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UV-B dependent and independent vitamin D synthesis in plants

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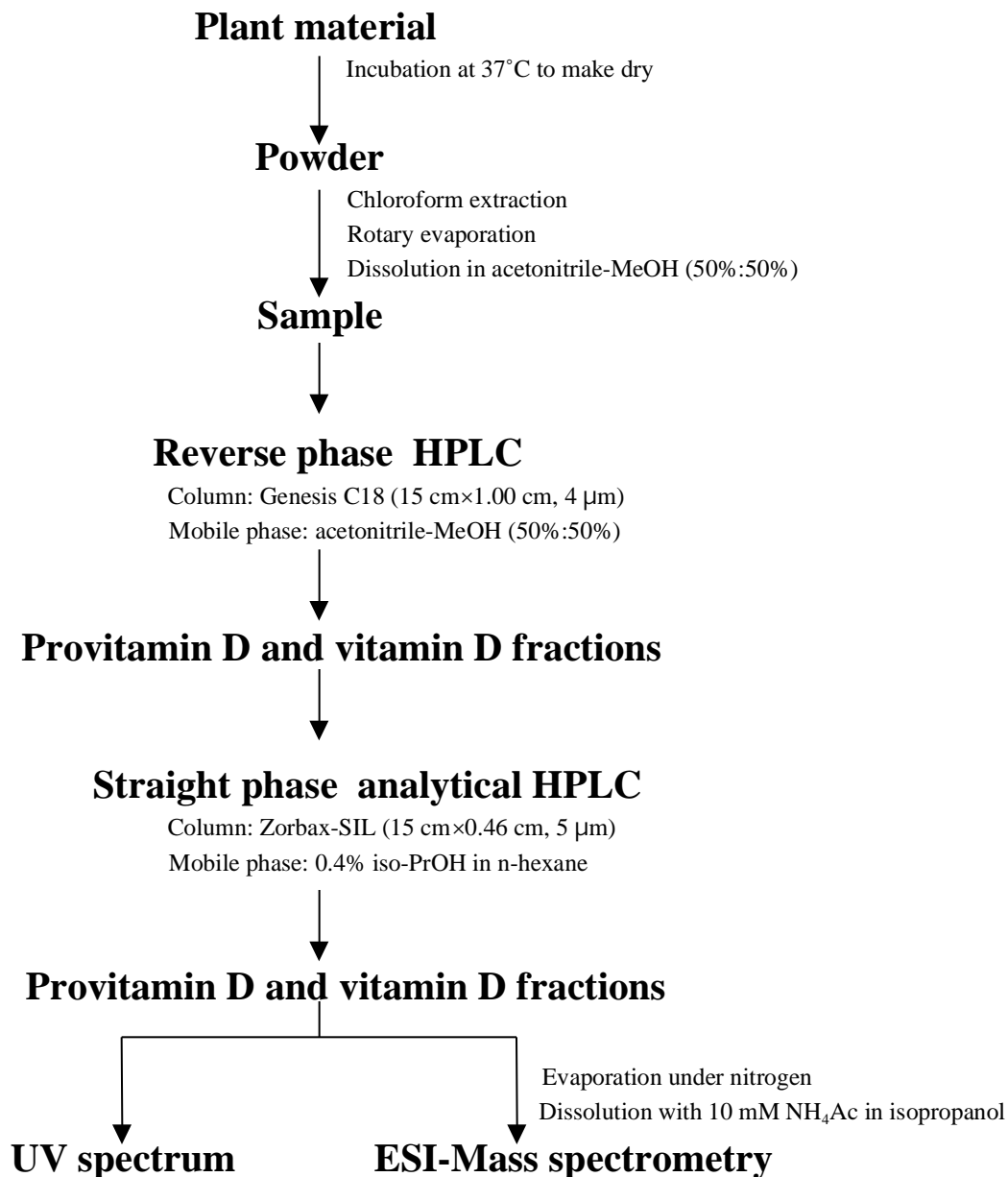
Introduction

- ✿ In the skin of vertebrate animals and humans, vitamin D is formed by the processes:
Provitamin D \rightleftharpoons Previtamin D \rightleftharpoons Vitamin D.
The conversion of provitamin D to previtamin D is a photochemical reaction requiring ultraviolet-B photons.
- ✿ Not only vitamin D₂ but also vitamin D₃ have been found in a variety of plants.
- ✿ The results of this study demonstrates that both UV-B dependent and independent pathways exist in plants for the biosynthesis of vitamins D.

Materials and Methods

Daucus carota (leaves and roots)

Lycopersicon esculentum (leaves)



Results

✿ Vitamin D₂ and D₃ in the root of carrot (*Daucus carota*) grown in absence of UV-B

Provitamin D₂, provitamin D₃, vitamin D₂ and vitamin D₃ were identified and determined

Chromatograms of reverse phase HPLC (Fig.1)

Chromatograms of straight phase HPLC (Fig.2, Fig.3)

UV-spectrum of the provitamin D₂ (Fig.4)

Electrospray ionisation (ESI) mass spectra (Fig.5, Fig.6)

✿ Contents of provitamins and vitamins D₂ and D₃ in tomato^a (*Lycopersicon esculentum*) leaves

Organism	Micrograms per gram dry weight			
	Provitamin D ₂	Provitamin D ₃	Vitamin D ₂	Vitamin D ₃
Tomato (-UV-B)	1.83	0.61	0	0
Tomato (+UV-B)	2.23	0.76	0.087	0.28

^aTomato plants were grown in a greenhouse with or without UV-B radiation (0.85 kJ plant weighted UV-B radiation per m² and day)

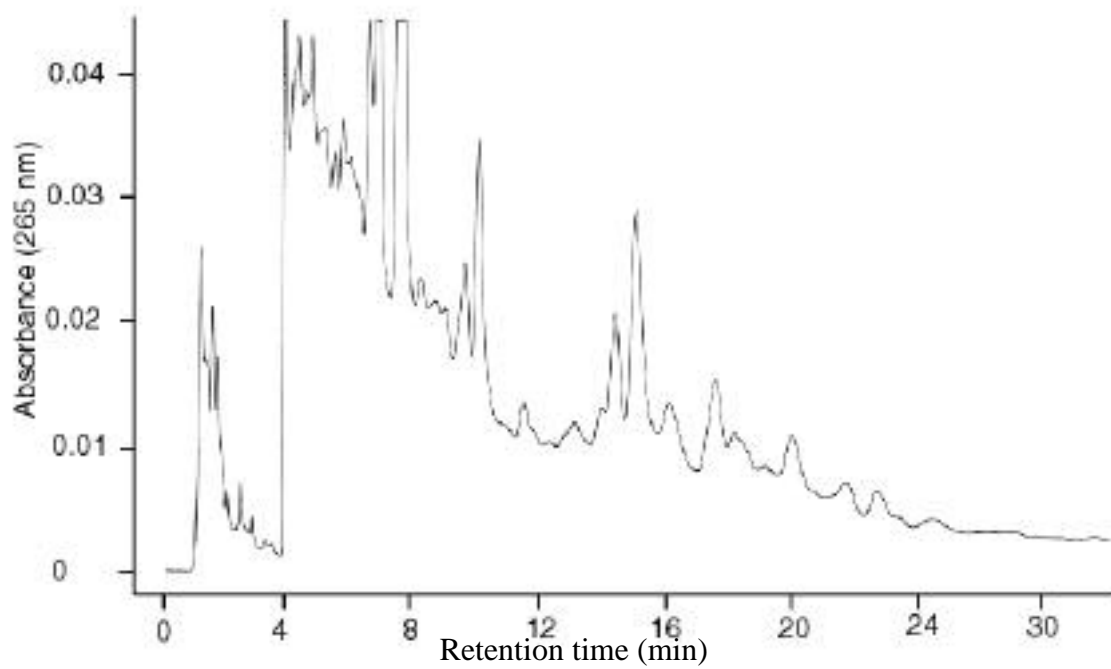


Fig. 1. Chromatogram of the first reverse phase HPLC

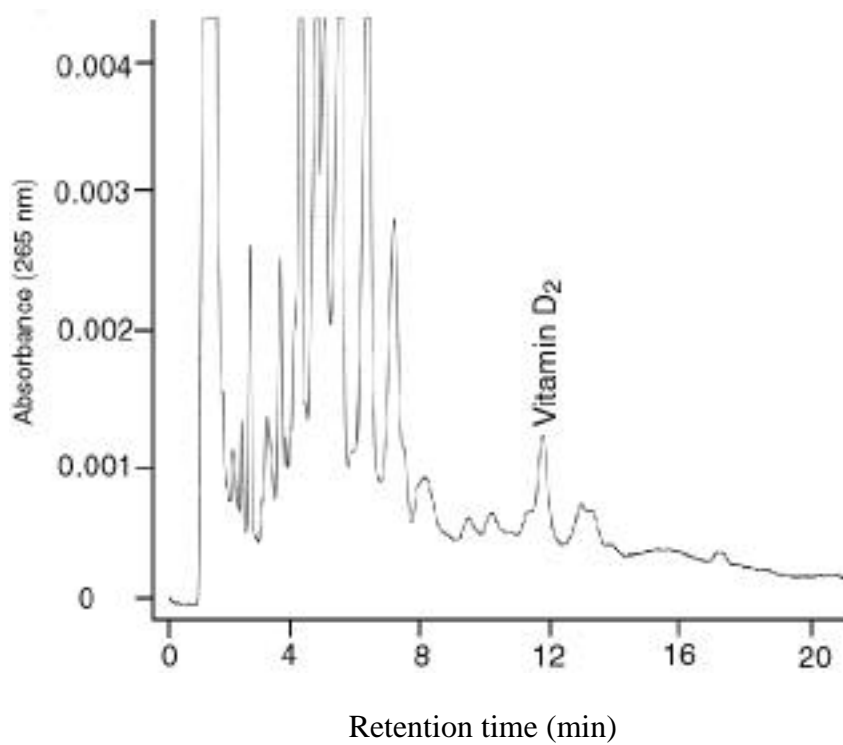


Fig. 2. Chromatogram of the straight phase HPLC

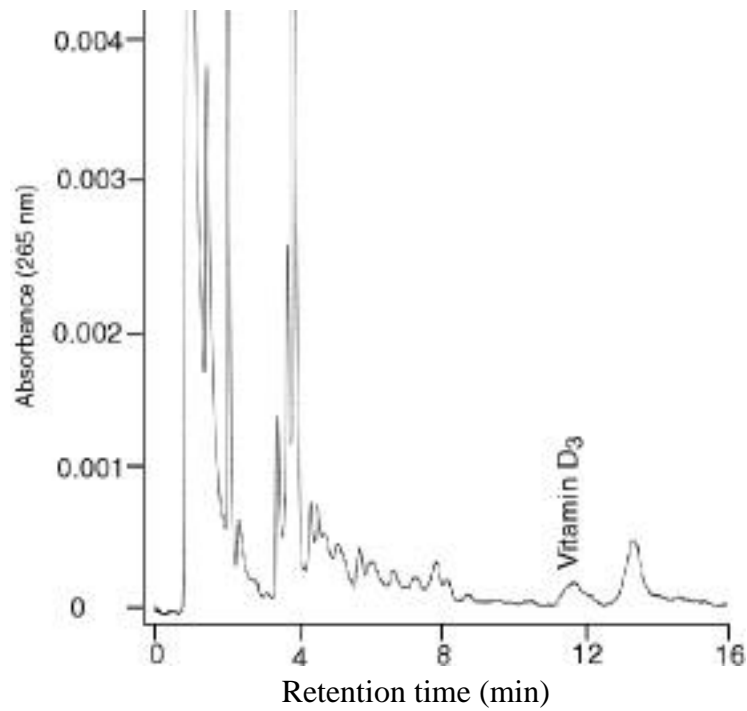


Fig. 3. Chromatogram of the straight phase HPLC

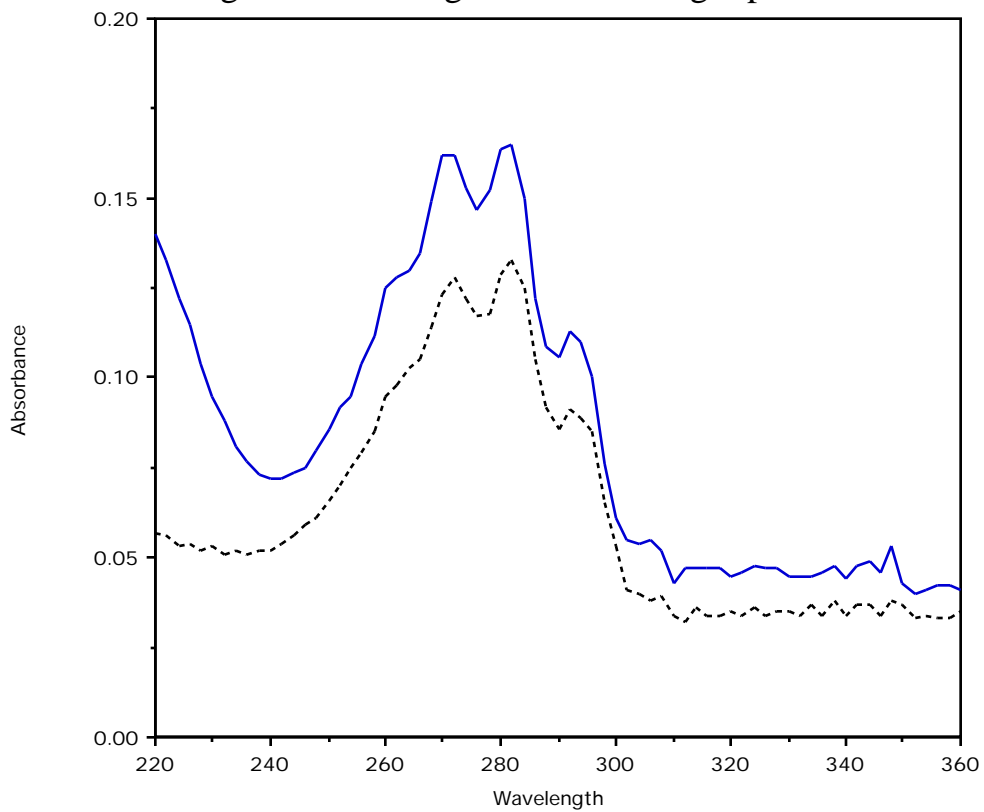


Fig. 4. Ultraviolet spectra of putative provitamin D₂ fraction from carrot purified by two successive steps of HPLC (solid line) and standard provitamin D₂ (dashed line).

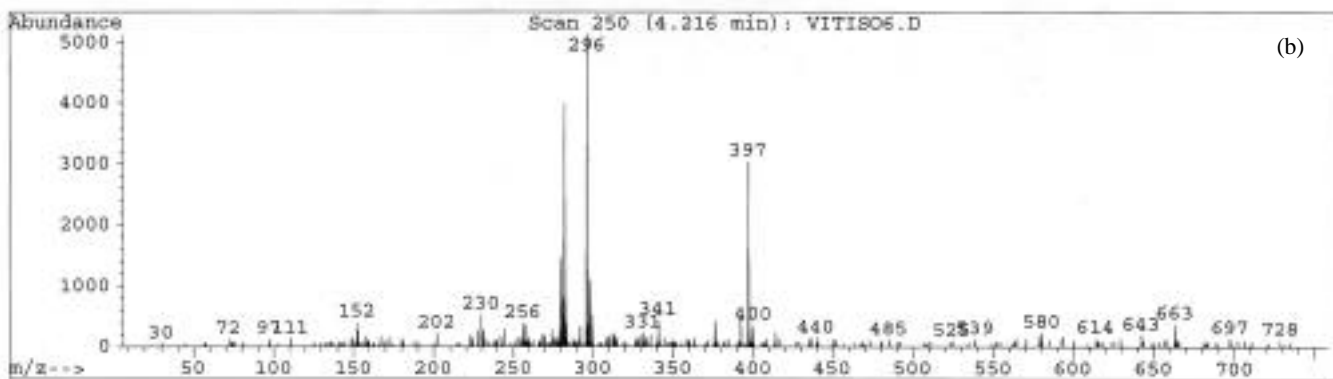
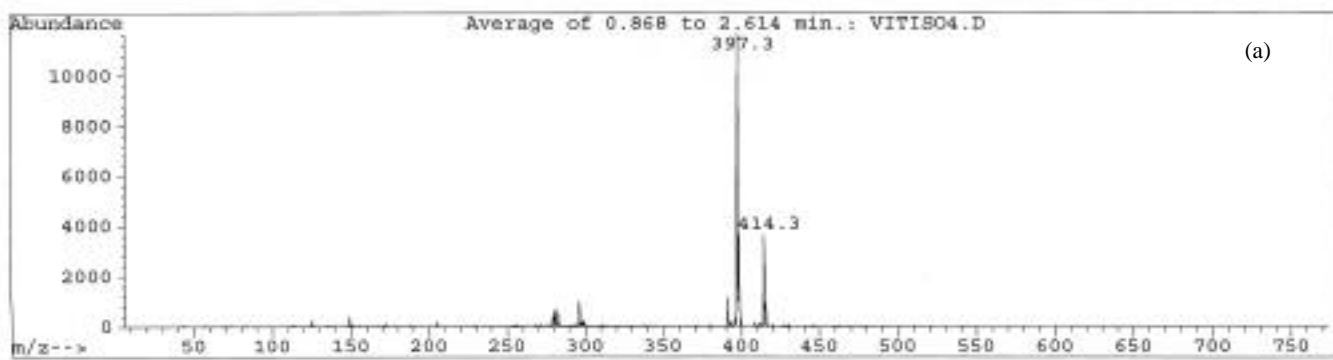


Fig. 5. ESI-MS analyses of standard vitamin D₂ and vitamin D₂ sample prepared from carrot. (a) standard vitamin D₂, peaks at m/z 397.3 and m/z 414.3 represent $[M-H]^+$ and $[M-NH_4]^+$, respectively; (b) vitamin D₂ sample, the peak at m/z 397 shows that the sample contains vitamin D₂.

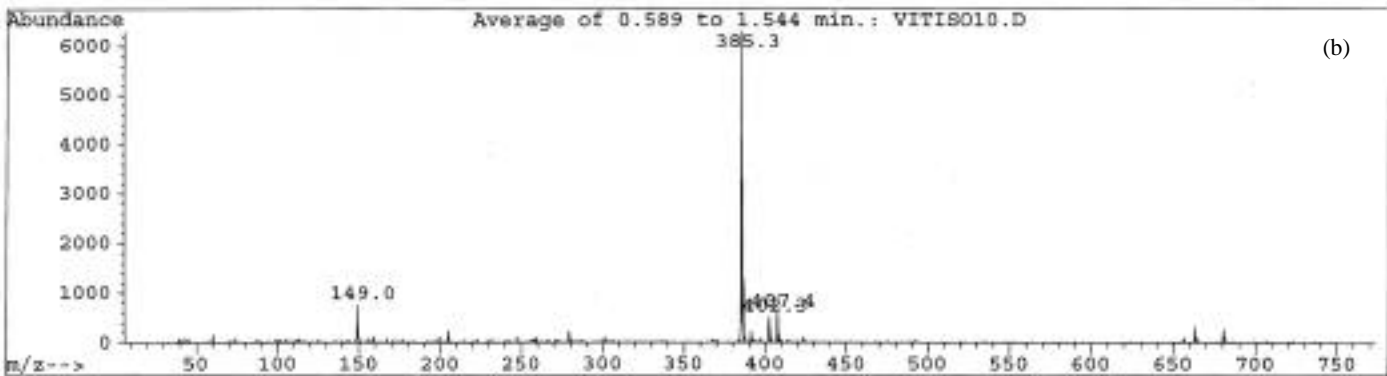
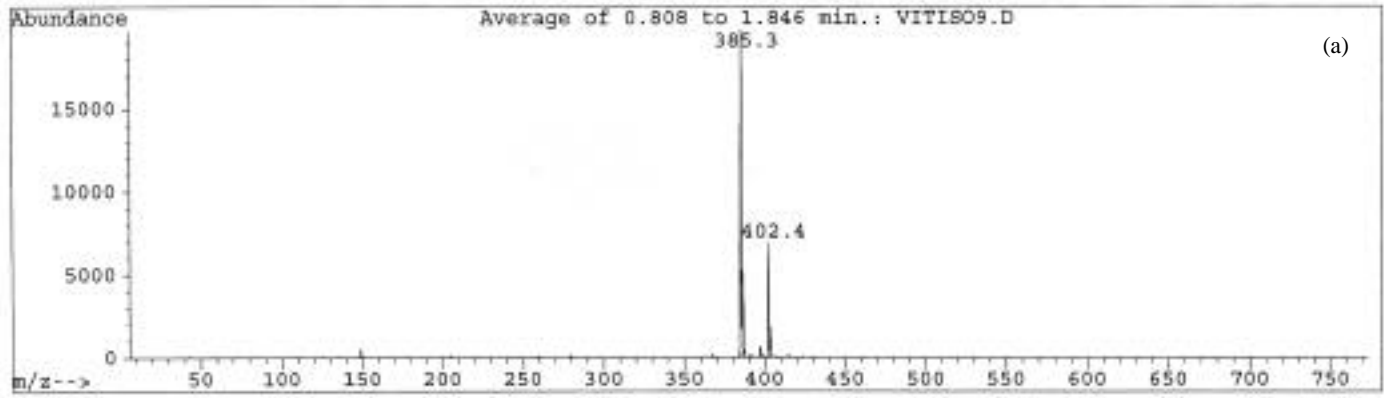


Fig. 6. ESI-MS analyses of standard vitamin D₃ and vitamin D₃ sample prepared from carrot. (a) standard vitamin D₃, peaks at m/z 385.3 and m/z 402.4 represent $[M-H]^+$ and $[M-NH_4]^+$, respectively; (b) vitamin D₃ sample, the peaks at m/z 385.3 and 402.3 show that the sample contains vitamin D₃.

☼ Is provitamin D a UV-B receptor?

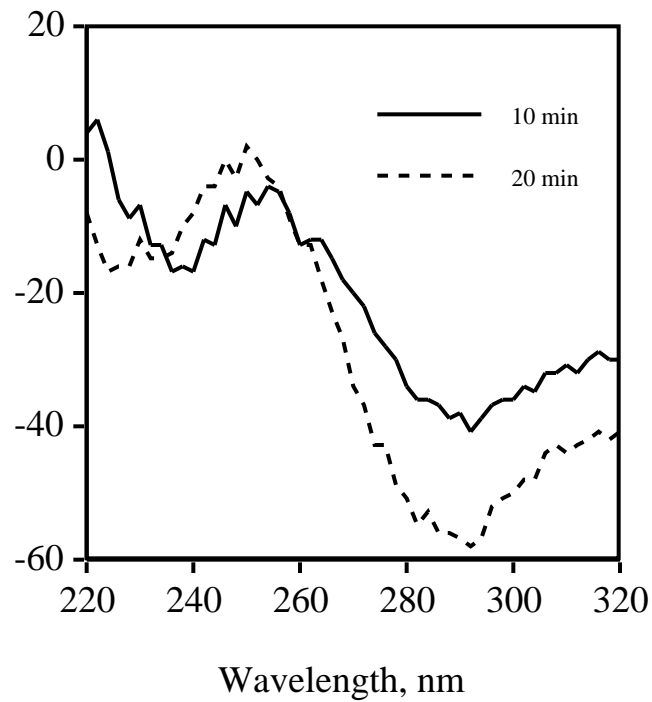


Fig.7. Absorbance change in outer cell membranes from tomato leaf cells irradiated for 10 and 20 minutes with radiation (mainly UV-B radiation, but also UV-C, UV-A and some visible light) from a Philips TL12 lamp. Note that the maximum absorbance decrease occurs where one would expect for a substance that acts as a UV-B receptor and disappears in the process.