Isolation of a New Indole Alkaloid, Roquefortine D, from the Cultures of *Penicillium roqueforti*

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Previously, we¹⁻³⁾ isolated three indole alkaloids, roquefortine A, B and C, from the cultures of *Penicillium roqueforti*, and made clear the structures of those alkaloids. Recently, we have isolated the unknown indole alkaloid "Z," which was found in the course of previous experiment,³⁾ from the cultures of the same *Penicillium* mold. This alkaloid "Z" was obtained in the yields of 50 mg together with 600 mg of roquefortine C from the mycelium and the culture filtrate, when a selected strain was grown at 30°C for 20 days in 100 Roux flasks (1 liter capacity), containing each 200 ml of the mannitol-glucose-ammonium succinate-medium.

The alkaloid "Z" crystallized from acetonitrile or methanol in colorless prisms; mp 153~154°C, $[\alpha]_D^{15} - 370^\circ$ ($c=0.23$, pyr.). The molecular formula was $C_{22}H_{25}N_5O_2$. Its IR-spectrum contained bands assignable to a diketopiperazine unit,⁴⁾ strong absorption at 3200 and 1660 cm^{-1} and absence of the amide II band in the region of 1515~1570 cm^{-1} (Fig. 1). Its NMR-spectrum possessed no singlet assignable to a vinyl proton of dehydrohistidine side chain of roquefortine C and had three double doublet at δ 2.88, δ 3.32 and δ 4.08, which are absent in the spectrum³⁾ of roquefortine C. The other signals of "Z" were all similar to those of roquefortine C. These data suggested that

FIG. 1. IR-spectra of Alkaloid "Z" (Roquefortine D) (a) and Isomer-1 of Dihydroroquefortine C (b) in KBr.

alkaloid "Z" might be a dihydro-derivative of roquefortine C in the part of dehydrohistidine side chain. This was supported strongly by the MS-spectrum. Namely, the primary fragmentation of "Z" was the loss of a C_6H_6 radical (mass 69) to give a prominent fragment (fragment A shown here) at m/e 322 (base peak). And fragment B (m/e 194), C (m/e 130) and D

Fragment A

(m/e 81), each originated from cleavage in the expected position α to the diketopiperazine ring of fragment A, appeared. Fragment E (m/e 157) and F (m/e 110), originated from cleavage of the diketopiperazine ring⁵⁾ of fragment A, also appeared. Comparing these fragments with those³⁾ of roquefortine C, fragment A, B and F were two mass units larger than those (m/e 320, 192 and 108) of roquefortine C, respectively. Also, fragment D is a unique fragment which can not appear in the spectrum of roquefortine C owing to the conjugated double bond.

Reduction of roquefortine C [I] with Zn in acetic acid caused hydrogenation in the part of dehydrohistidine side chain and afforded mixtures of two stereoisomers (isomer-1 and -2) of dihydroroquefortine C

[II]. The isomer-1 crystallized from methanol in colorless prisms; mp 154°C, $[\alpha]_D^{15} - 372^\circ$ ($c=0.3$, pyr.). The isomer-2 showed such physico-chemical properties as mentioned later.

The chemical and spectro-chemical properties of the alkaloid "Z" were all found to be identical with those of an isomer (isomer-1) of dihydroroquefortine C mentioned here. The thin-layer chromatographic behaviours of "Z" in different systems⁶⁾ were also corresponded well with those of the isomer-1 of dihydroroquefortine C. Finally, "Z" was found to be just identical with the isomer-1 of dihydroroquefortine C from the mixed melting point.

From these facts, the alkaloid "Z" was a new indole alkaloid having the structure [II], for which we propose the name roquefortine D.

By the way, the said isomer-2 of dihydroroquefortine C (stereoisomer of roquefortine D) could not be found in the cultures of the tested *Penicillium* strain.

EXPERIMENTAL

Fungus and cultivation. A strain of *Penicillium roqueforti*, which was selected in the previous experiments,¹⁻³⁾ was cultivated at 30°C for 20 days in 100 of 1 liter Roux flasks, containing each 200 ml of the medium composed of mannitol 30 g, glucose 10 g, succinic acid 10 g, KH_2PO_4 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3 g, NH_4OH (to pH 5.6) and tap water (1 liter).

Isolation of alkaloid "Z" (Roquefortine D). The alkaloids were extracted from the cultures with ethyl acetate in the presence of ammonia (pH ca. 10). The alkaloidal extract was dissolved in 3% aqueous solution of tartaric acid and reextracted with chloroform in the presence of ammonia. The alkaloidal solution thus obtained was concentrated to dryness and the residue was passed through a column (3.5 × 40 cm) containing 280 g of alumina (WAKO, activity II) suspended in chloroform. The column was first eluted with 600 ml of chloroform containing 1% ethanol (v/v) and next eluted with 1200 ml of chloroform containing 5% ethanol (v/v) at a rate of 20 g/tube/20 min. Alkaloid "Z" was contained in fractions No. 71~97 together with roquefortine C. These fractions were combined and evaporated to dryness under reduced pressure. The residue was next passed through a column (1.8 × 80 cm) containing 120 g of silica gel (WAKO, WAKOGEL Q-23) suspended in ammoniacal chloroform.* The column was in turn eluted with i) 500 ml of ammoniacal chloroform* containing 3% methanol (v/v), ii) 700 ml of ammoniacal chloroform* containing 5% methanol (v/v) and, iii) 800 ml of ammoniacal chloroform* containing 7% methanol (v/v)

at a rate of 20 g/tube/30 min. Alkaloid "Z" was contained in fractions No. 63~98. These fractions were combined and evaporated to dryness under reduced pressure. The residue gave crystals of "Z" from its benzene-acetonitrile solution (50 mg). "Z" was crystallized from acetonitrile in colorless prisms; mp 153~154°C, $[\alpha]_D^{15} - 370^\circ$ ($c=0.23$, pyr.), molecular formula $\text{C}_{22}\text{H}_{25}\text{N}_5\text{O}_2$ [Found: C, 67.62; H, 7.20; N, 16.92; m/e , 391 (M^+). Calcd. for $\text{C}_{22}\text{H}_{25}\text{N}_5\text{O}_2$: C, 67.50; H, 6.69; N, 17.89%; MW, 391.46]. Color reactions: modified Ehrlich's reagent⁹⁾ (yellow green, turning gradually into purplish blue), Pauly reagent⁷⁾ (reddish orange), Dragendorff reagent (orange), 2% KMnO_4 reagent (positive), 5% FeCl_3 reagent (negative). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 208 (5.29), 244 (4.55), 298 (4.18). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3200 (NH), 1660 (amide I), 1440, 1085, 750 (Fig. 1). MS m/e : 391 (M^+), 322 ($\text{M}^+ - \text{C}_2\text{H}_5$, 100%), 294, 241, 198, 194 (14%), 185, 157 (16%), 130 (52%), 110 (78%), 81 (23%), 69 (49%), 28. NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 0.98 (3H, s, $\text{CH}_3 - \text{C}-$), 1.09 (3H, s, $\text{CH}_3 - \text{C}-$), 2.26~2.54 (2H, m), 2.88 (1H, dd, $J=9$ and 17.5 Hz), 3.32 (1H, dd, $J=5$ and 17.5 Hz), 3.86 (1H, m), 4.08 (1H, dd, $J=5$ and 9 Hz), 4.85~5.10 (2H, m), 5.46 (1H, s), 5.7~6.1 (1H, m), 6.5~7.25 (4H, m, indole protons), 6.98 (1H, s, imidazole $-\text{C}=\text{CH}-$), 7.43 (1H, s, imidazole $-\text{C}=\text{CH}-$), 7.52 (1H, br. s, $-\text{NH}-$, disappeared on D_2O exchange). From the fractions No. 30~52 in the above column chromatography, roquefortine C was obtained in the yields of 600 mg.

Preparation of dihydroroquefortine C. Zinc powder (2 g) was added to a solution of roquefortine C (200 mg) in acetic acid (10 ml). After reflux for 3 hr at 70°C, 10-fold by volume of H_2O was added into reaction mixture, and then alkaloids were extracted with ethyl acetate at pH 10 (NaOH). Alkaloidal extract was purified by column chromatography on silica gel mentioned before. From the eluted fractions No. 60~93, 52 mg of one stereoisomer (isomer-1) of dihydroroquefortine C was obtained in crystalline powder (yield 26%); mp 154°C, $[\alpha]_D^{15} - 372^\circ$ ($c=0.3$, pyr.), molecular formula $\text{C}_{22}\text{H}_{25}\text{N}_5\text{O}_2$ [Found: C, 67.47; H, 6.74; N, 17.78%; m/e 391 (M^+). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 208 (5.28), 244 (4.55), 298 (4.18). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1660 (amide I) (Fig. 1). MS m/e : 391 (M^+), 322, 294, 241, 194, 185, 157, 130, 110, 81, 69, 28. From the fractions No. 104~140 in the same column chromatography, 50 mg of another stereoisomer (isomer-2) of dihydroroquefortine C was obtained in crystalline powder (yield 25%); mp 185°C, $[\alpha]_D^{15} - 137^\circ$ ($c=0.33$, pyr.), IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1665 (amide I), MS m/e : 391 (M^+), 322, 294, 241, 194, 185, 157, 130, 110, 81, 69, 28.

* Mixture of 10 parts of chloroform and 1 part of chloroform saturated with conc. ammonia.

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