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INFLUENCE OF ALTERATIONS IN THE PURINE RING ON BIOLOGICAL ACTIVITY OF CYTOKININS*

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Abstract—The 1-deaza-, 3-deaza-, 8-aza-1-deaza- and 8-aza-3-deaza-analogs of kinetin and 6-(3-methyl-2-butenylamino)purine and some of their ribosides were synthesized and their growth-promoting activities in the tobacco bioassay were determined and compared with those of the parent compounds. The replacement of nitrogen by carbon in the 1-position of the purine ring decreases cytokinin activity 15-fold for kinetin and 2-fold for 6-(3-methyl-2-butenylamino)purine (IPA); however, the replacement of nitrogen by carbon in the 3-position decreases the activity 2000 times for kinetin and 1000 times for 6-(3-methyl-2-butenylamino)-purine. The activity of 8-aza-1-deaza-analogs appears to be of the same order of somewhat lower than the corresponding 1-deaza-analogs. The corresponding 8-aza-3-deaza-analogs are less active than kinetin (400 times) and 6-(3-methyl-2-butenylamino)purine (40 times). However, they are more active than the corresponding 3-deaza-analogs. The concentration range in which the ribosides show activity is nearly the same as for the corresponding free bases, but the maximum yield of tobacco-callus for the riboside of the 3-deaza-analog of 6-(3-methyl-2-butenylamino)purine is very low.

INTRODUCTION

INVESTIGATIONS on structure-activity relationships of cytokinins indicate that an intact purine ring is necessary for high growth promoting activity.[1-4] Only a few cytokinin analogs with alterations in the purine ring have been tested for cytokinin activity.[1,3,4] It seemed interesting, therefore, to investigate several new analogs in order to try to elucidate the role of the purine ring in cytokinin action.

In this paper the growth promoting activity on tobacco callus tissue of eleven compounds is presented: 1-deaza- and 3-deaza-analogs of 6-(3-methyl-2-butenylamino)purine and of kinetin, their 8-aza-analogs and some of their ribosides. Eight of these compounds were newly synthesized.

* Part XI in the series "Deazapurine Derivatives". For Part X see SCHELLING, J. E. and SALEMINK, C. A. (1972) *Rec. Trav. Chim.* **91,** 650.

† The bioassays of the compounds presented in this paper were executed by Dr. J. H. Rogozińska during a 3 month stay in the Org. Chem. Lab., Utrecht.

[1] 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.

[2] SKOOG, F. and ARMSTRONG, D. J. (1970) *Ann. Rev. Plant Physiol.* **21,** 359.

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RESULTS AND DISCUSSION

Syntheses of Test Substances

The compounds shown in Table 1 were prepared by nucleophilic substitution of a chloro derivative by the appropriate amine (furfurylamine or 3-methyl-2-butenylamine). The reactivity of the chlorodeazapurines was much lower than that of the chloroazadeazapurines, which can be explained by the lack of one reaction-intermediate stabilizing nitrogen atom.[5] Compounds Ia, IIa and Va have been reported previously;[6,7] the yield of Ia could be increased by a longer heating period. Details of the syntheses and UV spectra for all the new compounds are provided in the Experimental.

TABLE 1. LIST OF COMPOUNDS TESTED FOR CYTOKININ ACTIVITY

Ia	7-Furfurylaminoimidazo[4,5-*b*]pyridine
Ib	7-(3-Methyl-2-butenylamino)imidazo[4,5-*b*]pyridine
IIa	7-Furfurylamino-*v*-triazolo[4,5-*b*]pyridine
IIb	7-(3-Methyl-2-butenylamino)-*v*-triazolo[4,5-*b*]pyridine
IIIa	4-Furfurylaminoimidazo[4,5-*c*]pyridine
IIIb	4-(3-Methyl-2-butenylamino)imidazo[4,5-*c*]pyridine
IVa	4-Furfurylamino-*v*-triazolo[4,5-*c*]pyridine
IVb	4-(3-Methyl-2-butenylamino)-*v*-triazolo[4,5-*c*]pyridine
Va	7-Furfurylamino-3-β-D-ribofuranosylimidazo[4,5-*b*]pyridine
Vb	7-(3-Methyl-2-butenylamino)-3-β-D-ribofuranosylimidazo[4,5-*b*]pyridine
VIb	4-(3-Methyl-2-butenylamino)-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine

Cytokinin Activity

The cytokinin activity of the analogs was determined on the basis of fresh weight yield in the tobacco bioassay (Fig. 1). The concentrations of the tested compounds ranged from 320 pM to 125 μM. The bars show the ranges in which tissue growth increases as a linear function of the log of concentration.

It can be seen from Fig. 1 that all kinetin analogs tested were less active than kinetin itself as is indicated by the start of the linear portions of growth curves. 6-(3-methyl-2-butenylamino)purine (IPA) analogs were also less active than the parent compound. The 1-deaza-analogs (Ia and Ib) were respectively 15 and 2 times less active than their corresponding adenine derivatives, whereas the 3-deaza-analogs (IIIa and IIIb) were respectively 2000 and 1000 times less active. This indicates that the nitrogen atom at the 3-position is very important for high cytokinin activity.

The 8-aza-1-deaza-analog (IIb) of IPA was about 2 times less active than its 1-deaza-analog (Ib). Besides cell division activity, bud formation occasionally was observed in some experiments at high concentrations. However, it is a potent cytokinin despite the two changes in the purine ring. In comparison the 8-aza-1-deaza-analog (IIa) of kinetin was nearly as active as the 1-deaza-analog (Ia) of kinetin. The 8-aza-3-deaza-analogs (IVa and IVb) are respectively 5 and 27 times more active than the corresponding 3-deaza-analogs

[5] MILLER, J. (1968) *Aromatic Nucleophilic Substitution*, p. 8, Elsevier, New York.
[6] DE ROOS, K. B. and SALEMINK, C. A. (1971) *Rec. Trav. Chim.* **90**, 1166.
[7] DE ROOS, K. B. and SALEMINK, C. A. (1971) *Rec. Trav. Chim.* **90**, 654.

(IIIa and IIIb). This indicates that substitution of a nitrogen atom in the 8-position of the purine ring does not always decrease* the cytokinin activity.

The ribosides (Va, Vb and VIb) were active in the same concentration ranges as their corresponding free bases (Ia, Ib and IIIb). The optimum yield for the riboside of the 3-deaza-analog of 6-(3-methyl-2-butenylamino)purine was much lower than that of its free base (IIIb) (Fig. 2).

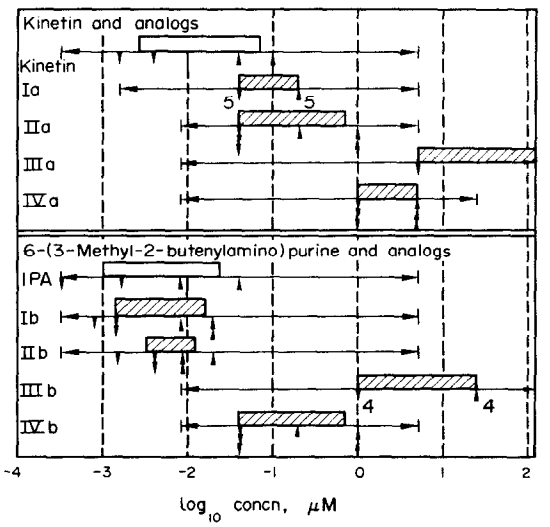

FIG. 1. RELATIVE CYTOKININ ACTIVITIES OF 1-DEAZA- AND 3-DEAZA- AND 8-AZA-1-DEAZA- AND 8-AZA-3-DEAZA-ANALOGS OF KINETIN AND 6-(3-METHYL-2-BUTENYLAMINO)PURINE.
The bars represent average values for the range in which growth increases as a linear function of the log of each compound concentration. The base lines represent tested concentration ranges, and the arrows under the base lines represent the start and end-points of the linear growth in individual experiments. Numbers have been substituted when more than three arrows occur at one point.

In view of the low activity of 3-deaza-compounds and the structural resemblance to some cytokinin inhibitors[8] the interaction between those substances and cytokinins was tested. The 3-deaza-analog (IIIb) of 6-(3-methyl-2-butenylamino)purine was tested in combination with 6-(3-methyl-2-butenylamino)purine and the 3-deaza-analog (IIIa) of kinetin in combination with kinetin. The concentration of 3-deaza-analogs ranged from 1·6 nM to 1·0 μM and that of cytokinins from 0·04 to 25·0 μM, and no anti-cytokinin activity was detected. Thus those substances neither inhibit nor augment the growth promoting effect of the cytokinins.

Only a few analogs of cytokinins with close structural relationship to the natural compounds have previously been tested: 6-(3-methyl-2-butenylamino)-8-aza-7-deazapurine and 6-(3-methyl-2-butenylamino)-8-aza-purine were reported to be about 100 times less active than 6-(3-methyl-2-butenylamino)purine[2] and 6-(3-methyl-2-butenylamino)-8-aza-9-deazapurine and closely related derivatives showed slight or no activity.[3] Some corresponding kinetin and 6-benzylaminopurine analogs show the same general range of activity.[1]

* It was reported[2] that substitution of a nitrogen atom in the 8-position of 6-(3-methyl-2-butenylamino)-purine lowered the activity 100 times.

[8] HECHT, S. M., BOCK, R. M., SCHMITZ, R. Y., SKOOG, F. and LEONARD, N. J. (1971) *Proc. Natl. Acad. Sci. U.S.* **68**, 2608.

8-Benzylamino-2-methyl-*s*-triazolo [1,5-*a*]pyrazine (6-benzylamino-8-methyl-4-aza-3-deazapurine) and 4(7)-benzylaminobenzimidazole (6-benzylamino-1,3-dideazapurine) show optimum growth response at 10 μM,[4] thus being about 100 times less active than 6-benzylaminopurine. None of these analogs, however, show such a high activity as 7-(3-methyl-2-butenylamino)imidazo[4,5-*b*]pyridine (Ib).

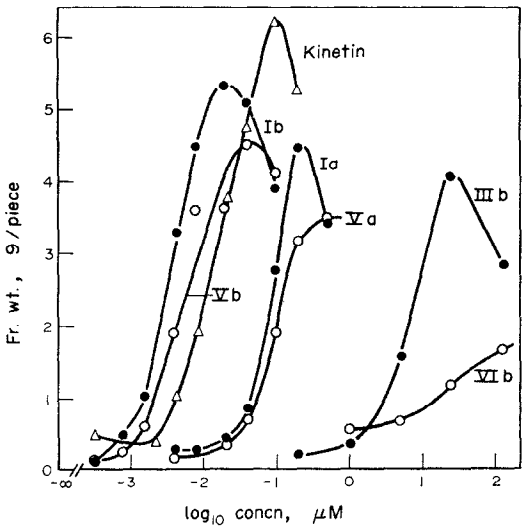

FIG. 2. EFFECT OF Ia, Ib, IIIb, Va, Vb, VIb AND KINETIN ON YIELD OF TOBACCO CALLUS, COMPARING THE CYTOKININ ACTIVITY OF RIBOSIDES AND THEIR CORRESPONDING BASES.

It is reported that several cytokinin analogs substituted at the 1 or 3 position of the purine ring have little or no activity.[1] 3-Benzyl-6-benzylaminopurine and 6-benzylamino-1-methylpurine give optimum growth at 12·5 μM and 1-benzyl-6-benzylaminopurine and 6-benzylamino-3-methylpurine give optimum growth at 0·1 μM. It was assumed that the compounds themselves were more or less inactive and that removal of the substituent attached to the ring nitrogen gave the activity found. The experiments with the deaza-analogs reported here more clearly show the role of the nitrogen atoms since there is only a slight steric change in the molecule and no chemical degradation can take place to give an active compound.

It has been suggested[9] that cytokinins, located next to anti-codons in certain *t*RNA species, influence the binding of the *t*RNAs to ribosomes. Letham[10] found a positive correlation between ribosomal binding and biological effects of various cytokinin analogs. Because of the 1-deaza-cytokinin analogs have relatively high activity and those with substitution of nitrogen by carbon in the 3-position are very low—it is attractive to suggest that the nitrogen in the 3-position must be more important with regard to the above mentioned binding to plant ribosomes than the nitrogen in the 1-position. Further work in this direction is under way.

[9] GEFTER, M. L. and RUSSELL, R. L. (1969) *J. Mol. Biol.* **39**, 145.
[10] BERRIDGE, M. V., RALPH, R. K. and LETHAM, D. S. (1970) *Biochem. J.* **119**, 75.

EXPERIMENTAL

Synthesis of test substances. 7-*Furfurylaminoimidazo*[4,5-b]*pyridine* (Ia) has been described previously.[6] The yield was increased by heating for a longer period (29% in 4 hr—67% in 3 days): UV, (EtOH) λ_{max} 220 nm (ε 25000), 267 (15000), 283 (15000); (1N NaOH) λ_{max} 225 (21000), 279 (17000); (1N HCl) λ_{max} 220 (21000), 266 (11500), 280 (23000).

7-(3-*Methyl*-2-*butenylamino*)*imidazo*[4,5-b]*pyridine* (Ib). To 350 mg (2·3 mmol) of 7-chloroimidazo[4,5]-pyridine[11] was added 450 mg (5·3 mmol) of 3-methyl-2-butenylamine and 1 ml of methylcellosolve. The mixture was refluxed with stirring in N_2 for 3 days, cooled and 5 ml of H_2O was added. The resulting colourless precipitate was filtered, washed (H_2O) and dried: yield 382 mg (82%). Chromatography over silica gel, elution with a gradient of EtOAc–EtOH (9:1–3:1) and recrystallization from acetone or H_2O yielded small needles; m.p. 179·2°. (Found: C, 65·0; H, 7·0; N, 27·9. $C_{11}H_{14}N_4$ requires: C, 65·3; H, 7·0; N, 27·7%); UV, (EtOH) λ_{max} 225 nm (ε 11 000), 267 (12 000), 284 (13 000); (1N NaOH) λ_{max} 226 (20 000), 282 (20 000), (1N HCl) λ_{max} 224 (14 000), 266 (9000), 293 (21 000).

7-*Furfurylamino*-v-*triazolo*[4,5-b]*pyridine* (IIa) has been described previously;[6] UV, (EtOH) λ_{max} 221 nm (ε 17 000), 267 (10 000), 310 (17 000), 318 sh; (1N NaOH) λ_{max} 224 (16 000), 265 (8000), 272 (9000), 299 (16 000); (1N HCl) λ_{max} 219 (20 000), 259 (8000), 315 (18 000).

7-(3-*Methyl*-2-*butenylamino*)-v-*triazolo*[4,5-b]*pyridine* (IIb). To 1 g (6·5) mmol of 7-chloro-triazolo-[4,5]pyridine[6] was added 1·5 g (17·6 mmol) of 3-methyl-2-butenylamine and 4 ml of methylcellosolve. The mixture was refluxed with stirring in N_2 for 4 hr, cooled and 7 ml of 1 N NaOH (H_2O) added. The solvent was evaporated *in vacuo*. The residue was recrystallized from H_2O–EtOH, giving slightly coloured needles: 950 mg (75%); m.p. 215·0°. (Found: C, 58·4; H, 6·5; N, 34·5. $C_{10}H_{13}N_5$ requires: C, 59·1; H, 6·5; N, 34·5%); UV, (EtOH) λ_{max} 223 nm (ε 12000), 269 (8000), 311 (17000), 321 sh; (1N NaOH) λ_{max} 226 (13000), 266 (7000), 273 (8000), 304 (15000); (1N HCl) λ_{max} 222 (14 000), 257 (6500), 318 (18 000). Picrate (H_2O), m.p. 183° (Found: C, 44·4; H, 3·8; N, 26·2. $C_{16}H_{16}N_8O_7$ requires: C, 44·4; H, 3·7; N, 25·9%).

4-*Furfurylaminoimidazo*[4,5-c]*pyridine* (IIIa). To 1 g (6·5 mmol) of 4-chloroimidazo[4,5]pyridine[11] was added 2·2 g (22·6 mmol) of furfurylamine and 3 ml of methylcellosolve. The mixture was refluxed (N_2) 2 days, cooled and basified. The resulting solid was purified by chromatography over silica gel: elution with EtOAc–EtOH (9:1). Yield 1·1 g (79%): recrystallization (H_2O) yielded slightly coloured needles; m.p. 134·0°. (Found: C, 60·7; H, 4·7; N, 26·4; O, 8·0. $C_{11}H_{10}N_4O$ requires: C, 61·7; H, 4·7; N, 26·2; O, 7·5%). NMR δ (DMSO-d_6): 4·77 (2H, *s*, $-CH_2$), 6·25 (1H, *q*), 6·37 (1H, *q*), 7·53 (1H, *q*), (furan H's), 6·83 (1H, *d*, pyridine βH), 7·78 (1H, *d*, pyridine αH), 8·13 (1H, *s*, imidazole H); UV, (EtOH) λ_{max} 220 nm (ε 13 000), 273 (15 000); (1N NaOH) λ_{max} 226 (16 000), 274 (14 000); (1N HCl) λ_{max} 216 (15 000), 265–279 (11 000); Picrate (H_2O), m.p. 214–216° (Found: C, 46·2; H, 3·0; N, 21·9. $C_{17}H_{13}N_7O_8$ requires: C, 46·1; H, 3·0; N, 22·1%).

4-(3-*Methyl*-2-*butenylamino*)*imidazo*[4,5-c] (IIIb). To 1 g (6·5 mmol) of 4-chloroimidazo [4,5]-pyridine[11] was added 1·5 g (17·6 mmol) of 3-methyl-2-butenylamine and 4 ml methylcellosolve. The mixture was refluxed 3 days (N_2), cooled and basified. The solvent was evaporated leaving an oily mass, which was purified by chromatography over silica gel: elution with EtOAc–EtOH (9:1) gave a nearly colourless oil (1 g, 76%). Picrate, recrystallized (H_2O) gave yellow needles: m.p. 203° (Found: C, 47·3; H, 4·1; N, 22·5. $C_{17}H_{17}O_7$ requires: C, 47·3; H, 4·0; N, 22·7%). The picrate in dry acetone, was treated with HCl. The precipitate was collected and carefully washed with acetone. 600 mg of the colourless solid was dissolved in 2 ml conc HCl and an excess of acetone was added. After a while, a colourless precipitate appeared. This solid was filtered and dried *in vacuo* (KOH, 80°); m.p. 210° (Found: Cl, 33·14. $C_{11}H_{14}N_4 . 3\,HCl$ requires: Cl, 35·5%). No stoichiometric ratio could be found. UV, (EtOH) λ_{max} 216 nm (ε 15 000), 269 (17 500); (1N, NaOH) λ_{max} 227 (16 500), 279 (10 500); (1N HCl) λ_{max} 209 (19 000), 263–277 (12 000).

4-*Furfurylamino*-v-*triazolo*[4,5-c]*pyridine* (IVa). To 1 g (6·5 mmol) of 4-chloro-*v*-triazolo[4,5]pyridine[12] was added 2·2 g (22·6 mmol) of furfurylamine and 3 ml of methylcellosolve. The mixture was refluxed for 2 hr (N_2), cooled and basified. The solvent was evaporated, leaving a dark solid. The solid was washed with H_2O and EtOH: yield 730 mg (52%) slightly coloured solid, m.p. 260° decomp. The solid could be crystallized from methylcellosolve. (Found: C, 54·3; H, 4·5; N, 32·1. $C_{10}H_9N_5O$ requires: C, 55·8; H, 4·2; N, 32·6%). UV, (EtOH) λ_{max} 221 nm (ε 20 000), 277 (21 000); (1N NaOH) λ_{max} 225 (22 000), 292 (15 000); (1 N HCl) λ_{max} 217 (12 000), 280 (12 000). Picrate (H_2O) m.p. 108° (Found: C, 43·1; H, 2·8; N, 24·9. $C_{16}H_{12}N_8O_8$ requires: C, 43·3; H, 2·7; N, 25·2%).

4-(3-*Methyl*-2-*butenylamino*)-v-*triazolo*[4,5-c]*pyridine* (IVb). To 1 g (6·5 mmol) of 4-chloro-*v*-triazolo-[4,5-*c*]pyridine[11] was added 1·2 g (14·5 mmol) of 3-methyl-2-butenylamine and 5 ml of methylcellosolve. The mixture was refluxed for 4 hr (N_2), cooled and basified. The solvent was evaporated, leaving a dark solid, recrystallized for H_2O–EtOH, yielding a light yellow substance (980 mg, 75%). (H_2O–EtOH, EtOAc); m.p. 210·3°. $C_{10}H_{13}N_5$ (M^+ Calc. 203·117. Found 203·119); UV, (EtOH) λ_{max} 224 nm (ε 14 000), 293 (10 000); (1 N NaOH) λ_{max} 226 (11 500), 294 (10 000); (1 N HCl) λ_{max} 213 (15 000), 281 (14 000). MS, *m/e* 203 (M^+), 188, 174, 160, 135, 107, 80, 69, 53, 41.

[11] DE ROOS, K. B. and SALEMINK, C. A. (1969) *Rec. Trav. Chim.* **88**, 1263.
[12] TALIK Z. and PŁAŻEK, E. (1956) *Roczniki Chem.* **30**, 1139.

7-*Furfurylamino*-3-β-D-*ribofuranosylimidazo*[4,5-b]*pyridine* (Va) has been reported previously.[7]

7-(3-*Methyl*-2-*butenylamino*)-3-β-D-*ribofuranosylimidazo*[4,5-b]*pyridine* (Vb). To 500 mg (1·75 mmol) of 7-chloro-3-β-D-ribofuranosylimidazo[4,5-*b*]pyridine[7] was added 1·3 g (15·3 mmol) 3-methyl-2-butenylamine and 2·5 ml methylcellosolve. The mixture was refluxed for 3 days (N_2), cooled and basified (NH_3). The mixture was evaporated to dryness *in vacuo*. The resulting syrup was purified by chromatography over silica gel: elution with EtOAc–EtOH (9:1) gave 300 mg (51%) crude product which was further purified by chromatography over silica gel with a gradient of $CHCl_3$–EtOH (9:1–5:1). Recrystallization (EtOH) yielded 95 mg colourless needles, m.p. 155°. (Found: C, 57·3; H, 6·9; N, 16·9. $C_{16}H_{22}N_4O_4$ requires: C, 57·5; H, 6·6; N, 16·8). Optical rotation (*c* 1·12, MeOH): $[\alpha]_{546}^{20}$ −83·0°, $[\alpha]_{578}^{20}$ −72·4°, $[\alpha]_{D}^{20}$ −68·5°. UV, (EtOH) λ_{max} 224 nm (ε 17 000), 267 (15 000), 287 (16 000); (1 N HCl) λ_{max} 223 (16 000), 265 (7500), 293 (20 000).

4-(3-*Methyl*-2-*butenylamino*)-1-β-D-*ribofuranosylimidazo*[4,5-c]*pyridine* (VIb). To 345 mg (1·21 mmol) of 4-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine[13] was added 1 g (11·8 mmol) of 3-methyl-2-butenylamine and 2 ml of methylcellosolve. The mixture was refluxed for 2 days (N_2), cooled and 25 ml of 1 N NH_4OH was added. The mixture was evaporated to dryness. The resulting syrup was chromatographed over silica gel: elution with a gradient of $CHCl_3$–EtOH (9:1–4:1). 340 mg (87%) light brown syrup resulted. Two crystallizations (EtOH) gave 85 mg colourless needles, m.p. 166°. (Found: C, 57·1; H, 6·6; N, 16·8. $C_{16}H_{22}N_4O_4$ requires: C, 57·5; H, 6·6; N, 16·8). Optical rotation (*c* 1·19, MeOH): $[\alpha]_{546}^{20}$ −53·2°, $[\alpha]_{578}^{20}$ −46·3°, $[\alpha]_{D}^{20}$ −43·7°. UV, (EtOH) λ_{max} 216 nm (ε 18 000), 274 (16 500); (1 N HCl) λ_{max} 212 (25 000), 267 (16 000).

Bioassay procedure. The cytokinin activity of the test compounds was determined by the tobacco bioassay.[14] The compounds in dimethylsulfoxide[15] were added in 25 μl aliquots to each Erlenmeyer flask with 50 ml sterilized agar medium. All bioassays were done at least 2×. After a growth period of 5 weeks the cultures were photographed and their fr. wt. was determined.

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[14] ROGOZIŃSKA, J. H., HELGESON, J. P. and SKOOG, F. (1964) *Physiol. Plant.* **17**, 165.
[15] SCHMITZ, R. Y. and SKOOG, F. (1970) *Plant Physiol.* **45**, 537.