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VITAMIN D_3 AND ITS METABOLITES IN TOMATO, POTATO, EGG PLANT AND ZUCCHINI LEAVES

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Key Word Index—*Lycopersicon esculentum*; *Solanum tuberosum*; *S. melongena*; Solanaceae; *Cucurbita pepo*; Cucurbitaceae; cholecalciferol; 25-hydroxycholecalciferol; 1α-hydroxycholecalciferol; calcitriol.

Abstract—The presence of vitamin D_3 (cholecalciferol) and its hydroxylated metabolite 25-hydroxyvitamin D_3 (25-OH D_3) was established in leaf extracts of *Lycopersicon esculentum*, *Solanum tuberosum*, *S. melongena* and *Cucurbita pepo*. Free vitamin D_3 was detected in all the leaves of these plants with the exception of *S. melongena*. 25-OH D_3 was detected only in the extract of *L. esculentum*. However, neither 1α-OH vitamin D_3 (1α-OH D_3) nor 1α,25$(OH)_2$ D_3 (calcitrol) was detected in any of the leaf extracts of these plants; no glycosides of either vitamin D_3 or its hydroxylated metabolites were found.

INTRODUCTION

Many plant species are known to cause calcinosis in grazing animals that ingest them. Calcinogenic activity is claimed to be attributed to the presence of vitamin D-like activity in these plants [1–3]. It is now well-established that the leaves of *Solanum malacoxylon* and *Cestrum diurnum*, two calcinogenic plants belonging to the family Solanaceae, contain the glycoside of calcitriol (1α,25-$(OH)_2$ D_3) which is the most potent hormonal form of vitamin D_3 (cholecalciferol) responsible for maintenance of calcium and phosphorus homeostasis in higher animals [4]. In addition to calcitriol glycoside, *S. malacoxylon* contains vitamin D_3 glycoside and the glycoside of 25-$(OH)D_3$ [5–7]. Prema *et al.* [8]. reported the occurrence of free vitamin D_3, its metabolites and their glycosides in *Cestruim diurnum* leaves. They also reported the presence of vitamin D-like activity in *Lycopersicon esculentum* leaves [9]. This activity is claimed to be due to the presence of vitamin D_3 and its hydroxylated metabolites 25-OH D_3 and 1α,25-$(OH)_2$ D_3 both free and in the glycosidic form. These effects were established in vitamin D_3-deficient rats.

In our previous work on cell cultures derived from *S. malacoxylon* leaves [10], we investigated the presence of different precursors of the vitamin D_3 biosynthtic pathway. The presence of cholesterol and 7-dehydrocholesterol was established. In addition, the occurrence of vitamin D_3 and its hydroxylated metabolites 25-OH D_3 and 1α,25-$(OH)_2$ D_3 was also reported. Most of the known calcinogenic plants so far screened belong mainly to the Solanaceae. In the present study, we investigated the occurrence of vitamin D_3 and its hydroxylated metabolites in leaves of some members of the Solanaceae. Among these are *Lycopersicon esculentum* (tomato), *Solanum tuberosum* (potato) and *Solanum melongena* (egg plant). Moreover, the presence of the above mentioned constituents was also investigated in *Cucurbita pepo* (zucchini), family Cucurbitaceae. The results show the presence of free vitamin D_3 in the leaves of tomato, potato, zucchini but not in egg plant leaves. Only tomato leaves were found to contain 25-OH D_3. However, neither the other vitamin D_3 hydroxylated metabolites, namely 1α-OH D_3 and 1α,25-$(OH)_2$ D_3, nor the glycosidic form of vitamin D_3 or any of its metabolites were detected in any of the above tested plant extracts.

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Table 1. Vitamin D_3, 25-OH D_3, 1α-OH D_3 and 1α,25$(OH)_2$ D_3 contents in different species ($\mu g\ g^{-1}$ leaves fr. wt)

Plant extract constituent	Plant ($\mu g/g^{-1}$ fr. wt)			
	Tomato	Potato	Egg plant	Zucchini
Vitamin D_3	1.1	0.15	*	0.23
25-OH D_3	0.15	*	*	*
1α-OH D_3	*	*	*	*
1α,25$(OH)_2$ D_3	*	*	*	*

* = Not detectable.

RESULTS AND DISCUSSION

Leaves of the four species were extracted with chloroform and then with ethanol. Ethanolic extracts expected to contain the glycosides of vitamin D_3 and/or any of its hydroxylated metabolites were hydrolyzed enzymically with mixed glycosidases prior to analysis as chloroform extracts. Chromatographic analysis on reverse-phase HPLC of the chloroform extracts of tomato, potato and zucchini but not egg plant revealed the presence of free vitamin D_3. The extract of each species was partially purified by silica gel column chromatography (chloroform–ethanol, 9.9:0.1). Fractions containing free vitamin D_3 were collected and further purified using preparative TLC (chloroform-methanol, 19: 1). The chemical identity of vitamin D_3 was established by HPLC, its ^1H NMR and mass spectra by comparison with an authentic sample. Concentrations of vitamin D_3 are shown in Table 1. Chromatographic analysis on TLC and reverse-phase HPLC also revealed the presence of 25-OH D_3 in the chloroform extract of tomato but not potato, egg plant or zucchini. Its identity was established by TLC, HPLC analysis, co-chromatography with authentic standard and mass spectra. The concentration of 25-OH D_3 in tomato leaves was 0.15 $\mu g\ g^{-1}$ fresh weight (Table 1).

Reverse-phase HPLC analysis revealed the absence of 1α,25 $(OH)_2$ D_3 in the chloroform extract of all the studied plants. Also absent was 1α-OH D_3.

The ethanolic extract of each plant treated with mixed glycosidases and partially purified by column chromatography was also analyzed by HPLC. No glycosidic forms of either vitamin D_3 or 25-OH D_3 were detected. Unhydrolyzed ethanolic extracts of the tested plants revealed no detectable levels of vitamin D_3 or any of its metabolites.

The present report confirms the occurrence of free vitamin D_3 in tomato, potato and zucchini leaves in accordance with data previously reported by Prema *et al.* [9]. Our results show that tomato leaves contain *ca.* five times more vitamin D_3 than zucchini leaves and twice that present in potato leaves. Tomato with the highest content of vitamin D_3 was the only plant found to contain 25-OH D_3.

The inability to detect the glycosidic form of vitamin D_3 or its 25 hydroxylated metabolite (25-OH D_3) in these plants could be due to the ontogeny of the plant, its presence in other parts of the plants and/or the time of collection of the leaves.

In our study, young apical leaves of the plants were extracted immediately after collection, which may explain the presence of only free vitamin D_3 and 25-OH D_3. This postulation is supported by the observation of Prema *et al.* [9]. that the content of free 1α,25$(OH)_2$ D_3 in *C. diurnum* leaves was decreased by *ca.* 30% on storage for 30 days. It seems fruitful therefore to extend our work in the near future to take into consideration studying the effect of storage, after collection for various times, on the contents of these compounds both in the free or the glycosidic form as it may be possible that the glycosidic content may increase with storage time on the expense of the contents of the free compounds.

In tomato leaves, it is clear that concentration of vitamin D_3 is *ca.* ten times higher than its corresponding 25-hydroxylated metabolite suggesting that vitamin D_3 may be synthesized first in this plant and then transformed into 25-OH D_3. In the other tested plants, inability to detect 25-OH D_3 metabolite may be due to insensitivity of the tested analytical methods because of their low concentration although with such method ngs of the standard can be detected.

The calcinogenic activity of some members of the Solanaceae is attributed to the presence of free vitamin D_3, 25-OH D_3 and 1α25$(OH)_2$ D_3 and/or their glycosides [1–3, 8, 10]. Our work confirmed that other members of Solanaceae contain some of these constituents. However, the presence of vitamin D_3 in zucchini indicates that other chemotaxonomically related families may contain vitamin D_3 or one or more of its hydroxylated metabolites. Moreover, the literature reported the presence of some of these constituents in other plants belonging to other families such as *Trisetum flavescence* (Gramineae) [11] and *Medicago sativa* (Leguminosae) [12]. The presence of these constituents could have a role in the growth and development of plants. Although this role in the plant is

not obvious, Buchala *et al* [13]. have shown that secosterols promote root differentiation.

EXPERIMENTAL

Extraction and analysis

Fresh young apical leaves (2 kg) of *L. esculeutum*, *S.*, *S.* melongena, and *C. pepo*, were cut into fine pieces and then extracted in the dark with $CHCl_3$ (0.6 l × 3) by percolation. The $CHCl_3$ extracts were dried (Na_2SO_4), filtered and the solvent removed under red. pres. The residues were then extracted with EtOH (0.6 l × 3). The EtOH extracts were dried (Na_2SO_4), filtered and the solvents were removed under red. pres. The EtOH extracts were hydrolyzed enzymically using a mixed glycosidases preparation derived from *Turbo cornutus* (ICN Biomedical) (15 mg for 100 mg EtOH extract) in pH 4.1 buffer at 37° for 18 hr, and then extracted with $CHCl_3$ (15 ml × 3). $CHCl_3$ extracts were dried (Na_2SO_4), filtered and the solvent removed under red. pres. Primary studies on both $CHCl_3$ extracts were analyzed by TLC (Merck silica gel 60 F254) using $CHCl_3$–EtOH–H_2O (183:16:1), $CHCl_3$–MeOH (49:1) and EtOAc–petrol (3:7 or 7:3). Spots were visualized by exposure to UV (254 nm) and by spraying with (a) 10% H_2SO_4 in MeOH and heating at 100° for 1 min, (b) anisaldehyde in H_2SO_4 (2%, w/v) mixed with HOAc (1:10, v/v) and warming at 50° for 60–90 secs and (c) phosphomolybdic acid in EtOH (10% v/v). Subsequently, the R_f of the different extract spots obtained were compared with those of authentic standards of vitamin D_3 and its hydroxylated metabolites analyzed under similar conditions. TLC analysis of the different extracts were confirmed by co-chromatography with authentic standards. Like TLC studies, primary HPLC studies were carried out on $CHCl_3$ extracts. HPLC analysis of $CHCl_3$ extracts dissolved in MeOH was carried out on reverse-phase column, Nova-pak C18 (150 × 3.9 mm). Gradient elution was performed {15 min isocratic conditions MeOH–H_2O (3:1) and 15 min to 100% MeOH} at a flow rate of 1 ml min^{-1}, with UV monitoring at 264 nm, comparing R_ts to that of a standard: vitamin D_3 (26.4 min), 25-OH D_3 (21.1 min), 1α-OH D_3 (20.5 min) and 1α,25(OH)$_2$ D_3 (10.35). HPLC analysis of the different extracts were confirmed by co-chromatography with authentic standards. Subsequently, the $CHCl_3$ extracts were partially purified by silica gel CC (Merck 70–230 mesh) using $CHCl_3$–MeOH (49:1). The frs containing spots corresponding to vitamin D_3 were collected, purified by prep. TLC ($CHCl_3$–MeOH, 49:1) and further purified by prep. TLC ($CHCl_3$–EtOH, 99:1). The chemical identity of vitamin D_3 was confirmed by ^{1}H NMR and MS. Frns from the CC column corresponding to 25-OH D_3 were collected and further analyzed by HPLC. The R_t at 264 nm was identical to that of an authentic sample. In addition to TLC, HPLC and co-chromatography, the chemical identity of 25-OH D_3 was further confirmed by MS. $CHCl_3$ extracts of the hydrolysis reaction mixts obtained from all tested plants were analyzed in a similar mannaer. TLC or HPLC analysis of these extracts did not reveal the presence of Vitamin D_3 or 25-(OH) D_3. The various vitamin D_3 metabolites identified were quantitated by comparison of the sample HPLC peak areas with standards.

Spectrometric methods

^{1}H NMR spectra were recorded on a Jeol Gx-270 spectrophotometer using TMS as int. standard.

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