of the Nicolet NT-300 spectrometer used to obtain some of the carbon spectra. Dr. K. Angelides kindly allowed the use of the phase fluorimeter; measurements were made by D. Marriott.

Registry No. $1 \cdot 2ClO_4^-$, 73333-47-8; $1 \cdot 2Cl^-$, 73333-48-9; **2**, 504-29-0; **3**, 86045-92-3; **3** conjugate acid, 86045-93-4; **5**, 86064-59-7; **6**, 86045-95-6; **6** conjugate acid, 86045-96-7; **7**, 86045-97-8; **8**, 86045-99-0; **8** conjugate acid, 86046-00-6; **9**, 86046-02-8; **9** conjugate acid, 86046-03-9; **10**, 86046-05-1; **11**, 86046-07-3; **12**, 86046-09-5; 2-methoxypyridine, 1628-89-3; 2-amino-6-methylpyridine, 1824-81-3; pyridine, 110-86-1; 2-amino-3-methylpyridine, 1603-40-3; 2-amino-4-methylpyridine, 695-34-1; 2-amino-5-bromopyridine, 1072-97-5; 2-aminothiazole, 96-50-4; thiourea, 62-56-6.

New Highly Fluorescent Derivatives of Cytidine and Cytosine

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Cytidine and cytosine react with 1′-methylthiaminium ion in methanol containing 2,4,6-trimethylpyridine catalyst. Products contain a tricyclic dipyrimido[1,6-*a*:4′,5′-*d*]pyrimidine ring which is highly fluorescent, having emission bands at 414 and 433 nm (shoulder) with the main excitation peak at 390 nm in water. The nucleosides inosine, guanosine, and xanthosine under the same conditions only alkylate, the first two at N-1 and N-7 and the latter at N-7. Uracil similarly gives the 1,3-dialkylated product. The alkyl group is the (4-amino-1,2-dimethyl-5-pyrimidinio)methyl portion of the thiamin.

Fluorescent compounds can often be detected in very low concentrations. Consequently, many seek to convert nonfluorescent natural products which may be present only at low levels into fluorescent derivatives in order to facilitate detection and even quantitation.[1]

Some success has been achieved in converting nucleic acid components, most of which are nonfluorescent under ambient conditions,[2] into fluorescent derivatives. Most notable are the light-emitting derivatives of adenosine.[3-5] Although cytidine (**1**) and cytosine (**2**) (Chart I) have been converted to fluorescent analogues,[3,6,7] these derivatives have limitations.

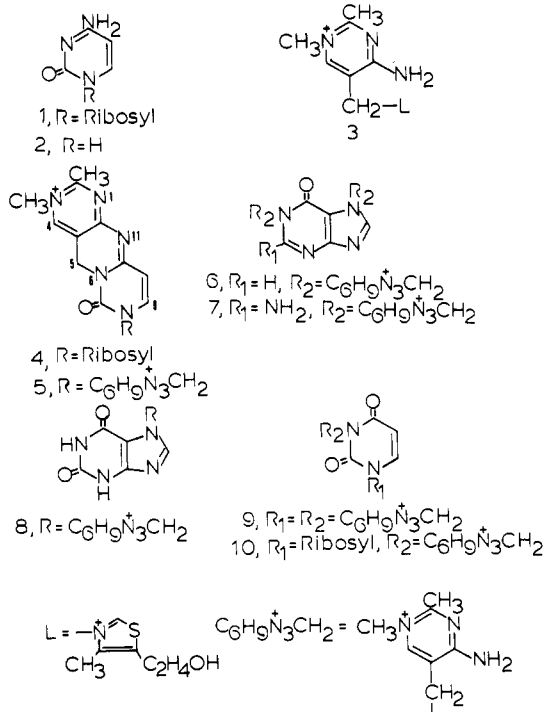
We report very promising results which may remedy deficiencies for both **1** and **2**. Both are converted to highly fluorescent linear tricyclic derivatives in a reaction with 1′-methylthiaminium ion (**3**).[8]

Results and Discussion

Cytidine (**1**) or cytosine (**2**) when heated in methanol containing 2,4,6-trimethylpyridine catalyst and the vitamin B_1 derivative 1′-methylthiaminium ion[8] (**3**) rapidly react to yield tricyclic fluorescent products.

The structures of these products may be assigned from a knowledge of the way in which they are formed. Previously, we have shown that a wide variety of nucleophiles reacts with **3** in substitution reactions which lead to the displacement of the thiazole leaving group (L).[9] When the nucleophile is ambident as in the case of adenosine[5] or 2-aminopyridines,[10] the annular nitrogen atom of the nucleophile, not the adjacent amino group, reacts first in an alklation process. This substitution then is followed by cyclization which proceeds by the loss of an amino group as ammonia. Thus, tricyclic structures **4** and **5** may be assigned, respectively, to the products from **1** and **2**.

Chart I



(1) Seitz, W. R. *CRC Crit. Rev. Anal. Chem.* **1980**, *8*, 367.
(2) Gueron, M.; Eisinger, J; Lamola, A. A. In "Basic Principles in Nucleic Acid Chemistry"; Ts'O, P. O. P., Academic Press: New York, 1974; Chapter 4, Vol. I.
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(9) Zoltewicz, J. A. *Synthesis* **1980**, 218.
(10) Zoltewicz, J. A.; Baugh, T. D.; Paszyc, S.; Marciniak, B. *J. Org. Chem*; preceding paper in this issue.

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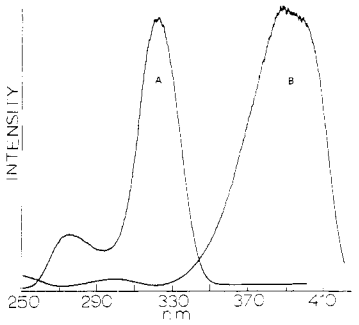

Figure 1. Excitation spectra of 2,3-dimethyl-8-ribosylcytosichrominium ion (**4**) in water: A, 1.1×10^{-3} M; B, 1.4×10^{-7} M. The emission detector is at 435 nm for both. Relative intensities are not to scale.

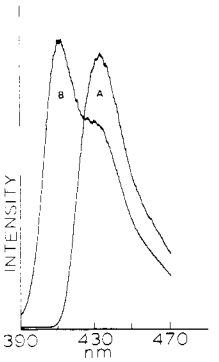

Figure 2. Emission spectra of **4** in water. Concentrations of A and B are the same as those in Figure 1. Sample A was excited at 325 nm and B at 360 nm. Relative intensities are arbitrary.

Cytosine product **5** also contains a quarternized pyrimidinylmethyl group ($C_6H_9N_3^+CH_2$) resulting from reaction with a second equivalent of **3** acting as an alkylating agent.

Structures **4** and **5** are completely consistent with their proton and carbon NMR chemical shifts[11] and with their absorption, excitation, and fluorescence emission spectra. Similar proton and carbon chemical shifts are observed for our fluorescent adenosine derivative[5] which has two pyrimidine rings, one quaternized and the other dihydro, common to **4** and **5**.

Systematic names for **4** and **5** are indicated in the Experimental Section. Because they are so cumbersome we propose a trivial name of cytosichromine for the parent tricyclic ring, reflecting its formation from the cytosine ring and its fluorescent property. Thus **4** might be named 2,3-dimethyl-8-ribosylcytosichrominium ion. Such a name would join thiochrome[12] for the uncharged tricyclic fluorescent derivative of thiamin and also 9,10-dimethyl-3-ribosyladenichrominium ion[5] for the fluorescent derivative of adenosine.

The fluorescence of **4** exhibits considerable self-quenching when solutions are concentrated. Thus, the spectra of 1.1×10^{-3} M **4** in water show excitation bands at 275 and 323 nm and emission at 434 nm. On dilution to 1.4×10^{-7} M in the same solvent these change to 299 and 393 nm for excitation and 413 and 430 nm (shoulder) for emission (Figures 1 and 2). The spectra are not highly solvent dependent; a 1.4×10^{-7} M solution of **4** in acetonitrile has excitation bands at 295 and 393 nm and emission at 415 and 435 nm (shoulder). The fluorescence from a 3×10^{-9} M solution in water could easily be detected, exciting at 390 nm. Moreover, the excitation bands for a 7.0×10^{-6} M solution of protonated **4** in 1 M perchloric acid shift to 227 and 353 nm, but the emission is unchanged, suggesting that the excited state of protonated **4** is more acidic than the ground state and that emission comes from the unprotonated excited state.

Similar observations have been made on aqueous solutions of **5**. A 6.7×10^{-4} M solution has excitation and emission bands at 320 and 433 nm, respectively. However, a 3.8×10^{-6} M solution shows excitation at 249 and 390 nm and emission at 415 nm with a shoulder at 433 nm. This excitation spectrum is similar to the absorption spectrum. Further dilution brings about no additional changes. The detection limit of **5** seems to be slightly higher than that of **4**, about 8×10^{-9} M.

Other Heterocycles. Inosine, guanosine, and xanthosine nucleosides react with **3** in the presence of the pyridine catalyst to give alkylation products **6**–**8**, respectively. Cyclization did not follow N-alkylation. Deribosidation took place when products were being purified.[13,14] Hypoxanthine **6** and guanine **7** are dialkylated while xanthine **8** is monoalkylated with the (4-amino-1,2-dimethyl-5-pyrimidinio)methyl group. Uracil similary yields dialkylated product **9**.

The classical problem of proving the site of alkylation of purine and pyrimidine rings has now been solved many ways, magnetic resonance techniques being expecially powerful. The N-3 position of the nucleosides is too sterically hindered by the N-9 ribosyl group to allow reaction with the bulky electrophile formed as an intermediate from **3**.[16] Therefore, potential reactive sites include annular nitrogen atoms (1 and 7 of the purines and 1 and 3 of the pyrimidine), the carbonyl oxygen atom, and the amino group of guanosine. The high-field position, about 42 ppm, for the $^{13}CH_2$ portion of the $C_6H_9N_3^+CH_2$ group of the purine products eliminates O-alkylation as a possibility.[17] Therefore, inosine must have undergone alkylation at N-7 and N-1, giving rise to hypoxanthine **6** on deribosidation. The similarity in the ^1H and ^{13}C chemical shifts of the two CH_2 groups found with the hypoxanthine and guanine dialkylation products **6** and **7** indicate that guanosine also alkylated at N-7 and N-1.[14]

The site of monoalkylation of xanthosine can be assigned by a consideration of the chemical shift of the CH_2 protons and literature data. Proton shifts associated with NCH_3 groups bonded to xanthine[18] and hypoxanthine rings[19] are characteristic of the site of attachment. Thus, the signal of N_7CH_3 is deshielded with respect to that of N_1CH_3.[18,19] Therefore, we may assign the low field signals at δ 5.44 and 5.42 to the N_7CH_2 group of **6** and **7**, respectively, and those at δ 5.04 and 4.98 to the N_1CH_2 substituent of **6** and **7**. Consequently, the signal at δ 5.35 for the monoalkylated xanthine indicates bonding to N-7 to give **8**.

Dialkylated uracil must have structure **9**. Proton chemical shifts of the two CH_2 groups are identical, eliminating mixed O- and N-alkylation as a possibility. The high-field positions, δ 45.24 and 37.40, for the $^{13}CH_2$ group

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(17) The ^{13}C shift of the CH_2 group of $C_6H_9N_3^+CH_2$ bonded to the oxygen atom of phenols[9] is about 61–63 ppm.
(18) Lichtenberg, D.; Bergman, F; Neiman, Z. *J. Chem. Soc. C* **1971**, 1676. Bergman, F.; Chen, G. W.; Rahat, M. *J. Chem. Soc., Perkin Trans. 1* **1976**, 90.
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are only consistent with N-alkylation.[17] Again, only N-alkylation is observed for the ambident nucleophile.

Spectra. Of the four alkylated bases **6–9** only a sample of guanine derivative **7** demonstrates significant fluorescence. A 1.0×10^{-5} M solution of **7** in water has an excitation peak at 365 nm and a very broad emission band with a maximum at about 445 nm, both being very weak. Acidification of a more concentrated sample with perchloric acid results in some quenching. Although alkylated guanines are known to fluoresce,[20] the emission from our sample probably comes from an unidentified impurity. (1) The product was difficult to purify to a constant melting point. (2) The emission occurs at a considerably longer wavelength than that of methylated guanines.[20] (3) Excitation and absorption spectra differ considerably. (4) When the concentration of purified **7** was increased so that the short wavelength absorption was off scale, an absorption peak at 363 nm, corresponding to the excitation peak, could be detected.

The contaminant has spectral properties similar to those of an aqueous solution of thiochrome, a thiamin oxidation product, excitation and emission falling at 365 and 450 nm, respectively.[21] But acidification of thiochrome leads to strong quenching[21] and shifting of excitation[12] and emission peaks. The impurity in **7** does not show such changes and therefore is not thiochrome.

Hydrolysis. In order to determine the stability of the fluorescent derivatives toward hydrolysis, we conducted some preliminary experiments. Tricyclic compound **4** is slowly attacked by aqueous alkali. A borate pH 9.18 buffer at 24 °C caused the intense broad absorption peak at 380 nm to become very weak in intensity. The half-life is 1.4 h. Under similar conditions **5** demonstrated similar spectroscopic changes and has a half-life of 33 min; a second slower process follows. Therefore, high pH should be avoided.

The alkaline hydrolysis products have not yet been identified, but spectral changes suggest that dialkylated uracils **9** and **10** may form from **5** and **4**, respectively. Addition of hydroxide ion to the C-10a position of fluorescent tricycle is expected to give rise to the suggested uracils. Although the short wavelength portion of the spectrum of the hydrolysis mixture does resemble that of authentic **9**, another band of very low intensity is present at 370 nm from **4** and **5**. Uracil **9** does not contain such an absorption peak. This absorption slowly disappears from the hydrolysis spectrum of **5**.

Uracil **9** also is slowly attacked by alkali. Under the same conditions used to examine the tricyclic materials, **9** has a half-life of 5.6 h. Comparision with 1,3-dimethyluracil, another N,N-dialkylated relative, reveals that **9** is far more labile toward alkaline hydrolysis.[22]

Outlook. Examination of the reactions of **3** with the purine and pyrimidine rings commonly found in nucleic acids shows that only the ribosides and presumably the deoxyribosides of adenine[5] and cytosine give products which are highly fluorescent under ambient conditions. The guanine ring which might have formed a fluorescent derivative in a cyclization reaction involving the 2-amino group following N-1 alkylation[23,24] did not do so to give a major product. Clearly, cyclization with **3** serves as a useful method of converting cytosine, cytidine, and adenosine[5] into highly fluorescent derivatives. Fluorescent products from the latter two can be detected easily at the 5×10^{-9} M level in water.

Tricycle **4** may well be the best fluorescent derivative of cytidine yet prepared. It clearly is superior to $3,N^4$-ethenocytidine,[3] a derivative produced by cyclization with toxic, carcinogenic chloroacetalydehyde,[25] in detection limits, in its stability toward hydrolysis, and in its emission spectrum.[26] Whereas **4** emits in the visible region well away from other natural products such as proteins, the ethenocytidine does not.[3] Moreover, the reagent **3** used to form **4** is easily prepared[8] and is nontoxic.

It would be worthwhile to test natural materials such as plant and animal tissue with **3** to determine whether cytosichrominium ions **4** and **5** may be formed and their presence quantified.

Experimental Section

2,3-Dimethyl-7-oxo-8-ribosyl-7,8-dihydro-5*H*-dipyrimido[1,6-*a*:4′,5′-*d*]pyrimidinium Perchlorate (4). A mixture of 2.43 g (10.0 mmol) of cytidine, 6.2 g (13 mmol) of 1′-methylthiaminium diperchlorate,[8] 4 mL of 2,4,6-trimethylpyridine, and 125 mL of methanol was heated at reflux for 2 h. The precipitated yellow solid was filtered and washed with absolute ethanol to give 4.7 g (9.8 mmol, 98%) of raw product, mp 183–186 °C. Recrystallization from 50% ethanol–water gave the analytical sample of **4** (mp 193–195 °C) which was dried at room temperature under vacuum: ^{1}H NMR (Me_2SO-d_6–D_2O, Me_4Si) δ 8.32 (H-4), 8.24 (d, H-9, J = 8 Hz), 6.14 (d, H-10, J = 8 Hz), 5.75 (d, 1′-ribose), 5.10 (H-5), 4.0–3.7 (band, NCH_3 and ribose), 2.66 (CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 163.9, 160.4, 147.7 (3 s), 145.8, 142.2 (2 d), 112.5 (s, C4a), 101.9 (d, C10), 90.1, 84.4, 73.8, 68.6 (4 d, ribose), 59.6 (t, CH_2OH), 42.8 (q, NMe), 41.4 (t, NCH_2), 21.8 (q, CMe); one C signal in the δ 148–164 region either is overlapped or of insufficient intensity to be detected; UV (pH 6.86) 220 nm (log ϵ 4.25), 235 (4.15), 250 (3.94), 382 (4.53); UV (0.1 M $HClO_4$) 223 nm (log ϵ 4.31), 347 (4.50); UV (CH_3CN) 390 nm (log ϵ 4.52). Anal. Calcd for $C_{16}H_{20}N_5O_9Cl \cdot H_2O$: C, 40.05; H, 4.62; N, 14.60. Found: C 40.02; H, 4.63; N, 14.60.

8-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]-2,3-dimethyl-7-oxo-7,8-dihydro-5*H*-dipyrimido[1,6-*a*:4′,5′-*d*]pyrimidinium Diperchlorate (5). A mixture of 0.154 g (1.39 mmol) of cytosine, 1.0 g (2.1 mmol) of 1′-methylthiaminium diperchlorate,[8] 0.5 mL of 2,4,6-trimethylpyridine, and 20 mL of methanol was heated at reflux for 2.5 h. The precipitated yellow solid was filtered and washed with absolute ethanol to give 0.68 g (1.17 mmol, 84%) of crude product, mp 250–256 °C dec. Recrystallization from water gave the analytical sample of **5** (mp 255–257 °C dec) which was dried at room temperature under vacuum: ^{1}H NMR (Me_2SO-d_6–D_2O, Me_4Si) δ 8.15 (H-4), 7.98 (d, H-9, J = 8 Hz), 6.21 (d, H-10, J = 8 Hz), 5.10, 4.86 (5-CH_2 and N_8CH_2), 3.90, 3.79 (2 NCH_3), 2.68, 2.59 (2 CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 164.0, 163.5, 162.0, 161.3, 161.1, 148.6, 146.8, 146.2, 146.1, 112.2, 110.0, 102.9, 45.9, 43.0, 41.7, 41.6, 22.0, 21.4; no assignments are made because only a decoupled spectrum was taken; UV (pH 6.86) 223 nm (log ϵ 4.14), 239 (4.23), 247 (4.22), 385 (4.43); UV (0.1 M $HClO_4$) 222 nm (log ϵ 4.23), 250 (4.15), 347 (4.33), 385 (sh, 3.59). Anal. Calcd for $C_{18}H_{22}N_8O_9Cl_2 \cdot H_2O$: C, 37.06; H, 4.15; N, 19.21. Found: C, 37.06; H, 4.16; N, 19.19.

1,7-Bis[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]hypoxanthine Diperchlorate (6). A mixture of 0.268 g (1.00 mmol) of inosine, 1.0 g (2.1 mmol) of 1′-methylthiaminium diperchlorate,[8] 0.5 mL of 2,4,6-trimethylpyridine, and 20 mL of methanol was heated at reflux for 16 h. The precipitated white solid was filtered and washed with absolute ethanol to give 0.28 g of crude product, mp 175–185 °C (probably contains riboside).

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Recrystallization from water gave the analytical sample of **6** (mp 258–260 °C) which was dried under vacuum at room temperature: ^{1}H NMR (Me_2SO-d_6–D_2O, Me_4Si) δ 8.50, 8.42, 8.01, 7.93 (2 H-6′ of pyrimidine and H-2 and H-8 of hypoxanthine), 5.44, 5.04 (2 CH_2N), 3.74, 3.72 (2 NCH_3), 2.58 (2 CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 162.2, 162.0, 161.3, 161.0, 156.8, 154.0 (6 s), 147.4, 147.0, 146.3 (3 d), 114.7, 111.4, 111.2 (3 s), 42.7, 41.8 (2 t, NCH_2), 41.7, 41.6 (2 q, NMe), 21.4, 21.3 (2 q, CMe); one CH signal in the δ 146–147 region either overlapped another or had insufficient intensity to be detected. Anal. Calcd for $C_{19}H_{24}N_{10}O_9Cl_2 \cdot H_2O$: C, 35.47; H, 4.39; N, 21.77. Found: C, 35.42; H, 4.32; N, 21.97.

1,7-Bis[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]guanine Diperchlorate (7). A mixture of 0.295 g (1.05 mmol) of guanosine, 1.0 g (2.1 mmol) of 1′-methylthiaminium diperchlorate,[8] 0.5 mL of 2,4,6-trimethylpyridine, and 30 mL of methanol was heated at reflux for 16 h. The solvent then was removed on an evaporator, and the residue was washed with absolute ethanol and was recrystallized twice from 0.1 M perchloric acid. The third recrystallization from water gave 0.18 g (0.27 mmol, 26%) of the analytical sample of **7** (mp 246–248 °C dec) which was dried at room temperature under vacuum: ^{1}H NMR (Me_2SO-d_6–D_2O, Me_4Si) δ 8.35, 8.05, 7.88 (H-8 guanine and 2 H-6′ of pyrimidine), 5.42, 4.98 (2 NCH_2), 3.88 (2 NCH_3), 2.65 (2 CCH_3); UV (pH 6) 219 nm (log ε 4.48), 249 (4.49). Anal. Calcd for $C_{19}H_{25}N_{11}O_9Cl_2 \cdot H_2O$: C, 34.66; H, 4.44; N, 23.40. Found: C, 34.60; H, 4.42; N, 23.39.

7-[(4-Amino-1,2-dimethyl-5-pyrimidinio)methyl]xanthine Perchlorate (8). A mixture of 0.394 g (1.39 mmol) of xanthosine, 1.0 g (2.1 mmol) of 1′-methylthiaminium diperchlorate,[8] 0.5 mL of 2,4,6-trimethylpyridine, and 20 mL of methanol was heated at reflux for 2.5 h. The precipitated white solid was filtered and washed with absolute ethanol. Two recrystallizations from boiling 0.1 M perchloric acid (with filtration of undissolved material) gave 0.30 g (0.77 mmol, 55%) of the analytical sample of **8** (mp >300 °C) which was dried at room temperature under vacuum: ^{1}H NMR (Me_2SO-d_6–D_2O, Me_4Si) δ 8.13 (H-8 and H-6′), 5.35 (CH_2N), 3.77 (NCH_3), 2.60 (CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 162.2, 161.1, 155.7, 151.1, 149.5 (5 s), 147.2, 142.7 (2 d), 111.1, 106.3 (2 s), 42.2 (t, CH_2N), 41.8 (q, NMe), 21.5 (q, CMe). Anal. Calcd for $C_{12}H_{14}N_7O_6Cl$: C, 37.17; H, 3.64; N, 25.29. Found: C, 37.16; H, 3.62; N, 25.26.

1,3-Bis[(4-amino-1,2-dimethyl-5-pyrimidinio)methyl]uracil Diperchlorate (9). A mixture of 0.112 g (1.00 mmol) of uracil, 1.0 g (2.1 mmol) of 1′-methylthiaminium diperchlorate,[8] 0.5 mL of 2,4,6-trimethylpyridine, and 20 mL of methanol was heated at reflux for 16 h. The white solid was collected and washed with absolute ethanol to give 0.20 g (0.34 mmol, 34%) of crude product. Recrystallization from water gave the analytical sample of **9** (mp 258–260 °C) which was dried at room temperature under vacuum: ^{1}H NMR (Me_2SO-d_6) 9.19, 8.40 (4 NH), 8.12 (2 H-6′), 7.87 (d, H-6, J = 8 Hz), 6.01 (d, H-5, J = 8 Hz), 4.78 (2 CH_2N), 3.78 (2 NCH_3), 2.58 (2 CCH_3); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 162.8, 161.8, 161.7, 161.2, 151.4 (5 s), 145.7, 145.5, 144.2 (3 d), 111.0 (2 s, C-5′), 101.7 (d, C-5), 45.2 (t, NCH_2), 41.7, 41.6 (2 q, NMe), 37.4 (t, NCH_2), 21.4 (2 q, CMe); one C signal in the δ 161–163 region either had insufficient intensity to be detected or overlapped another peak. Anal. Calcd for $C_{18}H_{24}N_8O_{10}Cl_2$: C, 37.06; H, 4.15; N, 19.21. Found: C, 37.08; H, 4.17; N, 19.25.

Fluorescence Spectra. Corrected spectra were taken with a Perkin-Elmer MPF-44A instrument. Detection of fluorescence of the most dilute aqueous solutions was faciliated when the correcting unit was not in use. Glass-distilled air-equilibrated water was employed.

Kinetics of Hydrolysis of 4, 5, and 9. A few microliters of a 1.4×10^{-3} M solution of **4** in acetonitrile was added to 3 mL of 0.01 M borax buffer pH 9.18 at 24 ± 1 °C in a Cary 17D spectrophotometer. The region 440–250 nm was scanned repeatedly, showing an isosbestic point at 290 nm. A first-order plot of the very large absorbance change at 385 nm was liner over 5.0 half-lives with a rate constant of 1.35×10^{-4} s^{-1} (half-life 1.4 h). Under the same conditions **5** underwent multiple absorption changes, and the initial isosbestic point at 290 nm became distorted. The absorbance at 250 nm which grew markedly eventually drifted downward somewhat. The first-order plot using the large absorbance change at 385 nm was linear over 4.6 half-lives. The rate constant is 3.55×10^{-4} s^{-1} (half-life 32.5 min) for **5**.

In the same borate buffer **9** underwent a change, showing an isosbestic point at about 275 nm. The small absorbance decrease at 250 nm was used to construct a pseudo-first-order plot which was linear over 2.1 half-lives. The rate constant is 3.43×10^{-5} s^{-1} (5.62-h half-life).

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Registry No. **4**, 86013-89-0; **5**, 86013-93-6; **6**, 86013-95-8; **7**, 86013-97-0; **8**, 86013-99-2; **9**, 86014-01-9; cytidine, 65-46-3; 1′-methylthiaminium diperchlorate, 73333-47-8; 2,4,6-trimethylpyridine, 108-75-8; cytosine, 71-30-7; inosine, 58-63-9; guanosine, 118-00-3; xanthosine, 146-80-5; uracil, 66-22-8.

Preparation of Optically Pure (*R*)-(+)-Nicotine. Studies on the Microbial Degradation of Nicotinoids

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(*R*)-(+)-Nicotine (**1a**) of high optical purity (average 99.6%) has been obtained from (*R,S*)-nicotine by stereoselective microbial degradation of the (*S*)-(−)-nicotine with use of the microorganism *Pseudomonas putida*. Liquid culture results indicated that this organism growing on (*S*)-(−)-nicotine can utilize **1a** but at a slower rate. Studies on related nicotinoids showed the microorganism to be primarily specific for (*S*)-(−)-nicotine.

Introduction

Numerous studies on the physiological and insecticidal properties of the optical enantiomers of the alkaloids of *Nicotiana tabacum* L. have been undertaken in an effort to better understand the actions of the naturally occurring alkaloids.[1,2] These investigations have been limited to the

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