Phytochemistry, Vol 28, No 10, pp 2603–2607, 1989
Printed in Great Britain

0031–9422/89 $3.00 + 0.00
Pergamon Press plc

2′-DEOXYZEATIN RIBOSIDE AND OTHER CYTOKININS IN CULTURE FILTRATES OF *PSEUDOMONAS AMYGDALI*

ANTONIO EVIDENTE, NICOLA S IACOBELLIS,* RITA VELLONE,† ANGELO SISTO* and GIUSEPPE SURICO‡

Istituto di Chimica, Università della Basilicata, 85100 Potenza, Italy, *Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, 70126 Bari, Italy, †Dipartimento di Chimica Organica e Biologica, Università di Napoli, 80134 Napoli, Italy; ‡Istituto di Patologia e Zoologia Forestale ed Agraria, Università di Firenze, 55144 Firenze, Italy

(*Received* 19 *December* 1988)

Key Word Index—*Pseudomonas amygdali*, almond, hyperplastic bacterial canker; plant growth regulators; cytokinins, adenine and 2′-deoxyadenosine derivatives

Abstract—A new cytokinin has been isolated from the culture filtrate of *Pseudomonas amygdali* together with isopentenyladenine, dihydrozeatin and *trans*-zeatin. The structure of the new cytokinin was assigned on the basis of spectroscopic data and by its partial synthesis starting from the *trans*-zeatin and the 2-deoxy-D-ribose. It was identified as the 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)-9-β-2′-deoxy-D-ribofuranosylpurine

INTRODUCTION

The hyperplastic bacterial canker is a serious disease of almond in the Mediterranean (Greece and Turkey) [1, 2] as well as in other geographical areas (Afghanistan) [3]. The pathogen responsible for the disease is *Pseudomonas amygdali* Psallidas & Panagopoulos, a bacterium which is reported to be highly host specific, infecting only almond.

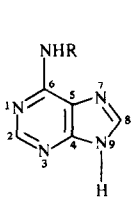
The development of the symptoms on branches, twigs and trunks and the final appearance of the canker suggest the possible involvement of phytohormones in the disease process of *P. amygdali* [4]. In fact, *P. amygdali* produces in culture several plant growth substances two of which have been isolated and identified as the *trans*-zeatin (**1**) and the indole-3-acetic acid (IAA) [4]

This paper reports on the structural elucidation of a new cytokinin and on the identification of two additional known cytokinins, isolated from the culture filtrate of *P amygdali*.

RESULTS AND DISCUSSION

The material obtained from the lyophilization of filtrates from five-day-old cultures of *P. amygdali* was taken up in water, acidified (pH 2.5) and partitioned with ethyl acetate to extract the acidic substances including indole-3-acetic acid (IAA) and other 3-substituted indoles [4]

The resulting aqueous phase was alkalinized (pH 8 5) and extracted with the same solvent The residue left from the organic extracts was active in the cucumber cotyledon bioassay for cytokinin activity. Preparative TLC (silica gel, eluent A) of active extracts yielded three UV-absorbing bands, but only two of them showed cytokinin activity. In particular, one (bands B) had a zeatin-like R_f value and the other (band A) a higher R_f value than *trans*-zeatin. A further fractionation of mixture B, by three analytical TLC steps (using reverse phase, eluent B and silica gel, eluent C), yielded two UV-absorbing substances (B2 and B3) both stimulating the synthesis of chlorophyll in the cucumber cotyledons bioassay. Compound B3 (1 7 mg/l) was identified as *trans*-zeatin (**1**) [4], while compound B2 (**5**, 0 28 mg/l) apparently did not correspond to any known cytokinin. In fact, its chromatographic behaviour was different from that of 11 cytokinins [*trans*-zeatin (Z), zeatin riboside (ZR), dihydrozeatin (diHZ), dihydrozeatin riboside (diHZR), isopentenyladenine (iP), isopentenyladenosine (iPA), 2-methylthioZR (2MeZR), 2-methylthioiP (2MeiP) 2-methylthioiPA (2MeiPA), 1′-methylzeatin (1′MeZ) and 1″-methylzeatin riboside (1″MeZR)] used as reference substances in TLC and HPTLC analysis (silica gel, eluents A and C) and by

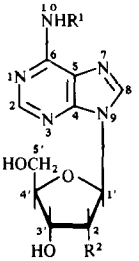


1 R = $CH_2CH{=}C(Me)CH_2OH$

2 R = $CH_2CH{=}C(Me)Me$

3 R = $CH_2CH_2CH(Me)CH_2OH$

4 R = $CH_2CH_2{=}C(Me)CH_2OAc$



5 R^1 = $CH_2CH{=}C(Me)CH_2OH$ R^2 = H

6 R^1 = $CH_2CH{=}C(Me)CH_2OH$, R^2 = OH

7 R^1 = R^2 = H

TLC (reverse phase, eluent B) However, pure **5** exhibited a UV spectrum characteristic of N^6-9-disubstituted purines [5–7].

An inspection of its ^{1}H NMR spectrum (Table 1) showed a very close structural correlation with zeatin riboside (**6**) The proton shifts were assigned by integration, multiplicity and by evidence from a series of ^{1}H-decoupling experiments The ^{1}H NMR spectra of **5** and **6** [8] (Table 1) exhibited the same signal pattern of the purine moiety and the side chain residue In particular, two singlets, assigned to H-8 and H-2, were present at δ8.26 and 8 22, respectively Moreover, a broad triplet, attributed to the olefinic proton (H-2″), appeared at δ5 63, a multiplet, due to the methylene group (H_2C-1″) attached to the purine moiety, was present at δ4 27, and two broad singlets, assigned to the $HOCH_2$-4″ and the Me-5″ group, appeared at δ3 97 and 1 78, respectively

The examination of the signal systems of the sugar moiety in **5** revealed the presence of a pentose residue linked to N^9 different from that in compound **6**. In fact, the anomeric proton (H-1′) appeared as a double doublet at δ6 42 in **5** and as a doublet at δ5 94 in **6** This typical downfield shift ($\Delta\delta$ 0.48) [9, 10] and the multiplicity of H-1′ suggested a 2′-deoxyribose *N*-glycosyl nature for **5** As expected, the signal of H-2′, present in **6** at δ4.73 as a double doublet, was absent in **5**, while in the spectrum of this new cytokinin two doublets of double doublets appeared at δ2 81 (H-2′A) and 2 40 (H-2′M) as the AM part of an AMXY system The protons assigned to the X and Y part of the latter system were present both as a doublet of double doublet, at δ4 57 (H-3′) and 4.07 (H-4′), respectively. Consequently, the H-3′ showed a more complex signal (a doublet of a double doublet) with respect to the double doublet observed for the same proton in the ^{1}H NMR spectrum of **6** Finally, the two double doublets due to the protons of the $HOCH_2$-5′ group were observed at δ3 85 (H-5′A) and 3 72 (H-5′B), respectively Further, the very close proton pattern (chemical shifts and coupling constants) resulting from the comparison between the ^{1}H NMR spectrum of **5** and that of 2′-deoxyadenosine (**7**) (Table 1), was consistent with the presence of the same nucleoside moiety in both compounds and in agreement with the literature [11] These findings indicated that the new cytokinin had a 2′-deoxyzeatin riboside structure (**5**)

The ^{13}C NMR spectrum of **5** (Table 2) was consistent with the structure proposed A detailed examination showed that the signal pattern of the furanosylpurine residue was very close to that of the 2′-deoxyadenosine, recorded in the same conditions (Table 2) and in agreement with the previously reported data [11, 12] In particular, the two protonated carbons of the purine moiety appeared at δ153 6 (C-2) and 140 9 (C-8), while the signals of the quaternary carbons were observed at δ156.1, 146 7 and 121 2 for C-6, C-4 and C-5 respectively The signal of the anomeric carbon appeared at δ87 2 together with the other three oxygenated carbons of the sugar ring present at δ89 9, 73 1 and 63.7, for C-4′, C-3′ and C-5′, respectively The H_2C-2′ group appeared at δ41 6 Moreover, the nucleoside signal pattern of the ^{13}C NMR spectrum of **5**, compared to that of the adenosine [12] and that of cytokinins containing a ribosyl residue [8], showed the upfield shift ($\Delta\delta \sim 30$) of the C-2′, as the main difference, and the upfield ($\Delta\delta$1 4) and the downfield ($\Delta\delta$1 5) shifts, respectively, of C-1′ and C-3′ and C-4′, as the minor differences [12] In fact, besides the above cited signals, the chemical shift values of the carbon of the side chain of **5** were very close to those reported for zeatin-like cytokinins [8, 13] In particular, the signals of the olefinic carbons of the side-chain appeared at δ140.0 (C-3″) and 121.7 (C-2″), the methylene (H_2C-1″) linked to the purine moiety was present at δ34 7, while the hydroxymethylene ($HOCH_2$-4″) and the methyl (Me-5″) were observed at δ68 2 and 13 7, respectively

Table 1 ^{1}H NMR data of 2′-deoxyzeatin riboside (**5**), zeatin riboside (**6**) and 2′-deoxyadenine (**7**)

	5*	6†	7*
	Chemical shifts are in δ-values (ppm) from TMS		
H-2	8 22 *s*	8 23 *s*	8 17 *s*
H-8	8 26 *s*	8 24 *s*	8 30 *s*
H-1′	6 42 *dd*	5 94 *d*	6 43 *dd*
H-2′A	2 81 *ddd*	4 73 *dd*	2 81 *ddd*
H-2′M	2 40 *ddd*		2 40 *ddd*
H-3′	4 57 *ddd*	4 31 *dd*	4 58 *ddd*
H-4′	4 07 *ddd*	4 16 *ddd*	4 08 *ddd*
H-5′A	3 85 *dd*	3 88 *dd*	3 85 *dd*
H-5′B	3 72 *dd*	3 74 *dd*	3 75 *dd*
2H-1″	4 27 *m*	4 27 *m*	
H-2″	5 63 *br t*	5 64 *br t*	—
2H-4″	3 97 *br s*	3 97 *br s*	—
3H-5″	1 78 *br s*	1 78 *br s*	

J(Hz) **5**, 7 1′,2′A = 8 0, 1′,2′M = 6 1, 2′A,2′M = 13 6, 2′A,3′ = 5 9, 2′M,3′ = 2 8, 3′,4′ = 4′, 5′A = 4′,5′B = 2 9, 5′A,5′B = 12 3, **5**, **6** 1″,2″ = 7 0, 2″,4″ = 1 5, 2″,5″ = 1 1, **6**, 1′,2′ = 6 4, 2′,3′ = 5 2, 3′,4′ = 4′, 5′A = 4′,5′B = 2 4, 5′A,5′B = 12 5

* † Assignments made in agreement with data reported in ref [11] and [8], respectively

Table 2 ^{13}C NMR data of 2′-deoxyzeatin riboside (**5**) and 2′-deoxyadenine (**7**)

	5*	7*
	Chemical shifts are in δ-values (ppm) from TMS	
C-2	153 6 *d*	153 5 *d*
C-4	146 7 *s*	149 9 *s*
C-5	121 2 *s*	120 8 *s*
C-6	156 1 *s*	157 5 *s*
C-8	140 9 *d*	141 5 *d*
C-1′	87 2 *d*	87 1 *d*
C-2′	41 6 *t*	41 5 *t*
C-3′	73 1 *d*	73 0 *d*
C-4′	89 9 *d*	89 9 *d*
C-5′	63 7 *t*	63 6 *t*
C-1″	34 7 *t*	
C-2″	121 7 *t*	
C-3″	140 0 *s*	
C-4′	68 2 *t*	
C-5″	13 7 *q*	

*Assignments consistent with data reported for reference compounds [8, 11, 12]

The fast atom bombardment (FAB) mass spectrum of **5** showed the occurrence of a peak at *m/z* 358 $[M+Na]^+$ and a pseudomolecular ion at *m/z* 336 $[MH]^+$. The fragmentation peaks, observed at *m/z* 341 $[M+Na-OH]^+$, 283 $[M+Na-H_2O-C_3H_5O]^+$, 261 $[MH-H_2O-C_3H_5O]^+$, 227 [zeatin + Na − Me]$^+$, 205 [zeatin + H − Me]$^+$, 197 [zeatin + Na − Me − CH_2O]$^+$ and 173 [zeatin − Me − CH_2OH]$^+$ are characteristic of 6-alkylaminopurines [14–17]. The peaks at *m/z* 249 [alkylaminoadenine residue + H + 30]$^+$ and 220 [alkylaminoadenine residue + 2H]$^+$, arose from a well known fragmentation processes involving the sugar moiety and indicated the presence of a 2′-deoxyribosyl residue while further supported the identity of the base [15, 16, 18]. These results made it possible to assign the structure of 6-(4-hydroxy-3-methylbut-*trans*-2-enyl)-9-β-2′-deoxy-D-ribofuranosylpurine to the new cytokinin (**5**).

Confirmation of the structure assigned to **5** was obtained from its partial synthesis starting from *trans*-zeatin (**1**) and 2-deoxy-D-ribose. The acetyl-*trans*-zeatin (**4**) and the 1,3,5-triacetyl-2-deoxy-D-ribose, prepared by the usual acetylation of **1** and 2-deoxy-D-ribose, respectively, were converted, according to a procedure described for the synthesis of purine nucleosides [19], into the 3′,5′,4″-triacetylderivative of **5** and its corresponding α-anomer The hydrolysis of the synthetic derivatives, simply performed with ethanol and 0.2 M sodium hydroxide, yielded a complex mixture, whose fractionation by TLC (reverse phase, eluent B) led to the purification of a compound having the same TLC and HPTLC behaviour (in three different systems) as **5**. Moreover, the $[\alpha]_D^{25}$, and UV, ^{1}H NMR and FAB mass spectra of this synthetic cytokinin were identical to those of the 2′-deoxyzeatin riboside (**5**) isolated from the culture filtrate of *P. amygdali*

Band A, obtained from the initial purification of the basic organic extract of the culture filtrate of *P amygdali*, was further fractionated by two TLC steps. This procedure made it possible to isolate another pure compound (A2, 1 5 mg/l) which proved to be active in the cytokinin bioassay This cytokinin showed the same R_f value, also by co-chromatography, by TLC and HPTLC (silica gel, eluent A) and by TLC (reverse phase, eluent B) as an authentic sample of isopentenyladenine (**2**) This evidence, together with the data from the UV, ^{1}H NMR and CI mass spectra, considered in comparison with the data of *trans*-zeatin [4, 13, 17], permitted the identification of compound A2 as isopentenyladenine (iP) (**2**).

Finally, a different cytokinin was produced by the *P amygdali* strain NCPPB* 2610 when grown on a minimal medium supplemented with tryptophan. One TLC step, on reverse phase (eluent B), of the alkaline EtOAc extracts of *P amygdali* culture filtrates led to the isolation of the *trans*-zeatin (0.24 mg/l) and another compound (2 mg/l) which had a lower R_f value (0 64) than **1** (0 66) This substance tested on cucumber cotyledons showed a clear cytokinin activity.

This cytokinin was identified as dihydrozeatin (**3**). In fact, it showed the same R_f values in three different TLC systems, and identical spectroscopic data (UV, ^{1}H NMR and EIMS spectra) as an authentic reference sample. These spectroscopic properties were also consistent with those reported in the literature for **3** [20, 21]

Moreover, the monoacetyl derivatives prepared from natural **3** and standard dihydrozeatin showed the same R_f values in three different TLC and HPTLC systems (silica gel, eluent C and D; reverse phase, eluent B). Finally, dihydrozeatin does not seem to be produced by *P amygdali* grown in Woolley's medium.

Besides identifying some already known cytokinins, our report focuses on the isolation and characterization of 2′-deoxyzeatin riboside as a naturally occurring new cytokinin We have demonstrated that *P. amygdali*, like other plant pathogenic microorganisms which cause growth disorders on their hosts, accumulate several cytokinins and auxins in culture (see ref [4]) It must now be directly demonstrated whether cytokinins and IAA of bacterial origin are the causative agents in the formation of hyperplastic cankers on almond. If this is the case, investigations on the factors, such as environmental conditions, which can affect the establishment and the development of the disease in almond and in particular the production or plant growth substances *in planta*, would help in an understanding of the pathogenic behaviour of *P. amygdali*

EXPERIMENTAL

General Optical rotations were measured on EtOH soln, UV spectra were recorded on EtOH soln; ^{1}H and ^{13}C NMR spectra were recorded at 300 and/or 270 and 75 46 MHz, respectively, in CD_3OD, and using TMS as int standard Analytical and prep TLC were carried out on silica gel (Merck, Kieselgel 60 F_{254}, 0 25 and 2 0 mm, respectively) and on reverse phase (Whatman, Stratocrom SIF_{254}, C-18, 0 2 mm) plates, HPTLC was carried out on silica gel plates (Merck, Kieselgel, 60 F_{254} *S*, 0 2 mm), the spots were visualized by exposure to UV radiations or by spraying with 10% H_2SO_4 in MeOH and then heating at 110° for 10 min EIMS were recorded at 70 eV and CIMS at 250–300 eV, using *iso*-butane as reagent gas FABMS were determined with a double-focusing mass spectrometer on samples dissolved in glycerol–thioglycerol on a Cu probe tip and inserted into the source at 10^{-4} Torr pressure of Xe The sample was bombarded with Xe atoms of 9 5 kV energy and the spectra were recorded on UV-paper Solvent systems (A) *n*-BuOH–HOAc–H_2O (20 5 8), (B) H_2O–EtOH (3 2), (C) $CHCl_3$–EtOAc–MeOH (2 2 1), (D) $CHCl_3$–*iso*-PrOH (4·1), (E) $CHCl_3$–*iso*-PrOH (19·1). Authentic sample of *trans*-zeatin (Z), dihydrozeatin (diHZ), 9-β-D-ribofuranosylzeatin (ZR), dihydrozeatin riboside (diHZR), isopentenyladenine (iP), isopentenyladenosine (iPA) (all purchased from Sigma, St Louis, U S A) and 2-methylthiozeatin riboside (2MeSZR), 2-methylthio-iP (2MeiP), 2-methylthio-iPA (2MeiPA) were used as standards Pure samples of 1′-methylzeatin (1′MeZ) and 1″-methylzaetin riboside (1″MeZR) were isolated from the culture filtrates of *Pseudomonas syringae* pv *savastanoi* [8, 13] 2′-deoxyadenosine and 2-deoxy-D-ribose were purchased from Fluka A. G Bush, Switzerland, and peptone from Difco, Laboratories, Detroit, U.S A

Bioassay The cytokinin activity of extract and purified fractions from *P amygdali* liquid cultures was determined on cucumber cotyledons according to the method of ref [22].

Cytokinin production and purification P amygdali strain NCPPB 2610 was grown in Woolley's [23] medium supplemented with 1 5% peptone under agitation for 5 days at 20°

*National Collection Plant Pathogenic Bacteria, Harpenden, U K

The culture filtrate (18 5 l) was lyophilized, the residue redissolved in one-tenth of the original vol of H_2O, was acidified to pH 2 5 (1M HCl) and then extracted with EtOAc to remove the acidic substances including indole compounds [4] The resulting water phase was adjusted to pH 8 5 (1M NaOH) and extracted with EtOAc (4 × 1 85 l) The combined organic extracts were dried (Na_2SO_4) and evapd under red pres The oily residue (935 5 mg), sepd on prep TLC (silica gel, eluent A), yielded three UV-absorbing bands (A–C) which were scraped off the plates, eluted with EtOH and dried under red pres The residue left from bands A (326 8 mg) and B (357 mg) exhibited cytokinin activity The residue from band B, which presented a zeatin-like R_f, was further fractionated by TLC (reverse phase, eluent B) to give 5 fractions (B1–B5) Fraction B3 (30 1 mg) consisted essentially of *trans*-zeatin (**1**) (R_f 0 65 and 0 28, by TLC on silica gel, eluent A and C, respectively, and 0 57 by TLC on reverse phase, eluent B) while fraction B2 (40 4 mg) (R_f 0 66 and 0 31 by TLC on silica gel, eluent A and C, respectively, and 0 62 by TLC on reverse phase, eluent B) was further purified by TLC (silica gel, eluent C) to give a further small amount of *trans*-zeatin (2 7 mg), for a total of 32 8 mg (1 7 mg/l), and crude component B2 which, in turn, was chromatographed on reverse phase plates (eluent B) to obtain cytokinin **5** as pure oil (5 2 mg, 0 28 mg/l) Compound **5** showed $[\alpha]_D^{25}$ −12 1 (*c* 0 19), UV λ_{max} nm (log ε) 268 (3 74), ^1H and ^{13}C NMR data are reported in Tables 1 and 2 respectively, FABMS, *m/z* (rel int) 358 $[M+Na]^+$ (76) 336 $[MH]^+$ (63), 341 $[M+Na-OH]^+$ (63), 283 $[M+Na-H_2O-C_3H_5O]^+$ (53), 261 $(MH-H_2O-C_3H_5O]^+$ (63), 249 [alkylaminoadenine residue + H + 30]$^+$ (60), 227 [zeatin + Na - Me]$^+$ (100), 220 [alkylaminoadenine residue + 2H]$^+$ (33), 205 [zeatin + H − Me]$^+$ (61), 197 [zeatin + Na − Me − CH_2O]$^+$ (72), 173 [zeatin − Me − CH_2OH]$^+$ (30) Fractions *B1*, *B4* and *B5* did not contain any cytokinins

The residue (326 8 mg) left from band A was further fractionated by TLC (silica gel, eluent C) to obtain 3 UV-absorbing fractions A1–A3 Fraction A2 (64 5 mg) was purified by TLC reverse phase, eluent B) to yield a pure cytokinin A2 (**2**) (27 5 mg, 1 5 mg/l) Bands A1 and A3 did not contain any cytokinins Compound **2** had UV λ_{max} nm (log ε) 265 (3 31), ^1H NMR, δ 8 23 (1H, s, H-2), 8 06 (1H, s, H-8), 5 40 (1H, *br t*, *J* = 6 9, 1 7 and 1 7 Hz, H-2'), 4 17 (2H, *m*, H-1'), 1 77 (6H, *br s*, *J* = 1 7 Hz, 3H-4' and 3H-5', respectively), CIMS, *m/z* (rel int) 204 $[MH]^+$ (11), 188 $[M-Me]^+$ (0 7), 160 $[M-C_3H_7]^+$ (2 4), 148 $[M-C_4H_7]^+$ (2), 136 [adenine + H]$^+$ (2 9), 43 (100)

Partial synthesis of 2'-deoxy zeatin riboside (**5**) *trans*-Zeatin (5 mg) was acetylated with pyridine (200 μl) and Ac_2O (200 μl) at room temp After 12 hr, the reaction was stopped by the addition of MeOH (1 ml) at 0°, the pyridine was removed by evapn under red pres, as it forms an azeotrope with C_6H_6 The resulting oily product (5 2 mg) (**4**) proved to be homogeneous by TLC analysis (silica gel, eluents C and D) The 2-deoxy-D-ribose (15 mg) was acetylated with pyridine (600 μl) and Ac_2O (600 μl) in accord with the procedure used to obtain the acetyl-*trans*-zeatin (**4**) from **1** The 1,3,5-triacetyl-2-deoxy-D-ribose obtained as a pure oil (27 mg) was homogeneous by TLC analysis (silica gel, eluent E) The acetyl-*trans*-zeatin (**4**) (5 2 mg) and the 1,3,5-triacetyl-2-deoxy-D-ribose (15 mg) were employed in the fusion synthesis of nucleoside performed at 145° according to the method described in ref [19] After 20 min, the reaction mixture was cooled at 100° and the clear brown melt was dissolved in EtOAc (10 ml) The soln was successively washed with ice-cold said $NaHCO_3$ and ice-water and then dried (Na_2SO_4) The TLC analysis (silica gel eluent, E) of the oily residue, obtained after the evapn of the solvent, showed the absence of both the starting acetyl-*trans*-zeatin (R_f 0 1) and the 1,3,5-triacetyl-2-deoxy-D-ribose (R_f 0 82) and the presence of two main compounds with similar chromatographic behaviour (R_f 0 28 and 0 25, respectively). This mixture (12 mg), dissolved in EtOH (2 ml), was hydrolysed with 0 2 M NaOH (600 μl), at room temp, and the reaction monitored by TLC (silica gel, eluents A and D) After 2 hr, the reaction was stopped by neutralization with 2 M HCl at 0° The mixture was evapd under red pres, and the residue dissolved in a minimal amount of MeOH was filtered through cotton wool The residue (8 mg) left by the evapn of the solvent, fractionated by TLC (silica gel, eluent A), yielded three UV-absorbing bands A, B, C Zone A, having a 2'-deoxy-ZR-like R_f value (0 66), was scraped off the plates, eluted with EtOH and dried under red pres Further purification of band A by TLC (reverse phase, eluent B) yielded pure cytokinin (1 9 mg) which showed the same R_f values as natural **5**, by TLC and HPTLC (silica gel, eluents A and C) and by TLC (reverse phase, eluent B), as well as by co-chromatography The synthetic 2'-deoxy-ZR showed $[\alpha]_D^{25}$ −10 7 (*c* 0 14), UV, ^1H NMR and FABMS spectra identical to those of natural **5**

Production and isolation of dihydrozeatin (**3**) *P amygdali*, strain NCPPPB 2610, was grown at 20° on minimal medium [K_2HPO_4, 10 5 g/l, KH_2PO_4, 4 5 g/l, $(NH_4)_2SO_4$, 1 g/l, Na-citrate, 0 5 g/l, glucose, 5 g/l, $MgSO_4$ $7H_2O$, 0 2 g/l] supplemented with tryptophan (final concentration 0 5 mM) The extraction of cytokinins from the culture filtrates (530 ml) was as described above The residue (5 6 mg) left from the evapn of the solvent was purified by TLC (reverse phase, eluent B), yielding two UV-absorbing bands A and B, which were scraped off the plates, eluted with EtOH and dried under red pres The residue obtained from band A (0 24 mg/l by HPLC analysis [24]) by TLC and HPTLC (silica gel, eluents A and C) and by TLC (reverse phase, eluent B) showed the same R_f values as an authentic sample of *trans*-zeatin (**1**) Band B yielded another pure cytokinin (1 0 mg/l) exhibiting by TLC and HPTLC, in the three usual systems, the same chromatographic behaviour as an authentic sample of reference dihydrozeatin (diHZ, **3**) The cytokinin yielded from band B showed $[\alpha]_D^{25}$ −9 0° (*c* 0 16); UV λ_{max} nm (log ε) 268 (3 86) (EtOH), 270 (3 92) (EtOH + 0 1 N HCl), 273 (3 92) (EtOH + 0 1 N NaOH), ^1H NMR, δ 8 22 (1H, s, H-2), 8 06 (1H, s, H-8) 3 66 (2H, *m* H-1'), 3 48 (1H, *dd*, *J* = 11 0 Hz, *J* = 6 0 Hz, H-4 A), 3 44 (1H, *dd*, *J* = 11 0 Hz, *J* = 6 0 Hz, H-4'B), 1 85 (1H, *m*, H-3'), 1 75 (1H, *m*, H-2'A), 1 51 (1H, *m*, H-2'B), 1 00 (3H, *d*, *J* = 6 8 Hz, H-5'), EIMS, *m/z* (rel int) 221 $[M]^+$ (8), 204 $[M-OH]^+$ (4), 190 $[M-CH_2OH]^+$ (5 4), 162 $[M-C_3H_7O]^+$ (43), 148 $[M-C_4H_8O]^+$ (100), 135 [adenine]$^+$ (24), 108 [adenine − HCN]$^+$ (5 4) The $[\alpha]_D^{25}$, the UV, ^1H NMR and mass spectra of this cytokinin were identical to those, recorded in the same conditions, as an authentic sample of dihydrozeatin (**3**) and very close to those reported in refs [20, 21]

Acknowledgements We wish to thank the Italian Ministry of Education for its financial support and Professor N Leonard (University of Illinois, U S A), and Dr R Horgan (University of Wales, U K), for generously providing samples of cytokinin not commercially available, and Mrs S L Lonigro for her technical assistance

REFERENCES

1 Psallidas, P G and Panagoupoulos, C G (1975) *Ann Inst Phytopath Benaki*, (N S) **11**, 94
2 Gundoğdu, M and Kaya, S J (1976) *J Turkish Phytopath* **5**, 87
3 Ercolani, G L and Ghaffar, A (1985) *FAO Plant Prot Bull* **33**, 37
4 Iacobellis, N S, Evidente, A and Surico, G (1988) *Experientia* **44** 70

5 Scott, A I (1964) in *Interpretation of the Ultraviolet Spectra of Natural Products*, Vol 7, pp 197–201 Pergamon Press, Oxford
6 Rao, C N R. (1961) in *Ultra-Violet and Visible Spectroscopy—Chemical Applications*, pp. 56–58. Butterworths, London.
7 Letham, D S (1973) *Phytochemistry* **12**, 2445
8 Surico, G., Evidente, A, Iacobellis, N S and Randazzo, G (1985) *Phytochemistry* **24**, 1499
9 Gatlin, L. and Davis, J C Jr (1962) *J. Am Chem Soc* **84**, 4464
10 Schweizer, M P, Broom, A D, Ts'o, P O. P and Hollis, D P. (1968) *J. Am. Chem Soc* **90**, 1042
11 Pathak, T, Bazin, H and Chattopadhyaya, J (1986) *Tetrahedron* **42**, 5427
12 Jones, A J, Grant, D M, Winkley, M W and Robins, R K (1970) *J Am Chem Soc* **92**, 4079
13 Evidente, A, Iacobellis, N. S, Surico, G and Randazzo, G (1986) *Phytochemistry* **25**, 525.
14 Porter, Q. N and Baldas, J (1971) in *Mass Spectrometry of Heterocyclic Compounds* (Weissberger, A and Taylor, E C, eds), pp 479, 483–487 Wiley-Interscience, New York
15. Hignite, C (1972) in *Biochemical Applications of Mass Spectrometry* (Waller, G R, ed), pp. 434–441. Wiley-Interscience, New York
16. Engel, L L. and Orr, J C (1972) in *Biochemical Applications of Mass Spectrometry* (Walles, G R ed), pp 567–568, Wiley-Interscience, New York
17 Letham, D S, Shannon, J S and McDonald, I R. C (1967) *Tetrahedron* **23**, 479
18 Biemann, K and McCloskey, J A (1962) *J Am Chem Soc* **84**, 2005
19. Robins, M J and Robins, R K (1968) in *Synthetic Procedures in Nucleic Acid Chemistry* (Zorbach, W and Tipson, R S, eds), Vol 1, p. 246 Wiley, New York.
20 Koshimizu, K, Kusaki, T Mitsui, T and Matsubara, S (1967) *Tetrahedron Letters* **14**, 1317
21 Fujii, T and Ogawa, N (1972) *Tetrahedron Letters* **30**, 3075.
22 Fletcher, R A, Kallidumbil, V and Steele, P (1982) *Plant Physiol* **69**, 673
23. Wooley, D W, Shaffner, G and Braun, A G (1955) *J Biol Chem* **215**, 485
24 Iacobellis, N S, Surico, G, Evidente, A, Iasiello, I and Randazzo, G (1985) *Phytopath Medit* **24**, 315