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CONFORMATIONAL STUDIES OF 3'-C-METHYL AND 2'-C-METHYL ANALOGUES OF CORDYCEPIN

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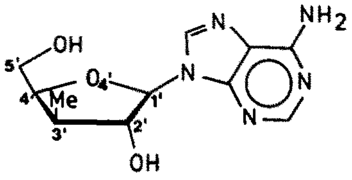
Abstract - A high resolution ^{1}H NMR conformational analysis study of a 3'-C-methyl (compound (1)) and a 2'-C-methyl (compound (2)) analogue of cordycepin, a naturall occurring anitibiotic, has been performed. For compound (1) it is found that the methyl group on $C_{3'}$ leads to an entirely different molecular conformation, which is determined primarily by a strong intramolecular hydrogen bond between $O_{5'}$ and of the *syn*-oriented adenine base. This particular conformation results in very unusual broadening of the $H_{5''}$ resonances in the case of $CDCl_3$ as solvent. Furthermore, the synthesis of compound (2) via a regiospecific Grignard-type reaction is described. Conformational analysis of compound (2) revealed that the methyl group on $C_{2'}$ shifts the conformational equilibrium of the furanose ring towards South.

Cordycepin (3'-deoxyadenosine) is a naturally occurring cytostatic antibiotic and an isomer of the DNA constituent 2'-deoxyadenosine[1]. It is a functional analogue of adenosine since its toxicity in eucaryotes and procaryotes can be reversed by adenosine[2], but not by 2'-deoxyadenosine. Cordycepin is also an important nucleoside antimetabolite[3], because it inhibits post-transcriptional processing of cytoplasmatic mRNA and acts as a potent feedback inhibitor of purine biosynthesis *de novo*[4,5]. Conformational analyses by ^{1}H NMR spectroscopy[6-8] have shown that the sugar conformation of cordycepin is more similar to adenosine, rather than to 2'-deoxyadenosine[9], which may explain the reversal of toxicity of cordycepin by adenosine, and not by its isomer 2'-deoxyadenosine. We have therefore been interested in the investigation of substituting a proton at the 2' or 3' carbon of cordycepin and its isomer (3) with a methyl group in order to explore and compare its effect on the conformation of the furanose moiety which has been shown by us to be controlled to a large extent by gauche- and



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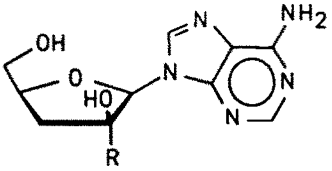


2 : R = Me
3 : R = H

anomeric effects[8]. We have already reported the synthesis of (1) through a ring-opening reaction of N^6,5'-bis-(4-methoxytriphenylmethyl)-2',3'-*anhydro*-adenosine with CH_3MgI and CuI in dry THF[10]. We report herein that the methyl group in compound (1) actually fixes the furanose ring in a South-type conformation, via the formation of a thermodynamically stable $O_{5'}$-H...N_3 intramolecular hydrogen bond. It is shown that the conformations of (1) and cordycepin are entirely different. We also report that compound (2) can be stereospecifically prepared in high yield by a straightforward Grignard reaction of CH_3MgI and an appropriately functionalized and protected derivative of cordycepin. High resolution ^1H NMR experiments pointed out that the methyl group in compound (2) directs the furanose conformational equilibrium towards the South form.

Conformation of compound (1)

Table I summarizes the spectral parameters that were determined for compound (1) in the solvents D_2O, C_5D_5N, and $CDCl_3$. The conformation of the furanose ring in nucleosides and nucleotides is generally described according to the pseudorotation concept[11] i.e., the ring structure is fully characterized by a phase angle (P) which has a cyclic nature adopting values between 0° and 360°, and a maximum puckering amplitude (ν_m) which is confined to a relatively narrow range around 39°. It is known that the furanose ring is highly flexible in solution. Generally, the ring is involved in a rapid two-state equilibrium between a puckered ring form with a P-value of approximately 19° (North), and a puckered ring form with a P-value of approximately 162° (South)[12]. In the case of compound (1) we determined the furanose conformation from

TABLE I. NMR spectral data measured for (1) in D_2O, C_5D_5N, or $CDCl_3$

Solvent	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$
D_2O	7.0	10.0	8.8	2.9	3.6
C_5D_5N	7.1	10.3	8.8	2.4	2.1
$CDCl_3$	7.2	10.6	8.9	2.0	2.1[a]

a: estimated from the total width of the 4'-pattern.

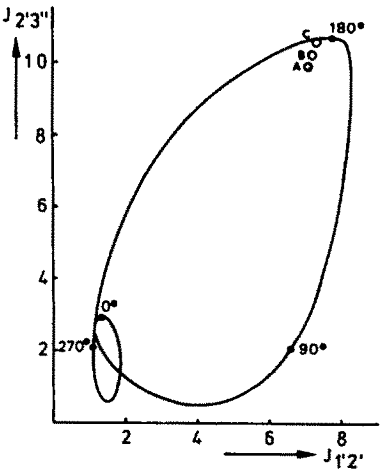


Figure 1. Calculated variation of the vicinal coupling constants $J_{1'2'}$ and $J_{2'3''}$ with the phase angle of pseudorotation ($\nu_m = 48^o$) for compound (1). The experimental data points (A: D_2O; B: C_5D_5N; C: $CDCl_3$) are found in the upper right part of the graph, indicating that the furanose conformation is heavily biased toward the South form.

the relationship between the coupling constants of the vicinal ring protons (i.e., $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$), and the pseudorotational parameters of the ring. Using the generalized Karplus equation of Altona et al.[13] we calculated the variation of these three coupling constants over the entire pseudorotation circuit, for a puckering amplitude of 48^o (vide infra). A convenient representation of the conformational equilibrium of the furanose ring is given in Figure 1, which shows the variation of $J_{1'2'}$ and $J_{2'3'}$ with P. The experimental data points are found in the upper right region of the plot, i.e. the ring conformation is heavily biased toward the South form. In the case of the solvent $CDCl_3$, a virtually complete conformational lock of the furanose ring is found ($P = 190^o$, $\nu_m = 48^o$).

The $C_{4'}$-$C_{5'}$ conformation was analyzed on the basis of the vicinal proton-proton coupling constants $J_{4'5'}$ and $J_{4'5''}$. For the solvents D_2O and C_5D_5N, these couplings could be readily extracted from the 200 MHz ^1H NMR spectra (Figure 2). Calculation of the relative populations of the three staggered conformations around the $C_{4'}$-$C_{5'}$ bond (γ^+, γ^t, γ^-) reveals that the γ^+ conformation is highly dominant (Table I). In the case of the solvent $CDCl_3$, a substantial broadening of the $H_{5''}$ resonances is observed (Figure 2), which prevents the precise determination of $J_{4'5''}$. It should be noted that analogous broadening of the $H_{5''}$ resonances was observed previously for 8-bromo-2',3'-O-isopropylidene adenosine in apolar solvents[14]. In that case, it was shown tha the conformation is determined by a strong intramolecular hydrogen bond between $O_{5'}$ and N_3 of the *syn*-oriented adenine base. It was proven that the broadening effect arises from long-range spin-spin interaction between $H_{5''}$ and N_3 *via* the planar coupli path $H_{5''}$-$C_{5'}$-$O_{5'}$-H...N_3. It can be concluded from the present data that the conformation in (1) is also determined by strong intramolecular $O_{5'}$-H...N_3 hydrogen bonding, which fixes the conformations of the furanose ring ($P = 170^o$, $\nu_m = 48^o$) and the $C_{4'}$-C bond (γ^+), as well as the orientation of the adenine base in the *syn*-domain.

Independent corroboration for this conclusion could be obtained via a one-dimensional proton nuclear Overhauser experiment, in which H_8 of the adenine base was specificall saturated. Figure 3 shows the NOE difference spectrum of $H_{1'}$. Clearly, a substantial

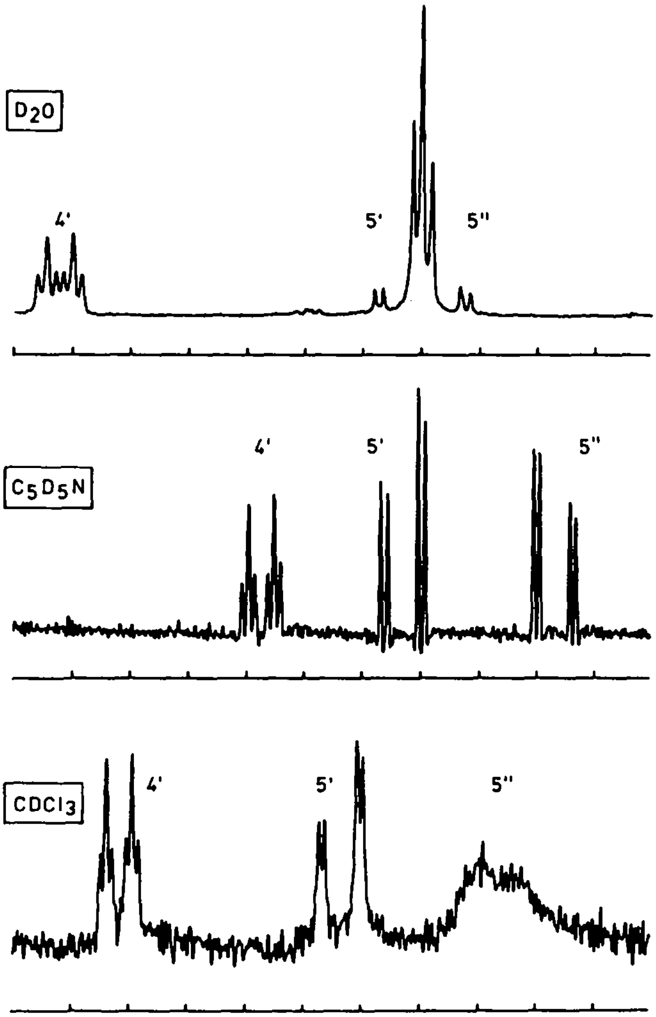


Figure 2. Expansions of the 4'/5'/5" patterns in the 200 MHz proton NMR spectra of (1) in D_2O C_5D_5N, of $CDCl_3$. The broadening of the $H_{5''}$ resonances in the case of $CDCl_3$ as the solvent is clearly visible.

NOE contact exists between H_8 and $H_{1'}$, proving that the base resides predominantly, if not exclusively, in the *syn*-domain[15,16]. The role of the methyl group on $C_{3'}$ in determining the (South, γ^+, *syn*) conformation of compound (1) could be clarified by means of a comparison with the conformational properties of cordycepin, and β-D-*xylo*-adenosine. Evidently, cordycepin corresponds with substitution of the methyl group in (1) by a hydrogen atom. The X-ray crystal structure of cordycepin shows a North-type furanose ring ($P = 19^\circ$, $\nu_m = 32^\circ$), γ^t conformation around the $C_{4'}$-$C_{5'}$ bond, and *anti*-orientation of the base[17]. Also, it is well-established that the solution conformation of cordycepin is predominantly North for the sugar ring and γ^t around the $C_{4'}$-$C_{5'}$ bond[8,18]. The compound β-D-*xylo*-adenosine in fact represents replacement of the methyl in (1) by a hydroxyl group. Like cordycepin, β-D-*xylo*-adenosine shows a marked preference for the (North, γ^t) conformation in solution[18]. The preference of cordycepin and β-D-*xylo*-

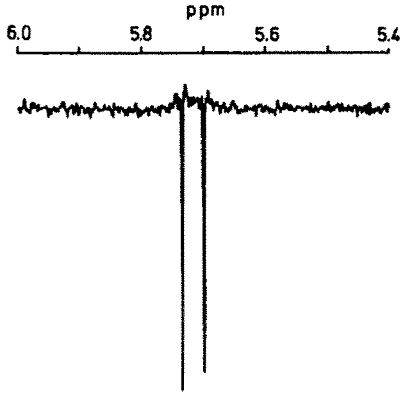


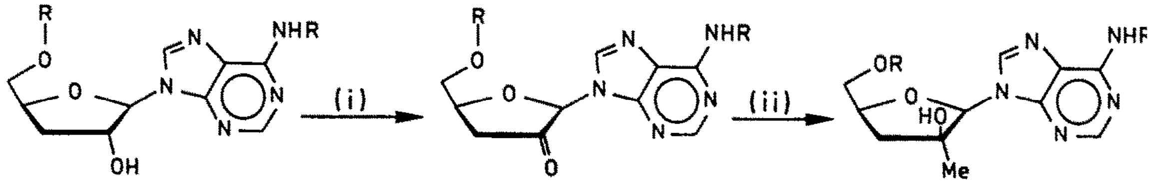
Figure 3. Signal of $H_{1'}$ in the NOE difference spectrum of (1), obtained after specific irradiation of H_8. The NOE contact between $H_{1'}$ and H_8 strongly indicates that the adenine base resides predominantly in the *syn*-conformation.

adenosine for a North-type sugar conformation is readily explained on the basis of the gauche effect, which states that $O_{2'}$ and/or $O_{3'}$ tend to be in a gauche arrangement wit respect to the endocyclic oxygen $O_{4'}$. This gauche orientation corresponds with axial location of $O_{2'}$ and/or $O_{3'}$ on the furanose ring, which is achieved in the North conformation[8].

The comparison of compound (1), cordycepin, and β-D-*xylo*-adenosine reveals that the methyl group on $C_{3'}$ in (1) induces a drastic change of the furanose conformation from North toward South. The South conformation in (1) places the methyl group in an equatorial location (which is sterically favoured), and also facilitates the stabilizing intramolecular $O_{5'}$-H...N_3 hydrogen bond. The only disadvantage of the South conformati seems to be the equatorial location of the 2'-hydroxyl group, which is "trans" with respect to $O_{4'}$ and hence unfavourable according to the gauche effect.

Preparation and conformation of compound (2)

The synthesis of compound (2) from N^6,5'-bis-(4-methoxytriphenylmethyl) cordycepin (4) is given in Scheme I. The first step is the oxidation of the 2'-hydroxyl into a keto-function (compound (5)) in dry dichloromethane at 20 °C. Then, a stereospecific Grigna reaction of CH_3MgI and (5) in dry THF yielded compound (6). Deprotection of (6) with 80 % acetic acid readily afforded the desired compound (2).



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Scheme I. Essential steps in the synthesis of compound (2). R = 4-methoxytriphenylmetl
Reagents: (i) CrO_3-Ac_2O-pyridine complex in CH_2Cl_2 at 20 °C; (ii) CH_3MgI in THF at 20

TABLE II. NMR spectral data measured for (2) in D_2O

$J_{3'4'}$	$J_{3''4'}$	$J_{3'3''}$	$J_{4'5'}$	$J_{4'5''}$
7.9	6.9	13.7	3.2	4.9

The NMR spectral data that were obtained for compound (2) in D_2O are listed in Table II. In this case, only the coupling constants $J_{3'4'}$ and $J_{3''4'}$ provide information on the North - South conformational equilibrium of the furanose ring. Under the reasonable assumption that the experimental couplings reflect rapid equilibration over a North form with $P = 19^o$, $\nu_m = 39^o$, and a South form with $P = 162^o$, $\nu_m = 39^o$ (vide supra), it follows from the PSEUROT procedure[19] that x(South) = 0.55. With respect to the $C_{4'}$-$C_{5'}$ conformation it follows from the experimental couplings $J_{4'5'}$ and $J_{4'5''}$ that $x(\gamma^+) = 0.55$, $x(\gamma^t) = 0.37$, and $x(\gamma^-) = 0.08$, i.e., the γ^+ rotamer is dominant. In order to assess the role of the methyl group in determining the conformation of compound (2), we made a comparison with compound (3) in which the methyl group is in fact replaced by hydrogen. Application of the PSEUROT procedure under the restictions as described above revealed that x(South) = 0.28 for compound (3)[8]. Thus, replacement of $H_{2''}$ by a methyl group results in an increase in the population of the South conformer. This seems plausible, since the South form corresponds with the methyl group in equatorial location, which is preferred for steric reasons. With respect to the $C_{4'}$-$C_{5'}$ conformation of (3) it was found previously[8] that $x(\gamma^+) = 0.53$, $x(\gamma^t) = 0.41$, and $x(\gamma^-) = 0.06$. These data show that the methyl group in (2) has virtually no effect on the $C_{4'}$-$C_{5'}$ conformation.

Experimental

^1H NMR spectra were run in the Fourier Transform mode at 200 or 300 MHz on a Bruker AC 200, or a Bruker CXP 300 spectrometer, respectively. ^1H NMR spectra at 90 MHz and ^{13}C NMR spectra at 23.7 MHz were recorded on a Jeol 90Q FT spectrometer. Tetramethylsilane was used as the internal standard and chemical shifts are reported in ppm (δ scale). For the NMR experiments in the solvent D_2O, we used the residual HDO resonance for δ-calibration (δ 4.68 ppm). Melting points were uncorrected. UV absorption spectra were recorded with a Varian-Cary 2200 instrument and Jeol DX 303 instrument was used for recording the mass spectra. Thin-layer chromatography (t.l.c.) was performed on Merck precoated 60 F_{254} plates. Merck Kieselgel G was used for short column chromatography. IR spectra were recorded on a Perkin-Elmer 298 spectrometer, and rotation on a Perkin-Elmer 241 polarimeter.

9-(5'-O-(4-monomethoxytriphenylmethyl)-3'-deoxy-β-D-glycero-pentofuran-2'-ulosyl)-6-N-(4-methoxytriphenylmethyl)adenine (5)

Dry pyridine (2.5 ml, 31.5 mmol) was slowly added to a fine suspension of CrO_3 (1.5 g, 15 mmol) in dry dichloromethane (35 ml), cooled at 0 °C, followed by acetic anhydride (1.5 ml, 15.9 mmol). After stirring for 5 min at 0 °C, a solution of compound (4) (3.9 g, 3.77 mmol) in dichloromethane (ca. 15 ml) was added dropwise in the latter reagent. After 50 min at 0 °C, pyridine (2.5 ml) was added and the reaction mixture was directly loaded onto a silica gel column and eluted with ethylacetate, the eluent was collected in a beaker containing saturated sodium bicarbonate (400 ml) and ethylene diamine tetracetate (3.1 g). The organic phase was collected and the aqueous phase was reextracted with ethylacetate (3 x 100 ml). All organic extracts were pooled and evaporated. The residue was dissolved in dichloromethane-petroleum ether mixture and was precipitated from petroleum ether to give 2.43 g (82 %) of the title compound.

M.S. (FAB): calc. 794.3343 for $(M+H)^+$, found 794.3377. I.R. (KBr): 1770 cm^{-1} (carbonyl) U.V. (95 % ethanol): λ_{max} = 275 nm (ε = 22.900).
^1H NMR ($CDCl_3$): δ 7.89 (s, 1H) H_8, 7.82 (s, 1H) H_2, 7.29 (m, 24H) MMTr, 6.92 (s, 1H) NH, 6.81 (m, 4H) MMTr, 5.87 (s, 1H) $H_{1'}$, 4.63 (m, 1H) $H_{4'}$, 3.74 (s, 6H) MMTr, 3.45 (m, 2H) $H_{5'/5''}$, 3.15 (dd, 1H, $J_{3'4'}$ = 8.5 Hz, $J_{3'3''}$ = 18.8 Hz) $H_{3'}$, 2.71 (dd, 1H, $J_{3''4'}$ = 7.1 Hz) $H_{3''}$.
^{13}C NMR ($CDCl_3$): δ 206.7 ($C_{2'}$), 86.3 ($C_{1'}$), 81.6 (O-MMTr), 75.0 ($C_{4'}$), 70.7 (N-MMTr), 64.9 ($C_{5'}$), 37.7 ($C_{3'}$).

9-(5'-O-(4-methoxytriphenylmethyl)-3'-deoxy-β-D-2'-C-methyl-*threo*-pentofuranosyl)-6-N-(4-methoxytriphenylmethyl) adenine (6)

To a solution of compound (5) (320 mg, 0.4 mmol) in a mixture of dry diethylether (15 m and THF (2 ml), cooled in an ice bath, was added MeMgI (20 equiv.) and stirred at 0 °C for 8 h, and then for 22 h at 20 °C. The supernatant was decanted and hydrolyzed with 10 % aqueous ammonium chloride solution. The reaction mixture was partitioned with dichloromethane (4 x 40 ml). Organic extracts were collected and evaporated to give a glass which was purified on a short silica gel column using dichloromethane as the eluent. Appropriate fractions were collected and evaporated to give a glass, 226 mg (70 %).

M.S. (FAB): calc. 810.3656 for $(M+H)^+$, found 810.3724. U.V. (95 % ethanol): λ_{max} = 275 nm (ε = 18.300).
^1H NMR ($CDCl_3$): δ 8.35 (s, 1H) H_8, 8.02 (s, 1H) H_2, 7.29 (m, 24H) MMTr, 6.82 (m, 5H) NH, MMTr, 5.93 (s, 1H) $H_{1'}$, 5.38 (brs, 1H) OH, 4.32 (m, 1H), $H_{4'}$ 3.76,3.75 (2xs, 6H) $2xOCH_3$, 3.58 (dd, 1H, $J_{4'5'}$ = 3 Hz, $J_{5'5''}$ = 10.3 Hz) $H_{5'}$, 3.22 (dd, 1H, $J_{4'5''}$ = 3.5 Hz $H_{5''}$, 2.22 (m, 2H) $H_{3'/3''}$, 1.38 (s, 3H) CH_3.

^{13}C NMR (CD_3COCD_3): δ 89.9 (d, J_{CH} = 163 Hz) $C_{1'}$, 86.6 (O-MMTr), 76.7 (d, J_{CH} = 149 Hz) $C_{4'}$, 76.7 ($C_{2'}$), 70.8 (N-MMTr), 66.5 (t, J_{CH} = 141 Hz) $C_{5'}$, 41.6 (t, J_{CH} = 132 Hz) $C_{3'}$, 24.3 (CH_3).

9-(3'-deoxy-β-D-2'-C-methyl-*threo*-pentofuranosyl)adenine (2)

Compound (6) (90 mg, 0.11 mmol) was deprotected in 80 % acetic acid (25 ml) for 12 h at 20 °C. The reaction mixture was evaporated and coevaporated with water, the residue was partitioned between water (20 ml) and chloroform (10 ml) the water phase was washed with ether (10 ml) and evaporated. Compound (2) was crystallized by diffusion with methanol-ether. Yield 25 mg (86 %). A sample was recrystallized from methanol m.p. 212 °C. $[\alpha]_D^{20}$ = + 27° (c = 0.25).

M.S. (FAB): calc. 266.1253 for $(M+H)^+$, found 266.1263. U.V. (95 % ethanol): λ_{max} = 259 nm (ε = 13.900).
^1H NMR (CD_3OD): 8.52 (s, 1H) H_8, 8.28 (s, 1H) H_2, 6.10 (s, 1H) $H_{1'}$, 4.45 (m, 1H) $H_{4'}$, 4.00 (dd, $J_{4'5'}$ = 2.9 Hz, $J_{5'5''}$ = 11.9 Hz, 1H) $H_{5'}$, 3.81 (dd, $J_{4'5''}$ = 3.8 Hz, 1H) $H_{5''}$, 2.51 (dd, $J_{3'4'}$ = 7.8 Hz, $J_{3'3''}$ = 13.4 Hz, 1H) $H_{3'}$, 2.31 (dd, $J_{3''4'}$ = 6.3 Hz, 1H) $H_{3''}$, 1.48 (s, 3H) CH_3.
^{13}C NMR (CD_3OD): δ 157.2 (C_6), 153.6 (C_2), 150.9 (C_4), 142.3 (C_8), 119.6 (C_5), 91.4 (d, J_{CH} = 161 Hz) $C_{1'}$, 79.2 (d, J_{CH} = 149 Hz) $C_{4'}$, 77.6 ($C_{2'}$), 64.5 (t, J_{CH} = 142 Hz) $C_{5'}$, 41.5 (t, J_{CH} = 133 Hz) $C_{3'}$, 23.9 (CH_3).

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