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3-METHYLINOSINE: SYNTHESIS, ACID-CATALYZED CLEAVAGE OF THE GLYCOSYL BOND, BASE-CATALYZED RING-OPENING, AND HYDROGENATION OF THE PYRIMIDINE RING

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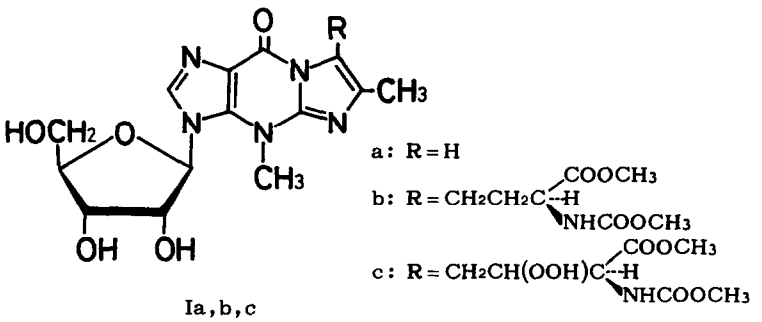
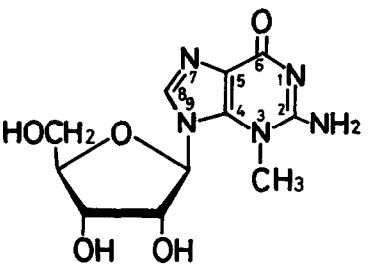
The natural occurrence of 3-alkylpurine ribosides has not been reported, although many modified nucleosides are known to occur in RNA.[1] However, the proposed structures[2] for the fluorescent Y nucleosides [wyosine (Ia); wybutosine (Ib); wybutoxosine (Ic)],[2b] which were found in $tRNA^{Phe}$s,[3] show that they are the derivatives of 3-methylguanosine (II). Nakatsuka and Goto recently mentioned the synthesis of Ia and II.[4] We also reported a simpler synthesis of II.[5] The prominent feature common between I and II is unusual lability of the nucleosidic linkage under acidic conditions.[2,3b,4,5] For elucidation of questions of such easy acidic hydrolysis, there is a need for some other 3-methylpurine ribosides. In this communication, we wish to report the synthesis and chemical properties of hitherto unknown 3-methylinosine (VIa).



Ia,b,c



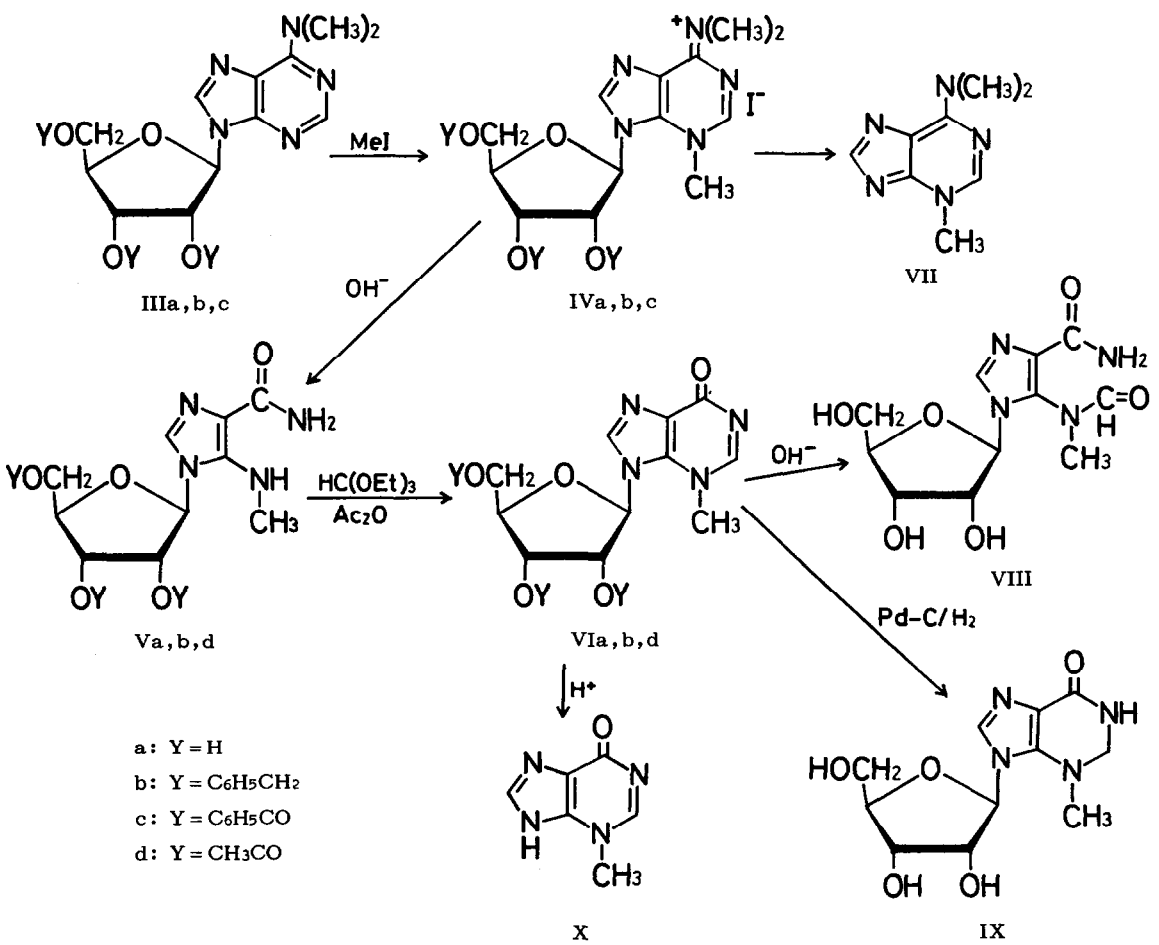
II

Since we already reported the synthesis of 3,9-dialkylhypoxanthines,[6] we undertook to apply the method of obtaining these model compounds to the nucleoside level as shown in the annexed formulae (III → IV → V → VI). But, treatment of N,N-dimethyladenosine (IIIa)[7] with an excess of CH_3I in DMAc at 40° gave a mixture of three main compounds besides the starting material. The products isolated were only the deglycosylated compounds, N,N,3-trimethyladenine (VII)[8] and N,N,3,9-tetramethyladeninium iodide.[9] The cleavage of the nucleosidic linkage might be rationalized by the nucleophilic displacement by I^- at the 1′-position of IVa. If it is the case, the ribosyl bond should be protected against the backside attack of I^- by a bulky substituent at the 2′-position.

We, therefore, tried the methylation of 2′,3′,5′-tri-O-benzyl-N,N-dimethyladenosine (IIIb: mp 88–89°; obtained from Ia in 91% yield according to the method of Michelson and Todd[10])[11] with a large excess of CH_3I in DMAc at 40° for 8 days and obtained the expected 2′,3′,5′-tri-O-benzyl-N,N,3-trimethyladenosine iodide [IVb: 81% yield; mp 143–144° (dec.); UV $\lambda_{max}^{95\%EtOH}$ 291 nm (ε 18500)]. The structure of IVb was established by the formation of VII on heating IVb in acetic acid.[12] Alkaline hy-

drolysis[6,13] took place on heating IVb at reflux in a mixture of 1 N NaOH and EtOH (1:1, v/v) for 2 hr and 5-(methylamino)-1-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)imidazole-4-carboxamide (Vb: a heavy oil) was produced. Heating Vb in a mixture of $HC(OEt)_3$ and Ac_2O[6] afforded 2′,3′,5′-tri-O-benzyl-3-methylinosine [VIb: 37% overall yield from IVb; mp 150–151°; UV $\lambda_{max}^{95\%EtOH}$ 259 nm (ε 11800)]. The structure of VIb was confirmed by the formation of 3-methylhypoxanthine (X)[14] on heating VIb in AcOH.[12]

Since the catalytic hydrogenation of VIb with 10% Pd on C resulted in reduction of the purine ring rather than debenzylation, we substituted the starting material by 2′,3′,5′-tri-O-benzoyl-N,N-dimethyladenosine (IIIc: obtained as a caramel by treating IIIa with C_6H_5COCl in pyridine). Methylation of IIIc under reaction conditions similar to those for IIIb furnished 2′,3′,5′-tri-O-benzoyl-N,N,3-trimethyladenosine iodide [IVc: 83% overall yield from IIIa; mp 189–190°(dec.); UV $\lambda_{max}^{95\%EtOH}$ 285 nm (ε 19900)]. Heating IVc at reflux in a mixture of 2 N NaOH and EtOH (2:1, v/v) for 2 hr gave 5-(methylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide [Va: 72% yield; mp 182–184°; UV $\lambda_{max}^{H_2O}$ (pH 1) 259 nm (ε 7600); $\lambda_{max}^{H_2O}$ (pH 7) 267 (8300); $\lambda_{max}^{H_2O}$ (pH 13) 268 (8500); PMR (DMSO-d_6) δ:[15] 2.82 (d, J = 5 Hz, CH_3), 3.56 (m, CH_2), 3.88, 4.04, and 4.28 (m each, C(4′)-H, C(3′)-H, and C(2′)-H), 5.02, 5.15, and 5.42 (OH), 5.48 (d, J = 5.5 Hz, C(1′)-H), 5.64 (dull q, CH_3NH), 6.85 and 6.98 (NH_2), 7.55 (s, C(2)-H); $[\alpha]_D^{26}$ −62.6° ± 1.3° (c 0.637, H_2O)].[16] This compound (Va) can be regarded as a versatile intermediate for the syn-



thesis of various 3-methylpurine ribosides and the utility of Va has been realized in the synthesis of II.[4,5]

Treatment of Va with Ac_2O in pyridine at room temperature gave 5-(methylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide [Vd: a caramel; PMR (DMSO-d_6) δ:[15] 2.13 (3H, s, CH_3CO), 2.09 (6H, s, CH_3CO), 2.81 (d, J = 5.5 Hz, $HNCH_3$)].[16] Cyclization of Vd in a similar manner to that described for Vb afforded 2′,3′,5′-tri-O-acetyl-3-methylinosine [VId: mp 161.5–162.5°; UV $\lambda_{max}^{H_2O}$ (pH 1) 253 nm (ε 11500); $\lambda_{max}^{H_2O}$ (pH 7) 259 (13000); $\lambda_{max}^{H_2O}$ (pH 13) unstable]. Removal of the acetyl groups of VId with cold NH_3–MeOH gave 3-methylinosine [VIa: $C_{11}H_{14}N_4O_5 \cdot H_2O$; 21% overall yield from IVc; mp 172–173°; UV $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 259 nm (ε 13900); $\lambda_{max}^{H_2O}$ (pH 13) unstable PMR (DMSO-d_6) δ:[15] 3.38 (H_2O), 3.64 (broad, CH_2), 3.85–4.24 (m, C(4′)-H and C(3′)-H), 4.00 (s, CH_3), 4.42 (m, C(2′)-H), 5.13, 5.30, and 5.74 (OH), 6.07 (d, J = 5 Hz, C(1′)-H), 8.12 and 8.30 (s each, purin protons); $[\alpha]_D^{26}$ −44.1°±0.1° (c 0.368, H_2O)]. The structures of VIa and VId were supported by close resemblance to 3,9-dimethylhypoxanthine[6] in UV spectrum. Further support for the structures is based on the chemical properties of VIa as described below.

In analogy with 3,9-dialkylhypoxanthine,[6] VIa underwent ring-opening at the pyrimidine moiety under alkaline conditions. When VIa was dissolved in 0.01 N NaOH at room temperature, it disappeared in 1 hr and 5-(formylmethylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide [VIII: mp 183–184° (dec.); PMR (DMSO-d_6) δ:[15] 3.02 and 3.24 (9/4H and 3/4H, s each, CH_3),[17] 5.25 (d, J = 5 Hz, C(1′)-H), 7.19 and 7.36 (NH_2), 7.98 (s, C(2)-H), 8.02 and 8.20 (3/4H and 1/4H, s each, formyl proton)[17]] was produced.

The high reactivity of the pyrimidine moiety of VIa was also realized in the catalytic hydrogenation. When VIa was treated with hydrogen and 10% Pd on C at atmospheric pressure and room temperature, the reaction smoothly took place to give stable 1,2-dihydro-3-methylinosine [IX: 83% yield; mp 189–190° (dec.); UV $\lambda_{max}^{H_2O}$ 261 nm (ε 4860); $\lambda_{max}^{H_2O}$ (pH 7) 267 (4840); $\lambda_{max}^{H_2O}$ (pH 13) 268 (4910); PMR (DMSO-d_6) δ:[15] 2.76 (s, CH_3), 4.38 (slightly dull,[18] C(2)-H_2, overlapped with the signal of C(2′)-H), 5.39 (d, J = 6 Hz, C(1′)-H, overlapped with the signal of C(2′)-OH), 7.25 (broad, NH), 7.82 (s, C(8)-H)] The signal of the methyl group shifts to higher field and the chemical shift for the proton at C-1′ is similar in magnitude to that of the imidazole nucleoside, Va or VIII. On the basis of these facts, an alternative 7,8-dihydro structure for IX is unlikely. For comparison, 3,9-dimethylhypoxanthine was reduced under similar conditions and 1,2-dihydro-3,9-dimethylhypoxanthine [77% yield; mp 200–201.5°; UV $\lambda_{max}^{H_2O}$ (pH 1) 267 nm (ε 4240); $\lambda_{max}^{H_2O}$ (pH 7) 269 (4860); $\lambda_{max}^{H_2O}$ (pH 13) 269 (4880); PMR (DMSO-d_6) δ:[15] 2.74 (s, 3-CH_3), 3.67 (s, 9-CH_3), 4.38 (slightly dull,[18] C(2)-H_2), 7.18 (broad, NH), 7.49 (s, C(8)-H)] was obtained.

Finally, the ribosyl bond of VIa proved unusually susceptible to acidic hydrolysis. When a solution of VIa in H_2O was kept at pH 3 and 40°, the reaction was completed within 24 hr and 3-methylhypoxanthine (X)[14] was obtained. At pH 1 and room temperature, the reaction was completed in 10 min. The rate of this reaction is more than 1000 times faster than the hydrolysis rate of inosine in 1 N HCl at 25°.[19] Such lability of the ribosyl bond may be comparable to that of 3-methylguanosine (II)[4,5] and of Y nucleosides (I).[2,3b,4] We anticipate from this result that alkylation of 9-β-D-ribofuranosylpurine at the 3-position markedly weakens the ribosyl bond in general. To know more about the destabilizing effect of 3-alkyl group on the glycosyl bond, we are planning the synthesis of 3-alkyl derivatives of other purine ribosides, e.g., adenosine, xanthosine, and isoguanosine.

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16. Nakatsuka and Goto gave a rough outline of the six-step synthesis of Vd from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide via Va.[4]
17. The appearance of the paired signals is probably due to cis-trans isomerism caused by restricted rotation about the C-N bond in the formamido group.
18. This signal sharpened on addition of D_2O, indicating the coupling of $C_{(2)}$-H with $N_{(1)}$-H.
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