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SHORT COMMUNICATION

CYTOKININ ACTIVITIES OF COMPOUNDS RELATED TO ZEATIN*

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Abstract—The growth promoting activities of 17 zeatin analogues, including 13 compounds not previously tested for cytokinin activity, were assessed using the excised radish cotyledon assay. The activities of the analogues indicated that the allylic hydroxyl group, the olefinic double bond and the methyl group are structural features which contribute to the high activity of zeatin. Only one compound, *O*-acetylzeatin, was more active than zeatin. In contrast, *O*-alkylzeatins were much less active than zeatin.

INTRODUCTION

THE PHYTOHORMONE zeatin,[1] a naturally occurring cytokinin, exhibits very high activity in certain cytokinin bioassays.[2–5] A number of analogues of this compound have been synthesized and tested for ability to induce cell division in plant tissue cultures.[6–8] The allylic hydroxyl group and olefinic double bond of zeatin were found to be structural features which are necessary for high activity in these tissue-culture assays. In a bioassay of a different type involving promotion of expansion of excised radish cotyledons, the activities of 17 zeatin analogues, including 13 compounds not previously tested for cytokinin activity, have been determined and are now presented.

RESULTS AND DISCUSSION

The radish cotyledon assay was used to assess the cytokinin activities of 6-(substituted amino)purines related to zeatin by the presence of either a hydroxyl group or unsaturation in the substituent (Table 1). Compounds not previously tested for cytokinin activity are marked by an asterisk in Table 1.

6-Allylaminopurine (I) and 6-(prop-2-ynylamino)purine (VIII) both exhibited only very weak activity. The diallyl (III) and diprop-2-ynyl (VII) compounds possessed activity similar

* Part XIII in the series "Regulators of Cell Division in Plant Tissues". For Part XII see D. S. LETHAM, *Physiol. Plant.* in press.

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TABLE 1. INCREMENTS INDUCED IN COTYLEDON WEIGHT BY ZEATIN ANALOGUES

Compound	Substituent at position 6 of purine	Increment induced in cotyledon weight (%)†	
		10 μM	1 μM
6-Alkenylaminopurines			
6-Allylaminopurine (I)	$CH_2{=}CH{-}CH_2{-}NH{-}$	8	0
6-(*N*-Allyl-*N*-methylamino)-purine* (II)	$CH_2{=}CH{-}CH_2{-}N{-}CH_3$	7	3
6-Diallylaminopurine* (III)	$CH_2{=}CH{-}CH_2{-}N{-}CH_2{-}CH{=}CH_2$	9	2
6-(3-Methylbut-2-enylamino)-purine (IV)	$CH_3{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	73	48
6-(3-Methylpent-*trans*-2-enylamino)purine* (V)	$CH_3{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	66	25
6-Cinnamylaminopurine* (VI)	$Ph{-}CH{=}CH{-}CH_2{-}NH{-}$	11	0
6-Alkynylaminopurines			
6-(Diprop-2-ynylamino)-purine* (VII)	$CH{\equiv}C{-}CH_2{-}N{-}CH_2{-}C{\equiv}CH$	6	0
6-(Prop-2-ynylamino)purine* (VIII)	$CH{\equiv}C{-}CH_2{-}NH{-}$	5	3
6-Hydroxyalkyl- and 6-Hydroxyalkenylaminopurines			
6-(6-Hydroxyhexylamino)-purine* (IX)	$HO{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}NH{-}$	20	3
6-(4-Hydroxy-2-methylbut-*trans*-2-enylamino)-purine* (X)	$HO{-}CH_2{-}CH{=}C(CH_3){-}CH_2{-}NH{-}$	51	34
6-(4-Hydroxy-3-methylbutyl-amino)purine (XI)	$HO{-}CH_2{-}CH(CH_3){-}CH_2{-}CH_2{-}NH{-}$	32	10
6-(5-Hydroxypentylamino)-purine* (XII)	$HO{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}NH{-}$	26	5
Zeatin (XIII)	$HO{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	100	73
O-Substituted Hydroxyalkenylaminopurines			
O-Acetylzeatin (XIV)	$CH_3COO{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	120	110
O-Butylzeatin* (XV)	$CH_3CH_2CH_2CH_2O{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	23	3
O-Ethylzeatin* (XVI)	$CH_3CH_2O{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	29	19
O-Methylzeatin* (XVII)	$CH_3O{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	59	38
O-Propylzeatin* (XVIII)	$CH_3CH_2CH_2O{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}NH{-}$	20	11

* Compounds marked with an asterisk have not been tested previously for cytokinin activity.
† Increments are expressed as a percentage of the increment induced by zeatin at 10 μM.

to that of the monosubstituted analogues (I and VIII). Addition of two methyl groups to the terminal carbon in the allyl substituent of I, yielding 6-(3-methylbut-2-enylamino)-purine (IV), markedly enhanced activity. This compound with a $\gamma\gamma$-dimethylallyl substituent is also highly active in tissue culture assays.[2,4,7] In the radish assay, IV was more effective than the closely related compound V with a γ-ethyl-γ-methylallyl substituent and markedly more active than VI containing a γ-phenylallyl grouping. Hence, in 6-(γ-substituted allylamino)purines, the size of a hydrocarbon substituent in the γ position can markedly affect activity.

As in certain other assays,[2,7] 6-(4-hydroxy-3-methylbutylamino)purine (XI) and 6-(3-methylbut-2-enylamino)purine (IV) were both considerably less effective than zeatin. The compound 6-(4-hydroxy-2-methylbut-*trans*-2-enylamino)purine (X), which differs from zeatin in the position of the methyl group, was also noticeably less active than zeatin. Compound XI, with a γ-methyl group, was more active than the corresponding straight-chain compound XII. These observations indicate that the allylic hydroxyl group, the olefinic double bond and the methyl group are structural features which contribute to the high activity of zeatin in the radish assay.

The activities of a series of *O*-substituted zeatins were determined with the radish cotyledon assay. *O*-Acetylzeatin, which was more active than zeatin, was the most active compound listed in Table 1. Recently *O*-acylzeatins, including *O*-acetylzeatin, have been reported[8] to be slightly more active than zeatin in the tobacco-pith tissue culture assay. The activities of four *O*-alkylzeatins (XV–XVIII) are defined in Table 1. Unlike *O*-acylzeatins, all were less effective than zeatin and activity decreased with increasing size of the substituent. Hydrolysis of *O*-acylzeatins, but not *O*-alkylzeatins, would readily yield zeatin. Possibly because of such hydrolysis within plant tissue, *O*-acylzeatins are more active than *O*-alkylzeatins.

EXPERIMENTAL

The excised radish cotyledon bioassay was performed as previously described.[5,9] Twelve of the 13 compounds (Table 1) not previously tested for cytokinin activity were synthesized by methods already published;[10] the remaining compound (VI) was kindly supplied by Dr. G. Shaw (Bradford).

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Key Word Index—Cytokinin activities, zeatin derivatives; *O*-acetylzeatin.