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SYNTHESIS AND CYTOKININ-LIKE ACTIVITY OF 7-CHLORO-IMIDAZO[1,2-*c*]PYRIMIDINES

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Key Word Index - 7-Chloro-imidazo[1,2-*c*]pyrimidines; cytokinin activity; chlorophyll retention; leaf expansion; chlorophyll production; root growth inhibition.

Abstract—Some 5-amino substituted 7-chloro-imidazo[1,2-*c*]pyrimidines and 7-chloro-8-methylthio-imidazo[1,2-*c*]pyrimidines were synthesized for further information on the role of the purine ring in cytokinin structure/activity relationships. Their cytokinin-like activity was examined using four different tests: expansion of cucumber etiolated cotyledons; formation of chlorophyll in etiolated cotyledons of cucumber; preservation of chlorophyll breakdown in sections of barley leaves; inhibition of root growth in intact wheat seedlings. Compounds with the imidazopyrimidine ring were generally less active than those with the purine ring, with the exception of the pentenylamino derivatives, which showed an activity comparable with that of the control, kinetin.

INTRODUCTION

The cytokinins and the other cytokinin-like compounds promote many different processes such as seed germination [1-3], expansion of cotyledons and leaves [4, 5], growth of lateral branches [6], the retardation of chlorophyll degradation [7] and induction of chlorophyll synthesis [8]. At low concentration they induce cell division in excised tissues [9, 10]. Skoog *et al.* [11] have reported that the intact purine ring is necessary for cytokinin activity, and Matsubara [12] confirmed that any modification of this structure results in a decrease in cytokinin activity. An exception to this rule seems to be 1-deazapurine derivatives, because they show a high cytokinin activity [13, 14]. When N is in the bridgehead position, deazapurine activity increases [15]. N^6-Substituted adenines with 4-7 carbon atoms in the side chain tend to be highly active. The introduction of a double bond, an aromatic ring or a heteroatom in the side chain further modifies the activity. Previously we reported [16] that some imidazo[1,2-*a*]pyrimidine derivatives are able to retard the chlorophyll degradation in barley leaves, and that halogenation of the imidazopyrimidine ring increases the activity of the compound and modifies the influence of the lateral chain. Therefore, we synthesized some 7-chloro-imidazo[1,2-*c*]pyrimidine and some 7-chloro-8-methylthio-imidazo[1,2-*c*]pyrimidine compounds (Scheme 1) in order to test their cytokinin activity and to see if this was modified by the presence of a thyomethyl group or lateral chains. The 7-chloro-8-methylthio-imidazo[1,2-*c*]pyrimidine compounds were synthesized in analogy with 2-methylthiozeatin, which was obtained from *s*RNA of plants [17, 18] and bacteria [19]. The alkylamino groups were introduced at the 5-position in analogy with the more active cytokinins [11, 12]. The activity of these compounds was assayed in four specific tests for cytokinins: expansion of cucumber etiolated cotyledons [20], induction of chlorophyll formation in etiolated cucumber cotyledons [10], preservation of chlorophyll breakdown in barley leaf sections [9] and inhibition of root growth in intact wheat seedlings [21]. Kinetin (6-furfurylaminopurine) was used as a control.

RESULTS AND DISCUSSION

Expansion of etiolated cotyledons

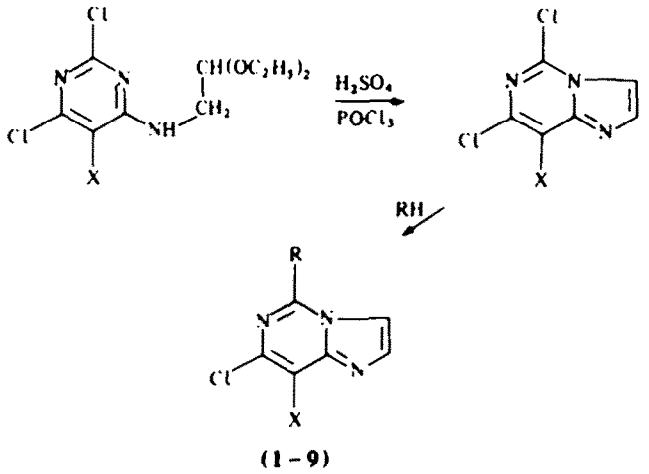
The activity of imidazo[1,2-*c*]pyrimidines (Table 1) are clearly less active than kinetin, also their isopentenyl derivatives (**3**, **8**), which show a slightly stronger effect, are still 57 and 64% less active than kinetin.

Induction of chlorophyll formation

In this case (Table 1) imidazopyrimidines are generally more active, either when the free amino group is present in the 5-position, or when it is partially replaced by an alkylamine (**2**, **3**, **7**, **8**) or arylalkylamine chain (**5**). Here too the isopentenyl derivatives (**3**, **8**) are more effective, though always less effective than kinetin. On the contrary, furfuryl compounds (**4**, **9**) show the opposite activity, inhibiting chlorophyll synthesis.

Preservation of chlorophyll breakdown

Imidazopyrimidines which are not thiomethylated in the 8-position (compounds **1**, **5**) have about the same effect as kinetin, while pentenyl and isopentenyl derivatives (**2**, **3**) are more effective by 132 and 264‰ respectively (Table 1). On the contrary, furfuryl derivatives (**4**, **9**) activate chlorophyll breakdown. Generally we noted a negative effect of the thiomethyl group in the 8-position of the imidazopyrimidine ring (**6**-**8**).



(1–9)

1 X = H, R = NH_2
2 X = H, R = $NH(CH_2)_2CH(CH_3)_2$
3 X = H, R = $NHCH_2CH{=}C(CH_3)_2$
4 X = H, R = $NHCH_2$–furyl
5 X = H, R = $NHCH_2C_6H_5$
6 X = SCH_3, R = NH_2
7 X = SCH_3, R = $NH(CH_2)_2CH(CH_3)_2$
8 X = SCH_3, R = $NHCH_2CH{=}C(CH_3)_2$
9 X = SCH_3, R = $NHCH_2$–furyl

Scheme 1.

Inhibition of root growth

The inhibiting activity of imidazopyrimidines on root growth (Table 1) is clearly stronger than that of kinetin, except for furfuryl derivatives (**4**, **9**) which induce root growth.

On the whole, our findings agree with published data on the correlation between chemical structure and cytokinin activity [11, 12, 22]. For all compounds tested, the dose–effect relationship is linear: the effect decreases as doses decrease. Briefly the imidazo[1,2-*c*]pyrimidine ring generally inactivates the cytokinin-like effect, except for inhibiting root growth in intact wheat seedlings. Since at the end of the treatment the root tip generally becomes brown, it seems likely that the compounds are toxic to roots. The relationship between the presence of a lateral chain and activity is worth noting. In our case, the unsaturated alkylamine residues seem the best, in fact isopentenyl derivatives (**3**, **8**) were by far the most active in all our tests, as they reached and in some cases exceeded kinetin activity (inhibition of root growth, preservation of chlorophyll breakdown). As to aminofurfuryl derivatives (**4**, **9**), in three out of four tests they showed the opposite activity when compared to similar compounds. In this case an anti-cytokinin action could be hypothesized. Finally, the 8-methylthio group (**6**–**9**) has an inhibitory effect analogous to that of natural compounds; in fact 8-SMe-imidazopyrimidines are always less active than the corresponding non-substituted compounds with the same lateral chain.

EXPERIMENTAL

The various reactions were checked by TLC on silica gel (eluent $CHCl_3$–*EtOH*, 9:1 or 98:2). Mps were determined in open capillary tubes and are uncorr. Only the most significant peaks of the IR (KBr) spectra are reported. The ^{1}H NMR spectra were recorded using $CHCl_3$ and DMSO-d_6 as solvents and tetramethylsilane (TMS) as internal standard. The spectra of the compounds were consistent with the assigned structure in all cases. The results of elemental analysis (C, H, N) were within ± 0.3 of the theoretical values.

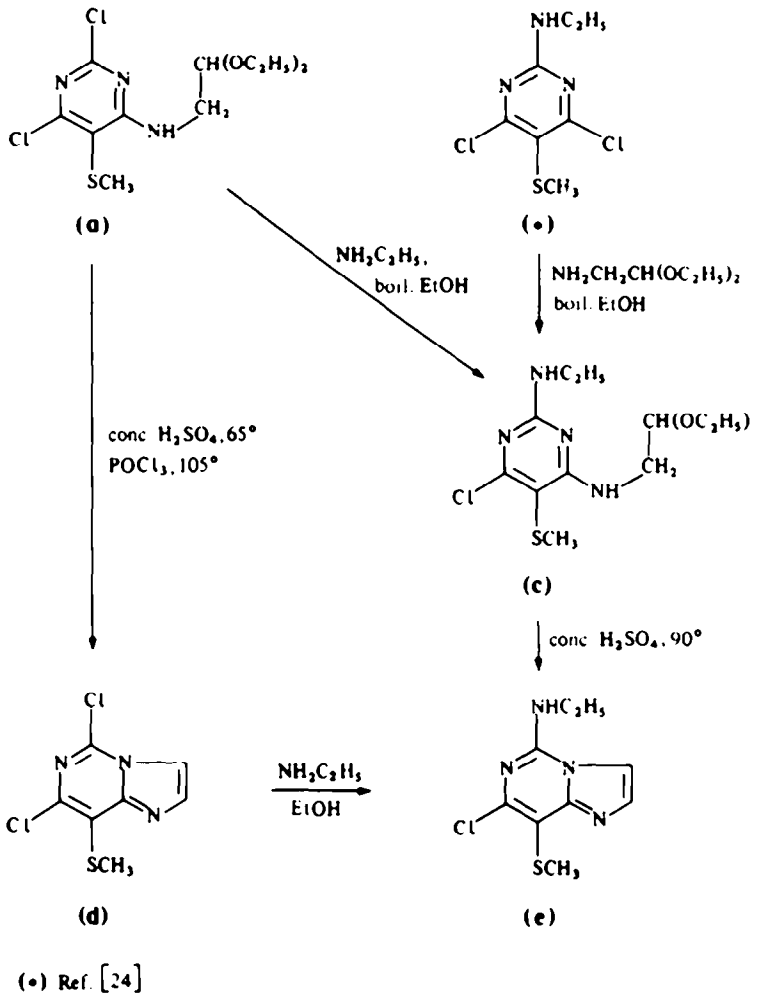
2,6-Dichloro-4-(2,2-diethoxyethyl)amino-5-methylthiopyrimidine (**a**) *and 4,6-dichloro-2-(2,2-diethoxyethyl)amino-5-methylthiopyrimidine* (**b**). 5-Methylthio-2,4,6-trichloropyrimidine (5.75 g) [23] was dissolved in EtOH (250 ml), 2-aminoacetaldehyde diethyl acetal (8 ml) was added and the soln stirred at room temp. for 2 hr, then evaporated. The residue was suspended in H_2O and exhaustively extracted with $CHCl_3$. The $CHCl_3$, previously dried on Na_2SO_4, was removed by evaporation and the crude isomeric mixture (7.6 g; 93%) was eluted, with $CHCl_3$–CCl_4 (1:2) on a neutral Al_2O_3 chromatographic column (Merck, Brockmann activity III; 650 g). Two fractions were collected in this order: 4.9 g of 4-aminoderivate (**a**), 2.0 g of the isomer (**b**). Both these compounds were recrystallized from petrol. The structures of (**a**) and (**b**) were proved by an unequivocal synthesis (Scheme 2), since 4,6-dichloro-2-ethylamino-5-methylthiopyrimidine [24] reacted with 2-aminoacetaldehyde diethyl acetal, in boiling EtOH, to give (**c**), identical with the compound prepared, in the same conditions, from (**a**) and ethylamine. Analytical data: (**a**) crystallized from petrol, mp 72–73° (found: C, 40.63; H, 5.24; N, 12.98; $C_{11}H_{19}Cl_2N_3O_2S$ requires: C, 40.49; H, 5.25; N, 12.88%); IR ν_{max}^{KBr} cm^{-1}: 3280, 1580; ^{1}H NMR ($CDCl_3$): δ 1.22 (6H, *t*, acetal), 2.28 (3H, *s*, SMe), 4.54 (1H, *t*, $CHCH_2NH$), 6.72 (1H, *br t*, NH); (**b**) crystallized from petrol, mp 88–89° (found: C, 40.21; H, 5.17; N, 12.62; $C_{11}H_{19}Cl_2N_3O_2S$ requires: C, 40.49; H, 5.25; N, 12.88%); IR ν_{max}^{KBr} cm^{-1}: 3280, 1560; ^{1}H NMR ($CDCl_3$): δ 1.22 (6H, *t*, acetal), 2.32 (3H, *s*, SMe), 4.52 (1H, *t*, $CHCH_2NH$), 5.72 (1H, *br t*, NH).

Table 1. Effects of 7-chloro-imidazo[1,2-c]pyrimidines (1-5) and 7-chloro-8-SMe-imidazo[1,2-c]pyrimidines (6-9) on the expansion of etiolated cotyledons, induction of chlorophyll formation, preservation of chlorophyll breakdown and inhibition of root growth. The results are expressed as a percentage of the control (phosphate buffer alone) ± s.e. by the formula $(T\text{-}C/C) \times 100$

Compound	Dose (molar concentration) 1×10^{-4}	1×10^{-5}	1×10^{-6}	1×10^{-7}
Cucumber cotyledon expansion				
K	*397.5 ± 40.1	*226.5 ± 25.3	*78.0 ± 6.3	0.0 ± 0.0
(1)	*32.6 ± 3.5	*18.1 ± 2.3	15.6 ± 1.4	10.1 ± 1.3
(2)	*73.9 ± 9.6	8.2 ± 0.9	6.2 ± 0.8	0.9 + 0.1
(3)	*172.4 ± 15.3	*133.2 ± 11.8	27.3 ± 3.1	0.7 ± 0.1
(4)	*26.0 ± 2.1	7.7 ± 1.9	2.4 ± 0.4	1.6 ± 0.2
(5)	*25.1 ± 2.4	16.4 + 1.3	10.2 ± 1.1	0.2 ± 0.03
(6)	*23.2 ± 1.9	9.9 ± 1.1	5.6 ± 0.1	4.3 ± 0.3
(7)	*89.3 ± 9.6	*29.6 ± 3.6	*18.2 ± 1.9	13.2 ± 0.9
(8)	*143.6 ± 12.3	*29.4 ± 2.8	*23.1 ± 2.6	2.1 ± 0.1
(9)	*25.8 ± 3.1	*19.6 ± 2.3	*18.1 ± 1.9	13.9 ± 1.1
Chlorophyll formation in cucumber cotyledons				
K	*100.6 ± 9.9	*70.6 ± 8.1	*20.6 ± 2.7	*19.4 ± 1.9
(1)	*23.9 ± 3.0	15.8 ± 2.0	5.3 ± 0.6	1.8 ± 0.2
(2)	*61.1 ± 5.3	*27.0 ± 3.1	*17.6 ± 2.3	15.8 ± 1.1
(3)	*75.2 ± 8.0	*67.1 ± 5.9	*40.1 ± 3.9	*32.6 ± 2.8
(4)	* − 16.3 ± 2.1	− 13.1 ± 1.9	− 8.2 ± 0.5	− 3.4 ± 0.1
(5)	*39.6 ± 4.2	*33.5 ± 3.1	*20.4 ± 1.9	7.0 ± 0.6
(6)	*16.6 ± 1.9	10.3 ± 1.1	7.5 ± 0.5	4.9 ± 0.3
(7)	*46.9 ± 4.7	*27.0 ± 2.8	10.2 ± 0.9	4.8 ± 0.3
(8)	*66.8 ± 5.4	*57.0 ± 4.7	*37.1 ± 3.8	*20.0 ± 1.7
(9)	* − 21.1 ± 2.3	* − 17.4 ± 1.9	− 11.1 ± 0.7	− 2.6 ± 0.1
Preservation of chlorophyll breakdown				
K	*42.1 ± 4.7	*41.9 ± 4.3	*34.5 ± 3.2	12.4 ± 1.3
(1)	*40.1 ± 4.1	13.1 ± 2.0	3.1 ± 0.4	2.0 ± 0.1
(2)	*55.5 ± 5.9	*21.0 ± 2.1	11.7 ± 1.2	9.8 ± 0.6
(3)	*111.1 ± 9.9	*64.7 ± 7.0	*31.8 ± 3.6	*24.8 ± 1.9
(4)	* − 31.4 ± 3.6	* − 23.4 ± 2.7	* − 21.2 ± 1.9	− 10.1 ± 0.7
(5)	*20.8 ± 1.9	5.0 ± 0.6	3.6 ± 0.2	1.6 ± 0.1
(6)	15.4 ± 1.3	14.5 ± 1.3	13.3 ± 0.9	3.7 ± 0.1
(7)	*44.9 ± 4.6	*33.8 ± 3.1	*26.4 ± 2.5	*16.2 ± 1.1
(8)	*41.3 ± 3.9	*40.2 ± 4.0	15.8 ± 1.7	13.6 ± 1.4
(9)	* − 24.4 ± 2.0	* − 14.3 ± 1.2	− 11.9 ± 0.9	0.0 ± 0.0
Inhibition of root growth				
K	* − 29.2 ± 2.6	* − 28.7 ± 2.6	* − 25.3 ± 1.9	− 12.4 ± 1.5
(1)	* − 37.6 ± 3.1	* − 17.6 ± 1.4	− 12.9 ± 0.8	− 3.4 ± 0.1
(2)	* − 55.1 ± 4.3	* − 29.3 ± 1.0	* − 16.3 ± 0.3	− 5.6 ± 0.1
(3)	* − 55.6 ± 6.1	* − 48.0 ± 2.7	* − 45.0 ± 2.5	* − 44.7 ± 2.4
(4)	* + 38.0 ± 1.6	+ 17.2 ± 1.1	+ 9.2 ± 1.0	+ 7.2 ± 0.9
(5)	* − 47.1 ± 3.1	* − 31.1 ± 2.1	− 18.4 ± 1.1	− 1.8 ± 0.5
(6)	* − 51.2 ± 3.1	* − 23.4 ± 1.5	− 3.3 ± 0.1	0.0 ± 0.1
(7)	* − 53.3 ± 2.9	* − 35.9 ± 1.9	* − 30.2 ± 1.9	* − 24.4 ± 1.3
(8)	* − 53.3 ± 3.7	− 16.2 ± 1.2	− 15.2 ± 1.4	− 13.0 ± 0.5
(9)	* + 41.0 ± 3.1	+ 5.6 ± 0.3	+ 2.1 ± 0.2	+ 0.9 ± 0.1

6-Chloro-4-(2,2-diethoxyethyl)amino-2-ethylamino-5-methylthiopyrimidine (c). Crystallized from petrol, mp 93–95° (found: C, 46.77; H, 6.91; N, 16.74: $C_{13}H_{23}ClN_4O_2S$ requires: C, 46.62; H, 6.92; N, 16.73%); IR ν_{max}^{KBr} cm^{-1}: 3290, 1600; ^{1}H NMR (DMSO-d_6): δ1.1 (9H, *t*, acetal, ethyl), 2.18 (3H, *s*, SMe), 4.70 (1H, *t*, $CHCH_2NH$), 6.90 (1H, *br t*, NH), 7.20 (1H, *br t*, NH).

5,7-Dichloro-8-methylthioimidazo[1,2-c]pyrimidine (d) (*Scheme* 2). A mixture of (a) (20 g) and conc. H_2SO_4 (12 ml) was heated at 65° for 2 hr. The resulting soln was cooled and poured on ice (35 g), then treated, at 0–5°, with 30% aq. NaOH, to pH 5–6. The solid was removed by filtration, dried and exhaustively extracted with hot $CHCl_3$–EtOH (9:2). The product obtained (1.2 g) was suspended in $POCl_3$ (12 ml) and the mixture heated at 105° for 48 hr. The excess $POCl_3$ was removed by evaporation at red. press. and the residue poured on to ice (15 g); the suspension was treated, at 0–5°, with 30% aq. NaOH, to pH 6, then rapidly extracted with Et_2O–petrol. The solvent was evaporated and 0.8 g (60%) of the compound (d) were obtained. Crystallized from Et_2O–petrol, mp 118–120° (found: C, 36.17; H, 2.35; N, 18.16; $C_7H_5Cl_2N_3S$ requires: C, 35.91; H, 2.15; N, 17.94%); IR ν_{max}^{KBr} cm^{-1}: 1580, ^{1}H NMR (DMSO-d_6): δ2.42 (3H, *s*, SMe), 7.75 and 7.85 (2H, *d*, ar).



Scheme 2.

5-Amino-7-chloroimidazo[1,2-c]*pyrimidines* (**1**–**5**). A soln of 5,7-dichloroimidazo[1,2-c]pyrimidine [25] (0.01 mole) in EtOH was added, at room temp., to the suitable amine (0.025 mole). To prepare **1** dichloropyrimidine was directly treated with excess conc. NH_4OH; 1-amino-3-methyl-2-butene was prepared by the method of Desvages and Olomuchi [26], the other amines were commercially available. The reaction mixture was evaporated to dryness and the residue suspended in H_2O. The solid that separated was removed by filtration and recrystallized. Yield 65–85%. The structures of **1**–**5** were assigned in analogy with some compounds previously studied [25].

5-Amino-7-chloroimidazo[1,2-c]*pyrimidine* (**1**) crystallized from DMF–H_2O, mp > 320° (found: C, 43.06; H, 3.27; N, 32.90; $C_6H_5ClN_4$ requires: C, 42.74; H, 2.98; N, 33.23%); IR ν_{max}^{KBr} cm^{-1}: 3330, 3170, 1600, ^{1}H NMR (DMSO-d_6): δ 6.92, 7.50 and 7.92 (3H, *s*, ar), 8.02 (2H, *br s*, NH_2); *7-chloro-5-i.pentylaminoimidazo*[1,2-c]*pyrimidine* (**2**) crystallized from EtOH–H_2O, mp 173–174° (found: C, 55.43; H, 6.27; N, 23.67; $C_{11}H_{15}ClN_4$ requires: C, 55.34; H, 6.33; N 23.47%); IR ν_{max}^{KBr} cm^{-1}: 3230, 1630, 1580; ^{1}H NMR (DMSO-d_6): δ 0.93 (6H, *d*, *i*.propyl), 6.82 (1H, *s*, ar) 7.47 and 7.98 (2H, *d*, ar), 8.02 (1H, *br t*, NH); *7-chloro-5-i.pent-2-enylamino)imidazo*[1,2-c]*pyrimidine* (**3**) crystallized from EtOH–H_2O, mp 198–199° (found: C, 55.54; H, 5.50; N, 23.44; $C_{11}H_{13}ClN_4$ requires: C, 55.81; H, 5.53; N, 23.67%); IR ν_{max}^{KBr} cm^{-1}: 3220, 1620, 1580; ^{1}H NMR (DMSO-d_6): δ 1.70 (6H, *s*, propenyl), 6.80 (1H, *s*, ar), 7.40 and 7.95 (2H, *d*, ar), 8.15 (1H, *br t*, NH); *7-chloro-5-furfurylaminoimidazo*[1,2-c]*pyrimidine* (**4**) crystallized from EtOH–H_2O, mp 182–183° (found: C, 53.32; H, 3.80; N, 22.60; $C_{11}H_9ClN_4O$ requires: C, 53.12; H, 3.64; N, 22.53%); IR ν_{max}^{KBr} cm^{-1}: 3230, 1620, 1580; ^{1}H NMR (DMSO-d_6): δ 4.70 (2H, *d*, CH_2), 6.95 (1H, *s*, ar), 6.45, 7.55, 7.65, and 8.05 (5H, *d*, ar), 8.70 (1H, *br t*, NH); *5-benzylamino-7-chloroimidazo*[1,2-c]*pyrimidine* (**5**) crystallized from EtOH–H_2O, mp 183–184° (found: C, 60.45; H, 4.35; N, 21.35; $C_{13}H_{11}ClN_4$ requires: C, 60.35; H, 4.28; N, 21.65%); IR ν_{max}^{KBr} cm^{-1}: 3220, 1630, 1590; ^{1}H NMR (DMSO-d_6): δ 4.70 (2H, *d*, CH_2), 6.86 and 7.30 (6H, *s*, ar), 7.38 and 7.90 (2H, *d*, ar), 8.53 (1H, *br t*, NH).

5-Amino-7-chloro-8-methylthioimidazo[1,2-c]*pyrimidines* (**6**–**9**). These compounds were obtained from 5,7-dichloro-8-methylthioimidazo[1,2-c]pyrimidine (**d**), by the method reported for compounds **1**–**5**. The structure assigned to compounds **6**–**9**, in analogy with that of some imidazo[1,2-c]pyrimidine derivatives previously studied [25], was also confirmed by an unequivocal synthesis of 7-chloro-5-ethylamino-8-methylthioimidazo[1,2-c]pyrimidine (**e**) (Scheme 2).

5-Amino-7-chloro-8-methylthioimidazo[1,2-c]*pyrimidine* (**6**) crystallized from benzene–ligroin, mp 175–176° (found: C, 38.89; H, 3.52; N, 25.87; $C_7H_7ClN_4S$ requires: C, 39.16; H, 3.28; N, 26.10%); IR ν_{max}^{KBr} cm^{-1}: 3240, 3140, 1590, 1540; ^{1}H NMR (DMSO-d_6): δ 2.50 (3H, *s*, SMe), 7.55 and 7.87 (2H, *d*, ar), 8.10

(2H, *br s*, NH_2); *7-chloro-5-i.penthylamino-8-methylthioimidazo*[1,2-c]*pyrimidine* (7) crystallized from benzene–ligroin, mp 185 187° (found: C, 50.36; H, 5.97; N, 19.79; $C_{12}H_{17}ClN_4S$ requires: C, 50.60; H, 6.01; N, 19.67%); IR ν_{max}^{KBr} cm^{-1}: 3240, 1610; ^{1}H NMR (DMSO-d_6): δ0.85 (6H, *d*, *i*.propyl), 2.45 (3H, *s*, SMe), 7.42 and 7.92 (2H, *d*, ar), 8.05 (1H, *br t*, NH); *7-chloro-5-(i.pent-2-enylamino)-8-methylthioimidazo*[1,2-c]*pyrimidine* (8) crystallized from benzene ligroin, mp 141 143° (found: C, 50.96; H, 5.37; N, 19.56; $C_{12}H_{15}ClN_4S$ requires: C, 50.96; H, 5.34; N, 19.81%); IR ν_{max}^{KBr} cm^{-1}: 3200, 1620; ^{1}H NMR (DMSO-d_6): δ1.70 (6H, *s*, propenyl), 2.47 (3H, *s*, SMe), 7.40 and 7.95 (2H, *d*, ar), 8.20 (1H, *br t*, NH); *7-chloro-5-furfurylamino-8-methylthioimidazo*-[1,2-c]*pyrimidine* (9) crystallized from benzene–ligroin, mp 248 251° (found: C, 48.79; H, 3.82; N, 18.72; $C_{12}H_{11}ClN_4S$ requires: C, 48.89; H, 3.76; N, 19.00%); IR ν_{max}^{KBr} cm^{-1}: 3060, 1560; ^{1}H NMR (DMSO-d_6): δ2.42 (3H, *s*, SMe), 4.50 (2H, *d*, CH_2), 6.30, 7.50, 7.55 and 8.05 (5H, *d*, ar), 8.75 (1H, *br t*, NH); *7-chloro-5-ethylamino-8-methylthioimidazo*[1,2-c]*pyrimidine* (e) crystallized from benzene, mp 211-213° (found: C, 44.21; H, 4.77; N, 22.85; $C_9H_{11}ClN_4S$ requires: C, 44.53; H, 4.56; N, 23.08%); IR ν_{max}^{KBr} cm^{-1}: 3230, 1620; ^{1}H NMR (DMSO-d_6): δ1.23 (3H, *t*, ethyl), 2.48 (3H, *s*, SMe), 7.51 and 7.95 (2H, *d*, ar), 8.15 (1H, *br t*, NH).

Cucumber cotyledon expansion. Cucumber seeds (*Cucumis sativus* L.) were soaked for 4 hr in tap water, and germinated on moist filter paper in darkness at 28° for 4–5 days. In these conditions completely expanded cotyledons were excised in dim green light and floated in Petri dishes containing 6.7 mM Na K phosphate buffer pH 6.7. Groups of 10 cotyledons were weighed and floated with the adaxial face down in 3 cm Petri dishes containing 3 ml of test soln. Incubation was in darkness at 28° for 20 hr. After incubation each group was re-weighed [20].

Chlorophyll formation in cucumber cotyledons. Groups of 10 cotyledons, obtained as described above, were floated in 3 cm Petri dishes containing 3 ml of test soln and incubated at 25° in the dark for 17 hr, then moved to light (3200 lux) at 25° for 3 hr. At the end of the treatment, the chlorophyll from 10 weighed cotyledons was extracted with 80% EtOH; chlorophyll retention was expressed by measuring the optical density of the extracts at 665 and 643 nm [10].

Preservation of chlorophyll breakdown. Barley seeds (*Hordeum vulgare* L.) were soaked for 8 hr in running tap water and grown in compost at 20° under continuous light for 13 days. At this time the first leaf was fully expanded and the second had just begun to appear. Plants were selected for uniformity, and a piece of each leaf was cut between the 3rd and 4th cm from the tip of the blade. The sections were aged for 24 hr by floating them on distilled water at 25° in darkness. After this period they were blotted and transferred to screwcap vials containing 1 ml of test soln and 250 units of penicillin G. Four sections were floated in each vial, and each fraction was tested in duplicate or triplicate samples. After 48 hr of incubation at 25° in the dark, the sections of each vial were extracted with 80% EtOH and chlorophyll retention was determined as described above [9].

Inhibition of root growth. Wheat (*Triticum vulgare* L.) grains were soaked in H_2O for about 18 hr, sown on filter paper moistened with H_2O and incubated in the dark for 48 hr at 22°. Plants with a central root 0.5 cm long were selected and, in groups of 10, put on a grid with the root immersed in the different solns. After 18–20 hr at 22° in the dark, the length of the central root was measured. All experiments were done in triplicate and repeated three times [21]. The results were expressed as a percentage of the control (6.7 mM phosphate buffer pH 5.9) ± s.e. by the formula $(T-C/C)\times 100$. The difference between each mean and the control (phosphate buffer alone) was significant at 1% (•) or 5% (Student's *t* test). The tested compounds were dissolved in 6.7 mM Na–K phosphate buffer, pH 5.9, at various concentrations.

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