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THE STRUCTURE OF HITACHIMYCIN, A NOVEL MACROCYCLIC LACTAM INVOLVING β-PHENYLALANINE

Satoshi Ōmura*, Akira Nakagawa, Kiyoshi Shibata and Hiroshi Sano

School of Pharmaceutical Sciences, Kitasato University and The Kitasato Institute
Minato-ku, Tokyo 108, Japan

Summary: The structure of hitachimycin (1) has been determined by NMR spectrometry and chemical reactions. The antibiotic is a novel 19-membered macrocyclic lactam possessing a β-phenylalanine moiety.

Hitachimycin[1] is an antiprotozoal antibiotic isolated from the culture broth of actinomycete strain No. KM-4927. Later, the antibiotic was found to be identical with an antitumor antibitic stubomycin.[2] The structure (1) assigned for hitachimycin possesses a novel 19-membered ring lactam skeleton which is built up biosynthetically from malonate, methylmalonate and phenylalanine *via* "polyketide pathway". The antibiotic could be substantially distinguishable with ansamycin antibiotics such as geldanamycin,[3] herbimycin,[4] actamycin[5] and ansatrienin[6] in the point that hitachimycin possesses no aromatic or quinoid nucleus in the ansa-chain moiety. In this paper we describe the structural determination of hitachimycin by means of chemical degradation, NMR spectrometry and biosynthesis using ^{13}C labeled precursors.

The ^{13}C NMR spectrum of hitachimycin (1), mp. 234.5°C, $[\alpha]_D^{20}$ +246° (*c* 0.5, DMSO), $C_{29}H_{35}NO_5$, *m/z* 477 (M^+), UV λ_{max}^{MeOH} 301 nm (ε 46,000) suggested the presence of an amide carbonyl carbon (δ 167.6), the enol form carbons of a β-diketone (δ 196.5, 185.5 and 112.6), a phenyl group, eight olefinic carbons, two oxycarbons (δ 81.2 and 68.1), a methoxyl (δ 58.1), two methines (δ 52.4 and 35.1), five methylenes (δ 29.4, 35.5, 39.1, 41.6 and 46.5) and a methyl (δ 20.0). Acetylation of 1 with acetic anhydride in pyridine afforded a diacetate 2, mp. 281°C, $[\alpha]_D^{20}$ +47.1° (*c* 0.5, $CHCl_3$), $C_{33}H_{39}NO_7$, *m/z* 561 (M^+), ^1H NMR ($CDCl_3$); δ 1.96 and 2.23 (OAc), IR (KBr); ν_{CO} 1770, 1730 and 1720 cm^{-1}. Methylation of 1 with diazomethane afforded a monomethyl ether 3, mp. 293°C, $[\alpha]_D^{20}$ +207° (*c* 0.5, $CHCl_3$), $C_{30}H_{37}NO_5$, *m/z* 491 (M^+), ^1H NMR ($CDCl_3$); δ 4.00 (CO_2CH_3), UV λ_{max}^{MeOH} 302 nm (ε 45,700), indicating the existence of the enol form of a β-diketone in its molecule. This was also confirmed from the fact that the three carbon signals due to the β-di-

ketone of 1 were not observed and additional two oxycarbons [δ 74.7 (d) and 73.8 (d)] and methine carbon [53.9 (d)] appeared in tetrahydrohitachimycin 4, mp. 186°C, $[\alpha]_D^{20}$ +165.0° (c 0.5, DMSO), $C_{29}H_{39}NO_5$, m/z 481 (M^+), UV λ_{max}^{MeOH} 301 nm (ε 19,900), obtained by the $NaBH_4$ reduction of 1. Hydrogenation of 1 over PtO_2 afforded an octahydro compound 5, mp. 155°C, $[\alpha]_D^{20}$ -43.2° (c 0.5, $CHCl_3$), $C_{29}H_{43}NO_5$, m/z 485 (M^+), UV λ_{max}^{MeOH} 291 nm (ε 10,400), which means the presence of four olefins in addition to the enolic double bond.

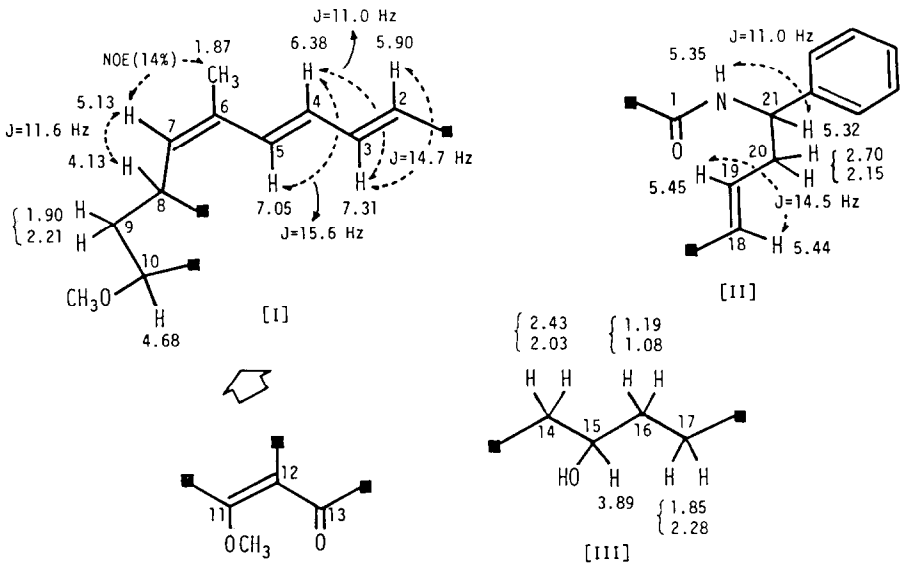

Chart 1

Homonuclear proton spin decoupling experiments at 400 MHz of 3 proposed conveniently three structural segments [I], [II] and [III], as shown in Chart 1. All three double bonds in segment [I] possess triene structure, trans-trans-cis-configuration from the coupling constants among each proton, the observation of nuclear Overhauser effect (14%) for the methyl proton at the C-6 and H-7, and C-H long range selective decoupling between a methyl carbon at 6 position at H-5. The evidence for segment [II] was obtained by the formation of a S-β-phenylalanine methyl ester by the oxidative ozonolysis of 1 followed by hydrolysis with 6N-HCl and treatment with diazomethane. The presence of a trienamide moiety in which the amide carbonyl carbon in segment [II] is linked to the C-2 position of the triene moiety in segment [I] was confirmed from the fact that the signal of the amide carbonyl carbon appearing at δ 167.5 in 1 shifted to a lower field region at δ 177.3 in the octahydro compound 5. On the other hand, another ozonolysis product of 1 was a methyl ester 6 of 5-membered ring lactone, $[\alpha]_D^{20}$ +19.6° (c 0.5, $CHCl_3$), $C_6H_8O_4$, m/z 144

(M^+), IR ($CHCl_3$); 1770, 1715, 1180 cm^{-1}, which implies the connection of the methylene at 17 position in segment [III] with the olefinic carbon at 18 position in [II].

The location of the remaining β-diketone moiety [-C(=O)-C=C(OH)-] was determined by the following chemical transformation. Catalytic hydrogenation of 1 over PtO_2 followed by $NaBH_4$ reduction and acetylation with acetic anhydride in pyridine afforded a decahydro triacetate 7, mp. 156°C, $[\alpha]_D^{20}$ +25.2° (c 0.5, $CHCl_3$), mass; M^+ m/z 613 (613.363, Calcd. for $C_{35}H_{51}NO_8$, 613.363), ^1H NMR ($CDCl_3$); δ 2.00, 2.10 and 2.11 (OAc) and the dodecahydro triacetate 8, $[\alpha]_D^{20}$ -52.0° (c 0.5, $CHCl_3$), mass; M^+ m/z 615 (615.374, Calcd. for $C_{35}H_{53}NO_8$, 615.374), ^1H NMR ($CDCl_3$); δ 2.01, 2,07 and 2.09 (OAc). The ^1H NMR spectral data (an olefinic proton at 7 position; δ 5.05, d, $J_{7,8}$=8.8 Hz and a methyl proton at 6 position; δ 1.70, s) of compound 7 indicate the presence of one non-reduced double bond. The proton signal (δ 5.37, dd, $J_{10,11}$=9.0 Hz, $J_{11,12}$=5.5 Hz) assignable to the base of an acetoxyl group collapsed from a double doublet to a doublet ($J_{10,11}$= 9.0 Hz) upon irradiating the proton signal (δ 3.70, m) at the base of a methoxyl group. This

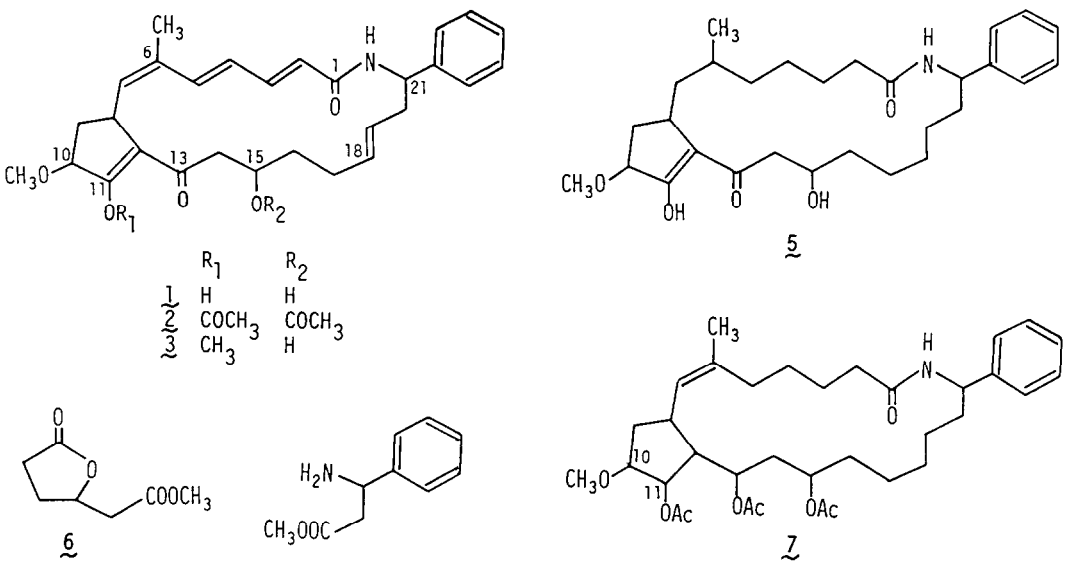

Chart 2

indicates that the methoxyl group should be located adjacent to the acetoxyl group at C-11 position. The further detailed homonuclear spin decoupling data at 400 MHz, in conjunction with the above results established the structure 1 for hitachimycin.

From the structural feature of hitachimycin, it was speculated that the antibiotic is biosynthetically derived from eight malonates, one methylmalonate and one phenylalanine. Then, we

carried out the feeding experiments using ^{13}C labeled precursors.[7] The feeding experiment of [1-^{13}C]sodium acetate indicated the enrichment for the signals at δ 196.5, 185.5, 167.6, 141.9, 134.9, 68.1, 35.5 and 29.4 in the ^{13}C NMR spectrum of hitachimycin. On the other hand, [1-^{13}C]-sodium propionate was incorporated to hitachimycin to show a strong enrichment for only one olefinic carbon at δ 136.9. In the feeding experiment of DL-[1-^{13}C]phenylalanine, the high incorporation to the carbon at δ 126.9 suggests that phenylalanine would be converted to β-phenylalanine by amino mutase in a hitachimycin producing strain. It is the first report that phenylalanine is incorporated into a polyketide chain presumably as a starter unit.

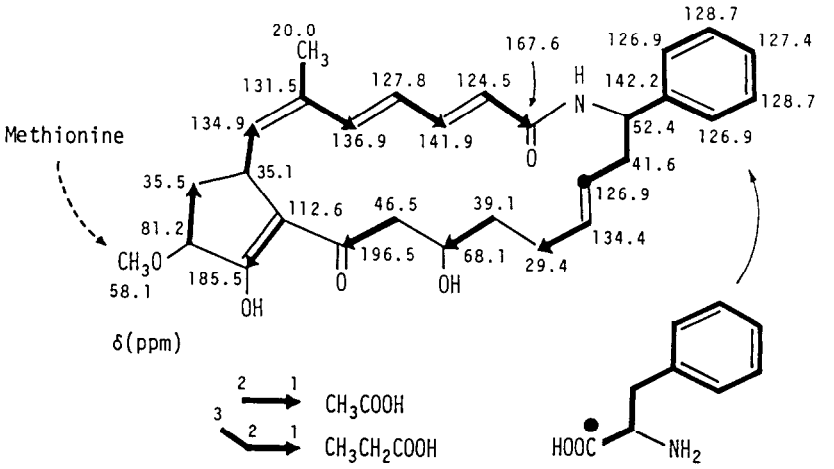

Fig. 1. Biosynthetic origins of hitachimycin and δ_C assignments (in $CDCl_3$)

References and Footnotes

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7) The ^{13}C precursors (0.05-0.2%, w/v), 90% enriched [1-^{13}C]acetate, [1-^{13}C]propionate, DL-[1-^{13}C]phenylalanine were added to 6 day fermentation broth (media: glucose 1.5%, soybean meal 1.0%, peptone 0.3%, NaCl 0.3%, $CaCO_3$ 0.3%) and the cultivations were continued at 27°C for 5 days. In the feeding experiment of phenylalanine, casamino acids eliminated phenylalanine and tyrosine was used instead of peptone. ^{13}C Labeled hitachimycins were isolated by solvent extraction, followed by silica gel column chromatography from the broth filtrate.

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