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EFFECT OF SUBSTITUENTS AT THE 9-POSITION ON CYTOKININ ACTIVITY*†

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Key Word Index—*Glycine max*; Leguminosae; soyabean; cytokinin activity; effect of 9-substitution; 6-benzylaminopurine.

Abstract—The preparation and properties of cytokinins substituted in the 9-position by methoxymethyl, propyl and cyclohexyl are described. Each is less active in the soybean and tobacco tests than the unsubstituted cytokinin from which they were derived.

INTRODUCTION

WHETHER or not the 9-position of purines is involved in cytokinin activity subsequent to the entry of cytokinins into the cell is a continuing controversy. A number of 9-substituted cytokinin analogs, including the methyl,[1] butyl,[2] and tetrahydropyran[3] derivatives of 6-benzyl-aminopurine, the methyl derivative of 6-(4-*trans*-hydroxy-3-methyl-2-butenylamino) purine,[4] and the cyclohexyl derivative of 6-(3-methyl-2-butenylamino) purine[5] have been reported to have cytokinin activity equal to or only slightly less than the parent compound. However, it was subsequently demonstrated in this laboratory[6] that in a least one instance the 9-substituent is metabolically removed by the plant tissue within a few minutes of incubation thus casting doubt on the metabolic stability of all such 9-substituted compounds in plant tissues.

By contrast, it has been reported that 6-benzylamino-9-(3-hydroxypropyl) purine[7] is considerably less active than 6-benzylaminopurine. Recently, it was demonstrated that a portion of an exogenously supplied cytokinin is converted to its corresponding 7-glucoside,[8] which exhibits strong cytokinin activity and a high degree of metabolic stability. If glycosidation at the 7 position is required for cytokinin activity, then a metabolically stable, 9-

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substituted cytokinin derivative should be either inactive or act as a cytokinin antagonist. In an effort to find such a compound, we have synthesized and ascertained the cytokinin activity of several substituted derivatives of 6-benzylaminopurine.

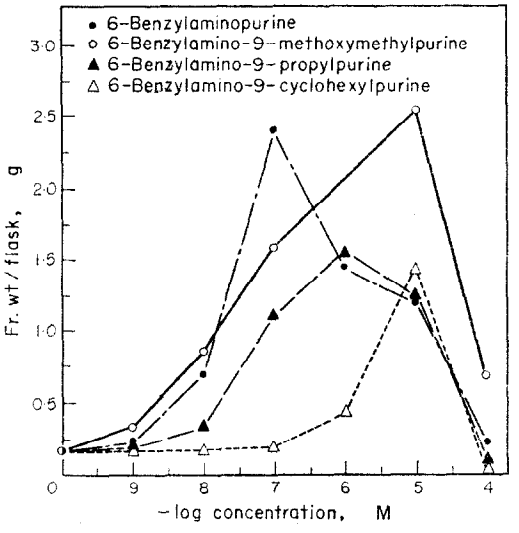

FIG. 1. THE GROWTH OF CYTOKININ REQUIRING SOYBEAN TISSUE ON A SERIES OF 9-SUBSTITUTED CYTOKININS.
Fresh point represents the average fr. wt of four flasks of tissue grown three pieces per flask for 21 days on a standard tissue culture medium (see text).

RESULTS AND DISCUSSION

The cytokinin activities of 6-benzylaminopurine (I), 6-benzylamino-9-methoxymethylpurine (II), 6-benzylamino-9-propylpurine (III) and 6-benzylamino-9-cyclohexylpurine (IV) were compared in the soybean and tobacco tissue bioassays. The results (Fig. 1) indicate that substituents at the 9 position reduce the activity of otherwise active cytokinins. Although the data in Fig. 1 are for soybean tissue, nearly identical results were obtained with two cytokinin requiring strains of tobacco tissue, and the data substantiate earlier reports from this laboratory[6,7] regarding the inhibitory effects of substituents at the 9-position. Compounds II, III and IV all have respectable cytokinin activity but reach their optimum activity only at concentrations ranging from 10 to 100 times greater than the parent compound from which they are derived. In view of the metabolic instability of the 9-methyl analog[6] it may be reasonably supposed that the activity of these compounds is related to the ease with which the tissues convert them to 6-benzylaminopurine (I). The relative lack of activity in IV is surprising in view of the report that the cyclohexyl derivative of zeatin exhibits activity equal to that of zeatin.[5] The difference may be related to a variation from tissue to tissue in the ability to remove substituents at the 9-position of the purine ring.

EXPERIMENTAL

Biological tests. The origin of the soybean and tobacco tissues used here and their growth on various cytokinin levels have been previously reported.[6] Test substances were cold-sterilized by filtration.

Synthesis of test substances. 6-Benzylamino-9-methoxymethylpurine (II) and 6-benzylamino-9-cyclohexyl-purine (IV). Benzylamine was refluxed in H_2O with the appropriate 6-chloro derivative for 6 hr, the

reaction mixture dried *in vacuo*, the product redissolved in EtOH, and twice-recrystallized from 50% EtOH. At this stage the yields were 65% (II) and 90% (IV). For further characterization and biological assay the products were further purified by sublimation. IR and MS confirmed their identity. (II) m.p. 118–8·5°, λ_{max} pH 6·0 H_2O 270 nm, λ_{max} pH 1·0 HCl 265·5 nm, λ_{max} pH 11·0 NH_4OH 270 nm (*Anal.* Calcd. for $C_{12}H_{15}N_5O$: C, 62·45; H, 5·58; N, 26·02. Found: C, 62·38; H, 5·81; N, 26·32%. (IV) m.p. 132–2·5° λ_{max} pH 6·0 H_2O 273 nm, λ_{max} pH 1·0 HCl 271 nm, λ_{max} pH 11·0 NH_4OH 273 nm (*Anal.* Calcd. for $C_{16}H_{21}N_5$: C, 70·35, H, 6·84; N, 22·80. Found: C, 69·61; H, 6·85; N, 22·66%).

6-*Benzylamino*-9-*propylpurine* (III) was synthesized from the potassium salt of 6-benzylamino-purine and iodopropane in a manner analogous to that previously described for the methyl derivative.[6] The product was purified by TLC on alumina as previously described[6] and recrystallized from 50% EtOH (34% yield). IR and MS studies confirmed the identification of this compound. (III) m.p. 112–3°, λ_{max} pH 6·0 H_2O 273 nm, λ_{max} pH 1·0 HCl 271 nm, λ_{max} pH 11·0 NH_4OH 273 nm (*Anal.* Calcd. for $C_{13}H_{17}N_5$: C, 67·41; H, 6·37; N, 26·27; Found: C, 65,36; H, 6·53; N, 25·49%).