analysis. The mean values of three rat experiments are shown in Fig. 2, comparing with those following injection of the same amount of unconjugated (free) mitomycin C. The excretion rate of mitomycin C following injection of conjugate was slow and gradually decreased to a small amount, whereas mitomycin C administered as a free form was excreted rapidly and no antimicrobial activity could be detected in the urine samples after 24 hr. These results indicate the sustained release of mitomycin C from agarose bead conjugate in the hypodermic spaces of the rat. In this case, the average amount of mitomycin C recovered in urine within 15 days was only 12 μg, owing to a metabolic inactivation of the liberated drug.

At the end of the *in vivo* experiment, the conjugate was collected from the hypodermic spaces of the sacrificed animals and the amount of remaining drug was determined also from the successive *in vitro* release experiment. One milligram conjugate obtained from the animal at the 16th day after injection liberated 0.34 μg of antimicrobially active mitomycin C during 24 hr incubation in buffer solution. This is almost identical to the results of *in vitro* release experiment of the 16th day shown in Table I. These results suggest that mitomycin C was released from the conjugate in the body at almost similar rate to the *in vitro* experiment.

Present results prove the feasibility of mitomycin C-agarose bead conjugate as an injectable delivery system for supplying mitomycin C to a tumor site at a controlled rate over a long period. Further examination concerning the antitumor activity of this system is now in progress.

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Synthesis of D-Glucuronic Acid Derivatives of 5-Fluorouracil having O-Glycosidic Linkage

5-Fluorouracil-4-O-glucuronic acid derivatives (**V**, **VII**) were synthesized by a condensation reaction of the silver salt of 2-benzyloxy-5-fluoro-4-pyrimidone with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate and subsequent careful removal of the protecting group.

Keywords——5-fluorouracil; D-glucuronic acid; 5-fluorouracil-O-glucuronide; anticancer activity; ultraviolet spectra; nuclear magnetic resonance spectra

Since Duschinsky, *et al.* reported the first synthesis of 5-fluorouracil (5-FU) as a potential anticancer agent in 1957,[1] a number of its derivatives have been synthesized and their anticancer activities have been investigated.[2]

It is interesting from the viewpoint of drug design of anticancer agents that the pH value in normal tissue is approximately 7.3, whereas in tumour tissue it is relatively lower

1) R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
2) C. Heidelberger, "Progress in Nucleic Acid Research and Molecular Biology," vol. 4 Academic Press, New York and London, 1965, p. 1.

having a value of about 6[3–5] in the presence of glucose. It is also very interesting that the glucuronidase activity of tumours is selectively enhanced by previous administration of glucose.[6] From these facts, it might be expected that O-β-D-glucuronide derivatives of 5-FU could be selectively activated in tumour tissue by the presence of glucose.

Rogers, *et al.* reported the synthesis of unprotected 2,4-bis-O-glucoside of uracil and thymine.[7] However, unprotected 2-O- or 4-O-glycosides of pyrimidine derivatives have never been reported hitherto.

Now we wish to report the first synthesis of a new class of 5-FU derivatives of D-glucuronic acid having O-glycosidic linkage.

Methyl 1-(2-benzyloxy-5-fluoro-pyrimidine-4-yl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (III) was obtained by condensation reaction of the silver salt of 2-benzyloxy-5-fluoro-3H-4-pyrimidone (II) with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (I)[8] in xylene. The compound (III) has a melting point of 120—121° and an ultraviolet (UV) absorption maximum at 271 nm (ε=8000) in 0.1 N HCl, 271 nm (ε=7300) in MeOH and 270 nm (ε=9000) in 0.1 N NaOH. The structure of the compound (III) was determined by its elemental analysis and its reasonable resemblance to the UV spectra of 2,4-dimethoxy-5-fluoropyrimidine.[9] The nuclear magnetic resonance (NMR) spectrum of the compound (III) was consistent with the structure shown in Chart 1. The anomeric C-1′ proton appeared as a doublet at δ 6.53 with a coupling constant, $J_{1'-2'}$=7.0 Hz, that indicated a trans-diaxial structure between C1′ and C2′ proton. Hence the compound (III) is the β-anomer. The

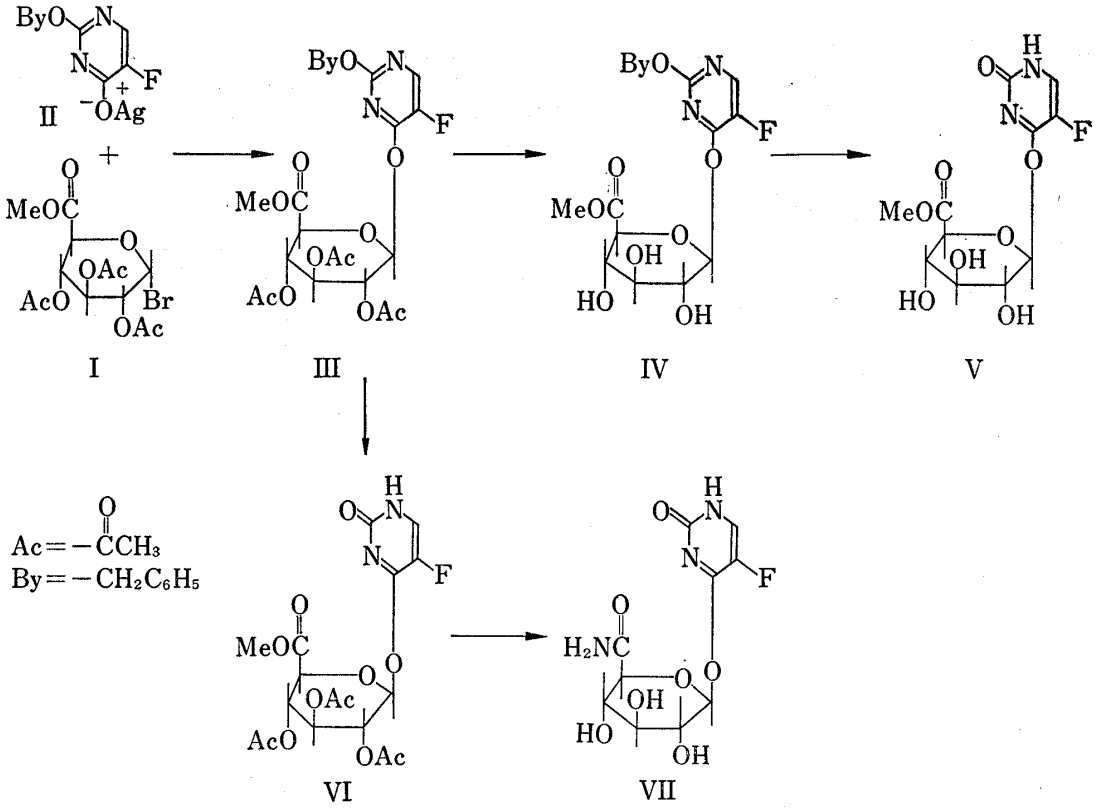

Chart 1

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5) T. Baba and N. Kimura, *Japanese Scientific Monthly*, **28**, 773 (1976).
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7) G.T. Rogers, R.S. Shadbolt, and T.L.V. Ulbricht, *J. Chem. Soc.* (*C*), **1969**, 203.
8) G.N. Bollenback, J.W. Long, D.G. Benjamin, and J.A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).
9) H. Prystas and F. Sorm, *Collection Czech. Chem. Commun.*, **30**, 1900 (1965).

proposed structure of III was also supported by the fact that the UV spectrum of the debenzylated compound (VI) whose UV absorption maximum appeared at 288 nm (ε=4800) in MeOH was also reasonably similar to that of 5-fluoro-4-methoxy-2-pyrimidone.[10]

Deacetylation of III was performed by careful addition of sodium methoxide solution and stirring of the solution for 40 min at 0°. The reaction solution was worked up as usual and methyl-1-(2-benzyloxy-5-fluoro-pyrimidine-4-yl)-β-D-glucopyranuronate (IV) was obtained as white crystals which melted at 159—160° and had a UV absorption maximum at 269 nm (ε=7100) in 0.1 N HCl, 270 nm (ε=7300) in water and 269 nm (ε=7700) in 0.1 N NaOH. Debenzylation of IV by catalytic hydrogenation with 10% palladium-carbon in methanol gave methyl 1-(5-fluoro-1*H*-2-oxo-pyrimidine-4-yl)-β-D-glucopyranuronate (V) which melted and solidified at 134—136° and decomposed at 149—157°. The UV spectrum of the compound (V) showed a maximum at 288 nm (ε=5700) in 0.02 M phosphate buffer (pH 6.86).

Treatment of the compound (VI) with methanolic ammonia for 16 hr at 0—5° gave 1-(5-fluoro-1*H*-2-oxo-pyrimidine-4-yl)-β-D-glucopyranuronamide (VII) which decomposed at 146—150° and had its UV maximum at 286 nm in 0.02 M phosphate buffer (pH 6.86).

It became apparent that the compounds (V) and (VII) had potential antitumour activities and expected lower toxicity from the result of primary screening tests.[11] Furthermore, it became clear that these compounds were activated selectively in tumour cells by the presence of glucose.[12]

The details of the antitumour activities of these compounds and their related derivatives will be published elsewhere.

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