REVISED STRUCTURE AND THE CHEMICAL TRANSFORMATIONS OF FR900148

NOBUYOSHI YASUDA† and KAZUO SAKANE*

New Drug Research Laboratories,
Fujisawa Pharmaceutical Co., Ltd.,
2-1-6 Kashima, Yodogawa-ku,
Osaka 532, Japan

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The structure of an antibiotic FR900148, which was isolated from *Streptomyces xanthocidicus* No. 301 in 1980, was reported as a pyrrolidine derivative as shown in Fig. 1[1,2]. In 1984, CHAIET *et al.*[3], isolated 4-amino-3-chloro-2-pentenedioic acid and they suggested that the structure **1** of FR900148 was questionable. This prompted us to reexamine the previous conclusions[2] and has led us to conclude that the correct structure of FR900148 is represented by the open-chain acid (**2**).

FR900148 was reisolated from the same strain

Fig. 1. Previous structure of FR900148.

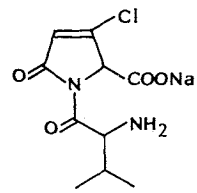

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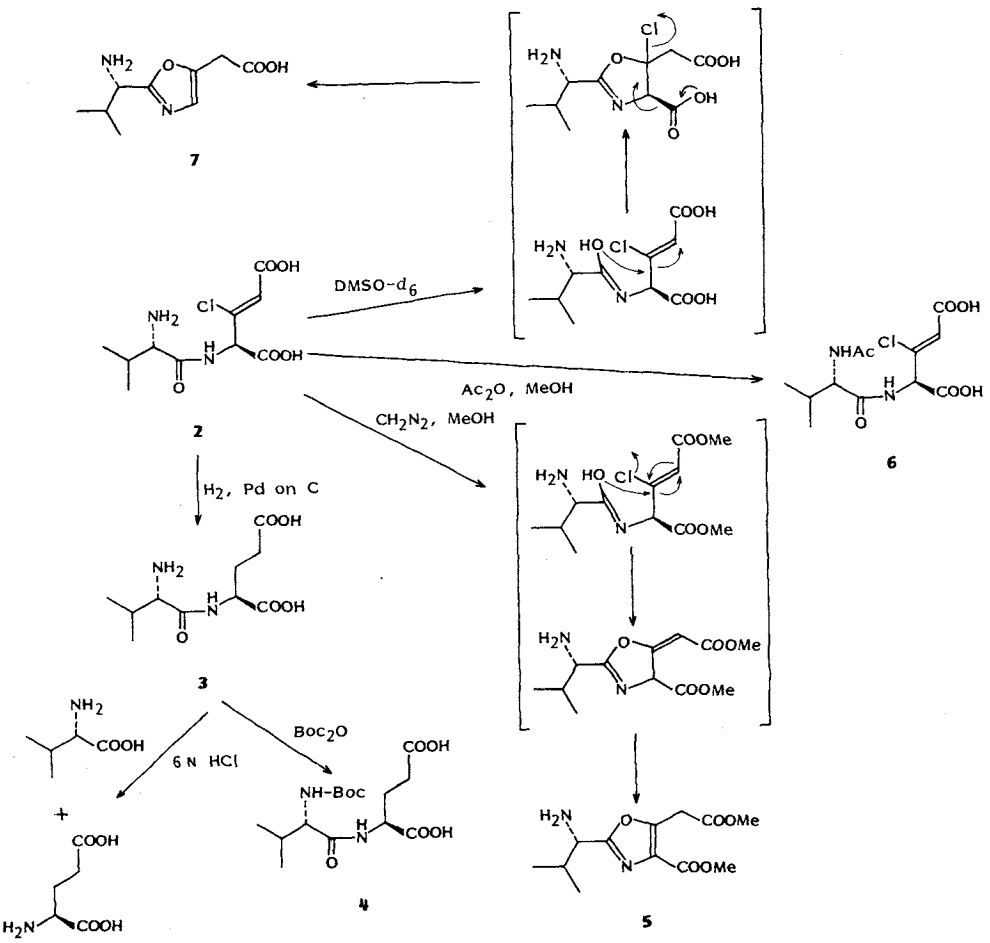
Fig. 2. Chemical modification of the free derivative (**2**) of FR900148.



† Present address: Process Research Labs., Merck Sharp & Dohme, P. O. Box 2000, Rahway, NJ 07065, U.S.A.

and its biological and physical data were exactly the same as in the previous isolation. In order to confirm the presence of a chlorine atom in the antibiotic, free acid derivative (**2**) was isolated from the fermented broth filtrate by successive column chromatography on activated carbon, DEAE-Sephadex, CM-Sephadex and Sephadex G15. The ^{13}C NMR of **2** in D_2O showed 10 carbons (δ 17.383 (q), 18.218 (q), 30.484 (d), 59.105 (d), 61.927 (d), 126.511 (d), 134.919 (s), 169.068 (s), 171.948 (s), and 172.668 (s)). FAB-MS data of the sodium salt of **2** showed strong signals at *m/z* 279 $(M+H)^+$, 301 $(M+Na)^+$, and 323 $(M+2Na)^+$ and each signal displayed the usual one chlorine isotope pattern. FAB-MS data of **2** (free acid) showed signals at *m/z* 199 ($(M+H)^+$ of **7**), 235 ($(M+H)^+-COO$), 245 ($M-Cl^-$), and 279 $(M+H)^+$, and two signals at 235 and 279 displayed the usual one chlorine isotope pattern. Titration of **2** showed p*K*a 1=2.00, p*K*a 2=3.37 and p*K*a 3=7.97. (In the previous report[1], the titration was reported as p*K*a 1=3.25 and p*K*a 2=7.90). Therefore, **2** must have two carboxylic acid moieties and one amino group. From these data and also elemental analysis of **2** ($C_{10}H_{15}ClN_2O_5 \cdot 2H_2O$: Calcd: C 38.16, H 6.08, Cl 11.26, N 8.90, Found: C 37.87, H 5.08, Cl 10.79, N 9.23), the formula of **2** is established to be $C_{10}H_{15}ClN_2O_5$.

Then we reexamined the chemical transformations of **2**[2]. Hydrogenation[2] of **2** gave a dipeptide **3**. Acid hydrolysis[2] of **3** gave L-Val and L-Glu, whose absolute stereochemistries were determined by chiral HPLC. FAB-MS and 2D-NMR data of the Boc derivative (**4**) of **3** clearly revealed that **4** was Boc-L-Val-L-Glu, which was converted into the corresponding dimethyl ester by treatment with diazomethane. The fact that no racemization had occurred during these reactions suggested that each corresponding carbon in **2** to the α-carbons in L-Val and L-Glu should be an optically active secondary carbon. Treatment of **2** with diazomethane in MeOH[2] gave an oxazole compound **5**, whose structure was confirmed by ^1H NMR ($CDCl_3$) δ 0.94 (3H, d, J=7 Hz), 0.98 (3H, d, J=7 Hz), 2.16 (1H, m), 3.74 (3H, s), 3.91 (3H, s), and 4.13 (2H, s), ^{13}C NMR ($CDCl_3$) δ 18.082 (q), 18.917 (q), 31.982 (t), 33.343 (d), 52.203 (q), 52.577 (q), 55.802 (d), 129.053 (s), 151.541 (s), 162.339 (s), 165.996 (s), and 168.040 (s), and UV (λ=213 nm, $\varepsilon=1.10\times10^4$ in EtOH)[4]. ^1H NMR of the acetylated compound **6**[2] in THF-d_8 and THF-d_8-D_2O were very similar to that of **2**. Two amide protons of **6** were assigned from a decoupling experiment as follows; δ 8.08 (1H, d, J=7.92 Hz, D-Val–CONH–) and 7.24 (1H, d, J=7.90 Hz, AcNH–D-Val–), and showed that acetylation had occured at only the amino moiety of the valine group. Interestingly, **2** spontaneously decarboxylated upon dissolution in DMSO-d_6 and gave the oxazolylacetic acid derivative **7** (^1H NMR (DMSO-d_6) δ 0.84 (3H, d, J=7 Hz), 0.99 (3H, d, J=7 Hz), 2.27 (1H, m), 3.80 (2H, s), 4.32 (1H, d, J=6 Hz), and 7.08 (1H, s); ^{13}C NMR (DMSO-d_6) δ 17.189 (q), 18.543 (q), 30.780 (d), 31.212 (t), 53.037 (d), 124.561 (d), 147.279 (s), 158.969 (s), and 169.565 (s).

From these data and ^{13}C-^1H shift collation by long-range coupling (COLOC) of **2**, the structure of **2** and the chemical transformations were revised as shown in Fig. 2. We could not determine the geometry of the double bond, however, from CHAIET's data[3], we assumed that it is the *Z* form.

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