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SHORT COMMUNICATION

# CYTOKININ ACTIVITY OF $N^6$,$O^{2'}$-DIBUTYRYL CYCLIC AMP AND $N^6$-BUTYRYLADENINE

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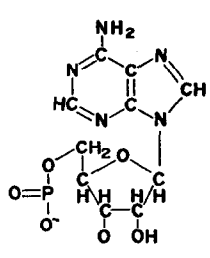
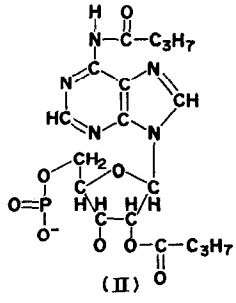
**Abstract**—Adenosine 3',5'-cyclic monophosphate (cAMP) and $N^6$,$O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP) were tested for relative growth promoting activity in the soybean callus bioassay. In contrast to cAMP, DBcAMP showed cytokinin activity. $N^6$-butyryladenine and DBcAMP were found to be equally active. Cytokinin activity of DBcAMP apparently does not depend on the ribosyl 3,5-cyclic monophosphate moiety of the molecule but is determined by the $N^6$-butyryl side chain. In a series of $N^6$-acyladenines ($C_2$–$C_6$) the optimum activity was obtained with a chain length of 4 carbon atoms.

## INTRODUCTION

ADENOSINE 3',5'-cyclic monophosphate (cAMP,I) acts as a mediator of several hormone-induced changes in vertebrates and invertebrates,[1,2] the hormones stimulating the enzyme, adenyl cyclase, to form cAMP from ATP. In recent years the possible role of cAMP as a mediator in plant hormone effects has been investigated. Adenosine diphosphate and cAMP can mimic gibberellin (GA) action in promoting α-amylase synthesis in barley aleurone layers[3,4] and GA stimulates the incorporation of radioactively labeled adenine into cAMP.[5] Similarly in Bengal gram seeds, in the presence of IAA the incorporation of 8-$^{14}$C-adenine into cAMP was twice that of the control without IAA.[6] In addition, auxin induced growth



(I)



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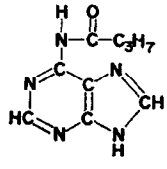

(III)

* Part of the work has been carried out at the Centre for Plant Physiological Research, Wageningen.

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of Jerusalem artichoke tuber tissue by 2,4-dichlorophenoxyacetic acid (2,4-D) is known to be synergistically accelerated by addition of GA or cAMP.[7]

Acyl derivatives of cAMP substituted at the 6-amino and 2'-*O*-positions (II) were often found to be more active than the parent compound when applied to intact animal cells. Recently, it has been found that $N^6$,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (DBcAMP) (II) has a stronger effect than cAMP in simulating the action of GA in Jerusalem artichoke tuber.[7] The derivatives are more resistant to the action of phosphodiesterase than the parent compound[8] and it has been assumed that they penetrate cell membranes more easily; properties which would explain their greater potency. Our interest in cytokinins,[9] plant hormones which stimulate cell division, prompted us to consider possible effects of cAMP, DBcAMP and $N^6$-butyryladenine (III) in the soybean tissue culture.

## RESULTS AND DISCUSSION

In contrast to 6-(furfurylamino)purine (kinetin), cAMP assayed in a concentration range of $10^{-5}$ to 1 mM did not show a significant stimulation of callus growth. Filter sterilization of cAMP before addition to the medium did not change this negative result. DBcAMP, however, stimulated growth vigorously. Maximum yield was found to be equal to that obtained with kinetin. However, the optimum concentration ($5 \times 10^{-2}$ mM) for DBcAMP was 50 times that for kinetin. The great difference between the activity of cAMP and DBcAMP may, as in animal systems, be explained in terms of differences in penetration into the soybean cells. However, it is well known that naturally occurring cytokinins which have been isolated and identified so far from plants, animals and bacteria are $N^6$-substituted adenines.[10] Therefore the effect of $N^6$-butyryladenine (III) was studied in the soybean callus assay. From the results shown in Fig. 1 it is clear that $N^6$-butyryladenine and DBcAMP show equal growth activity. Similar results have recently been obtained by Berridge *et al.*[11] In contrast to cAMP, DBcAMP and $N^6$-butyryladenine stimulated cell enlargement of Chinese cabbage leaf discs.

To determine the effect of the length of the $N^6$-side chain on cytokinin activity, a series of $N^6$-acyladenines were tested in the soybean callus assay. Propionyladenine was found to be far less active than butyryladenine. At low concentrations, valeryladenine stimulated growth better than butyryladenine but the amount of tissue produced by butyryladenine at its optimum concentration was markedly higher than that produced by valeryladenine (Fig. 2). Acetyladenine and caproyladenine had negligible activity. Thus at its optimum concentration butyryladenine was found to be superior to all other analogs tested so far.

Cytokinin activity of purine compounds with a carbonyl group directly attached to the 6-amino group had earlier been reported only for 6-ureidopurines.[12,13]

Apparently cytokinin activity of (DBcAMP) is associated primarily with the presence of a butyryl side chain attached to the 6-amino position of adenine and does not depend on the presence of the ribosyl 3,5-cyclic monophosphate group with the butyryl chain at the 2-*O* position. Whether the latter group is split from DBcAMP in the plant is not known.

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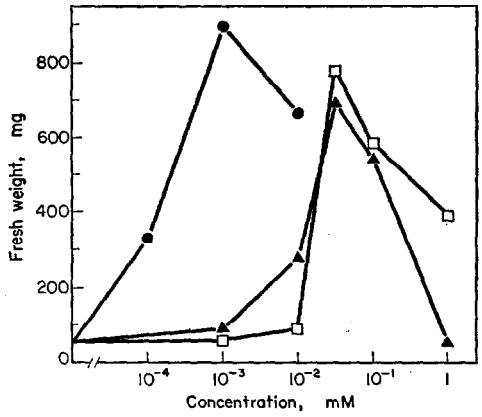


FIG. 1. THE EFFECT OF A CONCENTRATION RANGE OF DBcAMP, KINETIN AND OF $N^6$-BUTYRYLADENINE ON GROWTH OF SOYBEAN CALLUS.
Average fresh weight of duplicate experiments after a growth period of 4 weeks. ●—kinetin; □—DBcAMP; ▲—$N^6$-butyryladenine.

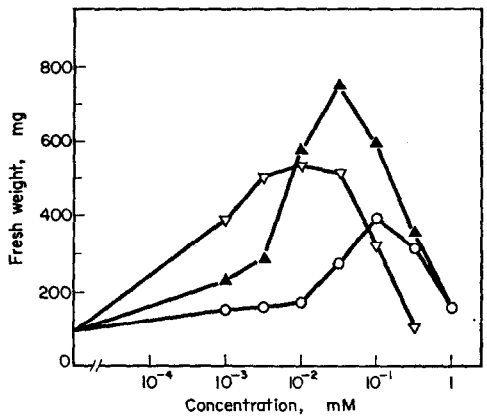


FIG. 2. THE EFFECT OF A CONCENTRATION RANGE OF $N^6$-ACYLADENINES ON GROWTH OF SOYBEAN CALLUS.
Average fresh weight of duplicate experiments after a growth period of 4 weeks. ○—$N^6$-propionyladenine; ▲—$N^6$-butyryladenine; ▽—$N^6$-valeryladenine.

According to Swislocki[14] DBcAMP decomposes in buffers in part into cAMP and $N^6$-butyryl cAMP.

As has already been reported, growth of Jerusalem artichoke tuber tissue effected by 2,4-D can be accelerated by addition of GA or cAMP. The fact that DBcAMP has a greater effect than cAMP may well be due to the combination of an auxin (2,4-D) together with that of a cytokinin (DBcAMP).

The present results indicate that the activity of DBcAMP in stimulating plant cell division in the presence of NAA and its ability to mimic hormone effects in animal systems do not depend on the same chemical property of the molecule. The presence of the acylamino group appears to be a prerequisite for obtaining a cytokinin effect in the soybean callus assay, whereas the ribosyl 3,5-cyclic monophosphate group is required for the activity in animal systems. However, $N^6$-substituted derivatives of cAMP are often far more active than unsubstituted cAMP in animal systems.[1,8,15,16] Henion *et al.*[15] tested $N^6$-acetyl, $N^6$-caproyl, $N^6$-caprylyl and $N^6$-dodecanoyl derivatives of cAMP. The $N^6$-butyryl derivative was found to be the most active compound in the activation of liver glycogen phosphorylase *in vitro*, whereas the $O^{2'}$-butyryl derivative of cAMP was far less active. It is known that the $N^6$-substituted compounds are more resistant to inactivation by phosphodiesterase.[8] It is, however, conceivable that, secondary to the ribosyl 3,5-cyclic monophosphate group, the $N^6$-butyryl side chain plays also another, so far unknown, role in evoking the physiological effects of DBcAMP in animals. It may therefore be worthwhile to compare the effect of DBcAMP with that of $N^6$-butyryladenine in both animal and plant systems.

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## EXPERIMENTAL

*Bioassay procedures.* Cytokinin activity was determined in the soybean callus bioassay.[17] The agar medium supplemented with 2 ppm α-naphthaleneacetic acid (NAA) was sterilized by heating twice at 100° for 30 min. One day was allowed between treatments. In a few experiments cAMP was filter sterilized and added to the cooling agar media.

*Synthesis of* $N^6$*-acyladenines.* $N^6$-Acyladenines were prepared from adenine and the appropriate acid anhydrides.[18] $N^6$-Acetyladenine was crystallized from glacial HOAc, the other $N^6$-acyladenines were crystallized twice from EtOH. Purity was checked by TLC and NMR spectroscopy.

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*Key Word Index—Glycine soja*; Leguminosae; bioassay; cyclic adenosine monophosphate; butyryladenine; kinetin.