# TRICHOMYCIN B, A POLYENE MACROLIDE FROM *STREPTOMYCES*

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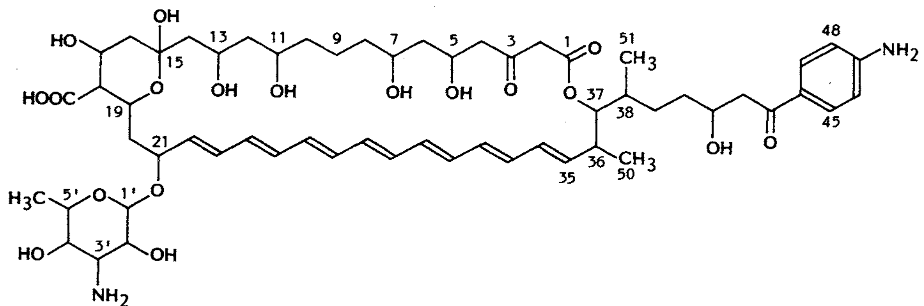
Two polyene macrolide, trichomycins A and B, were compared by physico-chemical and microbiological methods. The two antibiotics were found to have the same molecular formula, $C_{58}H_{84}N_2O_{18}$ (MW 1,096), by elemental analysis and FAB-MS. However, $^1H$ and $^{13}C$ NMR spectrometry studies indicated that the hydroxyl at C-5 of trichomycin A located on C-9 in trichomycin B.

Trichomycin B possessed lower activities against fungi and yeasts than those of trichomycin A.

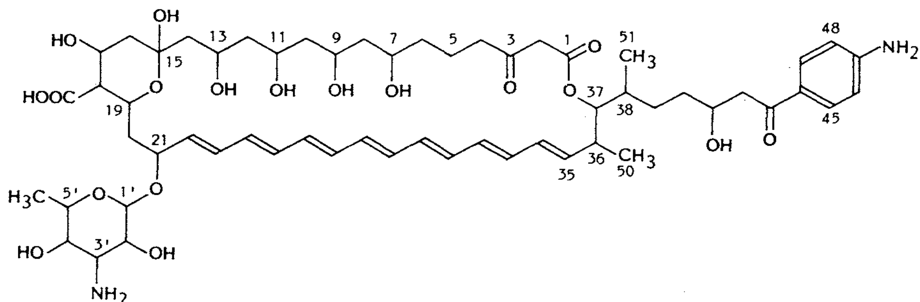
Trichomycin, a potent and clinically useful antifungal drug, especially as the trichomonacide, is produced by *Streptomyces hachijoensis*. The structure determination, isolation and characterization of trichomycin A (**1**), a major constituent, and the isolation of related compounds by HPLC were reported by us[1~3].

This paper represents the fermentation, isolation and also brief discussion on the structure of trichomycin B (**2**), the second major constituent, in comparison with trichomycin A.

Fig. 1. Structure of trichomycins A (**1**) and B (**2**).



**1**



**2**

## Fermentation

Ten to fifteen grains of *S. hachijoensis* on a malted rice were inoculated to one 500-ml Erlenmeyer flask containing 80 ml of sterile seed medium. The flask was incubated at 30°C for 2 days on a reciprocal shaker. A 30-liter fermenter with 20 liters of seed medium was inoculated with 1.2% of the mature seed broth. The seed culture was carried out at 27°C for 2 days under aeration of 20 liters/minute and agitation of 300 rpm.

A 30-liter fermenter with 20 liters of production medium was inoculated with 6% of the mature seed broth. The compositions of the seed and production media are in Table 1. The fermentation was carried out at 27°C for 5 days under agitation (350 rpm) and aeration (8.2 liters/minute). The progress of the fermentation was monitored by *Candida albicans* as a test organism.

## Isolation

The flow diagram of the isolation procedure is shown Fig. 2. Most of the activity was present in the mycelium extracts.

The acetone extracts (0.8 liter) from the mycelium were concentrated to an aqueous oil (100 ml), which was stood one overnight in the room temperature. The precipitates were filtered off and washed with water to yield crude powder. The powder was dissolved in the mixture of *N,N*-dimethylformamide - 5 N NaOH - acetone - water (6 : 2 : 27 : 3). The insoluble material was removed off by filtration and the filtrate was passed through an alumina (Woelm, acidic) containing the mixture of a solvent of acetone - water (9 : 1). The alumina was washed with 1/15 M phosphate buffer, followed with water and eluted with butanol - pyridine - water (4 : 3 : 7). The eluate was concentrated to dryness in the dark to yield an orange yellow powder. The powder was dissolved in the lower phase of a mixture of chloroform - methanol - water (2 : 2 : 1). The solution was loaded on a silica gel (Merck) containing the same solvent mentioned above. Developing with this solvent, four bands were generally appeared. The desired compounds were contained among the third and the forth bands. The fractions were combined and concentrated to an aqueous solution in the dark. Thus obtained the precipitate was again dissolved in 160 ml of the lower phase of a mixture of a solvent of chloroform - methanol - water (2 : 2 : 1). The 1/4 volume of solutions were applied to a silica gel column packed with Fuji gel CQ-3 (particle size 30 ~ 50 μm). Two peaks were obtained. The first peaks included trichomycin B, the second included trichomycin A. Each fraction was submitted to preparative HPLC. μBondapak C18 (Waters, 5 μm) column of dimensions 150 × 19 mm i.d. was used for preparative HPLC. The solvent system, acetonitrile - phosphate-citrate buffer (36 : 64, pH 4.75 ~ 4.85), was used as the mobile phase. The main peak, monitoring at 355 nm, was collected and concentrated *in vacuo* in the dark until no odor of acetonitrile. The concentrate was extracted twice with 20% of its volume of

Table 1. Media used for production of trichomycins A and B.

| Seed medium | | Production medium | |
|---|---|---|---|
| Glucose | 0.5 % | Starch | 2.2 % |
| Starch | 3 % | Gluten meal | 3.18% |
| Dried yeast | 1 % | Corn steep liquor | 0.38% |
| Peptone | 1 % | Soybean oil | 0.23% |
| Meat extracts | 0.25% | NaCl | 0.23% |
| Soybean oil | 0.25% | $CaCO_3$ | 0.32% |
| NaCl | 0.3 % | $MnCl_2 \cdot 4H_2O$ | $7 \times 10^{-4}$ |
| pH adjusted to | 7.4 to 7.6 | $CuSO_4 \cdot 5H_2O$ | $7 \times 10^{-4}$ |
| | | $ZnSO_4 \cdot 7H_2O$ | $27 \times 10^{-4}$ |

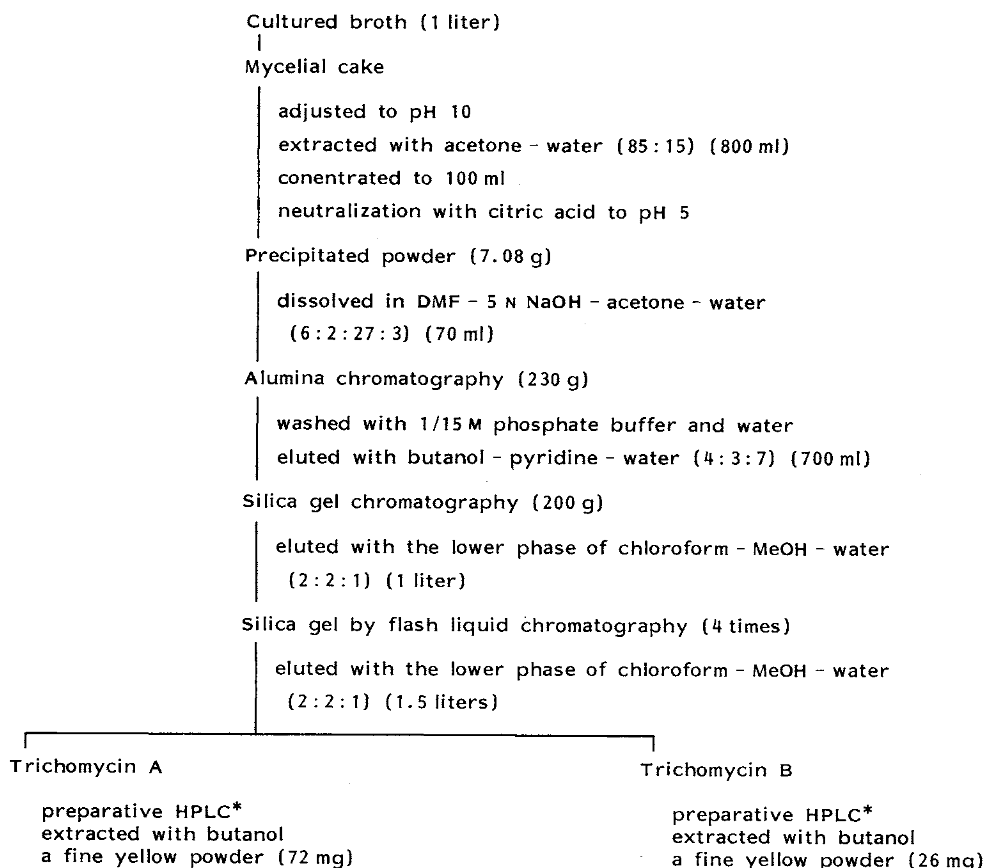



Fig. 2. Isolation procedure for trichomycins A and B.

*μBondapak C18 developed with acetonitrile - phosphate-citrate buffer (36 : 64, pH 4.75 ~ 4.85), flow rate 5.0 ml/minute.

butanol. The butanol extracts were washed twice with water, and then were concentrated, adding water, to syrup in the dark. The precipitated fine yellow powder was filtered off, washed with water and dried *in vacuo* over $P_2O_5$ to give trichomycins A (72 mg) and B (26 mg), respectively.

## Properties

The physico-chemical properties of trichomycin B are shown in Table 2. Trichomycin B exhibited one-eighth lowered activities against fungi and yeasts *in vitro* compared with those of trichomycin A (Tables 3 ~ 5). However, its efficacy was superior to miconazole against candidas, trichomonas and fungi except some strains.

## Structure

Trichomycin B, a fine yellow powder, gave microanalyses and a FAB-MS spectrum agreeing with the formula $C_{58}H_{84}N_2O_{18}$. The two antibiotics, trichomycins A and B, possess the same formula, which suggested an isomer.

$^{13}C$ NMR spectra of trichomycins A and B were recorded in DMSO-$d_6$ solution, using a JNM-FX 270 spectrometer. Spectra of the two compounds are virtually superimposable in many regions; a striking

Table 2. Physico-chemical properties of trichomycin B.

| | |
|---|---|
| Rt[a] (minutes) | 14.2 |
| Appearance | Fine yellow powder |
| MP | >300°C (dec) |
| UV-VIS $\lambda_{max}^{pH\,2.08}$ nm ($\varepsilon$) | 235 (23,674), 289 (14,796), 344 (37,593), 359 (49,210), 379 (58,636), 400 (46,032) |
| $\lambda_{max}^{pH\,6.81}$ nm ($\varepsilon$) | 236 (22,249), 341 (32,990), 358 (33,428), 379 (31,455), 400 (22,687) |
| $\lambda_{max}^{pH\,12.44}$ nm ($\varepsilon$) | 234 (27,510), 289 (24,880), 343 (40,114), 360 (60,390), 379 (81,762), 401 (67,514) |
| Elemental composition | $C_{58}H_{84}N_2O_{18}$ |
| FAB-MS (positive ion mode) | 1,097 $(M+H)^+$ |
| *pKa* In 66% DMF | 6.1 (-COOH) |
| | 9.0 ($-NH_2$) |

[a] HPLC was carried out by using Nucleosil 5C8 (5 μm, 150×4 mm i.d.). Mobile phase: acetonitrile-phosphate-citrate buffer (32.5:67.5, pH 4.6). Detection: 360 nm. Flow rate: 1.0 ml/minute.

Table 3. Anticandidal activity of trichomycins A, B and miconazole.

| Organism[a] | MIC (μg/ml) | | |
|---|---|---|---|
| | Trichomycin | | Miconazole |
| | A | B | |
| *Candida albicans* (eight strains) | Mean 0.034 | Mean 0.275 | Mean 9.76 |
| *C. tropicalis* FP583 | 0.1 | 0.78 | 1.56 |
| *C. pseudotropicalis* FP584 | <0.025 | 0.025 | <0.025 |
| *C. krusei* FP585 | 0.1 | 0.78 | 1.56 |
| *C. parakrusei* FP586 | 0.2 | 1.56 | 6.25 |
| *C. guilliermondii* FP587 | 0.1 | 0.78 | 50 |
| *C. stellatoidea* FP588 | <0.025 | 0.025 | 0.05 |

[a] Sabouraud agar.

Table 4. Antifungal activity of trichomycins A, B and miconazole.

| Organism[a] | MIC (μg/ml) | | |
|---|---|---|---|
| | Trichomycin | | Miconazole |
| | A | B | |
| *Aspergillus niger* FP1398 | 0.1 | 0.78 | 50 |
| *A. flavus* FP1022 | 6.25 | 50 | 3.13 |
| *A. fumigatus* FP1305 | 6.25 | 50 | 3.13 |
| *Trichophyton rubrum* FP596 | 12.5 | 100 | 0.78 |
| *T. metagrophytes* FP594 | 6.25 | 50 | 0.39 |
| *Cryptococcus neoformans* FP1551 | <0.025 | 0.025 | 0.39 |

[a] Sabouraud agar.

difference can be noticed in the behavior of carbons at C-4~C-12. Especially, the methylene carbon signal C-4 (δ 51.1, t) inserted between C-3 carbonyl and C-5 hydroxyl was deleted and a signal of δ 28.9 (t) was newly generated in the spectrum of trichomycin B. This may indicate that the hydroxyl at C-5 of trichomycin A located on C-9 in trichomycin B. $^1$H NMR spectra of the both compounds also supported this elucidation. There-

Table 5. Antitrichomonas activity of trichomycins A, B and miconazole.

| Organism[a] | MIC (μg/ml) | | |
|---|---|---|---|
| | Trichomycin | | Miconazole |
| | A | B | |
| *Trichomonas vaginalis* 4FM | 0.2 | 1.56 | >100 |
| *T. vaginalis* ATCC 30001 | 0.2 | 1.56 | >100 |
| *T. vaginalis* PCL110108 | 0.2 | 1.56 | >100 |

[a] V-Bouillon.

Table 6. Comparison of $^{13}C$ NMR chemical shifts ($\delta$, ppm) for trichomycins A and B.

| Carbon No. | Trichomycin A | Trichomycin B | Carbon No. | Trichomycin A | Trichomycin B |
|---|---|---|---|---|---|
| 1 | 166.3 | 166.5 | 36 | 38.2 | 38.2 |
| 2 | 49.5 | 48.8 | 37 | 79.1 | 79.2 |
| 3 | 201.7 | 202.2 | 38 | 32.8 | 32.8 |
| 4 | 51.1 | 28.9 | 39 | 29.7 | 29.7 |
| 5 | 67.5 | 19.3 | 40 | 37.3 | 37.3 |
| 6 | 38.2 | 34.6 | 41 | 67.4 | 67.3 |
| 7 | 70.6 | 70.4 | 42 | 45.3 | 45.3 |
| 8 | 34.6 | 42.4 | 43 | 196.2 | 196.2 |
| 9 | 22.1 | 69.3 | 44 | 137.2 | 137.8 |
| 10 | 37.9 | 44.3 | 45 | 130.4 | 130.4 |
| 11 | 71.0 | 67.3 | 46 | 112.4 | 112.4 |
| 12 | 43.7 | 44.7 | 47 | 153.4 | 153.4 |
| 13 | 67.0 | 66.9 | 48 | 112.4 | 112.4 |
| 14 | 46.2 | 45.3 | 49 | 130.4 | 130.4 |
| 15 | 96.9 | 96.9 | 50 | 16.4 | 16.6 |
| 16 | 44.1 | 44.3 | 51 | 12.5 | 12.5 |
| 17 | 65.5 | 65.7 | 52 | 176.7 | 176.7 |
| 18 | 57.9 | 57.5 | 53 (1′) | 96.2 | 96.7 |
| 19 | 65.5 | 65.5 | 54 (2′) | 68.0 | 68.2 |
| 20 | 36.7 | 36.9 | 55 (3′) | 56.0 | 56.0 |
| 21 | 74.6 | 74.8 | 56 (4′) | 70.0 | 69.8 |
| 22 | 133.8 | 134.0 | 57 (5′) | 72.7 | 72.7 |
| 23~34 | 124.4~133.5 | 124.3~133.3 | 58 (6′) | 17.8 | 17.8 |
| 35 | 134.5 | 134.4 | | | |

Solvent: DMSO-$d_6$, assignments to the numbering system shown in Fig. 1.

fore, from these results the structure of trichomycin B was deduced to be **2** shown in Fig. 1.

Assignments in $^{13}C$ NMR chemical shifts for trichomycins A and B are compared in Table 6.

Acknowledgments

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## References

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