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Liberation of Xanthoxin

By

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With 3 Figures

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Summary

UMRATH K. 1983. Liberation of xanthoxin. — Phyton (Austria) 24 (1): 79—85, with 3 figures. — English with German summary.

The liberation of xanthoxin was juged by the bendings of seedlings caused by inhibition of growth following unilateral application of the inhibiting substance in lanoline-water-paste.

The liberation of xanthoxin by cinnamic acid derivates shows a dependance of the fitting together of the cis- and trans-positions. This becoms clear if the influence of OH-groups is also considered. OH-groups on corresponding places of the liberating substance and of xanthoxin hinder the lineration completely, on neighbouring places of both substances they hinder, but less.

Zusammenfassung

UMRATH K. 1983. Freisetzung von Xanthoxin. — Phyton (Austria) 24 (1): 79—85, mit 3 Figuren. — Englisch mit deutscher Zusammenfassung.

Die Freisetzung von Xanthoxin wurde nach den Krümmungen von Keimlingen beurteilt, welche durch die Wachstumshemmungen bedingt waren, die auf einseitige Anbringung der freisetzenden Substanz in Lanolin-Wasser-Paste folgten.

Die Freisetzung von Xanthoxin durch Zimtsäure-Derivate zeigte eine enge Beziehung zum Zusammenpassen der cis- und trans-Stellungen. Das wird deutlich, wenn der Einfluß von OH-Gruppen auch berücksichtigt wird.

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OH-Gruppen an korrespondierenden Stellen der freisetzenden Substanz und von Xanthoxin verhinderten die Freisetzung vollkommen, an benachbarten Stellen beider Substanzen hinderten sie, aber weniger.

Introduction

BRUINSMA, FRANSSEN & KNEGT 1980 found, that *Helianthus* seedlings bend to the light without alteration in the distribution of auxin. As they found, the cause of the bending is an unequal distribution of xanthoxin, a substance inhibiting growth. As SOLTYS, UMRATH & UMRATH 1938 showed, that in *Mimosaceae* and in *Fabaceae* the respective own excitatory substance is an antagonist of auxin, one may take xanthoxin as the excitatiry substance of the *Asteraceae.*

On the other hand DÖRFLING 1978 showed, that xanthoxin is found also in *Pisum sativum,* being responsible for the reduced growth of dwarf cultivars.

As the biological active xanthoxin has a cis- and a trans-position in the side chain of a ring with six C-atoms, the liberating power of some cis- and trans-derivates of cinnamic acid were investigated.

Methods

Liberating substances were applied to seedlings unilaterally in lanoline-water-paste in 15, 75 and 450 mM concentrations, as described by UMRATH & THALER 1980. Bendings to the side of the applied paste were designated as positive, bendings to the other side as negative. In the results the difference of the two values is given in per cent of the toatl number of seedlings. Positive bendings are ascribed to the liberation of xanthoxin and in the case of *Fabaceae* also to their excitatory substance. Negative bendings are ascribed to the prevailing liberation of auxin or, in the case of applied auxin, to its own activity exceeding the effect of the liberated xanthoxin.

Results

Helianthus annuus hypocotyls

On hypocotyls of *Helianthus* the bendings with cis-4-hydroxy-cinnamic acid in 15, 75 and 450 mM paste were — 50%, + 37% and + 100% on the first day and on the second day with 75 and 450 mM paste + 18% and + 100%. In fig. 1 the formula of xanthoxin is at the top, that of cis-4-hydroxy-cinnamic acid is below. It is to be seen, that a translation of this formula upwards into the xanthoxin formula would cover the side chain of xanthoxin and one side of its ring. With trans-4-hydroxy-cinnamic acid (p-cumaric acid) the bendings were on the first day — 9%, — 54% and — 18% and on the second day

— 45%, — 64% and — 9%. In this case the rings could cover one another and the side chain of xanthoxin could be partly covered. But an union in this way would be hindered by the OH-groups on corresponding places. With trans-3-hydroxy-cinnamic acid (m-cumaric acid) the OH-groups of the two substances are at neighbouring positions. The bendings were — 20%, — 50% and 0% on the first day and + 20%, — 50% and — 33% on the second. Finally with trans-2-hydroxy-cinnamic acid (o-cumarie acid) the OH-groups are as far from each other as possible. The bendings were — 10%, + 36% and + 33% on the first day and — 33%, + 9% and + 45% on the second.

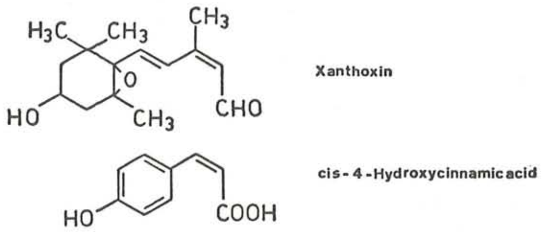


Fig. 1. Above the formula of xanthoxin, below that of cis-4-hydroxycinnamic acid. A translation of this formula upwards into that of xanthoxin would cover the side chain of xanthoxin and one side of its phenol-ring

With trans-2-methoxy-cinnamic acid the bendings were + 27%, + 55% and + 78% on the first day and + 27%, + 11% and + 45% on the second. A possible interaction of trans-2-methoxy-cinnamic acid with xanthoxin is illustrated in fig. 2. Cis-2-methoxy-cinnamic acid gave bendings of — 20%, + 30% and + 66% on the first day and — 46%, + 31% and + 45% on the second. A possible interaction of cis-2-methoxy-cinnamic acid with xanthoxin is illustrated in fig. 3.

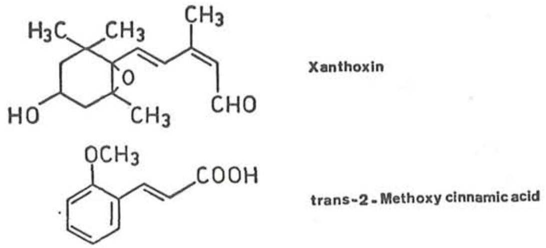
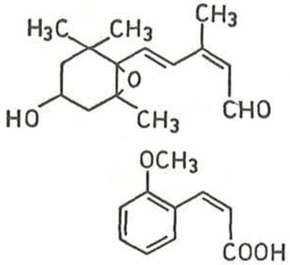


Fig. 2. Above the formula of xanthoxin, below that of trans-2-methoxycinnamic acid. A translation of this formula upwards into that of xanthoxin would cover the ring and a part of the side chain of xanthoxin

With trans-cinnamic acid in 15 and 75 mM paste the bendings were — 7% and + 38% on the first day and 0% and — 25% on the second day.



Xanthoxin

cis-2-Methoxycinnamic acid

Fig. 3. Above the formula of xanthoxin, below that of cis-2-methoxycinnamic acid. A translation of this formula upwards into that of xanthoxin would cover the side chain of xanthoxin and one side of its ring

Indolyl-3-acetic acid induced in *Helianthus* hypocotyls bendings of — 22%, + 100% and + 100% on the first day and — 33%, + 100% and + 100% on the second. 5-Hydroxy-indolyl-3-acetic acid is much less effective, so that its auxin action prevails. The bendings were — 86%, — 61% and — 10% on the first day and — 88%, — 40% and + 30% on the second.

Pisum sativum epicotyls

From *Pisum sativum* two cultivars were available, Imperial, a dwarf form, reaching about 20 cm, and cv. Telephon, reaching about 140 cm.

On epicotyls of cv. Imperial with cis-4-hydroxy-cinnamic acid the bendings were + 67%, + 100% and + 100% on the first day and 0%, + 100% and + 100% on the second. With trans-4-hydroxy-cinnamic acid the bendings were + 50%, + 40% and 0% on the first day and 0%, 0% and 0% on the second.

On epicotyls of cv. Telephon only 15 and 75 mM pastes were used. With cis-4-hydroxy-cinnamic acid the bendings were + 14% and + 80% on the first day and 0% and + 10% on the second. With trans-4-hydroxy-cinnamic acid they were + 57% and + 63% on the first day and + 57% and + 42% on the second.

For comparision analogous experiments with an other fabaceous plant, *Lupinus albus*, are mentioned. With cis-4-hydroxy-cinnamic acid the bendings were — 18%, — 46% and — 27% on the first day and — 61%, — 32% and — 82% on the second. With trans-4-hydroxy-cinnamic acid the bendings were + 46%, + 64% and + 42% on the first day and + 54%, + 62% and + 55% on the second.

Conclusions

Umrath 1930 found the excitatory substance different in different plant families or subfamilies. Soltys, Umrath & Umrath 1938 demonstrated an antagonism between the excitatory substance and auxin in *Mimosaceae* and *Fabaceae*. The antagonism was to be seen only on plants of the own subfamily.

Bruinsma, Franssen & Knegt 1980 found that xanthoxin besides inhibiting growth of *Helianthus* hypocotyls initiates their bending to the light by its unequal distribution on the lighted and on the shaded side. These properties of xanthoxin are what one would expect from the excitatory substance of the *Asteraceae*.

Xanthoxin has the peculiarity of inhibiting growth also in other families as the *Asteraceae* and it is responsible at least for dwarf cultivares of *Pisum sativum* (Dörfling 1978).

The present investigation shows by liberation experiments that in *Pisum* epicotyls growth is inhibited by the excitatory substance of the Fabaceae, as trans-4-hydroxy-cinnamic acid inhibits growth, and by xanthoxin, as cis-4-hydroxy-cinnamic acid inhibits growth especially in a dwarf cultivar. Umrath & Watanabe 1983 found that the coiling of *Pisum* tendrils is initated exclusively by substances liberating the excitatory substance of the *Fabaceae* and not at all by substances liberating specifically xanthoxin. It may be mentioned here, that they had tendrils of the dwarf cultivar Imperial from *Pisum sativum*. From this it can be concluded, that either xanthoxin is present only in the stem of *Pisum* and not in the tendrils or that xanthoxin does not induce coiling in *Pisum* tendrils.

Fig. 1 shows how cis-4-hydroxy-cinnamic acid fits to a part of the xanthoxin molecule and in the results it was shown to induce plenty of positive bendings of *Helianthus* hypocotyls, what means a good liberation of xanthoxin. On the other hand trans-4-hydroxy-cinnamic acid induces only negative bendings of *Helianthus*, indicating that only auxin is liberated and no xanthoxin. Trans-4-hydroxy-cinnamic acid and xanthoxin would fit to each other if the rings and parts of the side chains would lie on each other. But in this case the OH-groups also come together and they would hinder a close contact between the two substances, as it seems needed for a liberation. Trans-2-methoxy-cinnamic acid and cis-2-methoxy-cinnamic acid are both effective in liberating xanthoxin in *Helianthus* and the fitting of their formulas to that of xanthoxin is illustrated in figs. 2 and 3. This shows conclusively that in trans-4-hydroxy-cinnamic acid the OH-group in position 4 is the hindering element for the liberation of xanthoxin.

The fact that cis-4-hydroxy-cinnamic acid has a higher liberating efficacy as cis-2-methoxy-cinnamic acid is difficult to explain. The xanthoxin skeleton is to complicated at the point where the OH-group of cis-4-hydroxy-cinnamic acid comes to lie. In any case cis-4-hydroxy-cinnamic acid is the best substance to show by liberating the presence of xanthoxin in a tissue where it inhibts growth or has other physiological effects.

As shown in the results, the bendings of *Helianthus* hypocotyls with trans-3-hydroxy-cinnamic acid are not so exclusively negative as these with trans-4-hydroxy-cinnamic acid and the OH-group of trans-3-hydroxy-cinnamic acid and of xanthoxin are in neighbouring positions. trans-2-Hydroxy-cinnamic acid has the OH-group still farther from that of xanthoxin and its liberating efficiency is about the same as that of trans-cinnamic acid. Therefore the OH-group of trans-2-hydroxy-cinnamic acid is not appreciably hindering the liberation. Trans-2-methoxy-cinnamic acid and cis-2-methoxy-cinnamic acid are more effective in liberating xanthoxin as is trans-cinnamic acid, as can be seen from the figures in the results. From this it can be concluded, that a OCH_3-group on liberating substances corresponding to places of xanthoxin without an OH-group or a close neighbourhood to it are favorable for the liberation of xanthoxin.

Xanthoxin of the *Asteraceae* is the only known excitatory substance with a cis- and trans-position. For the *Mimosaceae* and *Fabaceae*, both subfamilies of the *Leguminosae*, BIELENBERG *et al.* 1983 found that the excitatory substances of both of them have a carboxylic acid group in trans- position and the same was found by UMRATH & WATANABE 1983, using tendrils of *Pisum (Fabaceae)*. In the other families investigated till now the excitatory substance has no cis- or trans-position. These families are the *Vitaceae* (UMRATH & WATANABE 1983), the *Cucurbitaceae* (unpublished results) and the *Hydrocharitaceae* with 1-methyl-histidine as the excitatory substance (FITTING 1936, UMRATH & WATANABE 1982).

The high effectiveness of indolyl-3-acetic acid in liberating the excitatory substance in all families investigated was founf also here for the liberation of xanthoxin in *Helianthus*.

SOLTYS, UMRATH & UMRATH 1938 found an anatogonism between the excitatory substance and indolyl-3-acetic acid. UMRATH & WATANABE 1983 proposed, that the physiological importance of this antagonism may have brought about by natural selection chemical similarities of these two substances. These similarities are responsible for the high liberating power of indolyl-3-acetic acid for the excitatory substance of every family.

5-hydroxy-indolyl-3-acetic acid is in most plant families much less effective in liberating the excitatory substance. In the *Fabaceae* it is completely ineffective (UMRATH & THALER 1981). BIELENBERG *et al.* 1983 ascribe in the excitatory substance of the *Fabaceae* OH-groups to the fifth and sixth C-atom from the carboxylic acid C counted as one. 5-hydroxy-indolyl-3-acetic acid has the OH-group on the sixth C-atom. This coincidence obviously hinders the liberation. In comparing the formulas in the case of xanthoxin it seems likely that the OH-groups are neighbouring positions on the two substances. In accordance with this there is only little liberation of xanthoxin in *Helianthus* by 5-hydroxy-indolyl-3-acetic acid.

References

BIELENBERG W., ESTERBAUER H., HAYN M. & UMRATH K. 1983. The excicatory substance of the *Mimosaceae*. — Phyton (Austria) 24 (1): 1—10.

BRUINSMA J., FRANSSEN J. M. & KNEGT E. 1980. Phototropism as a phenomenon of inhibition. — In SKOOG F. (ed.) Plant growth substances 1979. — Springer Verlag Berlin, Heidelberg, New York.

DÖRFLING K. 1978. The possible rôle of xanthoxin in plant growth and development. — Phil. Trans. R. Soc. Lond. B. 284: 499—507.

FITTING H. 1936. Über Auslösung von Protoplasmaströmung bei *Vallisneria* durch einige Histidinverbindungen. — Jb. wiss. Bot. 82: 613—624.

SOLTYS A., UMRATH K. & UMRATH CH. 1938. Über Erregungssubstanz, Wuchsstoff und Wachstum, — Protoplasma (Berl.) 31: 454—480.

UMRATH K. 1930. Über Erregungssubstanzen. — Jb. wiss. Bot. 73: 759—769.

— & THALER I. 1980. Auslösung von Blattbewegungen bei *Mimosa* und von Krümmungen von *Lupinus*-Hypokotylen, gedeutet durch Freisetzung von Erregungssubstanz und Auxin. — Phyton (Austria) 20: 333—348.

— & —. 1981. 5-Hydroxyindolyl-3-essigsäure als ein Auxin. — Ber. Deutsch. Bot. Ges. 94: 143—149.

— & WATANABE SH. 1982. The interaction of the excitatory substance with Auxin in *Elodea*. — Phyton (Austria) 22: 143—148.

— & —. 1983. The hormonal basis of tendril coiling. — Phyton (Austria) 23 (2): 307—312.