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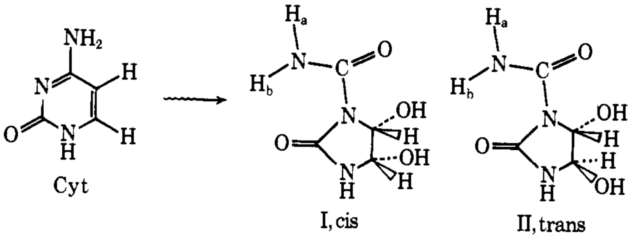
Radiation Chemistry of Nucleic Acids. Isolation and Characterization of Glycols of 1-Carbamylimidazolidone as Products of Cytosine[1]

Sir:

We wish to report the isolation of two major isomeric products (*g* value 0.075) obtained from the radiolysis of cytosine in aerated aqueous solution. These products characterized by spectral and X-ray diffraction analyses are identified as *cis*- (I) and *trans*- (II) 1-carbamylimidazolidone-4,5-diols.



A 1.9 × 10^{-2} *M* aqueous solution of Cyt[2] (375 ml, pH ~6) was saturated initially and at 2-hr intervals with oxygen and irradiated for 10 hr.[3] The absorbancy decrease was ~5% at λ_{max} 267 nm. The irradiated solution was evaporated (<35°) until dry, and the residue extracted with methanol. The combined extracts were concentrated, applied on Whatman No. 3 paper, and eluted with *n*-propyl alcohol–water (10:3). Material with an R_f value of 0.41 gave a positive Fink's test[4] and was rechromatographed with *sec*-butyl alcohol–water (5:2). Materials with R_f 0.26 (II) and 0.35 (I) were each twice rechromatographed, both fractions remaining somewhat impure. II was purified by washing the residue with a small amount of absolute methanol to eliminate the cis product and was then recrystallized from absolute methanol–chloroform (9:1). The crystals of I were obtained from concentrated methanolic solution after long standing.

The cis isomer melts at 119–122° dec and II at 175–176° dec. Uv spectra of both have only end absorption and show no appreciable changes after refluxing for up to 8 hr at pH ~2 or ~12. The ir (KBr) shows peaks ν_{OH} 3320; ν_{C-OH} 1150, 1115 (1095), 1075, and 1060 (1055); $\nu_{C=O}$ 1667; δ_{NH_2} 1582; and $\nu_{C=O}$ ureide 1739 (1751) cm^{-1} for cis and for trans except where indicated by parentheses.

The nmr ((CD$_3$)$_2$SO) has peaks of δ 4.94 (s, 1, C$_5$H), 5.55 (s, 1, C$_4$H), 6.66 (s, 1, C$_5$OH), and 6.96 (s, 1, C$_4$OH) for I; of δ 4.91 (s, 1, C$_5$H), 5.51 (s, 1, C$_4$H), 6.60 (s, 1, C$_5$OH), and 6.90 (s, 1, C$_4$OH) for II; and of δ 7.39 (s, 1 CONH$_a$), 7.90 (s, 1, CONH$_b$), and 8.70 (s, 1, NH) for both. The addition of D$_2$O eliminates all signals except C$_5$H and C$_4$H. Since NH$_2$ proton signals have a difference of ~0.5 ppm in chemical shifts, the two are nonequivalent. However, H bonding between CONH$_b$ and C$_2$O is probably weak (*cf.* X-ray diffraction study), since a strong intramolecular H bonding usually entails a difference of ~2 ppm in chemical shifts.[5]

The mass spectra of both fail to give the parent ions. However, their silyl derivatives, obtained by treatment with F$_3$CON[Si(CH$_3$)$_3$]$_2$ in pyridine (Regisil No. 270001), gave virtually indistinguishable spectra with molecular ions occurring at mass 449, along with abundant M − 15 ions at mass 434. This indicates a tetratrimethylsilyl derivative of C$_4$H$_7$N$_3$O$_4$ which was further confirmed by elemental analysis.

This suggests that both isomers have a glycol moiety and behave like a hCyt.[6] Yet, the NH$_2$ group, being stable, differs from the expected property of facile deamination observed for hCyt derivatives.[6] Although the nmr spectra also indicate the presence of glycol and an NH$_2$ group, they do not have the pattern displayed by analogous glycols of Thy,[7] a six-member heterocyclic derivative.

The data being insufficient for an unequivocal structural assignment, analysis by X-ray diffraction was required. Data for a single crystal of the trans isomer were collected on a four-circle fully automated computer-controlled diffractometer. The space group is *Pbca* (orthorhombic) with a = 13.289 (8), b = 13.260 (8), and c = 7.139 (4) Å. There is one molecule per asymmetric unit corresponding to a calculated crystal density of 1.71 g/cm^3. The structure was solved using the symbolic addition procedure for centrosymmetric crystals.[8] The atomic coordinates and thermal parameters were refined by least-squares methods and all seven hydrogen atoms were located in a difference map. The *R* factor (agreement between observed and calculated structure factors) for the full set of 1024 independent reflections is presently 6.5%.

The molecular conformation is illustrated in the stereodiagram in Figure 1 which was drawn by a computer using program ORTEP.[9] The C$_4$OH and C$_5$OH are trans to one another. The five-membered ring (excluding C$_5$) plus the carbonyl oxygen on C$_2$ are planar to within ±0.05 Å with C$_5$ being 0.18 Å out of the plane. The plane through the amide group is at an angle of ~13° to the above plane. The molecules are held together by an extensive system of H bonding.

Reviewing the uv, ir, nmr, and mass spectral data and elemental analyses of I and II in light of the assigned structure, we found them logically compatible.

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(2) Abbreviations following the IUPAC–IUB Commission on Biochemical Nomenclature recommendations [*J. Mol. Biol.*, **55**, 299 (1971)] are used throughout.

(3) For irradiation conditions see B. S. Hahn and S. Y. Wang, *J. Amer. Chem. Soc.*, **94**, 4764 (1972).

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