

The activity of deaza- and aza-purine analogues of cytokinins in gametophore induction in protonema cultures of *Funaria hygrometrica*

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Abstract

Cytokinin activities of the 1-deaza-, 3-deaza-, 1-deaza-8-aza- and of 3-deaza-8-aza-purine analogues of kinetin and of 6-(3-methyl-2-butenylamino) purine were determined in gametophore induction in protonema cultures of *Funaria hygrometrica* and compared with those of the parent compounds. A relatively high activity of the 1-deaza-analogues and very low activity of the 3-deaza-analogues were found. The results confirm the suggestion that the nitrogen in the 3-position of the purine ring is more important with regard to the cytokinin activity of a compound than the nitrogen in the 1-position.

INTRODUCTION

The cytokinin activity of N⁶-substituted adenines and derivatives is influenced by the structure of the side chain, its position on the ring and the purine ring itself (Skoog et al. 1967, 1973, 1975, Torigoe et al. 1972, Rogozińska et al. 1973, Hecht et al. 1975, Henderson et al. 1975, Sugiyama et al. 1975). The modification of the heterocyclic nucleus reduces to various degree the activity as compared with intact adenine cytokinins. As shown by Rogozińska et al. (1973), the 1-deaza-cytokinin analogues have relatively high activity and the activity of those with substitution of nitrogen by carbon in the 3-position is very low. It was suggested that the nitrogen in the 3-position must be of particular importance with regard to cytokinin activity.

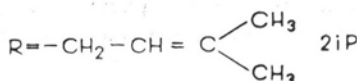
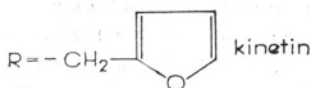
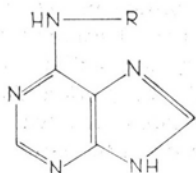
A survey using a second test system for cytokinin activity based on gametophore bud formation in the moss protonema (Hahn and Bopp

1968, Brandes and Kende 1968, Szweykowska et al. 1969) seemed to be of interest for the comparison of the cytokinin activities of 1-deaza-, 1-deaza-8-aza-, 3-deaza- and 3-deaza-8-aza-analogues of kinetin and of 6-(3-methyl-2-butenylamino) purine (2iP) tested previously with the tobacco bioassay (Rogozińska et al. 1973).

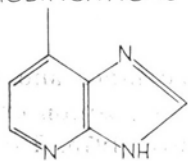
MATERIAL AND METHODS

Small samples of spores of the moss *Funaria hygrometrica* (L.) Sibth. were spread under sterile conditions on cellophane disks placed on the surface of a mineral medium (Szweykowska et al. 1971) supplied additionally with 1% glucose and solidified with 0.7% agar. After five days, the excess of germinating spores was removed, leaving approximately 1 spore/4 cm². Protonemata of about 5 mm diameter (after about 20 days of growth under light of ca. 1000 lux and a temp. of ca. 25°C) were transferred on mineral solutions with the addition of test substances (Fig. 1): the 1-deaza-, 3-deaza-, 1-deaza-8-aza- and 3-deaza-8-aza-analogues of kinetin and of 6-(3-methyl-2-butenylamino) purine (2iP), resp., at concs. of 0.01 — 500 µM. As controls served incubations with kinetin at 0.5 µM,

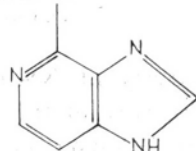
THE CYTOKININS



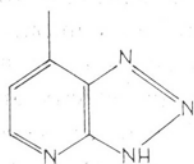
MODIFICATIONS IN THE PURINE RING:



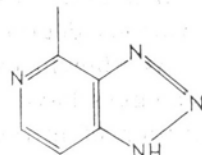
1-deaza-analogue



3-deaza-analogue



1-deaza-8-aza-
-analogue



3-deaza-8-aza-
-analogue

Fig. 1. The compounds tested for cytokinin activity

with 2iP at $0.05 \mu\text{M}$, and without any additions (basal control). After 3 days, the protonemata were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer of pH 7.4 for 24 h. They were then stored in phosphate buffer at 4°C . Gametophore buds were counted under a microscope.

The bioassays were 3 times repeated.

RESULTS AND DISCUSSION

No buds were ever present in the basal control, whereas numerous buds appeared in media with kinetin and 2iP, resp.

All analogues of kinetin were less active than their parent compound (Fig. 2). The activity of 1-deaza-kinetin was about 6 times lower from

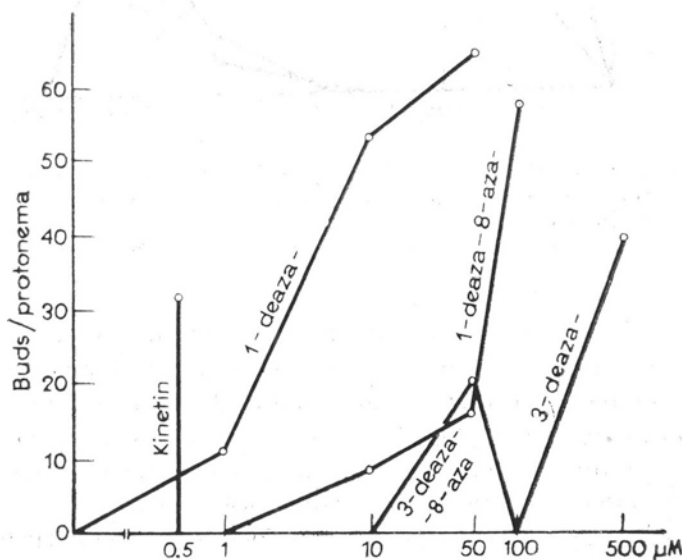


Fig. 2. Comparison of the effects of kinetin and its four analogues on gametophore bud initiation in the protonema of *Funaria hygrometrica*

The compounds are indicated as in Fig. 1. Bar represents the activity of kinetin. Conditions of bioassay as described under „Material and methods”. Test period: 12–15 July, 1975.

that of kinetin. The replacement of 8-C atom by N in 1-deaza-8-aza-kinetin led to further lowering of activity by a factor of about 7. The 3-deaza-analogue was about 1000 times less active than kinetin. It showed bud-inducing activity only at as high a concentration as $500 \mu\text{M}$. The activity of the 3-deaza-8-aza-kinetin started at 10 times lower concentration ($50 \mu\text{M}$), but was completely reduced at $100 \mu\text{M}$.

The 2iP is a more active cytokinin than kinetin and so were its analogues (Fig. 3). Besides that, the activity of 2iP was less sensitive to

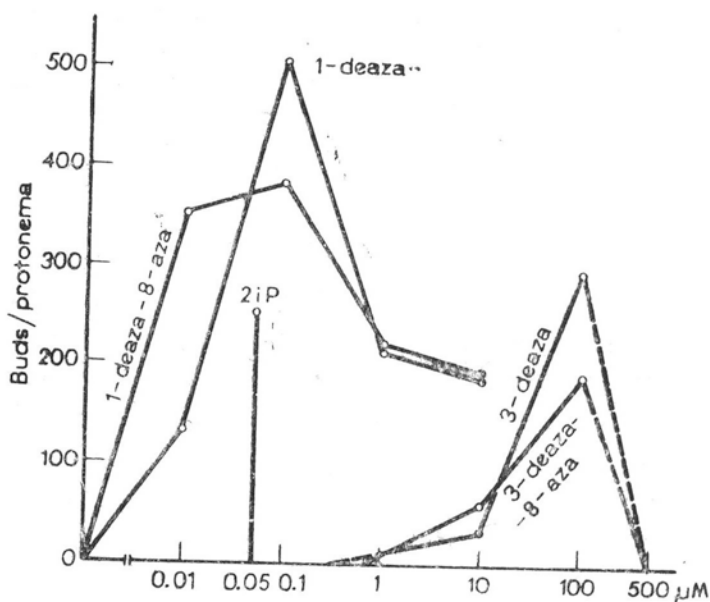


Fig. 3. Comparison of the effects of 2iP and its four analogues on gametophore bud initiation in the protonema of *Funaria hygrometrica*

The compounds are indicated as in Fig. 1. Bar represents the activity of 2iP. Conditions of bioassay as described under „Material and methods”. Test period: 28–31 May, 1975

modifications in the purine nucleus. In fact, activities of the 1-deaza- and 1-deaza-8-aza-analogue, resp., appeared even to be higher than that of the parent compound. Both analogues induced bud formation at 0.01 μM, with a maximum at 0.1 μM. There was no significant lowering of the activity by introducing the second modification to the 1-deaza-2iP in form of substitution the 8-C with N in the 1-deaza-8-aza-2iP.

The 3-deaza- and 3-deaza-8-aza-analogues were about 2000 times less active than 2iP. There was again not much difference between the two analogues. Their activity increased from 1 to 100 μM; the concentration of 500 μM showed toxic effects, with the protonema becoming completely white.

In general, kinetin was found to be more sensitive to modifications in the purine ring than was the natural cytokinin, 2iP. The results also showed good activity of the 1-deaza- and 1-deaza-8-aza-compounds, whereas the activity of 3-deaza- and 3-deaza-8-aza-compounds was relatively low. This is in agreement with the results obtained using the tobacco bioassay (Rogozińska et al. 1973), though the sensitivity of the *Funaria* test is about 5 times lower. One can assume that the analogues have similar receptor sites for affecting bud formation in mosses and in initiating cell divisions in tobacco callus. In a simplest model system, the cytokinin and a receptor in the cell are supposed to

interact, and the complex so formed (if active) initiates the response. The observed low activity of the 3-deaza- and 3-deaza-8-aza-analogues may result from steric changes in the molecules preventing them from assuming the proper conformational relationship with the receptor. The 1-deaza- in comparing with the 3-deaza-cytokinin-analogues seem to have the features more suitable for attachment and for the formation of an active complex.

The conclusion that a nitrogen atom at the 3-position in the purine ring plays an important role in conferring high cytokinin activity was recently supported by Sugiyama et al. (1975). Since cytokinins have been found to bind with ribosomes (Berridge et al. 1970, Fox and Erion 1975), it may be possible that the nitrogen in the 3-position is more important with regard to this binding than the nitrogen in the 1-position.

We are indebted to professor C. A. Salemink for the supply of the deaza- and deaza-aza-analogues synthesized by dr C. Kroon at the Organic Chemistry Laboratory, State University, Utrecht.

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*Aktywność deaza- i aza-purynowych analogów cytokinin w indukowaniu
gametoforów w kulturach splotka Funaria hygrometrica.*

Streszczenie

Oznaczano aktywność cytokininową 1-deaza-, 3-deaza-, 1-deaza-8-aza- i 3-deaza-8-aza-purynowych analogów kinetyny i 6-(3-metylo-2-butenylamino) puryny w indukowaniu gametoforów w kulturach splotka *Funaria hygrometrica* i porównywano ją z aktywnością macierzystych cytokinin. Stwierdzono stosunkowo wysoką aktywność 1-deaza-analogów i bardzo niską aktywność analogów 3-deaza-purynowych. Wyniki potwierdzają przypuszczenie, że azot w położeniu 3-im pierścienia purynowego ma większe znaczenie dla aktywności cytokininowej niż azot w położeniu 1-ym.