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A CONVENIENT NEW SYNTHESIS OF THE ALLYLIC PYROPHOSPHATE OF TRANS-ZEATIN

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Abstract: Allylic phosphonate of *trans*-zeatin was converted into the corresponding phosphoromorpholidate, which on treatment with the mono-(tri-n-butylammonium) phosphate gave the allylic pyrophosphate of *trans*-zeatin **1c**.

INTRODUCTION

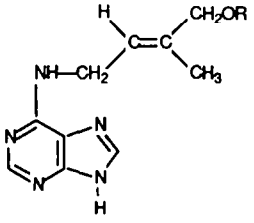
Zeatin, the highly active stimulant of cell division in plant tissue cultures, was first isolated from zeamays, and has the structure 6-(4-hydroxy-3-methyl-E-but-2-enylamino) purine **1a**[1-5]; both E- and Z- isomers have been synthesized [6-10].

In a quite recent paper[11] the unique importance of a new zeatin metabolite i.e. **1b** has been established in plants. The chemical preparation of this metabolite has been reported[12].

The synthesis of the allylic pyrophosphate of *trans*-zeatin **1c** however, has not been published yet. We now wish to report on the synthesis of this allylic pyrophosphate **1c.** This synthetic study is 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.

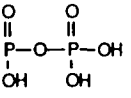
Difficulties encountered in the synthesis of allylic pyrophosphates and subsequent purification can be traced to the fact that the pyrophosphate residues are superb leaving groups, especially when they are protonated[13]. Up to now there are two procedures for the synthesis of allylic pyrophosphate. The first procedure was reported in 1959[14] and has not been altered significantly since then. This one-pot sequence involves treatment of a mixture of an allylic alcohol and inorganic pyrophosphate with trichloroacetonitrile to generate a complex mixture of organic and inorganic mono-, di-, and triphosphates. Yield of the desired products rarely exceeds 30% and further losses are usually encountered during purification. In addition, the procedure becomes difficult to manage if more than 50 mg of the products are desired[15]. The second procedure, which was described by Poulter et al.[15] is based on activation of an allylic alcohol by conversion into an allylic halide. The activated intermediates are treated with the tris-(tetra-n-butylammonium) hydrogen pyrophosphate to obtain the allylic pyrophosphate ester. Yields vary between 34-80%, depending on the structure of the allylic alcohol. In order to examine the

last possibility for the preparation of the allylic pyrophosphate of *trans*-zeatin attemps were made to halogenate the allylic hydroxy group in *trans*-zeatin **1a** by using either phosphorus tribromide, or N-chlorosuccinimide[15]. Both attempts gave rise to the formation of a lot of coloured products. It occurred to us that the above-mentioned problems inherent in the synthesis of allylic pyrophosphate of *trans*-zeatin could be circumvented by using the methodology which was originally devised by Khorana et al[16]. for the preparation of nucleoside-5'-pyrophosphate and which is based on the reaction between nucleoside-5'-phosphoromorpholidates and bis-(tri-n-butylammonium) phosphate. In this paper this approach will be described.



(**1a**) R = H

(**1b**) R = 

(**1c**) R = 

RESULTS AND DISCUSSION

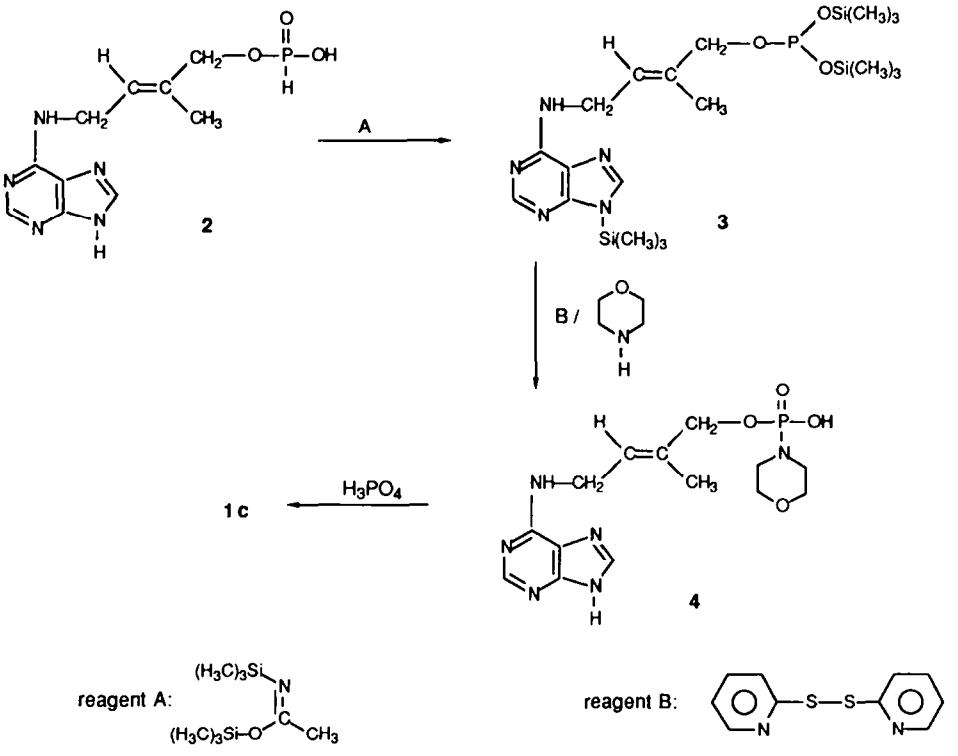
The strategy we have adopted in order to examine this possibility consisted of the following steps (see scheme 1): i. the synthesis of allylic phosphoromorpholidate of *trans*-zeatin i.e. **4**; ii. the conversion of **4** into *trans*-zeatin pyrophosphate **1c**.

The synthesis of allylic phosphoromorpholidate **4** was performed according to a slight modification of the procedure of Hata et al.[17]. Thus, allylic phosphonate of *trans*-zeatin **2** (being prepared by a procedure described before)[12] in acetonitrile was treated with N-O-bis-(trimethylsilyl) acetamide in the presence of N,N-diisopropylethylamine to give intermediate bis-(trimethylsilyl) phosphite **3**. Attemps to isolate **3** were unsuccessful. Without further purification **3** was treated with 2,2'-dipyridyldisulfide and the reaction solution was then treated with an excess of dry morpholine to give the allylic phosphoromorpholidate of *trans*-zeation **4** as identified by ^{1}H NMR, ^{13}C NMR and ^{31}P NMR[18].

The conversion of **4** into the allylic pyrophosphate **1c** was succesfully effected by treatment of a solution of **4** in dimethylformamide with mono-(tri-n-butylammonium) phosphate for 18 h at 45° C. After work-up and purification the allylic pyrophosphate **1c** was obtained in good yield (70%). The identity of *trans*-zeatin allylic pyrophosphate was ascertained by ^{1}H NMR, ^{13}C NMR

and ^{31}P NMR-spectroscopy (see experimental part).

The ^{1}H NMR and ^{13}C NMR-data of this compound are almost identical with those of *trans*-zeatin **1a** [10,19,21] with exception of the ^{1}H-absorption of CH_2-O, which in zeatin is found as singlet at 3.8 ppm., and in zeatin pyrophosphate **1c** as doublet at about 4.34 ppm., caused by the coupling of ^{1}H with $^{31}P(^{3}J_{H\text{-}P})$.



Scheme 1

In the ^{13}C NMR-spectra, the difference between zeatin **1a** and zeatin pyrophosphate **1c** is obvious in the absorption of $CH_3\underline{C}$=C. The ^{13}C singlet of $CH_3\underline{C}$=C in **1a** becomes a doublet in the zeatin pyrophosphate. The doublet is caused by the coupling of ^{13}C with $^{31}P(^{3}J_{C\text{-}P})$. Proton decoupled ^{31}P NMR-spectroscopy revealed the presence of two doublets, one doublet at -10.10 ppm., (J=20.2 Hz) and another doublet at -7.00ppm., (J= 20.2 Hz). The doublets are caused by the coupling of ^{31}P with ^{31}P. The ^{31}P chemical shifts and to a lesser extent ^{31}P-^{31}P coupling constants are dependent on pH, counterion and concentration[15].

From these results it can be unequivocally concluded that above mentioned procedure is a fast and simple method for the preparation of allylic pyrophosphate of zeatin. The reaction proceeded smoothly and the pure compound **1c** was isolated in a satisfactory yield.

EXPERIMENTAL PART

General procedures

N,N-diisopropylethylamine, acetonitrile and toluene were dried by refluxing with CaH_2 for 16 h and then distilled. Dimethylformamide was dried by stirring overnight at room temperature with CaH_2 and then distilled under reduced pressure; morpholine was distilled from sodium. All liquids were stored under nitrogen. N,O-bis-(trimethylsilyl) acetamide and 2,2'-dipyridyldisulfide were purchased form Janssen Chemica (Belgium). Mono-(tri-n-butylammonium) phosphate was prepared as described previously[16]. Triethylammonium bicarbonate 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 the solution became neutral. Scheicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC. The following solvent systems were used:
System A (chloroform/methanol, 80:20,v/v) and System B (isopropyl alcohol/concentrated ammonium hydroxide/water, 7:1:2, v/v). Short-column chromatography was performed on silanized silicagel RP 18 (Merck) 70-230 mesh ASTM. The column was eluted with water applying a methanol gradient (0→50%), unless otherwise mentioned. DEAE Sephadex A25 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, 1.5 x 5 cm) followed by washing of the column with sterile water until pH=7. ^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-spectrometer, chemical shifts are in ppm. relative to 85% H_3PO_4 as external standard.

Synthesis of *trans*-zeatin pyrophosphate **1c**

First the Na^+-salt of *trans*-zeatin phosphonate **2**[12](0.20 g, 0.5 mmol) was repeatedly coevaporated with acetonitrile (4x10 ml), the residue was diluted with acetonitrile (5 ml) and this mixture was treated with N,N-diisopropylethylamine (0.35 ml, 2 mmol) and N,O-bis-(trimethylsilyl) acetamide (0.5 ml, 2 mmol) and reacted for 15 min at 20°C. Then the solution was treated with 2,2'-dipyridyldisulfide (130 mg, 0.6 mmol). After 1 h at 20°C TLC analysis system B showed complete absence of **2**. Then an excess of morpholine was added (1 ml). After 18 h at 20°C TLC (system B) showed the formation of **4**[18] (Rf=0.85). Water was added (1 ml) and the reaction solution was left for 10 min at 20°C. The reaction solution was concentrated to a small volume and coevaporated with toluene (4x10 ml). The latter product was dissolved in dimethylformamide (5 ml) and a solution of mono-(tri-n-butylammonium) phosphate[16] in dimethylformamide (0.5M, 5 ml) was added. The reaction mixture was rendered anhydrous by repeated coevaporation with toluene (3x10 ml) and left at 45°C; after 18 h TLC (system B) showed the complete conversion of **4** into **1c**. The reaction mixture was treated with tri-n-butylamine until the pH had become 8 and concentrated to a small volume (1 ml) and applied to a column of DEAE Sephadex A25 (HCO_3-form) suspended in triethylammonium bicarbonate buffer (0.05 M). The column was eluted with

the same buffer (linear gradient 0.05→1.0 M) for 18 h with a flow rate of 35 ml/h. Fractions of 10 ml were collected and the UV-positive eluates containing only **1c**, [Rf = 0.3, (system B)] were pooled. They were concentrated to a small volume, coevaporated with water (4x50 ml) and lyophilized from H_2O. The residue was dissolved in water (1 ml) and applied to a column of Dowex 50 Wx-8 cation-exchange resin (Na^+-form, 1.5x5 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 **1c** as white solid. Yield 155 mg (70% based on **2**), Rf=0.3 (system B).
Compound **1c** ^1H NMR (D_2O): δ 8.17, s, 1H, H-8; 8.06, s, 1H, H-2; 5.72, t, J=7.2 Hz, 1H, CH=C; 4.34, d, $^3J_{P-H}$=6.9 Hz, 2H, CH_2-O-P; 4.12, d, J=6.4 Hz, 2H, CH_2-N; 1.76, s, 3H,CH_3; ^{13}C NMR (D_2O) : 154.1, s, C_6; 153.2, s, C_2; 150.9, s, C_4; 141.5, s, C_8; 137.3, d, $^3J_{C-P}$=5.5 Hz, CH_3-C=C;122.8, s,CH=C;116.9, s, C_5; 70.5, s, CH_2-O-P; 39.4, s, CH_2-N; 14.0, s, CH_3; ^{31}P NMR (D_2O): δ_P=-10.10, d, J=20.2 Hz, P(1);δ_P=-7.00, d, J=20.2 Hz, P(2).

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18. Compound **4** was identified after purification by using RP 18; the purified compound was

converted into its sodium salt by passing it over Dowex 50 Wx-8 (Na^+-form) (see General Procedures). Compound **4**, ^1H NMR (D_2O): δ 8.15, s, 1H, H-8; 8.06, s, 1H, H-2; 5.68, t, J=6.8 Hz, 1H, CH=C; 4.22, d, $^3J_{H-P}$=7.4 Hz, 2H, CH_2-O-P; 4.16, d, J=6.0 Hz, 2H, CH_2-N; 3.50, m, 4H, morpholine; 2.85, m, 4H, morpholine; 1.77, s, 3H, CH_3; ^{13}C NMR (D_2O): δ 154.7, s, C_6; 153.6, s, C_2, 141.6, s, C_4; 137.3, d, $^3J_{C-P}$=6 Hz, CH_3-C=C; 122.8, s, CH=C; 120.7, s, C_5, 70.2, s, CH_2-O-P; 67.7, d, $^3J_{C-P}$=6.0 Hz, morpholine CH_2-O-CH_2; 45.5, s, morpholine CH_2-N-CH_2; 39.1, s, CH_2-NH; 14.00, s, CH_3; ^{31}P NMR (D_2O): δp=9.2 ppm.

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