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THE INVOLVEMENT OF SUCROSE, GLUCOSE AND OTHER METABOLITES IN THE SYNTHESIS OF TRITERPENES AND DOPA IN THE LATICIFERS OF *EUPHORBIA LATHYRIS*

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Key Word Index—*Euphorbia lathyris*; Euphorbiaceae; biosynthesis; triterpenes; DOPA; laticifers; seedlings.

Abstract—A quantitative triterpene analysis was made of latex and stem tissue of *Euphorbia lathyris*. Young plants and seedlings of *E. lathyris* were incubated with various labelled precursors. Incorporation into triterpenes was obtained from [2-^{14}C]mevalonic acid, [1-^{14}C]acetate, [3-^{14}C]pyruvate, [U-^{14}C]sucrose, [U-^{14}C]-glucose, [U-^{14}C]xylose, [U-^{14}C]glyoxylate, [2,3-^{14}C]succinic acid, [1-^{14}C]glycerol and [U-^{14}C]serine. Both sugars and tyrosine appeared to be effective precursors in DOPA synthesis inside the laticifers. Exogenously supplied mevalonic acid was only involved in triterpene synthesis outside the laticifers. GC-RC of triterpenes synthesized from [U-^{14}C]glucose revealed the origin of these compounds in the latex. The labelled triterpenes obtained after incorporation of the other mentioned labelled precursors were only partly synthesized in the laticifers. For quantitative data on latex triterpene synthesis seedlings were incubated with [U-^{14}C]sucrose, [U-^{14}C]glucose, [U-^{14}C]xylose and [1-^{14}C]acetate in the presence of increasing amounts of unlabelled substrate. From the amount of ^{14}C incorporated into the triterpenes the amount of substrate directly involved in triterpene synthesis was calculated, as was the absolute triterpene yield. Sucrose showed the highest triterpene yield, equivalent to the daily increase of the triterpene content of growing seedlings. The possible significance of the other precursors in triterpene synthesis in the laticifers is discussed.

INTRODUCTION

Triterpenes and triterpene esters are common constituents of many plant latices. They occur together as massive lipid particles in the large central vacuole of the non-articulated laticifers [1] of, for example, *Euphorbia lathyris*. The wall-lining cytoplasm is supposed to be the site of synthesis prior to the final secretion as a lipid particle into the central vacuole [2, 3].

Many compounds, such as acetate, glyoxylate, leucine, valine, serine and CO_2 are reported to be effective precursors of isoprenoids [3–7]. Sugars were effective precursors in the synthesis of triterpenols and triterpene esters in the laticifers of *Hoya* and *Euphorbia* species [3, 8].

In these laticifers glycolysis was shown to be the major biochemical pathway in substrate breakdown and the required NADPH for squalene synthesis from acetyl CoA is possibly yielded by the pentose phosphate cycle [9]. In *Hoya* sugars are the exclusive precursors of latex triterpene synthesis. In *Euphorbia* intermediates of glycolysis may also be incorporated, to a certain extent, into latex triterpenes [3].

In the laticifers of *Euphorbia lathyris*, triterpenes (including triterpene esters) and DOPA are major constituents [10, 11]. The present paper deals in the first place with the possible involvement of various intermediates of primary metabolism in the synthesis of *Euphorbia lathyris*. Secondly, this paper is concerned with the triterpene yield (triterpenols and esterified triterpenes) from several intermediates.

RESULTS AND DISCUSSION

Lipid analysis

The lipid composition of the latex from *Euphorbia lathyris* was investigated by Nielsen *et al.* [10]. The non-saponifiable part of this milky fluid was shown to contain (in order of abundance) 24-methylene cycloartanol, lanosterol (including an isomer of lanosterol), cycloartenol, euphol and an unidentified terpenoid compound. About 35% of the triterpenes occur as triterpene esters [9]. Both the triterpene moiety of the esters and the free occurring triterpenols co-chromatographed in GLC with authentic compounds mentioned by Nielsen *et al.* [10]. In addition, argentation-chromatography of the triterpene acetates in combination with GLC revealed a more complex triterpene composition in both esterified triterpenes and triterpenols. Apart from euphol, which occurs in trace amounts, the compounds mentioned by Nielsen were detected as major constituents, but a different order of abundance was obtained. Lanosterol (including an isomer) was the major constituent in both the

Table 1. Triterpenes from latex and stem of *Euphorbia lathyris* seedlings and plants

	Relative amount of esterified triterpenes				Relative amount of triterpenols			
	Lanosterol	Cycloartenol	24-Methylene cycloartanol	Unknown	Lanosterol	Cycloartenol	24-Methylene cycloartanol	Unknown
Plant latex	1.0	0.49	0.33	0.16	1.0	0.40	0.65–1.14	0.16
Plant stem	1.0	0.51	0.36	0.16	1.0	0.42	0.65–1.14	0.15
Seedling latex	1.0	0.62	0.35	0.16	1.0	0.62	0.90–1.10	0.15
Seedling stem	1.0	0.65	0.37	0.16	1.0	0.56	0.90–1.10	0.15

Table 2. Incorporation of ^{14}C into the esterified triterpenes and triterpenols of *Euphorbia lathyris* plants after injection of several labelled precursors into the stem. Incubation time: 3 days

Labelled compound (sp. act. in Ci/mol, radioactivity added in μCi)	Distribution of radioactivity in dpm			
	Tapped latex		Remaining stem tissue	
	Esterified triterpenes	Triterpenols	Esterified triterpenes	Triterpenols
[U-^{14}C]Sucrose (5.1, 12.5)	18300	39200	30600	57200
Sodium [3-^{14}C]pyruvate (8.1, 7.5)	26040	49900	84700	98500
Sodium [1-^{14}C]acetate (55.7, 12.5)	14100	32000	79800	78200
DL-[2-^{14}C]MVA (10.0, 5.0)	—	—	361700	309500

esterified triterpenes and triterpenols (Table 1). In the course of this study a variable triterpene composition of the latex was observed; cycloartenol appeared to be more abundant in seedling latex than in the latex of older plants and overall a variable amount of 24-methylene cycloartanol as the free alcohol was observed.

Latex is not completely removed from laticifer-containing tissue after tapping. The triterpenes obtained from stem tissue are similar, as judged by GLC, to those obtained from the latex. Consequently, these triterpenes are assumed to represent the triterpenes of the remaining latex. In seedlings the triterpene fractions of the stem tissue are not completely consistent with those of the latex, but more than 95% appeared to originate from the laticifers.

Incorporation experiments

Labelled precursors were injected into the uppermost part of the hollow stem of 4-month-old plants. After 3 days latex was collected and the ^{14}C content of the triterpenes of this latex and the remaining stem tissue was assayed. The results, presented in Table 2, clearly show that exogenously supplied sucrose, pyruvate and acetate were involved in triterpene synthesis in the laticifers. However, MVA was not incorporated. The mass ratio between esterified triterpenes and triterpenols (1:2) was reflected in the ^{14}C ratio of both constituents as far as the tapped latex was concerned. This ratio was also found in the sucrose incorporated stem tissue, indicating that these [^{14}C]triterpenes mainly represent the latex triterpenes which remained after tapping. The [^{14}C]triterpenes extracted from the stem parts after [1-^{14}C]acetate or [3-^{14}C]pyruvate administration apparently are not all of latex origin as the ^{14}C content in the esterified triterpenes was relatively high. The exogenously supplied [2-^{14}C]MVA was not metabolized by the latex cytoplasm. The non-saponifiable lipids made from this precursor represent triterpenes synthesized outside the laticifers.

DOPA is another major constituent exclusively located in the laticifers of *Euphorbia lathyris* (up to 1.7% fr. wt) [11]. Haupt [12] observed incorporation of [^{14}C]tyrosine into DOPA in derooted shoots of this plant. She concluded from her experiments two possible sites of DOPA synthesis: either the wall-lining cytoplasm or more likely the leaf parenchyma from which a selective transport into the laticifers should take place. Results in Table 3 show that this constituent was synthesized in the stem from sucrose. This observation points to the wall-lining cytoplasm as the site of synthesis rather than the leaf parenchyma. In addition a small amount of [^{14}C]triterpenols and esterified [^{14}C]triterpenes was isolated from the tapped latex after [^{14}C]tyrosine administration.

Glucose incorporation experiments with excised defoliated stem tip parts revealed a high ^{14}C incorporation into the triterpenes. The considerable loss of latex, inevitable when excised stem parts are used, finally resulted in a high specific activity of [^{14}C]-lipids. GC-RC of the esterified triterpenes and triterpenols revealed four radioactive peaks co-chromatographing with the triterpenes. For quantitative data

Table 3. Incorporation of ^{14}C into DOPA and triterpenes from [U-^{14}C]tyrosine and [U-^{14}C]sucrose by *Euphorbia lathyris* plants. Data refer to [^{14}C]constituents obtained from the latex, tapped 5 days [^{14}C]precursor injection into the hollow stem

Fractions	Distribution of radioactivity after incorporation with: [U-^{14}C]Tyrosine* (dpm)	[U-^{14}C]Sucrose (dpm)
Esterified triterpenes	5970	114180
Triterpenols	9100	226020
Serum	58500	207500
DOPA†	56200	103750
DOPA‡	53240	93380

*Added: 5 μCi [U-^{14}C]tyrosine, sp. act. 513 Ci/mol; 25 μci [U-^{14}C]sucrose, sp. act. 10 Ci/mol.

†Purified by TLC.

‡Purified by Al_2O_3-adsorption chromatography.

Table 4. Incorporation of ^{14}C from D-[U-^{14}C]glucose into the esterified triterpenes and triterpenols of stem tip parts of *Euphorbia lathyris* plants

	Radioactivity and sp. act.			
	Esterified triterpenes		Triterpenols	
	(dpm)	(dpm/μg)	(dpm)	(dpm/μg)
Lanosterol	48080	160	136820	180
Cycloartenol	27210	159	64960	190
24-Methylene cycloartanol	13730	161	62540	125
Unknown	5320	142	16070	173

Added: 5 μCi D-[U-^{14}C]glucose, sp. act. 10 Ci/mol incubation time: 46 hr.

the [^{14}C]triterpenes were trapped via a splitter for subsequent ^{14}C assays. Results of a typical incorporation experiment, presented in Table 4, show a nearly complete synthesis of the triterpene mixture of the latex. This strongly indicates that both the labelled esterified triterpenes and free triterpenols were synthesized in the laticifers. Incorporation of ^{14}C into 24-methylene cycloartanol was relatively low, which may be related to the observed quantitative variability of this constituent.

To investigate the possible involvement of precursors other than sucrose and glucose in triterpene synthesis inside laticifers, groups of five derooted seedlings were placed in 100 μl solutions of various labelled precursors. After 2 days incubation the ^{14}C content of the non-saponifiable fraction was assayed. Results of a typical experiment, presented in Table 5, show that both sterols and triterpenes were actively synthesized from all the supplied compounds. Sterol synthesis from [U-^{14}C]glucose was relatively low.

The ^{14}C content of the triterpenes synthesized from this substrate was relatively the highest (in general 70–85% of the non-saponifiable ^{14}C lipids) and the ^{14}C distribution was consistent with the mass distribution of the latex triterpenes in GC-RC. Apparently after 52 hr of incorporation labelled intermediates of sterol synthesis such as cycloartenol and 24-methylene cycloartanol were not present in sufficient amounts to alter significantly the ^{14}C distribution which was characteristic for latex triterpenes. Intermediates of sterol synthesis may have been present in the radioactive fractions co-chromatographing (on TLC and Al_2O_3 adsorption chromatography) with 4α-methyl sterols (in general 8–18% of the non-saponifiable lipids). Pyruvate, acetate and MVA were metabolized efficiently and incorporated into the sterols. Their co-occurring triterpenes contained about 40% of the ^{14}C in the non-saponifiable fraction (Table 5). The ^{14}C distribution amongst these triterpenes was not consistent with the mass distribution in GLC. This was not the case with the [^{14}C]glyoxylate and [^{14}C]glycerol incorporations. Apart from glucose the other ^{14}C precursors used appeared to be mainly involved in a co-occurring triterpene synthesis outside the laticifers.

To investigate the possible use of the free triterpenols or the esterified triterpenes as a reference of latex lipid synthesis, both fractions were analysed separately. The results are presented in Table 6. Using the [^{14}C]triterpenes of the [U^{14}C]glucose incubated stem parts as a reference, only the [U-^{14}C]-xylose incorporated seedlings had a similar ^{14}C distribution over the free triterpenols and the esterified triterpenes. All the other precursors yielded a different ^{14}C distribution in both groups of triterpenes as revealed by GC-RC. The labelled lipid yield after incubation with [1-^{14}C]glycerol, [2, 3-^{14}C]succinic acid and [U-^{14}C]serine was too low for GC-RC analysis. Trapping the separate triterpenes after GLC

Table 5. Distribution of the radioactivity in the non-saponifiable lipids of *Euphorbia lathyris* seedlings after incubation with various ^{14}C-labelled compounds. Data refer to the triterpenes, 4α-methyl sterols and sterols extracted from the incubated stems and separated by TLC after saponification of the apolar lipid extract. Incubation time: 52 hr

Labelled compound (sp. act. in Ci/mol; radioactivity added in μCi)	Radioactivity (dpm) in the non-saponifiable lipids (% total fraction)		
	Triterpenes	4α-Methyl sterols	Sterols
D-[U-^{14}C]Glucose	98720	22880	14210
(3.1, 5.0)	(72.8)	(16.9)	(10.3)
Sodium [3-^{14}C]pyruvate	207500	112630	185880
(15.8, 5.0)	(41.0)	(22.2)	(36.8)
[1-^{14}C]Glycerol	65410	20870	11730
(23.7, 5.0)	(66.7)	(21.3)	(12.0)
Sodium [1-^{14}C]acetate	144230	51560	112100
(58.7, 5.0)	(41.1)	(18.6)	(40.3)
Sodium [U-^{14}C]glyoxylate	230320	74490	76200
(5.8, 5.0)	(60.4)	(19.6)	(20.0)
DL-[2-^{14}C]MVA	802000	562160	730800
(22.0, 5.0)	(38.3)	(27.0)	(34.9)

Table 6. Incorporation of ^{14}C from several labelled compounds into esterified triterpenes and triterpenols of *Euphorbia lathyris* seedlings

Labelled compound (sp. act. in Ci/mol, radioactivity added in μCi)	Incorporation into esterified triterpenes in dpm				Incorporation into triterpenols in dpm			
	Lanosterol	Cycloartenol	24-Methylene cycloartanol	Unknown	Lanosterol	Cycloartenol	24-Methylene cycloartanol	Unknown
D-[U-^{14}C]Glucose	15590	6730	5650	2440	29060	12160	17870	3640
(3.1, 25)	(1.0)	(0.43)	(0.36)	(0.16)	(1.0)	(0.42)	(0.62)	(0.13)
D-[U-^{14}C]Xylose	18850	7600	6510	3170	20910	8200	12420	3200
(90, 20)	(1.0)	(0.40)	(0.34)	(0.17)	(1.0)	(0.39)	(0.60)	(0.15)
[1-^{14}C]Glycerol	3590	2080	1520	540	15620	6630	6840	1590
(23.6, 7.5)	(1.0)	(0.60)	(0.42)	(0.15)	(1.0)	(0.42)	(0.44)	(0.10)
Sodium [1-^{14}C]acetate	41230	25280	19580	5040	71160	18070	35410	4640
(58.7, 12.5)	(1.0)	(0.61)	(0.47)	(0.12)	(1.0)	(0.25)	(0.50)	(0.06)
Sodium [3-^{14}C]pyruvate	9100	4760	3590	880	32630	8220	16730	1880
(15.8, 7.5)	(1.0)	(0.52)	(0.40)	(0.10)	(1.0)	(0.25)	(0.51)	(0.06)
[2, 3-^{14}C]Succinic acid	7910	5150	3080	1160	18070	6600	8160	1940
(50, 10)	(1.0)	(0.65)	(0.40)	(0.15)	(1.0)	(0.36)	(0.45)	(0.11)
Sodium [U-^{14}C]glyoxylate	8180	4960	2850	990	48840	22240	23460	3720
(5.8, 4)	(1.0)	(0.60)	(0.34)	(0.12)	(1.0)	(0.46)	(0.48)	(0.08)
L-[U-^{14}C]Serine	5010	3070	2270	570	20760	7390	11640	1840
(10, 10)	(1.0)	(0.61)	(0.45)	(0.12)	(1.0)	(0.36)	(0.56)	(0.09)
DL-[2-^{14}C]MVA	66940	59800	53630	3720	201530	42190	125690	7790
(22, 3.7)	(1.0)	(0.90)	(0.80)	(0.06)	(1.0)	(0.21)	(0.62)	(0.04)

separation revealed a ^{14}C distribution which was not consistent with the separate triterpenes (Table 6). Neither the triterpenols nor the esterified triterpenes can be used as a reference of latex lipid synthesis from non-sugar substrates in stem tissue. The above-mentioned results all indicate that sugars were exclusively incorporated into latex triterpenes as far as the 4, 4-dimethyl sterols are concerned. MVA was only involved in triterpene synthesis outside the laticifers, the other precursors may be partly involved in latex lipid synthesis. DOPA synthesis from sucrose emphasizes the importance of sugars as substrates in the metabolism of these laticifers.

The latex triterpenes (both esterified and free) are the major lipids of *E. lathyris* seedlings (Table 1). If sugars may be considered as the main substrates in latex lipid synthesis, they must account for a substantial part of this synthesis. Quantitative data on the conversion of sucrose, glucose, xylose and acetate to triterpenes were obtained from incorporation experiments using these labelled substrates in the presence of various amounts of unlabelled substrate. Data relate to a single defoliated stem tip or defoliated and derooted seedling. Triterpenes were no longer separated into esterified triterpenes and triterpenols. Detailed information on the calculation of substrate incorporation and lipid yield is given in the Experimental section.

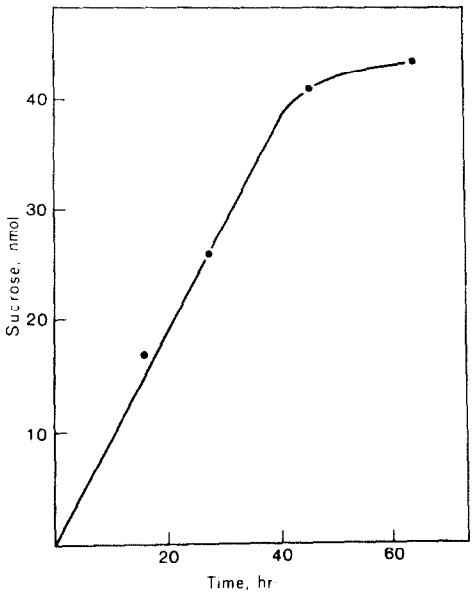

Fig. 1. Time-course of sucrose conversion to latex triterpenes by defoliated apical stem parts (12 cm) of *Euphorbia lathyris*; 1.25 μmol sucrose added per stem tip.

Conversion of sucrose and glucose to triterpenes by stem tips

Excised defoliated stem tips (12 cm) were placed in 50 μl solutions containing 426 μg [U-^{14}C]sucrose which was absorbed within 3 hr. After different time intervals the incubated stem parts were extracted and the ^{14}C content of the triterpenes assayed. The amount of sucrose directly involved in triterpene synthesis was calculated. The results given in Fig. 1 show a continuous synthesis of triterpenes from exogenously supplied sucrose. After 50 hr incorporation a small reduction in substrate conversion was observed, probably due to exhaustion of the substrate. Without considering a possible dilution by endogenous precursor about 43 nmol sucrose was used for triterpene synthesis (3.5% of the administered substrate). In a similar experiment [U-^{14}C]-glucose was used as substrate. Solutions (150 μl) containing 4, 15.1, 20.7, 26.3, 31.8 or 59.6 μmol [U-^{14}C]glucose were absorbed by defoliated stem tips (5.5 cm). The ^{14}C content of the triterpenes was assayed after 21 and 42 hr incorporation and the conversion of glucose to triterpenes was calculated. The results presented in Fig. 2 show that nearly all the triterpenes were synthesized from the 4 μmol aliquot in 21 hr. Increasing amounts of glucose produced higher yields of latex lipids. Apparently neither the glucose uptake nor the capacity for triterpene synthesis were limiting factors in these experiments.

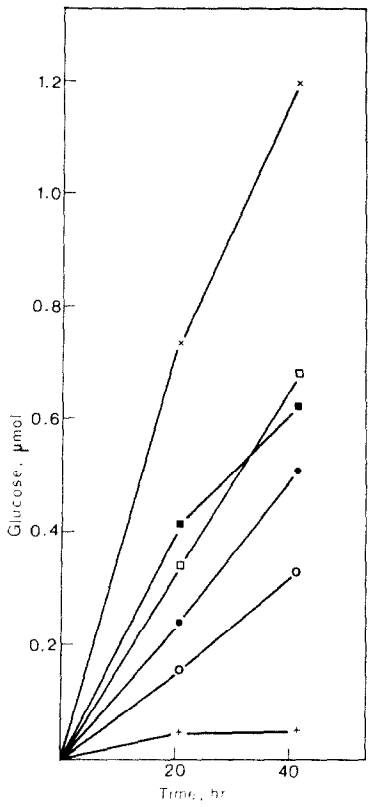

Fig. 2. Time-course of glucose conversion to latex triterpenes by defoliated stem tips (5 cm) of *Euphorbia lathyris*. Glucose added per stem tip: 4.0 μmol (+), 15.1 μmol (○), 20.7 μmol (●), 26.3 μmol (□), 31.8 μmol (■) and 59.6 μmol (×) glucose.

Conversion of sugars to triterpenes by seedlings

A value for the synthesis of triterpenes from glucose was obtained by incubating young, derooted seedlings with [U-^{14}C]glucose in the presence of increasing proportions of unlabelled glucose. The

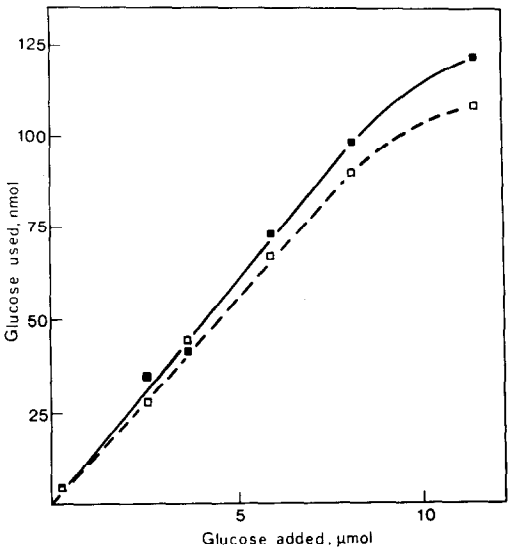

Fig. 3. Conversion of glucose to latex triterpenes from increasing proportions of glucose supplied to the excised stem of a *Euphorbia lathyris* seedling. Green (■) or etiolated (□) tissue. Incorporation time: 2 days.

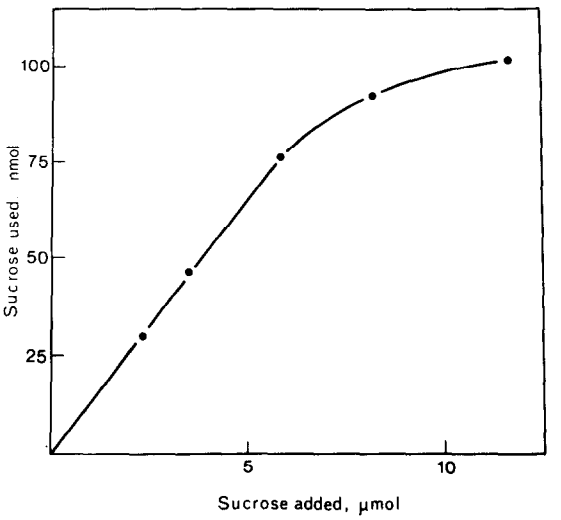

Fig. 4. Conversion of sucrose to latex triterpenes from increasing proportions of sucrose supplied to the excised stem of a *Euphorbia lathyris* seedling. Incorporation time: 2 days.

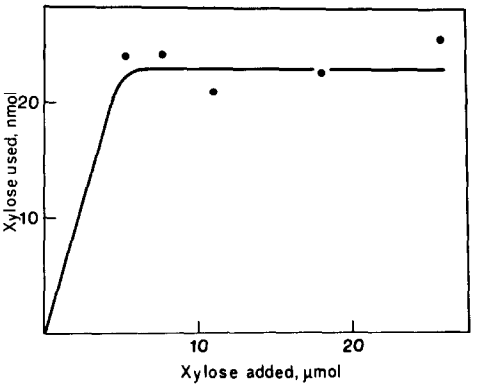

Fig. 5. Conversion of xylose to latex triterpenes from increasing proportions of xylose supplied to the excised stem of a *Euphorbia lathyris* seedling. Incorporation time: 2 days.

results presented in Fig. 3 show that the amount of glucose used for the synthesis of these lipids increased proportionally to the amount added. Complete substrate saturation was not obtained. More sugar could not be supplied to these seedlings; the highest amount of glucose was supplied as a 0.47 M solution (11.4 µmol in 24 µl, a greater volume caused an unacceptable delay in substrate uptake). Substrate saturation of glucose conversion to triterpenes either by glucose uptake or by triterpene synthesis is assumed to occur at proportions greater than 12 µmol glucose/seedling. These results were obtained with etiolated and green seedlings of nearly the same size. When sucrose was used as a substrate for triterpene synthesis more substrate could be taken up from the same small volume of equal molarity. Saturation was observed at about 12 µmol sucrose/seedling (Fig. 4).

Xylose also appeared to be an effective precursor in latex lipid synthesis (Table 6). Dilution with unlabelled substrate reduced the incorporation of ^{14}C and 4 µmol xylose/seedling was found to be the substrate saturating value in these experiments (Fig. 5). As the glycolytic sequence is assumed to be the main route in glucose catabolism prior to triterpene synthesis from acetyl-CoA, the conversion of xylose via xylulose-5-phosphate to glucose-6-phosphate (pentose phosphate cycle) might be the limiting factor in this sequence. Comparable data were obtained with acetate as substrate. Addition of unlabelled acetate resulted in a considerable reduction of the ^{14}C incorporation into the non-saponifiable lipids. Administration of 4 µmol acetate provided substrate saturation conditions for triterpene synthesis (Fig. 6).

The data for the conversion of sucrose, xylose, glucose and acetate to triterpenes relate solely to the uptake of tracer without reference to a possible dilution by endogenous precursor. From these data the maximum amount of triterpenes synthesized was calculated and the values obtained must be considered as minimum values (Table 7). As far as the seedlings are concerned, sucrose yields the highest amount of triterpenes per seedling per day. Glucose and sucrose are converted in equal molar proportions but the triterpene yield from sucrose is twice the yield from glucose. However, xylose was less effective giving at best one-tenth of the value obtained from sucrose. Similar data were obtained with acetate as the precursor, but in this case only a small part of the synthesized triterpenes may be of latex origin (Table 6). GC-RC of these [^{14}C]triterpenes showed that at best 20% of the non-saponifiable [^{14}C]lipids could represent the latex triterpenes. Therefore the amount of latex triterpenes synthesized from exogenously supplied acetate is at most 0.09 µg/seedling per day under these experimental conditions.

A value for the normal daily triterpene synthesis was derived from the increase of the triterpene con-

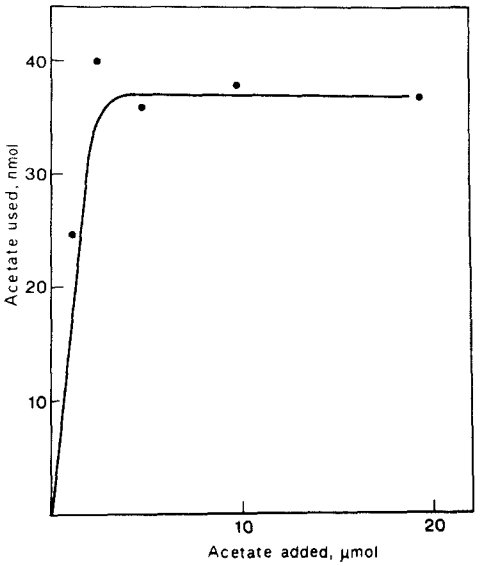

Fig. 6. Conversion of acetate to triterpenes from increasing proportions of acetate absorbed by the excised stem of a *Euphorbia lathyris* seedling. Incorporation time: 2 days. Carrier acetate was supplied as NaOAc–HOAc buffer, pH 7.

Table 7. Triterpene synthesis from various exogenously supplied precursors by excised stems of *Euphorbia lathyris* seedlings; incubation time: 2 days

Seedling age (days after germination)	Precursor	μmol added	Triterpene synthesized (μg/day per stem)
22	Sucrose	11.4	4.8
14	Glucose	11.4	2.9
11*	Glucose	11.4	2.6
22	Xylose	4–26	0.48
15	Acetate	4–19	0.44

*Etiolated seedlings.

Table 8. Triterpene content of the stems of *Euphorbia lathyris* seedlings before and after excision

Seedling age (days after germination)	μg Triterpene per stem	
	Before excision	After excision*
13	37–39	14–16
18	66–70	35–39
25	84–90	43–49

*These triterpenes account for remaining latex, see also Table 1.

tent of growing seedlings (Table 8). In the third week an average daily increase of about 6 μg triterpene/day was assayed. This value decreased to about 2.7 μg/day after 3.5 weeks. Compared with these figures the triterpene yield from the [^{14}C]sucrose incorporation was in the range of the normal daily synthesis. Glucose administration to green, as well as to etiolated plants, resulted in a lower lipid yield than that found with intact seedlings. Xylose was less effective and the contribution of acetate to the latex lipid yield was far from adequate at 0.09 μg/day (2% compared with sucrose).

It must be remembered that the derooted seedlings and stem parts used in the incorporation experiments inevitably have lost a considerable amount of latex triterpenes (up to 60%, Table 8). A declining growth of the derooted seedlings is another important difference compared with intact seedlings. The present data are calculated without reference to a possible dilution by endogenous precursors and the participation of these precursors is difficult to estimate. However, a contribution of endogenous sucrose and glucose is not required and the daily increase of latex lipids can be obtained from exogenously supplied sugars only. If the daily synthesis of the latex triterpenes depends solely on acetate uptake and conversion, then exorbitantly high endogenous concentrations of this substrate are required. Therefore sucrose, and to a lesser extent glucose, may be considered as the main substrates in latex triterpene synthesis in *Euphorbia lathyris* seedlings. The uptake of acetate by the laticifers from the adjacent cells and subsequent conversion to non-saponifiable lipids is of minor importance.

These findings are in contrast with those obtained with *Hevea* [13]. An unsuspected deficiency in sucrose catabolism assayed *in vitro* in *Hevea* latex suggested that there is no real basis for the assumption that all polyisoprene (rubber) comes from sugars, although it is most likely that carbohydrate is the main source of carbon utilized by the latex vessel [14]. To account for this discrepancy a substantial contribution of other metabolities (e.g. malate, leucine, citrate) in isoprenoid synthesis was proposed [13]. Our experiments with *Euphorbia lathyris* seedlings demonstrate that carbohydrate is the main source of carbon utilized by the laticifers in triterpene synthesis. Apart from MVA, the participation of other metabolites entering the latex vessels from the adjacent tissues is not excluded. However, their contribution to latex triterpene synthesis is to be considered as minimal.

EXPERIMENTAL

Plants. Euphorbia lathyris seeds were soaked for 24 hr in H_2O and germinated for 6 days in darkness at 25°. Seedlings were grown in the greenhouse. The seedlings used were 3–5 weeks old and 13–15 cm tall, having 2–3 leaf pairs. After 5 weeks plants were transferred to an environmental chamber and cultivated for 3 months at 18°.

Incorporation experiments. Labelled compounds were fed to 4-month-old plants either by injection (50 μl soln) into the apical stem part of intact plants or 100–120 μl soln were absorbed by excised, defoliated apical stem parts (5–12 cm long). Groups of five derooted and defoliated seedlings absorbed 100–120 μl soln of labelled substrates within 8 hr. All the incubations were carried out at 25°.

DOPA extraction. Freshly tapped latex was diluted with 1 M sucrose containing 0.05 M cysteine (1:1) and centrifuged 45 min at 120 000 *g*. The clear serum was diluted with 20 vol. 80% EtOH, containing 0.05 M cysteine and cleared

by centrifugation (2500 *g*, 5 min). DOPA was isolated from the clear supernatant by Al_2O_3 adsorption chromatography [15] or by TLC on cellulose–Si gel (10:4), developed in *n*-BuOH–HOAc–H_2O (15:3:5) or EtOH–HOAc–H_2O (15:15:10).

Lipid extraction. Triterpenol and triterpene ester extraction. Latex (0.2 ml) was suspended in 2 ml 0.2 M phosphate buffer, pH 8 and subsequently extracted with 4 ml petrol–MeOH (1:1). Stem tissue was extracted with Me_2CO in a Soxhlet and the neutral lipids were isolated with petrol [16]. The petrol fractions were separated by Al_2O_3 adsorption chromatography [17, 18] into the triterpene ester and sterol ester containing fraction, the (free) triterpenols, the 4α-methyl sterols and the sterols. The esters were saponified and chromatographed on Al_2O_3 yielding triterpenols, 4α-methyl sterols and sterols. A similar separation was obtained by TLC on Si gel G developed in cyclohexane–EtOAc (5:1).

GLC, GC-RC. Triterpenes and their prepared acetates were separated and quantified by GLC. The column was 180 cm × 0.3 cm, 3% SE-30. [^{14}C]Compounds were trapped in a glass capillary via a splitter (1:1) and counted in a liquid scintillation counter. In GC-RC [^{14}C]compounds were combusted to $^{14}CO_2$ and subsequently measured in a proportional counter.

Calculation of the triterpene yield from labelled substrates. Sugars involved in latex triterpene synthesis are assumed to be catabolized (in the wall-lining cytoplasm of the laticifers) via glycolysis [9] to acetyl–CoA which in turn is used as a substrate in triterpenol synthesis. The glycolytic breakdown of nine molecules of glucose yields 18 molecules of acetyl-CoA, which are used for the synthesis of one molecule of triterpenol ($C_{30}H_{50}O$). Only 30 of the 54 carbon atoms of glucose are incorporated into the triterpenol skeleton, 24 carbon atoms disappear as CO_2. The amount of [U-^{14}C] glucose directly used in triterpenol synthesis can be calculated as follows:

$$\frac{\text{Dpm [}^{14}\text{C]triterpenol}}{2.22 \times 10^6} \times \frac{54}{30} \times \frac{1}{\text{sp. act.}} = \mu\text{mol glucose}$$

(if sp. act. in Ci/mol).

The triterpenol yield is then calculated as

$$\frac{\mu\text{mol glucose used}}{9} \times 426 = \mu\text{g triterpenol synthesized.}$$

When sucrose and xylose are used as a substrate, they are assumed to be converted to glucose. A similar calculation can be made for [1-^{14}C]acetate:

$$\frac{\text{Dpm [}^{14}\text{C] triterpenol}}{2.22 \times 10^6} \times \frac{18}{12} \times \frac{1}{\text{sp. act.}} = \mu\text{mol [1-}^{14}\text{C]acetate used}$$

and

$$\frac{\mu\text{mol [1-}^{14}\text{C]acetate used}}{18} \times 426 = \mu\text{g triterpenol synthesized.}$$

In these formulae 426 represents the MW of a triterpenol ($C_{30}H_{50}O$). The proportion of glucose used in the required NADPH production is omitted in these calculations.

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