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DINOSTEROL SIDE CHAIN BIOSYNTHESIS IN A MARINE DINOFLAGELLATE, *CRYPTHECODINIUM COHNII*

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Key Word Index—*Crypthecodinium cohnii*; dinoflagellate; dinosterol; methionine-[CD_3]; side chain biosynthesis.

Abstract—The heterotrophic dinoflagellate, *Crypthecodinium cohnii*, cultured in a nutrient medium containing methionine-[CD_3] incorporated deuterium into the newly synthesized 4α-monomethyl compound dinosterol (4α,23,24-trimethylcholest-22-en-3β-ol). The MS fragmentation pattern indicated that the C-23 methyl group contained three deuterium atoms and was introduced intact by transmethylation from methionine. The C-24 methyl group contained only two deuterium atoms which is consistent with the production of a 24-methylenesterol intermediate which is subsequently reduced to give the 24-methyl side chain. Mechanisms are proposed to account for the production of the dinosterol side chain.

INTRODUCTION

Studies on the mechanisms of phytosterol side chain alkylation at C-24 have been facilitated by the use of the deuterium labelled methyl group donor, methionine-[CD_3] added to the growth media and subsequent mass spectroscopic examination of newly synthesized sterols to determine their deuterium content [1, 2]. In this way the formation of the C-24 ethyl or methyl groups of the sterols produced by chrysophyte [3], bacillariophyte [4] and chlorophyte [5, 6] algae have been studied.

The structural elucidation of dinosterol (**1**), isolated from a dinoflagellate *Gonyaulax tamarensis*, as (22*E*, 24*R*)-4α-23,24-trimethyl-5α-cholestan-22-en-3β-ol [7, 8], revealed that this alga produced a C-23 methylated sterol. The only other examples of C-23 methylated sterols are the marine invertebrate sterols gorgosterol and acanthasterol isolated from soft corals [9] and an echinoderm [10], respectively. Our demonstration of the production of dinosterol (**1**) in *Crypthecodinium cohnii* [11], a heterotrophic dinoflagellate which can be cultured on a defined synthetic medium [12], has enabled us to study the mechanism of alkylation at C-23 and C-24 in this unique phytosterol.

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RESULTS AND DISCUSSION

Examination by MS of the sterols isolated from *C. cohnii* cultured in the presence of methionine-[CD_3] revealed that over 60% of the newly synthesized 4α-

Table 1. MS fragmentation ions observed for dinosterol (**1**) isolated from *Crypthecodinium cohnii* grown in the presence of methionine-[CD_3]

Fragmentation*	Parent molecular ion species			
	M^+	$M^+ + 2D$	$M^+ + 3D$	$M^+ + 5D$
M^+	428	430	431	433
$M^+ - a(43)$	385	387	388	390
$M^+ - a\text{-}H_2O$	367	369	370	372
$M^+ - b(71)$	357	357	360	360
$M^+ - b\text{-}H_2O$	339	339	342	342
$M^+ - c(111)\text{-}H$	316	316	316	316
$M^+ - d\text{-}2H$	287	287	287	287

* See Fig. 1. for manner of fragmentation.

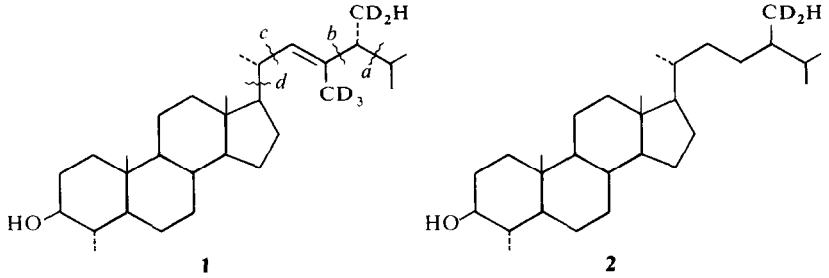

Fig. 1. Dinosterol (**1**) and 4α,24-dimethylcholestan-3β-ol (**2**) showing deuterium labelling patterns obtained from culture of *C. cohnii* in the presence of methionine-[CD_3]. The MS fragmentation of the side chain of **1** is indicated (see Table 1).

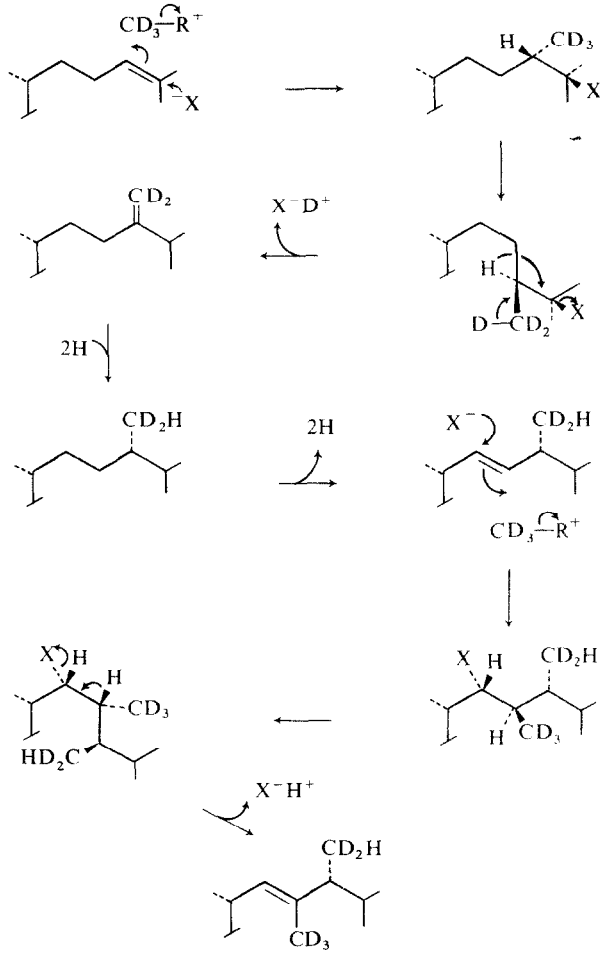

Fig. 2. Postulated mechanisms for biosynthetic side chain alkylations at C-23 and C-24 in dinosterol (**1**) production in *C. cohnii*.

monomethyl sterols contained deuterium atoms whereas no deuterium was incorporated into cholesterol or cholesta-5,7-dien-3β-ol which are the major 4-demethyl-sterols in this dinoflagellate [11].

The MS of the 4-monomethyl compound dinosterol (**1**) showed it to contain molecular species possessing two, three and five deuterium atoms (Table 1). The fragmentations at *a* and *b* (Fig. 1) produced ions containing a maximum of five and three deuteriums, respectively (Table 1), thus revealing the presence of $-CD_2H$ at C-24. This is compatible with the production of a 24-methylene intermediate in the C-24 alkylation sequence which is subsequently reduced to produce the C-24 methyl group of dinosterol (**1**). This mechanism has been reported previously to operate in fungi [1], a chrysophyte alga [3] and a diatom [4]. The fragmentations at *b* and *c* or *d* gave ions containing three and no deuterium atoms respectively which therefore demonstrated that the C-23 methyl group was derived intact by transmethylation from the methionine donor with retention of all three deuterium atoms. A second 4α-methyl-sterol from *C. cohnii*, which we have tentatively identified [11] 4α,24-dimethyl-cholestan-3β-ol (**2**), showed MS molecular ions at *m/e* 416 and 418, the retention of two deuterium atoms being in accord with a 24-methylene intermediate as concluded above for production of the C-24 methyl group of dinosterol (**1**).

A suggested biosynthetic scheme based upon the present results for the elaboration of the dinosterol (**1**) side chain is presented in Fig. 2. The alkylation steps involved in dinosterol (**1**) side chain biosynthesis may be relevant to considerations concerning the biosynthetic origins of the side chain of gorgosterol, (22*R*, 23*R*, 24*R*)-22,23-methylene-23,24-dimethylcholest-5-en-3β-ol [9], which is found in gorgonian corals containing symbiotic zooxanthellae which are apparently dinoflagellates [13].

EXPERIMENTAL

Crypthecodinium cohnii (Woods Hole Strain d) was grown in 1 l. of modified MLH medium [12] with reduced betaine (0.15 g/l) and added methionine-[CD_3] (1.5 g/l). Sterols were isolated and analysed as described previously [11]. MS were determined by direct probe at 70 eV in an AEI MS 12 instrument.

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