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6-[SUBSTITUTED AMINO]PURINES: SYNTHESIS BY A NEW METHOD AND CYTOKININ ACTIVITY*

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Abstract—The anion of 9-(tetrahydropyran-2-yl)adenine was reacted with benzylic and allylic halides to yield 6-(substituted amino)-9-(tetrahydropyran-2-yl)purines. The reaction provides a new route from adenine to 6-(substituted amino)purines. The cytokinin activities of a number of new 6-(substituted amino)-9-substituted-purines are reported.

INTRODUCTION

IN RECENT years, 6-(substituted amino)purines have assumed considerable biochemical significance. Some compounds of this type promote plant growth and belong to the group of growth regulators termed cytokinins.[1] In cytokinin bioassays based on induction of cell division in plant tissue cultures, the most active compound is the naturally occurring cytokinin termed zeatin, 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine.[2,3] Cytokinins closely related to zeatin occur as bases in soluble RNA;[4-6] in the serine and tyrosine transfer RNAs of yeast, the cytokinin is adjacent to the anticodon.[7,8] The growth of mammalian cell cultures is inhibited by certain 6-(substituted amino)purines with cytokinin activity.[9]

With stem segments,[10] leaf cuttings [11] and developing grapes,[12] 6-benzylamino-9-(tetrahydropyran-2-yl)purine (I) has been reported to evoke greater growth than the cytokinin 6-benzylaminopurine. In a tissue-culture bioassay, however, 6-benzylaminopurine is slightly more active than I.[3] These observations suggested that for induction of some growth responses, a 9-(tetrahydropyran-2-yl) group is a desirable structural feature. I has been synthesized by acid-catalysed condensation of 6-benzylaminopurine and 2,3-dihydropyran.[13]

* Part IX in the series "Regulators of Cell Division in Plant Tissues"; for Part VIII see D. S. LETHAM and M. W. WILLIAMS, *Physiol. Plantarum*, in press.

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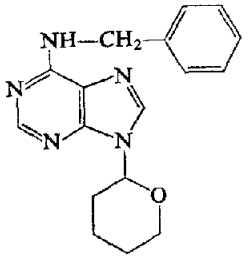
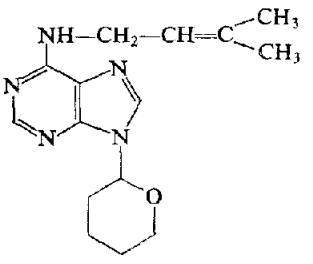
This communication reports the synthesis of 6-(substituted amino)-9-(tetrahydropyran-2-yl)-purines with high cytokinin activity directly from 9-(tetrahydropyran-2-yl)adenine, a compound which can be readily prepared from adenine.[14] The biological activities of some of the resulting new compounds are reported.



(I)



(II)

RESULTS AND DISCUSSION

Unlike adenosine which condenses with 3-methylbut-2-enyl bromide to yield 1-(3-methylbut-2-enyl)-9-β-D-ribofuranosyladenine,[15] 9-(tetrahydropyran-2-yl)adenine was found to react with the allylic bromide to give 1-(3-methylbut-2-enyl)adenine. It was hoped that the 9-tetrahydropyranyl group would not have been cleaved and that the product could then have been rearranged to the 6-(substituted amino)-9-(tetrahydropyran-2-yl)purine. In an attempt to prevent the hydrolysis of the tetrahydropyranyl group, triethylamine or solid sodium carbonate was added to the reaction mixture. Both experiments resulted in complete recovery of the 9-(tetrahydropyran-2-yl)adenine. However if 9-(tetrahydropyran-2-yl)-adenine was converted to the anion form with sodium hydride, potassium *t*-butoxide or preferably sodium ethoxide and then reacted with 3-methylbut-2-enyl bromide, a moderate yield of the new compound 6-(3-methylbut-2-enylamino)-9-(tetrahydropyran-2-yl)purine (II) was obtained. Direct alkenylation of the exocyclic nitrogen atom appeared to have occurred. The other major product isolated from the reaction mixture was 6-bis-(3-methylbut-2-enyl)amino-9-(tetrahydropyran-2-yl)purine. By analogous reactions a number of new 6-(substituted amino)-9-(tetrahydropyran-2-yl)purines have been prepared. Mild acid hydrolysis can be used to cleave the tetrahydropyranyl group to give 6-(substituted amino)-purines unsubstituted at position 9.

Condensation of 3-methylbut-2-enyl bromide with the sodium salt of 6-(acetylamino)-9-(tetrahydropyran-2-yl)purine yielded 6-(*N*-acetyl-*N*-3-methylbut-2-enylamino)-9-(tetrahydropyran-2-yl)purine. Alkaline hydrolysis yielded II, but, on the basis of overall yield, there was no advantage in this alternative method of synthesis.

6-(Substituted amino)purines with cytokinin activity have previously been synthesized from 6-chloropurine,[16] 6-methylthiopurine,[17] and adenine.[18–20] Because adenine is the least

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costly starting material, syntheses based on it are desirable, but the two previously used methods have undesirable features. Thus, reduction of 6-*N*-($\alpha\beta$-unsaturated acyl)adenines proceeds with hydrogenolysis[21,22] and has failed to yield the highly active substituted but-2-enylaminopurines, such as zeatin and 6-(3-methylbut-2-enylamino)purine; exchange amination[20] is only satisfactory with highly stable amines. The reaction described in the present report therefore provides a new useful route from adenine to 6-(substituted amino)-purines, and also yields, as synthetic intermediates, 9-(tetrahydropyran-2-yl) derivatives with high cytokinin activity.

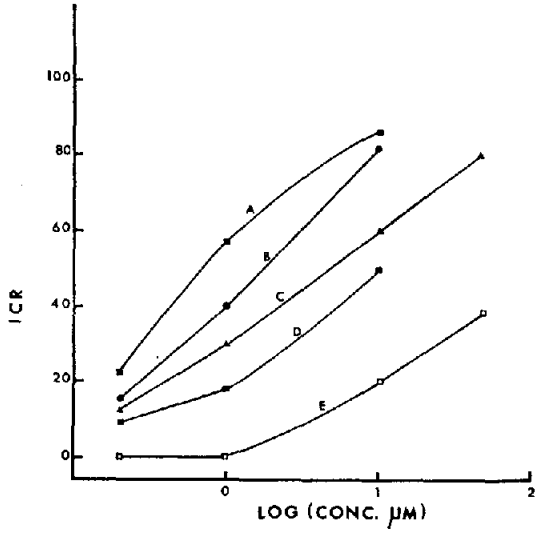

FIG. 1. COMPARISON OF THE ACTIVITIES OF CYTOKININS IN CAUSING CHLOROPHYLL RETENTION IN TOBACCO LEAF DISKS. THE INDEX OF CYTOKININ-INDUCED CHLOROPHYLL RETENTION (ICR, SEE BIOASSAY METHODS) IS PLOTTED AGAINST CONCENTRATION.

A 6-Benzylamino-9-(tetrahydropyran-2-yl)purine.
B 6-(3-Fluorobenzylamino)-9-(tetrahydropyran-2-yl)purine.
C 6-(3-Methylbut-2-enylamino)-9-(tetrahydropyran-2-yl)purine.
D Zeatin.
E 6-(3-Methylbut-2-enylamino)purine.

By the reaction outlined, two new 6-(substituted amino)-9-(tetrahydropyran-2-yl)purines of high cytokinin activity were prepared. These were 6-(3-methylbut-2-enylamino)-9-(tetrahydropyran-2-yl)purine (II) and 6-(3-fluorobenzylamino)-9-(tetrahydropyran-2-yl)-purine (III). When these and related compounds were compared in the tobacco leaf senescence assay, the cytokinins in order of decreasing activity were (see Fig. 1): I, III, II, zeatin and 6-(3-methylbut-2-enylamino)purine. The last-mentioned compound has previously been reported to be only weakly active in this assay;[2] the introduction of a tetrahydropyranyl group to give II is seen to enhance activity markedly. In the excised radish cotyledon assay, the cytokinins in order of decreasing activity were (see Fig. 2): III, I, II and 6-(3-methylbut-2-enylamino)purine. In this assay, I, II and III did not differ greatly in activity, all being highly active and much more effective than kinetin. Zeatin and III were

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approximately equally effective. III was considerably more active than the related new compounds, 6-(2-fluorobenzylamino)-9-(tetrahydropyran-2-yl)purine, 6-(4-fluorobenzylamino)-9-(tetrahydropyran-2-yl)purine and 6-(2-methylbenzylamino)-9-(tetrahydropyran-2-yl)purine.

When applied to the cotyledons of intact radish seedlings (cf. Okumura, Kusaka and Takematsu[23]), II was more effective than 6-(3-methylbut-2-enylamino)purine, but less active than I, in promoting cotyledon enlargement. Application of cytokinin to apple flowers induces development around the calyx of the fruitlets.[24] In induction of this growth in the varieties Jonathan and Sturmer, I and III were more effective than II.

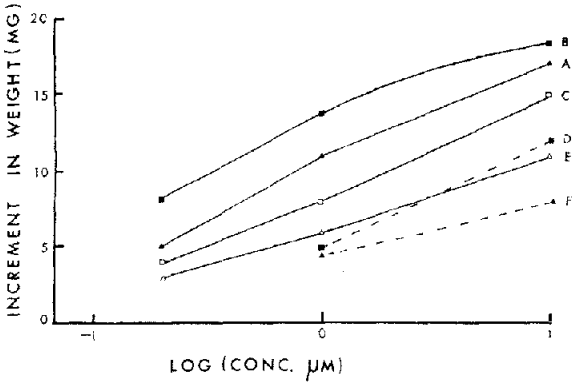

FIG. 2. INCREMENTS INDUCED IN WEIGHT OF EXCISED RADISH COTYLEDONS BY CYTOKININS AT VARIOUS CONCENTRATIONS.

A 6-Benzylamino-9-(tetrahydropyran-2-yl)purine.
B 6-(3-Fluorobenzylamino)-9-(tetrahydropyran-2-yl)purine.
C 6-(3-Methylbut-2-enylamino)-9-(tetrahydropyran-2-yl)purine.
D 9-Methoxymethyl-6-(3-methylbut-2-enylamino)purine.
E 6-(3-Methylbut-2-enylamino)purine.
F 9-Cyclohexyl-6-(3-methylbut-2-enylamino)purine.

The tetrahydropyranyl group is readily cleaved from the purine ring by mild acid hydrolysis and could be subject to chemical and enzymic cleavage in plant tissues. In an attempt to ascertain whether 9-(tetrahydropyran-2-yl)purines are active *per se* or active only after such cleavage, 9-methoxymethyl-6-(3-methylbut-2-enylamino)purine and 9-cyclohexyl-6-(3-methylbut-2-enylamino)purine were synthesized and tested in the excised radish cotyledon assay. The 9-cyclohexyl derivative exhibited appreciable cytokinin activity but was less active than the methoxymethyl derivative which was not as active as II (see Fig. 2). In the carrot-phloem cytokinin bioassay, the 9-cyclohexyl derivative also showed definite activity. Since the cyclohexyl group is resistant to chemical hydrolytic cleavage and is unlikely to be cleaved enzymically, 9-substituted purines probably possess some activity *per se*. However, the chemical lability of the 9-substituent (tetrahydropyranyl > methoxymethyl ≫ cyclohexyl) and cytokinin activity are positively correlated. Hence the responses evoked by compounds such as I, II and III are possibly partly due to the 9-substituted purines themselves, and partly to the action of hydrolysis products with enhanced intracellular activity.

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EXPERIMENTAL

Bioassay Methods

The excised radish cotyledon bioassay[25] and the tobacco leaf disk senescence assay[2] were performed as previously described. In the latter assay, absorptivities of 80% ethanol extracts of the senesced leaf disks were determined at 665 nm. The difference (multiplied by 10^3) between the absorptivity of extract from cytokinin-treated disks and that of extract from water-treated disks was taken as an index of cytokinin-induced chlorophyll retention (termed ICR).

Synthesis of 6-(Substituted amino)-9-(tetrahydropyran-2-yl)purines

NaOEt (1·3 mmoles) was reacted under N_2 with 9-(tetrahydropyran-2-yl)adenine (1 mmole) in 1,2-dimethoxyethane (10–15 ml) purified by distillation from $LiAlH_4$. The reaction mixture was stirred continuously and the solvent slowly distilled to remove the ethanol produced. Fresh dry solvent was distilled in to maintain a constant volume. When no ethanol could be detected in the distillate (GLC), the appropriate halide (1·3 mmole) was added and the mixture was stirred either at room temperature for about 24 hr (allylic halide) or under reflux for about 8 hr (benzylic halide). When TLC indicated the reaction had stopped, the reaction mixture was cooled and filtered. The products were separated by preparative TLC (Merck silica gel, PF_{254}; ether* and then purified by crystallization. M.ps and yields for the two highly active compounds already mentioned were: 6-(3-*methylbut*-2-*enylamino*)-9-(*tetrahydropyran*-2-*yl*)*purine*, m.p. 104·5–105·5°; yield 59 per cent. 6-(3-*fluorobenzylamino*)-9-(*tetrahydropyran*-2-*yl*)*purine*, m.p. 121–122°; yield 37 per cent. Products of the above and related reactions will be detailed in a later paper.

The tetrahydropyranyl group was cleaved in methanol–N HCl (2:1) at room temperature for 48 hr to yield 6-(substituted amino)purines.

Synthesis of 9-Methoxymethyl-6-(3-methylbut-2-enylamino)purine and 9-Cyclohexyl-6-(3-methylbut-2-enylamino)purine

A solution of 6-chloro-9-methoxymethylpurine in dioxan was reacted with a three-fold excess of 3-methylbut-2-enylamine at room temperature for 18 hr. Crystallization of the product from ether gave 9-*methoxymethyl*-6-(3-*methylbut*-2-*enylamino*)*purine* (75 per cent yield), m.p. 91–91·5° (Found: C, 58·2; H, 7·0; N, 28·5; O, 6·3. $C_{12}H_{17}N_5O$ required: C, 58·3; H, 6·9; N, 28·3; O, 6·5 per cent). NMR spectrum ($CDCl_3$): δ 1·74 (6H, broad singlet, $=C(CH_3)_2$), 3·38 (3H, singlet, $-OCH_3$), 4·30 (2H, triplet, $J=6$ c/s, $-NH-CH_2-$), 5·40 (1H, triplet, $J=6$ c/s, $-CH=C$), 5·55 (2H, singlet superimposed on low-field peak of triplet at 5·40, $-CH_2-OCH_3$), 6·30 (1H, broad signal absent in presence of D_2O, $-NH-$), 7·89 (1H, singlet, purine $-CH=$) and 8·48 (1H, singlet, purine $-CH=$) ppm.

Similarly 9-*cyclohexyl*-6-(3-*methylbut*-2-*enylamino*)*purine* was prepared (40–50° for 24 hr) in 65 per cent. yield from 6-chloro-9-cyclohexylpurine. The product, after elution from alumina with ether and crystallization from ether, had m.p. 105–106° (Found: C, 67·1; H, 8·2; N, 25·1. $C_{16}H_{23}N_5$ required: C, 67·3; H, 8·1; N, 24·5 per cent).

* The plates were developed twice with ether.

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