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A NOVEL PRODUCT FROM THE REACTION OF p-METHYLBENZYL CHLORIDE WITH GUANOSINE IN NEUTRAL AQUEOUS SOLUTION

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An unanticipated product from reaction of guanosine with p-methylbenzyl chloride in neutral aqueous solution has been identified as 4-(p-methylbenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole.

Reaction of benzyl chloride with guanosine in neutral aqueous media yields three products; $\underline{N}^2$-benzylguanosine, $\underline{O}^6$-benzylguanosine, and 7-benzylguanosine in a ratio of 6.6:1.0:14.4, respectively (1). However, treatment of [5'-^{3}H]guanosine (21 Ci/mmol, 5×10^{-7} M) with p-methylbenzyl chloride (1.7×10^{-2} M) in neutral aqueous solution at 40° for 24 hours produced an unanticipated product in addition to the expected $\underline{N}^2$-, $\underline{O}^6$-, and 7-(p-methylbenzyl)guanosine derivatives (2) in a ratio of 1.4:6.1:1.0:1.0, respectively (3). A non-radioactive compound which cochromatographs with the unanticipated radioactive product in several chromatographic systems was isolated from a large-scale guanosine-p-methylbenzyl chloride reaction (4) and its spectroscopic properties are described herein.

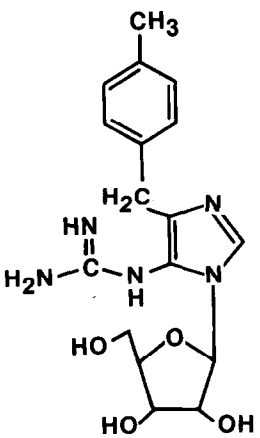
The ultraviolet absorption spectrum for this material differs substantially from the spectra of other benzylated guanosines (1) and shows at pH 6.9 a shoulder at 215 nm (ε 15900) and absorption bands of lower intensity at 258 nm (ε 416), 263 nm (ε 469), 265 nm (sh) (ε 451), and 272 nm (ε 434). These latter bands are characteristic of the weakly absorptive p-methylbenzyl chromophore. In 0.01 N NaOH, the spectrum shows shoulders at 215 nm (ε 13700) and 240 nm (ε 7960), and a weak band at 272 nm (ε 1380) is superimposed on end absorption in the 250 to 290 nm region. These spectral characteristics indicate a significant alteration to the structure of the guanine moiety and suggest loss of aromaticity in the pyrimidine ring of the original guanosine chromophore.

The 100 MHz proton magnetic resonance spectrum for this material in DMSO-d_6 (5) indicates it is a p-methylbenzyl-substituted ribonucleoside and three features of the spectrum are noteworthy. First, the chemical shift of δ 7.50 for a single, non-exchangeable proton is farther upfield than expected for the chemical shift of the 8-proton on an intact guanosine nucleoside and is closer to the chemical shift reported for the 2-proton of some substituted imidazole

ribonucleosides (6). Second, a total of seven D_2O-exchangeable protons are accounted for by a broad peak which extends from δ 4.90-6.30. Since three of these protons may be assigned to the ribose moiety, the "base" portion of the nucleoside must possess four exchangeable protons. Third, the chemical shift of δ 3.57 for the methylene protons of the p-methylbenzyl group is farther upfield than expected if this group were attached to a heteroatom of the nucleoside (7). This feature of the spectrum suggests that the methylene group is attached to a carbon atom of the "base" rather than to a nitrogen or oxygen atom.

Additional support for carbon attachment of the methylene group is provided by the ^{13}C nuclear magnetic resonance spectrum for this material in D_2O (pD 6) (8). The peak for the methylene carbon appears at 32.5 ppm. Chemical shifts for a benzylic carbon attached to nitrogen or oxygen are generally at substantially lower field (9). Although the ^{13}C spectrum shows only 15 well-resolved peaks, the chemical shift of at least two carbons may be assigned to each of the two most intense peaks in the spectrum at 129.6 and 130.1 ppm. If the peaks at 119.2, 135.5, 136.1 and 158.1 ppm (8) are assigned to chemical shifts of carbons of the "base", the ^{13}C spectrum indicates that the nucleoside contains only 17 carbon atoms and not the 18 carbons required for an intact (p-methylbenzyl)guanosine. A comparison of the infrared spectrum (KBr wafer) of guanosine with that of the isolated adduct shows that the carbonyl stretch of guanosine at 1730 cm^{-1} is absent in the spectrum of the isolated material and suggests that if any carbon of the original guanine moiety is not present in the adduct, then carbon-6 of guanine is most likely absent.

The high resolution electron impact mass spectrum for the adduct shows a molecular ion at m/z 361.177 requiring an elemental composition of $C_{17}H_{23}N_5O_4$ (10). The next largest peak in the spectrum appears at m/z 319.153 corresponding to M^+-H_2NCN or $C_{16}H_{21}N_3O_4$. The base peak in the spectrum appears at m/z 229.133 ($C_{12}H_{15}N_5$) and represents the intense $(B+1)^+$ fragment typical of ribonucleosides (11). The only peaks in the field desorption mass spectrum for this material appear at m/z 361 and 362 corresponding to M^+ and $(M+H)^+$, respectively. Taken together, these data lead us to propose the following structure for this nucleoside.



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This aralkylation product is most unusual and we are unaware of any published reports of production of a similar compound in guanosine reactions with any other alkylating or aralkylating agent. Since we failed to detect formation of this material when neutral aqueous solutions of either $\underline{N}^2$-, $\underline{O}^6$- or 7-(p-methylbenzyl)guanosine were incubated at 40° for 24 hours, we conclude that its formation is not a result of decomposition of any of these other reaction product Additionally, it seems improbable that this product is a result of reaction with an impurity in our guanosine preparations since it is produced in reactions with both highly purified radiolabeled guanosine and unlabeled guanosine and it is unlikely that the same contaminant would be present in both starting materials. Finally, because the extent of formation of this adduct is similar to that of both the $\underline{O}^6$- and 7-(p-methylbenzyl)guanosine, we conclude that this material is a true product of reaction between p-methylbenzyl chloride and guanosine in neutral aqueous solution.

Since the available data do not allow us to entirely rule out the possibility of an isomeric structure having the p-methylbenzyl group attached to position-2, the above structural assignment must be somewhat tentative. However, the alternative structure for this adduct is less tenable since its formation would involve the unlikely fission of the pyrimidine ring of an 8-substituted guanosine under mild aqueous conditions. Fission of the pyrimidine ring is more readily envisaged for a 5-substituted guanosine if we invoke a mechanism similar to that proposed by Leonard and co-workers (12,13) for rearrangement of a transiently formed 5-substituted purine. Thus, if after attachment of the p-methylbenzyl group to C-5 of guanosine the pyrimidine ring undergoes electrocyclic ring opening to an isocyanate derivative, then hydrolysis and loss of C-6 as CO_2 would result in formation of a product with the structure we present above.

Further investigations on the structure and mechanism of formation of this adduct are in progress but it is important to point out that such an adduct is more readily detected in guanosine reactions involving p-methylbenzyl chloride than the unsubstituted chloride (see above). Thus it may be that formation of such products is more general and typical of guanine nucleoside reactions with aralkylating agents whose reactivities show greater S_N1 character in neutral aqueous solution.

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2. These last three derivatives have been completely characterized by mass-, ultraviolet-, and nuclear magnetic resonance spectroscopy as well as elemental analysis.

3. Product separation by Aminex A-5 column chromatography and quantification by liquid scintillation counting were carried out as described previously for the benzylation of guanosine (ref. 1). The chromatographic order of elution of the p-methylbenzyl derivatives is the same as that for the benzylated derivatives but the former analogues have larger retention volumes. The unanticipated radioactive compound elutes ahead of 7-(p-methylbenzyl)guanosine when the solvent is changed to 1 M $NH_4^+HCO_2^-$ in DMF/H_2O (3:7), pH 7 (ref. 1).

4. Guanosine (6 g, 0.02 mol) was treated with 14 g (0.1 mol) of p-methylbenzyl chloride in 1 L H_2O containing 13.6 g (0.1 mol) KH_2PO_4 and 42.6 g (0.3 mol) Na_2HPO_4 at 50° for 24 hr with vigorous stirring. The neutral reaction solution was then concentrated to 100 mL under reduced pressure, was extracted 3X with an equal volume of $CHCl_3$, and was evaporated to dryness. The dry residue was extracted with MeOH (100 mL) and the extract was evaporated to dryness. The resulting residue was redissolved in 50 mL MeOH/H_2O/concentrated ammonium hydroxide solution (NH_4OH) (30:70:3) and was loaded on a 2.8 x 71 cm Sephadex LH-20 column and eluted with the same solvent at 1mL /min. Ultraviolet absorption was monitored continuously at 254 nm and fractions (10 mL) were collected. The reaction product eluted in nearly homogeneous form in fractions 42-50 following the imidazole "ring-opened" 7-substituted guanosine (frns. 16-22), unreacted guanosine (frns. 23-29) and N^2-(p-methylbenzyl)guanosine (frns. 36-41). Fractions 42-50 were pooled, evaporated to dryness, and the residue was redissolved in 2 mL H_2O. This solution was loaded on a 0.72 x 30 cm Aminex A-5 column (NH_4^+ form) which was eluted with 0.3 M $NH_4^+ HCO_2^-$ in CH_3CN/H_2O (3:7), pH 4.5, 40°, at 0.5 mL/min. When 50 mL of this solvent had passed through the column, elution was continued with 40 mL CH_3CN/H_2O (4:6) and finally with $CH_3CN/H_2O/NH_4OH$ (5:4:1). Ultraviolet absorption was continuously monitored at 254 nm and fractions (1 mL) were collected. The new reaction product eluted in 30 1-mL fractions after approximately 20 mL of this latter solvent had passed through the column. Yield: 0.05 g.

5. ^1H NMR (DMSO-d_6, 0.5% $(CH_3)_4Si$): δ2.24 (s,3,Ar-CH_3), 3.57 (s,4,H-5'+Ar-CH_2-), 3.80 (q,1, H-4') 4.06 (t,1,H-3'), 4.25 (t,1,H-2'), 5.37 (d,1,H-1'), 4.90-6.30 (broad, 7,OH-2', OH-3', OH-5', + 2(NH_2)), 7.08 (q, 4, Ar), 7.50 (s,1,H-2).

6. L.B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry, Vol. 2", W.W. Zorbach and R.S. Tipson (eds.); Wiley-Interscience: New York, 1973, p. 267.

7. The chemical shift for the methylene protons of 7-(p-methylbenzyl)-, N^2-(p-methylbenzyl)- and O^6-(p-methylbenzyl)guanosine in DMSO-d_6 are δ5.62, 4.46, and 5.48, respectively.

8. ^{13}C NMR (D_2O, pD 6, dioxane as internal standard): δ20.9 (Ar-CH_3), 32.5 (Ar-CH_2), 61.8 (C-5'), 70.8(C-3'), 75.0(C-2'), 85.5(C-4'), 88.5(C-1'), 119.2, 129.6(Ar), 130.1(Ar), 135.5, 136.1, 137.5(Ar), 138.9(Ar), 158.1.

9. For representative ^{13}C NMR chemical shift values, see: E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 NMR Data, Vols. 1 and 2", Heyden and Son, Ltd., Philadelphia, 1979.

10. Elemental Analysis: Calcd. for $C_{17}H_{23}N_5O_4 \cdot 1\frac{1}{2} H_2O$: C, 52.56; H, 6.75; N, 18.03. Found: C, 52.65; H, 6.94; N, 18.06.

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