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Comparative Investigations on the Excitatory Substances of Some Plant Families According to Experiments with Tendrils

By

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Summary

UMRATH K. 1984. Comparative investigations on the excitatory substances of some plant families according to experiments with tendrils. — Phyton (Austria) 24 (2): 321—331. English with German summary.

In many plant families the excitatory substance is an aromatic one. The Leguminosae with aliphatic excitatory substances in their tree subfamilies are an exception. In most families the excitatory substance is an acid. Only in the Leguminosae the carboxylic acid group was found to be in transposition. Only in the Asteraceae the excitatory substance Xanthoxin, is an aldehyde instead of an acid and the aldehyde group is in cis-trans-position. In most families the excitatory substance is easily liberated by indolyl-3-acetic acid, but much less by 5-hydoxyindolyl-3-acetic acid, suggesting hydroxyl groups on the excitatory substance. Exceptions are the Hydrocharitaceae, with 1-methylhistidin as the excitatory substance, and the Passifloraceae, both with equal liberating efficiency of the two auxins.

Zusammenfassung

Umrath K. 1984. Vergleichende Untersuchungen über Erregungssubstanzen einiger Pflanzenfamilien nach Versuchen mit Ranken. — Phyton (Austria) 24 (2): 321—331. — Englisch mit deutscher Zusammenfassung.

In vielen Pflanzenfamilien ist die Erregungssubstanz aromatisch. Die Leguminosen mit alipathischen Erregeungssubstanzen in ihren drei Unterfamilien sind eine Ausnahme. Bei den meisten Familien ist die Erregungssubstanz eine Säure. Bisher wurde nur bei den Erregungssubstanzen der Leguminosen die Carboxylgruppe in Trans-Stellung gefunden. Nur bei den Asteraceen ist die Erregungssubstanz, das Xanthoxin, ein Aldehyd statt

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einer Säure und die Aldehydgruppe ist in cis-trans-Stellung. Bei den meisten Pflanzenfamilien läßt sich die Erregungssubstanz durch Indolyl-3-essigsäure sehr leicht freisetzen, durch 5-Hydroxyindolyl-3-essigsäure aber viel schwerer. Das deutet auf Hydroxylgruppen in ähnlicher Stellung an der Erregungssubstanz. Ausnamen hievon sind die Hydrocharitaceen, mit 1-Methylhistidin als Erregungssubstanz, und die Passifloraceen, bei denen die beiden Auxine gleiche freisetzende Wirkung haben.

Introduction

The excitatory substance in a leaf extract, which induces movements in sensitive plants is different for different plant families (UMRATH 1930). UMRATH & WATANABE 1983 found, that the excitatory substance induces also coiling of tendrils. It was hoped to learn something more about the chemical nature of excitatory substances if the leaf extracts were tested also after passing them through filters retaining certain chemical substances and by investigating the efficiency of liberating substances to liberate the excitatory substance in the tendrils, as it was done by UMRATH & THALER 1980 and by BIELENBERG & al. 1983 on hypocotyls of Lupinus and on sprouts of Mimosa, both Leguminosae.

Material and Methods

The tendrils were cut with scissors near their origin on the stem and than the apical part of the tendril was cut to a length fitting to the Petri-dish used. For tendrils of Vitis vinifera dishes of 6,5 cm diameter with 10 ml solution were used, for tendrils of Cucumis melo dishes of 9 cm diameter with 20 ml solution. Tendrils of Passiflora coerulea were tested in extracts of their leaves in 6.5 cm dishes and in liberating substances in 9 cm dishes. From Vitis and Cucumis 4 to 8 tendrils were put in a dish, from Passiflora 2 to 5 because only few plants were available.

Leaves were extracted in destilled water by heating it to boil a few sec. The leaves remained half an hour in the water and were then removed with as little water as possible.

Extracts were passed through columns of anion exchange resin (Amberlite CG 400 II), 0,6 cm in diameter and 7 cm long, to retain acids and through such columns of polyamide (Woelm) to retain phenols and possibly other aromatic compounds.

In the experiments on *Vitis* and on *Cucumis* three dishes with tendrils were compared, one with the original extract, one with the filtrated extract and one with water. The hebavior of the tendrils was noted at least over two hours, often over a longer time. In the experiments on *Passiflora* with quickly reacting tendrils mostly 2 to 4 tendrils came in the original extract and 3 to 7 in the filtrated extract.

The behavior of the tendrils was noted every 30 min. After half an hour or one hour the tendrils from the filtrated extract came in a dish with the original extract and the behavior of all tendrils was noted over the following two hours.

The experiments on *Vites* tendrils were done in May, these on *Cucumis* tendrils from June to August and these on *Passiflora* tendrils from July to early in September.

As it got warmer and the temperature in the greenhouse reached 30° C the tendrils of *Cucumis melo* coiled better, but the difference between the coiling in an extract and that in water diminished. Therefore from the $4^{\rm th}$ of July the experiments on *Cucumis* tendrils were done outside the greenhouse on a shady place. On the other hand the experiments on the tendrils of *Passiflora* gave better results at high temperature and were performed in the greenhouse on the afternoon.

The experiments with substances supposed to liberate the excitatory substance were performed on tendrils of *Vitis* and of *Cucumis* im comparing the effect of different millimolar (mM) concentrations with that of water. To spare *Passiflora* tendrils they were observed in solutions of different substances in equal mM concentrations.

The experiments on hypocotyls of *Cucurbita pepo* and these on sprouts of *Mimosa pudica* were performed as described by Umrath & Thaler 1980.

Results

a) Tendrils of Vitis vinifera

Extracts of leaves were made in the tenfold amount of destilled water as g of leaves were used. In four experiments the extracts were filtrated through anion exchange resin. In all experiments a test was made on the first day, in two experiments another on the third day. In all these tests the filtrated extracts were distinctly less effective in inducing coiling or bending of tendrils as the unfiltrated extracts. Filtrated extracts were as effective as water or scarcely more. Also destilled water passed through a column of anion exchange resin got sometimes a little more effective.

In three experiments extracts were filtrated through polyamide (Woelm). The filtrated extracts were much less effective as the unfiltrated ones and scarcely more than water. Two of these experiments were tested on the day of the extraction, one on the next day.

Extracts in the dilution 1:40 induced bendings of tendrils sooner than water. The limit of effectiveness may be the dilution 1:100.

The substances supposed to liberate the excitatory substance in the tendrils were tested as sodium salts. In table 1 are listed the mM concentrations which had a good effect on the bending of the tendrils.

Concentrations of some phenolic compounds (mM) for inducing bending of tendrils of Vitis vinifera in neutralized solutions

benzoic acid	40
salicylic acid (= 2-hydroxybenzoic acid)	40
2,3,4-trimethoxybenzoic acid	40
3,4,5-trimethoxybenzoic acid	> 40
phenylacetic acid	20
mandelic acid (= 2-hydroxyphenylacetic acid)	10
phenylpropionic acid (= hydrocinnamic acid)	10
phenyllactic acid (= 2-hydroxyphenylpropionic acid)	10
3,4-dihydroxyphenylpropionic acid (= hydrocoffeic acid)	5
phenylbutyric acid	5
phenylvalerianic acid	5

b) Tendrils of Cucumis melo

Leaves of *Cucumis melo* were extracted in the tenfold amount of ml destilled water as g of leaves were used.

In two experiments the extract was filtrated through anion exchange resin. The behavior of the tendrils was noticed through 1½ respectively 3½ hours. Bendings of tendrils in water diminished in number sooner than in the native extract. In both experiments the filtrated extract had approximately the same effect on the tendrils as water, whereas the unfiltrated extract induced much more coiling.

In five experiments the extract was filtrated through polyamide (Woelm) columns. The extract was yellow. Throuh the polyamide filter it passed at first pale but later, if a greater amount of extract had passed the filter, the filtrate was yellow only a little clearer than the original extract.

In two experiments the 20 ml needed for a dish were filtrated through two columns of polyamide, so that none of them was overloaded and the filtrate was pale. One of these experiments was tested twice. In all these three tests the tendrils in the filtrated extract behaved very like as these in water, wheras in the unfiltrated extract they showed distinctly more coiling.

In three experiments the filtrated extract was yellow. In one of these experiments the filtrated and the unfiltrated extract were equally effective and distinctly more than water. In the remaining two experiments the filtrated extract was a little more effective as the unfiltrated one and both were distinctly more effective than water. This suggests the retaining of an inhibitory substance in the filter.

As abscisic acid is expected to be retained in both filters used, its action as a sodium salt was investigated in 0,01 and 0,1 mM concentration in water and in leaf extracts 1:10. In water the bending and

coiling of the tendrils was a little augmented, but the efficienty of an extract of leaves was somewhat diminished. This can be the reason, that in the above mentioned experiments the filtrated extracts were a little more effective.

The liberating substances were used in solutions neutralized with NaHCO₃. The effective concentrations are listed in table 2.

Table 2

Concentrations of some compounds (mM) for inducing bending of tendrils of Cucumis melo (neutralized solutions)

benzoic acid	20
phenylacetic acid	20
phenylpropionic acid	20
trans-cinnamic acid	40
3,4-dihydroxyphenylpropionic acid	40
phenylbutyric acid	10
phenylvalerianic acid	20
butyric acid	10
4-aminobutyric acid	10
2-hydroxybutyric acid	10
3-hydroxybutyric acid	10-20
2-amino-3-hydroxybutric acid	10-20
4-hydroxybutyric acid	20
valerianic acid	20
hexanoic acid (= caproic acid)	20

c) Hypocotyls of Cucurbita pepo

The liberating substances were applied to the hypocotyls unilaterally in lanoline-water-paste. Bendings were noted one and two days later. Bendings to the side of the paste, indicating a liberation of the excitatory substance, were designated as positive, bendings away from the side of the paste as negative, indicating a liberation of auxin or a prevailing auxin action of the applied substance. The following substances with exception of the auxins were tested only in 75 mM paste.

3-Phenylpropionic acid (= hydrocinnamic acid) induced + 100% bendings on both days. Trans-cinnamic acid induced + 43% bendings on the first day and + 14% on the second. Trans 2-menthoxycinnamic acid induced + 50% bendings on both days, cis 2-methoxycinnamic acid + 43% on both days. Mandelic acid (2-hydroxyphenylacetic acid) induced + 89% bendings on the first day and + 67% on the second, whereas 3,4-dihydroxymandelic acid induced 9% bendings on both days.

With indolyl-3-acetic acid in 15 mM paste the bendings were $\pm 100\%$ on the first day and $\pm 88\%$ on the second. With 75 and 450 mM paste they were $\pm 100\%$ on both days and very intensive with 450mM paste.

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On the contrary, 5-hydroxyindolyl-3-acetic acid induced in all three concentrations on both days only beedings away from the paste, $-100^{\circ}/_{\circ}$ and the hypocotyls were thickened.

d) Tendrils of Passiflora coerulea

Extracts of leaves were made in the twentyfold amount of destilled water as g of leaves were used.

In two expriments the extract was filtrated through anion exchange resin (Amberlite). In both experiments the tendrils in the original extract bended and coiled more as these in the filtrated extract. In the second experiment after one hour all tendrils were transfered to the original extract. The reaction of the tendrils which were from the beginning in this extract diminished a little, whereas the tendrils which came from the filtrated extract reached about the degree of coiling that the others had before.

Five experiments were performed in the same manner with a polyamide filter to retain phenols. In four of these experiments the tendrils in the original extract coiled distinctly more as these in the filtrated extract. After about one hour all tendrils were put in an unifiltrated extract. In the tendrils which were in this extract from the begining the coiling decreased a little, whereas the tendrils coming from the filtrated extract coiled increasingly during one hour. In one experiment the filtrated extract received two very old tendrils, which coil most easly, so it was an exeptional coiling in the filtrated extract. In an additional experiment dishes with the original extract, the filtrated extract and with water received each 5 tendrils. The tendrils in the filtrated extract were about as these in water, whereas the tendrils in the original extract were distinctly more bent and coiled.

Liberating substances were applied in solutions neutralised with NaHCO₃. Indolyl-3-acetic acid and 5-hydroxyindolyl-3-acetic acid were equally effective. bothe had a good efficacy in 1,5 mM solution and a minor one in 0,5 mM solution. Indolyl-3-propionic acid had about one third of this efficiency.

The following substances were tested in 40 mM and the last mentioned also in 30 mM solution. Phenylacetic acid was a little less effective as phenylpropionic acid. Phenyl-2-hydroxypropionic acid (phenyl-lactic acid) and 3,4-phenylpropionic acid (hydrocoffeic acid) were about equally effective as phenylpropionic acid, whereas trans-cinnamic acid was much less effective. Phenylbutyric acid and especially phenyl-valerianic acid seemed to be a little more effective as phenylpropionic acid.

e) Leaf movements of Mimosa pudica

Cut sprouts of *Mimosa pudica* were fixed on stages with their cut ends dipping in water. When the leaves were reexpanded the water was replaced by the solution to be tested. In table 3 are listed the mM concentrations of substances, which in solutions neutralised with NaHCO₃, induce leaf movements.

Table 3

Concentrations of some compounds (mM) inducing leaf movements of Mimosa pudica (neutralized solutions)

butyric acid	20
valerianic acid	40
hexanoic acid (= caproic acid)	40
phenylbutyric acid	20
phenylvalerianic acid	10
decanoic acid (= capric acid)	5
dodecanonic acid (= lauric acid)	2
dodecandioic acid	2
trans-2-dodecandioic acid (= traumatic acid)	1
chinolin-2-carbonic acid	2
chinolin-4-carbonic acid	2
9-anthracencarbonic acid	1
9-fluorencarbonic acid	2
9-fluorenon-4-carbonic acid	0,5

Conclusions

Now we know something about the excitatory substance of 7 plant groups. The Hydrocharitaceae with 1-methylhistidine as the excitatory substance (Fitting 1936, Umrath & Watanabe 1982), the Vitaceae, the Cucurbitaceae, the Passifloraceae and the Asteraceae with Xanthoxin as the excitatory substance (Bruinsma, Franssen & Knegt 1980, Umrath 1983) have aromatic excitatory substances. For the Vitaceae, Cucurbitaceae and Passifloreae this follows from the statements in the results, that leaf extracts of these plants lose their exciting power in passing though a polyamide filter. Only in the subfamilies of the Leguminosae, the Mimosoidae and the Faboidae, is the excitatory substance an aliphatic compound (Bielenberg et al. 1983). The excitatory substances of these two subfamilies of the Leguminosae are very similar to one another (Umrath & Soltys 1936) and that of the Caesalpinioidae is probably identical with that of the Faboidae (Umrath & Watanabe 1983).

In most plant families the excitatory substance is an acid. For the Vitaceae, Cucurbitaceae and Passifloreae this follows from the statements in the results, that their leaf extracts lose their exciting power

in passing through an anion exchange filter. For the *Leguminosae* the same was reported by Bielenberg *et al.* 1983. Only Xanthoxin of the *Asteraceae* is no acid, but an aldehyde.

In the Leguminosae the excitatory substance has a carboxylic acid group in trans position, as all tested liberating substances with a carboxylic acid group in trans position were much more effective as corresponding substances without a double bond (Bielenberg et al. 1983). On the contrary on Vitis tendrils phenylpropionic acid (= hydrocinnamic acid) was more effective than trans-cinnamic acid and than both trans-2-methoxycinnamic acid and cis-2-methoxycinnamic acid (Umrath & Watanabe 1983). Table 2 shows that on the tendrils of Cucumis phenylpropionic acid is more effective than trans-cinnamic acid and for the tendrils of Passiflora the same is reported in the text of the results. In Xanthoxin of the Asteraceae the aldehyde group is in cis-trans-position.

Besides the properties mentioned the excitatory substance has its peculiarity in every plant family. The excitatory substance of the *Passifloreae* has the peculiarity to be liberated equally well by indolyl-3-acetic acid and by 5-hydroxyndolyl-3-acetic acid and also equally well by phenylpropionic acid and by 3,4-dihydroxyphenylpropionic acid. This suggests, that the excitatory substance has no hydroxylgroups, at least not on corresponding places with these of the just mentioned substances. Besides this an equal effect of these two auxins in liberating the excitatory substance is only known of the *Hydrocharitaceae* with 1-methylhistidine as the excitatory substance, which has no hydroxylgroups (Umrath & Thaler 1981).

The excitatory substance of *Cucumis* has the peculiarity, that it is only loosly bound in the polyamide filter, so that it is liberated from the polyamide by a coloured substance in the leaf extracts. This suggests, that the aromatic ring in the excitatory substance is very different from a phenol ring. The relativly high liberating efficiency of phenylbutyric acid and of butyric acid may be a hint at a similar side chain of the excitatory substance.

Different hydroxy-substituents of butyric acid were tested for the following reason. In yet unpublished experiments about the liberation of acetylcholine in the quinea pig's cornea, as described by Umrath & Thaler 1980, the just effective liberating concentrations were found to be for 2-hydroxybutyric acid 5 mM, for 3-hydroxybutyric acid 1 mM, for 4-hydroxybutyric acid 20 mM, for 2-amino-3-hydroxybutyric acid 2 mM and for 2-amino-4-hydroxybutyric acid 20 mM. It is to be seen, that the 3-hydroxy-compounds are most effective and the 4-hydroxy-compounds least. On *Lupinus* hypocotyls it was found in yet unpublished experiments that acetylcholine unilaterally applied in lanoline-

waterpaste of 15 or 75 mM concentration induced about 50% bendings to the treated side. This indicates a growth inhibition, as it is also induced above all by the excitatory substance. As Umrath & Thaler 1980 described, may liberating substances induce by unilateral application, on Lupinus hypocotyls on the first day bendings to the side of application and on the second day these bendings are reduced or bendings away from the side of the paste appear. This was interpreted as a liberation of the excitatory substance and of auxin, the auxin acting preponderantly on the second day. Table 4 shows this effect for 4-hydroxybutyric acid and 2-amino-4-hydroxybutyric acid, whereas with 3-hydroxybutyric acid and 2-amino-3-hydroxybutyric acid the bendings to the paste augment from the first to the second day. The same can be seen in table 2 of Umrath & Thaler 1980 with citric acid and serine, the only two substances with a hydroxyl group on the second C-atom from the carboxylic acid group as it is true also of 3-hydroxybutyric acid.

Table 4
Bendings of Lupinus hypocotyls (%, bendings to the paste are counted as positive)

substance and concentration in the paste	1. day	2. day
4-hydroxybutyric acid 75 mM	63	25
4-hydroxybutyric acid 450 mM	25	-37
3-hydroxybutyric acid 75 mM	25	72
3-hydroxybutyric acid 450 mM	0	50
2-amino-4-hydroxybutyric acid 75 mM	70	30
2-amino-4-hydroxybutyric acid 450 mM	0	17
2-amino-3-hydroxybutyric acid 75 mM	50	60
2-amino-3-hydroxybutyric acid 450 mM	8	15

It is reasonable to assume that substances with a hydroxylgroup on the second C-atom from the carboxylic acid-C liberate acetylcholine. This acetylcholine inhibits growth and thereby strengthens bendings to the paste on the second day.

The figures in table 2 suggest, that acetylcholine is not involved in tendril coiling of *Cucumis*.

The excitatory substance of *Vitis* is easier liberated by indolyl-3-acetic acid as by 5-hydroxyindolyl-3-acetic acid (Umrath & Watanabe 1983), whereas phenylpropionic acid is rather less effective as 3,4-dihydroxypenylpropionic acid, as can be seen in table 1. A possible explanation would be, that the excitatory substance has two condensed rings and the auxins connect in liberating with both rings, the indols only with that which bears the side chain.

The experiments on sprouts of *Mimosa pudica* are summarized in table 3. The aliphatic liberating substances have an increasing efficiency with increasing length of the carbon-chain. The highest efficiency has trans-2-dodecandioic acid (= traumatic acid). Phenylbutyric acid and phenylvalerianic acid fit into this arrangement, if the length of the side chain plus that of the circumference of the ring is taken as total length. This is in accordance with the view, that in liberating the two substances lay themselves together in this manner.

Trans-2-dodecandioic acid (= traumatic acid) is twice as effective in liberating the excitatory substance of *Mimosa* as is dodecandioic acid. In table 1 of Bielenberg et al. 1983 refering to *Mimosa* and Lupinus, the latter belonging to the *Faboidae* phenyl compounds with a carboxylic acid group in trans-position are two and a half times to twenty times, in the average seven times as effective as corresponding compounds with no double bond or with a carboxylic acid group in cisposition.

FITTING 1930 found 0,3 mM franguloemodin effective on *Mimosa* and as well some other anthrachinon derivatives with three condensed rings. Umrath & Thaler 1980 found reserpine and some thymoleptics with many condensed rings effective on *Mimosa* in 2 mM solutions. Therefore five acids with two to three condensed rings were tested on *Mimosa* and listed in table 3 as the five last substances. They were all very effective.

The name traumatic acid was given to trans-2-dodecendioic acid by English, Bonner & Haagen-Smit 1939. They found, that the substance in combination with a co-factor induced cell enlargement and cell division on not wounded pericarp-parenchym of a certain cultivar of Phaseolus vulgaris. The authors found substances with a carboxyl group in transposition more effective as analog substances without a double bond. The same is true for substances liberating the excitatory substance of the Mimosidae and that of the Faboidae (Bielenberg et al. 1983). English, Bonner & Haagen-Smit 1939 found lauric acid with only one carboxyl group completely inactive in the bean test and after their opinion two carboxyl groups appear to be essential for wound hormone activity in the bean test. Table 1 of UMATH & THALER 1980 shows substances with acid groups on both ends, citric acid and cysteic acid, highly effective in liberating the excitatory substance of the Mimosiadae and that of the Faboidae. For substances with long carbonchains this does not hold for Mimosa, as shown in table 3, but experiments on Faboidae are missing. For traumatic acid English, Bonner & HAAGEN-SMIT 1939 described also the induction of cell divisions in potato tubers and the desinhibition of the germination of tomato seeds inside the fruit pulp. In these cases nothing is said about the efficacy of related substances.

The initiation of cell divisions by the excitatory substance was shown by Umrath 1930 and by Umrath and Soltys 1936, using the bean test.

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