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ON THE REACTION OF VILSMEIER-HAACK REAGENT WITH NUCLEOSIDE: A CONVENIENT SYNTHESIS OF 2,2'-CYCLOCYTIDINE

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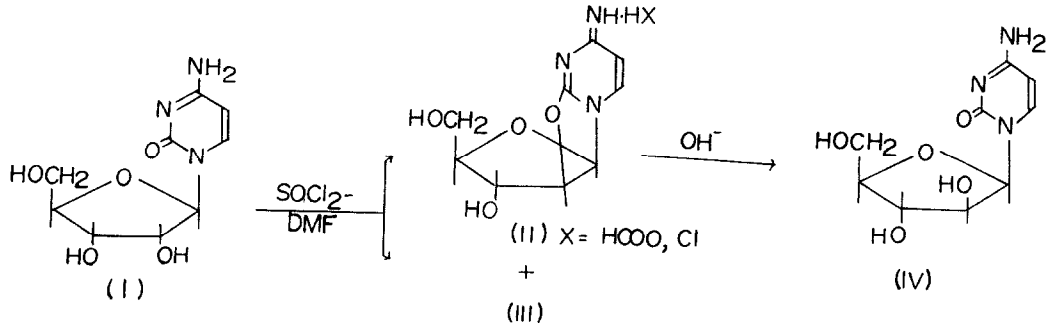
Inorganic acid halides ($SOCl_2$, $POCl_3$, PCl_3 and $COCl_2$) have been reported to react with N,N'-dimethylformamide (DMF) to form an active reagent, chloromethylene dimethylammonium chloride (Vilsmeier-Haack reagent) (1), which was initially found useful as formylating, halogenating and dehydroxylating agents (2). The reactions of the reagent with nucleosides to obtain several halogenated nucleosides such as 6-chloro inosine (3), 4-chloro uridine (4) and 5'-chloro-5'-deoxyuridine (5) derivatives were studied by several researchers.

Now, the reagent was first applied to cytidine (I), and it was found that the reagent afforded 2,2'-cyclocytidine (II) in a fairly good yield under rather mild reaction conditions. This cyclonucleoside (II) has been appreciated to be a novel intermediate in the chemical modification of the ribose or cytosine moiety of cytidine (6), but the tediousness in the preparation of this cyclonucleoside has prevented the current utilization of this compound. E. R. Walwick et al. (7) synthesized II from cytidine by heating it with polyphosphoric acid, followed by dephosphorylation of one of the reaction products, 2,2'-cyclocytidine-3',5'-diphosphate. However, because of the troublesome purification of the diphosphate and the poor yield in the enzymatic dephosphorylation of the diphosphate produced, this method is not satisfactory for the preparation of II. We found an improved and novel convenient method to preparate the compound (II), which will be reported in this paper.

Thionyl chloride was used as the inorganic acid halide and $SOCl_2$-DMF mixture was directly used in the following reaction without isolating an active species, chloromethylene dimethylammonium chloride.

Thionyl chloride (3.0 ml) was dissolved in 20 ml of DMF and the mixture was set aside at room temperature for 30 minutes. To the solution was added 2.0 g of cytidine (I) and the mixture was stirred at room temperature for 3 hours, then poured into about 50 ml of water and the aqueous solution was stirred for one hour to remove sulfur dioxide evolved from the reagent. Paper chromatography (solvent system, iPrOH-1 M NH_4OAc (pH 4.0), 7:3) of the aqueous solution revealed three main spots. The slow moving UV-absorbing substance (Rf, 0.49) corresponded to the starting material; thus the Rf value and the ultra-



violet absorption spectrum of the aqueous extract of the spot were coincided with those of cytidine (I). The major product (II) having a Rf value of 0.58 and the minor product (III) having a Rf value of 0.67 showed the same characteristic ultraviolet absorption spectra ($\lambda_{max}^{pH\ 1-7}$ 231 and 262 mμ) corresponding to that of the known 2,2'-cyclocytidine (7,8,9). In order to separate these three compounds, the aqueous reaction mixture was applied to a Dowex 50 X 4 (pyridinium form) column (2.5 X 40 cm). The column was eluted with 0.1 M pyridinium formate (pH 4.8) to give cytidine (I) in the first 2000 ml-fraction and the product (II) in the second 2000 ml-fraction. The product (III) was eluted when the molar concentration of the buffer was raised to 0.4 M.

The fraction containing the major product (II) was evaporated to dryness after pH of the solution was adjusted to 4.0 with formic acid in order to avoid the degradation of the product. Crystallization of the residual gum from ethanol gave II (X=HCOO) as granules which melted at 173-177° (decomp., uncorr.) and weighed 705 mg (yield, 30.4 %). UV; $\lambda_{max}^{pH\ 1-7}$ 231.5 and 263 mμ. It was redissolved in 20 ml of water and passed through

a Dowex 1 (chloride form) column (2 X 3 cm). The combined effluent and washings were evaporated to dryness and crystallization of the residual gum from ethanol gave II (X=Cl) as white needles which melted at 262-264° (decomp., uncorr.) and weighed 560 mg. UV; $\lambda_{max}^{pH\ 1-7}$ 231 (ε;9600), 262.5 (ε;10800) mμ, $\lambda_{min}^{pH\ 1-7}$ 218 (ε;7100), 243 (ε;6700) mμ, and Shoulder(pH 1-7) 282 (ε;3300) mμ. $[\alpha]_D^{20°}$: -22.0° (C; 2.0 in water). Paper chromatography; Rf, 0.58 (iPrOH-1 M NH_4OAc(pH 4.0), 7:3) and 0.05 (BuOH-H_2O, 84:16). Anal. Calcd for $C_9H_{13}O_4N_3 \cdot HCl$, C;41.32, H;4.63, N;16.07 %. Found, C;40.96, H;4.53, N;16.27 %. Other properties of the product (II) were also quite identical to those of the authentic 2,2'-cyclocytidine hydrochloride (7).

As the further proof of the structure, the compound (II) was converted to 1-β-D-arabinofuranosyl cytosine by treatment of it with slightly alkaline solution. Thus, 100 mg of the compound (II, X=Cl) was dissolved in 2 ml of 1 N NH_4OH and heated at 80° for 5 minutes. After the mixture was adjusted to pH 1 with hydrochloric acid, it was absorbed to Dowex 50 (H^+ form) column (1 X 1.5 cm) and the well washed column was eluted with 1 N NH_4OH. The effluent was evaporated in vacuo and crystallization of the residue from ethanol afforded the pure compound (IV), m.p. 208-212° (decomp., uncorr.), 80 mg (yield, 89 %). UV; $\lambda_{max}^{pH\ 1}$ 282 mμ, $\lambda_{min}^{pH\ 1}$ 242 mμ, $\lambda_{max}^{pH\ 7}$ 272 mμ, $\lambda_{min}^{pH\ 7}$ 251 mμ. $[\alpha]_D^{20°}$; +158° (C; 0.5 in water). The Rf values and the paper electrophoretic mobility of the compound (IV) in the solvent systems containing borate (10,11) were well coincided with those of 1-β-D-arabinofuranosyl cytosine (12).

Although the structure of the side product (III) which moved fast on paper chromatogram was not established, judging from the characteristic ultraviolet absorption spectrum it must have the 2,2'-cyclo structure. This product was found to be obtained when 2,2'-cyclocytidine (II) was dissolved in $SOCl_2$-DMF.

The reaction mechnism to afford 2,2'-cyclocytidine (II) and its derivative (III) from cytidine (I) is now under investigation and will be described elsewhere.

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