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SHORT REPORTS

1′-METHYL-ZEATIN, AN ADDITIONAL CYTOKININ FROM *PSEUDOMONAS SYRINGAE* PV. *SAVASTANOI**

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Key Word Index—*Pseudomonas syringae* pv. *savastanoi*; cytokinin; phytohormones; adenine derivatives.

Abstract—The isolation and structural elucidation of 1′-methyl-zeatin, a novel cytokinin, is reported.

INTRODUCTION

The culture filtrate of *Pseudomonas syringae* pv. *savastanoi* NCPPB 640‡ contains at least four cytokinins [1, 2] when assayed according to the method described in ref. [3]. Three of the cytokinins have been identified as the new cytokinin 6-(4-hydroxy-1,3-dimethylbut-*trans*-2-enylamino)-9-β-D-ribofuranosylpurine (**1**), zeatin (**2**) and zeatin riboside (**3**). The fourth, an additional new cytokinin, has now been assigned the structure of 6-(4-hydroxy-1,3-dimethylbut-*trans*-2-enylamino)purine (1′-methyl-zeatin, 1′MeZ) (**4**).

RESULTS AND DISCUSSION

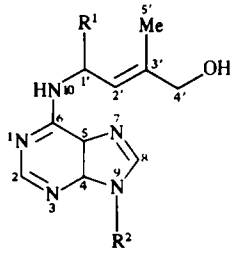
Compound **4**, $[\alpha]_D^{25}$ −52.6° (EtOH; *c* 0.13), gave UV absorption spectra [λ_{max}^{EtOH} nm (ε): 270 (11 359); $\lambda_{max}^{H_2O}$ nm (ε): 269 (11 842) and 275 (11 912) at pH 7.0 and 10.0, respectively] characteristic for a N^6-substituted adenine derivative [4]. Its mass spectrum (EI, 70 eV) exhibited peaks at *m/z* (rel. int.): 233 $[M]^+$ (20), 216 (100), 202 (80), 174 (25), 162 (20), 160 (18), 148 (10), 136 (60) and 135 (50). The occurrence of ions at *m/z* 216, 202, 174 and 162 was consistent with a fragmentation pathway of a 1′-methyl derivative of zeatin [5].

Its ^{1}H NMR spectrum (Table 1) revealed the presence of two singlets at δ8.22 and 8.05, typical for a 6-substituted purine system [6], a complex doublet at δ5.53 and two broad singlets at δ3.95 and 1.78. Moreover, the presence of a doublet, due to a secondary methyl group, was observed at δ1.36; in fact, this latter signal collapsed into a singlet upon irradiation of the multiplet present at δ5.24 (H-1′). In this experiment we also observed the coupling of H-1′ with the olefinic proton (H-2′).

All the above data suggested that compound **4** is 1′-methyl-zeatin.

In agreement with these findings, the ^{1}H NMR spectrum of compound **4** differed from that of compound **1**, only in the absence of the signals assigned to the ribose moiety. Similarly the ^{1}H NMR data of **4** were very close to those of zeatin (**2**) (Table 1), except for the presence of the signal attributed to the secondary methyl group. In addition the olefinic proton, a broad triplet in the spectrum of zeatin, appeared as a complex doublet in the spectrum of **4**. The ^{13}C NMR spectrum of **4** (Table 2) indicated the presence of 11 carbons, the chemical shifts of which were in agreement with the proposed structure.

Finally, we showed that **4** was the aglycone of cytokinin **1**, by the demonstration that the product present



	R^1	R^2
1	6′Me	β-D-ribosyl
2	H	H
3	H	β-D-ribosyl
4	6′Me	H

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Table 1. ^{1}H NMR spectral data of compounds **2** and **4** (270 MHz, CD_3OD as solvent and int. standard) (δ)

H	2	4
2*	8.23 *s*	8.22 *s*
8*	8.05 *s*	8.05 s
1′	4.26 *br d*	5.24 *m*
2′	5.65 *br t*	5.53 *br d*
4′ (2H)	3.98 *br s*	3.95 *br s*
5′ (3H)	1.79 *br s*	1.78 *br s*
6′ (3H)	—	1.36 *d*

J (Hz): **2**, **4**: 2′,4′ = 1.5; 2′,5′ = 1.1; **2**: 1′,2′ = 6.6; **4**: 1′,2′ = 8.5; 1′,6′ = 6.6.

*Assigned in agreement with literature data [6].

Table 2. ^{13}C NMR chemical shifts of compound **4** (67.88 MHz, CD_3OD as solvent and int. standard)

C	δ	C	δ
2*	153.9	2′	127.8
4*	149.9	3′	138.3
5*	116.4	4′	68.0
6*	156.1	5′	21.8
8*	140.6	6′	14.1
1′	45.5		

*Assignments made by reference to the data reported in the literature for adenine derivatives [7].

in the basic extract of an acid hydrolysate (0.5 M HCl, at 95° for 6 hr) of **1** had the same R_f values as natural **4**, with which it chromatographed, in three different TLC systems [silica gel, *n*-BuOH–HOAc–H_2O (4:1:1.6) and $CHCl_3$–EtOAc–MeOH (2:2:1); reverse phase H_2O–EtOH (1.5:1)].

All these results suggest structure **4** for the new cytokinin.

EXPERIMENTAL

Isolation. Compound **4** was obtained as described previously [1] by chromatographic fractionation of an EtOAC extract (514 mg) of a basified culture filtrate (34 l.) of pv. *savastanoi*. Cytokinin fractionation was performed by a combination of TLC on silica gel (Merck, Kieselgel 60, F_{254}, 0.25 and 2 mm) and on reverse phase (Stratocrom C-18, Whatman 0.2 mm). In particular, fractionation on silica gel (*n*-BuOH–HOAc–H_2O, 4:1:1.6) followed by purification on reverse phase plates (H_2O–EtOH, 1.5:1) yielded **1**, chromatographically pure (13 mg), and **4** in mixture. A further purification on silica gel plates ($CHCl_3$–EtOAc–MeOH, 2:2:1) gave **4** as an oil (1.3 mg) which resisted crystallization.

Identification. Compound **4** was identified as 1′MeZ on the basis of ^{1}H and ^{13}C NMR and MS. Results of analyses performed have been discussed above and are reported in Tables 1 and 2.

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