MICROBIAL TRANSFORMATION OF IMMUNOSUPPRESSIVE COMPOUNDS

II. SPECIFIC DESMETHYLATION OF 13-METHOXY GROUP OF FK 506 AND FR 900520 BY *Actinomycete* sp. ATCC 53828

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FK 506 and FR 900520 are a new class of fermentation products, isolated from *Streptomyces tsukubaensis*/*Streptomyces hygroscopicus* species *ascomyceticus* MA6475[1~5], showing potent inhibition of T cell activation[6]. In the course of our recent investigations on microbial modification of FK 506/FR 900520, several species of actinomycetes were found capable of transforming these drugs. The previous paper[7] described the microbial desmethylation of FK 506/FR 900520 to 31-desmethyl, 13,31-bisdesmethyl, 15,31-bisdesmethyl, and 13,15,31-tridesmethyl derivatives using *Actinoplanacete* sp. ATCC No. 53771. The present report describes the specific desmethylation of 13-methoxy group of FK 506 and FR 900520 by *Actinomycete* sp. ATCC No. 53828.

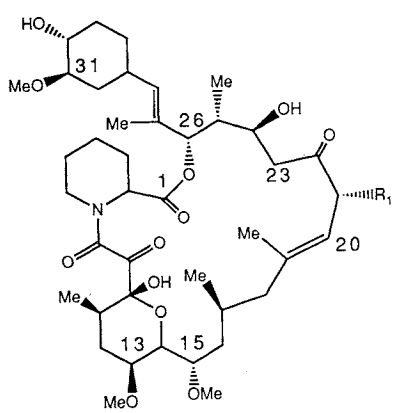
Fermentation Conditions for Conversion

Actinomycete sp. ATCC No. 53828 was stored and maintained on skim milk in the culture collection of MSDRL. The organism was inoculated into a seed medium which contained (in g/liter) dextrin 10.0, glucose 1.0, beef extract 3.0, Ardamine PH (Yeast Products, Inc.) 5.0, N-Z Amine type E 5.0, $MgSO_4 \cdot 7H_2O$ 0.05, KH_2PO_4 0.37 and $CaCO_3$ 0.5. The medium was adjusted to pH 7.1 with NaOH and was autoclaved for 20 minutes. The inoculated 50 ml volumes of seed medium were shaken in 250-ml baffled flasks at 220 rpm for 72 hours at 27°C. The mature seed cultures were used as the source of inoculum (10% inoculum) for the fermentation medium. The fermentation medium consisted of (in g/liter) glucose 10.0, yeast extract 1.0, beef extract 1.0, MOPS 11.6. After formulation the medium was adjusted to pH 7.2 with NaOH and sterilized by autoclaving. The medium was employed in the manner described for seed medium. FK 506/FR 900520 was dissolved in dimethyl sulfoxide and added to the fermentation at 0 hour to achieve final concentrations ranging between 50 to 100 μg/ml. The fermentations were continued for 3 days using the conditions described. These fermentations were the source of 13-desmethyl FK 506/FR 900520 (Fig. 1).

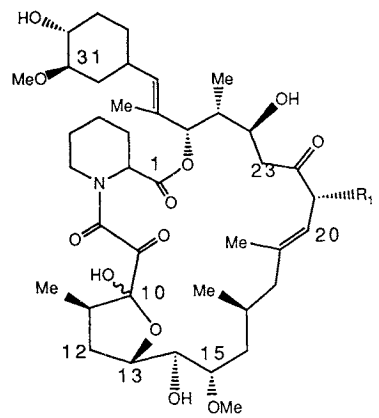
Isolation and Characterization of Conversion Products

Isolation and purification of the 13-desmethyl FK 506 and FR 900520 derivatives were performed as described in our previous publication[7]. The HPLC chromatographic study shows the same isomeric/tautomeric characteristics as 13,31-bisdesmethyl and 13,15,31-tridesmethyl derivatives due to

Fig. 1. The structure of FK 506/FR 900520 and 13-desmethyl derivatives.

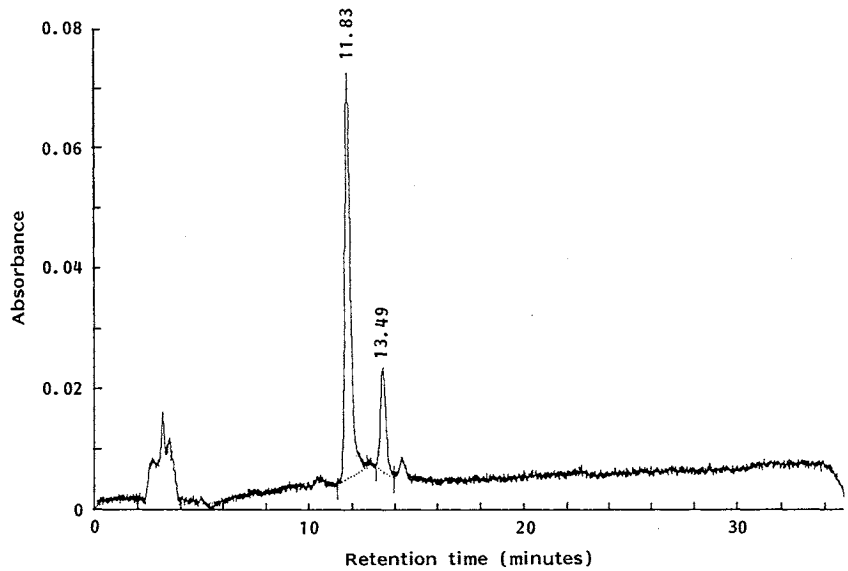


FK 506 $R_1 = CH_2CH=CH_2$
FR 900520 $R_1 = CH_2CH_3$



L-683,519 $R_1 = CH_2CH=CH_2$
L-685,487 $R_1 = CH_2CH_3$

Fig. 2. HPLC of 13-desmethyl FR 900520.



the desmethylation of C-13 methoxy group. The desmethylation of C-13 methoxyl group can result in isomers/tautomers that differ in the point of formation of hemiketal as reported previously[7]. The HPLC chromatogram of 13-desmethyl FR 900520 using a Whatman Partisil 10 ODS-3 column at 50°C and a 30-minute linear gradient of 45% to 80% acetonitrile in 0.1% phosphoric acid is shown in Fig. 2. The FAB mass spectra gave an M+Li of 796 and 784 which correspond to a loss of 14 mass units from FK 506 and FR 900520, respectively. The structure determination was based on virtually complete analysis of 400 MHz proton NMR. The proton resonances were assigned by using a combination of NMR techniques including COSY, NOESY, decoupling deference, NOE difference, proton-proton decoupling, solvent effects, and temperature effects. The proton NMR spectra showed many of the same major differences from FK 506/FR 900520 (Table 1) as the 13,31-bisdesmethyl derivatives which had undergone further structural rearrangement[7]. This sharing of distinctive features implies that the same compositional changes in the C-13~C-15 region have occurred. C-13 is selected as the desmethylation site, since desmethylation of either the C-15 or C-31 methoxy would not lead to rearrangement of the tetrahydropyran to the tetrahydrofuran, and proton signals from 2-H to 28-H would be virtually superimposable with those of the parent compounds, the changes in the proton NMR spectra must arise from desmethylation of the C-13 methoxy group[7].

Table 1. Select proton chemical shifts in FK 506 and 13-desmethyl FK 506 (L-683,519).

H	FK 506 (ppm)	L-683,519 (ppm)	$\Delta\delta$ (ppm)
2	4.62	5.20	0.58
6	4.43	4.50	0.07
11-CH_3	1.00	1.06	0.06
11	2.08	3.02	0.94
12	1.75, 1.4~1.5	2.45, 1.80	0.70, 0.3~0.4
13	3.40	4.43	1.03
14	3.69	3.82	0.13
15	3.58	3.25	−0.33

Table 2. Inhibition of T-cell proliferation by 13-desmethylated FK 506 and FR 900520.

Compounds	IC_{50} (nM)
FK 506	0.4
FR 900520	0.8
13-Desmethyl FK 506 (L-683,519)	2.2
13-Desmethyl FR 900520 (L-685,487)	5.0

The proton and carbon NMR spectra are shown in Figs. 3 and 4, respectively. The detailed analysis of all the isomers existing in equilibrium is in progress.

Biological Characterization

The biological effects of 13-desmethyl derivatives

Fig. 3. ^{1}H NMR spectrum (400 MHz) of 13-desmethyl FR 900520 in $CDCl_3$.

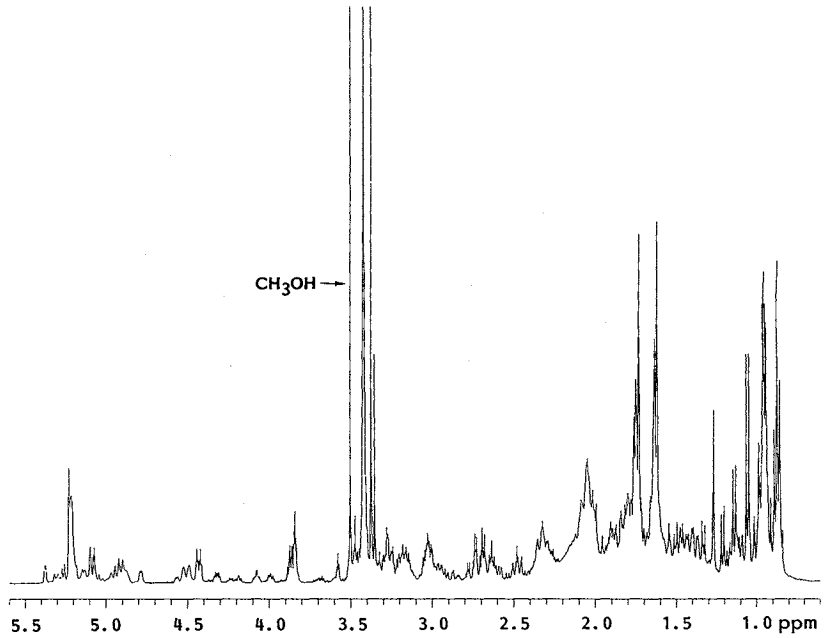
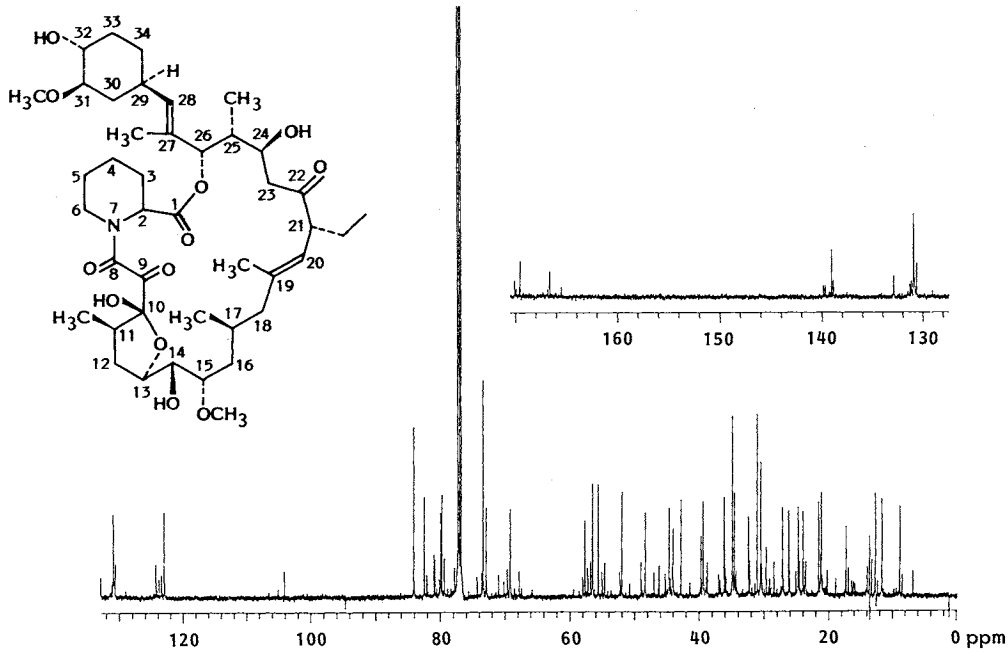


Fig. 4. ^{13}C NMR spectrum (125 MHz) of 13-desmethyl FR 900520 in $CDCl_3$.



were tested in comparison with their parent compounds for their immunosuppressive effects on murine T cells.[8] Data presented in Table 2 for the 13-desmethyl derivatives show a 5-fold reduction in ability to inhibit T-cell proliferation following the removal of methyl of C-13 methoxy group, suggesting that structural and conformational changes caused by the rearrangement of the tetrahydropyran to the tetrahydrofuran do not have significant impact on the biological actions of these

drugs.

Acknowledgment

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