of 1.5 μ 48 hours after induction. Although the gas vacuoles do develop in the chromoplasm, there is never any evident connection with the photosynthetic membranes or other cellular membranes.

Because the youngest gas vacuoles are short cylinders with conical ends, and the older gas vacuoles have similar ends but are longer in the middle, the new components of the membrane may be added at the middle of the cylinder as it increases in overall length. In some micrographs there is one band which stands out slightly from the others (Fig. 4, b and c); this could be the growing point of the gas vacuole.

It is unlikely that existing gas vacuoles were overlooked in the hundreds of uninduced and 4-hour-induced cells we examined. The unique banding pattern of gas vacuole membranes is readily identifiable in both the inflated and collapsed states. Whether unassembled gas vacuole membrane components existing in the cell prior to induction assemble, or whether synthesis of these components is initiated by induction is not known. In any case, as there are no parent gas vacuoles present at the time of induction, we conclude that the appearance of the assembled gas vacuole membranes in preexisting, nonvacuolate cells demonstrates their *de novo* origin.

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Uracil Photoproducts from Uracil Irradiated in Ice

Abstract. *Two new products were isolated from uracil irradiated with ultraviolet light in frozen aqueous solution. As judged by mass, nuclear magnetic resonance, and ultraviolet and infrared spectra, one is a photopolymer,* U_3, *and the structure of the other is probably 6-4′-[pyrimidin-2′-one]-uracil. Formations of these products between pyrimidine bases are apparently common photoreactions, and may be important to the study of the photochemistry and photobiology of nucleic acids.*

Dimerization occurs when uracil and a number of its derivatives are irradiated (254 nm) in ice or as solid films (*1, 2*). The major product, cyclobutyl type homodimer (U = U), has *cis-syn* configuration (*2, 3*). The formation of *cis-syn* U = U as the major product is explicable from the crystalline structure of uracil (*2, 4*). We now report photoproducts other than cyclobutyl dimers.

An aqueous solution of chromatographically pure uracil (2 mmole/liter) was irradiated in a frozen state for 2 hours at a distance of 7 cm from the G.E. germicidal lamps of an irradiator (*1*). This caused a decrease of 65 percent of the absorbancy at 260 nm. The solution was evaporated to dryness, and the residue was washed with portions of warm water until the washings showed no absorption above 300 nm. The washings were filtered and concentrated until precipitation started. A trace of precipitate was removed by filtration, and the filtrate was applied to a column of 100 to 200 mesh Dowex 50W X-12 (2.5 by 60 cm).

The residue was dissolved in boiling water for recrystallization. This

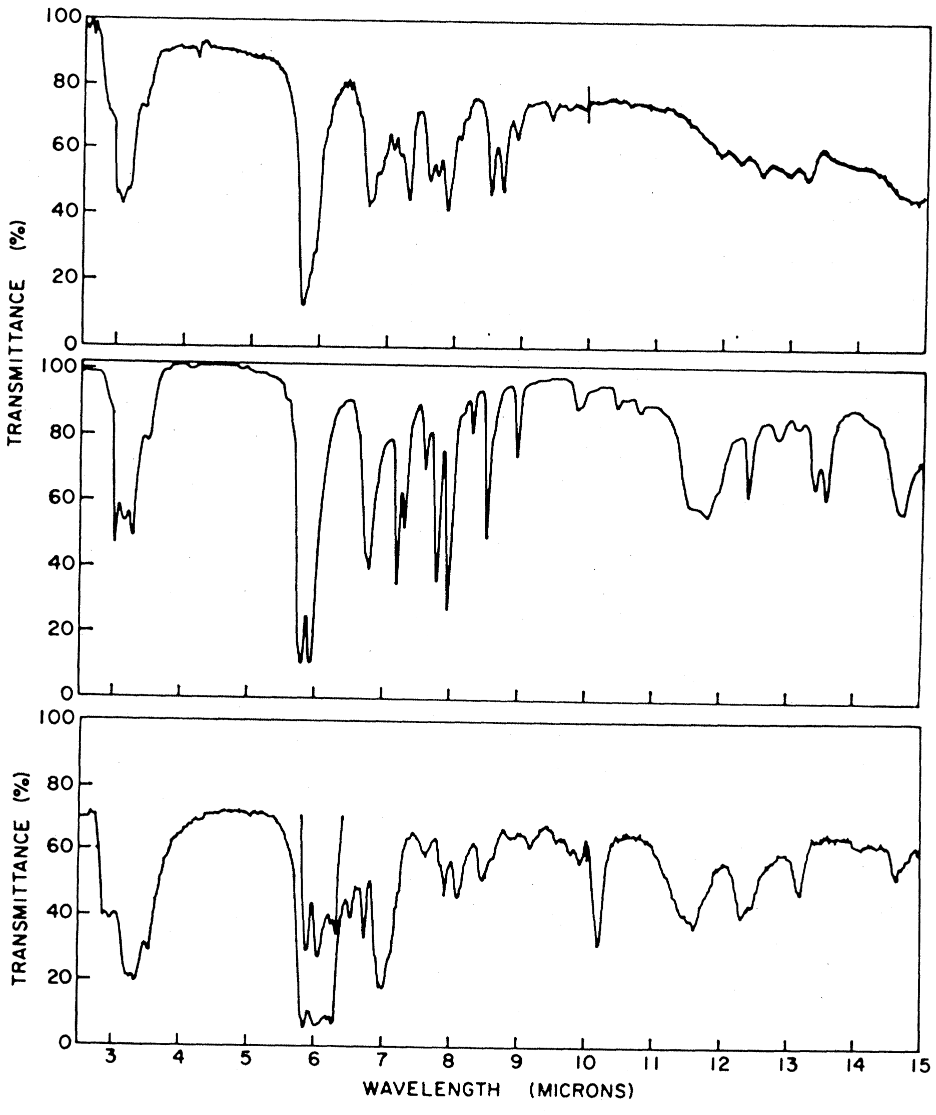


Fig. 1. Infrared spectra in KBr pellets. Top, U_3; middle, U = U; bottom, PO-U.

Table 1. The R_F values of uracil and its photoproducts.

Compound	R_F in solvent systems			
	A	B	C	D
Uracil	0.40	0.47	0.55	0.60
cis-syn U = U*	.06	.18	.23	.24
U_3*	.05	.18	.23	.25
PO-U	.10	.24	.15	.20

* Dark spot appears after irradiation of the dried plate with 254 nm.

product, sparingly soluble in most solvents, corresponds to the *cis-syn* U = U since it has infrared and ultraviolet spectra and photoreversibility identical to U = U.

The products present in the filtrate were eluted from the column with water, and fractions of 50 ml were collected. Fractions 2 to 6 contained a photoproduct like U = U having only an end absorption and reversing to uracil when irradiated in an aqueous solution or on paper (*5*). The residue from these fractions was recrystallized from boiling water as shining crystals. Under standard conditions, its mass spectrum is similar to that of uracil with molecular ion peak at 112. However, when the ion source temperature was lowered to 150°C, a very complex mass spectrum with a high mass peak at 370 was obtained (*6*). This information indicates that it is a polymeric product of uracil (U_3) and cannot be a structural isomer of U = U. The infrared spectra of U = U and U_3 also display characteristic difference in the 6-μ region (Fig. 1). The nuclear magnetic resonance spectrum of U_3 varies from those compounds with cyclobutyl structures.

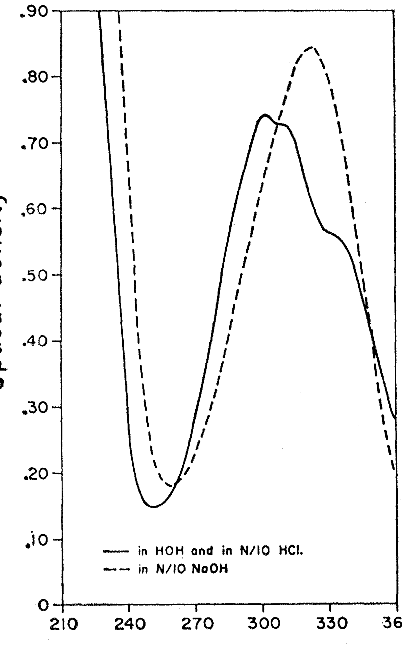
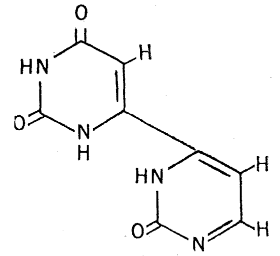


Fig. 2. Ultraviolet spectra of PO-U.

Another photoproduct (Fig 2) was isolated from fractions 10 to 20. The residue was dissolved in boiling water, and yellow crystals formed on cooling. Further recrystallization gave a purified product. Its spectra indicate that the yellow product is probably 6-4′-[pyrimidin-2′-one]-uracil (PO-U). Its infrared spectrum (Fig. 1) does not have the band at about 5.70 μ which is usually observed in dihydrouracil derivatives (*7*). The ultraviolet spectrum with absorbancy maximum at 332 nm suggests that the two chromophores may interact in coplanar molecules, in contrast to those of the analogous thymine photoproducts (*7, 8*) 6-4′-[pyrimidin-2′-one]-thymine (PO-T) and 6-4′-[5′-methylpyrimidin-2′-one]-thymine (MPO-T). Because of the methyl groups, the two components of the molecules derived from thymine are in skew conformations. The mass spectrum of PO-U shows a molecular ion peak at 206, corresponding to the molecular weight of $C_8H_6N_4O_3$. The nuclear magnetic resonance spectrum of PO-U in $(CD_3)_2SO$ at 100 Mc/sec shows a singlet at δ (parts per million from tetramethylsilane) 6.744 (1H) indicating a vinyl proton at C(5) with no proton on C(6). Two other protons at δ 7.370 (1H, doublet) and δ 8.516 (1H, doublet) with spin-spin coupling constant equal to 3 cycle/sec are due to the —CH = CH— as observed in pyrimidin-2-one (*9*). A broad peak at δ 11.70 is from the protons of NH groups. These data indeed conform with the suggested structure below.



The purity of these photoproducts was further examined by thin-layer chromatography (Eastman chromatogram sheets, No. 6471 cellulose-coated) with four solvent systems: (A) *n*-butanol and water (84:16); (B) *n*-butanol, acetic acid, and water (80:12:30); (C) isopropanol, ammonia, and water (70:20:10); and (D) *n*-propanol and water (75:25). Each developed as single spots with R_F values given in Table 1.

Studies of the irradiation of uracil-2-C^{14} in ice (*10, 11*) have revealed two radioactive areas besides that of uracil in the paper chromatograms developed with solvent system A. It was suggested (*10*) that one corresponded to U = U and the second to a water addition product of uracil. However, PO-U has the same R_F value in solvent A as the second product. In studies with oligo- and polynucleotides (*12*), the formation of photodimer of uracil was observed. Studies (*13*) with P^{32}-labeled UpU, dUpU, and uracil-2-C^{14} resulted in the isolation of four isomeric dimers.

The formation of PO-U, U-U adduct, or adducts between pyrimidine bases is apparently a common photoreaction. However, the possible biological importance of these adducts in the photochemistry of RNA remains to be seen (*14*).

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