UCH9, a New Antitumor Antibiotic Produced by *Streptomyces*
II. Structure Elucidation of UCH9 by Mass and NMR Spectroscopy

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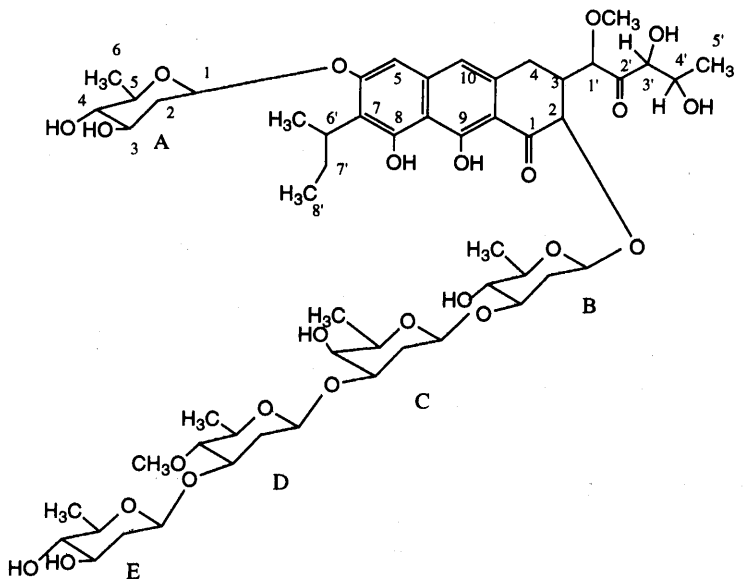
The structure of UCH9, which is a novel antitumor agent, was determined by spectroscopic methods. UCH9 consists of an aglycone and five 2,6-dideoxy sugars (three D-olivoses, one 4-*O*-methyl-D-olivose and one D-oliose). Four of the five sugars are sequentially connected through a β1→3 linkage (olivose-1→3-4-*O*-methyl-olivose-1→3-oliose-1→3-olivose). On the basis of the results of spectroscopic analysis, it was found that UCH9 belongs to the aureolic acid family of antibiotics. The structure of UCH9 is unique in that mono- and tetrasaccharide moieties, and a long hydrophobic side chain (*sec*-butyl group) are attached to the aglycone, while di- and trisaccharide moieties and a methyl or a hydrogen are attached in the case of the known aureolic acid analogs. It is known that aureolic acid analogs form a dimer in the presence of Mg^{2+}. NMR, FAB-MS and atomic absorption analysis revealed that UCH9 isolated from *Streptomyces* also forms a dimer, containing one equivalent molar Mg^{2+}.

In the preceding paper, we described the producing organism, and the fermentation, isolation and biological activities of the novel antitumor agent, UCH9[1] (Figure 1, **1**). In this paper, we report the structure elucidation of UCH9, including determination of the absolute configuration of the sugars. The spectroscopic analysis revealed that UCH9 belongs to the aureolic acid family of antibiotics. For aureolic acid group analogs, such as chromomycin A_3, the addition of Mg^{2+} results in the formation of a Mg^{2+}-coordinated dimer[2~6]. The dimer

Fig. 1. Structure of UCH9 (**1**).



Experimental

General

Physico-chemical and spectral data were obtained with the following instruments: MP, Yanaco micro melting point apparatus; $[\alpha]_D$, Jacso DIP-370 digital polarimeter; UV, Shimadzu UV-2200 UV-VIS spectrophotometer; CD, Jasco J-500A spectropolarimeter; IR, JEOL JIR-RFX3001 spectrophotometer; NMR, Bruker AM500 spectrometer; and FAB-MS, JEOL JMS-HX/HX110A spectrometer. Atomic absorption analysis was carried out with a Hitachi Z-8100 atomic absorption analyzer.

Methanolysis of UCH9 (1)

46.0 mg of UCH9 (**1**) was dissolved in 1 N methanolic HCl and then stirred for 3.5 hours at room temperature. The mixture was evaporated to dryness, and the yellow solid was chromatographed on silica gel (Wakogel C-200 25 ml, $CHCl_3$: MeOH 99 : 1 ~ >95 : 5, stepwise elution), which yielded 5.9 mg of methyl 4-*O*-methyl-α-D-olivoside (**2**), 14.1 mg of mixture of methyl-α/β-D-olivoside/olioside, and 1.8 mg of methyl β-D-olivoside. The mixture of methyl-α/β-D-olivoside/olioside was further chromatographed on silica gel (Wakogel C-200 12 ml, *n*-hexane : EtAc 1 : 2 ~ > 1 : 3, stepwise elution) to yield 7.6 mg of methyl α-D-olivoside (**3**) and 2.4 mg of methyl α-D-olioside.

Methyl 4-*O*-methyl-α-D-olivoside (**2**). $[\alpha]_D^{31} = +81°$ (*c* 0.07, $CHCl_3$); HR-EI-MS *m/z* 145.0850 $(M-OCH_3)^+$, $\Delta -1.5$ mmu for $C_7H_{13}O_3$, ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (3H, d, $J=6.1$, 5-CH_3), 1.68 (1H, ddd, $J=3.7$, 11.6 and 13.1 Hz, H2a), 2.13 (1H, ddd, $J=1.2$, 5.2 and 13.1 Hz, H2e), 2.71 (1H, t, $J=9.0$ Hz, H4), 3.30 (3H, s, OCH_3), 3.57 (3H, s, 4OCH_3), 3.60 (1H, dq, $J=6.1$ and 9.2 Hz, H5), 3.94 (1H, m, H3), 4.71 (1H, dd, $J=3.7$ and 1.2 Hz, H1).

Methyl α-D-olivoside (**3**). $[\alpha]_D^{26} = +156°$ (*c* 0.38, acetone), HR-EI-MS *m/z* 131.0717 $(M\text{-}OCH_3)^+$, $\Delta +0.9$ mmu for $C_6H_{11}O_3$, ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, d, $J=6.2$, 5-CH_3), 1.68 (1H, ddd, $J=3.7$, 11.5 and 12.9 Hz, H2a), 2.13 (1H, ddd, $J=1.2$, 5.1 and 12.9 Hz, H2e), 3.09 (1H, t, $J=9.3$ Hz, H4), 3.32 (3H, s, OCH_3), 3.61 (1H, dq, $J=6.2$ and 9.3 Hz, H5), 3.88 (1H, m, H3), 4.73 (1H, dd, $J=3.7$ and 1.2 Hz, H1).

Hydrolysis of UCH9

31.7 mg of UCH9 (**1**) was dissolved in 50% aq. AcOH and then stirred for 18 hours at 60°C. After cooling, the reaction mixture was extracted with ethyl acetate, which yielded 25.2 mg of sugar-containing products. The sugar-containing products were chromatographed on silica gel (Wakogel, C-200 10 ml, *n*-hexane : EtOAc 1 : 2 ~ > 0 : 1 stepwise elution), which yielded 2.0 mg of 4-*O*-methyl-D-olivose, 6.4 mg of D-olivose and 1.9 mg of D-oliose (**4**).

D-Oliose (**4**). $[\alpha]_D^{29} = +44°$ (*c* 0.19, H_2O), FAB-MS *m/z* 131 $(M+H-H_2O)^+$, HR-EI-MS *m/z* 131.0686 $(M-OH)^+$, $\Delta -2.3$ mmu for $C_6H_{11}O_3$, ^{1}H NMR (400 MHz, CD_3OD): δ 1.19 (3H, d, $J=6.6$, 5-$CH_{3\alpha}$), 1.25 (3H, d, $J=6.4$, 5-$CH_{3\beta}$), 1.65 (1H, m, H2a$_\beta$), 1.70 (1H, m, H2a$_\alpha$), 1.83 (1H, m, H2e$_\beta$), 1.85 (1H, m, H2e$_\alpha$), 3.43 (1H, dd, $J=3.2$ and 1.0 Hz, H4$_\beta$), 3.51 (1H, dq, $J=1.0$ and 6.4 Hz, H5$_\beta$), 3.52 (1H, dd, $J=3.7$ and 0.5 Hz, H4$_\alpha$), 3.67 (1H, m, H3$_\beta$), 3.97 (1H, m, H3$_\alpha$), 4.06 (1H, dq, $J=0.5$ and 6.6 Hz, H5$_\alpha$), 4.66 (1H, dd, $J=2.2$ and 9.8, H1$_\beta$), 5.22 (1H, dd, $J=3.7$ and 1.2 Hz, H1$_\alpha$).

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