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TRITERPENE ESTER SYNTHESIS IN LATEX OF *EUPHORBIA* SPECIES

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Key Word Index—*Euphorbia*; Euphorbiaceae; latex; biosynthesis; triterpene esters.

Abstract—Purified particles isolated from the latex of *Euphorbia* species catalyse the synthesis of triterpene esters from endogenous triterpenol and free fatty acid as the acyl donor. Exogenous ATP and CoA did not stimulate the enzyme activity. The enzyme is probably located in the protein layer that surrounds the particle. The experimental results indicate that the triterpenol esterification occurred via a triterpenol esterase reaction. The esterase exhibits fatty acid, and to a lesser extent, triterpenol specificity. Short-chain and polyunsaturated fatty acids are preferred to longer chain and less unsaturated acids. Among the various free triterpenols present in *Euphorbia* latex particles, cycloartenol was the favoured substrate for the esterase.

In vitro incubations with whole latex and labelled acetate showed that newly synthesized labelled triterpenols are absorbed by the particles as esters of almost unlabelled fatty acids. The possible physiological significance of the latex particle triterpenol esterase is discussed.

INTRODUCTION

Esters of triterpenoids with long-chain fatty acids probably occur in all higher plants. They have been found in small amounts in all parts of the plant. The composition of the steryl esters may vary with the tissue or organelle investigated [1, 2]. The fatty acid moieties of the steryl esters in plants are mainly palmitate, oleate, linoleate and linolenate. Triterpene esters are present in relatively large amounts in the latex particles of most latex plants [3]. The acids esterified with latex triterpenols are often less common acidic components [4–7].

Latex particles are solid terpenoid globules coated by a thin protein layer [8, 9] which are dispersed in the latex serum. The particles are probably synthesized by the cytoplasm and subsequently excreted into the vacuolar system of the laticifers. Lipid globules in which steryl and triterpene esters accumulate are also known from some non-latex bearing higher plants and from lower plants as well [10, 11].

Hitherto, the function of steryl and triterpene esters has been speculative. Sterols might be stored as fatty acid esters in order to regulate the amount of sterols in the cell membranes of yeast cells [12]. By contrast, the latex triterpene esters are excluded from metabolism after synthesis and their composition does not change during plant growth [13].

Esterification mechanisms of steryl and triterpene esters have generally been studied in mammalian tissues [14, 15], in bacteria [16], and in fungi [17, 18]. On the contrary, little is known about the biosynthesis of steryl esters in tissues of higher plants. Various 1,2-diacylglycerols [19] or triglycerides [20] could be utilized for steryl ester synthesis as acyl donor with plant tissue enzyme preparations.

Phospholipids were suggested to be efficient acyl donors in triterpene synthesis in laticifers of *Euphorbia lathyris* [21]. In the present paper data are presented concerning the identification and localization of an enzymatic mechanism involved in the biosynthesis of triterpene and steryl esters in latices of *Euphorbia* species.

RESULTS AND DISCUSSION

Table 1 shows the triterpenoid composition of the latex particles of the *Euphorbia* species used in the present investigations. Each species appeared to have its own

Table 1. Triterpenoids from the latices of *Euphorbia* species determined by gas chromatography (percentages are based upon weight)

Species	Free triterpenols (%)	Triterpene acetates (%)	Triterpene esters with fatty acids (%)	mg lipid/g latex
E. characias	96.5	0.5	3.0	73.5
E. lathyris	74.0	1.0	25.0	62.0
E. pulcherrima	24.5	41.0	34.5	43.5

specific composition, which was not affected by plant growth circumstances during the experiments. All latices contained triterpene esters with fatty acids, but in *E. characias* they were only minor components. Microphosphate analysis and TLC of polar lipid extracts showed phospholipids as minor components in *E. lathyris* (50–60 nmol/g latex), contrary to Nemethy *et al.* [21] who isolated phospolipids as a major lipid class.

In most experiments the latex of *E. pulcherrima* was used. Therefore, the main triterpenols and fatty acids of this species were analysed. The most abundant triterpenol of the free triterpenol fraction was cycloartenol (63%), whereas germanicol (86%) and β-amyrin (75%) predominated in the triterpene acetate and the triterpene fatty acid ester fraction, respectively. The fatty acid moiety of the triterpene esters consisted of caprylate (10%), caprate (12%), conjugated decatrienoates (63%) [5], palmitate (7%), palmitoleate (2%), oleate (1.5%), and linoleate (4%).

Incorporation of [1-^{14}C]*acetate into latex triterpenols*

The percentage incorporation of acetate per incubation period of 18 hr differed markedly for the two species investigated. For *E. pulcherrima* an incorporation was found from 2.5 to 3.6% and for *E. lathyris* it was 8.2–13.2%. For both species, most of the radioactivity was found in the triterpene ester fraction, especially in the long-chain fatty acid fraction (Table 2). In the triterpene acetate fraction of *E. lathyris* no radioactivity was found at all. As far as the triterpene esters are concerned, most acetate was incorporated into the triterpene moiety. For *E. lathyris* 1.8% and for *E. pulcherrima* 6.1% of the radioactivity was present in the fatty acid moiety, while these compounds represented 25–30% of the carbon atoms of the triterpene esters [5, 6]. Radio-GC of the triterpenols liberated by saponification showed that the distribution of radioactivity of the individual components was proportional to their mass. The amount of radioactivity in the fatty acid fraction was too small for radio-GC to be performed.

In vitro experiments with labelled acetate in latex of *Euphorbia* species were described for the first time in ref. [22]. They found that the triterpenols were synthesized by the latex bottom fraction. Our experiments demonstrated an unequal distribution of radioactivity over the fatty acid and triterpenol moieties of the newly synthesized triterpene esters. Most of the acetate was incorporated into the triterpenol part of the esters, which indicates that these labelled triterpenols were esterified with non-labelled medium and long-chain fatty acids from the latex particles. This phenomenon was found for both species examined. As free fatty acids were not extracted from the latices in detectable quantities, one can conclude that the esterification reaction is a transesterification, involving other triterpene or diterpene esters present in the particles. Another explanation is that the labelled triterpenols are esterified during absorption into the particles with free fatty acids which are bound to the protein layer surrounding the particles. The observations that newly formed triterpenols present in the particles were esterified mainly with fatty acids and that exogenously added free triterpenol was not absorbed by the particles, lead to the conclusion that esterification of triterpenols must have occurred before they are absorbed into the particles.

Synthesis of triterpene esters from fatty acids in freshly tapped latex

Freshly tapped latex of *E. pulcherrima* has the ability to catalyse the esterification of triterpenols present in the latex particles with [1-^{14}C]palmitic acid. Time-course experiments demonstrating labelled triterpene palmitate synthesis are shown in Fig. 1. Latex was added to labelled palmitic acid and at different times aliquots of 10 μl were pipetted from the incubation mixture. Thin layer chromatography of the different CG fractions showed that all radioactivity was incorporated exclusively into the long-chain fatty acid fraction and radio-GC demonstrated that the acid occurred unaltered in the triterpene esters. These experiments showed that the reaction is in equilibrium when only 25% of the fatty acid has been esterified. In spite of a considerable difference in the triterpenol/triterpene ester ratio in *E. characias* and *E. lathyris*, the two species exhibited roughly the same percentage of triterpene esterification.

The esterase esterified both emulsified and non-emulsified fatty acid substrate to the same extent, which might mean that it acts at a fatty acid : water interface at the surface of the latex particle. Studies on steryl esterase from mammalian sources showed that its activity depends on the used material if the substrates have to be emulsified [23, 24] or not [25] for sterol esterification. Bhat and Brockman [26, 27] reported that hydrolysis of cholesteryl esters by an esterase occurs at a fatty acid : esterase interfacial layer.

Table 2. Incorporation of [1-^{14}C]acetate into the latex triterpenoids of *E. lathyris* and *E. pulcherrima* (*in vitro*). Latex (200 μl) was incubated with 5 μCi sodium [1-^{14}C]acetate for 18 hr

	E. pulcherrima		*E. lathyris*	
Compounds	dpm.10^4	%	dpm.10^4	%
Free triterpenols	1.7	15.0	9.0	17.5
Triterpene acetates	1.3	11.0	—	—
Triterpenols	0.8	7.0		
Acetic acid	0.5	4.0		
Triterpene esters with fatty acids	8.5	74.0	42.5	82.5
Triterpenols	8.0	69.0	41.5	81.0
Fatty acids	0.5	5.0	1.0	1.5

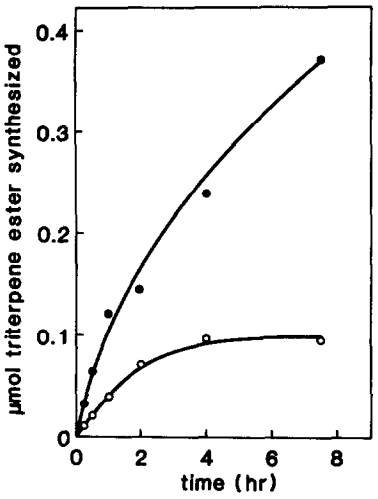

Fig. 1. Time-course of triterpene ester biosynthesis in latex of *E. pulcherrima*. Latex (200 μl) was incubated with 0.4 μmol (○---○) or 2.0 μmol (●---●) palmitic acid containing 0.5 μCi[1-^{14}C]palmitic acid.

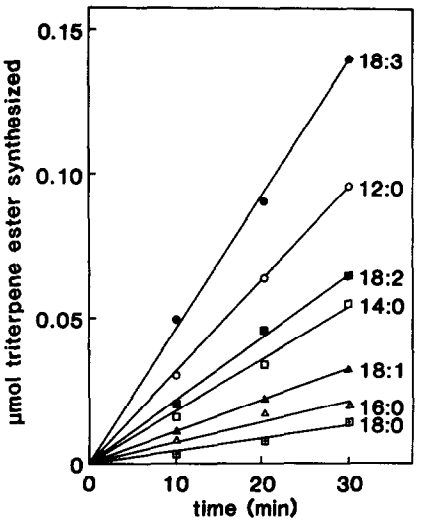

Fig. 2. Triterpenol esterification rates for various fatty acids. Fatty acids (0.5 μmol) were incubated with 150 μl latex of *E. pulcherrima*.

Incubations with different fatty acids

The fatty acid specificity of the triterpene ester catalysing system was investigated by incubation of the latex of *E. pulcherrima* with a series of [1-^{14}C]fatty acids ranging from C_{12} to C_{18}. The enzyme exhibited a chain-length specificity. Short-chain saturated fatty acids were better esterified than saturated acids with longer chains. Similar results have been reported for the formation of steryl esters from free sterol and 1,2-diacylglycerol [19] and for pancreatic cholesteryl esterase [14]. The fatty acids used in the present specificity experiments differed from the acids naturally esterified with the latex particle triterpenols, e.g. conjugated decenoic acids, which are unstable in air and are not commercially available in [^{14}C] form.

The enzyme also exhibited a double bond specificity. The overall fatty acid preference is demonstrated in Fig. 2. The fatty acids were added to the reaction mixture as a solid film on the bottom of the reaction tube. Experiments carried out with a fatty acid Triton X-100 micellar solution (0.1% Triton-X100 in 50 μl aqueous solution) did not increase the rate of enzyme activity.

Effect of fatty acid concentrations on triterpene ester biosynthesis

The specificity of the enzyme for the various fatty acids was examined at several concentrations. Increasing the fatty acid concentrations could saturate the system completely and double reciprocal plots fitted single straight lines. The apparent K_m and V_{max} values under standard assay conditions were estimated (Table 3). The data showed that the ratio of the fatty acids incorporated into the triterpenols present in the latex particles was determined by the ratio of the V_{max} for the fatty acids and showed that the triterpenols were present at saturating concentrations.

Table 4 illustrates the fatty acid selectivity of the triterpenol esterase when lauric acid and palmitic acid were added together to the reaction mixture in different concentrations. Acyl selectivities of the enzyme can be explained in terms of mutual competition, indicating that one enzyme is probably involved in triterpenol esterification.

Table 3. Apparent maximum velocity and apparent Michaelis constant values for triterpenol esterase with respect to different fatty acids. Latex (150 μl) samples of *E. pulcherrima* were incubated with fatty acid for 30 min. The values were determined by Lineweaver–Burk plots

Fatty acid	K_m (M.10^{-3})	V_{max} μmol/l. latex/min
12:0	2.0	37.4
14:0	2.1	23.7
16:0	3.0	11.4
18:0	2.4	6.5
18:1	1.4	11.9
18:2	1.4	21.6
18:3	2.9	69.0

Triterpenol specificity on triterpene ester biosynthesis

As mentioned above, the free triterpenol fraction of the latex of *E. pulcherrima* consists mainly of cycloartenol. Germanicol and β-amyrin were present in smaller amounts [28]. Table 5 shows the triterpene synthetase activity of *E. pulcherrima* latex incubated with capric acid at saturated concentration. It can also be seen that 0.70 μmol triterpenol was converted to 0.63 μmol triterpene caprate per g fresh latex. After incubation 14% of the combined β-amyrin and germanicol and 33% of cycloartenol were converted into triterpene caprates. Therefore, it

Table 4. Competitive inhibition of lauric acid and palmitic acid for triterpenol esterase. Latex (150 μl) of *E. pulcherrima* was incubated with fatty acids for 45 min. Triterpene esters were saponified and the methylated fatty acids were determined by radio-GC

Fatty acid	v^* Triterpene esters formed	
	Theoretical	Measured
3.0 μmol 12:0	33.8	41.7
0.5 μmol 16.0	5.9	5.2
3.0 μmol 12:0 + 0.5 μmol 16:0	30.7 + 1.0	26.6 + 1.1

v^*: μmol/l. latex/min.

Table 5. Specificity of triterpene ester synthetase from latex for triterpenols. Capric acid (4 μmol) was incubated with 200 μl latex of *E. pulcherrima* for 1 hr. Weight data were measured by GC. RR_t values are related to 5α-cholestane.

RR_t	Compound	μg g/fresh latex	
		−10:0	+10:0
1.99	β-Amyrin	457.5	392.5
1.94	Cycloartenol	708.0	474.5
2.21	Germanicol acetate	1542.0	1596.0
2.27		100.0	99.0
2.34		148.5	148.0
2.40		91.5	89.5
2.49		169.0	168.0
3.48	β-Amyrin caprylate	303.5	293.0
4.01		230.0	227.0
4.08	β-Amyrin caprate and cycloartenol caprate	95.5	459.0
4.16		82.5	94.5

can be concluded that the triterpenol specificity of the esterase bears more resemblance to the triterpenols of the free triterpenol fraction than to those of the triterpene ester fraction in the latex particles.

Incubations of latex with both capric acid and β-amyrin, however, were unsuccessful. Exogenously added triterpenol was neither esterified nor absorbed by the latex particles. This was shown by purification of the latex after incubation by CG followed by GC of the extracted lipids. The gas chromatography results were identical with those of incubations with capric acid only.

Characterization and isolation of triterpenol esterase in latex

Centrifugation of *E. lathyris* or *E. pulcherrima* latex (0.6 ml) at $1.3 \times 10^5 g$ for 1 hr after incubation with fatty acids gave three distinct fractions, a top fraction of packed latex particles, a clear serum, and a small amount of precipitate (bottom fraction). Labelled triterpene esters were found almost exclusively in the top layer; the bottom fraction contained 4–5 % of the label. Centrifugation over a discontinuous sucrose gradient (0.5–2.0 M) of *E. lathyris* latex incubated with 0.1 μmol palmitic acid showed that only 0.8 % of the labelled esters was present in the bottom fraction. It is concluded from these experiments that for triterpenol esterase there is no particular latex fraction with a high esterase activity. Purified latex particles of *E. characias* were incubated with fatty acid in different buffers. A particle-bound triterpenol esterase with an optimum pH of 4.5 for esterification was found. During incubation periods of 30 min the enzyme was stable up to 55°, but at higher temperatures it rapidly lost its activity. The effect of pH on triterpenol esterification in freshly tapped latex of *E. lathyris* was measured by adding aliquots of latex to different buffers in which fatty acid was dispersed ultrasonically. The latex appeared to be a very weakly buffered system which hardly affected the pH of the buffer used. The maximum rate of esterification occurred in this system at a somewhat higher pH than was found for isolated *E. characias* particles. The optimum pH coincided with the pH of pure latex, which was found to be 5.8.

An acetone powder preparation of purified *E. characias* latex particles was incubated with free triterpenols and [1-^{14}C]palmitic acid dispersed in buffer, yielding an incorporation activity of 85 nmol of fatty acid (see Experimental for details). A boiled enzyme control showed no triterpenol esterase activity at all. The esterase may consequently be regarded as located in the proteins present in or bound to the purified latex particles.

The cofactor requirement was examined by adding ATP and Co-enzyme A to latex (200 μl) of *E. pulcherrima*, which was incubated with 1.0 μmol (0.5 μCi) [1-^{14}C]palmitic acid. ATP (5 μmol) and Co-enzyme A (2 μmol) in 50 μl water were added and the mixture was incubated for 30 min. Compared with a control of water only, ATP and Co-enzyme A did not stimulate triterpenol esterase activity. High-energy intermediates, such as acyl-CoA, therefore do not seem to be involved in the esterification. These findings indicate that the enzyme present in the latex particles is probably a steryl esterase (E.C. 3.1.1.13). This was also indicated by the relatively low pH optimum of the enzyme.

Fatty acid binding to latex particles

Fatty acid esterification by latex particles was accompanied by a binding or an association of free fatty acids with the particles. The word absorption is used in this study for this phenomenon, since it is apparent from the amount of fatty acid bound by the particles that the acids have moved mainly into the particles after passing through the proteinaceous boundary of the particles. The fatty acid absorption to latex particles was studied for lauric acid and palmitic acid and the results are presented in Fig. 3. The absorption of fatty acids in the particles was proportional with time until about 50 % of the fatty acid had become associated with the latex particles. The rate of absorption depends on the chain-length of the fatty acid, just as was found for fatty acid/triterpenol esterification.

Figure 4 shows the amounts of the free fatty acid absorbed and esterified by *E. pulcherrima* latex. The ratio of free fatty acid to triterpenyl ester appears to depend on the amount of fatty acid added to the latex. A similar behaviour of fatty acid absorption to latex particles was also observed for *E. lathyris* latex (Fig. 5). After a rapid initial rate, the rate of binding appeared to be proportional to the incubation time.

Free fatty acid absorption probably influences the

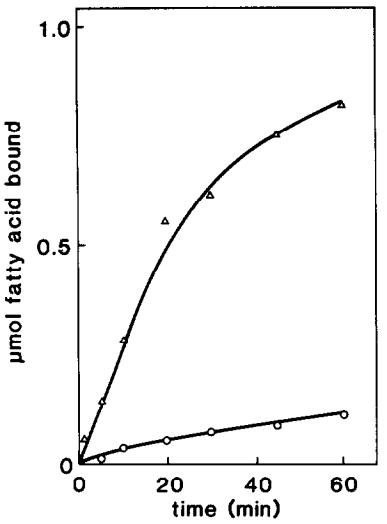

Fig. 3. Time-course of total fatty acid binding to latex particles of *E. pulcherrima*. Latex (200 µl) was incubated with 1 µmol (0.5 µCi) of [1-^{14}C]lauric acid (△---△) or palmitic acid (○---○). After the incubation period 50 µl samples were purified by CG and analysed for fatty acid binding.

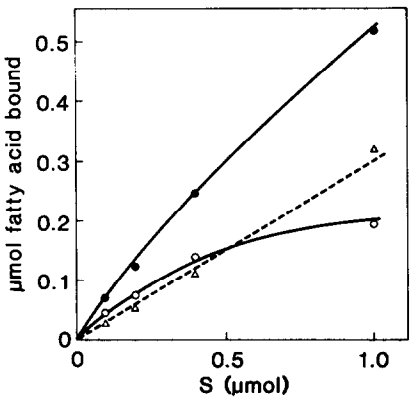

Fig. 4. Lauric acid binding to latex particles of *E. pulcherrima*. Increasing amounts of [1-^{14}C]lauric acid were incubated with 200 µl latex samples for 45 min. Total fatty acid binding (●---●) is separated in free fatty acid absorption (△---△) and triterpenol esterification (○---○) by the latex particles.

esterification rate in the time-course experiments (Fig. 1), since the reaction enters the stationary phase when only 25% of the substrate is esterified. As it is generally assumed that steryl esterase action is virtually irreversible and that product inhibition does not occur, it can be concluded that free fatty acids that have moved into the inner part of the particles do not participate in the esterification reaction.

Total fatty acid uptake experiments showed (Fig. 5) an initial rapid binding of fatty acid followed by a long-lasting slower phase of absorption with a constant rate, the latter is correlated to the amount of fatty acid added to the reaction mixture. This small burst of fatty acid binding is strongly reminiscent of the observations with pancreatic

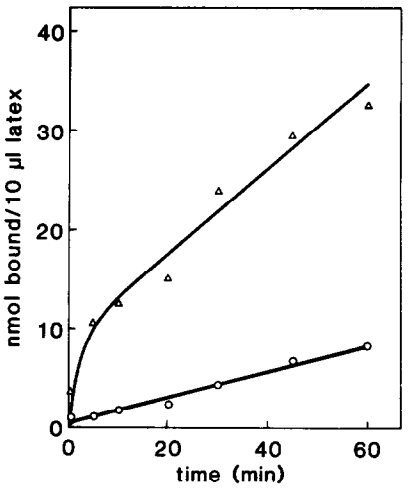

Fig. 5. Time-course of total fatty acid binding to latex particles in *E. lathyris*. Latex (200 µl) was incubated with 1 µmol (0.5 µCi) of [1^{14}C]lauric acid (△---△) or [1-^{14}C]palmitic acid (○---○).

cholesterol esterase [26, 27]. These authors found a small rapid association followed by a much greater, although slower adsorption of enzyme with the free fatty acid part of a substrate film. This process was thought to initiate the enzyme reaction. This adsorption is a complex process which depends on the concentration of the fatty acid in the incubation system. The pancreatic esterase was thought to function at a lipid–water interface and fatty acid adsorption to the enzyme was assumed to be reversible.

In the present work it is proposed that free fatty acid uptake by latex particles can be divided into a small rapid adsorption of free fatty acid to the protein layer surrounding the particles and which contains triterpenol esterase, subsequently followed by a slower phase of absorption of free fatty acid together with esterified fatty acids. The esterase activity itself was found to be independent of the rate of absorption of the free fatty acid. The free fatty acid absorption rate is probably diffusion controlled, because it is dependent on the amount of fatty acid added to the reaction mixture.

Free fatty acids adsorbed in small amounts to the protein layer surrounding the particles may also be present *in vivo*. These acids may therefore be involved in the esterification process of triterpenols synthesized *in vitro* from labelled acetate. That would provide an explanation for the relatively poor labelling of the fatty acid moiety of the formed triterpene esters in these experiments.

Incubations with [U-^{14}C]*phosphatidylcholine and glycerol tri*[1-^{14}C]*palmitate*

Latex of *E. pulcherrima* was incubated with PC or glycerol tri-[1-^{14}C]palmitate (20 nmol) for different times (0.5–4 hr). For both substrates, all experiments gave negative results for the incorporation of label into the triterpene esters. The radioactive components detectable after incubation were the unchanged substrates. This indicates that the enzymes using these substrates for esterification are not active or present in the latex. For

transesterification from phosphatidylcholine an optimum pH of 7 has been reported [18, 29]; the pH of *Euphorbia* latex is around 5.8. Triterpenol esterification with triglycerides [20] has been studied at pH 5.8, so it seems probable that this enzyme does not occur in *Euphorbia* latex.

Incubations with [1-^{14}C]*palmitoyl-CoA*

Although cofactor administration did not stimulate the esterification rate of triterpenols in latex, incubations with palmitoyl-CoA were carried out. Figure 6 shows the results of incubations of latex with increasing concentrations of palmitoyl-CoA. For comparison, latex samples were also incubated with palmitic acid only. Figure 6 demonstrates that triterpene ester was synthesized from the substrate but, above all, that 50–70% of the palmitoyl-CoA was rapidly hydrolysed to free fatty acid and CoA. Because of the strong acyl-CoA hydrolase activity in the latex it could not be completely ruled out in these experiments that some of the enzyme involved in the formation is acyl-CoA: sterol *O*-acyltransferase. However, it is very likely that the triterpene esters synthesized are made from the liberated fatty acids by triterpenol esterase. This view is strengthened by comparing the esterification rates of fatty acid hydrolysed from palmitoyl-CoA and those of free fatty acid (Fig. 6).

In developing non-articulated laticifers such as in *Euphorbia* species particle synthesis is thought to occur in small endoplasmic reticulum vacuolar-like vesicles present in the cytoplasm [30–33]. From cell-free experiments it is known that the site of triterpenoid biosynthesis is located in the endoplasmic reticulum [34]. It has been suggested that the newly synthesized triterpenols are transported intracellularly in the form of triterpene esters [35–37], as occurs in mammalian systems. Just like triterpenoid cyclase the enzyme sterol esterase is located in the microsomal fraction, where it functions mainly as a hydrolase [38, 39]. Optimum hydrolase activity has usually been found at pH 6.5–7.0 for mammals [40] and micoorganisms [16, 41].

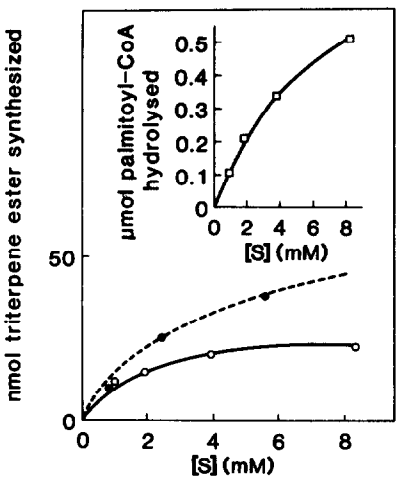

Fig. 6. Effect of increasing concentrations of palmitoyl-CoA and palmitic acid on triterpene ester biosynthesis in latex of *E. pulcherrima*. The experimental data for incubation with [1-^{14}C]-palmitoyl-CoA are described in the experimental section. [1-^{14}C]-Palmitic acid was dispersed by sonification into 50 µl 0.1 M MOPS buffer pH 7.0 prior to incubation. Triterpene esterification with palmitic acid (●---●), with palmitoyl-CoA (O---O), and palmitoyl-CoA hydrolase (□---□) were determined after an incubation period of 30 min.

In spite of its synthetic activity demonstrated in the present study, we propose that the triterpenol esterase in intact *Euphorbia* latices mainly acts as a hydrolase during latex particle synthesis. At the site of particle development a part of the intracellular transported triterpene esters is probably hydrolysed by the triterpenol esterase and subsequently added to the growing particle. This proposal explains our findings that (1) free triterpenols were not measurably absorbed by the latex particles, (2) triterpenols which were synthesized *in vitro* from acetate were found mainly as esters in the particles, and (3) the triterpenol specificity of the esterase corresponded with the free triterpenol composition of the particles; this composition differed distinctly from that of the experimentally synthesized ester fraction. Such differences between the triterpenol composition of the free triterpenol fraction of the particles and the triterpene ester fraction can be explained by assuming a selective hydrolytic step at the site of particle synthesis, where a part of the triterpene esters becomes hydrolysed in accordance with the triterpenol and fatty acid specificity of the enzyme. A fatty acid specificity for steryl ester hydrolase has also been found in roots of *Sinapis* seedlings [39]. After the latex particles have been synthesized, they are transferred to the developing central vacuole [30–32, 41–43]. On the contrary, Fineran [33] hypothesizes that portions of cytoplasm in which particles are synthesized become isolated within vacuoles. This sequestration of cytoplasm, followed by lytic breakdown in the vacuole, eliminates large volumes of cytoplasm. At the same time the latex particles contained within the smaller vacuoles of the sequestered cytoplasm can be released into the developing central vacuole.

Independent of the mechanism of latex particle transfer to the vacuole, the latex particles probably take with them the triterpenol esterase in the protein layer that surrounds them. The small part of the triterpenol synthesizing system [23, 44] is probably also of cytoplasmic origin and, just like the latex particles, is transferred to the central vacuole. In the latex the triterpenol esterase acts as a synthetase due to the low pH of the latex serum.

EXPERIMENTAL

Euphorbia plants were cultivated in the greenhouse on a Hoagland nutrient solution (*E. pulcherrima* Wildd.) or in the field (*E. characias* L. and *E. lathyris* L.). Latex was obtained from the petioles (*E. pulcherrima*) or from the stem tips by incision.

Chemicals. [1-^{14}C]Fatty acids were purchased by The Radiochemical Centre, Amersham, England. The Li(tri)salt of CoA was obtained from Boehringer, Mannheim, Germany. [1-^{14}C]-Palmitoyl-CoA was synthesized by the method of Al-Arif and Blecher [45]. [U-^{14}C]Lecithin (1.3 mCi/mmol) was prepared biochemically from [1-^{14}C]acetate according to ref. [46].

Isolation of latex particles. Latex (0.3 ml) of *E. characias* was diluted with 0.1 M K-Pi buffer (pH 8.0) to a vol of 1.0 ml and chromatographed on Sephadex G-100 using the same buffer. The eluate (2.5 ml) containing particles was desalted with 0.01 M K-Pi buffer (pH 7.0) and concd to a final vol. of 0.5 ml using a PM 10 membrane (Amicon). Latex particles of *E. pulcherrima* were chromatographed on Sephadex G-100 with 0.2 M KCl containing 0.01% Tween 80 [47].

Incubation conditions with latex. Incubations with freshly tapped latex were carried out in small (30 × 5 mm ID) glass test tubes. The other assays were performed in 8 ml test tubes. All incubations were done at 30°, unless mentioned otherwise.

Experiments with fresh latex. Sodium[1-^{14}C]acetate (57.2 mCi/mmol) in 50 μl H_2O was mixed with 150–200 μl latex and incubated in the dark. Fatty acid (0.5 μCi) dissolved in petrol was added to the test tube and evapd with N_2. Latex (150 μl) was added to it and incubated by gentle shaking. [1-^{14}C]Palmitoyl-CoA in 50 μl 0.1 M MOPS–Tris buffer (pH 7.0) was mixed with 150 μl latex and incubated for 30 min. Glycerol tri[1-^{14}C] palmitate (1 μCi) in toluene was added to the test tube and evapd. Latex (150 μl) was added and the mixture was subsequently incubated. Lecithin (0.53 μCi) was sonicated with 50 μl of 0.1 M K-Pi buffer (pH 7.0) and incubated with 200 μl latex.

Experiments with isolated latex particles. Fatty acid was dispersed by sonication in a bovine serum albumin (BSA) soln in H_2O. The reaction mixture consisted of 0.25 ml 0.1 M of a suitable buffer, 0.10 ml particle suspension and 0.05 ml substrate soln containing 1 μmol [1-^{14}C]palmitic acid (0.5 μCi). The enzymatic processes were stopped by 1 ml Me_2CO. Extraction, purification and saponification of the lipids was carried out as described before [4, 7]. The free triterpenols and fatty acids were extracted from the saponification mixture [5] and their radioactivities were measured. The fatty acids were methylated with either CH_2N_2 or BF_3 [4].

Incubation with Me_2CO powder preparation. Purified particles in 1.0 ml buffer were extracted × 4 with petrol. The resulting water phase was mixed with 9 ml Me_2CO (−30°) and the sediment was collected by centrifugation, washed twice with cold Me_2CO–Et_2O (1:1) and subsequently dried under N_2. The residue was dissolved in 1 ml 0.1 M citrate–K-Pi buffer (pH 5.5) and used for enzyme assay. A mixture of triterpenol and fatty acid in 50 μl Et_2O was sonicated in 0.1 M citrate–K-Pi buffer (pH 5.5) with 4 mg/ml defatted BSA. The reaction mixture consisted of 0.3 ml latex enzyme preparation and 0.7 ml substrate soln containing 5 μmol triterpenol and 1 μmol fatty acid (0.5 μCi). The mixtures were incubated for 2 hr with shaking.

Chromatography. Triterpenols and their esters were determined quantitatively by GC with FID on a 3% SE-30 column, operating at 200–300° (2°/min), with 5α-cholestane as int. stand. Fatty acid Me esters were analysed on a 10% Ap-L column (140 or 200°). GC–MS was carried out at 70 eV.

The distribution of radioactivity over the different compounds after incubation was detected by TLC-radioscanning. TLC of the neutral lipids was performed on silica gel-G developed with cyclohexane-EtOAc (9:1). Radio-GC was performed using a splitter after the GC-column, 50% of the flow was to a combustion furnace and the $^{14}CO_2$ was measured with a proportional counter.

Enzyme activity was expressed as μmol of triterpene ester synthesized by 1 l of latex per min.

REFERENCES

1. Kemp, R. J. and Mercer, E. I. (1968) *Biochem. J.* **110**, 111.
2. Kemp, R. J. and Mercer, E. I. (1968) *Biochem. J.* **110**, 119.
3. Van Die, J. (1955) *Ann. Bogor.* **2**, 1.
4. Warnaar, F. (1976) *Anal. Biochem.* **71**, 533.
5. Warnaar, F. (1977) *Lipids* **12**, 707.
6. Warnaar, F. (1981) *Phytochemistry* **20**, 89.
7. Warnaar, F. (1984) *Phytochemistry* **23**, 1049.
8. Moyer, L. S. (1935) *Am. J. Botany* **22**, 609.
9. Resing, W. L. (1955) Ph.D *Thesis*, Bandung.
10. Hegnauer, R. (1964) in *Chemotaxonomy der Pflanzen* Vol. III, p. 163. Birkhauser Verlag, Basel.
11. Clausen, M. K., Christiansen, K., Jensen, P. K. and Behnke, O. (1974) *FEBS Letters* **43**, 176.
12. Taylor, F. R. and Parks, L. W. (1978) *J. Bacteriol.* **136**, 531.
13. Warnaar, F. (1982) *Z. Pflanzenphysiol.* **105**, 307.
14. Hyun, J., Kothari, H., Herm, E., Mortenson, J., Treadwell, C. R. and Vahouny, G. V. (1969) *J. Biol. Chem.* **244**, 1937.
15. Norum, K. R. and Gjone, E. (1974) *Scand. J. Clin. Lab. Invest.* **33**, 191.
16. Okawa, Y. and Yamaguchi, T. (1977) *J. Biochem.* **81**, 1209.
17. Taketani, S., Nishino, T. and Katsuki, H. (1979) *Biochim. Biophys. Acta* **575**, 148.
18. Bartlett, K., Keat, M. J. and Mercer, E. I. (1974) *Phytochemistry* **13**, 1107.
19. Garcia, R. E. and Mudd, J. B. (1978) *Arch. Biochem. Biophys.* **190**, 315.
20. Zimowski, J. and Wojciechowski, Z. A. (1980) *Bull. Acad. Pol. Sci., Ser. Sci.* **28**, 203.
21. Nemethy, E. K., Skrukrud, C., Piazza, G. J. and Calvin, M. (1983) *Biochim. Biophys. Acta* **760**, 343.
22. Ponsinet, G. and Ourisson, G. (1968) *Phytochemistry* **7**, 757.
23. Kothari, H. V., Miller, B. F. and Kritchevsky, D. (1973) *Biochim. Biophys. Acta* **296**, 446.
24. Stokke, K. T. (1972) *Biochim. Biophys. Acta* **270**, 156.
25. Johnson, R. C. and Shah, S. N. (1978) *Lipids* **13**, 777.
26. Bhat, S. G. and Brockman, H. L. (1981) *J. Biol. Chem.* **256**, 3017.
27. Bhat, S. G. and Brockman, H. L. (1982) *Biochemistry* **21**, 1547.
28. Baas, W. J. (1977) *Planta Med.* **32**, 1.
29. Yao, J. K. and Dyck, P. J. (1977) *Clin. Chem.* **23**, 447.
30. Marty, F. (1968) *C. R. Acad. Sci. Ser. B* **267**, 299.
31. Marty, F. (1970) *C. R. Acad. Sci. Ser. B* **271**, 2301.
32. Marty, F. (1971) *C. R. Acad. Sci. Ser. B* **272**, 399.
33. Fineran, B. A. (1983) *Ann. Botany* **52**, 279.
34. Heintz, R. and Benveniste, P. (1970) *Phytochemistry* **9**, 1499.
35. Kemp, R. J., Goad, L. J. and Mercer, E. I. (1967) *Phytochemistry* **6**, 1609.
36. Goodwin, T. W. (1967) in *Terpenoids in Plants* (Pridham, J. B., ed.) p. 1. Academic Press, London.
37. Grunwald, C. (1971) *Plant Physiol.* **48**, 653.
38. Grunwald, C. (1975) *Annu. Rev. Plant Physiol.* **26**, 209.
39. Kalinowska, M. and Wojciecowski, Z. A. (1983) *Phytochemistry* **22**, 59.
40. Sakamoto, T., Okuda, H. and Fujii, S. (1974) *J. Biochem.* **75**, 1073.
41. Heinrich, G. (1970) *Protoplasma* **70**, 317.
42. Giordani, R. (1978) *Biol. Cell.* **33**, 253.
43. Wilson, K. J. and Mahlberg, P. G. (1980) *Am. J. Botany* **67**, 1160.
44. Groeneveld, H. W. (1976) *Acta Bot. Neerl.* **25**, 459.
45. Al-Arif, A. and Blecher, M. (1969) *J. Lipid Res.* **10**, 344.
46. Galliard, T. (1972) *Biochim. Biophys. Acta* **260**, 541.
47. Groeneveld, H. W. (1976) *Acta Bot. Neerl.* **25**, 459.