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Vitamin D_3 in plants – effect of UVB exposure

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Introduction

As a surprise for many not only vitamin D_2, but also vitamin D_3 can be found in plants. Vitamin D_3 is formed in the skin of vertebrates by exposure to UVB light (Fig. 1). The synthesis of vitamin D_3 in plants is on the other hand unresolved and contradicting results regarding the dependence of UVB-light has been presented (1,2,3). The aim of this study was, therefore, to investigate vitamin D_3 synthesis and metabolism in plants and how it changes upon UVB-exposure. Most work on vitamin D_3 in plants has been done with non-selective methods such as bioassays, but this study utilizes LC-MS/MS with derivatization to improve sensitivity and selectivity.

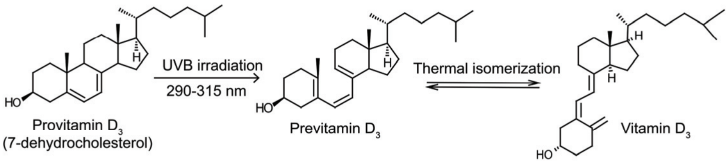
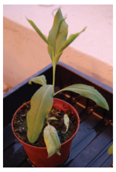


Fig. 1. Biosynthesis of vitamin D_3 from 7-dehydrocholesterol. UVB exposure of 7-dehydrocholesterol breaks the B-ring to form previtamin D_3, which undergoes thermally induced rearrangement to vitamin D_3.

Material

Plants were grown in growth chambers with or without UVB light. Three *Solanaceous* species were used:



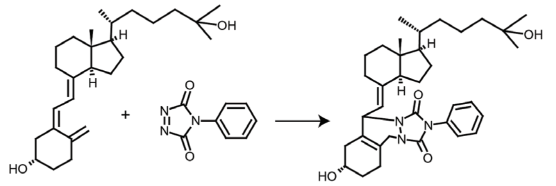
Solanum glaucophyllum Desf.
(waxy leaf nightshade)

Solanum lycopersicum L.
(tomato)

Capsicum annuum L.
(pepper)

Method

The leaves were harvested, freeze-dried and saponified overnight. The vitamin D_3 metabolites were extracted from the non-saponified matter followed by solid phase clean-up. Further clean-up was performed with semi-preparative HPLC. Fractions of vitamin D_3, 25-hydroxy vitamin D_3 and 1,25-dihydroxy vitamin D_3 were collected separately and derivatized with 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) to increase sensitivity (Fig. 2). The derivatized extracts were subsequently analyzed by LC-ESI-MS/MS. The vitamin D_3 metabolites were quantified using their deuterated form as internal standard.



25OHD3 PTAD

Fig. 2. The vitamin D_3 metabolites, as here 25-hydroxy vitamin D_3, were derivatized with 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) to enhance sensitivity in ESI-MS/MS.

Results

Vitamin D_3 was identified in *S. glaucophyllum*, *S. lycopersicum* and *C. annuum* (Table 1). The vitamin D_3 content in the UVB-exposed plants was 18-64 times higher than for the not UVB-exposed plants. 25-hydroxy vitamin D_3 was only identified in the UVB-exposed plants, whereas 1,25-dihydroxy vitamin D_3 only was found in UVB-exposed *S. glaucophyllum* (Table 1).

Table 1. Content of vitamin D_3, 25-hydroxy vitamin D_3 and 1,25-dihydroxy vitamin D_3 in plants grown with (+UVB) or without (-UVB) UVB light

	ng per gram dry weight		
Plant	**D_3**	**$25OHD_3$**	**$1,25(OH)_2D_3$**
S. glaucophyllum (+UVB)	200	31	32
S. glaucophyllum (-UVB)	3.2	0.8	<0.1
S. lycopersicum (+UVB)	100	4.3	<0.1
S. lycopersicum (-UVB)	1.7	<0.02	<0.1
C. annuum (+UVB)	2.9	0.5	<0.1
C. annuum (-UVB)	<0.02	<0.02	<0.1

Conclusion

It is remarkable that the leaves of the *Solanaceous* family contain high amounts of vitamin D_3 bearing in mind that the fruits from, e.g. tomato is an important food for humans. Thus, the potential of plants as a vitamin D_3 source exists. This study demonstrates that both UVB-dependent and independent pathways for biosynthesis of vitamin D_3 exist in plants.

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