Unusual Photochemical Transformation of 5-Ethylorotate to 5-Ethylidenehydroorotate

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Ultraviolet irradiation of 5-ethylorotate in aqueous medium led to formation of 5-ethylidenehydroorotate *via* a 1,3-hydrogen rearrangement from the ethylene group of the 5-substituent to the ring C_6. The same product was also obtained by an alternate pathway *via* a hitherto unidentified intermediate.

The structure of this unusual pyrimidine analogue, established in the solid state by X-ray diffraction, was shown with the aid of spectroscopic methods to be the same in solution, and some of its properties are described.

Heating of 5-ethylidenehydroorotate in the solid state led by direct conversion to a mixture of the parent 5-ethylorotate and 5-ethyluracil. By contrast, ultraviolet irradiation in aqueous medium resulted in quantitative photochemical conversion of the photoproduct to 5-ethyluracil.

Introduction

The biological significance of the photochemical transformations of the pyrimidine constituents of nucleic acids has been extensively documented. Although ultraviolet irradiation of 5-methyl-2,4-diketopyrimidines, or their nucleosides and nucleotides, leads largely to formation of 5,5′-6,6′ cyclobutane dimers, such dimer formation is only partial when the 5-methyl is replaced by a 5-ethyl or higher alkyl substituents[1]. The predominant reaction in such instances is an intramolecular cyclization, with formation of a cyclobutane ring on the 5,6 bond, the resulting products being 5,6-dihydro-5,6-cyclobutanylpyrimidines, or their nucleosides[2]. These photoproducts, which may be considered formally as models of a "half" pyrimidine cyclobutane photodimer, also readily undergo photodissociation in high quantum yield to release the parent dealkylated pyrimidine or nucleoside.

During attempts to examine the behaviour of 5-ethylorotic acid under similar conditions, quite different photoproducts were obtained, probably as a result of steric hindrance by the 6-carboxylate group to intramolecular photocyclization. This communication describes the isolation and identification of one of the major photoproducts, as well as some of its properties. It should be recalled that whereas orotic acid itself undergoes photodimerization to a cyclobutane dimer in high yield[3], *via* a triplet state[4, 5], 5-methylorotic acid is highly resistant to irradiation in aqueous medium, but does photodimerize in an ice matrix[3]. Both dimers readily photodissociate to the parent monomers, also with high quantum yield.

Experimental

Published procedures were employed for the synthesis of 5-ethylorotate (as the free acid)[6] and 5-ethyluracil[7]. Catalytic reduction of 5-ethylorotic acid (obtained from Sigma Chemical Co.) on a rhodium catalyst[8] provided 5,6-dihydro-5-ethylorotate, m.p. 236 – 238 °C, and with spectral properties in neural and alkaline media identical with those for other 2,4-diketo-5,6-dihydropyrimidines[9].

Thin-layer chromatography made use of commercial Merck Silica gel F254 5737/0025 plates for analytical purposes. Merck PF254 Silica gel was employed for preparative work. The solvent system used throughout for both purposes was chloroform-methanol (1:1, v/v).

Irradiation at 254 nm was with a Philips 6-watt germicidal lamp, surrounded by a 5 mm layer of 33% acetic acid to eliminate radiation below 230 nm; the intensity of this source 1.4 cm from the filter surface was about 3×10^{16} quanta/cm^2/min, measured actinometrically with the use of an aqueous solution of uridine for which the quantum

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yield for photohydration is 0.021[10]. For irradiation at wavelengths to the red of 265 nm, a Zeiss (Jena, GDR) medium-pressure therapeutic 700-watt mercury lamp with a Zeiss WG7 cut-off filter was employed. Solutions were irradiated in 1 mm and 10 mm pathlength spectral cuvettes.

Irradiations on a preparative scale made use of the Zeiss 700-watt lamp as part of a reactor with three concentric layers. The inner consisted of a 2-mm layer of a formamide solution of adenine to cut off at 265 nm[11]. The middle layer (2 mm) was for the solution to be irradiated, with an inlet and outlet allowing passage of nitrogen for mixing. The outer layer consisted of a water jacket for cooling purposes.

UV absorption spectra were run on a Zeiss VSU-2 manual instrument and a Unicam SP-8000 recording spectrophotometer, using 1-, 2-, and 10-mm pathlength cuvettes.

Melting temperatures (uncorrected) were measured on a Boetius microscope hot stage.

^1H NMR spectra were run on a Bruker 60 MHz spectrometer employing solutions in D_2O (Merck, 99.7 mol% ^2H), with tetradeutero-3(trimethylsilyl)-propionic acid as internal standard.

Mass spectra were obtained with the aid of an LKB-9000 spectrometer.

Atomic absorption spectrophotometry made use of Pye Unicam 1900 instrument.

Results and Discussion

Irradiation at 254 nm of a 10^{-4} M neutral aqueous solution of 5-ethylorotate was accompanied by a decrease in optical density of the principal long wavelength absorption band at 273 nm ($\varepsilon_{\max}$ 6.0×10^3), with an initial quantum yield of 0.03, which decreased during the course of the reaction. Following about 60% photolysis, the absorption began to increase; simultaneously the 273 nm band shifted hypsochromically to 265 nm. Irradiation at longer wavelengths (>265 nm) gave qualitatively analogous behaviour. The course of the reaction was unaffected by a 10-fold increase in concentration, or by removal of oxygen from the system.

Chromatography of the photolysate on Merck Silica gel plates led to isolation of a strongly absorbing product (R_F 0.90) identified spectrally as 5-ethyluracil ($\lambda_{\max}$ 265 nm, $\varepsilon_{\max}$ 8.7×10^3), which accounted for the hypsochromic shift of the 273 nm band of 5-ethylorotate to 265 nm during irradiation; and a product, PII, (R_F 0.32) with only end absorption in the quartz ultraviolet. Closer examination revealed the presence of an additional weakly absorbing product, PI (R_F 0.00).

Preparative isolation of PI and PII: Neutral 10^{-2} M solutions of 5-ethylorotate were irradiated at $\lambda > 265$ nm in a photochemical reactor for various time intervals and the yield of products monitored by TLC as above. Trials showed optimal yields of PI and PII[12] following about 80% photolysis. Larger volumes of 10^{-2} M solutions were then irradiated portionwise under these conditions, the pooled photolysates brought to dryness under reduced pressure, and the residue taken up in water and chromatographed on PF 254 Silica gel. The bonds corresponding to PI (R_F 0.00) and PII (R_F 0.32) were eluted with methanol (yield about 7% for each), the eluates brought to dryness, and the residues crystallized several times from 30% aqueous methanol for PI and 20% methanol for PII.

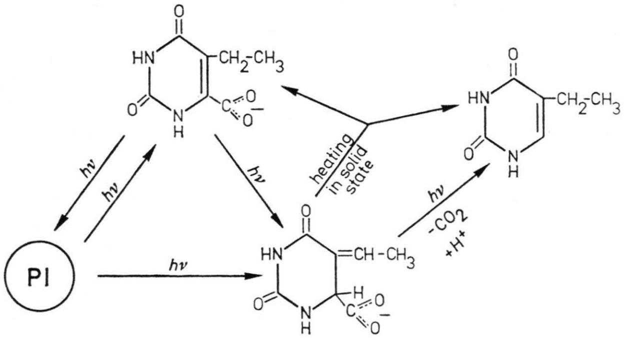
Properties of PI: This intermediate, obtained in the form of fine needles, exhibited only low end absorption in the quartz ultraviolet in neutral aqueous medium. It was relatively resistant to irradiation at $\lambda > 265$ nm. But on irradiation at 254 nm, it was converted to a mixture of 5-ethylorotate and 5-ethyluracil, the former *directly* from PI, and the latter *via* initial transformation to PII, as shown by chromatography. Efforts to identify the structure of the intermediate PI, or to obtain crystals suitable for X-ray diffraction studies, have hitherto been unsuccessful.

Properties of PII: Formation of PII occurs not only indirectly, *via* PI, but also directly from 5-ethylorotate. This follows from the fact that irradiation of 5-ethylorotate at $\lambda > 265$ nm leads to appearance of comparable amounts of PII and PI, whereas the latter, due to its low absorbance at this wavelength, is negligibly affected under these conditions.

The isolated crystalline photoproduct was soluble in water and less so in alcohols. Chromatographically it separated into two components, separated by fractional crystallization from 20% methanol, and initially suspected of being isomers. When X-ray diffraction analysis showed one of these to be the calcium salt[13] (see below), the other was then found to be free acid, both confirmed by atomic absorption spectrophotometry. The calcium obviously originated from the Silica gel employed to isolate PII, and its presence explained initial difficulties in obtaining a suitable elementary analysis of PII.

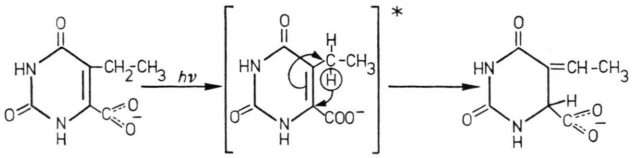
In the neutral aqueous medium, PII exhibited end absorption in the quartz ultraviolet, with a band at about 207 nm, suggesting saturation of the 5,6 bond as for 2,4-diketo-5,6-dihydropyrimidines [9], 2,4-diketopyrimidine cyclobutane photodimers, including those of orotic acid and 5-methylorotic acid [3, 14], and the intramolecular photocyclization products of 5-alkylpyrimidines [2]. The extinction coefficient of the 207 nm band ($\varepsilon \sim 11 \times 10^3$) pointed to the existence of a conjugated double bond system, probably involving $C_4 = 0$ and a double bond in the chain at C_5. The existence of such a double bond was shown by the positive reaction of PII with bromine water at room temperature; 5,6-dihydro-5-ethylorotate did not react with bromine. In alkaline medium, probably leading to formation of the anion, the 207 nm band shifted to about 265 nm ($\varepsilon \sim 4.7 \times 10^3$)[15] suggesting the presence in the anion of at least three conjugated double bonds.

Structure of PII: The foregoing data, even when supplemented with ^{1}H NMR, and mass spectroscopic data (see below), proved inadequate in our hands for resolution of the structure of PII. The solid state structure was finally elucidated by means of X-ray diffraction, described in detail elsewhere [13], as 5-ethylidenehydroorotate (see Schemes 1 and 2), and consisting of the two enantiomers.



PII

Scheme 1.



Scheme 2.

NMR spectrum of PII: The ^{1}H NMR spectrum of PII in D_2O which was difficult to interpret prior to obtaining the X-ray data, is fully consistent with the structure observed in the solids tate. The methyl group on the ethylidene chain appeared as a doublet at 2.0 ppm, and the methine hydrogen as a quartet at 7.1 ppm, while the ring H(6) showed up as a singlet at 4.9 ppm on the shoulder of a signal originating from traces of water in solution. The spectra of the free base and the calcium salt were identical.

Mass spectrum of PII: The mass spectrum of PII (either free acid or calcium salt) included a major peak with $m/e = 44$, corresponding to CO_2, and originating from the ready thermal decarboxylation of the photoproduct, so that the PII ion itself was not observed. The resulting product, $C_6H_8N_2O_2$ appeared at $m/e = 140$. Subsequent fragmentation probably proceeds *via* elimination of CH_3 to give $C_5H_5N_2O_2$ with $m/e = 125$; and this is succeeded by elimination of HNCO tog ive C_4H_4NO ($m/e = 82$), in agreement with the initial stage of fragmentation of thymine [16], uracil [17] and 6-methyluracil [18].

Phototransformation of PII: On irradiation of PII in neutral, aqueous medium at 254 nm (or at wavelengths to the red of 265 nm), the end absorption was gradually replaced by a band with λ_{max} 265 nm. The reaction proceeded with maintenance of an isosbestic point at 250 nm, pointing to conversion of PII to a single new UV-absorbing product, identified spectrally and chromatographically as 5-ethyluracil. Conversion of PII to 5-ethyluracil was virtually quantitative [19], and at 254 nm proceeded with a quantum yield of 0.20, value comparable with that for photodissociation of 5,6-dihydro-5,6-cyclobutanylpyrimidine derivatives [2], and not far below that for photodissociation of pyrimidine cyclobutane photodimers [10].

Thermal decomposition of PII: The isolated product PII did not have a defined melting point. The calcium salt, at temperatures exceeding 280 °C, decomposed to a mixture of 5-ethylorotate and 5-ethyluracil; for the free base this thermal decomposition process started at 230 °C. The process was followed by TLC. The formation of 5-ethylorotate and 5-ethyluracil from PII each involved a separate pathway, since free 5-ethylorotic acid underwent thermal decarboxylation only at temperatures above 320 °C. It follows that PII must possess a carboxyl group, and that elimination of this group occurs during photochemical conversion of PII to 5-ethyluracil, described in the previous paragraph.

Mechanism of formation of PII: Having established the structure of PII in solution as 5-ethylidenehydroorotate, it is now possible to represent the various photochemical and thermal rearrangements described above as in Scheme 1.

Furthermore, bearing in mind that one of the pathways for formation of 5-ethylidenehydroorotate is a *direct* photorearrangement (Scheme 1), it becomes pertinent to examine the possible mechanism of this reaction. One possibility is a 1,3-migration of a hydride ion from the α-carbon of the 5-ethyl side chain to the pyrimidine C_6, with displacement of an electron pair from the 5,6 bond to the α-carbon (Scheme 2). Such a mechanism would be most unlikely in the ground state because of the strong positive inductive effect of the carboxyl group at C_6. In the excited state, however, the pK of the carboxyl group may be appreciably altered. Furthermore, the absence of any effect of the state of dissociation of the carboxyl group in the ground state is furnished by the observation that the course of photolysis of 5-ethylorotate is unaltered at pH 1 (pK of carboxyl group 1.4).

Finally it should be noted that the photochemical rearrangement of 5-ethylorotate, as described in Scheme 2, bears some resemblance to the 1,3 hydrogen rearrangement which results from irradiation of the 3-methyl derivatives of 2-cyclohexenones[20]. In this latter instance, however, replacement of the 3-methyl by higher alkyl groups, such as ethyl or isopropyl, no longer gave this reaction.

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