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EFFECT OF 6-(SUBSTITUTED)THIO- AND AMINO-PURINES ON GERMINATION OF LETTUCE SEED[1]

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The biological activity of 6-(2-furfuryl)aminopurine (kinetin), a substance which has been found to effect increased cell division in tobacco "callus" tissue (8), has led to the synthesis and biological study of a number of 6-(substituted)purine derivatives. Several of these compounds have recently been found to have pronounced biological activity in both plant and animal systems. For example, in the moss *Tortella caespitosa*, 6-(2-thenyl)aminopurine, 6-benzylaminopurine and 6-*n*-pentylaminopurine have been found to have effects equal to or slightly greater than that of kinetin on the development of gametophores (5). Further, some of the purine analogues such as 6-(ω-phenylheptyl)aminopurine were found to be several thousand fold more active than adenine and several hundred fold more active than kinetin in retarding tentacle regeneration in hydra (6, 10).

The use of various organic compounds (especially auxins) in an attempt to increase the rate of germination of seeds has been studied for many years (1); however, only limited success has been obtained (3). In view of the biological activity of these purine derivatives, a study was made of their effects on seed germination.

The effect of several compounds, especially thiourea, upon lettuce seed germination has been reported by Thompson and Kosar (13), and during the final stages of preparation of this manuscript an article appeared (7) which presented some data on stimulation of lettuce seed germination by 6-(2-furfuryl)-aminopurine as well as some other 6-(substituted)-aminopurines. These latter results were interrelated to the red light effect previously observed as a requirement for lettuce seed germination (2). We have also observed such an effect with 6-(substituted)thiopurines (12).

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MATERIALS AND METHODS

Several types of field variety seed were initially studied in an effort to determine the effect of pre-soaking in 6-(substituted)purine solutions on their germination. In several instances enhanced rates of germination were noted; however, the most readily adaptable seed found for this study was a variety of lettuce (Early Curled Simpson).

Most of the compounds studied are relatively new, and their method of synthesis are reported elsewhere (4, 6, 9 to 12).

Unless otherwise noted, 100 or more seeds were pre-soaked in 100 ml of each purine solution, made up in distilled water at a concentration of 10 μgm/ml, for a period of 8 hours. In order to minimize light effects, the soaking period was uniformly overnight, and a water control was always included under identical experimental conditions as a standard. The seeds were drained of excess solution on filter paper, and finally placed on filter paper, wet with the corresponding purine solution, in Petri dishes and allowed to germinate in a dark room at 30° C. The same relative results were obtained whether the initial manipulation of seeds was carried out in diffuse light or in blue light (fluorescent light filtered through four layers of du Pont 300 MSC dark blue cellophane). The temperature of 30° C was chosen because these lettuce seed, soaked in water alone, germinate very slowly at this temperature in the dark. In order to show that the inactive compounds were not inhibiting germination, a corresponding experiment was performed so that germination was allowed to proceed in the presence of continuous fluorescent light at 25° C.

The light and temperature requirements for this variety of lettuce seed, with respect to rate of germination using several of the more "active" compounds, was studied. The seeds were pre-soaked in water, or

TABLE I

EFFECT OF VARIOUS 6-(SUBSTITUTED)PURINES ON THE RATE OF GERMINATION OF LETTUCE SEED

COMPOUND **	% SEED GERMINATED AT GIVEN NO. OF HRS* 24	36	48	60	NO. HRS REQUIRED FOR 30% GERMINATION IN THE DARK
6-(n-Alkyl)thiopurines					
(Water blank control)	0	0	9	11	>84
	(39)		(71)		
Butyl-	6	11	17	22	84
	(34)		(72)		
3-Methylbutyl-	1	7	11	18	>84
	(62)		(94)		
Pentyl-	31	55	73	77	24
	(59)		(81)		
Hexyl-	29	59	75	79	24
	(55)		(85)		
Heptyl-	5	10	14	18	>84
	(44)		(79)		
6-(n-Alkyl)aminopurines					
(Water blank control	0	2	2	5	>84
	(38)	(70)	(71)	(78)	
Propyl-	39	62	69	72	20
	(67)	(83)	(89)	(92)	
Butyl-	65	83	86	90	11
	(58)	(78)	(82)	(82)	
Pentyl-	55	72	85	89	12
	(60)	(78)	(79)	(83)	
Hexyl-	59	76	79	83	12
	(57)	(79)	(81)	(86)	
Heptyl-	50	67	77	82	14
	(52)	(70)	(74)	(80)	
6-(Di-alkyl)aminopurines					
(Water blank control)	1	1	2	3	>84
	(17)	(58)	(83)	(88)	
Di-propyl-	11	27	41	47	38
	(42)	(66)	(80)	(82)	
Di-butyl-	7	17	25	30	60
	(38)	(65)	(84)	(89)	
Di-pentyl-	9	10	19	22	>84
	(31)	(53)	(75)	(81)	
6-(Phenylalkyl)thiopurines					
(Water blank control)	0	3	3	6	>60
	(49)	(79)			
Phenylmethyl-	3	55	63	72	30
	(63)	(82)			
2-Phenylethyl-	9	33	41	48	34
	(50)	(72)			
3-Phenylpropyl-	5	45	55	62	31
	(50)	(73)			
5-Phenylpentyl-	1	2	3	3	>60
	(50)	(79)			

* The values given in parentheses were obtained using continuous fluorescent light during germination; the other values were obtained after germination in the dark.

** The following 6-(substituted)thiopurines were tested and found to be relatively inactive in stimulating seed germination under the test conditions: methyl-, ethyl-, propyl-, 2-methylpropyl-, octyl-, decyl-, cetylthiopurine; the following 6-(substituted)aminopurines were also relatively inactive: methyl-, ethyl-, decyl-, dimethyl-, diethyl-, 5-phenylpentyl-, 7-phenylheptyl-, and 11-phenylundecyl-aminopurine.

TARLE I (*Continued*)

COMPOUND **	% SEED GERMINATED AT GIVEN NO. OF HRS* 24	36	48	60	NO. HRS REQUIRED FOR 30% GERMINATION IN THE DARK
6-(Phenylalkyl)aminopurines					
(Water blank control)	0	3	3	6	>60
	(49)	(79)			
Phenylmethyl-	35	86	87	87	22
	(62)	(75)			
2-Phenylethyl-	35	70	77	81	23
	(52)	(76)			
3-Phenylpropyl-	23	71	77	83	26
	(53)	(72)			
4-Phenylbutyl-	1	5	5	10	>60
	(30)	(66)			
6-(Substituted)aminopurines					
(Water blank control)	0	0	0	0	>84
	(29)		(50)		
α-Naphthyl-	17	71	87	89	26
	(67)		(83)		
α-Naphthyl-methyl-	25	78	86	88	25
	(72)		(85)		
2-α-Naphthyl-ethyl-	0	0	1	1	>84
	(35)		(52)		
5-α-Naphthyl-pentyl-	0	2	3	5	>84
	(36)		(59)		
2-Furfuryl-	30	56	81	82	24
	(58)		(78)		
2-Thenyl-	17	56	85	88	28
	(71)		(83)		
2-Pyridyl-methyl-	24	67	88	89	25
	(52)		(74)		
3-Pyridyl-methyl-	17	69	89	94	27
	(66)		(83)		
4-Pyridyl-methyl-	4	56	78	79	30
	(53)		(75)		

the purine solution, in total darkness for eight hours; after which, they were transferred to Petri dishes in the presence of blue light, and the rate of germination at 22° and 30° C in total darkness and in the presence of continuous fluorescent light determined.

The number of seeds germinated at various time intervals was determined by visual observation of the appearance of the radicle, and all of the "dark germination" readings were made in the presence of blue light. The germinated seeds were removed from the Petri dishes after each time interval to facilitate counting.

RESULTS

From the germination data presented in table I it is apparent not only that certain 6-(substituted)-aminopurines possess a "dormancy breaking" activity, but that these effects are also produced by 6-(substituted)thiopurines as well. Further, at a concentration of 10 μgm/ml, the activity within the thio- and amino- series both reach peak activity near the same carbon length of the side chain. The activity of the compounds in the alkylthio- series peaks much more sharply with increase in chain length than in the alkylamino- series; however, the most active com-

Table II

Effect of Various Concentrations of 6-(Alkyl)Thiopurines on Rate of Germination of Lettuce Seed in the Dark

Compound, 6-(alkyl)thiopurine	Conc. μgm/ml	% Seed germinated at given no. of hrs				
		22	46	69	90	141
(Water blank control)	..	0	6	9	10	12
Butyl-	1	2	7	9	11	12
	3	0	6	7	9	10
	10	5	16	19	21	22
	30	4	55	67	71	75
Pentyl-	1	2	5	8	11	13
	3	3	25	29	31	32
	10	18	65	71	77	77
	30	3	71	84	86	87
Hexyl-	1	2	6	7	9	12
	3	3	13	15	18	20
	10	12	57	69	76	80
	30	16	74	83	90	91
Heptyl-	1	0	0	2	4	8
	3	0	3	7	9	10
	10	1	5	9	11	11
	30	3	10	15	20	20

pounds in both series are centered at the pentyl- and hexyl- carbon chain length.

The 6-(phenylalkyl)thio- and 6-(phenylalkyl)-aminopurines are comparable in their effect on altering the rate of seed germination under the experimental conditions studied. The most active compounds in both instances were the phenylmethyl-, phenylethyl- and phenylpropyl- compounds. When the phenyl- group was replaced by an α-naphthyl- radicle only the 6-(α-naphthylmethyl)aminopurine was appreciably active in stimulating the rate of lettuce seed germination at a concentration of 10 μgm/ml.

Substitution of a heterocyclic group for the aromatic nucleus in these 6-(substituted)aminopurines resulted in an increased germination rate of the same order of magnitude as obtained in the alkyl- and phenylalkyl- series above. The active compounds include 6-(2-furfuryl)aminopurine, 6-(2-thenyl)aminopurine and 6-(α-, β- and γ-pyridylmethyl)aminopurines.

The effect of concentration of the purine solutions on the rate of germination was not studied with every compound. A concentration range of three-fold increments was tested in the alkylthio- series, and the results indicated that a concentration of 10 μgm/ml of purine solution was adequate to initiate activity. At higher concentrations, as noted in table II, the analogues with shorter or longer chain length than the more active compounds possessed some effect on the rate of germination.

The light and temperature requirement for this variety of lettuce seed, with respect to rate of germination after pre-soaking in water and in the purine solutions, has been demonstrated with several of the more "active" compounds. In the dark at 22° C, seeds pre-soaked in a 10 μgm/ml solution of 6-benzylaminopurine, for example, were found to be 71 % germinated at 60 hours compared to 76 % germination for seeds soaked in water alone. However, at 30° C the corresponding values were 59 and 9 %, respectively. A similar increased rate in germination at the higher temperature in the absence of light was noted with several of the other compounds (table III). Further, some of these compounds appear to increase the rate of germination of these seeds even in the presence of light. For example, after 48 hours

Table III

Study of Light-Temperature Effects of Certain 6-(Substituted)Purines on Germination of Lettuce Seed

Compound, 6-(substituted)purine	Temp, °C	% Seed germinated at given no. of hrs							
		24		36		48		60	
		Lt.	Dk.	Lt.	Dk.	Lt.	Dk.	Lt.	Dk.
(Water blank control)	22	1	4	18	17	45	57	67	76
	30	0	0	3	1	8	5	30	9
Pentylthio-	22	4	5	13	28	45	56	54	65
	30	7	1	27	8	48	28	58	59
Butylamino-	22	14	10	29	24	54	53	66	59
	30	25	2	44	13	60	46	64	59
Benzylthio-	22	3	8	8	19	33	51	46	59
	30	9	4	33	11	55	44	63	60
Benzylamino-	22	15	21	25	40	43	61	48	71
	30	8	18	29	36	43	48	51	59
α-Naphthylmethylamino-	22	11	11	30	34	44	55	51	61
	30	17	0	27	9	46	38	56	52
2-Furfurylamino-	22	15	9	27	21	41	43	54	58
	30	7	10	29	30	45	49	60	57

lettuce seed pre-soaked in water and kept at 22° C were 45 % germinated compared to only 8 % germination for seeds kept at 30° C; which compares with 43 % germination at both temperatures for seed pre-soaked in 6-benzylaminopurine (table III).

The unsubstituted parent compounds, 6-mercaptopurine and adenine, had no appreciable effect on the germination of the lettuce seed over that of those seeds soaked in water alone.

Discussion

Many of the 6-(substituted)purines studied appreciably increased the rate of germination of lettuce seeds under the conditions of the experiments. That the "inactive" compounds were not toxic was indicated by the control experiment in the presence of light which allowed germination after pre-soaking in these compounds at approximately the same rate as the water controls.

The "active" compounds appear to have the property of replacing, or, in some manner, overcoming the light requirement in stimulating lettuce seed germination. The stimulation of germination by these compounds is most readily observed in the dark at 30° C. In the presence of light or at a lower temperature (22° C) lettuce seed germinate so rapidly that only moderate stimulatory effects of the purine derivatives are observed under these conditions.

These compounds probably exert some definite control relative to the biological effects of light and change of temperature. An increased rate of germination at 30° C was observed by increasing the concentration level of the more active purine analogues, resulting in very good dose-response curves of the data (table II). Also, in the presence of light, the rate of germination observed with lettuce seeds soaked in several of the more "active" purine solutions was increased compared with that of the water controls at 30° C, as indicated in tables I and III.

The fact that both the 6-(substituted)thio- and aminopurines affect the rate of germination of lettuce seed indicates that structural specificity necessary for this biological activity is not very exacting. It was observed that the number of "active" 6-(substituted)-purine analogues, within a homologous series, was greater in the amino- than in the thio- series suggesting that the 6-(substituted)aminopurines are possibly more closely related to the natural substance(s), which are involved in the normal seed germination, than are the thio-homologues. The maximum activity in both series was obtained with alkyl chain lengths of 5 to 6 carbon atoms or with an aromatic or heterocyclic substituent connected by a one or two carbon side chain.

Summary

Several 6-substituted amino- and thiopurines have been found to stimulate the rate of lettuce seed (Early Curled Simpson) germination. The size of the substituent group on the aminopurines is less specific for this activity than is the size of the group on the thiopurine; however, the maximum activity in both series is obtained with alkyl chain lengths of 5 to 6 carbons or with an aromatic or heterocyclic substituent connected by a methyl or ethyl group. The stimulation of lettuce seed germination by these compounds is most readily observed in the dark at 30° C. In the presence of light or at a lower temperature (22° C), lettuce seed germinate so rapidly that only moderate effects of the purines derivatives are observed under these conditions.

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