-NMR spectra on a Hitachi R-24 using tetramethylsilane as internal standard. Analysis of amino acid was carried out with a JEOL JLC-6AH amino acid analyzer. Column chromatography was carried out on silic AR CC-4 (Mallinckrodt, 200-325 mesh). TLC was performed silica layer GA (Nakarai Kagaku Yakuhin Co.). For the citation of NMR data the following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet).

**Isolation of the metabolite**

Aspergillus chevalieri (Mangin) Thom et Church IFO 4090 was grown as a surface culture for 3 weeks at 24°C on a malt extract medium. The culture filtrate (20 liters) was adjusted to pH 3 with 10% hydrochloric acid and stirred with activated charcoal. The adsorbed metabolites were eluted with acetone. The acetone solution was concentrated in vacuo to give a brown residue. The residue was adsorbed on a small amount of silic AR CC-4 and applied to the top of a column of silic AR CC-4. After elution of oil with benzene, elution with benzene-acetone (2:1, v/v) gave 150 mg of crude white solid. The solid was recrystallized from methanol to give colorless needles of the metabolite (I), mp 294-296°C, \([\alpha]_D^{24} +46^\circ\) (AcOH).

UV \(\lambda_{max}\) nm (ε): 223 (36,000), 283 (8100), 292 (7100); IR \(\nu_{max}\) cm\(^{-1}\): 3380, 1670, 1655; NMR (DMSO-\(d_6\), 60 MHz) \(\delta\): 1.22 (3H, d, J=7 Hz), 1.48 (6H, s), 3.00 (1H, dd, J=10 and 15 Hz), 3.28 (1H, dd, J=5 and 15 Hz), 3.80 (1H, q, J=7 Hz), 4.02 (1H, dd, J=5 and 10 Hz), 4.93 (1H, dd, J=2 and 10 Hz), 4.98 (1H, dd, J=2 and 17 Hz), 6.12 (1H, dd, J=10 and 15 Hz), 6.74 (1H, dd, J=1.5 and 6 Hz), 6.91 (1H, sextet, J=2, 6 and 10 Hz), 7.13-7.55 (3H, m), 8.02 (1H, s), 10.42 (1H, s); MS m/e (% ler. Int.): M+ 325 (8), 198 (100), 183 (22), 168 (10), 154 (3), 143 (2), 130 (4), 69 (3); Anal. Found: C, 69.85; H, 7.16; N, 12.89; Calcd. for C\(_{19}\)H\(_{23}\)O\(_2\)N\(_3\): C, 70.13; H, 7.12; N, 12.91 %; M.W., 325.

This compound is soluble in methanol, glacial acetic acid, and concentrated hydrochloric acid, slightly soluble in ethyl acetate and insoluble in benzene and chloroform. It gave a purple color with Ehrlich’s reagent.

**Hydrogenation of the metabolite**

A solution of 39 mg of the metabolite in 30 ml of methanol was stirred for 1.5 hr over hydrogen atmosphere over palladium-charcoal (5 mg). The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was chromatographed on a column of silic AR CC-4. Elution with benzene-acetone (4:1, v/v) gave 26 mg of dihydro-derivative (II). The dihydro-derivative was recrystallized from acetone to give colorless needles, mp 284-285°C; NMR (DMSO-\(d_6\), 60 MHz) \(\delta\): 0.70 (3H, t, J=8 Hz), 1.25 (3H, d, J=7 Hz), 1.40 (6H, s), 1.72 (2H, q, J=8 Hz), 3.05 (1H, dd, J=10 and 15 Hz), 3.32 (1H, dd, J=5 and 15 Hz), 3.79 (1H, q, J=7 Hz), 3.95 (1H, dd, J=5 and 10 Hz), 6.80 (1H, dd, J=1.5 and 6 Hz), 6.91 (1H, sextet, J=2, 6 and 10 Hz), 7.15-7.55 (3H, m), 8.10 (1H, s); Anal. Found: C, 69.36; H, 7.49; N, 12.62; Calcd. for C\(_{19}\)H\(_{25}\)O\(_2\)N\(_3\): C, 69.70; H, 7.70; N, 12.84 %.

**Hydrolysis of the metabolite**

A solution of 15 mg of the metabolite in 10 ml of 6 N
hydrochloric acid was stirred for 2 hr at 130°C. The solution was concentrated in vacuo to give a yellow viscous residue. The residue was analyzed on amino acid analyzer. Two peaks were detected and identified as those of alanine and tryptophan by comparison with authentic L-alanine and L-tryptophan.

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REFERENCES
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