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# A MIXED PHOTOPRODUCT OF THYMINE AND CYSTEINE: 5-S-CYSTEINE, 6-HYDROTHYMINE

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SUMMARY

A mixed photoproduct of thymine and cysteine has been produced by irradiating a solution of thymine-2-$^{14}$C (2.5 mM) and cysteine·HCl (10 mM) with ultraviolet light (2537Å). On the basis of chemical and NMR studies its structure has been assigned as 5-S-cysteine, 6-hydrothymine. The mechanism favored for the formation of this photoproduct involves the addition of a thiyl radical to the 5-position of a thymyl radical.

Ultraviolet light induces the cross-linking of DNA and protein both in vivo (1,2) and in vitro (3,4). The biological importance of this lesion in vivo has been demonstrated (2,5). The first direct evidence for the photochemical interaction of amino acids and pyrimidines came with the isolation of 5-S-cysteine, 6-hydrouracil from a UV irradiated (2537Å) solution containing uracil and cysteine (6). Kinetic studies with polynucleotides indicated that ($^{35}$S)-cysteine reacted photochemically with poly rU, poly rC, poly dC, poly dT and RNA and DNA (7). The reaction of cysteine with poly dT was unexpected. We therefore attempted the isolation and identification of the mixed photoproduct of ($^{14}$C)-thymine and cysteine. This report describes the experiments which led to the assignment of the structure of this compound as 5-S-cysteine,6-hydrothymine.

## EXPERIMENTAL

Bulk Isolation of a Mixed-Photoproduct of Thymine and Cysteine. The irradiation conditions (2537Å) and apparatus are the same as used previously for the production of the mixed photoproduct of uracil and cysteine (6).

The final L-cysteine-HCl concentration was 10 mM (Eastman). The final thymine concentration was 2.5 mM (Calbiochem) with the addition of 100 μCi per liter of (2-$^{14}$C)-thymine (final concentration: 0.004 mM at 24.4 mCi/mM; Calbiochem). The final specific activity of the thymine was ~ 6.5 x $10^4$ cpm/μM. The irradiated (60 min) solution was poured through a Dowex-50 (HCl) column; the column was washed with water; eluted with 2 N HCl; evaporated to dryness; disolved and treated with cuprous oxide to remove free cysteine (8); chromatographed on paper sheets in n-butanol-acetic acid-water (80:12:30) (9); eluted and treated with $H_2S$ and dried as previously described (6). The yield was about 2.5% or about 7.8 mg of thymine combined with cysteine (from one liter of irradiated solution). The $R_F$ of this material on paper in several solvents is given in Table 1. The product was ninhydrin positive and was faintly UV absorbing when the chromotograms were photographed with UV (10).

$R_F$ of 5-S-Cysteine,6-Hydrothymine

| Solvent* | $R_F$ | $R_T$** |
|---|---|---|
| n-Butanol-water (86:14) | 0.02 | 0.04 |
| n-Butanol-acetic acid-water (80:12:30) | 0.12 | 0.18 |
| Isopropanol-acetic acid-water (60:30:10) | 0.17 | 0.28 |
| sec-Butanol saturated with water | 0.24 | 0.32 |
| Methanol-HCl-water (70:20:10) | 0.46 | 0.66 |

*Descending chromatography on 1.5 in. strips of Whatman No. 1 paper. Solvent proportions are given as volume per volume.
**Chromatographic movement relative to thymine as 1.0.

When a solution of thymine-($^{14}$C) and cysteine was UV irradiated a broad peak of radioactive material with spikes at $R_F$ 0.13, 0.11 and 0.08 was produced. All of this material stuck to a Dowex-50 column and was eluted (along with free cysteine) by 6 N HCl and again chromatographed as it did originally. When this column eluate was treated with CuO to remove the free cysteine, only the $R_F$ 0.13 material was recovered.

Raney Nickel Desulfurization. If the cysteine were attached by a sulfur linkage to the 5 (or 6) carbon of thymine then treatment of the photoproduct with Raney nickel should yield dihydrothymine and alanine. A small sample of the ($^{14}$C)-thymine labeled photoproduct was treated with a ca. 10 times its weight of Raney nickel (W. R. Grace Co.) for various times at room temperature. The sample was filtered and the filtrate chromatographed in n-butanol-acetic acid-water. The ninhydrin positive spot appeared at $R_F$ 0.24, consistent with it being alanine (6).

After a 3 hr treatment of the photoproduct with Raney nickel about 20% of the radioactivity appeared as dihydrothymine ($R_F$ 0.58) and gave a positive test for dihydropyrimidines (11) as did an unidentified spot at $R_F$ 0.68 (containing 80% of the radioactivity). After a 1 hr treatment about 20% of the radioactivity chromatographed at approximately the $R_F$ of the starting material, 46% appeared as dihydrothymine with only about 7% at $R_F$ 0.68. Treatment of authentic dihydrothymine for 2.5 hr with Raney nickel yielded the $R_F$ 0.68 material (detected by the p-dimethylaminobenzaldehyde spray, ref. 11). Raney nickel treatment for 2.5 hr had no effect on $^{14}$C-thymine but did have a small effect after 5 hr.

Effect of Heat, Acid and Alkali on the Photoproduct. Heating for 15 min in a sealed capillary at 100°C had no effect upon the chromatographic properties of the photoproduct ($^{14}$C-thymine labeled). Fifteen hours at room temperature in concentrated $NH_4OH$ caused essentially a complete breakdown of the product to two equal products (containing radioactivity) at $R_F$ 0.41 and 0.48 (n-butanol-acetic acid-water; 80:12:30). Fifteen hours in 0.01 N NaOH destroyed only a few percent of the product. Fifteen hours in 0.1 N NaOH changed about 60% of the product to material with an $R_F$ of 0.25. Heating in concentrated trifluoroacetic acid for 60 min at 155°C (conditions normally used to hydrolyze DNA to free bases) destroyed about 85% of the product with new radioactive material appearing chiefly at $R_F$ 0.46, 0.65, and 0.77 (major peak). The $R_F$ of thymine under these conditions was 0.64.

Proton Magnetic Resonance Spectra of Photoproduct. The 100 MHz spectrum (Fig. 1) is consistent with the principle photoproduct being the enantiomeric

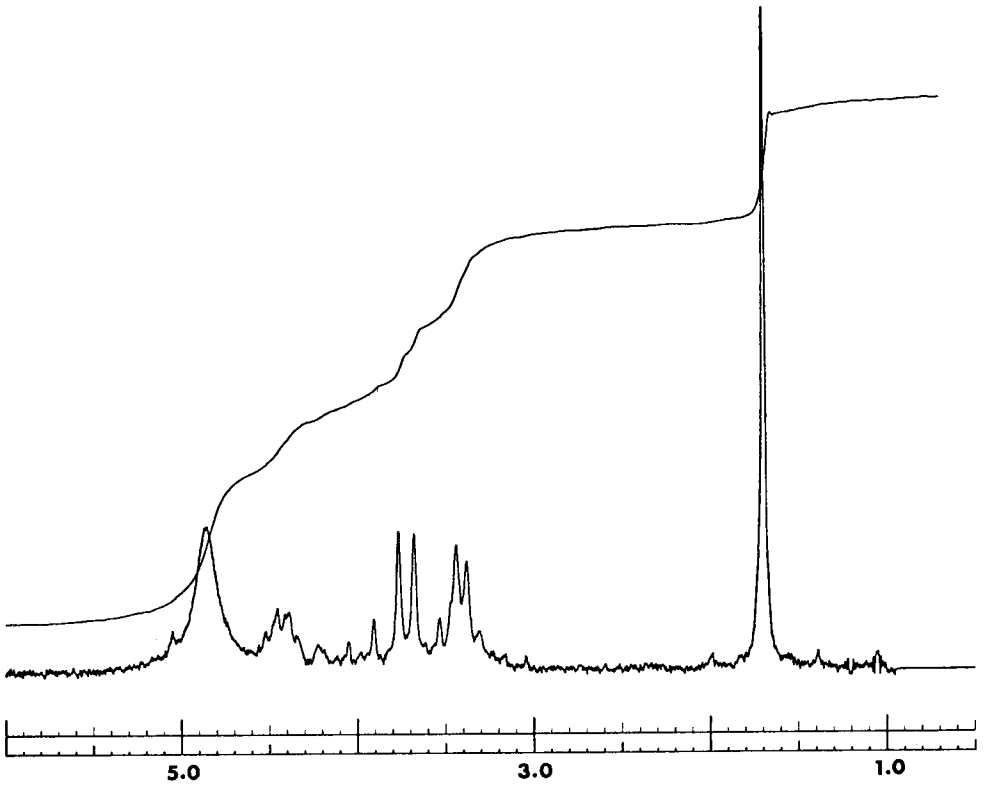


Fig. 1. Proton magnetic resonance spectrum of the mixed photoproduct of thymine and cysteine. The spectrum was measured at 100 MHz on a Varian HA 100 spectrometer. Hexamethyldisilazane was used as an external standard. The solvent was $D_2O$.

mixture of 5-S-cystinyl-dihydrothymines. The characteristic methyl doublet present in dihydrothymine (Fig. 2) has become a singlet owing to the 5-S substituent and exhibits a characteristic downfield shift consistent with the sulfur substituent. The 5a and b protons appear as two doublets at 3.66δ and 3.80δ (J=14 cps). The cysteine methyl group appears as a complex AB portion of an ABX pattern at ~ 3.4δ and methine H as the X portion centered at 4.43δ. Spectral lines due to impurities are apparent but cannot be assigned employing available models.

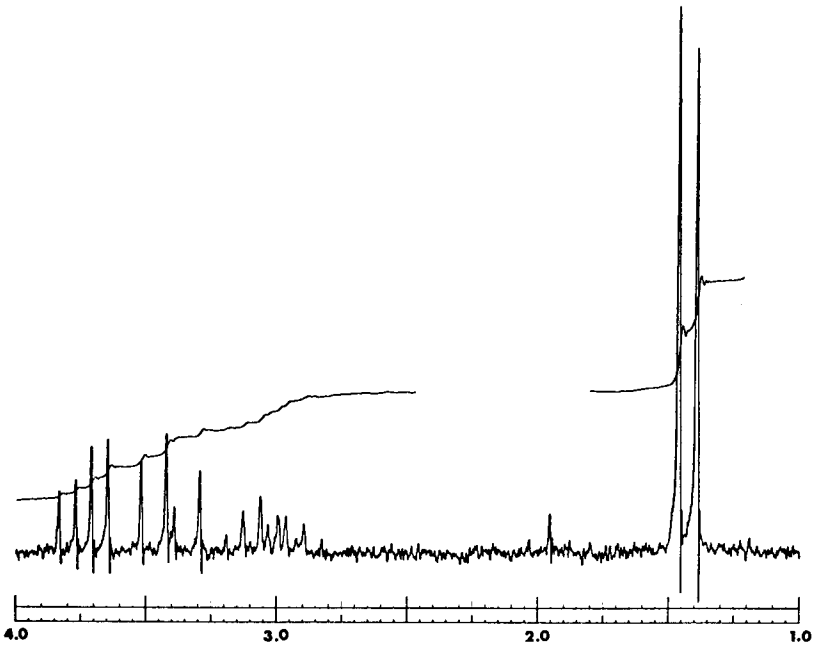


Fig. 2. Proton magnetic resonance spectrum of dihydrothymine. Conditions are as in Fig. 1.

## CONCLUSIONS

The proton magnetic resonance spectrum and the results with Raney nickel are consistent with the mixed-photoproduct of thymine and cysteine being 5-S-cysteine,6-hydrothymine (Fig. 3). Two other mixed photoproducts of about equal yield were lost during the CuO precipitation step for the removal of free cysteine. Their loss could be due to simple adsorption on the voluminous cuprous-cysteine precipitate or it may imply that these mixed products are joined such that their sulfur groups are free and thus are themselves precipitable by CuO.

The indication that thymine and cysteine can form more than one type of photochemical addition product comes from our earlier observation (7) that there was a 16% release of radioactivity when the photoproduct of $^{35}$S-cysteine and poly dT was heated at 65°C for 60 min. There was a 49% release of

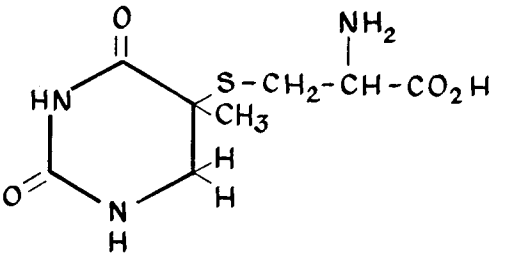

Fig. 3. 5-S-Cysteine,6-Hydrothymine.

radioactivity from the photoproduct of $^{35}$S-cysteine and poly dC but no release from the corresponding poly rU photoproduct under these conditions (7).

It is proposed that 5-S-cysteine,6-hydrothymine is formed by the addition of a thiyl radical to the 5-position of a thymyl radical. Thymyl radicals are formed when thymine or DNA is irradiated with UV (12). Thiyl radicals of cysteine can be formed by the direct absorption of UV (13) or by hydrogen abstraction by triplet excited state uracil (14). The addition of a thyminyl radical to the 5-position of a thymyl radical has recently been proposed as the mechanism for the formation of 5-thyminyl-5,6-dihydrothymine (15).

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