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PREPARATION OF 2'-DEOXYXANTHOSINE BY NITROSATIVE DEAMINATION OF 2'-DEOXYGUANOSINE UNDER ALKALINE AQUEOUS CONDITIONS

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Abstract: 2'-Deoxyxanthosine (dXao) has been prepared by treating 2'-deoxyguanosine (dGuo) with basic nitroprusside. Successful conversion appears to be favored by ionizing both dGuo (increasing its nucleophilicity) and dXao (protecting it from protolysis).

Nitrous acid deamination of the common nucleic acid purine and pyrimidine nucleosides is a well known reaction,[1] but the acid lability of dXao[1,2] makes its preparation from unprotected dGuo by this route effectively unusable.[3] Small quantities of dXao have been prepared enzymatically[2,4] and a multistep synthetic approach was used to prepare a protected derivative,[5] yet dXao remains commercially unavailable and seldom mentioned in the literature.

We postulated that nitrosative deamination of dGuo under alkaline conditions might lead to a useful preparation of dXao by introducing two favorable factors. Firstly, we expected the reactivity of dGuo's exocyclic nitrogen toward electrophilic deaminating agents to be increased on conversion to the anion, just as guanosine anion is more reactive than neutral guanosine toward other electrophiles.[6] In addition, it is known[2] that anionic dXao is stable but that it depurinates rapidly under mildly acidic conditions, suggesting that keeping the reaction mixture basic throughout should optimize the stability of the product.

In accord with these considerations, treatment of dGuo with an excess of the known *N*-nitrosating agent, sodium nitroprusside,[7] gave no reaction in neutral aqueous solution but led smoothly to the formation of the sodium salt of dXao in appropriately basic solution. In a typical reaction, 2 g of dGuo•H_2O (Pharmacia LKB) in 30 mL of aqueous 1 N NaOH was treated with 7 g of $Na_2Fe(CN)_5NO•2H_2O$ (Baker) at 70 °C with stirring for 4 h, whereupon the rust colored suspension was allowed to cool to 25 °C and was diluted to 60 mL with H_2O. The pH was lowered to 3.8 with ~6 mL of glacial acetic acid under constant stirring. When effervescence ceased, 30 mL of MeOH was added with stirring and the suspension was suction filtered. The filtrate was chromatographed on a column of Dowex 1-X2 (BioRad) 200-400 mesh anion exchange resin (30 g dry weight, acetate form) that had been equilibrated with 10 mM aqueous HOAc:MeOH (7:3). The product was eluted with the same solvent mixture (200 mL). The eluate was evaporated to produce a crusty yellow-brown residue that was stirred efficiently overnight in 500 mL of EtOH to remove the majority of contaminating NaOAc and other impurities. The resulting material was filtered, washed with EtOH and Et_2O, and dried under vacuum at 25 °C to yield 1.3 g (56%) of light brown, chromatographically homogeneous sodium salt of dXao. An analytical sample of this material was prepared by dissolving the solid in 10 mL of MeOH:H_2O (3:7) and loading it onto a 2.8 x 71 cm Sephadex LH-20 column

eluted with MeOH:H_2O (3:7) at a flow rate of 1 mL/min. UV-absorbing (280 nm) product eluted in fractions (10 mL) 24-36. These were evaporated overnight in vacuo to afford 1.0 g of the pure dihydrate as a pale brown glass; UV λ_{max}(pH 3.2) 235(ϵ=9.67x10^3), 264(ϵ=1.05x10^4); λ_{max}(pH 8) 248(ϵ=1.14x10^4), 278(ϵ= 1.01x10^4); ^{1}H NMR (DMSO-d_6/TMS) δ2.00(dd,1,H-2'α), 2.71(m,1,H-2'β), 3.53(dq,2,H-5'), 3.85(s,1,H-4'), 4.33(d,1,H-3'), 5.19(br s,1,OH-3'),[8] 6.03(dd,1,H-1'), 6.81(br s,1,OH-5'),[8] 7.48(s,1,H-8), 9.06(s,1,NH-1);[8] negative ion fast atom bombardment mass spectrum (FAB MS) (glycerol) *m/z* 289([$NaC_{10}H_{10}N_4O_5$]$^-$), 267([$C_{10}H_{11}N_4O_5$]$^-$), 151([$C_5H_3N_4O_2$]$^-$). Anal. Calcd. for $C_{10}H_{11}N_4O_5Na\cdot 2H_2O$: C,36.82;H,4.63;N,17.17. Found: C,36.94;H,4.39;N,17.17.

Pure dXao was obtained in 33% yield when a solution of 0.1 g of the sodium salt in 2.5 mL of MeOH:H_2O (3:7) was acidified to pH 4 with 0.2 mL of glacial acetic acid. After chilling to 4 °C overnight, 0.03 g of dXao precipitated as short, pale yellow needles; ^{1}H NMR (DMSO-d_6/TMS) δ2.29(m,2,H-2'), 3.63(d,2,H-5'), 3.91(d,1,H-4'), 4.34(d,1,H-3'), 5.35(d,1,OH-3'),[8] 6.20(t,1,H-1'), 7.91(s,1,H-8), 10.86(s,1,NH),[8] 11.52(br s,NH),[8] 13.31(br s,NH);[8] positive ion FAB MS (dithiothreitol/dithioerythritol) *m/z* 269([$C_{10}H_{12}N_4O_5$+H]$^+$), 153([$C_5H_4N_4O_2$+H]$^+$). Anal. Calcd. for $C_{10}H_{12}N_4O_5\cdot 1.5H_2O$: C,40.68;H,5.12;N,18.98. Found: C,40.65;H,4.67;N,19.20. Acidification to pH 4 of a solution of the sodium salt in H_2O alone led only to the precipitation of xanthine.

Our preparative procedure should allow ready access to authentic specimens of dXao for use as an analytical standard, for further physicochemical characterization, and for biological testing. Additionally, it should be pointed out that 2'-deoxycytidine and 2'-deoxyadenosine are not similarly deaminated by sodium nitroprusside under these alkaline aqueous conditions. It is possible, therefore, that sodium nitroprusside in alkali may be a useful reagent for selective modification of guanine residues in nucleic acids.

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References and Notes

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8. This resonance disappears on addition of D_2O.

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