IDENTITY OF IMMUNOSUPPRESSANT FR-900520 WITH ASCOMYCIN

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(Received for publication June 7, 1991)

Ascomycin is an antifungal antibiotic isolated from *Streptomyces hygroscopicus* var. *ascomyceticus* (ATCC 14891), and its physico-chemical and biological properties were reported by ARAI *et al.* in 1962[1] and 1963.[2] The immunosuppressive activity of ascomycin together with immunomycin was later described by a Merck group.[3] FR-900520 was described as a member of the immunosuppressive 23-membered macrolide antibiotics by a Fujisawa group in 1988.[4] In this paper, are reported additional physico-chemical data and data on the antifungal activity of ascomycin, and the identity of FR-900520 with ascomycin.

Ascomycin was crystallized as colorless plates from acetonitrile: $C_{43}H_{69}NO_{12}$; FAB-MS (Fig. 1), *m/z* 830 (M+K), 814 (M+Na), 774 (M−OH), 756 (M−OH−H_2O); mp 148~152°C; $[\alpha]_D^{25}$ −96.2° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectra including DEPT of ascomycin are shown in Figs. 2 and 3, respectively. They were found to be superimposable with those of FR-900520, which was obtained as described.[4] These physical data of ascomycin are fully consistent with the proposed structure (Fig. 4) of FR-900520.[4] ^{13}C NMR also indicated ascomycin to occur in solution as a mixture of two conformational isomers (approximately 2:1), as observed with other macrolide immunosuppressants FK-506[5] and rapamycin.[6] The Rf values of ascomycin on silica gel TLC developed with ethyl acetate, dichloromethane-2-propanol (9:1) and chloroform-methanol (9:1) were 0.44, 0.62 and 0.50, respectively, and

Table 1. Antifungal activity of ascomycin.

Test organisms	MIC (μg/ml)
Candida albicans 7N	>100
C. albicans IFM 40001	>100
C. albicans IFM 40002	100
C. albicans IFM 40003	>100
C. guilliermondii	100
C. kurusei	>100
C. tropicalis	>100
Cryptococcus neoformans	>100
Saccharomyces cerevisiae	>100
Penicillium expansum	>100
P. chrysogenum	>100 (50)[a]
Aspergillus flavus	50 (0.2)[a]
A. niger	100 (12.5)[a]
A. oryzae	50 (0.2)[a]
Sporothrix shenckii	>100
Trichophyton mentagrophytes	100 (50)[a]

[a] Incomplete inhibition.

Fig. 1. FAB mass spectrum of ascomycin.

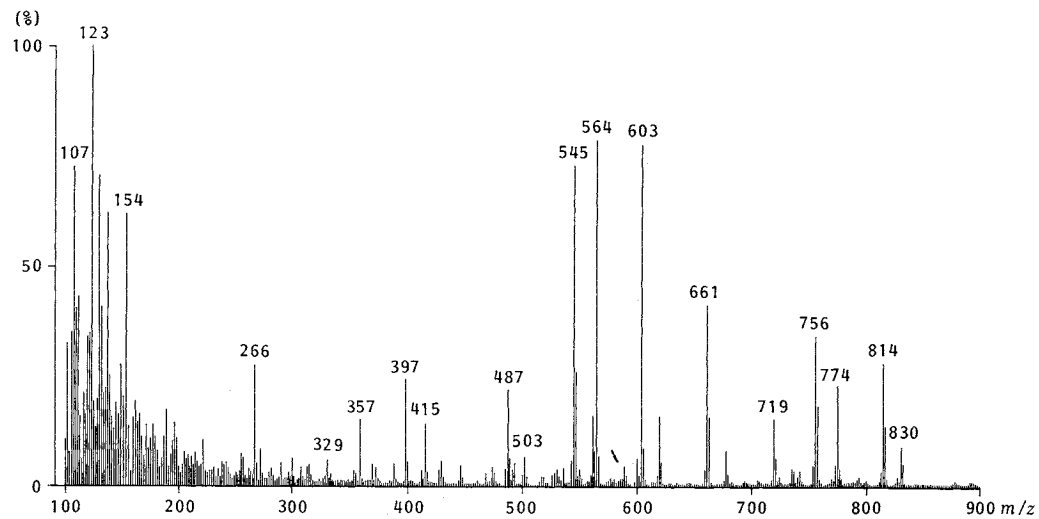


Fig. 2. ^{1}H NMR spectrum of ascomycin.

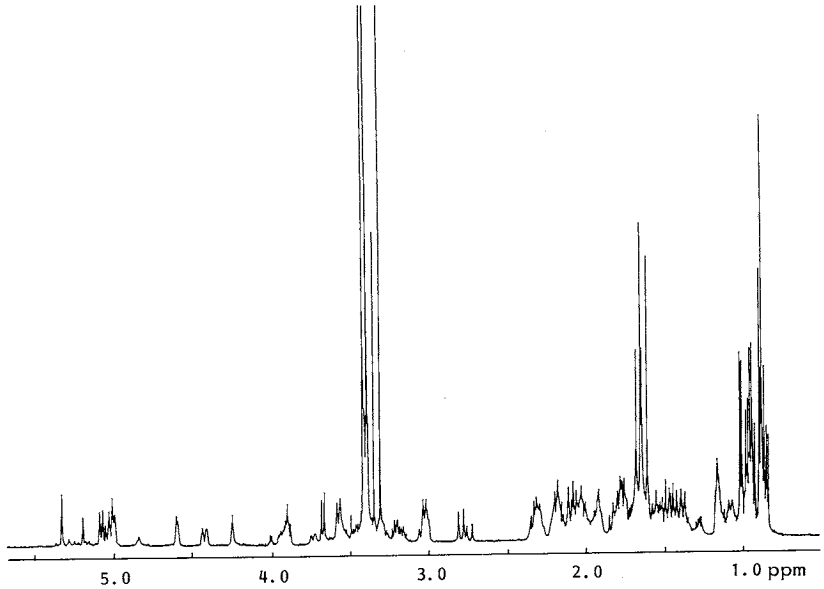


Fig. 3. ^{13}C NMR spectrum of ascomycin.

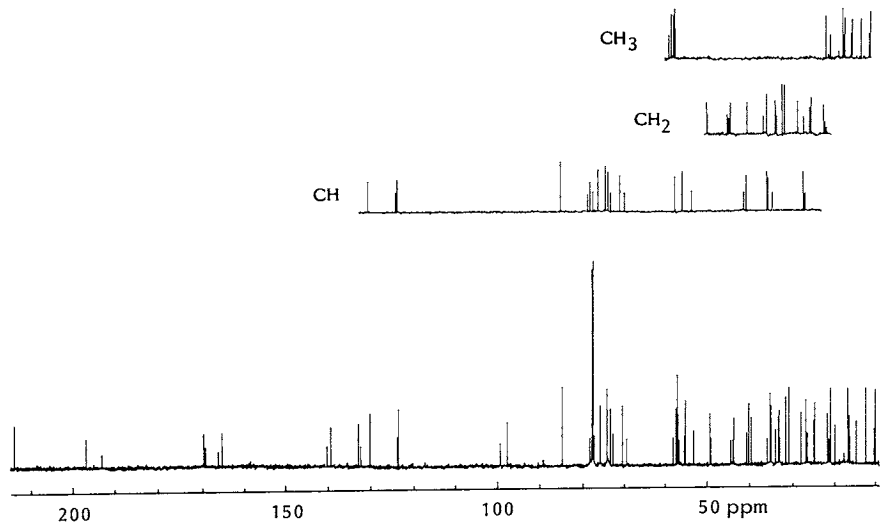
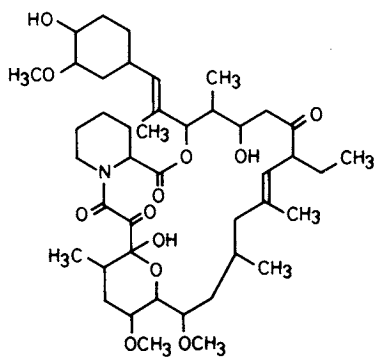


Fig. 4. Proposed structure of FR-900520.[4]



were identical with those of FR-900520. Further, ascomycin and FR-900520 comigrated on HPLC (Zorbax SB CN column, 4.6 × 250 mm, methanol-water (7:3), 1.0 ml/minute, Rt 9.1 minutes). These HPLC revealed a small peak (approximately 3% of ascomycin and FR-900520) at a Rt of 8.2 minutes, which was probably due to FR-900523.[4]

From all of these data, FR-900520 should be identical with ascomycin. In this connection, it is interesting to note that the 29-membered macrolide immunosuppressant rapamycin,[7] as well as the immunosuppresive macrocyclic peptide cyclosporin A[8] had originally been described as an antifungal

antibiotic.

The antifungal activity of ascomycin was studied by the agar streak method using SABOURAUD dextrose agar. The antibiotic was not active against yeast form fungi but incompletely inhibited the growth of some species of *Aspergillus* (Table 1). It did not inhibit respiration, DNA, RNA or protein synthesis of *Aspergillus niger* at concentrations below 1 μg/ml.

Acknowledgment

We thank Kyowa Hakko Kogyo Co., Ltd. for the fermentation of ascomycin. Thanks are also due to Central Research, Pfizer Inc. for spectroscopic analyses.

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