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Identification of Roquefortine C Produced by *Penicillium roqueforti*

Sadahiro OHMOMO, Teiichi UTAGAWA and Matazo ABE*

Laboratory of Industrial Microbiology, Faculty of Agriculture, Tokyo University of Education, Komaba, Meguro-ku, Tokyo
**Faculty of Agriculture, Tamagawa University, Machida-shi, Tokyo*

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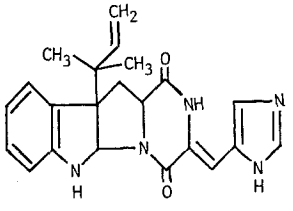
Previously, we[1,2] isolated festuclavine and three new indole alkaloids, roquefortine A, B and C, from the cultures of *Penicillium roqueforti* and found that these alkaloids are contained also in various roquefort-type blue cheeses on the market in a very small quantity. At the same time, we propose the structures of roquefortine A (ROQ-A) and B (ROQ-B) on the basis of the data of spectral studies. As to the structure of roquefortine C (ROQ-C), we could not give any comment because the amounts were very small.

Later, P. M. Scott *et al.*[3] reported that they isolated an indole alkaloid, roquefortine, having the very interesting structure [I] from the cultures of *Penicillium*



I

roqueforti and sent the alkaloidal sample to the authors for the identification with ROQ-C mentioned above.

Thereupon, we have prepared ROQ-C newly from the cultures of *Penicillium roqueforti* for comparing it with Scott's roquefortine. For this purpose, the cultural conditions for increasing the yield of ROQ-C were investigated because ROQ-C was a minor alkaloid in the previous experiments.[1,2] This investigation has resulted in attaining the purpose, especially by the delicate control of the culture temperature. As shown in Fig. 1, the cultivation at 30°C favored extremely the formation of ROQ-C and, by contraries, hindered remarkably the formation of ROQ-A, a major alkaloid in the previous experiments. Besides, it was found that in the case of the cultivation at 30°C, a hitherto unknown indole alkaloid "Z" was produced in a small quantity. The structure of this new alkaloid is now under examination.

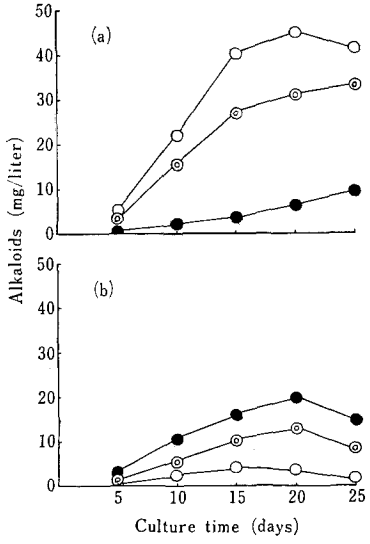
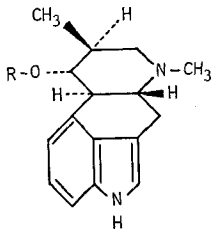


FIG. 1. Effect of Culture Temperature on the Formation of ROQ-C (a) as Well as of ROQ-A (b) in Surface Cultures of *Penicillium roqueforti*.

The mold was grown at 22, 26 and 30°C, respectively, in 1 liter Roux flasks, containing each 200 ml of a modification of MGS-medium. The total alkaloids were extracted with ethyl acetate in the presence of ammonia and the extract was concentrated to dryness. The alkaloidal residue was dissolved in a definite amount of methanol and subjected to thin layer chromatography[4] using chloroform–ethanol [10: 1.5, v/v] and acetone–chloroform [3 : 2, v/v] as solvent. The amounts of ROQ-C as well as of -A were determined with a chromatogram-scanner, OZUMOR 82, after treating with a Pauli reagent[5] and a modified Ehrlich's reagent,[4] respectively. Culture temperature: ●—●, 22°C; ◎—◎, 26°C; ○—○, 30°C.

The cultivation and identification of ROQ-C were carried out as follows: The test mold was cultivated at 30°C for 20 days in 100 of 1 liter Roux flasks, containing each 200 ml of a modification of MGS-medium[6] composed of mannitol 30 g, glucose 10 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, succinic acid 10 g, NH_4OH (to pH 5.6) and tap water (1 liter). After incubation, the culture filtrate (*ca.* 18 liter) was extracted with ethyl acetate in the presence of ammonia (*ca.* pH 10) and the ethyl acetate phase was concentrated to dryness. The alkaloidal residue was dissolved in 3% aqueous solution of tartaric acid and extracted with chloroform in the presence of ammonia (pH 10). The alkaloidal solution thus obtained was concentrated to dryness and the residue was dissolved in a definite amount of methanol. This methanol solution gave ROQ-C in

crystalline state in the yield of 600 mg. It crystallized from methanol as colorless needles; mp 225 to 228°C (decomp.), $[\alpha]_D^{15}$ −764° (c=0.50, pyr.). Further, it gave the following data: MS m/e: 389 (M^+), 320 (M^+-C_5H_9), 292, 198, 192, 185, 157, 130, 108, 69, 28. NMR $\delta_{TMS}^{CDCl_3}$: 1.00(3H, s, CH_3–C–), 1.12 (3H, s, CH_3–C–), 2.3~2.7 (2H, m), 4.02 (1H, dd, J=7 and 12 Hz), 5.02 (1H, dd, J=1.5 and 9 Hz), 5.05 (1H, br.s, >NH, disappeared on D_2O exchange), 5.18 (1H, s), 5.66 (1H, s), 5.8~6.2 (1H, m), 6.38 (1H, s, >C=CH–), 6.5~6.9 (2H, m), 7.0~7.3 (2H, m), 7.22 (1H, s, imidazole >C=CH–), 7.66 (1H, s, imidazole >C=CH–), 10.21 (1h, br. s, >NH, disappeared on D_2O exchange). These data were all found to be almost identical with those published for roquefortine by P. M. Scott *et al.*[8,7] The thin layer chromatographyic behavior of Scott's roquefortine in different systems[4] was also corresponded well with that of our ROQ–C. Finally, ROQ–C was found to be identical with Scott's roquefortine from the mixed melting point.



II

R=Ac. : ROQ–A

R=H : ROQ–B

(configulation shown is relative)

Previously, we[1,2] presumed that the structures of ROQ–A and –B might be 6,9-dimethyl-7-O-acetyl-ergoline and 6,9-dimethyl-7-hydroxyl-ergoline, respectively. However, J. Clardy[8] in Iowa State University has announced us recently that these two alkaloids were found to have the structural formulae [II] by the X-ray crystallography.

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