(d, J = 8 Hz, 1 H, Ar H), 8.9 (s, 1 H, Ar H), 12.9 (s, 1 H, OH).

Anal. Calcd for $C_{26}H_{20}O_5$: C, 75.72; H, 4.85. Found: C, 75.81; H, 4.96.

2-Methyl-7,11-dimethoxy-12-(benzyloxy)-6*H*-benzo[*b*]naphtho[2,3-*d*]pyran-6-one (4g). A magnetically stirred solution of monobenzyl ether **4e** (1.66 g, 4.05 mmol), K_2CO_3 (10 g), and excess dimethyl sulfate (2 mL, 21 mmol) in acetone (350 mL) was heated at reflux under nitrogen overnight. The solution was cooled, filtered, and evaporated at reduced pressure. The residue was taken up in ether and triethylamine (10 mL) was added. After standing for 1 h, the mixture was washed repeatedly with water, 10% HCl, and brine, then dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed (silica gel, CH_2Cl_2) to afford 1.21 g of pure **4g** which was recrystallized from acetone: mp 158–60 °C; ^{1}H NMR ($CDCl_3$) 4.8 (s, 2 H, OCH_2Ar), 7.0 (d, J = 8 Hz, 1 H, Ar H), 7.1 (s, 2 H, Ar), 7.3–7.5 (m, 6 H, Ar H), 7.97 (d, J = 8 Hz, 1 H, Ar H), 8.86 (s, 1 H, Ar H).

Anal. Calcd for $C_{27}H_{22}O_5$: C, 76.06; H, 5.16. Found: C, 75.81; H, 5.12.

Methyl 3-(2-Methoxy-5-methylphenyl)-1,5-dimethoxy-4-(benzyloxy)-2-naphthoate (5a). Dimethoxybenzyloxy compound (**4g**) (0.605 g, 1.42 mmol) was suspended in 2 equiv of sodium hydroxide (0.114 g, 2.45 mmol) in water (100 mL) and heated on a steam bath overnight. The sides of the flask were washed down with acetone at irregular intervals. The homogeneous solution was evaporated to dryness and the residue suspended in acetone. Potassium carbonate (5 g) and dimethyl sulfate (2 mL) were added and the mixture was heated at reflux overnight. The reaction mixture was filtered and evaporated and the residue taken up in diethyl ether. Triethylamine (5 mL) was added and the mixture allowed to stand for 1 h before being washed repeatedly with water, dilute HCl, and brine. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 0.825 g of crude **5a**. Chromatography on silica gel afforded 0.58 g of an oil: ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3 H, Ar CH_3), 3.47 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 4.56 (d'd, J = 9 Hz, 2 H, OCH_2Ph), 6.6–7.2 (m, 9 H, Ar H), 7.32 (t, J = 8 Hz, 1 H, Ar H), 7.72 (d, J = 8 Hz, 1 H, Ar H).

Methyl 3-(2-Methoxy-5-methylphenyl)-1,5-dimethoxy-4-hydroxy-2-naphthoate (5b). Trimethoxy(benzyloxy)-3-aryl-2-naphthoate ester **5a** (0.580 g, 1.23 mmol) dissolved in absolute ethanol (100 mL) containing palladium on carbon (10%, 300 mg) was shaken under 20 psi of H_2 gas for 2.5 h in a Parr hydrogenator. The solution was filtered through Celite and the filtrate evaporated. The residue was chromatographed (silica gel, CH_2Cl_2) to afford 0.350 g of **5b**: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3 H, Ar CH_3), 3.5 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 3.9 (s, 6 H, OCH_3), 6.65–7.2 (m, 4 H, Ar H), 7.3 (t, J = 8 Hz, 1 H, Ar H), 7.7 (d, J = 8 Hz, 1 H, Ar H), 9.4 (s, 1 H, OH); UV (EtOH) λ_{max} 345 nm, 330, 317, 291, 230 (ϵ 2175, 1720, 1565, 1490, 25 000).

Anal. Calcd for $C_{22}H_{22}O_6$: C, 69.11; H, 5.76. Found: C, 69.07; H, 5.83.

Chartreusin Aglycon (1b). To naphthoate **5b** (0.323 g, 0.846 mmol) dissolved in benzene (100 mL) and cooled in an ice bath was added cold condensed phosgene (20 mL) and pyridine (2 mL) while the mixture was stirred vigorously. The mixture was allowed to come to room temperature and stir for 1 h before the phosgene was removed by boiling on a steam bath under a hood. The benzene was removed under reduced pressure and the residue suspended in carbon disulfide (200 mL). An excess of aluminum chloride (5 g) was added and the mixture heated at reflux overnight. The solvent was evaporated and the dark green residue suspended in water and methylene chloride. The aqueous layer was removed and the organic layer dried ($MgSO_4$) and filtered. The filtercake was washed repeatedly with hot methylene chloride until the green color vanished. The filtrate was concentrated to 100 mL and cooled, yielding a green amorphous powder. Further concentration gave a second crop of material. The two crops were sublimed (250 °C, 0.03 mm) separately and found to have ultraviolet–visible spectra identical with the natural chartreusin aglycon (**1b**); mass spectrum, m/z 334 ($M^{+\cdot}$), with fragmentation pattern the same as the authentic material.

The mother liquor from the above precipitation was evaporated to dryness and the dark residue dissolved in 100 mL of acetic acid saturated with hydrobromic acid. The mixture was heated at reflux for 24 h and the green solution evaporated to dryness under reduced pressure. The residue was suspended in dilute HCl solution and heated at reflux overnight. The solvent was removed at reduced pressure and the residue was dissolved in hot methylene chloride. The green powder which precipitated upon cooling was sublimed (250 °C, 0.05 mm). The amorphous sublimate, while too insoluble for NMR spectroscopy, had ultraviolet, visible (EtOH), infrared ($CHCl_3$), and mass spectra (m/z 334 ($M^{+\cdot}$)) identical with natural chartreusin aglycon (**1b**). The melting point of the synthetic material (mp 302 °C) was undepressed when a mixture melting point[11] with an authentic sample (mp 313–314 °C) was taken, giving a value of 308–309 °C. The overall yield of aglycon from **5b** was 48%.

Acknowledgment. We are grateful to Dr. T. Ross Kelly of Boston College for providing a generous sample of natural chartreusin aglycon and for his extremely helpful comments concerning the purification of the final product. This work was generously supported by the National Cancer Institutes of the Department of Health, Education, and Welfare, Grant CA 18141.

Registry No. 1b, 34170-23-5; **2a**, 91-64-5; **2b**, 92-48-8; **3a**, 65131-08-0; **3b**, 74724-81-5; **4a**, 74724-82-6; **4b**, 74724-83-7; **4c**, 74724-84-8; **4e**, 74724-85-9; **4f**, 74744-16-4; **4g**, 74724-86-0; **5a**, 74724-87-1; **5b**, 74724-88-2; **5c**, 74724-89-3; phosgene, 75-44-5.

(11) The mixture melting point was taken in a sealed capillary on a Thomas-Hoover melting-point apparatus.

Structural Biochemistry. 20. Methylation of Purine Nucleosides[1]

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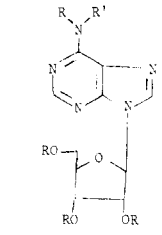
Experimental methods have been devised for the permethylation of both adenosine (**1a** → **1b**) and guanosine (**2a** → **2b** + **3a**) employing trimethylanilinium methoxide (TMAM). Reaction of the TMAM reagent with inosine (**5a**) and xanthosine (**11**) was found to promote imidazole ring and/or riboside cleavage (e.g., **5a** → **7a** and **10**). While methylation of inosine resulted in a variety of such reaction pathways, xanthosine followed essentially a single direction, leading to caffeine (**12**) as the major product. Reaction conditions developed for permethylation of these purine nucleosides with the TMAM reagent should prove useful with other such heterocyclic glycoside systems.

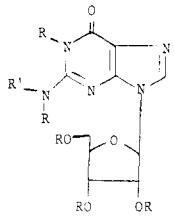
The selective methylation of tRNA and DNA by species specific methyl transferases may provide some of the structural integrity (by conformational changes) that inhibits ready incorporation of similar nucleic acids from

0022-3263/80/1945-4073$01.00/0

Chart I



1a, R = R' = H
b, R = R' = CH_3
c, R = CH_3, R' = H



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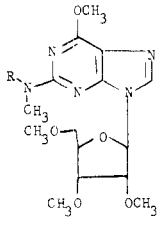
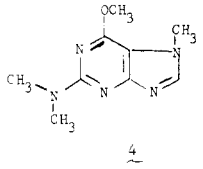
b, R = R' = CH_3
c, R = CH_3, R' = H



3a, R = CH_3
b, R = H



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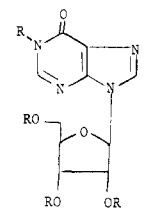
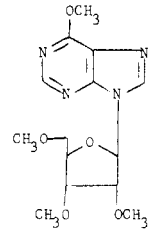


5a, R = H
b, R = CH_3

Chart II



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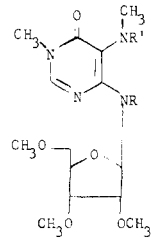
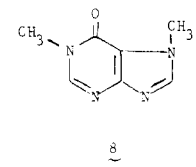


7a, R = R' = H
b, R = H, R' = CH_3
c, R = CH_3, R' = H



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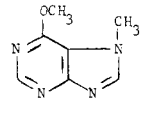

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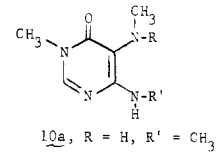
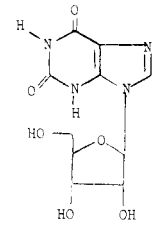
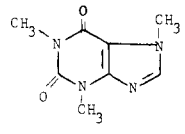


10a, R = H, R' = CH_3
b, R = CH_3, R' = H



11



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other organisms and invading viruses.[2] Interestingly, an abnormally high level (and/or site selection) of nucleoside methylation has been found to occur in certain neoplastic tissue.[3] The daily excretion (urine) of N^2,N^2-dimethylguanosine by some human cancer patients has been estimated to increase up to tenfold (20 mg/day) as compared to disease-free controls.[4] Other such potential markers of cancer progression include methylinosine, -adenosine, and -guanosine (N^2). Interest in both devising synthetic approaches to *N*- and *O*-methyl nucleosides[1,2,5a] of biosynthetic origin[6] and development of a permethylation procedure applicable to mass spectral characterization of nucleosides[5a] and small ribonucleosides[5b] led us to undertake the present investigation of purine nucleoside permethylation.

Previously we reported[1,2,5a] the pyrolytic methylation of pyrimidine nucleosides using trimethylanilinium hydroxide (TMAH). For initial attempts at exhaustive methylation of purine nucleosides we employed silver oxide–methyl iodide (Hakomori reaction), dimethyl sulfate, or diazomethane (in the presence of a Lewis acid catalyst such as tin(II) chloride). Permethyladenosine (**1b**) was isolated albeit in poor yield (1%) from a single treatment of adenosine (**1a**) with silver oxide–methyl iodide (see Chart I). With diazomethane–tin(II) chloride as the methylating agent, only partially alkylated products[2] were detected. Both the yield and extent of alkylation were markedly improved when, e.g., adenosine was heated with trimethylanilinium methoxide. Alkylation (**1a** → **1b**) of both hydroxyl and amino groups was realized. Other trimethylanilinium nucleoside salts were also prepared by mixing the respective nucleoside with strongly basic *N*,*N*,*N*-trimethylanilinium methoxide (TMAM) in anhydrous methanol. The anilinium methoxide reagent was prepared by the reaction of *N*,*N*,*N*-trimethylanilinium iodide with silver oxide in anhydrous methanol.[7,8]

Permethylation of guanosine (**2a**) proved to be much more difficult as compared to that of adenosine due to lower solubility in methanol and a greater number of side reactions. Both dimethyl sulfate and TMAM easily methylated guanosine at the N-7 position. But when the quaternary ammonium cation formed, glycoside cleavage occurred, and experiments were undertaken to the improve yields of the guanosine methylation products. Yield improvement was obtained when the reaction mixture was concentrated in vacuo prior to heating.[9] When the concentrated mixture was heated at 135 °C for 30 min, permethylguanosine was not obtained. Instead, substantial quantities of partial methylation products were observed. Other reaction temperatures from 105 to 200 °C did not

(1) For part 19 in the Structural Biochemistry series see: G. R. Pettit, K. Yamauchi, and J. J. Einck, *Synth. Commun.*, **10**, 25 (1980). The present paper is based on the Ph.D. dissertations of R. M. Blazer and J. J. Einck, submitted to the Graduate School, Arizona State University, 1976.

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(4) C. W. Gehrke, K. C. Kuo, T. P. Waalkes, and E. Borek, "Biological Markers of Neoplasia: Basic and Applied Aspects", N. Ruddon, Ed., Elsevier, Amsterdam, 1978, pp 559–567.

(5) G. R. Pettit, P. Brown, J. J. Einck, K. Yamauchi, and R. M. Blazer, *Synth Commun.*, **7**, 449 (1977); G. R. Pettit, J. J. Einck, and P. Brown, *Biomed. Mass Spectrom.*, **5**, 153 (1978).

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(7) E. Brochmann-Hanssen and T. O Oke, *J. Pharm. Sci.*, **58**, 370 (1969).

(8) Examination of the reaction stoichiometry, $2I^- + Ag_2O + CH_3OH \rightarrow 2AgI + CH_3O^- + OH^-$, indicates that an equal mixture of *N*,*N*,*N*-trimethylanilinium hydroxide (TMAH) and methoxide (TMAM) should be produced. Since the mixture was dried over 3-Å molecular sieves which retain water but not methanol, the product should be the more basic methoxide reagent (TMAM).

(9) Removal of methyl alcohol caused a shift of the acid–base equilibrium toward nucleoside anion formation (eq 1). Pyrolysis of the resulting salt mixture showed an enhancement of methylation of nucleoside (eq 2) and a reduction of reagent decomposition caused by methoxide ion attack to form dimethyl ether (eq 3): $RYH + CH_3O^- \rightleftharpoons RY^- + CH_3OH$ where Y = N, O (1); $PhN^+(CH_3)_3RY^- \xrightarrow{\Delta} RYCH_3 + PhN(CH_3)_2$ (2); $PhN^+(CH_3)_3OCH_3^- \xrightarrow{\Delta} CH_3OCH_3 + PhN(CH_3)_2$ (3).

lead to any noticeable improvement. However, when the TMAM/methanol reagent was concentrated to a syrup and added over a 12–20-min period to guanosine either in the solid state or suspended in xylene, *o*-dichlorobenzene, or nitrobenzene comparable, moderate quantities of permethylguanosine isomers (**2b** and **3a**) were produced. The hexamethylguanosine isomers **2b** and **3a** are also formed (mass spectral evidence) by using the Hakomori methylation technique[10] but were not heretofore isolated. The best yields (48%) of permethylguanosines **2b** and **3a** were obtained by employing a 200% excess of TMAM reagent. The crystalline products proved to be quite hygroscopic and were characterized as tetrafluoroborate salts.

The ability of crown ethers[11] to complex and thereby dissolve cations suggested the possibility of methylating guanosine with methyl iodide–silver oxide (in dimethylformamide) in the presence of 18-crown-6. Although a reasonable yield (19%) of the permethylguanosine was obtained, this promising approach was not further developed as part of the present investigation.

Methylation of inosine (**5a**) was conducted as summarized above for guanosine with the TMAM reagent. However, only products resulting from opening of the purine ring were obtained. Evidently alkylation occurred at N-7, followed by nucleophilic attack at C-8. After ring cleavage, base hydrolysis of the formamide would result in the various imidazole ring-opened products (**7a–c** and **10a–b**, Chart II) apparently formed. In a separate experiment at a lower temperature 1-methyl-6-oxo-5-(methylamino)-4-[($O^{2',3',5'}$-trimethylribosyl)amino]pyrimidine (**7a**) was isolated and characterized. Under similar conditions (200% excess TMAM and pyrolysis at 220 °C), methylation of xanthosine (**11**) gave almost exclusively caffeine (**12**).[12]

In summary, the first isolation and characterization of guanosine permethylation products was achieved. The TMAM reagent was found very useful for permethylation (and cleavage) of such purine heterocyclic systems. Further exploration of a crown ether technique for such methylation reactions should prove useful.

Experimental Section[13]

Trimethylanilinium Methoxide. The trimethylanilinium hydroxide (TMAH) reagent[7] was prepared from trimethylanilinium iodide (26.3 g, 100 mmol) and silver oxide (17.4 g, 150 mmol, 50% excess) in dry methanol (150 mL) as previously summarized.[2] After the silver salts were removed (filtration) and washed with dry methanol the final volume was 160 mL. The reagent was dried over 3-Å molecular sieves to promote further production of trimethylanilinium methoxide (TMAM). An ^{1}H NMR analysis employing the methyl signal of mesitylene as an internal standard allowed the trimethylanilinium ion content to be estimated as 0.61 M (97% of theoretical).

Methylation of Adenosine (1a). Method A. Silver Oxide–Methyl Iodide.[14] A mixture of adenosine (**1a**; 1.0 g, 3.8 mmol), methyl iodide (16.2 mL, 36.9 g, 260 mmol), silver oxide (17.2 g, 75 mmol) and dimethylformamide (DMF, 20 mL) was placed in a heavy-walled Pyrex tube (6 in. × $^1/_2$ in. o.d., sealed under vacuum at liquid nitrogen temperature) and the tube shaken at room temperature for 3 days. After the solvent was removed in vacuo (rotary evaporator) extraction with hot chloroform (2 × 50 mL) and evaporation of solvent yielded a light brown waxy solid (100 mg, 8%). Silica gel (25 g) chromatography and elution with ligroin/benzene, benzene/chloroform (4:1 to 1:4), and chloroform afforded 15 mg (1%) of permethyladenosine (**1b**): mp 74.5–76.5 °C; $[\alpha]_D$ −21.2° (*c* 0.880, CH_3OH); UV (H_2O, pH 6.6) λ_{max} 211 nm (ϵ 16 300), 272 (ϵ 19 400); IR (KBr) 3000, 2940, 2910, 2840, 1605, 1570, 1485, 1430, 1378, 1340, 1302, 1240, 1230, 1190, 1125, 1090, 1045, 995, 980, 940, 930, 892, 817, 795, 765, 730, 680, 650, 610, 600 cm^{-1}; ^{1}H NMR (CCl_4) δ 8.08 (s, 1, 8-H), 7.93 (s, 1 2-H), 6.00 (d, 1, J = 2.2 Hz, 1′-H), 3.5–4.3 (m, 5, 2′-, 3′-, 4′-, 5′-H), 3.54 (s, 3, OCH_3), 3.47 (s, 6, $N(CH_3)_2$), 3.45 (s, 3, OCH_3), 3.35 (s, 3, OCH_3); mass spectrum, *m/e* (relative intensity) 337 (60, M$^+$), 322 (33, M − CH_3·), 306 (62, M − OCH_3·), 292 (4, M − CH_3OCH_2·), 276 (11), 274 (11, M − CH_3OH − CH_3O·), 232 (6), 220 (29, base + 58), 192 (73, base + 30), 190 (15), 174 (72, sugar − H), 165 (100), 163 (64, base + H), 143 (63, sugar − CH_3OH), 134 (84), 129 (55), 114 (66), 111 (4, 143 − CH_3OH), 101 (48), 71 (29), 45 (65).

Anal. Calcd for $C_{15}H_{23}N_5O_4$: C, 53.40; H, 6.87; N, 20.76. Found: C, 53.49; H, 6.68; N, 20.90.

Method B. Trimethylanilinium Methoxide. To a mixture of adenosine (2.0 g, 7.5 mmol) and dimethylaniline (0.5 g) heated to 180 °C was added (dropwise) trimethylanilinium methoxide (250 mL of a 0.5 M solution in methanol, 125 mmol)[2,8] over 4 h with stirring. After 3 days at 180 °C dimethylaniline was removed under reduced pressure and the residue chromatographed (see method A) on a silica gel column (2 × 70 cm). After elution of residual dimethylaniline with benzene, permethyladenoside was eluted with chloroform–acetone (5:1). Recrystallization from hexane provided 1.3 g (50%) of pure permethyladenosine identical (spectroscopically and by mixture melting point) with that prepared by the silver oxide–methyl iodide method A.

Continued elution gave a brown solid which was recrystallized from hexane to yield N^6,$O^{2',3',5'}$-tetramethyladenosine (**1c**): 0.17 g (7%); mp 92 °C; $[\alpha]_D$ −16.70° (*c* 0.900, CH_3OH); UV λ_{max} 261 nm (ϵ 15 700, pH 1.8), 265 (14 600, pH 7), 265 (14 600, pH 13); IR (KBr) 3420 (NH), 2960, 1630, 1470, 1300, 1220, 1080, 935 cm^{-1}; mass spectrum, *m/e* (relative intensity 323 (M$^+$), 292 (M − OCH_3), 149 (base + H), 124 (sugar − H).

Anal. Calcd for $C_{14}H_{21}N_5O_4$: C, 51.99; H, 6.54; N, 21.35. Found: C, 51.96; H, 6.50; N, 21.17.

Methylation of Guanosine (2a). Method A. In Xylene. To a suspension of dry guanosine (**2a**; 1.13 g, 4.0 mmol) in xylene (10 mL) at 130 °C was added (dropwise over 15 min) the TMAM–methanol reagent[2,8] (30 mmol, 25% excess). The solution was filtered, and evaporation (in vacuo) of the solvent afforded a yellow residue (81 mg, 7.2%). Silica gel preparative layer and thin-layer chromatography (chloroform–methanol of varying composition) gave three fractions containing methylated guanosines. The first fraction (9.4 mg, 0.6%) was a mixture of $N^{2,2}$,$O^{6,2',3',5'}$-hexamethylguanosine (**3a**)[10] and a pentamethylguanosine: mass spectrum, *m/e* (relative intensity) 367 (34, M$^+$), 353 (50, M′$^+$), 250 (3, base + 58), 236 (8, base′ + 58), 222 (8, base + 30), 208 (20, base′ + 30), 194 (39, base + 2 H), 193 (67, base + H), 180 (64, base′ + 2), 179 (76, base′ + H), 178 (20, 193 − CH_3), 174 (39, sugar − H), 164 (29, 193 H_2C=NH, m*) 150 (21, 179 − 29 and/or 193 − 43), 143 (47), 129 (46), 115 (40), 111 (31, 143 − CH_3OH, m*), 101 (100), 99 (49), 45 (98), 43 (54). The next fraction (35.2 mg, 2.4%) was shown to be 1,$N^{2,2}$,$O^{2',3',5'}$-hexamethylguanosine (**2b**):[10] ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1, 8-H), 6.06 (d, 1, J = 3 Hz, 1′-H), 3.8–4.4 (m, 3, 2′-, 3′-, 4′-H), 3.5–3.8 (m, 2, 5′-H), 3.57 (s, 3, CH_3), 3.55 (s, 3 CH_3), 3.47 (s, 3, CH_3), 3.45 (s, 3, CH_3), 2.90 (s,

(10) D. L. von Mindend and J. A. McCloskey, *J. Am. Chem. Soc.*, **95**, 7480 (1973).

(11) Cf.: S. C. Shah and J. Smid, *J. Am. Chem. Soc.*, **100**, 1426 (1978); G. W. Gokel and H. D. Durst, *Aldrichimica Acta*, **9**, 3 (1976).

(12) Methylation of xanthine with trimethylselenonium hydroxide has been found to yield (85%) caffeine: K. Yamauchi, K. Nakamura, and M. Kinoshita, *Tetrahedron Lett.*, 1787 (1979).

(13) Solvents were distilled prior to use. Nucleosides were obtained from the Nutritional Biochemicals Division of ICN. Silica gel column chromatography was performed with either E. Merck silica gel (0.05–0.20 mm) or E. Merck silica gel 60 prepacked columns. Thin-layer chromatography (TLC) was conducted with either Analtech silica gel GF (250 μm) or E. Merck silica gel F-254 (0.25 mm) plates. Silica gel preparative layer plates were obtained from Machery, Nagel, and Co. (Düren) and E. Merck (Darmstadt). Thin-layer and preparative layer chromatography plates were developed with ultraviolet light. Sephadex LH-20 was supplied by Pharmacia Fine Chemicals. Low-resolution mass spectrometry was performed by Mr. E. Kelley with an Atlas CH-4B single-focussing mass spectrometer. The high-resolution mass spectra were obtained by Mr. R. Scott using an Altas SM-1B spectrometer. Nuclear magnetic resonance spectra were recorded by Miss K. Reimer and Dr. J. Witschel, Jr., using Varian A60, XL-100, and T60 instruments. Tetramethylsilane was employed as an internal standard. Infrared spectra were obtained by Miss K. Reimer and Dr. J. Witschel, Jr., employing a Beckman IR-12 spectrophotometer.

(14) We wish to thank Miss Mary Ann Heiselman for assistance with development of this procedure.

6, N^2-CH_3); mass spectrum, m/e (relative intensity) 367 (100, M^+), 262 (1, base + 70), 250 (3, base + 58), 222 (8, base + 30), 194 (22, base + 2 H), 193 (38, base + H), 178 (4, 193 - CH_3), 174 (17, sugar - H), 164 (9, 193 - $H_2C{=}NH$, m*), 150 (20, M - $H_2C{=}NCH_3$, m*), 143 (17), 129 (23), 115 (8), 111 (16, 143 - CH_3OH, m*), 101 (23), 99 (8), 70 (25), 58 (31), 45 (21), 43 (47). Fraction three (5.8 mg, 0.5%), a pentamethylguanosine [either 1,N^2,$O^{2',3',5'}$ (**2c**) or N^2,$O^{6,2',3',5'}$ (**3b**)] was crystallized from chloroform–hexane to give colorless prisms: mp 201.5–202.5 °C; ^1H NMR ($CDCl_3$) δ 7.85 (s, 1, 8-H), 5.9–6.5 (m, 2, 1′-, 2′-H), 3.6–4.4 (m, 5, N^2-, 3′-, 4′-, 5′-H), 3.58 (s, 3, CH_3), 3.52 (s, 3, CH_3), 3.47 (s, 3, CH_3), 3.44 (s, 3, CH_3), 3.04 (d, 3, J = 4 Hz, N^2-CH_3); mass spectrum, m/e (relative intensity) 353 (74, M^+), 208 (3, base + 30), 180 (16, base + 2 H), 179 (35, base + H), 174 (14, sugar - H), 164 (5, 179 - CH_3), 150 (6, 179 - 29, m*), 143 (10), 129 (11), 115 (11), 111 (4, 143 - CH_3OH, m*), 101 (28), 99 (6), 58 (63), 43 (100).

Anal. Calcd for $C_{15}H_{23}N_5O_5$: C, 50.98; H, 6.56; N, 19.82. Found: C, 51.19; H, 6.37; N, 19.74.

Method B. Without Solvent. The concentrated (in vacuo) TMAM–methanol mixture (96 mmol, 300% excess) was added dropwise over 4.5 min to dry guanosine (1.132 g, 4.0 mmol) at 220 °C (Wood metal bath). After a further 0.5 min the reaction mixture was cooled and chromatographed on a column of silica gel (50 g). Elution with chloroform and chloroform–methanol (96:4) afforded 1,$N^{2,2}$,$O^{2',3',5'}$-hexamethylguanosine (**2b**) as an orange foam (0.71 g, 48%). Crystallization from hexane gave colorless, extremely hygroscopic crystals.

Crude permethylguanosine from a similar methylation reaction was subjected to Sephadex LH-20 partition chromatography using chloroform–methanol (2:3) as eluent. Fractions collected following elution of permethylguanosine were found to contain a tetramethylguanine assumed to be the 7,$N^{2,2}$,O^6 isomer **4** (32 mg, 4%). Two recrystallizations from benzene–hexane afforded colorless needles: mp 154.5–155.5 °C; ^1H NMR ($CDCl_3$) δ 7.70 (s, 1, 8-H), 4.03 (s, 3, CH_3), 3.58 (s, 3, CH_3), 2.88 (s, 6, N^2-$(CH_3)_2$); mass spectrum, m/e (relative intensity) 207 (100, M^+), 192 (14, M - CH_3), 178 (23, M - $H_2C{=}NH$, m*), 164 (81, M - $H_2C{=}NCH_3$, m*), 163 (43, 192 - $H_2C{=}NH$, m*), 136 (47, 164 - CO?, m*), 123 (54, 164 - MeNC?, m*), 67 (30).

Method C. Using 18-Crown-6. A suspension of guanosine (**2a**; 56.6 mg, 0.2 mmol), silver oxide (0.42 g, 1.8 mmol, 200% excess), methyl iodide (227 μL, 0.51 g, 3.6 mmol, 200% excess), and 18-crown-6 (0.95 g, 3.6 mmol, 200% excess) in dimethylformamide (4.0 mL) was stirred at ambient temperature for 3 days. After 2.3 h, thin-layer chromatography showed no increase in permethylguanosine formation. The reaction mixture was diluted with methanol and centrifuged to collect the voluminous suspended white solid, and the solvent was removed (in vacuo). Preparative layer chromatography employing chloroform–methanol (9:1) led to permethylguanosine (**2b**): 14.3 mg (19%); R_f 0.53 [TLC on silica gel using chloroform–methanol (9:1)].

1,$N^{2,2}$,$O^{2',3',5'}$-Hexamethylguanosine Tetrafluoroborate. To a solution of permethylguanosine (**2b**; 0.20 g, 0.54 mmol) in 95% ethanol (2.0 mL) was added 49% aqueous tetrafluoroboric acid (123 μL, 0.68 mmol). The solution was saturated with diethyl ether, and the light tan tetrafluoroborate crystallized (0.14 g, 52%). Three recrystallizations from 95% ethanol–diethyl ether gave colorless flat prisms: mp 199–201 °C; $[\alpha]^{25}_D$ + 48.0° (c 0.008, CH_3OH); ^1H NMR (MeOH-d_4) δ 9.04 (s, 1, 8-H), 6.16 (d, 1, J = 2 Hz, 1′-H), 4.40 (dd, 1, J = 2, 4 Hz, 2′-H), 3.7–4.3 (m, 4, 3′-, 4′-, 5′-H), 3.62 (s, 3, CH_3), 3.54 (s, 3, CH_3), 3.44 (s, 3, CH_3), 3.38 (s, 3, CH_3), 3.00 (s, 6, N^2-$(CH_3)_2$).

Anal. Calcd for $C_{16}H_{26}BF_4N_5O_5$: C, 42.22; H, 5.76; B, 2.38; F, 16.69; N, 15.38. Found: C, 42.09; H, 5.51; B, 2.02; F, 16.91; N, 15.45.

Methylation of Inosine (5a). Method A. Inosine (**5a**; 53.6 mg, 0.20 mmol) was heated in a test tube to 220 °C (Wood metal bath). To the hot inosine was added 1.56 M TMAM–methanol (1.54 mL, 2.4 mmol, 200% excess) reagent over a 2-min period. Heating was continued for a further 0.5 min. Following removal of dimethylaniline in vacuo silica gel preparative layer chromatography using chloroform–methanol (8:2) afforded three main fractions. Fraction 1 (28.4 mg, 43%) contained two ring-opened compounds: mass spectrum, m/e (relative intensity) 342 (29, M'^+), 328 (99, M^+), 175 (79, sugar), 168 (28, base′ + H), 154 (42, base + H), 143 (100), 115 (49), 101 (66), 99 (47), 85 (39), 58 (42), 43 (90); high-resolution mass spectrum, m/e 342.1907 (calcd for $C_{15}H_{26}N_4O_5$, 342.1903), 328.1758 (calcd for $C_{14}H_{24}N_4O_5$, 328.1747), 154.0853 (calcd for $C_6H_{10}N_4O$, 154.0855). Fraction 2 (23.0 mg, 35%) consisted of two ring-opened compounds (**7a** and either **7b** or **7c**, vide supra) and permethylinosine (**5b** or **6**): mass spectrum, m/e (relative intensity) 342 (11, M''^+, ring-opened), 328 (80, M'^+, ring-opened), 324 (81, M^+, permethylinosine), 309 (4, M - CH_3), 219 (16, base + 70), 207 (10, base + 58), 179 (17, base + 30), 175 (74, sugar), 174 (52, sugar - H), 168 (20, base″ + H), 154 (30, base′ + H), 151 (63, base + 2 H), 150 (13, base + H), 143 (100, sugar - MeOH, m*), 129 (28), 115 (74, 143 - CO, m*), 111 (66, 143 - MeOH, m*), 101 (84), 99 (74), 89 (44), 71 (47), 58 (49), 45 (72), 43 (70). Fraction 3 (3.2 mg, 10%) was principally dimethylhypoxanthine (**8** or **9**) plus a ring-opened product (**10a** or **10b**): mass spectrum, m/e (relative intensity) 168 (19, M'^+, ring-opened compound), 164 (100, M^+, dimethylhypoxanthine), 163 (72, M - H, m*), 149 (9, M - CH_3), 136 (15, M - CH_2N), 135 (10, M - 29, m*), 110 (15), 43 (35); high-resolution mass spectrum, m/e 168.1003 (calcd for $C_7H_{12}N_4O$, 168.1011), 164.0674 (calcd for $C_7H_8N_4O$, 164.0698), 163.0624 (calcd for $C_7H_7N_4O$, 163.0620), 136.0511 (calcd for $C_6H_6N_3O$, 136.0511).

Method B. Trimethylanilinium hydroxide (0.5 M in methanol)[2] was added (dropwise over 4 h) to inosine (**5a**; 0.7 g, 2.6 mmol) at 75–100 °C. After reagent addition the reaction mixture was heated to 180 °C, the methylation was repeated two additional times, and dimethylaniline was removed under reduced pressure. The residue was column chromatographed on silica gel with chloroform as eluant. The first substance eluted was distilled at 1 μm in a heating block (~140 °C) to give 1-methyl-6-oxo-5-(methylamino)-4-[($O^{2',3',5'}$-trimethylribosyl)amino]pyrimidine (**7a**) as a light viscous oil: <10% yield; IR (KBr) 3350 (m, OH), 2960 (s), 1700 (w), 1625 (s), 1580 (s), 1200 (br), 1100 (br), 955 (m), 815 (m); UV (H_2O) λ_{max} 259; ^1H NMR ($CDCl_3$) δ 6.97 (br q, 1, NH), 5.70 (d, 1, 1′-H), 5.20 (br s, 1, NH), 3.75–4.25 (m, 4, 2′-, 3′-, 4′-H), 3.50–3.65 (br m, 2, 5′-H), 3.43 (s, 3, OCH_3), 3.40 (s, 3, OCH_3), 3.39 (s, 3, OCH_3), 2.92 (d, $NHCH_3$), 2.85 (s, 3, 1-CH_3); mass spectrum, m/e 328 (M^+), 175 (sugar - H), 143 (sugar - CH_3OH), 154 (base + H).

Methylation of Xanthosine (11). To dry xanthosine (**11**; 56.8 mg, 0.20 mmol) in a test tube heated at 220 °C (Wood metal bath) was added dropwise over 2 min 1.56 M TMAM–methanol solution (1.92 mL, 3.00 mmol, 200% excess). After 0.5 min the reaction mixture was cooled, and dimethylaniline was removed (in vacuo). Silica gel preparative layer chromatography with chloroform–methanol (85:15) as eluant provided a crude sample of caffeine (**12**, 40.7 mg) as major product: mass spectrum, m/e (relative intensity) 194 (100, M^+), 193 (15, M - H, m*), 165 (6, M - CH_3N), 137 (7, M - MeNCO, m*), 136 (6), 109 (49, 137 - CO, m*), 82 (18, 109 - HCN, m*), 67 (20, 109 - $H_2NC{\equiv}N$), 55 (27, 82 - HCN).

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