Communication to the Editor

REVISED STRUCTURE OF THE ANTIBIOTIC GE 2270A

Sir:

GE 2270A[1] is a new antibiotic produced by *Planobispora rosea* ATCC 53773. It inhibits Gram-positive bacteria and anaerobes by acting on bacterial protein synthesis[2,3]. It was isolated by extraction from the mycelium of the fermented organism and purified by silica gel chromatography.

Ge 2270A (**1**) is a highly modified peptide, belonging to the thiazolyl peptide antibiotic, whose structure was determined by physico-chemical methods applied to the intact molecule and to the main hydrolysis products[4]. They reported that overnight acid hydrolysis (HCl 6 N, 100 ~ 120°C) of the natural antibiotic produces the fragments shown in Fig. 1.

We have found recently that the asparagine thiazole amino acid (**2**) and the valine thiazole amino acid (**3**), as verified by chiral HPLC[5], undergo racemization during the hydrolysis process. In the attempt of establishing the chirality of both asparagine and valine thiazole amino acids, we analyzed the time course of the hydrolysis (HCl 6 N, 100° ~ 120°C) of GE 2270A. The HPLC profile, after two hours hydrolysis, showed several peaks whose UV spectra indicated the presence of the thiazole ring (Diode Array analysis). The reaction mixture was cooled, diluted with water, taken to pH 3.3

Fig. 1. GE 2270A structure and acid hydrolysis fragments.

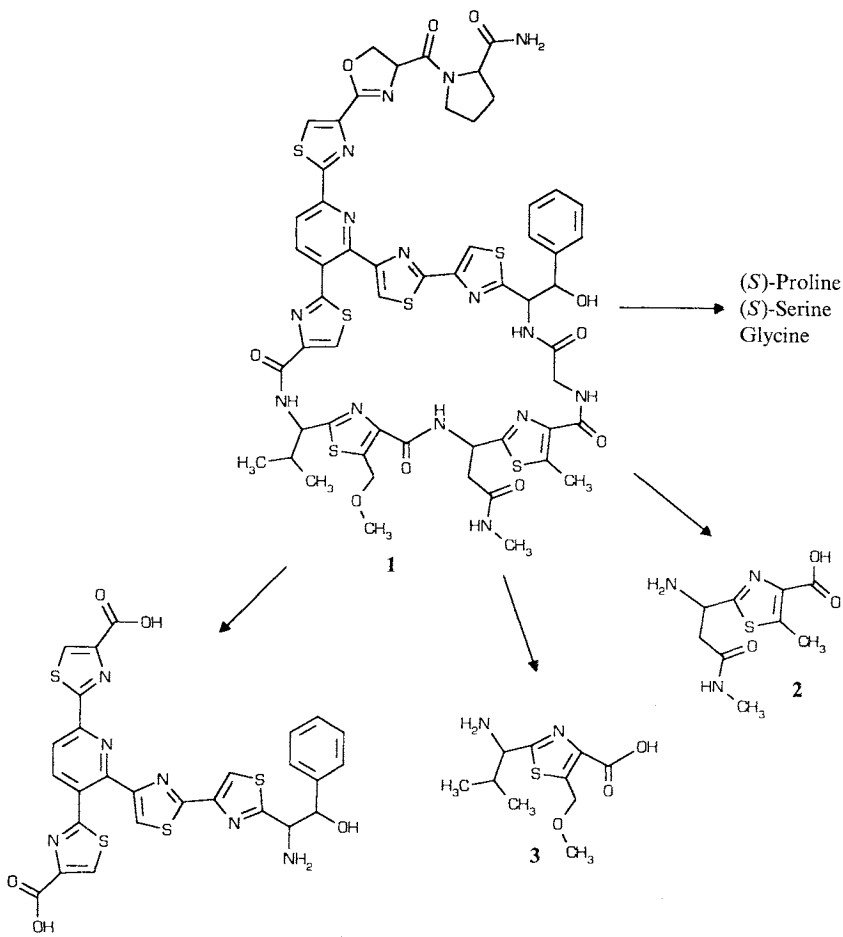
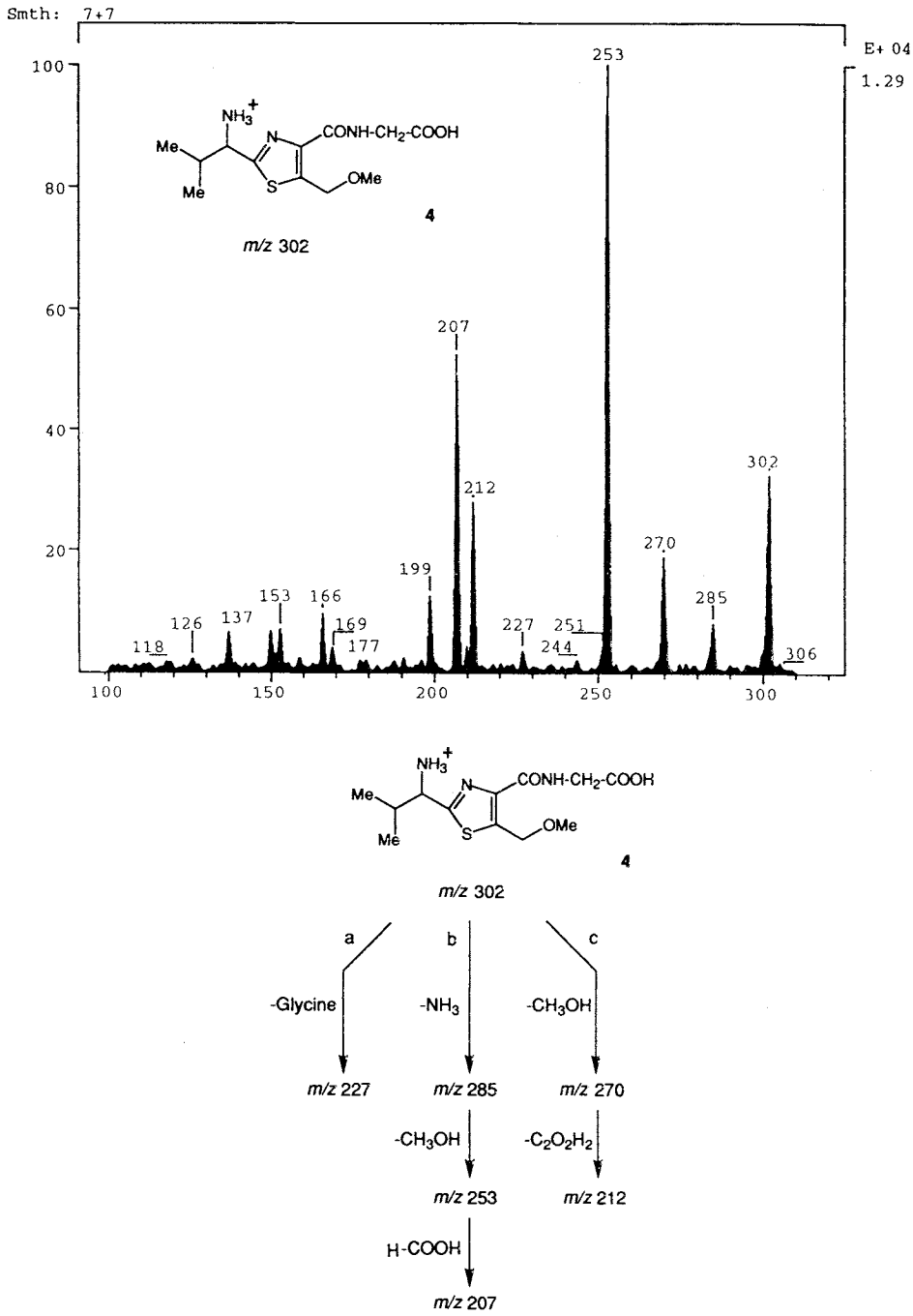
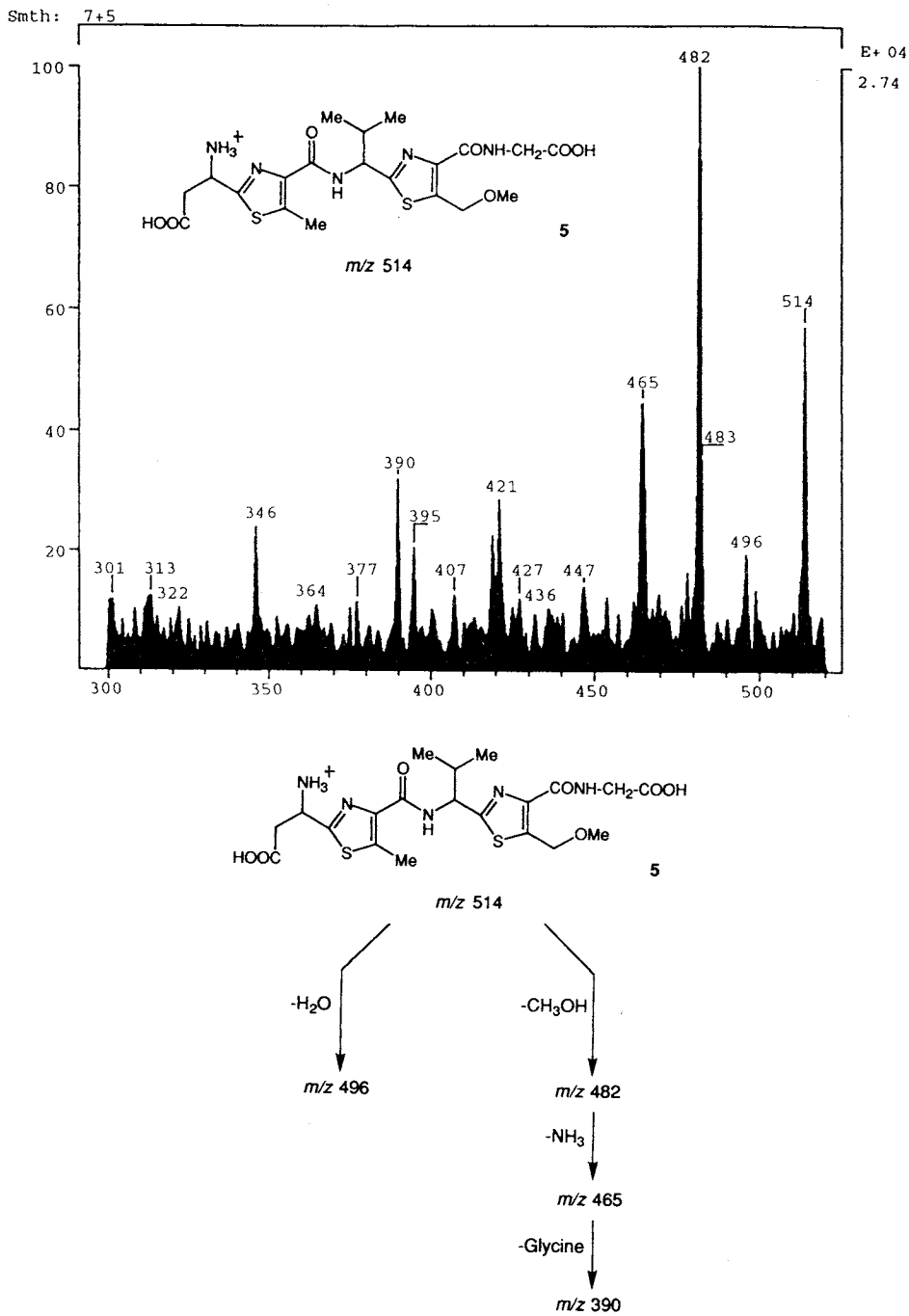


Fig. 2. FAB-MS and MS/MS fragmentation pattern of the first fraction.



and left at 4°C overnight. The precipitate was filtered and the filtrate purified on a S112 column using a gradient of methanol (0 to 50%) in water. The fractions, which contained two thiazolyl fragments were collected, lyophilized and then analyzed by Mass spectrometry. The FAB-MS and MS/MS measurement were performed on a triple stage quadrupole mass spectrometer TSQ 700 Finnigan fitted with an ion-Tek neutral atom gun, operated with Xenon gas at 8 Kv. The sample was dissolved in thioglicerol immediately before the analysis. Positive daughter ions of the protonated molecular

Fig. 3. FAB-MS and MS/MS fragmentation pattern of the second fraction.

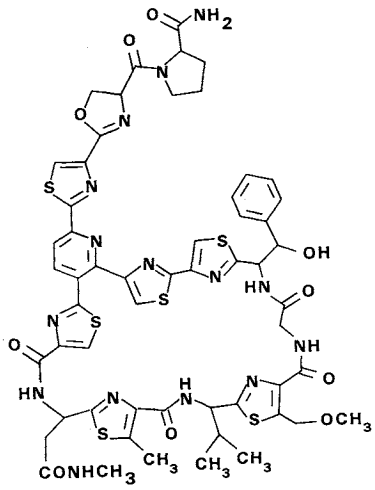


ion were recorded using the second quadrupole as collision cell (collision gas: Argon, collision energy: 20 eV).

The structures of the two fragments were not consistent with the structure of GE 2270A originally proposed. Indeed, the first fraction showed a FAB-MS spectrum in which the peak at *m/z* 302 corresponds to the molecular weight of a veline-thiazole-glycine fragment (**4**). MS/MS experiments, as shown in Fig. 2, confirmed the structure of **4**.

The FAB-MS of the second fraction showed a molecular weight of 514 that corresponds to an

Fig. 4. GE 2270A revised structure.



asparagine-thiazole-valine-thiazole-glycine fragment (**5**). The FAB-MS spectrum on the quasi molecular ion at *m/z* 514 and the fragmentation pattern are shown in the Fig. 3. MS/MS experiment on the daughter ion at *m/z* 465 confirmed the structure. The ^{1}H NMR spectra of the two fractions were in accordance with the structure of the fragments suggested by mass results.

These data clearly proves that the sequence of the thiazole amino acids is reversed with respect to the previously proposed structure leading to the revised structure as shown in Fig. 4. Additional evidences of the revised structure of GE 2270A and the absolute configuration of the thiazole amino acids will be reported later.

PAOLO TAVECCHIA*
PATRIZIA GENTILI
MICHAEL KURZ
CRISTINA SOTTANI
RICCARDO BONFICHI
SERGIO LOCIURO
ENRICO SELVA

Marion Merrell Dow Research Institute,
Lepetit Research Center,
Via R. Lepetit 24, 21040
Gerenzano (VA), Italy

(Received August 18, 1994)

References

1) SELVA, E.; G. BERETTA, N. MONTANINI, L. GASTALDO, P. FERRARI, R. LORENZETTI, B. P. GOLDSTEIN, M. BERTI, L. MONTANARO & M. DENARO: Antibiotic GE 2270A: A novel inhibitor of bacterial protein synthesis. I. Isolation and characterization. J. Antibiotics 44: 693~701, 1991
2) ANBORG, P. H. & A. PARMEGGIANI: New antibiotic that acts specifically on the GTP-bound form of elongation factor Tu. The EMBO Journal 10: 779~784, 1991
3) LANDINI, P.; M. BANDERA, B. P. GOLDSTEIN, F. RIPAMONTI, A. SOFFIENTINI, K. ISLAM & M. DENARO: Inhibition of bacterial protein synthesis by elongation-factor-Tu-binding antibiotics MDL 62,879 and efrotomycin. J. Biochem. 283: 649~652, 1992
4) KETTENRING, J.; L. COLOMBO, P. FERRARI, P. TAVECCHIA, M. NEBULONI, K. VÉKEY, G. G. GALLO & E. SELVA: Antibiotic GE 2270A: A novel inhibitor of bacterial protein synthesis. II. Structure elucidation. J. Antibiotics 44: 702~715, 1991
5) PIRKLE, W. H.; D. W. HOUSE & J. M. FINN: Broad spectrum resolution of optical isomers using chiral HPLC bonded phases. J. of Chromatography 192: 143~158, 1990