CYCLONUCLEOSIDE DERIVATIVES OF FORMYCIN

Sir:

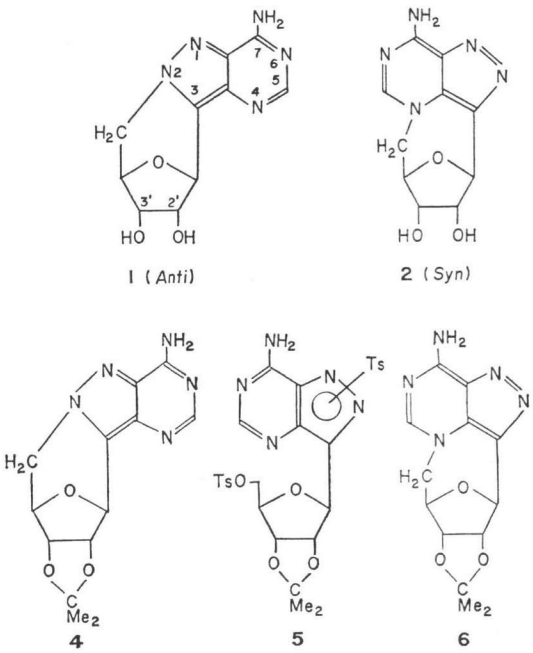
Formycin[1] is a representative pyrazolopyrimidine nucleoside that contains the unusual *C*-riboside linkage and its biological properties[2] have been intensely investigated ever since its discovery by H. UMEZAWA and coworkers.[1] Formycin is a cytotoxic analog of adenosine and it has been shown that adenosine deaminase[3,4,5,6] and adenosine kinase[6,7,8] are two important enzymes in tumors responsible for resistance to formycin. On the other hand, the *syn-anti* conformations of formycin have become of interest in view of the relationship between substrate conformation and the specificity of enzymes.[9] The X-ray crystallographic study[10] of formycin hydrobromide revealed that it possesses a *syn* conformation, whereas unprotonated adenosine possesses an *anti* conformation.[11] PRUSINER *et al.*[12] have recently reported that formycin monohydrate possesses an intermediate (or high *anti*) conformation in the solid state, and this fact suggests that the high *anti* conformation renders it susceptible to the action of adenosine deaminase which is found to be inactive to the systems of *syn* conformation.[13] The high *anti* conformation of formycin monohydrate has been attributed[12] to the electrostatic repulsion between the *N*-2 (which replaces the C-8 of adenosine) and the sugar *O*-5′ atom. Recently, TOWNSEND *et al.*[14] prepared the *N*-1 and *N*-2 methyl derivatives of formyicn and observed that the latter derivative, in which the rotation around the glycosyl (carbon-carbon) bond is hindered by the presence of the *N*-2 methyl group, exhibited a higher *T/C* value against leukemia L-1210 than the *N*-1 methyl derivative, although no further detail was described. In this paper we report the preparation of two anhydroformycins (**1**, **2**) in which the above-mentioned *syn* and *anti* conformations are fixed by cyclization.

Isopropylidenation of formycin with 2,2-dimethoxypropane in acetone in the presence of 5 equiv. *p*-toluenesulfonic acid gave 2′,3′-*O*-isopropylideneformycin (**3**), a colorless powder, in 94 % yield; mp 134~138°C; $[\alpha]_D^{20}$ −88° (*c* 0.5, dimethylsulfoxide). Found: C, 50.46; H, 5.65; N, 22.49 %. Calcd. for $C_{13}H_{17}O_4N_5$: C, 50.81; H, 5.58; N, 22.79 %.

Cyclization of **3** was achieved by the use of triphenylphosphine and diethylazodicarboxylate which is a dehydrating reagent between alcohols and active hydrogen compounds reported by WADA and MITSUNOBU.[15] The reaction in dry dioxane at room temperature for 2 hours gave 2′, 3′-*O*-isopropylidene-N^2,5′-anhydroformycin (**4**) (*anti* conformation) in 93 % yield; mp 223°C (subl.); $[\alpha]_D^{20}$ −54° (*c* 0.5, dimethylsulfoxide), −44° (*c* 0.5, dimethylformamide). Found: C, 53.69; H, 5.24; N, 24.35 %. Calcd. for $C_{13}H_{15}O_3N_5$: C, 53.97; H, 5.23; N, 24.21 %.

Treatment of **4** with 1 N hydrochloric acid-dioxane (1:2) at room temperature afforded the monohydrochloride of N^2,5′-anhydroformycin (**1**) in 83 % yield; mp < 300°C; $[\alpha]_D^{20}$ −75° (*c* 0.5, 0.1 N HCl). Found: C, 42.10; H, 4.26; N, 24.69; Cl, 12.41 %. Calcd. for $C_{10}H_{11}O_3N_5 \cdot HCl$: C, 42.04; H, 4.23; N, 24.52; Cl, 12.41 %.

Scheme 1.



Treatment of **3** with 3 equiv. *p*-toluensulfonyl chloride in pyridine at room temperature for 4 hours gave 2′,3′-*O*-isopropylidene-N^1 (or N^2)-*p*-toluenesulfonyl-5′-*O*-*p*-toluenesulfonylformycin (**5**) in 67 % yield; mp 134~138°C; $[\alpha]_D^{20}$-16° (*c* 0.5, chloroform). Found: C, 53.11;

Table 1. UV Spectral data

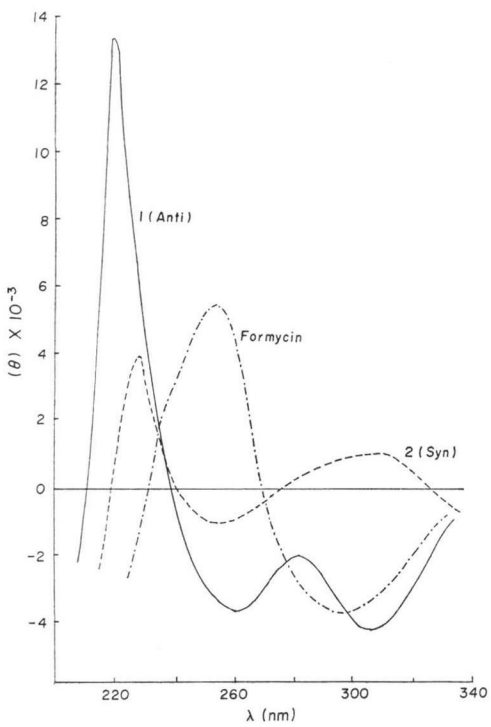
Compound	pH 1		pH 11		pH 7	
	λ_{max} nm	ε	λ_{max} nm	ε	λ_{max} nm	ε
1	304	11,200	305	12,500	305	12,100
	260	5,630			257	4,980
	231	13,700	234*	9,700	231*	11,400
2	304	14,300	317	7,990	304	14,000
			272	9,780		
	245	7,930	253	9,860	247	9,000

* Shoulder.

H, 4.89; N, 10.89; S, 10.10 %. Calcd. for $C_{27}H_{29}O_8N_5S_2$: C, 52.67; H, 4.75; N, 11.38; S, 10.41 %.

The structure of the ditosyl derivative (**5**) was supported by a downfield shift of the methylene protons at *C*-5′ (to δ 4.22) in the NMR spectrum and by the presence of an absorption (at 1630 cm^{-1}) of the aromatic amino group in the IR spectrum (KBr).

Fig. 1. CD Spectra of **1**, **2** and formycin in water.



It should be noted that similar treatment of **3** with 1 equiv. *p*-toluenesulfonyl chloride gave the *N*-1 (or *N*-2) tosyl derivative which, by treatment with ammonia, reverted to formycin.

Cyclization of **5** was readily effected[16] by refluxing it in dry dioxane for 7 hours. The IR spectrum of the product showed an ionic tosylate absorption at 1010 cm^{-1}. The product was then treated with concentrated ammonia at room temperature for 2 hours to give the 2′,3′-*O*-isopropylidene-N^4,5′-anhydroformycin (*syn* conformation) (**6**) in 39 % yield, a colorless powder; mp 272°C (decomp.); $[\alpha]_D^{20}$ −44° (*c* 0.5, dimethylsulfoxide). Found: C, 53.44; H, 5.29; N, 23.72 %. Calcd. for $C_{13}H_{15}O_3N_5$: C, 53.97; H, 5.23; N, 24.21 %.

Finally, acid hydrolysis of **6** afforded the monohydrochloride of N^4,5′-anhydroformycin (**2**) in 73 % yield; mp 263°C (decomp.); $[\alpha]_D^{20}$ −0.5° (*c* 0.6, 0.1 N HCl). Found: C, 41.89; H, 4.24; N, 24.29; Cl, 12.13 %. Calcd. for $C_{10}H_{11}O_3N_5 \cdot HCl$: C, 42.04; H, 4.23; N, 24.52; Cl, 12.41 %.

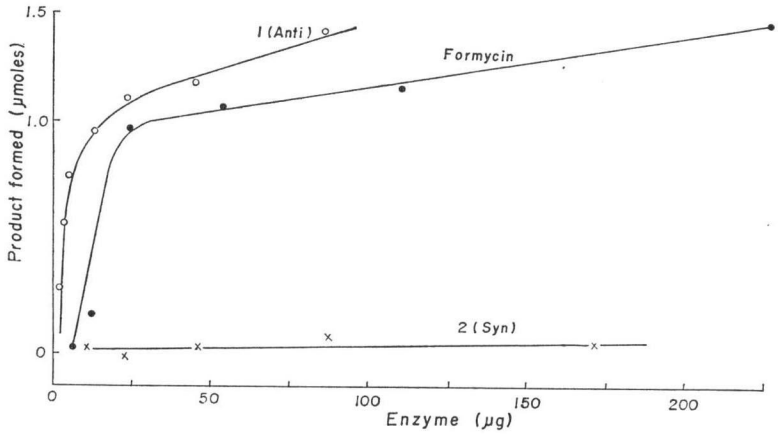
The *anti-syn* conformations of the anhydroformycins (**1**, **2**) are unequivocal from the

Table 2. NMR Spectral data*

Compounds	H-1′, δ	$J_{1',2'}$ Hz	H-5, δ	7-NH_2, δ
Formycin monohydrochloride	5.27	6	8.68	10.0
1 hydrochloride	5.73	0	8.65	10.0
2 hydrochloride	5.47	0	8.88	9.95

* Determined in DMSO-d_6.

Fig. 2. Deamination of **1** (*anti*), **2** (*syn*) and formycin by Takadiastase adenosine deaminase.
A reaction mixture (0.2 ml) containing 1.5 μmole of **1** (and **2** or formycin), 5 μmoles of PBS (pH 6.6) and a variable quantity of enzyme was incubated at 45°C for 60 minutes.

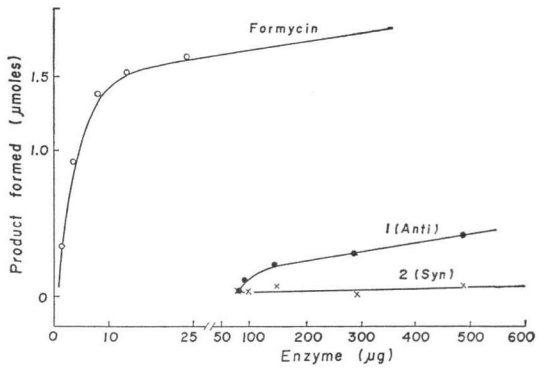


above syntheses and are supported by the UV, CD and NMR spectra. The UV spectrum of **1** was compatible with the absorption pattern of 2-*N*-methylformycin[14] (Table 1). The CD spectrum of the hydrochloride of **1** in water showed a negative COTTON effect at 304 nm, whereas that of **2** showed a positive COTTON effect at about 300~310 nm, and formycin showed a negative COTTON effect at about 300 nm, indicating that the unprotonated formycin has an *anti* conformation in water. In the NMR spectra, the H-5 proton of the hydrochloride of **2** was observed at lower field than formycin hydrochloride and the hydrochloride of **1**, and the anhydroformycins (**1**, **2**) showed $J_{1',2'}=0$ Hz. The difference in the chemical shift of 7-NH_2 between the formycin hydrochloride and anhydroformycin hydrochlorides was hardly discernible.

The deamination activity of the anhydroformycins (**1**, **2**) by Takadiastase adenosine deaminase (*Aspergillus oryzae*, 17.1 U/mg) and calf intestinal mucosa adenosine deaminase (purchased from Sigma Chemical Company, 2 U/mg) are shown in Figs. 2 and 3.

A reaction mixture (0.2 ml) containing 1.5 μmoles of **1** (and **2** or formycin), 5 μmoles of phosphate buffer (PBS) (pH 6.6) and a variable quantity (μg) of Takadiastase adenosine deaminase was incubated at 45°C for 60 minutes. The reaction was stopped by adding 0.2 ml of methanol. After centrifugation at 3,000 rpm for 10 minutes, 40 μl of the supernatant was spotted on a filter paper (Toyo Roshi No. 51)

Fig. 3. Deamination of **1** (*anti*), **2** (*syn*) and formycin by calf intestinal mucosa adenosine deaminase.
A reaction mixture (0.2 ml) containing 1.8 μmoles of **1** (and **2** or formycin), 10 μmoles of PBS (pH 7.4) and a variable quantity of enzyme was incubated at 37°C for 60 minutes (20 minutes in the case of formycin).



and it was subjected to high voltage electrophoresis under 3,500 V for 20 minutes. The spot of **1** (and **2** or formycin) was cut and extracted with 2 ml of 0.1 N HCl at 37°C for 30 minutes. The optical density of the extract at 304 nm (295 nm in the case of formycin) was measured and the amount of **1** (and **2** or formycin) was calculated from the standard curve (Fig. 2).

A reaction mixture (0.2 ml) containing 1.8 μmoles of **1** (and **2** or formycin), 10 μmoles of phosphate buffer (pH 7.4) and a variable quantity of calf intestinal mucosa adenosine

deaminase was incubated at 37°C for 60 minutes (20 minutes in the case of formycin), and worked up in a similar manner as described above (Fig. 3).

From the above results, it became clear that the *syn* compound (**2**) is resistant to both deaminases and that the *anti* compound (**1**) and formycin are deaminated. Though the *anti* compound (**1**) is less susceptible to calf intestinal mucosa adenosine deaminase than formycin, in the case of Takadiastase adenosine deaminase, the former is more deaminated than the latter.

The anhydroformycins (**1**, **2**) were found to be inactive against EHRLICH ascites tumor and mouse leukemia L-1210 ascites tumor *in vivo*.

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