Purines, Pyrimidines, and Imidazoles. Part XXIV.* Syntheses of Zeatin, a Naturally Occurring Adenine Derivative with Plant Cell-division-promoting Activity, and its 9-β-D-Ribofuranoside

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6-(4-Hydroxy-3-methylbut-*trans*-2-enylamino)purine (zeatin) a naturally occurring kinetin-like cell-division-promoting factor and its 9-β-D-ribofuranosyl derivative have been synthesised by reaction of *trans*-4-amino-2-methylbut-2-en-1-ol with 6-methylthiopurine (or 6-chloropurine) and 6-chloro-9-β-D-ribofuranosylpurine, respectively. The aminobutenol was prepared by reaction of methyl γ-bromotiglate with sodium azide and reduction of the resulting methyl γ-azidotiglate with lithium aluminium hydride. Some derivatives of the aminobutenol and zeatin, and the ozonisation of zeatin to methylglyoxal, are described.

A FACTOR capable of inducing cell-division in carrot phloem explants and resembling the synthetic compound kinetin (I) was first isolated in crystalline form from immature sweet corn (*Zea mays*) kernels and named zeatin.[1] The compound, which is considerably more active than kinetin in growth tests, has also been detected in plum fruitlets and sunflower seeds and is probably of widespread occurrence.[2,3] From chemical, spectroscopic, and enzymic evidence zeatin was formulated as a geometrical isomer of either 6-(4-hydroxy-3-methylbut-2-enylamino)- or 6-(4-hydroxy-2-methylbut-2-enylamino)-purine.[4] We now report an unambiguous synthesis of the *trans* form (II) of the first of these compounds, which proves to be identical with zeatin. A preliminary account of our initial experiments has been recorded.[5]

Reaction of methyl γ-bromotiglate[6] (the *trans* structure of which has been confirmed by its use in a synthesis of crocetin[7]) with sodium azide in hot methyl cyanide gave the γ-azidotiglate (III). Reduction of this with lithium aluminium hydride in ether for 1 hour gave the corresponding aminobutenol (IV), which was readily converted into a crystalline hemisulphate and bisacyl

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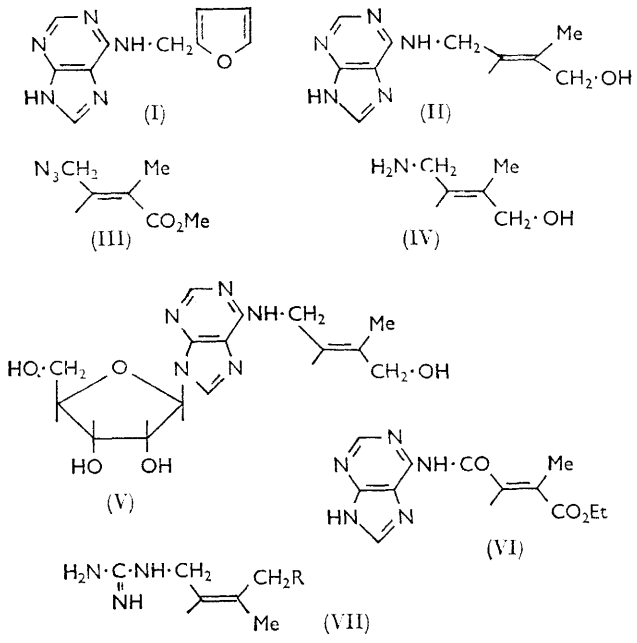
derivatives. Reaction of the crude amine with 6-methylthiopurine (chosen initially in place of the more reactive 6-chloropurine to avoid overacidity which might have isomerised an allylic system) at 134° overnight gave zeatin (II) which was isolated as the cystalline picrate



and converted into the free base by careful treatment of an aqueous solution with a Dowex 1 × 8 (OH^-) resin or, better, by conversion into the hydrochloride, reaction of this with sodium hydroxide, and subsequent ethanol extraction of the free base after evaporation. Zeatin was, however, obtained in much better yield (88%) by reaction of the pure aminobutenol (IV) sulphate with 6-chloropurine and triethylamine in hot n-butanol for 1 hour and crystallised directly from the reaction mixture. It readily gave a crystalline mono-*O*-acetate, and ozonisation in acetic acid gave methylglyoxal which was isolated as the bis-2,4-dinitrophenylhydrazone, so providing additional confirmation of the position of the exocyclic double bond.

Natural and synthetic zeatin were shown to be identical by comparison of melting points and mixed melting points of the bases and of derived picrates and 3-iodo-2,4,6-trinitrophenates, by paper and thin-layer chromatography, by comparison of proton magnetic resonance, mass, and ultraviolet absorption spectra, and by comparison of cell-division-promoting activity.[4,8]

In a similar manner reaction of the aminobutenol (IV) with 6-chloro-9-β-D-ribofuranosylpurine [9] and triethylamine in butanol readily gave the crystalline zeatin riboside (V) in excellent yield. This may be identical with a naturally occurring zeatin glycoside which gives zeatin and ribose on hydrolysis and was recently detected in *Zea mays* var. Golden Cross Bantam.[10] Suggestions have been made that methods used to isolate zeatin, especially the use of resins such as Dowex 50 (H^+), would lead to hydrolysis of nucleoside and nucleotide derivatives and that zeatin may not therefore be a native substance.[11] However, subsequent isolation and detection of zeatin using mild extraction procedures have confirmed the natural occurrence of the base [10] but it seems likely that several derivatives of the base in addition to the nucleoside will ultimately be isolated from various plant sources.

An early attempt to prepare the aminobutenol (IV) by reduction of 3-carbamoyl-2-methylprop-2-enyl acetate led to the dihydro-derivative.[12] In our hands, a similar attempt to prepare zeatin by reduction of the acyl adenine (VI), prepared by acylation of adenine with 3-ethoxycarbonyl-2-methylbut-*cis*-2-enoyl chloride,[13] resulted mainly in hydrogenolysis, since adenine was the main reaction product. Since our preliminary report appeared, an alternative route to compound (IV) has been described, *viz.*, oxidation of *N*-(γγ-dimethylallyl)phthalimide with selenium dioxide and hydrolysis of the product.[14] The amine was used in an attempt to synthesise 4-hydroxygalegin (VII; R = OH) the only naturally occurring compound other than zeatin known to contain the γ-hydroxymethyl-γ-methylallyl group attached to nitrogen. However, the synthetic compound was not identical with 4-hydroxygalegin and it is suggested that, in this case, the natural product has the *cis* configuration (VII; R = OH) and this has been confirmed by proton magnetic resonance spectra.[14] The two naturally occurring compounds, galegin (VII; R = H) and hydroxygalegin (VII; R = OH) provide an interesting parallel analogy with zeatin and the corresponding 6-*N*-(γγ-dimethylallylamino)purine which has been synthesised recently.[15] The latter compound has been reported to be some ten times more active than kinetin in the tobacco bioassay,[16] and zeatin, which in recent tests has shown measurable growth responses in concentrations as low as 5×10^{-11}M, appears to be even more active, suggesting that in this series of compounds the exocyclic $CH_2{\cdot}CH{:}C{\cdot}C_2$ group, which occurs in the more active compounds, is an important prerequisite for enhanced biological activity.

We have repeated the preparation of the aminobutenol (IV) by the foregoing method [14] and shown that the compound is identical with material obtained by our method and that it gives zeatin after condensation with 6-chloropurine.

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EXPERIMENTAL

Ultraviolet spectra were measured on a Perkin-Elmer 137UV recording spectrophotometer, and infrared spectra on a Perkin-Elmer 237 spectrophotometer, the potassium bromide disc technique being used. Evaporations were carried out in a Buchi rotary evaporator, under water-pump vacuum, with a flask temperature of 40° or less.

Methyl γ-Azidotiglate.—A mixture of methyl γ-bromotiglate [6] (63 g.), sodium azide (105 g.), and methyl cyanide (250 ml.) was boiled under reflux for 2½ hr. The cooled mixture was filtered and the filtrate evaporated to an oil which was distilled *in vacuo* to give the *azide* (30 g.), b. p. 65—68°/2 mm. (Found: C, 46·25; H, 5·7; N, 22·25. $C_6H_9N_3O_2$ requires C, 46·45; H, 5·8; N, 27·1%), $\nu_{max.}$ 2100 cm.$^{-1}$. The analysis corresponds to a product about 82% pure.

trans-4-*Amino*-2-*methylbut*-2-*en*-1-*ol*.—A solution of the foregoing azide (5·5 g.) in ether (200 ml.) was added slowly with stirring to lithium aluminium hydride (2·2 g.) suspended in ether (300 ml.). The mixture was set aside for 1 hr. then water (12 ml.) added dropwise with stirring. After 30 min. the mixture was filtered and the dried (Na_2SO_4) filtrate evaporated to an oil (3 g.). A suspension of the crude amine in water was treated with N-sulphuric acid to pH 3 and the solution evaporated to dryness. The residue was evaporated with ethanol then rubbed with ether to give a gummy solid. This was dissolved in hot ethanol to give after cooling, a crystalline precipitate. The amine sulphate (1·5 g.) recrystallised from ethanol as needles, m. p. 108—110° (lit.,[14] 108—109°) (Found: C, 39·85; H, 7·95; N, 8·8; S, 10·1. Calc. for $(C_5H_{11}NO)_2,H_2SO_4$: C, 40·0; H, 8·05; N, 9·35; S, 10·65%).

The foregoing crude amine in aqueous sodium hydroxide with 2·1 mol. equivalents of *p*-chlorobenzoyl chloride gave the *di*-p-*chlorobenzoyl derivative* which crystallised from ethanol as needles, m. p. 117—119° (Found: C, 60·25; H, 4·65; Cl, 18·6; N, 3·6. $C_{19}H_{17}Cl_2NO_3$ requires C, 60·3; H, 4·5; Cl, 18·8; N, 3·7%). The *di*-p-*nitrobenzoate* was similarly prepared, and separated from ethanol as needles, m. p. 163—166° (Found: C, 57·25; H, 4·3; N, 10·35. $C_{19}H_{17}N_3O_7$ requires C, 57·1; H, 4·25; N, 10·55%).

Zeatin.—(*a*) A mixture of the foregoing amine sulphate (0·25 g.), 6-chloropurine (0·257 g.), triethylamine (0·5 ml.), and n-butanol (3 ml.) was boiled under reflux for 1 hr. Evaporation of the solution to a small volume gave, on cooling, a crystalline precipitate. 6-(4-*Hydroxy*-3-*methylbut*-trans-2-*enylamino*)*purine* (zeatin) (0·32 g., 88%) recrystallised from water as needles, m. p. 207—208° (Found: C, 54·75; H, 6·1; N, 32·15. $C_{10}H_{13}N_5O$ requires C, 54·8; H, 5·95; N, 31·95%). It had R_F 0·78 in butanol–acetic acid–water (12 : 3 : 5), R_F 0·45 in butanol saturated with 1·5N-hydrochloric acid, R_F 0·78 in propan-2-ol–water (7 : 3) and ammonia solution (0·35 ml.; *d* 0·88), and R_F 0·66 in water saturated with n-butanol. In addition it had $\lambda_{max.}$ 207 (ε 14,500) and 275 mμ (ε 14,650) and $\lambda_{min.}$ 235 mμ in 0·1N-hydrochloric acid; $\lambda_{max.}$ 212 (ε 17,050) and 270 mμ (ε 16,150) and $\lambda_{min.}$ 233 mμ at pH 7·2; $\lambda_{max.}$ 220 (ε 15,900) and 276 mμ (ε 14,650), $\lambda_{min.}$ 242 mμ in 0·1N-sodium hydroxide. The principal infrared spectral peaks were at 792m, 846m, 900m, 938m, 988m, 1000w, 1048w, 1074m–s, 1092s, 1132w, 1145s, 1160w, 1226m, 1278m, 1304vs, 1348s, 1380m, 1393s, 1440m, 1471w, 1510m, 1543m, 1590s, 1600s, 1645vs, 1655vs, 1813w, 2832m, 2887m, 3050s, and 3308s cm.$^{-1}$. The last four peaks were superimposed on a broad band 3650—2500 cm.$^{-1}$. The same material was similarly obtained in slightly less yield by condensation of the foregoing crude amine with 6-chloropurine.

(*b*) 6-Methylthiopurine (0·25 g.) and the foregoing crude amine (0·65 g.) were heated together at 130—140° for 16 hr. in a sealed tube. The product was treated with water (15 ml.) and 2N-hydrochloric acid (2 ml.) and the mixture filtered. The filtrate with aqueous picric acid gave a yellow precipitate. This was collected and washed with acetone. The residual *zeatin picrate* (0·2 g.) recrystallised from acetone as yellow needles, m. p. 187—188° (Found: C, 42·85; H, 4·1; N, 24·85. $C_{16}H_{16}N_8O_8$ requires C, 42·8; H, 3·55; N, 25·0%). It had $\lambda_{max.}$ 269 mμ in ethanol. Hydrogen chloride was passed through a suspension of the picrate (0·208 g.) in acetone (15 ml.) until the yellow solid was replaced by a white precipitate. Ether (25 ml.) was added and the solid (0·14 g.) collected and washed with ether. The hydrochloride (0·125 g.) in water (25 ml.) was adjusted to pH 7 with N-sodium hydroxide and the solution evaporated to dryness. The residue was extracted with hot ethanol and the extract evaporated until crystals appeared. Zeatin (0·073 g.) was obtained and, after recrystallisation from water, had m. p. and mixed m. p. 207—208°.

6-(4-*Acetoxy*-3-*methylbut*-trans-2-*enylamino*)*purine* (*Mono*-O-*acetylzeatin*).—A mixture of zeatin (0·1 g.), acetic anhydride (0·2 ml.) and pyridine (5 ml.) was shaken for 30 min. to give a clear solution. This was set aside overnight, evaporated, and the residue evaporated several times with ethanol to leave a solid residue. *Mono*-O-*acetylzeatin* (0·06 g.) recrystallised from water as needles, m. p. 168—169° (Found: C, 55·4; H, 5·95; N, 27·0. $C_{12}H_{15}N_5O_2$ requires C, 55·15; H, 5·75; N, 26·8%). The *O*-acylation was confirmed by strong bands in the infrared spectrum at 1740 and 1240 cm.$^{-1}$.

Ozonolysis of Zeatin.—Ozonised oxygen (from the Towers ozoniser operating at 7500 v with a flow rate of about 500 ml. of gas per min.) was passed through a solution of zeatin (0·07 g.) in acetic acid (10 ml.) for 5 min. Water and a little zinc dust were added to the solution and products volatile in steam collected in a solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid. A yellow precipitate, m. p. 300—302° (decomp.), was obtained which, after crystallisation from nitrobenzene, was identical (mixed m. p., paper chromatography, and comparison of infrared spectra) with the bis-2,4-dinitrophenylhydrazone of methylglyoxal.

6-(4-*Hydroxy*-3-*methylbut*-trans-2-*enylamino*)-9-β-D-*ribofuranosylpurine* (9-β-D-*Ribofuranosylzeatin*).—A mixture of the foregoing amine sulphate (0·085 g.), 6-chloro-9-β-D-ribofuranosylpurine (0·143 g.), triethylamine (0·25 ml.), and n-butanol (4 ml.) was boiled under reflux for 1 hr. The solution was evaporated to about half volume and cooled, when crystals separated. The *riboside* (0·08 g.) recrystallised from methanol in small clusters of needles, m. p. 180—182° (Found: C, 51·75; H, 5·9; N, 19·65. $C_{15}H_{21}N_5O_5$ requires C, 51·3; H, 6·0; N, 19·95%). A further quantity (0·04 g.) of riboside was recovered from the mother-liquors. It had R_F 0·69 in butanol–acetic acid–water (12 : 3 : 5) and R_F 0·82 in water saturated with n-butanol. In addition it had $\lambda_{max.}$ 207—209 (ε 19,800) and 266 mμ (ε 18,550), $\lambda_{min.}$ 235 mμ in 0·1N-hydrochloric acid; $\lambda_{max.}$ 210—212 (ε 19,300) and 269—271 mμ (ε 17,800),

$\lambda_{min.}$ 232—234 mμ at pH 7·2 (M/30 phosphate buffer); $\lambda_{max.}$ 215 (ε 18,050) and 269—271 mμ (ε 18,300), $\lambda_{min.}$ 235 mμ in 0·1N-sodium hydroxide.

6-N-(3-*Ethoxycarbonylbut*-cis-2-*enoyl*)*adenine*.—A solution of ethyl 3-chlorocarbonyl-2-methylprop-*cis*-2-enoate (1·05 ml.) in ether was added slowly to a stirred solution of adenine (0·67 g.) in 2N-sodium hydroxide (5 ml.). After 30 min. at room temperature the organic layer was removed and discarded. A solid precipitate in the aqueous layer was collected, washed with water, and dissolved in hot ethanol (*ca.* 180 ml.). The solution was filtered and cooled to give a precipitate of the *acyl adenine* (0·3 g.) as needles, m. p. 166° (Found: C, 52·4; H, 4·8; N, 25·5. $C_{12}H_{12}N_5O_3$ requires C, 52·4; H, 4·75; N, 25·45%).

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