

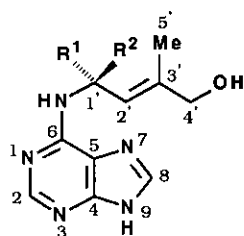
1'-METHYLZEATIN AND ITS 9- β -D-RIBOFURANOSIDE: THE CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA REVISITED

Masashi Ohba,[†] Tozo Fujii,^{*,†} Antonio Evidente,[‡] Giuseppe Surico,[§] and Nicola S. Iacobellis[¶]

[†]Faculty of Pharmaceutical Sciences, Kanazawa University, Takaramachi, Kanazawa 920, Japan; [‡]Istituto di Chimica Agraria, Università di Napoli "Federico II", Via Università 100, 80055 Portici, Italy; [§]Istituto di Patologia e Zoologia Forestale e Agraria, Università di Firenze, Piazzale delle Cascine, 28, 50144 Firenze, Italy; [¶]Istituto Tossine e Micotossine da Parassiti Vegetali del C.N.R., Via Amendola 197/F, 70126 Bari, Italy

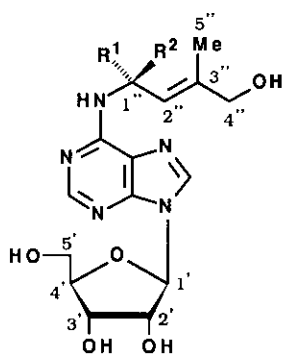
Abstract — Revised carbon-13 chemical shift assignments are reported for the cytokinins (1'R)-1'-methylzeatin [(1'R)-**1**] and its 9- β -D-ribofuranoside [(1''R)-**2**]. Previous shift assignments for the two methyl carbons in the purine N⁶ side chain of (1'R)-**1** and (1''R)-**2**, as well as those for the C(2') and C(3') carbons of (1''R)-**2**, have now been reversed by comparison with the ¹³C nmr data for the diastereomeric nucleoside (1''S)-**2** and the *cis* isomers **3** and **4**.

In the mid 1980's, some of us reported the isolation of two new cytokinins, 1'-methylzeatin¹ and its 9- β -D-ribofuranoside,² from the culture filtrate of *Pseudomonas syringae* pv *savastanoi*. Their structures and absolute configurations were rigorously established as (1'R)-**1** and (1''R)-**2**, respectively, as a result of the chiral syntheses of both enantiomers [(1'R)-**1** and (1'S)-**1**] of 1'-methylzeatin and their 9- β -D-ribofuranosides [(1''R)-**2** and (1''S)-**2**].³ Recently, Fujii's group also synthesized (1'R)-1'-methyl-*cis*-zeatin (**3**) and its 9- β -D-ribofuranoside (**4**) in order to compare the cytokinin activities of both geometrical isomers of (1'R)-1'-methylzeatin at the aglycone [(1'R)-**1** and **3**] and the glycoside [(1''R)-**2** and **4**] levels.⁴ Comparison of the ¹³C nmr spectra of (1'R)-**1**¹ and (1''R)-**2**² reported in the literature with those of the *cis* isomers **3** and **4** revealed several disagreements existing in the shift assignments for the former two compounds. Herein we describe the reassignment of the ¹³C nmr signals observed for 1'-methylzeatin [(1'R)-



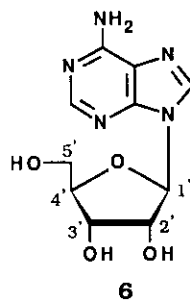
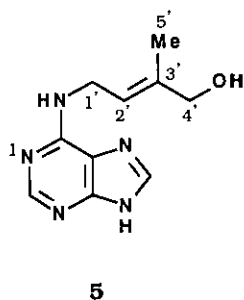
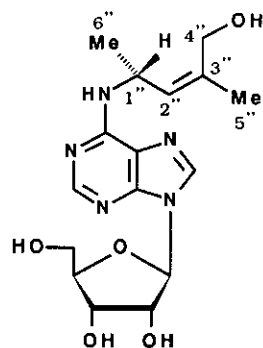
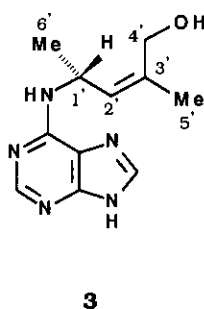
(1'*R*)-**1**: R¹ = Me(6'); R² = H

(1'*S*)-**1**: R¹ = H; R² = Me(6')



(1''*R*)-**2**: R¹ = Me(6''); R² = H

(1''*S*)-**2**: R¹ = H; R² = Me(6'')



1] and its 9-β-D-ribofuranoside [(1'*R*)-**2**].

Table I lists the chemical shifts for all carbons of 1'-methylzeatin [(1'*R*)-**1**] and the related cytokinins in CD₃OD. Assignments are made largely by comparison with the spectra of zeatin (**5**)⁵ and adenosine (**6**)⁶ reported in the literature. It may be seen that the C(4') carbon of **3** resonated at higher field than the corresponding carbon of (1'*R*)-**1** by 5.8 ppm owing to the γ effect.⁷ A similar upfield shift of the C(5') carbon signal of (1'*R*)-**1**, relative to that of **3**, would be expected. Actually, the C(5') carbon signal of zeatin (**5**) is observed at δ 13.6 in CD₃OD-D₂O (2 : 1)^{5a} or at δ 13.5 in (CD₃)₂SO^{5b} because of the shielding effect based on its *cis* relationship between the methyl and C(1') methylene groups. Therefore, the resonance at δ 14.1, instead of δ 21.8 as assigned previously,¹ of (1'*R*)-**1** should be assigned to the C(5') carbon. A selective decoupling experiment on the C(5') proton [δ 1.79 (3H, d, *J* = 1 Hz)]^{3b} of (1'*R*)-**1** unambiguously confirmed this revised assignment, and hence the resonance at δ 21.8 should be assigned to the C(6') carbon. From the same reason as described above, previous shift assignments² for the two methyl carbons [C(5'') and C(6'')] in the purine N⁶ side chain of 1''-methylzeatin-9-β-D-ribofuranoside [(1''*R*)-**2**] are also reversed.

In addition, the revision of previous shift assignments² for the C(2') and C(3') carbons of (1''*R*)-**2** may deserve particular mention. In 1970, among the C(2') and C(3') carbon resonances of

Table 1. ^{13}C Chemical Shifts of 1'-Methylzeatin [(1'R)-1] and Related Cytokinins in CD_3OD

Carbon	Chemical shifts ^{a)}			Carbon	Chemical shifts ^{a)}			
	(1'R)-1		3		(1''R)-2		(1''S)-2	4
	Reported ^{b)}	Revised			Reported ^{c)}	Revised		
C(2)	153.9	153.8	153.5	C(2)	153.6	153.5	153.2	153.4
C(4)	149.9	151.1	151.0	C(4)	149.9	149.3	149.0	149.1
C(5)	116.4	118.8	118.8	C(5)	116.7	121.3	121.0	121.0
C(6)	156.1	154.7	154.5	C(6)	157.1	155.3	155.0	155.2
C(8)	140.6	140.4	140.6	C(8)	141.4	141.3	141.0	141.5
C(1')	45.5	45.5	45.4	C(1'')	45.5	45.6	45.5	45.5
C(2')	127.8	127.8	130.5	C(2'')	127.7	127.7	127.4	130.4
C(3')	138.3	138.3	137.9	C(3'')	138.3	138.3	138.0	138.0
C(4')	68.0	68.0	62.2	C(4'')	68.0	68.0	67.9	62.2
C(5')	21.8	14.2 ^{d)}	21.7 ^{d)}	C(5'')	21.8	14.1 ^{d)}	14.2	21.7 ^{d)}
C(6')	14.1	21.9 ^{d)}	22.1	C(6'')	14.1	21.8 ^{d)}	21.8	22.1
				C(1')	91.3	91.3	91.1	91.4
				C(2')	72.7	75.4 ^{d)}	75.3	75.5
				C(3')	75.4	72.7 ^{d)}	72.5	72.7
				C(4')	88.2	88.2	88.1	88.2
				C(5')	63.5	63.5	63.4	63.5

a) The ^{13}C nmr spectra were recorded on a JEOL JNM-FX-100 nmr spectrometer (25.0 MHz) at 25°C by using Me_4Si as an internal standard ($\delta = 0$ ppm).

b) Data taken from ref. 1.

c) Data taken from ref. 2.

d) This assignment is based on a selective proton decoupling experiment.

adenosine (6) found in the range 70–75 ppm, Jones *et al.*^{6a} assigned the one observed at higher field to the C(2') carbon. However, five years later, Chenon *et al.*^{6b} reversed the assignments for the C(2') and C(3') carbons of 6 according to the work of Mantsch and Smith,⁸ which unequivocally confirmed the chemical shifts for the C(2') and C(3') carbons of uridine derivatives. The earlier assignments² of the C(2') and C(3') resonances for (1''R)-2, based on the work of Jones *et al.*, are therefore incorrect and should be reversed. The correctness of the C(2') and C(3') shift assignments for (1''R)-2 was also supported by selective decoupling experiments on the C(2') proton [δ 4.74 (1H, dd, $J = 6.5$ and 5 Hz)]^{3b} and C(3') proton [δ 4.32 (1H, dd, $J = 5$ and

2.5 Hz)]^{3b} of (1''R)-**2**.

Thus, all the carbon signals of (1'R)-1'-methylzeatin [(1'R)-**1**] and its 9-β-D-ribofuranoside [(1''R)-**2**] are newly assigned as listed in the 3rd and 7th columns of Table I. Table I also includes the ¹³C chemical shifts of the diastereomeric nucleoside (1''S)-**2** (*vide supra*).

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