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# Free Sterols of the Sea Urchin *Echinometra lucunter*

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The sterol components of the Colombian sea urchin *Echinometra lucunter* were fractionated by high performance liquid chromatography (HPLC) and analyzed by gas chromatography–mass spectrometry (GC–MS) and 360 MHz proton magnetic resonance ($^1$H-NMR) spectroscopy. The sea urchin contains mainly $\Delta^5$-$C_{27}$ sterols with cholesterol as the main constituent. Various $\Delta^5$-$C_{26}$, -$C_{28}$, and -$C_{29}$ sterols were also found. This is the first report of the presence of 24ξ-27-nor-24-methylcholesta-5,22-dien-3β-ol (**2**), (22*E*,24*S*)-24-methylcholesta-5,22-dien-3β-ol (**6**), 24-methylcholesta-5,24-dien-3β-ol (**7**) and 5α-cholestan-3β-ol (**13**) in sea urchin.

**Keywords**——marine sterol; sterol; sea urchin; Echinoid

Marine invertebrates contain a very complex mixture of sterols, often including sterols with unusual alkyl side chains and/or unconventional steroid rings.[1,2] Although the marine sterol field is perhaps the oldest and best studied aspect of marine natural products chemistry, there still remains an incredible number of species which have never been studied. In the class Echinoid, which comprises approximately 850 species, only 1.2% has been analyzed for sterol components.[3] The species studied contained predominantly $\Delta^5$ sterols such as cholesterol, desmosterol, 22-dehydrocholesterol and some C-24 alkylated sterols.[4–6] However, the absolute stereochemistry at C-24 of those alkylated sterols was not determined.

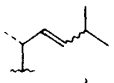
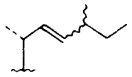
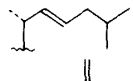
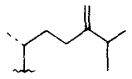
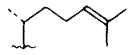
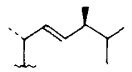
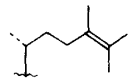
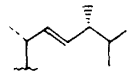
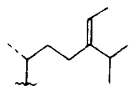
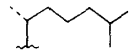
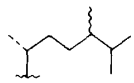
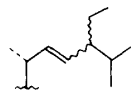
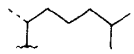
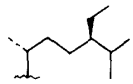
In view of our interest in possible missing links in the study of sterol biosynthesis and as a first stage in a sequential study of sterols from marine invertebrates collected off Colombian coasts we have examined the free sterol fraction of the sea urchin *Echinometra lucunter*.

The sterol fraction was obtained from the acetone extract of the animal gonads and was purified by column chromatography and thin layer chromatography (TLC) on silica gel. Reversed phase high performance liquid chromatography (HPLC) yielded at least 14 sterols (Table I). The identification of these compounds was based on relative gas liquid chromatography (GLC) and HPLC retention times and on a careful analysis of the gas chromatography–mass spectrometry (GC–MS) spectral data. High resolution proton magnetic resonance ($^1$H-NMR, 360 MHz) was also used for structural assignment of those sterols isolated in sufficient amount.

One sterol having 26 carbon atoms was characterized as 24-norcholesta-5,22-dien-3β-ol (**1**). This sterol, whose biosynthetic origin is still unknown, seems to be widely distributed in the marine environment.[2]

The $C_{27}$-sterols comprised about 75% of the sterol mixture with cholesterol (**10**) as the major sterol. 24ξ-27-Nor-24-methylcholesta-5,22-dien-3β-ol (**2**), (22*E*)-cholesta-5,22-dien-3β-ol (**3**) and cholesta-5,24-dien-3β-ol (**5**) accounted for only 5.8% of the total sterols. The stereochemistry of the $\Delta^{22}$ double bond of **3** was determined by inspection of its $^1$H-NMR spectrum. The C-21 methyl group signal appears at 1.00—1.01 ppm when it occurs together with a $\Delta^{22}$-*trans* double bond, whereas a *cis* double bond shifts it to 0.94—0.95 ppm.[7,8] Our value of 1.007 ppm clearly showed a *trans* stereochemistry in compound **3**. The $\Delta^{22}$ stereochemistry of **1** and **2** has so far remained undetermined. 5α-Cholestan-3β-ol (**13**) was the sole sterol with a 5,6-dihydro structure present in *Echinometra lucunter*.

TABLE I. Sterlos from the Gonads of the Sea Urchin *Echinometra lucunter*

| Entry | Compound[a] | Mobility LC[b] | Mobility GC[b] | M⁺ *m/z* | % sterol fraction |
|---|---|---|---|---|---|
| **1** |  | 0.62 | 0.65 | 370 | 1.8 |
| **2** |  | 0.72 | 0.90 | 384 | 0.7 |
| **3** |  | 0.78 | 0.94 | 384 | 5.0 |
| **4** |  | 0.82 | 1.39 | 398 | 3.1 |
| **5** |  | 0.85 | 1.23 | 384 | 0.1 |
| **6** |  | 0.85 | 1.17 | 398 | 3.1 |
| **7** |  | 0.85 | 1.39 | 398 | 0.4 |
| **8** |  | 0.89 | 1.18 | 398 | 2.9 |
| **9** |  | 0.93 | 1.84 | 412 | 0.6 |
| **10** |  | 1.00 | 1.00 | 386 | 69.5 |
| **11** |  | 1.06 | 1.31 | 400 | 0.3 |
| **12** |  | 1.06 | 1.43 | 412 | 1.2 |
| **13** |  | 1.10 | 1.00 | 388 | 0.7 |
| **14** |  | 1.14 | 1.62 | 414 | 3.0 |

*a*) All the sterols have a $\Delta^5$ nucleus except for **13** which has a 5α-nucleus.
*b*) Retention time relative to cholesterol.

The presently identified $C_{28}$ sterols are: 24-methylene-cholest-5-en-3β-ol (**4**), (22*E*, 24*R*)-24-methylcholesta-5,22-dien-3β-ol (**8**), 24ξ-24-methylcholest-5-en-3β-ol (**11**), (22*E*, 24*S*)-24-methylcholesta-5,22-dien-3β-ol (**6**) and 24-methylcholesta-5,24-dien-3β-ol (**7**). As noted by Djerassi and coworkers,[9] the epimers **6** and **8** could be separated by reverse phase HPLC. The stereochemistry at the $\Delta^{22}$ double bond and at C-24 were determined by analysis of the $^1$H-NMR spectra.[7-9] The $\Delta^{22}$ *trans* bond in both compounds was ascertained by inspection of the chemical shift of the C-21 methyl signal as described above for the sterol **3**. By comparison of their spectra with the 360 MHz $^1$H-NMR spectra of authentic 24*R*- and 24*S*-methylcholesta-5,22-dien-3β-ol and with the $^1$H-NMR data for the $\Delta^{22}$-24-alkylated steroids, which always show a slight up-field shift for C-21 when the C-24 substituent is in the β position,[8,9] the sterols **6** and **8** were determined to be the 24*S* and 24*R* isomer, respectively.

Three $C_{29}$ sterols were detected: (24*Z*)-24-ethylcholesta-5,24 (28)-dien-3β-ol (**9**), 24ξ-24-ethylcholesta-5,22-dien-3β-ol (**12**) and (24*R*)-24-ethylcholest-5-en-3β-ol (sitosterol) (**14**). The 24*Z* isomer **9** (isofucosterol) can be effectively separated from the corresponding 24*E* isomer (fucosterol) by reverse phase HPLC[9] and GC with a glass capillary column.[9,10] In the sterol

**14**, the stereochemistry at C-24 was again deduced by comparing its $^1$H-NMR data with those of 24-ethyl sterols.[8] The major diagnostic feature to determine the *R* or *S* configuration at C-24 is the chemical shift of the C-29 methyl group, which is deshielded by 0.01 ppm in the 24*S* isomer. Our value of 0.844 ppm indicated the *R* stereochemistry at C-24 for compound **14**.

The sterol mixture of *Echinometra lucunter* described above showed considerable similarity to those obtained from other species of sea urichin.[4-6] However, in the present work the sterols **2**, **6**, **7**, and **13** were detected for the first time in sea urchins. According to published results[5] the major sterol **10** of sea urchin may arise by "*de novo*" biosynthesis. The other $C_{27}$ sterols **3**, **5** and **13** may be either precursors or metabolites of **10**, whereas the $C_{28}$ and $C_{29}$ sterols seem to be of dietary origin.[11] The coexistence of the $C_{26}$ sterol **1**, $C_{27}$ sterol **2** and $C_{28}$ sterol **6** seems to support the postulation[12] that occelasterol [(22*E*, 24*S*)-27-nor-24-methylcholesta-5,22-dien-3$\beta$-ol] is produced by demethylation of the $C_{28}$ sterol **6** and serves as the precursor of (22*E*)-24-norcholesta-5,22-dien-3$\beta$-ol.

## Experimental

**General Methods**——Analytical GLC was carried out on a Hewlett-Packard 5700A chromatograph using 1% OV-17 on GCQ in a 4 m × 4 mm i.d. glass column with a flame ionization detector. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min, and the oven temperature was 270°C.

Preparative HPLC was performed on a Whatman Partisil M9 10/50 ODS-2 column using a Waters Associates pump and dual cell refractometer with methanol as the mobile phase (flow rate 2 ml/min).

Combined GC/MS was carried out on a Ribermag R-10-10B spectrometer equipped with fused glass capillary column (28 m × 0.35 mm i.d.) containing SE-54 as the stationary phase.

$^1$H-NMR spectra were recorded in deuteriochloroform solution on a Bruker HXS-360 (360 MHz) spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). Column chromatography was carried out with Merck Kieselgel 60 and thin layer chromatography was done on Merck precoated Kieselgel 60 $F_{254}$ plates (0.25 mm thick).

**Isolation and Separation of the Sterol Mixture**——The animals were collected in the shallow waters of Santa Marta Bay, Columbia and identified as *Echinometra lucunter* by Dr. B. Werding of El Instituto de Investigaciones Marinas de Punta Betin. The gonads were taken from the whole body of the sea urchin and dried at 60°C. The dried tissues (2.6 g) were then transferred to a Soxhlet apparatus and continuously extracted with acetone for 36 h according to a described procedure.[13] The extract was concentrated under reduced pressure and the residue (398 mg) was subjected to $SiO_2$ column chromatography and preparative thin layer chromatography using benzene: ethyl acetate (5: 1) as the eluent to afford 30 mg of the free sterol mixture. The sterols thus obtained were analyzed by GLC. Euriched fractions for GC–MS analysis and pure compounds were obtained by subjecting the sterol mixture to preparative HPLC.

24-Nor-cholesta-5,22-dien-3$\beta$-ol (**1**): MS *m/z*: 370 (50%, $M^+$), 355 (8), 352 (5), 337 (11), 300 (28), 285 (17), 282 (8), 273 (14), 271 (30), 259 (6), 255 (25), 231 (6), 213 (14) and 55 (100).

24$\xi$-27-Nor-24-methylcholesta-5,22-dien-3$\beta$-ol (**2**): MS *m/z*: 384 (33%, $M^+$), 369 (6), 366 (7), 351 (8), 300 (31), 299 (9), 273 (14), 271 (26), 255 (34), 231 (4), 229 (7), 213 (14) 384 and 55 (100).

(22*E*)-Cholesta-5,22-dien-3$\beta$-ol (**3**): $^1$H-NMR $\delta$: 0.692 (3H, s, 18-Me), 0.857 (3H, d, $J$=6.57 Hz, 26-Me or 27-Me), 0.858 (3H, d, $J$=6.57 Hz, 26-Me or 27-Me), 1.007 (3H, d, $J$=6.48 Hz, 21-Me), 1.009 (3H, s, 19-Me), 3.53 (1H, m, 3$\alpha$-H), 5.3 (2H, m, 22-, 23-H) and 5.35 (1H, m, 6-H). MS *m/z*: 384 (92%, $M^+$), 369 (16), 366 (13), 351 (19), 300 (67) 299 (16), 273 (27), 271 (51), 255 (54), 231 (11), 229 (11) and 55 (100).

24-Methylenecholest-5-en-3$\beta$-ol (**4**): MS *m/z*: 398 (14%, $M^+$), 383 (11), 380 (5), 365 (8), 315 (38), 314 (100), 299 (38), 296 (11), 287 (5), 281 (27), 273 (8), 271 (59), 255 (8), 253 (14), 231 (22), 229 (24), 213 (22) and 221 (14).

Cholesta-5,24-dien-3$\beta$-ol (**5**): MS *m/z*: 384 (30%, $M^+$), 369 (7), 366 (8), 351 (7), 300 (19), 299 (5), 273 (12), 271 (12), 255 (32), 229 (8), 231 (5), 213 (13) and 55 (100).

(22*E*,24*S*)-24-Methylcholesta-5,22-dien-3$\beta$-ol (**6**): $^1$H-NMR $\delta$: 0.692 (3H, s, 18-Me), 0.816 (3H, d, $J$= 6.58 Hz, 27-Me), 0.834 (3H, d, $J$=6.48 Hz, 26-Me), 0.909 (3H, d, $J$=6.94 Hz, 28-Me), 1.001 (3H, d, $J$=6.11 Hz, 21 Me), 1.009 (3H, s, 19-Me), 3.53 (1H, m, 3$\alpha$-H), 5.15 (2H, m, 22-, 23-H), 5.35 (1H, m, 6-H). MS *m/z*: 398 (47%, $M^+$), 383 (5), 380 (7), 365 (7), 313 (5), 300 (17), 287 (3), 273 (8), 271 (25), 255 (27), 231 (5), 229 (7), 213 (15) and 55 (100).

24-Methylcholesta-5,24-dien-3$\beta$-ol (**7**): MS *m/z*: 398 (11%, $M^+$), 383 (11), 380 (6), 365 (8), 314 (78), 313 (6), 300 (20), 299 (33), 296 (11), 287 (6), 281 (28), 273 (6), 271 (42), 255 (8), 231 (14), 229 (28), 213 (17) and 55 (100).

(22*E*,24*R*)-24-Methylcholesta-5,22-dien-3$\beta$-ol (**8**): $^1$H-NMR $\delta$: 0.692 (3H, s, 18-Me), 0.816 (3H, d, $J$= 6.27 Hz, 26-Me), 0.834 (3H, d, $J$=6.13 Hz, 27-Me), 0.909 (3H, d, $J$=6.93 Hz, 28-Me), 1.009 (3H, s, 19-Me,)

1.010 (3H, d, $J$=6.32 Hz, 21-Me), 3.52 (1H, m, 3α-H), 5.18 (2H, m, 22-, 23-H), 5.35 (1H, m, 6-H). MS $m/z$: 398 (53%, $M^+$), 383 (7), 380 (5), 365 (8), 313 (5), 300 (32), 287 (3), 273 (8), 271 (30), 255 (33), 231 (3), 229 (5), 213 (17) and 55 (100).

(24*Z*)-24-Ethylcholesta-5,24(28)-dien-3β-ol (**9**): MS $m/z$: 412 (9%, $M^+$), 397 (3), 394 (1), 379 (2), 314 (100), 299 (25), 296 (8), 281 (33), 271 (17), 255 (8), 231 (8), 299 (35) and 213 (13).

Cholest-5-en-3β-ol (**10**): $^1$H-NMR δ: 0.677 (3H, s, 18-Me), 0.861 (3H, d, $J$=6.58 Hz, 26-Me), 0.865 (3H, d, $J$=6.63 Hz, 27-Me), 0.913 (3H, d, $J$=6.53 Hz, 21-Me), 1.007 (3H, s, 19-Me), 3.53 (1H, m, 3α-H), 5.35 (1H, m, 6-H). MS $m/z$: 386 (100%, $M^+$), 371 (30), 368 (50), 353 (28), 301 (55), 275 (58), 273 (19), 255 (25), 247 (14), 231 (17) and 213 (30).

24ξ-24-Methylcholest-5-en-3β-ol (**11**): MS $m/z$: 400 (58%, $M^+$), 385 (22), 382 (25), 367 (19), 315 (36), 289 (33), 273 (19), 255 (22), 231 (14), 213 (30) and 55 (100).

24ξ-24-Ethylcholesta-5,22-dien-3β-ol (**12**): MS $m/z$: 412 (100%, $M^+$), 397 (11), 394 (9), 379 (11), 327 (9), 301 (17), 300 (31), 273 (29), 271 (74), 255 (74), 231 (20), 229 (14) and 213 (51).

5α-Cholestan-3β-ol (**13**): MS $m/z$: 388 (89%, $M^+$), 373 (55), 370 (11), 355 (28), 257 (28), 233 (100) and 215 (83).

(24*R*)-24-Ethylcholest-5-en-3β-ol (**14**): $^1$H-NMR δ: 0.679 (3H, s, 18-Me), 0.812 (3H, d, $J$=7.25 Hz, 27-Me), 0.833 (3H, d, $J$=7.71 Hz, 26-Me), 0.844 (3H, t, $J$=7.64 Hz, 29-Me), 0.920 (3H, d, $J$=6.45 Hz, 21-Me), 1.008 (3H, s, 19-Me), 3.53 (1H, m,3α-H), 5.35 (1H, m, 6-H). MS $m/z$: 414 (75%, $M^+$), 399 (28), 396 (28), 381 (22), 329 (44), 303 (28), 273 (19), 255 (22), 231 (19), 213 (33) and 55 (100).

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