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Inclusion Complex of a New Orally Active Cephalosporin ME1207 with β-Cyclodextrin

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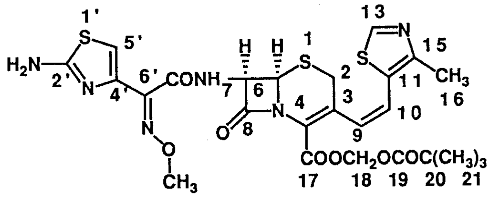
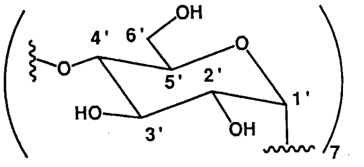
The structure of a 1:1 inclusion complex of β-cyclodextrin (β-CD) and a new orally active cephalosporin, pivaloyloxymethyl 7-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3(Z)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate (ME1207), was estimated by nuclear magnetic resonance (NMR) analysis. The experimental results indicated that the *tert*-butyl moiety of ME1207 was selectively inserted into the β-CD cavity.

Keywords cephalosporin; oral cephalosporin; inclusion complex; NMR analysis; β-cyclodextrin; dissociation constant

In a previous paper,[1,2] we reported that pivaloyloxymethyl 7-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3(Z)-(4-methylthiazol-5-yl)vinyl-3-cephem-4-carboxylate (ME1207) was a new orally active cephalosporin and that the free acid ME1206, an active form of ME1207, showed potent and broad antibacterial activity against both gram-positive and gram-nagative bacteria. In the course of studying the clinical evaluation of ME1207, it was found that the combination of ME1207 with β-cyclodextrin (β-CD) improves the urinary recovery of ME1207 after oral administration in dogs.[3] This result may come from the increasing solubility of ME1207 in water, presumably due to inclusion complex formation. In this paper, we report a structural evaluation of the complex of ME1207 and β-CD.[4]



ME1207



β-cyclodextrin (β-CD)

Fig. 1. Structure and Numbering of ME1207 and β-CD

Results and Discussion

Secondary ion mass spectrometry (SIMS) of the 1:1 complex of the β-CD–ME1207 using dithiothreitol–dithioerythriol (5:1) as a matrix revealed a distinct peak at m/z 1755 $(M+H)^+$ of the β-CD–ME1207 inclusion complex.

TABLE I. Chemical Shifts (δ_H ppm) of β-CD (0.065 N DCl Solution) and β-CD–ME1207 ([β-CD]/[ME1207] = 4.8 mM/4.8 mM, 0.065 N DCl Solution)

	β-CD	β-CD–ME1207	Δδ[a)]
H-1′	4.942	4.927	−0.018
H-2′	3.523	3.521	−0.002
H-3′	3.830	3.769	−0.061
H-4′	3.452	3.446	−0.006
H-5′	3.729	3.679	−0.050

a) Chemical shift displacement (δβ-CD–ME1207–δβ-CD).

TABLE II. Induced Chemical Shift Changes (δ_H ppm) of ME1207 at Various Molar Ratios ([ME1207]/[β-CD])

[ME1207]:[β-CD]	H-21	H-16	H-2	OCH_3	H-7	H-18-1	H-18-2	H-6	H-9	H-10	H-5′	H-13
1.00:0.00	1.000	2.429	3.348	3.948	5.279	5.682	5.767	5.764	6.607	6.705	7.034	9.580
1.00:0.25	1.027	2.435	3.354	3.950	5.282	5.687	5.757	5.773	6.607	6.706	7.035	9.584
Δδ (ppm)	0.027	0.006	0.006	0.002	0.003	0.005	−0.010	0.009	0.000	0.001	0.001	0.004
1.00:0.50	1.047	2.437	3.355	3.947	5.280	5.687	5.745	5.777	6.608	6.703	7.034	9.595
Δδ (ppm)	0.047	0.008	0.007	−0.001	0.001	0.005	−0.022	0.013	0.001	−0.002	0.000	0.015
1.00:0.75	1.057	2.439	3.357	3.947	5.280	5.688	5.739	5.779	6.609	6.702	7.031	9.596
Δδ (ppm)	0.057	0.010	0.009	−0.001	0.001	0.006	−0.028	0.015	0.002	−0.003	−0.003	0.016
1.00:1.00	1.067	2.440	3.359	3.947	5.281	5.688	5.734	5.782	6.609	6.702	7.033	9.600
Δδ (ppm)	0.067	0.011	0.011	−0.001	0.002	0.006	−0.033	0.018	0.002	−0.003	−0.001	0.020
1.00:1.25	1.070	2.440	3.359	3.946	5.280	5.688	5.732	5.783	6.608	6.702	7.032	9.600
Δδ (ppm)	0.070	0.011	0.011	−0.002	0.001	0.006	−0.035	0.016	0.001	−0.003	−0.002	0.020
1.00:1.50	1.074	2.442	3.359	3.947	5.281	5.689	5.731	5.783	6.609	6.702	7.033	9.601
Δδ (ppm)	0.074	0.013	0.011	−0.001	0.002	0.007	−0.036	0.019	0.002	−0.003	−0.001	0.021
1.00:2.00	1.078	2.442	3.361	3.946	5.280	5.689	5.728	5.785	6.608	6.701	7.032	9.600
Δδ (ppm)	0.078	0.013	0.013	−0.002	0.001	0.007	−0.039	0.021	0.001	−0.004	−0.002	0.020
1.00:2.50	1.081	2.442	3.360	3.945	5.280	5.688	5.727	5.786	6.607	6.701	7.031	9.602
Δδ (ppm)	0.081	0.013	0.012	−0.003	0.001	0.006	−0.040	0.022	0.000	−0.004	−0.003	0.022
1.00:3.00	1.084	2.444	3.362	3.947	5.282	5.690	5.727	5.787	6.609	6.702	7.034	9.603
Δδ (ppm)	0.084	0.015	0.014	−0.001	0.003	0.008	−0.040	0.023	0.002	−0.003	0.000	0.023
1.00:4.00	1.088	2.444	3.363	3.947	5.282	5.691	5.726	5.788	6.609	6.702	7.033	9.602
Δδ (ppm)	0.088	0.015	0.015	−0.001	0.003	0.009	−0.041	0.024	0.002	−0.003	−0.001	0.022

This data indicates that a β-CD–ME1207 inclusion complex exists as a stable 1 : 1 inclusion complex in the gaseous phase. The structure of β-CD–ME1207 in D_2O (0.065 N DCl solution, pD = about 1.2) was intensively studied by 400 MHz nuclear magnetic resonance (NMR) spectroscopy.

The chemical shift changes of β-CD in the β-CD–ME1207 complex are listed in Table I. H-3′ (0.06 ppm) and H-5′ (0.05 ppm) protons which are located inside the cavity of β-CD tours shift more than those of the other protons on the outer surface by the addition of a guest molecule. This implies that the guest molecule is inserted into the cavity of β-CD.[5)]

Table III. Chemical Shifts (δ_C ppm) of ME1207 (0.0656 N DCl Solution) and β-CD–ME1207 ([β-CD]/[ME1207] = 4.8 mM/4.8 mM, 0.065 N DCl Solution)

	ME1207	ME1207–β-CD	$\Delta\delta_C$ (ppm)
C-2	29.13	29.17	0.04
C-4	125.70	125.70	0.00
C-6	60.49	60.52	0.03
C-7	58.42	58.45	0.03
C-8	165.81	165.42	−0.39
C-9	132.01	132.16	0.15
C-10	120.63	120.67	0.04
C-11	132.99	132.55	−0.44
C-13	156.57	156.67	0.10
C-15	145.63	145.57	−0.06
C-16	12.94	13.01	0.07
C-17	163.09	163.13	0.04
C-18	81.90	81.65	−0.25
C-19	180.58	179.58	−1.00
C-20	39.62	39.78	0.16
C-21	27.09	27.75	0.66
C-2′	143.35	143.37	0.02
C-5′	112.16	112.19	0.03
C-6′	162.19	162.01	−0.18
C-7′	171.93	171.93	0.00
OCH_3	64.96	64.96	0.00

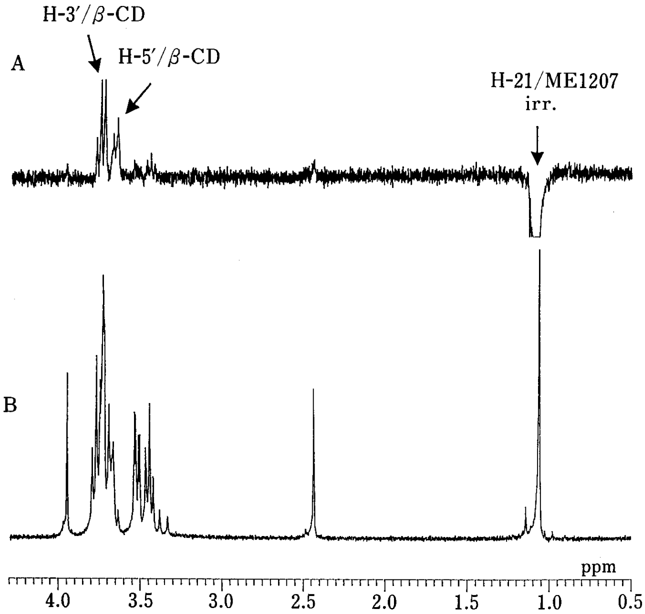


Fig. 2. ROE Difference Experiments with β-CD–ME1207 Complex

(A) Irradiation at H-21 of ME1207; (B) 400 MHz ^{1}H-NMR spectrum ([β-CD]/[ME1207] = 4.8 mM/4.8 mM, 0.065 N DCl solution).

The chemical shift changes of ME1207 induced by added β-CD are illustrated in Table II in various molar ratios ([ME1207]/[β-CD]). Upon the addition of β-CD, a downfield shift of the H-18 proton and an upfield shift of the H-21 proton in ME1207 were also observed. These shifts were larger in comparison with those of other protons in ME1207, suggesting that the pivaloyl group of C-4 of ME1207 is selectively inserted into the β-CD cavity. The magnitude of the shifts of H-18 and H-21 increased as a function of an increasing ratio of [β-CD]/[ME1207] until the ratio became 1/1, indicating that a rapid complexation process

$$\text{ME1207} + \beta\text{-CD} \rightleftharpoons [\text{ME1207–}\beta\text{-CD}]$$

was achieved. A modified Hildebrand-Benesi plot[5)] of H-21 shift data of ME1207 in the form of [β-CD]/$\Delta\delta$ *vs.* [ME1207] gave an excellent linear line. The dissociation constant of this inclusion complex, K_d, was calculated to be 8.06×10^{-4} M by a least-squares fitting procedure from the plots.

These results supported the proposal that β-CD and ME1207 form a 1 : 1 inclusion complex. In addition to the larger shifts of H-18 and H-21 in ^{1}H-NMR of the complex, ^{13}C-NMR revealed noticeably larger shifts of C-19 (upfield shift, 1.00 ppm) and C-21 (downfield shift, 0.66 ppm) of ME1207 compared to other carbons (Table III).

These larger shifts also imply selective insertion of a pivaloyl group into the β-CD cavity. This is supported from the NMR measurement using the rotating-frame Overhauser enhancement (ROE)[6,7)] technique. Selective irradiation at H-21 of ME1207 in the complex produced positive signals for both H-3′ and H-5′ of β-CD (Fig. 2), implying that the protons on *tert*-butyl (H-21) of the guest molecule underwent a comparable degree of intermolecular dipolar relaxation inside the β-CD cavity.

In conclusion, this data indicates that ME1207 forms a 1 : 1 inclusion complex with β-CD in which the *tert*-butyl entity of ME1207 is selectively inserted into the β-CD cavity.

Experimental

Materials β-Cyclodextrin (β-CD) was purchased from Tokyo Kasei Kogyo Co., Ltd. and used without further purification. ME1207 was synthesized by the method described in previous reports[1,2)] (95% purity). D_2O (minimum isotopic purity 99.96 atom% D) and 20% DCl solution in D_2O (99 atom% D) were used as solvents. These deuterated compounds were purchased from Aldrich Chemical Company, Inc.

Methods ^{1}H (resolution, 0.00065 ppm) and ^{13}C (resolution, 0.016 ppm) NMR spectra were taken on a JEOL GSX-400 spectrometer. Dioxane ($\sigma_C = 67.4$ ppm) and sodium 3-(trimethylsilyl)-1-propansulfonate ($\sigma_H = 0.00$ ppm) were used as internal standards for ^{13}C and ^{1}H-NMR in D_2O, respectively. Chemical shifts are recorded in σ values (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad) and coupling constants in hertz (Hz). ^{1}H–^{13}C NMR shift correlation spectra (COSY) were obtained with JEOL standard software. Assignments of all ^{1}H and ^{13}C chemical shifts of ME1207 in D_2O (pD = about 1.2) were unambiguously carried out by decoupling experiments including ^{1}H–^{1}H and ^{1}H–^{13}C COSY measurements. SIMS was taken with a Hitachi M-80B mass spectrometer.

Preparation of ME1207 and β-CD Inclusion Complex The inclusion complex of ME1207 and β-CD was prepared by the addition of equimolar ME1207 to β-CD in 0.065 N of a DCl solution (pD = about 1.2). From ^{1}H-NMR spectrum, ME1207 in this solution was stable for 1 d at room temperature. The 0.065 N DCl solution was prepared from 20% DCl solution in D_2O (99% atom% D) and D_2O (minimum isotopic purity 99.96 atom% D).

Determination of ME1207–β-CD Dissociation Constant by ^{1}H-NMR

The concentration of ME1207 was held constant at 4.8 mM, and β-CD concentration varied between 0.00—19.2 mM. The change in chemical shift of the H-21 proton of ME1207 was measured as a function of changing β-CD concentration. The data was treated according to a modified Hildebrand-Benesi equation.

References and Notes

1) K. Sakagami, K. Atsumi, A. Tamura, T. Yoshida, K. Nishihata, and S. Fukatsu, *J. Antibiot.*, **43**, 1047 (1990).
2) K. Sakagami, K. Atsumi, Y. Yamamoto, A. Tamura, T. Yoshida, K. Nishihata, and S. Fukatsu, *Chem. Pharm. Bull.*, **39**, 2466 (1991).
3) T. Kikkoji, T. Kobayashi, T. Sato, K. Watanabe, and K. Nishizawa, Eur. Patent Apple. EP 339465 (1989) [*Chem. Abstr.*, **113**, 65243*c* (1990)].
4) After this work, similar study of α-CD and penicillin V inclusion complexation was reported; H. Qi, V. Mark, L. Diaz, D. M. Grant, and C. Chang, *J. Org. Chem.*, **56**, 1537 (1991).
5) Y. Yamamoto and Y. Inoue, *J. Carbohyd. Chem.*, **8**, 29 (1989).
6) A. Bax and D. G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).
7) H. Kessler, C. Griesinger, R. Kerssebaum, K. Wagner, and R. R. Ernst, *J. Am. Chem. Soc.*, **109**, 607 (1978).