A NEW NATURALLY OCCURRING ERYTHROMYCIN: ERYTHROMYCIN F

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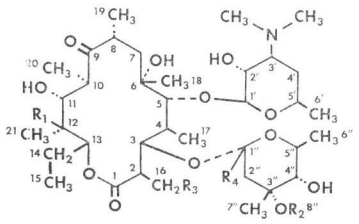
A new erythromycin, designated erythromycin F, was isolated from the mother liquors of an early commercial strain of *Streptomyces erythreus*. Although not established experimentally, erythromycin F may be a biosynthetic precursor of erythromycin E.

Erythromycins A~E (**1**~**5**) are composed of a 14-membered lactone ring, either erythronolide A or B, a common basic sugar, D-desosamine, and either of two branch-chain sugars, L-mycarose or L-cladinose. The sugars are normally attached *via* glycosidic linkage to the lactone ring; however, in erythromycin E (**5**), L-cladinose is attached by an *ortho* ester coupling.

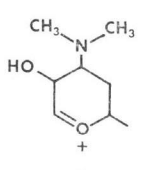
Although numerous erythromycin related glycosides have been isolated from fermentation broths of blocked mutants and producing strains of the erythromycin producing organism *Streptomyces erythreus*,[1~4] only the letter-designated erythromycins A~E (**1**~**5**) have been shown to be primary biosynthetic compounds. The other erythromycin structures are presumably derived from the letter-designated erythromycins by acid or base degradation or by non-specific enzymes of the producing organism.

We earlier reported the isolation and biosynthetic role of erythromycin D (**4**)[4] from a concentrate of the mother liquors remaining after the commercial crystallization of erythromycin A (**1**). We now report the isolation of an additional erythromycin, designated erythromycin F (**6**), from a similar mother liquor concentrate and suggest that erythromycin F (**6**) is a biosynthetic precursor of erythromycin E (**5**).

Erythromycin F (**6**) was isolated by HPLC from a mother liquor concentrate of an early commercial strain of the erythromycin producing organism *Streptomyces erythreus*.



Erythromycin		R_1	R_2	R_3	R_4
1	A	OH	CH_3	H	H
2	B	H	CH_3	H	H
3	C	OH	H	H	H
4	D	H	H	H	H
5	E	OH	CH_3	—O—	
6	F	OH	CH_3	OH	H



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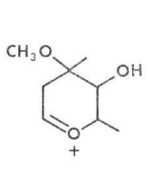

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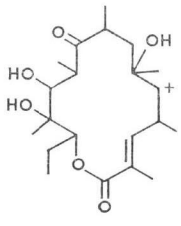

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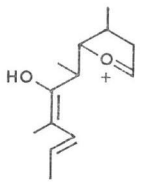

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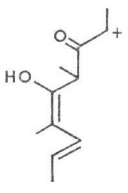

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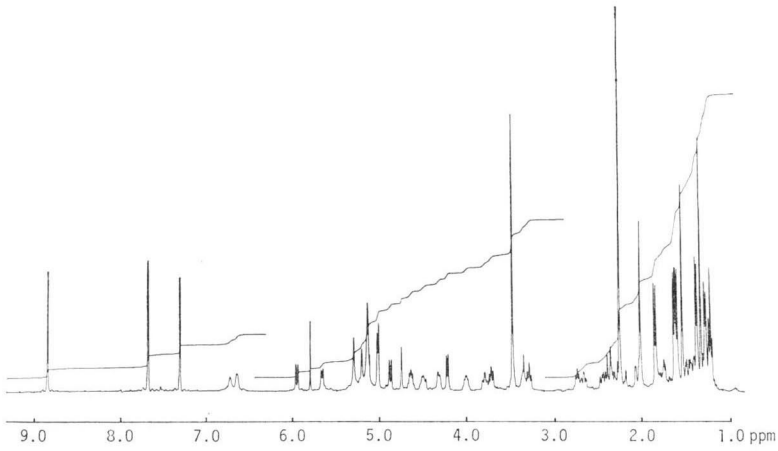
Fig. 1. The 400 MHz PMR spectrum of erythromycin F (**6**) determined in C_5D_5N.



The high resolution mass spectrum of erythromycin F (**6**) gave a weak molecular ion at *m/z* 749.4570, indicating a molecular formula of $C_{37}H_{67}NO_{14}$. This formula represents a compound having one more oxygen atom than is present in erythromycin A (**1**). Prominent ions attributed to desosamine and cladinose [*m/z* 158 (**7**) and 159 (**8**), respectively] were present, indicating that the additional oxygen atom was substituted on the erythronolide moiety. This was confirmed by an ion ascribed to the aglycone moiety in erythromycin A (**1**) [*m/z* 383 (**9**)] which appeared at *m/z* 399 in the spectrum of erythromycin F (**6**). As no smaller ion was evident that contained an additional 16 mass units, the mass spectrum did not allow assignment of the position of the additional oxygen. The spectrum did provide, however, some evidence that oxygen substitution was not at C-7 ~ C-13 of the erythronolide moiety by the presence of prominent ions at *m/z* 239 (**10**) and 181 (**11**) which were also observed in the spectrum of erythromycin A (**1**).

The PMR spectrum of erythromycin F (**6**) was recorded at 400 MHz (Fig. 1) as well as at 90 MHz (Table 1). The PMR spectra of erythromycin A (**1**)[5] and erythromycin E (**5**)[2] have been previously analyzed, allowing direct comparison with that of erythromycin F (**6**). In the spectrum of **6** the chemical shift and multiplicity of H-13 (5.68 ppm, $J_{13,14a}$=10 Hz and $J_{13,14a}$=2 Hz) and H-11 (4.49 ppm, $J_{10,11}$= ~1 Hz) indicate that erythromycin F (**6**) has a C-12 hydroxyl substituent. In erythromycin A (**1**) the resonance of H-2 appears at 3.48 ppm as a doublet of quartets of which only the four most intense lines are normally visible. The resonance of the corresponding proton of erythromycin F (**6**) also appears at 3.48 ppm but displays a multiplet, suggesting that H-2 is no longer coupled to a methyl group. Spin decoupling experiments established that, in the spectrum of **6**, H-2 is coupled to H-3 and to resonances at 4.22 ppm and 4.15 ppm. The latter protons, designated H-16a and H-16b ($J_{2,16a}$ unresolved, $J_{2,16b}$= 4 Hz), are, in turn, coupled to each other ($J_{16a,16b}$=10 Hz). The chemical shift of the latter protons suggests that they are attached to a carbon atom substituted by an electro-negative atom indicating that the C-2 methyl group had been oxidized. The oxidation of the C-2 methyl group was confirmed by the absence of one high field methyl doublet in the 1.0 ~ 1.5 ppm region of the spectrum. The remaining proton resonances of erythromycin F (**6**) were essentially unchanged from their corresponding chemical shifts and multiplicities seen in the spectrum of erythromycin A (**1**).

An analysis of the CMR spectrum of erythromycin F (**6**) gave further evidence for the structure.

Table 1. PMR parameters of erythromycin F (6)*.

Chemical shifts (ppm)				Coupling constants (Hz)	
H-2	3.48	H-1′	4.81	$J_{2,3}$	10.0
H-3	4.68	H-2′	3.54	$J_{4,5}$	8.0
H-4	2.32	H-3′	2.2	$J_{10,11}$	1.0
H-5	4.05	H-5′	3.82	$J_{13,14a}$	10.0
H-8	3.0	CH_3-6′	1.27	$J_{13,14e}$	2.0
H-10	3.35	$N(CH_3)_2$	2.23	$J_{2,16b}$	4.0
H-11	4.49			$J_{16a,16b}$	10.0
H-13	5.68				
H-16a	4.22				
H-16b	4.15				
CH_3-17	1.62	H-1″	5.05	$J_{1',2'}$	8.0
CH_3-18	1.81	H-4″	3.16	$J_{1'',2''}$	3.5
CH_3-19	1.20	H-5″	4.45		
CH_3-20	1.42	CH_3-6″	1.49		
CH_3-21	1.23	CH_3-7″	1.39		
CH_3-15	1.07	CH_3-8″	3.36		

* Chemical shifts were obtained from 400 MHz PMR spectra measured in C_5D_5N at 95°C with a Brucker WH 400 spectrometer. Spin-decoupling experiments were performed at 90 MHz in C_5D_5N at 95°C with a JEOL FX 90 Q spectrometer.

CMR chemical shifts of erythromycin F (**6**) and reference antibiotics erythromycins A (**1**) and E (**5**) are given in Table 2. In the spectrum of erythromycin F (**6**), the carbon resonance at 16.2 ppm, attributed to C-16 in erythromycin A (**1**) was absent. A signal at 62.4 ppm, which gave a triplet in an off-resonance spin decoupling experiment, was assigned to the C-16 methylene group of erythromycin F (**6**). In the spectrum of erythromycin E (**5**) a resonance with a similar chemical shift has been previously assigned to the C-16 CH_2O group.[2] When compared with the spectrum of erythromycin A (**1**), the presence of the CH_2O group in erythromycin F (**6**) gave a downfield α-shift of 46.2 ppm. This shift magnitude is consistent with published substituent effects of replacement of a proton by a primary hydroxyl group.[6] Also in agreement with the assigned structure was the 9.8 ppm downfield β-substituent effect at C-2. Upfield γ-shifts of 1.1

Table 2. CMR chemical shifts of erythromycin F (**6**), erythromycin A (**1**) and erythromycin E (**5**)*.

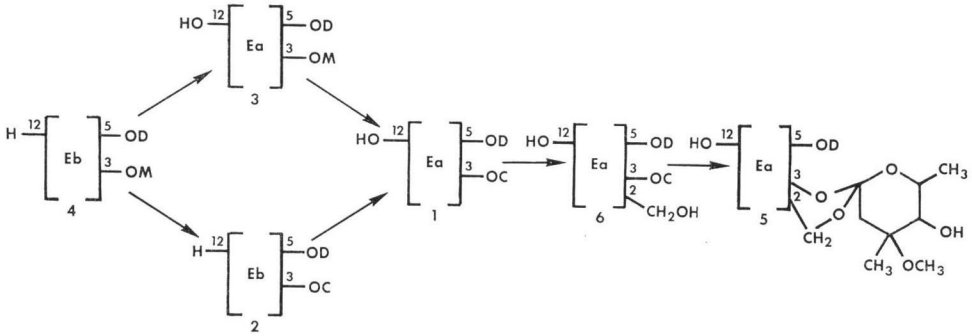
Carbon number	Chemical shifts		
	6	**1**	**5**
C_1	175.0	176.1	166.9
C_2	55.5	45.6	43.5
C_3	79.4	80.5	74.9
C_4	40.1	41.1	35.6
C_5	84.2	84.2	84.0
C_6	75.6	75.6	74.9
C_7	39.8	39.7	39.0
C_8	43.6	43.2	45.4
C_9	219.8	219.7	222.7
C_{10}	40.1	41.1	37.5
C_{11}	71.9	71.9	68.9
C_{12}	74.4	74.4	74.5
C_{13}	77.9	77.3	77.4
C_{14}	22.2	22.2	21.1
C_{15}	9.9	9.7	10.6
C_{16}	62.4	16.2	62.0
C_{17}	9.9	9.9	8.7
C_{18}	27.5	27.4	27.0
C_{19}	18.5	18.6	16.3
C_{20}	12.4	12.3	11.9
C_{21}	17.6	17.6	18.2
$C_{1'}$	103.7	103.8	101.5
$C_{2'}$	69.8	69.8	71.0
$C_{3'}$	66.3	66.1	65.5
$C_{4'}$	30.6	30.6	28.9
$C_{5'}$	68.4	68.4	68.9
$C_{6'}$	21.8	21.8	21.1
$N(CH_3)_2$	40.5	40.5	40.4
$C_{1''}$	96.7	97.0	109.3
$C_{2''}$	35.8	35.7	38.4
$C_{3''}$	73.4	73.4	74.3
$C_{4''}$	78.6	78.7	77.3
$C_{5''}$	65.7	65.6	68.5
$C_{6''}$	19.4	19.4	17.4
$C_{7''}$	21.5	21.5	22.3
$C_{8''}$	49.6	49.6	49.4

* CMR spectra of erythromycins A (**1**) and F (**6**) were measured at 22.5 MHz in C_5D_5N with a JEOL FX 90 Q spectrometer. Due to the instability of erythromycin E (**5**) in C_5D_5N the CMR spectrum of this antibiotic was recorded at 25.2 MHz in $CDCl_3$ with a Varian Associates/Nicolet Technology XL-100-15/TT-100 spectrometer system. All CMR spectra were recorded at ambient temperature.

Table 3. *In vitro* antibacterial activity of erythromycin F (**6**) compared to that of erythromycins A (**1**) and E (**5**).

Organism	Minimum inhibitory concentration (μg/ml)		
	1	**5**	**6**
Staphylococcus aureus 9144	0.1	1.56	0.78
Staphylococcus aureus Smith	0.2	1.56	1.56
Staphylococcus aureus Wise 155 (ER)	>100	>100	>100
Streptococcus faecalis 10541	0.02	0.39	0.78
Streptococcus pyogenes Roper (ER)	>100	>100	>100
Escherichia coli Juhl	50	>100	>100
Klebsiella pneumoniae 10031	3.1	6.2	6.2
Proteus vulgaris Abbott JJ	>100	>100	>100
Proteus mirabilis Fin #9	100	>100	>100
Pseudomonas aeruginosa BMH #10	50	>100	>100
Salmonella typhimurium ED #9	50	>100	>100
Shigella sonnei 9290	25	100	50

Scheme 1. Proposed pathway for the latter stages of erythromycin biosynthesis.
Brackets denote the erythromycin aglycone.
Abbreviations: Eb; erythronolide B, Ea; erythronolide A, M; mycarose, C; cladinose, D; desosamine, Numbers indicate lactone carbons.



ppm were observed for C-1 and C-3. Other chemical shifts in the CMR spectrum of erythromycin F (**6**) are nearly identical to those seen in the CMR spectrum of erythromycin A (**1**).

The antibacterial activity of erythromycin F (**6**) was determined against a variety of bacteria using a two fold dilution assay in brain heart infusion agar (Table 3). The activity of erythromycin F (**6**) was about equal to that of erythromycin E (**5**) or 2~8 fold less active than erythromycin A (**1**).

The position of erythromycin F (**6**) in the erythromycin biosynthetic scheme was not determined experimentally. Although no information is available concerning the biological formation of *ortho* ester groupings, inspection suggests that erythromycin F (**6**) may be an obligate precursor of erythromycin E (**5**). If this is correct, the latter stages of the erythromycin biosynthetic pathway may be depicted as shown in Scheme 1.

Experimental

General

The optical rotation was determined with a Perkin-Elmer Model 241 polarimeter. The IR spectrum

was recorded with a Perkin-Elmer Model 521 grating spectrometer. Mass spectra were obtained on an A. E. I. MS-902 spectrometer at 70 eV and 100~150°C using the direct probe insert. PMR and CMR spectra were recorded as noted in the tables of data.

Isolation of Erythromycim F (**6**)

Erythromycin F (**6**) was isolated from a concentrate of mother liquors using high pressure liquid chromatography. The mother liquor concentrate was obtained from an early high yielding commercial strain of *Streptomyces erythreus* after crystallization and removal of the majority of the erythromycin A. Samples (15 g) of the concentrate were chromatographed on a Waters Prep System 500 high pressure liquid chromatography instrument fitted with a single C_{18} prep column. With a solvent system consisting of 0.025 M dihydrogen potassium phosphate - acetonitrile - triethylamine (600: 400: 1, v/v) at a flow rate of 100 ml/minute, erythromycin F eluted cleanly approximately 12.5 minutes after sample application. Fractions containing erythromycin F from 10 purifications were pooled. After the majority of the acetonitrile was removed under diminished pressure the concentrate was adjusted to pH 9.4 with 1 N sodium hydroxide Extraction with methylene chloride followed by crystallization of the product from the same solvent gave 1.02 g of erythromycin F (**6**): mp 168~170°C; $[\alpha]_D^{24}$ −61.5° (*c* 1.0, CH_3OH); IR (KBr) 3445, 2965, 1700, 1435, 1372 and 1162 cm^{-1}; PMR (see Table 1); CMR (see Table 2); mass spectrum, *m/z* 749.4570 (M^+), calcd. for $C_{37}H_{67}NO_{14}$: 749.4562.

Anal. Calcd. for $C_{37}H_{67}NO_{14}$: C 59.26, H 9.00, N 1.87.
Found: C 59.00, H 9.03, N 1.90.

Acknowledgments

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References

1) MARTIN, J. R.; T. J. PERUN & R. L. GIROLAMI: Studies on the biosynthesis of the erythromycins. I. Isolation and structure of an intermediate glycoside, 3-α-L-mycarosylerythronolide B. Biochemistry 5: 2852~2856, 1966
2) MARTIN, J. R.; R. S. EGAN, A. W. GOLDSTEIN & P. COLLUM: Extension of the erythromycin biosynthetic pathway. Isolation and structure of erythromycin E. Tetrahedron 31: 1985~1989, 1975
3) COLLUM, P.; R. S. EGAN, A. W. GOLDSTEIN & J. R. MARTIN: 3-*O*-(2″,6″-Dideoxy-α-L-ribohexopyranosyl) erythronolide B and 3-*O*-(2″,6″-dideoxy-α-L-arabinohexapyranosyl)erythronolide B, aberrant erythromycin biogenetic metabolites with defective sugar moieties. Tetrahedron 32: 2375~2378, 1976
4) MAJER, J.; J. R. MARTIN, R. S. EGAN & J. W. CORCORAN: Antibiotic glycosides. 8. Erythromycin D, a new marcolide antibiotic. J. Amer. Chem. Soc. 99: 1620~1622, 1977
5) EGAN, R. S.: Conformation of erythromycin and related antibiotics. Ph. D. Thesis, University of Illinois at the Medical Center, Chicago, Ill., 1971
6) BREITMAIER, E. & W. VOELTER: "^{13}C-NMR Spectroscopy". H. F. EBEL *ed.*, p. 141, Verlag Chemie GmbH, Weinheisor, 1974