Note

Isolation of Biogenetically Correlated Four Alkaloids from the Cultures of *Penicillium corymbiferum*Sadahiro OHMOMO, Tsutomu OHASHI
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In the previous paper,¹⁻³⁾ we isolated four new indole alkaloids, roquefortine A, B, C and D together with festuclavine from the cultures of *Penicillium roqueforti*. Recently, we isolated a *Penicillium* strain having the ability to produce at least four alkaloids I, II, III and IV in the course of screening fungi for their alkaloid productivity. By close investigation on morphological characteristics, this strain was identified as *Penicillium corymbiferum*. In this paper, we report the isolation and identification of these four alkaloids.

Alkaloid I and II were isolated in crystalline form from the culture filtrates (ca. 5.4 liters) in the yields of 45 and 30 mg, respectively, by the routine procedure using silica gel column chromatography. Alkaloid III was obtained at first as crude powder containing alkaloid IV from the said culture filtrates by the same technique. This powder was purified repeatedly by the use of silica gel column chromatography, giving pure crystals of III and IV in the yields of 240 and 210 mg, respectively.

I and II were identical with roquefortine C^{1,2)} and D,³⁾ respectively, not only in the physico- and spectro-chemical properties but also in the mixed melting point with authentic samples.

III and IV were identical with cyclophenol^{4,5)} and cyclophenine,^{4,5)} respectively, which were isolated from the cultures of *Penicillium cyclopium*, not only in the physico- and spectro-chemical properties but also in the mixed melting point with authentic samples.

Judging from the assumed biosynthetic route⁶⁾ of roquefortine C and D, and from that⁷⁾ of cyclophenol and cyclophenine, these four alkaloids seem to be biosynthesized in used *Penicillium* in the correlation as shown in Fig. 1.

In further screening tests, it was found that roquefortine C was produced also by *Penicillium expansum* IFO 5854 and *Penicillium urtica* IFO 7010 together or independently with roquefortine D.

FIG. 1. Possible Biosynthetic Relationships among Roquefortine C, Roquefortine D, Cyclophenol and Cyclophenine in *Penicillium corymbiferum*.

EXPERIMENTAL

Fungus and cultivation. A strain of *Penicillium corymbiferum*, isolated from the soil in Tokyo, was cultivated at 30°C for 15 days in 1 liter Roux flasks containing each 200 ml of a modification of MGS-medium.⁸⁾ The medium was composed of mannitol 3%, glucose 1%, succinic acid 1%, KH_2PO_4 0.1%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.03%, NH_4OH to pH 5.6 and tap water.

Isolation of I (roquefortine C) and II (roquefortine D). The alkaloids were extracted from the culture filtrates (ca. 5.4 liters) of *P. corymbiferum* with ethyl acetate after made alkali with aqueous ammonia (pH ca. 10). The alkaloidal solution was concentrated to dryness and the residue was passed through a column (ϕ 2.0 × 80 cm) containing 150 g of silica gel (Wako, Wakogel Q-23) suspended in ammoniacal chloroform.* The column was first eluted with 500 ml of ammoniacal chloroform*¹ containing 1% ethanol (v/v), next eluted with 800 ml of ammoniacal chloroform* containing 3% ethanol (v/v) and last eluted with 800 ml of ammoniacal chloroform* containing 5% ethanol (v/v) at a rate of 20 g/tube/40 min. I was contained in fractions No. 42~54, III was in fractions No. 58~72 together with IV, and II was in fractions No. 80~107.

The fractions No. 42~54 were combined and evap-

* Mixture of 10 parts of chloroform and one part of chloroform saturated with conc. aqueous ammonia.

orated to dryness under reduced pressure. The residue gave crystals of **I** from methanol in colorless needles (45 mg): mp 225~228°C (decomp.), $[\alpha]_D^{25} - 763^\circ$ ($c=0.5$, pyr.), molecular formula $C_{22}H_{23}N_5O_2$ [Found: C, 67.08; H, 5.82; N, 17.01; m/e , 389 (M^+)].

On the other hand, the fractions No. 80~107 were combined and evaporated to dryness. The residue gave crystals of **II** from acetonitrile in colorless prisms (30 mg): mp 154°C, $[\alpha]_D^{25} - 371^\circ$ ($c=0.25$, pyr.), molecular formula $C_{22}H_{25}N_5O_2$ [Found: C, 67.74; H, 7.12; N, 17.03; m/e , 391 (M^+)].

Isolation of III (cyclophenol) and IV (cyclophenine). The fractions No. 58~72 of column chromatography mentioned before were combined and evaporated to dryness under reduced pressure. The residue was next passed through a column (ϕ 1.8 $\times$ 60 cm) containing 100 g of silica gel (Wako, Wakogel Q-23) suspended in chloroform. The column was in turn eluted with (i) 500 ml of chloroform containing 2% methanol (v/v), (ii) 300 ml of chloroform containing 5% methanol (v/v) and (iii) 500 ml of chloroform containing 10% methanol (v/v) at a rate of 15 g/tube/30 min. **III** was contained in fractions No. 43~60 and **IV** was in fractions No. 20~40.

These fractions No. 43~60 were combined and evaporated to dryness under reduced pressure. The residue gave crystals of **III** from methanol in colorless needles (240 mg): mp 217°C (decomp.), $[\alpha]_D^{20} - 250^\circ$ ($c=0.32$, pyr.), $[\alpha]_{5461}^{20} - 310^\circ$ ($c=1.0$, MeOH), molecular formula $C_{17}H_{14}N_2O_4$ [Found: C, 66.10; H, 4.52; N, 9.11; m/e , 310 (M^+). Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; MW, 310.28]. IR ν_{max}^{KBr} cm^{-1} : 3340, 3200, 1690, 1610. MS m/e : 310 (M^+), 253, 189, 161, 146, 119. NMR δ_{TMS}^{DMSO} : 3.10 (3H, s), 4.22 (1H, s), 6.7~7.7 (8H, m), 9.45 (1H, br. s), 10.60 (1H, s).

On the other hand, the fractions No. 20~40 were combined and evaporated to dryness. The residue gave

crystals of **IV** from methanol in colorless needles (210 mg): mp 182°C (decomp.), $[\alpha]_D^{20} - 231^\circ$ ($c=0.40$, pyr.), $[\alpha]_{5461}^{20} - 291^\circ$ ($c=1.0$, MeOH), molecular formula $C_{17}H_{14}N_2O_3$ [Found: C, 69.48; H, 5.05; N, 8.82; m/e , 294 (M^+). Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.37; H, 4.80; N, 9.52; MW, 294.28]. IR ν_{max}^{KBr} cm^{-1} : 3150, 1705, 1615. MS m/e : 294 (M^+), 237, 161, 146, 119. NMR $\delta_{TMS}^{CDCl_3}$: 3.22 (3H, s), 4.00 (1H, s), 6.5~7.6 (9H, m), 9.35 (1H, br. s).

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