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STUDIES ON THE IONOPHOROUS ANTIBIOTICS X[1]
THE ASSIGNMENT OF ^{13}C-NMR SPECTRUM OF SALINOMYCIN

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Although ^{13}C-nmr spectroscopy is becoming one of the most powerful tools for the structural determination of natural products, it has never found its practical use in the structural elucidation of ionophorous antibiotics. The lack of suitable model compounds as well as of degradation products hampered to obtain the basic chemical shift data necessary for the assignment of the ^{13}C-nmr spectra of the antibiotics.

Recently, Dorman et al.[2] reported the assignment of narasin(4-methylsalinomycin). Half of their assignment, however, were not made on one-to-one basis, and furthermore, in view of our experimental results, half of the signals were incorrectly assigned.

We wish to report herein the almost complete assignment of a polyether antibiotic, salinomycin[3], assisted by the use of ^{13}C-^{13}C coupling[4].

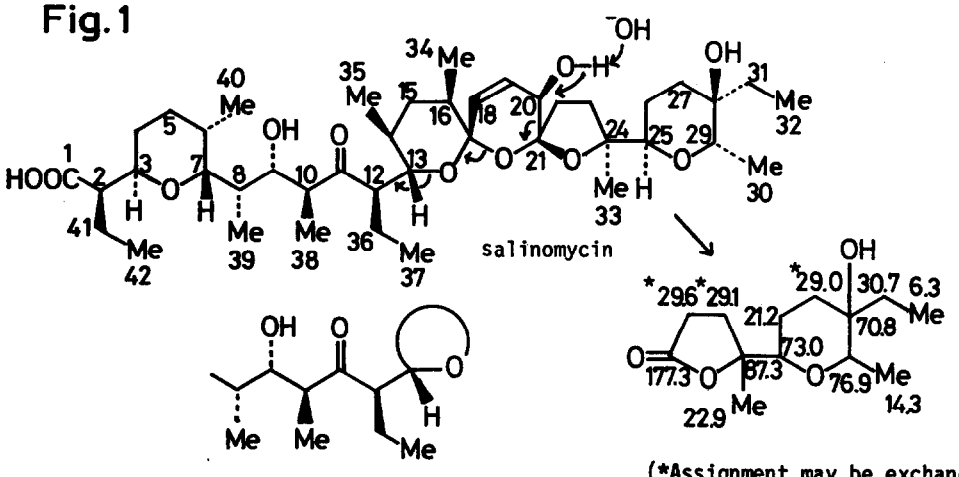
In the ^{13}C-nmr spectrum of salinomycin free acid[5](SLH), $C_{42}H_{70}O_{11}$(Fig. 1), all 42 carbon signals were well resolved (Table 1). Based on the chemical shift trend, these signals were assigned to a ketone (C_{11} 214.5), a carboxylic acid (C_1 177.2), two olefinic carbons (C_{18} and C_{19}, 121.6 and 132.4), two ketal carbons (C_{17} and C_{21}, 99.2 and 106.4), nine oxygenated carbons (88.5-67.2), three methines adjacent to a carbonyl group (56.5-48.9), four methines, ten methylenes and ten methyls.

The two olefinic and two ketal carbons were distinguished respectively by comparison of SLH and 20-deoxysalinomycin free acid[6](DSLH). (SLH → DSLH, C_{18} 121.6 → 121.8, C_{19} 132.4 → 125.6, C_{17} 99.2 → 99.0, C_{21} 106.4 → 105.0). In addition, the replacement of a sec-carbinyl carbon (67.2 in SLH) by a methylene (40.0 in DSLH) established the assignment of C_{20} in SLH.

The three methines adjacent to a carbonyl carbon were assigned as follows. The comparison[7] of SLH and its sodium salt (SLNa) indicated a signal at 48.9 in SLH to be assigned to C_2 which appeared at 51.1 in SLNa. The structural similarity around a ketone in common to SLH, lysocellin[8] and lasalocid[9] (Fig. 1) required signals at 49.2 and 56.5 to be assigned to C_{10} and C_{12}, respectively. These carbons had been differentiated based on the chemical shift of ketones obtained from lysocellin[10] and lasalocid[9,11] by retroaldol reaction.

Unlike to these two antibiotics, alkaline treatment of SLH gave, instead of the expected retroaldol ketone, a γ-lactone ($C_{13}H_{22}O_4$, M^+ m/e 242, $\nu_{max}^{CDCl_3}$ 3440, 1765cm^{-1}) via mechanism shown in Fig. 1. This γ-lactone, the assignment of which was made mainly based upon selective proton

Fig. 1



decoupling, assisted to establish the assignment of C_{24} to C_{33} in SLH.

Since several efforts to obtain degradation products or derivatives of SLH useful for the assignment were unsuccessful, we next turned to the use of the double labeling method[4] using sodium 1,2-^{13}C-propionate and sodium 1,2-^{13}C-acetate to advance further assignment of SLH.

In the ^{13}C-nmr spectrum of SLH labeled with 1,2-^{13}C-propionate, six pairs of ^{13}C-^{13}C couplings were observed. Among these, two AB-type couplings between a methylene and a methine had been previously assigned[1] to C_5(26.4)-C_6(28.0), $J_{C-C}\fallingdotseq$33Hz and C_{15}(38.6)-C_{16}(40.7), $J_{C-C}\fallingdotseq$32Hz by taking the chemical shift of cis-3,5-dimethyltetrahydropyran into consideration. A coupling between an oxygenated quartery carbon, C_{24}(88.5) and a methylene established the assignment of C_{23}(30.2, J_{C-C}=34.8Hz). Of the three pairs between a methine and an oxygenated methine, one is easily assigned to C_{10}(49.2) and C_9(68.7, $J_{C-C}\fallingdotseq$42Hz). On elimination of the already established relationships, the two remaining pairs, i.e. CH-O(75.2)-CH(32.6), $J_{C-C}\fallingdotseq$36Hz and CH-O(71.7)-CH (36.5), $J_{C-C}\fallingdotseq$41Hz were limited to C_7-C_8 and C_{13}-C_{14}. In salinomycin methyl ester[12] (SLMe), the signal at 75.2 moved downfield by 3.9ppm[13]. This signal is therefore assigned to C_7 due to its sterical proximity to C_1. Thus, the assignment of C_7, C_8(32.6), C_{13}(71.7) and C_{14}(36.5) was established. The relatioship of C_7-C_8 and C_9-C_{10} mentioned above were confirmed by ^{13}C-homo nuclear spin decoupling.

In the ^{13}C-nmr spectrum of SLH labeled with 1,2-^{13}C-acetate, 12 pairs of coupling with strong intensity were observed[14] and eight out of these pairs were found between carbon signals, the assignment of which had been already explained (C_1-C_2, C_{11}-C_{12}, C_{17}-C_{18}, C_{19}-C_{20}, C_{25}-C_{26}, C_{27}-C_{28}, C_{29}-C_{30} and C_{31}-C_{32}). The comparison of SLH and 4-methyl SLH[1] had revealed the relation of C_3(74.9)-C_4(20.1), J_{C-C}=35.9Hz.

Of three remaining pairs, one was easily assigned to C_{21}(106.4) and a methylene, C_{22}(36.2) due to its characteristic coupling constant (J_{C-C}=43.5Hz), leaving CH_2(22.7), CH_2(16.6), CH_3 (13.2) and CH_3(11.9) to be connected. Although the ^{13}C-^{13}C couplings of these four carbons were of the same magnitude ($J_{C-C}\fallingdotseq$35Hz), the AB-type coupling between CH_2(16.6)-CH_3(13.2) revealed the relationship of CH_2(22.7)-CH_3(11.9). These partial structures were only found at C_{36}-C_{37} and C_{41}-C_{42}. Of these, the signal at 16.6 was assigned to C_{41} since it moved downfield by 2.7ppm on

TABLE 1**

carbon	SLH*	SLNa*	DSLH*	SLMe*	J_{C-C}^{Pr*}	J_{C-C}^{Ac*}
1	177.2	184.8	177.1	175.8		56.0
2	48.9	51.1	48.2	47.8		55.6
3	74.9	75.8	74.7	74.9		35.9
4	20.1	19.7	20.1	19.7		35.7
5	26.4	26.8	26.6	26.2	33.2[+]	
6	28.0	28.0	28.1	28.1	32.6[+]	
7	75.2	75.6	75.6	79.1	35 [#,a]	
8	32.6	32.5	32.7	33.2	36.3[a]	
9	68.7	67.8	68.5	69.1	41 [#,b]	
10	49.2	49.6	49.6	48.4	43 [#,b]	
11	214.5	217.6	213.8	213.5		38.9
12	56.5	55.5	56.3	56.7		38.8
13	71.7	71.3	71.6	71.7	41.2	
14	36.5	35.9	36.2	36.5	40.9	
15	38.6	38.6	38.8	38.9	32.9[++]	
16	40.7	40.6	41.0	40.4	32.0[++]	
17	99.2	99.1	99.0	99.0		50.1
18	121.6	122.1	121.8	120.8		50.2
19	132.4	130.8	125.6	132.6		44.6
20	67.2	66.6	40.0	67.5		44.6
21	106.4	107.1	105.0	106.2		43.5
22	36.2	36.1	33.1	36.5		43.5
23	30.2	32.5	30.0	30.3	34.8	
24	88.5	88.3	88.5	87.8	34.8	
25	73.7	74.6	74.1	74.2		36.7
26	21.9	19.9	22.2	22.1		37.1
27	29.3	29.1	29.7	29.2		37.6
28	70.9	70.5	71.3	70.7		37 [#]
29	77.2	76.5	76.4	76.7		37.2
30	14.5	14.7	14.4	14.6		37.1
31	30.6	32.1	31.9	30.8		35.1
32	6.3	6.5	6.5	6.3		35.2
33	25.8	27.7	25.8	25.8		
34	17.9	17.6	17.9	17.6		
35	15.6	15.7	15.7	15.6		
36	22.7	23.7	22.7	22.6		34.7
37	11.9	12.4	11.9	11.8		35.2
38	12.8	12.1	13.2	12.9		
39	7.0	6.8	7.0	7.3		
40	11.2	10.7	11.2	11.0		
41	16.6	15.7	16.6	19.3		34.7[+++]
42	13.2	13.2	13.4	13.9		34.7[+++]
OCH_3				52.5		

**Determined on a JEOL FX-100 nmr spectrometer operating at 25.05MHz, in $CDCl_3$, relative to internal TMS. Data points 16K, flip angle ~70°, spectral width; for measurement of $^{13}C-^{13}C$ coupling constants, 2.5KHz, for whole spectra, 6KHz.

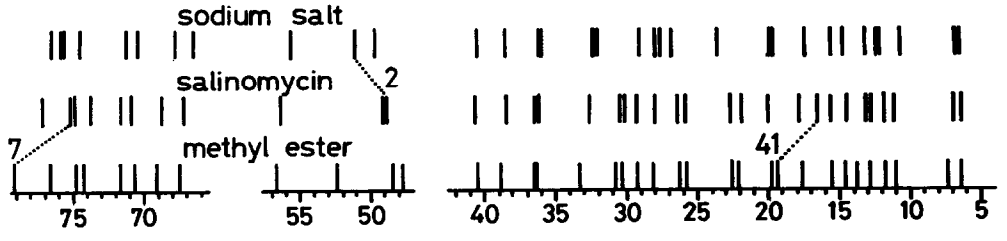
*abbreviations; SLH:salinomycin free acid, SLNa:salinomycin sodium salt, DSLH:20-deoxy salinomycin free acid, SLMe:salinomycin methyl ester, J_{C-C}^{Pr} and J_{C-C}^{Ac}:$^{13}C-^{13}C$ coupling constants in Hz observed with SLH labeled by 1,2-^{13}C-propionate and 1,2-^{13}C-acetate, respectively.

+,++, +++ AB-type $^{13}C-^{13}C$ coupling was observed between these signals. Weak intensities of the outer signals of these AB quartets prevented to obtain accurate coupling constants.

Due to the overlapping of other signals, the correct values could not be obtained.

a,b The relationship of these carbons were confirmed by $^{13}C-^{13}C$ spin decoupling experiments.

Fig.2



going from SLH to SLMe. Thus, the methyl at 13.2 must be assigned to C_{42}, the pair of C_{36}(22.7) -C_{37}(11.9) being established by elimination.

Now at this point, the unassigned are five methyl signals (C_{34}, C_{35}, C_{38}, C_{39} and C_{40}). C_{40} had been assigned to a signal at 11.2 based on the comparison with 4-methyl SLH[1]. The structural similarity of SLH, lasalocid and lysocellin suggested a signal at 12.8 to be due to C_{38}[15].

Among the three finally remained methyl signals (7.0, 15.6 and 17.9), one appeared due probably to a strong γ-effect at considerably higher field than usually observed for CH_3-CH< in polyether antibiotics[16]. This type of steric compression is expected between C_9-OH and C_{39}. Since the crystal structure of SLH itself was not determined, no evidence was available about the conformational disposition between C_{39} and C_9-OH in SLH. However, the crystal structures of p-bromophenacyl ester[3] of salinomycin and of lysocellin silver salt[8] showed such conformational relationships really existed[17] and the carbon corresponding to C_{39} in lysocellin sodium salt had been assigned to 5.4[10]. Of the two carbons (C_{34} and C_{35}), C_{35} is expected to move higher field due to the γ-effect by a substituent at C_{13}. Thus, signals at 15.6 and 17.9 were assigned to C_{35} and C_{34}, respectively.

The established assignment of SLH and SLNa has revealed the biosynthetic origin[18] of SLH and is expected to be of use for the conformational analysis in solution of polyether antibiotics as well as for the structural determination of salinomycin related compounds to be isolated in future.

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REFERENCES AND FOOTNOTES

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13) This downfield shift (79.2 in SLMe) of the signal at 75.2 in SLH and not of one at 77.2 was confirmed by comparison of the ^{13}C-nmr spectra of SLH and SLMe both labeled with 1-^{13}C-propionate, since the former signal was ^{13}C-enriched while the latter was not.
14) In addition, ^{13}C-^{13}C couplings with weaker intensity was also observed with the rest of the carbon signals due to randomization.
15) H. Seto and J. W. Westley; manuscript in preparation.
16) H. Seto; unpublished data.
17) The conformational relationship between C_{39} and C_9-OH in salinomycin was more accurately represented in ref. 3b than in ref. 3a.
18) The biosynthetic studies on salinomycin will be reported in a full paper of this work.