$C_4H_9Br$, 109-65-9; c-$C_6H_{11}CH_2Br$, 2550-36-9; benzylamine, 100-46-9; *N*-methyl-4-piperidone, 1445-73-4; acetic–formic anhydride, 2258-42-6; acetic anhydride, 108-24-7; benzoyl chloride, 98-88-4.

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# Reactions of Cytidine with 7-Bromomethylbenz[*a*]anthracene, Benzyl Bromide, and *p*-Methoxybenzyl Bromide. Ratio of Amino to 3 Substitution[1]

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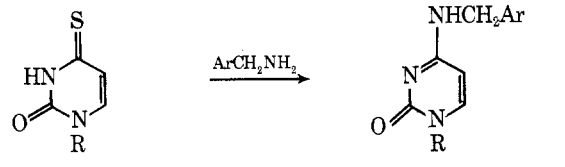
Alkylation of cytidine in dimethylacetamide by 7-bromomethylbenz[*a*]anthracene (1), benzyl bromide, and *p*-methoxybenzyl bromide afforded the 3-substituted cytidines in good yield. The identity of these products has been confirmed by spectroscopic studies and chemical transformations. Deamination of 3-(benz[*a*]anthryl-7-methyl)cytidine by nitrous acid or sodium bisulfite afforded the corresponding uridine derivative, which was also prepared from uridine and 1. Alkylation of cytidine by the above halides in aqueous solution led to the formation of 3- and $N^4$-substituted products. The structure of the latter was established by unequivoval synthesis from 2′,3′,5′-tri-*O*-benzoyl-4-thiouridine and the appropriate benzylic amines. The alkylation reactions in aqueous solution went in low overall yield. 3-Substitution of cytidine predominated in the case of benzyl bromide, and $N^4$ substitution with *p*-methoxybenzyl bromide, while both types of product were formed in almost equal amounts with 1. Substitution at $N^4$ of cytidine appears to be correlated with the ability of the reagent to accommodate a positive charge, which leads to thermodynamic rather than kinetic control of the reaction.

Alkylation of the heterocyclic bases of the nucleic acids generally proceeds most rapidly at the pyridine-type ring nitrogen atoms. The most reactive positions in nucleosides are the 7 position of guanosine, 1 position of adenine, and 3 position of cytosine. Similar results are obtained in single stranded nucleic acids.[2,3] One striking exception to this rule was reported by Dipple and co-workers in studies with the carcinogenic alkylating agent, 7-bromomethylbenz[*a*]anthracene.[4] Alkylation by this reagent in dimethylacetamide was in accord with the usual pattern described above. In aqueous solution, however, alkylation of nucleosides and polynucleotides proceeded mainly on the amino group of guanine, adenine, and possibly cytosine. The possible importance of amino group alkylation to carcinogenesis was pointed out by the above authors. More recently, another author has suggested that amino group substitution is a significant process in the reaction of another carcinogen, *N*-acetoxy-*N*-acetyl-2-aminofluorene, with DNA.[5]

We have further investigated the reactions of cytidine with 7-bromomethylbenz[*a*]anthracene (1) and with benzyl bromide and *p*-methoxybenzyl bromide. One objective was to confirm the structure of the reaction product formed by 1 in an aqueous solution and in dimethylacetamide. The position of substitution on alkylation of a nucleic acid component is usually assigned on the basis of ultraviolet spectroscopy.[2] This technique is inapplicable in reactions in-

volving 1 because the reagent has its own very strong ultraviolet absorption. We have used this opportunity to demonstrate other methods of structure determination. Another purpose of this study was to understand more about the factors that direct a particular alkylating agent to the amino group of a nucleic acid component, rather than to a pyridine-type ring nitrogen. Cytidine was selected as a model in this study because of its simplicity—it contains just one of each type of reactive site.

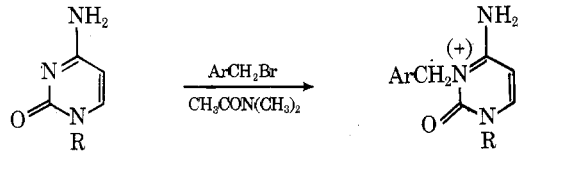
**Syntheses of $N^4$- and 3-Substituted Cytidines.** It was desirable to have authentic samples of $N^4$-substituted cytidines as reference compounds. These compounds were prepared by reaction of 2′,3′,5′-tri-*O*-benzoyl-4-thiouridine (**2**)[6] with benzylamine, *p*-methoxybenzylamine, and 7-aminomethylbenz[*a*]anthracene. In the case of the synthesis of **3c**, a subsequent treatment with ammonia was necessary to complete the removal of the *O*-benzoyl groups. It was also possible to prepare **3a** directly from cytidine by bisulfite-catalyzed transamination with benzylamine. This procedure failed in the case of **3c**.




**2**, R = 2′,3′,5-tri-*O*-benzoyl-β-D-ribofuranosyl

**3**, R = β-D-ribofuranosyl
**a**, Ar = $C_6H_5$
**b**, Ar = *p*-$CH_3OC_6H_4$
**c**, Ar = 7-benz[*a*]anthryl

The preparation of 3-benzylcytidine (**4a**) by direct reaction of cytidine with benzyl bromide in dimethylacetamide had previously been reported.[8] The assignment of structure was based largely on ultraviolet spectroscopic properties. We now found that the reaction of cytidine with *p*-methoxybenzyl bromide went similarly to give a compound with properties closely comparable to those of **4a**. We assigned the structure 3-*p*-methoxybenzylcytidine (**4b**) to this product. The reaction product of cytidine with 7-bromomethylbenz[*a*]anthracene[4] could not be characterized by ultraviolet spectroscopy, for reasons discussed above. It differed in its properties from the authentic sample of **3c**, prepared as described above.




R = β-D-ribofuranosyl

**4a**, Ar = $C_6H_5$
**b**, Ar = *p*-$CH_3OC_6H_4$-
**c**, Ar = 7-benz[*a*]anthryl

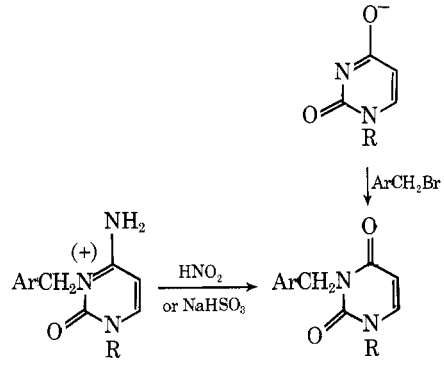
To demonstrate a chemical method for distinguishing ring- from amino-substituted product, applicable when reference compounds are not available, the following transformations were run. Deamination of **4c** was successful, using either nitrous acid[9] or sodium bisulfite.[10] The product retained the benzanthryl residue, and was also preparable by the reaction of 7-bromomethylbenz[*a*]anthracene with uridine. It was assigned structure **5**.

The assignment of **4c** and **5** as 3-substituted nucleosides was further supported by the following evidence. Their NMR spectra show the retention of H-5 and H-6. Compound **4c** migrated as a cation upon paper electrophoresis at pH 7. The infrared spectra of **4a–c** were quite similar in the carbonyl region, all three compounds containing a band at 5.75–5.8 μ, not present in **2a–c**. The absorbance due to the $CH_2$ group in the NMR spectra of **4a–c** was about 0.6–0.75 τ further downfield than the corresponding absorbance in **3a–c**. An additional structural possibility, not completely excluded by this evidence, is O-2 substitution in **4c** and **5**. Reaction at this position of cytosine has no precedent in nucleic acid chemistry, however,[2] and this structure is quite unlikely as a major reaction product.

**Table I. Yield of Substituted Cytidine Derivatives**[a]

| Alkylating agent | Position substituted | Solvent, % Dimethylacetamide | Solvent, % Aqueous buffer |
|---|---|---|---|
| Benzyl bromide | 3 | 95 | 0.62 |
| | $N^4$ | 0 | 0.09 |
| *p*-Methoxybenzyl bromide | 3 | 83 | 0 |
| | $N^4$ | 0 | 0.14 |
| 7-Bromomethylbenz[*a*]anthracene | 3 | 51 | 0.18 |
| | $N^4$ | 1.1 | 0.14 |

[a] The conditions (temperature, reactant ratio) used in the aqueous reactions differed from those in dimethylacetamide. See the Experimental Section for complete details.




**4c**

**5**, R = β-D-ribofuranosyl; Ar = 7-benz[*a*]anthryl

**Product Distribution on Alkylation in Dimethylacetamide and Water.** With authentic samples now available as reference compounds, it was possible to study the relative yields of 3- vs. $N^4$-substituted cytidine upon alkylation of cytidine in dimethylacetamide, and in a buffered aqueous solution, pH 5.5. With our technique, we were able to detect as little as 0.1% yield of a product. The results are presented in Table I. It can be seen that the total yield of products was good in the reactions conducted in dimethylacetamide. In water, however, the yield of alkylated products was less than 1%, which undoubtedly is due to competition by the solvent for the alkylating agent. These results are comparable to those recently reported for alkylation of cytidine by ethyl methanesulfonate and diethyl sulfate in aqueous solution.[11]

The results of the alkylation study in dimethylacetamide with all three bromides was generally in accord with observation previously reported.[4] The sole new feature was that a small amount of $N^4$ substitution (relative to 3 substitution) was observed in the case of 7-bromomethylbenz[*a*]anthracene. The results in aqueous solution provide a striking contrast. The introduction of a methoxyl group into the para position of benzyl bromide altered the product distribution from 7:1 in favor of 3 substitution to exclusive (within the limits of our technique) $N^4$ substitution. The case of bromomethylbenz[*a*]anthracene fell in between

these extremes, with 3 substitution slightly ahead of $N^4$ substitution. This contrasts with the results of Dipple et al.,[4,12] who suggested that amino substitution was the principal reaction, at the nucleoside level as well as with nucleic acids.

Some studies were also performed on the methylation of cytidine in an organic solvent, and aqueous buffer. This was done to compare our results with those of other workers, who generally have not observed monoalkylation at $N^4$, except in strongly alkaline solution. In fact, even when a large excess of dimethyl sulfate was used, no $N^4$-methylcytidine was detected. 3-Methylcytidine was obtained in 77% yield in dimethylformamide, and in 64% yield in an aqueous buffer, under these conditions.

The results of Sun and Singer[11] on the ethylation of cytidine should be considered in connection with our results. The ratio of 3- to $N^4$-substituted product was about 20:1 in dimethyl sulfoxide, and 5.5:1 in neutral aqueous solution.

We wished to consider the possibility that the $N^4$-substituted products observed by us were formed by intramolecular Dimroth rearrangement[13] of the 3-substituted products. This process has been observed when 3-substituted cytosines were heated in alkali,[14] or in a mixture of acetic anhydride and acetic acid.[15] This process seemed unlikely in our case, as it would also have been expected in the methylation reaction, where no amine-substituted product was observed. In fact, no rearrangement was observed when **4b** and **4c** were allowed to stand under the aqueous reaction conditions. At 85%, pH 4.7, a small amount of rearrangement to **3b** and **3c** was observed. The major product, under those conditions, was cytidine. This formation of cytidine suggested that the rearrangement observed was not Dimroth, but by an intermolecular path, similar to that produced on heating 3-benzylhypoxanthine.[16]

It is apparent that the conditions which favor substitution on the amino group relative to the ring nitrogen in cytidine are those which favor the development of a positive charge on the alkylating agent: stabilization of the developing carbonium ion (compare *p*-methoxybenzyl bromide to benzyl bromide) and an aqueous rather than a less polar solvent. As suggested by Dipple and co-workers,[4] this first-order process resembles acylation. The thermodynamically more stable amino-substituted product is formed. The neutral product formed after 3 substitution is in the less stable amino tautomeric form of cytidine.

Our results provide additional information on the factors that control amino group substitution in nucleosides. The biological significance of this event remains undetermined.

## Experimental Section

Melting points are uncorrected. Ultraviolet spectra were determined on a Cary 15 spectrophotometer, infrared spectra with a Perkin-Elmer Model 137 spectrophotometer, and NMR spectra with a Varian XL-100 instrument using tetramethylsilane ($\tau$ 10.00) as standard. Mass spectra were determined at 70 eV with Varian M-66 and CEC 21-110B spectrometers. Thin layer chromatography was performed on Avicel mecrocrystalline cellulose TG-101 (FMC Corp.) and on precoated silica gel plates (E. Merck). Plates were visualized with a uv lamp equipped with a short-wavelength filter. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

**Preparation of $N^4$-Benzylcytidine (3a). A. From 2′,3′,5′-Tri-*O*-benzoyl-4-thiouridine.** A mixture of 228 mg (0.396 mmol) of 2′,3′,5′-tri-*O*-benzoyl-4-thiouridine[6] (**2**) and 2.26 ml (24.0 mmol) of benzylamine in 6 ml of absolute ethanol was heated for 22 h at 100 °C in a sealed vial. The solvents were then removed under vacuum, and 40 ml of water was added to the resulting heavy liquid. The aqueous layer was extracted repeatedly with benzene, then freeze dried to afford a light yellow solid. This product was worked up by preparative TLC in methanol–benzene (3:7). The major band ($R_f$ 0.20) afforded 111 mg of a light yellow glass. This material was dissolved in methanol–2-propanol (2:3) and filtered. The solvents were evaporated, and the solid triturated with ether to afford 40 mg (29%) of an amorphous solid, which could not be crystallized: $\lambda_{max}$ (pH 2) ($\epsilon$) 285 nm (16 400), $\lambda_{max}$ (pH 7) 238, 273 nm; NMR ($CD_3OD$) $\tau$ 2.07 (1 H, d, $J$ = 8 Hz, $H_6$) 2.71 (5 H, s, $C_6H_5$), 4.00–4.20 (2 H, m, $H_5$, $H_{1'}$), 5.42 (2 H, s, $CH_2$), 5.78–6.08 (3 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 6.10–6.30 (2 H, m, $H_{5'}$); ir (KBr) 6.03, 6.36, 6.60 $\mu$; mass spectrum *m/e* calcd for $C_{16}H_{19}N_3O_5$, 333.1325; found, 333.1303.

Anal. Calcd for $C_{16}H_{19}N_3O_5 \cdot H_2O$: C, 54.70; H, 6.03; N, H.95. Found: C, 55.39; H, 5.65; N, 11.99.

**B. Preparation of 3a from Cytidine.** Cytidine (48.6 mg, 0.20 mmol), benzylamine (0.44 ml, 4.0 mmol), and 5 ml of aqueous sodium bisulfite were combined. The pH was adjusted to 7.2 with 6 N NaOH, and the final volume of solution was brought to 12.5 ml by addition of water. The solution was heated at 45 °C for 138 h. The pH was adjusted to 10.7 by addition of concentrated $NH_4OH$, and the solution was stirred for 10 min. The solvents were evaporated to give a white powder. The powder was dissolved in water, and the solution was absorbed onto 100 ml of cation exchange resin (Dowex A.G. 50W-X8), $H^+$ form. The column was washed with water and then eluted with 1.5 N $NH_4OH$. The purity of fractions was checked by TLC on cellulose in acetonitrile–0.1 M $NH_4Cl$ (9:1) ($R_f$ 0.13 for cytidine, 0.67 for **3a**). Earlier fractions were contaminated with cytidine, later fractions with a fluorescent impurity. The pure middle fractions were evaporated to afford 17.3 mg (26%) of **3a** as a glass. The $R_f$ (above) of **3a** and NMR were identical with those of the product prepared by another route.

**$N^4$-(*p*-Methoxybenzyl)cytidine (3b).** The first procedure listed above for **3a** was employed, starting from 230 mg (0.40) mmol of 2′,3′,5′-tri-*O*-benzoyl-4-thiouridine and 3.28 ml (25.2 mmol) of *p*-methoxybenzylamine. After preparative TLC on silica in methanol–benzene (3:7), the product ($R_f$ 0.37) was recrystallized from ethanol–ethyl acetate to yield 67 mg (44%) of a solid: $\lambda_{max}$ (pH 2) ($\epsilon$) 283 nm (16 600); $\lambda_{max}$ (pH 7) 273 nm; NMR ($CD_3OD$) $\tau$ 2.10 (1 H, d, $J$ = 8 Hz, $H_6$), 2.76 (2 H, d, $J$ = 8 Hz, aromatic), 3.16 (2 H, d, $J$ = 8 Hz, aromatic), 4.06–4.20 (2 H, m, $H_5$, $H_{1'}$), 5.50 (2 H, s, $CH_2$), 5.78–6.08 (3 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 6.10–6.28 (5 H, m, $OCH_3$, $H_{5'}$); ir (KBr) 6.03, 6.36, 6.60 $\mu$; mass spectrum *m/e* 231 (base + $H^+$), 121 ($CH_3OC_7H_6^+$).

Anal. Calcd for $C_{17}H_{21}N_3O_6 \cdot H_2O$: C, 53.53; H, 6.08; N, 11.02. Found: C, 53.79; H, 6.09; N, 10.50.

**$N^4$-(Benz[a]anthryl-7-methyl)cytidine (3c).** A mixture of 115 mg (0.20 mmol) of 2′,3′,5′-tri-*O*-benzoyl-4-thiouridine[6] (**2**) and 257 mg (1 mmol) of 7-aminomethylbenz[a]anthracene[4] in 8 ml of absolute ethanol was heated for 42 h at 100 °C in a sealed vial. The solvent was evaporated and the residue was dissolved in 8 ml of methanol. Dry ammonia gas was passed through the solution at 25 °C for 3 h. The suspension was filtered and washed with methanol. The filtrate was concentrated and worked up by preparative TLC on silica in methanol–benzene (3:7). The product ($R_f$ 0.45) was triturated with ethyl acetate, washed with ether, and recrystallized from ethanol–water to yield 44 mg (45%) of light yellow crystals: mp 175–177 °C; $\lambda_{max}$ (MeOH) ($\epsilon$) 260 nm (38 700), 270 (52 800), 280 (88 700), 291 (106 000), 319 (5100), 335 (8600), 351 (12 300), 369 (7900), 388 (900); NMR ($CD_3OD$) $\tau$ 0.6–2.6 (12 H, m, $H_6$ + aromatic), 4.05 (1 H, d, $J$ = 3 Hz, $H_{1'}$), 4.20 (1 H, d, $J$ = 8 Hz, $H_5$), 4.55 (2 H, s, $CH_2$), 5.60–6.60 (5 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$); ir (KBr) 6.01, 6.40, 6.62 $\mu$.

Anal. Calcd for $C_{28}H_{25}N_3O_5 \cdot H_2O$: C, 67.05; H, 5.43; N, 8.38. Found: C, 66.95; H, 5.35; N, 8.28.

**3-Benzylcytidine Hydrobromide (4a).** This compound was prepared by the method of Brookes et al.[8] and had its melting point and uv in accord with those listed: NMR ($CD_3OD$) $\tau$ 1.47 (1 H, d, $J$ = 8 Hz, $H_6$), 2.65 (5 H, s, $C_6H_5$), 3.71 (1 H, d, $J$ = 8 Hz, $H_5$), 4.10 (1 H, d, $J$ = 2 Hz, $H_{1'}$), 4.67 (2 H, s, $CH_2$), 5.73–6.03 (3 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 6.04–6.21 (2 H, m, $H_{5'}$); ir (KBr) 5.75, 6.01, 6.46 $\mu$.

**3-*p*-Methoxybenzylcytidine Hydrobromide (4b).** To a suspension of cytidine (243 mg, 1 mmol) in 4 ml of dry dimethylacetamide was added 605 mg (3 mmol) of *p*-methoxybenzyl bromide.[17] The mixture was heated in a sealed tube at 32 °C for 72 h. To the mixture were added acetone (8 ml), ethyl acetate (80 ml), and then ether, to the point where the solution became cloudy. The solution was allowed to stand at 25 °C for 16 h. The gummy solid which deposited was triturated with ether, then worked up by preparative TLC on cellulose in 2-propanol–water (8:2). The product ($R_f$ 0.66) was purified by recrystallization from ethanol–ethyl acetate to yield 228 mg (51%) of **4b**: mp 176–177°; $\lambda_{max}$ (pH 2) ($\epsilon$) 280 nm (13 500); $\lambda_{max}$ (pH 11.5) 268 nm; NMR ($CD_3OD$) $\tau$ 1.47 (1 H, d, $J$ = 8 Hz, $H_6$), 2.69 (2 H, d, $J$ = 8 Hz, aromatic), 3.07 (2 H, d, $J$ = 8 Hz, aromatic), 3.73 (1 H, d, $J$ = 8 Hz, $H_5$), 4.08 (1 H, d, $J$ = 2 Hz,

$H_{1'}$), 4.75 (2 H, s, $CH_2$), 5.70–6.04 (3 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 6.05–6.33 (5 H, m, $OCH_3$ at 6.23, $H_{5'}$); ir (KBr) 5.75, 6.03, 6.50, 6.60 $\mu$.

**3-(Benz[a]anthryl-7-methyl)cytidine Hydrobromide (4c).** This compound was prepared by the method of Dipple et al.[4] from 300 mg (1.23 mmol) of cytidine and 450 mg (1.4 mmol) of 7-bromomethylbenz[a]anthracene.[18] Purification of the product was performed by preparative TLC on cellulose with 1-butanol–water (86:14). The yield of recrystallized product was 242 mg (35%): mp 177–179 °C (lit.[4] 177–179 °C); $\lambda_{max}$ (MeOH) ($\epsilon$) 260 nm (37 100), 270 (45 400), 281 (74 100), 292 (89 000), 320 (5800), 336 (8300), 353 (9800), 370 (7400), and 388 (2500); NMR ($Me_2SO$-$d_6$ + $D_2O$) $\tau$ 0.36–2.46 (12 H, m, aromatic and $H_6$), 3.50 (1 H, d, $J$ = 8 Hz, $H_{5'}$), 3.92 (2 H, s, $CH_2$), 4.62 (1 H, d, $J$ = 3 Hz, $H_{1'}$), 5.96–6.46 (5 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$); ir (KBr) 5.80, 6.00, 6.52 $\mu$.

**Preparation of 3-(Benz[a]anthryl-7-methyl)uridine (5) from 4c. A. Nitrous Acid.** Sodium nitrite (560 mg, 8.12 mmol) was added over 30 min at 25 °C to a solution of 70 mg (0.124 mmol) of **4c** in 7 ml of aqueous acetic acid. The reaction mixture was stirred at 5 °C for 16 h. The precipitate that appeared was filtered and washed with water. This product was separated from contaminating starting material by preparative TLC on cellulose using 1-butanol–water (86:14), and recrystallized from absolute ethanol to give 21 mg (35%) of **5**: no sharp melting point; $\lambda_{max}$ (MeOH) 260, 270, 280, 291, 321, 336, 352, 369, and 388 nm; NMR ($Me_2SO$-$d_6$) $\tau$ 0.4–2.60 (12 H, m, aromatic + $H_6$), 3.96 (2 H, s, $CH_2$), 4.13 (1 H, d, $J$ = 8 Hz, $H_5$), 4.36 (1 H, d, $J$ = 3 Hz, $H_{1'}$), 4.60–5.20 (3 H, m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5.90–6.60 (5 H, m, $H_{5'}$ + OH); ir (KBr) 5.82, 6.05 $\mu$; mass spectrum $m/e$ 484 ($M^+$); TLC on silica, $R_f$ 0.41 (methanol–benzene, 3:7), 0.26 (acetone); TLC on cellulose, $R_f$ 0.69 (2-propanol–water, 8:2).

Anal. Calcd for $C_{28}H_{24}N_2O_6 \cdot H_2O$: C, 66.92; H, 5.22; N, 5.58. Found: C, 66.57; H, 4.80; N, 5.71.

**B. Sodium Bisulfite.** A suspension of 10 mg (0.017 mmol) of **4c** in 2.5 ml of 2.26 M $NaHSO_3$ solution, pH 5.0, was stirred at 37 °C for 22 h. The pH was adjusted to 11.5 by addition of $Na_2HPO_4$ and NaOH, and the reaction mixture stirred for an additional 1 h. The pH was then adjusted to 1 by addition of 6 N HCl, and a stream of nitrogen was passed through to remove sulfur dioxide. NaOH was added, to change the pH to 7, and the precipitate, which contained starting material and product, was filtered. The work-up by preparative TLC was conducted in the same manner as in the nitrous acid reaction. The $R_f$ values and uv spectrum of the product were identical with those of the product in the nitrous acid reaction. The yield (estimated spectrophotometrically) was 53%.

**Preparation of 3-(Benz[a]anthryl-7-methyl)uridine (5) from Uridine.** To a suspension of 20 mg (0.41 mmol) of sodium hydride (50% dispersion in oil) in 1 ml of dry dimethylformamide was added 100 mg (0.41 mmol) of uridine. The mixture was heated under $N_2$ for 3 h at 70 °C, and 158 mg (0.49 mmol) of 7-bromomethylbenz[a]anthracene in 2 ml of dry dimethylformamide was added. The reaction mixture was stirred under $N_2$ at 70 °C for 20 h, and the solvents were evaporated under vacuum. The residue was worked up by preparative TLC on silica in methanol–benzene (3:7). A crude yield of 87 mg (44%) of yellow, partly crystalline material, $R_f$ 0.48, was obtained. A pure sample, identical in ir (KBr) and $R_f$ with **5** preprared from **4c**, was obtained.

**Reaction of Cytidine with Benzylic Bromides in Aqueous Buffer. Product Distribution Studies.** To 100 mg (0.41 mmol) of cytidine dissolved in 45 ml of 0.01 M sodium acetate buffer, pH 5.5, was added 0.5 mmol of either benzyl bromide (as a solution), p-methoxybenzyl bromide (as a solution), or 7-bromomethylbenz[a]anthracene (as a suspension, in 5 ml of acetone), over a 30-min period. The reaction mixtures were stirred at room temperature for 7 days and then freeze dried. The resulting powders were extracted in methanol and worked up by preparative thin layer chromatography: on cellulose in 2-propanol–water (8:2) for the benzyl bromide reaction, on cellulose in acetonitrite–0.1 M $NH_4Cl$ (9:1) for the p-methoxybenzyl bromide reaction, and on silica with methanol–benzene (3:7) for the 7-bromomethylbenz[a]anthracene reaction. The following are the $R_f$ values of the marker compounds run to locate the products: **4a**, 0.45; **3a**, 0.78; **4b**, 0.25; **3b**, 0.42; **4c**, 0.25; **3c**, 0.48. The identity of each compound was confirmed by its uv spectrum and $R_f$ on TLC in a different solvent system.

**Reaction of Cytidine with Benzylic Bromides in Dimethylacetamide. Product Distribution Studies.** Cytidine (20 mg, 0.8 mmol) and an approximately twofold excess of each of the benzylic halides were allowed to react for 24 h at 38 °C in dry dimethylacetamide. The reaction mixtures were worked up by preparative TLC following the procedures used in the aqueous reactions.

**Reaction of Cytidine with Dimethyl Sulfate in Dimethylformamide.** To a suspension of 400 mg (1.64 mmol) of cytidine in 4 ml of dry dimethylformamide was added 1.6 ml (16 mmol) of dimethyl sulfate. The mixture was heated for 45 min at 40 °C. On addition of 10.4 ml of methanol and 25 ml of ethyl acetate, a precipitate appeared. The melting point, $R_f$, and uv spectrum of this substance were identical with those reported for 3-methylcytidine methosulfate,[19] yield 450 mg (77%). The mother liquor was concentrated under vacuum, neutralized with dilute ammonia, and worked up by preparative TLC on cellulose in 1-butanol–water–concentrated $NH_4OH$ (86:14:1). A number of weak bands were observed by uv, but none corresponded in $R_f$ to an authentic marker of $N^4$-methylcytidine.[7]

**Reaction of Cytidine with Dimethyl Sulfate in Aqueous Buffer.** To a solution of 50 mg (0.21 mmol) of cytidine in 4 ml of 0.05 M sodium acetate buffer, pH 5.5, was added 0.4 ml (4.3 mmol) of dimethyl sulfate. The reaction mixture was stirred at 25 °C for 1 h, with occasional addition of NaOH to maintain the pH at 5.0. The reaction mixture was examined by TLC on cellulose in 1-butanol–water–concentrated $NH_4OH$ (86:14:1). Spots were observed that were identified by $R_f$ and uv as 3-methylcytidine ($R_f$ 0.34, yield 64%) and unreacted cytidine ($R_f$ 0.15). No spot corresponding in $R_f$ to a marker of $N^4$-methylcytidine was observed.

**Registry No.**—1, 24961-39-5; **2**, 15049-50-0; **3a**, 58343-13-8; **3b**, 58343-14-9; **3c**, 58343-15-0; **4a**, 22423-32-1; **4b**, 58343-16-1; **4c**, 58343-17-2; **5**, 58343-18-3; benzylamine, 100-46-9; cytidine, 65-46-3; p-methoxybenzylamine, 2393-23-9; 7-aminomethylbenz[a]anthracene, 58343-19-4; p-methoxybenzyl bromide, 2746-25-0; uridine, 58-96-8; benzyl bromide, 100-39-0; dimethyl sulfate, 77-78-1.

## References and Notes

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