COMMUNICATION TO THE EDITOR

Deacylation of the Glycopeptide Antibiotic A40926 by *Actinoplanes teichomyceticus* ATCC 31121

Sir:

The antibiotic A40926 is a complex of three main factors, designated A (**1**), B (**2**) and B1 (**3**) in Fig. 1, which are produced by *Actinomadura* sp. ATCC 39727[1,2]. A similar complex of antibiotics was also found in the broths of *Actinomadura parvosata* ATCC 53463[3]. Factors A, B and B1 have a core glycopeptide structure with an aminoglucuronyl sugar acylated with *n*-undecanoic, 10-methylundecanoic and *n*-dodecanoic acids, respectively.

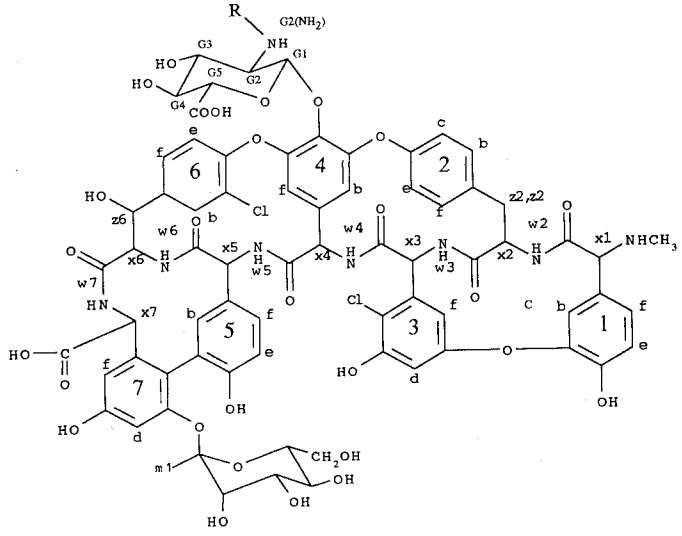
Some microbial transformations of glycopeptide antibiotics described in the literature[4~6] have furnished derivatives difficult to obtain by means of chemical reactions. We found that *Actinoplanes teichomyceticus* ATCC 31121, the producer of teicoplanin, converted the A40926 complex into the deacyl derivative **4**. In this paper we describe the production of deacyl A40926 and its characterization.

A. teichomyceticus ATCC 31121 was grown aerobically at 28°C in a medium containing 0.5% glucose, 0.4% malt extract, 0.4% peptone, 0.1% yeast extract, 1% soybean meal, 0.25% NaCl and 0.5% $CaCO_3$. After incubation for 48 hours the glucose was completely consumed. A40926 complex (200 μg/ml) was then added to the medium. The transformation was monitored by affinity adsorption[7] followed by HPLC analysis on a Beckmann ODS 4.6 × 250 mm column. The column was eluted at 1.5 ml/minute in 40 minutes with a linear gradient from 5% to 65% of phase B. Phase A was 18 mM sodium phosphate buffer pH 6.0 : acetonitrile (98 : 2) and phase B was 18 mM sodium phosphate buffer pH 6.0 : acetonitrile (30 : 70). UV detection was at 283 nm.

The complex (Rt 27~29 minutes) was gradually converted into a single more hydrophilic compound **4** showing Rt 7.4 minutes. The broth was harvested after 192 hours of incubation when about 80% of A40926 complex was transformed. The pool of glycopeptide antibiotics was recovered from the supernatant by adsorption to sepharose-D-alanyl-D-alanine affinity resin[8] and, after washing with 0.1 M Tris-HCl pH 7 buffer, was eluted with 1% NH_4OH. The eluates were brought to pH 3.5 with H_2SO_4 to precipitate the remaining unaltered A40926 factors and teicoplanin. The supernatant contained deacyl A40926 which was then purified by a second round of adsorption at neutral pH to sepharose-D-alanyl-D-alanine resin[8], washing with distilled water, and elution with 1% NH_4OH and lyophilization.

4 shows UV absorption maxima at 282 and 300 nm at neutral and basic pH, respectively, which are characteristic of the glycopeptide chromophore. The main ^1H

Fig. 1. Structures of A40926 complex and deacyl factor.



		R
A40926 Factor A	(**1**)	$CO(CH_2)_9CH_3$
A40926 Factor B	(**2**)	$CO(CH_2)_8CH(CH_3)_2$
A40926 Factor B1	(**3**)	$CO(CH_2)_{10}CH_3$
A40926 Deacyl	(**4**)	H

Table 1. Assignment of the main signals of the ^1H NMR spectrum† of deacyl A40926.

Proton	(ppm)
x1	5.07 (d)
x2	4.88 (m)
x3	6.04 (d)
x4	5.62 (d)
x5	4.40 (d)
x6	4.10 (m)
x7	4.40 (d)
z2	2.80 (m)
z'2	3.30
z6	5.07
4b	5.86 (s)
4f	5.07 (s)
6b	7.73 (s)
m1	5.26 (s)
7f	6.45 (s)
g1	5.35 (d)
g2	2.78 (d)
NCH_3	2.30 (s)

† Chemical shifts in ppm are down field from $(CH_3)_4Si$. The spectrum was measured in DMSO-d_6 with a Bruker 250 MHz instrument on phase sensitive double quantum filter COSY mode (Bruker COSYPHDQ microprogram).

NMR assignments were made (Table 1). They show that the fatty acid 0.8 ~ 2.0 ppm signals of the A40926 factors were not present in the deacyl A40926 whereas its core structure was not modified (Table 1). The MW of 1548, determined by FAB-MS, in agreement with the molecular formula $C_{71}H_{66}N_8O_2Cl_2$ of **4**.

Compound **4** was less active *in vitro* than the A40926 complex against Gram-positive bacteria and *Neisseria gonorrhoeae* (Table 2) and was less efficacious in protecting mice infected with *Streptococcus pyogenes* L49. The ED_{50} of **4** was 2.33 mg/kg as compared with 0.35 mg/kg for A40926 complex. The products were administered subcutaneously once, immediately after infection as described by BERTI *et al*[9].

A. teichomyceticus ATCC 31121 can also deacylate aridicin[6], but not teicoplanin, its own product. Among other producers of glycopeptide antibiotics that we tested, *Actinoplanes missouriensis* ATCC 23342[10] and *Actinoplanes* sp. NRRL 3884[11] were able to deacylate the A40926 factors, whereas various *Nocardia*, *Actinomadura*, *Micromonospora*, *Kibdelosporangium*, *Streptomyces* strains did not. *Actinoplanes utahensis* NRRL 12052, which is not a producer of glycopeptide antibiotics, but has been used to deacylate other classes of antibiotics[12,13], was unable to deacylate A40926.

Table 2. Antibacterial activity of deacyl A40926.

Strain	MIC (μg/ml)	
	Deacyl A40926	A40926
Staph. aureus L165	12	0.06
Staph. aureus (30% bovine serum)	2	0.13
Staph. epidermidis ATCC 12228	2	2
Staph. haemolyticus L602	2	0.06
Strep. pyogenes L49 C203	32	4
Strep. pneumoniae L44 UC41	0.25	0.06
E. faecalis ATCC 7080	0.25	0.06
Strep. mitis L796	2	0.13
Clostridium perfringens L290	0.5	0.06
Neisseria gonorrhoeae L997	0.13	0.008
Haemophilus influenzae ATCC 19418	64	1
Escherichia coli SKF 12140	128	64
Proteus vulgaris ATCC 881	>128	>128
Pseudomonas aeruginosa ATCC 10145	>128	>128
Ureaplasma urealyticum L1479	>128	>128
Klebsiella pneumoniae L142	>128	>128
	>128	>128

MICs were determined by broth microdilution. Culture media and growth conditions were as follows: Iso-Sensitest broth (Oxoid), 24 hours, for staphylococci, *E. faecalis* and Gram-negative bacteria *E. coli*, and *K. pneumoniae*; Todd-Hewitt broth (Difco), 24 hours for other streptococci; GC Base broth (Difco) + 1% (v/v) IsoVitaleX (BBL), 48 hours, CO_2-enriched atmosphere for *N. gonorrhoeae*; Brain Heart Infusion broth (Difco) + 1% (v/v) Supplement C (Difco), 48 hours, CO_2-enriched atmosphere for *H. influenzae*; AC broth (Difco), 24 hours, anaerobic atmosphere for *C. perfringens*; PPLO broth with supplements as in R. T. EVANS and D. TAYLOR-ROBINSON[14], 24 hours for *U. urealyticum*. Incubation was at 37°C. Inocula were as follows: 10^4 color-changing units/ml for *U. urealyticum*; about $10^4 \sim 10^5$ colony-forming units/ml for other microorganisms.

ANGELO BORGHI
FRANCA SPREAFICO
GRAZIA BERETTA
PIETRO FERRARI
BETH P. GOLDSTEIN†
MARISA BERTI
MAURIZIO DENARO††
ENRICO SELVA

Marion Merrell Dow Research Institute,
Lepetit Research Center,
Gerenzano (VA), Italy

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† Present address: Applied Microbiology Inc., New York, NJ 10016, U.S.A.
†† Present address: Amylin Pharmaceuticals, San Diego, CA 92121, U.S.A.

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