Photochemistry of *N*-[9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]-pyridinium Chloride in Aqueous Solutions. Mechanism of the Formation of Tri-*O*-acetylluminarosine

Bohdan Skalski,[†] Ronald P. Steer,[‡] and Ronald E. Verrall*,[‡]

Contribution from the Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Poland, and the Department of Chemistry, University of Saskatchewan, Saskatoon, Canada S7N0W0.
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Abstract: The photochemistry of *N*-[9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium chloride (**1**) has been investigated in aqueous solutions in the pH range 6–8 under aerobic and anaerobic conditions. A multistep mechanism of the photochemical transformation of **1** into the highly fluorescent nucleoside 2′,3′,5′-tri-*O*-acetylluminarosine (**2**) is proposed in which, as the first step, **1** undergoes light-induced, hydrolytic ring opening in the imidazole portion of the purine ring to form *N*-[5-formamido-6-[(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)amino]pyrimidin-4-yl]pyridinium chloride (**3**). At pH >7, **3** exists partly in the electrically neutral, zwitterionic form, which undergoes electron-transfer-induced ring closure to give luminarosine **2**. This process is sensitized by the excited triplet state of **1**, which serves as an electron acceptor. Under anaerobic conditions, the resulting pyridinyl radicals undergo dimerization.

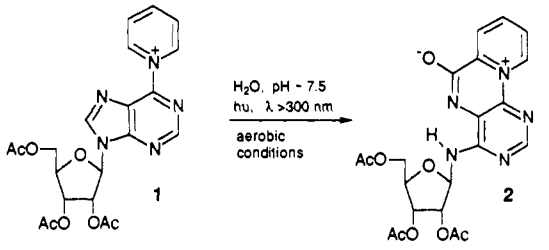
Introduction

Chemical[1] or photochemical[2] modifications of nucleic acid bases which give rise to derivatives that fluoresce at room temperature are always of interest since they often provide the opportunity to use fluorescence techniques as a means to study aspects of nucleic acids including their structure and dynamics[3] and nonradioactive detection.[4] Although a number of modified, fluorescent nucleosides have been synthesized,[5] there is a growing need for new derivatives, especially those emitting in the visible region, since they can be easily monitored in biological systems.

In this regard, we have reported recently[6] the photochemical transformation of water-soluble, blue-emitting *N*-[9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium chloride[7] (**1**) into another nucleoside that emits intense green fluorescence, namely 4-[(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)amino]pyrido-[2,1-*h*]pteridin-11-ium-5-olate (**2**), termed 2′,3′,5′-tri-*O*-acetyl-β-luminarosine (cf. Scheme I). The α-anomer of **2** and its aglycon, luminarine, have also been obtained.[6] The photophysical studies[8] of these new fluorophores have shown their great potential for use in a wide range of chemical applications. Consequently, attempts were also made to convert some other pyridinium salts derived from various 9-substituted hypoxanthine[7,9] and guanine[10] derivatives into related analogues of luminarosine by using methods and conditions previously established[6] as optimal for preparation of **2**. However, these initial attempts were unsuccessful, as most of those salts appeared to be photochemically stable.[11] This prompted us to study the detailed photophysical and photochemical properties of these salts to better understand the differences between their photochemical reactivity and to solve the problem of the mechanism of the formation of luminarosine. In a previous paper[12] concerning the photophysical properties of a series of purinylpyridinium salts we have suggested that the high photochemical reactivity of salt **1**, compared with other salts studied, and formation of luminarosine were associated with an efficient intersystem crossing to the excited triplet state in this particular compound.

In this paper, we present and discuss the results of the detailed photochemical studies of **1** in aqueous solutions under aerobic and anaerobic conditions and as a function of pH. It will be shown that luminarosine **2** is formed from **1** in a chain of reactions, where the latter also acts as an electron-transfer sensitizer in one of the steps of the mechanism.

Scheme I



Results and Discussion

Irradiation of 1 under Various Conditions. Analytical Scale Experiments. An aqueous solution of **1** (2×10^{-4} M) was irradiated with near-UV light (λ > 300 nm) under the following conditions: (i) in the presence of $NaHCO_3$ (1 mmol, pH ≃7.5), aerobic conditions; (ii) same pH as in (i) but in the absence of oxygen; and (iii) at pH 5.8–6.0 (no $NaHCO_3$ added), in the absence of oxygen.

[†] A. Mickiewicz University.
[‡] University of Saskatchewan.

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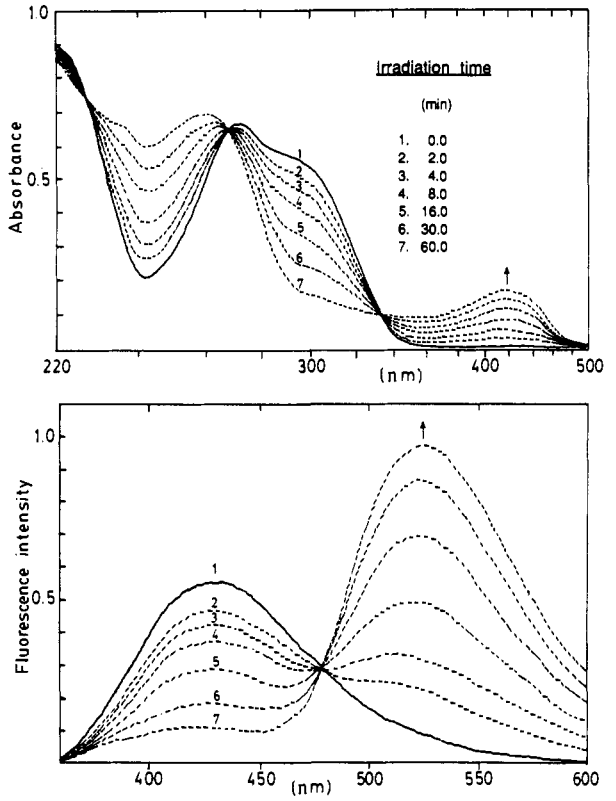


Figure 1. Changes in the absorption and fluorescence spectra of an aqueous solution of **1** (pH 7.5, adjusted with $NaHCO_3$) during irradiation under aerobic conditions.

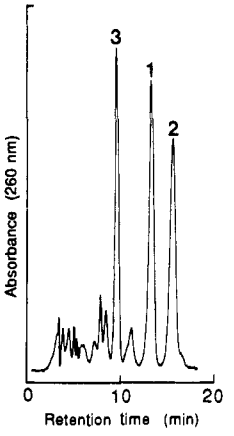


Figure 2. HPLC analysis of the solution of **1** irradiated under conditions (i) to ca. 63% conversion. HPLC conditions are described under Experimental Section. Retention times: **1**, 12.7 min; **2**, 15.1 min; **3**, 9.1 min.

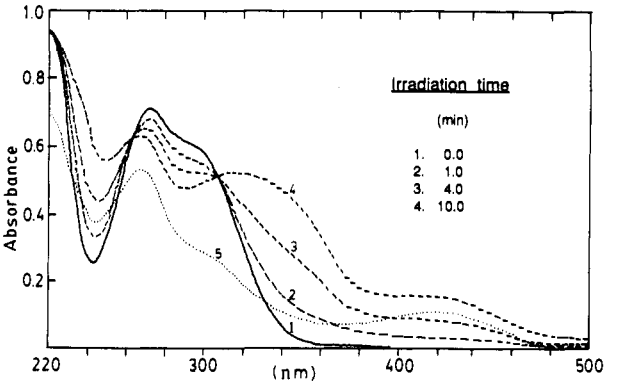


Figure 3. Changes in the absorption spectra of an aqueous solution of **1** (pH 7.5) during irradiation in the absence of oxygen. Spectrum 5 was obtained after filtration of solution 4 through a membrane filter (0.45 μm).

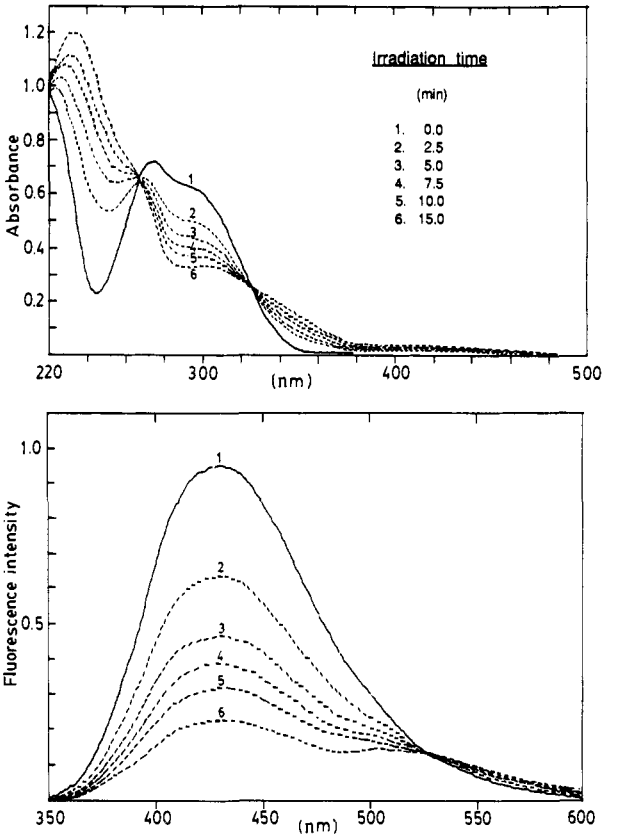


Figure 4. Changes in the absorption and fluorescence spectra of an aqueous solution of **1** (pH 5.8–6.0) during irradiation in the absence of oxygen.

(i) As seen in Figure 1, irradiation of an aqueous solution of **1** at pH $\simeq$7.5, under aerobic conditions, i.e., the conditions previously established as optimal for preparation of luminarosine,[6] results in the gradual disappearance of the absorption band at $\lambda \simeq 300$ nm, characteristic of **1**,[12] with the concomitant formation of a new absorption band in the visible region of the spectrum at $\lambda \simeq 420$ nm, characteristic of luminarosine **2**. Analogously, in the fluorescence spectra, the decrease in emission of **1** at $\lambda \simeq 430$ nm is accompanied by the formation of a new, intense fluorescence band with a maximum at $\lambda = 528$ nm, characteristic of luminarosine.[8] The HPLC analysis of the irradiated solution revealed, besides the unreacted **1** and luminarosine **2**, the presence of a substantial amount of another photoproduct which we assigned tentatively as **3** (cf. Figure 2).

(ii) Irradiation of the same solution of **1** in the absence of oxygen results in a much faster loss of substrate (cf. Figure 5). The irradiated solution became turbid, indicating the formation of a photoproduct, assigned here as **4**, which is water insoluble. This is clearly visible in the absorption spectra, which show the formation of a new band at ca. 330 nm (Figure 3). Filtration of the irradiated solution through a membrane filter (0.45 μm) or its centrifugation removes, completely, this photoproduct, and the absorption spectrum of the filtrate (cf. spectrum 5 in Figure 3) now resembles those shown in Figure 1; i.e., it shows a maximum at ca. 430 nm, characteristic of luminarosine. Changes in the fluorescence spectra (not shown) are qualitatively identical with those shown in Figure 1, further indicating that the formation of luminarosine occurs under these experimental conditions. Both the quantitative fluorescence measurements and HPLC analysis showed that the amount of luminarosine formed (i.e., the chemical yield) was practically the same as for the conditions in (i). Furthermore, HPLC analysis also revealed the presence of the photoproduct **3** in the irradiated solution, but in much smaller amount than in the previous case.

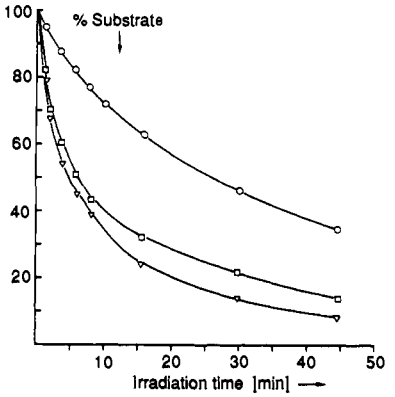
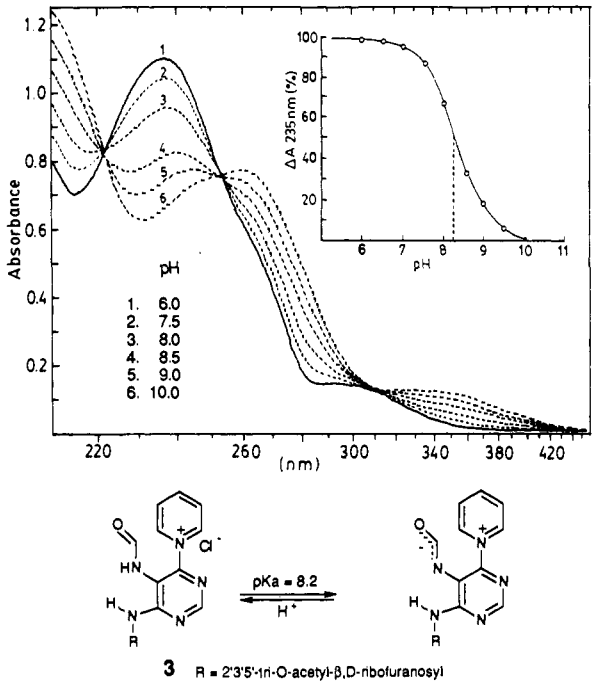


Figure 5. Comparison of the rates of disappearance of **1** under various conditions of irradiation: (○) pH 7.5, aerobic conditions; (□) pH 7.5, anaerobic conditions; (▽) pH 5.8–6.0, anaerobic conditions.



3 R = 2'3'5'-tri-O-acetyl-β,D-ribofuranosyl

Figure 6. pH dependence of the absorption spectra of photoproduct **3** and proposed ground-state prototropic equilibrium. (Inset) Spectrophotometric titration curve.

(iii) Figure 4 shows the changes in absorption and fluorescence spectra of **1** irradiated in an aqueous solution in the absence of $NaHCO_3$ (pH ≃6) and of oxygen. In this case, the disappearance of the absorption band of **1** is accompanied by formation of an intense absorption band in the short-wavelength region at ca. 235 nm, whereas the fluorescence spectra show only a gradual decrease in intensity. Since both the absorption and fluorescence spectra of luminarosine do not change in this pH range,[8] one can conclude that luminarosine is not formed in this case. Indeed, the HPLC analysis revealed that, under these conditions of photolysis, **1** is almost quantitatively converted into the photoproduct **3**. No formation of **4** occurred, and only traces of **2** could be detected in the irradiated solution.

The measured rates of disappearance of substrate during irradiation under conditions described above are compared in Figure 5. The role of oxygen in the photochemical conversion of **1** will be discussed later.

Identification of Photoproduct 3. On the basis of the observation that **3** was the only product formed when **1** was irradiated at pH 6, in the absence of oxygen, a preparative scale irradiation of **1** was performed under these conditions and **3** was isolated as described under Experimental Section and identified as *N*-[5-formamido-6-[(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)amino]pyrimidyn-4-yl]pyridinium chloride (Figure 6). The identification of this photoproduct was done on the basis of ^1H NMR and UV spectra, elemental analysis, and its chemical properties. An examination of the ^1H NMR spectrum (CD_3CN, TMS) showed that both the pyridinium ring and the per-O-acetylated ribosyl moiety remained intact within the structure and that the major change occurred in the imidazole part of the purine ring (see Experimental Section for assignment of the ^1H NMR spectrum). The low-field region ($\delta > 9$) consists of two signals, a doublet at 9.95 ppm (J = 8 Hz) and a singlet at 11.62 ppm. The doublet appeared to be coupled with a signal of ribose 1′-H, which appears in the spectrum at 6.07 ppm as a doublet of doublets. When D_2O was added to the solution, both the singlet and the doublet disappeared and the doublet of doublets at 6.07 ppm collapsed into a doublet (J = 3 Hz), indicating the presence of a C1′–H–NH glycosidic linkage.[6,13] The singlet at 11.62 ppm was assigned to the pyrimidine C5-formamido-NH hydrogen.

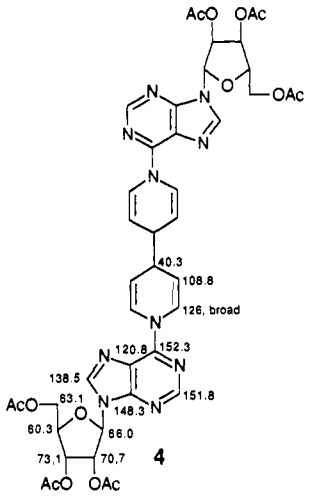


Figure 7. Structure of the photoproduct **4** and graphic assignment of its ^{13}C NMR spectrum.

The proposed structure of **3** is also consistent with the observed pH dependence of its absorption spectra (cf. Figure 6). In the pH range 1–6 the absorption spectrum of **3** shows two overlapping bands, a low-intensity one at ca. 300 nm and an intense one in the short-wavelength region (λ_{max} = 235 nm). Increasing the pH from 6 to 10 results in a gradual decrease in the intensity of the low-wavelength band and a red shift of the two bands. These changes are assigned to the ground-state prototropic equilibrium between the cation and zwitterion, shown in Figure 6, and are similar to that observed previously for other purinylpyridinium salts.[9,12] Thus, the spectrum at pH <6 corresponds to the cationic and that at pH 10 to the zwitterionic form of **3**. The ground-state ionization constant, pK_a = 8.2, is obtained from the spectrophotometric titration curve (cf. Figure 6, inset). It has also been found during this study that **3** undergoes an efficient reversal to the parent pyridinium salt **1** (ca. 70% yield, HPLC) when heated in 85% aqueous acetic acid. In slightly alkaline solution (pH 7.5–8.0) at room temperature a slow transformation of **3** into another compound was observed (HPLC, ca. 40% conversion). The UV absorption spectrum of the solution did not change during this transformation, indicating that structural change(s) occurred beyond the chromophoric part of the molecule. This suggests that **3** undergoes a $\beta \rightleftharpoons \alpha$ anomerization process similar to that previously found in the case of luminarosine[6,8] and encountered in other glyco-[13] and glucosyloamines.[14] Indeed, an analytical scale experiment (HPLC) with a separated fraction of the new product showed that it undergoes reversal to **3** under the same conditions. The anomerization process was further proved by photochemical conversion of a mixture of the two anomers into a mixture of β-

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Table I. ^{1}H NMR Spectral Assignment of the Photoproduct **4**[a]

protons	DMSO		$CDCl_3$	
	345 K	295 K	292 K	220 K
2-H	8.49s	8.58s	8.44s	8.46s
8-H	8.44s	8.45s	8.00s	8.10s
Py-αH	8.18d ($J_{\alpha,\beta}$ = 7 Hz)	8.20b	8.08b	7.90d, 8.56d ($J_{\alpha,\beta}$ = 7 Hz)
Py-βH	5.14d	5.12d	5.12m	5.18m
Py-γH	3.10o	3.13s	3.24s	3.32s
1′-H	6.27d ($J_{1,2}$ = 5 Hz)	6.25d	6.22d	6.28d, 6.30d
2′-H	5.63t ($J_{2,3} = J_{3,4}$ = 6 Hz)	5.61t	5.62t	5.70m
3′-H	6.00t	5.99t	5.84t	5.84m
4′-H, 5′-H_2	4.2–4.45m	4.2–4.45m	4.2–4.5m	4.25–4.7m

[a] Chemical shifts in ppm vs TMS; s, singlet; d, doublet; t, triplet; m, multiplet; b, broad, o, overlap. Py, pyridine protons.

Table II. Distribution of Photoproducts at ca. 90% Conversion of **1** under Various Conditions of Irradiation

exptl conditions	% product		
	2[a]	**3**[a]	**4**[b]
i	34	43	0
ii	36	8	37
iii	>1	86	0

[a] Based on HPLC analysis. [b] Based on spectrophotometric analysis.

and α-luminarosine (vide infra).

No fluorescence or phosphorescence emission could be detected from **3** either at room temperature or in ethanol glass at 77 K.

Identification of Photoproduct 4. To identify photoproduct **4**, which is insoluble in water, a preparative scale irradiation of a deoxygenated aqueous solution of **1** (2×10^{-4} M, pH 7.5) was carried out (see Experimental Section) and the product was isolated by filtration on a membrane filter (37% yield). Its structure was established as the 4,4′-dimeric, reduced pyridine compound (Figure 7) on the basis of ^{1}H NMR spectra at different temperatures in $CDCl_3$ and DMSO-d_6 and ^{13}C NMR spectroscopy; these results were further supported by results of EI and FAB MS spectra and elemental analysis.

The assignments of the ^{1}H NMR spectra are summarized in Table I. According to these data the ribose and the purine rings of the starting pyridinium salt **1** remained intact during the photoreaction; however, an addition reaction occurred at the γ position of the pyridine ring. Characteristic dynamic behavior of the ^{1}H NMR spectra can be observed as the temperature is changed. The α-hydrogens of the pyridine ring give two separate signals at 220 K. At room temperature these signals are broadened by coalescence, while at 345 K they give a sharp, exchange average doublet. This can be explained by restricted rotation around the purine–pyridine C6–N1 single bond at low temperature. Some conformational changes also slow down in the ribose ring at low temperatures, as is indicated by the separation of the 1-H signal into two doublets. The measured coupling constants of the ^{1}H NMR spectra are also in agreement with the 1,4-dihydropyridine structure. The reported $J_{\alpha,\beta}$ coupling constants in similar structures[15] are 8–9 Hz, whereas the $J_{\beta,\gamma}$ and $J_{\alpha,\gamma}$ values are much smaller, 3–4 and 1–2 Hz, respectively. In the spectra reported here, however, the γ-H signal is a broadened singlet and the small coupling constants could not be resolved. The molecular ion and the fragmentation pattern in the EI MS spectrum of the photoproduct are in agreement with the structure of the starting material, indicating that the dimer cleaves easily under these conditions. In a FAB MS spectrum, however, low-intensity signals appear at m/z 914 and 911, corresponding to M + 2 and M − 1, respectively.

Photochemical Pathways of 1. Mechanism of Formation of Luminarosine 2. If one compares the structure of luminarosine **2** with its precursor, i.e., the pyridinium compound **1**, then it is quite reasonable to assume that formation of the former must proceed via several steps. These include opening of the imidazole moiety of the purine ring of **1**, with formation of an intermediate compound of a structure analogous to that of the photoproduct **3**, followed by recyclization of the latter. As shown in Table II, **3** is the only photoproduct formed under all the experimental conditions of photolysis used in this work. The direct involvement of oxygen in the formation of **3** can be excluded since the latter is also formed in its absence. Thus, it can be concluded that in the excited state **1** undergoes a nucleophilic attack by water at the 8-position of the purine moiety followed by opening of the imidazole ring to give **3**. The susceptibility of purine nucleosides containing electron-withdrawing substituents in the 6-position to a chemical, hydroxide-induced ring opening in the imidazole moiety of the purine ring is well-known.[16] In the case of **1** the electron density of the imidazole moiety of the purine ring is decreased strongly upon excitation due to the intramolecular charge-transfer interaction[9,12] between the purine part (donor) and the pyridinium substituent (acceptor) which labilizes the imidazole ring to such an extent that opening occurs under mild basic or acidic conditions. Opening of the imidazole ring previously has been observed to occur in ionizing radiation studies of aqueous solutions of purine nucleosides and nucleotides.[17]

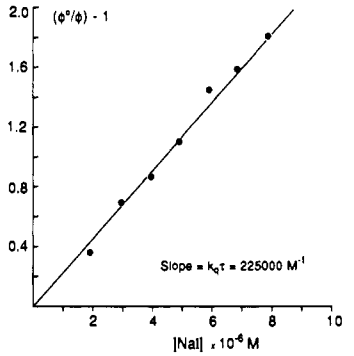

Figure 8. Stern–Volmer plot for quenching of photochemical formation of **4** by NaI.

As shown in Figure 5, the rate of disappearance of substrate decreases strongly in the presence of oxygen. The quantum yield for the loss of **1** increases by a factor of 10 in the absence of oxygen (Φ = 0.0042 and 0.041 under aerobic and anaerobic conditions, respectively), whereas the fluorescence quantum yield does not change upon deoxygenation. This suggests that formation of **3** occurs via the excited triplet state of **1**, and it is consistent with the efficient $T_1 \leftarrow S_1$ intersystem crossing detected previously in this compound by flash photolysis and low-temperature luminescence studies.[12] Transformation of **1** into **3** is also strongly inhibited by addition of small amounts of sodium iodide ($<10^{-5}$ M). The relevant Stern–Volmer plot for quenching the formation of **3** by NaI is shown in Figure 8. It should be noted that, at the concentrations of NaI used in this experiment, it had no effect on the fluorescence of **1**.[12] Assuming $k_q \simeq k_d \simeq 10^{10}$ M^{-1} s^{-1}, which is the upper limit for a diffusion-controlled bimolecular

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quenching process,[18] an approximate value of 2.2×10^{-5} s for the lifetime of the reactive species is obtained from the slope of the plot in Figure 8. This result further supports the involvement of a triplet state in this reaction. As is the case in its fluorescence quenching by NaI,[12] a mechanism involving electron transfer from I^- rather than a heavy atom effect might be expected for the observed excited triplet state quenching of **1**.

Since **3** was an expected intermediate in the photochemical transformation of **1** into **2**, its photochemical reactivity was tested under conditions (i) and (ii), i.e., the conditions at which formation of **2** occurs. However, no transformation of **3** into **2** could be achieved as **3** appeared to be stable under both conditions. The problem was solved by considering the mechanism of formation of the dimer **4**.

Pyridinium salts and related charged N-heteroaromatic compounds, being powerful electron acceptors,[19] participate in a variety of excited-state processes including reductive dimerization.[20] This process requires an electron transfer to the N-heteroaromatic salt and subsequent coupling of the intermediate radicals. In the case of pyridinium salts it can be stimulated by irradiation of the salts in the presence of good electron donors, such as diethylamine.[21] We have irradiated deoxygenated, aqueous solutions of **1** in the presence of triethylamine (pK_a = 11.01)[22] (0.01 M) buffered (CH_3COOH) to pH 9 (above pH 9.5 **1** undergoes a pyridinium ring opening reaction,[10] characteristic for pyridinium salts with unsubstituted α-hydrogens[23]). Under these conditions a quantitative transformation of **1** into a reduced dimeric compound identical with **4** occurred. An identical transformation also occurred when the same solution of **1** containing triethylamine was neutralized to pH 7 with acetic acid, indicating that both triethylamine and acetate ion may serve as electron donors in this reaction. It has been verified from lifetime[12] and steady-state measurements (Stern–Volmer plots not shown) that the latter quench the fluorescence of **1** with a quenching constant $k_q = 3.2 \times 10^9$ M^{-1} s^{-1}. As in the case of other carboxylic anions and other pyridinium compounds,[24] quenching via an electron-transfer mechanism can be anticipated.

Thus, assuming that an analogous, single electron-transfer mechanism occurs for formation of **4** under experimental conditions (ii), the electron donor(s) must be present in the solution. The most likely candidates are the photoproducts **2** or **3**. Note that **4** is formed at pH >7.0, i.e., the pH at which the zwitterionic forms of **2** and **3** exist in solution [pK_a (S_0) of **2** = 3.1].[8] It can be further assumed that electron transfer from **3** to **1** also induces ring closure in the former to form luminarosine.

To test these assumptions, *N*-(9-methyl-purin-6-yl)pyridinium chloride[7] (**5**) was used as an electron acceptor instead of **1**. Contrary to **1**, this compound was found to be practically unreactive, photochemically,[11,12] and no transformation to the appropriate 9-methyl analogues of the photoproducts **2**–**4** could be observed under similar conditions (i, ii, and iii) of irradiation. It should be pointed out also that, similar to **1**, **5** undergoes intersystem crossing to the excited triplet state, however, with much lower efficiency.[12] When a deoxygenated, aqueous solution of **5** (3×10^{-4} M, pH 7.5 maintained with $NaHCO_3$) was subjected to irradiation in the presence of **3** (10^{-4} M), a quantitative transformation of the latter into **2** occurred as revealed by

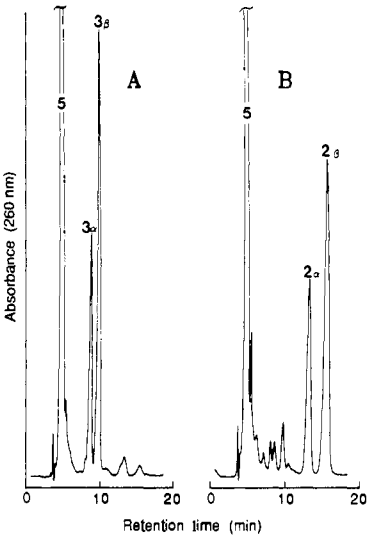
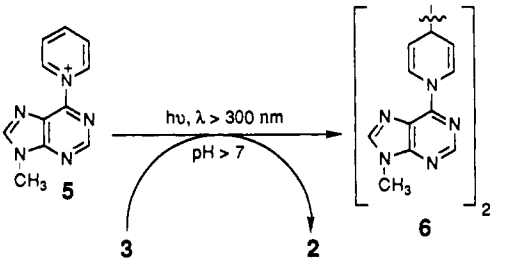


Figure 9. HPLC analysis of an aqueous solution of **3** (10^{-4} M, pH 7.5), in which an anomeric equilibrium $\alpha \rightleftharpoons \beta$ was established, and **5** (3×10^{-4} M). (A) Before irradiation and (B) after irradiation in the absence of oxygen. Retention times: **5**, 4.1 min; **3α**, 7.9 min; **3β**, 9.1 min; **2α**, 12.6 min; **2β**, 15.1 min.

Scheme II



spectrophotometric and HPLC analyses (cf. Figure 9). As well, there was concomitant formation of a water-insoluble photoproduct, which was indicated by a characteristic turbidity of the irradiated solution. The identical, water-insoluble photoproduct was formed quantitatively when **5** was irradiated in deoxygenated aqueous solution in the presence of triethylamine (pH 9). The photoproduct was isolated as in the case of **4** (vide infra) and identified as a reduced pyridine, dimeric compound, **6**, having a structure similar to that of **4** (cf. Scheme II). Its UV absorption spectra in $CHCl_3$ and CH_3CN are almost identical with those of **4**. In the ^1H NMR spectrum ($CHCl_3$) the signal of the two methyl groups appears as a singlet at 3.84 ppm, while the chemical shifts of both the purine and pyridine protons (data shown under Experimental Section) are in excellent agreement with the assignments made previously for **4** (cf. Table I). The molecular structure of **6** is further supported by the FAB MS spectrum, which shows signals at m/z 425, 424, and 423, corresponding to ($M^+ + 1$), (M^+), and ($M^+ - 1$), respectively.

Considering the fact that in the photochemical experiment with **5** and **3** (Scheme II) the exciting light ($\lambda > 300$ nm) was almost completely absorbed by **5** and that **3** had no effect on the fluorescence of **5** (τ_f = 5 ns),[12] one can conclude that the formation of **2** and **6** is associated with an electron-transfer process between the excited triplet state of **5** (acceptor) and a ground state of **3** (donor). Furthermore, this experiment also proved the earlier assumption that **3** undergoes an anomerization process in basic, aqueous solution (vide supra). As shown in Figure 9, irradiation of a mixture of the two interconvertible, isomeric forms of **3** resulted in formation of both α and β anomers of tri-*O*-acetylluminarosine (**2**) as checked by comparison (HPLC, UV, and fluorescence spectra) with authentic samples of these two compounds obtained in previous work.[6,8]

In an analogous experiment with **5** in which **2** was used as an electron donor, **5** appeared to be moderately stable and only small

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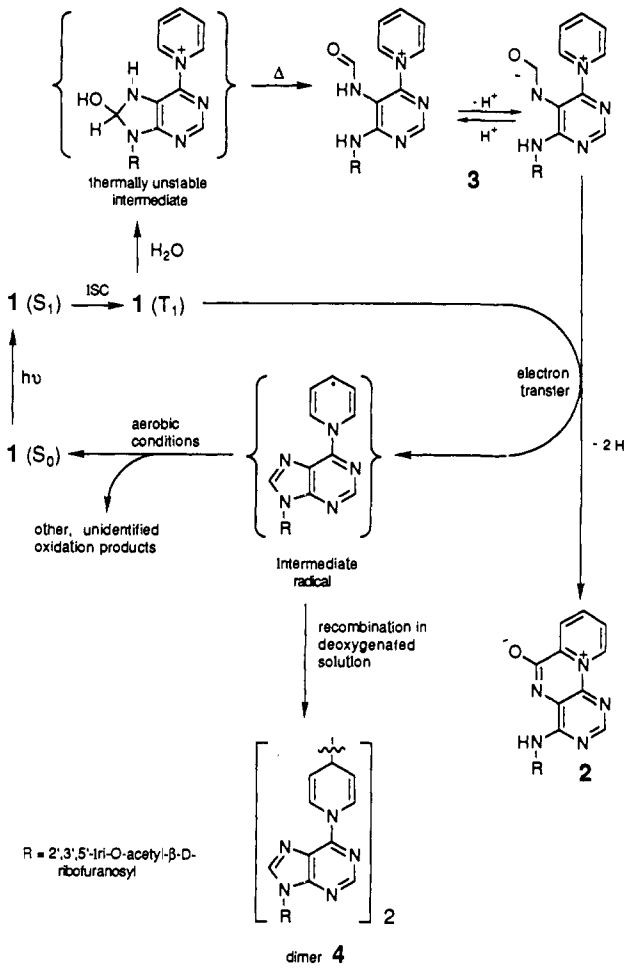
Scheme III



amounts of **6** could be detected after prolonged irradiation.

On the basis of the above observations, the following mechanism (Scheme III) for photochemical transformation of **1** under experimental conditions (i–iii) is proposed. In the first step a partial conversion of **1** into **3** occurs as a result of photochemically induced, hydrolytic ring opening in the imidazole portion of the purine ring of **1**. This transformation involves an excited triplet state of **1** and is common for all three conditions. At pH 6, in the absence of oxygen, this process is continued until there is complete loss of substrate. At pH >7, **1** may interact, in the excited triplet state, with **3**, in the ground state, which at this pH exists partly in the electrically neutral, zwitterionic form. During this interaction an electron is transferred from **3** to **1**, resulting in reduction of the latter to form a pyridinyl radical and concomitant ring closure in the former to give luminarosine **2**. Pyridinyl radicals formed in this way may participate in a number of chemical transformations. In the absence of oxygen, they can undergo a coupling reaction with formation of the dimeric compound **4**. In the presence of oxygen, they can undergo various oxidation processes observed previously in other pyridinyl radicals,[25] including partial reversal to form the original pyridinium salt **1**. When **4** was resuspended in water and irradiated in the presence of oxygen, the pyridinium salt **1** was formed as one of the major photodecomposition products, further supporting the validity of the above assumption.

Conclusion

We have shown that the primary photochemical reaction of *N*-[9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium chloride (**1**) in aqueous solution, at pH 6–8 in the absence of electron donors, involves an excited triplet state mediated, hydrolytic opening of the imidazole moiety of the purine ring which results in the formation of *N*-[5-formamido-6-[(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)amino]pyrimidyn-4-yl]pyridinium chloride (**3**), a key intermediate in the transformation of **1** into tri-*O*-acetylluminarosine (**2**). The ring-opening process must be facilitated by the inductive, electron-withdrawing effect of the per-O-acetylated ribose substituent since no ring opening occurs under analogous conditions of photolysis in the case of the 9-methyl analogue of **1**, i.e., the pyridinium salt **5**. Note that, like **1**, **5** also undergoes intersystem crossing to the excited triplet state. This assumption is also consistent with the high photochemical stability of other purinylpyridinium salts containing electron-donating substituents observed previously.[11,12]

In the presence of suitable electron donors such as triethylamine or acetate ion under anaerobic conditions, **1** undergoes photoreduction via a single electron-transfer process followed by dimerization of the resulting pyridinyl radicals.

The results of this work show that purinylpyridinium salts, which have already proven valuable intermediates in purine nucleoside chemistry,[27] may also find application in the photochemical synthesis of modified purine nucleosides. We anticipate that the reactions illustrated in Scheme III should provide a general approach to the synthesis of various analogues of luminosarine which might find an application as fluorescent probes in biological systems.

Experimental Section

Methods. ^1H and ^{13}C NMR spectra were recorded on Bruker AM 300 and JEOL 90 FX spectrometers. All chemical shifts (δ) are reported in parts per million. Mass spectra were obtained by fast atom bombardment technique with a VG 7070 HE mass spectrometer. UV absorption spectra were recorded in 0.5- or 1-cm quartz cuvettes, using Zeiss M-40 and Perkin-Elmer Lambda 17 spectrophotometers. Microanalyses were performed on a Perkin-Elmer 2400 analyzer. Fluorescence spectra were obtained on Spex Fluorolog 222 or Perkin-Elmer MPF 66 spectrofluorometers in a 1-cm cell. The concentrations of the solutions were adjusted so that the absorption at the excitation wavelength did not exceed 0.05.

Thin-layer chromatography (TLC) analyses were made on precoated silica gel plates (Merck) using a mixture of 95% ethanol and 1 M aqueous ammonium acetate (7/3 v/v, system A). High-performance liquid chromatography (HPLC) was performed with a Waters 600 E programmer and multisolvent delivery system and a 481 variable-wavelength UV detector using a reversed-phase column, Delta Pak C-4, 100 Å (Waters-Millipore). The column was eluted isocratically with an acetonitrile–water mixture (23/77 v/v) containing 0.25 M ammonium acetate (flow rate 0.8 mL/min).

Analytical scale irradiations were carried out either in a quartz cuvette (1 cm path length) on an optical bench or in cylindrical, Pyrex tubes (0.5 cm internal diameter) placed in a "merry-go-round" system. Samples were irradiated with a high-pressure mercury lamp (HBO 200, Narva) through a Pyrex cutoff filter (λ > 300 nm). For preparative scale irradiations an immersion apparatus (450 mL) equipped with an Original Hanau TQ-150 high-pressure mercury lamp and a cylindrical, Pyrex filter was used. Deoxygenation was carried out by passing purified nitrogen through the solutions for 30 min prior to irradiation. In the case of preparative scale experiments the nitrogen purge was continued throughout the irradiation. The photochemical reaction was monitored by means of UV–visible absorption and fluorescence emission spectroscopy as well as by HPLC analyses of irradiated solutions. The quantum yields of photochemical reactions were measured at λ_{313} excitation (313-nm interference filter, Zeiss) by using an uranyl oxalate actinometer.[26]

Materials. The pyridinium salts, *N*-[9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium chloride (**1**) and *N*-(9-methylpurin-6-yl)pyridinium chloride (**5**), were synthesized and purified according to procedures described previously.[5] HPLC grade solvents (Aldrich) and all the other chemicals (Merck) were used as received. Water used in the preparation of aqueous solutions for photochemical experiments was either triply distilled or purified by using a Millipore Super-Q system.

Preparation of 3. **1** (0.15 g, 0.3 mmol) was dissolved in water (1.5 L) and the pH of the solution adjusted to ca. 5.8–6.0 with dilute aqueous

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$HClO_4$. The solution was placed in a photochemical reactor (ca. 420-mL aliquots), purged with nitrogen for 30 min, and then irradiated under a continuous flow of nitrogen until HPLC analysis revealed the almost complete disappearance of **1**. The irradiated solution was concentrated under vacuum (at 30 °C) to a volume of ca. 5 mL and chromatographed on a reversed-phase silica gel column (Merck). The column was eluted initially with water and then with a mixture of water-acetonitrile (95/5 v/v). The fractions containing pure photoproduct (TLC, R_f = 0.5 in system A) were combined, concentrated under vacuum to a small volume, and passed through a Dowex (Cl^-) column to give, after lyophilization, 0.13 g of **3**, 85% yield: ^{1}H NMR (CD_3CN, TMS) δ 11.62 (s, 1, C5-NH), 9.95 (d, 2, C6-NH), 9.06 (m, 2, Py^+-αH), 8.67 (m, 1, Py^+-γH), 8.53 (s, 1, C2-H), 8.18 (m, 2, Py^+-βH), 7.98 (m, 1, CHO), 6.07 (dd, 1, C1′-H), 5.56 (m, 2, 2′-H and 3′-H), 4.28 (m, 3, 4′-H and 5′-H_2), 2.05 (s, 9, $3CH_3$); UV (H_2O, pH 6.0) nm (ϵ) 300 (sh, 2200), 235 (max, 15300), 215 (min, 9960); UV (H_2O, pH 10.0) 260 (max, 10700), 232 (min, 8600). Anal. Calcd for $C_{21}H_{24}N_5O_8Cl$: C, 49.4; H, 4.7; N, 13.7. Found: C, 48.9; H, 4.6; N, 13.4.

Preparation of 4. **1** (0.3 g, 0.61 mmol) was dissolved in a freshly prepared 1 mM aqueous solution of $NaHCO_3$ (3 L, pH $\simeq$7.8). The solution was deoxygenated and irradiated under conditions similar to those described for preparation of **3**. Irradiation was continued until 90% of **1** was reacted as revealed by HPLC analysis. A resulting suspension of water-insoluble photoproduct was filtered through a membrane filter (0.45 μm). The solid material was washed well with water and dried under vacuum over P_2O_5 to give **3**, 0.102 g, as a red powder (37% yield): UV (CH_3CN) nm (ϵ) 327 (max, 38400), 258 (min, 7000); UV ($CHCl_3$) 334 (max, 40600); FAB MS, m/z (rel intensity) 914 (0.3, M^+ + 2), 911 (0.3, M^+ - 1), 457 (13), 456 (45), 198 (100); EI MS (70 eV), m/z (rel intensity) 457 (10), 456 (8), 414 (4), 396 (3), 354 (1), 336 (1), 259 (5), 198 (82). Anal. Calcd for $C_{42}H_{44}N_{10}O_{14}$: C, 55.2; H, 4.8; N, 15.3. Found: C, 54.8; H, 4.8; N, 15.1.

Preparation of 6. **5** (0.1 g, 0.4 mmol) was dissolved in water (2 L) containing triethylamine (0.01 M) buffered to pH 9.0 with CH_3COOH. The solution was deoxygenated and irradiated as in the case of **3** to ca. 90% conversion of substrate. Centrifugation of the resulting suspension gave a red solid material, which was washed with water and dried under vacuum to give **6**, 0.054 g (64% yield) as a red powder: ^{1}H NMR ($CHCl_3$, TMS) δ 8.48 (s, 2, C2-H), 8.22 (b s, 4, Py-αH), 7.82 (s, 2, C8-H), 5.14 (m, 4, Py-βH), 3.84 (s, 6, N9-CH_3), 3.26 (s, 2, Py-γH); UV (CH_3CN) nm (ϵ) 327 (max, 37200), 257 (min, 4300); UV ($CHCl_3$) 336 (max, 40100), 264 (min, 6500); FAB MS, m/z (rel intensity) 425 (0.8, M^+ + 1), 424 (0.8, M^+), 423 (2.30 (M^+ - 1), 212 (100). Anal. Calcd for $C_{22}H_{20}N_{10}$: C, 62.2; H, 4.7; N, 33.0. Found: C, 61.7; H, 4.6; N, 32.7.

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Enthalpies of Solvation of Ions. Aliphatic Carboxylic Acids: Steric Hindrance to Solvation?

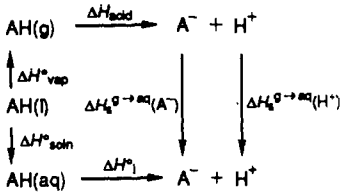
Burton Wilson,[†] Rosina Georgiadis,[‡] and John E. Bartmess*[,§]

Contribution from the Department of Chemistry, Cumberland College, Williamsburg, Kentucky 40769, Department of Chemistry, University of Oregon, Eugene, Oregon 97403, and Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996-1600. *Received February 5, 1990. Revised Manuscript Received November 5, 1990*

Abstract: By use of solution calorimetry, plus literature data such as enthalpies of vaporization and gas-phase acidities, a thermochemical cycle is used to evaluate the relative enthalpies of solvation of carboxylate anions from the gas phase into aqueous solution. It is found that the weaker solution-phase acidity of the larger carboxylic acids arises from a complex mixture of entropic and enthalpic effects on the solvation of the neutral acids and the anions. An increase in steric bulk results in an *increase* in the enthalpy of solvation of both the acids and anions, but the neutral acid is more sensitive to the steric effect than the anion is. Solvation enthalpy thus is the opposite predicted by the usual concept of "steric hindrance to solvation"; it is the entropy of solvation that makes the larger acids more weakly acidic in terms of free energy in aqueous solution.

The relationships that chemists have perceived between structure and reactivity were altered in the late 1960s with the advent of modern gas-phase ion/molecule chemistry. Many "well-known" structural trends, such as the nonmonotonic change in the basicities of the multiply methylated amines[1] and the decrease in acidity of the aliphatic alcohols with increasing alkyl group size,[2] were shown to be due in large part to the solvation of the species involved. In the gas phase, where only the intrinsic structure of the molecule controls the reactivity, different trends were found. Notably, in the work of Brauman and Blair,[2] the importance of polarizability as a controlling effect in alcohol acidities was shown. It was also postulated[2] that the reversal of acidities for the alcohols on going to the condensed phase was due to "steric hindrance to solvation" of the alkoxides. This concept is one widely used in organic chemistry[3] to explain how a change in alkyl group structure affects reactivity trends, generally by increasing the energy of an ionic species in solution more than of some neutral species in equilibrium with it.

Scheme I



[†] Cumberland College.
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[§] University of Tennessee.

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