NEW FLUORINATED ERYTHROMYCINS OBTAINED BY MUTASYNTHESIS

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Following the previously described semisynthetic preparation of new aglycones (8*S*)-8-fluoroerythronolide A (I), (8*S*)-8-fluoroerythronolide B (II) and the monoglycoside 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (III), their conversion into new fluoroerythromycins was attempted by mutational biosynthesis. The strain *Streptomyces erythraeus* ATCC 31772, a mutant blocked in the biosynthesis of erythromycin, was employed in the present investigation. Four new antibiotics, (8*S*)-8-fluoroerythromycin A (IV), (8*S*)-8-fluoroerythromycin B (V), (8*S*)-8-fluoroerythromycin C (VI) and (8*S*)-8-fluoroerythromycin D (VII) were successfully derived by such an approach. The result is also discussed in terms of the substrate specificity of the enzymes involved in the biosynthesis of erythromycins. The new antibiotics exhibited promising biological properties.

During the last twenty years advances in organic fluorine chemistry have been responsible for the development of a large number of new compounds of importance in biology and medicine. Replacement of an atom of hydrogen by one of fluorine in organic molecules may profoundly change their biological properties[1,2]. The rationale employed is that, since hydrogen and fluorine are nearly isosteric, the fluoro analog would be expected to have little difficulty in fitting onto active sites of receptors. At the same time, the strong electronegativity of the fluorine atom, when strategically placed, could be used to make defined alterations in the biological activity.

As part of a general strategy of our laboratories aimed at the synthesis of fluorinated organic derivatives designed as potential drugs, we have undertaken a program involving the synthesis and the biological evaluation of new fluoroerythromycins.

In previous papers[3,4] we have reported the fluorination of enol ether groups in 8,9-anhydroerythronolide A and B 6,9-hemiketals and 3-*O*-mycarosyl-8,9-anhydroerythronolide B 6,9-hemiketal with trifluoromethyl hypofluorite. This reaction, already employed for the introduction of fluorine into naturally occurring products[5~10], was of interest *per se* and also as model to be used in the erythromycin series. These fluorinations were achieved under mild conditions to give (8*S*)-8-fluoroerythronolides A (I) and B (II) and 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (III). Moreover, in contrast to ery-

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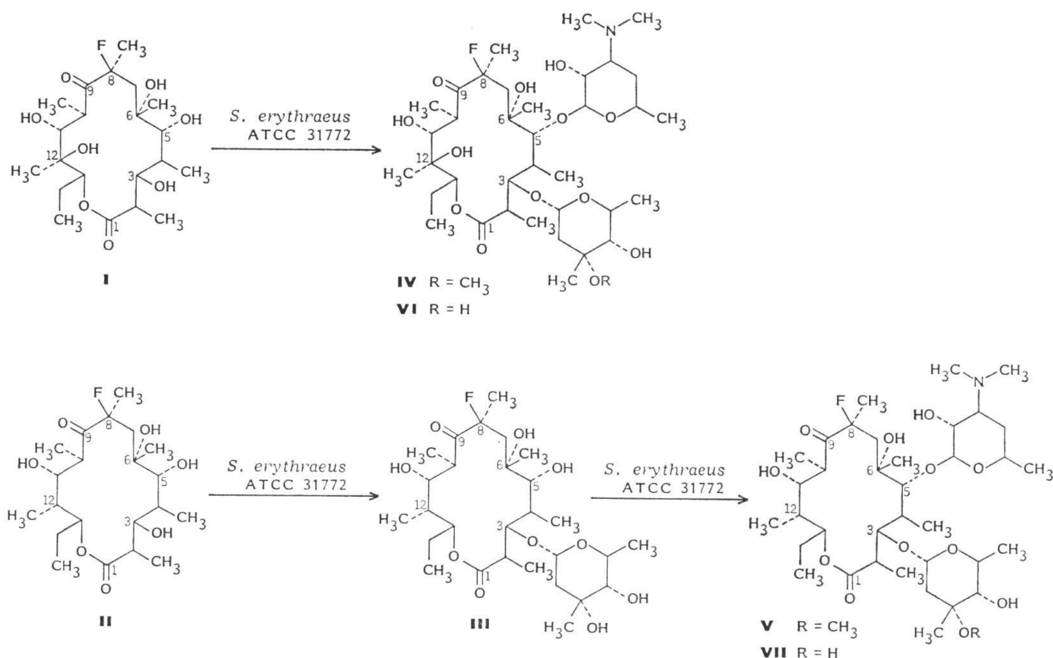
Scheme 1.



thronolide A, (8*S*)-8-fluoroerythronolide A (**I**) was shown to be stable in mineral acid solution[3]. Since the acid degradation pattern of erythromycin A[11] closely resembles that of erythronolide A[12], we thought to synthesize the (8*S*)-8-fluoroerythromycin A (**IV**) with the aim of obtaining a compound with same antibacterial activity as erythromycin A but with an enhanced stability to acids[13]. However, when we carried out an analogous fluorination[4] on the enol ether group of 8,9-anhydroerythromycin A and B 6,9-hemiketals[11], the complete range of the reaction products could not be characterized because of difficulties encountered in the attempted isolation.

Instead, the new (8*S*)-8-fluoroerythromycins **IV**, **V**, **VI** and **VII** (Scheme 1) could be obtained by a mutasynthetic approach[14], individually adding the fluoro-compounds **I**, **II**, **III**, in the fermentation medium of *Streptomyces erythraeus* ATCC 31772, a mutant blocked in erythromycin biosynthesis. This strain was previously[15] shown to produce erythromycins A and B when fed erythronolides A and B.

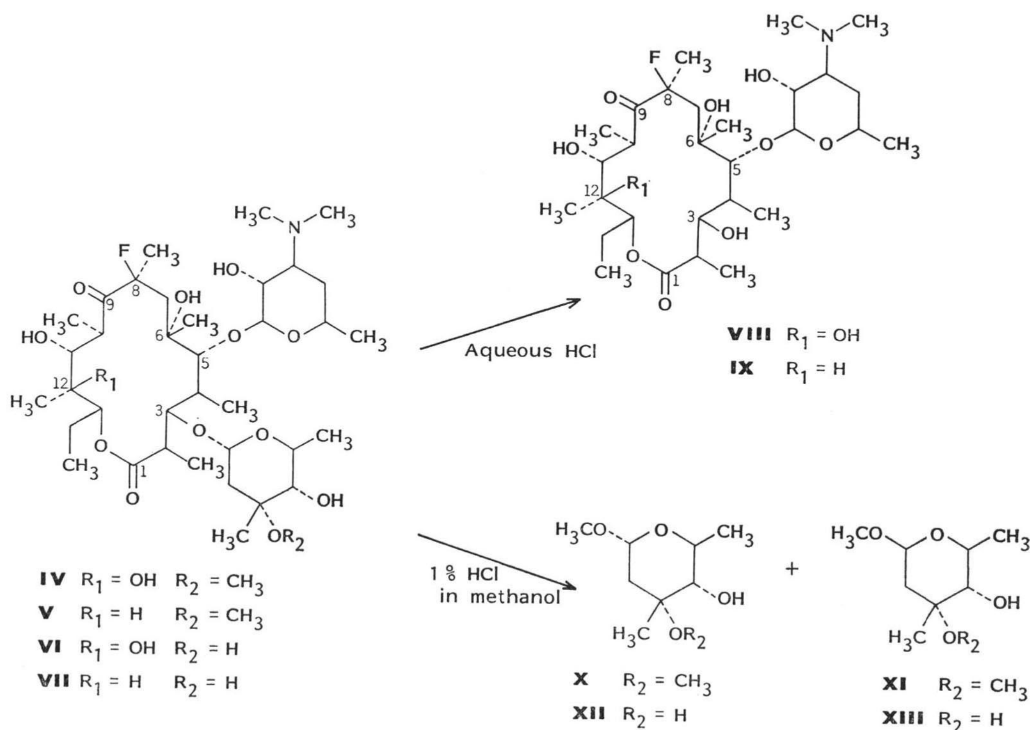
In this paper the fermentative production, isolation, physico-chemical properties, antibacterial activity and some preliminary absorption studies in rats of these antibiotics are presented.

Results and Discussion

Bioconversion of (8*S*)-8-Fluoroerythronolide A (I)

Two active compounds **IV** and **VI** (Scheme 1) were obtained when (8*S*)-8-fluoroerythronolide A (**I**) was added to the fermentation broth of *S. erythraeus* ATCC 31772, a blocked mutant of an erythromycin-producing strain. The structures of **IV** and **VI** were determined to be (8*S*)-8-fluoroerythromycin A and (8*S*)-8-fluoroerythromycin C, respectively, on the basis of elemental analyses, fast atom bombardment (FAB) mass spectral data and behavior to the acids. FAB-mass spectrometry (MS) has been shown[16~18] to be the method of choice in determining molecular weights of a number of non-volatile or

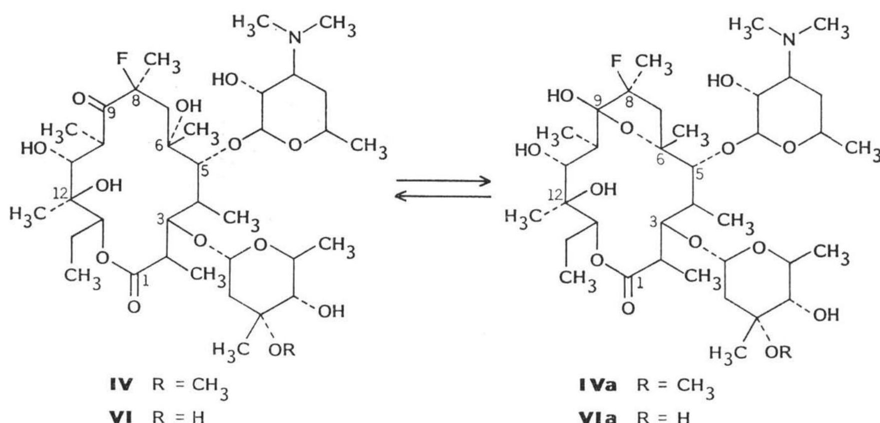
Scheme 2.



thermally unstable antibiotics.

The microanalyses (C, H, F and N) of **IV** and **VI** were in agreement with the molecular formulas $C_{37}H_{66}FNO_{13}$ and $C_{36}H_{64}FNO_{13}$, respectively. FAB-MS of **IV** and **VI** showed good pseudomolecular ion sensitivity and fragmentations that could be interpreted on the basis of chemical ionization or electronic impact mass spectral data reported[19~21] before for natural erythromycins. FAB-MS of **IV** gave the protonated molecular ion at *m/z* 752 that was also the base peak. The fragmentation pattern showed the presence of peaks at *m/z* 734 ($MH^+ - H_2O$), and *m/z* 594 and 576 accounting for the removal of cladinose from the protonated molecular ion, with or without the glycosidic oxygen atom, respectively. The glycosidic fragmentations were substantiated by metastable ion peaks at the appropriate mass values (469.2 and 441.2). The fragments at *m/z* 174 and 158 indicated the presence of desosamine, while those at *m/z* 159, 127 and 109 were characteristic of cladinose. FAB-MS of **VI** gave the protonated molecular ion at *m/z* 738, while the peak at *m/z* 720 was due to ion $MH^+ - H_2O$. The fragmentation pattern was analogous to that showed by (8*S*)-8-fluoroerythromycin A. Peaks at *m/z* 145, 127 and 109 showed that the sugar at C-3 was mycarose. When **IV** and **VI** were treated with dilute hydrochloric acid (Scheme 2), according to the method described in previous papers[22,23], they afforded a common monoglycoside, whose elemental analysis, FAB-mass and ^{1}H NMR spectral data were consistent with structure **VIII** of 5-*O*-desosaminyl-(8*S*)-8-fluoroerythronolide A. Acid-catalyzed methanolysis of **IV** led to the mixture of the α- and β-anomers (**X** and **XI**) of methyl cladinoside[24] as with erythromycin A. In an analogous way, both **VI** and 3-*O*-mycarosylerythronolide B afforded the α- and β-anomers (**XII** and **XIII**) of methyl mycaroside[25].

Scheme 3.



Although (8*S*)-8-fluoroerythromycin A (**IV**) showed a single spot in several TLC systems, its ^{1}H NMR and ^{13}C NMR spectra in pyridine-d_5 and deuteriochloroform, respectively, revealed a duality of resonances indicative of a mixture of two components. In both solvents, and particularly in pyridine-d_5 where the effect was most pronounced, a temperature dependent reversible change in the proportions of the two components was observed. In aqueous acid solution, HPLC analysis (mobile phase 2) evidenced an analogous phenomenon with formation of two peaks at 4.01 (UV maximum at 288 nm) and 3.44 minutes (no UV maximum). These and previously reported results[26~29] suggested that the two components of (8*S*)-8-fluoroerythromycin A were the fluoroketone **IV** and the hemiketal **IVa** (Scheme 3). The structure of the hemiketal **IVa** was determined by ^{13}C NMR analysis. In deuteriochloroform solution, where the hemiketal form was predominant, the formation of a 6,9-oxygen bridge resulted in C-6 and C-9 chemical shifts at 83.0 and 106.3 ppm (J_{FC-9}=20.6 Hz, characteristic[30] carbon-fluorine coupling constant for the β-carbons), reminiscent of the corresponding chemical shifts observed for the 6,9-methylketal of (8*S*)-8-hydroxyerythromycin A[31] and the 6,9-hemiketal of 5-deoxy-5-oxoerythronolide B[32]. Analogous fluoroketone (**VI**) - hemiketal (**VIa**) tautomerism was exhibited by (8*S*)-8-fluoroerythromycin C (Scheme 3).

The mutasynthetic approach using modified lactones derived from the aglycone of erythromycin was rarely successful, mainly because of the specificity of the desosamine-binding enzyme[33~35]. To our knowledge, only the 11-*O*-methyl ether of erythronolide B was previously reported[36] to yield a bisglycoside, the 11-*O*-methylerythromycin B. The successful conversion of (8*S*)-8-fluoroerythronolide A (**I**) indicates that the fluorine atom at C-8 does not introduce significant steric and conformational changes in the structure of the aglycone, which still has a facile permeation through the cell membrane and is recognized by the glycosidating enzymes. Before this experiment, we already described[15] the obtainment of a bisglycoside by feeding erythronolide A to *S. erythraeus* ATCC 31772. The latter substrate was quantitatively converted to erythromycin A. In the present experiment, a mixture of (8*S*)-8-fluoroerythromycins A (**IV**) and C (**VI**) in approximatively equimolar amounts was produced when (8*S*)-8-fluoroerythronolide A (**I**) was added to a culture of that same strain. This suggests that *O*-methylation is a rate-limiting step due to the high substrate specificity of the *S*-adenosyl-L-methionine transmethylase and this activity is negatively affected by the fluorine atom in the substrate.

Bioconversion of (8*S*)-8-Fluoroerythronolide B (II) and 3-*O*-Mycarosyl-(8*S*)-8-fluoroerythronolide B (III)

Two active bioconvertants **V** and **VII** (Scheme 1) were produced when either (8*S*)-8-fluoroerythronolide B (**II**) or 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (**III**) was added to the culture of *S. erythraeus* ATCC 31772. On TLC and HPLC analysis of the broth culture fed **II**, it was possible to detect a compound with same chromatographic behavior as **III**, which disappeared with increasing time of incubation. In analogy to the corresponding biosynthesis of erythromycin[37], it is reasonable to assume that **III** is a biosynthetic intermediate in the transformation of **II** to **V** (Scheme 1). The structures of **V** and **VII** were deduced from their elemental analyses, FAB-mass spectral data and behavior to the acids.

The elemental analysis (C, H, F and N) of **V** accorded with the formula $C_{37}H_{66}FNO_{12}$.

Its FAB-MS gave the protonated molecular ion at *m/z* 736. A prominent ion at *m/z* 718 attributed to MH^+-H_2O was present. Metastable ion peaks at the appropriate mass values (453.9 and 426.0) were also present, indicating the direct loss of cladinose from the protonated molecular ion, either with (*m/z* 578) or without (*m/z* 560, base peak) its glycosidic oxygen atom. The occurrence of peaks corresponding to those observed for (8*S*)-8-fluoroerythromycin A, proved that the sugars at C-3 and C-5 were cladinose and desosamine, respectively.

The elemental analysis (C, H, F and N) of **VII** was consistent with the formula $C_{36}H_{64}FNO_{12}$.

Its FAB-MS showed the protonated molecular ion at *m/z* 722 and the ion MH^+-H_2O at *m/z* 704. The mass fragmentation pattern was similar to that showed by (8*S*)-8-fluoroerythromycin B. Peaks at *m/z* 145, 127 and 109 showed that the sugar at C-3 was mycarose.

Treatment of **V** and **VII** in dilute hydrochloric acid (Scheme 2) yielded the same monoglycoside, whose structure was inferred to be 5-*O*-desosaminyl-(8*S*)-8-fluoroerythronolide B (**IX**) on the basis of elemental analysis, FAB-mass and ^1H NMR spectral data. Complementarily, the same mixture of α- and β-anomers (**X** and **XI**) of methyl cladinoside was obtained by acid-catalyzed methanolysis of **V** and erythromycin A, whereas the α- and β-anomers (**XII** and **XIII**) of methyl mycaroside were derived from both **VII** and 3-*O*-mycarosylerythronolide B. In contrast to (8*S*)-8-fluoroerythromycins A (**IV**) and C (**VI**), which exist as an interconvertible mixture of tautomers, (8*S*)-8-fluoroerythromycins B (**V**) and D (**VII**) exist exclusively as the ketonic forms.

In agreement with the results of the biotransformation of (8*S*)-8-fluoroerythronolide A (**I**), we demonstrate that the glycosidases accept both (8*S*)-8-fluoroerythronolide B (**II**) and 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (**III**) as possible substrates. It is worth noting that, also in this case, the *O*-methylation step is rate-limiting, leading to accumulation of (8*S*)-8-fluoroerythromycin D (**VII**) which is not completely converted to (8*S*)-8-fluoroerythromycin B (**V**). Moreover, another enzyme, the C-12 hydroxylase[15], is clearly shown to possess a very strict substrate specificity, since (8*S*)-8-fluoroerythromycin C (**VI**) and (8*S*)-8-fluoroerythromycin A (**IV**) are not produced. It is obvious from the results that this enzyme either does not recognize (8*S*)-8-fluoroerythromycin D (**VII**) or is not induced by it. This specificity, which seems to be the strictest among those of the enzymes of the last steps in the biosynthesis of erythromycins, also came out from the before mentioned conversion of 11-*O*-methyl ether of erythronolide B yielding only the 11-*O*-methylerythromycin B, and not the A-form.

Acid Stability

The importance of the fluorine atom at C-8 is clearly illustrated by the data of Table 1 where the acid stability at pH 2, 3 and 4 of the new (8*S*)-8-fluoroerythromycins is compared with that of erythromycin

Table 1. Acid stability at 25°C.

Compounds	$t_{\frac{1}{2}}$*		
	pH 2	pH 3	pH 4
IV**	9	82.5	>100
V	3	45	>100
VI**	1.65	41.5	>100
VII	0.4	10	>100
Erythromycin A	0.05	0.1	2

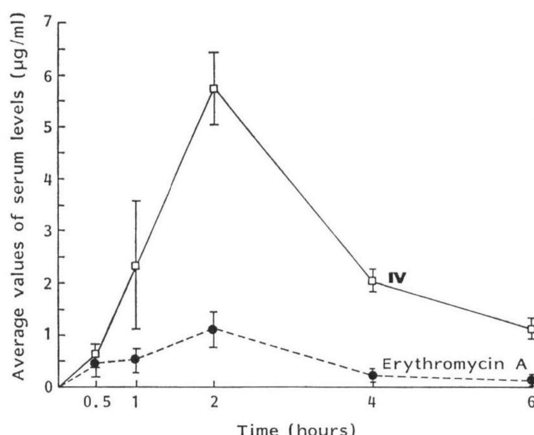
* The half-life in hours.

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

A[18]. As reported before, (8*S*)-8-fluoroerythromycins A (**IV**) and C (**VI**) display a fluoroketone-hemiketal tautomerism in these environments (Scheme 3).

Fig. 1. Average values of serum levels of erythromycin A and **IV** in rats after a single oral dose of 100 mg/kg.

The standard error of the mean is represented by the vertical bars.



Biological Activity of the Conversion Products

Tables 2~4 summarize the antimicrobial activity *in vitro* of the new fluoroerythromycins in com-

Table 2. Antibacterial activity of compounds **IV**, **V**, **VI**, **VII** compared to that of erythromycin A and erythromycin B (agar dilution method).

Organism	MIC (μg/ml)					
	IV	**V**	**VI**	**VII**	Erythromycin A	Erythromycin B
Staphylococcus aureus ATCC 6538P	0.097	0.097	0.097	0.097	0.049	0.097
Staphylococcus aureus ATCC 14154*	>25	>25	>25	>25	>25	>25
Staphylococcus aureus PRL 14**	0.097	0.097	0.097	0.097	0.049	0.195
Streptococcus pyogenes ATCC 8668	0.012	0.024	0.024	0.024	0.012	0.024
Streptococcus pneumoniae ATCC 6303	0.024	0.049	0.024	0.024	0.012	0.024
Streptococcus faecalis subsp. *zymogenes* ATCC 12958	0.195	0.195	0.097	0.097	0.097	0.195
Corynebacterium diphteriae PRL 24	0.012	0.006	0.012	0.006	0.006	0.012
Micrococcus luteus ATCC 9341	0.006	0.012	0.012	0.006	0.006	0.006
Micrococcus luteus ATCC 15957*	>25	>25	>25	>25	>25	>25
Bacillus subtilis ATCC 6633	0.049	0.049	0.097	0.049	0.049	0.049
Haemophilus influenzae ATCC 19418	3.12	6.25	12.5	6.25	3.12	6.25
Neisseria gonorrhoeae ATCC 19424	0.049	0.097	0.195	0.097	0.049	0.097
Escherichia coli PRL 50	12.5	25	12.5	25	6.25	25
Klebsiella pneumoniae PRL 54	12.5	>25	25	>25	12.5	25
Proteus vulgaris ATCC 6380	>25	>25	25	>25	>25	>25
Salmonella typhi PRL 8	12.5	25	12.5	12.5	12.5	25
Shigella sonnei PRL 5	25	25	25	>25	12.5	>25
Pseudomonas aeruginosa PRL 9	>25	>25	25	25	>25	>25
Clostridium perfringens ATCC 3624	0.78	0.39	6.25	3.12	1.56	1.56
Bacteroides fragilis ATCC 23745	0.195	0.195	0.78	0.39	0.195	0.195
Fusobacterium necrophorum ATCC 27852	1.56	3.12	12.5	6.25	3.12	6.25

* Erythromycin-resistant

** Penicillin-resistant

Table 3. Antibacterial activity of compounds **IV**, **V**, **VI**, **VII** compared to that of erythromycin A and erythromycin B (broth dilution method).

Organism	MIC (μg/ml)					
	IV	V	VI	VII	Erythromycin A	Erythromycin B
Staphylococcus aureus ATCC 6538P	0.195	0.097	0.195	0.195	0.097	0.195
Streptococcus pyogenes ATCC 8668	0.097	0.024	0.049	0.049	0.024	0.024
Streptococcus pneumoniae ATCC 6303	0.049	0.049	0.097	0.049	0.024	0.049
Streptococcus faecalis subsp. *zymogenes* ATCC 12958	0.39	0.39	0.39	0.39	0.39	0.39
Corynebacterium diphteriae PRL24	0.012	0.012	0.049	0.012	0.012	0.012

Table 4. Bactericidal activity of compounds **IV**, **V**, **VI**, **VII** compared to that of erythromycin A and erythromycin B.

Organism	MBC (μg/ml)					
	IV	V	VI	VII	Erythromycin A	Erythromycin B
Staphylococcus aureus ATCC 6538 P	3.12	3.12	3.12	3.12	1.56	6.25
Streptococcus pyogenes ATCC 8668	0.78	0.195	0.78	0.39	0.39	0.195
Streptococcus pneumoniae ATCC 6303	0.195	0.049	0.195	0.195	0.097	0.195
Streptococcus faecalis subsp. *zymogenes* ATCC 12958	6.25	3.12	3.12	3.12	6.25	6.25
Corynebacterium diphteriae PRL 24	0.097	0.049	0.39	0.195	0.049	0.195

parison with erythromycins A and B. The convertants (8*S*)-8-fluoroerythromycins A (**IV**) and B (**V**) showed the same spectrum and were active to the same extent as compared with the natural occurring erythromycins. When their potency was estimated[38] against *Micrococcus luteus* ATCC 9341, (8*S*)-8-fluoroerythromycin B (**V**) had about 110% of the activity of erythromycin B, whereas (8*S*)-8-fluoroerythromycin A (**IV**) had about 70% of the activity of erythromycin A. The lower potency in this latter case may be attributable to the fluoroketone-hemiketal tautomerism, should a minor activity of **IVa** be hypothesized. Comparison between **V** and erythromycin B shows that replacement of hydrogen by fluorine at C-8 does not affect the antibacterial activity, contrary to what happens with a corresponding hydroxyl substituent[28,39]. To our knowledge, this is the first time that macrolide antibiotics derived by mutasynthesis are as active as the analogous natural antibiotics.

The high acid stability of (8*S*)-8-fluoroerythromycins prompted us to further biological investigations. In Fig. 1 some preliminary data of the serum levels of (8*S*)-8-fluoroerythromycin A (**IV**) in rats are reported in comparison with those of erythromycin A. In view of these results, (8*S*)-8-fluoroerythromycin A (**IV**) could be an attractive alternative to erythromycin A 2′-esters which, despite their good oral absorption, possess undesirable side effects[40] and must be hydrolyzed *in vivo* to be therapeutically effective[41]. More complete studies *in vivo* will be presented in a forthcoming paper.

Experimental

Materials

Erythromycin A was purified from a commercial product by crystallization from chloroform. Erythromycin B was prepared as reported previously[39] from a mother liquor concentrate. The latter was

obtained from an industrial strain of *S. erythraeus* after crystallization and removal of the majority of the erythromycin A. 3-*O*-Mycarosylerythronolide B[37] was isolated from the fermentation beers of LMC 1198, a blocked mutant of *S. erythraeus*.

Analysis

Elemental analyses were performed by Alfred Bernhardt Microanalytical Laboratories, Elbach über Engelskirchen, West Germany. All melting points were taken in open capillary tubes using a Tottoli apparatus (N. Büchi, Flawil, Switzerland) and are uncorrected. Optical rotations were determined at 20°C in 1% methanol solutions with a Schmidt-Haentsch polarimeter.

UV spectra were measured in methanol using a Varian Cary 210 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 577 spectrophotometer for KBr disks (0.001 g of substance in 0.2 g of KBr). ^{1}H NMR spectra were obtained on a Varian T-60A spectrometer at room temperature in pyridine-d_5 (*c* 0.05 g/ml). Chemical shifts are reported in ppm from tetramethylsilane (TMS) as internal reference. ^{13}C NMR spectra were recorded with a Varian XL-200-FT spectrometer at 50.3 MHz in proton decoupled conditions at room temperature. Samples were dissolved in deuteriochloroform (*c* 0.2 g/ml) containing TMS as internal reference. FAB-MS were carried out on VG Analytical 7070E mass spectrometer equipped with a FAB source and operating with a 6kV accelerating potential. Approximatively 0.5~1 μg of sample dissolved in glycerol was used.

Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates (Merck) using acetone - chloroform - methanol - 10% ammonium hydroxide (50: 50: 1.4: 2) as developing solvent system (three runs). Compounds were visualized by spraying the plates with anisaldehyde - acetic acid - methanol - sulfuric acid (1: 5: 90: 2). Colors developed after a few minutes at 80°C. Antibiotics were also detected by bioautography on *Micrococcus luteus* ATCC 9341 seeded agar. This same strain was used as test organism to determine the antibiotic levels in the cultured broths and the biological potency of the isolated compounds by a standard agar diffusion method.

High performance liquid chromatography (HPLC) analyses were carried out according to a modification of a described procedure[42]. A Hewlett-Packard 1084 B liquid chromatograph equipped with a variable-wavelength detector at 210 nm and a Lichrosorb RP8 10 μm stainless steel column, 250×4.6 mm i.d., was used. Flow rate of the mobile phase was 2.0 ml/minute and the column was operated at 40°C. Two mobile phases were employed: mobile phase 1 consisted of acetonitrile - 0.01 M phosphate buffer pH 7.0 (40: 60); mobile phase 2 consisted of acetonitrile - 0.01 M phosphate buffer pH 7.0 (64: 36). Acid stability trials of new antibiotics were carried out according to the method described in a previous paper[43]. Gas liquid chromatographies (GLC) were carried out on a Perkin-Elmer Model 900 B equipped with a glass column, 2,000×2 mm i.d., packed with 4.3% Silicone OV 25 on 80~100 mesh HP Chromosorb W and heated from 110°C to 160°C (6°C/minute). Nitrogen gas was used as a carrier at 30 ml/minute and 2.8 kg/cm^2 inlet pressure.

Column Chromatography

Partition column chromatography was carried out in conformity with a reported method[44] using a column (740×24 mm i.d.) packed with silica gel 60, 70~230 mesh (Merck). Fifteen-milliliter fractions were collected at a flow rate of 1.0 ml per minute and were tested by TLC and HPLC (mobile phase 2). Fractions containing one product only were combined and concentrated to dryness under reduced pressure. Residual buffer salts were removed from products by washing their chloroform solutions with water. Sephadex LH-20 (Pharmacia Fine Chemicals), particle size 25~100 μm, was refluxed three times for 30 minutes in a mixture of chloroform and methanol (1: 1), filtered and dried at 40°C before beeing used for column chromatography[45]. Preparation and elution of the column was performed with a mixture of chloroform - hexane (1: 1). Homogeneous fractions were combined and evaporated under reduced pressure.

Microorganism and Fermentation

The strain employed in the present investigation was *Streptomyces erythraeus* ATCC 31772 which was cultured as described in a previous paper[15]. After 24 hours of cultivation, 500 μg/ml of either substrate, (8*S*)-8-fluoroerythronolide A (I)[3] or B (II)[3] or 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (III)[4], was added into the culture and the cultivation was continued for a further 96 hours. Antibiotic

production generally reached a maximum after 120 hours (HPLC, mobile phase 2), corresponding to the total conversion of the added substrate (HPLC, mobile phase 1). TLC and bioassay were also used to monitor the process.

Isolation of (8*S*)-8-Fluoroerythromycins A (IV) and C (VI)

The broth (2.1 liters) to which 1.0 g of (8*S*)-8-fluoroerythronolide A (I) had been added, was filtered over Celite to remove mycelia and the filtrate was clarified by the addition of equal volumes of a 10% aqueous solution of zinc sulfate and a 4% solution of sodium hydroxide. After centrifugation, the clear supernatant was extracted with ethyl acetate at pH 9.8. The organic extract was washed with water and dried on anhydrous sodium sulfate. Evaporation of the ethyl acetate under reduced pressure left 1.36 g of yellow foam. Partition column chromatography of this material gave 0.35 g of (8*S*)-8-fluoroerythromycin A (**IV**, fractions 90~174) and 0.345 g of (8*S*)-8-fluoroerythromycin C (**VI**, fractions 280~400). Crystallization from ethanol yielded 0.230 g of pure **IV**, as prisms: mp 183~184°C; $[\alpha]_D$ −54.9°; UV 283 nm (ε 17.9); ^{1}H NMR δ 2.15 (NMe_2 of **IV**), 2.20 (NMe_2 of **IVa**), 3.36 (OMe of **IVa**), 3.45 (OMe of **IV**); IR 3520, 3480 (sh.), 3250 (broad), 1735, 1720, 1460, 1380, 1345, 1330, 1305, 1170 cm^{-1}; FAB-MS *m/z* 752 (MH^+).

Anal. Calcd. for $C_{37}H_{66}FNO_{13}$: C 59.10, H 8.85, F 2.52, N 1.86.
Found: C 59.09, H 8.89, F 2.59, N 1.88.

Pure **VI** (0.145 g) was obtained as prisms by crystallization from ethanol: mp 217~218°C; $[\alpha]_D$ −42.4°; UV 284 nm (ε 23.2); ^{1}H NMR δ 2.10 (NMe_2 of **VI**), 2.18 (NMe_2 of **VIa**); IR 3550, 3500, 3440 (sh), 3300 (broad), 1730, 1455, 1410, 1380, 1360, 1340, 1330, 1305, 1170 (broad) cm^{-1}; FAB-MS *m/z* 738 (MH^+).

Anal. Calcd. for $C_{36}H_{64}FNO_{13}$: C 58.60, H 8.74, F 2.57, N 1.90.
Found: C 58.47, H 8.87, F 2.60, N 1.82.

Isolation of (8*S*)-8-Fluoroerythromycins B (V) and D (VII)

The broth (2.1 liters) to which 1.0 g of (8*S*)-8-fluoroerythronolide B (II) had been added, was clarified, extracted and purified by partition column chromatography as reported before. Fractions 18~32 (0.35 g) were crystallized from ethanol to give 0.15 g of pure (8*S*)-8-fluoroerythromycin B (**V**) as prisms: mp 164~166°C; $[\alpha]_D$ −63.1°; UV 285 nm (ε 29.5); ^{1}H NMR δ 2.15 (NMe_2) 3.45 (OMe); IR 3480 (broad), 1735, 1465, 1385, 1330, 1305, 1170 cm^{-1}; FAB-MS *m/z* 736 (MH^+).

Anal. Calcd. for $C_{37}H_{66}FNO_{12}$: C 60.39, H 9.04, F 2.58, N 1.90.
Found: C 60.31, H 9.09, F 2.60, N 1.88.

(8*S*)-8-Fluoroerythromycin D (**VII**) was eluted in subsequent fractions (55~105) and isolated as a glass (0.32 g). Crystallization from ethanol afforded 0.15 g of an analytical sample: mp 213~215°C; $[\alpha]_D$ −59.9°; UV 285 nm (ε 30.8); ^{1}H NMR δ 2.13 (NMe_2); IR 3600, 3520, 3300 (broad), 1730, 1460, 1420, 1385, 1370, 1310, 1160 cm^{-1}: FAB-MS *m/z* 722 (MH^+).

Anal. Calcd. for $C_{37}H_{66}FNO_{12}$: C 59.89, H 8.94, F 2.63, N 1.94.
Found: C 59.87, H 8.85, F 2.63, N 1.88.

The culture (1.0 liter) fed 0.50 g of 3-*O*-mycarosyl-(8*S*)-8-fluoroerythronolide B (III), was processed in the same way as reported above. Partition column chromatography of the residue from the solvent extraction, gave 0.115 g of (8*S*)-8-fluoroerythromycin B (**V**) and 0.095 g of (8*S*)-8-fluoroerythromycin D (**VII**), identical with those prepared as described before.

Acid Cleavage

1) Isolation of 5-*O*-Desosaminyl-(8*S*)-8-fluoroerythronolide A (**VIII**): A solution of 0.752 g (0.001 mol) of (8*S*)-8-fluoroerythromycin A (**IV**) or 0.738 g (0.001 mol) of (8*S*)-8-fluoroerythromycin C (**VI**) in 75 ml of a pH 2 hydrochloric acid buffer, was allowed to stand at room temperature for 24 hours. The complete disappearance of **IV** and **VI** was monitored by TLC and HPLC (mobile phase 2). The reaction mixture was poured into a saturated sodium hydrogen carbonate solution, and the product was extracted with ethyl acetate. The combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting solid residue was purified on Sephadex LH-20 to yield, after crystallization from ethyl ether - hexane, 0.145 g of 5-*O*-desosaminyl-(8*S*)-8-fluoroerythronolide A (**VIII**): mp 120~124°C; $[\alpha]_D$ −18.5°; UV 287 nm (ε 22.5); ^{1}H NMR δ 2.12 (NMe_2); IR 3460, 1725, 1460, 1380, 1350, 1325, 1170 cm^{-1}; FAB-MS *m/z* 594 (MH^+).

Anal. Calcd. for $C_{29}H_{52}FNO_{10}$: C 58.67, H 8.83, F 3.20, N 2.36.
Found: C 58.65, H 8.91, F 3.03, N 2.28.

2) Isolation of 5-*O*-Desosaminyl-(8*S*)-8-fluoroerythronolide B (IX): A solution prepared from 0.736 g (0.001 mol) of (8*S*)-8-fluoroerythromycin B (V) or 0.722 g (0.001 mol) of (8*S*)-8-fluoroerythromycin D (VII) and 75 ml of a pH 2 hydrochloric acid buffer, was kept at room temperature 19 hours, until the complete disappearance of the starting product was checked by TLC and HPLC (mobile phase 2). The reaction solution was adjusted to pH 9.0 with 5% sodium hydroxide and immediately extracted with ethyl acetate. The organic layers were combined, washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The resulting colorless foam was purified on Sephadex LH-20 to yield 0.215 g of pure 5-*O*-desosaminyl-(8*S*)-8-fluoroerythronolide B (IX): mp 116~120°C; $[\alpha]_D$ −43.1°; UV 284 nm (ε 35.6); ^{1}H NMR δ 2.10 (NMe_2); IR 3450 (broad), 1725, 1460, 1380, 1330, 1170 cm^{-1}; FAB-MS *m/z* 578 (MH^+).

Anal. Calcd. for $C_{29}H_{52}FNO_9$: C 60.29, H 9.07, F 3.29, N 2.42.
Found: C 60.23, H 9.09, F 3.26, N 2.31.

3) Methanolysis of (8*S*)-8-Fluoroerythromycins A (IV) and B (V): A solution of 0.037 g (0.05 mmol) of (8*S*)-8-fluoroerythromycin A (IV) or B (V) in 2 ml of methanol containing 1% hydrogen chloride, was allowed to stand at room temperature for 22 hours. The reaction solution was poured into a saturated sodium hydrogen carbonate solution, concentrated under reduced pressure to remove methanol and then extracted with ethyl acetate. After drying on anhydrous sodium sulfate, the extract was concentrated under reduced pressure. GLC analysis of the residue showed two components with retention times of 3.8 (18%) and 4.5 (82%) minutes, identical to those obtained from the corresponding cleavage of erythromycin A.

4) Methanolysis of (8*S*)-8-Fluoroerythromycins C (VI) and D (VII): GLC analysis of the mixture obtained by methanolysis of (8*S*)-8-fluoroerythromycins C (VI) and D (VII) showed two components with retention times of 3.2 (35%) and 6.7 (65%) minutes, identical to those obtained from the corresponding cleavage of 3-*O*-mycarosylerythronolide B.

Antibacterial Activity *In Vitro*

Minimal inhibitory concentrations (MIC) were determined by the standard two-fold dilution method using Müller Hinton (MH) medium (Difco). For the genera *Streptococcus* and *Corynebacterium* the MH medium was added with 5% horse serum. The genera *Haemophilus*, *Neisseria* and the anaerobes were tested on GC Completed Medium (Difco). Incubation was performed in gas pack anaerobic jar (BBL) for anaerobes, in candle jar for the others. Bacterial strains used for susceptibility determinations were cultures regularly employed in our screening tests. The strains coded PRL (Pierrel Research Laboratories) have been collected and identified by standard criteria in these laboratories. Bacterial cultures containing approximatively $10^6 \sim 10^7$ viable cells/ml were prepared from overnight cultures. One loopful of the cultures was inoculated on the agar plates containing antibiotics. The agar plates were incubated at 37°C for 24 hours, and MIC was defined as the lowest concentration that prevented visible growth. The usual diffusion cylinder method[38] was used to estimate the potency of the new antibiotics against *Micrococcus luteus* ATCC 9341. For a selected number of bacterial strains the MIC were determined in MH broth. For these strains the minimal bactericidal concentrations (MBC) were also determined by further subculturing in the medium without antibiotic the cultures that after 24 hours did not show visible growth.

Absorption Studies in Rats

Female Sprague-Dawley rats weighing 160~180 g were given 100 mg/kg of antibiotic orally. Six rats were sacrificed at each interval, blood samples were withdrawn and sera separated by centrifugation. The sera were stored at −20°C until assay. Serum levels were determined by the usual diffusion cylinder plate method[38] using *Micrococcus luteus* ATCC 9341.

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