Conversion of Formycin into the Fluorescent Isoguanosine Analogue 7-Amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one

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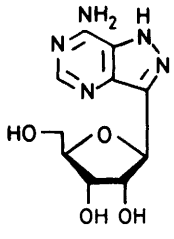
On u.v. irradiation, in aqueous solution, formycin *N*(6)-oxide (**3**) undergoes photorearrangement to give 5-cyano-3-(β-D-ribofuranosyl)-4-ureido-1*H*-pyrazole (**5**) as the major product; small amounts of 7-amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (**4**) and formycin (**1**) are also formed. When treated with aqueous ammonia, compound (**5**) cyclizes to the isoguanosine analogue (**4**). The latter compound is strongly fluorescent and its c.d. spectrum resembles that of formycin. Its u.v. absorption and fluorescence characteristics are compared with those of the corresponding guanosine analogue 5-amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**2**).

The pyrazolopyrimidine nucleoside formycin (**1**) is a *C*-nucleoside analogue of adenosine which exhibits wide ranging biological activity[1,2] and possesses distinctive conformational[3,4] and spectroscopic properties.[5] These features have led to a sustained interest in the chemistry of formycin, its naturally occurring derivatives formycin B and oxoformycin,[1] and other synthetic pyrazolopyrimidine nucleosides. Recently, Lewis and Townsend[6] described the synthesis of the guanosine analogue 5-amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**2**) from formycin by a route involving acid-catalysed ring opening of formycin *N*(6)-oxide (**3**) as a key step. Here, we report that ultraviolet photolysis of (**3**) affords a straightforward and efficient means for preparing the corresponding isoguanosine analogue 7-amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-5(4*H*)-one (**4**).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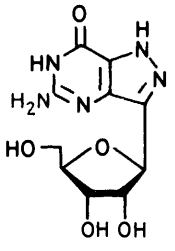
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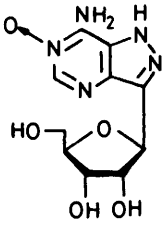
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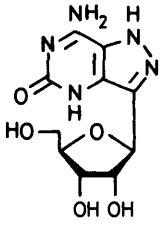
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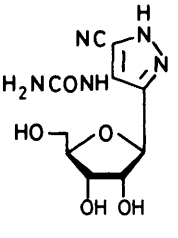
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Results and Discussion

U.v. irradiation of (**3**), in aqueous solution, caused a marked reduction in its intense absorption at 231 nm characteristic of the *N*-oxide chromophore. For preparative purposes, irradiation was carried out at wavelengths >290 nm, by using a medium-pressure mercury lamp with a Pyrex filter, though the same photoreaction occurs on irradiation at 254 nm with a low-pressure mercury lamp. Analysis by high-voltage paper electrophoresis revealed the formation of one major and two minor photoproducts. The major photoproduct was isolated, in 50% yield, by silica gel chromatography and identified as 5-cyano-3-(β-D-ribofuranosyl)-4-ureido-1*H*-pyrazole (**5**). The minor photoproducts were separated on an analytical scale by high-voltage paper electrophoresis and identified as formycin (**1**), recovered in *ca.* 5% yield, and the isoguanosine analogue (**4**), recovered in *ca.* 1% yield. The formation of these products from (**3**) parallels the behaviour of adenosine *N*(1)-oxide when it is irradiated under similar conditions.[7–9] Thus, adenosine *N*(1)-oxide undergoes photorearrangement to give both 5-cyano-3-(β-D-ribofuranosyl)-4-ureido-imidazole and -isoguanosine as well as photoreduction to adenosine.[8] Possible mechanisms for these processes have been discussed[9] and the same considerations should apply to formycin *N*(6)-oxide.

The pyrazole *C*-nucleoside (**5**), isolated by chromatography on silica gel, was homogeneous on t.l.c. and no signals attributable to contaminants were evident in its ^{1}H n.m.r. spectrum. Attempts to purify the material further by recrystallization from non-aqueous solvents were unsuccessful. Aqueous solvents are not suitable for crystallizing (**5**) because of its tendency to cyclize to (**4**) when heated in water. In keeping with its ring-opened structure, (**5**) exhibits only weak u.v. absorption, with steadily decreasing intensity and no defined peak, in the range 225—300 nm. The presence of a nitrile function is indicated by an i.r. absorption peak at 2 254 cm^{-1}.

On being heated in water, (**5**) cyclizes to give the isoguanosine analogue (**4**), the reaction being complete in 4 h at 100 °C. The cyclization reaction is base-catalysed and the conversion of (**5**) into (**4**) was most conveniently achieved by treating (**5**) with conc. aqueous ammonia at room temperature. This transformation allows the isoguanosine analogue (**4**) to be prepared from formycin *N*(6)-oxide (**3**) in an overall yield of *ca.* 40%.

Compounds (**4**) and (**5**) were characterized by high-resolution mass spectrometry of their respective pentakis- and hexakis-trimethylsilyl derivatives.[10] Besides confirming their expected elemental compositions, the mass spectra of both compounds showed fragmentation patterns characteristic of *C*-nucleosides, in which ions resulting from glycosidic bond cleavage (base + H; *m/z* 259) are greatly reduced in abundance,[10–12] and base + 30 and base + 102 ions[10] are relatively prominent. A major fragment ion at *m/z* 189 in the spectrum of hexakistrimethylsilylated (**5**) is not common to nucleoside mass spectra, and appears to be diagnostic of the ureido side-chain. Its measured molecular mass of 189.0881 is exactly that calculated for $C_6H_{17}N_2OSi_2$ and is consistent with the structure $Me_2Si^+NHC(NH)OSiMe_3$.

In common with formycin[5] and oxoformycin,[13] the isoguanosine analogue (**4**) is strongly fluorescent under physiological conditions. At pH 7, the corrected excitation and emission maxima (Table) are at 300 and 380 nm respectively, and its fluorimetric estimation is feasible at nanomolar concentrations in solution. Measurements on the guanosine analogue (**2**) (Table) showed that, at pH 7, it has an almost identical fluorescence spectrum with that of (**4**) but the intensity of emission is approximately halved. The u.v. absorption spectra of (**2**) and (**4**) at pH 7 are also very similar but they differ markedly at pH 1 (see Table).

The c.d. spectrum of (**4**) in aqueous solution at pH 7 closely resembles that of formycin[14] in water. A negative band at 292 nm ($\Delta\varepsilon$ −1.13) and cross-over at 264 nm is followed by a positive band at 239 nm ($\Delta\varepsilon$ + 1.69) and a further cross-over to negative dichroism at 223 nm. The same alternating pattern of dichroic absorption bands is observed with formycin B and has been correlated[14] with an *anti* orientation about the glycosidic bond in these compounds. The conformational preference of the ribofuranose ring of (**4**) can be assessed from the magnitude of the coupling constant $J_{1'2'}$ derived from its ^1H n.m.r. spectrum. According to the analysis of Altona and Sundaralingam,[15] the percentage of *S*-type [C(2′)-*endo* puckering domain] conformers at equilibrium should be given approximately by $10 \times J_{1'2'}$. For compound (**4**) in perdeuteriodimethyl sulphoxide solution the observed value of $J_{1'2'}$ was 7.0 Hz, which corresponds to a 7:3 equilibrium mixture of *S*-type and *N*-type [C(3′)-*endo* puckering domain] conformers. Similar conformer ratios have been reported[4] for formycin in D_2O and ND_3.

The reaction sequence described here should also be applicable to formycin nucleotides and to formycin derivatives with modified sugar residues. The isoguanosine analogues prepared in this way may have interesting biochemical properties in view of the diverse biological activities of isoguanosine (crotonoside) and the pyrazolopyrimidine *C*-nucleosides.[1]

Table. U.v. absorption and fluorescence characteristics of the pyrazolopyrimidine *C*-nucleosides (**2**) and (**4**)

		U.v.		Fluorescence	
Compound	pH	$\lambda_{max.}$/nm	ε/ dm^3 mol^{-1} cm^{-1}	Excitation $\lambda_{max.}$/nm	Emission $\lambda_{max.}$/nm
(**2**)	1[a]	282	7 020		
	7	242sh	8 960	300	377
		299	6 680		
(**4**)	1	253	5 270		
		310	5 170		
	7	250	9 590	300	380
		299	6 620		
	13	266	9 090		
		304	5 050		

[a] Data taken from ref. 6.

Experimental

U.v. spectra were recorded on a Cary 118 spectrophotometer, i.r. spectra on a Perkin-Elmer Model 257 grating infrared spectrophotometer, fluorescence spectra on a Perkin-Elmer MPF-44B spectrofluorimeter with a DCSU-2 differential corrected spectra attachment, and c.d. spectra on a Cary 61 instrument. ^1H N.m.r. spectra were obtained for solutions in [$^2H_6$]dimethyl sulphoxide with a Bruker WM 250 Fourier transform spectrometer; Me_4Si was used as internal standard.

Trimethylsilyl (TMS) derivatives for mass spectrometry were prepared by heating *ca.* 10 μg of vacuum-dried material with *N,O*-bis(trimethylsilyl)acetamide, trimethylchlorosilane, and dimethylformamide (100:1:10) in a total volume of 10 μl, in a sealed m.p. capillary tube for 1 h at 100 °C.

Electron-impact mass spectra were recorded using a Varian MAT 731 mass spectrometer under the following conditions: ionizing energy 70 eV, ion source temperature 250 °C. Samples were introduced by direct probe after removal of reagent in the probe vacuum lock. Exact mass values were determined by peak matching at resolution 12 000, using perfluoroalkanes as internal standard.

High-voltage electrophoresis was performed at pH 2.5 on Whatman 3 MM paper strips using a Shandon apparatus (Model L24). The buffer was prepared by adjusting an aqueous solution of 15mM ammonium formate to pH 2.5 with formic acid. T.l.c. was carried out on precoated (0.2 mm) silica gel 60 F_{254} plastic sheets (E. Merck, Darmstadt, Germany). The following solvent systems were used: A, butan-1-ol–water–acetic acid (86:30:12 v/v); B, ethyl acetate–water–propan-1-ol (4:2:1 v/v; upper phase). Solutions were concentrated under reduced pressure, at 40 °C, on a rotary evaporator.

Formycin (formycin A) was purchased from Sigma Chemical Co. The guanosine analogue, 5-amino-3-(β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**2**) was kindly supplied by Professor L. B. Townsend.[6]

Formycin N(6)-*Oxide* (**3**).—This was prepared from formycin (**1**) using the procedure described by Lewis and Townsend.[6] The product was homogeneous on t.l.c. [R_F(A) 0.23, R_F(B) 0.04]; $\lambda_{max.}$(water) 231 (ε 23 400 dm^3 mol^{-1} cm^{-1}), 244sh (14 000), and 298 nm (6 000).

U.V. Irradiation of Formycin N(6)-*Oxide and Analysis of Photoproducts*.—A solution of formycin *N*(6)-oxide (**3**) (300 mg, 1.06 mmol) in water (1 l) was irradiated under nitrogen in a Hanovia 1-l photochemical reactor with a 100-W medium-pressure mercury lamp through a Pyrex glass filter. The progress of the photoreaction was monitored by the decrease in absorbance of the solution at 231 nm and irradiation was continued until no further change was observed (6 h, *ca.* 75% decrease in original intensity).

After concentration of the irradiated solution, analysis of a small sample by high-voltage paper electrophoresis showed the presence of three discrete photoproducts with apparent electrophoretic mobilities of 1.1, 5.9, and 6.5 cm h^{-1} kV^{-1}. The individual photoproducts were extracted from the electrophoretogram with water and identified as compounds (**5**), (**4**), and (**1**) respectively from their spectroscopic properties and their mobilities on t.l.c. The yield of each of the products from the photoreaction was estimated from u.v. absorbance measurements on the aqueous extracts. The pyrazole nucleoside (**5**) was the major photoproduct formed in 62% yield. Formycin (**1**) and the isoguanosine analogue (**4**) were formed in much smaller amounts with yields of 4.7 and 1.2% respectively.

5-Cyano-3-(β-D-ribofuranosyl)-4-ureido-1H-pyrazole (**5**).—The irradiated solution of formycin *N*(6)-oxide (**3**) (300 mg, 1.06 mmol) was concentrated to *ca.* 1 ml and mixed with silica gel (5 g; 60—120 mesh from BDH) in a round-bottomed flask on a rotary evaporator. Excess of water was removed under reduced pressure and the silica gel was then introduced onto the top of a column (30 × 1.5 cm) of silica gel in chloroform–methanol (4:1). This solvent was used to elute selectively the major photoproduct. Evaporation of the chloroform–methanol (4:1) eluate gave the *pyrazole nucleoside* (**5**) as an amorphous white solid (150 mg, 50%) which was homogeneous on high-voltage paper electrophoresis and t.l.c. [R_F(A) 0.50, R_F(B) 0.62]. On being heated, it charred above 140 °C; $\lambda_{max.}$ (pH 7) 240infl nm (ε 3 100 dm^3 mol^{-1} cm^{-1}); $\nu_{max.}$(KBr) 2 254m (CN), 1 667s, and 1 596s cm^{-1}; δ 13.62 [1 H, br s, N(1)H, D_2O-exchangeable], 7.94 (1 H, br s, N*H*CONH_2, D_2O-exchangeable), 6.00 (2 H, s, NHCON*H*$_2$, D_2O-exchangeable), 5.2—4.9 (3 H, m, ribose OH groups, D_2O-exchangeable), 4.70 [1 H, d, *J* 6.5 Hz, C(1′)H], and 4.0—3.5 (multiplets due to remaining ribose protons); mass spectrum of TMS_6 derivative: *m/z* 715.3256 (M^+), $C_{28}H_{61}N_5O_5Si_6$ requires *M*, 715.3287; *m/z* 700.3016 ($M^+ - CH_3$), $C_{27}H_{58}N_5O_5Si_6$ requires *m/z*, 700.3053; intensities relative to B + 30 (100%): *M* (60), *M* − 15 (18), B + 102 (7.8), B + 1 (7.8); *m/z* 259 (4.3) and 189 (105).

7-Amino-3-(β-D-ribofuranosyl)-1H-pyrazolo[4,3-d]*pyrimidin-5(4H)-one* (**4**).—The pyrazole nucleoside (**5**) (20 mg, 0.07 mmol) was stirred in conc. (0.88 g cm^{-3}) ammonia solution (10 ml) at room temperature for 10 h. Evaporation of the reaction mixture to dryness gave the *isoguanosine analogue* (**4**) (16 mg, 80%). The same yield of (**4**) was obtained when (**5**) (20 mg, 0.07 mmol) was dissolved in water (2 ml) and the solution then heated at 100 °C for 4 h in a sealed tube. The crude product was recrystallized from aqueous acetonitrile to give white microcrystals which were dried at 110 °C *in vacuo* prior to analysis (Found: C, 42.3; H, 4.65; N, 24.45. $C_{10}H_{13}N_5O_5$ requires C, 42.4; H, 4.65; N, 24.75%). On being heated, compound (**4**) darkened and decomposed without melting above 270 °C; t.l.c. mobility: R_F(A) 0.27, R_F(B) 0.10; $\nu_{max.}$(KBr) 1 691m and 1 620s cm^{-1}; δ 10.09 [1 H, br s, N(4)H, D_2O-exchangeable], 7.37 [2 H, br s, C(7)NH_2, D_2O-exchangeable], 5.7—5.0 (3 H, 3 br peaks, ribose OH groups, D_2O-exchangeable), 4.83 [1 H, d, *J* 7.0 Hz, C(1′)H], and 4.1—3.5 (5 H, m due to remaining ribose protons); mass spectrum of TMS_5 derivative: *m/z* 643.2879 (M^+), $C_{25}H_{53}N_5O_5Si_5$ requires *M*, 643.2892; *m/z* 628.2655 ($M^+ - CH_3$), $C_{24}H_{50}N_5O_5Si_5$ requires *m/z*, 628.2658; intensities relative to B + 30 (100%): *M* (13), *M* − 15 (11), B + 102 (26), B + 1 (2.5); *m/z* 259 (3.2). U.v., corrected fluorescence, and c.d. data for (**4**) are given in the main text.

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