either completely (plot C), or almost completely (plots A and B), after being suspended with a gamone preincubated with the corresponding 5-fold diluted immune serum. There was no prevention of conjugation only when tester cells were suspended with a gamone preincubated with the corresponding 640-fold diluted antiserum, and then maximal mating reactions were observed. However, a certain degree of shared antigenicity characterizes the different types of immune sera. Tester cells did not perform maximal mating reactions until they were suspended with a gamone preincubated with a 160-fold diluted immune serum elicited against another type of gamone. Cells in pairs were uniformly found to be approximately 20–30% lower than the respective control (shown in plot D of the figure).

Therefore, it is probable that the antisera raised against the different types of gamones, each of which distinguishes a different mating type, are directed not only at gamone-specific epitopes, but also at other immunogenic determinants common to all gamones. This possibility is substantiated by the results of immunoenzymatic assays (reported in the section on materials and methods) where the intensity of the reaction of each type of antiserum with the corresponding gamone was virtually identical to that shown by the same antiserum in cross-reactions with other types of gamones. It might be also possible that the cross-reactivity between the antibodies contained in an immune serum and non-corresponding gamones is, to some extent, the result of a differential binding at the gamone-specific epitopes. At least some of the epitopes might have slight variations in the amino acid sequences.

Until now, efforts to elicit mating type-specific antibodies in ciliates have all been concentrated on *Paramecium* which, however, does not appear to be an encouraging system for this purpose. The mating type substances of *Paramecium* consist, in fact, of proteins which are intrinsic in the membranes of a small number of cilia and coexist with other powerful immunogens such as immobilization antigens[10]. Therefore, apart from a partial, preliminary success by Hiwatashi[11] in obtaining mating type-specific differences between two types of antisera in *P. caudatum*, the other antisera that have been produced are effective in blocking cell mating but lack mating type specificity[12]. On the other hand it appears to be readily possible to define the antigenic properties of the gamones of *E. raikovi*. The knowledge of such properties is important for tracing both the activity of these mating signals in target cells and for providing insights into their molecular details. This should help towards a better understanding of basic mechanisms in cell-cell recognition of ciliate conjugation.

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Epicatechin can cause the seedling growth inhibitor, nagilactone E, to induce growth stimulation

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Summary. A possible new role for the flavonoid (−)-epicatechin (**II**) is described. It has no growth effects on its own, but when it is added to lettuce and rice seeds together with the known seedling growth inhibitor nagilactone E (**I**), the growth inhibitor activity of **I** can cease and growth stimulation can be observed.
Key words. Flavonoids; growth inhibition; seedling growth; lettuce seedling; rice seedling.

We recently investigated the chemistry of several species of *Podocarpus*[1]. These species are known for their strong resistance to insect attack and their ability to control other plant species in their surroundings[2]. During the course of our bioassay-directed isolation of chemical components it was noticed that there was a discrepancy in the strength of plant growth inhibition. The norditerpenoid dilactone nagilactone E (**I**) was isolated as the major component of a *P. nagi* root bark extract and was found to be responsible for the observed plant growth inhibitory activity[3]. However, the activity of the crude extract of *P. nagi* was less active in seed germination and growth tests than could be explained when the amount of **I** present in the crude extract and strength of the activity of pure **I** was considered. This led to the examination of other components of the crude extract and their effect, when combined with **I**, in seedling growth studies.

The growth bioassays were carried out according to the method of Kamikawa et al.[4], employing seeds of lettuce (*Lactuca sativa*, L., cv Grand Rapids) and rice (*Oryza sativa*, L., cv Norin 20). Lettuce and rice seeds were placed on two layers of filter paper in a 9-cm petri dish containing 4 ml of the test solution. The lettuce seeds were allowed to germinate and grow under continuous fluorescent light (3000 lux at plant level) at 25 °C. Rice seeds were allowed to germinate and grow in the dark at 30 °C. After 5 days the lengths of the lettuce hypocotyl, or the rice shoot, and their roots were measured and an average was taken of 30 seedlings from three petri dishes. One of the major components of the crude mixture was isolated and identified as the known flavonoid (−)-epicatechin (**II**)[5]. The test compounds **I** and **II** showed no visible effects on the germination of both plants except at the highest concentration of **I** used, where 100 µg/ml of **I** resulted in 80% and 50% germination inhibition of lettuce and rice, respectively. Growth effects can be seen in tables 1 and 2.

When **II** was tested alone it elicited little change in the growth of lettuce or rice seedlings. Only at 100 µg/ml does it cause any

Table 1. Combined effect of nagilactone E with (−)-epicatechin on growth of rice seedlings

Nagilactone E (μg/ml)	Length (mm)* Root (−)-Epicatechin (μg/ml)				Shoot (−)-Epicatechin (μg/ml)			
	0	3	30	100	0	3	30	100
0	92.6 ± 5.0	91.6 ± 7.6	91.9 ± 12.6	94.2 ± 4.2	30.4 ± 2.7	30.9 ± 4.2	30.9 ± 4.7	31.8 ± 3.2
0.1	85.9 ± 7.0	101.2 ± 10.5	104.4 ± 8.9	95.5 ± 8.5	32.2 ± 4.2	47.7 ± 4.3	36.9 ± 3.3	34.0 ± 3.1
1	74.1 ± 9.1	89.6 ± 5.7	104.5 ± 8.7	105.6 ± 12.9	29.7 ± 4.9	35.1 ± 4.7	40.8 ± 4.2	45.1 ± 5.5
10	32.0 ± 1.7	35.5 ± 2.2	32.9 ± 2.7	33.1 ± 2.3	21.4 ± 3.7	19.4 ± 3.3	31.1 ± 3.7	30.4 ± 4.0
100	1.4 ± 0.9	2.4 ± 1.6	1.5 ± 1.0	1.6 ± 1.0	17.1 ± 6.3	17.2 ± 5.9	19.8 ± 7.4	17.2 ± 6.3

* Mean ± SE.

Table 2. Combined effect of nagilactone E with (−)-epicatechin on growth of lettuce seedlings

Nagilactone E (μg/ml)	Length (mm)* Root (−)-Epicatechin (μg/ml)				Hypocotyl (−)-Epicatechin (μg/ml)			
	0	3	30	100	0	3	30	100
0	42.7 ± 4.0	43.6 ± 2.2	41.4 ± 3.0	36.6 ± 3.0	4.1 ± 0.2	4.1 ± 0.3	4.0 ± 0.3	4.3 ± 0.4
0.1	44.9 ± 5.2	44.6 ± 3.6	39.4 ± 3.2	35.9 ± 4.6	4.3 ± 0.4	4.0 ± 0.2	4.8 ± 0.4	4.9 ± 0.3
1	19.3 ± 3.1	21.9 ± 2.8	22.1 ± 3.4	18.3 ± 2.1	3.7 ± 0.3	3.7 ± 0.3	4.7 ± 0.4	4.9 ± 0.3
10	4.1 ± 0.8	6.0 ± 0.3	4.7 ± 0.7	4.2 ± 0.8	1.9 ± 0.3	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.4
100	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2

* Mean ± SE.

significant effect; at this level a 14% decrease in lettuce root growth can be seen. Assays using **I** alone showed an increase in growth inhibition with an increase in the concentration of **I**. Most of the growth of both lettuce and rice seedlings was impeded at 100 μg/ml of **I**. However, when **I** and **II** are applied together a rather remarkable observation can be made. The co-presence of **II** can remove the inhibitory effect of **I** and in some cases even cause growth stimulation to take place.

The effect is strongest in the case of rice (see table 1). Concentrations of 0.1 and 1.0 μg/ml of **I** had little effect on the growth of rice seedlings. However, when **I** was combined with **II**, at a respective concentration of 0.1 μg/ml and 3 μg/ml, the rice shoot was seen to grow 48% longer than was the case with only **I** present. A similar effect could be seen when **I** and **II** were applied at 1.0 and 100 μg/ml, respectively. A 52% increase in the length of shoots was seen over those with only 1.0 μg/ml of **I**. Although **I** at 10 μg/ml was responsible for a 30% decrease in shoot growth, when 30 μg/ml of **II** was added the growth inhibition was removed.

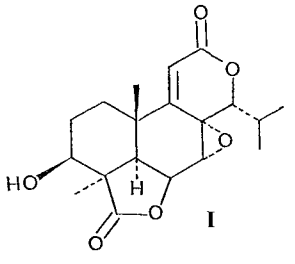
Rice root growth was more sensitive to **I** than shoot growth. Growth inhibition of the root was present even at 0.1 and 1.0 μg/ml of **I**. However, the 20% root growth inhibition due to **I** (1.0 μg/ml) was removed by the added presence of **II** (3 μg/ml), and at higher concentrations of **II** (30 μg/ml) the growth inhibition was actually reversed to give a 14% stimulation of root growth. This is a 41% increase in root growth over the inhibited length due to **I** (1.0 μg/ml).

Lettuce seedling hypocotyl growth behaved in a similar way to rice root growth. Increasing the concentration of **I** gave an increasingly larger growth inhibition of the hypocotyl. **II** had little effect on the hypocotyl growth when used alone. **I** alone gave a slight growth inhibition at 1.0 μg/ml (10%). However, the combination of **I** (1.0 μg/ml) and **II** (100 μg/ml) gave a 20% growth stimulation over the control which contained neither **I** nor **II**, and a 32% increase in hypocotyl growth over the inhibited case (**I**, 1.0 μg/ml).

Lettuce seedlings showed only a slight inhibition in root growth due to **II**. Increasing concentrations of **I** showed an increasingly stronger growth inhibition of the root. However, unlike the effect on rice seedling shoots and roots, and lettuce hypocotyls, there was no change in the effect of **I** and **II** on lettuce root growth when they were combined.

The interaction of nagilactone E (**I**) and (−)-epicatechin (**II**) is difficult to classify as the two usual categories, antagonist or synergist, do not apply. The original bioactivity of **I** is neither enhanced nor merely decreased, but is actually reversed.

The mode of action of **I** and **II**, separate or combined, is unknown. **II** is widely distributed among fruit-bearing plants. It is usually described as a precursor of condensed procyanidins. As a flavan-3-ol, it is generally implicated in antifungal activity and considered capable of complexing with proteins[6]. The related tannins have been known to have an antagonistic effect on GA_3 activity[7]. It is possible that *P. nagi* could utilize a specific concentration of **II** to act as an antidote to the growth inhibitor it normally produces. However, the present study used only lettuce and rice seedlings and seedlings of *P. nagi* were not tested. A study of the effect of **II** on other plant-chemical interactions is now in progress, in the hope that it will lead to a better understanding as to the origin of the activity.



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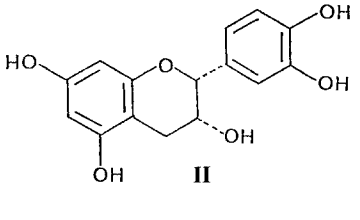

II

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