crystallites. We could obtain an estimate of the degree of crystallinity of the monolayers at different pH's and at a different state of compression. It was concluded that the bilayer formed at high basic pH (pH ≥ 11.2) is the most stable one; the K^+ ions are arranged in a hexagonal cell commensurate with the monolayer cell. The crystalline order in uncompressed PFA monolayers is greatly enhanced in this case. Very recently, we observed similar results with a different system: uncompressed arachidic acid monolayers spread over $CdCl_2$ subphases at 5 °C self-assembled into large crystalline clusters, whereas in the uncompressed state, over pure water, the crystalline order was very low.[40] The X-ray diffraction data also gave an indication of an ordered cadmium ion layer beneath the monolayer.

The obtained structures for the crystallites are in agreement with expectations from NaCl epitaxial crystal nucleation. This confirmation implies that epitaxial crystallization can be a sensitive method for the detection of structured aggregates, as was proposed from studies on short-chain hydrophobic α-amino acids,[30] systems which are not yet amenable to GID measurements.

The results presented in this study open new possibilities for studying nucleation and growth of self-assembling crystals of model systems on a molecular level in two dimensions. The aim would be to monitor the growth of the crystallites from their inception to their mature form, gleaning information on the size and structure of their nuclei.

Acknowledgment. We are grateful to Isabella Weissbuch for assistance, together with Ronit Popovitz-Biro for valuable discussions, and Edna Shavit for synthesizing and purifying the compounds. We thank Ada Yonath at the Max Planck Unit for Structural Molecular Biology for use of laboratory facilities. We acknowledge financial support from U.S./Israel Binational Science Foundation, Jerusalem, the Petroleum Fund administered by the American Chemical Society, the fund for Basic Research of the Israel Academy of Sciences and Humanities, the Minerva Foundation and the Danish Foundation for Natural Sciences, and beam time at the HASYLAB, DESY, Hamburg, FRG.

Supplementary Material Available: Table of PFA atomic fractional coordinates and a structure for PFA (2 pages). Ordering information is given on any current masthead page.

(40) Leveiller, F.; Jacquemain, D.; Lahav, M.; Leiserowitz, L.; Deutsch, M.; Kjaer, K.; Als-Nielsen, J. manuscript in preparation.

(41) The cell axes of various crystal structures were relabeled a', b', and γ' to be consistent with the a, b, γ system of the monolayer.

3-Ureidoacrylonitriles: Novel Products from the Photoisomerization of Cytosine, 5-Methylcytosine, and Related Compounds

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Abstract: During studies of the effects of far-ultraviolet light on DNA and its components, we have discovered that cytosine and 5-methylcytosine, as well as their nucleosides and *N*1-methyl derivatives, undergo photoisomerization reactions to yield the corresponding *cis*- and *trans*-3-ureidoacrylonitriles. For example, *N*1-methylcytosine reacts to give *cis*- and *trans*-3-[*N*3-methylureido]acrylonitrile. These products have been characterized through use of one- and two-dimensional high-resolution ^1H and ^{13}C NMR spectroscopy, ultraviolet and infrared spectroscopy, electron impact and liquid secondary ion mass spectrometry, and, in some cases, synthesis by an alternate route. Detailed ^{13}C NMR data for the parent compounds are presented for comparison purposes. These photoisomerization reactions take place cleanly in acetonitrile; the corresponding reactions occur in aqueous solution as well. For cytosine and 5-methylcytosine the photoreaction in acetonitrile is slow; however, the rate of reaction is greatly enhanced by N1 substitution. We determined the quantum yields for formation of the products derived from 2′-deoxycytidine to be 2.44×10^{-4} in water and 1.34×10^{-3} in acetonitrile. The mechanism of formation of the 3-ureidoacrylonitriles is proposed to involve initial formation of a N3–C6 Dewar structure, followed by a ring-opening rearrangement.

Introduction

Over the past 30 years, a significant amount of research effort has been devoted to determining the mechanisms by which absorption of ultraviolet (UV) light causes harmful effects on living tissues. It was clear early on that DNA is a primary cellular target of far-UV radiation; photochemical damage to DNA is believed to be an important contributor to UV-induced cell death, mutagenesis, and carcinogenesis.[1] Many studies concerning photoinduced reactions of nucleobases and related compounds have been published in efforts to identify the in vivo photoproducts responsible for these adverse effects.[1] A major breakthrough came with the isolation of the cyclobutane dimer of thymine by Beukers and Berends[2a] and Wang,[2b] which has since been isolated from the DNA of far-UV-irradiated cells[2c] and for which elaborate enzymatic repair mechanisms have been identified.[2d] Since then, a variety of other lesions involving DNA bases have been identified, including, for example, cytosine-derived cyclobutyl dimers,[3] pyrimidine hydrates,[4] and pyrimidine–pyrimidone photoconjugates.[5]

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In addition, the nucleobases are known to photoreact with a variety of small organic molecules including amino acids and their analogues.[6] It has also been shown that nucleobases incorporated into DNA or into polynucleotides are reactive with amino acids.[7] With particular reference to cytosine and 5-methylcytosine, products from photoreactions of the nucleobases with alcohols,[8] aliphatic amines,[9a] and the amino acid L-lysine[9b] have been isolated and characterized. (The nucleobase 5-methylcytosine is found as a minor constituent in most eukaryotic DNA and is thought to play an important role in transcriptional regulation.[10])

We have recently discovered that cytosine and 5-methylcytosine, as well as their *N*1-methyl derivatives and nucleosides, undergo an interesting and previously unreported photoinduced isomerization reaction. The products of these reactions, which have been identified as ureidoacrylonitriles, are the only ones detectable when the parent compounds are irradiated in acetonitrile. They are also formed in aqueous media; in these cases, however, the photoisomerization reactions compete with other photoreactions of the parent compounds.

There are several references to 3-ureidoacrylonitrile (Ia and Ib) in the literature, including those dealing with synthetic procedures,[11] and describing its role as a possible cytostatic asparagine analogue[12] and as a hypothermia-potentiating drug in rats.[13] However, the various substituted 3-ureidoacrylonitriles described in this paper evidently have not been reported in the literature.

Experimental Section

General Procedures. Irradiations of cytosine and 5-methylcytosine and their *N*1-methyl derivatives and nucleosides were carried out in a cold room at 4 °C in a 650-mL quartz vessel placed in a Rayonet RPR-100 photoreactor equipped with Rayonet 2537 lamps emitting principally at 254 nm, all supplied by Southern New England Ultraviolet Co. The reaction vessel was surrounded by a Vycor shield. The volumes of the irradiated solutions were generally 500 mL. Deoxygenations, where necessary, were carried out by bubbling 99.997% pure nitrogen through the solution for 10 min.

UV-absorption measurements were made with a Cary 118C spectrophotometer or a Hewlett-Packard HP8452A diode array spectrophotometer. Liquid secondary ion mass spectrometry (LSIMS) measurements were made in the positive-ion mode with a Kratos MS50 mass spectrometer; samples were run in a thioglycerol matrix. Proton and ^{13}C NMR data were obtained by use of General Electric GN500 and QE300 spectrometers. Infrared absorption data were obtained in a Nujol mull on KBr plates with a Nicolet 5DX FT-IR spectrophotometer.

Photoproducts were purified by silica gel column chromatography or high-performance liquid chromatography (HPLC). The HPLC system used consisted of two Beckman 110A pumps, with a Beckman Model 421 controller coupled to a Gilson Holochrome variable-wavelength detector working at 255 nm. The column used for purification was a Whatman Partisil 5 ODS-3 RaC semipreparative column of 10-cm length and was packed with particles of 5-μm diameter. Thin-layer chromatography (TLC) was carried out by use of polyester plates with a 200-μm coating of highly purified silica gel (particle size 5–20 μm) supplied by Sigma Chemical Co.

All organic solvents were obtained from Fisher Scientific. Other chemicals were obtained from Sigma (cytosine, 5-methylcytosine hydrochloride, 1,5-dimethylcytosine, cytidine, 2′-deoxycytidine, and 5-methyl-2′-deoxycytidine), Vega-Fox Biochemicals (1-methylcytosine), Aldrich (*N*-methylurea, 3-bromo-2-methylacrylonitrile, methyl iodide, 2-aminopyrimidine, and hydrogen peroxide), and Fluka (3-ethoxy-2-methylacrolein).

Irradiation and Photoproduct Isolation. Irradiations were done in aerated solution at a concentration of 2 mM in parent compound. For cytosine (I) and 5-methylcytosine (II), 15% and 5% water, respectively, were necessary to ensure sufficient solubility in acetonitrile. All other derivatives were irradiated in acetonitrile containing 1% water.

In the cases of I and II, the samples were irradiated for 72 h and then evaporated to dryness on a rotary evaporator. The residue was taken up to 20 mL of ethyl acetate and applied to a 20 cm × 2.5 cm column composed of 30 g of silica gel (0.063–0.2 mm, 70–230 mesh, Merck) preequilibrated with the eluent ethyl acetate. The eluent was collected in 20-mL fractions. The cis isomers eluted within 3–5 fractions, and the trans isomers within 12–15 fractions. The fractions were analyzed by TLC (see Table I for R_f values), and those containing pure product were combined and evaporated to dryness. Overall conversion of starting material to products was of the order of 5–10%.

All other cytosine derivatives were irradiated for 24–48 h and the products isolated according to the following protocol. (After 48-h irradiation, the overall conversion to products was of the order of 10–15%.) After the irradiation stopped, samples were evaporated down to a volume of 20 mL, the precipitated parent compound was removed by filtration, and the filtrate was evaporated to dryness. The residue was taken up in 5 mL of water, and the products were purified by reversed-phase HPLC with aqueous eluents containing 0–5% methanol. Typical elution orders were starting material followed by the trans isomer of the 3-ureidoacrylonitrile derivative; the cis isomer eluted last. It was found necessary to store the products of 2′-deoxycytidine in the dry or frozen aqueous state to avoid a fairly rapid rearrangement of the sugar moiety to the α and β pyranoid and the α furanoid forms.

Synthesis of Photoproducts by Alternative Routes. Syntheses of the 3-ureidoacrylonitrile derivatives resulting from irradiation of cytosine (I), 5-methylcytosine (II), and 1-methylcytosine (III) were accomplished by alternative routes starting from the appropriate 2-aminopyrimidine derivatives. These were converted to the corresponding *N*1-oxides, after which rearrangement was induced by irradiation at λ > 300 nm, as described by Streith et al.,[11a] to give the desired products. Synthesis of the corresponding photoproducts of 1,5-dimethylcytosine (IV) was accomplished by nucleophilic substitution of 3-bromo-2-methylacrylonitrile with *N*-methylurea, as described in the following text.

A. Synthesis of 2-Aminopyrimidines. Irradiation of 1-methylcytosine gives rise to both *cis*- and *trans*-3-(*N*-methylureido)acrylonitrile, which necessitates the synthesis of 2-(methylamino)pyrimidine as a starting material. This was prepared via the methiodide,[14] with subsequent Dimroth rearrangement[15] to the desired product. Synthesis of the *cis*-

Table I. UV Absorbance and Silica Gel TLC Data[a]

compd	λ_{max} (nm)	ε (mol^{-1} cm^{-1} dm^3)	R_f
Ia	251	19900	0.55
Ib	251	24800	0.28
IIa	254	19000	0.56
IIb	250	23800	0.28
IIIa	255	21700	0.63
IIIb	254	25800	0.34
IVa	260	19400	0.65
IVb	254	27600	0.37
Va	253		
Vb	253		
VIa	254		
VIb	254		
VIIa	257		
VIIb	252		

[a] UV data recorded in water at pH 7. The TLC developing solvent was ethyl acetate.

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and *trans*-3-ureido-2-methylacrylonitriles (IIa and IIb), the products from reaction of 5-methylcytosine, required preparation of 2-amino-5-methylpyrimidine. This was prepared by a slight modification of the method of Tjoeng et al.[16] in which 3-ethoxy-2-methylacrolein, rather than 3-amino-2-methylacrolein, was treated in basic solution with guanidine hydrochloride. To sodium ethoxide (32.6 g) in 200 mL of absolute ethanol was added 3-ethoxy-2-methylacrolein (22.8 g, 0.2 mol) followed by guanidine hydrochloride (22.9 g, 0.24 mol) with stirring. The mixture was heated under reflux for 24 h. It was filtered through a preheated Buchner funnel to remove salt. The filtrate was cooled in a cold room (4 °C), and the colorless plates were harvested by filtration. After the plates were washed with cold ethanol, 8.7 g of white platelets were obtained, giving spectroscopic data identical with literature values.[16] Yield: 40%. Cytosine photoreacts to give *cis*- and *trans*-3-ureidoacrylonitrile for which 2-aminopyrimidine, the required starting material, is commercially available.

B. Synthesis of *N*-Oxides. The *N*-oxides of the various 2-aminopyrimidine derivatives were prepared by treatment with hydrogen peroxide in glacial acetic acid according to literature methods.[17] The products were purified on silica gel with ethyl acetate/methanol mixtures and recrystallized from chloroform/cyclohexane. Yield: 9–18%.

C. Irradiation of *N*-Oxides. Typically, the *N*-oxide was dissolved in acetonitrile (8 mM, 150 mL), placed in a 180-mL quartz vessel, and irradiated with use of a Pyrex filter. The reaction was followed by UV spectrophotometry. An irradiation time of 100 h yielded quantitative conversion to product, predominantly in the cis form. Where desired, 2-h irradiation at 254 nm, with a Vycor filter, resulted in a more even distribution of cis and trans isomers. The isomers were readily separated on a silica gel gravity-flow column (30 g, 20 cm × 2.5 cm) with ethyl acetate as eluent. The overall yield of purified product was typically 80%.

D. Preparation of IVa and IVb, the 1,5-Dimethylcytosine Photoproducts. Compounds IVa and IVb were prepared by nucleophilic substitution of 3-bromo-2-methylacrylonitrile with *N*-methylurea as follows. After addition of *N*-methylurea (2.22 g, 30 mmol) to 25 mL of butanol, the mixture was stirred for 10 min. At this time, 3-bromo-2-methylacrylonitrile (1.46 g, 10 mmol), as a mixture of cis and trans isomers, was added. The mixture was heated under reflux for 16 h, cooled, and evaporated to a viscous oil on a rotary evaporator. The oil was taken up in 20 mL of ethyl acetate and chromatographed on a silica gel column (30 g, 25 cm × 2.5 cm) with ethyl acetate as eluent. The eluate was collected in 20-mL fractions. The cis isomer eluted in fractions 3 and 4 (40 mg), and the trans isomer eluted in fractions 6–9 (65 mg). Overall yield: 8%.

Quantum Yield Measurements. The quantum yields for formation of the cis (VIa) and trans (VIb) isomers of the product resulting from irradiation of 2′-deoxycytidine (VI) in water and in acetonitrile were measured. Aqueous 1,3-dimethyluracil (DMU) was used as an actinometer in both cases. Samples of 3-mL volume were irradiated in stirred, matched quartz cuvettes with a path length of 10 mm; the cuvettes were placed at equivalent positions adjacent to a Vycor-shielded germicidal lamp. A cuvette containing DMU was placed on each side of a solution of VI. The concentrations used were 3.36 mM in the case of DMU and 2.10 mM for VI.

A 50-μL aliquot of each solution was removed after 30-, 60-, 90-, and 120-min irradiation. HPLC analysis was carried out with a Rainin dual-pump gradient system controlled by a Macintosh computer running the Dynamax Method Manager. A Whatman ODS3 RaC 5-μm analytical column was used for separations. The eluent was passed through a Kratos Spectroflow 783 detector, and the peaks were integrated by use of the data analysis module of the Dynamax Method Manager. For the 2′-deoxycytidine samples, the eluent was aqueous 10 mM LiCl and the detector was set to 254 nm. The eluent for the DMU samples was water methanol (85/15), and detection was at 220 nm. By use of authentic samples of VIa, VIb, and DMU hydrate (5,6-dihydro-6-hydroxy-1,3-dimethyluracil), calibration curves of detector response versus concentration for a 10-μL injection were calculated. The ε values for VIa and VIb were approximated as those calculated for products IIIa and IIIb, namely 2.17×10^4 and 2.58×10^4 mol^{-1} cm^{-1} dm^3, respectively. The ε value for the DMU hydrate at its λ_{max} of 220 nm was determined as follows. The absorbance of 3 mL of aqueous DMU hydrate at pH 7.0 was determined at 220 nm. The pH was then adjusted to pH 11.0 by addition of 2.0 μL of aqueous 1 N NaOH. The solution was heated at 100 °C for 10 min, which caused quantitative decomposition of the hydrate to DMU.[18] The pH was then adjusted back to pH 7.0 by addition of 20 μL of 0.1 N HCl(aq) and the absorbance determined at 266 nm. By multiplying the ratio of these absorbance values by the reported ϵ_{266} value of 8.6×10^3 mol^{-1} cm^{-1} dm^3 for DMU,[18] we calculated ϵ_{220} for DMU hydrate to be 6.2×10^3 mol^{-1} cm^{-1} dm^3.

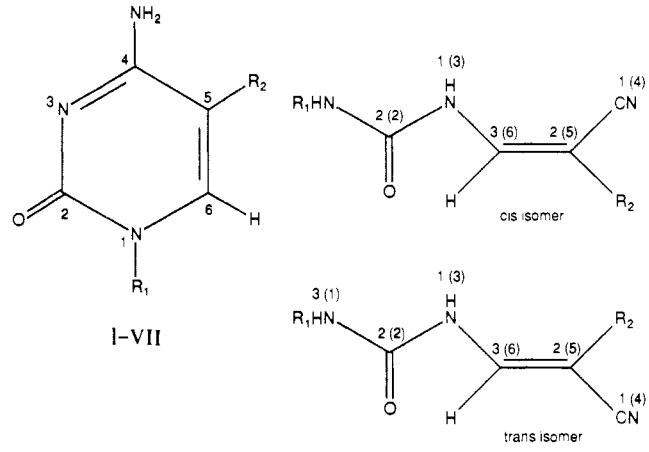

Figure 1. Key: I, $R_1 = R_2 = H$; II, $R_1 = H$, $R_2 = CH_3$; III, $R_1 = CH_3$, $R_2 = H$; IV, $R_1 = R_2 = CH_3$; V, R_1 = ribosyl, $R_2 = H$; VI, R_1 = 2′-deoxyribosyl, $R_2 = H$; VII, R_1 = 2′-deoxyribosyl, $R_2 = CH_3$. Cis (a) and trans (b) isomers: Ia, $R_1 = R_2 = H$; Ib, $R_1 = R_2 = H$; IIa, $R_1 = H$, $R_2 = CH_3$; IIb, $R_1 = H$, $R_2 = CH_3$; IIIa, $R_1 = CH_3$, $R_2 = H$; IIIb, $R_1 = CH_3$, $R_2 = H$; IVa, $R_1 = R_2 = CH_3$; IVb, $R_1 = R_2 = CH_3$; Va, R_1 = ribosyl, $R_2 = H$; Vb, R_1 = ribosyl, $R_2 = H$; VIa R_1 = 2-deoxyribosyl, $R_2 = H$; VIb, R_1 = 2-deoxyribosyl, $R_2 = H$; VIIa, R_1 = 2-deoxyribosyl, $R_2 = CH_3$; VIIb, R_1 = 2-deoxyribosyl, $R_2 = CH_3$. Ribosyl and 2′-deoxyribosyl are systematically β-D-*erythro*-pentofuranosyl and 2′-deoxy-β-D-*erythro*-pentofuranosyl, respectively.

For each irradiated sample, the detector response for a 10-μL injection was converted into a solution concentration by use of the appropriate calibration curve. The actinometric responses from the two DMU samples were averaged for each irradiation time. The φ values for formation of VIa and VIb were calculated by dividing the concentration of product by the concentration of DMU hydrate for each time point and multiplying by 0.0106, the value of φ for formation of DMU hydrate.[19] Over the range of doses used, the value of φ for each isomer remained the same within experimental error.

Oxygen Quenching and Acetone Photosensitization. The HPLC conditions used were the same as those used for the quantum yield determinations, and yields were again determined on the basis of HPLC peak areas.

i. Duplicate samples of 2′-deoxycytidine (VI) (2 mM, 10 mL) in either water or acetonitrile containing 1% water were prepared. One sample for each solvent was degassed by passage of nitrogen for 10 min, and the other was left aerated. The four samples were irradiated for 1 h at 254 nm in Vycor-shielded quartz tubes in a Rayonet RP-100 A equipped with a merry-go-round.

ii. Two samples for each solvent, water and acetonitrile, were prepared (9.5 mL, 2.1 mM). One sample of each solvent was kept as a control by addition of 0.5 mL of solvent, and to the second was added 0.5 mL of acetone, so as to yield final concentrations of approximately 2 mM in VI. The four samples were irradiated for 1 h in Pyrex-shielded quartz tubes in the RP-100 A reactor equipped with a merry-go-round and RPR-3000A lamps.

Results and Discussion

We have isolated and characterized the products obtained from irradiating cytosine (I), 5-methylcytosine (II), 1-methylcytosine (III), 1,5-dimethylcytosine (IV), cytidine (V), 2′-deoxycytidine (VI), and 5-methyl-2′-deoxycytidine (VII) in acetonitrile. The photoreaction, in each case, leads to two products. For each parent cytosine or 5-methylcytosine derivative, these products are the cis and trans isomers of ureidoacrylonitriles. The evidence for

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Table II. ^{1}H NMR Data Recorded versus TSP Internal Standard in D_2O at 295 K and 300 MHz

a. Chemical Shifts (ppm)

compd	H6	H5	CH_3	NCH_3	H1′	H2′	H2″	H3′	H4′	H5′	H5″
Ia	7.44	4.62									
Ib	7.54	4.93									
IIa	7.27		1.87								
IIb	7.41		1.81								
IIIa	7.47	4.55		2.79							
IIIb	7.68	4.87		2.76							
IVa	7.30		1.85	2.76							
IVb	7.68		1.79	2.77							
Va	7.49	4.67			5.41	4.10		4.18	4.01	3.75	3.66
Vb	7.69	5.00			5.39	4.08		4.17	4.00	3.74	3.65
VIa	7.49	4.65			5.81	2.13	2.27	4.39	3.95	3.67	3.62
VIb	7.69	4.98			5.78	2.11	2.24	4.37	3.92	3.66	3.60
VIIa	7.32		1.87		5.79	2.11	2.26	4.38	3.93	3.67	3.62
VIIb	7.45		1.80		5.80	2.11	2.26	4.38	3.94	3.67	3.60

b. Coupling Constants (J, Hz)

compd	H5H6	$H6CH_3$	1′2′	1′2″	2′2″	2′3′	2″3′	3′4′	4′5′	4′5″	5′5″
Ia	9.2										
Ib	14.5										
IIa		1.3									
IIb		1.3									
IIIa	9.1										
IIIb	14.5										
IVa		1.3									
IVb		1.3									
Va	9.2		5.6			5.3		4.1	3.5	4.5	−12.5
Vb	14.5		5.7			5.4		4.1	3.6	4.5	−12.4
VIa	9.1		7.8	6.2	−14.0	6.1	2.9	2.8	4.4	5.0	−12.2
VIb	14.7		7.7	6.2	−14.0	6.1	3.0	2.8	4.5	5.1	−12.1
VIIa		1.3	7.8	6.2	−14.0	6.2	2.9	2.7	4.4	5.0	−12.2
VIIb		1.3	7.9	6.1	−14.0	6.1	2.9	2.6	4.4	5.0	−12.2

the proposed structures is supported by a large body of NMR, mass spectrometric, UV, and IR data, as well as by independent synthesis of all products derived from the nonnucleosidic compounds. The products from the reaction of I, namely Ia and Ib, are known compounds whose preparation through a different synthetic route has been previously reported, along with the supporting UV and IR spectrometric data.[11]

Structural Analysis. For the purposes of our discussion of structural assignments, we will refer to particular atoms as labeled in Figure 1 (parentheses), where product atoms keep the number they are assigned in the parent compound.

A. Ultraviolet Absorption Spectroscopy. All products isolated give UV absorption spectra showing single bands of high molar extinction coefficient with λ_{max} in the region 251–260 nm. They also showed characteristic λ_{min} in the region 210–215 nm. The products for which molar extinction coefficients could be measured had values of ϵ_{max} in the range of 1.9–2.8 × 10^4 mol^{-1} cm^{-1} dm^3. For cytosine products Ia and Ib the UV absorption data were in agreement with literature values for *cis*- and *trans*-3-ureidoacrylonitrile.[11] The UV data are listed in Table I, and Figure S1 in the supplementary material shows the UV absorption spectrum for compound VIa. The single band in each case is very sharp and shows a λ_{min} in the region 210–218 nm with an ϵ of around 2 × 10^3 mol^{-1} cm^{-1} dm^3. The profiles for all of these compounds are markedly different from those obtained for other nucleic acid photoproducts, such as pyrimidine cyclobutane dimers and hydrates, and will serve as a fairly unique and unambiguous marker for these compounds in further studies.

B. Infrared Absorption Spectroscopy. The IR spectra of compounds Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, and IVb, recorded in Nujol mulls, showed sharp bands at around 2200 cm^{-1} characteristic of a nitrile group. Supportive of the proposed urea residue are bands at around 1640 and 1560 cm^{-1} for the carbonyl group and at around 3300 cm^{-1} for the NH frequencies.[20]

C. Proton NMR Spectroscopy. The ^{1}H NMR data for each of the isolated photoproducts are highly supportive of the proposed structures. The chemical shift and coupling constant data are listed in Table II parts a and b, while a representative ^{1}H NMR spectrum, that of Vb, is given in Figure S2 of the supplementary material. For each compound, we detect a vinylic proton signal downfield in the region of 7.5–8 ppm, consistent with the chemical shift usually observed for the H6 proton of the corresponding cytosines and assigned as that of the proton attached to C3 of the acrylonitrile residue. For the products of cytosine and its derivatives, namely Ia, Ib, IIIa, IIIb, Va, Vb, VIa, and VIb, the signal corresponding to the adjacent vinylic proton is at unusually high field, compared to that observed for the H5 of the parent cytosine. In the case of the cis isomers, this chemical shift is in the region of 4.5 ppm, while the corresponding resonance for the trans isomers is in the neighborhood of 4.9 ppm. This is in agreement with the data reported for *cis*- and *trans*-3-aminoacrylonitrile,[21] where the resonances for the vinylic proton adjacent to the nitrile group were observed at 3.72 and 4.05 ppm, respectively, when measured in acetonitrile-d_3. For the products of 5-methylcytosine and its derivatives (IIa, IIb, IVa, IVb, VIIa, and VIIb) the H6 vinylic proton signal downfield was observed in each case as a tight quartet, through long-range coupling to the methyl group with a coupling constant of 1.3 Hz, further supporting the proposal of a carbon–carbon double bond.

When recorded in acetonitrile-d_3 versus TMS, two NH resonances became apparent. All products showed a broad, one-proton resonance signal at around 8 ppm, just downfield of the vinylic resonance. This signal was assigned as the N3 amino proton resonance of the urea residue with a coupling constant of 11–12 Hz to the downfield vinylic resonance (H6). For compounds Ia, Ib, IIa, and IIb, the NMR spectra showed a broad two-proton singlet in the region of 5.3 ppm, assigned as the N1 urea NH_2 signal. For compounds IIIa, IIIb, IVa, and IVb, we observed a broad one-proton signal in the region of 5.3–5.5 ppm assigned as

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(21) Hong, J. H.; Pownall, H. J.; Becker, R. S. *Photochem. Photobiol.* **1976**, *24*, 217–222.

Table III. ^{13}C NMR Chemical Shift Data (ppm) versus TMS with 1,4-Dioxane Internal Standard ($\delta_{TMS} = \delta_{diox} - 67.4$ ppm)

compd	C2	C4	C5	C6	CH_3	NCH_3	C1′	C2′	C3′	C4′	C5′
I[a]	160.2	168.3	95.9	143.9							
Ia[c]	154.1	117.2	70.6	144.3							
Ib[c]	154.3	119.5	74.0	145.3							
II[b]	160.2	168.4	103.4	141.4	13.0						
IIa[b]	156.9	119.0	82.5	140.0	16.5						
IIb[b]	156.6	122.7	83.9	139.3	12.6						
III[a]	159.8	167.3	96.1	148.4		38.3					
IIIa[c]	154.4	117.3	68.9	144.2		26.9					
IIIb[c]	154.5	119.7	73.4	145.3		27.0					
IV[b]	159.7	167.6	104.0	145.7	13.0	37.6					
IVa[b]	156.5	119.1	81.7	140.0	16.4	26.8					
IVb[b]	156.2	122.8	83.1	139.4	12.6	26.9					
V[a]	158.3	166.8	96.9	142.4			91.2	74.9	70.1	84.6	61.6
Va[a]	156.7	118.2	73.0	143.8			85.4	74.6	71.1	84.3	62.2
Vb[a]	155.6	121.1	75.8	145.5			85.4	74.4	71.0	84.2	62.2
VI[a]	158.2	166.8	96.9	142.3			86.9	40.2	71.2	87.5	62.1
VIa[a]	155.3	118.3	72.7	143.9			82.3	39.6	72.3	86.7	62.8
VIb[a]	155.2	121.2	75.5	145.6			82.3	39.4	72.2	86.6	62.8
VII[a]	158.2	166.6	105.4	139.3	13.2		86.6	40.0	71.3	87.3	62.0
VIIa[a]	155.7	119.8	84.3	139.2	16.1		82.2	39.5	72.3	86.6	62.7
VIIb[a]	155.6	123.6	85.4	138.8	12.6		82.3	39.5	72.3	86.6	62.7

[a] Recorded in D_2O. [b] Recorded in methanol-d_4. [c] Recorded in acetone-d_6.

the N1 urea proton resonance. The attached methyl group gave a resonance signal at approximately 2.7 ppm with a coupling constant of 4.7 Hz to the N1 amino proton. For products Va, Vb, VIa, VIb, VIIa, and VIIb, the corresponding amino proton shows coupling to the H1′ proton of the sugar residue with a coupling constant of around 9 Hz. The connectivities of the amino protons on N3 and N1 to the H6 vinylic proton and H1′ sugar proton, respectively, were confirmed by a two-dimensional correlation spectroscopy (COSY) experiment carried out on compound Va. It is worth noting that the relative chemical shifts of the H2′ and H3′ signals of compounds Va and Vb are reversed compared to those of V, the H3′ signal now appearing at lower field than the H2′ signal. This may be explained by loss of the diamagnetic effect of the pyrimidine base subsequent to photoinduced ring opening.

D. ^{13}C NMR Spectroscopy. The ^{13}C NMR data for all the products isolated are listed in Table III. Also included in this table, for comparison, are data obtained for the parent cytosine derivatives. Because of the very different solubility properties of the free nucleobase products, as compared to the starting materials, we could not always be consistent with the solvents used to record the spectra of parent compounds and products. The solubilities of cis and trans isomers of a particular product differed greatly for a particular solvent. The osidic moiety present in all nucleosidic compounds afforded significant water solubility, and therefore all such compounds were run in D_2O against 1,4-dioxane as internal standard.

The evidence for the presence of a nitrile group is clear. Compared to the starting material in each case, the C4 carbon resonance moves from the region of 167 ppm to around 120 ppm. The nitrile resonances for 3-aminocrotonitrile (Aldrich) measured in acetonitrile were found to be 122.5 ppm (trans), 120.4 ppm (cis), and 118.4 ppm for the solvent relative to TMS. The structurally similar 1-methyl-1,4,5,6-tetrahydronicotinonitrile gave a nitrile carbon resonance at 122.9 ppm,[22] Just as one of the vinylic proton signals moved upfield compared to the corresponding proton signals of the parent compounds, the C5 resonance in each case moved 15–20 ppm upfield. We might also note that the C6 carbon resonances in the region of 145 ppm showed no significant change in chemical shift in the photoproduct compared to the parent compound, which was also found to be the case for the corresponding proton resonances.

For products IIIa, IIIb, IVa, and IVb, the signals corresponding to the *N*1-methyl carbon resonances make dramatic upfield shifts in the products compared to the starting compounds, which would be expected following ring-opening. Similarly, for Va, Vb, VIa, VIb, VIIa, and VIIb, the C1′ osidic carbon signals also move upfield compared to the corresponding signals for the parent nucleosides. All nucleoside ^{13}C resonances, apart from those of compounds VIIa and VIIb, were assigned by two-dimensional ^{13}C–^{1}H heteronuclear correlation spectroscopy. The resonances of the latter two compounds were assigned with reasonable confidence by comparison with those obtained for the parent nucleoside VII, and, in particular, the C5 resonance could be assigned unambiguously because it is a quarternary carbon atom.

E. High-Resolution Mass Spectrometry. All products obtained from nonnucleosidic cytosine derivatives were analyzed by electron impact mass spectrometry which, in each case, gave molecular formulas identical with those of the parent compounds. The molecular ions were pronounced in each case (30–60%). The base peak ion for every compound corresponded to cleavage between the N1 nitrogen and carbonyl carbon atoms of the urea residue, the charge remaining with the vinyl residue, and indeed this was the only significant fragmentation. For example, for compound Ia, the only significant peaks were m/z 111 (M), 68 (B), and 44. For the nucleosidic compounds, which are generally too fragile for electron impact mass spectrometry, high-resolution LSIMS(+) was performed with the parent nucleosides as molecular mass standards. In each case, the cis and trans isomers of the substituted acrylonitriles gave molecular masses identical with the parent compounds within an error margin of 5 ppm, confirming identical molecular formulas. The pseudomolecular ions (MH^+) were confirmed in each case by sodium adduct ions ($M + Na^+$), and fragmentation was negligible.

Assignment of Cis and Trans Configurations. The assignment of cis and trans configuration for compounds Ia, Ib, IIIa, IIIb, Va, Vb, VIa, and VIb was made on the basis of the magnitude of the H5H6 coupling constant. A coupling constant of around 9 Hz indicated a cis configuration, and a value of around 14 Hz suggested the trans configuration. For C5-substituted products, relative chromatographic properties and ^{1}H and ^{13}C chemical shift data provide good evidence for the configurations assigned. For the above compounds, the cis isomer always had a greater R_f value than the corresponding trans isomer for silica gel T.L.C. For reversed-phase HPLC, the trans isomer always eluted first. For ^{1}H NMR chemical shift data, the H6 resonance was at 0.1–0.2 ppm toward lower field for the trans isomer than for the cis isomer. The ^{13}C NMR signal for the nitrile carbon was consistently 2–3 ppm toward the lower field end for the trans isomers. For compounds IIa, IIb, IVa, IVb, VIIa, and VIIb, the same combined trends were observed for each pair of compounds and therefore we feel reasonably confident in our assignments for the latter compounds.

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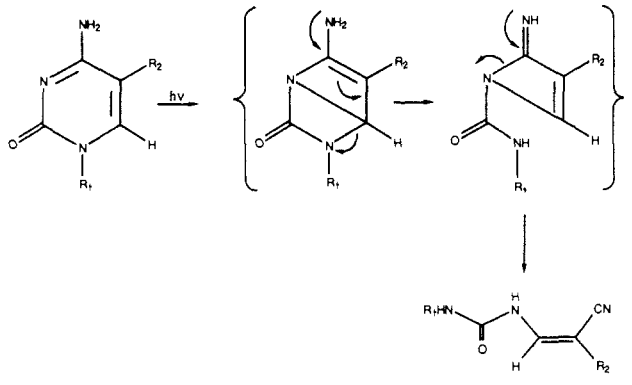
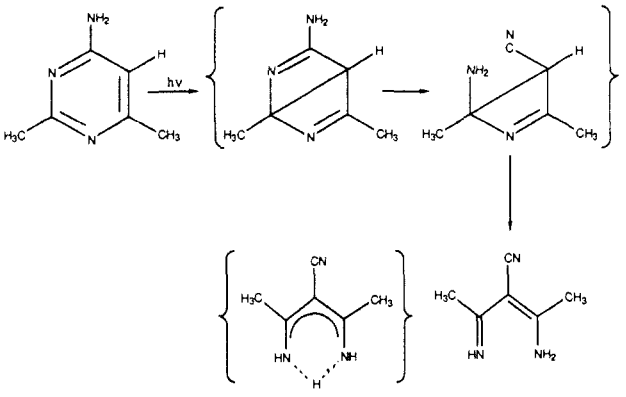
Scheme I. Proposed Mechanism for the Formation of Substituted 3-Ureidoacrylonitriles



Scheme II. Mechanism Proposed by Wierzchowski and Shugar[31] To Explain the Formation of 2-Amino-3-cyanopent-2-en-4-imine via Photolysis of 2,6-Dimethyl-4-aminopyrimidine in Aqueous Buffer



Independent Synthesis of 3-Ureidoacrylonitriles. The products resulting from irradiation of cytosine, 1-methylcytosine, 5-methylcytosine, and 1,5-dimethylcytosine, namely compounds Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, and IVb, were synthesized with slightly modified literature methodologies. It has been reported[12] that, in the presence of aqueous base, 3-ureidoacrylonitrile is rapidly hydrolyzed via the amide to 3-ureidoacrylic acid. We therefore decided to use a method that avoided the use of aqueous solvents. According to Streith et al.,[11a] irradiation of 2-aminopyrimidine *N*-oxide in acetonitrile at $\lambda > 300$ nm leads to the formation of *cis*-3-ureidoacrylonitrile in quantitative yield. We therefore synthesized the *N*1-oxides of 2-aminopyrimidine, 2-amino-5-methylpyrimidine, and 2-(methylamino)pyrimidine, which, upon irradiation behind Pyrex in acetonitrile, gave the predominantly cis isomers of the products expected from irradiation of I, II, and III, respectively. Irradiation of the cis products in acetonitrile at 254 nm gave a mixture of the cis and trans isomers. Products IVa and IVb were prepared by heating a solution of 3-bromo-2-methylacrylonitrile and *N*-methylurea in butanol under reflux for 24 h. Gardner et al.[23] had carried out a similar reaction using diethylamine instead of *N*-methylurea. In each case, the spectrometric and chromatographic data obtained for the products prepared by synthesis were identical with those obtained for the products isolated from the corresponding photoreaction in acetonitrile at 254 nm.

Quantum Yield Studies. We determined the quantum yield for formation of products VIa and VIb via photolysis of 2′-deoxycytidine (VI) in both aqueous solution at pH 7.0 and in acetonitrile containing 1% water. In the the latter solvent, the reaction goes cleanly and the substituted 3-ureidoacrylonitriles are the only photoproducts detectable at the doses used. In water, however, significant side reactions occur, predominantly hydration to form 6-hydroxy-5,6-dihydro-2′-deoxycytidine,[4] for which the reported quantum yield is 8.6×10^{-3} at pH 7.1.[24] The combined ϕ (VIa + VIb) in water was measured at 2.44×10^{-4} and that in acetonitrile at 1.34×10^{-3}. In both cases, there was a very slight preference for the trans form (cis/trans = 47/53).

We also performed less detailed experiments to compare the rates of formation of the products resulting from irradiation of 2′-deoxycytidine (VI) and 5-methyl-2′-deoxycytidine (VII) in acetonitrile, both being pyrimidine nucleosides occuring naturally in DNA. Our preliminary results showed that the rate of formation of products VIIa and VIIb was approximately two-thirds (0.65) that of products VIa and VIb for given dose rate.

Mechanism of the Photoreaction Probably Involves an N3–C6 Dewar Product as an Intermediate. The simplest and most plausible mechanism for the formation of the substituted 3-ureidoacrylonitriles from cytosines is via initial formation of an N3–C6 Dewar structure, followed by a ring-opening rearrangement (Scheme I). From the literature, it is clear that both C2–C5 or N3–C6 Dewar products can be formed from pyrimidines, depending on the nature and position of substituents. It has been shown that 1,4,6-trialkyl-2-pyrimidones[25] form N3-C6 Dewar structures upon UV irradiation. Analogously, with compounds more relevant to DNA photochemistry, Taylor and coworkers[26,27] have shown that the pyrimidone ring of the (6–4) products of thymidylyl-(3′-5′)-thymidine (TpT) and thymidylyl-(3′-5′)-2′-deoxycytidine (TpC), which are both 2-pyrimidones, quantitatively photoisomerize to the N3-C6 Dewar form upon irradiation at 313 nm. The conversion is completely reversible by irradiation at 254 nm. These Dewar structures are apparently quite stable, and indeed the former has been detected by radioimmunoassay in DNA irradiated first at 254 nm and subsequently at 340 nm.[28] On the basis of analogy to the few examples available, formation of the N3-C6 Dewar structure certainly appears to be plausible in the photoreactions of cytosines, which are also substituted 2-pyrimidones.

It has been shown that 2,3,6-trialkyl-4-pyrimidones photochemically react to form C2–C5 Dewar structures.[29] A similar type of C2–C5 Dewar structure was postulated to be an intermediate in the photoreaction of 1,3-dimethyluracil in methanol to form 2-(methoxycarbonyl)-*N*-methyl-3-(methylamino)-propenamide.[30]

Although the Dewar photoproducts resulting from photolysis of the pyrimidine-(6-4)-pyrimidone products obtained from TpT and TpC are stable, at least at neutral or slightly acidic pH, other pyrimidone Dewar photoproducts are unstable and decompose to give final products involving ring opening. For example, the C2–C5 Dewar product from the irradiation of 1,3-dimethyluracil was proposed to undergo subsequent decomposition and addition of solvent to give the propenamide referred to in the previous paragraph.[30] Similarly, the C2–C5 Dewar products obtained from 2,3,6-trialkyl-4-pyrimidones undergo reactions that, depending on the reaction conditions, lead to opening of one or both rings,[29]

Formation of nitriles in the ring-opening reaction of Dewar structures arising from photochemical reaction of 4-aminopyrimidines also has precedent. In 1963, Wierzchowski and Shugar[31] reported the formation of an open-chain nitrile from irradiation of 2,6-dimethyl-4-aminopyrimidine in phosphate buffer at pH 8.6, namely 2-amino-3-cyanopent-2-en-4-imine, shown in Scheme II. They based their assignment on UV and IR spectroscopic data and independent synthesis. In order to confirm

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(28) Mitchell, D. L. *Mutat. Res.* **1988**, *194*, 227–237.
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(31) Wierzchowski, K. L.; Shugar, D. *Photochem. Photobiol.* **1963**, *2*, 377–391.

their findings, and at the same time rule out a similar type of structure for the products we obtained in the present study, we repeated their experiment and obtained a white crystalline product with UV absorption data and melting point identical with those reported by Wierzchowski and Shugar. To further test their structural assignment, we analyzed the compound by NMR. On the NMR time scale we would expect the product to appear to have the symmetrical structure shown in braces in Scheme II. The ^{1}H NMR spectrum recorded in acetonitrile-d_3 (TMS standard) showed a six-proton singlet at 2.18 ppm (2 CH_3), a broad two-proton singlet at 7.73 ppm (2 NH), and a fairly sharp one-proton resonance at 11.50 ppm (N··H··N). This supports their structural assignment. As Wierzchowski and Shugar point out, the simplest and most plausible route of formation of this compound proceeds through a C2–C5 Dewar intermediate. Although the intermediate Dewar structures for 2,6-dimethyl-4-aminopyrimidine and those we propose as intermediates in the reactions of cytosine derivatives are different, the presence of the 4-amino group common to both appears to allow facile decomposition of the Dewar structures to form open-chain nitriles.

Photoreaction of 2′-Deoxycytidine To Form VIa and VIb Is Insensitive to Oxygen and Is Not Photosensitized by Acetone. One piece of information of photochemical interest is the nature of the excited-state precursor(s) of the ureidoacrylonitriles. In particular, it is desirable to know whether excited singlet, triplet, and/or "hot" (highly vibrationally excited) ground states of the parent cytosines are involved in the photoreaction. We have studied the effect of oxygen quenching and acetone photosensitization on the reaction of 2′-deoxycytidine (VI) to form VIa and VIb, in order to gain information about the possible involvement of the triplet state of VI. Deoxygenation of either an aqueous or an acetonitrile solution of 2′-deoxycytidine (2 mM) and irradiation for 1 h at 254 nm showed no significant change in yield of formation of products VIa and VIb as compared to aerated samples, based on HPLC measurements. Furthermore, we did not observe reaction to produce VIa and VIb when VI was irradiated in the presence of 5% acetone at $\lambda > 300$ nm for 1 h. Both of these observations suggest that a triplet state is not involved; however, in drawing this conclusion, we have to make assumptions that the energy level for the triplet state of acetone is higher than that of 2′-deoxycytidine and that the rate constant of oxygen quenching is sufficiently large to inactivate the triplet state before the putative Dewar intermediate forms. The first assumption is probably valid; although the triplet energy of 2′-deoxycytidine is evidently not known, the triplet energy level of cytidine 5′-monophosphate lies below the triplet state of acetone.[32] The second assumption, however, is more questionable as the intramolecular rearrangement of the triplet state of VI to form a Dewar intermediate could be extremely fast.

Conclusions

We have reported here the isolation and characterization of a novel class of photoproducts produced when cytosine, 5-methylcytosine, and related compounds are irradiated with ultraviolet light, namely the 3-ureidoacrylonitriles. The simplest and most plausible mechanism for formation of these compounds involves the initial formation of the N3–C6 Dewar valence isomer, followed by rearrangement to the final product. In addition to their photochemical interest, these findings may be relevant to understanding the effects of ultraviolet radiation on DNA and, thus, could have significant photobiological importance.

Abbreviations

Key: TSP, 2-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography; COSY, two-dimensional correlation spectroscopy; LSIMS, liquid secondary ion mass spectrometry; T.L.C., thin-layer chromatography.

Acknowledgment. Research support from the NIH (Grant GM 23526) is gratefully acknowledged. Also acknowledged is the Bio-organic, Biomedical Mass Spectrometry Resource (A. L. Burlingame, Director), supported by NIH Division of Research Resources Grant RR01614.

Supplementary Material Available: UV absorbance spectrum of compound VIA and 300-MHz ^{1}H NMR spectrum of Vb measured in acetonitrile-d_3 against TMS (2 pages). Ordering information is given on any current masthead page.

(32) Reference 3, Table I, p 239 and references therein.

Application of the Structural Correlation Method to Ring-Flip Processes in Benzophenones

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Abstract: Thirty-eight crystallographically independent structures of thirty-two benzophenones were retrieved from the Cambridge Structural Data Base. All except one show an helical propeller conformation. The torsional angles of the two rings ϕ_1 and ϕ_2 were plotted one against the other in order to identify the threshold enantiomerization mechanism by applying the structural correlation method to the potential ring-flip processes. Molecular mechanics (MM) calculations on benzophenone gave the corresponding calculated potential energy surface. An excellent agreement with the calculated route for a one-ring flip was obtained from the conformational map of the crystallographic data, especially in benzophenones where the C=O bond is involved in intramolecular hydrogen bonding. The strong preference for this route is rationalized by the tendency to maximize the Ar—C=O conjugation interaction during the rotation. The structural correlation method can be used also to evaluate trends in the changes of the structural parameters, such as bond lengths and angles in approaching the rotational transition state. Similar trends are obtained from analysis of the X-ray data and from MM calculations on the one-ring flip of benzophenone.

Introduction

By using literature data we recently[1] applied the structural correlation method[2-4] to assess the most feasible ring flip routes in 1,1-diaryl- and 1,1,2-triarylvinyl systems.[1] Both the one- and the two-ring flips were found to be feasible for 1,1-diarylvinyl

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