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BIOSYNTHETIC STUDIES OF MARINE LIPIDS 15.[1]

CONVERSION OF PARKEOL (LANOSTA-9(11),24-DIEN-3ß-OL) TO 14α-METHYLCHOLEST-9(11)-EN-3ß-OL IN THE SEA CUCUMBER *HOLOTHURIA ARENICOLA*

M. Lucinda Cordeiro, Russell G. Kerr and Carl Djerassi*

Department of Chemistry, Stanford University, Stanford, CA 94305

Abstract: Tritium labeled parkeol (2), but not lanosterol (4) or cycloartenol (3), was transformed by the sea cucumber *Holothuria arenicola* into 14α-methyl-5α-cholest-9(11)-en-3ß-ol (5), thus implying that squalene-2,3-oxide cyclizes directly to parkeol (2).

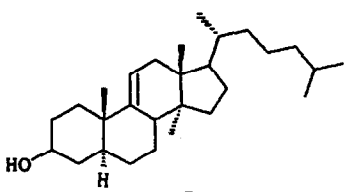
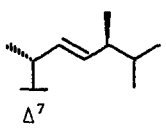
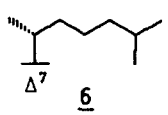
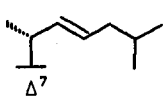
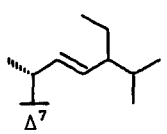
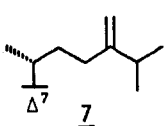
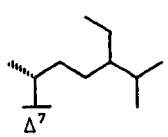
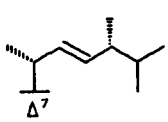
Biosynthetic work[2] in this laboratory has focused on the genesis of the unusual side chains found in sponge sterols, but recently attention has also been paid to the earlier stages of sponge sterol biosynthesis.[3] In this connection we have re-examined some earlier work on the sea cucumber *Stichopus californicus*[4] found near Stanford University's Hopkins Marine Station in Monterey Bay. In addition to conventional Δ^7-sterols (cf. Table 1), we isolated from the free sterol fraction (without prior saponification or acid hydrolysis) the biosynthetically intriguing 14α-methylcholest-9(11)-en-3ß-ol (5).[5] This same sterol, along with conventional Δ^7-sterols (see table 1), was also isolated from several other sea cucumbers including the Caribbean *Holothuria arenicola*, with which this biosynthetic study is concerned.

There are few other instances of the occurrence of the rare $\Delta^{9(11)}$-14α-methyl nuclear substitution pattern in nature: 14α-methyl-5α-ergosta-9(11),24(28)-dien-3ß-ol from a member of the Cucurbitaceae;[7a] 4α,14α-dimethyl-5α-cholest-9(11)-en-3ß-ol from a member of the Solanaceae;[7b] and 14α-methyl-5α-cholest-9(11)-en-3ß-ol (5) from the sea cucumbers *Psolus phantapus*[7c] and *Psolus fabricii*.[7d] Goad *et al.*[7d] speculated that this latter sterol (5) may arise by cyclization of squalene-2,3-oxide to an intermediate cation 1, which subsequently gives rise to both lanosterol (3) and parkeol (2). They further suggest that 3 is the immediate precursor of the major sterol lathosterol (6), which possesses the conventional sterol skeleton (without any methyl groups at C-4 and C-14), whereas parkeol (2) is metabolized via loss of the two C-4 methyl groups to the 14-methylated sterol (5). In the latter case, loss of the C-14 methyl group presumably does not occur due to lack of activation by an 8,9 double bond.[8] By contrast, Akhila *et al.*[9] suggested that 5α-stigmast-9(11)-en-3ß-ol, a $\Delta^{9(11)}$ sterol lacking the 14α-methyl group arises by cyclization of squalene oxide to parkeol (2), possibly via prior formation of cycloartenol (4).

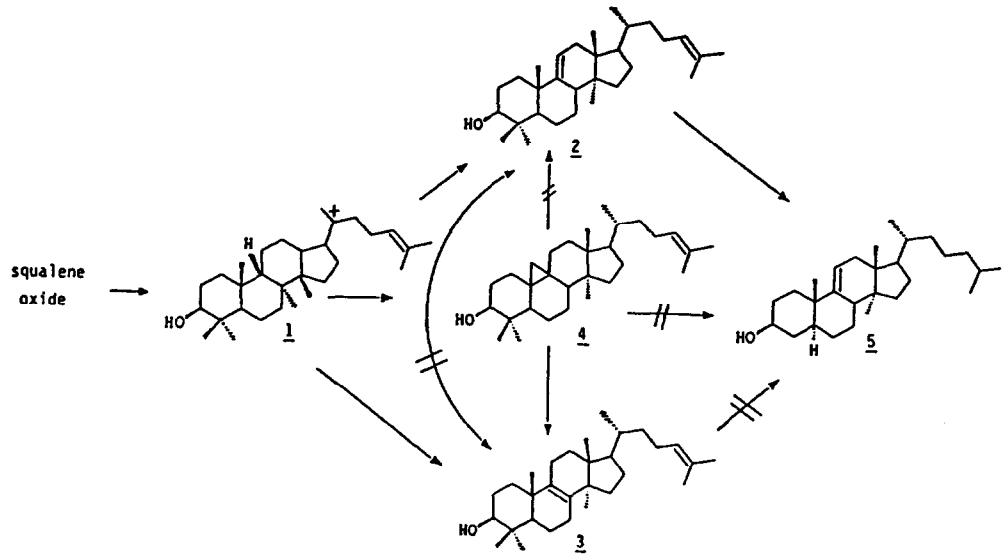
In order to distinguish between these various metabolic possibilities, we fed [24-^{3}H]-labeled[10] lanosterol (3), cycloartenol (4) and parkeol (2) to *Holothuria arenicola*,[10] and examined the incorporation of radioactivity among its sterols (Table 1). In each instance,

approximately 20 μCi (44 x 10^6 dpm) of precursor in 1 mL of 70% ethanol was administered to the Holothurian by injection into the coelemic cavity and the animal maintained for 7 days in an aquarium in circulating seawater with aeration before sacrifice. The sterols were purified by normal phase HPLC (6% ethyl acetate in hexanes) followed by reverse phase HPLC using methanol and then 30 mM $AgNO_3$ in 97% methanol. The results were quite unambiguous. No radioactivity was found in any of the eight sterols listed in Table 1 when labeled lanosterol (3) or cycloartenol (4) were fed, although 7.7 x 10^5 dpm and 6.2 x 10^5 dpm were noted in the lanosterol and cycloartenol fractions, respectively, showing that the precursors were taken up by the sea cucumber. When parkeol (2) was administered, significant radioactivity (45,500 dpm after repeated purification to constant radioactivity using cold 5 as carrier during HPLC) was encountered in 14α-methyl-5α-cholest-9(11)-en-3ß-ol (5), while all the other seven sterols (Table 1) were cold. We conclude that (a) the $\Delta^{9(11)}$-14α-methyl sterol (5) is the only *bona fide* sterol metabolite in the sea cucumber and (b) that the remaining Δ^7-14α-desmethyl sterols (e.g. 6 or 7) are dietary constituents or late metabolites (e.g. side chain alkylation) of a dietary precursor which already possesses a standard 4,4,14-desmethyl sterol skeleton.

TABLE 1. FREE STEROLS IN *HOLOTHURIA ARENICOLA*

Sterol[a]	Relative Abundance (%)	Sterol[a]	Relative Abundance (%)
 5	7.8	 Δ^7	6.4
		 Δ^7 6	22.5
 Δ^7	3.8	 Δ^7	14.9
 Δ^7 7	24.1	 Δ^7	12.4
 Δ^7	6.8	unidentified trace sterols	1.3

[a] "Δ^7" denotes the 5α-androst-7-en-3ß-ol nucleus.



Scheme 1

In photosynthetic organisms, squalene oxide is known to cyclize to cycloartenol (4),[8] while in non-photosynthetic organisms, lanosterol (3)[8] is the primary isolable cyclization product. Scheme 1 depicts the three possible products of rearrangement of cation 1, derived from squalene oxide, and their modes of interconversion. Cycloartenol (4) can be metabolized to lanosterol (3) in some photosynthetic organisms[11] and thus conceivably to parkeol (2). In addition, parkeol could arise from the isomerization of lanosterol (3). Our incorporation results definitely exclude these possibilities in the sea cucumber, since radioactive 3 and 4 were not metabolized further.

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