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A REINVESTIGATION OF THE BIOSYNTHESIS OF LANOSTEROL IN *EUPHORBIA LATHYRIS*

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Key Word Index—*Euphorbia lathyris*; Euphorbiaceae; biosynthesis; steroids; oxidosqualene cyclase; lanosterol.

Abstract—The latex of *Euphorbia lathyris* was shown to be capable of producing lanosterol, as well as cycloartenol, butyrospermol and hopenol B, via the enzymatic cyclization of 2,3-oxidosqualene. The conversion of cycloartenol to lanosterol could not be demonstrated using the same latex. The triterpene composition of the latex of *Euphorbia lathyris* is reported.

INTRODUCTION

The biosynthesis of sterols in plants diverges from that in animals at the stage of squalene oxide (**1**) cyclization [1, 2]. While lanosterol (**2**) is the product of the oxidosqualene cyclase in animals, the plant cyclase produces cycloartenol (**3**) (Scheme 1). This difference requires an extra step in the biosynthetic pathway to phytosterols, namely the opening of the cyclopropyl ring of cycloartenol (**3**).

During our investigation of the biosynthesis of marine sterols [3, 4], it was found that sponges were able to utilize both lanosterol and cycloartenol as sterol precursors [5]. While this was surprising, other nonphotosynthetic organisms have been shown to utilize the cycloartenol pathway of plants [6]. However, further investigation using sponge cell-free extracts showed not only that sponges produce lanosterol (**2**) from the cyclization of squalene oxide (**1**), but also that sponges are capable of opening the cyclopropyl ring of cycloartenol (**3**) to give lanosterol (**2**) [7]. Dinoflagellates also possess this ability [7]. This ability is not found in animals, but it also differs from the situation in plants and algae, since in these organisms, removal of the 4β-methyl group of cycloartenol is required before cyclopropyl ring-opening can take place [8].

Only one other example of the direct conversion of cycloartenol (**3**) to lanosterol (**2**) has been reported—in the *Euphorbia* species, *E. lathyris* [9]. This plant, in common with other *Euphorbia*, contains in its latex a complex mixture of triterpenes, the study of which has been used in the taxonomic classification of the Euphorbiaceae [10]. Lanosterol (**2**), which is typically found in animals, is found among the triterpenes of some *Euphorbia* where its presence represents a scientific oddity. We report here our reinvestigation of the biosynthesis of lanosterol (**2**) in *E. lathyris*.

RESULTS AND DISCUSSION

An analysis of the triterpenes of *E. lathyris* latex using HPLC separation and ^{1}H NMR spectroscopic analysis was carried out (Table 1). The triterpenes were largely those previously identified by GC–mass spectrometry analysis [11], with the exception of euphol, which we did not detect. Lanosterol (**2**) was found, and the product of its biomethylation, 24-methylenelanosterol, was detected [12]. Hopenol B, which had not yet been identified in this species of *Euphorbia*, was also isolated [13].

Upon assaying the oxidosqualene cyclase activity of the latex with ^{3}H-labelled squalene oxide (**1**), all the triterpenes, including lanosterol (**2**), were labelled (Table 1) with the exception of those requiring subsequent SAM-dependent methylation. In order to determine whether cycloartenol (**3**) represented a precursor to lanosterol (**2**) in this plant, the latex was incubated with ^{3}H-labelled cycloartenol (**3**). No radioactivity other than starting material was detected in any HPLC fraction.

These results clearly demonstrate that lanosterol (**2**) is formed directly from squalene oxide (**1**) in *E. lathyris* latex. In contrast to an early study in plant triterpene biosynthesis [9], we were unable to detect the isomerization of cycloartenol (**3**) to lanosterol (**2**). Because the cyclopropyl ring-opening in plants and algae has since been shown to require removal of the 4β-methyl group [8], we believe the direct isomerization of cycloartenol (**3**)

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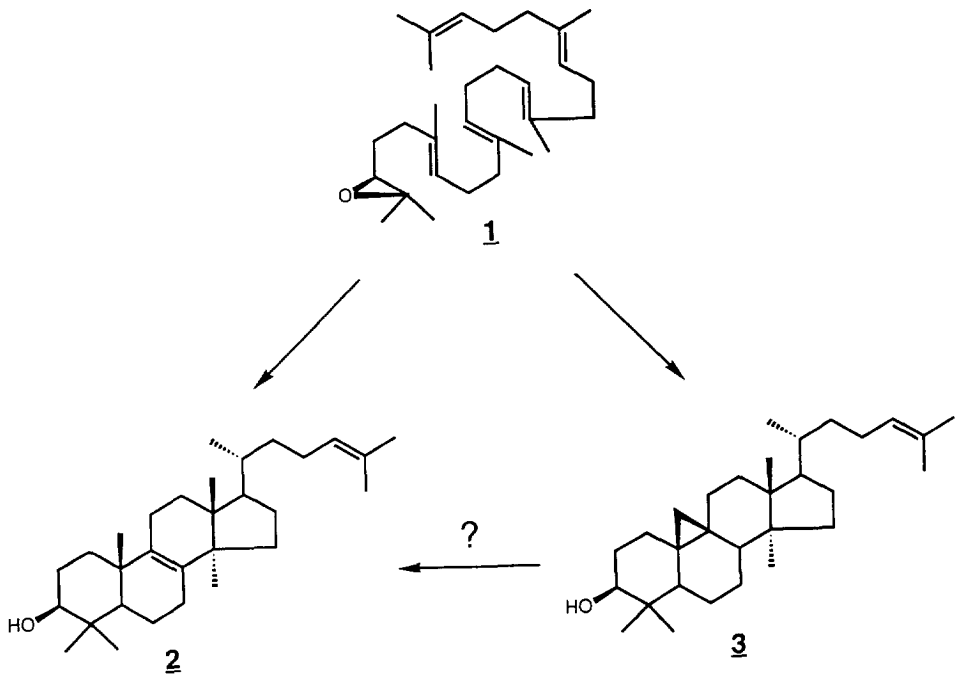

Table 1.

Triterpene	*E. lathyris* latex composition (%)	Products of ^{3}H-oxidosqualene cyclization (%)*
Lanosterol (**2**)	16	7
Butyrospermol	23	65
24-Methylenelanosterol	3	—
Cycloartenol (**3**)	10	19
24-Methylenecycloartenol	40	—
Hopenol B	8	9

*Total radiochemical yield = 0.2%.

to lanosterol (**2**) to be limited to sponges and dinoflagellates [7].

It is of interest that cyclization to lanosterol (**2**) results from the same chair–boat–chair–chair folding of squalene oxide (**1**) as does cycloartenol (**3**) [14]. In addition, a derivative of butyrospermol has been produced by a cyclase that uses the chair–chair–chair–chair conformation found in hopenol biosynthesis [15]. While it is presently unknown if the different latex triterpenes are produced by different cyclases, the mechanistic similarity of their biosyntheses suggests cyclase nonspecificity [16] as a possible cause for some of the triterpene diversity found in *Euphorbia*.

EXPERIMENTAL

Radiolabelled substrates. [24-^{3}H]-Labelled cycloartenol (**3**) (150 mCi mmol^{-1}) and [3-^{3}H]-labelled squalene oxide (**1**) (200 mCi mmol^{-1}) were prepd as previously described [17]. To solubilize the substrate, Tween 80 (2 mg) was mixed in $CHCl_3$ with substrate (20 μCi [24-^{3}H]-cycloartenol or 200 μCi [3-^{3}H]-squalene oxide). After evaporating the solvent, the mixt. was dissolved in 0.2 ml H_2O.

HPLC. A Waters Associates HPLC system (M 6000 pump, R403 differential refractometer) was used equipped with 2 Altex Ultrasphere ODS 5-μm columns (10 mm i.d. × 24 cm) in series.

Triterpene analysis. The triterpenes of an EtOAc extract of 0.5 ml latex were first isolated by silica gel TLC (hexane–Et_2O, 1:1) and then purified by reversed phase HPLC. The retention times (MeOH, 4 ml min^{-1}) were: lanosterol (**2**) 42 min; butyrospermol 43 min; 24-methylenelanosterol 45 min; cycloartenol (**3**) 46.5 min; 24-methylenecycloartenol 49 min; hopenol B 56 min. Lanosterol (**2**) and butyrospermol were further sepd using MeCN–MeOH–EtOAc (3:1:1, 4 ml min^{-1}): lanosterol (**2**) 33 min; butyrospermol 32 min. The triterpenols were identified from their ^{1}H NMR (400 MHz) spectra by comparison with authentic samples and published values [18].

Oxidosqualene cyclase assay. Freshly collected latex (0.6 ml) from young *E. lathyris* plants (30–40 cm) obtained from a commercial greenhouse was mixed on ice with 1 ml of a 100 mM Na HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid hemisodium salt) buffer (pH 7.5), 2 mM in $MgSO_4$, 1 mM in DTT (Dithiothritol), to which 170 μg PMSF (phenylmethylsulphonyl fluoride) was added in 5 μl EtOH. Oxidosqualene cyclase activity could be improved by concentrating the latex membranes by centrifugation. Thus the white gummy pellet after centrifugation (350 000 *g*, 30 min) was resuspended, on ice, with a glass-Teflon homogenizer in 100 μl of the same buffer. This latex membrane preparation (20 μl) was incubated with the [3-^{3}H]-squalene oxide (**1**)-Tween 80 suspension (10 μl, 10 μCi) at 27° for 4 hr. The mixture was extracted with 1 ml EtOAc, unlabelled triterpenes were added, the products purified by TLC (hexane–Et_2O, 1:1), and analysed by HPLC and liquid scintillation counting.

Cycloartenol (**3**)*-lanosterol* (**2**) *isomerization assay.* A modification of the above procedure was used with the substitution of [24-^{3}H]-labelled cycloartenol (**3**)-Tween 80 suspension (5 μl, 0.5 μCi) for [3-^{3}H]-squalene oxide (**1**). Only the cycloartenol fraction contained radioactivity. No radioactivity was detected in the lanosterol (**2**) fr. within the limits of detection (200 dpm).

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