REGULATORS OF CELL DIVISION IN PLANT TISSUES

VII.* THE SYNTHESIS OF ZEATIN AND RELATED 6-SUBSTITUTED PURINES

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Summary

6-(4-Hydroxy-3-methylbut-*trans*-2-enylamino)purine (zeatin), a cytokinin isolated from *Zea mays*, has been synthesized by a new route. β-Methylcrotononitrile was brominated with *N*-bromosuccinimide yielding γ-bromo-β-methylcrotononitrile, from which *trans*-γ-acetoxy-β-methylcrotononitrile was prepared. The alcohol derived from this acetate was converted into *trans*-β-methyl-γ-(tetrahydropyran-2-yloxy)crotononitrile. Reduction and acid hydrolysis gave 4-amino-2-methylbut-*trans*-2-en-1-ol which was made to react with 6-chloropurine to yield zeatin.

Several γ-alkoxy-β-methylcrotononitriles were prepared and reduced by aluminium chloride–lithium aluminium hydride to the corresponding unsaturated amines. Saturated nitrile formation also occurred in these reductions. The amines prepared were condensed with 6-chloropurine to yield a series of *O*-alkylzeatins. A number of other zeatin analogues were also synthesized. Two 6-methoxyalkylaminopurines were cleaved by sodium borohydride in the presence of iodine to 6-hydroxyalkylaminopurines.

INTRODUCTION

Cytokinins, a type of phytohormone, have been detected by bioassay methods in extracts of many plant species.[1] Cytokinins also occur as minor bases in certain transfer ribonucleic acids.[1] At extremely low concentrations, cytokinins induce cell division in certain plant tissue cultures and in addition cause many other growth and physiological responses.[1] The first naturally occurring cytokinin to be isolated from a plant tissue was termed zeatin,[2] and was identified as 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine[3] (I). Zeatin induces cell division in excised carrot-phloem tissue at concentrations less than 5×10^{-11}M, and, as a stimulant of cell division in plant tissue cultures, zeatin is more effective than any other known compound.[4,5]

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[1] Letham, D. S., *A. Rev. Pl. Physiol.*, 1967, **18**, 349.

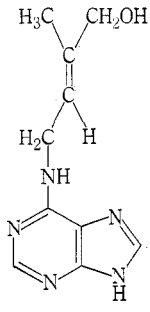
[2] Letham, D. S., *Phytochemistry*, 1966, **5**, 269.

[3] Letham, D. S., Shannon, J. S., and McDonald, I. R. C., *Tetrahedron*, 1967, **23**, 479.

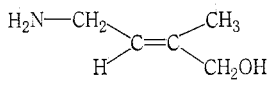
[4] Letham, D. S., *Planta*, 1967, **74**, 228.

[5] Skoog, F., Hamzi, H. Q., Szweykowska, A. M., Leonard, N. J., Carraway, K. L., Fujii, T., Helgeson, J. P., and Loeppky, R. N., *Phytochemistry*, 1967, **6**, 1169.

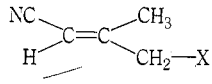
Zeatin has been synthesized by Shaw *et al.*[6] Methyl γ-bromotiglate was treated with sodium azide to yield methyl γ-azidotiglate. This was reduced to 4-amino-2-methylbut-*trans*-2-en-1-ol (II) which was then condensed with either 6-methylthiopurine or 6-chloropurine to yield zeatin. An alternative synthesis of zeatin and the preparation of certain zeatin analogues are detailed in the present communication. A preliminary brief account of some aspects of this work has been published previously.[7]



(I)



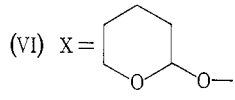
(II)



(III) X = Br (VII) X = OCH_3

(IV) X = OAc (VIII) X = $OCH_2CH_2CH_3$

(V) X = OH (IX) X = $O(CH_2)_3CH_3$



(VI) X =

Results and Discussion

4-Amino-2-methylbut-*trans*-2-en-1-ol (II) was first prepared from β-methylcrotononitrile and then condensed with 6-chloropurine to yield zeatin. Bromination of β-methylcrotononitrile with *N*-bromosuccinimide in the presence of benzoyl peroxide as free-radical initiator yielded γ-bromo-β-methylcrotononitrile, which was hydrolysed by alkali to the known compound *trans*-γ-hydroxy-β-methylcrotonic acid.[8] Since the n.m.r. spectrum of the nitrile exhibited two signals for each type of proton present, the product was a mixture of the *cis* and *trans* isomers. In α,β-unsaturated nitriles, a β group *cis* to the nitrile group would be expected to experience a larger deshielding effect than one *trans* to the nitrile group. Hence the lower-field methyl signal and the higher-field methylene signal were assigned to the *trans* isomer (III). The spectral integral indicated that the *trans* to *cis* isomer ratio was approximately 2 : 1 and that the lower-field olefinic proton signal was given by the *trans* isomer. The assignment of stereochemistry to other γ-substituted β-methylcrotononitriles mentioned later in this section was also based on n.m.r. data and the reasoning outlined above. Such reasoning is supported by the very limited n.m.r. data in the literature for α,β-unsaturated nitriles of known configuration.[9,10] In the γ-bromo-β-methylcrotononitriles and in the series of γ-alkoxy-β-methylcrotononitriles detailed in the present paper, the coupling of the methyl allylic protons with the α olefinic proton consistently followed the trend often observed in olefinic systems, namely that cisoid allylic coupling is larger than transoid.[11] Coupling between the methylene

[6] Shaw, G., Smallwood, B. M., and Wilson, D. V., *J. chem. Soc.* (*C*), 1966, 921.

[7] Cebalo, T., and Letham, D. S., *Nature*, 1967, **213**, 86.

[8] Halmos, V. M., and Mohacsi, T., *J. prakt. Chem.*, 1960, **12**, 50.

[9] Reddy, G. S., Goldstein, J. H., and Mandell, L., *J. Am. chem. Soc.*, 1961, **83**, 1300.

[10] Bullock, E., and Gregory, B., *Can. J. Chem.*, 1965, **43**, 332.

[11] Emsley, J. W., Feeney, J., and Sutcliffe, L. H., "High Resolution Nuclear Magnetic Resonance Spectroscopy." Vol. 2, p. 735. (Pergamon Press: Oxford 1966.)

allylic protons and the olefinic proton also followed this trend. However, such coupling differences are not sufficiently reliable for assignment of configuration.[11]

When treated with potassium acetate in acetic acid solution, γ-bromo-β-methylcrotononitrile (*cis–trans* mixture) yielded an acetate. The n.m.r. spectrum indicated this was *trans*-γ-acetoxy-β-methylcrotononitrile (IV) containing only a very small amount of *cis* isomer as impurity. The nitrile (IV) was also obtained in much lower yield by oxidation of 3-methylcrotononitrile with selenium dioxide in acetic anhydride. When hydrolysed with dilute potassium hydroxide, (IV) yielded *trans*-γ-hydroxy-β-methylcrotononitrile (V). An attempt to convert (III) directly into (V) by alkaline hydrolysis was unsuccessful. Reaction of (V) with 2,3-dihydropyran in the presence of *p*-toluenesulphonic acid yielded the acetal *trans*-β-methyl-γ-(tetrahydropyran-2-yloxy)crotononitrile (VI) which was reduced by lithium aluminium hydride in ether at 0° to the corresponding unsaturated amine. Some saturated amine formation accompanied the reduction. Hydrolysis of the crude amine with dilute acid at room temperature yielded 4-amino-2-methylbut-*trans*-2-en-1-ol which was allowed to react with 6-chloropurine to give a product identical with authentic zeatin. The structure already assigned to zeatin was thus confirmed. Reduction of (VI) with an excess of lithium aluminium hydride in ether (volume less than a third of that used in previous reduction) at room temperature or with a slight excess of hydride in refluxing tetrahydrofuran, followed by acid hydrolysis, yielded principally the saturated amine, 4-amino-2-methylbutan-1-ol. This amine, previously obtained as the picrolonate by Schreiber *et al.*[12] by reduction of 4-acetoxy-3-methylbut-2-enamide, was also prepared by reduction of (IV) with lithium aluminium hydride. Condensation of the amine with 6-chloropurine yielded 6-(4-hydroxy-3-methylbutylamino)purine, a cytokinin recently isolated from lupin seeds and synthesized by catalytic hydrogenation of zeatin.[13,14]

The synthesis of zeatin devised by Shaw *et al.*[6] was repeated to compare it with the procedure outlined above. The procedure of Shaw *et al.* requires a more expensive starting compound (methyl tiglate), but fewer steps are involved. The overall yield of (II) was the same in both procedures. If only a small quantity of product is required, the method of Shaw *et al.* is probably the one of choice.

An attempt to synthesize zeatin from 6-(3-methylbut-2-enylamino)purine by allylic oxidation with selenium dioxide in acetic anhydride–acetic acid followed by alkaline hydrolysis was unsuccessful. 6-(3-Methylbut-2-enylamino)purine occurs as a minor base in certain transfer ribonucleic acids.[1] This purine exhibits high cytokinin activity, but is less active than zeatin, and has been prepared by condensation of 6-chloropurine with 3-methylbut-2-enylamine.[4] An alternative synthesis was attempted. Adenine was made to react with β-methylcrotonoyl chloride at low temperature to give 6-*N*-(3-methylbut-2-enoyl)adenine. However, reduction of this amide at room temperature in tetrahydrofuran with lithium aluminium hydride or with aluminium chloride–lithium aluminium hydride (an equimolar mixture) yielded adenine as the main product. Shaw *et al.*[6] have reported that reduction of 6-*N*-(3-ethoxycarbonylbut-*cis*-2-enoyl)adenine with lithium aluminium hydride also results in hydrogenolysis to adenine. Thus, although a number of 6-*N*-acyladenines have

[12] Schreiber, K., Pufahl, K., and Brauniger, H., *Liebigs Ann.*, 1964, **671**, 154.

[13] Koshimizu, K., Kusaki, T., Mitsui, T., and Matsubara, S., *Tetrahedron Lett.*, 1967, 1317.

[14] Koshimizu, K., Matsubara, S., Kusaki, T., and Mitsui, T., *Agric. biol. Chem.*, 1967, **31**, 795.

been reduced to 6-(substituted amino)purines by lithium aluminium hydride,[15,16] it appears that this reduction may not be possible if the amide is α,β-unsaturated. An attempt to effect allylic oxidation of 6-*N*-(3-methylbut-2-enoyl)adenine with selenious acid in dioxan resulted principally in degradation to adenine.

β-Methylcrotononitrile, the starting compound in the zeatin synthesis detailed in this paper, was prepared by condensing acetone with cyanoacetic acid in cyclohexylamine as solvent. Because of its low cost, cyclohexylamine was used in preference to piperidine, the solvent commonly used for this type of condensation.[17] Triethylamine was also tried as solvent in this condensation but poor yields resulted. Condensation of butan-2-one with cyanoacetic acid in cyclohexylamine yielded a product (b.p. 159–161°/760 mm) with physical properties identical to those recorded for *trans*-β-ethylcrotononitrile.[18] However, an n.m.r. spectrum indicated that the product obtained was a *cis–trans* (1 : 2) mixture. The purity of *trans*-β-ethylcrotononitrile previously prepared and the identity of the compound (b.p. 142–143°/765 mm) regarded as *cis*-β-ethylcrotononitrile[18,19] may therefore be questioned. Condensation of methoxyacetone with cyanoacetic acid in cyclohexylamine yielded a mixture of *trans*-γ-methoxy-β-methylcrotononitrile (VII), the anticipated product, and 3-cyano-2-methylpropanal, a nitrile previously obtained by hydrolysis of 3-cyano-2-methylprop-1-enyl acetate.[20] Condensation in piperidine as solvent yielded a similar mixture of products. Formation of 3-cyano-2-methylpropanal is attributed to isomerization of γ-methoxy-β-methylcrotononitrile to the corresponding enol ether and subsequent hydrolysis. When γ-alkoxy-β-methylcrotononitriles were heated with aqueous piperidine, the formation of 3-cyano-2-methylpropanal was demonstrated by infrared spectroscopy.

Condensation of γ-bromo-β-methylcrotononitrile with sodium or potassium alkoxides at −5° to 0° and careful fractionation of the products yielded *trans*-γ-methoxy-β-methylcrotononitrile (VII), *trans*-β-methyl-γ-propoxycrotononitrile (VIII), and *trans*-γ-butoxy-β-methylcrotononitrile (IX). The ethoxy homologue was obtained as a *cis–trans* mixture. Condensation of γ-bromo-β-methylcrotononitrile with sodium methoxide in refluxing dry methanol yielded a mixture of (VII) and 3-cyano-2-methylpropanal.

Reduction of the γ-alkoxy-β-methylcrotononitriles with lithium aluminium hydride was next investigated. Reduction of γ-methoxy-β-methylcrotononitrile (*cis–trans* mixture) with lithium aluminium hydride at room temperature gave polymeric compounds of high boiling point as the main product and also a low yield of a mixture of saturated and unsaturated amines. The principal amine appeared to be 4-methoxy-3-methylbutylamine, which was purified by fractional distillation. Reduction of β-ethylcrotononitrile also gave polymeric products in addition to the unsaturated amine, 3-methylpent-2-enylamine. Reductive polymerization of α,β-unsaturated nitriles by lithium aluminium hydride has been reported by at least one

[15] Baizer, M. M., Clark, J. R., Dub, M., and Loter, A., *J. org. Chem.*, 1956, **21**, 1276.
[16] Rothwell, K., and Wright, S. T. C., *Proc. R. Soc.* B, 1967, **167**, 202.
[17] Trakhtenberg, D. M., and Shemyakin, M. M., *J. gen. Chem. USSR*, 1943, **13**, 477.
[18] "Beilsteins Handbuch der Organischen Chemie." Drittes Ergänzungswerk, Vol. 2, p. 1326. (Springer-Verlag: Berlin 1961.)
[19] Bruylants, P., *Bull. Acad. r. Belg. Cl. Sci.*, 1931, **17**, 1008.
[20] Pino, P., Gaudiano, G., Cecchetti, M., and Piacenti, F., *Annali Chim.*, 1961, **51**, 785.

other investigator.[21] In an attempt to improve the yield of unsaturated amines in such reductions, the reduction of β-ethylcrotononitrile was carried out under various conditions. The best yield (47%) of pure 3-methylpent-2-enylamine was obtained by reduction with a mixture of aluminium chloride and lithium aluminium hydride (equimolar amounts) at about 0°. These conditions were then used for the reduction of (VII) and gave a low yield of the desired product, 4-methoxy-3-methylbut-*trans*-2-enylamine. A similar reduction procedure was used for the remaining γ-alkoxy-β-methylcrotononitriles yielding 3-methyl-4-propoxybut-*trans*-2-enylamine, 4-butoxy-3-methylbut-*trans*-2-enylamine, and 4-ethoxy-3-methylbut-2-enylamine (*cis–trans* mixture). In these reductions, it was surprising to find that saturated nitriles appeared to be formed in appreciable amounts as a side reaction. Three of these were isolated in a state of purity, namely, γ-methoxy-β-methylbutyronitrile, β-methyl-γ-propoxybutyronitrile, and γ-butoxy-β-methylbutyronitrile. The identity of the first of these was confirmed by frequency sweep n.m.r. spectra with double and triple irradiation. The formation of such products was unexpected, especially as reduction of β-ethylcrotononitrile under similar conditions did not result in appreciable formation of saturated nitrile. However, it is noteworthy that saturated amide formation has been observed in lithium aluminium hydride reductions of certain α,β-unsaturated amides;[22] reduction of β-methoxy-α,β-diphenylacrylonitrile yielded the saturated nitrile, α,β-diphenylpropionitrile.[23] The amines prepared as outlined above, and also some previously known unsaturated amines, were condensed with 6-chloropurine to yield zeatin analogues which are detailed in Table 1.

TABLE 1

MELTING POINTS AND ELEMENTARY ANALYSES FOR ZEATIN ANALOGUES [6-(SUBSTITUTED AMINO)PURINES]

Substituent(s) at Amino Group of 6-Aminopurine	M.P.	Formula	Found (%)				Calc. (%)			
			C	H	N	O	C	H	N	O
N-Allyl-*N*-methyl	208–209°	$C_9H_{11}N_5$	57·4	5·95	36·9		57·1	5·9	37·0	
Diallyl	164–165	$C_{11}H_{13}N_5$	61·7	6·3	32·4		61·4	6·1	32·5	
3-Methylpent-2-enyl*	192–193	$C_{11}H_{15}N_5$	60·9	7·3	32·2		60·8	7·0	32·2	
4-Methoxy-3-methylbutyl†	176–177	$C_{11}H_{17}N_5O$	56·4	7·4	29·5	6·75	56·15	7·3	29·8	6·8
4-Methoxy-3-methylbut-*trans*-2-enyl†	197–198	$C_{11}H_{15}N_5O$	56·85	6·6	29·95	—	56·6	6·5	30·0	
4-Ethoxy-3-methylbut-2-enyl‡	169–173	$C_{12}H_{17}N_5O$	58·0	7·1	28·1	6·6	58·3	6·9	28·3	6·5
3-Methyl-4-propoxybut-*trans*-2-enyl†	176–177	$C_{13}H_{19}N_5O$	59·6	7·3	27·0	6·3	59·75	7·3	26·8	6·1
4-Butoxy-3-methylbut-*trans*-2-enyl	154–155	$C_{14}H_{21}N_5O$	61·1	7·6	25·3	6·0	61·1	7·7	25·4	5·8
Diprop-2-ynyl§	171–172‖	$C_{11}H_9N_5$	62·5	4·5	33·0		62·55	4·3	33·2	
4-Hydroxy-3-methylbutyl†	164–165	$C_{10}H_{15}N_5O$	54·2	6·8	31·7		54·3	6·8	31·65	
4-Hydroxy-2-methylbut-*trans*-2-enyl	179–181	$C_{10}H_{13}N_5O$	54·95	6·1	31·9		54·8	6·0	31·9	
5-Hydroxypentyl	176–177	$C_{10}H_{15}N_5O$	54·1	6·9	31·5		54·3	6·8	31·7	
6-Hydroxyhexyl	175–176	$C_{11}H_{17}N_5O$	56·0	7·4	29·7		56·15	7·3	29·8	

* This compound is very probably the *trans* isomer. † The n.m.r. spectra of these compounds were determined and were consistent with the assigned structure. ‡ *cis–trans* mixture. § ν_{max} (mull) 2108 cm^{-1} (C≡CH). ‖ Decomposition.

6-(4-Hydroxy-2-methylbut-*trans*-2-enylamino)purine was prepared by a series of reactions analogous to those used by Shaw *et al.*[6] in their synthesis of zeatin. Methyl γ-bromo-β-methylcrotonate was synthesized as described by Halmos and

[21] Boyer, N. E., *Tech. Apsk.*, 1960, **23**, 8 (*Chem. Abstr.*, 1963, **58**, 10309).

[22] Snyder, H. R., and Putnam, R. E., *J. Am. chem. Soc.*, 1954, **76**, 1893.

[23] Reynaud, P., and Matti, J., *C. r. hebd. Séanc. Acad. Sci., Paris*, 1952, **235**, 1230.

Mohacsi[8] but the n.m.r. spectrum established that this product was a *cis–trans* mixture. Reaction with sodium azide yielded methyl *trans*-γ-azido-β-methylcrotonate. Reduction with lithium aluminium hydride yielded 4-amino-3-methylbut-*trans*-2-en-1-ol which on condensation with 6-chloropurine gave 6-(4-hydroxy-2-methylbut-*trans*-2-enylamino)purine (Table 1). An attempt to prepare the *cis* isomer of zeatin and zeatin riboside by a similar series of reactions starting with methyl γ-bromoangelate[24] was unsuccessful. Geometrical isomerization occurred at some stage during the reaction sequence as the products possessed the properties of zeatin and zeatin riboside. The attempted synthesis of 6-(4-hydroxybut-*trans*-2-enylamino)-purine by the same route starting with methyl *trans*-γ-bromocrotonate was also not successful. Reduction of the azido ester was accompanied by considerable saturation of the double bond and formation of a mixture of products difficult to separate by fractional distillation. Methyl γ-bromo-β-methylcrotonate (*cis–trans* mixture) was also treated with silver nitrite to give methyl β-methyl-γ-nitrocrotonate (*cis–trans* mixture) which was reduced to give 4-amino-3-methylbut-2-en-1-ol, but in much lower yield than obtained above.

A method for cleaving ethers at low temperature with diborane in the presence of a halogen has been devised by Long and Freeguard.[25] By a modification of this procedure, two 6-(methoxyalkylamino)purines, 6-(3-methoxypropylamino)purine and 6-(4-methoxy-3-methylbutylamino)purine, were cleaved to the corresponding 6-(hydroxyalkylamino)purines in good yield. An alternative synthetic route to the naturally occurring cytokinin, 6-(4-hydroxy-3-methylbutylamino)purine, was thus provided. An attempt to convert 6-(3-methyl-4-propoxybut-*trans*-2-enylamino)-purine into zeatin by this ether cleavage procedure was also made. Thin-layer chromatography of the crude reaction product indicated the presence of zeatin in low yield together with other unidentified compounds. Because of the low zeatin yield, the crude product was not further purified.

The biological activity of the zeatin analogues detailed in the present paper will be reported in a later communication in this series.

Experimental

Melting points are corrected. Infrared spectra were obtained with a Perkin–Elmer 237 spectrophotometer; liquids were examined as thin films while solids were studied either as paraffin mulls or KBr disks. The 60-Mc/s nuclear magnetic resonance spectra were obtained either with a Varian A60 or a Varian DP60 spectrometer, using tetramethylsilane as an internal standard. In brackets immediately following each chemical shift (τ value) is listed number of protons, peak multiplicity, coupling constant, and structural assignment, in that order, with use of the following abbreviations: s, singlet; s-b, singlet broadened by allylic coupling; d, doublet; t, triplet; m, multiplet.

All amines were chromatographed on Whatman No. 3MM paper as hydrochlorides in n-butanol saturated with water and in n-butanol–acetic acid–water (12 : 3 : 5 by volume) as solvents. To detect the amines, the developed chromatograms were sprayed with ninhydrin solution and then heated at 80° for 5–10 min. β,γ-Unsaturated amines were revealed as yellow spots which turned to purple when left at room temperature for about 24 hr. Saturated amines were revealed as purple spots after heating at 80°. β,γ-Unsaturated amines were also distinguished from saturated amines by their reaction to a permanganate spray (a 0·25% aqueous solution

[24] Korte, F., and Behner, O., *Chem. Ber.*, 1956, **89**, 2675.

[25] Long, L. H., and Freeguard, G. F., *Nature*, 1965, **207**, 403.

of potassium permanganate containing sodium carbonate (5 g/l.)); the unsaturated amines gave yellow spots while the saturated amines did not discolour the spray. Amine tetraphenylborates were converted into amine hydrochlorides for chromatography by stirring solutions in 10–20% ethanol with the anion-exchange resin De-Acidite FF (chloride form). Water solutions of chloroplatinates were similarly treated.

For separation of liquids with similar boiling points by fractional distillation, either a column packed with Dixon gauze or a Nester–Faust spinning-band column (23 theoretical plates) was used.

Ether for lithium aluminium hydride reductions was dried first with sodium wire and then distilled from lithium aluminium hydride directly into the reaction flask under anhydrous conditions.

All crystalline compounds were dried to constant weight at 60° and 2 mm pressure before elementary analysis. The boron content of amine tetraphenylborates was calculated from the u.v. absorption spectrum; ϵ for the tetraphenylborate ion was determined with a pure sample of 3-aminopropan-1-ol tetraphenylborate (purity checked by n.m.r. spectroscopy) using the same u.v. spectrophotometer. A similar method has been used for the determination of potassium tetraphenylborate.[26] All other elementary analyses were performed by A. Bernhardt, Max-Planck-Institut für Kohlenforschung, Mülheim, West Germany.

(*a*) *γ-Bromo-β-methylcrotononitrile*

(i) *Synthesis.*—*N*-Bromosuccinimide (319 g, 1·8 mole) and benzoyl peroxide (30 g, 0·12 mole) were added to a solution of β-methylcrotononitrile (203 g, 2·5 mole) in dry carbon tetrachloride (1·3 l.) and the resulting mixture was gently heated. This initiated a very vigorous exothermic reaction which was moderated, so that the solvent refluxed gently, by temporarily placing the reaction flask in an ice-bath. As the vigour of the initial reaction declined, the flask was further heated and the solvent refluxed for 2·5 hr. The succinimide formed was filtered off and washed with carbon tetrachloride. Evaporation of the combined carbon tetrachloride solutions under reduced pressure gave a liquid residue which was fractionally distilled in vacuum to yield *γ-bromo-β-methylcrotononitrile* (*cis–trans* mixture) (211 g, 73%), b.p. 50–54°/0·3 mm, n_D^{24} 1·5170 (Found: C, 37·4; H, 3·8; Br, 50·0; N, 8·7. C_5H_6BrN requires C, 37·5; H, 3·8; Br, 49·9; N, 8·75%). ν_{max} 2223 (nitrile), 1626 and 818 cm^{-1} (trisubstituted double bond). The n.m.r. spectrum (CCl_4) indicated that the product was a mixture of geometrical isomers, the *cis* to *trans* isomer ratio being approximately 1 : 2. Signals assigned to the *cis* isomer: τ 4·75 (1H, m, C=CHCN), 5·86 (2H, s, CH_2Br), and 7·91 (3H, d, *J* 1·5 c/s, CH_3). Signals assigned to the *trans* isomer (III): τ 4·53 (1H, m, C=CHCN), 6·05 (2H, d, *J* 0·7 c/s, CH_2Br), and 7·80 (3H, d, *J* 1·0 c/s, CH_3).

It should be emphasized that this bromonitrile is highly lachrymatory.

(ii) *Hydrolysis.*—The foregoing bromonitrile (8·5 g) was stirred with NaOH solution (2N, 94 ml) at 65–70° for 4 hr. The resulting aqueous solution was separated from an oil which formed during the reaction, neutralized, concentrated to 60 ml, filtered, and extracted with ether. The solution was then acidified (pH to 2·5) and re-extracted with ether (10 times with equal volume). Evaporation of the dried extracts yielded an oil (2·5 g) which would not crystallize and which co-chromatographed on paper with *trans*-γ-hydroxy-β-methylcrotonic acid.[8] The isolated acid was converted into the *S*-benzylisothiuronium salt which was recrystallized from water to give *S-benzylisothiuronium* trans-*γ-hydroxy-β-methylcrotonate*, m.p. and mixed m.p. 139° (Found: C, 55·2; H, 6·3; N, 9·9; O, 17·2; S, 11·5. $C_{13}H_{18}N_2O_3S$ requires C, 55·3; H, 6·4; N, 9·9; O, 17·0; S, 11·4%).

(*b*) trans-*γ-Acetoxy-β-methylcrotononitrile* (*IV*)

(i) *Synthesis from γ-bromo-β-methylcrotononitrile.*—A mixture of γ-bromo-β-methylcrotononitrile (130 g), glacial acetic acid (120 ml), and freshly fused potassium acetate (120 g) was heated and stirred under reflux for 1 hr, cooled, poured into water, and then extracted with ether. The combined extracts were washed successively with water, aqueous $NaHCO_3$ solution, and water. The residue obtained by evaporation of the dried (Na_2SO_4) ether extracts was distilled under vacuum, yielding trans-*γ-acetoxy-β-methylcrotononitrile* (74 g, 66%), b.p. 59–60°/0·08 mm,

[26] Pflaum, R. T., and Howick, L. C., *Analyt. Chem.*, 1956, **28**, 1542.

n_D^{21} 1·4565 (Found: C, 60·3; H, 6·5; N, 10·2; O, 23·2. $C_7H_9NO_2$ requires C, 60·4; H, 6·5; N, 10·1; O, 23·0%). ν_{max} 2223 (nitrile), 1750 and 1230 cm^{-1} (acetate); λ_{max} (ethanol) 210 mμ, ϵ 12930. N.m.r. spectrum ($CDCl_3$): τ 4·53 (1H, s-b, C=CHCN), 5·33 (2H, s-b, CH_2O), 7·85 and 7·90 (6H, two overlapping s, CH_3C=C, CH_3CO). A very weak signal at τ 5·13 was assigned to the methylene group of the *cis* isomer present as impurity.

(ii) *Synthesis from β-methylcrotononitrile.*—A stirred mixture of the nitrile (12 g), acetic anhydride (40 ml), and selenium dioxide (8·3 g) was heated under reflux for about 4 hr. The cooled reaction mixture was filtered onto crushed ice and the resulting aqueous solution extracted with ether. The extracts were washed successively with water, aqueous $NaHCO_3$ solution, and water. The dried (Na_2SO_4) extracts were concentrated and the residue fractionally distilled, yielding *trans-γ*-acetoxy-*β*-methylcrotononitrile (1·6 g, 8%), b.p. 70–72°/0·4 mm, n_D^{21} 1·4566 (Found: C, 60·2; H, 6·5; N, 9·9; O, 23·1. $C_7H_9NO_2$ requires C, 60·4; H, 6·5; N, 10·1; O, 23·0%). The i.r. and n.m.r. spectra were identical with those of (IV) prepared as previously described. Oxidation with selenium dioxide in acetic acid–acetic anhydride (1 : 4 by volume) for 1 hr gave a similar yield.

(*c*) trans-*γ-Hydroxy-β-methylcrotononitrile* (*V*)

trans-γ-Acetoxy-*β*-methylcrotononitrile (23 g) was stirred vigorously at 60° with potassium hydroxide solution (1N, 300 ml) until a homogeneous reaction mixture was obtained (about 4 min). The resulting aqueous solution was immediately cooled to 0°, saturated with NaCl, and extracted with ether. The residue obtained by evaporation of the dried (Na_2SO_4) ether extracts was vacuum distilled, yielding trans-*γ-hydroxy-β-methylcrotononitrile* (8·1 g, 51%), b.p. 74–75°/0·05 mm, n_D^{25} 1·4768 (Found: C, 61·8; H, 7·3; N, 14·25; O, 16·6. C_5H_7NO requires C, 61·8; H, 7·3; N, 14·4; O, 16·5%). ν_{max} 3400 and 1079 (hydroxyl), 2223 cm^{-1} (nitrile); λ_{max} (ethanol) 212 mμ, ϵ 12500. N.m.r. spectrum ($CDCl_3$): τ 4·33 (1H, s, C=CHCN), 5·70 (2H, s, CH_2OH), 6·62 (1H, s, OH), 7·88 (3H, s, CH_3); addition of D_2O eliminated the signal at τ 6·62.

The ether-extracted aqueous solution obtained above was adjusted to pH 2·0 and extracted continuously with chloroform for 12 hr. Evaporation of the extract yielded a residue (9·9 g) which was chromatographed on paper with ethyl acetate–acetic acid–water (10 : 3 : 4 by volume; upper phase) as solvent. Spraying with iodide–iodate–starch reagent[27] revealed two acids; one (R_F 0·87, spot intense) co-chromatographed with *trans-γ*-hydroxy-*β*-methylcrotonic acid while the other (R_F 0·96, spot faint) co-chromatographed with trans-*γ-acetoxy-β-methylcrotonic acid.* The latter (m.p. 53–54°) was prepared by acetylation of *γ*-hydroxy-*β*-methylcrotonic acid with acetic anhydride–pyridine at room temperature and recrystallization from ethyl acetate–hexane (Found: C, 53·4; H, 6·2; O, 40·4. $C_7H_{10}O_4$ requires C, 53·2; H, 6·4; O, 40·5%). Attempts to isolate in crystalline form the two acids detected in the above hydrolysate yielded only uncrystallizable oils.

The yield of (V) was increased to about 60% when (IV) was hydrolysed at 25°.

(*d*) trans-*β-Methyl-γ-(tetrahydropyran-2-yloxy)crotononitrile* (*VI*)

To a vigorously stirred mixture of *trans-γ*-hydroxy-*β*-methylcrotononitrile (10 g) and 2,3-dihydropyran (16 ml; dried and purified by distillation from lithium aluminium hydride), a few crystals of *p*-toluenesulphonic acid were added. After a few minutes, the reaction mixture became hot and was then heated under reflux for 1 hr. After addition of anhydrous K_2CO_3 (0·5 g), the cooled reaction mixture was filtered. Vacuum distillation of the filtrate yielded trans-*β-methyl-γ-(tetrahydropyran-2-yloxy)crotononitrile* (16·9 g, 91%), b.p. 84°/0·08 mm, n_D^{25} 1·4756 (Found: C, 66·25; H, 8·5; N, 7·8; O, 17·8. $C_{10}H_{15}NO_2$ requires C, 66·3; H, 8·3; N, 7·7; O, 17·7%).

(*e*) *4-Amino-2-methylbut*-trans-*2-en-1-ol* (*II*)

A solution of *trans-β*-methyl-*γ*-(tetrahydropyran-2-yloxy)crotononitrile (16·0 g) in dry ether (75 ml) was added over a period of 40 min to a stirred suspension of lithium aluminium hydride (3·6 g) in dry ether (600 ml) maintained at about −5°. The mixture was stirred at 0°

[27] Wood, T., in "Chromatographic Techniques." (Ed. I. Smith.) p. 206. (Heinemann: London 1958.)

for 4 hr. Stirring was continued while the resulting suspension warmed to room temperature over a 1-hr period. The reaction mixture was then cooled to 0° and 2·5N NaOH added until evolution of hydrogen ceased and granular salts were obtained. The resulting suspension was filtered and the salts were washed thoroughly with ether. Evaporation of the combined dried (K_2CO_3) ether extracts yielded crude amine (16·1 g, 98%). An attempt to distil this amine in vacuum was unsuccessful due to decomposition. Addition of an aqueous solution of sodium tetraphenylborate to an aqueous solution of the crude amine (pH adjusted to 7 with dilute HCl) and crystallization of the resulting precipitate from cold absolute ethanol yielded the *tetraphenylborate* of *3-methyl-4-(tetrahydropyran-2-yloxy)but*-trans-*2-enylamine*, m.p. 68–70° (Found: C, 80·4; H, 8·1; B, 2·1. $C_{34}H_{40}BNO$ requires C, 80·8; H, 8·0; B, 2·1%).

The foregoing crude amine (15 g) was suspended in water and HCl solution slowly added till the solution pH was 0·6. The resulting solution was first kept at 25° for 10 hr, then extracted with ether and finally adjusted to pH 12 and extracted continuously with chloroform. The oil obtained by evaporation of the chloroform extracts was distilled rapidly under vacuum to yield crude 4-amino-2-methylbut-*trans*-2-en-1-ol (II) (2·4 g, 29%), b.p. 106–108°/0·08 mm. The rather low yield was apparently partly due to decomposition during distillation. Paper chromatography indicated that the above product contained a saturated amine as an impurity. This was confirmed by the n.m.r. spectrum, which, in addition to the anticipated peaks, exhibited weak signals at τ 9·13 (d) and τ 8·50 (m). Addition of an absolute ethanol solution of chloroplatinic acid to an absolute ethanol solution of crude (II) and recrystallization of the resulting crystalline precipitate from ethanol–water yielded the *chloroplatinate* of (II) which decomposed on heating at 160–165° (Found: C, 19·3, 19·5; H, 4·2, 4·0; N, 4·6; O, 5·3; Pt 31·9, 31·9; duplicate values refer to different preparations. $(C_5H_{11}NO)_2,H_2PtCl_6$ requires C, 19·6; H, 4·0; N, 4·6; O, 5·2; Pt, 31·9%).

By a procedure involving fractional distillation followed by fractional precipitation with sodium tetraphenylborate, the saturated amine in crude (II) was isolated as the *tetraphenylborate*, m.p. 122–124° (Found: B, 2·50. $C_{29}H_{34}BNO$ requires B, 2·55%). Paper chromatography of the derived hydrochloride and spectroscopy (n.m.r. and i.r.) established that this tetraphenylborate and the tetraphenylborate of 4-amino-2-methylbutan-1-ol were identical.

(f) Zeatin (I)

A solution of the foregoing crude aminobutenol (II) (1·1 g) in n-butanol (15 ml) was heated under reflux with 6-chloropurine (0·68 g) for 3 hr. The reaction solution was then concentrated under vacuum to about 6 ml, seeded with zeatin, and left at 2° for several days. The precipitate which formed was filtered off, washed with ether, dried, and finally washed with water (2 ml) at 0°. One recrystallization from ethanol yielded needles (0·36 g), m.p. 205–207°; a second recrystallization elevated the m.p. to 209–209·5°, unaltered on admixture with authentic zeatin (m.p. 208–209°). The residue obtained by evaporation of the butanol filtrate, wash liquid, and recrystallization mother liquids was dissolved in formic acid (0·5N) and an excess of a saturated aqueous solution of picric acid added. The resulting precipitate was converted into free base using ion-exchange resin as previously described.[2] Recrystallization from ethanol yielded a further 60 mg of product.

The u.v., i.r., and n.m.r. spectra of the synthetic compound were identical with those of authentic zeatin. The two compounds could not be distinguished by paper chromatography or by silica-gel thin-layer chromatography. Both exhibited identical activity in the carrot phloem cytokinin bioassay.[4] On oxidation with permanganate, the synthetic compound yielded the products previously obtained by oxidation of zeatin.[3]

The following derivatives recrystallized from water were prepared from the synthetic compound: picrate, m.p. and mixed m.p. 192–194°; 3-iodo-2,4,6-trinitrophenolate, m.p. and mixed m.p. 204–205°.

(g) 4-Amino-2-methylbutan-1-ol

(i) *Synthesis from* trans-*γ-acetoxy-β-methylcrotononitrile.*—A solution of this nitrile (21·5 g) in dry ether (75 ml) was slowly added to a stirred suspension of lithium aluminium hydride (12·5 g)

in dry ether (600 ml) maintained at 0°. The resulting mixture was allowed to warm to room temperature and was then refluxed for 1 hr. Sodium hydroxide solution (2·5N) was added to the cooled reaction mixture until evolution of hydrogen ceased and granular salts were obtained. The resulting suspension was filtered and the insoluble material washed thoroughly with ether and then chloroform. The combined dried (Na_2SO_4) ether and chloroform solutions were evaporated, yielding an oil which was distilled rapidly under vacuum to yield crude amine (3·0 g, 20%), b.p. 120–130°/0·05 mm. Paper chromatography indicated that this product contained one ninhydrin-reacting component (a saturated amine) but the i.r. spectrum suggested the presence of a carbonyl impurity. Addition of an absolute ethanol solution of chloroplatinic acid to an ethanol solution of the crude amine yielded the crystalline *chloroplatinate* of 4-amino-2-methylbutan-1-ol, m.p. 178–179° (dec.) (Found: C, 19·55; H, 4·6; N, 4·7; O, 5·1; Pt, 31·4. $(C_5H_{13}NO)_2,H_2PtCl_6$ requires C, 19·5; H, 4·6; N, 4·55; O, 5·2; Pt, 31·7%). N.m.r. spectrum (D_2O): τ 6·57 (2H, d, J 6 c/s, CH_2OH), 7·00 (2H, t, J 7 c/s, CH_2NH_2), *c.* 8·4 (3H, broad m, $>CHCH_2$), 9·03 (3H, d, J 6 c/s, CH_3).

(ii) *Synthesis from* trans-*β-methyl-γ-(tetrahydropyran-2-yloxy)crotononitrile.*—A solution of this nitrile (9·7 g) in dry ether (50 ml) was added to a stirred suspension of lithium aluminium hydride (3·4 g) in dry ether (75 ml), the reaction temperature being maintained at 15°. The resulting mixture was stirred at room temperature for 24 hr. Water was then added at 0° until evolution of hydrogen ceased and granular salts resulted. These were washed with ether. Evaporation of the combined, dried (Na_2SO_4) ether solutions yielded an oil which was dissolved in dilute hydrochloric acid to give a solution of pH 1. This was left at 25° overnight, adjusted to pH 6, extracted with ether, adjusted to pH 12, and finally extracted with chloroform. Evaporation of the dried (K_2CO_3) chloroform extracts yielded crude 4-amino-2-methylbutan-1-ol (4·4 g, 81%). Paper chromatography showed that this product was a homogeneous saturated amine which co-chromatographed with the 4-amino-2-methylbutan-1-ol previously prepared. Identity was established by n.m.r. and i.r. spectra.

(*h*) *6-N-(3-Methylbut-2-enoyl)adenine*

β-Methylcrotonoyl chloride (6·2 g) was gradually added to a stirred suspension of adenine (2 g) in dry pyridine (20 ml) maintained at about −3°. After the resulting mixture had been stirred at 4° overnight, it was diluted with 95% ethanol (30 ml) and then centrifuged. The very dark supernatant liquid was discarded, while the crude acyl adenine which had been centrifuged down was washed successively with 95% ethanol, 0·5N formic acid, and water. The resulting product (1·75 g, 55%) was recrystallized first from ethanol–acetone and then from acetonitrile yielding *6-N-(3-methylbut-2-enoyl)adenine*, m.p. 219–220° (Found: C, 55·2; H, 5·2; O, 7·9. $C_{10}H_{11}N_5O$ requires C, 55·3; H, 5·1; O, 7·4%).

(*i*) *Preparation of α,β-Unsaturated Nitriles by Condensation of a Ketone with Cyanoacetic Acid*

General procedure.—To a stirred solution of cyanoacetic acid (1·3 mole) in the appropriate ketone (1·0 mole), cyclohexylamine (185 ml) was added dropwise over a 2-hr period. The mixture was refluxed for 5 hr, cooled, and poured into water (500 ml) which was then extracted with ether (400 ml). The ether solution was washed sequentially with water (3 × 200 ml), 2N HCl cooled to 0° (till washings became acidic), and water. The residue obtained by evaporation of the dried (Na_2SO_4) ether solution was fractionally distilled to yield the unsaturated nitrile. By the above procedure, the following known nitriles were prepared:

β-Methylcrotononitrile: b.p. 141–142°/765 mm (lit.[28] 140–142°/760 mm), yield 38%.

β-Ethylcrotononitrile, *cis–trans* (1 : 2) mixture: b.p. 64–65°/27 mm, 159–161°/760 mm (lit.[18] for *trans* isomer, 158–160°/760 mm); n_D^{21} 1·4438 (lit.[18] for *trans* isomer, n_D^{20} 1.4445); n.m.r. spectrum ($CDCl_3$): τ 4·90 (m, olefinic protons), 7·67 (two overlapping quartets, methylene protons), 7·95 (s, $CH_3C{=}C$ of *trans* isomer), 8·08 (d, J 1·5 c/s, $CH_3C{=}C$ of *cis* isomer), and 8·90 (two overlapping triplets, CH_3CH_2 of both isomers). The integral of the spectrum was in accord with this assignment. The yield was 51%.

[28] "Beilsteins Handbuch der Organischen Chemie." Hauptwerk, Vol. 2, p. 433. (Springer: Berlin 1920.)

Condensation of methoxyacetone (prepared according to Kanella and Leech[29]) and cyanoacetic acid by the above method gave a liquid (yield 53 g per mole of ketone), b.p. 92–94°/26 mm, ν_{max} 2247 (saturated nitrile), 2220 (α,β-unsaturated nitrile), 1692 cm^{-1} (carbonyl). This product (41 g) was added to a 2,4-dinitrophenylhydrazine solution prepared by addition of conc. H_2SO_4 (100 ml) to 2,4-dinitrophenylhydrazine (50 g) suspended in stirred ethanol (1·5 l.). The resulting mixture was slowly heated to 38° while being stirred and then left at 4° overnight. The crystalline precipitate was filtered off (filtrate retained and termed A) and washed with dilute H_2SO_4 and then with water. Thin-layer chromatography on silica gel indicated that the product was homogeneous. Recrystallization from ethanol yielded 3-cyano-2-methylpropanal 2,4-dinitrophenylhydrazone (66 g), m.p. 152–153° (lit.[20] 149°) (Found: C, 47·8; H, 4·1; N, 25·2; O, 22·9; *m/e* 277; OCH_3, 0·0. $C_{11}H_{11}N_5O_4$ requires C, 47·65; H, 4·0; N, 25·3; O, 23·1%; mol. wt., 277). ν_{max} (mull) 2247 cm^{-1} (saturated nitrile). The three principal ions in the mass spectrum occurred at *m/e* 277, 237 (loss of CH_2CN), and 231 (loss of NO_2, base peak). In addition to the peaks arising from aromatic protons, the n.m.r. spectrum (pyridine-d_5) exhibited signals at τ 2·09 (1H, d, *J* 4 c/s, CH=N), 6·8–7·5 (3H; a broad complex signal with a very prominent peak at τ 7·15; $\geq CHCH_2CN$), and 8·75 (3H, d, *J* 6 c/s, CH_3). The doublet centred at τ 2·09 partly overlapped the doublet signal from the aromatic proton at position 6 of the 2,4-dinitrophenyl moiety. In a spectrum in dimethyl sulphoxide-d_6 the two signals were completely resolved.

Filtrate A was extracted with ether. The extracts were washed with water, dried (Na_2SO_4), and evaporated yielding a residue which was fractionally distilled to give trans-*γ-methoxy-β-methylcrotononitrile* (VII) (3·7 g), b.p. 90–92°/26 mm (Found: C, 64·8; H, 8·3; N, 12·6; O, 14·6. C_6H_9NO requires C, 64·8; H, 8·2; N, 12·6; O, 14·4%). The i.r. and n.m.r. spectra were identical with those of (VII) prepared as described in (*j*).

(*j*) *Preparation of γ-Alkoxy-β-methylcrotononitriles*

The preparation of the propoxynitrile exemplifies these syntheses. Potassium (22·7 g, 0·580 g-atom) was dissolved under anhydrous conditions in n-propanol (270 ml, 3·6 mole) dried by refluxing with magnesium propylate. The cooled solution was slowly added to stirred γ-bromo-β-methylcrotononitrile (91·0 g, 0·568 mole), the reaction temperature being maintained at −5° by cooling in an ice–salt bath. The resulting mixture was stirred for 2 hr at 0° before being poured into water (3 l.; cooled to 0°) which was then extracted with ether (4 × 600 ml). The combined extracts were filtered, washed with water (3 × 300 ml), dried (Na_2SO_4), and evaporated. Distillation of the residue yielded *β-methyl-γ-propoxycrotononitrile* (*cis–trans* mixture), yield 34·5 g (44%), b.p. 49–53°/0·5 mm (Found: C, 69·1; H, 9·2; N, 10·3; O, 11·7. $C_8H_{13}NO$ requires C, 69·0; H, 9·4; N, 10·1; O, 11·5%). The n.m.r. spectrum indicated that the ratio of *trans* isomer to *cis* isomer was approx. 2 : 1.

The above *cis–trans* mixture was fractionated in a Nester–Faust spinning-band distillation column yielding pure trans-*β-methyl-γ-propoxycrotononitrile* (VIII), b.p. 37°/0·08 mm, n_D^{23} 1·4489; ν_{max} 2220 (α,β-unsaturated nitrile), 1640, 810 (trisubstituted double bond), 1115 cm^{-1} (ether). N.m.r. spectrum (CCl_4): τ 4·53 (1H, s-b, C=CHCN), 6·03 (2H, s-b, $OCH_2C=C$), 6·60 (2H, t, *J* 6·5 c/s, CH_2CH_2O), 8·00 (3H, s, $C=CCH_3$), 8·40 (2H, sextet, *J* 7 c/s, $CH_2CH_2CH_3$), and 9·06 (3H, t, *J* 7 c/s, CH_2CH_3). The *cis* isomer was also obtained in 85% purity, b.p. 34°/0·08 mm, n_D^{23} 1·4520. N.m.r. spectrum (CCl_4): τ 4·77 (1H, s-b, C=CHCN), 5·81 (2H, s, $OCH_2C=C$), 6·60 (2H, t, *J* 6·5 c/s, CH_2CH_2O), 8·05 (3H, d, *J* 1·5 c/s, $C=CCH_3$), 8·40 (2H, sextet, *J* 7 c/s, $CH_2CH_2CH_3$), and 9·07 (3H, t, *J* 7 c/s, CH_2CH_3) and also weak signals from the *trans* isomer present as impurity.

The new compounds detailed below were similarly prepared. In these condensations, it is important to maintain the reaction temperature at about 0° and to add the alkoxide solution to the nitrile. It is also important that all traces of base be removed before the products are distilled. In preparing the methoxy and ethoxynitriles, the sodium alkoxides were used. All products exhibited the expected n.m.r. and i.r. spectra.

γ-Methoxy-β-methylcrotononitrile (*cis–trans* mixture, 1 : 1), yield 73%, b.p. 87–92°/28 mm, n_D^{25} 1·4465 (Found: C, 64·7; H, 8·15; N, 12·6; O, 14·65. C_6H_9NO requires C, 64·8; H, 8·2; N, 12·6; O, 14·4%). trans-*γ-Methoxy-β-methylcrotononitrile* (VII), b.p. 91–92°/28 mm, n_D^{25} 1·4443.

γ-Ethoxy-β-methylcrotononitrile (*cis–trans* mixture, 1 : 1·5), yield 77%, b.p. 92–98°/26 mm, n_D^{12} 1·4523 (Found: C, 66·8; H, 9·0; N, 11·1. $C_7H_{11}NO$ requires C, 67·2; H, 8·9; N, 11·2%).

[29] Kanella, R. P., and Leech, J. L., *J. Am. chem. Soc.*, 1949, **71**, 3558.

γ-Butoxy-β-methylcrotononitrile (*cis–trans* mixture, 1 : 2), yield 48%, b.p. 55–60°/0·3 mm, n_D^{25} 1·4478 (Found: C, 70·0; H, 9·9; N, 9·25. $C_9H_{15}NO$ requires C, 70·55; H, 9·9; N, 9·1%).

trans-*γ-Butoxy-β-methylcrotononitrile* (IX), b.p. 59–60°/0·3 mm, n_D^{25} 1·4470.

An attempt to prepare γ-isopropoxy-β-methylcrotononitrile under the above conditions was unsuccessful.

(*k*) *Reduction of β-Ethylcrotononitrile and γ-Methoxy-β-methylcrotononitrile with Lithium Aluminium Hydride*

(i) *β-Ethylcrotononitrile* (cis–trans *mixture*).—The nitrile (24 g, 0·25 mole) in dry ether (100 ml) was added slowly to a stirred suspension of lithium aluminium hydride (10 g, 0·26 mole) in dry ether (400 ml) maintained at −5 to 0°. When the addition was complete, the mixture was stirred for 2 hr at room temperature. Water was then slowly added to the resulting suspension cooled to 0° till evolution of hydrogen ceased and granular salts resulted. The ether was decanted and the salts were stirred with three 100-ml volumes of ether. The combined dried (Na_2SO_4) ether solutions were evaporated and the residue distilled at 28 mm yielding crude amine (12 g) which was then fractionally distilled to give *3-methylpent-2-enylamine* (8·3 g, 33%), b.p. 43–45°/28 mm, n_D^{26} 1·4444 (Found: C, 72·7; H, 13·0; N, 14·0. $C_6H_{13}N$ requires C, 72·7; H, 13·2; N, 14·1%). ν_{max} 3360, 3280, 1597, and 845 (primary amine), 1665 cm^{-1} (double bond).

The *chloroplatinate* of the foregoing amine was prepared and recrystallized from ethanol, yielding yellow plates, m.p. 200–201° (dec.) (Found: C, 23·8; H, 4·8; N, 4·65; Pt, 31·9. $(C_6H_{13}N)_2,H_2PtCl_6$ requires C, 23·7; H, 4·6; N, 4·6; Pt, 32·1%).

(ii) *γ-Methoxy-β-methylcrotononitrile* (cis–trans *mixture*).—The reduction was carried out under the conditions just described (i), giving a low yield (1·1 g from 11·0 g of nitrile) of amine (b.p. 58–66°/27 mm). Paper chromatography and i.r. spectroscopy indicated that this was a mixture of a saturated amine and an unsaturated amine with the former predominating. Fractional distillation yielded *4-methoxy-3-methylbutylamine* (b.p. 59–63°/27 mm) which was converted into the *chloroplatinate*, m.p. 186–187° (dec.) (Found: C, 22·4; H, 5·15; N, 4·4; O, 5·0; Pt, 30·25. $(C_6H_{15}NO)_2,H_2PtCl_6$ requires C, 22·4; H, 5·0; N, 4·35; O, 5·0; Pt, 30·3%).

(*l*) *Reduction of γ-Alkoxy-β-methylcrotononitriles with Aluminium Chloride–Lithium Aluminium Hydride*

The reduction of one nitrile is detailed; the remainder were reduced with this reagent under identical conditions. A solution of anhydrous aluminium chloride (13·3 g, 0·10 mole) in dry ether (50 ml) was added to a stirred suspension of lithium aluminium hydride (3·78 g, 0·10 mole) in dry ether (25 ml). The mixture was cooled under anhydrous conditions and added over a period of 1 hr to a stirred solution of γ-butoxy-β-methylcrotononitrile (*cis–trans* mixture) (15·2 g, 0·10 mole) in dry ether (125 ml) maintained at −10°. The resulting suspension was stirred for 3 hr at about −4° and then allowed to warm to room temperature (20°) over a 1·5-hr period. Water was then slowly added to the cooled (ice-bath) suspension until granular salts resulted. The supernatant ether was decanted and the salts were then stirred with three 200-ml volumes of ether. The combined dried (Na_2SO_4) ether solutions were evaporated yielding a liquid residue (5·0 g) termed A. A suspension of the salts in water (500 ml; suspension pH about 5) was adjusted to pH 12 with 6N KOH and extracted with five 200-ml volumes of ether followed by three 200-ml volumes of chloroform. Evaporation of the dried extracts yielded a liquid (10·1 g) termed B.

Fractional distillation of liquid A yielded *γ-butoxy-β-methylbutyronitrile* (1·53 g, 10%), b.p. 54°/0·4 mm, n_D^{22} 1·4112 (Found: C, 70·0; H, 11·1; N, 8·8; O, 10·0. $C_9H_{17}NO$ requires C, 69·6; H, 11·0; N, 9·0; O, 10·3%). ν_{max} 2245 (saturated nitrile), 1115 cm^{-1} (ether). The i.r. spectrum did not show bands indicative of a trisubstituted double bond. A 100-Mc/s n.m.r. spectrum ($CDCl_3$) exhibited signals at τ 6·70 (4H, m, CH_2OCH_2), 7·62 (2H, m, CH_2CN), 7·83 (1H, broad m, methine proton), 8·53 (4H, m, $CH_3CH_2CH_2CH_2$), 8·94 (3H, d, *J* 6 c/s, CH_3CH), and 9·09 (3H, t, *J* 6·5 c/s, CH_3CH_2).

Fractional distillation of liquid B yielded *4-butoxy-3-methylbut-2-enylamine* (*cis–trans* mixture) (4·5 g, 29%), b.p. 44–48°/0·2 mm, n_D^{22} 1·4490; ν_{max} 3360, 3290, 1596, and 845 (primary amine), 1678 (double bond), and 1095 cm^{-1} (ether). For elementary analysis the *chloroplatinate*

(m.p. 183–184° (dec.); crystallized from ethanol) was prepared (Found: C, 29·9; H, 5·8; N, 4·1; O, 4·8; Pt, 27·2. $(C_9H_{19}NO)_2,H_2PtCl_6$ requires C, 29·85; H, 5·6; N, 3·9; O, 4·4; Pt, 26·9%).

Reduction of *trans*-γ-butoxy-β-methylcrotononitrile under identical conditions yielded *4-butoxy-3-methylbut*-trans-*2-enylamine*, b.p. 48–49°/0·2 mm, n_D^{22} 1·4515; the *chloroplatinate* (correct elementary analyses) was crystallized from ethanol, m.p. 186–188° (dec.). N.m.r. spectrum ($CDCl_3$) of the amine: τ 4·48 (1H, t broadened by allylic coupling, J 6·5 c/s C=CH), 6·17 (2H, s, $OCH_2C{=}C$), 6·65 (4H, m, CH_2CH_2O and CH_2NH_2), 8·10–8·90 (9H, complex), 9·07 (3H, t, J 6·5 c/s, CH_3CH_2). The complex (τ 8·10–8·90) appeared to comprise two sharp prominent singlet peaks (τ 8·33, $CH_3C{=}C$; τ 8·70, eliminated by D_2O, NH_2) superimposed on a broad multiplet due to two methylene groups.

By the reduction procedure described above, the new amines detailed below were prepared. All exhibited the expected i.r. and n.m.r. spectral characteristics.

4-Methoxy-3-methylbut-trans-*2-enylamine* (10% yield), b.p. 69°/26 mm, n_D^{24} 1·4401; *chloroplatinate*, m.p. 179·5–181° (dec.) (Found: C, 22·5; H, 4·7; N, 4·4; Pt, 30·9. $(C_6H_{13}NO)_2,H_2PtCl_6$ requires C, 22·5; H, 4·4; N, 4·4; Pt, 30·5%).

4-Ethoxy-3-methylbut-2-enylamine (*cis–trans* mixture) (12% yield), b.p. 77–80°/26 mm, n_D^{17} 1·4480; *chloroplatinate*, m.p. 182–183° (dec.) (Found: C, 24·8; H, 4·75; N, 4·2; O, 4·8; Pt, 29·4. $(C_7H_{15}NO)_2,H_2PtCl_6$ requires C, 25·2; H, 4·8; N, 4·2; O, 4·8; Pt, 29·2%).

3-Methyl-4-propoxybut-trans-*2-enylamine* (25% yield), b.p. 34°/0·1 mm, n_D^{25} 1·4490; *chloroplatinate*, m.p. 184–186° (dec.) (Found: C, 27·5; H, 5·4; N, 4·1; O, 4·7; Pt, 28·2. $(C_8H_{17}NO)_2,H_2PtCl_6$ requires C, 27·6; H, 5·2; N, 4·0; O, 4·6; Pt, 28·0%).

Two saturated nitriles produced in the above reductions were purified and are detailed below.

γ-Methoxy-β-methylbutyronitrile (16% yield), b.p. 74–75°/27 mm, n_D^{22} 1·4108 (Found: C, 63·6; H, 9·8; N, 12·2; O, 14·3. $C_6H_{11}NO$ requires C, 63·7; H, 9·8; N, 12·4; O, 14·1%). N.m.r. spectrum (CCl_4): τ 6·5–7·0 (5H; a sharp methoxyl singlet, τ 6·68, superimposed on a multiplet; CH_3OCH_2CH), 7·63 (2H, m, CH_2CN), 7·95 (1H, broad m, $-CH<$), and 8·92 (3H, d, J 6·5 c/s, CH_3CH). Frequency sweep spectra using moderate perturbing fields provided strong evidence in support of the assigned structure. Irradiation of the suspected methine proton simplified the multiplet signals at τ 6·5–7·0 and τ 7·63, and also the doublet signal at τ 8·92. Irradiation on the methylene adjacent to the methoxyl group and on the methyl adjacent to the methine proton simplified the signal centred at τ 7·95 to a broad triplet, but did not affect the signal at τ 7·63.

β-Methyl-γ-propoxybutyronitrile (11% yield), b.p. 39°/0·1 mm, n_D^{21} 1·4200 (Found: C, 68·0; H, 10·8; N, 9·9. $C_8H_{15}NO$ requires C, 68·0; H, 10·7; N, 9·9%). The n.m.r. spectrum was analogous to that given by the methoxyl analogue.

(*m*) *Methyl γ-Azido-β-methylcrotonate*

(i) *Preparation.*—Methyl γ-bromo-β-methylcrotonate was first prepared according to Halmos and Mohacsi.[8] The n.m.r. spectrum indicated that this product was a *cis–trans* (1 : 2) mixture. A continuously stirred mixture of methyl γ-bromo-β-methylcrotonate (*cis–trans*) (17·3 g, 0·09 mole), dry sodium azide (22·5 g, 0·35 mole), and acetonitrile (60 ml) was heated under reflux for 3 hr. The cooled reaction mixture was filtered and the filtrate evaporated under reduced pressure, yielding a liquid residue which was fractionally distilled to give *methyl γ-azido-β-methylcrotonate* (*cis–trans* mixture, 1 : 2) (10·3 g, 74%), b.p. 47–52°/0·15 mm (Found: C, 46·7; H, 5·7; N, 27·0; O, 20·7. $C_6H_9N_3O_2$ requires C, 46·45; H, 5·85; N, 27·1; O, 20·6%). ν_{max} 2100 (azide), 1720 cm^{-1} (ester). N.m.r. spectrum ($CDCl_3$): τ 4·05 (m, C=CH of both isomers), 5·53 (s, CH_2N_3 *cis* isomer), 6·12 (s, CH_2N_3 *trans* isomer), 6·27 (s, $COOCH_3$ of both isomers), 7·82 (d, J 1·2 c/s, $CH_3C{=}C$ *trans* isomer), 8·00 (d, J 1·4 c/s, $CH_3C{=}C$ *cis* isomer). The integral of the spectrum was in accord with these assignments.

(ii) *Reduction.*—Fractional distillation of the above *cis–trans* mixture yielded *trans* isomer over 80% pure. A solution of this *trans* azido ester (5·5 g) in dry ether (220 ml) was added over 1 hr to a stirred, uncooled suspension of lithium aluminium hydride (2·2 g) in dry ether (330 ml). After the resulting mixture had been stirred for 1 hr, water (about 15 ml) was slowly added to yield granular salts. The resulting ether solution was filtered, dried (Na_2SO_4), and evaporated

yielding a liquid (2·6 g) which was distilled rapidly under vacuum giving semi-pure *4-amino-3-methylbut*-trans-*2-en-1-ol* (1·62 g, 45%), b.p. 77–80°/0·04 mm. N.m.r. spectrum ($CDCl_3$): τ 4·40 (1H, t broadened by allylic coupling, J 6·5 c/s, C=CH), 5·78 (2H, d, J 6·5 c/s, CH_2OH), 6·77 (2H, s, CH_2NH_2), 7·12 (2H, s, NH_2), 8·17 (1H, s, OH), 8·30 (3H, s, CH_3). Addition of D_2O eliminated the signals at τ 7·12 and 8·17. Weak signals were also observed at τ 7·5 (d) and τ 8·85 (d). These are attributed to saturated amine present as impurity. This impurity was also detected by paper chromatography. In an attempt to suppress formation of saturated amine, the reduction was repeated at 0°. Under these conditions, reduction of the azido group was incomplete and saturated amine was still detectable in the product by n.m.r. spectroscopy.

(*n*) *Methyl β-Methyl-γ-nitrocrotonate*

Under anhydrous conditions, methyl γ-bromo-β-methylcrotonate (*cis–trans* mixture) (25 g, 0·13 mole) was added dropwise to a stirred suspension of silver nitrite (25 g, 0·16 mole) in dry ether (75 ml). The reaction temperature was maintained at −10° and the reaction flask was protected from light. When the addition of bromo ester was complete, the resulting mixture was stirred for 5 hr at −10° and then for 18 hr at 4°. The reaction solution was then filtered and evaporated giving a yellow liquid which was fractionally distilled, yielding *methyl β-methyl-γ-nitrocrotonate* (*cis–trans* mixture) (15·4 g, 75%), b.p. 73–78°/0·4 mm (Found: C, 45·5; H, 5·7; O, 40·4. $C_6H_9NO_4$ requires C, 45·3; H, 5·7; O, 40·2%). ν_{max} 1720 (ester), 1557 and 1378 cm^{-1} (nitro group).

Reduction of the nitro ester at 15° with a slight excess of lithium aluminium hydride (inverse addition) yielded semi-pure 4-amino-3-methylbut-2-en-1-ol in low yield (10%).

(*o*) *Condensation of Amines with 6-Chloropurine to yield the Zeatin Analogues of Table 1*

The first eight analogues listed in Table 1 were prepared by heating for 3 hr under reflux a mixture of n-butanol (6 ml), 6-chloropurine (1·5 mmole), and the appropriate amine (5·0–6·0 mmole). The n-butanol and excess amine were then evaporated under vacuum and water was added to the residue. Evaporation of the water gave a solid which was washed with water and then recrystallized from ethanol–water. The remaining compounds were prepared by modifications (outlined below) of the above general procedure.

6-(4-Hydroxy-3-methylbutylamino)purine, prepared from crude amine (see (*g*)(ii) above), required purification by ion-exchange chromatography before crystallization. An aqueous solution (pH 10·9) of the crude purine was passed through a column of the anion-exchange resin De-Acidite FF (acetate form). The purified purine was eluted with 1·5N acetic acid and was then recrystallized first from n-butanol and finally from methanol–ethyl acetate.

Because of the instability of diprop-2-ynyl amine, the condensation with 6-chloropurine to yield 6-(diprop-2-ynylamino)purine was performed in ethanol at 80°.

To prepare 6-(4-hydroxy-2-methylbut-*trans*-2-enylamino)purine, a mixture of n-butanol (5 ml), 6-chloropurine (2·0 mmole), the aminobutenol (4·0 mmole), and triethylamine (0·4 ml) was heated under reflux for 1 hr. The resulting solution was concentrated to 2 ml and left at 2° for 24 hr. The product was filtered off and recrystallized first from water and then from methanol–benzene.

To prepare 6-(5-hydroxypentylamino)purine and 6-(6-hydroxyhexylamino)purine, 6-chloropurine (1·5 mmole) was condensed with the appropriate amine (3·6 mmole) in refluxing n-butanol. The residue obtained by vacuum evaporation of the butanol was dissolved in water and the solution pH was adjusted to 7 with dilute HCl. The desired product separated from the concentrated solution after cooling and was recrystallized from ethanol–water.

(*p*) *Conversion of 6-Methoxyalkylaminopurines into 6-Hydroxyalkylaminopurines*

Iodine (0·75 g, 5·92 mg-atoms) was dissolved in a solution of the methoxyalkylaminopurine (0·97 mmole) in acetonitrile (5 ml). After addition of sodium borohydride (0·105 g, 2·77 mmole), the solution was shaken at 25° for 20 hr and then left at 40° for 5 hr. Water (10 ml) was then added. After the resulting solution had been extracted with ether to remove free iodine, an excess of a saturated aqueous solution of picric acid was added. After 24 hr, the precipitate of 6-hydroxyalkylaminopurine picrate was filtered off, washed with water, and then dissolved

in water at 50°. Anion-exchange resin (De-Acidite FF, acetate form, 100–200 mesh) was slowly added to the stirred solution until this was free from picrate ions. The resin was then filtered off and washed with water. The combined filtrate and wash liquids were concentrated and extracted with five equal volumes of water-saturated n-butanol. Evaporation of the combined extracts to dryness yielded the crude 6-(hydroxyalkylamino)purine which was recrystallized from ethanol.

By the above procedure *6-(3-methoxypropylamino)purine* yielded 6-(3-hydroxypropylamino)-purine (yield of crude 91%), m.p. 218–220°, unaltered on admixture with authentic compound (m.p. 219–220°). The i.r. spectra and chromatographic behaviour of the two compounds were identical. 6-(3-Methoxypropylamino)purine was prepared by the general procedure given under (*o*), m.p. 176° (Found: C, 52·4; H, 6·4; N, 33·8. $C_9H_{13}N_5O$ requires C, 52·2; H, 6·3; N, 33·8%).

Ether cleavage of 6-(4-methoxy-3-methylbutylamino)purine gave 6-(4-hydroxy-3-methyl-butylamino)purine (yield of crude 70%), m.p. and mixed m.p. 165–166°. N.m.r. spectrum (pyridine-d_5): τ 1·57 (1H, s, purine H), 1·77 (1H, s, purine H), 6·03 (2H, broad t, *J* 6 c/s, $\mathbf{CH_2}NH$), 6·30 (2H, d, *J* 5 c/s, $\mathbf{CH_2}OH$), 7·7–8·4 (3H, broad m, $>\mathbf{CHCH_2}CH_2$), and 8·92 (3H, d, *J* 6 c/s, CH_3). The identity of the product was further confirmed by the i.r. and u.v. spectra and by co-chromatography with authentic compound prepared as described under (*o*).

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