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CYTOKININ ACTIVITY OF *N*-PHENYL-*N'*-1, 2, 3-THIADIAZOL-5-YLUREA (THIDIAZURON)

M. C. MOK, D. W. S. MOK, D. J. ARMSTRONG,* K. SHUDO,† Y. ISOGAI‡ and T. OKAMOTO†

Department of Horticulture and Genetics Program and *Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, U.S.A.; †Laboratory of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan; ‡Department of Biology, College of General Education, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

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Key Word Index—*Phaseolus lunatus*; Leguminosae; plant tissue culture; Dropp; SN 49537; *N,N'*-diphenylurea; *N*-phenyl-*N'*-4-pyridylurea; zeatin.

Abstract—The plant growth regulator *N*-phenyl-*N'*-1, 2, 3-thiadiazol-5-ylurea (Thidiazuron) displayed high activity in promoting the growth of cytokinin-dependent callus cultures of *Phaseolus lunatus* cv. Kingston. The cytokinin activity of Thidiazuron was similar to that of the highly active *N*-phenyl-*N'*-4-pyridylurea derivatives and to the most active cytokinins of the adenine type. Replacement of the phenyl ring of Thidiazuron with other ring structures resulted in a decrease in cytokinin activity.

INTRODUCTION

The ability of *N,N'*-diphenylurea and related compounds to substitute for cytokinin-active adenine derivatives has been demonstrated in callus culture bioassays[1–6] as well as in cytokinin bioassays based on chlorophyll retention, bud development, and seed germination[7]. Diphenylurea is a rather weakly active cytokinin[1, 3, 6], but particular derivatives of *N*-phenyl-*N'*-4-pyridylurea exhibit cytokinin activity equal to or exceeding that of zeatin in the tobacco callus bioassay[8]. Thus, cytokinin activity is a property of certain types of substituted urea compounds as well as of N^6-substituted adenine derivatives, and highly active cytokinins may be obtained with either type of structure.

The compound *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (Thidiazuron, Dropp, SN 49537; Fig. 1) is known to be a plant growth regulator and has been used as a cotton defoliant in experimental tests (Nor-Am Agricultural Products, Inc., Technical Information Bulletin, 1976). However, the biological properties of this compound have not previously been reported to include cytokinin activity. As part of a continuing study of cytokinin metabolism in *Phaseolus* species[4, 9–11], we have examined the effects of Thidiazuron and a number of other substituted urea compounds on the growth of cytokinin-dependent callus tissues of *Phaseolus lunatus* cv. Kingston. We report here that Thidiazuron is a highly active cytokinin with activity in this test system comparable with that of the *N*-phenyl-*N'*-4-pyridylurea derivatives and of the most active cytokinins of the adenine type.

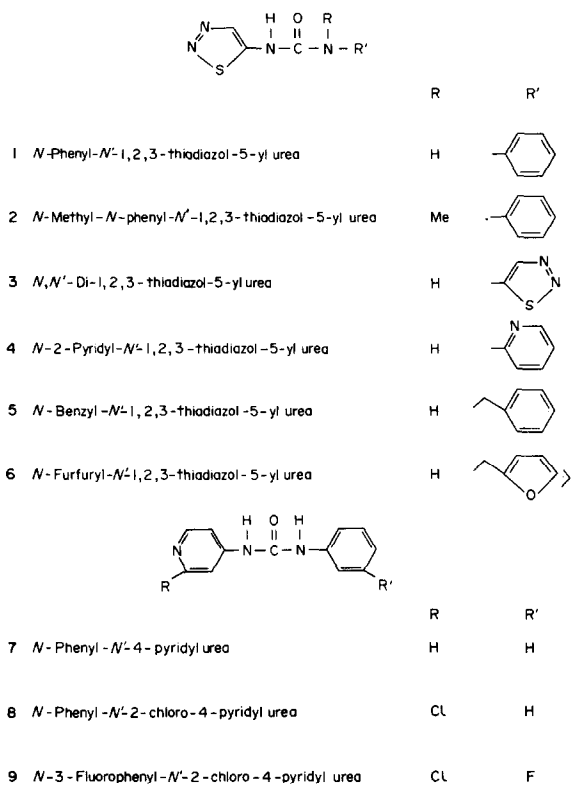

Fig. 1. Chemical structures of thiadiazolylurea- and 4-pyridylurea-derivatives tested for cytokinin activity.

RESULTS AND DISCUSSION

We have earlier reported a comparison of the relative activities of eight cytokinin-active adenine derivatives in promoting the growth of callus tissues of *P. lunatus*[10]. Among these compounds, zeatin was the most active, and it has been included in the present tests as a convenient reference compound.

The effects of Thidiazuron and several other thiadiazolylurea derivatives on the growth of callus cultures of *P. lunatus* cv. Kingston are shown in Fig. 2. The cytokinin activity of Thidiazuron (**1**) was the

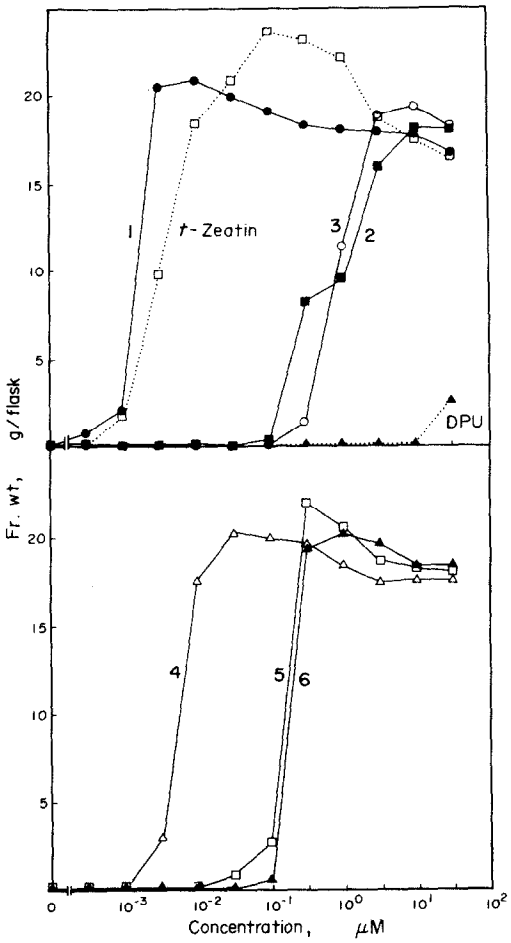

Fig. 2. Comparison of cytokinin activity of thiadiazolylurea derivatives and zeatin.

highest of the thiadiazolylurea derivatives tested and exceeded that of zeatin. Replacement of the phenyl group of Thidiazuron with other ring structures resulted in a reduction in activity in the following order: phenyl (**1**) > 2-pyridyl (**4**) > benzyl (**5**) = furfuryl (**6**) > thiadiazolyl (**3**) ring. The 2-pyridyl derivative was *ca* three-fold less active than Thiadiazuron, and the corresponding benzyl- and furfuryl-derivatives were *ca* 100-fold less active. Although some benzylurea derivatives have been reported to act as cytokinin antagonists [12], this is not the case in the thiadiazolylurea series. The symmetrical *N,N'*-dithiadiazolylurea (**3**) was the least active compound of the series (*ca* 300- to 1000-fold less active than Thidiazuron), but even this compound was considerably more active than *N,N'*-diphenylurea (DPU). Finally, methylation of the urea bridge of Thidiazuron (**2**) resulted in a marked reduction in cytokinin activity.

The *N*-phenyl-*N'*-4-pyridylurea derivatives that exhibit high cytokinin activity in the tobacco callus bioassay [8, 13] had not previously been tested in the *P. lunatus* callus system, and it was of interest both to examine their activities in this test system and to compare their activities to that of Thidiazuron. The results of tests with these compounds are shown in Fig. 3. The relative activities of the three pyridylurea

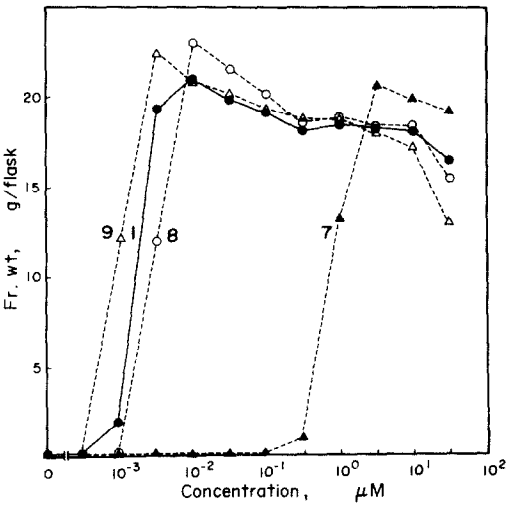

Fig. 3. Comparison of cytokinin activity of 4-pyridylurea derivatives and Thidiazuron.

derivatives were the same as in the tobacco callus bioassay [8, 13]. The compound *N*-3-fluorophenyl-*N'*-2-chloro-4-pyridylurea (**9**) was the most active cytokinin tested. The corresponding compound with an unmodified phenyl ring (*N*-phenyl-*N'*-2-chloro-4-pyridylurea; **8**) was slightly less active and exhibited activity approximately equivalent to that of zeatin. The parent compound bearing unmodified phenyl and pyridyl rings (*N*-phenyl-*N'*-4-pyridylurea; **7**) was the least active compound of the series, with activity approximately equal to that of *N,N'*-di-1, 2, 3-thiadiazol-5-ylurea. Thidiazuron was intermediate in activity between the two most active pyridylurea derivatives.

We have not conducted extensive tests of Thidiazuron in other cytokinin bioassay systems, but preliminary results indicate that it is also highly active in the tobacco callus bioassay. Thus, there are at least two classes of urea derivatives, the pyridylureas and the thiadiazolylureas, that provide examples of compounds with cytokinin activity equivalent to or exceeding that of the most active cytokinins of the adenine type.

EXPERIMENTAL

Chemicals. *N*-Phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (Thidiazuron, Dropp, SN 49537), *N*-methyl-*N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (SN 69330), *N,N'*-di-1,2,3-thiadiazol-5-ylurea (SN 85721), *N*-2-pyridyl-*N'*-1,2,3-thiadiazol-5-ylurea (SN 72115), *N*-benzyl-*N'*-1,2,3-thiadiazol-5-ylurea (SN 72501), and *N*-furfuryl-*N'*-1,2,3-thiadiazol-5-ylurea (SN 85719) were kindly provided by Schering AG. The synthesis of *N*-phenyl-*N'*-4-pyridylurea, *N*-phenyl-*N'*-2-chloro-4-pyridylurea and *N*-3-fluorophenyl-*N'*-2-chloro-4-pyridylurea has been described previously [8, 13]. *N,N'*-Diphenylurea (DPU) and *trans*-zeatine were obtained from Sigma. Picloram (4 - amino-3, 5, 6 - trichloropicolinic acid) was a gift from Dow Chemical.

Plant material. Seeds of *P. lunatus* L. cv. Kingston were obtained locally.

Tissue culture medium. The tissue culture medium consisted of the mineral nutrients described in ref. [14] with the

following organic substances added: sucrose (30 g/l), myo-inositol (100 mg/l), thiamine · HCl (1 mg/l), nicotinic acid (5 mg/l), pyridoxine · HCl (0.5 mg/l) and picloram (2.5 μM). (The latter compound is used to supply the auxin requirement of *Phaseolus* tissue cultures [15].) Kinetin (5 μM) was included in medium used for callus initiation and stock cultures. The pH of the medium was adjusted to 5.7 and Difco Bacto-agar (10 g/l) was added. The medium was dispensed into 125-ml conical flasks (50 ml/flask) and autoclaved at 120° for 15 min. The compounds tested for cytokinin activity were dissolved in DMSO [16] and added to the autoclaved tissue culture flasks prior to solidification of the medium (The final amount of DMSO in the tissue culture medium was 0.025 ml/flask.)

Growth and harvest of P. callus *cultures. Phaseolus* callus cultures were established from the hypocotyls of 5-day-old seedlings as described previously [15]. The callus tissue that formed on the initial explants was transferred once (first passage) on medium containing 5 μM kinetin. Tests for cytokinin activity were performed in the second passage of the callus tissue using 4-week-old first-passage cultures as stock tissue. Three pieces of callus weighting *ca* 10 mg each were planted per flask. Four replicate flasks were used per treatment. Tissues were harvested and weighted after 35 days of growth at 28° in the dark. All tests were repeated at least once.

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