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ASTEROID STEROLS

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It has been reported by many workers that sterols of asteroids are composed of a mixture of Δ^7-mono- and -diunsaturated sterols.[1,2] The distribution of Δ^7-sterols is regarded now as one of the evidences of phylogenetic relationship of asteroids and holothurians.[3]

Through the investigation of the sterol components of 6 species of asteroids collected in Hokkaido, we found that asteroids generally contain new C_{26} sterol, asterosterol, as a minor component.[4] This sterol was also detected in a trace amount in holothurian, Stichopus japonicus by gas liquid chromatography (GLC).

The unsaponifiable matters were obtained by the usual procedure from the asteroids, Asterina pectinifera, Asterias amurensis, Distolasterias sticantha, Certonardoa semiregularis, Lysastrosoma anthostictha, and Solaster paxillatas and the sterol fractions were separated by preparative thin-layer chromatography. Each showed almost the same peaks in GLC (peaks 1 to 7) except for their relative abundances.[5] Identification of these peaks was made by combined GLC-mass spectrometry (GLC/MS) using free sterols, methyl ether and trimethylsilyl (TMS) ether derivatives from Distolasterias sticantha as a typical one.[6]

Peak 1 (asterosterol and its methyl and TMS ether) showed molecular ions (M) at m/e 370, 384, 442[7] and other fragment ions at a (M-Me) m/e 355, 369, 427; b (M-ROH) 352; c (M-Me-ROH) 337; d (M-side chain) 273, 287, -; e (M-side chain-ROH) 255; f (M-side chain-42) 231, 245, -; g (M-side chain-42-ROH) 213; h (M-side chain-27) 246, 260, 318; i (M-side chain-27-RO) 229. The molecular ions and ions a to c show that asterosterol is diunsaturated C_{26} sterol.

The ions d to i are the common fragments of 3ß-hydroxy Δ^5- and Δ^7-sterol and their derivatives[8], and accordingly, asterosterol has unsaturated C_7H_{13} side chain. It was confirmed by prominent ions j (M-side chain-2H) at m/e 271, 285 and 343, characteristic of the sterols of unsaturated side chain,[9] and at m/e 97, derived from side chain fragment.[10] The ions k (cleavage of C-20 and C-22 with a one hydrogen transfer) at m/e 300, 314 and 372 show that the double bond is located at C-22.[9] The Δ^5-unsaturation was excluded by the absence of ions at m/e 129 and M-129 in TMS ether, and other fragments derived from the fragmentation of A and B rings.[8] Treatment of 20ß-methyl-pregn-7-en-3ß-ol-21-carboxaldehyde acetate[11] with isobutyl triphenyl phosphonium bromide in hexane and subsequent hydrolysis afforded 22-*cis* and -*trans* mixture of 24-nor-cholesta-5,22-dien-3ß-ol, resistant to separation. The retention time of more volatile peak agreed with that of asterosterol on 1.5% SE-30 and 1.5% OV-17, showing that asterosterol is 24-nor-cholesta-7,22-dien-3ß-ol.[12] Till the present time, we have not succeeded to define the configuration at C-22.

The retention time and cracking pattern of peak 2 agreed with that of the mixture of cholesterol and cholestanol supposed to be of dietary origin.

Other peaks showed sufficient fragments of 3ß-hydroxy Δ^7-sterol ring (ions a to i). The peaks 3 and 5 showed ions j and k compared with ions j and l (M- C-23 to C-27 -1H, characteristic of the Δ^{24}-sterols[9]) at m/e 314, 328, 386 of peaks 6 and 7. From these and their molecular ions, peaks 3 to 7 were identified as shown in the table. It should be pointed out that α-spinasterol, reported to be obtained by fractional crystallization from *Asterina pectinifera* and *Asterias amurensis*,[13] is not at least a major sterol in these and other species of asteroids studied.

The novel C_{26} sterol, 22-*trans*-24-nor-cholesta-5,22-dien-3ß-ol was isolated first by Idler from mollusc of the class Pelecypoda,[10] and recently synthesized by Unrau.[14] We detected the sterol as a minor component in Gastropoda, *Littorina brevicula*; holothurian, *Stichopus japonicus*, and annelida of class Polychaeta, *Pseudopotamilla occelata*. The sterol was isolated as acetate, mp 141-142° (reported,[14] mp 142.5-143°) from *Pseudopotamilla occelata* and the spectral data was in good agreement with Idler's C_{26} sterol. This sterol had a retention time relative to cholestane of 1.20 compared with 1.31 of asterosterol.[5] The GLC of the sterol mixture of *Distolasterias sticantha* showed a peak (less than 0.03% of total sterol) whose retention time is identical to this sterol.

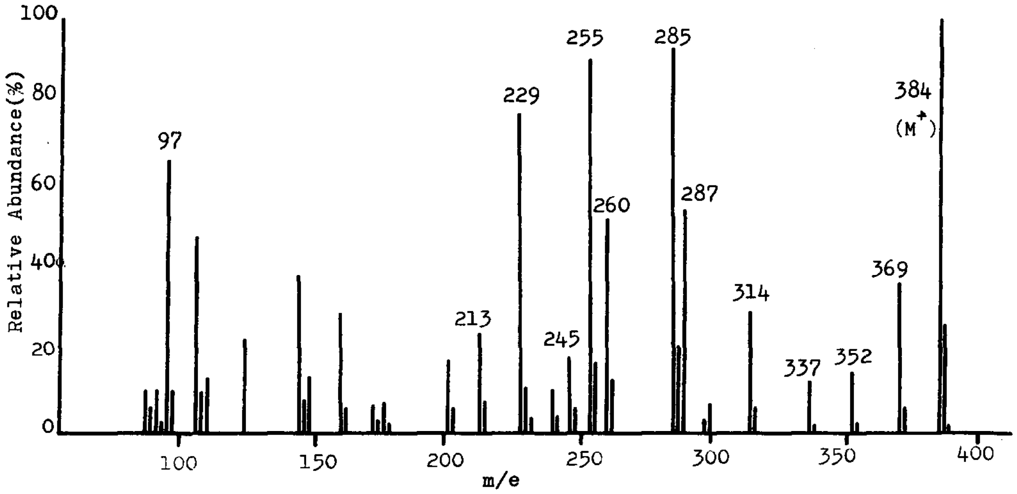
The distribution of C_{26} sterols in mollusca, arthropoda,[10] echinodermata, annelida, and protochordata[15] shows that C_{26} sterols occur rather widely in marine invertebrates.

TABLE. Components of free sterol

Peak	Relative abundance	Structure	Retention time relative to cholesterol	Mol. wt of sterol and derivatives
1	0.6%	Asterosterol	0.82	370, 384, 442
2	3.5%	Cholesterol Cholestanol	1.00	386, 400, 458 388, 402, 460
3	11.8%	Cholesta-7,22-dien-3ß-ol	1.08	384, 398, 456
4	34.2%	Cholest-7-en-3ß-ol	1.15	386, 400, 458
5	15.6%	24-Methyl-cholesta-7,22-dien-3ß-ol	1.28	398, 412, 470
6	28.2%	24-Methyl-cholest-7-en-3ß-ol 24-Methylene-cholest-7-en-3ß-ol	1.48	400, 414, 472 398, 412, 470
7	6.1%	24-Ethyl-cholest-7-en-3ß-ol 24-Ethylidene-cholest-7-en-3ß-ol	1.74	414, 428, 486 412, - , 484

2% OV-17 column, 2 m x 3 mm i.d.; He carrier gas at 30 ml/min; isothermal at 280°.

FIGURE. Mass spectrum of asterosterol methyl ether



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6. The GLC/MS was carried out using 2% OV-17 column, 2 m x 3 mm i.d.; He carrier gas at 30 ml/min; isothermal at 280° (free), 265° (methyl ether), and 270° (TMS ether).
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