Bioconversion of Lanosterol into Holotoxinogenin, a Triterpenoid from the Sea Cucumber *Stichopus Californicus*

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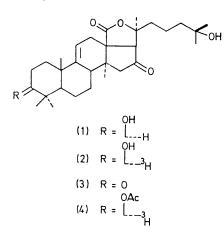
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Summary Labelled lanosterol is converted 200 times more efficiently than labelled acetate into holotoxinogenin when administered to the sea cucumber Stichopus californicus.

SEA cucumbers (Holothurians) of the phylum echinodermata possess toxic triterpenoid saponins¹ but there is conflicting evidence whether the echinoderms in general and holothurians² in particular are capable of *de novo* synthesis of sterols and triterpenoids (trimethylsteroids) from small precursors (e.g., acetate or mevalonate). We now report that the genin derived from *Stichopus californicus* (collected in Monterey Bay) is identical with holotoxinogenin³ (1) (identical with stichopogenin A_4 derived⁴ from *S. japonicus*) and that it can be biosynthesized *de novo* from acetate and [3-³H]lanosterol (5).

An aqueous solution of tritiated potassium acetate $(25 \,\mu\text{Ci})$ was injected directly into the abdominal cavity of two sea cucumbers (250–300 g each). [3-³H]Lanosterol (5) prepared by reduction of lanostenone (6) with LiAl³H₄

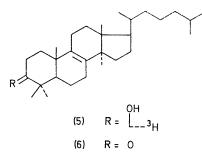
 $(25 \,\mu\text{Ci})$ was dissolved in 2 ml each of 95% ethanol and neat Tween 60 and was injected into the abdominal cavity of two sea cucumbers. The incubations were conducted in sea water (300 ml) at 10 °C for 72 h with filtered air being bubbled through the incubation medium in the presence of



solid calcium carbonate (20 mg). The incubation was stopped by pouring the animal and sea water mixture into 95% ethanol and the partially purified (t.l.c.) genin was hydrolysed with 2N HCl. Purification by t.l.c. and column chromatography with benzene-acetone (9:1) gave holotoxinogenin (R_f 0.43 vs. 0.67 for lanosterol) which was crystallised twice from hexane-benzene and identified by comparison of n.m.r., c.d., i.r., and mass spectral properties with those of an authentic sample.³

The 3-position of tritium in genin (2) derived from the $[3-^{3}H]$ lanosterol incubation could be established by oxidation to a non-radioactive 3-keto-derivative (3) or by conversion into a radioactive monoacetate (4) which in turn

could be hydrolysed, with retention of tritium, to (2). It is clear from our results (Table) that *Stichopus californicus* can biosynthesize triterpenoid genins both from acetate[†] and



lanosterol. Since the conversion efficiency of labelled lanosterol (5) into holotoxinogenin (2) is 200 times higher than that of the acetate, it is likely that in nature holotoxin originates in part from dietary lanosterol or a closely related precursor derived from lanosterol. This is the first experi-

TABLE.	Incorporation of C ³ H ₃ CO ₂ K and [3α- ³ H	I]lanosterol in sea
	cucumber Stichopus californicus	
	Incorporation /0/	Dadiogativity/

	Incorporation/%		Radioactivity/
Substrate	(Total genin)	Product	$d.p.m./mg^{-1}$
C ³ H ₈ CO ₂ K	0.08	(2)	$7.02 imes 10^{5}$
[3α- ³ H]Lanosterol	18.00	(2)	$1.97 imes 10^5$
		(4) (from 2)	$1.80 imes10^{5}$
		(2) (from 4)	$1.90 imes10^{5}$

mental demonstration that the highly functionalized triterpenoid (trimethylsteroid) glycosides of sea cucumbers are biosynthesized from lanosterol.

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 \dagger Similar low level incorporation of ¹⁴C labelled acetate into stichopogenin A₄ (1) by Stichopus japonicus has been reported (ref. 2b).

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