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Formycin Anhydronucleosides. Conformation of Formycin and Conformational Specificity of Adenosine Deaminase[1]

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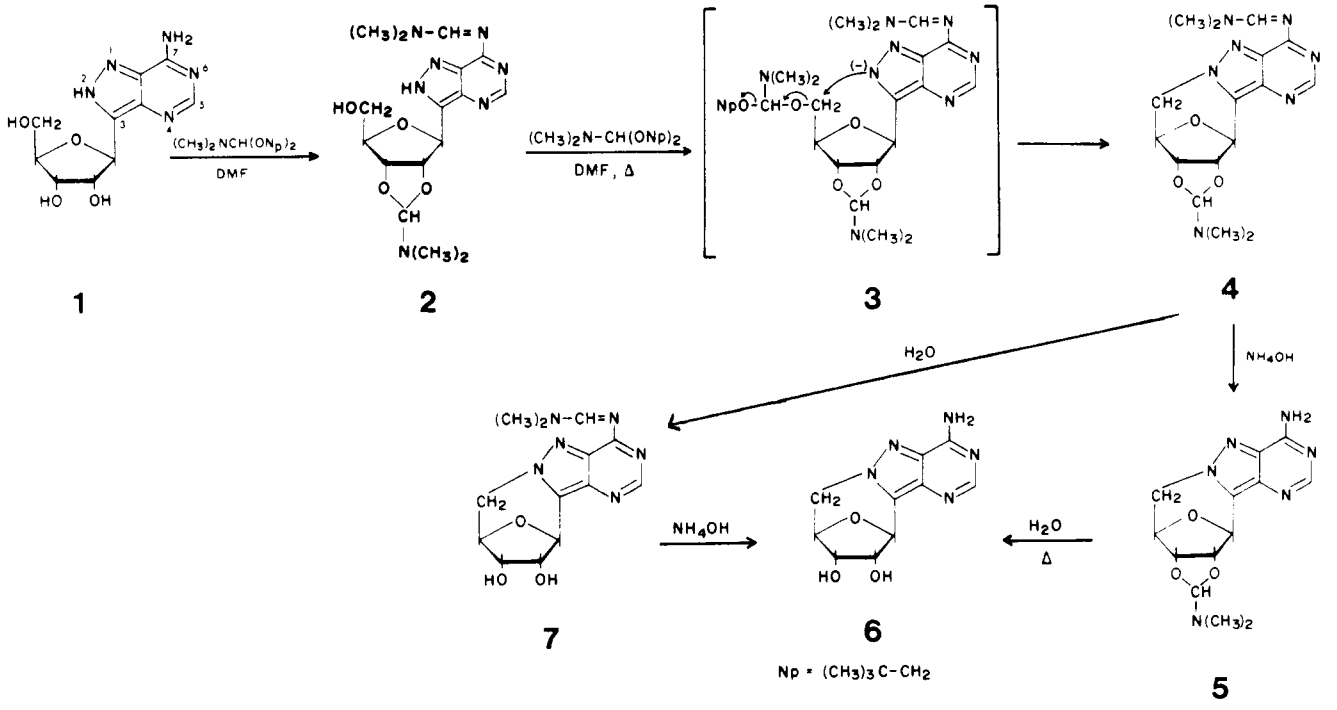
Abstract: The synthesis and CD spectra of 2,5′-anhydroformycin (**6**), 2,5′-anhydroformycin B (**9**), and 4,5′-anhydroformycin (**14**) are described. Treatment of formycin (**1**) with dimethylformamide dineopentyl acetal at 130° for 14 hr gave, depending on the work-up of the reaction mixture, compound **6**, *N*-dimethylaminomethylene derivative **7**, or 2′,3′-*O*-dimethylaminomethylene derivative **5**. Similarly, formycin B (**8**) afforded 2,5′-anhydroformycin B (**9**). The reaction of **1** with dimethylformamide dimethyl acetal gave selectively, after ammonolysis, 1-methylformycin (**10**). The reaction of 2′,3′-*O*-isopropylideneformycin (**13**) with *p*-toluenesulfonyl chloride in pyridine gave, after deblocking with 90% CF_3COOH, compounds **6** and **14**. The CD spectra of **6** and **9** in water and 0.01 *N* HCl show a negative Cotton effect at ca. 300 nm whereas that of **14** exhibits in water a positive Cotton effect of low intensity at ca. 280 nm which is considerably enhanced in 0.01 *N* HCl (at ca. 300 nm). Formycin (**1**) and formycin B (**8**) have a negative Cotton effect in water at ca. 290 and 270 nm, respectively. The magnitude of the Cotton effect of **1** in 0.01 *N* HCl is markedly decreased whereas that of **8** remains essentially unchanged. The results can be interpreted by assuming that the anti conformation of **1** and **8** is preponderant in water and that there is a higher proportion of **1** in the syn form in 0.01 *N* HCl. Compound **6** is deaminated by calf intestine adenosine deaminase to give **9** whereas **14** is completely resistant. This indicates that the substrate must be in (or approximately in) an anti conformation. The diastereoisomeric composition of **2, 4, 5, 12a,** and **12b** was determined by NMR spectroscopy.

Formycin (**1**) is a pyrazolopyrimidine C-nucleoside antibiotic which exhibits distinct cancerostatic activity.[2] X-Ray studies of formycin dihydrobromide hydrate have shown that **1** has a β configuration and a syn conformation.[3] More recent studies using formycin hydrate have suggested[4] a conformation intermediate between syn and anti.[5] A syn conformation of formycin units in the corresponding polynucleotide (poly F) has also been postulated[5a,6a] to explain the anomalous behavior of this polymer toward nucleases.[5a,6] It was, therefore, of interest to study some formycin derivatives fixed in either a syn or anti conformation or some approximation thereof. The present work presents the results of the synthesis and CD studies of both possible 5′-anhydronucleosides of **1**—2,5′-anhydroformycin (**6**) and 4,5′-anhydroformycin (**14**)—derived from anti and syn conformations of **1**. A method of preparation of 2,5′-anhydroformycin B (**9**) and selective N-1 methylation of **1** are also reported.

Results and Discussion

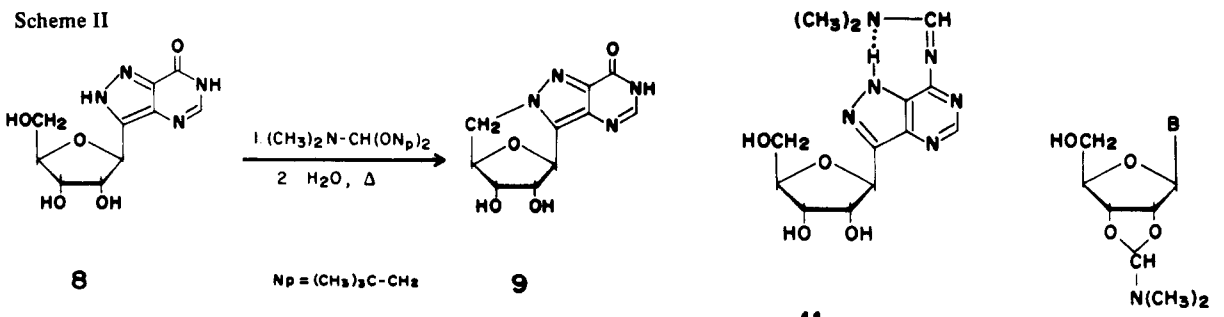
The interaction of **1** and excess dimethylformamide dineopentyl acetal in dimethylformamide (DMF) at 130° for 14 hr gave the anhydronucleoside **6** in almost 80% yield. Although intermediate **3** was not isolated,[7] *N*-dimethylaminomethylene-2,5′-anhydroformycin (**7**) was obtained merely by hydrolysis of **4** with water, and the product **7** was characterized by uv and NMR spectra. Previous studies[8,9] have indicated that a mixed dimethylformamide acetal of an aliphatic alcohol and nucleoside hydroxyl function can serve as an effective leaving group. It is, therefore, likely that intermediate **3** gives **4** via intramolecular nucleophilic attack at C-5′ by N-2 which is presumably ionized under the reaction conditions (Scheme I). Similarly, when formycin B (**8**) was heated with dimethylformamide dineopentyl acetal in DMF for 5 hr at 130°, the corresponding anhydro derivative **9** was obtained in 78% yield after briefly heating the crude reaction product (presumably the 2′,3′-*O*-dimethylaminomethylene derivative of **9**) in water (Scheme II). The formation of 3,5′-anhydroxanthosine from xanthosine and dimethylformamide dineopentyl acetal has been similarly explained.[8] The pK_a of the pyrazole portion of **1** (9.5)[10] is reasonably close to those compounds that undergo smooth N-alkylation with dimethylformamide dialkyl acetals.[7a,11] The reason for using dimethylformamide dineopentyl acetal was to suppress intermolecular alkylation.[7a,11] Thus, when **1** was treated with excess dimethylformamide dimethyl acetal a nearly quantitative yield of 1-methylformycin (**10**) was obtained and **6** could not be detected (Scheme III). By contrast, both intra- and intermolecular alkylation were observed on treatment of xanthosine with

Scheme I



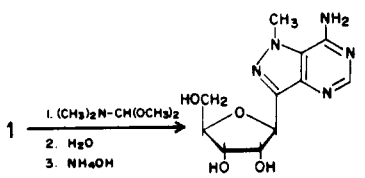
Np = $(CH_3)_3C-CH_2$

Scheme II



Np = $(CH_3)_3C-CH_2$

Scheme III



12a: B = N-dimethylamino-methyleneadenine

12b: B = N-dimethylamino-methylene-4-amino-5-cyanopyrrolo[2,3-d]-pyrimidine

the above reagent.[8] The structure of **10** was confirmed by NMR, CD, and uv spectra. The latter was virtually identical with that reported for **10** prepared by another route but different from the 2-methylformycin.[12] It is also of interest to note that intermolecular alkylation (formation of **10**) proceeds at appreciably lower temperature (70°) than the intramolecular process (formation of **6**). Thus, the sterically less crowded transition state for methylation[13] apparently offsets the favorable proximity effect of C-5′ in intermediate 3. The selectivity of the alkylation for N-1 is also surprising in view of the fact that methylation of formycin (**1**) with CH_3I in the presence of C_2H_5ONa gives both 1- and 2-methyl derivatives, the latter being the predominant product.[14] A plausible explanation of a high selectivity of alkylation for N-1 observed in the present study could be the result of the interposition of structure **11** where the hydrogen bonding would make the N-1 more nucleophilic than N-2.

Still another interesting facet of the reaction of **1** and dimethylformamide dineopentyl acetal was detected following prolonged treatment (54 hr) of the crude product with ammonium hydroxide. Thus, a crystalline solid was obtained (80% yield) with spectral (uv, NMR) properties consistent with the 2′,3′-*O*-dimethylaminomethylene derivative **5** (Scheme I). The latter was readily converted to the deblocked anhydronucleoside **6** on brief reflux in water.

This transformation (**4** → **5**) is the first example of the selective removal of an *N*-dimethylaminomethylene function from a ribonucleoside in the presence of a 2′,3′-*O*-dimethylaminomethylene group. The reverse transformation, i.e., removal of the 2′,3′-*O*-dimethylaminomethylene group to give selectively *N*-dimethylaminomethylene derivatives of ribonucleosides, has been described.[7] The 2′,3′-*O*-dimethylaminomethylene group is very labile and is readily hydrolyzed at room temperature in water. The surprising stability of the 2′,3′-*O*-dimethylaminomethylene group in **5** can probably be attributed to the fact that the 1,3-dioxolane ring in the latter forms a part of a stable pentacyclic system although other factors such as relatively rapid deposition of the product in high yield during the course of the reaction may also be an important consideration. Thus, when *N*:-

Table I. Diastereoisomeric Composition of 2′,3′-*O*-Dimethylaminomethylene Derivatives of Some Ribonucleosides

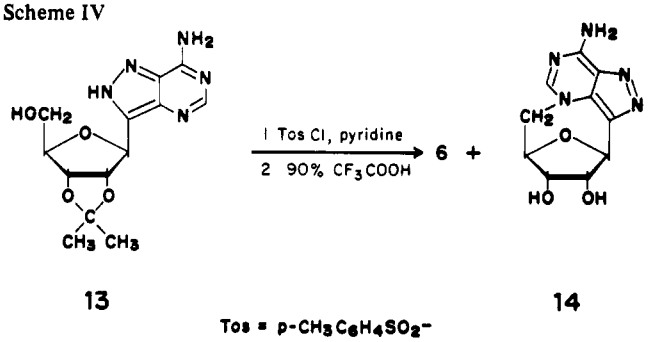
Compd	Composition determined[a] from proton signals			
	Heterocycl CH	$H_{1'}$	CH of acetal	$N(CH_3)_2$ of acetal
2			68.4	
			31.6	
12a	*b*	63	62.5	59
	b	37	37.5	41
12b	62 (H_2)	61	67.0	61
	38 (H_2)	39	33.0	39
5	*c*	*c*	67.5	67
			32.5	33
4	*c*	*c*	66.3	
			33.7	

[a] From the height of the corresponding signal in CD_3COCD_3. [b] Overlapped signals. Better resolution was obtained in CD_3SOCD_3. [c] Single signal for both diastereoisomers.

2′,3′-*O*-bis(dimethylaminomethylene)formycin (**2**) was dissolved in concentrated NH_4OH and the solution was held at room temperature for 3 days, the only isolable product was formycin. A similar result (formation of adenosine as the only product) was observed with compound **12a.** This may indicate that the 2,5′-anhydro bond plays a significant role in the stability of **5**. NMR spectrometry has shown that compound **5** is a mixture of two diastereoisomers of a composition close to that observed in some related derivatives, e.g., **2, 4, 12a,** and **12b** (Table I). Although it is not possible at the present time to assign rigorously the configuration of both diastereoisomers, it appears likely that the less abundant diastereoisomer with the more deshielded $H_{1'}$ and less deshielded CH of the acetal grouping will have the dimethylamino (acetal) function in the endo position. This is supported by examination of framework molecular models which show that the distance between $H_{1'}$ and the dimethylamino portion of the acetal grouping is smaller for an endo than for an exo diastereoisomer. Support for this assumption is derived from the fact that the acetal proton in 2′,3′-*O*-benzylideneribonucleosides is less deshielded in a phenyl endo stereoisomer than in the corresponding exo counterpart.[15] It is of interest to note that the NMR spectra of anhydronucleosides **4** and **5** show only one signal for the heterocyclic proton H_5 and for $H_{1'}$ although the CH (acetal) and $N(CH_3)_2$ (acetal) signals indicate a diastereoisomeric mixture (Table I). On the other hand, in **2, 12a,** and **12b** the signals of heterocyclic CH, acetal CH, $H_{1'}$, and $N(CH_3)_2$ belonging to both diastereoisomers are resolved.[16] The CH (heterocyclic) signals of **12a** and **12b** and the $N(CH_3)_2$ signals of **2, 5, 12a,** and **12b** of the less abundant (presumably endo) diastereoisomer are more deshielded than the corresponding signals of the more abundant (exo) isomer.

In contrast with the reaction of **1** with dimethylformamide dineopentyl acetal which gave **6** as the sole product, the interaction of 2′,3′-*O*-isopropylideneformycin (**13**) with *p*-toluenesulfonyl chloride (TosCl) in pyridine for 24 hr at room temperature gave, after deblocking of the isopropylidene group with 90% CF_3COOH, both anhydronucleosides **6** and **14** (Scheme IV) which were separated by preparative thin-layer chromatography on microcrystalline cellulose (Avicel). The formation of **14,** which is the predominant product, would be expected by analogy with the similar behavior of 2′,3′-*O*-isopropylideneadenosine which also gives the anhydronucleoside derived from the syn conformer of adenosine.[17] Nevertheless, the simultaneous formation of two products, each of which is derived from a different rotameric form of a nucleoside, represents a unique situation

Scheme IV



13 14

Tos = p-$CH_3C_6H_4SO_2$-

in the chemistry of nucleosides. It is also of interest to note while in the case of 2′,3′-*O*-isopropylideneadenosine the corresponding 5′-*O*-*p*-toluenesulfonyl derivative is first isolated and then converted to the anhydronucleoside by refluxing in acetone,[17] treatment of **13** with TosCl gives directly the 2′,3′-*O*-isopropylidene derivatives of the anhydronucleosides **6** and **14.** To explain the difference in the reaction product composition between the reaction of **1** with dimethylformamide dineopentyl acetal and the interaction of **13** with TosCl, two factors can be invoked: (a) difference in the reaction conditions (absence of a strong tertiary base in the latter conversion) and (b) different leaving groups in both cases (mixed acetal vs. *p*-toluenesulfonyl). An additional point of interest is the unusual stability of 2′,3′-*O*-isopropylidene derivatives of **6** and **14.** The general procedure[18] for hydrolysis of the 2′,3′-*O*-isopropylidene group from ribonucleosides in 90% CF_3CO_2H (30 min at room temperature) was not satisfactory. Prolonged treatment (19 hr at room temperature) led to nearly complete removal of the isopropylidene group though chromatography of the crude reaction mixture revealed still some unreacted isopropylidene derivatives of **6** and **14.** The rather unique stability of the latter toward hydrolysis is reminiscent of a similar unreactivity of the 2′,3′-*O*-dimethylaminomethylene derivative **5** (vide infra).

The structures of **6, 9,** and **14** are supported by NMR, uv, and CD spectra. Thus, the NMR spectra exhibit $H_{1'}$ as a singlet typical for purine and pyrimidine 5′-anhydronucleosides.[8,19] It should be noted, however, that whereas anhydronucleoside **14** is a seven-membered ring system, the "anhydro ring" in compounds **6** and **9** comprises only six atoms. Yet, neither the position nor the pattern of $H_{1'}$ in all three anhydro derivatives changes appreciably (δ 5.65–5.73) although it is shifted considerably downfield from that observed in formycin (**1**) or formycin B (**8**) (δ 5.29 and 5.27, respectively). The H_5 signal, however, is more deshielded in **14** than in **6**. Paper chromatography of anhydro derivatives **6, 9,** and **14** (Table II) reveals some interesting qualitative information as to their relative polarities. Thus, compound **14** derived from the syn conformer of **1** is considerably more polar than the corresponding "anti" derivative **6**. In addition, **14** is more fluorescent than **6** as judged qualitatively from the behavior of the two compounds toward uv light after paper chromatography in S_4.

The uv spectra of compounds **6, 9,** and **14** exhibit some important differences both in water and 0.01 *N* HCl. The bathochromic shift of the main (B_{2u}) absorption band relative to **1** or **8** is consistent with the formulation of **6, 9,** and **14** as N-alkylated derivatives of formycin or formycin B (Figures 1, 2, and 5). The uv spectra of **8** and **9** in water are essentially identical with those in 0.01 *N* HCl (Figure 5). The shape of the B_{2u} band of **1, 6,** and **14** is also similar in both solvents. All compounds show three peaks or shoulders (water) or one major peak followed by a shoulder (0.01 *N* HCl). In water, however, the region 230–280 nm which does not show a significant band for either **1** or **6** exhibits

Table II. Paper Chromatography of Starting Materials and Reaction Products

Compd	$R_f(S_4)$	$R_f(S_5)$
Formycin (1)	0.43	0.29
2,5′-Anhydroformycin (6)	0.42	0.30
Formycin B (8)	0.45	0.29
2,5′-Anhydroformycin B (9)	0.38	0.28
1-Methylformycin (10)	0.58	0.36
4,5′-Anhydroformycin (14)	0.23	0.24

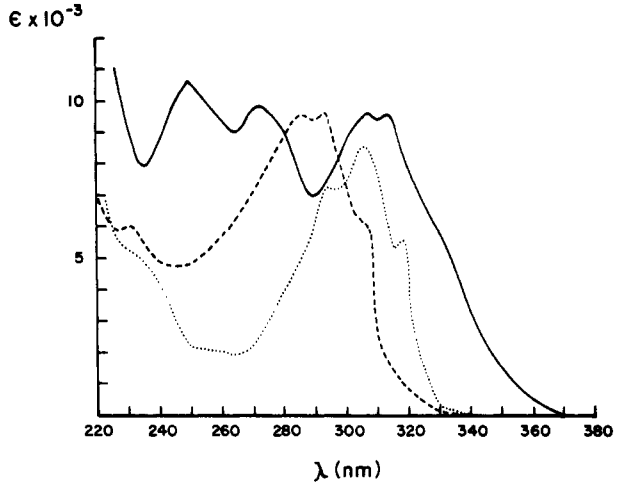


Figure 1. Uv spectra of formycin (**1**, - - -), 2,5′-anhydroformycin (**6**, • • • •), and 4,5′-anhydroformycin (**14**, —) in water.

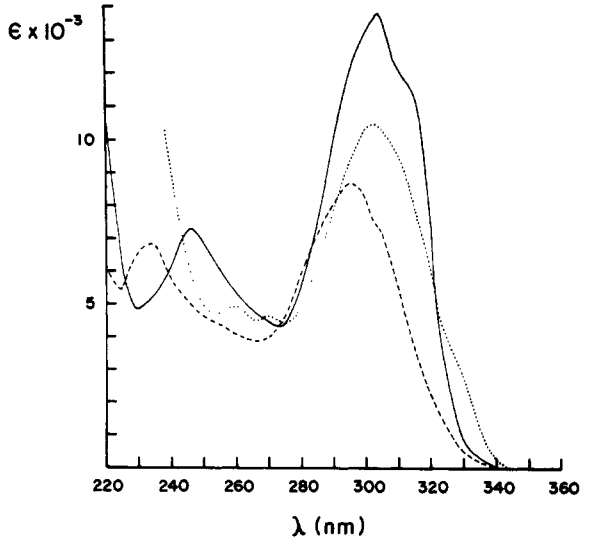


Figure 2. Uv spectra of formycin (**1**, - - -), 2,5′-anhydroformycin (**6**, • • • •), and 4,5′-anhydroformycin (**14**, —) in 0.01 *N* HCl.

two high-absorbancy peaks in **14**. Contrastingly, in 0.01 *N* HCl the region 230–280 nm contains only one significant maximum in both **1** and **14** at 235 and 250 nm, respectively, whereas **6** exhibits only two minor peaks at 260 and 270 nm. In this respect, the uv spectrum of **6** resembles formycin (**1**) more in water than in 0.01 *N* HCl while the opposite is true for compound **14**. A very important feature which further corroborates the structure of **6** is the similarity of the uv spectrum to that of 2-methylformycin. Thus, the principal peaks which comprise the uv spectrum of **6** in acid are also present in 2-methylformycin.[12]

The CD spectra of **6**, **9**, and **14** are also in agreement with proposed structures (Figures 3–5). Thus, the CD spectrum of **6** in water exhibits two negative (B_{1u} and B_{2u}) Cot-

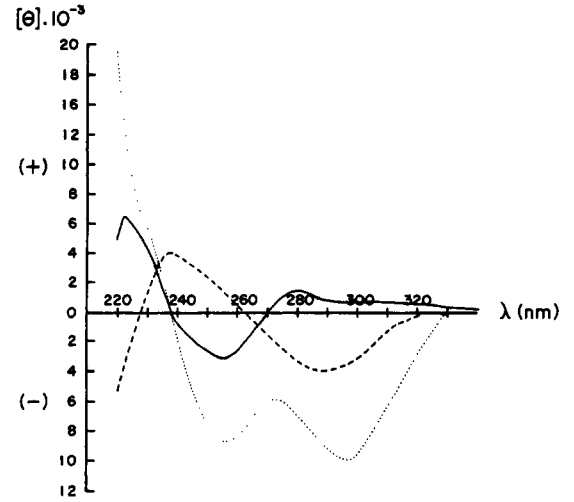


Figure 3. CD spectra of formycin (**1**, - - -), 2,5′-anhydroformycin (**6**, • • • •), and 4,5′-anhydroformycin (**14**, —) in water.

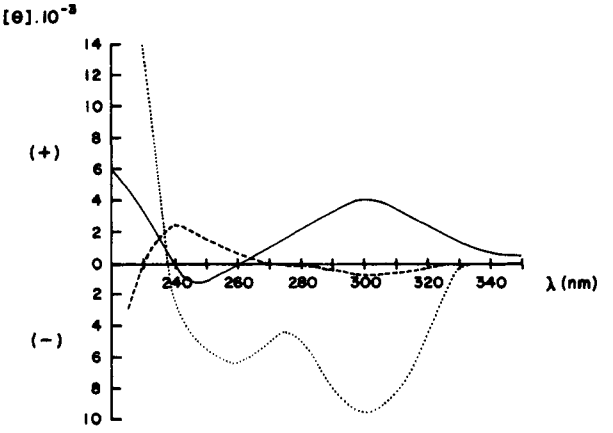


Figure 4. CD spectra of formycin (**1**, - - -), 2,5′-anhydroformycin (**6**, • • • •), and 4,5′-anhydroformycin (**14**, —) in 0.01 *N* HCl.

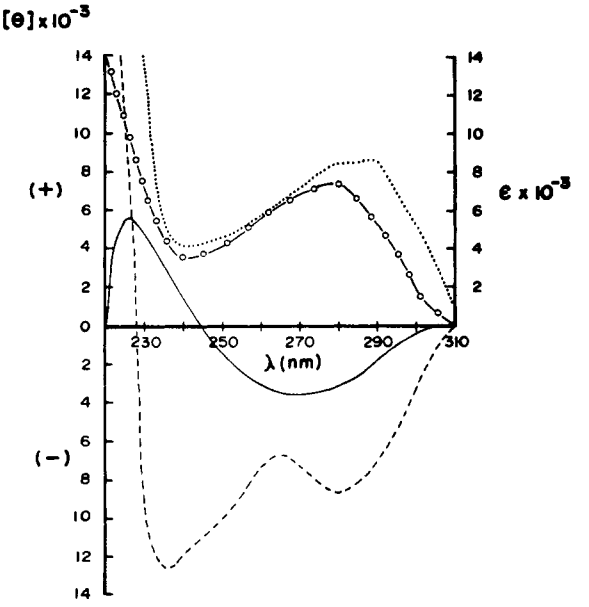


Figure 5. Uv and CD spectra of formycin B (**8**) and 2,5′-anhydroformycin B (**9**) in water. Spectra in 0.01 *N* HCl are identical with those in water: (-O-O-), **8** (uv); (• • • •), **9** (uv); (—), **8** (CD); (- - -), **9** (CD).

Table III. Paper Electrophoresis of Anhydronucleosides **6**, **9**, and **14** in Borate Buffer (pH 9)

Compd	Spot	%[a]	Mobility[b]
6	A	38.5	0.58
	B	61.5	1.35
9	A	41.0	0.69
	B	59.0	1.07
14			1.00

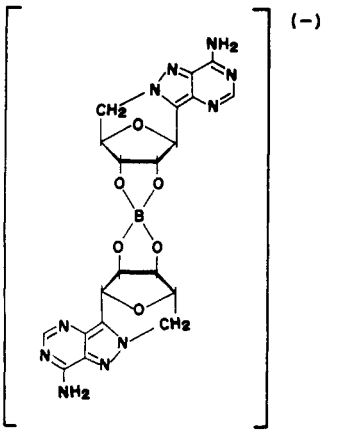
[a] Determined by elution of the corresponding spots with 0.01 *N* HCl and uv spectrophotometry of the eluates. [b] Relative to formycin (**1**) = 1.00 for compound **6** and **14** or to formycin B (**8**) = 1.00 for **9**.

ton effects at 255 and 300 nm, respectively (Figure 3). On the other hand, the CD spectrum of formycin (**1**) has a negative B_{2u} Cotton effect at 290 nm. In this respect it is quite similar to that of adenosine[20] which also exhibits a negative B_{2u} Cotton effect. Unlike adenosine, compound **1** has a positive B_{1u} band at ca. 240 nm. The CD spectra of **8** and **9** are very similar to those of formycin (**1**) and compound **6**, respectively (Figure 5). Thus, formycin B (**8**) exhibits a negative B_{2u} Cotton effect at ca. 270 nm whereas **9** has two negative B_{1u} and B_{2u} Cotton effects at 235 and 280 nm. On the other hand, anhydronucleoside **14** has a positive low-intensity B_{2u} Cotton effect at 280 nm and a negative B_{1u} Cotton effect at 255 nm (Figure 3).

Some interesting changes in the CD spectra of **1** and **14** are produced in 0.01 *N* HCl. Thus, the B_{2u} Cotton effect at 300 nm in **1** is greatly decreased (Figure 4) relative to that in water. Anhydronucleoside **14** exhibits a distinct positive B_{2u} Cotton effect at 300 nm whereas the CD curves of **6**, **8**, and **9** are essentially unchanged (Figures 4 and 5). It is of interest to note that 3,5′-anhydroxanthosine which is, as compound **14**, also derived from a syn conformation of corresponding nucleoside (xanthosine) has a positive B_{2u} Cotton effect.[21]

The comparison of the CD spectra of formycin (**1**) and formycin B (**8**) with those of anhydronucleosides **6**, **9**, and **14** is of interest in view of the problem of the conformation of **1** and **8** in solution. Thus, in water both **1** and **6** exhibit negative B_{2u} Cotton effects whereas the CD curve of **14** is roughly opposite to that of **1** (Figure 3). The fact that both **1** and **6** have a negative B_{2u} Cotton effect is in accord with the preponderance of the anti conformation of **1** in water. A substantial decrease of the intensity of the B_{2u} Cotton effect of **1** in 0.01 *N* HCl (Figure 4) may then be accounted for in terms of a conformational shift toward the syn form. It is also of interest to note that the differences in uv spectra between **1**, on one hand, and compounds **6** and **14** on the other (vide infra) seem to lend further support to such an assumption. The tendency of the protonated form of formycin (**1**) to attain a conformation closer to syn may also be the reason for a different conformation of formycin (**1**, situation between syn and anti)[4] and the corresponding dihydrobromide (syn)[3] in a solid state. The comparison of CD spectra of **8** and **9** in both water and 0.01 *N* HCl (Figure 5) leads to a similar conclusion—suggestion of the anti conformation for **8**.

Of interest is the behavior of 2,5′-anhydroformycin (**6**) and 2,5′-anhydroformycin B (**9**) during paper electrophoresis in sodium borate (pH 9). Two distinct spots (ratio ca. 60:40, Table III), which exhibited identical uv spectra, were obtained from all preparations of **6** and **9**. Their mobilities indicate that both are borate complexes of a vicinal glycol arrangement in the product. Eluates of both spots were deionized and evaporated and the residues were subjected to paper electrophoresis in borate to again give (each) two spots in the same ratio as in the original compound **6**. This result excludes the possibility of the contamination of **6** with some product containing a vicinal diol and strongly suggests that two distinct borate complexes of **6** are involved. The structure of such complexes remains a matter of speculation although, e.g., two stereoisomers of **15** represent a likely possibility.[23] Interestingly, isomeric anhydronucleoside **14** travels in paper electrophoresis in borate buffer as a single spot.



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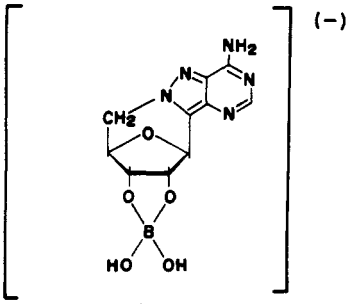

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Of interest from both the chemical (further confirmation of the proposed structures) and biochemical viewpoints is the behavior of compounds **6** and **14** toward adenosine deaminase. Formycin (**1**) itself is readily deaminated with the latter enzyme.[24] Some recent results have indicated that an anti conformation of the substrate can be an important requirement. Thus, 8-bromoadenosine which is known to be in a syn conformation[25] is not deaminated.[26] On the other hand, 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine which approximates a situation close to an anti conformation[27] is readily deaminated. The use of compounds **6** and **14** as models for investigation of substrate specificity of adenosine deaminase has some advantages over the aforementioned derivatives (both compounds are derived from the same parent nucleoside and approximate better the extreme situations in a rotameric composition of **1**—syn and anti conformation). We have found that anhyhdronucleoside **14** is completely resistant to adenosine deaminase whereas **6** was deaminated to **9** as shown by uv spectrum and paper chromatography. These results thus present additional evidence that adenosine deaminase requires the substrate to be in or close to an anti conformation. It is believed[28] that the 5′ or 3′-hydroxy group (the latter in the configuration cis to the base) is essential for activity in the adenosine deaminase-catalyzed deamination. The ready deamination of **6** along with the fact that adenine itself is deaminated[29] shows that neither the presence of the 5′ nor the 3′ ("up") hydroxy group consitutes an absolute requirement for activity. On the other hand, the anti conformation may well be such a requirement because an example of the substrate in a syn conformation is yet to be found.

It should also be stressed that formation of both anhydronucleosides **6** and **14** constitutes a further and unambiguous proof of the β configuration of formycin (**1**).

Experimental Section

General Methods. Evaporations were carried out in a Büchi rotary evaporator in vacuo at a bath temperature below 40° unless stated otherwise. Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Samples for analysis were dried for 8 hr at 10^{-3} mm over P_2O_5 at room temperature unless stated otherwise. Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. Thin-layer chromatography (TLC) was performed on 6 × 2 cm precoated silica gel F-254 aluminum foils (Merck, Darmstadt, Germany) in dichloromethane–methanol mixtures [9:1 (S_1), 4:1 (S_2), 1:1 (S_3)] and on glass plates covered with Avicel microcrystalline cellulose (see ref 9) (Brinkman Instruments, Inc., Westbury, N.Y.) containing 1% of fluorescent indicator (Leuchtpigment ZS Super, Riedel-De Haën, Hannover, Germany) in solvents S_4 (2-propanol–ammonium hydroxide–water, 7:1:2), S_5 (1-butanol–acetic acid–water, 5:2:3), and S_6 (1-butanol saturated with water). The solvents S_4 and S_5 were also used for descending chromatography on a Whatman No. 1 paper. Uv-absorbing compounds were detected using a Mineralight lamp. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Uv absorption spectra were obtained by using a Cary recording spectrophotometer or Beckman grating spectrophotometer Model DB-GT. The ir spectra were measured in a Perkin-Elmer Model 21 spectrometer (KBr pellets). NMR spectra were obtained using a Varian A-60A spectrometer with $(CH_3)_4Si$ as an internal standard in CD_3COCD_3 and as an external reference in CD_3SOCD_3. CD spectra were determined with a Jasco optical rotatory dispersion recorder, Model ORD/UV-5 in a CD modification SS-10 (Sproul Scientific, Boulder Creek, Calif.), between 500 and 200 nm in a 1-cm cell. Dimethylformamide dimethyl and dineopentyl acetals were products of Aldrich, Milwaukee, Wis. Formycin (formycin A) and formycin B were supplied by Meiji Seika Kaisha, Ltd., Tokyo, Japan.

2′,3′-*O*-Isopropylideneformycin (13). The described procedure[30] was modified[31] as follows. The solution of formycin (**1**, 1.07 g, 4 mmol), acetone (0.5 ml, 6.9 mmol), and ethyl orthoformate (1.2 ml, 8 mmol) in DMF (25 ml) was treated with 3.05 *M* HCl in DMF (2.62 ml, 8 mmol) and the solution was kept for 25 hr at room temperature. TLC (S_1) showed after elution and spectrophotometry of the corresponding spots a 68% conversion of **1** to **13**. After 4 days acetone (0.5 ml, 6.9 mmol) and ethyl orthoformate (0.6 ml, 4 mmol) were added followed by 3.05 *M* HCl in DMF (0.5 ml, 1.5 mmol) and the solution was kept for 2 days at room temperature. TLC (S_1) showed a quantitative conversion to **13**. The solution was then cooled to 0°, triethylamine (2 ml, 20 mmol) was added, and precipitated triethylamine hydrochloride was filtered off and washed with DMF (10 ml). The filtrate was evaporated at 0.1 mm and room temperature to a syrup which was then partitioned between water (25 ml) and dichloromethane (50 ml). The aqueous layer was extracted with dichloromethane (25 ml) and the combined organic layers were dried ($MgSO_4$) and evaporated to give a syrup, **13**. The aqueous layer was passed through the Dowex AG 1 (X2, 200–400 mesh, HCO_3^- form) column (5.5 × 3 cm) which was then eluted with 50% ethanol (400 ml). The eluate was evaporated to give a glassy (**13**) homogeneous compound, identical (S_1) with the syrup obtained from the organic layer. Both portions were combined and dissolved in dioxane (50 ml) and the solution was lyophilized. The resultant amorphous residue was dissolved in acetone (20 ml). Addition of ether (150 ml) precipitated 0.42 g (33%) of **13**. Further addition of petroleum ether gave 0.66 g (52%) of the same material. The melting point of both portions was not sharp and indicated an amorphous compound [lit.[27] gives 172–182° (hydrochloride)]: NMR (CD_3COCD_3) δ 8.08 (s, 1, H_5), multiplet centered at 5.03 (3, $H_{1'}$, $H_{2'}$, $H_{3'}$), 4.27 (m, 1, $H_{4'}$), 3.67 (t, 2, $H_{5'}$), 1.55 and 1.32 (2 s, 6, isopropylidene). Anal. Calcd for $C_{13}H_{17}N_5O_4 \cdot 0.5H_2O$: C, 49.36; H, 5.74; N, 22.14. Found: C, 49.39; H, 5.77; N, 21.51.

***N*:2′,3′-*O*-Bis(dimethylaminomethylene)adenosine (12a)** was prepared according to the described procedure[7b] as a syrup containing, according to the NMR, 1 mol of DMF: NMR (CD_3COCD_3) δ 8.92 [s, 1, CH= of $(CH_3)_2NCH$=], 8.42 and 8.38 (2 s, 2, H_8 and H_2, H_8 signal of exo diastereoisomer overlapped with H_2), 7.96 (s, 1, CH= of DMF), 6.33 and 6.17 (2 d, 1, $J_{1',2'}$ = 3 Hz, $H_{1'}$ of endo and exo diastereoisomer), 5.83 and 5.58 (2 s, 1, CH of acetal grouping, exo and endo diastereoisomers, respectively), 5.35 (m, 1, $H_{2'}$), 5.05 (m, 1, $H_{3'}$), 4.30 (m, 1, $H_{4'}$), 3.73 (d, 2, $H_{5'}$), 3.22 and 3.16 [2 s, 6, $(CH_3)_2N$ of $(CH_3)_2NCH$=N–], 2.90 and 2.77 [2 s, 6, $(CH_3)_2N$ of DMF], 2.40 and 2.30 [2 s, $(CH_3)_2N$ of acetal grouping belonging to endo and exo diastereoisomers, respectively].

***N*:2′,3′-*O*-Bis(dimethylaminomethylene)toyocamycin (12b) and *N*-dimethylaminomethylenetoyocamycin** were prepared according to the procedure[7b] described for **12a**. The solution of toyocamycin (0.38 g, 1.3 mmol) and dimethylformamide dimethyl acetal (0.6 ml, 6 mmol) in DMF (10 ml) was kept for 28 hr at room temperature whereupon it was evaporated at 0.1 mm and 50° (bath temperature) to a syrupy **12b** which according to NMR contained 1 mol of DMF: uv max (95% ethanol) 323, 231 nm; NMR (CD_3COCD_3) δ 8.84 [s, 1, CH= of $(CH_3)_2NCH$=N–], 8.33 (s, 1, H_6), 8.18 and 8.15 (2 s, 1, H_2 of endo and exo diastereoisomers), 7.95 (s, 1, CH= of DMF), 6.35 and 6.24 (2 d, 1, $H_{1'}$ of endo and exo diastereoisomers), 5.85 and 5.58 (2 s, 1, CH of acetal grouping of exo and endo diastereoisomers), 5.12 (m, 2, $H_{2'}$ overlapped probably with OH), ca. 4.82 and 4.32 (poorly resolved m, 2, $H_{3'}$ + $H_{4'}$), 3.78 (d, 2, $H_{5'}$), 3.23 [2 poorly resolved s, 6, $(CH_3)_2N$ of $(CH_3)_2NCH$=N–], 3.07 and 2.93 [2 s, 6, $(CH_3)_2N$ of DMF], 2.42 and 2.29 [2 s, 6, $(CH_3)_2N$ of acetal grouping of endo and exo diastereoisomers]. The syrup **12b** was kept overnight at room temperature. Ethanol (10 ml) followed by ether (50 ml) was added and the crystalline *N*-dimethylaminomethylenetoyocamycin was filtered off: 0.415 g (88%); mp 212°; homogeneous on TLC (S_2); uv max (95% ethanol) 322 nm (ϵ 23,900); ir (KBr) 2260 cm^{-1} (C≡N). Anal. Calcd for $C_{15}H_{18}N_6O_4 \cdot 0.75H_2O$: C, 50.06; H, 5.46; N, 23.36. Found: C, 49.70; H, 5.03; N, 23.23.

2,5′-Anhydroformycin (6). The solution of formycin (**1**, 325 mg, 1.22 mmol) was heated with dimethylformamide dineopentyl acetal (1 ml) in DMF (10 ml) for 5 hr at 125–130° (bath temperature). After cooling, the reaction mixture was evaporated at 0.1 mm and 30°, the whole procedure was repeated (9 hr), and the solution again evaporated. The residue was dissolved in dioxane (10 ml); Dry Ice (excess) followed by water (10 ml) was added. The resultant solution was kept for 1 hr at room temperature and then lyophilized. The resultant crude **4** was dissolved in a mixture of methanolic ammonia (saturated at 0°, 10 ml) and concentrated NH_4OH (30 ml). The solution was kept for 19 hr at room temperature whereupon it was evaporated to give solid **6** which was filtered off after addition of acetone (20 ml), 0.24 g (79%). Products from the two experiments had melting points above 265 and 295° dec, respectively; their uv, ir, and NMR spectra were identical. Recrystallization (105 mg) from water gave the analytical sample (67 mg), mp >305° dec which was dried at 100° and 10^{-3} mm: $[\alpha]^{24}D$ −73.4°, $[\alpha]^{24}_{436}$ −221.6°, $[\alpha]^{24}_{365}$ −633.8° (*c* 0.5, DMF); uv and CD (see Figures 1–4); NMR (CD_3SOCD_3) δ 8.48 (s, 1, H_5), 7.88 (br s, 2, NH_2), 5.91 and 5.50 (2 br m, 2, OH), 5.73 (s, 1, $H_{1'}$), ca. 4.8 (m, 5, ribose protons). Anal. Calcd for $C_{10}H_{11}N_5O_3$: C, 48.19; H, 4.45; N, 28.10. Found: C, 47.94; H, 4.73; N, 27.96.

***N*-Dimethylaminomethylene-2,5′-anhydroformycin (7).** The solution of formycin (**1**, 60 mg, 0.225 mmol) in DMF (3 ml) was heated with dimethylformamide dineopentyl acetal (0.3 ml) for 8 hr at 120° (bath temperature). After cooling, the reaction mixture was evaporated at 0.1 mm, the residue was dissolved in water (5 ml), and the solution was lyophilized. The resultant foam was dissolved in ethanol (1 ml), ether (5 ml) was added, and the solution cooled to −20° to give **7**: 5 mg (7.6%); mp 300–302° dec. Evaporation of the mother liquors and treatment of the residue with water gave an amorphous solid which was filtered off. The filtrate deposited on cooling to 0° an additional portion of **7**: 10 mg (15%); mp 308–310° dec; uv max (95% ethanol) 345, 278, 222 nm (ϵ 18,500, 4900, 16,000); NMR (CD_3SOCD_3) δ 9.27 [s, 1, CH= of $(CH_3)_2NCH$=N–], 8.70 (s, 1, H_5), 5.73 (s, 1, $H_{1'}$, partially overlapped with a broad band of 2-OH groups), ca. 4.7 (m, 5, ribose protons), 3.57 and 3.48 [2 s, 6, $(CH_3)_2N$].

2′,3′-*O*-Dimethylaminomethylene-2,5′-anhydroformycin (5). The solution of formycin (**1**, 270 mg, 1 mmol) in DMF (10 ml) was heated with dimethylformamide dineopentyl acetal (1 ml) for 10 hr at 130° (bath temperature). The reaction mixture was evaporated at 0.1 mm and the whole procedure was repeated (5.5 hr). After evaporation the residue was dissolved in concentrated

NH_4OH (25 ml) and the solution was kept for 54 hr at room temperature. The separated fine needles of **5** were filtered off and washed with water: 195 mg (78%); mp 230–231° dec. The sample for analysis was dried at 10^{-3} mm and 100°: mp 232–233° dec; uv was essentially identical with that of **6** (cf. Figure 2); NMR (CD_3SOCD_3) δ 8.47 (s, 1, H_5), 7.88 (br s, 2, NH_2), 6.12 and 5.85 (2 s, 1, CH of acetal grouping, exo and endo diastereoisomers), 5.93 (s, 1, $H_{1'}$), 5.47–4.88 (m, 5, $H_{2'}$, $H_{3'}$, $H_{4'}$, and $H_{5'}$), 3.66 (s, 2, H_2O), 2.70 and 2.59 [2 s, 6, $(CH_3)_2N$ of endo and exo diastereoisomers]. Anal. Calcd for $C_{13}H_{16}N_6O_3 \cdot H_2O$: C, 48.43; H, 5.63; N, 26.08. Found: C, 48.55; H, 5.19; N, 26.35. After drying at 100° immediately before the analysis a loss of 0.75 mol of H_2O was observed. Anal. Calcd for $C_{13}H_{16}N_6O_3 \cdot 0.25H_2O$: C, 50.56; H, 5.39; N, 27.22. Found: C, 50.32; H, 5.15; N, 27.37.

Conversion of 5 to 6. Compound **5** (175 mg, 0.543 mmol) was refluxed for 5 min in water and the filtered solution was kept overnight at room temperature to deposit 105 mg (78%) of **6**: mp 306–308° dec; ir, NMR, uv, and CD spectra correspond to those of an authentic sample.

***N*:2′,3′-*O*-Bis(dimethylaminomethylene)formycin (2) and *N*:2′,3′-*O*-Bis(dimethylaminomethylene)-2,5′-anhydroformycin (4).** Compound **2** was prepared according to a described[7a] procedure. Formycin (**1**, 50 mg, 0.19 mmol) was dried by evaporation with DMF (5 ml) at 0.05 mm and room temperature, the residue was dissolved in DMF (2 ml), dimethylformamide dineopentyl acetal (0.2 ml) was added, and the solution was kept for 16 hr at room temperature. Evaporation at 0.04 mm and room temperature afforded a syrup (**2**) which was coevaporated with DMF (5 ml) and dried at 0.05 mm at room temperature overnight. NMR (CD_3COCD_3) showed the presence of ca. 1 mol of DMF: δ 8.92 [s, 1, CH= of $(CH_3)_2NCH{=}N$-], 8.40 and 8.37 (2 s, 1, H_5 of exo and endo diastereoisomers, respectively), 7.95 (s, 1, CH= of DMF), 5.85 and 5.58 (2 s, 1, CH of acetal grouping, exo and endo diastereoisomers, respectively), multiplet centered at 5.03 (3, $H_{1'}$, $H_{2'}$, and $H_{3'}$), 4.26 (1, m, $H_{4'}$), 3.74 (2, t, $H_{5'}$), 3.27 and 3.18 [6, 2 s, $(CH_3)_2N$ of $(CH_3)_2NCH{=}N$-], 2.93 and 2.80 [6, 2 s, $(CH_3)_2N$ of DMF], 2.45 and 2.32 [6, 2 s, $(CH_3)_2N$ of acetal grouping, endo and exo diastereoisomers, respectively]. Product **2** was dissolved in DMF (2 ml), dimethylformamide dineopentyl acetal (0.2 ml) was added, and the solution was heated at 125–130° (bath temperature) for 5 hr whereupon it was evaporated and the whole procedure was repeated. The residue was evaporated after dissolving in DMF (10 ml) and the NMR spectrum of the resultant foam was taken in CD_3COCD_3: δ 8.84 [s, 1, CH= of $(CH_3)_2NCH{=}$], 8.32 (s, 1, H_5), 7.93 (s, CH= of DMF), 5.78 and 5.49 (2 s, 1, CH- acetal, exo and endo diastereoisomers), 5.58 (s, 1, $H_{1'}$), multiplet centered at 4.74 (5, $H_{2'}$, $H_{3'}$, $H_{4'}$, and $H_{5'}$), 3.28 and 3.22 [2 s, 6, $(CH_3)_2N$ of $(CH_3)_2NCH{=}N$-], 2.93 and 2.77 [2 s, $(CH_3)_2N$ of DMF], 2.40 and 2.28 [2 s, 6, $(CH_3)_2N$ of acetal grouping, endo and exo disastereoisomers].

2,5′-Anhydroformycin B (9). Formycin B (**8**, 0.27 g, 1 mmol) dried by dissolving in warm DMF (10 ml) and evaporating the cooled solution at 0.05 mm at room temperature was heated with dimethylformamide dineopentyl acetal (1 ml) in DMF (10 ml) for 5 hr at 125–130° (bath temperature). The solution was evaporated in vacuo as above and the residue was refluxed 5 min in water (10 ml). After cooling the separated white solid **9** was washed with water (10 ml) and dried over concentrated H_2SO_4 at room temperature and 0.02 mm: yield 195 mg (78%); mp >320°; chromatographically (S_4 and S_5) homogeneous; $[\alpha]^{25}D$ −48°, $[\alpha]^{25}_{436}$ −136°, $[\alpha]^{25}_{365}$ −324° (*c* 0.05, DMF); uv and CD spectra, see Figure 5; NMR (CD_3SOCD_3) δ 8.13 (s, 1, H_5), 5.65 (s, 1, $H_{1'}$), multiplet centered at 4.70 (5, $H_{2'}$, $H_{3'}$, $H_{4'}$, and $H_{5'}$). Anal. Calcd for $C_{10}H_{10}N_4O_4 \cdot 0.25H_2O$: C, 47.15; H, 4.16; N, 22.00. Found: C, 47.09; H, 4.34; N, 22.00. After drying at 100° immediately before the analysis, an anhydrous compound **9** was obtained. Anal. Calcd for $C_{10}H_{10}N_4O_4$: C, 48.00; H, 4.03; N, 22.39. Found: C, 48.24; H, 4.24; N, 22.12.

1-Methylformycin (10). The solution of formycin (**1**, 108 mg, 0.4 mmol) in DMF (5 ml) was heated with dimethylformamide dimethyl acetal (0.8 ml, 8 mmol) at 65–70° for 14 hr. The solution was evaporated, the syrupy residue was dissolved in concentrated NH_4OH (10 ml), and the solution was kept for 3 days at room temperature. After evaporation, crude **10** was coevaporated twice with ethanol and then with water to give a white solid: 105 mg (94%); mp 96–98° (transition point) [lit.[12] 170–173° (*foaming*)]; dec >200°; TLC (S_4, S_5) homogeneous. Crystallization from ethanol afforded 70 mg (62%) of **10**: melting point unchanged; homogeneous on TLC (S_2, S_3) and paper chromatography (S_4, S_5); $[\alpha]^{25}D$ −28.9°, $[\alpha]^{25}_{436}$ −61.3°, $[\alpha]^{25}_{365}$ −101.4° (*c* 0.49, CH_3OH); uv max (0.01 *N* HCl) 302, 238 nm (ϵ 10,300, 8900) [lit.[12] 302, 236 nm (ϵ 7030, 6320) at pH 1, 2-methylformycin 305, 270, 260, 231 (ϵ 11,240, 5900, 6050, 10,950) at pH 1]; NMR (CD_3SOCD_3) δ 8.38 (s, 1, H_5), 7.65 (br s, 2, NH_2), 5.23 (d, partially overlapped with OH groups, $J_{1',2'}$ = 7 Hz, $H_{1'}$), 4.53 (s, 3, partially overlapped with ribose protons, NCH_3), 4.9–3.9 ($H_{2'}$, $H_{3'}$, $H_{4'}$, and $H_{5'}$, poorly resolved); CD max (H_2O) 294, 240 nm ($[\theta]_{max}$ −3200, +3700). Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot 2H_2O$: C, 41.64; H, 6.04; N, 22.07; H_2O, 11.36. Found: C, 41.91; H, 5.62; N, 22.45; H_2O, 10.96. After drying at 80° and 10^{-3} mm anhydrous compound **10** was obtained. Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.72; H, 5.30; N, 25.09.

4,5′-Anhydroformycin (14) and 2,5′-Anhydroformycin (6). The solution of 2′,3′-*O*-isopropylideneformycin (**13**, 0.65 g, 2.12 mmol) in pyridine (10 ml) was treated with *p*-toluenesulfonyl chloride (freshly distilled, 0.44 g, 2.33 mmol) and the resultant mixture was kept for 24 hr at room temperature. Water (2 ml) was then added and the solution was evaporated at 0.1 mm and room temperature. The residue was dissolved in 50% ethanol (20 ml), the solution was put on the top of a Dowex 1, X2, 200–400 mesh column (HCO_3^- form, 16 × 1 cm), and the column was eluted with 50% ethanol (50 ml) and 50% pyridine (50 ml). The combined eluates were evaporated, and the residue was dissolved in acetone to give solid A (70 mg) which was shown (paper chromatography, S_4) to contain the 2′,3′-*O*-isopropylidene derivative of **14** (major product), the 2′,3′-*O*-isopropylidene derivative of **6**, and a trace of **14**. The filtrate was applied on one loose layer of silica gel and chromatographed in S_2. Isopropylidene derivatives of **6** and **14** failed to separate, giving a broad uv-absorbing and fluorescent band which was eluted with the solvent, S_2. The uv-absorbing materials from the origin and front were not further investigated. Both portions (solid A and the material from the preparative TLC) were combined and dissolved in 90% CF_3COOH (5 ml) and the solution was kept for 19 hr at room temperature and then lyophilized. The residue was dissolved in methanol (50 ml) and the solution was passed through a Dowex 1 column (HCO_3^- form, 3.5 × 2 cm) which was eluted with water. The residue was dissolved in warm 50% ethanol and applied on 11 plates of Avicel (20 × 20 cm) which were developed with the solvent S_4. Bands of **14** (slower, strongly fluorescent) and **6** (faster, fluorescent) were eluted with water; traces of the isopropylidene derivatives of **14** and **6** traveling near the solvent front were discarded. The eluates were evaporated to give 100 mg (19%) of **14** and 35 mg (7%) of **6**, both as chromatographically (S_4) homogeneous solids. For analysis **14** was crystallized from 50% ethanol (7 ml, turbidity was removed by hot filtration through Celite) to give 60 mg (11%): mp 295° dec; $[\alpha]^{25}D$ −55°, $[\alpha]^{25}_{436}$ −209.3°, $[\alpha]^{25}_{365}$ −171.3° (*c* 0.047, DMF); uv and CD spectra, see Figures 1–4; NMR (CD_3SOCD_3) δ 8.64 (s, 1, H_5), 5.70 (s, 1, $H_{1'}$), 5.03–4.12 (the rest of ribose protons and OH groups, poorly resolved). Anal. Calcd for $C_{10}H_{11}N_5O_3 \cdot 0.5H_2O$: C, 46.51; H, 4.68; N, 27.12. Found: C, 46.87; H, 4.62; N, 27.30. After drying at 100° immediately before the analysis 0.25 mol of H_2O was lost. Anal. Calcd for $C_{10}H_{11}N_5O_3 \cdot 0.25H_2O$: C, 47.33; H, 4.57; N, 27.60; H_2O, 1.78. Found: C, 47.05; H, 4.54; N, 27.70; H_2O, 1.88.

Crystallization of **6** from water (8 ml) gave 8 mg (1.5%): mp >295° dec; identical (paper chromatography, uv, CD) with an authentic specimen.

Behavior of Anhydronucleosides 6 and 9 in Borate Buffer. Compounds **1**, **6**, **8**, and **9** were subjected to paper electrophoresis in 0.02 *M* $Na_2B_4O_7$ (pH 9) on a Whatman No. 1 paper for 1 hr at 40 V/cm. Anhydronucleosides **8** and **9** afforded two distinct spots, A and B; for mobilities and ratios, see Table III. Compound **14** gave only a single spot. The ratio of A and B (obtained from **6**) was unchanged irrespective to the origin of the sample (prepared either directly from **1** or from the isopropylidene derivative **13**). In a micropreparative experiment compound **6** (1.4 mg) was subjected to electrophoresis under the above conditions on a 17 cm wide strip of paper. The bands of A and B were eluted with water, the eluates lyophilized, the residues dissolved in 50% pyridine, and the solutions passed through Dowex 50 W X4 columns (200–400 mesh, pyridinium form). The eluates were evaporated and the products chromatographed in S_4 on Whatman No. 1 paper. The single uv-

absorbing compounds have uv and CD spectra identical with those of **6**. Each of them, on paper electrophoresis in borate (see above), gave two spots of the same ratio as the original compound **6**.

Deamination of 6 with Calf Intestine Adenosine Deaminase. Compound **6** (0.05 mg, 2 μmol) was dissolved[32] in 0.05 *M* K_2HPO_4 (pH 7.5, 0.2 ml). Enzyme (Type II, Sigma Chemical Co., St. Louis, Mo.) stock solution was then added (0.2 ml, 2 mg of enzyme per 1 ml of 0.02 *M* ammonium acetate, pH 8.1) and the mixture was incubated at 37° for 24 hr. The whole solution was then applied on Whatman 3 MM paper and chromatographed in S_4 (double development). The spots of the product **9** and starting **6** were eluted with 0.01 *N* HCl. Uv spectrophotometry of the eluates established the degradation was ca. 85% complete. Under the same conditions compound **14** remained unchanged. Using a more active enzyme preparation (Calbiochem, Los Angeles, Calif., 0.4 mg of protein) the deamination of **6** was quantitative after 6 hr under otherwise identical conditions. The product **9** was identical (uv) with that of an authentic sample.

Note Added in Proof. Our CD spectrum of formycin B (Figure 5) differs considerably from that reported in the literature. The latter shows a positive Cotton effect at ca. 250 nm (W. Guschlbauer, *Jerusalem Symp. Quantum Chem. Biochem.*, **4,** 297 (1972). Although the reason for this discrepancy is not yet clear, it should be pointed out, however, that the reported CD_{max} does not correlate with the uv_{max} of formycin B.

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References and Notes

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(21) The reported ORD curve[22a] at pH 7.6 shows a small positive Cotton effect whereas CD spectra at pH 7[22b] show a strong positive Cotton effect at ca. 265 nm. Furthermore, it has been reported[22a] that 3,5′-anhydro-2′,3′-*O*-isopropylideneadenosine iodide (also derived from the syn conformer of adenosine) has a negative Cotton effect at pH 7.6 although in an earlier paper[22c] the same compound was found to exhibit a positive Cotton effect in water. These differences are difficult to explain although the latter compound may not be sufficiently stable above pH 7 (cf. ref 22a). It is also of interest to note that 8,5′-anhydroadenosine which structurally resembles 2,5′-anhydroformycin (**6**) and in which the anti conformation of the base residue is fixed also exhibits a negative Cotton effect.[22c]

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