Phytochemistry, 1972, Vol. 11, pp. 1019 to 1021. Pergamon Press. Printed in England.

SHORT COMMUNICATION

CYTOKININ-INDUCED SHOOT FORMATION

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(*Received* 13 *July* 1971; *in revised form* 15 *September* 1971)

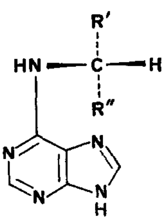
Abstract—Zeatin, (±)-dihydrozeatin and optically active cytokinins (asymmetric carbon α to the exocyclic nitrogen) were tested for their ability to induce development of shoots in tobacco callus. Zeatin and dihydrozeatin were equally active. The levorotatory compounds tested were active in inducing shoot formation but the corresponding dextrorotatory compounds were inactive at all concentrations tested. These findings suggest that the group attached to the N^6 position of cytokinins binds to a receptor site to bring about organ formation.

INTRODUCTION

AFTER the synthetic compound kinetin[1] was found to possess cytokinin activity, the naturally occurring cytokinins zeatin[2] (6-(4-hydroxy-3-methylbut-*trans*-2-enyl-amino)purine) and (—)-dihydrozeatin[3] ((—)-6-(4-hydroxy-3-methylbutyl-amino)purine) were isolated from *Zea mays* and *Lupinus luteus*, respectively, These compounds promote cell division in tobacco callus tissue. Kinetin also induces organ formation in this tissue but it is not known whether the naturally occurring cytokinins affect organogenesis in tissue cultures.

Dihydrozeatin is optically active, although kinetin is not; of the two possible enantiomers, one mirror image may be more active than the other. Unfortunately, both optically active dihydrozeatins are not available for study. Therefore, optically active N^6-substituted adenines[4] with asymmetric carbons adjacent to exocyclic nitrogen atoms (Table 1) were tested for their ability to induce organ development in tobacco callus tissue.

TABLE 1. OPTICALLY ACTIVE CYTOKININS



Code name	Compound	R′	R″
R–PE	*R*-(−)-6-(α-methyl-α-phenylmethylamino)purine	$-CH_3$	−Ph
S–PE	*S*-(+)-enantiomer	−Ph	$-CH_3$
S–PA	*S*-(−)-6-(α-hydroxymethyl-β-phenylethylamino)purine	$-CH_2OH$	$-CH_2Ph$
R–PA	*R*-(+)-enantiomer	$-CH_2Ph$	$-CH_2OH$

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[1] C. O. MILLER, F. SKOOG, F. S. OKUMURA, M. H. VON SALTZE and F. M. STRONG, *J. Am. Chem. Soc.* **77**, 2662 (1955); *ibid.* **78**, 1375 (1956).

[2] D. S. LETHAM, *Proc. Chem. Soc.* 230 (1964).

[3] K. KOSHIMIZU, T. KUSAKI, T. MITSUI and S. MATSUBARA, *Tetrahedron Letters* 1317 (1967); K. KOSHIMIZU, S. MATSUBARA and T. MITSUI, *Agric. Biol. Chem.* **31**, 795 (1967).

[4] K. KOSHIMIZU, A. KOBAYASHI, T. FUJITA and T. MITSUI, *Phytochem.* **7**, 1989 (1968).

RESULTS AND DISCUSSION

Letham[5] tested the growth promoting ability of zeatin and kinetin in a number of axenic systems. In all systems zeatin was considerably more effective than kinetin. Table 2 shows the ability of zeatin and (±)-dihydrozeatin to promote callus growth and to induce shoot formation *in vitro* from tobacco callus. For both growth promotion and organ redifferentiation, zeatin and (±)-dihydrozeatin were equally active. Hence, zeatin and (±)-dihydrozeatin, like kinetin, have the ability to induce bud formation from tobacco callus. Nitsch[6] reported that zeatin induced vegetative bud formation on stem segments of *Plumbago indica* L. and was more active than 6-benzylaminopurine.

TABLE 2. EFFECTS OF ZEATIN AND (±)-DIHYDROZEATIN ON CALLUS GROWTH AND SHOOT FORMATION

Concn (M)	Zeatin		(±)-Dihydrozeatin	
	Callus growth	Shoot formation	Callus growth	Shoot formation
5×10^{-9}	+	−	+	−
5×10^{-8}	++	−	+	−
5×10^{-7}	+++	−	+	−
5×10^{-6}	++	+++	++	+++
5×10^{-5}	+	+++	++	+++

The abilities of optically active N^6-substituted adenines to induce shoot formation and to promote callus growth are compared in Table 3. Both *R*-(−)-6-(α-methyl-α-phenylmethylamino)purine (*R*–PE) and *S*-(−)-6-(α-hydroxymethyl-β-phenylethylamino)purine (*S*–PA) induced shoot formation. *R*–PE and *S*–PA were active in inducing shoot formation at concentrations of 4×10^{-5} M and 2×10^{-4} M, respectively. At 4×10^{-5} M of *R*–PE, shoots formed over the surfaces of the calluses. *S*–PA at 2×10^{-4} M also brought about shoot formation on the callus but it was less than that of *R*–PE. In contrast, *S*-(+)-6-(α-methyl-α-phenylmethylamino)purine (*S*–PE) and *R*-(+)-6-(α-hydroxymethyl-β-phenylethylamino)purine (*R*–PA) did not bring about organ development at any of the tested concentrations. The concentrations at which *R*–PE and *S*–PA induced shoot formation were higher than the effective concentrations of zeatin and dihydrozeatin.

TABLE 3. EFFECT OF N^6-(OPTICALLY ACTIVE ALKYL AND ARYLALKYL SUBSTITUTED) ADENINES ON CALLUS GROWTH AND ON REDIFFERENTIATION OF SHOOTS FROM TOBACCO CALLUS

	Compounds	Concn (M) 4×10^{-7}	4×10^{-6}	4×10^{-5}	2×10^{-4}
Callus growth	*R*–PE	++	++	++	+++
	S–PE	++	+	±	±
	S–PA	++	++	++	+++
	R–PA	++	++	++	++
Shoot formation	*R*–PE	−	−	S++++	−
	S–PE	−	−	−	−
	S–PA	−	−	−	S++
	R–PA	−	−	−	−

1. Number of + indicates the degree of callus growth.
2. Number of S+ indicates the degree of shoot formation.

[5] D. S. LETHAM, *Biochemistry and Physiology of Plant Growth Substances* (edited by F. WIGHTMAN and G. SETTERFIELD), p. 19, Runge Press, Ottawa (1968).
[6] C. NITSCH and J. P. NITSCH, *Planta* **72**, 355 (1967).

Although both dextrorotatory and levorotatory compounds induced callus growth (Table 3), only levorotatory structures brought about shoot formation. Since configuration markedly influences the ability to induce differentiation, binding of the cytokinin N^6-group to a receptor site is probably involved in organogenesis.

EXPERIMENTAL

Optically active cytokinins were prepared as reported in a previous paper.[4] Tobacco (*Nicotiana tabacum* var. bright yellow) callus was induced from pith tissue with a concentration of 10^{-5} M of indole-3-butyric acid (IBA) in Linsmaier and Skoog medium.[7] About 40 days after callus induction, this IBA callus was used in the test of zeatin and ($\pm$)-dihydrozeatin and in the test of optically active cytokinins. Shoot formation was observed 28 days after inoculation of callus on Linsmaier and Skoog media containing each optically active cytokinin, and 53 days after inoculation of callus with zeatin and ($\pm$)-dihydrozeatin.

[7] E. M. Linsmaier and F. Skoog, *Physiol. Planta.* **18**, 100 (1965).

Key Word Index—Nicotiana tabacum; Solanaceae; tobacco callus; cytokinins; structure–activity relationships; shoot formation.