STEROL COMPOSITION OF *CIONA INTESTINALIS*[1]

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In the course of isolation of sperm attractant(s) responsible for sperm chemotaxis in *Ciona intestinalis* (1) we also isolated the sterols and determined their structure and composition, the analytical tool being a high resolution-glass capillary-gas chromatography mass spectrometry-computer system. Since tunicates, which are considered to be chordate progenitors (2), occupy an interesting position from a phylogenetic point of view, their sterol composition may be useful as a parameter for chemotaxonomic classification (3).

Hence, we have examined the sterols of *Ciona intestinalis*, and their structures and composition are reported in table 1. The major sterols isolated were cholest-5-en-3β-ol (cholesterol), 38.0%; 5α-cholestan-3β-ol, 16.9% and 24ξ-methylcholest-5,22E-dien-3β-ol, 13.8%. It is interesting to note that this sterol composition is quite different from the eleven sterols identified from *C. intestinalis* collected at Naples, Italy, (4) (see table 1). This may be because different extraction procedures were used and because

TABLE 1. *Sterol composition of* Ciona intestinalis (%).

Sterol	California, USA[a]	Naples, Italy[b]
C-26 Sterols:		
24-Dimethylchol-5,22-dien-3β-ol	1.4	1.1
24-Dimethyl-5α-chol-22-en-3β-ol	0.2	
C-27 Sterols:		
Cholest-5-en-3β-ol	38.0	68.7
5α-Cholestan-3β-ol	16.9	
Cholesta-5,22E-dien-3β-ol	3.5	3.9
5α-Cholest-7-en-3β-ol	3.0	1.0
5α-Cholest-22E-en-3β-ol	0.6	
Cholesta-5,22Z-dien-3β-ol	0.5	1.4
C-28 Sterols:		
24ξ-Methylcholest-5,22E-dien-3β-ol	13.8	5.3
24-Methylcholesta-5,24(28)-dien-3β-ol	9.2	
24ξ-Methylcholest-5-en-3β-ol	6.2	7.3
24ξ-Methyl-5α-cholestan-3β-ol	1.6	
24-Methyl-5α-cholest-24(28)-en-3β-ol	0.2	
C-29 Sterols:		
24ξ-Ethylcholest-5,22E-dien-3β-ol	1.9	6.3
24ξ-Ethylcholest-5-en-3β-ol	1.7	3.9
24-Ethylcholesta-5,24(28)E-dien-3β-ol	0.3	0.9
24-Ethylcholesta-5,24(28)Z-dien-3β-ol	0.7	
Unknown	0.3	

[a]Average composition of four measurements.
[b]Reference 4.

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more than 1,000 animals were extracted in the current work, whereas only 69 were extracted by Voogt *et al.* (4). Although cholesterol is the major sterol in both cases, 24-methylcholest-5-en-3β-ol and 24-ethylcholesta-5,22-dien-3β-ol are present in the next highest percentages among the eleven sterols in the Italian species. Ten of the eleven sterols found in the Italian species have also been shown to occur in the U.S. species. We are in agreement with Voogt *et al.* (4) and Yasuda (5) that the sterol composition of these lower invertebrates is very complex. This complexity may be due to the diet (5) of these organisms in different localities in combination with their ability to synthesize some sterols (4).

EXPERIMENTAL[2]

EXTRACTION.—Mature *Ciona intestinalis* was collected in the summer of 1976 in Newport Bay near Corona Del Mar, California. The animals (>1000) were freed manually from adhering contaminants and the tunicates cut open. They were then extracted with 95% ethanol until a colorless extract was obtained. Evaporation under reduced pressure afforded a residue (154 g) which was taken up in 600 ml H_2O and extracted with ether (5 x 300 ml). Concentration of the ether extract yielded an orange oily mass (3.5 g).

The ether-soluble lipid was taken up in ether (20 ml) and chromatographed on a column of silica gel (2.0 x 70 cm; 120 g). Elution with hexane followed by increasing amounts of ether in hexane (5, 10 and 15%) furnished sterols in the last eluate. The crude mixture was recrystallized from methanol-ether (3:1), m.p. 130–132°.

The sterols were converted to their respective acetate derivatives with pyridine and acetic anhydride (Supelco, Inc., Bellfonte, PA) and analyzed on an SE-54 (20 m x 0.32 mm i.d.) glass capillary column in a Carlo Erba Fractovap 2150 gas chromatograph. The injector and detector were operated at 275°C; the derivatized samples (1–2 μl) were injected splitless at 150°C, heated to 250° at 4°/min. and held isothermally for 30 min.

Structural assignments were based on comparison of high resolution glass capillary gas chromatographic retention times with authentic standards and on comparison of mass spectra with authentic standards and published mass spectra (6). The gas chromatograph used for GC-MS work was a Varian Aerograph 1400 equipped with an SE-52 glass capillary column (17 m x 0.32 mm i.d.). The temperature program was the same as described earlier for the Carlo Erba instrument. The gas chromatograph was interfaced by glass capillary tubing to a Finningan 1015C quadrupole mass spectrometer. The mass spectrometer was run in the electron impact mode; the ionization potential was 70 eV. The data were collected and edited by a DEC PDP8-E (16 K core) computer equipped with a System Industries System 150 data system, Diablo Series 30 disc drive and Tektronix 4010 CRT display unit and 4610 copier.

[2]Column chromatography was carried out on Biosil-A 200-300 mesh (Bio-Rad Laboratories, Rockville Center, New York). Thin layer chromatography (tlc) was performed on precoated silica gel GF plates (Anal. Tech., Inc., Newark, Delaware) using as a developing solvent hexane-ether (14:1).

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