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FLUORESCENT NUCLEOSIDE DERIVATIVES OF IMIDAZO[1,2-C]PYRROLO[2,3-d]PYRIMIDINE

A NEW AND NOVEL HETEROCYCLIC RING SYSTEM (1).

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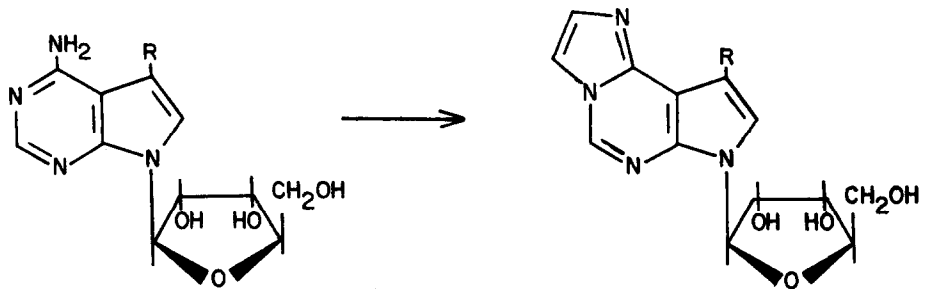
The synthesis of certain tricyclic nucleosides derived from the pyrrolo[2,3-d]pyrimidine ring system has been previously reported (2,3) from this laboratory. The significant antitumor activity (4) shown by these tricyclic compounds has stimulated the synthesis of new tricyclic ring systems from the nucleoside antibiotics tubercidin (1), toyocamycin (2) and sangivamycin (3). We now wish to report on the first synthesis of imidazo[1,2-c]pyrrolo[2,3-d]pyrimidine derivatives.

Reaction of 1 (1.0 g) with 15 ml of chloroacetaldehyde (6) at 30° for 18 hr afforded a 93% yield of a compound which was assigned the structure 7-(β-D-ribofuranosyl)imidazo[1,2-c]pyrrolo-[2,3-d]pyrimidine (2) (7), mp 202-204° (dec.) on the basis of the following data. That an initial alkylation and subsequent ring annulation had occurred was confirmed by pmr spectroscopy



1, R = H, Tubercidin

2, R = H, ϵ Tubercidin

3, R = CN, Toyocamycin

4, R = CN, ϵ Toyocamycin

5, R = C(=O)-NH2, Sangivamycin

6, R = C(=O)-NH2, ϵ Sangivamycin

(DMSO-d_6); two sets of doublets (2H, $J_{2,3}$ = 2 Hz) centered at δ 8.16 (H3) and δ 8.50 (H2), peaks for H5(1H, s, δ 9.4), H8(1H, d, $J_{8,9}$ = 3.5 Hz, δ 8.1) (8), H9 (1H, d, $J_{8,9}$ = 3.5 Hz,

δ 7.4), H1' (1H, d, J = 5.5 Hz, δ 6.4) and the peaks usually observed between δ 3.5 and δ 6.0 (9) for the remaining carbohydrate protons. Elemental analysis (10) provided further confirmation that cyclization of 1 to 2 had occurred. Similar treatment of 3 (1.0 g) produced a 91% yield of 9-cyano-7-(β-D-ribofuranosyl)imidazo[1,2-c]pyrrolo[2,3-d]pyrimidine (4), mp 211-212° (dec.). A pmr spectrum of 4 exhibited a pair of doublets ($J_{2,3}$ = 2 Hz) centered at δ 8.5 and δ 8.0 for H2 and H3, respectively. The ir spectrum of 4 (KBr pellet) confirmed that no modification of the cyano group had occurred (CN, 2210 cm^{-1}). Treatment of 5 using the same conditions afforded a 95% yield of 7-(β-D-ribofuranosyl)imidazo[1,2-c]-pyrrolo[2,3-d]pyrimidine-9-carboxamide (6), mp 264-265°. The pmr spectrum of 6 showed 2 doublets ($J_{2,3}$ = 2 Hz) at δ 7.99 (H3) and δ 8.60 (H2).

The close structural similarity (11) between the naturally occurring pyrrolopyrimidine nucleosides and adenosine suggested that 2, 4 and 6 might be fluorescent and this was subsequently established (Table I). It was assumed that 2, 4 and 6 could be distinguished

TABLE I

Technical Fluorescence Data[a]

Compound	pH	Fluorescence (Emission (nm)[b]) λmax	+ 1/2	-1/2	Fluorescence (Excitation (nm)[c])
2	H_2O	415	461	379	300
	pH 1[d]	415	480	374	370, 293
	pH 11	415	461	379	300
4	H_2O	410	462	373	295
	pH 1	410	478	372	295
	pH 11	415	464	374	295
6	H_2O	410	460	371	295, 265
	pH 1	415	481	368	290
	pH 11	410	456	372	300, 265

a) In water and uncorrected.

b) Fluorescence emission spectra were taken by fixing on the fluorescence excitation maximum.

c) Fluorescence excitation spectra were taken by fixing on the fluorescence emission maximum.

d) All pH 1 spectra were recorded on 10X scale.

from the 3-β-D-ribofuranosylimidazo[2,1-i]purine hydrochloride (12) by fluorescence emission spectra, however, it was established that all four compounds show emission maxima in the 410-415 nm region (Table I). Therefore, other methods were investigated in an effort to obtain an unequivocal difference between the imidazo[2,1-i]purine derivative and 2, 4 and 6. Three methods were found which differentiate between 2, 4 and 6 and the imidazo-[2,1-i]purine derivative.

Specific differences between 3(β-D-ribofuranosyl)imidazo[2,1-i]purine hydrochloride and 2, 4 and 6 are evident in the fluorescence excitation spectra (Table I) and thin-layer chromatographic behavior (13). However, 2, 4 and 6 may be most easily distinguished from the adenosine derivative by a comparison of uv spectra (Table II) with the most definitive feature being the large ε max observed for the pyrrolopyrimidine derivatives in the 240 nm

TABLE II

Ultraviolet Spectral Data

Compound	$\lambda_{max}^{pH\ 1}$	ε max[a]	λ_{max}^{ETOH}	ε max	$\lambda_{max}^{pH\ 11}$	ε max
2	281	9.2	292	6.4	287	6.4
	242	39.2	(281)[b]	5.5	272	6.4
			243	31.2	(247)	29.1
					242	32.8
4	279	8.2	289	4.6	283	6.0
	243	37.9	(256)	15.3	(256)	15.1
			242	38.2	241	38.2
6	280	7.5	290	4.4	285	5.0
	245	30.1	281	4.1	244	31.2
			246	27.1		

a) ε max x 10^{-3} b) ( ) = shoulder

region of the uv spectrum.

The reaction of chloroacetaldehyde with the naturally occurring nucleoside antibiotics tubercidin, toyocamycin and sangivamycin has furnished derivatives of the new tricyclic ring system imidazo[1,2-c]pyrrolo[2,3-d]pyrimidine which are fluorescent and should be substrates for anabolic enzymes while maintaining a high degree of resistance towards enzymatic catabolism. These compounds can be distinguished from the corresponding imidazo[2,1-i]-

purine derivative by tlc as well as uv and fluorescence excitation spectrometry. These fluorescent derivatives (2, 4 and 6) should prove to be valuable compounds as probes for studying the biological and chemotherapeutic function of the pyrrolo[2,3-d]pyrimidine nucleosides.

References

1. This research was supported by Research Contract NO1-CM-23710 from the Division of Cancer Treatment, National Cancer Institute, ational Institutes of Health, U. S. Public Health Service.

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4. Unpublished testing data from Drug Research and Development Branch of the Division of Cancer Treatment, N.C.I., N.I.H.

5. The chloroacetaldehyde was prepared as follows: Fluka chloroacetaldehyde 50% in water, practical grade, was distilled at 45 mm and the fraction distilling at 33-35° was collected in a round bottom flask which was immersed in a dry ice/acetone bath and used without further modification.

6. The use of chloroacetaldehyde to convert 9-methyladenine, 1-methylcytosine, adenosine, cytidine and their nucleotides to the tricyclic etheno derivatives has been reported by N. K. Kochetkov, V. W. Shibaev and A. A. Kost, Tetrahedron Letters, 1993 (1971) and J. A. Secrist III, J. R. Barrio, N. J. Leonard and G. Reber, Biochemistry 11, 3499 (1972).

7. Derived nomenclature would be 3,N^4-ethenotubercidin (ε tubercidin) (2), 3,N^4-etheno-toyocamycin (ε toyocamycin) (4) and 3,N^4-ethenosangivamycin (ε sangivamycin) (6).

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10. All new compounds were dried in vacuo at 110° for 18 hr to afford elemental analysis (C, H, N) within acceptable limits.

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13. Tlc was performed using SilicAr 7GF plates 0.25 mm thick with n-PrOH/EtoAC/H_2O (SSE) (1:4:2, v/v/v, upper phase) with conc. NH_4OH (1 ml) being added to 50 ml of SSE and this was then used as solvent. Rf: 2, 0.37 with tailing to 0; 4, 0.45; 6, 0.45; 3-(β-D-ribofuranosyl)imidazo[2,1-i]purine hydrochloride, 0.25 to 0.