Synthesis of rhodotorucine A, the inducing factor of mating tube formation of *Rhodosporidium toruloides*

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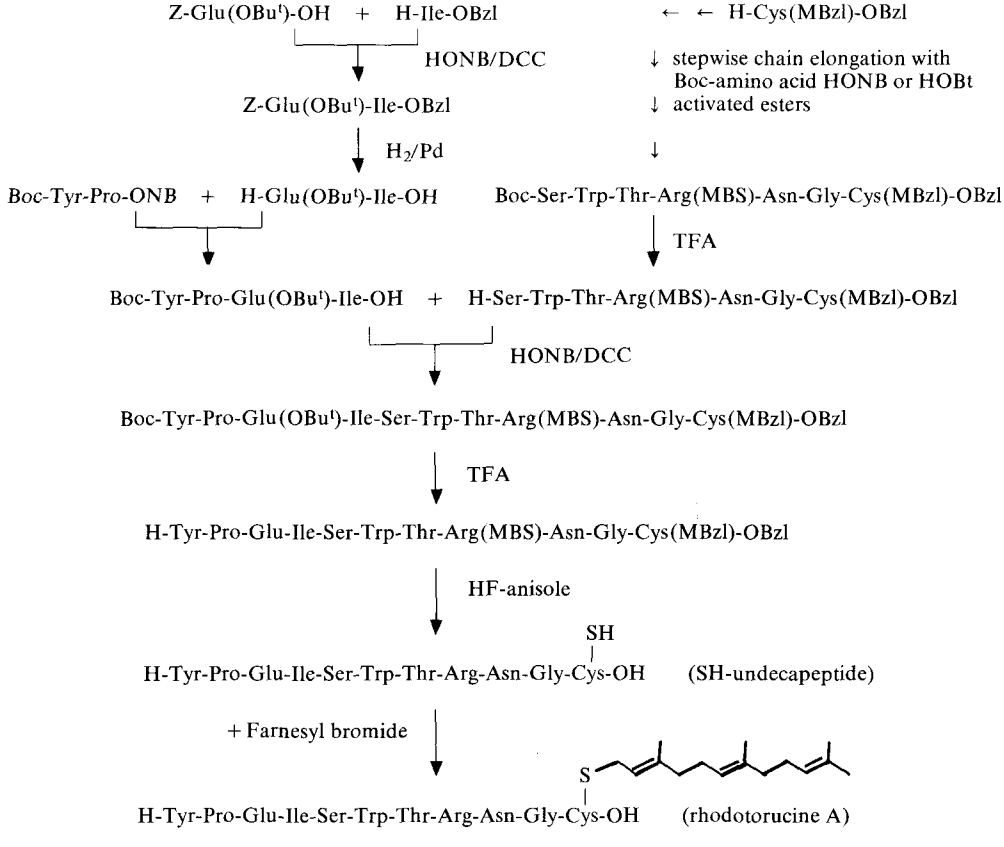
Summary. The inducing factor of mating tube formation of *Rhodosporidium turuloides,* named rhodotorucine A (H-Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys(S-farnesyl)-OH), has been synthesized to confirm the structure proposed for the natural lipopeptide. The synthetic S-farnesyl undecapeptide has identical Rf values on TLC using several different solvents, and also the same biological activity as the natural hormone.

A novel lipopeptidyl factor which induces mating tube formation of the yeast *Rhodosporidium toruloides* was isolated from the culture media of A type cells of *Rh. toruloides*[5] and its chemical structure was very recently elucidated to be H-Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys(S-farnesyl)-OH[6].

To confirm the chemical structure of this hormone, the S-farnesyl undecapeptide corresponding to the proposed structure was synthesized by the conventional solution method, and the synthetic S-farnesyl peptide was compared with the native hormone chromatographically and biologically.

Materials and methods. Natural rhodotorucine A was isolated from the culture filtrate of yeast strain IFO 0559-M-919 (A type cell) as described by Kamiya et al.[5]. Farensyl bromide was prepared by the PBr_3 method of Rüegg et al.[7] from all trans farnesol[8]. All melting points were taken in open capillaries and are uncorrected. Rotations were determined with a Perkin Elmer Model 141 polarimeter. Amino acid analyses were run on acid hydrolysates prepared in 5.7N HCl at 105 °C for 20 h under reduced pressure. The analyses were performed on a Hitachi KLA-3B amino acid analyzer. Catalytic hydrogenation was performed at room temperature with palladium black as catalyst. Where analyses are indicated only by symbols of the elements, analytical results were obtained for the elements within ±0.4 of the theoretical value. The purity of the intermediates and the products were tested by TLC using Merck precoated silica gel plate 60F-254 and/or cellulose plate Avicel. Solvent systems used were: $CHCl_3$-MeOH-AcOH (9:1:0.5, Rf^1), AcOEt-pyridine-AcOH-H_2O (60:20:6:11, Rf^2), n-BuOH-AcOEt-AcOH-H_2O (1:1:1:1, Rf^3), n-BuOH-pyridine-AcOH-H_2O (30:20:6:24, Rf^4). Enzymatic digestion of rhodotorucine A with trypsin (EC 3.4.21.4, Worthington Biochemical Co.), α-chymotrypsin (EC 3.4.21.1, Miles Research Products) or thermolysin (EC 3.4.24.4, Daiwakasei, Tokyo) was performed as described in a previous paper[6].

Synthesis of peptides. The undecapeptide was synthesized in solution as shown in the figure. The protected undecapeptide was prepared by fragment condensation between Boc-tetrapeptide[9] of the N-terminal part and C-terminal hep-



Synthesis of rhodotorucine A.

tapeptide benzyl ester using the HONB-DCC method[10] to minimize racemization at the activated carboxy terminus. Since the carboxy component has isoleucine as the C-terminus, if any unexpected racemization during the coupling process occurs, it can be detected by amino acid analysis of the product. The protected heptapeptide [m.p. 116–118 °C, $[\alpha]_D^{26} - 18.7°$ (c, 0.5 in DMF), $Rf^1 = 0.20$, $Rf^2 = 0.79$, Anal. ($C_{60}H_{78}O_{17}N_{12}S_2 \cdot H_2O$) C,H,N,S] was prepared in the stepwise chain elongation manner as shown in the figure. In this synthesis, the following protecting groups were used: Boc for the N^{α}-amino group, MBS[11] and MBzl for the side chain functional groups of arginine and cysteine, and benzyl for the C-terminal carboxy group. The N^{α}-Boc protecting group was usually removed by TFA treatment, except in the case of Boc-Trp-peptide, which was deblocked with 4 N HCl/dioxane in the presence of ethanedithiol. The N-terminal tetrapeptide [m.p. 104–108 °C (decomp.), $[\alpha]_D^{28} - 39.6°$ (c, 0.5 in MeOH), $Rf^1 = 0.66$, Anal. ($C_{34}H_{52}O_{10}N_4$), C,H,N] was prepared by fragment condensation of Boc-Tyr-Pro-ONB and H-Glu (OBu^t)-Ile-OH. The protected C-terminal heptapeptide was treated with TFA-containing anisole and ethanedithiol to give the corresponding partially protected heptapeptide as TFA salt, which was dissolved in DMF together with TEA (10 equiv.) and then precipitated by addition of dry ether to give the corresponding free base. The free base was condensed with the protected N-terminal tetrapeptide by the HONB-DCC method. The product was precipitated with ether and acetonitrile, and the crude product was treated with TFA to remove the Boc and OBu^t groups. Then the partially protected undecapeptide was purified by column chromatography on silica gel using AcOEt-pyridine-AcOH-H_2O (60:20:6:10) as solvent. The purified partially protected undecapeptide [m.p. 169–172 °C (decomp.), $[\alpha]_D^{26} - 24.4°$ (c, 0.7 in DMF), $Rf^2 = 0.17$, $Rf^3 = 0.66$, Anal. ($C_{80}H_{104}O_{22}N_{16}S_2$) C,H,N,S: Calcd, 3.76, Found, 3.35] was treated with HF at 0 °C for 50 min in the presence of anisole. After removal of the HF in vacuo under 0 °C, the residue was extracted with water and the solution was washed with ether. The aqueous solution was passed through a column of Amberlite IRA-410 (acetate form); then the effluent was lyophilized. The crude material was purified by gel filtration on Sephadex LH-20 using N-AcOH as solvent. The main fractions were collected and lyophilized to give the SH-undecapeptide: $[\alpha]_D^{26} - 63.5°$ (c, 0.6 in N-AcOH), Rf^4 (cellulose) = 0.64, amino acid analysis: Arg 1.14, Trp 0.60, Asp 1.00, Thr 1.00, Ser 0.95, Glu 0.95, Pro 1.02, Gly 1.00, Cys 0.86, Ile 0.96 (allo-Ile, less than 1.2%), Tyr 0.95 (average recovery, 80%).

Synthesis of rhodotorucine A. The SH-undecapeptide was dissolved in 50% aqueous DMF together with MgO (5 equiv.). To this solution was added all trans farnesyl bromide (1.5 equiv., 10% solution in isopropyl ether) and the mixture was stirred at room temperature for 16 h. The reaction mixture was passed through a column of Sephadex LH-20 with 85% aqueous n-butanol. The main fractions were collected and the product was further purified by gel filtration on Sephadex LH-20 using MeOH–0.01M ammonium acetate (6:4) as solvent. According to the procedure for isolation of native material[12], the main fractions were collected and the solution was stored in a refrigerator at 3 °C for 2 days to yield colorless precipitates of the product, which were collected by filtration: $[\alpha]_D^{25} - 49.7°$ (c, 0.1 in a mixture of MeOH, n-BuOH and H_2O, 1:2:1), amino acid analysis: Arg 0.99, Trp 0.79, Asp 1.02, Thr 0.93, Ser 0.87, Glu 0.97, Pro 1.02, Gly 1.00, Cys 0.39, Ile 0.98, Tyr 0.89.

Results and discussion. The synthetic material was chromatographically identical to native rhodotorucine A in 5 different solvent systems. Comparisons of chromatographic mobilities of synthetic and native rhodotorucine A were carried out by TLC (silica gel) in n-BuOH-AcOH-H_2O (4:1:5, upper phase) (Rf = 0.29), n-BuOH-n-PrOH-0.2M AcOH (2:1:3, upper phase) (Rf = 0.23), sec-BuOH saturated with H_2O (Rf = 0.25), Rf^3 (Rf = 0.63) and Rf^4 (Rf = 0.53). In every case, the synthetic material exhibited 1 spot of identical mobility with that of natural hormone. Moreover, the peptide fragments from trypsin, chymotrypsin or thermolysin digestion of synthetic and native rhodotorucine A yielded identical TLC patterns.

As shown in the table, synthetic hormone exhibits the same potency for induction on mating tube formation from a-cells, although SH-undecapeptide has no such activity[13]. This indicates clearly that the lipophilic nature of the farnesyl group in the hormone molecule has a very important role in the induction of mating tube formation. More detailed studies on the structure-activity relationships are in progress.

Inducing activity on mating tube formation from a-cells of natural and synthetic rhodotorucine A

Material	Minimum amount of inducing action on mating tube formation*
Natural rhodotorucine A	8 ng/ml
Synthetic rhodotorucine A	5–10 ng/ml
SH-undecapeptide	negative (> 100 µg/ml)

*Activity was determined by incubating different amounts of the products as described by Kamiya et al.[14], and the activity was expressed as the minimum amount of the products necessary for the formation of mating tubes from the cells.

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9 Abbreviations. Boc, tert-butoxycarbonyl; Z, benzyloxycarbonyl; OBzl, benzyl ester; OBu^t, tert-butyl ester; MBzl, p-methoxybenzyl; MBS, p-methoxybenzenesulfonyl; HONB, N-hydroxy-5-norbornene-2,3-dicarboximide; ONB, HONB ester; DCC, N,N′-dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; TEA, triethylamine.
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