Fluorescent Derivatives of Nucleosides

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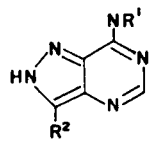
Fluorescence spectra of formycin anhydronucleosides **5**, **6**, **8** and of *N*-dimethylaminomethylene ribonucleosides **1b**, **2**, **3a-3c** and **4** in aqueous solution at 5-7 x 10^{-5} *M* are reported. Compounds **5** and **6** exhibit a very strong fluorescence emission, *ca.* 4 and 2 times more intense than that of formycin (**1a**) accompanied by a bathochromic shift of the emission maximum. Anhydronucleoside **8** also has an increased fluorescence intensity over the parent nucleoside **7**. The level of fluorescence emission is lower in **7** and **8** than in **1a**, **5** or **6**. Introduction of *N*-dimethylaminomethylene group into **1a** (compound **1b**) caused a decrease in the fluorescence intensity relative to **1a** but a bathochromic shift of the emission maximum. In other instances (compounds **2**, **3a-3c**, **4**) the introduction of *N*-dimethylaminomethylene group led also to fluorescent derivatives. This effect is most pronounced with **2**, whereas the fluorescence intensity of the rest of the group (**3a-3c** and **4**) is much lower. Compounds **3c** and **4** exhibit, however, a profound bathochromic shift in the fluorescence emission maximum relative to **2**, **3a** or **3b**. A possible relationship of the fluorescence emission to the base conformation in formycin and potential use of *N*-dimethylaminomethylene nucleoside and nucleotide derivatives as fluorescent probes are discussed.

J. Heterocyclic Chem., **14**, 135 (1977).

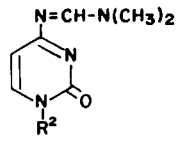
Current interest in fluorescent nucleosides stems in part from their utility in nucleic acid chemistry and biochemistry. Available fluorescent structures include: (a) naturally occurring compounds such as formycin (**1a**) (1) and the nucleoside of the Y base from tRNA (2) and (b) fluorescent derivatives obtained by a suitable chemical modification of the parent nucleoside such as 2-aminonebularine (1), 7-methylguanosine (3) and others (4,5). Formycin (**1a**) is the most widely used fluorescent nucleoside presumably because it is both readily available and can be relatively easily incorporated into nucleic acids (tRNA) (1,6). Among the compounds obtained by chemical modification are the etheno derivatives of adenosine, cytidine (7) and some related derivatives (8,9) which have found an extensive use (10,11). However, the choice of the proper fluorescent nucleosides is restricted and a clear need exists for derivatives which would supplement currently accessible compounds. The present work reports the fluorescence spectra of some additional modified nucleosides.



1

1a : $R^1 = H_2$

1b : $R^1 = (CH_3)_2N-CH=$



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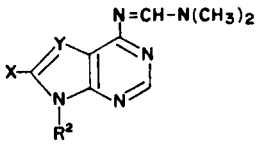
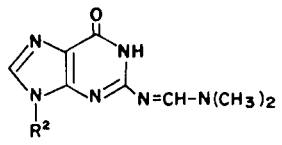


3

3a : X = H, Y = N

3b : X = Br, Y = N

3c : X = H, Y = CH

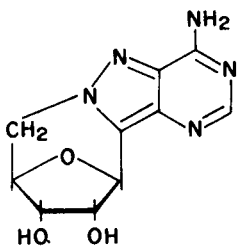


4

In formulae 1–4

R^2 = β-D-ribofuranosyl

Our interest centered on two groups of nucleoside derivatives: *N*-dimethylaminomethylene nucleosides (12) and formycin anhydronucleosides (13) **5**, **6** and **8**. In the latter case, additional stimulus for a study of fluorescence is provided by the fact that nucleosides **5** and **6** are models of formycin (**1a**) "frozen" in the *anti* and *syn* conformation, respectively (14). Compound **8** mimics the *anti* conformation of formycin B (**7**) (13,14).



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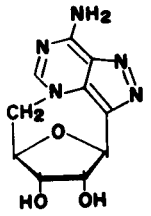
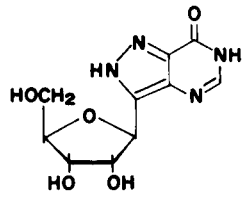


6



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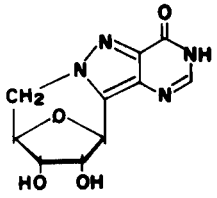

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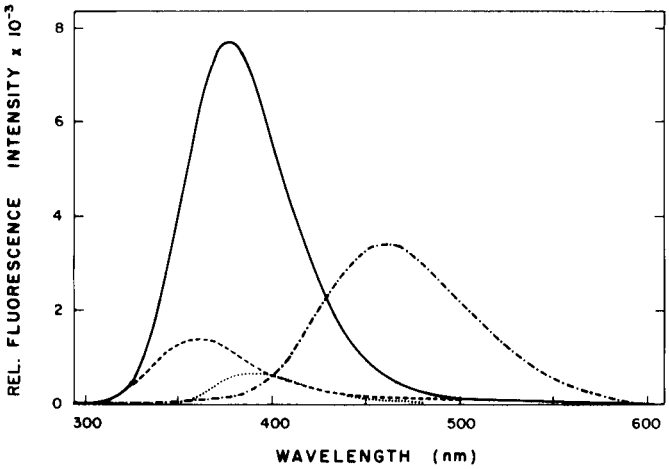

Figure 1. Water, ------- **1a**, · · · · **1b**, —— **5**, ·------· **6**.

Anhydronucleosides **5** and **6** gave the most intense fluorescence spectra of the group of compounds studied (Figure 1). Not only is the considerably enhanced intensity of the fluorescence emission of **5** and **6** relative to formycin (**1a**) of interest but also the fact that nucleoside **5** derived from the *anti* conformation of **1a** exhibits a much higher relative fluorescence intensity than its *syn* counterpart **6**. On the other hand, the fluorescence emission maximum of **6** shows a profound bathochromic shift relative to both **5** and formycin (**1a**) (15). The increased intensity of the fluorescence emission may be associated with the relative rigidity of the molecule (chromophore) with respect to formycin (**1a**) where the rotation of the base is hindered but, unlike in **5** or **6**, by no means precluded (13,14). It would be of particular interest to discuss whether the observed differences in fluorescence between **5** and **6** may be attributed, at least partly, to the changed orientation of the chromophore in both compounds. Other factors such as intramolecular alkylation by C-5′ in different positions of the base and differences in the strain and electron distribution in **5** and **6** must also be considered. It is important to note that introduction of the *N*-dimethylaminomethylene group into **1a** (compound **1b**) led to a distinct decrease of the fluorescence intensity, whereas the opposite effect was observed with other *N*-dimethylaminomethylene derivatives (*vide infra*). Formycin B (**7**) is also fluorescent (16) although to a lesser degree than **1a** (Figure 2). The corresponding anhydronucleoside **8**

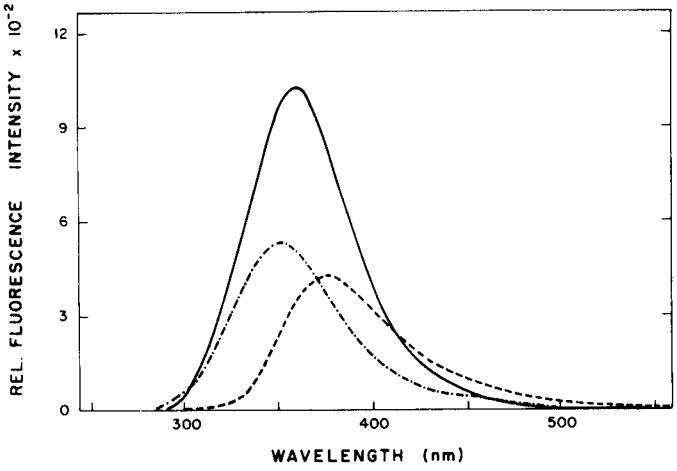

Figure 2. Water, ---------- **2**, ·---------· **7**, ———— **8**.

exhibits a slight bathochromic shift of the fluorescence emission maximum (this is similar to **5**) but the increase in the fluorescence intensity is smaller.

In acid (0.01 *N* hydrochloric acid) the fluorescence intensity of **5**, **6**, **7** and **8** (Figure 3) is decreased as observed with formycin (**1a**) (1). This effect is particularly pronounced in **1a**, **5** and **6** whereas in less basic nucleosides **7** and **8** the corresponding change is much smaller. In the case of **6**, the decrease in the fluorescence intensity is accompanied by the appearance of two fluorescence emission maxima. It is of interest to note that the fluorescence spectrum of formycin (**1a**) in water is more similar to that of anhydronucleoside **5** than to **6** (Figure 1). On the other hand, the situation in acid is reversed: the fluorescence spectrum of **1a** resembles more that of **6** than **5** (Figure 3). A similar pattern was observed previously (13) in the corresponding uv and CD spectra. It is possible that such a trend may reflect a conforma-

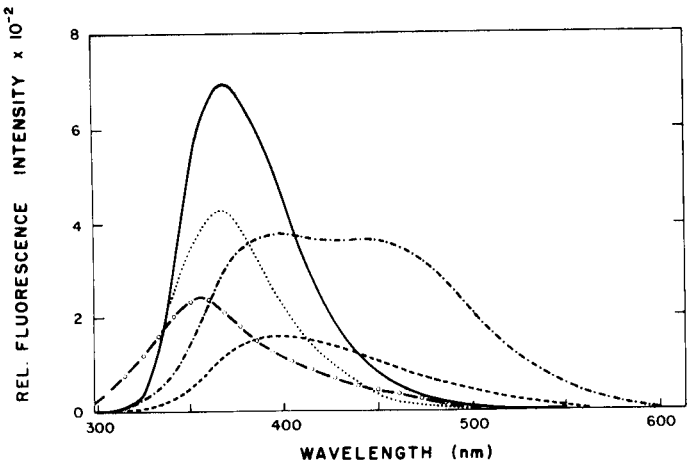

Figure 3. 0.01 *N* Hydrochloric acid, ------- **1a**, —— **5**, ... **6**, -O-O-O-O **7**, ... **8**.

tional shift of formycin (**1a**) toward the *syn* form in acid (13,17).

N-Dimethylaminomethylene derivatives gave all measureable fluorescence emissions at concentrations 5-7 x 10^{-5} *M* (Figures 2 and 4). However, with the exception of the formycin derivative **1b** the intensity of the fluorescence emission was considerably below the values observed with **1a**, **5** or **6**. Thus, *N*-dimethylaminomethylenecytidine (**2**) has *ca.* 4 times lower fluorescence intensity than formycin (**1a**). Even lower values were observed with other *N*-dimethylaminomethylene derivatives such as **3a-3c** and **4**. It should be noted, however, that compounds **3c** and **4**, despite a rather low fluorescence intensity, have their emission maxima at considerably longer wavelengths. They are also more stable (18) than *N*-dimethylaminomethylenecytidine (**2**). These considerations, along with the fact that a simple fluorescent modification for the guanine moiety would be highly desirable, make both derivatives attractive as possible fluorescent probes. It should also be noted that the preparation of a variety of *N*-dimethylaminomethylene derivatives of nucleotides and oligonucleotides is extremely simple (19-21). Of interest is a lack of substantial differences (Figure 4) in the intensity of fluorescence emission between **3a** and **3b** (the latter is assumed to be in a *syn* conformation (22) as compared with models with fixed *anti* and *syn* conformation **5** and **6**).

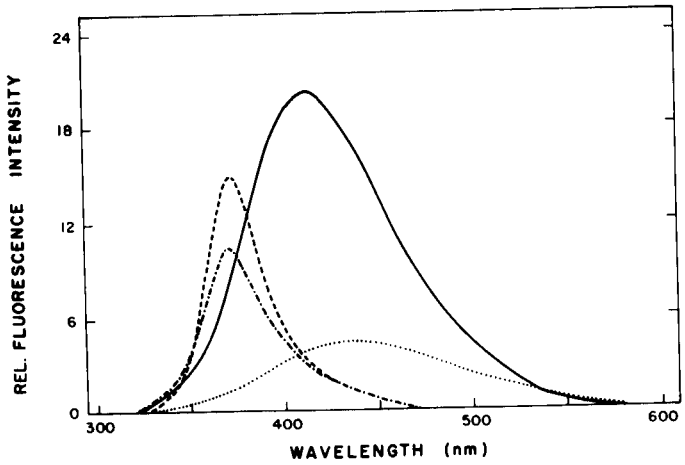

Figure 4. Water, ------- **3a**, ------- **3b**, —— **3c**, ... **4**.

EXPERIMENTAL

All compounds studied were prepared according to the described procedures (12,13,23,24). Formycin and formycin B were supplied by Meiji Seika Kaisha, Ltd., Tokyo, Japan. Uv spectra were obtained using a Cary recording spectrophotometer Model 11 or Beckman grating spectrophotometer Model DB-GT.

The fluorescence spectra were determined at concentrations 5-7 x 10^{-5} *M* in aqueous solutions at room temperature on a Baird-Atomic fluorescence spectrophotometer Model SF-100 equipped with a Houston Instrument Omnigraphic 2000 recorder. The fluorescence spectra of the studied compounds were obtained by excitation at their maximum excitation wavelengths except for compounds with low fluorescence intensities. For these compounds, the suitable excitation wavelengths were chosen in order to eliminate the Rayleigh and Raman scattering of the fluorescence spectra. The excitation and emission wavelengths and relative fluorescence intensities were corrected using those of quinine sulfate in 0.1 *N* sulfuric acid. The lack of solvent fluorescence and scattering was ascertained by control runs at the appropriate excitation wavelengths.

Uv spectra in water corresponded to those in the literature (12,13,23). Compound **1b** has λ max 333 and 272 nm, respectively.

Fluorescence spectra in 0.01 *N* hydrochloric acid were measured only with derivatives **5**, **6** and **8** and the corresponding reference compounds **1a** and **7** because of a limited stability of *N*-dimethylaminomethylene function in dilute acids (12,18). The results are summarized in Figures 1-4.

Acknowledgments

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(14) For a definition of the *syn* and *anti* conformation of formycin see: D. C. Ward and E. Reich, *Proc. Nat. Acad. Sci. USA*, **61**, 1494 (1968).

(15) This is probably the reason why according to our preliminary qualitative observations (13) made on paper chromatograms **6** seemed more fluorescent than **5**.

(16) Literature (1) reported that fluorescence of formycin (**1a**) was completely abolished by adenosine deaminase (formation of **7**).

(17) A referee suggested that the occurrence of two fluorescence maxima in the spectrum of **6** can be explained by an equilibrium between protonated and unprotonated forms.

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(24) Compound **1b** was first prepared in our laboratory by Dr. S. Chládek using the general method (12).