Phytochemistry, Vol 26, No 3, pp 619–620, 1987
Printed in Great Britain

0031-9422/87 $3.00 + 0.00
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THE GREEN ALGAL PIGMENT CAULERPIN AS A PLANT GROWTH REGULATOR*

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(*Received for publication* 26 *August* 1986)

Key Word Index *Caulerpa*, alga, caulerpin, auxin, plant growth regulator

Abstract—Caulerpin, a green algal pigment possessing a unique bis-indole structure, has been shown to be a plant growth regulator. Root growth assays were conducted with caulerpin and its hydrolysis product, and the results were compared with data obtained with indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPA) and indole-3-acrylic acid (IAcA). This study has indicated that caulerpin, in essence a dimer of indole-3-acrylic acid, behaves much like the indole auxins

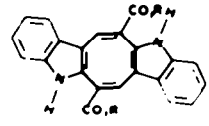
INTRODUCTION

Caulerpin, **1**, is a unique pigment found in some, but not all, species of the green alga *Caulerpa* [1, 2]. Our interest in the chemotaxonomic significance of **1** [3] led us to contemplate whether this seeming dimer of indole acrylic acid, a known auxin [4], might act as a plant growth regulator. Our investigations have revealed that **1** and its hydrolysis product **2** are, in fact, promotors of root growth. Details of these studies are described herein.

RESULTS AND DISCUSSION

We have isolated caulerpin from several species of *Caulerpa*, most frequently *C. sertularioides* and *C. racemosa*, by adsorption and gel permeation chromatography of the crude dichloromethane soluble extracts. Caulerpin can be crystallized from benzene–diethyl ether or dichloromethane. ^{1}H NMR and TLC analyses were employed to assure the purity of the compounds prior to bioassays.

Caulerpin is not particularly soluble in any medium, ethanol and dimethylsulphoxide seem to provide the best solubility for subsequent aqueous dilutions. Solvent blanks were included in the assays. In either distilled water or nutrient solution, DMSO exhibited no influence on roots of lettuce seedlings at concentrations $\leq 0.25\%$, and increasing inhibition of germination and growth at concentrations $> 1.0\%$. In the dilution scheme utilized, 1.0% DMSO corresponds to a 10^{-3} M solution of **1** or **2**, while 0.25% represents a 10^{-4} M solution of the test compound. The effect of ethanol is even more dramatic. In aqueous solutions containing 0.25% ethanol, germination is almost completely inhibited, while a 0.05% ethanol water mixture inhibits root growth, dilution to 0.025% ethanol eliminates any effect.



1 R = CH$_3$
2 R = H

The assays were tried in both nutrient solution and distilled water, although the use of nutrient solution resulted in much shorter average root lengths in both controls and test samples. In contrast to the report of Khan and Tolbert [5], the auxin standards (and **1** and **2**) exhibited only ~20% inhibition of germination at 10^{-3} M and none at 10^{-4} M. It is noteworthy that at 10^{-3} M germination proceeded slowly, with no germination in the first 24 hr and maximum (~80%) germination in 48–72 hr. This delayed germination may explain the apparent incongruity between our data and Khan and Tolbert's. The results of the root growth tests of **1** and **2** were indicative of auxin activity: growth inhibition at high concentrations and growth promotion at low concentrations [6].

A representative run with **1** indicated greatest growth promotion at 10^{-8} and 10^{-9} M with an increase in average root length over that of the controls of 21%. Results of the root elongation test for **2**, conducted either in water or in nutrient solution, parallel those of **1** (see Fig. 1).

Indole acetic acid exhibited effects similar to **1** and **2**, promoting growth at 10^{-8}/10^{-9} M to the extent of 130% of the controls on average. Indole-3-pyruvic acid also promoted root growth at 10^{-8} M, but only to 113% of the controls; indole-3-acrylic acid displayed 15% root growth promotion at 10^{-8} and 10^{-9} M (see Fig. 2).

The presence of indole auxins in green algae has been an object of some debate for a number of years, but Jacobs *et al.* [7] have recently confirmed the presence of indole-3-

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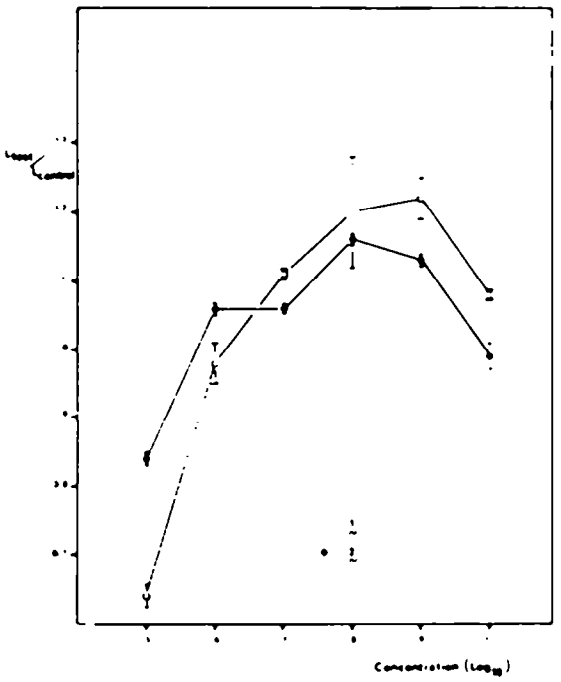

Fig. 1 Root growth assay results for caulerpin (1) and its hydrolysis product (2). Points represent the mean of three trials, s.d. is indicated by error bars.

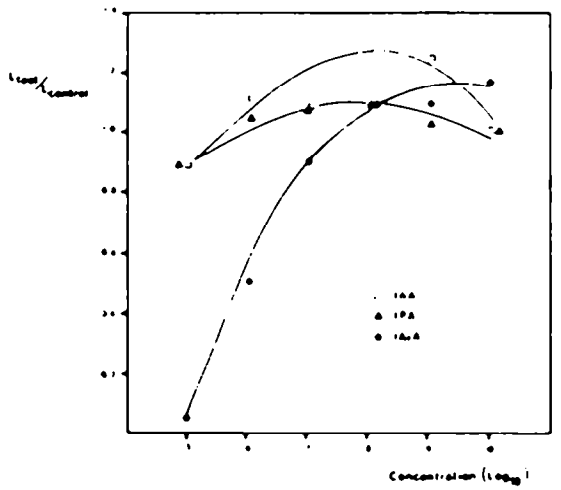

Fig. 2 Root growth assay results for indole acetic acid (IAA), indole pyruvic acid (IPA) and indole acrylic acid (IAcA). Curves were computer generated from means of three trials.

acetic acid in *Caulerpa paspaloides*, a species which does not contain caulerpin [3], and suggested that IAA stimulates blade assimilators and inhibits rhizome development. We did examine the polar extracts of *C. sertularioides* for the presence of known indole auxins, but found none, perhaps because the concentrations found by the Jacobs group were probably beyond the detection limits of our search.

In summary, caulerpin (1) and its hydrolysis product 2 are the first novel plant growth regulators identified from the marine biosphere. These unique indole derivatives exhibit root growth promotion activity comparable to that of indole-3-pyruvic and indole-3-acrylic acids, but somewhat less than indole-3-acetic acid. More recently, we have found plant growth promotors in extracts of two sponges [8].

EXPERIMENTAL

Isolation of caulerpin. *Caulerpa sertularioides* was collected from Mangrove Lake, Bermuda, in October, 1979. The alga was steeped in Me_2CO (twice) and then in CH_2Cl_2 (twice). The Me_2CO extracts were reduced, *in vacuo*, to an aqueous suspension which was then equilibrated with the CH_2Cl_2 extracts. The CH_2Cl_2 phase was reduced, *in vacuo*, to a dark green gum, 6.79 g. This crude extract was chromatographed on Florisil (200 g, column 4.5 × 40 cm) with a hexane–EtOAc–MeOH gradient; fourteen fractions were obtained. Fraction 9 (929 mg), eluted with EtOAc–MeOH (9:1), was permeated through Sephadex LH-20 (column 2 × 130 cm) with CH_2Cl_2–MeOH (1:1) to give caulerpin, 1, 133 mg, as the sixth of six fractions.

Hydrolysis of caulerpin. Caulerpin (26.9 mg) was added to a soln of 236 mg of $Ba(OH)_2\ 8H_2O$ in 10 ml 80% EtOH. The mixture was stirred under N_2 at 55° for 6 hr, then at room temp. overnight. The soln was acidified to pH 4.5 with HCl and the EtOH was removed at red. pres. The resulting aq. suspension was extracted with Et_2O. Evaporation of the organic phase gave 23 mg of a dark green solid. Gel permeation of this material through Sephadex LH-20 (*vide supra*) gave 21 mg of 2.

Assays. For the germination and root growth test, lettuce seeds (*Lactuca sativa*, Burpee Iceberg) were soaked in a 10% EtOH soln for 15 min, rinsed 5 times with dist. H_2O, dried with cheese cloth. Seed assayed at ≥ 95% germination. 5 ml of soln containing the test compound in distilled H_2O:DMSO were pipetted into small Pyrex Petri dishes. A square plastic screen supported on a Teflon ring was placed in each dish. 20 seeds were placed on the screens. Dish covers were replaced and the seeds were incubated under white light at 20–23°. The root lengths were measured after 3 days. Each test was repeated 3 times.

Acknowledgements—Fieldwork was supported by grants from the H. B. Wilkinson Foundation and the Continental Corporation. Research at Montana State University was supported by NSF-EPSCOR Grant ISP 8011449 and the Montana Agricultural Experiment Station. We thank Drs. Gordon Julian and Ralph Olsen for helpful suggestions.

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