The Structure of Galantinamic Acid, a New Amino Acid in a Peptide Antibiotic Galantin I

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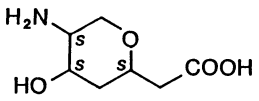
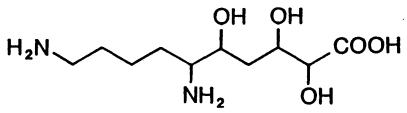
Synopsis. A new amino acid named galantinamic acid was found as one of the constituent amino acids in a peptide antibiotic galantin I. The primary structure of galantinamic acid was determined chemically and spectrometrically to be 6,10-diamino-2,3,5-trihydroxydecanoic acid.

A peptide antibiotic galantin I was isolated from a culture broth of *Bacillus pulvifaciens* by Shoji et al.[1] The structure of galantin I was determined as shown in Fig. 1, in which we recognized two new amino acids, i.e., galantinic acid (**1**) and galantinamic acid (**2**).[2] An absolute structure of galantinic acid was determined chemically, spectrometrically,[3] and finally synthetically[4] to be (2*S*,4*S*,5*S*)-5-amino-2-carboxymethyl-4-hydroxytetrahydropyran. In this paper, we describe the determination of primary structure of galantinamic acid.

An isolation of galantinamic acid was carried out by ion-exchange column chromatography of the acid hydrolyzate of galantin I. For preparation of galantinamic acid, the acid hydrolysis of the peptide was ceased for 10 h in order to avoid an easy decomposition of this amino acid during the hydrolysis.[5] The observed pK_a values, 3.2, 8.7, and 10.5, for galantinamic acid suggested that this amino acid must be a diamino monocarboxylic acid but not an α-amino acid whose pK_1' value is generally around 2.4. The molecular formula of galantinamic acid was deduced to be $C_{10}H_{22}N_2O_5$ from the results of an elemental analysis and a determination of molecular weight by FD-MS spectrometry (m/z=251 $(M+H)^+$). This fact suggested the presence of three hydroxyl, two amino and one carboxyl groups in the molecule.



1 Galantinic acid



2 Galantinamic acid

The result of ^{1}H NMR analysis of galantinamic acid was summarized in Table 1. Four methine carbon atoms must carry OH or NH_2 as judged from the values of chemical shifts of their protons. Of these methine proton signals, a characteristic one appearing as doublet at δ 3.91 was first assigned to be α-methine proton connected to a carboxyl group suggesting us a presence of a partial structure such as -CH(**Y**)-CH(**X**)-COOH (**X**,**Y**=OH or NH_2). Since galantinamic acid is not an α-amino acid as described above, **X** is now deduced to be OH. The other proton at the highest field (δ 3.39) was assigned to be -CH(NH_2)-, since an irradiation to this proton caused a collapse at regions of two methine protons centered at δ 4.13 and of methylene protons centered at about δ 1.8 corresponding to -C-CH_2-C-. However, this proton did not couple with α-methine proton at δ 3.91. Thus, we could assign OH for **Y** to lead another partial structures of -C-CH_2-CH(NH_2)-CH(OH)- as well as -CH(OH)-CH(OH)-COOH. Furthermore, methylene signal at δ 3.01 was assigned to be NH_2CH_2- adjacent to -CH_2-C- type of methylene from the value of chemical shift and the coupling mode.

When we prepared *N*-benzoyl derivative of galantinamic acid, the IR spectrum of the derivative showed the absorption at 1735 cm^{-1} corresponding to ester or δ-lactone. Since there was no possibility of ester formation, δ-lactone was assumed to be formed under the acidic conditions after the *N*-benzoylation. Therefore, a partial structure can now be extended to -CH(OH)-

Table 1. ^{1}H NMR of Galantinamic Acid in D_2O

δ	Multiplicity	Supposed partial structure
1.8 (8H)	Multiplet	-C-**CH_2**-C- (×4)
3.01 (2H)	Triplet	-C-**CH_2**-N-
3.39 (1H)	Multiplet	-**CH_2**-N-
3.91 (1H)	Doublet	-CH-**CH**-COOH
4.13 (2H)	Multiplet	-**CH**-O- (×2)

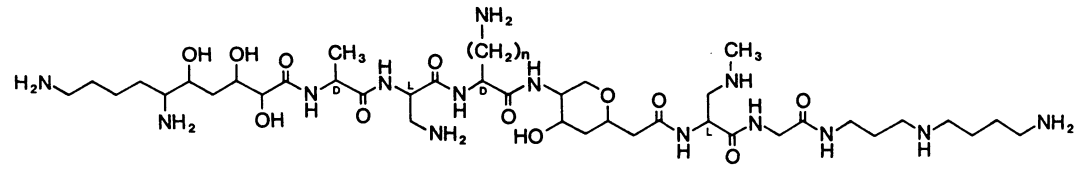


Fig. 1. The structure of galantin I which is a mixture of two congeners. Lysine (n=4) and ornithine (n=3) are found in a ratio of 9:1.

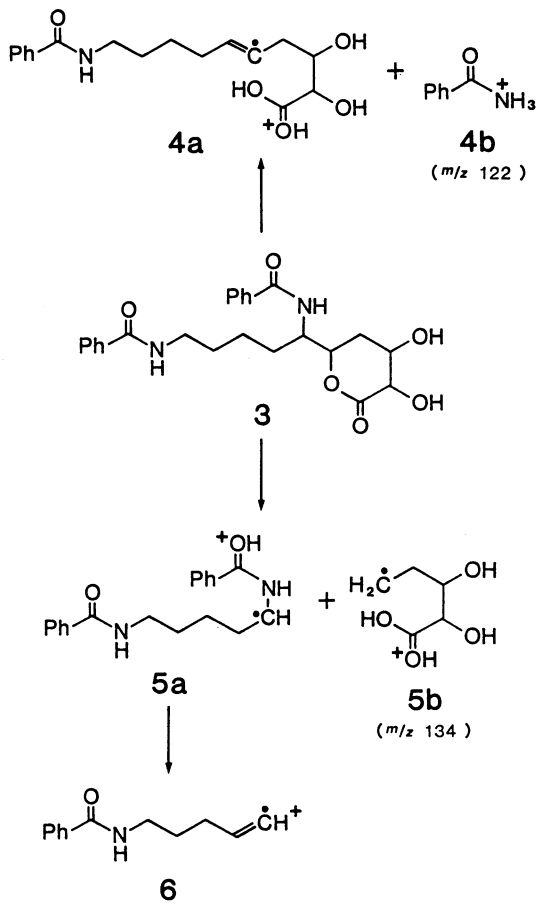


Fig. 2. Significant fragment ions observed in EI-MS spectrum of *N,N'*-dibenzoylgalantinamic acid lactone (**3**). The masses of fragments **4a**, **5a**, and **6** were exactly measured by high resolution method as summarized in Table 2.

Table 2. The Results of High-Resolution Mass Sprctrometry of Fragments **4a**, **5a**, and **6**.

Fragment	Molecular formula	EI-MS	
		Observed (*m/z*)	Calcd (M)
4a	$C_{17}H_{23}NO_5$	321.1590	321.1577
5a	$C_{19}H_{22}N_2O_2$	310.1710	310.1683
6	$C_{12}H_{14}NO$	188.1055	188.1076

C-CH(OH)-CH(OH)-COOH. As the results of above considerations, we can propose a following whole structure of galantinamic acid:

$NH_2CH_2CH_2CH_2CH_2CH(NH_2)$-
$CH(OH)CH_2CH(OH)CH(OH)COOH$.

The proposed structure was then confirmed chemically and spectrometrically. First, periodic acid oxidation followed by permanganate oxidation produced 5-aminovaleric acid which was derived from a part of $NH_2CH_2CH_2CH_2CH_2CH(NH_2)CH(OH)$-. Secondly, a study of high resolution EI-mass spectrometry of *N,N'*-dibenzoylgalantinamic acid lactone (**3**) gave quite important informations as shown in Fig. 2 and Table 2. For example, a fragment ion **5a** corresponding to $PhCONHCH_2CH_2CH_2CH_2CHNHC(=OH)^+Ph$ (*m/z* 310.1710) showed consistency with the molecular weight calculated as $C_{19}H_{22}N_2O_2$ (*m/z* 310.1683). Another two fragments, **4a** and **6**, also supported the proposed structure.

As described above, we could determine the primary structure of galantinamic acid, albeit the absolute structure was not assigned yet.[6]

Experimental

The NMR spectra were obtained with Varian XL-100-15 spectrometer for ^1H and JEOL JNM-FX-90Q spectrometer for ^{13}C in D_2O or CD_3OD. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as an external standard in D_2O or tetramethylsilane as an internal standard in CD_3OD. The chemical shifts were given in δ-value (ppm) from the standard. FD and EI mass spectra were measured with a JEOL JMS-O1SG-2 mass spectrometer. Amino acid analysis was carried out with a Hitachi KLA-5 amino acid analyzer.

Isolation of Galantinamic Acid. The acid hydrolyzate of galantin I (1.76 g)[3] was subjected to column chromatography over Amberlite IRC-50 (100—200 mesh, NH_4^+ form, 1.5×140 cm). Neutral amino acids were first eluted from the column by a gradient elution with 0.1—1.5% aqueous ammonia (1L). Basic amino acids were next separated by use of 1.5—5.6% aqueous ammonia (1L). The fractions containing galantinamic acid were concentrated in vacuo to obtain 160 mg of the crude material as hygroscopic powder. A part of crude galantinamic acid (110 mg) was dissolved in water and pH of the solution was adjusted to 6.5 with 0.01M HCl (1M=1 mol dm^{-3}). The neutralized solution was once concentrated in vacuo. To a solution of the residue in a small amount of water was added methanol. The methanolic solution was kept in a refrigerator to precipitate crystalline hydrochloride of galantinamic acid, yield 78 mg, mp 207.5—209 °C (decomp), $[\alpha]_D^{22}$ −0.4° (*c* 0.5, 1M HCl), amino acid analysis: 34 min (7.5 cm column, Hitachi #2611 resin, 0.35M citrate buffer (pH 5.28)). ^{13}C NMR (D_2O) δ=22.72 (C_8), 26.89 (C_9), 27.16 (C_7), 35.28 (C_4), 39.73 (C_{10}), 56.56 (C_6), 67.57 (C_5), 68.96 (C_3), 74.36 (C_2), 176.07 (C_1).

Found: C, 41.53; H, 8.23; N, 9.64; Cl, 12.10%. Calcd for $C_{10}H_{22}N_2O_5 \cdot HCl$: C, 41.88; H, 8.10; N, 9.77; Cl, 12.36%.

***N,N'*-Dibenzoylgalantinamic Acid δ-Lactone.** To a solution of free galantinamic acid (33 mg, 0.13 mmol) in water (4 mL) were added aqueous Na_2CO_3 (110 mg in 2 mL of H_2O) and benzoyl chloride gradually. A progress of the reaction was checked by TLC and addition of reagents was continued until the starting material disappeared. The alkaline solution was extracted with ether and ethyl acetate. Aqueous layer acidified with 1M HCl was extracted with ether twice and then concentrated in vacuo until colorless crystals deposited out. Crude product thus obtained was recrystallized from water, yield 42 mg (70%), mp 117.5—119.5°C. FD-MS: *m/z* 441 $[(M+H)^+]$, ^1H NMR(CD_3OD) δ=1.4—2.0 (m, 2H×4); 3.40 (t, 2H); 3.8—4.3 (m, 1H×4); 7.4 (m, 6H); 7.8 (m, 4H), IR (KBr): 1540, 1635, 1735 cm^{-1}.

Periodic-Permanganate Oxidation of Galantinamic Acid. To a solution of galantinamic acid (1.3 mg, 5.2 μmol) in 0.5 ml of water was added periodic acid ($HIO_4 \cdot 2H_2O$, 3.0 mg, 13 μmol) and the mixture was allowed to stand overnight at room temperature. To the solution was added 0.2% potassium permanganate solution until pale purple color remained. A small amount of MnO_2 was filtered off and the filtrate was used for TLC or amino acid analysis.

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5) Hydrolysis for 24 h as a general condition caused a decomposition of more than 50% of galantinamic acid.

6) Recently, (2*R*,3*S*,5*S*,6*R*)-configuration for galantinamic acid was suggested from the synthetic study by Dr. Y. Ohfune (private communication).