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UTILIZATION OF ^{13}C-^{13}C COUPLING IN STRUCTURAL AND BIOSYNTHETIC STUDIES. VII [1)]

THE STRUCTURE AND BIOSYNTHESIS OF VULGAMYCIN.

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Vulgamycin is an antibiotic produced by *Streptomyces hygroscopicus* No A-5294[2)] and is active against gram negative bacteria. The antibiotic has been proved to be identical with enterocin recently reported by Miyairi et al.[3)]

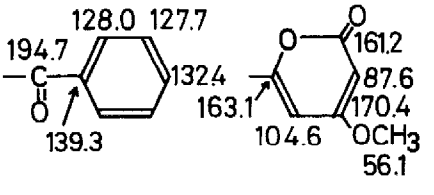
We wish to report the structural and biosynthetic studies of vulgamycin, the result of which is summarized in Fig. 1.

Vulgamycin I, $C_{22}H_{20}O_{10}$(M^+ m/e, found:444.1058, calcd:444.1055), m.p. 166-168°C, $[\alpha]_D^{21}$ -11° (c 1, MeOH), λ_{max}^{MeOH} 250nm(ε 16900) and 283(11100), gave a diacetate II, $C_{26}H_{24}O_{12}$(M^+ m/e, 528), on acetylation with acetic anhydride/pyridine and a dihydro derivative III, $C_{22}H_{22}O_{10}$(M^+ m/e 446) on reduction with $NaBH_4$.

The ^1H-nmr spectrum of I (d_6-DMSO, 100MHz) showed the presence of three tertiary alcohols (δ_H^{TMS}5.46,s, 5.85,s, and 5.90,s), one secondary alcohol(5.65,d,J=5.5Hz), mono-substituted benzene (7.45-7.85,5H,m), two *meta* coupled heteroaromatic protons(5.61,d, and 6.29,d,J=2.3Hz), a methylene(1.68,bd, and 2.27,bd, J_{gem}=14.4Hz), a methoxy(3.87,3H,s) and four protons(~4.47,2H,m, and ~4.66,2H,m), one of which(4.47) was coupled to the secondary hydroxy proton.

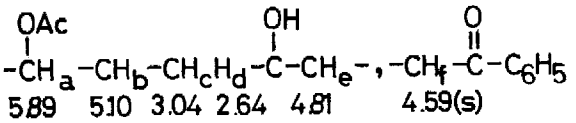
The ^{13}C-nmr spectrum of I (δ_C^{TMS}, d_6-DMSO, 25.05MHz) and known chemical shifts of acetophenone[4)] and 4-methoxy-2-pyrones[5)], together with the information described above revealed the following functionalities. The molecular formula of I indicated the identity of O_A and O_B and the presence of additional three ring structures.

CH_2	:35.5	COH	: 76.2
CH—	:52.9	COH	: 78.1
CH—	:54.6	COH	: 79.6
CHOH	:69.2	C-O_B—	:173.4
CHO_A—	:75.3	C=O	



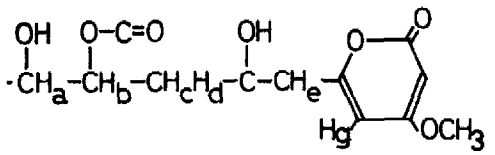
The spin decoupling experiments on II in $CDCl_3$ showed the

following partial structures. Since H_e(J=2.5Hz) was coupled to only one proton (H_c) of the

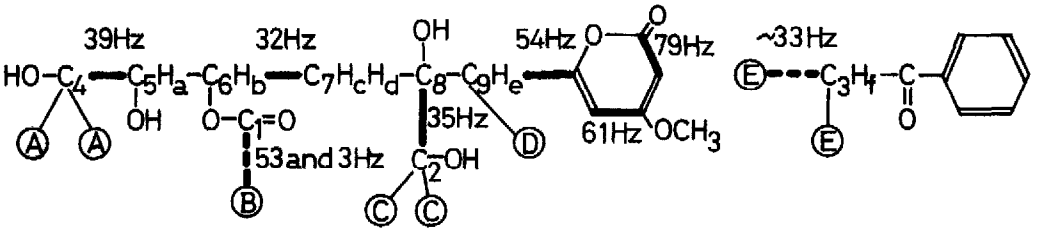


methylene and not to the other (H_d), H_e and H_c must be in a 1,3-diequatorial relationship[6] (W-form long range coupling) connected by a quartery hydroxylated carbon. In the ^{1}H-nmr spectrum of a $NaBH_4$ reduction product III, Hf which had appeared as a sharp singlet (4.59) in II, changed to a doublet (d_5-pyridine, 4.15, J=7.5Hz) coupled to a newly appeared doublet(6.02, J=7.5Hz). Thus, the relation $-CH_f-C(=O)-C_6H_5$ was established.

In the above partial structures, one of three methines (CH_b, CH_e and CH_f) remained to be oxygenated. The ^{13}C-nmr selective proton decoupling experiments on I taken in d_5-pyridine showed clearly CH_b to be an oxygenated one (δ_C^{TMS} CH_b 77.0, CH_e 54.1 and CH_f 56.2). The carbon chemical shift of CH_e is reasonably explained by binding the pyrone system to this carbon; in supporting this explanation, the long range coupling between H_e and H_g of the pyrone (J=1.0Hz) was confirmed by spin decoupling experiments on methyl vulgamycinate triacetate $C_{29}H_{28}O_{13}$(M^+-H_2O m/e, 584) which was prepared by mild alkaline hydrolysis of I followed by acetylation and methylation. Therefore, the partial structure was extended as shown below.



At this point, we turned to the use of the double labeling method[7] which utilizes ^{13}C-^{13}C coupling in structural elucidation. As expected, the ^{13}C-nmr spectrum of I (Fig. 3) labeled with $^{13}CH_3{}^{13}COO$ Na (without dilution of unlabeled acetate) showed several pairs of ^{13}C-^{13}C coupling which could be accommodated in the following partial structures representing all carbons of I.



The magnitude of the coupling constants of $-O-C_1=O$ and $-C_3H_f-$ (shown by▬▬) did not match to any other coupling constants observed. This phenomenon was believed to be caused by the coupling between different acetic acid molecules(*vide infra*).

In connecting the unpaired bonds A-E, the following combinations had to be excluded based on the reasons given below. (i) A-C. I consumed 2 moles of periodate. (ii) C-D. The combina-

tion of C and D would form a cyclopropane ring. However, the coupling constant ($J_{2,8}$=35Hz) is too large in magnitude as found for cyclopropane rings[8] (J=10-17Hz). (iii) D-E. H_e and H_f were not coupled to each other. (iv) A=C, C=E, or A=E. The ^{13}C-nmr spectral data showed that C_2, C_3 and C_4 are sp^3 carbons.

In addition to the above limitations, the ^{13}C-^{13}C coupling observed between C_2(OH) and C_3H_f ($J_{2,3}$=34Hz) in the ^{13}C-nmr spectrum of I derived from $^{13}CH_3COONa$ imposed the direct combination of these two carbons, leaving only one possible combination (A-D, A-E, B-C, and C-E) to give the structure as shown in Fig. 1. The W-form long range coupling observed between H_e and H_c, and not between H_a and H_e or H_c required H_e to be in an equatorial and H_a in an axial orientation.

Fig.1

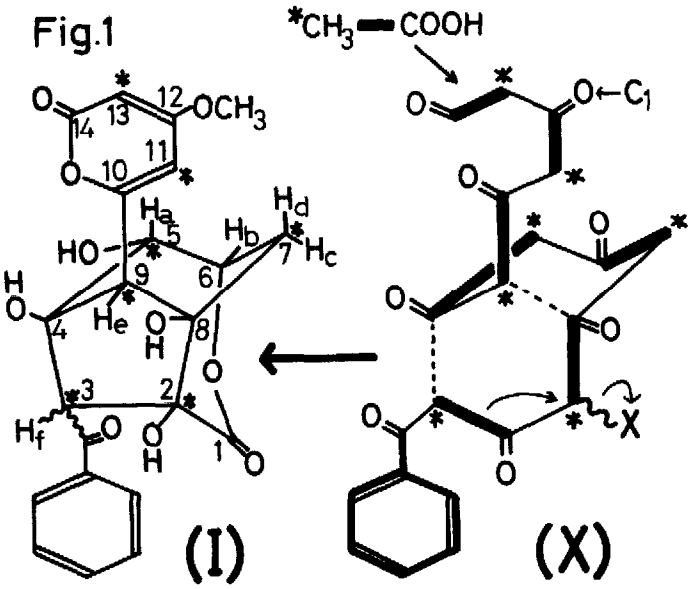
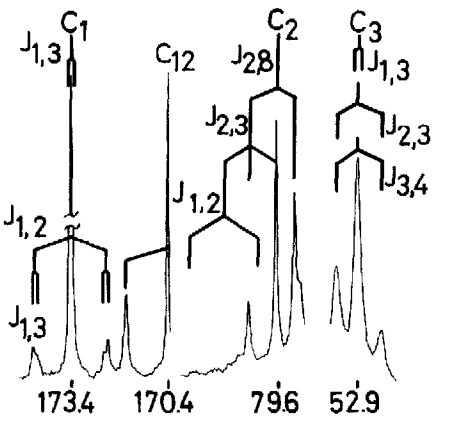
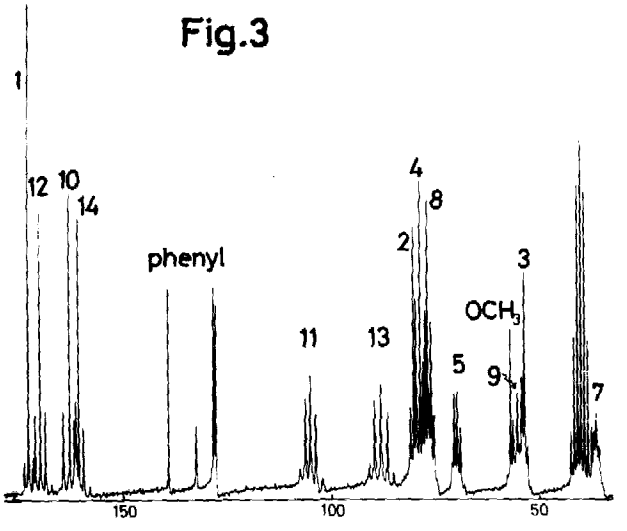


Fig. 2 Partial expansion of Fig. 3.
spectral width 5KHz, pulse width π/4
pulse repetition 2 sec.
data points 16K. in d_6-DMSO.



In the ^{13}C-nmr spectrum of I derived from $^{13}CH_3{}^{13}COONa$, the intensity of satellite peaks of C_1 and C_3 is considerably weaker than those in similar environments, indicating that the observed satellites were due to the coupling between different acetic acid molecules. Actually the satellite peaks of only C_1 and C_3 disappeared in the ^{13}C-nmr spectrum of I labeled with $^{13}CH_3{}^{13}COONa$ diluted three times with unlabeled acetate. The expanded spectrum together with the expected splitting pattern for C_1 and C_3, as well as C_2 which should be coupled to C_1 and C_3 is shown in Fig. 2. It should be noted in Fig. 2 that the satellite peaks of C_1 were further splitted to a doublet (J=3Hz) without being accompanied by a central peak. This pattern can only be explained by assuming that C_1 and C_3 derived from the same acetic acid molecule and that the bond having connected C_1 and C_3 in a biosynthetic intermediate (X in Fig. 1) was cleaved

during a biosynthetic process probably through a Favorskii type rearrangement. Similar precedents have been observed in the biosynthesis of a pyrone[9,10] and sterigmatocystin[10]. The satellite peaks with small ^{13}C-^{13}C coupling expected to appear on both sides of the C_1 central peak were obscured by the overlapping of the strong C_1 peak simply resulting in the line broadening.



Two separate experiments using $^{13}CH_3COONa$ and $CH_3{}^{13}COONa$ indicated that carbons 2, 3, 5, 7, 9, 11 and 13 derived from the former and that carbons 1, 4, 6, 8, 10, 12 and 14 from the latter. Uniformally ^{14}C-labeled benzoate (1 μCi) was incorporated efficiently and selectively into the benzoyl portion of I (incorporation 4.4%, location of the radioactivity 69%). The incorporation of [CD_3]-methionine into the methoxy group of the pyrone moiety was disclosed by mass spectrometry. Thus I is biosynthesized from methionine and seven acetate units with benzoate as the starter (Fig. 1).

Recently Miyairi has informed us that the absolute configuration of I (enterocin) was determined by an X-ray analysis as shown with S-configuration at C_3.[11]

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