Pure & Appl. Chem., Vol.52, pp.2705-2715.
Pergamon Press Ltd. 1980. Printed in Great Britain.

ORGANIC CHEMICAL APPROACH TO PHOTO-CROSS-LINKS OF NUCLEIC ACIDS TO PROTEINS

Teruo Matsuura, Isao Saito, Satoru Ito, Hiroshi Sugiyama and Tetsuya Shinmura

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University
Yoshida, Kyoto 606, Japan

Abstract — Various proteins are known to be linked covalently to nucleic acids under UV irradiation. The photo-cross-links occurs from the interacting states between nucleic acids and proteins. The present approach to this problem involves the characterization and identification of photoproducts obtained from model systems using simple pyrimidine bases and amino acids as well as some mechanistic aspects. 5-Bromouracil or its derivatives undergoes selective photocoupling to tryptophan, indoles and some electron-rich aromatics under various irradiation conditions, where four modes of reactions were observed: involvement of (1) triplet bromouracil, (2) a double electron transfer in the presence of an electron carrier, (3) mixed aggregate formation in aqueous frozen solution, and (4) fluorescence quenching of the aromatics by bromouracil. Other topics of the photochemistry of pyrimidine bases, including synthesis of fluorescent uracils using photo-cross-coupling with 5-chloro- and 5-iodouracils, photoreactions between a 4-thiouracil and L-lysine and between thymine and L-tryptophan, and synthesis and behaviors of model compounds for stacking interactions between 5-bromouracil (or thymine) and tryptophan.

INTRODUCTION

The active study of nucleic acid-protein interactions in recent years has emphasized that these interactions play essential roles in life processes and their controls. Thus the formation of specific complexes between nucleic acids and proteins is of central importance in molecular biology. More recently, many reports have indicated that stable covalent complexes between nucleic acids and proteins are formed *in vitro* and *in vivo*, when cells are irradiated with ultaviolet light, causing damages of the living systems such as aging, carcinogenic and lethal effects (Ref. 1). This type of photodamage is certainly of impotance as much as other known damages of nucleic acids caused by light irradiation such as the photodimerization (Ref. 2) and photohydration (Ref. 3) of pyrimidine bases and the photodynamic action (Ref. 4).

The photoinduced cross-links of nucleic acids to proteins have been found to require specific complex formation between the interacting biopolymers, indicating the non-random nature of the cross-linking reactions (Ref. 5). The tendency of nucleic acids and proteins to form specific covalent adducts by UV irradiation is also used as a probe for the investigation of the structure of native nucleic acid-protein complexes (Ref. 1, 2). Despite many approaches to solve the problem, little is yet known about the chemical nature of these photo-cross-links. We have approached to this problem utilizing the photochemistry of simple model systems to freeze existing contact points in the nucleic acid-protein complexes, which will allow us to contribute to the characterization and identification of the interacting residues as well as the understanding of certain kinds of photodamages of living systems. The present paper summarizes recent results obtained from our laboratory.

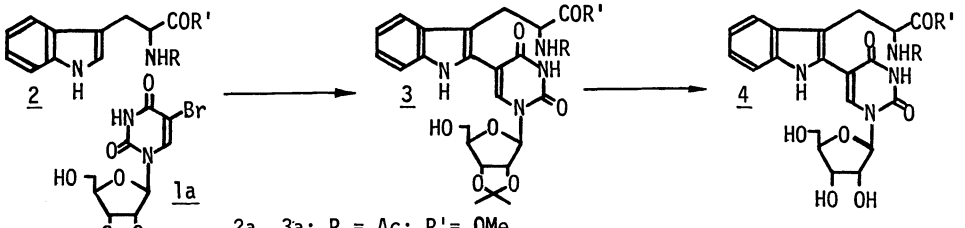
I. PHOTOCOUPLING OF 5-BROMOURACILS

When thymine is replaced by 5-bromouracil (BrU) in DNA, the sensitivity of certain cells to killing by UV radiation is remarkably increased (Ref. 6). Among three possible mechanisms proposed for this sensitizing effect, self-coupling of two BrU residues to form 5,5'-diuracilyl linkage (Ref. 6), induction of single-strand breaks in DNA (Ref. 7), and rate enhancement of production of DNA-protein cross-links in cells (Ref. 8), the third mechanism drew our particular attention. On the other hand, Varghese has shown that thiols such as cysteine undergo photocoupling to BrU (Ref. 9). The formation of 5,5'-diuracilyl and the thiol coupling products from BrU has been interpreted in terms of 5-uracilyl radical formed by C-Br linkage (Ref. 6b), probably via a singlet excited state.

As a model for the photo-cross-coupling of BrU-substituted DNA with proteins, we have investigated the photoreaction of 5-bromouracil derivatives with various aromatic amino acid derivatives and found that tryptophan derivatives undergo a facile and regiospecific photocoupling. Detailed studies of this photocoupling reaction with indoles other than tryptophan derivatives and with some electron-rich aromatic compounds, led us to obtain a finding that there are four modes of photocoupling reaction (eqs. 1 - 4).

(A) Triplet-mediated coupling of 5-bromouracils to indoles via electron-transfer process (Ref. 10, 11).

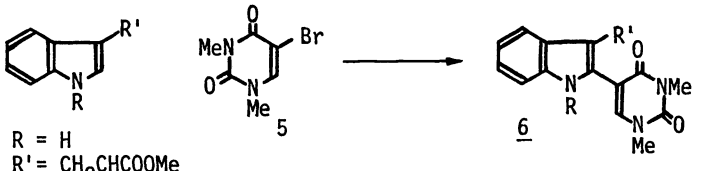
Acetone-sensitized irradiation of 2',3'-O-isopropylidene-5-bromouridine (1a) with Pyrex-filtered light in acetonitrile in the presence of N^b-acetyltryptophan methyl ester (2a) gave 3a in 70 % yield. A similar coupling product, 3b (80 %) or 3c (49 %), was obtained from 1a and 2b or 2c under the same irradiation conditions. During irradiation, part of the tryptophan derivatives gave rise to polymeric products, possibly originated from hydrogen abstraction from them by acetone triplet (Ref. 12). Mild acid treatment of 3a and 3b gave 4a (55 %) and 4b (56 %) respectively. Under the same irradiation conditions melatonin (N^b-acetyl-5-methoxytryptamine) was photolabile to decompose to polymeric products, but under direct irradiation with 254-nm light in the presence of 1a it gave a similar coupling product (13 %) together with a uridine (35 %), the debrominated product of 1a, which might arise via C-Br bond fission from the singlet excited state of 1a (Ref. 6).



2a, 3a: R = Ac; R'= OMe
2b, 3b: R = tBoc; R'= OMe
2c, 3c: R = tBoc;
R'= $NHCH(COOCH_2Ph)CH_2CHMe_2$

4a: R = Ac; R'= OMe
4b: R = H; R'= OMe

A mechanistic study of this coupling reaction was carried out using a simple analog, 5-bromo-1,3-dimethyluracil (5) in place of 1a. Acetone-sensitized irradiation of 5 in the presence of 2a, 3-methylindole, and 1,3-dimethylindole with Pyrex-filtered light in acetonitrile gave a coupling product 6a (67 %), 6b (66 %), and 6c (71 %) respectively. The coupling reaction of 5 with 2a leading to 6a was not sensitized by acetophenone (E_T = 74 kcal/mol), benzophenone (68 kcal/mol) and triphenylene (65 kcal/mol). Comparing these triplet energies with those known or estimated for 3-methylindole (68 kcal/mol), tryptophan (65 kcal/mol) and 5-bromouracil (∿74 kcal/mol), it seems reasonable that the triplet state of 5 formed from triplet acetone (79-82 kcal/mol) is responsible for the photocoupling reaction. This was also supported by the fact that the formation of 6a (13 %) from the unsensitized irradiation of 5 and 2a was inhibited by the addition of 1,3-pentadiene which did not affect the simultaneous formation of the debrominated product, 1,3-dimethyluracil (75 %).

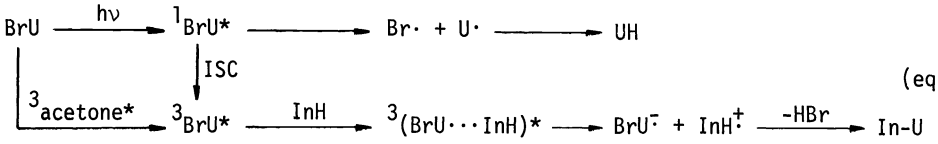


2a: R = H
R'= $CH_2CHCOOMe$
NHAc

7: R = H
R'= $CH_2CH_2NHCOOMe$

6a: R = H; R'= $CH_2CHCOOMe$
NHAc
6b: R = H; R'= Me
6c: R = R'= Me
6d: R = H;
R'= $CH_2CH_2NHCOOMe$

On the basis of the following facts, we proposed an electron-transfer mechanism of eq. 1., which involves interaction of the triplet state of 5 (BrU) with indoles (InH), possibly giving a triplet excited complex, followed by its dissociation into a radical ion pair. The anion radical of 5 ($BrU^{\cdot-}$) thus formed will release Br anion yielding a 5-uracilyl radical which combines with the indole cation radical ($InH^{\cdot+}$) followed by deprotonation to form the coupling product (In-U). Alternatively for the unsensitized photoreaction of 5 and 2a, bromouracil triplet may be formed via an inefficient intersystem crossing of the singlet excited BrU, which mainly undergoes debromination into the parent uracil (UH).

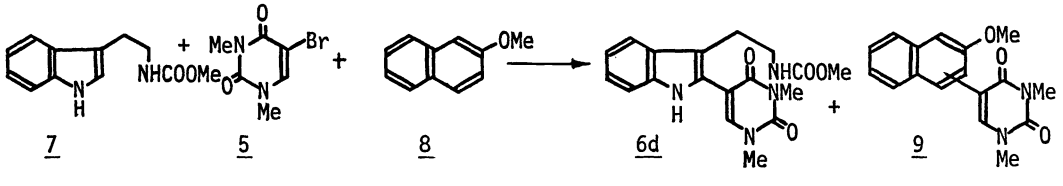


(eq. 1)

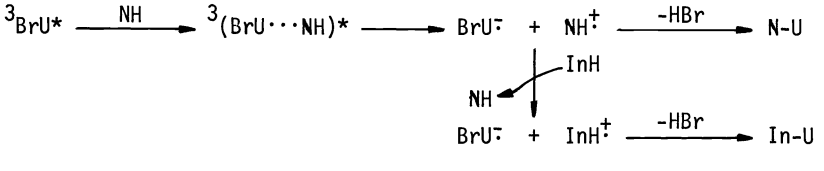
For the acetone-sensitized coupling between 5 and 2a giving rise to 6a, (1) the fact that the coupling reaction occurs regiospecifically on the 2-position of indoles, suggested that the reaction involves an electron-transfer process (for example, see Ref. 14). (2) The coupling reaction was quenched by adding a compound (0.1 equivalent to 5) such as N,N, N',N'-tetramethyl-*p*-phenylenediamine (TMPD; E^{ox} = 0.36 V vs. SCE) having a lower E^{ox} than that of 2a (E^{ox} = 0.82 V), while 1,4-dimethoxybenzene (E^{ox} = 1.34 V) and 1,2,4,5-tetramethoxybenzene (E^{ox} = 0.81 V), having a higher or comparable E^{ox} compared to that of 2a, did not inhibit the coupling reaction. (3) Addition of 1,2,4,5-tetracyanobenzene (TCNB) (0.1 equivalent to 5) having a higher reduction potential than that of 5 (E^{red} = -0.90 V) quenched the formation of 6a. The role of TMPD and TCNB in this quenching may be to undergo facile electron transfer from the intermediate radical ions, InH$^+$ and BrU$^-$ respectively, resulting in the formation of TMPD$^+$ and TCNB$^-$ respectively. (4) As will be described in the next section (B), N^b-methoxycarbonyltryptamine (7), which gave no coupling product 6d with 5 under acetone-sensitized irradiation, reacted smoothly with 5 in the presence of 2a or other electron carriers.

(B) Triplet-mediated coupling of 5-bromo-1,3-dimethyluracil (5) to N^b-methoxycarbonyltryptamine (7) involving a double electron transfer (Ref. 11, 15).

Among indole derivatives tested for the acetone-sensitized coupling of 5 yielding 6, 7 gave no coupling product 6d. However, this difficulty was overcome by adding an electron carrier such as 2a and 2-methoxy- (8), 2,3-dimethoxy- and 1,4-dimethoxynaphthalenes in the reaction system. For example, irradiation of a mixture of 5, 7, and 2a (1 : 2 : 2) in 1 : 3 acetone-acetonitrile with Pyrex-filtered light gave a mixture of the corresponding coupling products 6a (42 %) and 6d (22 %). Similar irradiation of a mixture of 5, 7, and 8 (1 : 2 : 1) gave 6d (38 %) and a mixture of two isomeric coupling products 9 (31 %). Under the conditions, about 80 % of the incident light was absorbed by acetone. Exclusive involvement of the triplet state of 5 is supported by the finding that neither 6d nor 9 was formed by sensitizing by acetophenone, benzophenone or triphenylene in place of acetone. Direct irradiation of a mixture of 5 and 8 in the absence of acetone gave 9 in only 12 % yield.



We proposed a mechanism involving a double electron transfer (eq. 2). According to this mechanism, triplet bromouracil (^{3}BrU*) interacts with an electron carrier (NH) to give a radical ion pair, possibly via a triplet excited complex. The NH$^+$ cation radical undergoes either coupling to the bromouracil anion radical (BrU$^-$) yielding the coupling product (N-U) such as 9 or an electron transfer from 7 (InH) followed by coupling of InH$^+$ thus formed to BrU$^-$ yielding another coupling product (In-U) such as 6d. The following experimental data



(eq. 2)

are consistent with this mechanism. (1) The competitive coupling of 5 to 7 (E^{ox} = 0.75 V) and 8 (E^{ox} = 1.52 V) or to 7 and 2a (E^{ox} = 0.82 V) showed a marked concentration dependency (Fig. 1). The results indicate that the presence of an electron carrier having an oxidation potential higher than that of 7 is necessary for the formation of 6d. (2) The coupling of 7 to 5 in the presence of 8 was quenched by adding catalytic amounts (0.1 equivalent to 5) of a quencher having a lower E^{ox} than that of 7 (E^{ox} = 0.75 V) such as TMPD and N,N-dimethyl-*p*-anisidine (E^{ox} = 0.50 V) but not as 1,2,4,5-tetramethoxybenzene. The result indicates that such a quencher undergoes selective electron quenching of the indole cation radical (InH$^+$). (3) When the coupling reaction was carried out using a 1 : 2 : 0.5 molar mixture of 5, 7 and 8 until 60 % of 5 was consumed, no naphthalene-uracil coupling product 9 was found and 80 % of 8 remained unreacted despite the formation of 6d in 38 % yield. The large recovery of 8 indicates that it apparently acts as a catalyzer.

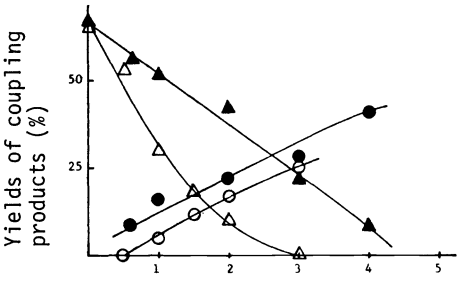
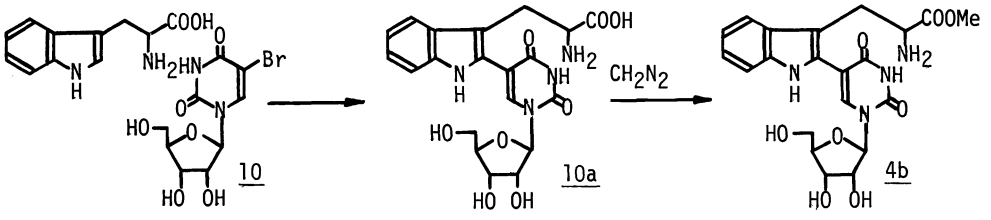


Fig. 1. Dependence of the yields of coupling products on the concentrations of 7. The plots (○) and (△) show the yields of 6d and 9 respectively in the system 7/8. The plots (●) and (▲) show the yields of 6d and 6a respectively in the system 7/2a.

(C) Coupling of 5-bromouridine (10) to tryptophan in aqueous frozen solution.[#] (Ref. 16)

The observation that mixed aggregates can be formed upon freezing aqueous solutions of two solute molecules, has allowed an extensive study of complex formation between necleic-acid bases and aromatic amino acids. Investigation of photoreactions occurring within such mixed aggregates in aqueous frozen systems is particular useful in understanding the nature of the photo-cross-links in nucleic acid-protein complexes (Ref. 17). In our attempts along this line, we found that a photocoupling of 5-bromouridine (BrUd) or 5-bromouracil to tryptophan (Trp) readily proceeds *only in aqeuous frozen soluiton* giving rise to a coupling product 10a not observed in the photoreaction in the aqueous fluid solution.

Irradiation of a 1 : 1 mixture of BrUd and Trp in unbuffered aqueous frozen solution at -78°C with light through a filter (>270 nm) gave a photoproduct which was separated by preparative high-pressure liquid chromatography. The structure of the photoproduct was assigned as 10a on the basis of its spectral properties, particularly UV, H-NMR and fluorescence spectra, which are characteristic of 3 and 4 previously described. Treatment with diazomethane in aqueous methanol gave a mixture of products, among which the methyl ester 4b was separated and identified. A similar photoproduct was obtained by the irradiation of a 1 : 1 mixture of 5-bromouracil and Trp in aqueous frozen solution.



The following observations suggest that the photocoupling reaction may proceed via mixed aggregate formation between BrUd and Trp, most probably originated from stacking interactions between them in aqueous frozen solution, as eq. 3, where BrU and InH denote BrUd (or bromo-uracil) and Trp respectively. (1) The fluorescence of Trp was efficiently quenched by only

$$BrU + InH \xrightarrow{\text{aq. frozen soln.}} [BrU\cdots InH] \xrightarrow{h\nu} BrU^{\cdot-} + InH^{\cdot+} \xrightarrow{-HBr} In\text{-}U \quad \text{(eq. 3)}$$

0.025 equivalent mole of BrUd in aqueous solution at 77 K, while it was not quenched appreciably by an equimolar amount of BrUd in aqueous solution at room temperature. (2) Addition of acetone or mineral salts, such as NaCl and K_2CO_3, to the frozen reaction system completely inhibited the formation of 10a, as the known prevention of aggregate formation by the addition of such substances in aqueous frozen solution. (Ref. 17). The observed quenching of Trp fluorescence indicates that a very efficient electron-transfer (or energy-transfer) from the excited state of Trp to BrUd occurs within the mixed aggregate. Stacking interactions between Trp and nucleosides leading to quenching of Trp fluorescence has been ascribed to a strong electron donor-acceptor interaction in the excited state of Trp acting as the electron donor (Ref. 18). It should be noted that no heavy-atom effect of the bromine atom was observed in the case of BrUd, in contrast to what has been reported in the case of 5-mercuripyrimidines (Ref. 19).

[#] This part of work was done in collaboration of C. Helene, Centre de Biophysique Moleculaire, CNRS, Orlean, France.

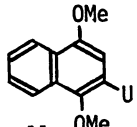
Photoreactions of BrUd and other aromatic amino acids such as phenylalanine, tyrosine and histidine were examined in aqueous frozen solution. However, no coupling product was detected by careful TLC analyses of the photolyzed mixture. Despite that irradiation of BrUd and cysteine in aqueous solution gives 5-cysteinyluracil (Ref. 9), we found that irradiation in an aqueous frozen solution with Pyrex-filtered light gave no coupling product. Thus, the photocoupling of BrUd is specific to Trp in aqueous frozen solution. A similar coupling may probably occur between BrU-substituted DNA and tryptophyl residues in a protein. In such cases, the coupling product may be readily detectable by its characteristic fluorescence emission (λ_{max} 460 nm excited at 360 nm).

(D) Coupling of 5-bromo-1,3-dimethyluracil (5) to naphthalenes via fluorescence quenching (Ref. 20).

In the course of our investigation on the sensitized coupling of 5 and 7 in the presence of naphthalene derivatives as the electron carrier (Section I-B), the coupling between 5 and the naphthalenes (eq. 2) draw our attention in view of the facile synthesis of fluorescent uracil derivatives. We found that in the absence of acetone sensitizer, 5 undergoes the fourth type of photocoupling to methyl- and methoxynaphthalenes (NH) via quenching of naphthalene fluorescence (eq. 4).

$$NH \xrightarrow{h\nu} {}^1NH^* \xrightarrow{BrU} {}^1(NH\cdots BrU)^* \longrightarrow NH^{\cdot+} + BrU^{\cdot-} \xrightarrow{-HBr} N\text{-}U \qquad \text{(eq. 4)}$$

Irradiation of an acetonitrile solution of 5 and naphthalene derivatives with Pyrex-filtered light gave coupling products 11 - 14, which have a fluorescence emission maximum at 418 - 457 nm (Table 1). Under the irradiation conditions, 75 - 80 % of the incident light was absorbed by the naphthalenes. Thus 1,4- and 2,3-dimethoxynaphthalenes gave a single coupling product 11 and 12 respectively, and 1-methoxy, 2-methoxy, 1-methyl- and 2-methylnaphthalenes gave an inseparable 1 : 1 mixture of two isomeric products, 13a, 14a, 13b and 14b respectively.



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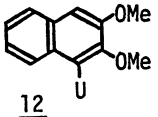

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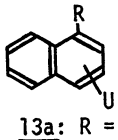
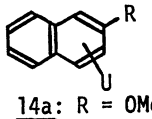


13a: R = OMe
13b: R = Me



14a: R = OMe
14b: R = Me

Table 1. Photo-coupling of 5-bromo-1,3-dimethyluracil (5) with methyl- and methoxynaphthalenes.

Naphthalenes	Products	Yield (*a*) unsens. irradn.	Yield (*a*) acetone-sens. irradn.	Fluorescence max. of product nm (*b*)	k_q (*c*) $M^{-1}sec^{-1}$
1,4-$(MeO)_2$	11	40	0	457	5.7×10^9
2,3-$(MeO)_2$	12	44	10	421	3.1×10^8
1-MeO	13a	41	0	426	3.0×10^9
2-MeO	14a	12	65	420	8.0×10^8
1-Me	13b	54	0	409	1.1×10^9
2-Me	14b	53	0	418	1.6×10^9

(*a*) Based on the consumed 5. (*b*) Excited at 320 or 330 nm. (*c*) Obtained from the observed $k_q\tau$ values.

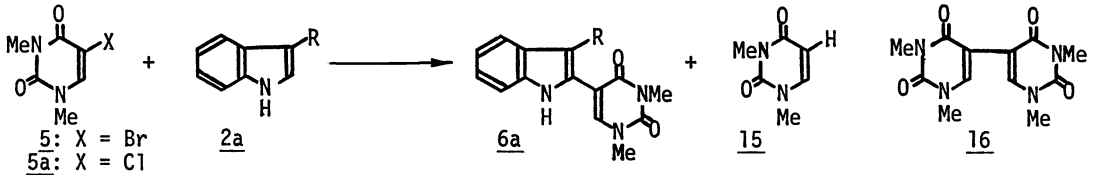
On acetone-sensitization only 2,3-dimethoxy- and 2-methoxynaphthalenes gave the coupling products 12 and 14a respectively, indicating that in other cases triplet states may not be involved in the formation of the coupling products. The fluorescence of the naphthalenes was found to be quenched by 5-bromo-1,3-dimethyluracil (5). The observed rate constants (k_q) for fluorescence quenching are fairly close to that of diffusion-controlled reaction (k_{dif} = 2.7×10^{10} $M^{-1}sec^{-1}$ in acetonitrile at 20°C) with the exception of 2,3-dimethoxy- and 2-methoxynaphthalenes, which have a smaller k_q value. Another significant finding in the quenching experiments is that the k_q values for the fluorescence -quenching of 1,4-dimethoxynaphthalene (k_q = 32.7 M^{-1}) and 1-methoxynaphthalene (40.5 M^{-1}) were comparable to those (44.4 and 35.0 M^{-1} respectively) obtained from the kinetics of their unsensitized coupling reaction. The results support that complexation of the naphthalene singlet with 5, presumably exciplex formation, followed by an electron transfer is a common process in both the fluorescence quenching and the coupling reaction.

In contrast to the above observations, the Stern-Volmer plot for the unsensitized coupling reaction between 2,3-dimethoxynaphthalene and 5 gave no linear relation, suggesting that the reaction may involve an electron-transfer process from the triplet naphthalene to some extent like eq. 1, in competion with the electron-transfer from the singlet naphthalene as eq. 4.

The k_q values for 9-methylanthracene (8.7×10^7 M^{-1}) and 9,10-dimethoxyanthracene (1.4×10^6) were significantly smaller than those for the naphthalenes, and they failed to undergo photocoupling to 5 but gave the corresponding 5,5'-diuracilyl (Cf. Ref. 6).

II PHOTOCOUPLING OF 5-CHLORO- AND 5-IODOURACILS (Ref. 21, 22)

Replacement of thymine by 5-chloro- and 5-iodouracils in DNA increases the UV-sensitivity of cells like the case of 5-bromouracil (Ref. 6). A comparative study was carried out the photocoupling reaction of 5-chloro (5a), 5-bromo- (5) and 5-iodo- (5b) 1,3-dimethyluracils with the tryptophan derivatives 2a under three irradiation conditions: acetone-sensitized irradiation with Pyrex-filtered light (>290 nm), unsensitized irradiation with Pyrex-filtered light and unsensitized irradiation with 254-nm light. As shown in Table 2, the chlorouracil 5a gave the coupling product 6a in all cases, whereas the major photoreaction of the iodouracil 5b was reduction into 1,3-dimethyluracil (15), most probably via the 5-uracilyl radical. In the absence of 2a, the iodouracil 5b again gave 15 exclusively in all the irradiation conditions, whereas the chlorouracil 5a gave rise to 1,3,1',3'-tetramethyldiuracilyl (16) but gave no reduction product. The chlorouracil 5a showed higher photoreactivity than the bromouracil 5 toward indole derivatives leading to coupling products of type 6a. Thus, a mixture of 5a and 7 yielded, under acetone-sensitized (54 %) and unsensitized (26 %) irradiation (>290 nm) conditions, the coupling product 6d, which could not be obtained from the bromouracil 5 and 7 under both conditions.



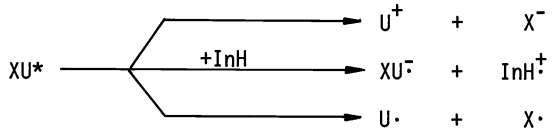
5: X = Br
5a: X = Cl
5b: X = I

2a

Table 2. Photoreaction of 5-halo-1,3-dimethyluracils, 5a, 5 and 5b.

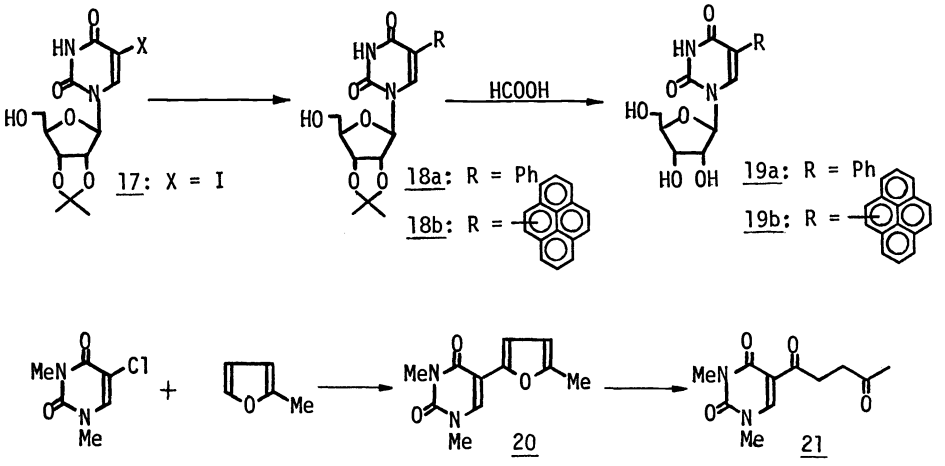
Irradiation conditions	Halogen	Products in the presence of 2a (% yield) 6a	15	Products in the absence of 2a (% yield) 15	recovd.
acetone-sensitized (>290 nm)	Cl	61	0	59(16)	41
	Br	67	0	2	94
	I	8	81	67	0
unsensitized (>290 nm)	Cl	31	0	0	100
	Br	0	0	0	100
	I	0	94	75	0
unsensitized (254 nm)	Cl	54	38	0	80
	Br	15	74	52	20
	I	6	81	99	0

The results suggest that the halouracils (XU) undergo three types of primary photoreaction in the presence and absence of an electron donor such as 2a, as eq. 5: (i) heterolytic fission of C-X bond, (ii) electron transfer from a donor (InH) and (iii) homolytic fission of C-X bond. The chloro-, bromo- and iodouracils seem to favorable undergo process (i), (ii) and (iii) respectively.



(eq. 5)

The photoreactions of the chloro- and iodouracils were utilized for the synthesis of some 5-substituted uracils, particularly of fluorescent uracil derivatives. For example, irradiation of 2',3'-O-isopropylidene-5-iodouridine (17) in 3 : 1 acetonitrile-benzene with Pyrex-filtered light gave 18a which was hydrolyzed to 5-Phenyluridine (19a). Under similar irradiation conditions 17 and pyrene in acetonitrile gave 18b which was derived to 5-pyrenyluridine (19b). While neither 5 and 5b produced the coupling products with furans upon sensitized and direct irradiation, direct irradiation of the chlorouracil 5a with an excess of 2-methylfuran in acetonitrile containing aqueous K_2CO_3 gave a coupling product 20 which was converted into 21. Most of the above products are fluorescent implying potential utility as a fluorescence probe. Emission max, nm (excitation wavelength, nm): 19a, 417 (310) in acetonitrile; 19b, 460 (360) in methanol; 20, 417 (346) in acetonitrile.

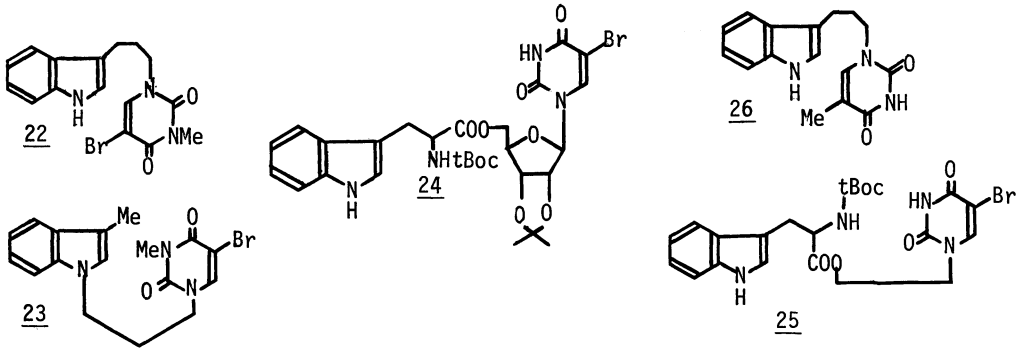


III. MODEL STUDY FOR INTERACTIONS BETWEEN PYRIMIDINE BASES AND TRYPTOPHAN

(A) Between 5-bromouracil and tryptophan. (ref. 22)

Among various types of photocoupling between 5-bromouracils and indoles, the coupling reaction of 5-bromouridine to L-tryptophan leading to the formation of 10a in aqueous frozen solution is of particular interest, because aggregate formation between them is essential for the photocoupling. In order to gain insight on the role of the geometry of the interacting molecules in their photochemical behaviors, several model compounds, 22 - 25, having a bromouracil ring and an indole nucleus in a molecule, were synthesized and their spectral and photochemical behaviors were examined.

Compounds 22 and 23 were synthesized by the condensation of 5-bromo-3-methyluracil with the corresponding bromides by modifying the reported procedure (Ref. 24, 25). Compounds 24 and 25 were synthesized from N^b-t-Boc-tryptophan anhydride and the corresponding alcohols by the reported procedure (Ref. 26).



Although the chemical shifts of the ring protons and the UV absorption maxima of these model compounds showed no significant shifts from those observed for the parent indole and bromouracil derivatives, we observed a hypochromic effect in their UV absorptions as exemplified by Fig. 2 for compound 24. The percent hypochromism *H* calculated (Ref. 27) are given in Table 3. All of the model compounds showed appreciable hypochromism indicating the occurrence of intramolecular interactions between the indole moiety and the bromouracil ring, corresponding to folded (or stacked) conformations.

In accordance with this indication, the fluorescence of the indole moiety was quenched by the bromouracil moiety in all of the model compounds, as exemplified by Fig. 3. The relative quenching efficiencies, q^{rel}, given in Table 3 were determined by comparing the fluorescence intensity of each model compound with that of a 1 : 1 mixture of the component compounds. From comparison of the hypochromism and quenching data of Table 3 with those for a model compound 26 (Ref. 24), it is reasonable to assume that 22, 23 and 25 have significantly folded (or stacked) conformations in acetonitrile solution to an extent comparable to 26, which is known to have 45 % of the degree of internal association (Ref. 24). Such folded conformations may not be significant for compound 24 which has a q^{rel} value higher by one order of magnitude than those for other model compounds.

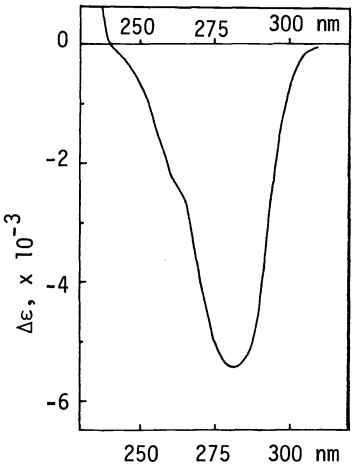


Fig. 2. Difference UV spectrum of 24 vs. a 1 : 1 mixture of 1a and t-Boc-tryptophan methyl ester in acetonitrile.

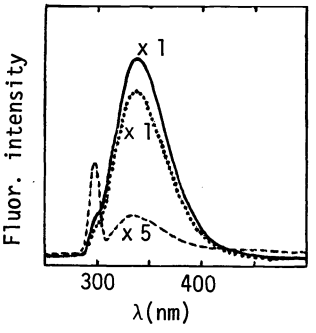


Fig. 3. Fluorescence spectra in acetonitrile solutions at room temperature of 24 ( ), an equimolar mixture of 1a and t-Boc-tryptophan methyl ester ( ), and t-Boc-tryptophan methyl ester alone ( ).

Table 3. Percent hypochromism for 235 - 300 nm absorption band and relative quenching efficiency for model compounds 22 - 25 (*a*)

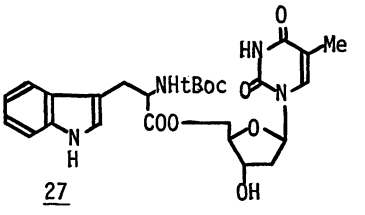
	22	23	24	25	26 (*b*)
% *H* (CH_3CN)	11.0	9.5	8.4	3.1	9.3
q^{rel}(CH_3CN)	0.010	0.009	0.125	0.053	0.030

(*a*) The parent components are: 5 and methyl 3(indol-3-yl)propionate for 22; 5 and methyl 3-(3-methylindol-1-yl)propionate for 23; 1a and t-Boc-tryptophan methyl ester for 24; and 5 and t-Boc-tryptophan methyl ester for 25. (*b*) From Ref. 24.

In the hope of the occurrence of intramolecular coupling between the bromouracil and indole moieties of the model compounds, photoreactions were carried out in acetonitrile. In all cases, however, we failed to get any intramolecular coupling product. On acetone-sensitization using Pyrex-filtered light, compounds 22 - 25 gave polymeric products and/or a complex mixture of products. Under unsensitized irradiation conditions the model compounds were largely recovered unchanged (50-80 %), while 22 was less recovered (14 %). The results suggest that the geometry of the model compounds is not favorable for internal coupling

(B) Between thymine and tryptophan (Ref. 23).

As will be described below, irradiation of thymine and L-tryptophan in aqueous solution gave two photoproducts, one of which was selectively obtained on irradiation in aqueous frozen solution. In order to gain insight on these phenomena, model compounds 27 - 30 were synthesized and their spectral and photochemical behaviors were examined.



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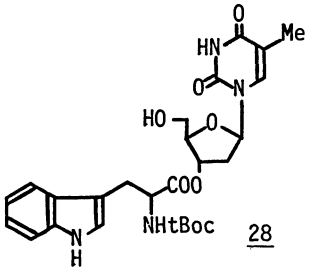

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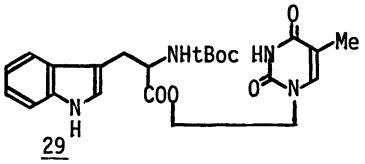

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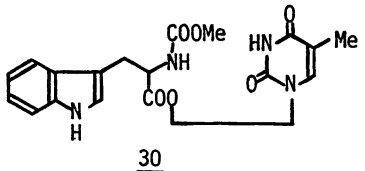

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Compounds 27 and 28 were synthesized by the condensation of t-Boc-tryptophan with thymidine using DCCD, and the condensation of t-Boc-tryptophan with 1-(2-hydroxyethyl)thymine gave 29 which was derived into 30 by selective hydrolysis followed by methoxycarbonylation.

All of the model compounds had a hypochromic effect in their UV spectra compared with those of their parent components. The percent hypochromism data are shown in Table 4, which also lists their relative quenching efficiencies obtained from quenching data of the fluorescence of the tryptophan moiety by the thymine moiety. The values for 27, 28 and 30 are comparable to those for 25 and 26 (Table 3) indicating that these three compounds may have also folded (or stacked) conformations.

Irradiation of compounds 27 - 30 in acetonitrile with 254-nm light yielded polymeric products but no internal photocoupling product was detected.

Table 4. Percent hypochromism and relative quenching efficiency for model compounds 27 - 30 (α)

	27	28	29	30
% H (CH_3CN)	6.0	3.0	22.9	28.1
q^{rel}(CH_3CN)	0.075	0.340	0.072	0.035

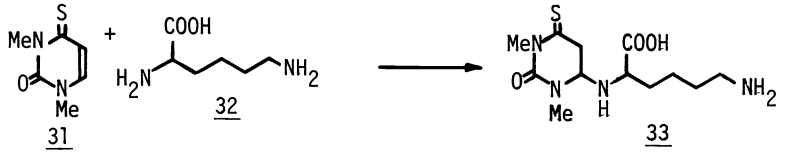
(α) The parent components are: t-Boc-tryptophan methyl ester and 3',5'-diacetylthymidine for 27 and 28; t-Boc-tryptophan methyl ester and 1-(2-acetoxyethyl)-thymidine for 29; and N^b-methoxycarbonyltryptophan methyl ester and 1-(2-acetoxyethyl)thymidine for 30.

IV. PHOTOREACTIONS OF OTHER PYRIMIDINE BASES TO AMINO ACIDS

(A) Photoaddition of L-lysine to 1,3-dimethyl-4-thiouracil (31) (Ref. 28).

Among the naturally occurring fluorescent minor bases, 4-thiouracil (4-SRra) is of particular interest because of its high photochemical reactivity (Ref. 29) and of a photochemical covalent binding between 4-thiouridine and RNase (Ref. 30). Considering the high photoreactivity of 4-Sr toward olefins, alcohols and alkyl amines (Ref. 31), it seems likely that selective excitation of 4-Sra (335 nm) in tRNAs induces a covalent link to an adjacent nucleophilic group of nuclear proteins. In this respect the following model reaction was examined.

Irradiation of an aqueous unbuffered solution of 1,3-dimethyl-4-thiouracil (31) in the presence of L-lysine (32) with Pyrex-filtered light gave a 1 : 1 mixture of two photoadducts (23 %). Recrystallization from aqueous ethanol gave one of the photoadducts as crystals, positive to the ninhydrin test. The structure of the crystalline photoproduct was assigned as 33 on the basis of its spectral and chemical properties. Attempts to isolate another photoproduct in a pure form were unsuccessful. However, the elemental analysis of the 1 : 1 mixture of photoproducts was the same as that of the crystalline photoproduct. The 13C-NMR spectrum of the 1 : 1 mixture shows twelve signals assignable as those of a diastereomer of the crystalline photoproduct, in addition to the signals due to the latter. Thus, it is reasonably concluded that the photoreaction gave a 1 : 1 mixture of two diastereomers of 33.



31 32 33

It should be noted that the attacking mode of the alkyl chain of lysine to photoexcited 31 is different from that reported by Forrey (Ref. 31) who demonstrated that irradiation of 31 with 1-aminobutane in dichloromethane gives two diastereomeric products 34, which may involve a radical process. The present reaction is apparently a nucleophilic ionic addition of the α-amino group of L-lysine to 31 rather than a radical addition reaction.

34

(B) Photoreaction of thymine with L-tryptophan (Ref. 32).

Recently, Reeve and Hopkins have reported that irradiation of thymine (or uracil) and L-tryptophan in aqueous buffered solution at pH 7.0 with filtered light (>260 nm) gave two thymine-tryptophan photoproducts (or four uracil-tryptophan photoproducts) (Ref. 33). We also independently found that irradiation of an aqueous unbuffered solution of a mixture of thymine and L-tryptophan with various ratios with Pyrex-filtered light or 254-nm light at room temperature yielded two photoproducts, A and B, in up to 50 % yield, as well as the formation of some minor products (Fig. 4). Although the structures of these products A and B are not elucidated yet, the elemental and spectral analyses indicate that both products are not simple adducts.

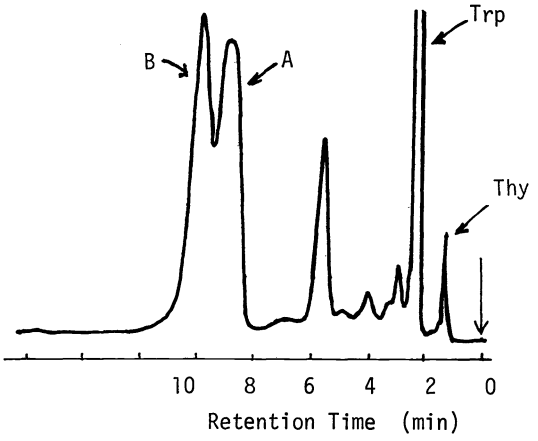


Fig. 4. High-pressure liquid chromatogram of the photolyzed mixture from thymine and L-tryptophan in aqueous fluid solution.

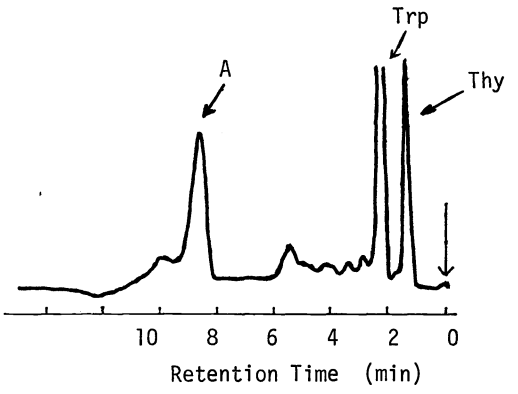


Fig. 5. HPLC of the photolyzed mixture from thymine and L-tryptophan in aqueous frozen solution.

It is remarkable that the product distribution of the photoreaction between thymine and L-tryptophan is quite different depending upon the state of solution. Thus, irradiation of an aqueous frozen solution of an equimolar mixture of thymine and L-tryptophan with Pyrex-filtered light gave rise to the selective formation of the photoproduct A (Fig. 5). The result suggests that aggregation formation between thymine and tryptophan in aqueous frozen solution as reported (Ref. 17) plays an important role in the selective formation of the product A.

Acknowledgement — The authors are indebted to the Ministry of Education for a Grant-in-Aid for Scientific Research and to the Yamada Science Foundation for a financial support to this research

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