might play a significant role in these facts, although the contribution due to changes in π-electron structures cannot completely be excluded. Further, this conclusion is supported by the similar behavior reported on $J_{^{31}P\text{-}C\text{-}H}$ and $J_{^{31}P\text{-}C\text{-}C\text{-}H}$,[10] when the reduced coupling constants are compared. The former J value decreases algebraically whereas the latter, which is always positive, increases as the ^{31}P atom becomes more cationic ($P \rightarrow P^+$).

It should also be noted that whenever the signals concerned were separately observed, the presence of weak couplings (about 1 Hz or less), although not exactly measured, were clearly discernible for $|J_{^{15}N,H(4)}|$ and $|J_{^{15}N,H(8)}|$, which are considerably smaller than $|J_{^{15}N,H(3)}|$ in all the cases examined (see Figure 1, for example).[11]

(10) (a) S. L. Manatt, G. L. Juvinall, and D. D. Elleman, *J. Am. Chem. Soc.*, **85**, 2664 (1963); (b) A. R. Cullingworth, A. Pidcock, and J. D. Smith, *Chem. Commun.*, 89 (1966); (c) S. L. Manatt, G. L. Juvinall, R. I. Wagner, and D. D. Elleman, *J. Am. Chem. Soc.*, **88**, 2689 (1966); (d) W. McFarlane, *Chem. Commun.*, 58 (1967).

(11) We thank the referee for his valuable comments on the sign of coupling constants.

K. Tori, M. Ohtsuru, K. Aono
Shionogi Research Laboratory, Shionogi & Co., Ltd.
Fukushima-ku, Osaka, Japan
Y. Kawazoe, M. Ohnishi
National Cancer Center Research Institute
Chuo-ku, Tokyo, Japan
Received February 27, 1967

The Synthesis of N^4-Acetyl-3,4,5,6-tetrahydrocytidine and Copolymers of Cytidylic Acid and N^4-Acetyl-3,4,5,6-tetrahydrocytidylic Acid

Sir:

Recent studies of the optical rotatory dispersion curves of poly C[1,2] and poly U[3] at neutral pH, and of the reaction of poly C[2] and poly A[4] with formaldehyde, led to the conclusion that intra- or interstrand hydrogen bonding plays a negligible role in the stabilization of the secondary structure of these polymers. It has been postulated for these polymers that a single-stranded helical structure is stabilized by interactions of the stacked bases. Polynucleotides containing residues with a saturated and therefore nonplanar heterocyclic portion are suitable model compounds for studying this hypothesis. Saturated nucleotides are unlikely to participate in base stacking due to their stereochemistry and their shortened π-electron system.[5] Copolymers containing saturated nucleotide residues are also interesting model compounds for yeast transfer RNA, where 5,6-dihydrouridine has been detected as a minor constituent.[6] We wish to report the synthesis of N^4-acetyl-3,4,5,6-tetrahydrocytidine and copolymers of cytidylic acid containing varying amounts of N^4-acetyl-3,4,5,6-tetrahydrocytidylic acid as nonplanar constituents.

While cytidine is completely resistant toward sodium borohydride in the dark,[7] N^4-acetylcytidine is reduced

(1) Abbreviations: polyuridylic acid, poly U; polycytidylic acid, poly C; polyadenylic acid, poly A.

(2) G. D. Fasman, C. Lindblow, and L. Grossman, *Biochemistry*, **3**, 1015 (1964).

(3) A. M. Michelson and C. Monny, *Proc. Natl. Acad. Sci. U. S.*, **56**, 1528 (1966).

(4) C. L. Stevens and A. Rosenfeld, *Biochemistry*, **5**, 2714 (1966).

(5) P. Cerutti, H. Miles, and J. Frazier, *Biochem. Biophys. Res. Commun.*, **22**, 466 (1966).

(6) J. T. Madison and R. W. Holley, *ibid.*, **18**, 153 (1965).

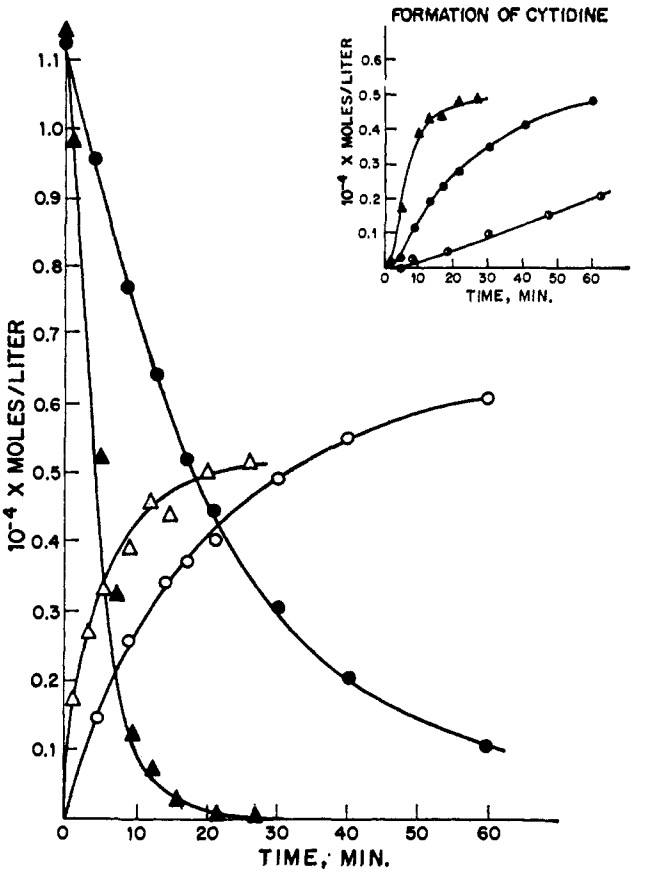


Figure 1. The reduction and deacetylation of N^4-acetylcytidine in the presence of sodium borohydride in the dark. Disappearance of N^4-acetylcytidine calculated from the absorption at 310 mμ in 1 *N* HCl (●, 14 *M* excess $NaBH_4$; ▲, 140 *M* excess $NaBH_4$; ϵ_{310} N^4-acetylcytidine 18 × 10^3); formation of N^4-acetyl-3,4,5,6-tetrahydrocytidine, orcinol assay (○, 14 *M* excess $NaBH_4$; △, 140 *M* excess $NaBH_4$). Inset: formation of cytidine calculated from the absorption at 310 mμ and 270 mμ in 1 *N* HCl (●, 14 *M* excess $NaBH_4$; ▲, 140 *M* excess $NaBH_4$; ϵ_{310} N^4-acetylcytidine 18 × 10^3, ϵ_{270} N^4-acetylcytidine 23 × 10^3, ϵ_{310} cytidine 0, ϵ_{270} cytidine 10 × 10^3); deacetylation of N^4-acetylcytidine in the absence of $NaBH_4$ at pH 10 (◑, from the disappearance of the absorption at 310 mμ measured in 1 *N* HCl; ϵ_{310} N^4-acetylcytidine 18 × 10^3).

to N^4-acetyl-3,4,5,6-tetrahydrocytidine and partially deacetylated to cytidine in the presence of sodium borohydride at pH 10. The rate of the reduction and deacetylation[8] are both dependent on the concentration of sodium borohydride. N^4-Acetylcytidine is also deacetylated at a slower rate at pH 10 in the absence of the reducing agent. The extent of the reduction can be determined with the orcinol assay for ribose due to the labilization of the N-glycoside bond upon saturation of the 5,6 double bond in N^4-acetylcytidine. The disappearance of N^4-acetylcytidine and the formation of cytidine can be followed spectrophotometrically and the composition of the reaction mixture calculated at each point of the reaction from the absorbance at 310 and 270 mμ in 1 *N* HCl[9] (see Figure 1). The final yield of N^4-acetyl-3,4,5,6-tetrahydrocytidine was 54% if a 14 *M* excess of sodium borohydride was used and 45% at a 140 *M* excess of the reducing agent. In both cases

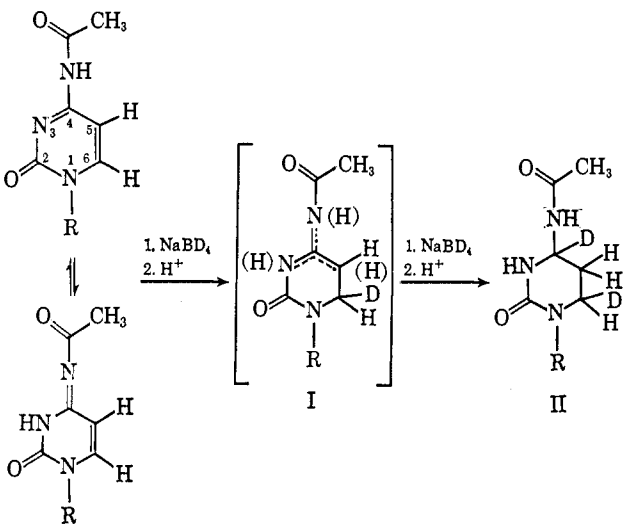
(7) P. Cerutti and N. Miller, *J. Mol. Biol.*, in press.

(8) The deacetylation reaction is reminiscent of the reductive deacylation of N-acylindoles and carbazoles with $NaBH_4$ (K. Banholzer, T. W. Campbell, and H. Schmid, *Helv. Chim. Acta*, **35**, 1577 (1952)).

(9) N^4-Acetyl-3,4,5,6-tetrahydrocytidine has no appreciable absorption at wavelengths longer than 250 mμ.

44% cytidine was formed. If the reaction was carried out under ultraviolet irradiation (2537 A) the yield for the reduction product was 76% and for cytidine 21% (14 *M* excess of $NaBH_4$).[10]

The two components of the reduction mixture, cytidine and N^4-acetyl-3,4,5,6-tetrahydrocytidine, were separated by column chromatography on Dowex 50W-X8 (H^+). The structure of the reduction product was derived from the nmr spectrum (in D_2O). A triplet at 5.30 ppm (J = 4.5 cps, 1 H) was attributed to the proton at C_4 and a multiplet centered at 3.39 ppm (2 H) to the methylene protons at C_6. A multiplet at higher field was superimposed by a singlet originating from the methyl group of the acetyl substituent (singlet at 1.99 ppm) and was attributed to the methylene protons at C_5. The signal at 5.30 ppm disappeared and the multiplet at 3.39 ppm was simplified and lowered in intensity if the reduction was carried out with sodium borodeuteride. The product of the reduction with sodium borodeuteride is therefore N^4-acetyl-3,4,5,6-tetrahydrocytidine-d_4,d_6 (II). The isotope distribution in II suggests the following reaction steps: (1) 1,4 addition of a hydride (deuteride) ion (at C_6) and a proton (at N^3 or N^4) to an α,β-unsaturated imine, and (2) further reduction of the intermediate N^4-acetyldihydrocytidine (I) by the attack of a second hydride (deuteride) ion at C_4.



R=β-D-ribofuranose

This novel reaction was used for the synthesis of copolymers of cytidylic acid and N^4-acetyl-3,4,5,6-tetrahydrocytidylic acid by the reduction of copolymers of cytidylic acid and N^4-acetylcytidylic acid.[11] The polymers were exposed to a large excess of sodium borohydride in 0.05 *M* sodium carbonate buffer at pH 9.8 for 40 min at room temperature. The polymers were purified by repeated precipitation with cold ethanol and by passage through Sephadex G-25. The composition of the polymers was determined spectrophotometrically and with the orcinol assay after total digestion with pancreatic ribonuclease. In contrast to the behavior of the monomer no significant deacetylation was observed. Polymers containing 8, 16, and 29% N^4-acetyl-3,4,5,6-tetrahydrocytidylic acid were prepared by this method, and their physical properties are now being investigated. The characterization of the polymers is given in Table I. The presence of N^4-acetyl-3,4,5,6-tetrahydrocytidine in the reduced polymer was demonstrated in experiments using sodium borotritiide as a reducing agent. The components of the ribonuclease digest were separated on Dowex 50W-X4 (H^+). The fractions containing radioactive material were treated with alkaline phosphomonoesterase and their content was compared to authentic N^4-acetyl-3,4,5,6-tetrahydrocytidine by thin layer chromatography (silica gel G, 85% 2-propanol). Identical R_f values were found for the radioactive compound derived from the polymer and for N^4-acetyl-3,4,5,6-tetrahydrocytidine obtained from the reduction of the monomer. No radioactivity was found in the eluates containing cytidylic acid.

Table I. Characterization of the Polymers

Compn before reduction, %		Compn after reduction, %	
Cytidine 3′-phosphate[a]	N^4-Acetylcytidine 3′-phosphate[a]	Cytidine 3′-phosphate[b]	N^4-Acetyl-3,4,5,6-tetrahydrocytidine 3′-phosphate[b]
68	26	73	29
81	18	84	16
90	7	90	8

[a] The amounts of cytidine 3′-phosphate and N^4-acetylcytidine 3′-phosphate were calculated from the absorbance of the nucleotide mixture obtained from the digestion of the polymers with pancreatic ribonuclease in 0.05 *M* NH_4HCO_3, pH 7.5, at 270 and 294 mμ (ϵ_{270} cytidine 3′-phosphate 9 × 10^3; ϵ_{294} cytidine 3′-phosphate 10^3; ϵ_{270} N^4-acetylcytidine 3′-phosphate 4.4 × 10^3; ϵ_{294} N^4-acetylcytidine 3′-phosphate 8.6 × 10^3). To accomplish complete deacetylation the samples were then kept for 6 hr at 65–70° and the cytidine 3′-phosphate content was determined from the absorbance at 280 mμ (ϵ 1.3 × 10^4, pH 1). This value was taken as 100% for the calculation of the base composition of the polymers. [b] The amount of cytidine 3′-phosphate was determined from the absorbance of the nucleotide mixture obtained from the digestion of the reduced polymers with pancreatic ribonuclease at 280 mμ (ϵ 1.3 × 10^4, pH 1). The content of N^4-acetyl-3,4,5,6-tetrahydrocytidine 3′-phosphate was measured with the orcinol assay. Total polymer phosphate was determined according to B. N. Ames and D. T. Dubin, *J. Biol. Chem.*, **235**, 769 (1960), and taken as the basis for the calculation of the base composition of the polymers.

Attempts are now being made to synthesize copolymers of cytidylic acid and 3,4,5,6-tetrahydrocytidylic acid by the reduction of N^4-formylated and N^4-trifluoroacetylated polycytidylic acid followed by deacylation.

Acknowledgment. This research was supported by Grants GB 4894 from the National Science Foundation, GM 14090-01 from the National Institutes of Health, and a grant from Hoffmann-La Roche Inc., Nutley, N. J.

Naishun Miller, Peter A. Cerutti
Department of Chemistry, Princeton University
Princeton, New Jersey 08540
Received March 16, 1967

(10) No spectral changes were detected if N^4-acetylcytidine was irradiated under the analogous conditions for 70 min in the absence of $NaBH_4$.

(11) A. M. Michelson and M. Grunberg-Manago, *Biochim. Biophys. Acta*, 91, 92 (1964).

Rate Constants and the Mechanism for the Transfer of Triplet Excitation Energy

Sir:

The intramolecular transfer of triplet excitation from one chromophore to another has been measured for