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Shoot Formation of Tobacco Callus by Various Cytokinin-Active Ureas and Pyrimidines

N-(2-Chloro-4-pyridyl)-N′-phenylurea was found to have the strongest shoot forming activity so far known in tobacco callus. Some characteristics on the shoot-forming activity of several ureas and aminopyrimidines were described.

Keywords——cytokinin; re-differentiation; shoot formation; pyridylurea; N-(2-chloro-4-pyridyl)-N′-phenylurea; 4-aminopyrimidine; tissue culture; tobacco callus

The mechanism and principle of governing organ formation or various morphogenetic phenomena of higher plants are quite interesting. Recently we reported a strong shoot-forming activity of N-(4-pyridyl)-N′-phenylurea (4PU, **1**) in tobacco callus and pith tissue.[1] An exceptionally strong cytokinin activity (promoting cell division of tobacco callus) of N-(2-chloro-4-pyridyl)-N′-phenylurea (**2**) was also found.[2] In this communication we wish to report a high shoot-forming activity of **2** and some characteristic shoot-forming phenomena by several synthetic chemicals.

The bioassay was performed by tissue culture using tobacco plant as described earlier.[1,3] The basal medium was Murashige–Skoog's. Always the second generation of callus was used in the experiments to get a more reproducible result. Because of experimental dispersion frequently encountered in shoot formation experiments, most of the results reported here were confirmed by more than three times. The activity was represented by shoot formation rate (number of calluses which formed shoots per number of total calluses), and a qualitative ovser vation designated by +, ++, +++, which corresponds to the number and size of shoots formed. Usually the judgement of the result was made on 30 days after inoculation.

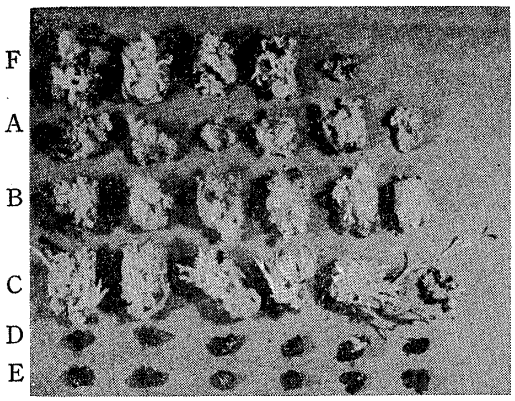
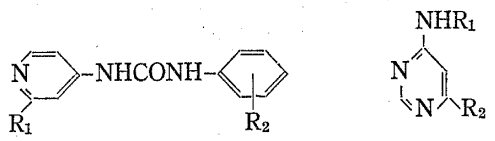


Fig. 1. Effect of N-(2-Chloro-4-pyridyl)-N′-phenylurea (**2**): A (10 ppm), B (1 ppm), C (0.1 ppm), D (0.01 ppm) and E (0.001 ppm) on the Tobacco Callus Wisconsin 38 in the absence of added auxin. F shows the effect of 4PU (**1**) (10 ppm).



The shoot-forming activity of N-(2-chloro-4-pyridyl)-N′-phenylurea (**2**), the most active compound which promotes cell division of tobacco callus so far known,[2] was found to be very high. The shoot formation was very vigorous and a large number of shoots were formed (Fig. 1). The optimum concentration for the shoot formation was 0.1 ppm (4×10^{-7} M) in the absence of added auxins. The concentration is about 10 times lower than that of N^6-benzyladenine, N^6-isopentenyladenine or zeatin under the same conditions.[4] Consequently, **2** is the strongest shoot-forming chemical. The activity of the mother compound 4PU (**1**), was about 1/100 as active as **2**. The presence of the chlorine atom at the α position to the

1) Y. Isogai, K. Shudo, and T. Okamoto, *Plant and Cell Physiol.*, **17**, 591 (1976).
2) S. Takahashi, K. Shudo, T. Okamoto, K. Yamada, and Y. Isogai, *Phytochem.*, **17**, 1201 (1978).
3) Y. Torigoe, M. Akiyama, M. Hirobe, T. Okamoto, and Y. Isogai, *Phytochem.*, **11**, 1623 (1972).
4) Y. Isogai, *Sci. Pap. Coll. Gen. Educ., University of Tokyo*, **27**, 9 (1977).

pyridine nitrogen resulted in the enhancement of the shoot-forming activity. The presence of auxin (indoleacetic acid, IAA) was always inhibitory to the shoot formation by **2** similarly to the case of **1**.[1] All the 4PU derivatives so far tested showed shoot-forming activity at about 100 times higher concentrations of the optimum concentrations for promotion of cell division. A part of experimental results is shown in Table I.

Table I. Result of Shoot Formation Experiments of Various Ureas in the Absence of Added Auxins

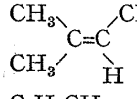
R_1	R_2	Conc. (ppm)	Shootformation rate[a]			Grade[b]
H	H (**1**)	10	6/6	6/6	6/6	###
Cl	H (**2**)	1	6/6	6/6	12/12	###
		0.1	6/6	6/6	7/12	
Cl	2-CH_3	1	5/6	5/6	8/12	###
		0.1	2/6	2/6	na[c]	
Cl	3-CH_3	10	6/6	6/6	11/11	###
		1	3/6	4/6	7/12	
		0.1	na	na	na	
Cl	4-CH_3	10	2/6	4/6	na	+
		1	1/6	1/5	na	
CH_3	H (**3**)	10	5/6	5/5	6/6	###
		10[d]	6/6	6/6	6/6	###
H	3-Cl (**4**)	10	5/6	2/5	na	+
		10[e]	6/6	6/6	6/6	##

a) Number of calluses which formed shoots per the total number of inoculated calluses.
b) +, ##, ###, see text.
c) na means not active.
d) In the presence of 2 ppm of added indoleacetic acid.
e) In the presence of 0.02 ppm of added indoleacetic acid.

N-(2-methyl-4-pyridyl)-N′-phenylurea (**3**) is an exceptional urea among some fifty 4PU derivatives; its shoot formation was not inhibited by the presence of IAA (up to 2 ppm). The rate of shoot formation and the growth of shoots were very vigorous and independent of the added IAA. Another characteristic 4PU derivative, N-(3-chlorophenyl)-N′-(4-pyridyl)-urea (**4**), showed a weak shoot-forming activity at 10 ppm in the absence of auxin, but rate of shoot formation increased in the presence of 0.02 ppm of added IAA.

Finally, shoot-forming effect of several pyrimidine derivatives, some of which showed a significant activity promoting cell division of tobacco callus,[5] was shown (Table II). Some

Table II. Result of Shoot Formation Experiments of Various Pyrimidines in the Absence of Added Auxins

R_1	R_2	Conc. (ppm)	Shoot formation rate[a]			Grade[b]
$C_6H_5CH_2$	CH_3NH (**5**)	10	6/6	6/6	4/6	##
		1	2/6	2/6	na[c]	
$C_6H_5CH_2$	NH_2	100	3/6	na	na	+
C_6H_5CO	Cl (**6**)	10	5/6	5/6	na	##
	Cl (**7**)	10	4/6	5/6	2/5	##
$C_6H_5CH_2$	H (**8**)	25	2/12	2/12	na	+
		10	8/11	na	na	
C_6H_5NHCO	Cl	10	6/6	6/6	3/5	###

a, b, c) see Table I.

5) S. Takahashi, T. Yatsunami, K. Shudo, T. Okamoto, K. Yamada, and Y. Isogai, *Chem. Pharm. Bull.* (Tokyo), **26**, 2286 (1978).

derivatives were found to be active. The optimum concentrations were rather lower than the expected ones from the relationship between the optimum concentration for shoot formation and that for promotion of cell division by 4PU derivatives and purine cytokinins. In particular, 4-benzylamino-6-methylaminopyrimidine (**5**), 4-benzoylamino-6-chloropyrimidine (**6**), and 4-chloro-6-isopentenylaminopyrimidine (**7**) were active at the same concentration as the optimum concentration for promotion of cell division in the absence of added auxin. 4-Benzylaminopyrimidine (**8**) which did not promote cell division showed a weak activity in the shoot formation experiment.

In summary, most of cytokinin-active pyridylureas and aminopyrimidines were proved to be active in the shoot formation of tobacco callus.

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Sanguirubine and Sanguilutine[1]

Structures of sanguirubine (3) and sanguilutine (4), a fully aromatized O_5-benzo[*c*]phenanthridine alkaloid, were established by synthesis through a newly developed pathway.

Keywords——O_5-benzo[*c*]phenanthridine alkaloid; synthesis; structural establishment; Bischler–Napieralski reaction; sanguirubine; sanguilutine

The natural occurrence[2] of four fully aromatized O_5-benzo[*c*]phenanthridine alkaloids has been known in *Papaveraceous* plants. We succeeded in establishing the structures of chelirubine[3] (**1**) and chelilutine[4] (**2**) by the syntheses of them and proposed tentative structures of sanguirubine[5] (**3**) and sanguilutine[5] (**4**) from diagnostic inspection of the reported NMR

1) This paper forms Part XXXIX of "Studies on the Chemical Constituents of *Rutaceous* Plants" by H. Ishii. Part XXXVIII; H. Ishii and T. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 2598 (1978).
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