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A Novel Method for Synthesis of *cis*-Zeatin and Its Valuable Precursor *(Z)*-4-Chloro-2-methyl-but-2-en-1-ol

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Cytokinins are one of the major groups of plant hormones. The vast majority of naturally occurring cytokinins are derivatives of zeatin and isopentenyladenine. Zeatin, 2-methyl-4-(7H-purin-6-ylamino)but-2-en-1-ol, occurs as two stereoisomers, *E* and *Z*, referring to the position of the terminal hydroxyl group on the isoprenoid side chain. Both isomers play key roles in plant development and occur ubiquitously in the plant kingdom.[1] However, whereas the chemistry of (*E*)-zeatin is well-developed and the compound is synthetically available by several methods,[2–4] the synthesis of pure (*Z*)-zeatin is problematic and in all cases generates low yield. These problems stem from difficulties connected with the synthesis and isolation of pure (*Z*)-4-amino-2-methylbut-2-en-1-ol (**4a**). To the best of our knowledge, only five methods have so far been described for the synthesis of this amino alcohol. Three of them[5–7] have been reported by Tolman[8] to be unreliable or unsuitable, because they yield a mixture of difficult isomers or only small amounts of the desired compound **4a**. Tolman further re-examined the synthesis described by Evidente[9] and found that this five-step reaction pathway gives good results, but it is very time- and labor-intensive with many scaling-up problems. Therefore, in an attempt to obtain better results, Tolman[8] modified one of the previous methods[5] based on a Diels–Alder strategy (*Scheme 1*, but note that benzyl was used originally as a protecting group instead of *tert*-butyl). The Tolman group used metastable nitrosoformates prepared by *in situ* oxidation from benzyl N-hydroxycarbamate in reaction with isoprene. The reaction product was a mixture of benzyl 5(and 4)-methyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylates in a total yield of 48% and a ratio of 30:70. Thus only a low proportion of the required isomer was formed. The Tolman

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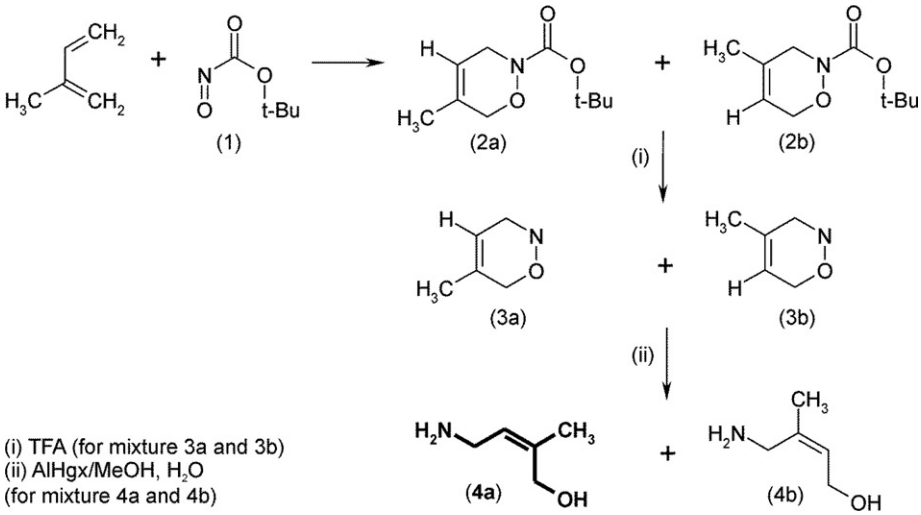
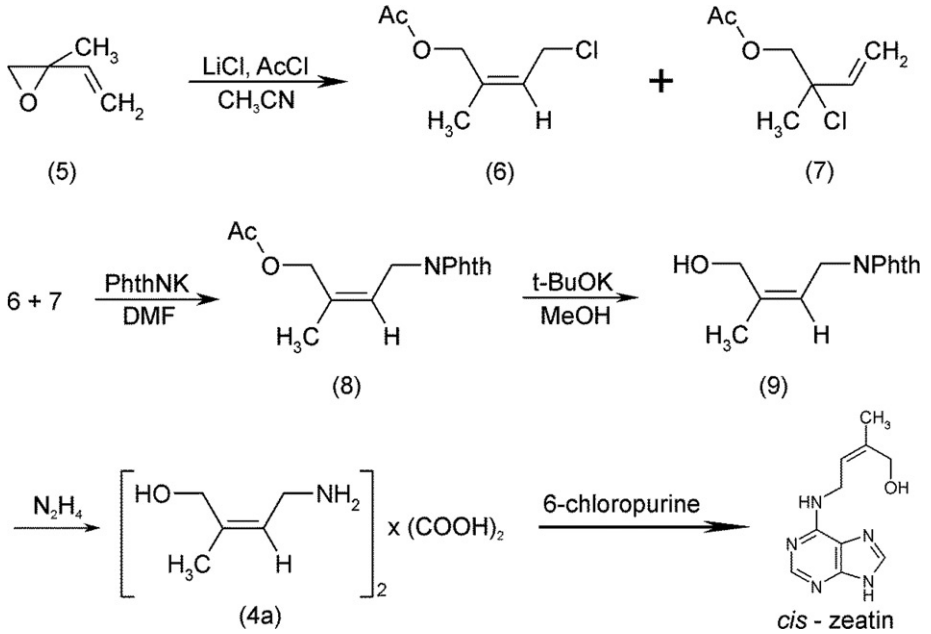


Scheme 1. Synthesis of (*Z*)-4-amino-2-methylbut-2-en-1-ol (**4a**) based on a Diels–Alder strategy according to Tolman.[8]

group then used (*Scheme 1*) the bulkier *tert*-butyl N-hydroxycarbamate (**1**) in the same reaction and obtained a mixture of *tert*-butyl 5(and 4)-methyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylates (**2a** and **2b**) in a yield of 52% and ratio of 55:45. This gave rise to a little more than 50% of the required isomer, an improvement. They further removed the ester groups from both isomers and without purification reduced the prepared 5(and 4)-methyl-3,6-dihydro-2H-1,2-oxazines (**3a** and **3b**) to the corresponding amino alcohols (**4a** and **4b**). The amino alcohols were subsequently separated by chromatography into **4a** and (*Z*)-4-amino-3-methylbut-2-en-1-ol (**4b**) with yields of 24% and 26%, respectively. The amino alcohol **4a** was then converted into a hemioxalate. By this method, Tolman[8] prepared amino alcohol **4a** *via* a three-step synthesis with separation by one column chromatography to give a total yield of ca. 10%.

Owing to the still rather low yield of compound **4a**, J. Hanuš, a former colleague of Tolman, developed a new method for the synthesis of **4a** in pure *Z*-form (*Scheme 2*).

Importantly, J. Hanuš had thus uncovered a convenient synthesis of *(Z)*-1-acetoxy-3-methylbut-2-enylchloride (**6**). The (*E*)-form of this chloroalcohol serves as a precursor for the chemical synthesis of (*E*)-4-hydroxydimethylallyl diphosphate[10,11] (HDMAPP). In the non-mevalonate (MEP) pathway HDMAPP is the last precursor of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) from which all the isoprenoids are synthesized. The alternative pathway for IPP and DMAPP is the mevalonic pathway.[12] Most importantly, the enzymes of the MEP pathway are essential for numerous pathogenic bacteria including Enterobacteria, *Mycobacterium tuberculosis* and *Plasmodium spp*.[13] These enzymes are excellent targets for the development of antimicrobial agents because there are no known human orthologs of them.[14] Therefore, (*E*)-1-acetoxy-3-methylbut-2-enyl chloride and HDMAPP and its derivatives have been extensively synthesized in the past decades; at first, this was for studying the enzymes of the MEP pathway and later for the synthesis of potential antimicrobial drugs.[15,16] Thus, *(Z)*-1-Acetoxy-3-methylbut-2-enylchloride (**6**) and its corresponding amine are very valuable building blocks for the synthesis of new isoprenoid compounds and of potential antibacterial and anti-parasitic agents.



Scheme 2. Synthesis of *cis*-zeatin from 2-methyl-2-vinyloxirane.

In our own synthesis, commercially available 2-methyl-2-vinyloxirane (**5**) was selected as the starting material. According to Yoo[17] cleavage of **5** with acetyl chloride gives a mixture of 1-acetoxychlorides (compounds **6** and **7**) in a ratio of 3:2 and in 60% yield. It was further indicated that opening of the oxirane ring proceeds by a *trans*-mechanism to generate the *E*-form of **6**, but evidence of this was not presented.[17] In our hands, the yield of **6** and **7** together was 86%, but the major product **6** was, surprisingly, only (*Z*)-1-acetoxy-3-methylbut-2-enylchloride (**6**), which demonstrates the opening of the oxirane to its (*Z*)-isomer.

In our synthesis, it was further expected that compounds **6** and **7** would react differently with potassium phthalimide (PhthNK). Compound **7** could react by eliminating HCl to form phthalimide, KCl and the de-halogenated product 1-acetoxy-2-methylbutadiene, which could dissolve in nonpolar solvents. On the other hand, **6** could react to form the expected phthalimide **8**. Indeed, 1-acetoxy-2-methylbutadiene generated from **7** gradually separated out during processing of the reaction mixture. The yield of crystallized phthalimide **8** (HPLC purity 96%) was 37% (based on the sum of the products **6** and **7**). To evaluate the reaction of the rearranged chloride **6**, the product formed after reaction with PhthNK (**8**) was compared with N-[(*E*)-3-acetoxy-2-butenyl]phthalimide, which was used as a standard for HPLC and was synthesized according to Mornet and Gouin.[4] The dominant product peak of **8** (retention time (Rt) = 24.0 min) had a retention time close to that of the standard (Rt = 22.5 min), but it was nonetheless distinctly different. For the phthalimido derivative **8**, the presence of the (*E*)-form was not shown in our sample. This demonstrates stereoselective rearrangement at the opening of oxirane **5** by acetyl chloride.

Alcoholysis of the acetoxy group of **8** generated the hydroxy derivative **9**. This derivative **9** was again compared to its (*E*)-form by HPLC. The (*Z*)-form and (*E*)-form

had Rt = 9.3 min and 8.2 min, respectively. N-[(*E*)-4-Hydroxy-3-methylbut-2-enyl]phthalimide was prepared from its acetylated precursor[4] by the method described herein for alcoholysis. The ^{1}H NMR spectrum of **9** had not been previously reported. We now provide it (see Experimental Section). Hydrazinolysis of **9** then yielded the corresponding amine, which, after processing, was precipitated as the hemioxalate **4a**. After crystallization, the pure hemioxalate **4a** was obtained in four-steps and the total yield was 17%. The reaction of **4a** with 6-chloropurine generated *cis*-zeatin identical to the material described by Tolman.[8] *cis*-Zeatin was characterized by ^{1}H NMR and melting point analysis. Its HPLC purity was 99.5% (with only 0.15% *trans*-zeatin).

Our result is consistent with that of Socolsky and Plietker,[18] who mentioned this reaction in supplementary material in their paper. They used the same reaction as us and obtained (*Z*)-1-acetoxy-3-methylbut-2-enyl chloride (**6**), compound **7** and one another chloride in a ratio 2.7:1.4:1 in 64% yield. However, the authors neither commented on the discovery nor described the significance of the reaction and the product. The only alternative synthesis of **6** in (*Z*)-form had been described previously.[19] Cereda[19] reacted acetoxyacetone with an unstabilized ylide, which was obtained from the dialkylamino phosphonium salt and potassium hexamethyldisilazide. Interestingly, the (*E*)-form of **6** is also synthesized by the opening of the 2-methyl-2-vinyloxirane ring, but in the presence of titanium tetrachloride. This is the most successful method for the synthesis of **6** in the (*E*)-form.[6] The (*E*)-geometry of the chloroalcohol is preferred (97:3).

In sum, we report here the stereoselective rearrangement of 2-methyl-2-vinyloxirane by acetyl chloride in the presence of lithium chloride and use of this reaction for the synthesis of (*Z*)-4-hydroxy-3-methyl-2-but-2-enyl amine, a side chain of cytokinin *cis*-zeatin. The synthesis comprises a simple and efficient procedure of five synthetic steps without the need for chromatographic separation of isomeric by-products, which is expected to facilitate scaling up the synthesis of *cis*-zeatin. Importantly, this method includes synthesis of (*Z*)-1-acetoxy-3-methylbut-2-enyl chloride, which may serve as a significant building block in the synthesis of new isoprenoid compounds, including antimicrobial drugs. To the best of our knowledge, this report is the first one highlighting the importance of the convenient synthesis of **6** and **4a** in (*Z*)-forms from 2-methyl-2-vinyloxirane.

Experimental Section

Solvents and reagents were of at least reagent grade. Melting points were determined on a Büchi-SMP 20 instrument and were uncorrected. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60 WF254 plates (230-400 mesh) and using the solvents indicated in the individual procedures below. HPLC analyses were performed on a reverse-phase RP Select B column (2 x 150 mm, 4μm, Merck) using a methanol - buffer mobile phase (40 mM CH_3COONH_4 buffer, pH 3.4). A gradient from 20:80 to 35:65 over 15 min and then to 50:50 over 10 min was used. NMR spectra were measured on a Varian VXR-400 or Inova-400 spectrometer (400 MHz) at 25 °C. Samples were prepared by dissolving the compounds in DMSO-d_6 or D_2O and tetramethylsilane (TMS) was used as an internal standard. The NMR spectra of **9** and its (*E*)-form were submitted for review by the editors and are available from the corresponding author upon request.

N-[(Z)-4-Acetoxy-3-methylbut-2-enyl]phthalimide (8)

2-Methyl-2-vinyloxirane (**5**, 22.2 g, 264 mmol) and dry LiCl (11.23 g, 265 mmol) were suspended in dry acetonitrile (85 mL) at -5 to 0 °C and 1.5 equivalent (28.2 mL, 396 mmol) of acetyl chloride was slowly added. The suspension was stirred for 30 min at 0 °C and then refluxed for 2 h. After cooling, water (170 mL) was added and then the mixture was extracted with diethyl ether (180 and 2 x 130 mL). The combined organic extract was washed with water (130 mL) followed by portions of saturated sodium bicarbonate solution (80 and 120 mL) and brine (40 mL) and then dried over anhydrous $MgSO_4$. After filtration and evaporation, 37 g (86%) of a mixture of chlorides **6** and **7** was isolated (compare with Yoo[17]).

The oily product was dissolved in DMF (130 mL) and dry PhthNK (37 g, ca. 200 mmol) and NaBr (1.5 g, 14.5 mmol) were added. The mixture was warmed to 45 °C for 1 h and then to 60 °C for 3 h. Afterwards, the mixture was poured into cooled water (500 mL) and immediately slightly acidified with conc. HCl. The resulting solution, with almost neutral pH, was placed in a refrigerator. Crystals formed, were filtered, washed with water and then dried in the air, then in a desiccator (41.9 g of impure product). The dry crystals were extracted with benzene (200 mL) and the insoluble phthalimide was removed. After evaporation of the solvent, the crude product (27 g) was purified by extraction in n-heptane (150 mL, 80-85 °C, 30 min). After cooling, the oily product (undissolved) yielded crystals that were filtered off, producing 22.2 g of the expected product **8** (m.p. 74-77 °C). HPLC analysis showed that the product had about 91% purity; the principal impurity was phthalimide (9%). The crystals were again extracted with n-heptane (2 x 150 mL). After cooling in a refrigerator, 20.2 g of (**8**) was obtained (m.p. 77-80 °C; HPLC ~ 96%, only 4% of phthalimide was found). The only available work[6] reported m.p. 89-90 °C. However, this work has been found unreliable by Tolman.[8] The authors of the same work[6] also stated that the ^{1}H NMR spectrum of **9** was very complex and was not interpreted.

N-[(Z)-4-Hydroxy-3-methylbut-2-enyl]phthalimide (9)

Compound **8** (20.2 g, 74 mmol) was dissolved in absolute methanol (200 mL, 60 °C), t-BuOK (1 g, 8.9 mmol) was added and the mixture was stirred overnight at r. t. under an inert atmosphere. TLC analysis (chloroform-ethyl acetate, 2:1, v:v) showed the presence of several percent unreacted starting material. Another portion (0.2 g) of *t*-BuOK was added and mixing was continued for another 3 h. Dowex 50 (H + in methanol, 10 mL) resin was added and the mixture was stirred for 2 h. Afterwards, the Dowex resin was filtered off and the solution was evaporated to dryness. The crystalline compound obtained was dissolved in hot benzene (70 mL) and n-hexane (50 mL) was added. The mixture was left in a refrigerator overnight and crystals (11.6 g) were then isolated. Benzene-hexane from the mother liquor was evaporated off and the residue was extracted with boiling n-hexane (3 x 150 mL). All together, 15.6 g of the crude product **9** (92%) was isolated. HPLC analysis (Rt = 9.3 min) showed only the presence of product **9** and phthalimide (max. 8%, Rt = 5.0 min). The product (after column chromatography and crystallization from n-heptane) showed a m.p. of 91-93 °C. Corse and Kuhnle[6] reported a different m.p. (109-111 °C). ^{1}H NMR (DMSO-d^6): δ 1.67 (3H, s, CH_3); 4.07 (2H, d, $J = 5.6$ Hz, CH_2OH); 4.20 (2H, d, $J = 6.8$ Hz, CH_2N); 4.70 (1H, t,

$J = 5.6$ Hz, OH) 5.21 (1H, t, $J = 6.8$ Hz, CH=), 7.82 (4H, m, ArH). The crude product (with m.p. 86-90 °C) was used in the next reaction.

(Z)-4-Hydroxy-3-methyl-2-but-2-enylamine hemioxalate (4a)

Compound **9** (15.2 g, 65 mmol) was dissolved in 80% ethanol (80 mL, 50 °C), then cooled, hydrate (Caution! Corrosive! 3.35 mL, 80 mmol) was added and the mixture was stirred at r. t. overnight before being heated to 60 °C (2 h). The reaction mixture was cooled to r. t., H_2SO_4 (35 ml, 2 M) was added and the mixture was stirred for 30 min. After cooling in a refrigerator, the phthalhydrazide deposit was filtered off. The filtrate was neutralized using NH_4OH. The resulting brownish oil was treated with methanol saturated with NH_4OH (20 ml), then mixed, chloroform (20 ml) was added and the solution was placed in a refrigerator overnight. Precipitated $(NH_4)_2SO_4$ was filtered off and the filtrate was washed 2x with absolute ethanol. A brown dense oil (7.3 g) was obtained. Oxalic acid (8.2 g, 65 mmol) was dissolved in water (80 mL) and slowly added to the crude product. The hemioxalate was immediately formed and, after being placed in a refrigerator for a few hours, the crystallized hemioxalate was filtered off. Additional hemioxalate was obtained by concentration of the solvent volume to about half. The resulting crystals were washed with ethanol and diethyl ether and dried at r. t. The hemioxalate (6.7 g, 45 mmol) was isolated and TLC analysis (chloroform-methanol-conc. NH_4OH, 6:4:0.4, v:v:v) showed one single spot. A sample of the product was re-crystallized from EtOH-water (10:1), m.p. 182-182.5 °C. Tolman[8] found m.p. 168–170 °C (dec.). ^{1}H NMR (D_2O): δ 1.84 (3H, dt, $J = 1.1$ Hz, 0.75 Hz, CH_3C=); 3.68 (2H, dq, $J = 7.5$ Hz, 1.1 Hz, CH_2N); 4.15 (2H, d, $J = 0.75$ Hz, CH_2OH); 5.46 (1H, ttq, $J = 7.5$ Hz, 1.1 Hz, 0.75 Hz, CH=).

cis-Zeatin

Hemioxalate (**4a**, 2.92 g, 20 mmol) obtained from the previous step was treated in n-propanol (80 mL) with 6-chloropurine (2.8 g, 18.1 mmol) and triethylamine (6.8 mL, 49 mmol). The mixture was heated under argon to 98 °C for 6 h. After placing the mixture overnight in a refrigerator, yellow crystals were filtered off, then washed with n-propanol (2 x 15 mL) and diethyl ether (10 mL). After drying in a desiccator, 3.2 g of crude product was isolated. The product was purified by heating with n-propanol (30 mL) to give a yield of 2.9 g (66%, based on the starting hemioxalate) of yellowish *cis*-zeatin, m.p. 213-214 °C (m.p. in ref.5,206–208 °C; in ref.9,212–214°C; in ref.8,214–215°C). ^{1}H-NMR (DMSO-d_6): δ 1.70 (3H, bs, =C-CH_3); 4.04 (2H, d, $J = 5.2$ Hz, CH_2OH); 4.13 (2H, m, NCH_2); 4.76 (1H, t, $J = 5.2$ Hz, OH), 5.35 (1H, t, $J = 6.8$ Hz, =CH-), 7.56 (1H, bs, NH), 8.06 and 8.16 (1H each, s, purine protons). HPLC: 99.5% of *cis*-zeatin, containing 0.15% of trans-zeatin.

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