4 Mitoma, C., and Smith, T.E., J. biol. Chem. *235* (1960) 426.
5 Gould, B.S., Int. Rev. Cytol. *15* (1963) 301.
6 Bourne, G.H., J. Physiol. Lond. *101* (1942) 327.
7 McLean, D.L., Sheppard, M., and McHenry, E.M., Br. J. exp. Path. *20* (1939) 451.
8 Dalldorg, G., J. Am. med. Assoc. *111* (1938) 1376.
9 Jowsey, J., Curr. Concepts Nutr. *4* (1976) 131.
10 Ringborn, A., in: Complexation in Analytical Chemistry, pp. 42, 63, 320. Interscience Publishers, New York 1963.
11 Handbook of Chemistry and Physics. The Chemistry Rubber Publishing Co., Cleveland, USA, 1961.
12 Craggs, A., Moody, G.J., and Thomas, J.D.R., Analyst *104* (1979) 961.
13 Instruction Manual, Calcium Ion Electrode Model 93-20. Orion Research Inc., Cambridge, MA, 1979.
14 Seamonds, B., Towfighi, J., and Arvan, D.A., Clin. Chem. *18* (1972) 155.
15 Tolbert, B.M., Downing, M., Carlson, R.W., Knight, M.K., and Baker, E.M., Ann. N.Y. Acad. Sci. *258* (1975) 48.
16 Pachla, L.A., and Kissinger, P.T., Meth. Enzymol. *62* (1979) 15.
17 Davies, C.W., and Hoyle, B.E., J. chem. Soc. *1951*, 233.
18 Free, H.M., and Free, A.H., Urinalysis in Clinical Laboratory Practice. CRC Press, 1976.
19 Handbook of Clinical Laboratory Data, 2nd ed., p. 6. Ed. R.C. Weast. The Chemical Rubber Co., Cleveland, OH 1968.

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The sterols of two hadromerida sponges[1]

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Summary. Sterols were extracted and identified from 2 marine sponges, *Aaptos aaptos* and *Suberites domuncula.* The sponges contained conventional C_{26}–C_{30} sterols with a saturated ring system. Minor amounts of cholest-7-en-3β-ol and cholesterol were also present. Cholestanol and 24-ethylcholestanol were the major components of the sterol mixtures.

Sponges are rich sources of complex mixtures of sterols and the components of the mixtures seem to be of dietary origin and products of chemical transformation subsequent to ingestion[2-4]. Stanols are found in sponges belonging to the family Suberitidae[5,6], Chondrosiidae[7], Stellettidae[8], Desmacidonidae[9], Hymeniacidonidae[10,11], Halichondriidae[11], Nepheliosphongiidae[12], Axinellidae[13,14] and Geodidae[15]. We report here the sterol composition of 2 marine sponges *Suberites domuncula* and *Aaptos aaptos* as a part of our work on the sterols from sponges[16].

Materials and methods. Sponges *Aaptos aaptos* (order Hadromerida, family Tethyidae) and *Suberites domuncula* (order Hadromerida, family Suberitidae) were collected in the Bay of Naples and supplied by the Zoological Station of Naples. Each sponge was extracted (3 times) with acetone for 3 days. Solvent was removed and the resulting suspension extracted with diethyl ether. After evaporation, the oily residue was chromatographed on a silica gel column using CH_2Cl_2 as eluent. The crude sterol fraction, after acetylation with Ac_2O-pyridine (1:1), was further purified by SiO_2 column using as eluent 40–70° light petroleum-C_6H_6 (7:3). Steryl acetates were separated by column chromatography on silica gel impregnated with $AgNO_3$ which was eluted with light petroleum containing increasing amounts of C_6H_6. The various column fractions were monitored by GLC and combined accordingly. Some fractions were further subjected to preparative silver nitrate-silica gel TLC. Each fraction was analyzed by capillary GLC, GC-MS, NMR and coinjection with previously identified sterols.

Combined GC-MS analysis was performed on a LKB 2091 S GC-MS instrument. An SE-30 (30 m) fused silica capillary column (J & W Scientific) programmed from 200 to 260 °C (6 °C/min) was used. Analytical GLC of steryl acetates was performed with a C. Erba Fractovap 4160 gas chromatograph using a DB-1 glass capillary column (30 m; J & W Scientific) at 270 °C. ^{1}H-NMR spectra were recorded with a Brucker WH-270 in $CDCl_3$ and TMS as internal reference.

Results and discussion. The table lists the sterols isolated

Sterol composition of sponges (%)

Sterol	RRT*	*S. domuncula*	*A. aaptos*
(22E)-24-nor-5α-Cholest-22-en-3β-ol	0.70	1.1	0.1
(22E)-27-nor-24-Methyl-5α-cholest-22-en-3β-ol	0.90	1.2	0.3
(22E)-5α-Cholest-22-en-3β-ol	0.94	4	0.9
5α-Cholestan-3β-ol	1.03	72.9	66.4
24-Methyl-5α-cholestan-3β-ol	1.31	2.3	2.1
(22E)-24-Methyl-5α-cholest-22-en-3β-ol	1.13	4	2
5α-Ergost-24(28)-en-3β-ol	1.26	3	0.3
24-Ethyl-5α-cholestan-3β-ol	1.59	6	25
(22E)-24-Ethyl-5α-cholest-22-en-3β-ol	1.41	0.8	0.4
(24E)-5α-Stigmast-24(28)-en-3β-ol	1.58	0.8	0.7
(24Z)-5α-Stigmast-24(28)-en-3β-ol	1.63	0.5	0.7
(24E)-24-Propylcholest-24(28)-en-3β-ol	1.78	0.1	0.1
(24Z)-24-Propylcholest-24(28)-en-3β-ol	1.88	0.3	0.2
5α-Cholest-7-en-3β-ol	1.12	2.5	0.6
Cholest-5-en-3β-ol (cholesterol)	1.00	0.4	0.1

* RRT, retention time of acetate derivatives relative to cholesteryl acetate.

from the sponges *A. aaptos* and *S. domuncula,* together with the relative retention times (RRT) in gas chromatograph and the percentage of the sterol components. From these results it may be seen that the sponges *A. aaptos* and *S. domuncula,* classified in the order Hadromerida[17], have rather similar sterol composition. They contain stanols widely distributed in the marine environment, cholestanol and 24-ethylcholestanol being the principal sterols present. Minor amounts of cholest-7-en-3β-ol and cholesterol were also present. Nuclear saturated sterols predominate in other species of Hadromerida order[5-7], and their origin is difficult to determine. It was pointed out that they may be arise from dietary Δ^5sterols[3].

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2 Goad, L.J., in: Marine Natural Products, vol. 2, p. 75. Ed. P.J. Scheuer. Academic Press, New York 1978.
3 Goad, L.J., Pure appl. Chem. *51* (1981) 837.
4 Djerassi, C., Pure appl. Chem. *53* (1981) 873.
5 Delseth, C., Tolela, L., Scheuer, P.J., Wells, R.J., and Djerassi, C., Helv. chim. Acta *62* (1979) 101.
6 Sica, D., De Simone, F., Senatore, F., and Zollo, F. Gazz. chim. ital. *110* (1980) 147.
7 Sica, D., De Simone, F., and Zollo, F., Gazz. Chim. ital. *108* (1978) 575.
8 Sheikh, Y.M., and Djerassi, C., Tetrahedron *30* (1974) 4095.
9 Kanazawa, A., Teshima, S., and Hyodo, S., Comp. Biochem. Physiol. *62B* (1979) 521.
10 Erdmann, T.R., and Thomson, R.H., Tetrahedron *28* (1972) 5163.
11 Sica, D., De Simone, F., Ramundo, E., and Zollo, F., Biochem. Syst. Ecol. *6* (1978) 77.
12 Khalil, M.W., Djerassi, C., and Sica, D., Steroids *35* (1980) 707.
13 Voogt, P.A., Neth. J. Zool *26* (1976) 84.
14 Dini, A., Minale, L., Pizza, C., Riccio, R., and Zollo, F., Comp. Biochem. Physiol. *71B* (1982) 285.
15 Ballantine, I.A., Lavis, A., and Morris, R.J., Comp. Biochem. Physiol. *63B* (1979) 119.
16 Di Giacomo, G., Dini, A., Falco, B., Marino, A., and Sica, D. Comp. Biochem. Physiol. *74B* (1983) 499.
17 Bergquist, P.R., Sponges. Hutchinson, London 1978.

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Induction of heart alterations by immunization with subcellular fractions from *Crithidia fasciculata*

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Summary. Immunization of mice with subcellular fractions of *C. fasciculata* led to myocarditis and electrocardiographic alterations similar to those induced by immunization with *T. cruzi,* the etiological agent of Chagas' disease, suggesting the presence of similar cardiotoxic antigens in both trypanosomatid flagellates.

It has been postulated that immunological mechanisms participate in the pathogenesis of tissue damage in Chagas' disease[1]. This opinion is partly supported by the fact that in the mouse[2] and rabbit[3] heart lesions, resembling those appearing in human Chagas' disease, can be induced by immunization with subcellular fractions of *Trypanosoma cruzi,* the etiological agent of South American Trypanosomiasis. In order to determine whether this ability is shared by other trypanosomatid flagellates, experiments were performed in which mice were immunized with subcellular fractions obtained from *Crithidia fasciculata,* an insect parasite not infectious for mammals.

For comparative purposes, *C. fasciculata* was grown in a biphasic culture medium developed for *T. cruzi*[4]. When cultures reached the late logarithmic phase, the cells were harvested by centrifugation at 5000 × g for 15 min at 4 °C, and washed twice in 5 mM KCl 0.25 M sucrose (SKS). The parasites were disrupted by compression-decompression in a Sorvall Ribi Cell Fractionator and the homogenate was fractionated by differential centrifugation, following the experimental protocol developed for *T. cruzi* epimastigotes[5]. The pellets obtained at 5000 × g and 105,000 × g, resus-

Histopathological and electrocardiographic results obtained from mice immunized 3 months before with subcellular fractions of *Crithidia fasciculata*

Immunogen	Number of pathologic electrocardiographs/ total number of mice	Number of mice with myocarditis/total number of mice studied
5,000 × g pellet	1/12	5/11
105,000 × g pellet	7/15	6/15
Cytosol	4/11	3/11
SKS	0/20	0/20
Serum albumin	2/10	0/10
Culture medium	2/13	0/13

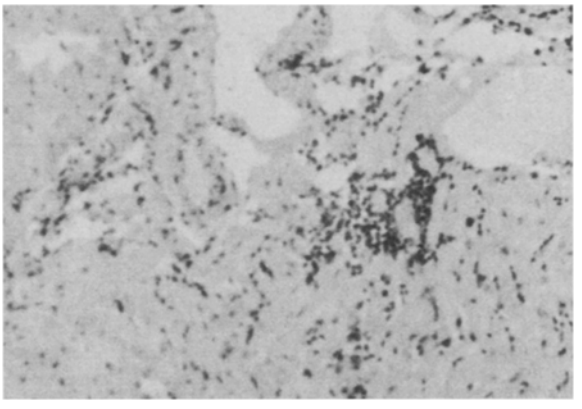

Figure 1. Low power view of atria showing a nodular mononuclear infiltrate in the interstitium. H and E, × 125.