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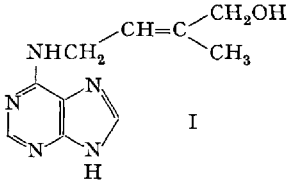
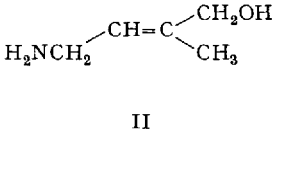
SPECIALIA

Synthesis and Cytokinin Activity of the 3-, 7- and 9-Methyl Derivatives of Zeatin

A substance that promotes growth (termed a cytokinin) was isolated from sweet corn (*Zea mays*) kernels and named zeatin[1]. Its structure was confirmed as 6-(4-hydroxy-3-methylbut-*Trans*-2-enylamino) purine (I) by synthesis from 6-chloropurine and the amino alcohol (II)[2]. Only a small proportion of the activity of sweet corn extracts is due to zeatin, since more of the total activity is attributable to the nucleotide (III) which occurs in much greater quantity than the aglycone or its riboside but is reported to be less active than zeatin at similar concentrations[3]. The synthesis of both the nucleoside[2] and nucleotide[4] have been described.



I



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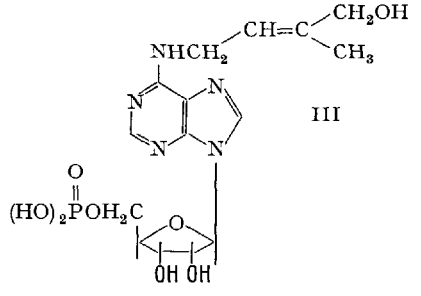
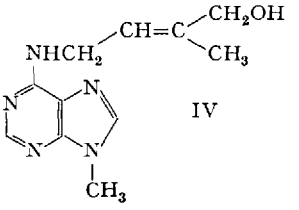


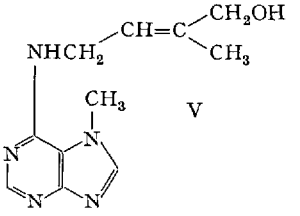
III

The natural occurrence of 9-β-D-ribofuranosyl derivatives of zeatin might suggest that the hormonal activity of the material is in some way connected with the nucleotide structure either as part of an enzyme co-factor unit or as a precursor to its introduction into a nucleic acid molecule. The related 6-N-γ, γ-dimethyl allyladenine which has high cytokinin activity is a known constituent of serine[5] and tyrosine[6] specific *t*-RNA but no correlation of this with cytokinin activity has yet been made.

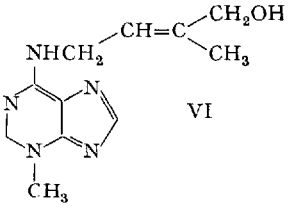
As part of a programme of experiments to relate structure and biological activity it was of interest to prepare zeatin analogues in which the ring proton was substituted, so making ribotide formation unlikely. The derivatives chosen were the 3-, 7- and 9-methyl zeatins. 9-Methyl zeatin (IV) was prepared by reaction of 9-methyl-6-chloropurine with the amine II, 7-methyl zeatin (V) by reaction of the amine II with the mixture of 6-chloro-7- and 9-methyl purine which results from methylation of 6-chloropurine with dimethylsulphate[7], and separation of the 2 (7- and 9-)methyl zeatins formed, and 3-methyl zeatin (VI) by methylation of zeatin with dimethyl sulphate in dilute aqueous sodium hydroxide solution.



IV



V



VI

The structures of the 3 methylated derivatives were confirmed in each case by elemental analysis and characteristic UV-absorption spectra (see Table I).

Tests of the activity of the 3 methylated zeatins in comparison with that of zeatin were made by the standardized procedures in use in the laboratory of one of us (F.C.S.). Standard explants are removed aseptically from a given carrot root at a given distance from the cambium. These are exposed in specially designed culture

Table I. UV-absorption spectra of some methylated adenine derivatives

Compound	λ_{max} (nm) at pH		
	1	7	11
Zeatin	274	269	275
Zeatin riboside	265	269	269
3-Methyl zeatin	286	290	290
7-Methyl zeatin	284	278	278
9-Methyl zeatin	267	270	270
1, N^6-Dimethyladenine[8]	261	276	274
3, N^6-Dimethyladenine[9]	281	–	287
7, N^6-Dimethyladenine[10]	280	–	275
9, N^6-Dimethyladenine[11]	265	–	268

Table II. Growth promoting activity of zeatin and its 3-, 7- and 9-methyl derivatives in the carrot assay test

Compounds tested Structure	Activity at 1.0 ppm indicated by Fresh weight/explant (mg)	Cells/explant (thousands)	Average cell size (μg/cell)	Activity at 0.1 ppm indicated by Fresh weight/explant (mg)	Cells/explant (thousands)	Average cell size (μg/cell)
3-Methyl zeatin (VI)	53.1	1386	0.04	65.3	1273	0.05
7-Methyl zeatin (V)	48.5	1012	0.05	44.0	813	0.06
9-Methyl zeatin (IV)	52.7	1295	0.04	61.3	1212	0.05
Zeatin (I)	42.6	1219	0.04	46.9	1356	0.04

Controls	Fresh weight/explant (mg)	Cells/explant (thousands)	Average cell size (μg/cell)
Initial explants	2.8	29	0.10
After growth in B + CH + IAA + inos	26.1	443	0.06
After growth in B + CH + CM	147.1	1620	0.09

B, basal medium; IAA, indoleacetic acid at 0.5 ppm; CH, casein hydrolysate at 250 ppm; inos, inositol at 25 ppm.

tubes to 10 ml of medium. The initial explants are described by their average fresh weight in mg, by the number of cells they contain (this being determined after maceration under standard conditions), and by the average size of the cells (μg/cell). The activity of the growth substances to which the carrot tissue readily responds is demonstrated in the basal medium, patterned after that of WHITE[12] but now modified for routine use in this work. The basal medium, however, is used with the addition of (a) indoleacetic acid (0.5 ppm), with which adenine growth substances are synergistic, (b) inositol (25 ppm), with which certain naturally occurring, non-adenine growth substances are synergistic, and (c) casein hydrolysate (250 ppm), which substantially accentuates the growth that occurs. Therefore, the essential control which describes the maximum capacity for growth in the absence of the growth promoting substance under test is the response of the tissue to the basal medium (B) + casein hydrolysate (CH) + indoleacetic acid (IAA) + inositol (inos). Finally, and as a measure of the reasonable maximum growth to be expected of the particular batch of carrot explants under test, their behaviour in the basal medium plus casein hydrolysate plus coconut milk (10% by vol.), is most useful. The relevant data are in Table II.

The points to be noted are:

(1) All 4 compounds tested caused a substantial response by growth of the explanted carrot tissue. Except in 1 case (7-methyl zeatin), the activity was somewhat greater at 0.1 ppm than at 1.0 ppm.

(2) All the methylated zeatins here tested gave activity of the same order as that due to zeatin, and the 3- and 9-methyl substituted compounds may even be more active than zeatin itself.

(3) Zeatin and its 3 methyl derivatives affected cell division conspicuously, but they did not promote subsequent cell enlargement to the same degree. This is a familiar feature of this sort of response, and the less active the compound is in fostering cell division the more likely it is to permit more cell expansion (cf. 7-methyl zeatin with zeatin).

(4) These adenine compounds (zeatin and its methyl substituents) are all active by promoting cell division in the carrot assay test along with the appropriate synergists (e.g. IAA). The complex of factors present in certain natural sources of growth promoting activity (e.g. coconut milk) induce greater growth rates than these substances seem able to induce.

The results imply that the mechanism of cytokinin activity in substituted adenines does not require prior formation of nucleotide derivatives[13].

Zusammenfassung. Die 3-, 7- und 9-Monomethylderivative des Cytokinins Zeatin wurden hergestellt und ihre Aktivität in der Karottenwurzel nachgewiesen. Die Ergebnisse zeigen, dass die Nukleotidderivative nicht notwendige Stufen im Wirkungsmechanismus darstellen.

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7 August 1968.

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[13] The chemical work was carried out in the School of Chemistry at the University of Bradford (G. S. and B. M. S.) and the biological tests at Cornell University by one of us (F. C. S.) with the technical assistance of Mrs. M. O. MAPES and made possible by a grant from the National Institute of Health, U.S. (No. G.M.09609).