Production of Erythromycin E by Pathogenic *Nocardia brasiliensis*

Sir:

Since the discovery of erythromycin A (**1**; Fig. 1)[1] more than 25 biosynthetic representatives of this family of 14-membered macrolide antibiotics have been reported. Most of erythromycins are produced by the genera *Saccharopolyspora* and *Streptomyces* but few by *Nocardia* strains[2]. Erythromycin E (**2**; Fig. 1) is distinguishable from all the other known macrolide antibiotics and other microbial metabolites by the presence of an ortho-carboxylic acid ester structure. This is supplied by the oxidized form of cladinose which is bonded vicinally to the 14-membered aglycone[3]. Formation of **2** was observed when submerged cultures of an idiotrophic mutant of *Streptomyces erythreus* (ABOTT 2NU153) were incubated with 0.5 g erythromycin A/liter. Obviously this strain possesses an early block in erythromycin production and is capable of converting erythromycin A (**1**) to **2** *via* an extension of the biosynthetic pathway[3].

However, the occurrence of erythromycin E (Fig. 1) has never been reported for non-idiotrophic producer strains of macrolides. Here we report the discovery and isolation of erythromycin E (**2**) from the culture broth of *Nocardia brasiliensis* IFM 0466.

The strain of *N. brasiliensis* IFM 0466, an isolate from nocardiosis patient, is kept in the culture collection of the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University and was used in this study.

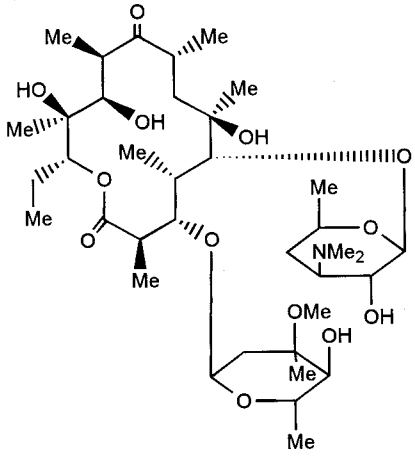
Isolation of erythromycins from a 96 hour-culture broth (20-liters) was done by chromatography with a Diaion HP-20 column (3 × 30 cm) and elution with MeOH : H_2O (4 : 1 and 100 : 0). The combined fractions active against *Bacillus subtilis* PCI 219 were further purified by preparative reverse-phase HPLC (Soken pack C18 Soken Co., Ltd., 2 × 30 cm, flow rate; 30 ml/minute, MeCN : 50 mM phosphate buffer pH 6.5 = 38 : 72). At the end of the purification process **2** was obtained in a yield of 65 mg as a pure compound in addition to coproduced erythromycin A (Fig. 1) and 8,9-anhydro-pseudo-erythromycin A-6,9-hemiketal[4]. The structure of **2** from *N. brasiliensis* IFM 0466 was readily inferred from mass spectrometry and one- and two-dimensional NMR measurements.

The electrospray mass spectrum of **2** displayed *m/z* 748.1 ($[M+H]^+$). Collision-induced dissociation (ESI-CID-MS/MS) of *m/z* 748 with argon gas afforded diagnostic fragment ions such as *m/z* 574.8 ion ([monoglycosylated aglycone, –O, –desosamine, $+H]^+$) and *m/z* 157.9 ([desosamine $-O]^-$).

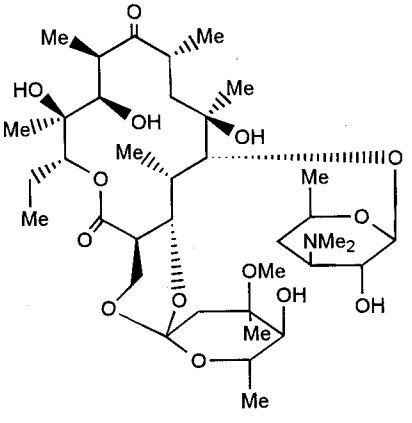
HRFAB-MS suggested the chemical formula $C_{37}H_{65}NO_{14}$ for **2** ($[M+H]^+$ *m/z* 748.4562 found, calcd. 748.4483). In addition, the same diagnostic fragments were observed as found for ESI-CID-MS/MS.

The ^1H and ^{13}C NMR spectra of **2** proved unambiguously the identity of the *Nocardia* metabolite **2** as erythromycin E[3]. Additional evidence was provided by the HSQC, TOCSY, NOESY and HMBC NMR spectra. Shown in Fig. 2 are diagnostic C, H long-

Fig. 1. Structures of erythromycins A (**1**) and E (**2**) from *Nocardia brasiliensis* IFM 0466.

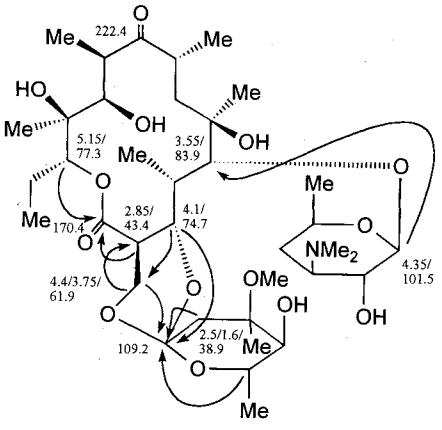


1



2

Fig. 2. Instructive C, H-long-range couplings (HMBC), confirming the presence of the ortho-carboxylic acid ester structure bound to the 14-membered aglycone (in $CDCl_3$; chemical shifts in ppm).



range couplings in the HMBC spectrum of **2** confirming the ortho-carboxylic acid ester structure, its unique structural feature.

Coproduced compounds **1** and 8,9-anhydro-pseudo-erythromycin A-6,9-hemi-ketal were identified readily *via* their MS spectra (ESI-CID-MS/MS) and an NMR spectroscopic investigation[4]. In the crude mixture of erythromycins **1**, **2** and 8,9-anhydro-pseudo-erythromycin A-6,9-hemiketal isolated from *N. brasiliensis* IFM 0466, the portion of erythromycin E (**2**) was estimated to be 64 % from the relative intensities of the $[M+H]^+$ ions (ESI-MS), which were 1, 2.78 and 0.57 for *m/z* 734, 748 and 716, respectively.

N. brasiliensis IFM 0466 was thus found to be a new producer of erythromycin E rendering this antibiotic available for normal fermentation processes which could supply this unusual structure for semisynthetic modifications. Only one case of the production of erythromycins from *Nocardia* (*Nocardia* sp. ATCC 59045) has been reported[5]. However, the present *Nocardia* strain was easily differentiated from strain ATCC 59045, which uses nitrogen or carbon sources such as adenine, xanthine and starch. Therefore, we believe that this is also the first isolation of erythromycins from pathogenic *N. brasiliensis*.

YUZURU MIKAMI
KATSUKIYO YAZAWA

Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan

AKIRA NEMOTO
HISAYUKI KOMAKI
YASUSHI TANAKA

Research Laboratory, Higeta Shoyu Co., Ltd., Chuo-cho, Choshi, Chiba 288, Japan

UDO GRÄFE

Hans-Knöll-Institute of Natural Products Research, Beutenberg-straße 11, Jena, Germany

(Received for October 20, 1998)

References

1) FLYNN, E. H.; M. V. SIGAL Jr., P. F. WILEY & K. GERZON: Erythromycin. I. Properties and degradation studies. J. Am. Chem. Soc. 76: 3121～3131, 1954
2) LAATSCH, H.: Antibase, database of microbial compounds. Chemical Concepts, Heidelberg, Germany, 1998
3) 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
4) KIBWAGE, I. O.; G. JANSSEN, R. BUSSON, J. HOOGMARTENS & H. VANDERHAEGHE: Identification of novel erythromycin derivatives in mother liquor concentrates of *Streptomyces erythraeus*. J. Antibiotics 40: 1～6, 1987
5) CELMER, W. D. (Pfizer Inc.): Erythromycin D and its ester derivatives. Japan 58-198499, Nov.18, 1983