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

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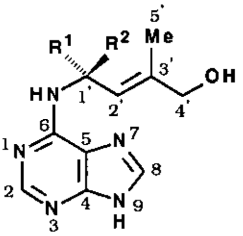
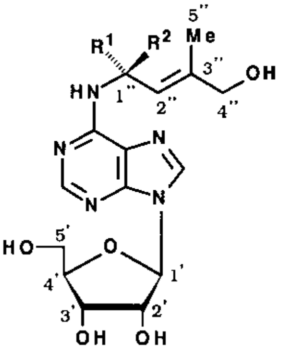
Abstract ——— Revised carbon-13 chemical shift assignments are reported for the cytokinins (1'*R*)-1'-methylzeatin [(1'*R*)-**1**] and its 9-β-D-ribofuranoside [(1"*R*)-**2**]. Previous shift assignments for the two methyl carbons in the purine N^6 side chain of (1'*R*)-**1** and (1"*R*)-**2**, as well as those for the C(2') and C(3') carbons of (1"*R*)-**2**, have now been reversed by comparison with the ^{13}C nmr data for the diastereomeric nucleoside (1"*S*)-**2** and the *cis* isomers **3** and **4**.

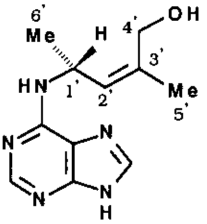
In the mid 1980's, some of us reported the isolation of two new cytokinins, 1'-methylzeatin[1] and its 9-β-D-ribofuranoside,[2] from the culture filtrate of *Pseudomonas syringae* pv *savastanoi*. Their structures and absolute configurations were rigorously established as (1'*R*)-**1** and (1"*R*)-**2**, 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 ^{13}C 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 ^{13}C nmr signals observed for 1'-methylzeatin [(1'*R*)-



(1'*R*)-**1**: R^1 = Me(6'); R^2 = H
(1'*S*)-**1**: R^1 = H; R^2 = Me(6')



(1''*R*)-**2**: R^1 = Me(6''); R^2 = H
(1''*S*)-**2**: R^1 = H; R^2 = Me(6'')



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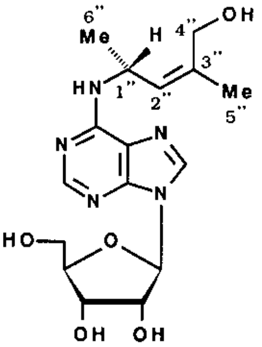

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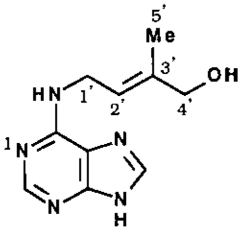

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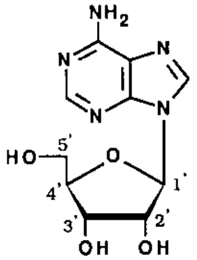

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1] and its 9-β-D-ribofuranoside [(1''*R*)-**2**].

Table I lists the chemical shifts for all carbons of 1'-methylzeatin [(1'*R*)-**1**] and the related cytokinins in CD_3OD. 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 CD_3OD–D_2O (2 : 1)[5a] or at δ 13.5 in $(CD_3)_2SO$[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 assignments[2] for the two methyl carbons [C(5'') and C(6'')] in the purine *N*^{6} side chain of 1''-methylzeatin-9-β-D-ribofuranoside [(1''*R*)-**2**] are also reversed.

In addition, the revision of previous shift assignments[2] 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 I. ^{13}C Chemical Shifts of 1'-Methylzeatin [(1'*R*)-**1**] and Related Cytokinins in CD_3OD

	Chemical shifts[a]				Chemical shifts[a]			
Carbon	(1'*R*)-**1**		**3**	Carbon	(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')	$\boxed{21.8}$	$\boxed{14.2^{d)}}$	21.7[d]	C(5")	$\boxed{21.8}$	$\boxed{14.1^{d)}}$	14.2	21.7[d]
C(6')	$\boxed{14.1}$	$\boxed{21.9^{d)}}$	22.1	C(6")	$\boxed{14.1}$	$\boxed{21.8^{d)}}$	21.8	22.1
				C(1')	91.3	91.3	91.1	91.4
				C(2')	$\boxed{72.7}$	$\boxed{75.4^{d)}}$	75.3	75.5
				C(3')	$\boxed{75.4}$	$\boxed{72.7^{d)}}$	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 (δ = 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 assignments[2] 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 ^{13}C chemical shifts of the diastereomeric nucleoside (1"S)-**2** (*vide supra*).

REFERENCES

1. A. Evidente, G. Surico, N. S. Iacobellis, and G. Randazzo, *Phytochemistry*, 1986, **25**, 525.
2. G. Surico, A. Evidente, N. S. Iacobellis, and G. Randazzo, *Phytochemistry*, 1985, **24**, 1499.
3. (a) T. Itaya, T. Fujii, A. Evidente, G. Randazzo, G. Surico, and N. S. Iacobellis, *Tetrahedron Lett.*, 1986, **27**, 6349; (b) T. Fujii, T. Itaya, and S. Matsubara, *Chem. Pharm. Bull.*, 1989, **37**, 1758.
4. T. Fujii, M. Ohba, and M. Sakari, *Heterocycles*, 1988, **27**, 2077.
5. (a) C. C. Duke and J. K. MacLeod, *Aust. J. Chem.*, 1978, **31**, 2219; (b) S. C. Chen and N. Cyr, *Agric. Biol. Chem.*, 1982, **46**, 2361.
6. (a) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Am. Chem. Soc.*, 1970, **92**, 4079; (b) M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *ibid.*, 1975, **97**, 4627; (c) S. Uesugi and M. Ikehara, *ibid.*, 1977, **99**, 3250; (d) G. Ah-Kow, F. Terrier, M.-J. Pouet, and M.-P. Simonnin, *J. Org. Chem.*, 1980, **45**, 4399; (e) C. Chang, L. E. Diaz, W. R. Woolfenden, and D. M. Grant, *ibid.*, 1982, **47**, 5318.
7. (a) J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, pp. 80–85; (b) E. Breitmaier and W. Voelter, 'Carbon-13 NMR Spectroscopy,' 3rd ed., VCH Verlagsgesellschaft, Weinheim, 1987, p. 192.
8. H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, 1972, **46**, 808.

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