UDC 612.398.145 : 547.965

50. **Mitsuji Sano** : Studies on Nucleosides and Nucleotides. V.[*1] Ultraviolet Spectrophotometric Studies on Pyrimidine Nucleosides and Isopyrimidine Nucleosides.

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Syntheses of hitherto unknown isopyrimidine nucleosides was successfully carried out and the fact that these nucleosides possessed specific characteristics different from the known pyrimidine nucleosides was reported in the preceding paper.[*1] Ultraviolet absorption spectra of these isopyrimidine and pyrimidine nucleosides were comparatively examined and some new observations were gained, which are reported herein.

In the first place, 1-β-D-glucopyranosyluracil[*3] and 3-β-D-glucopyranosyluracil were compared with their model substances, 1-methyluracil and 3-methyluracil. Based on their observations, 1-(β-D-glucopyranosyl)-2-thiouracil and 3-(β-D-glucopyranosyl)-2-thiouracil were respectively compared with 1-methyl- and 3-methyl-2-thiouracils, elucidating their ultraviolet absorption spectra.

Ultraviolet absorption spectra of 1-methyl- and 3-methyluracils have been examined in detail and these published reports gave very valuable suggestions in analysis of ultraviolet spectra of the pyrimidine and isopyrimidine nucleosides synthesized in series of the present work. Fox and others[1,2] compared ultraviolet spectra of 1- and 3-methyluracils with those of 1-glycosyluracils and pointed out the close similarity of the spectra of 1-glycosyluracils and 1-methyluracil. They stated that, since these compounds possessed one dissociable proton in their molecule, they would undergo keto-enol conversion with pH change and would take the enol form in alkaline state. On the other hand, Nakanishi and others[3] proved, from infrared spectral data, that 1-glycosyluracils were

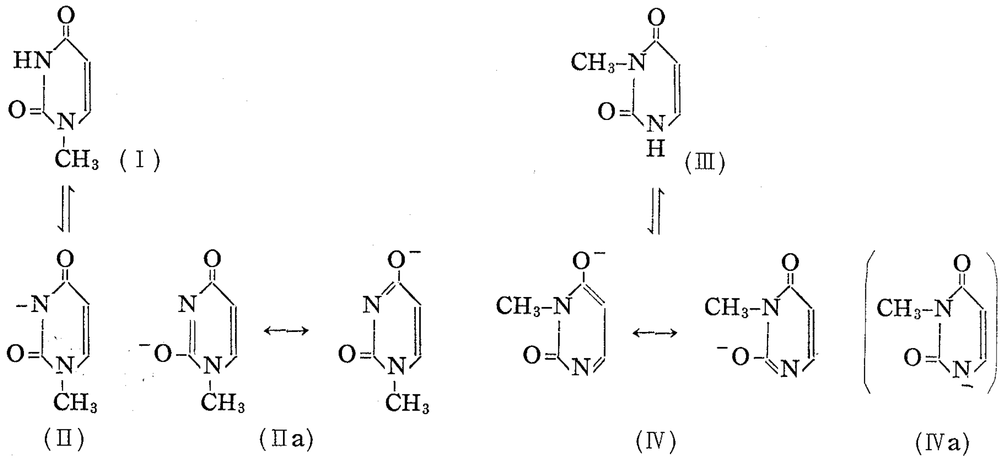
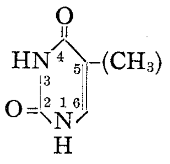


Chart 1.

[*1] Part IV : This Bulletin, **10**, 313 (1961).

[*2] Hirakawa-bashi, Sumida-ku, Tokyo (佐野光司).

[*3] Nomenclature for uracils and thymines used in the present report followed those used in the Chemical Abstracts, as shown.



1) D. Shugar, G. B. Fox : Biochem. et Biophys. Acta, **9**, 199 (1952).

2) *Idem* : *Ibid.*, **9**, 369 (1952).

3) K. Nakanishi, N. Suzuki, F. Yamazaki : Bull. Chem. Soc. Japan, **34**, 53 (1961).

in diketo form in neutral solution and their dissociation in alkaline solution was not merely in enol form. If 1-methyluracil (I) were to be dissociated into the enol form (IIa), it should show absorption at much longer wave-lengths region than the neutral species, because of a more extended conjugated system. Consequently, they gave the diketo form (II) with a negative charge on the nitrogen as an anionoid species. Nakanishi and others believe that 3-methyluracil (III) has the enol-form anionoid species (IV), since it shows the anticipated shift to a longer wave-length region and increased intensity of the absorption (cf. Chart 1) (Fig. 1 and 2).

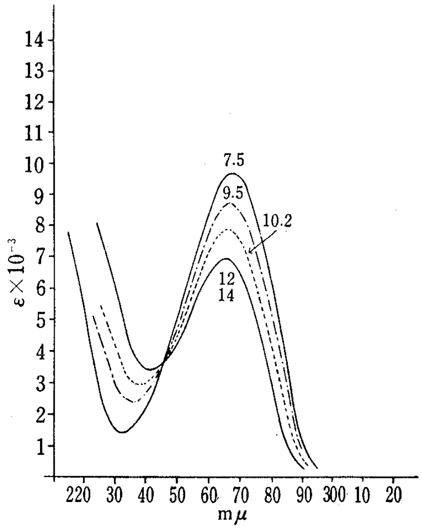


Fig. 1. Ultraviolet Spectra of 1-Methyluracil at pH Values indicated

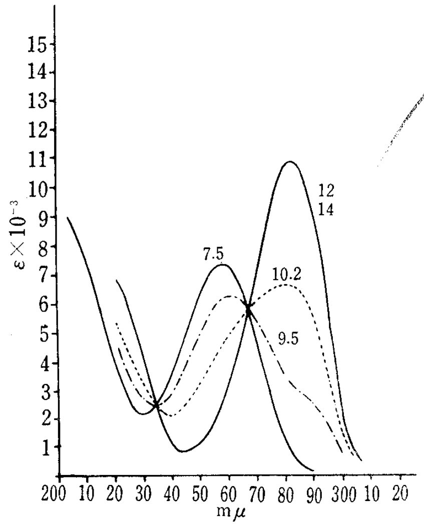


Fig. 2. Ultraviolet Spectra of 3-Methyluracil at pH Values indicated

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Comparison of the ultraviolet absorption spectra of 1-β-D-glucopyranosyluracil (V) (Fig. 3) and 3-β-D-glucopyranosyluracil (VII) (Fig. 4) with those of the foregoing model substances showed good agreement (Chart 2). The anionoid species of 1-β-D-glucopyranosyluracil showed almost no shift of the absorption and absorption intensity became weaker

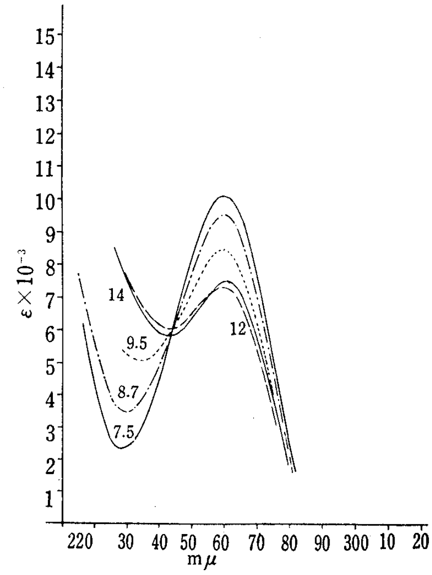


Fig. 3. Ultraviolet Spectra of 1-β-D-Glucopyranosyluracil at pH Values indicated

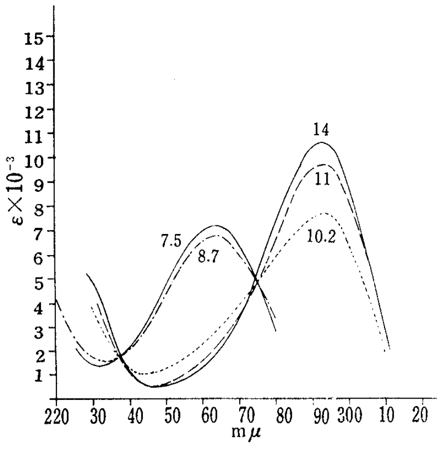
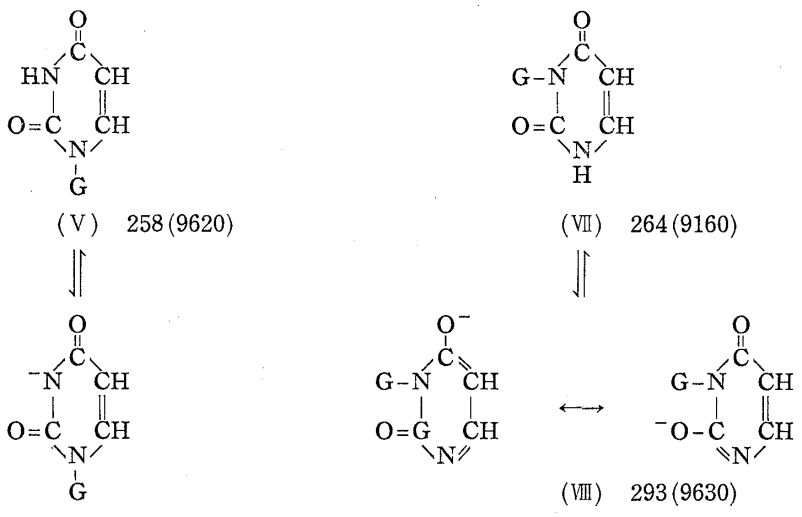


Fig. 4. Ultraviolet Spectra of 3-β-D-Glucopyranosyluracil at pH Values indicated

so that the compound is assumed to take the dissociated diketo from (VI) with a negative charge on the nitrogen, as in the case of 1-methyluracil. In the case of 3-β-D-glucopyranosyluracil (VII), there is a shift of absorptions to a longer wave-length region and increased intensity of absorptions, so that the compound is assumed to take the enol-form anionoid species (VIII). The data given by Nakanishi and others[3] for each state of the uracil and the data given in Chart 2 for glucopyranosyluracil show good agreement.



(V) 258(9620)

(VII) 264(9160)

(VI) 259(7260)

(VIII) 293(9630)

Chart 2.

Data for neutral molecules and anions are those at pH 7.5 and 11.0, respectively. Peak position (mμ) and intensity (ε) are indicated. G=β-D-Glucopyranosyl group.

Reliability of the structure of 3-β-D-glucopyranosyluracil can also be discussed from the agreement of its ultraviolet absorption spectrum with that of the model substance, 3-methyluracil. The dissociation of the sugar portion in the high alkalinity range, observed in 1-glycosylpyrimidines by Shugar and others,[2] was also observed in 3-β-D-glucopyranosyluracil, as indicated in Fig. 4.

The same comparison as above was carried out on 1-glycosyl-2-thiouracils and 3-glycosyl-2-thiouracils with 1-methyl-2-thiouracil and 3-methyl-2-thiouracil. Elion and others[4] examined the ultraviolet absorption spectra of 2-thiouracils and stated that it is difficult to discuss their dissociated structures from their ultraviolet spectral data alone because thiouracils show somewhat complicated dissociation states.

The ultraviolet absorption spectra of 1-methyl-2-thiouracil and 1-(β-D-glucopyranosyl)-2-thiouracil are almost the same in alkaline state. The absorptions appear at 236 and 266 mμ in 1-methyl-2-thiouracil (Fig. 5) and at 238.5 and 272 mμ in 1-(β-D-glycopyranosyl)-2-thiouracil (Fig. 6), there being no fundamental difference. In neutral solution, absorption of 1-methyl-2-thiouracil appears at 265 mμ, with a shoulder in the longer wave-length side and that of 1-(β-D-glucopyranosyl)-2-thiouracil at 278 mμ. If the observations on 1-substituted uracil were to be applied to 2-thiouracil, according to the report of Nakanishi and others,[3] the absorptions at 266 mμ in 1-methyl-2-thiouracil and at 272 mμ in 1-(β-D-glucopyranosyl)-2-thiouracil may be considered as these of the diketo-form anionoid species, (XI) and (XIII), respectively.

However, the fact that the absorption intensity of (XI) is stronger than that of (IX) in 1-methyl-2-thiouracil and that there is a shoulder in the longer wave-length side, it is assumed that the compound is present not only in a diketo form in the neutral region

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[4] G. B. Elion, W. S. Ide, G. H. Hitchings: J. Am. Chem. Soc., **68**, 2137 (1946).

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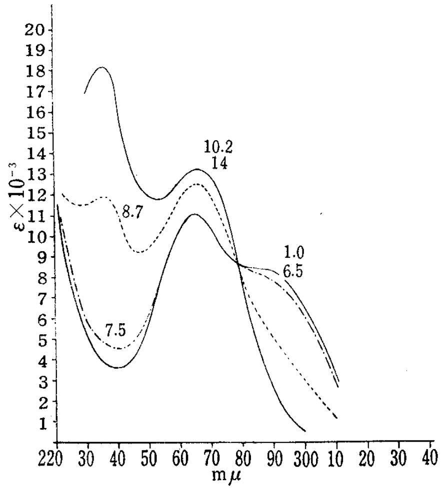


Fig. 5. Ultraviolet Spectra of 1-Methyl-2-thiouracil at pH Values indicaetd

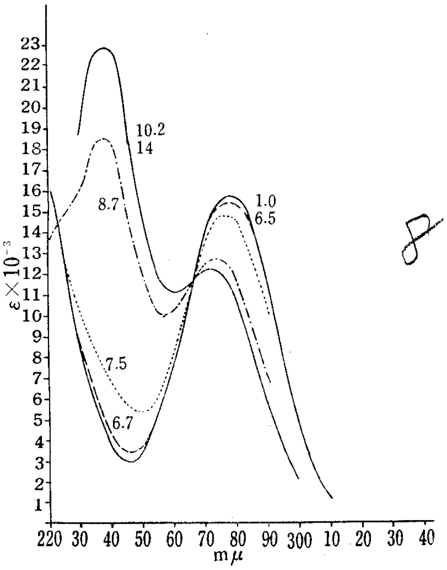
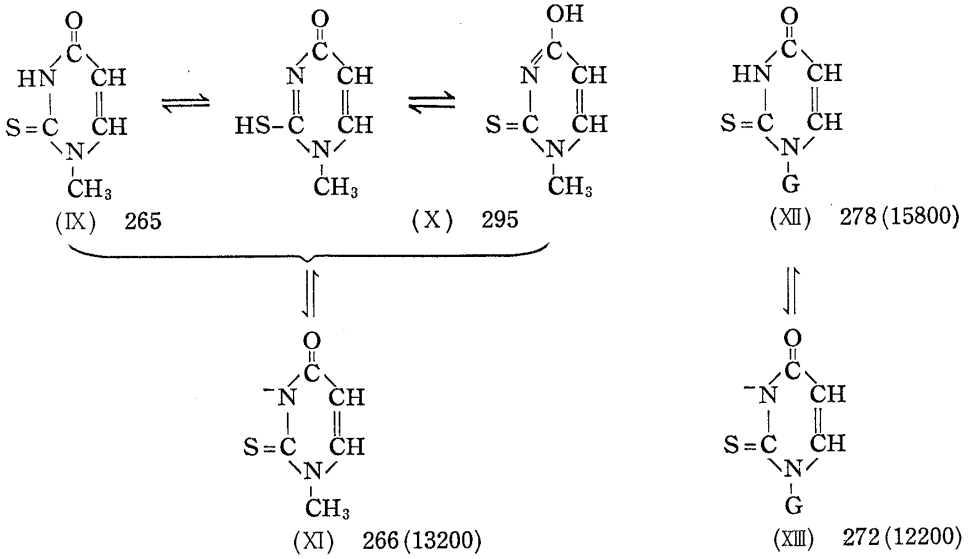


Fig. 6. Ultraviolet Spectra of 1-(β-D-Glucopyranosyl)-2-thiouracil at pH Values indicated


(IX) 265

(X) 295

(XII) 278(15800)

(XI) 266(13200)

(XIII) 272(12200)

Chart 3. All notations are the same as in Chart 2.

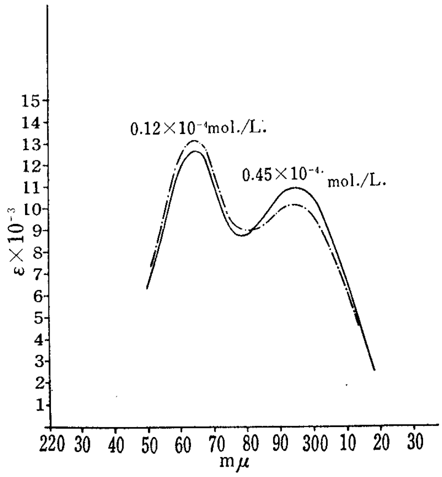


Fig. 7. Ultraviolet Spectra of 1-Methyl-2-thiouracil in Dioxane

but a part of it is enolized. The ultraviolet absorption of 1-methyl-2-thiouracil in dioxane (Fig. 7) clearly shows two peaks at 265 and 295 mμ, and intensity ratio of these two peaks varies with changes in the concentration. This facts seems to indicate the co-existence of (IX) and (X) and that the absorption at 295 mμ is that of the enolized form (X).

Summarizing the facts stated above, 1-methyl-2-thiouracil is present in both the diketo and enolized forms in neutral species, while 1-(β-D-glucopyranosyl)-2-thiouracil is present only in the diketo form, and both are in diketo form in anionoid species. These facts were observed not only in 2-thiouracils but also in 2-thiothymine, and the same phenomenon was also observed when ribofuranosyl as well as glucopyranosyl group is bonded (Figs. 8, 9, and 10).

Ultraviolet spectra of 3-substituted 2-thiouracils are almost the same in alkaline region. The absorptions appear at 257.5 and 310 mμ in 3-methyl-2-thiouracil (Fig. 11)

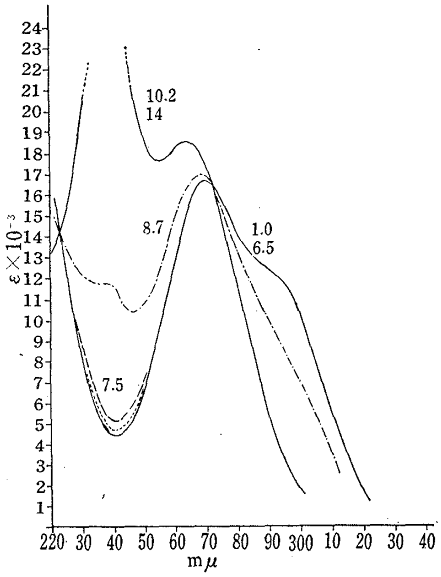


Fig. 8. Ultraviolet Spectra of 1-Methyl-2-thiothymine at pH Values indicated

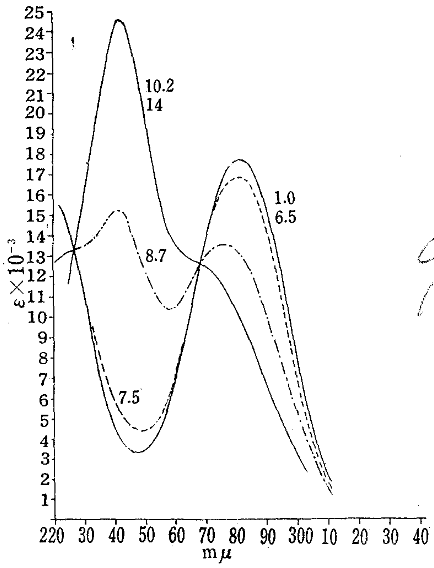


Fig. 9. Ultraviolet Spectra of 1-(β-D-Glucopyranosyl)-2-thiothymine at pH Values indicated

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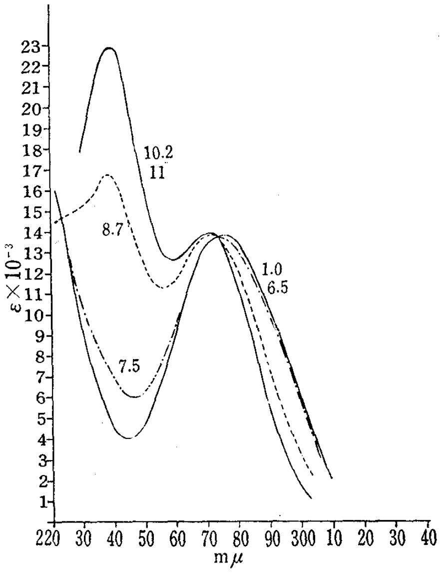

Fig. 10. Ultraviolet Spectra of 1-(β-D-Ribofuranosyl)-2-thiouracil at pH Values indicated

and at 259 and 327 mμ in 3-(β-D-glucopyranosyl)-2-thiouracil (Fig. 12), there being no fundamental difference. In a neutral region, the absorptions appear at 277 mμ in 3-methyl compound (Fig. 11) and at 270.5 and 300 mμ in 3-(β-D-glucopyranosyl) compound (Fig. 12). The large absorption in the longer wave-length region in alkaline solution signifies the formation of a conjugated system in the molecule and 3-methyl-2-thiouracil and 3-(β-D-glucopyranosyl)-2-thiouracil are considered to be dissociative species, (XV) and (XVIII), possessing an enolized form.

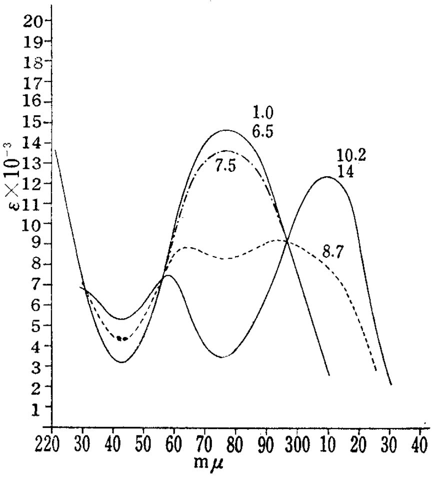

Fig. 11. Ultraviolet Spectra of 3-Methyl-2-thiouracil at pH Values indicated

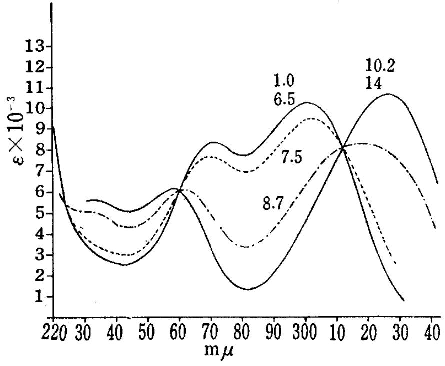

Fig. 12. Ultraviolet Spectra of 3-(β-D-Glucopyranosyl)-2-thiouracil at pH Values indicated

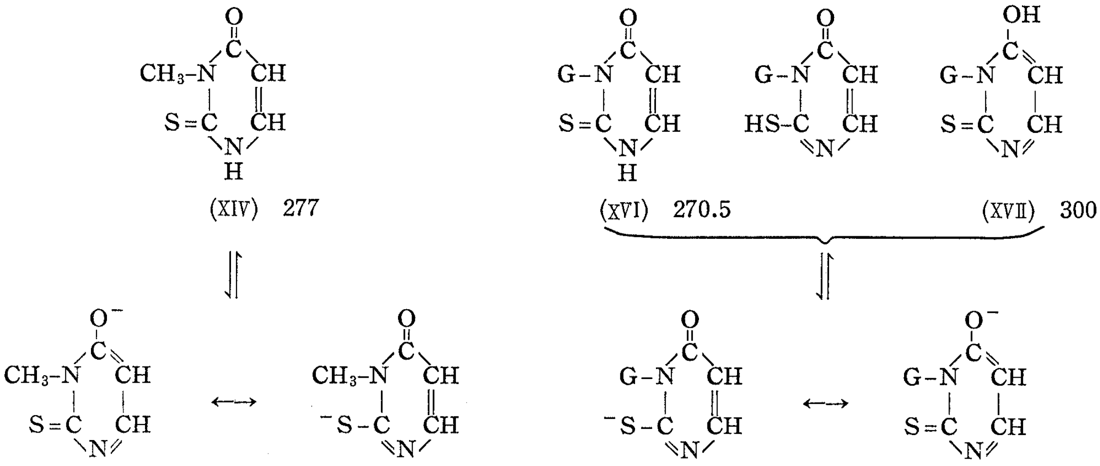

Chart 4.

Data for neutral molecules and anions are those obtained at pH 6.5 and 10.2, respectively. Ultraviolet peak position (mμ) and intensity (ε) are indicated. G=β-D-Glucopyranosyl group.

In the neutral region, 3-methyl-2-thiouracil shows only one absorption and judging from the position of this absorption, the molecule takes the diketo form (XIV). However, 3-(β-D-glucopyranosyl)-2-thiouracil shows two absorptions and, as indicated in Fig. 13, the intensity ratio of these two absorptions becomes different in dioxane. Since the intensity ratio also varies with changes in concentration, the absorptions at 270.5 and 300 mμ are considered to indicate the diketo form (XVI) and enolized form (XVII), respectively. Application of the relationship between absorptions of diketo and enol forms indicated by (IX) and (X) in Chart 3 to (XVI) and (XVII) gives a satisfactory agreement.

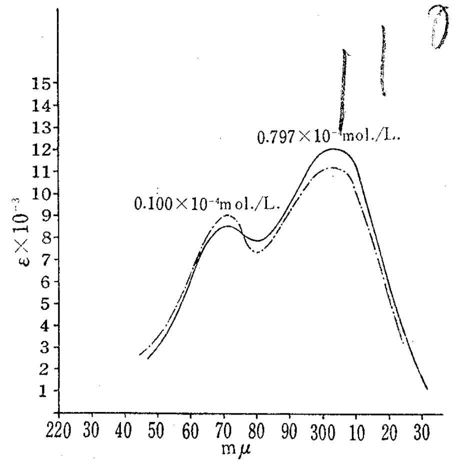

Fig. 13. Ultraviolet Spectra of 3-(β-D-Glucopyranosyl)-2-thiouracil in Dioxane

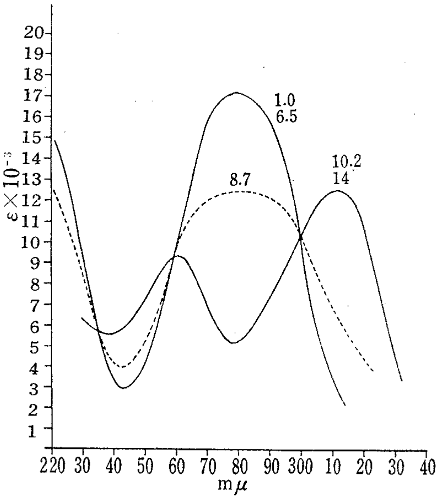

Fig. 14. Ultraviolet Spectra of 3-Methyl-2-thiothymine at pH Values indicated

Summarizing these data for 3-substituted 2-thiouracils, it is seen that in neutral species, 3-methyl-2-thiouracil is present in diketo form alone, while 3-(β-D-glucopyranosyl)-2-thiouracil is present in both diketo and enolized forms, and that both are present in enolized dissociated form in alkaline medium. These observations on 3-methyl-2-thiouracil and 3-(β-D-glucopyranosyl)-2-thiouracil were the same in 2-thiothymine derivatives as well as in ribofuranosyl derivatives (Figs. 14, 15, and 16). The dissociation of the sugar portion in high alkaline region, observed in glycosyluracils was not seen in glycosyl-2-thiouracils.

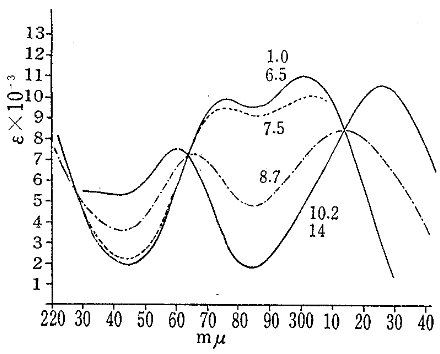

Fig. 15. Ultraviolet Spectra of 3-(β-D-Glucopyranosyl)-2-thiothymine at pH Values indicated

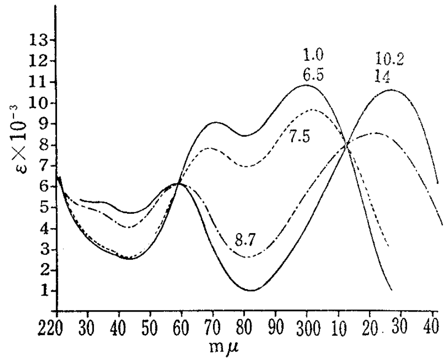

Fig. 16. Ultraviolet Spectra of 3-(β-D-Ribofuranosyl)-2-thiouracil at pH Values indicated

Comparison of the ultraviolet absorption spectra of 1-(β-D-glucopyranosyl)uracil and 3-(β-D-glucopyranosyl)uracil showed that they have almost similar spectra in the neutral region but the spectra are clearly different in alkaline solution due to different state of dissociation. Ultraviolet absorption spectra of 1-(β-D-glucopyranosyl)-2-thiouracil and 3-(β-D-glucopyranosyl)-2-thiouracil are entirely different in both the neutral and alkaline ranges because they show different states of dissociations in both cases. These facts were found to be true also in the case of thymines instead of uracils or ribosyl group instead of glucosyl group. It follows, therefore, that it is possible to clarify the structure of isopyrimidine nucleosides to a certain extent from their ultraviolet absorption spectra.

Experimental

Materials——Syntheses of the pyrimidine and isopyrimidine-nucleosides used in the present work have been reported in Part I to VI of this series, as follows: 1-Methyl-2-thiothymine and 3-methyl-2-thiothymine in Part I,⁵⁾ 1-(β-D-glucopyranosyl)-2-thiothymine in Part II,⁶⁾ 1-methyluracil, 1-methyl-2-thiouracil, 1-(β-D-glucopyranosyl)uracil, 1-(β-D-glucopyranosyl)-2-thiouracil, and 1-(β-D-ribofuranosyl)-2-thiouracil in Part III,⁷⁾ and 3-methyluracil, 3-methyl-2-thiouracil, 3-(β-D-glucopyranosyl)-uracil, 3-(β-D-glucopyranosyl)-2-thiouracil, 3-(β-D-glucopyranosyl)-2-thiothymine, and 3-(β-D-ribofuranosyl)-2-thiouracil in Part IV.*1

Buffer Solution——Solutions of 0.1*N* HCl as pH 1.0, 0.01*N* HCl as pH 2.0, and 0.025*N* solution of Walpole acetate buffer as pH 2.0~5.4 solutions. The solution of pH 6.0~8.7 was a Sörensen phosphate buffer of 0.015*M* concentration and Sörensen's glycocol buffer of 0.1*M* concentration was used for pH 9.0~11.0 solution. For solutions of pH 12.0, 0.001*N* NaOH was used, 0.1*N* NaOH for pH 13.0 and 1.0 *N*NaOH for pH 14.0 solution.

Spectral Measurement——Ultraviolet absorption spectra were measured with Hitachi Model EPS-2 spectrophotometer. For the measurement, 3.5 cc. of the buffer solution and 0.5 cc. of the aqueous solution of each sample were accurately measured directly into the cell.

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Summary

Ultraviolet absorption spectra of pyrimidine and isopyrimidine nucleosides were compared and their difference was examined. 1-(β-D-glucopyranosyl)uracil and 3-(β-D-glucopyranosyl)uracil show practically the same spectra in the neutral region while their spectra are clearly different in alkaline solution. The spectra of 1- and 3-(β-D-glucopyranosyl)-2-thiouracils are different in both neutral and alkaline regions, and the spectra in neutral region seemed to be somewhat lacking in uniformity. These facts were found to be true in the case of thymines instead of uracils or in the presence of ribosyl group instead of glucosyl group. It follows that pyrimidine and isopyrimidine nucleosides could clearly be differentiated from their ultraviolet absorption spectra, especially from those taken in alkaline solution.

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5) T. Naito, M. Hirata, T. Kawakami, M. Sano: This Bulletin, **9**, 703 (1961).
6) Part II. T. Naito, M. Sano: *Ibid*,, **9**, 709 (1961).
7) Part III. M. Sano: *Ibid.*, **10**, 308 (1962).