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PHOTOCHEMICAL REACTION BETWEEN TRYPTOPHAN AND THYMINE[1]

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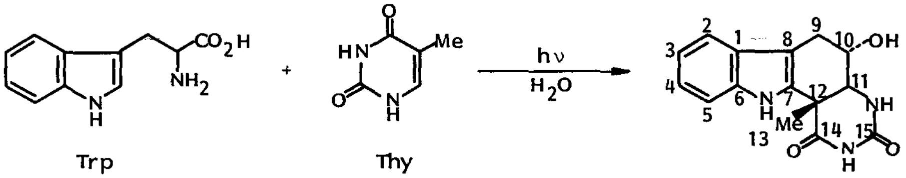
Summary: Irradiation of tryptophan and thymine in aqueous solution produced two photoadducts whose structures were determined by spectral data and X-ray crystallographic analysis.

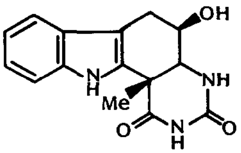
The photochemical cross-linking of proteins to nucleic acids has been observed in UV irradiation of many nucleic acid-protein assemblies.[2] DNA-protein cross-linking is presumed to be a major cause of UV-induced damage on living systems.[2] A number of model studies have been reported on the photoreactions between DNA components and amino acids.[2,3] In most of these studies light absorption by the nucleic acid bases appears to be the primary event.[2,3] The possibility that the absorption of light by aromatic amino acids might induce cross-linking with DNA has received only little attention. In our studies on the stacking interaction between tryptophan (Trp) and pyrimidine bases, we observed the adduct formation in irradiation of Trp in the presence of thymine (Thy).[4] Reeve and Hopkins also reported that irradiation of Trp and Thy in an aqueous buffered solution at pH 7 gives two Trp-Thy photoadducts each with a 1:1 molecular stoichiometry together with a dihydropyrimidine form of the base, although the structures of the adducts have not been clarified.[5,6] We now report the characterization of Trp-Thy photoaddcuts formed in irradiation of Trp in the presence of Thy.

A solution of L-Trp (5 mM) and Thy (1 mM) in unbuffered distilled water was irradiated with a 400 W high-pressure mercury lamp through Vycor filter (> 250 nm) for 10 h under nitrogen. The progress of the reaction was monitored by HPLC (reverse-phase Nucleosil 7C_{18}

column; acetonitrile-water 1:4) with a 254-nm detector to reveal the presence of two photoproducts together with unreacted starting materials.[7] The photoproducts were separated by preparative TLC (silica gel; water-saturated *n*-butanol; R_f 0.85) and purified with HPLC to give **1** (15%) and **2** (14%). The structure of **1** was assigned on the basis of spectral data[8] and the stereochemistry was determined by X-ray crystallographic analysis (Figure 1).[9]
The structure of **2** was tentatively assigned as the epimer at C-10 on the basis of spectral data[8] and elemental analysis. Chromatographic behaviours and UV spectral data of both photoproducts are identical with those reported by Reeve and Hopkins.[6] However, unlike their proposal, the photoproduct is not the Trp-Thy adduct with a exact 1:1 stoichiometry; the Trp moiety suffered deamination and decarboxylation.



1



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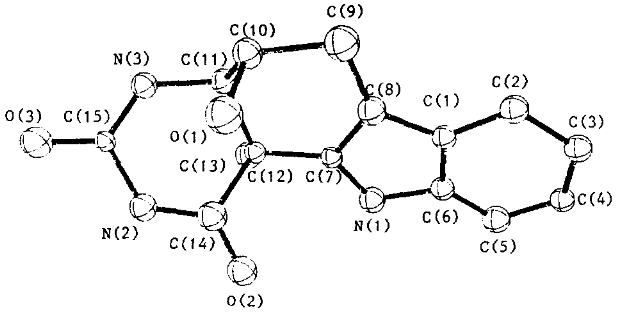
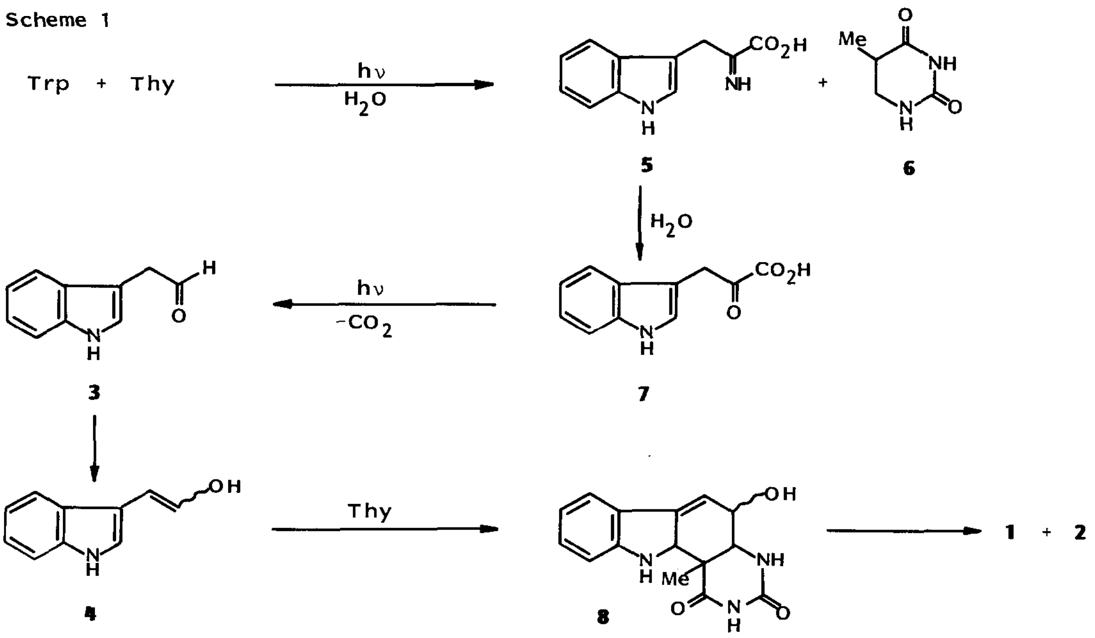


Figure 1 A computer generated perspective drawing of Trp-Thy photoadduct **1**. Hydrogens are omitted for clarity and no absolute configuration is implicated.

Reeve and Hopkins demonstrated the involvement of singlet excited Trp in the photoaddition and proposed a mechanism whereby Trp free radical formed by an electron-transfer from singlet Trp to Thy reacts with ground state Thy.[6] However, as apparent from the structure of the photoproduct 1, the mechanism of the adduct formation is more complicated, *i.e.*, Trp should undergo both deamination and decarboxylation prior to the adduct formation. A reasonable precursor for 1 is indole-3-acetaldehyde (3) which might be produced from the photoreaction of Trp. Diels-Alder type addition of the enol 4 to Thy followed by aromatization would produce 1 and 2. In order to probe this possibility, we have examined the irradiation of 3 (5 mM) and Thy (1 mM) in water at pH 7.0 under the same conditions. HPLC analysis of the mixture indicated the presence of 1 (20%) and 2 (20%) together with a considerable amount of polymeric products derived from 3. However, attempts to detect 3 in irradiation of Trp in the presence or absence of Thy have been unsuccessful presumably because of the susceptibility of 3 to polymerization under the reaction conditions.

The formation of 1 and 2 appears to involve a multistep photochemical and non-photochemical route and the mechanism of each step is by no means clear. Nevertheless, we speculate a following reaction scheme for the adduct formation (Scheme 1). The first step may involve a photochemical redox process between Trp and Thy to produce imine 5 and 5,6-dihydrothymine (6).[7]

Scheme 1



This process is probably initiated by an electron-transfer from singlet Trp to Thy.[10] Hydrolysis of 5 followed by photochemical decarboxylation of the resulting α-keto acid 7 would give 3. Indole-3-acetaldehyde (3) after enolization may undergo Diels-Alder addition to Thy to give 8 which isomerizes to 1 and 2 presumably by a photochemical 1,3-H-shift.

In summary we have characterized the structure of the photoproduct between Trp and Thy. A similar type of adduct formation has been observed in irradiation of Trp and uracil.[6] Such type of photoaddition may be relevant to the UV-induced damage on nucleic acids in the presence of Trp.[11, 12]

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(7) Formation of 6 was detectable by TLC analysis.

(8) Selected spectral data: 1, mp >250 °C; UV (MeOH) 292 nm (log ε 3.85), 283 (3.94); 400 MHz ^{1}H-NMR (DMSO-d_6) δ 1.61 (s, 3H), 2.76 (dd, 1H, J=12, 4 Hz), 2.82 (dd, 1H, J=12, 4 Hz), 3.45 (m, 1H), 4.13 (m, 1H), 5.14 (d, 1H, J=4 Hz, OH), 6.94 (dd, 1H, J=9,7 Hz), 7.04 (dd, 1H, J=8,7 Hz), 7.33 (d, 1H, J=8 Hz), 7.36 (d, 1H, J=9 Hz), 7.46 (s, 1H, NH), 9,88 (brs, 1H, NH), 10.88 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ 24.4 (C-13), 27.9 (C-9), 40.2 (C-12), 58.9 (C-11), 68.2 (C-10), 105.9 (C-8), 111.5 (C-5), 118.1 (C-2), 118.5 (C-4), 121.2 (C-3), 126.7 (C-7), 132.4 (C-1), 137.2 (C-6), 153.8 (C-15), 173.4 (C-14). 2; ^{1}H-NMR (DMSO-d_6) δ 1.64 (s, 3H), 3.04 (dd, 1H, J=15,5 Hz), 3.17 (dd, 1H, J=10,5 Hz), 3.22 (dd, 1H, J=15, 5 Hz), 3.74 (m, 1H), 5.48 (m, 1H, OH), 6.95 (t, 1H, J=8 Hz), 7.06 (t, 1H, J=8 Hz), 7.34 (d, d, 1H, J=8 Hz), 7.38 (d, 1H, J=8 Hz), 7.89 (d, 1H, J=5 Hz, NH), 10.15 (brs , 1H, NH), 11.07 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ 23.0 (C-13), 29.4 (C-9), 43.1 (C-12), 60.7 (C-11), 67.6 (C-10), 105.9 (C-8), 111.5 (C-5), 118.3 (C-2), 118.7 (C-4), 121.5 (C-3), 126.0 (C-7), 132.0 (C-1), 137.4 (C-6), 152.4 (C-15), 173.5 (C-14).

(9) Crystallized from acetic acid. Orthorhombic, space group $P2_12_12_1$ with a = 29.49 Å, b = 7.84 Å, c = 6.89 Å, Z = 4, V = 1609 Å. Details will be published elsewhere.

(10) Alternatively, hydrogen abstraction from α-methine of Trp side chain by photoexcited Thy followed by disproportionation of the resulting radicals may also produce 5 and 6.

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