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STEROLS AND TRITERPENOLS IN LATEX AND CULTURED TISSUES OF *EUPHORBIA PULCHERRIMA**

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Abstract—The sterol and triterpenol constituents of *Euphorbia pulcherrima* latex and cultured callus tissues were examined by GLC and mass spectrometry. Latex extracts from different varieties contained sitosterol, β-amyrin, germanicol, cycloartenol, β-amyrin acetate, and germanicol acetate. Capillary GC profiles of these varieties indicated that the triterpene content was essentially identical for examined latices. Cultured tissues derived from petioles and stem internodes synthesized only sitosterol in significant quantities, although trace amounts of several sterols that occur in latex were also detected in cultured tissues. This study supports the interpretation that the pattern of triterpene synthesis in the laticifer of the normal plant is a highly controlled and stable phenomenon among varieties of this species.

INTRODUCTION

The poinsettia, *Euphorbia pulcherrima* Willd. is an important horticultural species of the family Euphorbiaceae. As in other members of the genus, the plant produces copious amounts of latex from non-articulated laticifers present throughout the plant body. *Euphorbia* latex is usually milky-white in appearance and exudes from the plant when it is injured or when the plant surface is scarified.

Early workers demonstrated the presence of both tetracyclic and pentacyclic triterpenes and their esters in the latex of various *Euphorbia* species [1–3]. Using *E. pulcherrima*, germanicyl acetate and sitosterol have been found in stem extracts and whole plant extracts, respectively [4, 5]. Other workers [6] reported that only α-amyrin was present in the ester and free alcohol fractions of latex, whereas tetracyclic triterpenes were not detected. Baas [7] identified cycloartenol as the main free triterpene alcohol, a mixture of β-amyrin and germanicol as minor free alcohols, and possibly campesterol, stigmasterol, and sitosterol in the free sterol fraction of exuded latex.

The purpose of this study was to examine the triterpene composition of the laticifer cell of *E. pulcherrima* to determine the stability of the hydrocarbon spectrum in latex from several varietal forms of this plant, as well as to clarify ambiguities in the literature on the triterpene composition of the cytoplasm in the laticifer in this species. In addition, analyses were extended to cultured tissues of poinsettia to compare the capacity of triterpene synthesis between callus and laticifer cells. Capillary GLC, a technique not previously employed in studies of this species, was used to separate triterpene components.

RESULTS

Capillary GLC readily separated the various triterpenes present in the *E. pulcherrima* latex. The triterpenes detected and identified in *E. pulcherrima* var. Paul Mikkelsen latex are listed in Table 1. Germanicol was the main component, representing 49.3% of the total triterpene fraction. Both β-amyrin and germanicol occurred naturally as their acetates whereas sitosterol and cycloartenol were detected only as free alcohols.

Table 2 summarizes the compounds present in latices of the five examined poinsettia cultivars. Inspection of the capillary column and packed column chromatograms indicated that the latex composition of the examined cultivars was nearly identical. Comparison of sterol profiles by superposition of the chromatograms showed that very little quantitative or qualitative variation was detectable between these horticultural varieties. Other commonly occurring 4-demethyl sterols such as campesterol and stigmasterol were not detected in these plants.

Callus tissues synthesized sitosterol in significant amounts although trace quantities of other triter-

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Table 1. Tetracyclic and pentacyclic triterpenols in exuded latex of *E. pulcherrima* var. Paul Mikkelsen

Retention time (min)*	Compound	Concentration (mg/g dry wt)	Selected mass spectra
17.86	Sitosterol	28.5	414[M]$^+$, (100.0), 396.4 (51.5), 329.3 (58.8), 145.1 (48.9)
18.66	β-Amyrin	20.5	426.1[M]$^+$, (6.1), 218.0 (100.0), 203.1 (90.4), 190.0 (56.2), 189.3 (72.1)
19.67	Germanicol	75.0	426.25[M]$^+$ (11.4), 109.0 (67.1), 94.9 (86.1), 80.9 (58.2), 68.9 (75.9), 55.0 (91.1), 43.0 (100.0)
20.73	Cycloartenol	9.5	426.4[M]$^+$ (3.2), 107.1 (49.9), 95.1 (50.4), 93.4 (47.2), 69.1 (100)
21.23	β-Amyrin acetate	12.5	468.2[M]$^+$ (17.5), 464.0 (25.8), 408.1 (12.5), 218.0 (100.0), 189.2 (66.6), 203.0 (58.7)
21.80	Germanicol acetate	6.0	468.4[M]$^+$ (16.7), 218.2 (63.9), 204.2 (93.1), 189.2 (100.0), 177.2 (69.6)

*Retention times relative to eicosane.

penes, as found in latex, were present in callus (Table 1). Germanicol was not detectable in callus cells. Fig. 1 illustrates the synthesis of sitosterol in callus cultures of 'Paul Mikkelsen' over a period of 4 months. Growth was rapid with the callus being quite compact and bright-green in appearance. Sitosterol production, measured on a dry wt basis, remained rather stable over the 4 month experimental period (Fig. 1).

DISCUSSION

Results derived from this investigation indicate that there is a high level of stability for the pattern of sterol and triterpenol synthesis in latex from the non-articulated laticifer among varietal forms within a given taxon of *Euphorbia*. These analyses show that latex from five cultivars of poinsettia possess identical compositions for sterolic compounds. In another study of over 20 populations of *E. dentata* Michx. and several populations of *E. tirucalli* L., it has been reported that a given taxon possessed essentially identical profiles for sterol composition [8].

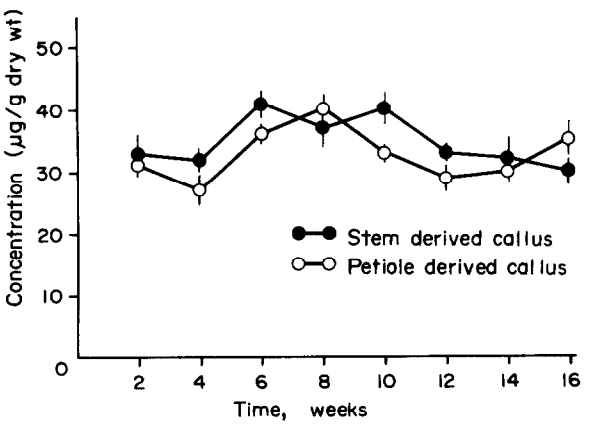

Fig. 1. Sitosterol concentration in stem- and petiole-derived callus of *E. pulcherrima* var. Paul Mikkelsen. Vertical lines represent s.d. values.

The differences in triterpene composition reported for *E. pulcherrima* may be reflective of different analytical procedures employed by various workers. Techniques employed in previous studies [4–7] lacked the resolving capacity of capillary GLC and mass spectrometry employed in this study. We found germanicol, sitosterol and β-amyrin as the major constituents of latex, with β-amyrin acetate, cycloartenol and germanicol acetate present in low concentrations. Baas [7] reported the presence of germanicol, β-amyrin and cycloartenol as major latex constituents and tentatively identified the presence of campesterol, stigmasterol and sitosterol. Our analyses, which included co-injection of campesterol and stigmasterol standards with latex extracts, did not indicate the presence of either of these free sterols.

α-Amyrin was absent from our examined extracts. These results contrast with the report [6] that α-amyrin and its esters were the sole triterpenes present in *E. pulcherrima* latex. Baas [7], who also noted its absence in poinsettia latex, suggested that the difference may be related to chemical variation in latex composition within this species. Our studies, however, show very little or no chemical variation for sterol composition among poinsettia cultivars examined in this study, or among populations of a latex-producing species [8].

Cultured tissues of *E. pulcherrima* produced only sitosterol in significant quantities. Germanicol, the most abundant triterpenol in latex was undetected in cultured cells; only trace amounts of other sterols appeared in these cultures. Sitosterol, along with stigmasterol and campesterol, were the three sterols most commonly detected in plant tissue culture systems. This was consistent with other observations that sitosterol may be present in cultures without being accompanied by these other common phytosterols [9]. Recently, it has been reported for *E. tirucalli* that cultured cells of this plant synthesized the same sterols, euphol and tirucallol, that are characteristic of the intact plant [10].

Similar studies of other *Euphorbia* species will provide more information on the composition and synthetic activity of laticifers for sterols, and the capacity of cultured cells to synthesize those sterols

Table 2. Summary of tetracyclic and pentacyclic compounds detected in exuded latex, callus and whole plant extracts of several *E. pulcherrima* cultivars. Values are the means of three determinations ±s.e.

Cultivar	Compound (mg/g dry wt)					
	Sitosterol	β-Amyrin	Germanicol	Cycloartenol	β-Amyrin acetate	Germanicol acetate
Latex						
Annette Hegg Super Star	27.2 ± 3.6	10.5 ± 0.5	68.2 ± 5.9	10.3 ± 1.0	10.5 ± 1.2	t
Eckespoint C-1	31.6 ± 5.1	12.9 ± 2.3	62.4 ± 6.2	4.2 ± 1.6	9.2 ± 0.8	5.2 ± 0.3
Paul Mikkelsen	28.5 ± 2.4	20.5 ± 1.9	75.0 ± 6.7	9.5 ± 1.3	12.5 ± 1.2	6.0 ± 0.7
Gutbier's V-14	19.9 ± 1.4	19.8 ± 10.9	78.1 ± 5.5	11.7 ± 1.2	6.1 ± 1.0	t
Annette Hegg Supreme	25.3 ± 2.3	17.6 ± 0.8	70.0 ± 7.1	11.1 ± 1.6	9.9 ± 1.4	4.7 ± 0.2
Callus tissues						
Paul Mikkelsen	0.037 ± 0.009	t	–	t	t	t

t = trace amount; – = absent.

unique to a species. These studies will contribute insight not only into the interpretation of the evolution of the sterol biosynthetic pathway, but also provide a basis for employing these data to interpret the progressive evolution of the laticifer both at the cell and at the species level within this genus.

EXPERIMENTAL

Plant materials. Cultivars of *E. pulcherrima* examined in this study included Gutbier's V-14, Annette Hegg Supreme, Annette Hegg Super Star, Eckespoint C-1, and Paul Mikkelsen. Latex was collected from greenhouse grown plants by excision of petioles near the basal region. Exuded latex was collected directly into clean tubes containing Me_2CO. The latex was refluxed for 30 min in a Soxhlet apparatus, evaporated to dryness with N_2, and dissolved in a small vol. of Me_2CO prior to injection into the GC. Tissue culture explants were obtained from both petioles of uppermost fully expanded leaves and from young, upper internodes of Paul Mikkelsen plants.

Tissue culture. Explants (10–15 cm) were sterilized by immersion in 70% EtOH for 5 min, rinsed once with sterile de-ionized H_2O, and placed into a 10% soln of commercial bleach (5.25% NaOCl) for 25 min. After three rinses in sterile, deionized H_2O both petioles and stems were cut into 1-cm long sections and placed horizontally on the medium surface. The medium [11] was supplemented with 5 g/l. inositol and 2% sucrose. Major salts were added at half-strength. Hormonal additions to the medium included 1.0×10^{-5} M N^6-(Δ^2-isopentenyl)-adenine and 5.0×10^{-7} M α-naphthaleneacetic acid for petioles and 5.1×10^{-6} M kinetin and 6.0×10^{-5} M indole-3-acetic acid for stem segments. All cultures were solidified with 0.7% agar. Media were adjusted to pH 5.5–5.7 with 0.1 N NaOH prior to autoclaving. Culture flasks (125 ml) were covered with sterile polypropylene [12] and placed in the light (petioles, 12 hr light, 12 hr dark) or into total darkness (stems) at 25 ± 1°. Cultures were transferred every 28 days to fresh media. Two replicate flasks were harvested at 2 week intervals for 16 weeks and examined for triterpene content.

Callus tissue was dried for 24 hr at 50°. The callus was ground to a fine powder using a mortar and pestle and subsequently extracted with Me_2CO in a Soxhlet apparatus for 24 hr. The solvent was evaporated with a Buchi Rotavapor R and a vacuum pump and the greenish residue subsequently taken up in Me_2CO for injection into the GC.

GLC. Extracted triterpenes were chromatographed as free alcohols or occasionally as acetates after acetylation in Ac_2O–pyridine (4:1) GLC analyses were performed on instruments equipped with a flame ionization detector and operated by temp. programming from 220 to 310° at 4°/min. N_2 (20 ml/min) was used as the carrier gas. The injection port and detector temps were 250 and 300°, respectively. Glass columns were pre-treated with 10% dimethyldichlorosilane in toluene and packed with 3% OV-1 or 3% SP-2250 on Supelcoport (80–100 mesh). Eicosane was used as an int. standard. Quantitative data were determined with an integrator.

For capillary GLC a 15 m × 0.28 mm i.d. soft glass capillary column coated with SE-52 was used. The temp. was programmed from 100 to 200° at 10°/min and from 200 to 250° at 4°/min. Column temps were: injection port 260° and detector 290°. Gas pressures (psi) were: air 60, hydrogen 40, and helium 60, respectively, which provided an overall column pressure of 10 psi or *ca* 56 cm/sec.

Mass spectrometry. Identification of sterols was made by co-injection of available standards and by comparison of their MS with those of unknowns. Spectra were obtained by injection of compounds into a Hewlett-Packard 5992A GC/MS.

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