STUDIES ON A NEW ALKALINE DEGRADATION PRODUCT OF JOSAMYCIN

Sir:

On the aglycon structure of macrolide antibiotics possessing an aldehyde function, WOODWARD once suggested a 17-membered lactone structure with a -CHO side chain for magnamycin by degradation studies[2], and later revised it to a 16-membered structure with $-CH_2-CHO$ after NMR study of its degradation product.[3] For spiramycin, PAUL *et al.* proposed an 18-membered lactone structure with a -CHO side chain by degradation studies[4], KUEHNE *et al.* amended it to a 17-membered ring with a -CHO based on MS and NMR studies[5], and later OMURA *et al.* revised it to a 16-membered lactone with a $-CH_2-CHO$ by studying a derivative which was also obtained from leucomycin[6]. For leucomycin, OMURA *et al.* assigned a 16-membered lactone structure with a $-CH_2-CHO$ side chain[7].

In the course of structure studies on josamycin[1], we found a new interesting product which was obtained by mild alkaline treatment of this antibiotic.

When josamycin (**I**) was refluxed in ethanol with equimolar lithium hydroxide ($LiOH \cdot H_2O$) for three hours, a new product was formed in good yield and was separated and purified by silica gel or alumina column chromatography. The purified substance (**II**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −20.7° (*c* 1, EtOH), λ_{max}^{MeOH} 234.5 mμ (ε 25,900), and gave a crystalline thiosemicarbazone (**III**) as needles, mp 153~155°C, λ_{max}^{MeOH} 232 mμ (ε 33,500) and 272 mμ (ε 28,400). **II** showed no antibacterial activity.

The elemental analysis of the crystalline thiosemicarbazone (**III**) showed excellent agreement for a formula $C_{40}H_{67}NO_{13} \cdot NNHCSNH_2$ (*Anal.* Calcd. for $C_{41}H_{70}N_4O_{13}S$: C 57.32, H 8.21, N 6.52, S 3.73. Calcd. for $C_{41}H_{68}N_4O_{12}S$ ($C_{41}H_{70}N_4O_{13}S-H_2O$): C 58.55, H 8.15, N 6.66, S 3.81. Found: C 57.17, H 8.01, N 6.47, S 3.92). The mass spectrum of **II** gave the peak of highest mass at *m/e* 767, which could be assigned to the M^+-18 peak derived from $C_{40}H_{67}NO_{14}$ (MW 785) in consideration of the formula of **III** above-assigned. Thus the molecular weight of **II** was determined to be 785, which showed a decrease of 42 mass units from josamycin (**I**) ($C_{42}H_{69}NO_{15}$, M^+ at *m/e* 827), suggesting hydrolytic removal of an acetyl from josamycin. In accordance with this, the NMR and IR spectra of **II** and its thiosemicarbazone (**III**) revealed loss of a signal at δ 2.28 (3H, s, $OCOCH_3$) and a band at 1234 cm^{-1} ($OCOCH_3$ ν_{c-o}), indicating absence of O-acetyl originally found in **I**. Catalytic

Fig. 1. ORD and CD curves of josamycin (**I**) and its alkaline degradation product (**II**)

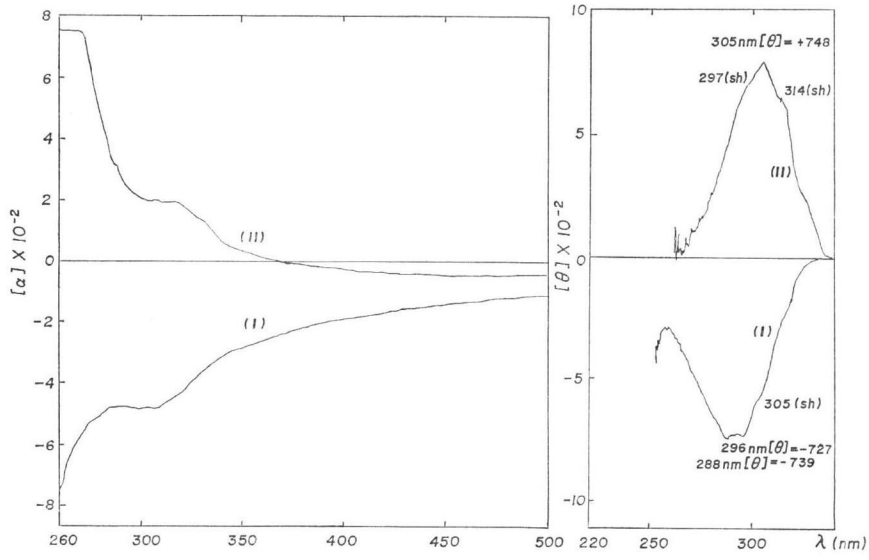


Fig. 2. NMR spectrum of **II** (100 MHz, $CDCl_3+D_2O$)

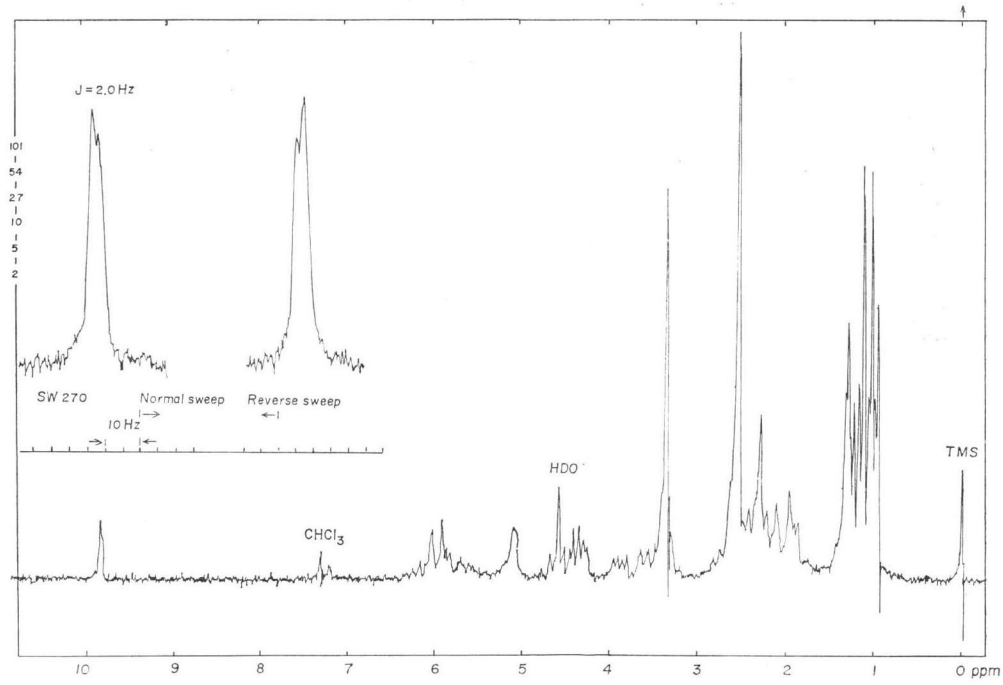
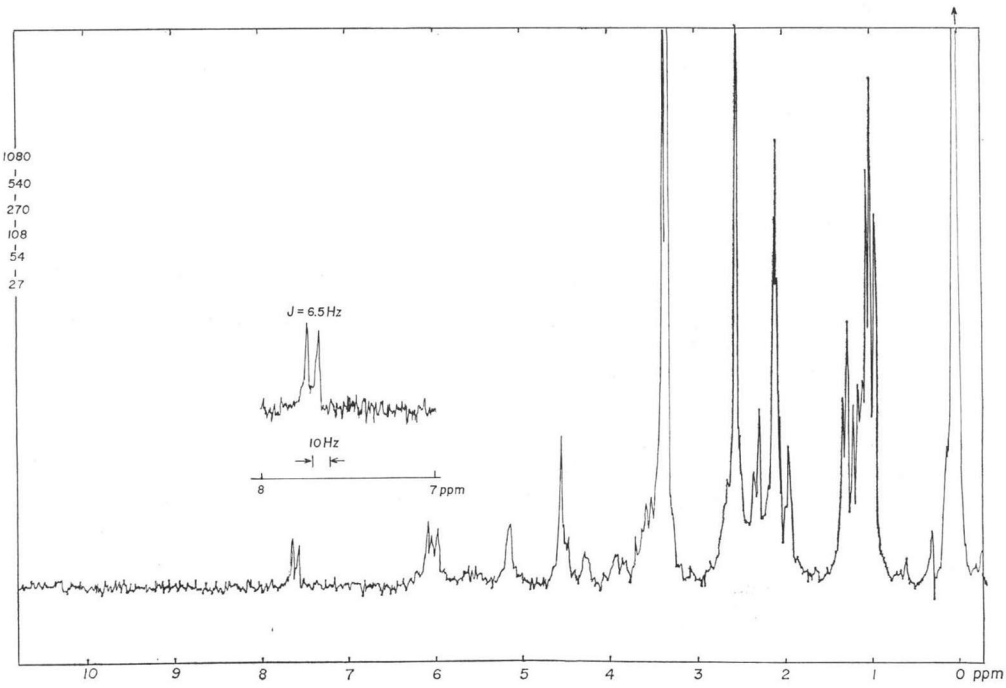


Fig. 3. NMR spectrum of thiosemicarbazone of **II** (**III**) (100 MHz, $CD_3COCD_3+D_2O$)



hydrogenation of **II** over palladium showed the same amount of hydrogen absorption as that in **I** (two molar equivalents), denying formation of any new double bond by deacetylation. This was further confirmed by a ^{13}C NMR spectrum of **II** giving signals for two ester carbonyl carbons (176.6 and 174.6 ppm) and four olefinic carbons (140.8, 135.9, 132.2 and 131.3 ppm), indicating a decrease of one ester carbonyl and no change in the number of double bonds compared with josamycin (**I**) (176.6, 174.5 and 173.6 ppm for ester carbonyls; 138.4, 135.3, 135.0 and 130.7 ppm for olefinic carbons). Thus **II** was shown to be a deacetylated josamycin.

Fig. 1 gives ORD and CD curves of **II** and josamycin (**I**). As seen in the figure, the ORD curves of **II** and **I** are almost symmetrical in the carbonyl region. **II** shows peaks at 319 mμ and 308 mμ, while **I** gives troughs at 308 mμ and 298 mμ, showing positive and negative COTTON effect curves respectively. The CD curve of **II** shows positive maxima at 314 mμ (sh), 305 mμ (θ= +748) and 297 mμ (sh), and that of **I** gives negative maxima at 305 mμ (sh), 296 mμ (θ= −727) and 288 mμ (θ= −739). These almost symmetrical ORD curves of **II** and **I** in the carbonyl region, suggested that **II** and **I** might have symmetrical partial structures in the vicinity of the aldehyde carbonyl group. This suggested the possibility of epimerization on the carbon atom adjacent to the aldehyde, indicating a 17-membered lactone aglycon in josamycin.

This epimer formation was also suggested by the 100 and 60 MHz NMR spectra of **II** and its thiosemicarbazone (**III**). The aldehyde proton of josamycin (**I**) gave a broad singlet signal at δ 9.64. **II** gave a doublet at δ 9.82 with a coupling constant of 2.0 Hz (cf. Fig. 2). In the thiosemicarbazone of **II** (**III**), the −CH=N− proton gave a well-resolved doublet at δ 7.60 (1 H, J=6.5 Hz). These J values were identical in 100 and 60 MHz spectra, and irradiation near 2.4 ppm caused collapse of the doublet at δ 7.60 into a singlet (Fig. 3). However, the ^1H FT-NMR spectrum of **II** on a Varian XL-100 NMR spectrometer kindly made by Dr H. NAGANAWA, Institute of Microbial Chemistry, Tokyo, showed that the aldehyde proton of **II** at δ 9.82 is a double-doublet with coupling constants of 2.0 and 0.5 Hz. Decoupling studies showed that irradiation at δ 2.44 collapsed the broad doublet of the aldehyde proton into a broad singlet and irradiation at δ 2.14 caused sharpening of the doublet signal. Josamycin X-ray crystallography is now under study.

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