ASPICULAMYCIN, A NEW CYTOSINE NUCLEOSIDE ANTIBIOTIC

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURAL ELUCIDATION

TATSUO HANEISHI, AKIRA TERAHARA and MAMORU ARAI

Fermentation Research Laboratories, Sankyo Co., Ltd.,
Tokyo, Japan

(Received for publication February 28, 1974)

Aspiculamycin (**I**), $C_{19}H_{30}N_8O_{10}$, is a water-soluble basic antibiotic with versatile biological activities. From its physico-chemical properties, it has been characterized as one of the cytosine-nucleoside antibiotics for which the structure, 1-(4-sarcosyl-D-seryl-D-serylamino-4-deoxy-β-D-glucopyranosyluronamide) cytosine, is proposed.

As previously described[1], aspiculamycin (**I**) was produced by submerged fermentation of *Streptomyces toyocaensis* var. *aspiculamyceticus*. Isolation of the antibiotic from the culture filtrate was carried out by column chromatography on a cation exchange resin due to its basic, water-soluble character.

Present studies of the physico-chemical properties of aspiculamycin (**I**) revealed its close resemblance to the known antibiotic, gougerotin (**II**)[2~6], a N_1-substituted cytosine nucleoside (Chart 1). Structural elucidation of the antibiotic, therefore, proceeded by comparative interpretation of the spectroscopic and chemical characteristics of aspiculamycin with those of gougerotin (**II**).

Physico-chemical Properties of Aspiculamycin

Aspiculamycin (**I**) was obtained as colorless needle-like crystals. It exhibited no sharp melting point but decomposed at about 205°C, and was optically active, $[\alpha]_D^{20}+54.9°$ (*c* 1, H_2O). It is readily soluble in water, slightly soluble in methanol, and practically insoluble in other organic solvents such as ethanol, acetone and ethylacetate. The antibiotic is a diacidic base (pKa′ 3.9 and 8.2) with a molecular weight of 490 determined by osmometry. The elementary analysis of the dipicrate of the antibiotic were as follows: Found, C, 37.65; H, 3.64; N, 19.80 %. Calcd. for $C_{19}H_{30}N_8O_{10}\cdot 2(C_6H_3N_3O_7)$, C, 37.60; H, 3 69; N, 19.89 %. The UV spectra showed maxima at 236 (ε 8,250) and 268 nm (ε 8,600) at pHs 6.8 and 11.0, and 276 nm (ε 11,300) at pH 3.0. These spectra suggest the presence of N_1-substituted cytosine moiety in the structure of the antibiotic. The IR spectrum in a KBr pellet revealed characteristic bands at 3400 and

Chart 1.

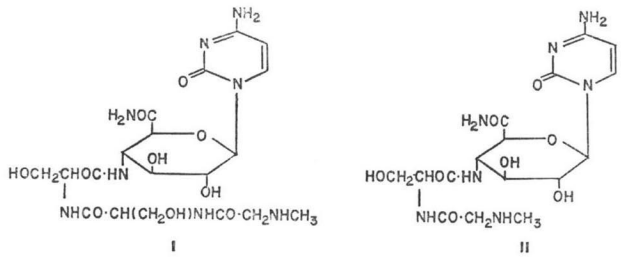


Fig. 1. Ultraviolet absorption spectra of cytidine and aspiculamycin (**I**)

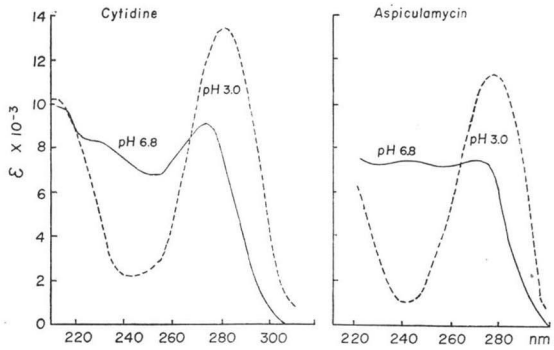


Fig. 2. Infrared absorption spectrum of aspiculamycin (**I**) (KBr)

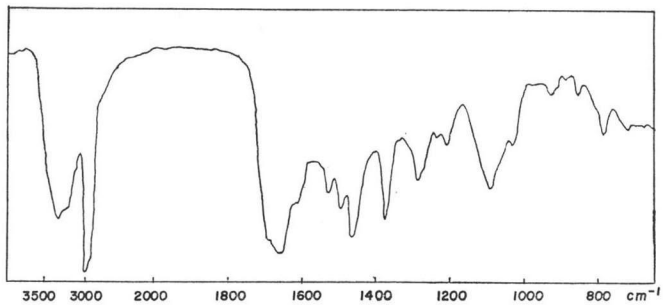


Fig. 3. NMR spectrum of compound **III** at 100 MHz (D_2O-DCl)

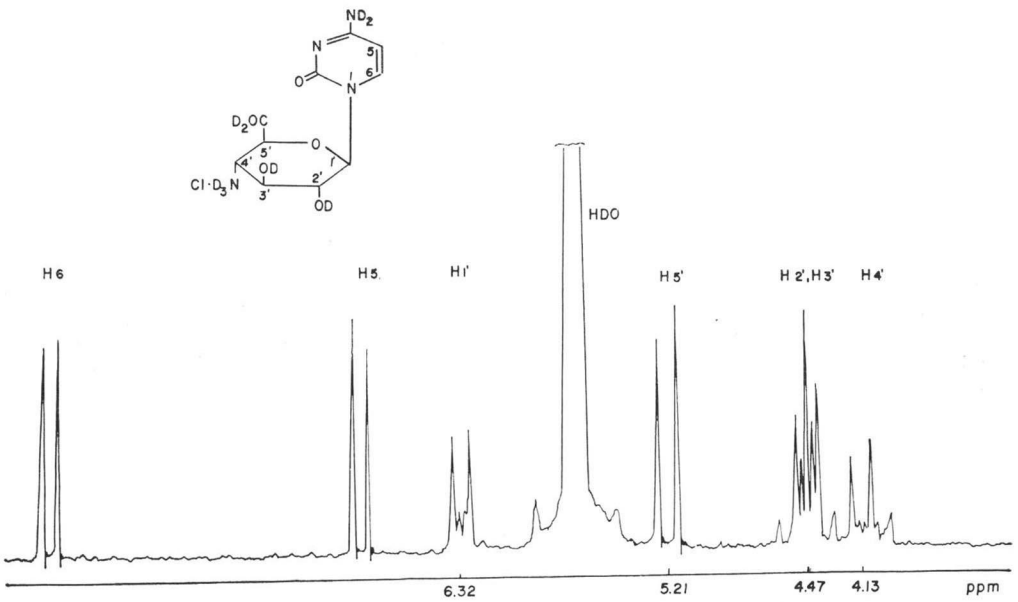
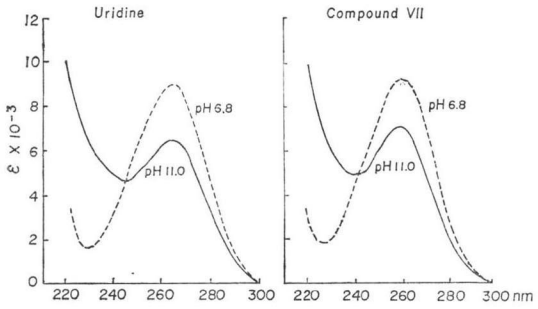


Fig. 4. Ultraviolet absorption spectra of uridine and compound **VII**



1690~1660 cm^{-1} indicating the existence of hydroxyl and amide carbonyl groups in aspiculamycin (Figs. 1 and 2).

The NMR spectrum (D_2O) of the antibiotic indicated the presence of a methyl group δ 2.32 (singlet), an isolated methylene δ 3.19 (singlet), an anomeric proton δ 5.60 (doublet, J=10.0 Hz), and heteroaromatic protons δ 5.90 (doublet, J=9.0 Hz) and δ 7.80 (doublet, J=9.0 Hz). Acid hydrolysis (6 N HCl, 5 hours at 110°C) of aspiculamycin (**I**) gave D-serine and sarcosine as amino acids. These physicochemical data of aspiculamycin suggested its chemical structure to be a N_1-substituted cytosine nucleoside antibiotic similar to a known antibiotic gougerotin (**II**). Further structural studies were, therefore, based on the comparative interpretation of spectroscopic and chemical data connecting to gougerotin (**II**).

Structual Studies

Acid hydrolysis (refluxed in 6 N HCl for 1.5 hours) of aspiculamycin (**I**) gave compound **III** $C_{10}H_{14}N_4O_6$, sarcosine (**IV**) and D-serine (**V**). Compound **III** was an amphoteric substance with pKa′ 2.87, 4.16 and 7.51. The UV and NMR spectra showed the presence of cytosine moiety. Both the IR absorption band at 2500 cm^{-1} in a KBr pellet and a newly detected dissociable function (pKa′ 2.87) indicated the presence of carboxyl group. The other two dissociable functions apparently belong to a cytosine nucleus (4.16) and an α-amino function (7.51). The NMR spectrum (D_2O, 100 MHz) of monohydrochloride of compound **III** is shown in Fig. 3.

The integration revealed five protons in the region at δ 4.0~6.5 ppm. The splitting pattern of the H-1′ and H-2′ protons ($J_{1'2'}$=9.0 Hz, $J_{2'3'}$=8.0~9.0 Hz) can be explained by the coupling constants of vicinal axial-axial protons. Similar relations were found among other four protons, such as H-3′ and H-4′ ($J_{3'4'}$= 10 Hz, $J_{4'5'}$=10.5 Hz), and H-5′ and H-6′ ($J_{5'6'}$=8.0 Hz).

These data indicated that these protons had an alternating axial-axial configuration, typical of the Cl conformation of glucopyranosides. All physical and chemical constants of compound **III** in aspiculamycin were identical with those of the C-substance of gougerotin, which was confirmed as glucopyranoside type by stereo-specific synthesis from α-D-galactose

Chart 2

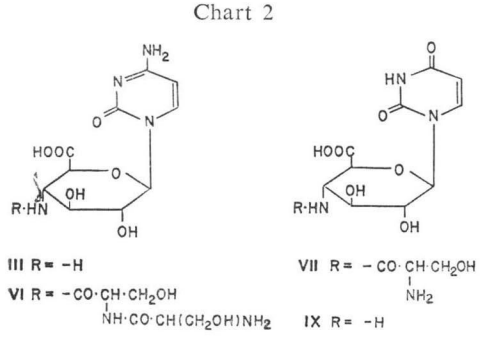
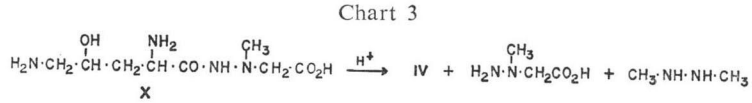


Chart 3



by WATANABE *et al.*[6]

When compound **I** was subjected to mild acid hydrolysis (6 N HCl for 60 hours at room temperature), it yielded crystalline compound **VI**, $C_{16}H_{24}N_6O_{10}$, mp 214~216°C (dec.); $[\alpha]_D^{20}$+16.8° (*c* 1, H_2O); pKa' 2.9, 4.0, 7.5; UV maxima at 235 (ε 9,500) and 268 nm (ε 10,000) at pH 6.8 and 11.0, 275 nm (ε 14,000) at pH 3.0, and compound **VII** as a minor component, besides compounds **IV** and **V**. The structure of compound **VI** was unambiguously confirmed to be a diseryl-C-substance by acid hydrolysis of its 2,4-dinitrophenyl derivative and methylester, affording compounds **III**, **V**, DNP-D-serine (**VIII**) and the methylester of these three compounds, respectively. Compound **VII** was characterized as $C_{13}H_{18}N_4O_9$; mp. 218~220°C (dec.); $[\alpha]_D^{20}O$ (*c* 1, 0.1 N HCl); pKa' 3.0, 7.6, 9.3; NMR (D_2O) heteroaromatic protons δ 5.60 (doublet, 1H, J=8.0 Hz), δ 7.80 (doublet, 1H, J=8.0 Hz), anomeric proton δ 5.35 (doublet, J=8.0 Hz). The UV spectrum of compound **VII** suggested the presence of uridilyic chromophore in its structure (Fig. 4). This was further supported by the difference in dissociation constant between **I** (pKa' 4.0) and **VII** (pKa' 9.3) which indicates the structural change from cytosine to uracil. These data are consistent with the results obtained by DUTTA *et al.*[7], in which cytidine or cytidilic acid changes to uridine or uridilyic acid, respectively, by acid hydrolysis under various conditions. It was determined that compound **VII** was constituted of compounds **V** and **IX** by further acid hydrolysis and by NMR analysis (Charts 2 and 3).

The experimental evidence and NMR analysis of aspiculamycin (**I**) indicated a lack of 1-methyl-hydrazino acetic acid moiety in its structure, unlike negamycin (**X**)[8], in which sarcosine (**IV**) together with 1,2-dimethyl hydrazine and 1-methyl-hydrazino acetic acid were derived from a novel intermolecular rearrangement under acid-hydrolysis condition. Therefore, it was concluded that the peptide side chain in aspiculamycin (**I**) was a tripeptide, sarcosyl-D-seryl-D-serine, in contrast to dipeptide of gougerotin (**II**), sarcosyl-D-serine.

Finally, structural correlation to biological activities was taken into consideration. The acid degradation products **III**, **VI** and **IX** possessed antifungal activity but their antibacterial activities were greatly depressed in comparison with aspiculamycin (**I**). It can be illustrated that the sarcosyl moiety of the tripeptide side chain plays a very important role for antibacterial activity and the tripeptide side chain may not be an essential function to antifungal activity.

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