Novel Electrochemically Derived Dimers of Methylated Uracils

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Electrolysis of acetic acid/sodium acetate solutions of N-methylated uracils results in the formation of 5-substituted methyl and acethoxy derivatives. Electrolysis of trifluoroacetic acid/potassium trifluoroacetate solutions of N-l- and N-3-methylated uracils provided, besides 5-trifluoromethyl derivatives, novel N-C uracil dimers. In the case of 1,3-dimethyluracil in trifluoroacetic acid, N-1 demethylathion was also observed.

Introduction

Pyrimidine cyclobutane dimers formed photochemically play an important role in the UV-induced cell mutagenesis [1]. Another type of pyrimidine dimers, the so-called 6–4 dimers, are also important determinants in the mutagenic and lethal effects of UV irradiation on biological systems (for review see [2]). Uracil molecules coupled by single C(5)–C(5') bond were isolated from UV-irradiated solutions of 5-bromouracil [3,4] and 1,3-dimethyl-5-bromouracil [5–7], and were also obtained by chemical synthesis [8]. Here we describe and characterize electrochemically derived new type of uracil dimers linked by N(l)-C(5') or N(3)-C(5') bond.

Electrochemical trifluoromethylation of unsaturated organic compounds provides products in generally good yields [9–11]. Trifluoroacetic acid is a very effective source of anodically generated trifluoromethyl radicals [12]. Earlier, a successful trifluoromethylation of 1-methyl-uracil (40%) was achieved on nickel electrodes due to the formation of a molecular complex between 1-methyluracil and NiF₂ on the anodic surface [13]. The same authors mentioned that experiments using platinum electrodes gave no satisfactory results [13]. Most likely, the yields of the products were limited by trapping of short-lived anodic products by uracil from the bulk.

Electrolysis of simple aliphatic acids is an interesting source of short-lived free radicals and carbocations formed on and nearby the anodic surface. The generally accepted anodic reaction of carboxylic acids [14] is shown below:

\[
\begin{align*}
\text{R} \overset{e}{\rightarrow} & \text{C(5') \rightarrow R'} \\
\text{R} \overset{e}{\rightarrow} & \text{C(5') \rightarrow R'} \\
& \text{− CO₂}
\end{align*}
\]

We investigated the reaction of methylated uracils with anodically formed species derived from acetic acid and trifluoroacetic acid. Our main interest was the distribution and type of products, and not the preparative utility of the method. We did not attempt to optimize experimental conditions by varying either the concentrations of salts in the electrolysis medium, or the concentration of methylated uracils in the bulk, or current density. While the results presented below are of rather informative character, the finding of non-typical dimers of methylated uracils encouraged us to report these preliminary data.

Importantly, out of five major nucleic acid bases uracil and thymine do not show polarographic reduction waves in aqueous solutions. However, our unpublished observations on electrolysis in undivided cells of uracil and thymine derivatives in organic solvents revealed decreases in total UV absorption of the reaction mixtures, in particular at high current densities. TLC patterns of the reac-

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tion mixtures appeared similar to those found by chemical oxidation [15]. In general, the presence of oxidation products significantly lowered the yields of desired derivatives.

**Results and Discussion**

Electrolysis of acetic acid/sodium acetate solution containing either 1- or 3-methyluracils (1a and 1b), or 1,3-dimethyluracil (1c), gives two defined products. HPLC patterns of the reaction mixtures (Fig. 1) indicate the presence of methylated thymines (2a–c) and the respective 5-acetoxuryacils (3a–c). In the case of 1a, the amount of 3a was higher than that of 2a, whereas in the experiment with 3-methyluracil the reverse was found. Retention times of 5-acetoxy derivatives (3a and 3b) were taken from HPLC pattern of the reaction mixture obtained by partial methylation of 5-acetoxyuracil with diazomethane. Exhaustive methylation of 5-acetoxyuracil gave 3c as expected (see Experimental section).

Using 1a as a substrate, we were able to isolate 3a (with ca. 90% purity), and determine its structure by NMR and mass spectroscopy. Similarity in chromatographic mobilities of 2a and 3a makes the column separation difficult. In the case of 1c, chromatographic separation of the two expected products 2c and 3c on XAD-4 column was successful. We gave up column isolation of 3b because of low yield of this product during electrolysis of 1b in acetic acid.

The above results indicate a radical substitution of uracil ring by the methyl and acetoxy radicals formed on the anodic surface (Scheme 1). Experiments using 1a and 1b in trifluoroacetic acid/potassium trifluoroacetate gave results which did not follow the above scheme. Beside the expected 5-trifluoromethyl-substituted derivatives of the respective uracils (4 and 6) (Fig. 2), and a number of unidentified products, two dimeric species (5 and 7) were found. In the case of 1a, the amount of dimer (5) formed was comparable to that of the 5-trifluoromethyl-substituted compound (4). In contrast, 1b gave mostly the corresponding 5-substituted derivative (6). Isolation of both these dimers (5 and 7) was achieved by XAD-column chromatography. However, the 3-methyluracil-derived dimer (7) required additional purification by preparative HPLC. Mass spectra of these dimers indicated that the products consist of two coupled methyluracil moieties. 1H NMR measurements in-

![Fig. 1. HPLC profiles of the reaction mixtures obtained by electrolysis of 1a, 1b (elucent: methanol/water, 10:90), and 1c (elucent: methanol/water, 20:80) in acetic acid/sodium acetate solution.](image-url)
The NMR spectra of 5 and 7 show a characteristic pattern of the uracil H(5)/H(6)-coupled spin system with J(5,6) = 7 Hz and 8 Hz, respectively, as well as one H(6) singlet (7.905 ppm in 5 and 7.925 ppm in 7). Each spectrum contains also two singlets corresponding to two N-substituted methyl groups (3.362 ppm, 3.252 ppm in 5, and 3.176 ppm, 3.164 ppm in 7), and a broad N-H signal at approximately 11.6 ppm. Due to poor solubility of the dimers in DMSO we gave up running their 13C NMR spectra.

The compounds 5 and 7 are unstable in alkaline medium (Fig. 3). This observation provides a hint regarding the structure of these derivatives. One fragment of each of the two dimers resembles a 1,3-disubstituted uracil. Such derivatives undergo ring opening in alkali. However, this reaction does not result in a complete decay of UV absorption which is typical of 1,3-dimethyluracil. The other fragment (1-methyl- or 3-methyluracil) of these dimers, as expected, is stable in alkaline solution. Slow rate of decomposition of these dimers (about 10 times lower than that of 1,3-dimethyluracil) can be explained by the fact that the decomposition results from hydroxyl anion attack on negatively charged dimer molecule. Both these dimers are UV-sensitive. Nevertheless, their UV-induced decomposition does not yield hydrated methyluracils exclusively. Irradiation of unbuffered aqueous solutions of the dimers showed decreases in UV absorption. However, boiling these irradiated solutions did not result in a full restoration of the original UV absorption. The pattern of photochemically induced decomposition of the dimers showed this reaction is not a simple cleavage of the dimer link followed by hydration of uracil moieties. Based on the above results, we conclude that the pyrimidine moieties are coupled by N(1)–C(5') or N(3)–C(5') bond (Scheme 2).

Earlier studies by Eberson group and others investigators have shown that trifluoroacetic acid can act as powerful one-electron oxidant (see [15a,b]). We suppose that methylated uracils can...
Scheme 2. Electrochemical conversion of 1a and 1b to, respectively, 4 and 5, and 6 and 7, in trifluoroacetic acid/potassium trifluoroacetate solution.

![Scheme 2](image-url)

indicates anodic oxidation of this substituted pyrimidine, while no voltamperometric curve can be obtained using 1-methyluracil (1a) in acetic acid/sodium acetate solution.

As described above, electrolysis of acetic acid/sodium acetate solution in the presence of 1,3-dimethyluracil yields two main products 3a and 3b which were isolated and characterized by spectral and chromatographic comparison with authentic samples. Unexpectedly, the same procedure using trifluoroacetic acid/potassium trifluoroacetate gave 3-methyl-5-trifluoromethyluracil (6, 4.5%) in addition to the expected 1,3-dimethyl-5-trifluoromethyluracil (8, 4.5%) (Fig. 2). Formation of the latter compound can be explained by radical substitution of 1c with trifluoromethyl radical. The only explanation for the formation of 6 we can offer at this time is instant hydrolysis of trifluoroacetoxy derivative (9) in aqueous medium. The intermediate 1-hydroxymethyluracil derivative which might be formed by hydrolysis of 9 would be unstable. This has clearly been shown in studies on formaldehyde reaction with uracil [16a,b]. Recently, we have reported that electrochemically obtained 1-acetoxy-methyluracil and 1-acetoxy-methylthymine hydrolyse to uracil and thymine, respectively [17]. Formation of 9 can be explained by the attack of trifluoroacetoxy radical on 1,3-dimethyluracil (1c), or, alternatively, oxidation of 1c to cation radical followed by deprotonation, isomerisation and reaction of isomeric cation radicals with either trifluoroacetoxy radical or trifluoroacetate (Scheme 3). However, neither proposal can explain why uracil demethylation takes
place at N-1 and not N-3. Notably, we have found in our studies the presence of 3-methylthymine among the products of electrolysis of trifluoroacetic acid, but not in the presence of 1,3-dimethylthymine (not shown).

Electrolysis on platinum electrodes of uracil in trifluoroacetic acid gives 5-trifluoromethyluracil among a number of products. Isolation of 5-trifluoromethyluracil on an XAD-4 column is simple, but the yield of electrolysis is low (<10%) and compares unfavorably with that reported by chemical methods. Our attempts to obtain the expected uracil dimer for physicochemical characterization were unsuccessful.

Materials and Methods

Electrolyses were performed in an undivided water-coated cell equipped with two 3 cm² platinum electrodes spaced 5 cm apart. Current density was 0.08 A/cm² in all experiments. Electrolysis time was 90 min. The electrolytic reaction mixture consisted of 15 ml of acetic acid or trifluoroacetic acid, 800 mg of sodium acetate or 450 mg of potassium trifluoroacetate, respectively, and 3 mmol of uracil derivative. Analytical TLC was performed on precoated silica gel 60 F254 (Merck). Melting points (uncorr.) were measured using Boetius microscope hot stage. HPLC was performed using a model LC 6A Shimadzu instrument (UV detector, λ = 254 nm) with a column C18 (4.6×25 mm) (Beckman) (water-MeOH solutions). Mass spectra (70 eV) were obtained with a model AMD-604 Intectra spectrometer. UV spectra were recorded with a model Kontron Uvikon 940 spectrophotometer. NMR spectra were measured with a Varian UNITY plus 500 MHz spectrometer. Chromatography was performed on a XAD-4 column (Serva) (2.5×40 cm) in water/isopropanol gradients.

Experimental

5-Acetoxy-1,3-dimethyluracil (3c): A suspension of 5-acetoxyuracil [18] (170 mg, 1 mmol) in methanol (15 ml) was treated with ether solution of diazomethane until a single product was found (TLC control). Partially methylated mixture was used for determination of HPLC retention times of 3a and 3b. Following the methylation, the reaction mixture was evaporated to dryness, and the residue was crystallized from small amount of water to give needles (120 mg, 61%) with m.p. 155–157 C. UV: λ max (water) 273 nm. TLC: Rf (CHCl₃–MeOH, 9:1) 0.73. 1H NMR (d₆-DMSO): 2.23 (s, CH₃-acetyl), 3.18 (s, NCH₃), 3.30 (s, NCH₃), 7.91 (6-H). MS: 198 (6), 156 (100), 127 (2), 99 (10).

Analysis for C₈H₁₀N₂O₄ (198.18)
Caled  C 48.48 H 5.09 N 14.14 %.
Found   C 48.41 H 5.19 N 14.06 %.

5-Acetoxy-1,3-dimethyluracil (3c) and 1,3-dimethylthymine (2c): Following electrolysis of 1,3-dimethyluracil in acetic acid (see Materials and Methods), the reaction mixture was evaporated to dryness, and the residue was dissolved in water (25 ml) and applied onto XAD-4 column. The elution using water/isopropanol gradient (total volume 2.1, final isopropanol concentration 25%) gave two peaks in addition to the starting compounds: 5-
acetoxy-1,3-dimethyluracil (74 mg, 13%, m.p. 154–155 °C) at ca. 20% isopropanol, and 1,3-dimethylthymine (4% calc. from optical units) at ca. 24% isopropanol concentration. The product containing fractions were evaporated to dryness and the residues were compared chromatographically and spectrally with authentic compounds.

5-Acetoxy-1-methyluracil (3a) obtained by electrolysis of 1-methyluracil was identified by MS and HPLC. The 2a and 3a peaks overlapped. Therefore, the first two thirds of the appropriate peak volume was taken to give the title compound of over 90% purity (HPLC). TLC: Rf (CHCl3–MeOH, 9:1) 0.46. UV: (pH 1 and 7) λmax 273 nm, (pH 12) λmax 270 nm. 1H NMR (d6-DMSO) 2.21 (s, CH3-acetyl), 3.22 (s, N′-CH3), 7.81 (s, 6-H), 11.61 (bs, N-H). MS: 184 (7), 142 (100), 99 (1), 71 (18).

5-Acetoxy-3-methyluracil (3b) formed with low yield during electrolysis of 3-methyluracil in acetic acid, and was not isolated. The identity of this compound was deduced from analysis of HPLC retention times of partially methylated products of 5-acetoxyuracil.

1-Methyl-5-trifluoromethyluracil (4) and 5-(1-methyluracil-3-yl)-1-methyluracil (5). Following electrolysis of 1a in trifluoroacetic acid, the reaction mixtures was evaporated to dryness and applied onto XAD column. The elution was performed using water/isopropanol gradient (total volume 21, final isopropanol concentration 15%). In addition to 1-methyluracil, two major peaks were found. The first one (eluted at about 10% isopropanol) contained the dimer (5), and the second one (obtained at ca. 13% isopropanol) carried 4. The fractions corresponding to the dimer peak were evaporated to dryness, the residue was taken into 2 ml of water, and the solid material was filtered and washed with small amount of cold ethanol and ether to obtain 34 mg (9%) of 5-Acetoxy-3-methyluracil-3-yl)-1-methyluracil (5). Following electrolysis of 1b in trifluoroacetic acid, the reaction mixture was evaporated to dryness and applied onto XAD column. Elution was performed with water/isopropanol gradient (total volume 21, final isopropanol concentration 20%). The dimer-containing minor peak obtained at ca. 10% isopropanol was evaporated to dryness, the residue was taken into 2 ml of water, and the solid material was filtered out (6 mg). This latter was further purified by preparative HPLC to give 4.5 mg (1.5%) of needles with m.p. >310 °C (decomp.). TLC: Rf (CHCl3–MeOH, 9:1) 0.27. 1H NMR (d6-DMSO): 3.16 and 3.17 (2s, N-CH3), 8.24 (d, 5-H), 7.51 (d, H-6), 7.93 (s, 6'-H), 11.61 (bs, N3'-H). MS: 250 (100), 193 (61), 165 (95), 138 (10). UV: (pH 1 and 7) λmax 264 nm, (pH 12) λmax 288 nm.

The main peak (obtained at ca. 18% isopropanol) was evaporated to dryness, and the residue was crystallized from water to give 6 (55 mg, 11%) with m.p. 183–186 °C (lit [20], m.p. 185–190 °C) TLC: Rf (CHCl3–MeOH) 0.49. 1H NMR (d6-DMSO): 3.14 (s, N3'-CH3), 8.07 (s, 6-H), 11.89 (bs, N1'-H). MS: 194 (100), 174 (42), 165 (4), 146 (13), 137 (32).

3-Methyl-5-trifluorouracil (6) and 5-(3-methyluracil-1-yl)-3-methyluracil (7). Following electrolysis of 1b in trifluoroacetic acid, the reaction mixture was evaporated to dryness and applied onto XAD-4 column. Two main products obtained were 3-methyl-5-trifluorouracil (6) (eluted at 18% isopropanol; 24 mg, 4%) and 1,3-dimethyl-5-trifluoromethyluracil (8) (eluted at 23% isopropanol; 21 mg, 4%) m.p. 101 °C (lit [20], m.p. 101–101.5 °C). TLC: Rf (CHCl3–MeOH) 0.74. 1H NMR (d6-DMSO): 3.17, 3.29 (2s, N-CH3), 8.24 (6-H).

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