

provides 1,2-bis-*p*-fluorophenyl-3,3-dichlorocyclopropene (2, Ar = *p*-F-C<sub>6</sub>H<sub>4</sub>-), m.p. 169–170°. *Anal.* Calcd. for C<sub>15</sub>H<sub>8</sub>Cl<sub>2</sub>F<sub>2</sub>: C, 60.63; H, 2.71; Cl, 23.86; F, 12.79. Found: C, 60.81; H, 2.95; Cl, 23.66; F, 12.91. This material shows strong infrared bands (Nujol mull) at 1600, 1515, 1360, 1351, 1232, 1150, 1120, 839, 689, and 660 cm.<sup>-1</sup> in the NaCl region. On standing in air this compound rapidly reverts to bis-*p*-fluorophenylcyclopropenone. It is remarkable that compounds of the type 2 hydrolyze exclusively to the cyclopropenone, whereas the monoaryl compounds 1, like tetrachlorocyclopropene, hydrolyze to give ring-opened products.<sup>7</sup>

Using the methods outlined herein we have also obtained examples of compounds 1, 2, and 3 in which Ar is phenyl, *p*-chlorophenyl, *p*-tolyl, and mesityl. Work on other functionally substituted benzene and polynuclear aromatic derivatives, and on the properties of the diarylcyclopropenones, is in progress.

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(7) S. W. Tobey and R. West, *Tetrahedron Letters*, 1179 (1963).

(8) Correspondence should be addressed to the Department of Chemistry, Purdue University, West Lafayette, Ind.

DEPARTMENT OF CHEMISTRY  
UNIVERSITY OF WISCONSIN  
MADISON 6, WISCONSIN

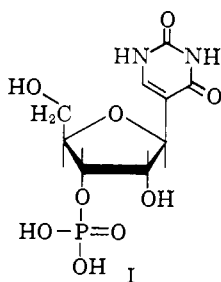
STEPHEN W. TOBEY<sup>8</sup>  
ROBERT WEST

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### Photolysis of Pseudouridine 3'-Phosphate and sRNA

Sir:

In the course of our studies on periodate oxidation of pseudouridine 3'-phosphate<sup>1</sup> (I, Ψ3'P), we routinely looked for 5-formyluracil (FoU) by spraying paper



chromatograms with the aldehyde reagent *o*-dianisidine.<sup>2</sup> We noticed that when the chromatogram was inspected over an ultraviolet light prior to spraying with *o*-dianisidine, Ψ3'P, applied as a standard, gave the yellow color characteristic of an aldehyde. Investigation of this peculiar observation led to the discovery of a new photolytic reaction which we wish to record in this communication.

An aqueous solution (10 ml.) of Ψ3'P<sup>3</sup> (1 *A*<sub>260</sub> unit/ml.)<sup>4</sup> was placed in a 1-l. quartz erlenmeyer flask and

(1) M. Tomasz and R. W. Chambers, *Abstr. Sixth Intern. Congr. Biochem.*, **1**, 90 (1964).

(2) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., New York, N. Y., 1954, pp. 148–151.

(3) W. E. Cohn, *Biochem. Prep.*, **8**, 116 (1961).

(4) *A*<sub>260</sub> = absorbance in a 1-cm. quartz cuvette at 260 mμ.

irradiated from below with a low pressure mercury lamp equipped with a filter passing mainly 253.7-mμ light.<sup>5</sup> The solution was stirred magnetically and cooled with a fan during the reaction (~27°). After 1.5 hr. the reaction mixture was analyzed by paper chromatography, paper electrophoresis, and ion-exchange chromatography. In addition to starting material (29%), inorganic phosphate (P<sub>i</sub>, 0.65 equiv.)<sup>6</sup> and 5-formyluracil<sup>7</sup> (FoU, 0.37 equiv.)<sup>8</sup> were found. A product of unknown structure (13% of the original *A*<sub>260</sub>) was also detected. This compound does not contain phosphate; it has a spectrum at pH 6 similar to that of Ψ3'P; at pH 2 or 12 the spectrum is similar to, but not identical with, that of FoU.

Irradiation of pseudouridine 3',5'-diphosphate<sup>9</sup> also gave FoU and P<sub>i</sub>. However, neither of these products was produced by irradiation of pseudouridine 2'-phosphate, pseudouridine 2',3'-cyclic phosphate, or pseudouridine itself.<sup>10</sup>

Irradiation of yeast uridylic acid under the same conditions resulted in a 79% loss in *A*<sub>260</sub> which was restored by treatment with acid as expected.<sup>11</sup> No P<sub>i</sub> was produced by this reaction nor by irradiation of a mixture of yeast adenylic, guanylic, cytidylic, and uridylic acids. However, when the mixture included Ψ3'P, 0.33 equiv. (based on Ψ3'P) of P<sub>i</sub> was formed in 1.5 hr.

These results suggested that it might be possible to cleave transfer RNA (sRNA) specifically at its pseudouridine residues. Accordingly, partially purified phenylalanine sRNA (40 *A*<sub>260</sub> units in 10 ml. of water) was irradiated for 3.5 hr. The ability to load phenylalanine was completely destroyed. The sedimentation coefficient, *S*<sub>20,w</sub>, of the irradiated product was 1.93 while that of the starting material was 3.01. This information plus some preliminary light-scattering measurements indicate that a decrease in molecular weight occurred during irradiation.<sup>12</sup>

Treatment of the irradiated product with crystalline *Escherichia coli* phosphatase<sup>13</sup> liberated 0.26 μmole of P<sub>i</sub> as compared to 0.12 μmole released from the un-irradiated control. Alkaline hydrolysis of the irradiated product and analysis of the nucleotides by ion-exchange chromatography indicated that 23% of the pseudouridine had been destroyed.<sup>14</sup>

The sum of the evidence strongly suggests that cleavage of the sRNA molecule occurred during irradiation. We have been unable to separate any fragments on columns of Sephadex or methylated albumin-

(5) Mineralite, Model SL 2537, Ultraviolet Products, South Pasadena, Calif.

(6) Determined directly on an aliquot from the reaction mixture by the Lowry-Lopez method [O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946)]. No evidence of any phosphate containing intermediate could be found by chromatography or electrophoresis.

(7) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

(8) In a separate experiment it was established that a 27% loss of *A*<sub>260</sub> occurs when FoU is irradiated under these conditions.

(9) M. Tomasz and R. W. Chambers, to be published.

(10) An 11–13% loss of *A*<sub>260</sub> occurred with these three compounds. Similar results were obtained with pseudouridine by Lis and Allen [A. W. Lis and F. W. Allen, *Biochim. Biophys. Acta*, **49**, 190 (1961)].

(11) R. L. Sinsheimer, *Radiation Res.*, **1**, 505 (1954).

(12) We are grateful to Dr. Robert C. Warner and Mr. Frank Zaboretzky for the ultracentrifuge data and to Dr. David B. Ludlum and Mr. Robert Pollet for the light-scattering measurements.

(13) We are indebted to Dr. Michael Malamy for a sample of this enzyme.

(14) This is in contrast to the findings of Lis and Allen,<sup>10</sup> who reported that "all but a trace" of the pseudouridine was destroyed by irradiation of "1 M NaCl soluble" RNA from wheat germ.

silicic acid,<sup>15</sup> but the behavior of irradiated sRNA on these columns was clearly different from the controls. We are also unable to present additional information on the specificity of the cleavage.

This reaction is of particular interest in view of the recent reports on the photoinactivation of sRNA<sup>16,17</sup> and the implication that either uridine or pseudouridine residues<sup>17</sup> may be involved in this loss of activity. Further work on the mechanism of the photolysis of  $\Psi 3'P$  and on the photolysis of sRNA is in progress.

**Acknowledgment.**—This work was supported by a grant from the United States Public Health Service (GM-072620-4).

(15) T. Okamoto and Y. Kawade, *Biochem. Biophys. Res. Commun.*, **13**, 324 (1963).

(16) P. A. Swenson and S. Nishimura, *Photochem. Photobiol.*, **3**, 85 (1964).

(17) F. Fawaz-Estrup and R. B. Setlow, *Biochim. Biophys. Acta*, **87**, 28 (1964).

(18) Recipient of an Investigatorship of the Health Research Council of the City of New York under Contract I-200.

DEPARTMENT OF BIOCHEMISTRY  
NEW YORK UNIVERSITY  
SCHOOL OF MEDICINE  
NEW YORK, NEW YORK 10016

MARIA TOMASZ  
ROBERT WARNER CHAMBERS<sup>18</sup>

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### Specifically Solvated Ion-Pair Intermediates in Nitrous Acid Deamination of an Axial Amine<sup>1</sup>

Sir:

Nitrous acid deamination of equatorial amines gives substitution products of very predominantly retained configuration,<sup>2</sup> while axial amines have been reported to give steric results which range from complete retention, as in the case of  $\delta\alpha$ -cholestan- $3\alpha$ -ylamine,<sup>3</sup> to very predominant inversion, as in the case of the structurally similar *trans,trans*-2-decalylamine (I).<sup>4</sup> The non-stereospecific results obtained with *cis*-4-*t*-butylcyclohexylamine has led to the postulation<sup>5</sup> that a free carbonium ion is an important reaction intermediate.

We now wish to demonstrate the value of a new technique for studying this type of reaction. It involves the determination of the proportion of alcohol and acetate in both the inverted and retained products of nitrous acid deamination of an amine in aqueous acetic acid mixtures of varying composition. When applied to the *axial* amine (I)<sup>7</sup> this procedure provides the data shown in Table I.<sup>8</sup>

The results almost certainly preclude a "free carbonium ion"<sup>5,6</sup> as the sole or even predominant inter-

(1) This work was supported by Grant AM 06419 from the National Institutes of Health and Grant B-19 from the Health Research and Services Foundation, Pittsburgh, Pa.

(2) A. K. Bose, *Experientia*, **9**, 256 (1953); J. A. Mills, *J. Chem. Soc.*, 260 (1953).

(3) C. W. Shoppee, D. E. Evans, and G. H. R. Summers, *ibid.*, 97 (1957).

(4) W. Hüchel, *Ann.*, **533**, 1 (1938).

(5) W. Hüchel and I. Heyder, *Ber.*, **96**, 220 (1963); a similar postulation has been put forth<sup>2</sup> to explain the steric results in other *axial* cases.

(6) W. G. Dauben, R. C. Tweit, and C. Mannerskantz, *J. Am. Chem. Soc.*, **76**, 4420 (1954).

(7) This was prepared in pure form by ammonolysis of the epimeric tosylate; see J. L. Pinkus, G. Pinkus, and T. Cohen, *J. Org. Chem.*, **27**, 4356 (1962).

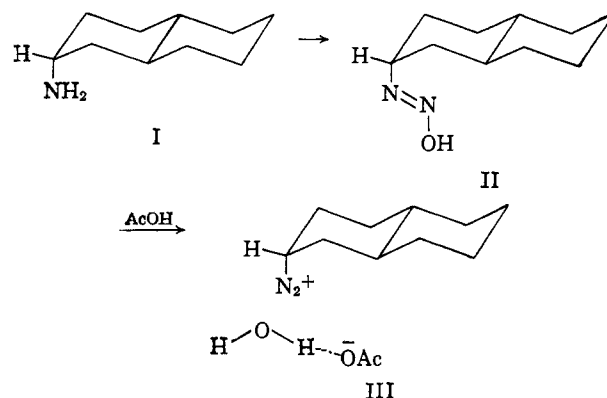
(8) Control tests established that olefins and alcohols are partially destroyed under the reaction conditions, but that the latter can be fully regenerated by ebullition of the reaction mixture with nitrogen until nitrous fumes are no longer evolved. Any nitrite esters formed by attack of nitrite ions on intermediates during the deamination reaction would be converted to alcohols by this procedure. However, nitrite ions are not expected to enter into the reaction significantly since acetate ions apparently do not do so (see below).

TABLE I  
DEAMINATION<sup>a</sup> OF I AT 30.0 ± 0.1°

Mole % acetic acid	% Inversion <sup>b</sup> (% alcohol) <sup>c</sup>	% Retention <sup>b</sup> (% alcohol) <sup>d</sup>
3.4	79.5 (65.8)	20.5 (70.3)
25.0	50.1 (53.2)	49.9 (65.6)
49.9	42.1 (29.9)	57.9 (56.9)
49.9 <sup>e</sup>	42.9 (29.5)	57.1 (57.4)
49.9 <sup>f</sup>	39.7 (29.0)	60.3 (56.8)
75.0	39.4 (12.9)	60.6 (51.7)
100	40.3 (3.7)	59.7 (45.6)

<sup>a</sup> Molar ratio of sodium nitrite to amine is 5. <sup>b</sup> % of substitution product. <sup>c</sup> % in inverted product; the remainder is acetate. <sup>d</sup> % in retained product; the remainder is acetate. <sup>e</sup> Sodium acetate (in same molar quantity as sodium nitrite) was added. <sup>f</sup>  $\alpha$ -Deuterio-*trans,trans*-2-decalylamine.

mediate, since the ratios of alcohol to acetate in the retained and inverted products are different throughout the range and their variations with solvent composition differ greatly. These ratios in the product of retained configuration are remarkably insensitive to solvent composition, changing only from 0.84 to 2.37 as the molar ratio of water to acetic acid varies from 0 to 28.4. This provides strong support for the existence in this reaction of a specifically solvated diazonium acetate ion-pair intermediate (III).<sup>9</sup> The bulk of retained product must arise by loss of nitrogen from III followed by rather nonselective attack of the internal water or acetate on the "front side" of the resulting carbonium ion.



Another striking fact is that the proportion of acetate in the inverted product is greater than the molar proportion of acetic acid in the solvent in each of the media used, except in acetic acid. The most dramatic example is in the case of 3.4 mole % acetic acid, in which 34% of the inverted product is acetate. The intramolecular inversion mechanism of White and Aufdermarsh<sup>11</sup> cannot explain this result over the whole range since the mechanism allows no greater proportion of acetate in the inverted than in the retained product. Indeed, an upper limit of about 8% (3.7 + 3.7 × 54.4/45.6) can be placed on the proportion of inverted product formed by this mechanism in pure acetic acid. Neither is this due to attack of acetate ions<sup>12</sup> on the back side

(9) This type of intermediate (III), presumably formed by reaction of the diazohydroxide II or its precursor with acetic acid, has been postulated<sup>10</sup> to account for the alcohol of retained configuration which is produced along with acetate in deaminations conducted in acetic acid. Our finding that a substantial proportion of the retained product is acetate, even in a solvent which contains very little acetic acid, is entirely consistent with this view.

(10) E. H. White and J. E. Stuber, *J. Am. Chem. Soc.*, **85**, 2168 (1963).

(11) E. H. White and C. A. Aufdermarsh, *ibid.*, **83**, 1179 (1961).

(12) These are produced during the deamination and by the reaction of excess nitrite ions with acetic acid.