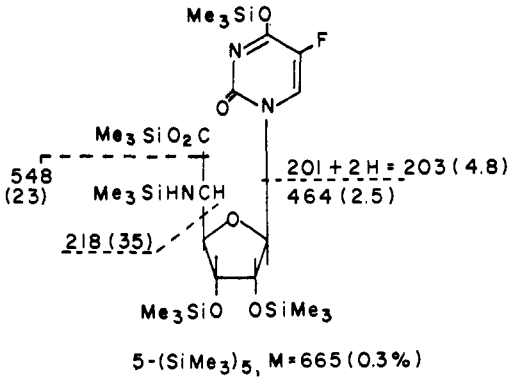
dec; uv_{max} (H_2O) 0.05 *N* HCl, 267 (ε 8500); 0,05 *N* NaOH, 269 (ε 7000); mass spectrum of the trimethylsilyl derivative,[5] found for M − CH_3, *m/e* 650.2400; calcd for $C_{24}H_{49}N_3O_7Si_5F$, 650.2401. Principal fragment ions[6] agree with structure **5** (see Chart II) and

Chart II



correlate with the spectrum of **6**, with all base-containing ions shifted 17.991 ± 0.003 mass units higher due to fluorine. The pmr spectrum (100 MHz) of **5** (compared with **6**) is shown in Table I. Close resemblance of chemical shifts and coupling constants of 1′, 2′, 3′, 4′, and 5′ protons strongly suggests the same configuration and conformation in **5** and **6**. Moreover, typical coupling of H-1′ and H-6 with 5-fluorine was observed.[7]

Table I. Nuclear Magnetic Resonance Spectral Data of 5-Fluorouracil Polyoxin C and Uracil Polyoxin C in 20% DCl in D_2O

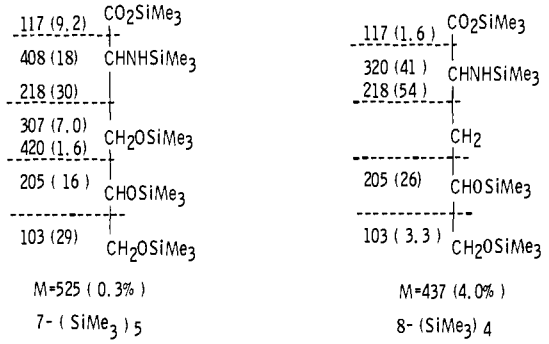
5-Fluorouracil polyoxin C (**5**)			Uracil polyoxin C (**6**)		
Assignment	Chem shift[a]	Coupling constant[b]	Assignment	Chem shift[a]	Coupling constant[b]
1′-H	5.81 (br d)	$J_{1',2'} = 4.0$[c]	1′-H	5.82 (d)	$J_{1',2'} = 3.8$[c]
2′-H	4.51 (q)	$J_{2',3'} = 6.2$	2′-H	4.54 (q)	$J_{2',3'} = 6.0$
3′-H	4.76 (q)	$J_{3',4'} = 6.6$	3′-H	4.77 (q)	$J_{3',4'} = 6.8$
4′-H	4.51 (q)	$J_{4',5'} = 3.0$	4′-H	4.52 (q)	$J_{4',5'} = 2.7$
5′-H	4.76 (d)		5′-H	4.75 (d)	
6-H	7.75 (d)	$J_{6\text{-F}} = 6.0$[d]	5-H	5.98 (d)	$J_{56} = 8.1$
		$J_{1'\text{-F}} \simeq 1$[d]	6-H	7.61 (d)	

[a] Shifts are given in ppm from DSS as an internal standard. [b] *J* values are in Hz; read from first-order splittings; d, doublet; q, quartet; br, broad. [c] Coupling was confirmed by the spin-decoupling experiment. [d] See ref for H_6–F and $H_{1'}$–F coupling of 5-fluorouracil nucleosides.

Compounds **7** and **8** were not crystallized. However, the R_f values of **7** and **8** were identical with authentic **7** and **8** (tlc; three solvents). Mass spectra (relative abundance) of the major ions shown below (Chart III) from gas chromatography–mass spectrometry[6] of the trimethylsilyl derivatives of **7** and **8** were the same as those derived from authentic **7** and **8** and clearly establish the overall structure as **1** and **2**.

Whereas the natural polyoxins only inhibit the fungi, **1** and **5** are extremely inhibitory to *Streptococcus faecalis* and *Escherichia coli* (10^{-5}–10^{-6} *M*). 5-Fluorouracil-2′-deoxyuridine 5′-monophosphate inhibits thymidylate synthetase.[8] With partially purified thymidylate synthetase from *E. coli*, the fluoropolyoxins (**1** and **5**) were not inhibitory. Therefore, the mode of action of the fluoropolyoxins is unclear. This mode of action is under study. Finally, **1** and **3** had the same inhibitory properties against purified chitin synthetase from *Saccaromyces cerevisiae* 5233C; **5** was not inhibitory.

Chart III



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(5) J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger, and R. N. Stillwell, *J. Amer. Chem. Soc.*, **90**, 4182 (1968).

(6) Mass spectra were recorded with 70-eV ionizing energy using LKB 9000 (low resolution) or CEC 21-110B (high resolution) instruments.

(7) R. J. Cushley, I. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, **90**, 709 (1968).

(8) (a) T. I. Kalman, *Ann. N. Y. Acad. Sci.*, **186**, 166 (1971); (b) R. J. Landenbach, P. V. Danenberg, and C. Heidelberger, *Biochem. Biophys. Res. Commun.*, **48**, 1565 (1972); (c) D. V. Santi and C. McHenry, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1855 (1972).

(9) This paper is part XVI of a series; the previous paper is T. Uematsu and R. J. Suhadolnik, *J. Med. Chem.*, in press.

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5-Chlorocytosine. Occurrence in Salmon Sperm Deoxyribonucleic Acid

Sir:

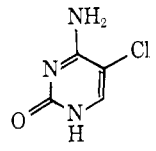
Fractionation of a deoxyribonucleic acid (DNA) hydrolysate from 900 kg of salmon sperm resulted in the isolation of 5-chlorocytosine (I). While numerous halogen-containing compounds have been isolated from marine organisms,[1] this is, to our knowledge, the first halogenated nucleic acid constituent to be isolated.[2,3]

(1) See, for example, J. J. Sims, W. Fenical, R. M. Wing, and P. Radlick, *J. Amer. Chem. Soc.*, **95**, 972 (1973); W. Fenical, J. J. Sims, and P. Radlick, *Tetrahedron Lett.*, 313 (1973); G. E. VanLear, G. O. Morton, and W. Fulmor, *ibid.*, 299 (1973); W. B. T. Cruse, M. N. G. James, A. A. Al-Shamma, J. K. Beal, and R. W. Doskotch, *Chem. Commun.*, 1278 (1971); J. J. Sims, W. Fenical, R. M. Wing, and P. Radlick, *J. Amer. Chem. Soc.*, **93**, 3774 (1971); S. Hunt and S. Breuer, *Biochem. Soc. Trans.*, **1**, 215 (1973).

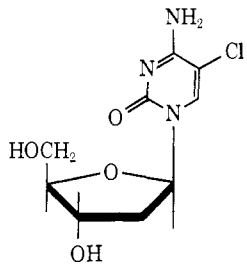
(2) Halogenated nucleosides, *e.g.*, 5-bromouridine, when administered to certain organisms are incorporated into nucleic acid chains. See P. Hackett and P. Hanawalt, *Biochim. Biophys. Acta*, **123**, 356 (1966).

(3) Nucleocidin, an antibiotic nucleoside produced by *Streptomyces calvus*, has a fluoro substituent at C-4 of the ribosyl moiety. See G. O. Morton, J. E. Lancaster, G. E. Van Lear, W. Fulmor, and W. E. Meyer, *J. Amer. Chem. Soc.*, **91**, 1535 (1969).

DNA was isolated from a 900 kg lot of salmon sperm by the method of Emanuel and Chaikoff[4,5] and hydrolyzed to the nucleoside level using snake venom diesterase and intestinal phosphatase. From the resulting hydrolysate, fractions enriched in each of the four principal nucleosides were obtained by ion exchange chromatography.[5] Each of these fractions was concentrated by distillation at 50° under reduced pressure and followed by crystallization of the principal nucleoside present in the respective fractions. The crystalline nucleosides were removed and the resulting filtrates were concentrated further and analyzed[6] by paper chromatography and electrophoresis.[7]



I



II

The filtrate obtained after removal of the bulk of deoxycytidine was examined using a number of chromatographic systems and was shown to contain several uv-absorbing components. Two of the components were successfully isolated and purified using ascending paper chromatography[8] and were characterized by spectrophotometric and chemical techniques. A mass spectrum of the compound with R_f values of 0.76, 0.34, and 0.36 in solvent systems A, B, and C,[8] respectively, exhibited molecular ions at *m/e* 145.0040 (calcd for $C_4H_4N_3O^{35}Cl$, 145.0042) and *m/e* 147.0010 (calcd for $C_4H_4N_3O^{37}Cl$, 147.0013) and a prominent fragment ion at *m/e* 110.0360 (calcd for $C_4H_4N_3O$, 110.0354) consistent with a chlorinated cytosine structure. The uv spectra of this compound, pH 1 λ_{max} 293.5 nm and pH 13 λ_{max} 295 nm, and paper chromatographic mobilities (see above) were identical with those of authentic 5-chlorocytosine[9] (I). Further confirmation was provided by deamination of the isolated compound with nitrous acid to 5-chlorouracil,[10] pH 1 λ_{max} 273 nm and pH 13 λ_{max} 289 nm.

The second isolated compound showing R_f values of 0.43, 0, and 0.21 in solvent systems A, B, and D,[8] respectively, exhibited uv spectra (pH 1 λ_{max} 297 nm; pH 13 λ_{max} 286 nm) consistent with assignment as 5-chlorodeoxycytidine (II).[11,12] A mass spectrum exhibited intense ions at *m/e* 110, 145, and 147[13] confirming the assignment of the base structure as 5-chlorocytosine (I). However, no molecular ions corresponding to 5-chlorodeoxycytidine (II) were observed; instead apparent molecular ions were present at *m/e* 225 and 227[13] corresponding to loss of two molecules of water from the expected molecular ions (*m/e* 161 and 163).

Although the complexities involved in processing 900 kg of salmon sperm to isolate very small samples (<1 mg) of minor DNA constituents make hazardous estimates of the quantities present, the amount of 5-chlorocytosine (I) in the DNA hydrolysate appears to be less than 0.01% of the cytosine content. It is exceedingly difficult to rule out absolutely the possibility that such small amounts of material are formed artifactually. However, during the process of isolation of DNA, enzymatic hydrolyses, and fractionation of the resulting constituents,[4,5,7,8] the material was never subjected to conditions known to effect chlorination of the pyrimidine nucleus.

The biological significance of the occurrence of 5-chlorocytosine (I) in salmon sperm DNA cannot be assessed at present. While "minor" bases are numerous in RNA[14] only 5-methylcytosine and N^6-methyladenine (6-methylaminopurine) are known to be widely distributed in DNAs.[15] 5-Chlorocytosine (I) may be a disfunctional derivative of cytosine resulting in some way from the high chloride concentration in the marine environment. However, the possibility that 5-chlorocytosine (I) or other minor bases occur as functional constituents of DNA cannot be ruled out. Prior to the present investigation, fractionations of DNA for the purpose of detecting minor bases have been carried out on sufficiently small scales that detection of a base occurring with a frequency of one or two residues per DNA molecule would be unlikely.[15-17]

(4) C. F. Emanuel and I. L. Chaikoff, *J. Biol. Chem.*, **203**, 167 (1953). Some modifications were required because of scale; however, the basic method and all reagents used were as described.

(5) The preparative steps involving the handling of the bulk substrate were carried out by Calbiochem Inc. of La Jolla, Calif. 92037. Their help and cooperation are gratefully acknowledged.

(6) A. W. Lis and D. I. McLaughlin, 15th Annual Meeting, Biophysical Society, New Orleans, La., Feb 16–18, 1971.

(7) A. W. Lis and W. E. Lis, *Biochim. Biophys. Acta*, **61**, 799 (1962); A. W. Lis and W. E. Passarge, *Arch. Biochem. Biophys.*, **114**, 593 (1966); S. J. Hayes and A. W. Lis, *Physiol. Chem. Phys.*, **3**, 517 (1971); A. W. Lis and S. J. Hayes, *ibid.*, **4**, 377 (1972).

(8) Solvent systems used were: A, isobutyric acid–0.5 *M* ammonium hydroxide (65:39); B, *n*-butyl alcohol–water (86:14), C, *n*-butyl alcohol–water–ammonia (86:14:5) D, *tert*-amyl alcohol–formic acid–water (90:19.2:39).

(9) I. Wempen and J. J. Fox, *J. Med. Chem.*, **6**, 688 (1963).

(10) I. Wempen and J. J. Fox, *J. Amer. Chem. Soc.*, **86**, 2474 (1964).

(11) D. M. Firsch and D. W. Visser, *J. Amer. Chem. Soc.*, **81**, 1756 (1959).

(12) Reference samples of synthetic 5-chlorocytidine and 5-chlorouridine were generously supplied by Dr. D. W. Hutchison, Department of Molecular Sciences, University of Warwick, Coventry CV4 7 Al, England.

(13) High-resolution studies confirm the assigned elemental compositions.

(14) R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, N. Y., 1971.

(15) See ref 14, Chapter 5.

(16) A highly sensitive immunochemical method for the detection of minor bases in DNA has been described: D. L. Sawicki, B. F. Erlanger, and S. M. Beiser, *Science*, **174**, 70 (1971).

(17) This work was supported in part by the Medical Research Foundation of Oregon.

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An Unusual Chelated *o*-Carborane Transition Metal Complex

Sir:

Lithium adducts of certain icosahedral carborane derivatives react with various transition metal salts to