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SHORT REPORTS

CYTOKININ ACTIVITY OF DISCADENINE: A SPORE GERMINATION INHIBITOR OF *DICTYOSTELIUM DISCOIDEUM*

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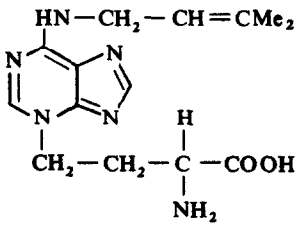
Key Word Index—*Dictyostelium discoideum*; slime mold; biological activity; cytokinins; discadenine.

Abstract—Discadenine, 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine, a spore germination inhibitor of the cellular slime mold *Dictyostelium discoideum* showed cytokinin activity in the tobacco callus bioassay.

INTRODUCTION

Spore germination of *Dictyostelium discoideum* is inhibited by compounds present in this slime mold [1–3]. Recently we have determined the structure of one inhibitor named discadenine as 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine [4]. This inhibitor is the first natural purine derivative possessing an α-amino acid residue on the *N*-3 position of the purine ring. This substance is a potent inhibitor of the spore germination of this organism and showed 100% inhibition at a concentration of 40 ng/ml. Cytokinins, an important class of plant growth hormones, generally have the N^6-alkyladenine moiety. Therefore, it is conceivable that discadenine has cytokinin or anti-cytokinin activity [5]. In this report, we show that discadenine has cytokinin activity, as determined on the basis of fr. wt yields in the tobacco callus bioassay.



RESULTS AND DISCUSSION

Figure 1 clearly shows that discadenine has potent cytokinin activity. At 5×10^{-7} M, its ability to form callus is *ca* one sixth that of kinetin. However, when the concentration of discadenine was increased to 1.6×10^{-5} M, its activity exceeded the highest activity of kinetin observed at 5×10^{-7} M, although the activity of discadenine is only about 1% that of N^6-isopentenyladenine, the corresponding compound without the 3-substituent. Our data concerning cytokinin activity of kinetin is consistent with that reported by Skoog *et al.* [6]. Skoog, Leonard and their collaborators also concluded that substitution at the 3 position on the purine ring strongly diminishes cytokinin activity on comparing cytokinin activities of 40 adenine derivatives. The discrepancy between their conclusions and our findings, which show that a 3 substituted adenine derivative has a potent cytokinin activity, can be interpreted as follows. Firstly Skoog *et al.* used 3-methyl-6-benzylaminopurine and 3-benzyl-6-benzylaminopurine as a 3 substituted model compound. On the other hand, discadenine is a N^6-isopentenyladenine derivative. In the case of N^6-isopentenyladenine derivatives, substitution at the 3

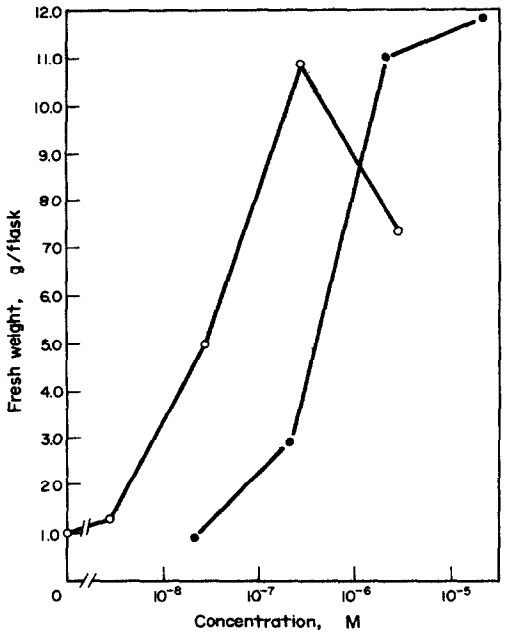


Fig. 1. Effects of discadenine and kinetin on growth of tobacco callus, ●——●; discadenine, ○——○; kinetin.

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position still allows a relatively high cytokinin activity. In this connection, it is of interest to note that in our preliminary experiment 3-methyl-N^6-isopentenyladenine that was chemically synthesized was as active as kinetin. Secondly, discadenine may be converted to N^6-isopentyladenine in the callus tissue by some unknown enzymatic systems. However, we presume that this latter possibility is very unlikely. Earlier [5], we showed that discadenine inhibits spore germination of *D. discoideum* at concentrations of *ca* 10^{-8} M. In the case of chemically synthesized zeatin and N^6-isopentenyladenine inhibition occurred at *ca* 10^{-5} M. These results suggest that substitution of the purine skeleton at the 3 position, possibly with an amino acid, is necessary for high inhibitory activity against spore germination. It was suggested that this inhibitor may act on the plasma membrane of the spores [5]. In this context, it should be noted that LéJohn and his associates reported that N^6-isopentenyladenine regulates uptake of amino acids, Ca^{2+}, nucleosides and glucose in the water mold *Achlya* [7, 8]. It was also shown that N^6-isopentenyladenine binds to a glycopeptide which is localized on the cell membrane of the sporangiospores of this organism [9].

EXPERIMENTAL

Discadenine was isolated from *Dictyostelium discoideum* (NC-4) by the procedure of refs [4, 10]. Discadenine crystallized twice was used in this expt. Callus, derived from the pith of tobacco plant (*Nicotiana tabacum* var. Wisconsin No. 38), was cultured aseptically on the revised agar medium (RM-1962) of ref. [11] with 2 mg/l. of IAA and the discadenine soln. The inhibitor soln was added through a sterilized millipore filter syringe to avoid breakdown by heating. Three pieces of callus, each *ca* 50 mg, were planted per flask with 20 ml of agar medium and 5 flasks were used for each expt. These flasks were incubated at 30° in the dark for 30 days and fr. wt yields of callus were determined.

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REFERENCES

1. Snyder, H. M. and Ceccarini, C. (1966) *Nature* 209, 1152.
2. Cotter, D. A. and Raper, K. B. (1966) *Proc. Natl. Acad. Sci. U.S.* **58**, 880.
3. Ceccarini, C. and Cohen, A. (1967) *Nature* **214**, 1345.
4. Abe, H., Uchiyama, M., Tanaka, Y. and Saito, H. (1976) *Tetrahedron Letters* 3807.
5. Tanaka, Y., Hashimoto, Y., Yanagisawa, K., Abe, H. and Uchiyama, M. (1975) *Agr. Biol. Chem. (Japan)* **39**, 1929.
6. Skoog, F., Hamzi, H. Q., Szweykowska, A. M., Leonard, N. J., Carraway, K. L., Fujii, T., Helgeson, J. P. and Loeppky, R. N. (1967) *Phytochemistry* **6**, 1169.
7. LéJohn, H. B., Cameron, L. E., Stevenson, R. M. and Meuser, R. U. (1974) *J. Biol. Chem.* **249**, 4016.
8. Singh, D. P. and LéJohn, H. B. (1975) *Can. J. Biochem.* **53**, 975.
9. LéJohn, H. B. and Cameron, L. E. (1973) *Biochem. Biophys. Res. Commun.* **54**, 1053.
10. Obata, Y., Abe, H., Tanaka, Y., Yanagisawa, K. and Uchiyama, M. (1973) *Agr. Biol. Chem. (Japan)* **37**, 1989.
11. Murashige, T. and Skoog, F. (1962) *Physiol. Plantarum* **15**, 473.

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α-METHYLENE-γ-AMINOBUTYRIC ACID FROM *MYCENA PURA**

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Key Word Index—*Mycena pura*; Tricholomataceae; Basidiomycetes; α-methylene-γ-aminobutyric acid.

Abstract—α-Methylene-γ-aminobutyric acid was isolated and characterized from fruit bodies of *Mycena pura*. It was the decarboxylation product of L-γ-methyleneglutamic acid by L-glutamic acid decarboxylase.

Previously we reported the isolation of 3 unsaturated amino acids, L-γ-methylene-, L-γ-ethylidene- and L-γ-propylideneglutamic acid from fruit bodies of *Mycena pura* (Fr.) Kummer [1]. During this work we observed the presence of another unsaturated amino acid (ninhydrin-yellow) in the neutral amino acid-fraction.

The result of elementary analysis, as well as the PMR spectrum of the isolate suggested the structure, α-methylene-γ-aminobutyric acid, which has been reported in groundnut (*Arachis hypogaea*) [2]. Our isolate was, as expected from the above structure, optically inactive and oxidation with $KMnO_4$ gave β-alanine.

For direct comparison, we prepared α-methylene-γ-aminobutyric acid from L-γ-methyleneglutamic acid by L-glutamic acid decarboxylase. Mp, IR, colour reaction with ninhydrin, as well as chromatographic behaviour of the decarboxylation product were in good agreement with those of the natural amino acid.

EXPERIMENTAL

Fungal material. The fruit bodies of *Mycena pura* (Fr.) Kummer were collected in Sept. 1974 in Nagano Prefecture. Voucher specimens are deposited in the Department of Biology, College of General Education, The University of Tokyo.

Isolation of amino acid. Fruit bodies (9.4 kg) were crushed and extracted × 4 with 80% EtOH and the filtered extract (80 l.) was treated with a column of Amberlite IR-120 (H^+, 1 l.). After the resin was washed with aq. EtOH and H_2O, successively, the amino acids were eluted with 2M NH_4OH (14 l.).

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