Tetrahedron Vol. 45, No. 12, pp. 3889 to 3896, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

THE SYNTHESIS OF ALLYLIC PHOSPHATE DERIVATIVES OF *TRANS* ZEATIN

BELAL SHADID AND HENK C. VAN DER PLAS*

Laboratory of Organic Chemistry, Agricultural University Wageningen, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

C.R. VONK, E. DAVELAAR AND S.A. RIBOT

Centre for Agrobiological Research, P.O. Box 14, 6700 AA Wageningen, The Netherlands

(*Received in UK* 29 *March* 1989)

Abstract: Phosphitylation of the allylic hydroxy group in 9-tetrahydropyranyl *trans*-zeatine with salicyl chlorophosphite gives the allyl phosphonate monoester **5**, which can be readily converted into the corresponding phosphate **7**, methylphosphate **8** or thiophosphate **9**. After cleavage of the tetrahydropyranyl group under mild acidic condition allylic phosphate derivatives of *trans*-zeatin are obtained.

INTRODUCTION

Progress in biochemical research during recent years has clearly pointed out the unique importance of cytokinins in biological systems. The cytokinins, a group of naturally occurring compounds which induce plant cell division, are N-6-substituted derivatives of adenine, which may occur as a free-base, as its ribonucleoside or ribonucleotide.

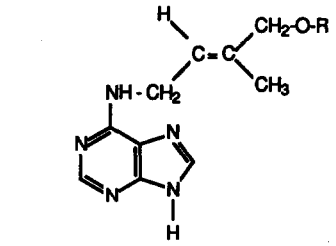
The first naturally occurring plantgrowth substance with pronounced cell division induction was isolated in pure form from immature sweetcorn kernels[1]. This compound was assigned structure Z_1, based on UV, NMR and Mass spectral data, enzymatic characterisation as well as independent synthesis.

Attempts were made to synthesize the allylic phosphate of zeatin i.e. Z_2, from Z_1 by using the powerful phosphorylating pyrophosphoryl chloride, or by applying a mixture of 2-cyanoethyl phosphate and dicyclohexylcarbodiimide in pyridine. Both attempts failed[2]. It has been reported[3] that phosphorylation of polyprenols, containing an allylic hydroxy group, by a reaction between the active intermediate polyprenol trichloroimidates and anhydrous orthophosphoric acid, was successful, but that in this reaction also rearranged products are obtained.

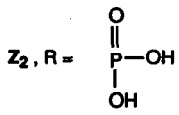
Recently a new and efficient method for the phosphorylation of an allylic hydroxy group via a phosphite intermediate has been found[4]. This method is based on the phosphitylation of an allylic hydroxy group with salicyl chlorophosphite and oxidation of the allylic phosphonate monoester, being formed to the corresponding phosphate monoester. Application of this phosphitetriester-approach was already demonstrated in the preparation of phosphonate and

phosphate monoesters of N-(4-hydroxy-3-methyl-E-but-2-enyl) phthalimide.

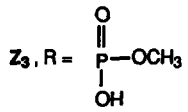
We will now report in detail the synthesis of the allylic phosphonate Z_5, the methylphosphate Z_3, and the thiophosphate Z_4 of *trans*-zeatin Z_1. This synthetic study was part of a program of cooperation between our laboratory and the Centre of Agricultural and Biological Research at Wageningen, directed to study the mechanism and physiological effects of cytokinins in plants[5].



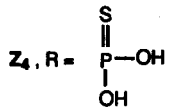
Z_1, R = H



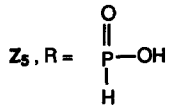
Z_2, R =



Z_3, R =



Z_4, R =

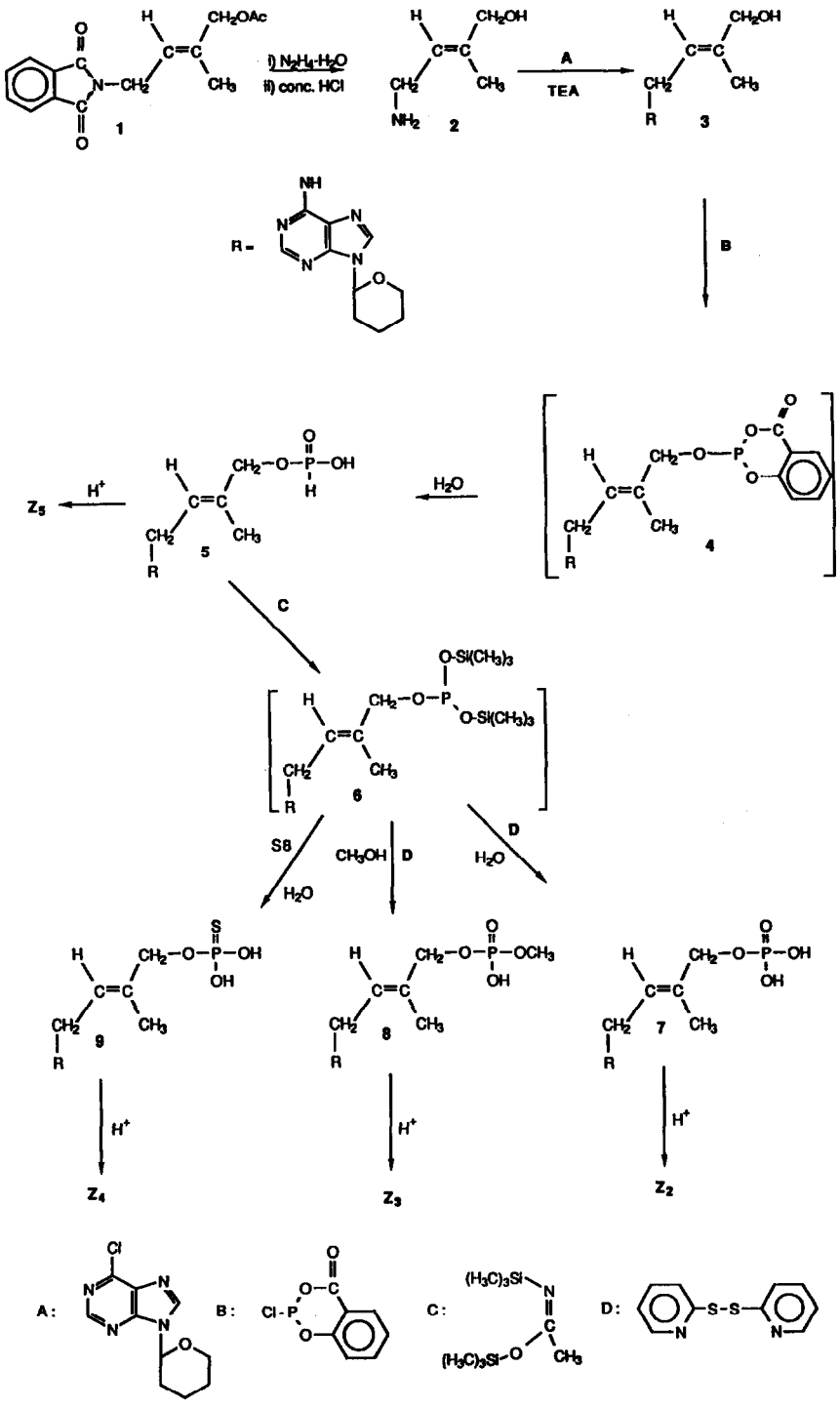


Z_5, R =

RESULTS AND DISCUSSION

The strategy we have adopted for the synthesis of these allylic phosphate derivatives of zeatin consisted of the following steps: i. the synthesis of *trans*-zeatin protected at N-9 with a tetrahydropyranyl (THP)group i.e. **3**; ii. the synthesis of N-9-protected *trans*-zeatin phosphonate **5**; iii. the conversion of **5** into the corresponding phosphate, methylphosphate or thiophosphate **7**, **8** and **9** respectively; iv. the removal of the N-9-protecting group.

Our approach to the synthesis of the N-9-THP *trans*-zeatin **3** is a modification of two syntheses, being already reported. N-(4-acetoxy-3-methyl-E-but-2-enyl) phthalimide **1**, being prepared by a procedure described before[6], was converted in a one-pot reaction into E-4-amino-2-methyl-2-buten-1-ol (**2**) (yield 85%) by treatment with 85% aqueous hydrazine (removal of the phthaloyl group) and subsequent heating with hydrochloric acid (hydrolysis of the acetoxy group). Coupling of **2** with 6-chloro-N-9-THP purine[7] (reagent A) in refluxing butanol in the presence of triethylamine gave N-9-THP *trans*-zeatin **3** (yield 76%). Protection of the N-9-position by the tetrahydropyranyl group was necessary to avoid side-reactions


i) $N_2H_4 \cdot H_2O$
ii) conc. HCl
A
TEA
B
C
D
H_2O
CH_3OH
S8
H^+
Z_5
Z_4
Z_3
Z_2
A:
B:
C:
D:

during the phosphitylation reaction. The protective THP group can later be removed under mild acidic conditions[8]. The next step is the synthesis of N-9-THP-*trans*-zeatin phosphonate **5**, being the key intermediate in the synthesis of the allylic phosphate derivatives **Z_2**, **Z_3**, **Z_4**, and **Z_5**. Treatment of N-9-THP-*trans*-zeatin **3** in dioxane with the monofunctional reagent 2-chloro-4H-1, 3, 2-benzodioxaphosphorin-4-one (reagent B, 1.4 equiv.) in the presence of N, N-diisopropylethylamine (1.4 equiv.) for 5 min gave phosphite triester **4**, which was hydrolyzed into **5** being isolated as Na^+-salt in a yield of 85%. By ^1H NMR, ^{13}C NMR and ^{31}P NMR data (see experimental part) its structure was proven.

The oxidation of N-9-*trans* zeatin phosphonate **5** into N-9-THP-*trans*-zeatin phosphate **7** was performed according to a slight modification of the procedure of Hata et al[9]. Thus, 1 equiv. of **5** in dioxane was treated with N, O-bis (trimethylsilyl) acetamide (reagent C, 2 equiv.) in the presence of N, N-diisopropylethylamine (2 equiv.) for 15 min. to give intermediate bis (trimethylsilyl) phosphite (**6**). Without further purification **6** was treated with 1.2 equiv. of 2,2'-dipyridyldisulfide (reagent D) for 1h, and the reaction mixture obtained was subsequently treated with water, yielding **7**. Treatment of **6** with dry methanol (8 equiv.) gave N-9-THP-*trans* zeatin methylphosphate **8**.

Sulfurization of **6** with powdered sulfur for 15 h and subsequent treatment with water gave N-9-THP-*trans*-zeatin phosphorothioate **9**. The cleavage of the acid-labile-THP-group in the phosphate derivatives **5**, **7**, **8** and **9** was effected by acid treatment at pH=2 for 3 h. After work-up *trans*-zeatin phosphonate **Z_5**, *trans*-zeatin phosphate **Z_2**, *trans*-zeatin methylphosphate **Z_3** and *trans*-zeatin thiophosphate **Z_4** were obtained. Over-all yield of these phosphate derivatives fluctuated between 70% and 83% as calculated from N-9-THP *trans*-zeatin **3**.

The identity of *trans*-zeatin allylic phosphate derivatives **Z_2**, **Z_3**, **Z_4** and **Z_5** was ascertained by ^1H NMR, ^{13}C NMR and ^{31}P NMR-spectroscopy (see Experimental Part). The ^1H NMR and ^{13}C NMR data of these compounds in D_2O are almost identical with those of *trans*-zeatin **Z_1**[10,11] with exception of the ^1H-absorption of CH_2-O which in zeatin is found as singlet at 3.8 ppm., and in the zeatin derivatives **Z_2**, **Z_3**, **Z_4** and **Z_5** as a doublet at about 4.2 ppm., caused by the coupling of ^1H with $^{31}P(^3J_{H\text{-}P})$.

In the ^{13}C NMR-spectra, the difference between zeatin and its phosphate derivatives is obvious in the absorption of CH_2O and $CH_3\underline{C}$=C. The ^{13}C-signal of the CH_2O group appears in zeatin (**Z_1**) as a singlet at 67 ppm. but in the zeatin phosphate derivatives (**Z_2**-**Z_5**) a doublet or a broad singlet at about 70 ppm is observed. The ^{13}C singlet of $CH_3\underline{C}$=C at about 137 ppm. in **Z_1** becomes a doublet in the zeatin phosphate derivatives (**Z_2**-**Z_5**). The doublets are caused by the coupling of ^{13}C with $^{31}P(^2J_{C\text{-}P}$ and $^3J_{C\text{-}P})$. The range of the coupling depends on the structure of the compound[12]. Proton decoupled ^{31}P NMR spectroscopy revealed the presence of only one resonance absorption which further evidences the structural identity and purity of the compounds.

From these results it can be unequivocally concluded that the method described above is a fast, simple and reliable procedure for the preparation of zeatin phosphate derivatives. These compounds are produced in satisfactory yields without the formation of any side-products.

EXPERIMENTAL

General procedures.

N, N-diisopropylethylamine, dioxane, triethylamine were dried by refluxing with CaH_2 for 16 h and then distilled. Dioxane was redistilled from $LiAlH_4$ (5 g/l). Methanol was dried by refluxing with magnesium methoxide and distilled before use. All liquids were stored under nitrogen. N,O-bis(trimethylsilyl)acetamide and 2,2'-dipyridyldisulfide were purchased from Janssen Chimica (Belgium). N-9-(tetrahydro-2-pyranyl)-6-chloropurine and 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one were prepared as described previously[7,13]. Triethylammonium bicarbonat buffer was prepared by passing a stream of CO_2 gas through a cooled (ice-water bath) 2M solution of triethylamine in deionized water until solution became neutral. Scheicher and Schüll DC Fertigfolien Fl 1500 LS 254 were used for TLC. The following solvent systems were used:
System A (chloroform/methanol, 85:15, v/v), system B (chloroform/methanol, 80:20, v/v), system C (isopropyl alcohol/concentrated ammonium hydroxide/water, 7:1:2, v/v).
Short column chromatography was performed on silicagel 60 (230-400 mesh A STM) suspended in CH_2Cl_2, or on Sephadex LH 20 suspended in CH_2Cl_2/CH_3OH (2:1, v/v), unless otherwise mentioned. DEAE Sephadex A 25 was purchased from Pharmacia (Uppsala, Sweden). Cation-exchange resin (Na^+-form): a solution of NaOH (2M; 100 ml) was passed over a column packed with cation-exchange resin (Dowex 50 Wx-8, 100-200 mesh; Fluka H^+-form, 1.5x5 cm) followed by washing of the column with sterile water until pH=7. Sterile water and glass were used during the whole deblocking and purification processes.
^1H NMR spectra were measured at 300 MHz using a Bruker CXP 300 spectrometer. ^{13}C NMR spectra were measured at 75.460 MHz using a Bruker CXP 300 spectrometer; Proton noise decoupling was used. ^{31}P NMR spectra were measured at 121.470 MHz using a Bruker CXP 300 spectrometer, chemical shifts are in ppm relative to 85% H_3PO_4 as external standard. Mass spectrum of **3** was measured using a AEI MS 902 spectrometer equipped with a VG ZAB console.

The synthesis of (E)-4-amino-2-methyl-2-buten-1-ol **2**
A mixture of N-(4-acetoxy-3-methyl-E-but-2-enyl) phthalimide **1** (3.30 g, 14.12 mmol), 85% aqueous hydrazine hydrate solution (0.85 ml, 14.14 mmol) and methanol (100 ml) was refluxed for 1 h with stirring. TLC analysis (system A) indicated complete conversion of the starting material into a product having Rf=0. After cooling of the solution water (8.7 ml) and conc. hydrochloric acid (8.7 ml) were added and the reaction mixture was heated under reflux for 1 h. After cooling to 0 C the phthalylhydrazide was removed by filtration and the filtrate was evaporated at 30°C in a rotary evaporator. Then the residue was dissolved in water (20 ml) and the insoluble material was removed by filtration. The filtrate was brought to pH=10 with 4N aqueous sodium hydroxide solution. The resulting solution was continuously extracted with chloroform. The organic extract was dried ($MgSO_4$) to give after evaporation the aminoalcohol **2**. Yield 1.21 gram (85%), oil, ^1H NMR-spectrum was identical to that described in the literature[6].

N-9-(Tetrahydro-2-pyranyl)-*trans*-zeatin **3**
A mixture of aminoalcohol **2** (1.21 g, 12 mmol), N-9-(tetrahydro-2-pyranyl)-6-chloropurine-(2.38 g, 10 mmol), anhydrous triethylamine (1.43 ml) and n-butanol (150 ml) was boiled under reflux for 3 h. TLC analysis (system A) indicated complete conversion of starting material. The reaction solution was concentrated under reduced pressure to an oil, which was chromatographed on a column of silicagel. Elution with CH_2Cl_2/CH_3OH (100:0 → 98:2, v/v) gave after evaporation 2.3 g (76%) of **3**, oil, Rf=0.72 (system A).
Compound **3**, ^1H NMR ($CDCl_3$): δ = 8.32, s, 1H, H–8; 7.91, s, 1H, H–2; 6.55, t, J = 5.16 Hz, 1H, CH_2-NH; 5.62, m, 2H, CH = C and 1H (THP); 4.22-4.06, m, 4H, CH_2-N, OH and 1H (THP); 3.90, s, 2H, CH_2-O; 3.72, m, 1H, 1H (THP); 2.05 – 1.92, m, 3H, 3H (THP); 1.67 – 1.58, m, 3H, 3H (THP); 1.60, s, 3H, CH_3; ^{13}C NMR: ($CDCl_3$): δ = 154.5, s, C_6; 153.2, s, C_2; 148.1, s, C_4; 139.9, s, C_8; 137.2, s, CH_3-C = C; 120.1, s, CH = C; 119.3, s, C_5; 81.6, 69.7, 67.1, 24.8 and 22.7 5 x s,(THP); 67.1, s, CH_2-OH; 31.8, s, CH_2-N; 13.8, s, CH_3; HRMS, found m/z 303.1703, $C_{15}H_{21}N_5O_2$ requires 303.1695.

N-9-(Tetrahydro-2-pyranyl)-*trans*-zeatin phosphonate **5**
To a solution of N-9-(tetrahydro-2-pyranyl)-*trans*-zeatin **3** (0.30 g, 1 mmol) and N, N-diisopropylethylamine (0.26 ml, 1.4 mmol) in dioxane (10 ml) was added 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one (0.28 g, 1.4 mmol). After reaction for 5 min at 20°C, it was found by TLC analysis (system A) that complete conversion of starting compound into a product

with zeromobility has taken place. Water was added (1 ml) and the solution was left for 10 min at 20°C. The reaction solution was concentrated to a small volume and triturated with diethylether, the precipitated oil (crude **5**) was dissolved in water and applied to a column of DEAE Sephadex A 25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05 M).
The column was eluted with a linear gradient of triethylammonium bicarbonate buffer (0.05 → 0.70 M) for 16 h with a flow rate of 35 ml/h, and fractions of 6 ml were collected. All UV-positive eluates, containing purine product Rf = 0 (system B), Rf = 0.75 (system C), were pooled and concentrated to a small volume; coevaporation with water (4 x 50 ml) removed most of triethylammonium bicarbonate. Then the solution was applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form, 1.5 x 5 cm). The column was eluted with water and all UV-positive eluates were collected and concentrated to a small volume. Lyophilization from D_2O gave **5** as white solid. Yield 0.33 g (85%), Rf = 0.73 (system C).
Compound **5**, ^1H NMR (D_2O): δ = 7.89, s, 1H, H-8; 7.84, s, 1H, H-2; 6.60, d, $^1J_{H\text{-}P}$ = 634.5 Hz, 1H, P-H; 5.44, m, 1H, 1H (THP); 5.27, t, J = 7.2 Hz, 1H, CH = C; 4.12, d, $^3J_{H\text{-}P}$ = 7.8 Hz, 2H, CH_2-O-P; 3.84, m, 3H, CH_2-N and 1H (THP); 3.56, m, 2H, 2H (THP); 1.82, s, 3H, CH_3; 1.49, m, 5H, 5H (THP); ^{13}C NMR (D_2O) : δ = 154.7, s, C_6; 153.2, s, C_2; 148.0, s, C_4; 137.3, s, C_8; 136.5, d, $^3J_{C\text{-}P}$= 7.3 Hz, CH_3-C = C; 123.4, s, CH = C; 119.4, s, C_5; 82.6, 67.4, 30.5, 25.10 and 23.0 5x s, (THP); 69.5, d, $^2J_{C\text{-}P}$ = 7.3 Hz, CH_2-O-P; 39.3, s, CH_2-N; 14.1, s, CH_3; ^{31}P NMR (D_2O); δp = 5.59 ppm, ($^1J_{H\text{-}P}$ = 620 Hz).

Trans-zeatin phosphate **Z2**
Crude **5** (synthesized from 1 mmol of **3**) was coevaporated with dioxane and dissolved in dioxane (10 ml). The solution was treated with N, N-diisopropylethylamine (0.35 ml, 2 mmol) and N, O-bis(trimethylsilyl) acetamide (0.5 ml, 2 mmol). After 15 min, ^{31}P NMR analysis of the solution indicated the complete conversion of **5** into **6**, (δp = 6.8 ppm → 118 ppm). The reaction solution was further treated with 2,2'-dipyridyldisulfide (0.26 g, 1.2 mmol) for one hour. After 1 hr the ^{31}P NMR absorption at 118 ppm was completely disappeared and the ^{31}P-spectrum features a new peak at δp = 9.8 ppm indicating that the conversion of **6** is complete (solution A). Then water was added (3 ml) and the solution was applied left for 2 h at 20°C. The solution was concentrated to an oil which was applied to a column of DEAE-Sephadex A 25 (HCO_3^--form) suspended in triethylammonium bicarbonate buffer (0.05 M).
The column was eluted with a linear gradient of triethylammonium bicarbonate buffer (0.05 → 0.7 M) for 18 h with a flow rate of 35 ml/h. Fractions of 10 ml were collected, and all UV-positive eluates, containing purine product **7**[14], [Rf = 0 (system B), Rf = 0.51 (system C)], were pooled, concentrated to a small volume and coevaporated with water (4 x 50 ml) to remove most of the triethylammonium bicarbonate and lyophilized from H_2O. The residue was dissolved in water (20 ml) and the pH was adjusted to 2 by the addition of HCl (0.1 N) After 3 h at 20°C TLC analysis (system C) showed that the cleavage of the tetrahydro-2-pyranyl group was completed.
The reaction solution was washed with diethylether (2 x 15 ml). The solution was neutralized with 25% aqueous amonia, concentrated to a small volume and applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form, 1.5 x 5 cm). The column was eluted with water and all UV-positive eluate, were collected, concentrated to a small volume and lyophilized from D_2O to give **Z2** as white solid. Yield 0.28 g (81%), Rf = 0.42.
Compound **Z2**, ^1H NMR (D_2O): δ = 8.20, s, 1H, H-8; 8.10, s, 1H, H-2; 5.60, t, J = 5.6 Hz, 1H, CH = C; 4.20, d, $^3J_{H\text{-}P}$ = 8.3 Hz, 2H, CH_2-O-P; 4.16 d, J = 6.4 Hz, 2H, CH_2-N; 1.75, s, 3H, CH_3; ^{13}C NMR (D_2O): δ = 161.5, s, C_6; 155.9, s, C_2; 152.1, s, C_4; 145.1, s, C_8; 138.9, d, $^3J_{C\text{-}P}$ = 9.2 Hz, CH_3-C = C; 122.3, s, CH = C; 118.6, s, C_5; 69.9, br s, CH_2-O-P; 39.8, s, CH_2-N, 14.4, s, CH_3; ^{31}P NMR (D_2O): δp = 2.15 ppm.

Trans-zeatin methylphosphate **Z3**
To solution A (see above) dry methanol (0.32 ml, 8 mmol) was added and this solution was left for 2 h at 20°C. Then solution was concentrated to a small volume and applied to a column of Sephadex LH 20. The column was eluted with (CH_2Cl_2/CH_3OH, 2:1, v/v). The fractions, containing product **8**[15] [Rf = 0 (system B), Rf = 0.67 (system C)] were pooled and concentrated to an oil. After dissolving the oil in water (20 ml), the pH was adjusted to 2 with an aqueous hydrogen chloride solution (0.1 N). After 3 h at 20°C TLC analysis (system C) showed that the cleavage of the tetrahydro-2-pyranyl group was complete. The reaction solution was washed with diethylether (2 x 15 ml). The solution was neutralized with 25% aqueous ammonia, concentrated to a small volume and applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form. 1.5 x 5 cm).
The column was eluted with water and all UV-positive eluates, were collected, concentrated to small volume and lyophilized from D_2O to give **Z3** as white solid. Yield 0.25 g (75%), Rf =

0.59 (system C).
Compound **Z3**, ^{1}H NMR (D_2O): δ = 8.12, s, 1H, H-8; 8.06, s, 1H, H-2; 5.68, t, J=5.5 Hz, 1H, CH = C; 4.30, d, $^3J_{H-P}$ = 7Hz, 2H, CH_2-O-P; 4.13, d, J = 5.8 Hz, 2H, CH_2-N; 3.55, d, $^3J_{H-P}$ = 10.7 Hz, 3H, CH_3-O-P; 1.78, s, 3H, CH_3; ^{13}C NMR (D_2O): δ = 155.3, s, C_6; 154,2, s, C_2; 152,7, s, C_4; 142.9, s, C_8; 137.8, d, $^3J_{C-P}$ = 5.5 Hz, CH_3-C = C; 123.9, s, CH = C; 118.1, s, C_5; 72.0, br s, CH_2-O-P; 54.6, br s, CH_3O-P; 40.4, s, CH_2-N; 14.0, s, CH_3; ^{31}P NMR (D_2O): δp = 2.10 ppm.

Trans-zeatin thiophosphate **Z4**
Crude **5** (synthesized from 1 mmol of **3**) was coevaporated with dioxane and dissolved in dioxane (10 ml). The solution was treated with N, N-diisopropylethylamine (0.35 mmol, 2 mmol) and N-O-bis (trimethylsilyl) acetamide (0.5 ml, 2 mmol). After 15 min powdered sulfur (1.0 g, 4 mmol) was added. TLC analysis (system C) revealed that it requires 16 h to complete the conversion of phosphonate **5**. Water was added and excess of sulfur was removed by filtration. The resulting solution was evaporated and dissolved in water and applied to a column of DEAE-Sephadex A 25 (HCO_3^--form), suspended in triethylammonium bicarbonate buffer (0.05 M).
The column was eluted with a linear gradient of triethylammonium bicarbonate buffer (0.05 → 0.7 M) for 18 h with a flow rate of 35 ml/h. Fractions of 10 ml were collected and all UV-positive eluates, containing product **9**[16], Rf = 0 (system B), Rf = 0.48 (system C), were pooled. They were concentrated to a small volumn, coevaporated with water (4 x 50 ml) to remove most of triethylammonium bicarbonate and lyophilized from H_2O. The residue was dissolved in water (20 ml) and the pH was adjusted to 2 by the addition of aqueous hydrogen chloride (0.1 N). After 3 h at 20°C, TLC analysis (system C) showed that the cleavage of the tetrahydro-2-pyranylgroup was complete.
The reaction solution was washed with diethylether (2 x 15 ml). The solution was neutralized with 25% aqueous ammonia, concentrated to a small volume and applied to a column of Dowex 50 Wx-8 cation -exchange resin (Na^+-form, 1.5 x 5 cm).
The column was eluted with water and all UV-positive eluates were collected, concentrated to a small volume and lyophilized from D_2O to give **Z4** as white solid. Yield 0.3 g (83%), Rf = 0.40 (system C).
Compound **Z4**, ^{1}H NMR (D_2O): δ 8.20, s, 1H, H-8; 8.10, s, 1H, H-2; 5.62, t, J= 6Hz, 1H, CH=C; 4.20, d, $^3J_{H-P}$ = 8Hz, 2H, CH_2-O-P; 4.15, d, J = 6 Hz, 2H, CH_2-N; 1.75, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 162.9, s, C_6; 154.7, s, C_2; 152.5, s, C_4; 147.1, s, C_8; 137.9, d, $^3J_{C-P}$=11.1 Hz, CH_3-C=C; 122.7, s, CH=C; 117.4, s, C_5; 70.0, d, $^2J_{C-P}$ = 11.1 Hz, CH_2-O-P; 39.4, s, CH_2-N; 14.0, s, CH_3; ^{31}P-NMR(D_2O): δp=40.00 ppm.

Trans-zeatin phosphonate **Z5**
Na^+-salt of **5** (0.33 g, 0.84 mmol) was dissolved in water 20 ml and the pH was adjusted to 2 with HCL (0.1 N). After 3 h at 20°C TLC analysis (system C) showed that the cleavage of the tetrahydro-2-pyranyl group was complete.
The reaction solution was washed with diethylether (2 x 15 ml). The solution was neutralized with aqueous ammonia (25%) and concentrated to a small volume, then applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form, 1.5 x 5 cm). The column was eluted with water and all UV-positive eluates were collected, concentrated to a small volume and lyophilized from D_2O to give **Z5** as white solid. Yield 0.25 g (82%), Rf = 0.7 (system C).
Compound **Z5**, ^{1}H NMR (D_2O): δ 8.28, s, 1H, H-8; 8.19, s, 1H, H-2; 6.76, d, $^1J_{H-P}$ = 636 Hz, 1H, P-H; 5.70, t, J = 7.2 Hz, 1H, CH = C; 4.31, d, $^3J_{H-P}$ = 9 Hz, 2H, CH_2-O-P; 4.24, d, J = 6.0 Hz, 2H, CH_2-N; 1.8, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 152.2, s, C_6; 149.0, s, C_2; 148.2, s, C_4; 139.4, s, C_8; 137.2, d, $^3J_{C-P}$ = 12.94, CH_3-C = C; 121.5, CH = C; 119.2, s, C_5; 70.5, br s, CH_2-O-P; 40.4, CH_2-N; 14.0, s, CH_3; ^{31}P NMR (D_2O): δp = 6.70 ppm, ($^1J_{H-P}$ = 633 Hz).

References and notes

1. D.S. Letham, J.S. Shannon and I.R. Mc Donald, *Proc. Chem. Soc.*, **1964**, *230*.
2. G. Shaw, B.M. Smallwood and D.V. Witson. *Experientia* **1967**, *23*, 515.
3. L.L. Danilov, V.N. Shibaev and N.K. Kochetkov, *Synthesis* **1984**, 404.
4. B.R. Shadid, H.C. van der Plas, E. de Vroom, G.A. van der Marel and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1987**,*106*, 509.
5. C.R. Vonk, E. Davelaar, S.A. Ribot, B.R. Shadid and H.C. van der Plas, *Plant Growth Regulation*, in press.
6 M. Ohsugi, I. Ichimoto and H. Ueda, *Agr. Biol. Chem.*, **1974**, *38* (10), 1925.
7. R.K. Robins, E.F. Godefroi, E.C. Taylor and A. Jackson, *J. Amer. Chem. Soc.*, **1961**, *83*, 2574.
8. Y.E. Sutcliffe and R.K. Robins, *J. Org. Chem.*, **1963**, *28*, 1662.

9. T. Hata and M. Sekine. *Tetrahedron Letters*, **1974**, 3943.
10. N.J. Leonard, A.J. Playthis, F. Skoog and R.Y. Schmitz, *J. Amer. Chem. Soc.* **1971**, *93*, 3056.
11 C.C. Duke and J.K. MacLeod, *Aust. J. Chem.*, **1978**, *31*, 2219.
12. ^{13}C-NMR-Spektroskopie. Hans-Otto Kalinowski, Stefan Berger, Siegmar Braun. *Georg Thieme Verlag Stuttgart/New York*, **1984**, p. 534-535.
13. J.E. Marugg, M. Tromp, E. Kuyl-Yeheskiely, G.A. van der Marel and J.H. van Boom. *Tetrahedron Letters*, **1986**, *27*, 2661. 2-Chloro-4H-1,3,2-benzodioxaphosphorin-4-one (2) is now commercially available from Aldrich.
14. ^{1}H NMR of compound **7** (Na^+-salt)(D_2O): δ= 8.00, s, 1H, H-8; 7.90, s, 1H-H-2; 5.50, t, J = 7.20 Hz, 1H, CH = C; 5.30, m, 1H, 1H(THP); 4.2, d, J = 8Hz, 2H, CH_2-O-P; 3.8, m, 3H, CH_2-N and 1H (THP); 3.60, m, 2H, 2H(THP); 2.10 – 1.61, m, 5H, 5H(THP); 1.76, s, 3H, CH_3; ^{31}P NMR (D_2O): δp = 1.80 ppm.
15. ^{1}H NMR of compound **8** (Na^+-salt)(D_2O) : δ = 8.05, s, 1H, H-8; 8.02, s, 1H, H-2; 5.55, t, J = 7.8 Hz, 1H, CH = C; 5.40, m, 1H, 1H(THP); 4.20, d, J = 5.8 Hz, 2H, CH_2-O-P; 4.15, m, 3H, CH_2-N and 1H(THP); 3.70, m, 2H, 2H(THP); 3.50, d, $^{3}J_{H\text{-}P}$ = 10 Hz, 3H, OCH_3; 2.00 – 1.40, m, 5H, 5H(THP); 1.7, s, 3H, CH_3; ^{31}P-NMR (D_2O) : δ_p = 2.50 ppm.
16. ^{1}H NMR of compound **9** (Na^+-salt) (D_2O): δ = 8.03, s, 1H, H-8; 8.00, s, 1H, H-2; 5.40, t, J = 6 Hz, 1H, CH = C; 5.20, m, 1H, 1H(THP); 4.20, d, J = 6.2 Hz, 4H, CH_2-N and CH_2-O-P; 4.00 - 3.80, m, 3H, 3H(THP); 2.20 – 1.50, m, 5H, 5H(THP); 1.70, s, 3H, CH_3; ^{31}P NMR (D_2O): δp = 45.00 ppm.