Note

Carbon-13 NMR Spectra of Zeatin and Related Compounds

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Zeatin (1) is the first known cytokinin among a group of naturally occurring N^6 -monosubstituted adenines which regulate cell division and cell differentiation.¹⁾ Because only minute amounts of cytokinins are isolatable from the plant materials and perhaps because of the limited use of and accessibility to ^{13}C NMR spectroscopy in its early stage of development, ^{13}C NMR spectra of this group of plant hormones have not yet been published.

As a result of remarkable advances in the Fourier-transform NMR technique in the last decade, ^{13}C NMR spectroscopy has now become an excellent tool for the structural characterization of natural products. During the course of synthesis of zeatin, dihydrozeatin (3) and their deuterated compounds,²⁾ we became interested in a systematic study of the ^{13}C NMR spectra of zeatin, its related natural cytokinins and the free isoprenoid amines of the purine side-chain (4, 5 and 6).

^{13}C spectra of structurally related compounds such as purine,³⁾ adenine⁴⁾ and their derivatives^{5,6)} have already been reported. On the basis of these published data, the assignments for the ^{13}C signals of the purine rings (110~160 ppm) in ($\pm$) dihydrozeatin, and those for the C-

6, C-2 and C-4 aromatic carbons in zeatin and 6-(3-methyl-2-pentenylamino)purine (2) were straightforward. However, the side-chain olefinic carbon signals in compounds 1 and 2 are not only close to the purine C-5 (*ca.* 120 ppm) and C-8 (*ca.* 140 ppm) carbon signals but the quaternary olefinic C-3 carbon exhibits a greater peak height than that of the purine C-8 tertiary carbon. The identification of these two nearby signals was made by using the multiplicity of the proton coupled spectra. For the assignment of two methyl carbons in compounds 2 and 5, the more deshielded signal was assigned to the one which is *trans* to the amino group.⁷⁾ This assignment agrees with the observed long range carbon-proton coupling to the C-2' vinyl proton shown in Table II, in which *trans*-methyl carbon gives a slightly larger coupling constant.⁸⁾

Carbon-proton coupling constants of compounds 4, 5 and 6 were obtained from proton-coupled spectra by the gated decoupling technique.⁹⁾ For determinations of the two and three-bond coupling constants, 2J and 3J , spectra of deuterium labelled compounds were used to confirm some of the assignments. Deuterium substitution virtually eliminates the small splittings in spectra because of the smaller gyromagnetic ratio of deuterium ($\sim 1/6.55$ of that of proton). Long range proton coupling constants to C-2 and C-3 in compounds 4 and 5 were not unequivocally determined since the signals were multiplets of seven or nine peaks with approximately equal separation of about 5 Hz.

The substituent effects on allylamines reported earlier by other workers,¹⁰⁾ are worth mentioning here. It is clear from Fig. 1 that *N*-substitution of allylamine causes a polarization of the olefinic carbon atoms, resulting in shielding of the proximal carbon (C-2') and deshielding of the distal carbon (C-3') to the amino group. These differences in the chemical shifts of the olefinic carbon, C-2' and

TABLE I. ^{13}C NMR CHEMICAL SHIFTS (ppm from TMS) OF COMPOUNDS 1~3 AND THEIR CORRESPONDING SIDE-CHAIN AMINES 4~6

Compound	Solvent	C-2	C-4	C-5	C-6	C-8	C-1'	C-2'	C-3'	C-4'	C-5'
1	DMSO- d_6	152.6	151.0	117.9	154.3	139.0	38.5	121.0	137.4	13.5	65.9
1- d_1	DMSO- d_6	152.6	150.0	118.0	154.1	139.2	— ^a	121.0	137.5	13.5	65.6 ^b
1- d_1	D $_2$ O	152.6	149.6	116.8	153.6	140.6	39.1	121.5	139.4	13.7	67.1
1- d_3	D $_2$ O	153.0	149.9	117.0	154.0	140.9	— ^c	121.5	139.5	13.8	67.1 ^b
2	DMSO- d_6	152.6	151.1	118.0	154.0	139.1	38.0	122.4	133.4	17.7	25.2
3	DMSO- d_6	152.6	150.5	118.0	154.0	139.1	39.5	38.1	33.2	16.7	66.2
4	CDCl $_3$						38.7	124.8	136.6	13.1	66.8
4- d_1	D $_2$ O						38.0	126.0	136.7	13.2	67.1 ^b
4- d_3	D $_2$ O						37.4 ^b	125.7	136.9	13.1	67.1 ^b
5	CDCl $_3$						39.3	126.2	132.2	17.1	25.1
6	CDCl $_3$						39.2	37.2	33.8	16.4	66.9

^a Overlap with DMSO- d_6 peaks.^b Center signal of the multiplets caused by ^2H coupling.^c Not observed.TABLE II. CARBON-PROTON COUPLING CONSTANTS $^nJ_{\text{CH}}$ (Hz) OF COMPOUNDS 4~6

Compound	Carbon	$^1J_{\text{CH}}$	$^2J_{\text{CH}}$	$^3J_{\text{CH}}$
4	1'	135	$^2J_{\text{CH}(2)} = 2$	—
	2'	153	$^2J_{\text{CH}(1)} \approx 4^a$	$^3J_{\text{CH}(4)} \approx ^3J_{\text{CH}(5)} \approx 4^a$
	3'	—	$^2J_{\text{CH}(2)} \approx ^2J_{\text{CH}(5)} \approx 4.5^a$	$^3J_{\text{CH}(1)} \approx 4.5^a$
	4'	126	—	$^3J_{\text{CH}(2)} = 8$; $^3J_{\text{CH}(5)} = 2.5$
	5'	141	—	$^3J_{\text{CH}(2)} = 7.5$; $^3J_{\text{CH}(4)} = 3.5$
5	1'	134	$^2J_{\text{CH}(2)} = 2.8$	—
	2'	152	$^2J_{\text{CH}(1)} \approx 5^a$	$^3J_{\text{CH}(4)} \approx ^3J_{\text{CH}(5)} \approx 5^a$
	3'	—	$^2J_{\text{CH}(2)} \approx ^2J_{\text{CH}(4)} \approx ^2J_{\text{CH}(5)} \approx 6^a$	$J_{\text{CH}(1)} = 6^a$
	4'	126	—	$^3J_{\text{CH}(2)} = 8$; $^3J_{\text{CH}(5)} = 4$
	5'	126	—	$^3J_{\text{CH}(2)} = 7$; $^3J_{\text{CH}(4)} = 4$
6	1'	135	$^2J_{\text{CH}(2)} = 0$	$^3J_{\text{CH}(3)} = 3.5$
	2'	~ 123		
	3'	~ 127		
	4'	123		
	5'	139	$^2J_{\text{CH}(3)} = 5$	$^3J_{\text{CH}(2)} = ^3J_{\text{CH}(5)} = 5$

^a Tentative assignments (see text for explanation).

C-3', are not as large as those of prop-2-enylamine and its alkylated compound.¹⁰ Comparisons of chemical shifts (measured in D $_2$ O solvent) show that the changes on *N*-alkylation of the amines are approximately -4.5 $+2.5$ ppm for C-2' and C-3', respectively.

As expected, mono-deuterated C-5' carbon showed a triplet. The di-deuterated C-1' carbon signal was not visible since the intensity of the components of its quintet was expected to be weak and lost in the noise. The observed deuterium isotope effect on these ^{13}C chemical shifts is in good accord with the literature.¹¹

EXPERIMENTAL

All the C-13 NMR spectra were measured on a Bruker WP-80 pulse FT spectrometer at 20.1 MHz in 5 mm O.D. tubes. Deuterium signals from solvents were used for the field-frequency lock. Typically, ^{13}C signals were accumulated overnight with a pulse width of about 30° and a pulse repetition time of 1.6 sec. Proton coupled spectra were obtained by the gated decoupling technique⁹ to take advantage of the Nuclear Overhauser effect. All compounds were synthesized as reported previously.²

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FIG. 1. Carbon-13 NMR Spectra (20.1 MHz) of (A) (*E*)-3-Hydroxymethyl-2-butenylamine (in CDCl₃) and (B) Zeatin (in DMSO-*d*₆).

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