Phytochemistry, Vol 24, No 5, pp 897–900, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

STIMULATION OF SOYBEAN CALLUS GROWTH BY *S*-LATHYRINE

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(*Revised received* 10 *August* 1984)

Key Word Index—*Glycine max*, *Lathyrus* spp, Papilionoideae, growth, cell division, cytokinins, amino acids, lathyrine

Abstract—*S*-Lathyrine stimulated growth of soybean callus tissue by cell division in the presence or absence of kinetin It was also active in the radish leaf senescence test, but was inactive in the tobacco callus assay and in five other tests for cytokinins

INTRODUCTION

In the course of studies on compounds that stimulate cell division in plants, we observed that the amino acid *S*-lathyrine (*S*-2-amino-3-(2-aminopyrimid-4-yl) propanoic acid), a constituent of some *Lathyrus* species [1], caused growth of soybean callus We report the effects of the isomers of lathyrine on soybean callus and in a range of other cytokinin bioassays We also describe the interactive effects of *S*-lathyrine and kinetin on the growth of soybean callus

RESULTS AND DISCUSSION

RS-Lathyrine hydrochloride was synthesized by a published route [2] and desalted Resolution was accomplished by conversion to the α-chloroacetyl derivative followed by selective deacylation of the *S*-antipode using hog acylase I and separation of the two compounds by cation-exchange chromatography The acylated *R*-lathyrine was hydrolysed in hydrochloric acid and desalted by cation exchange to give *R*-lathyrine The synthetic *S*-lathyrine was indistinguishable from lathyrine isolated from *Lathyrus tingitanus* L, supplied by Professor E A Bell

S-Lathyrine (10^{-6}–10^{-4} M) stimulated growth of soybean callus (Fig 1), with 3×10^{-6} M giving greatest yields Very similar callus yields were obtained when *S*-lathyrine was autoclaved in the growth medium or was added to the autoclaved medium by filter sterilization, indicating that lathyrine was stable to autoclaving *R*-Lathyrine did not cause significant callus growth The activity of *RS*-lathyrine compared with that of *S*-lathyrine suggested that *R*-lathyrine may have had an antagonistic effect on the callus growth

The maximum callus yields obtained with *S*- and *RS*-lathyrine were *ca* six times less than those obtained with similar concentrations of 6-benzylaminopurine (BAP) or kinetin *S*-Lathyrine (3×10^{-6} M) gave callus yields similar to those obtained with 10^{-8} M BAP or 10^{-7} M kinetin The appearance of callus grown on media containing lathyrine was similar to that of callus grown on kinetin Cell number and cell diameter determinations on callus grown on media containing either kinetin or *S*-lathyrine showed that callus weights were proportional to

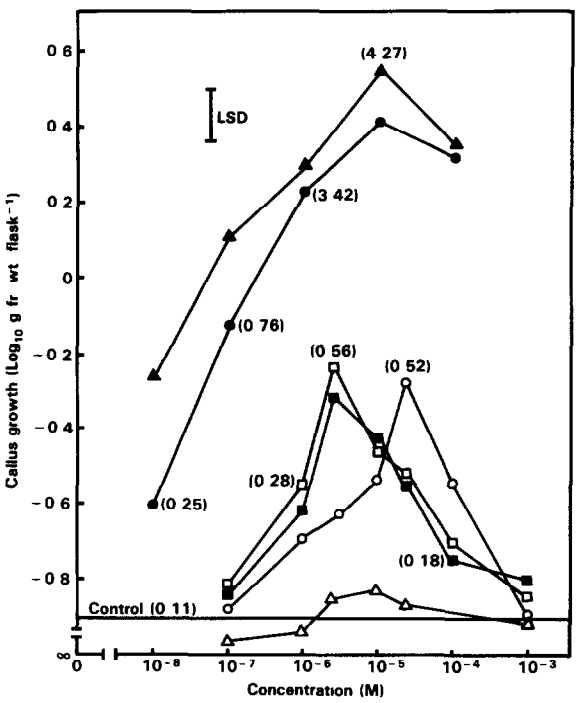

Fig 1 Growth of soybean callus in response to *RS*-lathyrine (○——○), *S*-lathyrine (□——□ autoclaved, ■——■ filter-sterilized), *R*-lathyrine (△——△), kinetin (●——●) and BAP (▲——▲) in combination with 1.1×10^{-5} M NAA Each point represents the logarithm of mean callus fresh weight (g) in eight flasks The data were transformed logarithmically before analysis LSD = 0 142 ($P = 0.05$) Figures in parentheses are de-transformed mean callus fresh weights (g)

cell numbers, and that cell diameters were similar in all treatments (Table 1) Thus, *S*-lathyrine promoted cell division and growth of soybean callus

S-Lathyrine (10^{-5} M and 3×10^{-5} M) interacted synergistically with kinetin (10^{-7}–10^{-5} M) in promoting soybean callus growth (Fig 2) *S*-Lathyrine (3×10^{-6} M)

Table 1 Cell numbers and mean cell diameters of soybean callus tissues grown on culture media containing 1.1×10^{-5} M 1-naphthylacetic acid and *S*-lathyrine or kinetin

Treatment		Callus fresh wt (mg)	Cell number (cells $\times 10^{-7} \pm$ SE)	Mean cell diameter (μm $\pm$ SE)
Control		121	1.74 ± 0.49	8.87 ± 0.24
S-Lathyrine	3×10^{-6} M	542	8.58 ± 1.05	9.05 ± 0.35
	1×10^{-5} M	353	5.90 ± 0.76	9.21 ± 0.35
	3×10^{-5} M	305	4.97 ± 0.84	9.28 ± 0.29
Kinetin	1×10^{-6} M	448	8.41 ± 1.36	9.34 ± 0.38
	1×10^{-5} M	980	15.6 ± 2.18	8.98 ± 0.31

SE = standard error

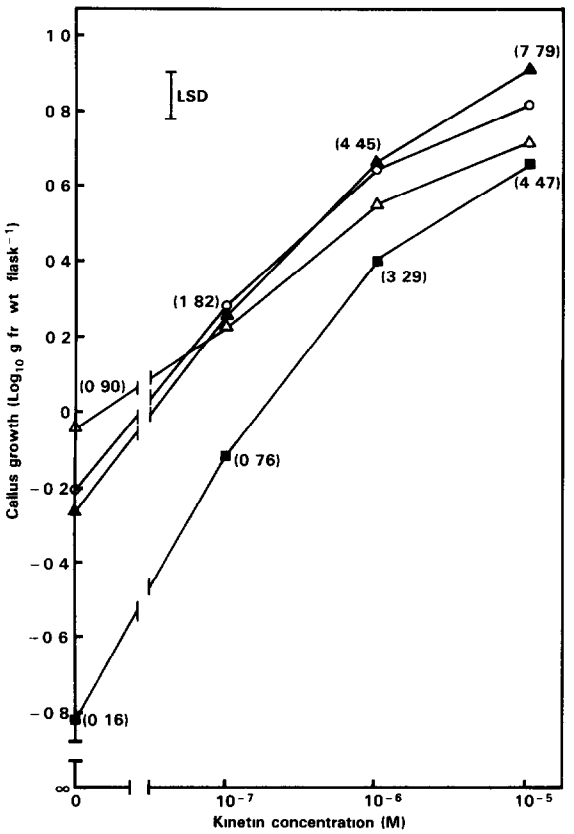

Fig 2 Effects of *S*-lathyrine on soybean callus growth in the presence of 1.1×10^{-5} M NAA and kinetin The concentrations of *S*-lathyrine tested were zero (■——■), 3×10^{-6} M (△——△), 10^{-5} M (○——○) and 3×10^{-5} M (▲——▲) Each point represents the logarithm of mean callus fresh weight (g) in eight flasks The data were transformed logarithmically before analysis LSD = 0.124 ($P = 0.05$) Figures in parentheses are de-transformed mean fresh callus weights (g)

also showed significant synergism with kinetin (10^{-7} and 10^{-6} M)

In order to obtain some indication of whether lathyrine was acting as a cytokinin, its activity in a range of cytokinin bioassays was studied *S*-Lathyrine (10^{-8}–10^{-3} M) did not stimulate growth of tobacco callus (results not shown) *S*-Lathyrine and *RS*-lathyrine were active in the radish leaf chlorophyll retention bioassay at 10^{-4}–10^{-2} M (Fig 3) *R*-Lathyrine (10^{-4}–10^{-2} M) was also active in this test, although it gave smaller responses than *S*- or *RS*-lathyrine

S-Lathyrine (10^{-8}–10^{-3} M) showed no significant activity in five other bioassays for cytokinins (results not shown) These assays were the *Amaranthus* betacyanin synthesis assay, the cucumber chlorophyll cotyledon greening assay, the cucumber and radish cotyledon expan-

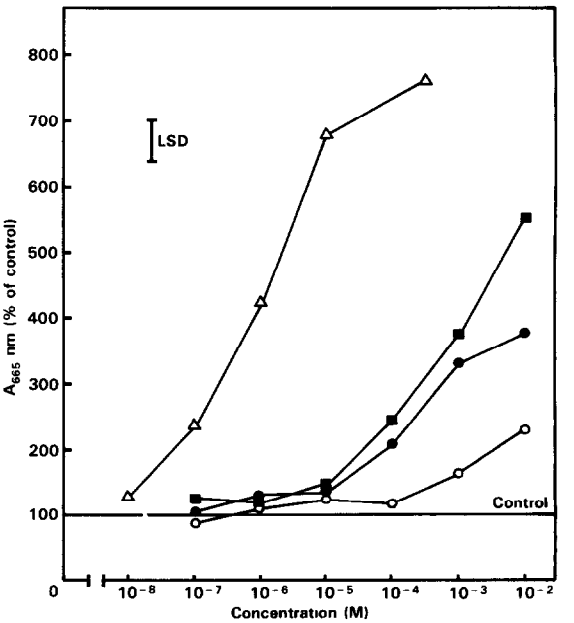

Fig 3 Effects of *S*-lathyrine (■——■), *RS*-lathyrine (●——●), *R*-lathyrine (○——○) and kinetin (△——△) on the retention of chlorophyll in excised leaf discs of radish After 4 days of incubation in the dark, the leaf discs were extracted with H_2O–EtOH (1 4) and the A_{665} of the solutions was determined Each point represents the mean A in five replicates LSD = 57% ($P = 0.05$)

sion assays, and the pea lateral bud outgrowth test In all these tests, kinetin gave the expected responses

Cytokinins are defined as compounds that act in a manner similar to kinetin, and in particular stimulate cell division in certain tissue cultures [3] The lack of activity of lathyrine in a number of cytokinin bioassays, and particularly the tobacco callus assay, indicates that it cannot be considered to be a cytokinin S-Lathyrine, despite its relatively low activity on soybean callus, enhanced kinetin-induced soybean callus growth This enhanced growth occurred even at 10^{-5} M kinetin, which gave maximum callus yields in the absence of lathyrine This may indicate that S-lathyrine acts in this tissue in a manner different from that of the purine cytokinins

The apparently limited distribution of lathyrine in plant species means that it cannot be considered to be a plant growth substance Whether the biological activity of lathyrine reported here reflects its role in the species in which it occurs, is unknown

EXPERIEMENTAL

Synthesis of lathyrine RS-Lathyrine HCl was synthesized by a published route [2] It was desalted by cation exchange RS-Lathyrine HCl (2 g) was dissolved in H_2O (7 ml) and loaded onto a cation-exchange column (Dowex 50W-X8, H^+, 450 × 20 mm) The column was washed with H_2O (600 ml) and then eluted with NH_4OH (1 M, 800 ml) The basic eluate was reduced to dryness *in vacuo* and the residue was recrystallized from H_2O to yield RS-lathyrine (1 4 g), mp ⩾ 248° (dec) (Found C, 46 0, H, 5 5, N, 30 9 $C_7H_{10}N_4O_2$ requires C, 46 1, H, 5 5, N, 30 8, O, 17 6%)

Synthesis of RS-2-(α-*chloroacetamide*)-3-(2-*aminopyrimid*-4-*yl*)*propanoic acid* A soln of α-chloroacetyl chloride (13 5 g) in Et_2O (50 ml) and an aq soln of NaOH (1 M, 30 ml) were alternately added over 30 min to a stirred soln of RS-lathyrine (5 g) in aq NaOH (1 M, 150 ml, 0°) The soln was evapd to dryness *in vacuo*, and the residue was recrystallized from H_2O to give RS-2-(α-chloroacetamido)-3-(2-aminopyrimid-4-yl)propanoic acid (5 5 g), mp ⩾ 300° (dec) (Found C, 41 8, H, 4 2, N, 21 8 $C_9H_{11}N_4O_3Cl$ requires C, 41 8, H, 4 3, N, 21 7, O, 18 6, Cl, 13 7%)

Resolution of RS-*lathyrine* A soln of DL-2-(α-chloroacetamido)-3-(2-aminopyrimid-4-yl)propanoic acid (5 g) in H_2O (1 l) was adjusted to pH 7 by addition of NH_4OH (1 M) Hog acylase I (EC 3 5 1 14, Sigma, grade II, 0 2 g) was added and the soln was stirred under N_2 for 24 hr at 37° The pH was re-adjusted to 7 and a further portion of acylase (0 2 g) was added The soln was stirred under the same conditions for 24 hr The pH was again adjusted to 7 and acylase (0 1 g) was added The incubation was continued for 14 hr, then EtOH (420 ml) was added and the soln concd *in vacuo* to 250 ml The denatured protein was removed by centrifugation, and the supernatant was reduced to dryness *in vacuo* The residue was dissolved in H_2O (25 ml) and loaded onto a cation-exchange column (Dowex 50W-X8, H^+, 600 × 45 mm) The column was eluted with 0 01 M Na citrate buffer, pH 5, at 43 ml/hr The eluate was monitored at 254 nm Fractions corresponding to the second peak to elute were combined and reduced to dryness *in vacuo* The residue was recrystallized from Me_2CO–H_2O to yield S-lathyrine (1 7 g), mp ⩾ 250° (dec), $[\alpha]_D^{25}$ − 63 0° (H_2O, *c* 23 5 mmol) Fractions corresponding to the first peak to elute were combined, reduced to dryness *in vacuo*, and the residue refluxed in aq HCl (1 M, 20 ml) for 1 5 hr The soln was loaded onto a column of Dowex 50W-X8, H^+ (150 × 25 mm) and washed with H_2O (450 ml) The column was then eluted with NH_4OH (1 M, 450 ml) and the eluate dried *in vacuo* The residue was recrystallized from Me_2CO–H_2O to give R-lathyrine (0 67 g), mp ⩾ 250° (dec), $[\alpha]_D^{25}$ + 64 1° (H_2O, *c* 22 4 mmol) A sample of S-lathyrine isolated from *Lathyrus tingitanus* L (supplied by Professor E A Bell) had mp ⩾ 250° (dec) and $[\alpha]_D^{25}$ − 63 9° (H_2O, *c* 2 13 mmol)

Bioassays The soybean and tobacco callus bioassays were carried out using the procedures of refs [4] and [5], respectively All compounds were autoclaved in the media, except where stated otherwise Assays were carried out using 3 pieces of callus on 25 ml medium, and the callus was weighed after 4 weeks growth Cell number determinations in soybean callus pieces were based on the technique of ref [6] Weighed callus pieces were suspended in 10 M HCl–chromic acid–H_2O (1 1 20) (5 ml/g) for 24 hr, and then shaken vigorously for 10 min The cell suspensions were repeatedly drawn into a Pasteur pipette, diluted suitably, and then counted on a Segwick Raphter slide The number of cells in a pieces of callus was calculated from the mean of the numbers of cells in 20 squares selected at random Mean cell diameters were calculated from two diameter measurements on each of 20 randomly selected cells Measurements were made using a microscope at 60 × magnification

The radish leaf chlorophyll retention assay was carried out using the method of ref [7], and the radish cotyledon expansion assay using the method of ref [8] Radish seeds (*Raphanus sativus* L cv Long Scarlet, Market Strain) were obtained from Yates and Co, New Zealand The *Amaranthus* betacyanin assay was carried out according to ref [9] Seeds of *Amaranthus candatus* L cv Love Lies Bleeding were obtained from Charles and Sharpe, Lincoln, U K The cucumber chlorophyll synthesis assay was performed according to ref [10], and the cucumber cotyledon expansion assay was carried out according to ref [11] The pea bud assay was carried out according to ref [12] Cucumber seeds (*Cucumis sativus* L cv Telegraph) and pea seeds (*Pisum sativum* cv Alaska) were obtained from Dickson, Brown and Tate, Llangollen, U K

The LSD, calculated using the studentized range *Q* method, was used to compare means of treatments at the $P = 0\,05$ level In some experiments the biological error was related to the magnitude of the results In such cases the data were first transformed using a logarithmic transformation in order to stabilize the variance

Acknowledgements—We thank Professors E A Bell and F M Strong for samples of authentic lathyrine, Mr W V McCarthy for advice on the statistical analyses, Shell Research Ltd for a studentship to T S Lee, and Professor P F Wareing and Dr R Horgan for advice and use of facilities in the Department of Botany, University College of Wales, Aberystwyth, where part of this work was undertaken

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