THE STRUCTURE OF HIDAMICIN, A POLYETHER ANTIBIOTIC

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Hidamicin, a member of polyether antibiotic,[1] was found in the fermentation broth of an actinomycetes, MI429-38F1, which had been isolated from a soil sample collected at Takayama-city, Gifu Prefecture, Japan. The strain MI429-38F1 was shown to be closely related to *Actinomadura verrucosospora*[2] by taxonomic studies. The antibiotic was purified using centrifugal partition chromatography and crystallized from acetone - hexane. The structure of hidamicin was determined by NMR spectrometry. We report the isolation and characterization of hidamicin and show the structure determination by the use of advanced techniques of NMR spectroscopy.[3]

The slant culture of the producing organism was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of a seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, corn steep liquor 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.32% (adjusted to pH 7.4 before sterilization). The inoculated medium was incubated at 30°C for 96 hours with a rotary shaker. The seed culture thus obtained was inoculated to 500-ml Sakaguchi flasks each containing 125 ml of a producing medium. The producing medium was composed of beef extract 0.3%, yeast extract 0.5%, Tryptose (Difco) 0.5%, glucose 1.0% and agar (Difco) 0.15% (adjusted to pH 7.0 before sterilization). The fermentation was carried out at 27°C for 96 hours with a reciprocating shaker.

The cultured broth thus obtained was separated to clear filtrate (8,440 ml) and mycelium cake. The broth filtrate was extracted with EtOAc (5,000 ml×2) and the organic layer was dried over Na_2SO_4. After filtration, the EtOAc layer was concentrated to dryness to give 390.6 mg of oily material. The oily material containing the antibiotic was subjected to centrifugal partition chromatography (CPC). The chromatography was performed using a CPC apparatus model NMF (Sanki Engineering Limited) with a solvent system of acetonitrile - hexane. The bioactive fraction against *Bacillus subtilis* PCI 219 was collected and concentrated to give 72.0 mg of a colorless solid, which was dissolved in 50 ml of EtOAc and washed with 0.5 M HCl (30 ml×2) followed by washing with saturated Na_2CO_3 (40 ml×2) to form the sodium salt of the antibiotic. The EtOAc solution was dried over Na_2SO_4, filtered and concentrated to dryness. The residual solid was crystallized from acetone - hexane to give 44.5 mg of hidamicin Na-salt (colorless prism).

The mycelium cake was extracted with 2,500 ml of MeOH and the MeOH solution was concentrated to about half of the volume giving white precipitate (517.4 mg) of crude hidamicin. The crude powder (460 mg) was changed to the Na salt in the same way described above and crystallized to give 356.0 mg of hidamicin Na-salt.

Physico-chemical properties of hidamicin Na-salt are summarized in Table 1. Chemical shifts in the ^{13}C and ^{1}H NMR spectra of hidamicin Na-salt in C_6D_6 are shown in Table 2. The connectivities between carbons and protons *via* one-bond J_{C-H} coupling were found from the ^{1}H-

Table 1. Physico-chemical properties of hidamicin Na-salt.

FD-MS	*m/z* 807 (M+1)
Analysis	
Calcd for $C_{42}H_{71}O_{13}Na$:	C 62.51, H 8.87
Found:	C 62.45, H 8.82
$[\alpha]_D^{22}$	−31.1° (*c* 1.0, $CHCl_3$)
MP	196~199°C (dec)
IR (KBr) cm^{-1}	3400, 2980, 2890, 1620, 1595, 1465, 1385, 1250, 1205, 1105, 960, 935, 740, 710

FD-MS: Field desorption MS.

Table 2. ^{13}C and ^{1}H NMR chemical shifts of hidamicin Na-salt in C_6D_6.

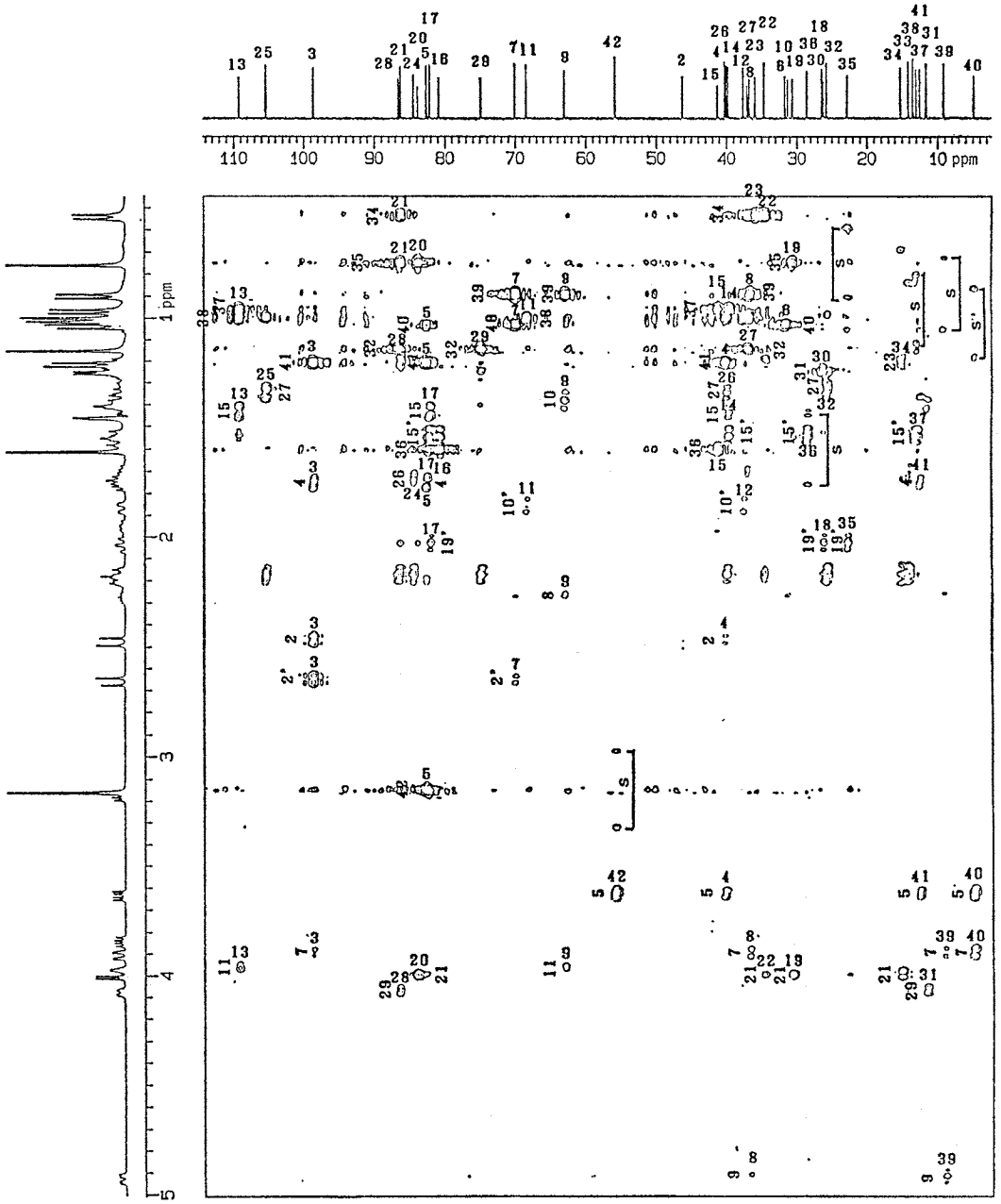
Position	δ_C (ppm)	δ_H (ppm)	Assignment
1	180.29		COONa
2	46.27	2.48 (d, 13.0),* 2.67 (d, 13.0)	CH_2
3	98.70		C(O) (O)
4	40.26	1.75	CH
5	82.67	3.64 (dd, 10.6, 4.6)	CH(O)
6	31.72	2.22	CH
7	70.09	3.91 (dd, 10.6, 1.8)	CH(O)
8	36.78	2.28	CH
9	63.07	4.93	CH(O)
10	31.34	1.39, 1.87	CH_2
11	68.48	3.97	CH(O)
12	37.62	1.54	CH
13	109.28		C(O) (O)
14	39.80	1.95	CH
15	41.33	1.42, 1.55	CH_2
16	80.89		C(O)
17	82.19	3.18	CH(O)
18	26.23	1.26, 1.57	CH_2
19	30.67	1.01, 2.04	CH_2
20	83.87		C(O)
21	86.33	4.01 (d, 4.2)	CH(O)
22	34.70	1.72	CH
23	35.98	1.20, 2.16	CH_2
24	84.49	3.85 (dd, 10.6, 5.8)	CH(O)
25	105.41		C(O) (O)
26	40.05	1.70	CH
27	37.07	1.35, 2.16	CH_2
28	86.58		C(O)
29	74.92	4.07 (br d, 7.8)	CH(O)
30	26.41	1.28, 1.42	CH_2
31	11.60	1.26 (m)	CH_3
32	25.80	1.16 (s)	CH_3
33	14.14	1.01 (d, 6.6)	CH_3
34	15.22	0.55 (d, 7.0)	CH_3
35	22.83	0.77 (s)	CH_3
36	28.57	1.62 (s)	CH_3
37	13.04	0.97 (d, 6.6)	CH_3
38	13.50	1.02 (d, 7.6)	CH_3
39	9.14	0.91 (d, 7.0)	CH_3
40	4.91	1.04 (d, 7.2)	CH_3
41	12.52	1.22 (d, 6.6)	CH_3
42	55.88	3.16 (s)	OCH_3

* Proton signal multiplicity and coupling constant (J=Hz).

^{13}C shift correlation spectrum. The multiplicity of each carbon signal in the ^{13}C NMR spectrum was determined by the distortionless enhancement by polarization transfer (DEPT) spectrum. The ^{13}C NMR data showed that this antibiotic belonged to the polyether antibiotics group 3a according to the classification proposed by SETO and ŌTAKE.[4]

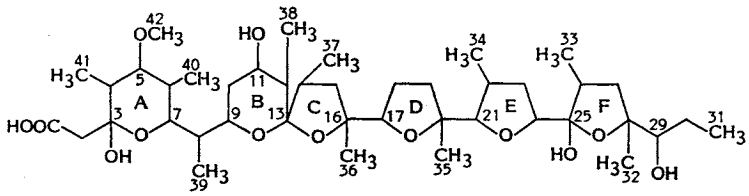
The result of the heteronuclear multiple-bond correlation (HMBC) spectrum is shown in Fig. 1. The HMBC correlations, which showed connectivities between carbons and protons *via* two- and three-bond J_{C-H} coupling, were useful in determining the structure of this antibiotic. As

Fig. 1. HMBC spectrum of hidamicin Na-salt in C_6D_6.
s: Incompletely suppressed signals from one-bond correlations.



shown in Fig. 1, the HMBC correlations were sufficient to connect C1-C2-C3-C4(C41)-C5(O-C42)-C6(C40)-C7-C8(C39)-C9, C11-C12(C38)-C13-C14(C37)-C15-C16(C36)-C17, C19-C20(C35)-C21-C22(C34)-C23 and C25-C26(C33)-C27-C28(C32)-C29-C30-C31. The following connectivities were elucidated by ^{1}H-^{1}H shift correlation spectrum (COSY); H9-H10/H10′-H11, H17-H18/H18′, H18′-H19/H19′, H23/H23′-H24. (In the case of two nonequivalent methylene protons, we refer to the proton in higher field as Ha and to the other one as Ha′.) Four OH signals were observed in ^{1}H NMR spectrum of hidamicin (6.22, 6.37, 7.38 and 8.44 ppm). One

Fig. 2. Structure of hidamicin.



drop each of H_2O and D_2O were added to the NMR sample in C_6D_6 to observe ^{13}C NMR deuterium induced upfield shift (DIS) by the exchange of OH and OD.[5] Splitting and broadening of ^{13}C NMR signals were observed at C2, C3, C4, C10, C11, C12, C24, C25, C29 and C30. Among these, C3, C11, C25 and C29 were thought to bear a hydroxyl group each as a result of their DIS values and chemical shifts.

In general, it is difficult to elucidate ether linkages in polyether antibiotics from three-bond J_{C-H} couplings by the HMBC method.[3] In the case of hidamicin, only one cyclic ether linkage in a six membered ring (A-ring) was deduced from the HMBC spectrum (C3-O-C7-H7). Other cyclic ether structures in this molecule were proposed in comparison with ^{13}C NMR chemical shifts of other known polyether antibiotics.[4] The connection of C9-O-C13 to form six membered cyclic ether B-ring was deduced from the chemical shift of C9 (63.07 ppm). The C13-O-C16 linkage to form five membered C-ring was deduced from C13 and C16 chemical shifts (109.28 and 80.89 ppm, respectively). The structures of D-ring and E-ring were proposed on the basis of the ^{13}C NMR chemical shifts of C17~C24, which were similar to those of the D- and E-ring in mutalomycin.[4] Finally, the ether linkage C25-O-C28 to form the five membered F-ring was proposed, because C29 was shown to bear a hydroxyl group from the deuterium induced shift experiment described above. The five membered structure of F-ring was supported by the ^{13}C NMR chemical shifts compared with the ^{13}C NMR chemical shifts of C21~C24 in portmicin.[3] Thus, the structure of hidamicin was proposed as shown in Fig. 2.

The planar structure of hidamicin determined by the present NMR studies was found to be identical with that of CP-51,532, of which the molecular formula was reported to be $C_{43}H_{68}O_{13}$.[6] Hidamicin seems to be identical with CP-51,532 from the physico-chemical and biological properties.[6,7] The molecular formula must be corrected as $C_{42}H_{72}O_{13}$.

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