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The Isolation of Oxamide and Parabanic Acid from the Products of Ultraviolet Irradiated Uracil[1]

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This communication is a report of part of a systematic investigation of the breakdown products of the irradiation of nucleic acid derivatives. The investigation is based on the fact that the "action spectrum," i.e., the wavelengths which are effective in producing mutational growth and other biological effects, including lethal ones, in many cases is similar to the ultraviolet absorption spectrum of the nucleotides comprising the nucleic acids. An attempt is being made to identify the irradiation breakdown products of the nucleic acids and their derivatives and to correlate the biologic effects of these products with those of irradiation itself. The present paper deals with the isolation and, where possible, the identification of substances produced on irradiating uracil, one of the pyrimidines contained in ribonucleic acid.

Some evidence exists that identifiable products can be demonstrated on ultraviolet irradiation of purines and pyrimidines. Canzanelli et al. (*1*) demonstrated the appearance of varying amounts of urea and ammonia on the irradiation of various purines and pyrimidines and their nucleosides and nucleotides. Scholes and Weiss (*2*) isolated oxalic acid from the X-irradiation product of adenine and guanine, and guanidine from the latter; they also found that oxalic acid could be isolated from the alkaline hydrolyzate of uracil irradiation products.

In the present work, ammonia and urea were found after irradiation in amounts similar to those previously found (*1*) when uracil was irradiated in 5×10^{-5} M concentration. Parabanic acid and oxamide were isolated and identified, and four other compounds were isolated in smaller amounts but not identified.

EXPERIMENTAL

Irradiation of uracil. The uracil (Schwarz Laboratories, Inc.) was irradiated in aqueous solution at a concentration of 10^{-3} M. The irradiation was carried out in flat-bottomed pans, each having an area of about 1350 cm². Each pan was irradiated with a bank of seven low-pressure mercury arc lamps (General Electric germicidal

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lamps), each of 15-watt capacity for 22 to 24 hours. The spectral extinction of the solution at 260 mμ during this time usually dropped to less than 1% of the original value. During irradiation the lamps were approximately 1 cm above the surface of the solution. The resulting solution was concentrated in a Precision Scientific Company vacuum evaporator with a capacity of 12 liters per hour. The temperature during evaporation was usually 35–40° C and never above 50° C. The total time of evaporation was about 10 hours.

Elementary analyses were performed by Weiler and Strauss Laboratories, Oxford, England. Infrared spectra were done in Nujol mulls by the Microanalytical Laboratory of the Massachusetts Institute of Technology. Melting points were determined on a Fisher-Johns block.

RESULTS

1. *Separation of Oxamide*

This substance crystallized out on cooling the concentrate from the uracil irradiation.

Analysis: Calc'd for $C_2H_4O_2N_2$: 27.26% C; 4.57% H; 31.8% N.
Found: 27.65% C; 4.77% H; 31.4% N.

Its identity was proved by elementary analysis; by the fact that the compound sublimed without melting at about 300° C, as does oxamide; by the fact that an aqueous solution gave a green precipitate with ammoniacal solutions of cupric nitrate similar to that with oxamide; and by the fact that the infrared spectrum of the unknown (bands at 2.94, 3.13, 5.98, 6.16, and 9.1 μ) was identical with that of known oxamide.

An analysis of the recovery of solids from the filtrate from the oxamide precipitation showed (Table I) that there was a loss of volatile material during either the irradiation or the subsequent concentration. As will be seen in the Table I, three-fourths of the nitrogen was recovered, but only one-fourth of the carbon. The volatile material has not been further investigated at the present time. Of

TABLE I

RECOVERY OF TOTAL SOLIDS, CARBON, AND NITROGEN AFTER IRRADIATION OF URACIL

	In uracil (*gm*)	*In irradiated uracil* (*gm*)	*Recovery* (%)
Total solids	24.0	14.6	61
Carbon	10.3	2.6	25.2
Nitrogen	6.05	4.59	76

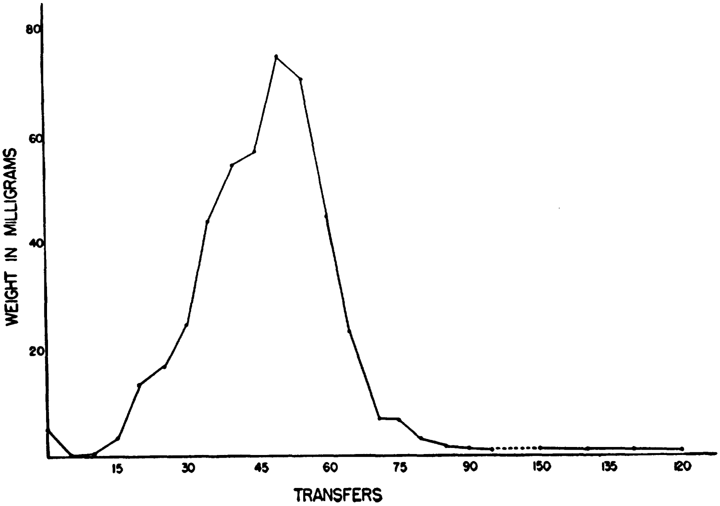

FIG. 1. Countercurrent distribution pattern of solids in uracil irradiation products in the first distribution (141 transfers).

the nonvolatile material, a portion was subjected to countercurrent distribution for separation.

2. *Countercurrent Distribution*

Two of the compounds were isolated in relatively pure form by countercurrent distribution, making use of an all-glass Craig (*3*) apparatus of 100 tubes, with the volumes of the upper and lower phases each equal to 15 ml. The solvent system consisted of 10:7:3 water, *n*-butanol, 95% ethanol mixture. The distribution was first carried to 141 transfers. At the end of the distribution, solids were present in the 5th through the 85th tube of the apparatus. Figure 1 gives the weight curve of this distribution. The 5th through the 36th tube of this distribution (containing approximately 25% of the solids in the distribution) were carried 159 transfers further. Figure 2 gives the weight curve of this distribution, together with the melting ranges of the solids in the tubes. It shows two peaks corresponding to two relatively pure compounds, designated in Table II as A, charring at 270° C, and B, m.p. 216–218° C, after recrystallization from water-ethanol to a constant melting point.

Compound B had bands in the infrared at 3.12, 5.73, 5.87 to 5.98, and 8.27 μ. On the basis of its analysis, it was suspected to be ammonium oxamate. The latter has never been reported. It was prepared by a slight modification of the method of Oelkers (*4*) for oxamic acid and was found to have a melting point of 216–218° C and bands in the infrared at 2.90, 3.12, 5.77, 5.91, 6.2, 7.6, and 9.1 μ. The mixed

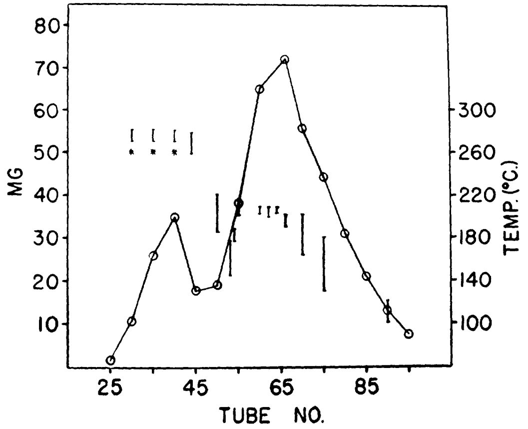

FIG. 2. Countercurrent distribution pattern of the solids in the uracil irradiation products with lowest distribution coefficient (material from tubes 5–36 of Fig. 1). Total number of transfers: 300. Vertical bars represent the melting ranges of the solids found in the tube. Starred bars represent charring ranges rather than melting points.

TABLE II

PURE COMPOUNDS ISOLATED FROM URACIL IRRADIATION PRODUCT

Name or designation of the compound if of unknown structure	*Amount isolated (mg)*	*From grams of UIP*	*% of UIP*	*Melting point*
Oxamide	300	14.6	2.1	300° C (sublimes)
Compound A	300	11.2	2.7	270° C (chars)
Compound B	570	11.2	5.1	216–18° C
Isolated by silica gel chromatography:				
Parabanic acid	370	6.09	6.1	235° C
Compound C	100	6.09	1.6	161–5° C
Urea				
Isolated by florisil chromatography:				
Compound D	25	12 gm	0.2	180° C
Calculated from previously published results (*1*)				
Urea			28	
Ammonia			3.5	
% of the UIP accounted for—49%				

melting point of the known ammonium oxamate with compound B was depressed to 195–200° C. The latter, therefore, is not ammonium oxamate, and its identity is unknown.

The remaining 75 % of the solids in the original distribution were treated similarly

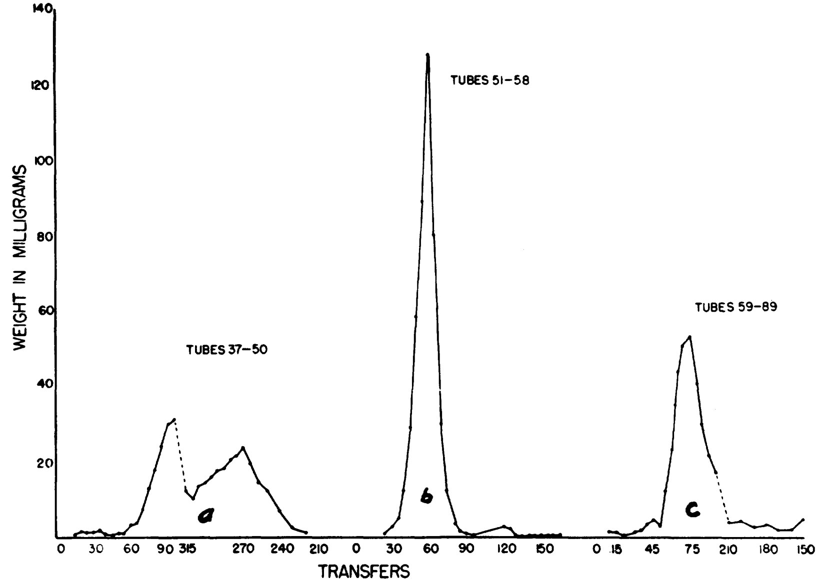

FIG. 3. Countercurrent distribution of solids in uracil irradiation products with subsequent distributions. Curve *a*, solids from tubes 37–50 of Fig. 1; curve *b*, solids from tubes 51–58 of Fig. 1; curve *c*, solids from tubes 59–89 of Fig. 1.

in three additional distributions. Figure 3 shows the weight curves obtained from these distributions, material from tubes 37 to 50 shown in curve *a*, from tubes 51 to 58 in curve *b*, and from tubes 59 to 89 in curve *c*. Urea and several other relatively pure compounds were isolated by this procedure, but the amounts of the latter were not large enough to permit satisfactory purification. The compound removed in transfer 60 (curve b, Fig. 3), representing the major component of the original distribution, had a melting point of 131–133° C. A mixed melting point of 131–134° C was obtained with urea, indicating its identity with urea.

3. *Silica gel chromatography.*

(*a*) *Separation of parabanic acid.* Another portion of 6.09 gm of uracil irradiation product (UIP) which had been lyophilized after concentration was extracted exhaustively with 3500 ml of ethyl acetate, which dissolved 2.08 gm of the material. This was diluted with 7 liters of petroleum ether (30–60° C) and run through a column of 180 gm of silica gel (Davison desiccant activated). The eluate from the solution contained 376 mg of a compound melting at 210–230° C, raised to 246–250° C after one recrystallization from water.

Analysis: Calc'd for $C_3H_2O_3N_2$: 31.6 % C; 1.77 % H; 24.7 % N.
Found: 32.1 % C; 2.02 % H; 24.2 % N.

Parabanic acid, m.p. 251–254° C, was prepared by two methods: (1) by oxidation of uric acid with alkaline peroxide solution (*5*); (2) by the action of oxalyl chloride on urea (*6*). The mixed melting point of the unknown with prepared parabanic acid was 247–253° C, and the infrared spectra of the two were practically identical (bands at 3.11, 3.63, 3.96, 4.10, 4.20, 4.50, 5.00, 5.73, 6.20, 8.81, 10.00, 10.14, and 12.40 μ). The identity of the compound may therefore be regarded as established.

(*b*) *Separation of compound C*. After elution of the parabanic acid, the column was washed with a 7:3 mixture of ethyl acetate and petroleum ether without eluting any significant amount of solids. On elution with 4 liters of 4:1 ethyl acetate-petroleum ether, 450 mg of material, m.p. 130–160° C, was obtained. On recrystallization, a small amount of material (compound C in Table II), m.p. 161–165° C, was obtained. Although the analysis of this material (28.08% C, 4.86% H, 30.8% N) checks well for formyl urea, m.p. 173° C. the mixed melting point was depressed to 144–154° C. The latter, therefore, is not formyl urea, and its identity is unknown.

The column was then eluted with 9:1 ethyl acetate-petroleum ether, and urea and oxamide were obtained.

4. *Chromatography on Florisil*

Fractional crystallization of 12 gm of the UIP yielded 1.8 gm of an alcohol-soluble, ethyl acetate-insoluble fraction which was distributed countercurrently to 148 transfers in a 10:7:3 water, butanol, ethanol system. At the end of the distribution, tubes 44 through 67, containing 613 mg of solids, were combined and evaporated to dryness. The material was extracted with 120 ml of absolute ethanol, which dissolved 450 mg; the ethanol extract was diluted with 3 volumes of benzene and chromatographed on a column of 35 gm of Florisil. Fractions of 25 ml were collected. A graph of the weight of solids in each fraction and the melting range of the solids is given in Fig. 4.

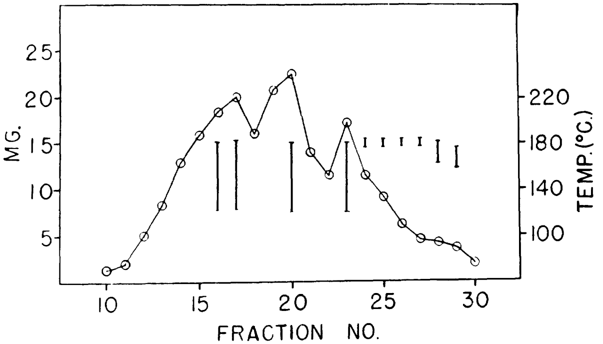

FIG. 4. Elution curve obtained on Florisil chromatography of a fraction of UIP. Vertical bars represent the melting range of the solids in the fraction.

Fractions 24 through 27 contained 25 mg of a compound (D in Table II), m.p. 180° C, with transformation into another substance which sublimes without melting at 270–300° C. Analysis: 25.7% C, 6.11% H, 39.0% N. Identity of this compound is therefore unknown.

All other fractions consisted of material with very wide melting ranges in amounts insufficient for adequate purification.

SUMMARY

Uracil has been irradiated with ultraviolet light and both volatile and nonvolatile degradation products obtained. The latter were subjected to countercurrent distribution and column chromatography. Two compounds were identified as parabanic acid and oxamide. Four other substances were isolated in relatively pure form, but the yields were small, and larger amounts will be needed for positive identification. The biological effects of the obtained products will be investigated.

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