[Chem. Pharm. Bull. 13(9)1108~1113(1965)]

UDC 612.398.145 : 541.144.8

145. Kazuko Zenda, Mineo Saneyoshi, and Goro Chihara : Biological Photochemistry. I. The Correlation between the Photodynamical Behaviors and the Chemical Structures of Nucleic Acid-bases, Nucleosides, and Related Compounds in the Presence of Methylene-blue.

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As a study on photodynamic inactivation mechanism in biological system with methylene-blue, recently, Simon, Van Vunakis[1] reported that only guanine moieties in DNA and its components preferentially underwent photosensitized reaction by visible light-irradiation in the presence of oxygene. After that, Sussenbach, *et al.*[2~4] investigated photodynamic degradation of guanine, and Wacker, *et al.*[5] investigated photodynamic reaction mechanism on purine bases with thiopyronine. But there have not been reported any systematic studies which reveal what kind of compounds undergo easily photochemical reaction in connection with their chemical structures.

This paper concerns the systematic study on the photochemical behaviours of 40 kinds of purine bases, nucleosides and their related compounds. Thus, the studies were made to examine whether or not these compounds were suffered from photosensitized degradation in presence of methylene-blue by visible light-irradiation. As a result, some informations were obtained on the correlation between the photochemical behavior and the chemical structure of the irradiated compounds.

Experimental

Materials——The compounds used here were 31 kinds of purine bases, purine nucleosides and thier related compounds, and 9 of pyrimidine and imidazol derivatives.

As follows :

2-Aminopurine-6-ol (Guanine) (I)
2-Amino-9-(β-D-ribofuranosyl)purine-6-ol (Guanosine) (II)
5-Amino-v-triazolo[4,5-*d*]pyrimidine-7-ol (8-Azaguanine) (III)
Purine-2,6-diol (Xanthine) (IV)
9-(β-D-Ribofuranosyl)purine-2,6-diol (Xanthosine) (V)
1,3,7-Trimethylpurine-2,6-diol (Caffeine) (VI)
1,3-dimethylpurine-2,6-diol (Theophylline) (VII)
Purine-2,6,8-triol (Uric acid) (VIII)
6-Aminopurine (Adenine) (IX)
6-Amino-9-(β-D-ribofuranosyl)purine (Adenosine) (X)
4-Amino-2-pyrimidinol (Cytosine) (XI)
1-(β-D-Ribofuranosyl)-4-amino-2-pyrimidinol (Cytidine) (XII)
2,4(1*H*,3*H*)-Pyrimidinedione (Uracil) (XIII)
1-(β-D-Ribofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione (Uridine) (XIV)
5-Methyl-2,4(1*H*,3*H*)-pyrimidinedione (Thymine) (XV)
1-(β-D-Ribofuranosyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (Thymidine) (XVI)
Purine-6-ol (Hypoxanthine) (XVII)
9-(β-D-Ribofuranosyl)purine-6-ol (Inosine) (XVIII)
3-(β-D-Ribofuranosyl)-4-aminoimidazole-5-carboxamide (AICA-riboside) (XIX)
2-Mercaptopurine-6-ol[6] (XX)†

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1) M. I. Simon, H. Van Vunakis : J. Mol. Biol., **4**, 488 (1962).
2) J. S. Sussenbach, W. Berends : Biochim. et Biophys. Acta, **76**, 154 (1963).
3) *Idem* : Biochim. Biophys. Res. Comm., **16**, 263 (1964).
4) *Idem* : Biochim. et. Biophys. Acta, **95**, 184 (1965).
5) A. Wacker, G. Türck, A. Gerstenberger : Naturwiss., **50**, 377 (1963).
6) Y. Mizuno, T. Ueda, M. Kobayashi, Y. Shimizu, T. Murakami : Yakugaku Zasshi, **77**, 686 (1957).

9-(β-D-Ribofuranosyl)purine[7] (Nebularine) (XXI)
Purine-6-thiol[8] (6-MP) (XXII)
9-(β-D-Ribofuranosyl)purine-6-thiol[7] (XXIII)
2-Amino-9-(β-D-ribofuranosyl)purine-6-thiol[7] (6-Thioguanosine) (XXIV)
9-(β-D-Ribofuranosyl)-2,6-diaminopurine[9] (XXV)
2,6,8-Trichloropurine[10] (XXVI)†
2-Amino-6-hydrazino-9-(β-D-ribofuranosyl)purine[8] (XXVII)
2-Amino-6-benzylthio-9-(β-D-ribofuranosyl)purine[9] (XXVIII)
Purine-2,6-dithiol[11] (XXIX)†
2-Amino-9-(β-D-ribofuranosyl)purine[7] (6-Deoxyguanosine) (XXX)
4-Aminopyrrolo[2,3-*d*]pyrimidine[12] (XXXI)
4-Chloropyrrolo[2,3-*d*]pyrimidine[12] (XXXII)
Pyrrolo[2,3-*d*]pyrimidine-4-thiol[12] (XXXIII)
2,6-Diamino-4-pyrimidinol[13] (XXXIV)
2,5,6-Triamino-4-pyrimidinol[13] (XXXV)
2-Amino-8-thiocyanato-9-(β-D-ribofuranosyl)purine-6-ol[14] (XXXVI)†
2-Thiocyanatopurine-6-ol[14] (XXXVII)
2,8-Dithiocyanatopurine-6-ol[14] (XXXVIII)†
2,6-Dithiocyanatopurine[14] (XXXIX)
2-Amino-6-thiocyanato-9-(β-D-ribofuranosyl)purine[14] (XL)

Among above substances, I～XVIII were offered from Ajinomoto Co., Ltd. (containing N. B. C. commercial substances) and XIX were offered from Fujisawa Pharm. Co., Ltd.

XX～XXXV were synthesized by the method described in the literatures,[5~13] and XXXVI～XL which are unknown compounds, were newly synthesized in our laboratory for this study and the details will be publishd elsewhere in near future.[14]

Methylene-blue (Merk, Medicinal) was used as its HCl salt.

Method——Toshiba photoreflector lamp (500 W, Spot type) was used as a light source, its UV region was filtered off by glass plate. The irradiation was carried out from a distance of ca. 20 cm. apart from the sample examined under ice-cooling.

Each sample was made into an aqueous solution of 10^{-3} mole/L.*2 and methylene-blue was added to 10^{-4} mole/L. The solution thus prepared was irradiated and sampled every two hours for the UV measurements. At the same time, the experiments were made without methylene-blue under the same condition as described above, and compared to examine whether such photochemical reaction was simple photodegradation or not. The solution was not buffered, but in all cases they indicated pH 5.4～5.6. On guanine and guanosine, experiment was also made under acid and alkaline solution.

Results and Discussion

As a result of above experiment, changes of ultraviolet spectra as a function of irradiation time can be classified in 4 types.

Type I : The type which leads to a complete loss of ultraviolet absorption by irradiation within 6 hours (Fig. 1).

Type II : The type which causes no change or a little in ultraviolet absorption in the same hours (Fig. 2).

Type III : The type, which a different ultraviolet spectrum appears from before irradiation (Fig. 3).

Type IV : The type which causes a change in ultraviolet spectra by irradiaton without methylene-blue (Fig. 4).

*2 In the case that materials were not soluble up to 10^{-3} mole/L., a mark "†" was put on the sample name in above material list. In these cases, the molar ratio of the sample to methylene-blue remained also to be about 10:1.

7) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr : J. Am. Chem. Soc., **80**, 1669 (1958).
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It can be said, that the compounds in Type I and Type III undergo photosensitized reaction in the presence of methylene-blue.

Typical examples of each type are exhibited in Figs. 1~4.

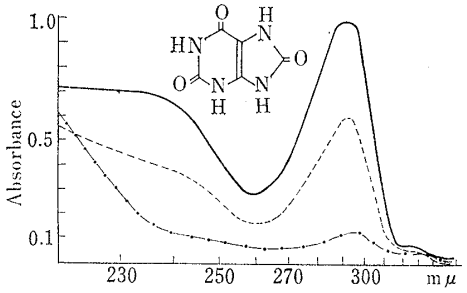


Fig. 1. Ultraviolet Absorption Spectra of Type I (Uric acid (VIII))

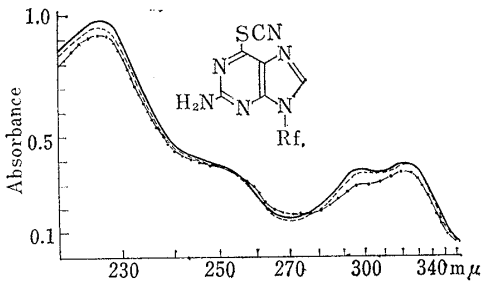


Fig. 2. Ultraviolet Absorption Spectra of Type II (2-Amino-6-thiocyanato-9-(β-D-ribofuranosyl)purine (XL))

before irradiation — — — — after 2 hr. —●—● after 6 hr.

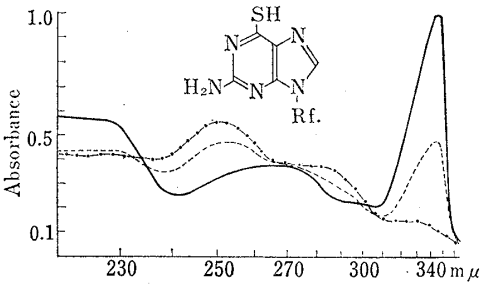


Fig. 3. Ultraviolet Absorption Spectra of Type III (6-Thioguanosine (XXIV))

——— before irradiation
— — — — after 2 hr.
—●—● after 6 hr.

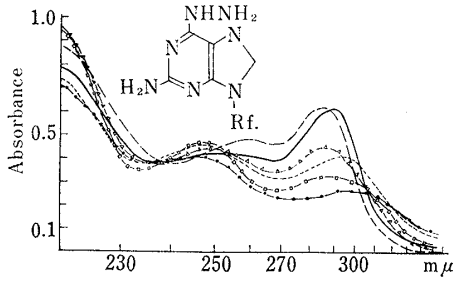


Fig. 4. Ultraviolet Absorption Spectra of Type IV (2-Amino-6-hydrazino-9-(β-D-ribofuranosyl)purine (XXVII))

with methylene-blue
——— before irradiation
- - - - - - - after 2 hr.
—●—● after 6 hr.
without methylene-blue
— — — — before irradiation
—△—△— after 2 hr.
—□—□— after 6 hr.

Compounds belonging to each type are shown in Charts 1~4, respectively.

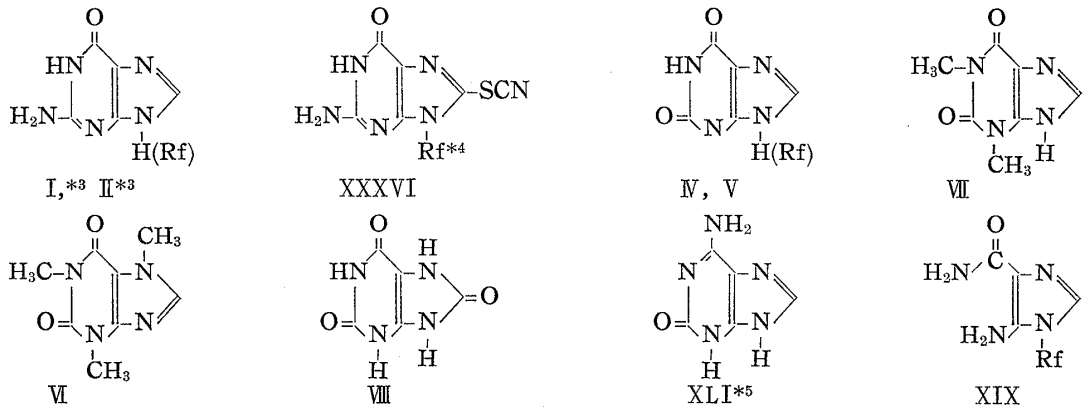


Chart 1. Compounds belonging to Type I

*3 The same results as those by Simon, *et al.*[1]

*4 Rf = β-D-ribofuranosyl.

*5 Cited from Wacker, *et al.*[5] data with thiopyronine.

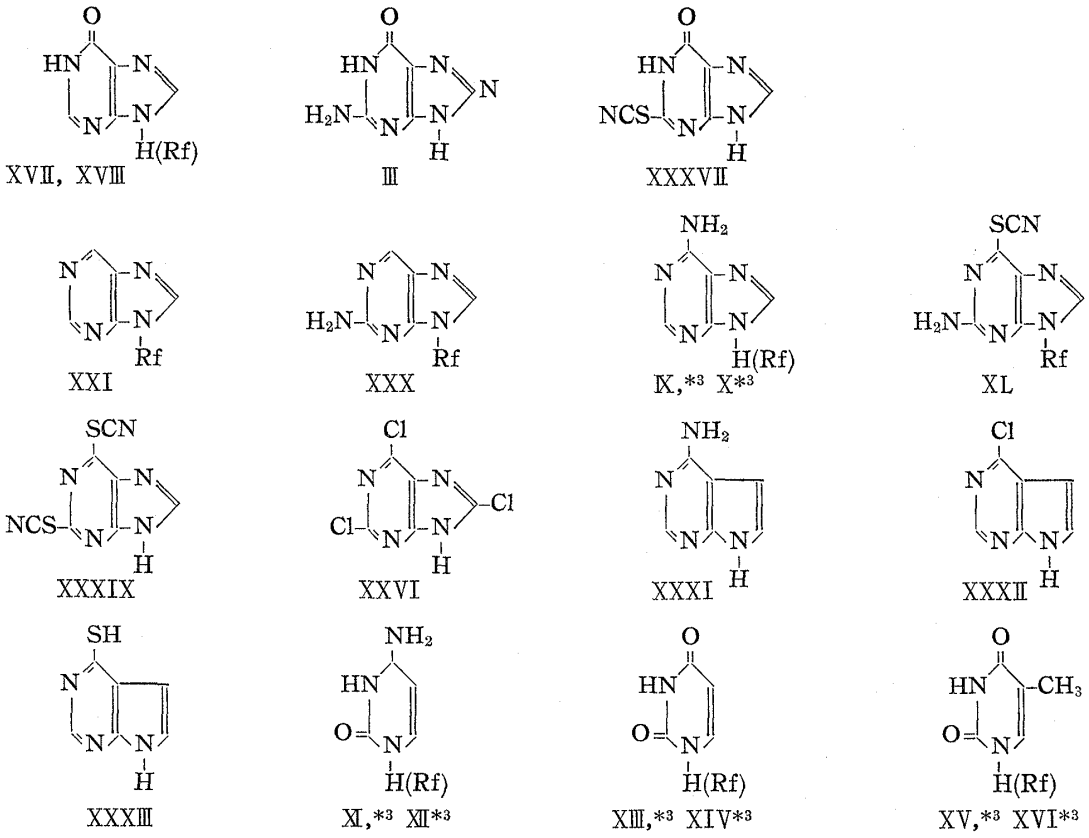


Chart 2. Compounds belonging to Type II

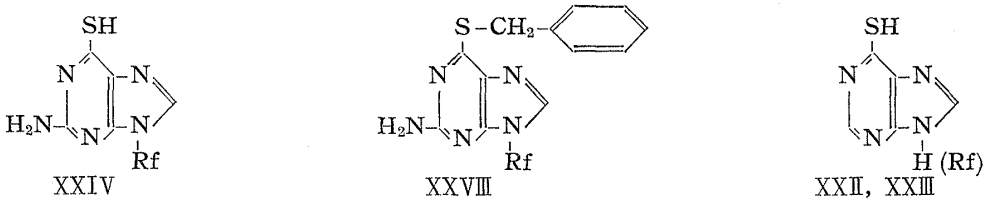


Chart 3. Compounds belonging to Type III

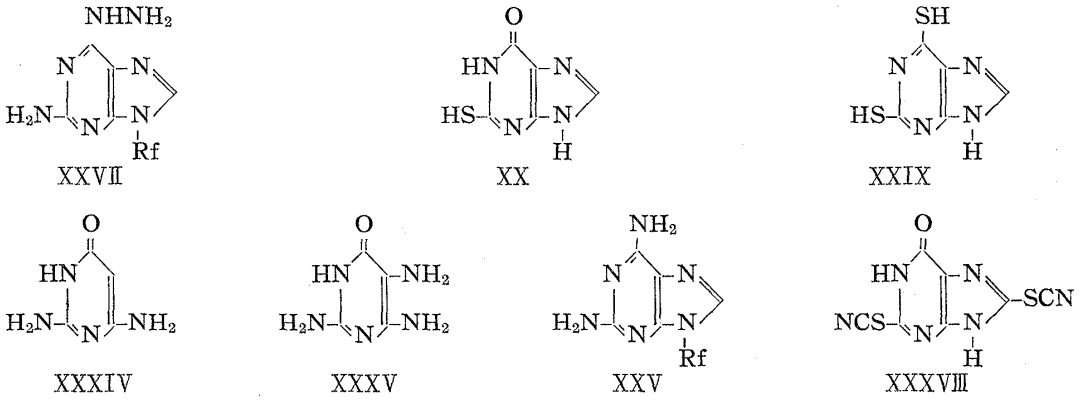


Chart 4. Compounds belonging to Type IV

From the results shown in the above charts, the following can be said.

1) Any of the compounds belonging to Type I as guanosine (II), that are respond to photosensitized reaction, has a carbonyl group at 6-position of purine ring or the corresponding position of other analogue skeletons (AICA-riboside (XIX)).

Further requirement for this photosensitized reactivity is that lactim form is possible as the resonance canonical formulas with respect to nitrogenes 1 and 3-position, in other words, the 2-carbon and its substituent is possibly doubly bonded.

The compounds which, even if have carbonyl group in 6-position, are not satisfied with the second requirement described above, as hypoxanthine (XVII), inosine (XVIII), 2-thiocyanate compound (XXXVII), are inactive in photosensitized reaction.

2) Furthermore, an imidazol ring is required for the photosensitized reactivity besides the essential structure described in 1). Thus, a triazol derivate, 8-azaguanine (III), is inactive, and pyrimidine derivatives such as cytosine (XI), uracil (XIII), thymine (XV) etc., which are satisfied with the requirement 1) are also inactive. A substituent at 8-position of purine ring does not interfere these photosensitized reactions unless a skeletal change occurs in the imidazol moiety. The presence of ribofuranosyl at 9-position does not seem to affect the reaction at all. All compounds belonging to Type II are not satisfied with the requirements of 1) and 2) in part or in all.

3) The compounds belonging to Type III undergo photosensitized reactions to show new types of spectra in ultraviolet region different from the original, in contrast to a complete loss of ultraviolet absorption in case of Type I compounds. For example, a strong absorption newly appeared at 250 mμ, in cases of 6-mercaptopurine (XXII) and 6-thioguanosine (XXIV), while those at 327 mμ and 342 mμ disappeared, respectively.

The results from the compounds belonging to Type IV should be excluded from the present discussion on photodynamic reactions by irradiation only without methylene-blue.

4) Wacker, *et al.*[5] reported on photosensitized reactions of adenine (IX), guanine (I), xanthine (IV), hypoxanthine (XVII), isoguanine (XLI), 2-aminopurine, 2,6-diaminopurine (XXV), and 8-azaguanine (III), using thiopyronine. As compared their results with our experiments, adenine, hypoxanthine, azaguanine are considered to be classified as Type II, and others as Type I from our experimental classification.

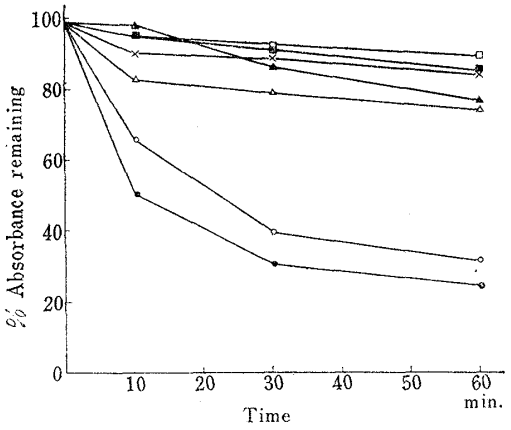


Fig. 5. The Photochemical behaviors of Nucleotides and Nucleoside with Riboflavin.*6

The percentage absorbance remaining was plotted against irradiated time.

—●— xanthosine (V) (255 mμ)
—○— 5′-guanylic acid (255 mμ)
—■— 5′-adenylic acid (260 mμ)
—□— 5′-uridylic acid (265 mμ)
—▲— 5′-inosinic acid (250 mμ)
—△— 5′-cytidylic acid (270 mμ)
—×— 5′-thymidylic acid (265 mμ)

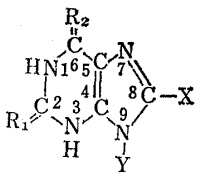
Authors did not make an experiment on isoguanine (XLI), but their results are completely agreed with ours with methylene-blue except for only one case of 2-aminopurine riboside which belongs to Type II in our experiments.

Uehara, *et al.*[15] reported that adenine, adenylyic acid, inosinic acid and guanylic acid were photochemically oxydized in the presence of riboflavin, and that, on the other hand, uridylic acid, thymidylic acid, and cytidylic acid were not oxydized. But according to our results, adenine, adenylic acid and inosine belong to Type II, the compounds difficult to be oxydized so that our results are different from theirs. Our results using riboflavin is shown in Fig. 5.

*6 The reaction mixtures containing 5×10^{-5} moles of each nucleotides, nucleoside and 5×10^{-6} mole of riboflavin in 1 L. of water were irradiated at 2 KW Xenon lamp (Ushio Xe 2000 Type), which were cut off below 350 mμ region by filter (Toshiba UV 35 Type).

15) K. Uehara, T. Mizoguchi, Y. Okada : J. Biochem. (Tokyo), 55, 685 (1964).

As a conculsion, it is revealed that among nucleic acid-bases, nucleosides and their related compounds, the compounds easily photodegradated with methylene-blue have a portial structure A and that they are not influenced by 8 and 9 substitutes of purine ring.



Portial structure A
R_1=O, NH
R_2=O, NH
Chart 5.

The authors are indebted to Dr. Waro Nakahara, Directer of this institute, for heartful encouragement.

Summary

As a study on mechanism of photodynamic action with methylene-blue, the authors examined structural correlation of 40 kinds of nucleic acid-bases, nucleosides and related compounds with their photodynamic degradation by visible light-irradiation in the presence of methylene-blue.

As a result, it became established that: 1) Above-mentioned compounds, in order to be easily photodynamically degradated, need to have the lactim structure with respect to N 1 and 3-position in pyrimidine moiety of purine ring.

2) Imidazol ring seems to be essential for photodegradation.

3) Existence of substituent in 8, 9-position in purine didn't affect on photochemical reaction.

(Received March 26, 1965)

[Chem. Pharm. Bull. 13(9)1113~1130(1965)]

UDC 615.41-014 : 612.398.145

146. Edward R. Garrett, Pramod B. Chemburkar,*1 and Tokuji Suzuki*2 : Prediction of Stability in Pharmaceutical Preparations. XIV.*3 The Complete pH Dependent Solvolytic Degradations of an Iodinated Nucleoside, the Antiviral 5-Iodo-2′-deoxyuridine.*4

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The biologically active nucleoside[1] 5-iodo-2′-deoxyuridine (IDU) is effective as an antitumor agent[2] and as an antiviral agent against herpes simplex in human keratitis.[3]

A complete knowledge of the stability of this compound in solution is vital for its pharmaceutical utility since a possible degradation product, iodouracil (IU), has greater toxicity and can inhibit the antiviral activity of IDU.[4] The observed reversal of the

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*3 This work constitutes a part of a series entitled "Prediction of Stability in Pharmaceutical Preparations" by E. R. Garrett. Part XIII: J. Pharm. Sci., 53, 917 (1964).
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3) H. E. Kaufman, E. L. Martola, C. Dohlman: Arch. Ophthalmol., 68, 235 (1962).
4) E. D. Maloney, H. E. Kaufman: Invest. Opthalmol., 2, 55 (1963).