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Sparsomycin Analogs. I. Synthesis of 5-Carboxy-6-methyluracil[1)]

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Methods for the synthesis of 5-carboxy-6-methyluracil (**9**), which is expected to be useful as an intermediate for the preparation of sparsomycin analogs, were investigated. A new route that leads to **9** from cyanoacetylurea (**5**) *via* 2-cyano-3-oxobutanoylurea (**6a**), 5-cyano-6-methyluracil (**7a**), and 5-carbamoyl-6-methyluracil (**8**), was developed. The total yield from **5** to **9** was 20.7%.

Keywords——5-carboxy-6-methyluracil; 5-cyano-6-methyluracil; 5-carbamoyl-6-methyluracil; 2-cyano-3-oxobutanoylurea; sparsomycin analogs

Sparsomycin (**1**) was isolated in 1962 from the culture filtrate of *Streptomyces sparsogenes* var. *sparsogenes* sp. n. by Argoudelis *et al.*[2)] This compound was subjected to several preliminary biological tests, and exhibited moderate to high activity against several *in vivo* tumor systems, such as the Walker carcinosarcoma 256 and the sarcoma 180 solid tumor, in addition to moderate *in vitro* activity against various bacteria, fungi, and viruses.[3)]

The structure of this antibiotic was proposed by Wiley and MacKellar in 1970[4a)] and recently a total synthesis of its enantiomer was independently reported by Ottenheijm *et al.*[5)] and Helquist *et al.*[6)] (Fig. 1).

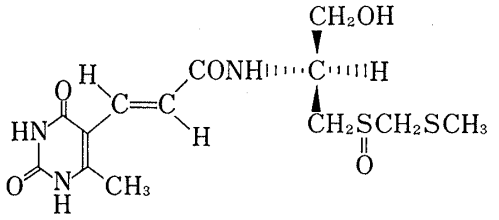

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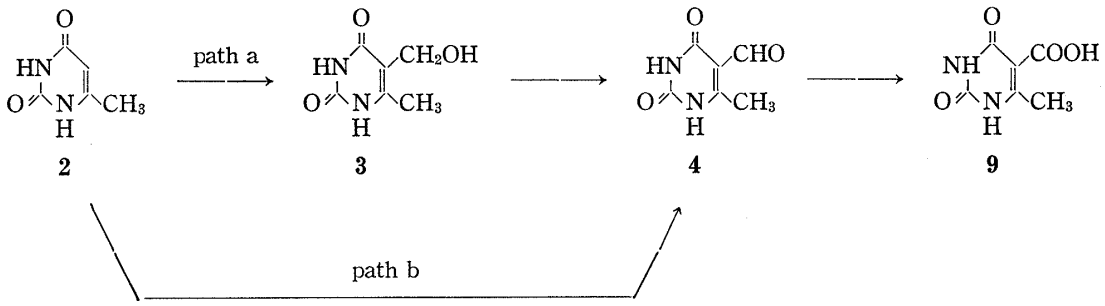
Fig. 1. Structure of Sparsomycin (**1**)

The unique structural and biological properties of sparsomycin prompted us to investigate the structure-activity relationship of sparsomycin analogs. This report describes the synthesis of 5-carboxy-6-methyluracil, which is expected to be an intermediate for syntheses of sparsomycin analogs lacking the ethylene moiety.



2 **3** **4** **9**

Chart 1

5-Carboxy-6-methyluracil (**9**) has been already prepared by the route shown in Chart 1 (path a) in Wiley and MacKellar's structural study[4b)] of sparsomycin. In this synthetic route, 6-methyluracil (**2**) was treated with formaldehyde to afford 5-hydroxymethyl-6-methyluracil (**3**), then **3** was oxidized with CrO_3 to afford 5-formyl-6-methyluracil (**4**), and finally **4** was oxidized with $KMnO_4$ in an alkaline medium to afford **9**, in 43%, 20%, and 16% yields, respectively. Consequently the total yield from **2** to **9** was 1.4%. **4** has also been prepared by the oxidation of **3** with $K_2S_2O_8$ containing $AgNO_3$ in 63% yield.[7)] When this figure is employed,

the total yield *via* path a is 4.3%. On the other hand, **4** has been directly prepared from **2** in 14% yield by the Reimer–Tiemann reaction (Chart 1, path b),[8] and, if this figure is employed, the total yield from **2** to **9** (path b) would be 2.2%.

These poor yields are inappropriate for a synthetic route to sparsomycin analogs. Therefore we developed another route, shown in Chart 2, making use of 5-cyano-6-methyluracil (**7a**) as an intermediate.

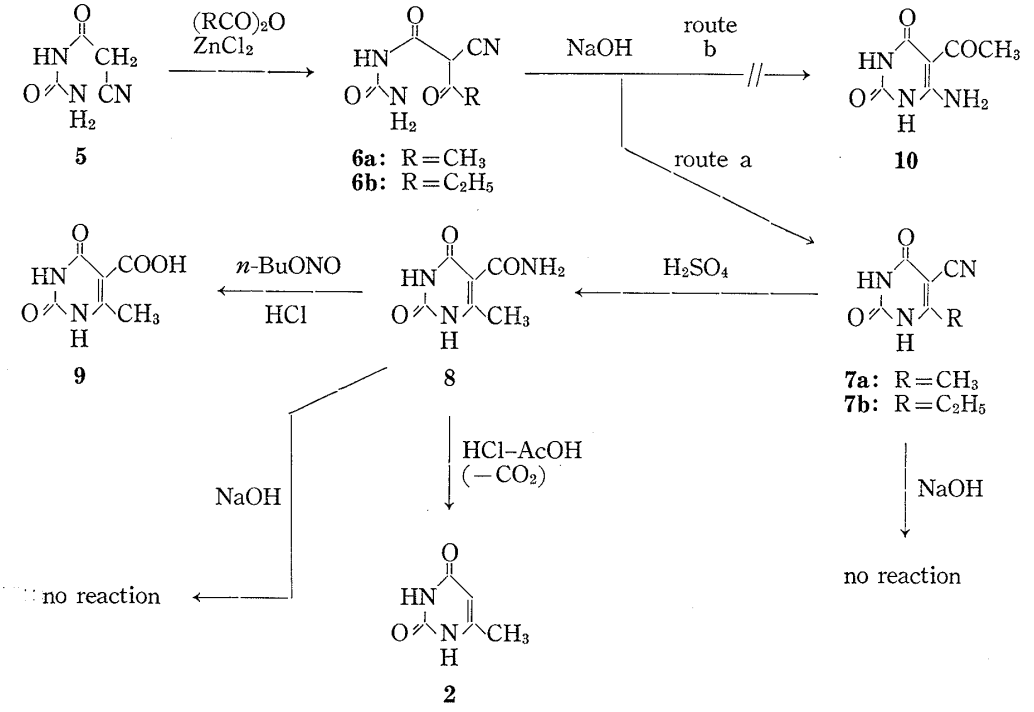


Chart 2

Cyanoacetylurea (**5**), prepared from cyanoacetic acid and urea by the method in the literature,[9] was treated with acetic anhydride in the presence of molten $ZnCl_2$ to give 2-cyano-3-oxobutanoylurea (**6a**) in 73% yield. The ureid **6a** was treated with 10% NaOH to give a cyclized product in 89% yield.

In this cyclization reaction, two kinds of route were anticipated, *i. e.* route a and route b. If the reaction involves the initial nucleophilic attack of the amide nitrogen on the carbon atom of the cyano group, 5-acetyl-6-aminouracil (**10**) should be formed. However, the infrared (IR) spectrum of our cyclization product showed a nitrile absorption band at 2230 cm^{-1} and its mass spectrum showed the molecular ion peak of 5-cyano-6-methyluracil (**7a**) at *m/e* 151. On the basis of these data, the structure of this product was assigned as **7a**. The elemental analysis data also supported this assignment.

An attempted hydrolysis of **7a** with 1 N NaOH at 100° for 10 hr resulted in a quantitative recovery of unchanged **7a**. **7a** was hydrolyzed by heating with 90% H_2SO_4 to afford 5-carbamoyl-6-methyluracil (**8**) in 68% yield. Attempted alkaline hydrolysis of **8** was also unsuccessful. On the other hand, when **8** was heated with HCl–AcOH, 6-methyluracil (**2**) was formed with evolution of carbon dioxide.

Thus, the amide **8** was treated with *n*-butylnitrite (*n*-BuONO) and HCl in acetic acid, and 5-carboxy-6-methyluracil (**9**) was successfully obtained in 47% yield. IR and NMR spectral data of **9** were identical with those of an authentic sample prepared from **2** according to the reported procedure.[4b]

Thus, we have developed a new route for the synthesis of 5-carboxy-6-methyluracil: the total yield from **5** to **9** was 20.7%.

Since 5-cyano-6-methyluracil (**7a**) and potent antitumor agents such as 5-fluorouracil (5-FU) and futraful are structurally closely related, both having an electron-withdrawing group at position 5 of the uracil nucleus, we tested **7a** for cytotoxic activity on HeLa cells by a colony formation method.[10] However, **7a** showed no activity at a concentration of 1, 10, or 100 μg/ml of the assay medium, while 5-FU as a control sample showed inhibition at a concentration of 1 μg/ml.

Synthesis of sparsomycin analogs lacking the ethylene moietyl is being conducted in this laboratory.

Experimental

All melting points are uncorrected. IR spectra were taken on a JASCO IRA-2 spectrometer, mass spectra on a JEOL JMS-D100 mass spectrometer, UV spectra on a Hitachi 323 recording spectrophotometer, and NMR spectra on a JEOL JNM-MH-100 spectrometer with TMS as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; b, broad. For thinlayer chromatography, silica gel GF_{254} (Merck) was used with the solvent system MeCOEt–Me_2CO–H_2O (7 : 2 : 1).

2-Cyano-3-oxobutanoylurea (6a)——A mixture of 5.0 g (0.039 mol) of **5** (prepared from cyanoacetic acid and urea in the presence of Ac_2O in 82% yield by the reported method[9]), 20 ml of Ac_2O, and 0.5 g of molten $ZnCl_2$ was heated gently over a small flame for several minutes until a solution was obtained. The solution was immediately cooled in an ice bath. The solidified product was filtered off, washed with Et_2O–EtOH, and dried *in vacuo*. The crude product was recrystallized from EtOH to yield 4.94 g (73%) of **6a**, colorless needles, mp 161° (dec.). *Anal.* Calcd for $C_6H_7N_3O_3$: C, 42.61; H, 4.17; N, 24.84. Found: C, 42.79; H, 4.23; N, 24.88. IR ν_{max}^{Nujol} cm^{-1}: 2300 (CN), 1730, 1675. UV $\lambda_{max}^{H_2O}$ nm (ε): 275 (80).

2-Cyano-3-oxopentanoylurea (6b)——Acylation of **5** (2.542 g, 0.02 mol) with $(EtCO)_2O$ (13 ml) and molten $ZnCl_2$ (0.25 g) afforded 1.5 g (41.6%) of **6b**, mp 141—144° (dec.).

5-Cyano-6-methyluracil (7a)——**6a** (5.0 g, 0.03 mol) was mixed with 10% NaOH (20 ml, 0.05 mol). The mixture was shaken to give a solution, which solidified after a few minutes. The solidified mixture was heated at 60° for 2 min on a water bath, cooled to room temperature and acidified with 50% AcOH. The resulting precipitate was filtered off with suction, washed with cold water, and dried *in vacuo*. The crude product was recrystallized from water to yield 4.03 g (89%) of **7a**. Colorless prisms, mp above 300°. *Anal.* Calcd for $C_6H_5N_3O_2$: C, 47.69; H, 3.33; N, 27.81. Found: C, 47.80; H, 3.08; N, 27.98. IR ν_{max}^{Nujol} cm^{-1}: 2230 (CN), 1720, 1658. MS *m/e*: 151 (M^+). UV $\lambda_{max}^{H_2O}$ nm (ε): 278 (7900). *Rf*: 0.80.

5-Cyano-6-ethyluracil (7b)——Treatment of **6b** (1.465 g, 8 mmol) as described for **7a** followed by recrystallization from water gave **7b** (139 mg, 11%) as colorless needles, mp 281° (dec.). *Anal.* Calcd for $C_7H_7N_3O_2 \cdot 1/6H_2O$: C, 50.00; H, 4.40; N, 24.99. Found: C, 50.04; H, 4.22; N, 24.81. IR ν_{max}^{Nujol} cm^{-1}: 2230 (CN), 1730, 1665. MS *m/e*: 165 (M^+). UV $\lambda_{max}^{H_2O}$ nm (ε): 273.5 (11670).

5-Carbamoyl-6-methyluracil (8)——**7a** (1.51 g, 0.01 mol) was heated with 1.1 ml of H_2O (0.061 mol) and 13.9 ml of conc. H_2SO_4 at 110° for 16.5 hr. After cooling, the reaction mixture was poured over ice in a beaker. The precipitate was filtered off with suction, washed with cold water and recrystallized from water to yield 1.08 g (62%) of **8**, colorless prisms, mp above 300°. *Anal.* Calcd for $C_6H_7N_3O_3 \cdot 1/3H_2O$: C, 41.15; H, 4.41; N, 23.99. Found: C, 41.42; H, 4.01; N, 23.92. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1665. MS *m/e*: 169 (M^+). NMR (DMSO-d_6): 2.41 (s, 3H, CH_3), 7.10 (b, 1H), 8.21 (b, 1H), 11.16 (b, 1H), 11.22 (b, 1H). UV $\lambda_{max}^{H_2O}$ nm (ε): 268 (9365). *Rf*: 0.60.

5-Carboxy-6-methyluracil (9)——Dry HCl gas was bubbled slowly through a solution of 0.169 g (0.001 mol) of the amide (**8**) in glacial acetic acid for 15 min. The solution was stirred and 0.210 g (0.002 mol) of *n*-BuONO was added to it. The reaction mixture was heated at 100° for 3 hr with stirring, then cooled. The solvent was removed *in vacuo*, and the residue was recrystallized from water to give 0.080 g (47%) of **9**. Colorless needles, mp 242° (dec.). *Anal.* Calcd for $C_6H_6N_2O_4 \cdot 1/3H_2O$: C, 40.92; H, 3.82; N, 15.91. Found: C, 41.27; H, 3.51; N, 16.34. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1610. MS *m/e*: 170 (M^+). NMR (DMF-d_7): 2.70 (s, 3H, CH_3), 12.10 (b, 2H, NH), 13.78 (b, 1H, COOH). UV $\lambda_{max}^{H_2O}$ nm (ε): 271 (8900). *Rf*: 0.30.

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References and Notes

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