shifts are arranged by the linear combination of the substituent constants σ_i and σ_π,[2] and linear relations among both α- and β-C-13 chemical shifts with respect to σ_i—0.3 σ_π are observed as shown in Fig. 1 and 2.

Our previous study[3] confirmed that the α-H-1 chemical shifts for substituted methyl and ethyl derivatives are linear with σ_i—0.25 σ_π, whereas those in the β- position, separated from the substituent group by three σ bonds, for ethyl and isopropyl derivatives are linear with σ_i, and from this fact it has been expected that the π-electronic effect, in other words, the delocalization effect, is effective through two σ bonds.

In the present, the same conclusion was verified for the β-C-13 resonance chemical shifts for substituted ethyl and isopropyl derivatives. Details of this work will be published in due time.

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Synthesis of 2-Thiouridine and 6-Methyl-3-(β-D-ribofuranosyl)-2-thiouracil

Recently several 2-thiopyrimidine nucleosides have been identified as the minor constituent of transfer ribonucleic acids (t-RNAs). 5-Methylaminomethyl-2-thiouridine and 2-thiocytidine were found in t-RNA of *E. coli*[1] and 5-methoxycabonylmethyl-2-thiouridine was in t-RNA of baker's yeast.[2] 2-Thiocytidine has been prepared by the extended Hilbert–Johnson procedure from 4-amino-2-methylthiopyrimidine and a ribosyl chloride *via* the ribosyl pyrimidinium intermediate[3] and by the mercuri–procedure starting from diacetyl-2-thiocytosine.[4] Mercuric cyanide procedure[5] has recently been applied.[6] 2-Thiouridine, which had been prepared by the transformation of uridine through anhydronucleoside,[7] has been reported to be prepared by the mercuri–procedure starting from acetylated 2-thiouracil.[4] More recently, the silyl–procedure has been reported to be effective for 2-thiouridine synthesis.[8] These recent developments to the synthesis of 2-thiopyrimidine nucleosides prompted us to

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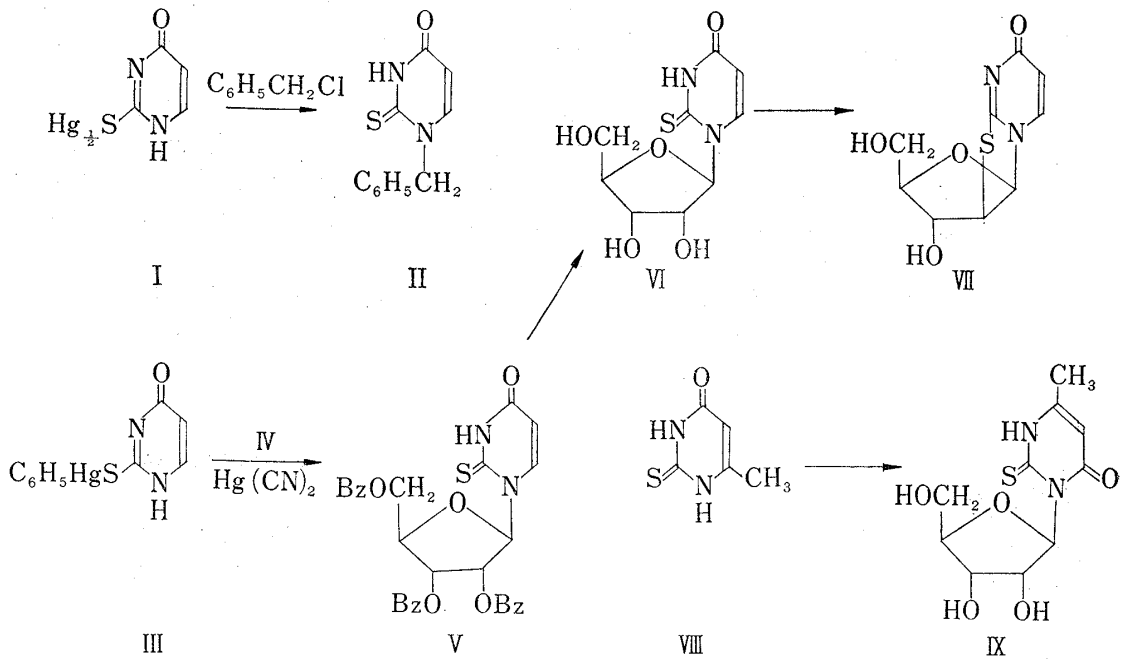

report a versatile method of preparation of 2-thiouridineand possibly of N-ribosyl derivative of thiolated nitrogen heterocycles.[9]

The present method is essentially an extension of mercuric cyanide procedure,[5] in which a mercuric ion serves as the protector of the thiol group as well as the activator of the ring nitrogen of 2-thiouracil. Bis(2-thiouracilyl)mercury (I) prepared by the usual procedure exhibited the ultraviolet (UV) spectra in neutral and alkaline media($\lambda_{max}^{H_2O}$ 292 mμ, $\lambda_{max}^{OH^-}$ 285 mμ) closely similar to those of 2-alkylthiouracil. In acidic medium, the spectra($\lambda_{max}^{H^+}$ 275 mμ) are almost identical with those of 2-thiouracil.[10] Therefore it is evident that the mercury–sulfur bond of I in neutral and alkaline media is stable but dissociates in acidic medium. Similar phenomena in UV spectra are observed with 4-thiouracil with mercuric chloride in aqueous solution. These findings suggest that mercury–sulfur bond in I should be stable under the condition of alkylation reaction. It is to be noted that bis(2-thiouracilyl)mercury (I) exists as a tautomeric mixture whose ratio is solvent dependent as revealed by UV spectral measurements.

The action of benzyl chloride and potassium carbonate with bis(2-thiouracilyl)mercuri (I) in dimethylformamide at 100° for 4 hours gave 1-benzyl-2-thiouracil(II)[11] as a main product with the concomitant formation of the 3-benzyl isomer. Phenylmercuric acetate in place of mercuric chloride gave also S-phenylmercuri-2-thiouracil(III). Treatment of III with 2,3,5-tri-O-benzoyl-D-ribosyl chloride(IV) in boiling nitromethane for 4 hours and the work-up (hydrogen sulfide was bubbled through the solution to remove mercuric ion) and silica gel chromatography afforded 2′,3′,5′-tri-O-benzoyl-2-thiouridine (V), mp~110°; *Anal.* Calcd. for $C_{30}H_{24}O_8N_2S$: C, 62.93; H, 4.23; N, 4.89; S, 5.60. Found: C, 62.97; H, 4.36; N, 5.14; S, 5.62. Debenzoylation with methoxide solution gave 2-thiouridine[7] (VI), mp 214—216.5° (decomp.), in 45% overall yield, whose configuration was confirmed by its conversion to a (*S*)-2,2′-anhydronucleoside (VII)[7b] with the action of diphenyl carbonate.[12] Direct condensation of 2-

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thiouracil and the ribosyl chloride (IV) in the presence of two equivalents of mercuric cyanide also afforded the nucleoside (V) in a slightly lower yield. Trace of the N-3 riboside[13] was detected.

6-Methyl-2-thiouracil (VIII) was next treated with the ribosyl chloride (IV) at the presence of one equivalent of mercuric cyanide and after the similar work-up a blocked nucleoside was obtained as a syrup in a 34% yield: *Anal.* Calcd. for $C_{31}H_{26}O_8N_2S$: C, 63.48; H, 4.47; N, 4.78; S, 5.46. Found: C, 63.30; H, 4.40; N, 4.58; S, 5.24. Debenzoylation afforded a crystal (IX), mp 208—210° (decomp.): *Anal.* Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.80; H, 5.15; N, 10.22; S, 11.67. Found: C, 43.79; H, 5.10; N, 10.17; S, 11.58. NMR (d-DMSO, ppm from TMS): 2.09(6-CH_3), 5.72(5-H), 6.95(1′-H, $J_{1',2'}$=3 cps). The UV spectra of IX ($\lambda_{max}^{H_2O}$, mμ 215, 275(shoulder), 296; $\lambda_{max}^{OH^-}$, mμ 213, 262, 321) resembled to those of 3-alkyl-2-thiouracils rather than 1-alkyl-2-thiouracils.[10] Therefore it was concluded that the ribosylation had occurred on N-3 of VIII to afford 6-methyl-3-(β-D-ribofuranosyl)-2-thiouracil (IX). The steric hindrance of 6-methyl group should be the main reflection of this result.

The present method of ribosylation and alkylation should have wider application to various N-heterocyclic bases possessing potential sulfhydryl group, which with the usual alkylation procedure, would give S-alkylated derivative. Further studies on the scope of this reaction are presently being undertaken.

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New Fluorescence Edman Reagent for Microanalysis of Peptides

Stepwise degradation from an N terminal by Edman[1,2] with phenylisothiocyanate is widely utilized for sequential analysis of peptides and proteines. As the sensitivity based on the ultraviolet (UV) absorption of phenylthiohydantoin ring at 250 mμ is not enough to practical use for microanalysis of peptides, some fluorescence reagents have been used to increase the sensitivity, however, fluorescein isothiocyanate[3,4] produced scarcely soluble thiohydantoin derivatives, and Edman-DNS method[5,6] is indirect and tedious.

We have developed a new Edman reagent, 4-(N,N-dimethylamino)-1-naphthyl isothiocyanate (DNTC), which is capable of stepwise degradation of minute quantities of peptides

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