NEW SEMISYNTHETIC FLUORINATED "HYBRID" MACROLIDES

Sir:

In a previous paper[1] we reported the biological transformation of erythronolide B into four new "hybrid" macrolide antibiotics by the mutant strain *Streptomyces antibioticus* ATCC 31771, isolated in our laboratories. The availability[2] of (8*S*)-8-fluoroerythronolides A (I) and B (II) and their successfull conversion to fluorinated erythromycins[3] by *Streptomyces erythraeus* ATCC 31772, prompted us to attempt the analogous transformation of these fluoro-compounds by the *S. antibioticus* ATCC 31771 strain.

In a representative experiment, 500 μg/ml of crystalline **I** and **II** were individually added to different flask cultures of *S. antibioticus* ATCC 31771 prepared as already reported[1], after 32 hours of cultivation. Disappearance of substrates **I** and **II**, and the concurrent production of the new compounds **III** and **IV** respectively (Scheme 1), was monitored by high performance liquid chromatography (HPLC), using the same conditions as already described[1]. The retention times of compounds **III** and **IV** relative to erythromycin A were 0.71 and 0.84 respectively (mobile phase: acetonitrile - 0.01 M phosphate pH 7.0, 64: 36). After 100 hours of cultivation, 2 liters of each culture to which 1.0 g of either substrate was added, were filtered through Celite, and the filtrate was extracted with ethyl acetate at pH 9.8. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated at reduced pressure.

The residue (0.98 g) coming from the culture fed the (8*S*)-8-fluoroerythronolide A was chromatographed on a silica gel partition column (740 ×

Table 1. Acid stability at 25°C.

Compounds	pH 2	pH 3	pH 4
	$t_{\frac{1}{2}}$*	$t_{\frac{1}{2}}$*	$t_{\frac{1}{2}}$*
III**	10	>100	>100
IV	25	>100	>100
Erythromycin A	0.05	0.1	2

* $t_{\frac{1}{2}}$ is the half-life in hours.

** Quantitative data were calculated by summing the areas of the two peaks corresponding to the ketonic and hemiketalic forms (HPLC analysis).

Scheme 1.

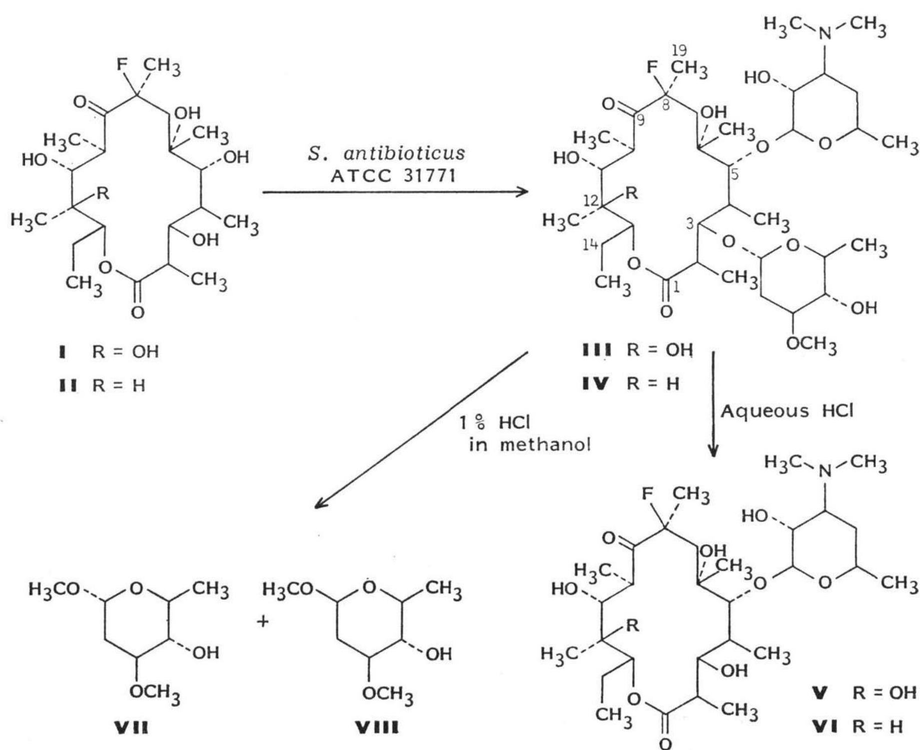

Table 2. *In vitro* antibacterial activity of compounds **III** and **IV** compared to that of erythromycin A, erythromycin B, oleandomycin and 3-*O*-oleandrosyl-5-*O*-desosaminylerythronolide B.

Organism	Minimum inhibitory concentration (μg/ml)					
	III	IV	Erythromycin A	Erythromycin B	Oleandomycin	3-*O*-Oleandrosyl-5-*O*-desosaminylerythronolide B
Staphylococcus aureus ATCC 6538 P	0.39	0.39	0.049	0.097	0.39	0.78
Staphylococcus aureus ATCC 14154*	>25	>25	>25	>25	>25	>25
Streptococcus pneumoniae ATCC 6303	0.049	0.049	0.012	0.024	0.195	0.097
Streptococcus pyogenes ATCC 8668	0.049	0.049	0.012	0.024	0.195	0.097
Corynebacterium diphteriae LRP 24	0.049	0.049	0.006	0.012	0.097	0.049
Micrococcus luteus ATCC 9341 LRP 6	0.049	0.049	0.006	0.006	0.049	0.097
Micrococcus luteus ATCC 15957*	>25	>25	>25	>25	>25	>25
Bacillus subtilis LRP 25	0.39	0.195	0.049	0.049	0.39	0.39
Haemophilus influenzae ATCC 19418	12.5	6.25	3.12	6.25	>25	6.25
Neisseria gonorrhoeae ATCC 19424	0.097	0.39	0.049	0.097	0.195	0.195
Escherichia coli LRP 50	>25	>25	6.25	25	>25	>25
Klebsiella pneumoniae LRP 54	>25	>25	25	25	>25	>25
Proteus vulgaris ATCC 6380	>25	>25	>25	>25	>25	>25
Pseudomonas aeruginosa LRP 9	>25	>25	>25	>25	>25	>25
Salmonella typhi LRP 8	>25	>25	12.5	25	>25	>25
Shigella sonnei LRP 5	>25	>25	12.5	>25	>25	>25
Acholeplasma laidlawii ATCC 23206	3.12	3.12	0.097	0.097	6.25	12.5
Mycoplasma hominis I ATCC 14027	>25	>25	>25	>25	>25	>25
Clostridium perfringens ATCC 3624	6.25	1.56	1.56	1.56	3.12	12.5
Bacteroides fragilis ATCC 23745	3.12	3.12	0.195	0.195	0.39	3.12
Fusobacterium necrophorum ATCC 27852	12.5	12.5	1.56	6.25	6.25	>25

* Erythromycin resistant

24 mm i.d.) according to a reported method[4]. Fifteen-milliliter fractions were collected at a flow rate of 1.0 ml per minute. Fractions 45~80, which contained **III** alone, were combined and concentrated to dryness *in vacuo* to afford, after crystallization from acetone - hexane, 0.18 g of **III**: mp 155~157°C; $[\alpha]_D^{20}$ −40.2° (*c* 1.0, methanol); UV (methanol) 283 nm (ε 20.3); IR (KBr) 3480 (broad), 1730, 1510, 1380, 1305, 1165 cm^{-1}; *Anal.* calcd. for $C_{36}H_{64}FNO_{13}$: C 58.60, H 8.74, F 2.57, N 1.90; found C 58.52, H 8.75, F 2.63, N 1.95.

From the culture fed the (8*S*)-8-fluoroerythronolide B, a yellow foam (1.2 g) was obtained, which underwent the same purification process on silica gel as described above. Combined frac-

tions 17~42 were evaporated *in vacuo* to yield 0.51 g of **IV**. A further chromatography on Sephadex LH-20 prepared[5] in chloroform - hexane (1:1), followed by crystallization from acetone - hexane, afforded an analytical sample: mp 195~196°C; $[\alpha]_D^{20}$ −48° (*c* 1.0, methanol); UV (methanol) 285 nm (ε 29); IR (KBr) 3400 (broad, irregular), 1730, 1455, 1380, 1305, 1180, 1160 cm^{-1}; *Anal.* calcd. for $C_{36}H_{64}FNO_{12}$: C 59.90, H 8.94, F 2.63, N 1.94; found C 59.94, H 9.06, F 2.69, N 1.83.

Structure assignments of the new antibiotics **III** and **IV** were made by following their behavior on acid-catalyzed hydrolysis (Scheme 1). By treatment of **III** with dilute hydrochloric acid, a product was isolated with the same physico-chemical properties as the 5-*O*-desosaminyl-(8*S*)-8-fluoroerythronolide A (**V**) reported elsewhere[2]. Analogously, 5-*O*-desosaminyl-(8*S*)-8-fluoro-erythronolide B (**VI**)[2] was obtained from the hydrolysis of **IV**. Furthermore, the same mixture of the α- and β-anomers (**VII** and **VIII**) of methyl oleandroside was obtained when the new antibiotics and oleandomycin were subjected to acid catalyzed methanolysis[6], as determined by a gas liquid chromatographic (GLC) procedure[1].

These results clearly showed that compounds **III** and **IV** were 3-*O*-oleandrosyl-5-*O*-desos-aminyl-(8*S*)-8-fluoroerythronolides A and B, respectively.

In analogy to (8*S*)-8-fluoroerythromycin A[3], compound **III** exists as ketonic form in the solid state, whereas it displays the hydroxyketone/hemiketal tautomerism in certain solvents.

Acid stabilities of the compounds **III**, **IV** and related erythromycin A in 0.1% buffered solutions at pH 2, 3, 4 and at 25°C, were evaluated by HPLC analysis (Table 1). In contrast to erythromycin A, the new antibiotics are remarkably stable in the acid environment.

The antibacterial activities *in vitro* of **III** and **IV** in comparison with those of erythromycin A, erythromycin B, oleandomycin and 3-*O*-oleandrosyl-5-*O*-desosaminylerythronolide B[1] are reported in Table 2. The two new convertants exhibit similar antibacterial properties, which are intermediate between those of erythromycins and oleandomycin. Comparison between **IV** and 3-*O*-oleandrosyl-5-*O*-desosaminylerythronolide B shows that replacement of hydrogen by fluorine at C-8 positively affects the antibacterial activity, contrary to what happens with the corresponding hydroxyl substitution[1]. The same structure-activity relationships were already observed for erythromycin B and its (8*S*)-8-fluoro-[3] and (8*S*)-8-hydroxy-analogue[7,8].

In contrast to the similar conversion of erythronolide B, from which four compounds were derived[1], transformation of (8*S*)-8-fluoroerythronolide B (**II**) yielded essentially one product (**IV**). The fluorine atom clearly prevented the hydroxylation at C-8 and the further (8*R*)-8,19-epoxide formation. Besides **IV**, a trace amount of a more polar active compound was detected in the fermentation broth (retention time 0.49 relative to erythromycin A, determined as reported above), but our attempts to isolate it by partition column chromatography failed due to its high polarity. In light of our previous findings[1], however, we can argue that this product results from the 14-methyl hydroxylation of **IV**.

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