Tetrahedron Vol. 29, pp. 1193 to 1196 Pergamon Press 1973. Printed in Great Britain

MARINE STEROLS—II[1]

ASTEROSTEROL, A NEW C_{26} STEROL FROM *ASTERIAS AMURENSIS* LÜTKEN

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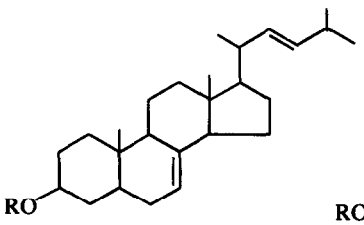
(*Received in Japan* 1 *November* 1972; *Received in UK for publication* 20 *December* 1972)

Abstract—Asterosterol, a new marine C_{26} sterol, was isolated from the asteroid, *A. amurensis*, and its structure was characterized as 22-*trans*-24-nor-5α-cholesta-7,22-dien-3β-ol (1). Episterol (9), 22-*trans*-(24R)-24-methylcholesta-7,22-dien-3β-ol (stellasterol, 3) and 22-*trans*-cholesta-7,22-dien-3β-ol (7) were also isolated and their structures were confirmed.

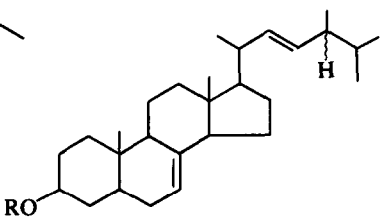
The asteroid, *Distolasterias sticantha*, contains a new sterol in 0·6% of the total sterol mixture.[2] Other components characterized by combined GLC-mass spectrometry were cholesterol and cholestanol (3·5%), cholesta-7,22-dien-3β-ol (11·8%), cholest-7-en-3β-ol (34·2%), 24-methylcholesta-7,22-dien-3β-ol (15·6%), 24-methyl- and 24-methylene-cholest-7-en-3β-ol (28·2%), and 24-ethylidene- and 24-ethyl-cholest-7-en-3β-ol (6·1%). The new sterol seemed fairly widely distributed in asteroids and was named asterosterol (1). From the mass spectral data and comparison of the GLC retention time with the synthetic mixture from ergosterol, 1 was tentatively assigned the structure of 24-norcholesta-7,22-dien-3β-ol. It was also found in trace amounts in Japanese holothurian, *Stichopus japonicus*. Essentially the same sterol composition was observed in other asteroids, *A. amurensis*, *A. pectinifera*, *Certonardoa semiregularis*, *Lysastrosoma anthostictha*, and *Solaster paxillatas*, except the absence of 1 in *S. paxillatas*.[2] C_{30} sterols such as acanthasterol[3] were not major sterols in the asteroids studied. The present paper describes the isolation and characterization of 1.

Column chromatography of the acetate of the crude sterol obtained from *A. amurensis* over silver nitrate-impregnated silicic acid[4] with hexane-benzene gave (1) cholestanol and a mixture of Δ^7-sterols with a saturated side chain; (2) a mixture of asterosterol acetate (2), 24-methylcholesta-7,22-dien-3β-ol acetate (4), and cholesta-7,22-dien-3β-ol acetate (8). Further elution gave 24-methylene-cholest-7-en-3β-ol acetate (episterol acetate[5]) (10), m.p. 143–145·5°, $[\alpha]_D$ +4·5°, ν_{max} 1665, 1643, 889 cm^{-1}; NMR (δ) 4·66 and 4·71 (each 1H, broad s, terminal methylene), 5·15 (1H, broad s, 7-H). Hydrolysis of 10 gave the free sterol (9), m.p. 127·5–128·5°, $[\alpha]_D$ +5·4°. This sterol has been isolated by Fagerlund and Idler from the asteroid, *Pisaster ochraceus*.[5a] Column chromatography of above diunsaturated sterol fraction over silver nitrate-silicic acid gave 4, m.p. 181–182°, $[\alpha]_D$ +6·4°, ν_{max} 1663, 967 cm^{-1} (*trans*-disubstituted double bond[6]); NMR (δ) 0·540 (18-Me), 0·805 (19-Me), 0·815 (3H, d, J = 6·7 Hz, 28-Me), 0·995 (3H, d, J = 6·7 Hz, 21-Me), 2·00 (OAc), 4·40–4·84 (1H, m, acetoxy methine), 4·96–5·24 (3H, m, 7- and 22,23-H). Hydrolysis of 4 gave the free sterol (3), m.p. 159·5–161°, $[\alpha]_D$ +7·8°. This sterol corresponds to the stellasterol designated by Bergmann *et al.* as the one of unresolved sterol components from *A. forbesi*.[7] The weak but distinct dextrorotatory specific rotations differ considerably from those of authentic 22-*trans*-(24S)-24-methylcholesta-7,22-dien-3β-ol (5, m.p. 173·5–174°, $[\alpha]_D$ −22·8°; acetate (6), m.p. 183·5–184°, $[\alpha]_D$ −23·4°) prepared from ergosterol, though the IR and NMR spectra, and the GLC retention time were almost indistinguishable. Since GLC resolves the epimers at C-20 but hardly those at C-24 in the condition employed,[8] the discrepancy indicates that the configuration at C-24 of our sample is *R* or, it is at least a mixture of epimers in which 24*R* epimer predominates. It also supports Bergmann's conclusion that the configuration at C-24 in stellasterol is *R* from the ozonization experiment of the mixture of stellasterol and stellastenol, the C-22, 23 dihydro derivative of stellasterol.[7] Further elution gave cholesta-7,22-dien-3β-ol acetate (8), m.p. 140–142°, $[\alpha]_D$ −5·8°, ν_{max} 1663, 967 cm^{-1}; NMR (δ) 0·537 (18-Me), 0·805 (19-Me), 0·853 (6H, d, J = 6·7 Hz, 26,27-Me), 1·001 (3H, d, J = 6·7 Hz, 21-Me), 2·00 (OAc), 4·48–4·84 (acetoxy methine), 4·96–5·40 (3H, m, 7- and 22,23-H). Hydrolysis of 8 gave the free sterol (7), m.p. 129–130·5°, $[\alpha]_D$ −4·2°. Synthesized 7 was reported to melt at 123–124° and to show a rotation of −13·3°.[9] Although the occurrence of 7 in asteroids has been known,[2,3a,10] the isolation and characterization from

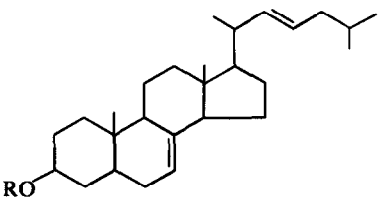
asteroids or other living organisms has not been recorded in literature. Further elution gave a mixture containing *ca* 60% of 2. Two separations on a column of silver nitrate-silicic acid, followed by fractional recrystallization gave 2, m.p. 134–136·5°, $[\alpha]_D$ −2·8°. Hydrolysis of 2 gave the free sterol (1), m.p. 129–130°, $[\alpha]_D$ ±0°, $C_{26}H_{42}O$, from the elemental analysis and mass spectrum.



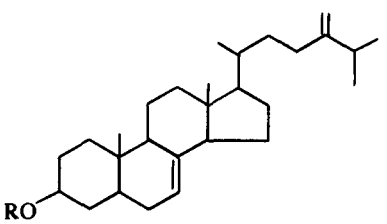
1: R = H
2: R = Ac



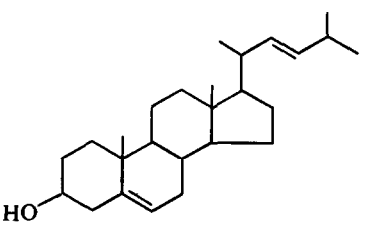
3: R = H, 24R
4: R = Ac, 24R
5: R = H, 24S
6: R = Ac, 24S



7: R = H
8: R = Ac



9: R = H
10: R = Ac



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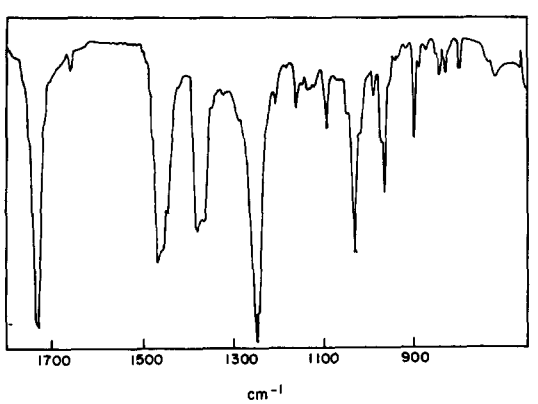

Fig 1.

Compound 1 showed a deep green color in the Liebermann-Burchard test. Its mass spectrum showed molecular ion (M^+) at *m/e* 370 and other prominent ions at *m/e* 355 (M^+-Me), 352 (M^+-H_2O), and 337 (M^+-Me and H_2O) indicating that 1 is a C_{26} sterol and diunsaturated. The monounsaturated steroid ring was established by the ions at *m/e* 273 (M^+-side chain), 255 (M^+-side chain and H_2O), 231 (M^+-side chain and ring D cleavage), 213 (M^+-side chain and H_2O, and ring D cleavage), 213 (M^+-side chain and H_2O, and ring D cleavage), 246 (M^+-side chain and C-16 to C-17), and 229 (M^+-side chain and C-16 to C-17 and HO).[11a] The presence of an unsaturated C_7H_{13} side chain was supported by its intense fragment ion at *m/e* 97[12] and by the ion at *m/e* 271 (base peak, M^+-side chain, and 2H), characteristic of sterols with an unsaturated side chain.[11b] The ion at *m/e* 300, derived from the allylic cleavage with one hydrogen transfer, is characteristic of C-22 unsaturated sterols with a nuclear double bond. The ions at *m/e* 246 and 271 were far more intense than those observed for $\Delta^{5,22}$-sterols.[1] The absence of ions at *m/e* 129 and M^+-129 in the mass spectrum of TMS ether excludes the possibility of Δ^5-unsaturation.[11a] The NMR spectrum of 2 showed signals of 18-Me (δ 0·539, 3H, s), 19-Me (0·811, 3H, s), terminal dimethyl (0·941, 6H, d, J = 6·7 Hz), 21-Me partially enveloped by dimethyl signal (0·997, 3H, d, J = 6·7 Hz), acetyl (2·02), acetoxy methine (4·45–4·85, 1H, m), and three olefinic protons at δ 5·15 (1H, broad s, >C=CH—) and 5·20 (2H, ill-defined m). The presence of a terminal dimethyl and a secondary Me groups establishes the 24-norcholestane skeleton and the side chain double bond at C-22. The position of angular Me groups indicates the trisubstituted nuclear double bond at C-7 and rules out the $\Delta^{9(11)}$ unsaturation which would cause downfield shift of 18- and 19-Me signals by *ca* 0·06

and 0·16 ppm, respectively.[13] The broad nature of acetoxy methine signal shows the *trans* juncture of A and B rings. The IR spectrum of **2** (Fig 1) is superimposable with that of **8** including the finger print region. It showed absorptions at 967 cm^{-1} (*trans*-disubstituted side chain double bond) and at 797, 827 and 843 cm^{-1} (Δ^7).[14] From these results, it was concluded that asterosterol is 22-*trans*-24-nor-5α-cholesta-7,22-dien-3β-ol, the second C_{26} sterol whose structure has been fully characterized. The first C_{26} sterol, 22-*trans*-24-norcholesta-5,22-dien-3β-ol (**11**) was isolated by Idler *et al.*[12] from the scallop, *Placopecten magellanicus*, and was subsequently found in many marine invertebrates.[1,15] GLC of **1** on 1·5% SE-30 column at 250° showed retention time relative to cholestane of 1·30 compared with 1·79 for **7**, 2·18 for **3**, and 1·20 for **11** which we isolated from the annelida of the class polychaeta, *Pseudopotamilla occelata*.[1] GLC of the asteroid sterols showed a very small peak which corresponds to **11** or its C-5 saturated analog. The biotransformation of Δ^5-sterol into Δ^7-sterol through the C-5 saturated sterol was demonstrated to occur in asteroids.[10] From the biogenetic point of view, it is suggested that asterosterol may be the sterol modified by asteroids from digested $\Delta^{5,22}$-C_{26} sterol (**11**) whose origin and biogenesis remain unsettled.

EXPERIMENTAL

M.ps were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCl_3$ soln. NMR spectra were determined on a JEOL PS100 spectrometer operating at 100 MHz, in $CDCl_3$ soln with TMS as internal standard. Mass spectra were determined on a Hitachi RMU-7 mass spectrometer. IR spectra were taken in Nujol mull on a Hitachi 215 spectrometer. GLC was carried out on a Shimadzu CG4BPF gas chromatograph using a glass column (1·8 m × 3 mm i.d.) packed with 1·5% SE-30 on 60–80 mesh Chromosorb-W at 250°, with N_2 carrier gas flow-rate of 60 ml/min. Column chromatography was carried out on a 100 mesh silicic acid (Mallinckrodt) unless otherwise specified.

Isolation of crude sterol. The whole body of the starfish, *A. amurensis*, collected at the coast of Tokoro, Hokkaido, in July 1970 was used as the material. The minced starfish (35 kg) was extracted exhaustively with hexane and ether. The combined extract was concentrated, suspended in $CHCl_3$, and washed thoroughly with water. The organic layer was evaporated and the residue was refluxed with 5% KOH in MeOH for 3 hr. The non-saponifiable matter was extracted with ether, the extract was washed with H_2O, and sat NaCl aq, and evaporated. The oily residue (250 g) was chromatographed over 1 kg of silica gel, eluted with benzene and $CHCl_3$, and the sterol fraction was combined and crystallized from MeOH to give 7 g of needles, m.p. 128–133°. Sterol acetate was obtained in a usual manner with Ac_2O in pyridine.

Column chromatography of sterol acetate. A soln of sterol acetate (6·5 g) in hexane was applied to a column of $AgNO_3$-impregnated silicic acid (20%, 750 g) and eluted with a mixture of benzene and hexane. The fractions (50 ml) were collected automatically and monitored by GLC and combined accordingly (Table 1).

Recrystallization of fraction 2 several times from MeOH gave cholestanol acetate as plates, m.p. 114–115°. Mass spectrum: *m/e* 430 (M^+). Recrystallization of fraction 7 from MeOH gave **10** (450 mg), shown to be pure by GLC, as plates, m.p. 143–145·5°, $[\alpha]_D$ +4·5° (*c*, 1·5) (lit.,[5a] m.p. 140°, $[\alpha]_D$ +6·0°). NMR (δ): 0·535 (3H, s, 18-Me), 0·805 (3H, s, 19-Me), 1·02 (9H, d, J = 6·7 Hz, 21, 26, 27-Me), 1·99 (OAc), 4·45–4·84 (1H, m, 3α-H), 4·66 and 4·71 (each 1H, broad s, terminal methylene), 5·15 (1H, broad s, 7-H); IR: 1735, 1665, 1643, 899, 889, 843, 828, 797 cm^{-1}. Hydrolysis of **10** gave **9** as needles, m.p. 127·5–128·5° from MeOH, $[\alpha]_D$ +4·4° (*c*, 2·28) (lit.,[5a] m.p. 131°, $[\alpha]_D$ +6·4°). (Found: C, 84·24; H, 11·53. Calc. for $C_{28}H_{46}O$: C, 84·35; H, 11·63%); IR: 3300, 1665, 1645, 890, 847, 828, 797 cm^{-1}.

Column chromatography of fraction 6. Fraction 6 was applied to a column of $AgNO_3$-silicic acid (20%, 400 g) and eluted with benzene-hexane (1:5). Elution with 9 l of solvent gave at first a mixture of cholest-7-en-3β-ol acetate and **4** (75 mg), then 111 mg of **4** shown to be pure by GLC, as plates from MeOH, m.p. 181–182°, $[\alpha]_D$ +6·4° (*c*, 2·38). (Found: C, 82·02; H, 10·86. $C_{30}H_{48}O_2$

Table 1.

Fraction	Solvent (benzene:hexane)	Amount (g)	and composition of material
1	1:5 (5 l)		
2	1:5 (0 5 l)	0·0083	Cholestanol acetate (88%)
3	1:5 (5 l)	3·29	Cholest-7-en-3β-ol acetate (47%), 24-methylcholest-7-en-3β-ol acetate (19%), 24-ethylcholest-7-en-3β-ol acetate (23%) and acetate of $\Delta^{7,22}$-C_{27}, C_{28}, C_{29} sterols (11%).
4	1:5 (2·5 l) and 3:10 (2·5 l)	1·33	**4** (41%), **8** (11%), 24-ethylcholesta-7,22-dien-3β-ol acetate (5%) and acetate of Δ^7-C_{27}, C_{28}, C_{29} sterols (43%).
5	3:10 (0·5 l)	0·182	**4** (61%), **8** (21%) and cholest-7-en-3β-ol acetate (8%)
6	3:10 (3 l)	0·70	**2** (5%), **4** (40%) and **8** (43%)
7	3:10 (2 l) and 1:1 (2 l)	0·72	mainly **10**

requires: C, 81·76; H, 10·98%). NMR and IR: see Discussion. Hydrolysis of **4** gave **3** as needles from MeOH, m.p. 159·5–161°, $[\alpha]_D$ +7·8° (*c*, 1·30); IR: 3300–3400, 970, 850, 830, 800 cm^{-1}; Mass spectrum: *m/e* 398 (M^+), 383 (M^+-Me), 365 (M^+-Me and H_2O), 300 (M^+-C-22 to C-28 and 1H), 271 (base peak, M^+-side chain and 2H), 255 (M^+-side chain and H_2O), 246 (M^+-side chain and C-16 to C-17). Further elution with 7 l of benzene-hexane (3:5) gave a mixture of **4** and **8** (253 mg), then 115 mg of pure **8** as plates from MeOH, m.p. 140–142·5°, $[\alpha]_D$ −5·8° (*c*, 3·26). (Found: C, 81·73; H, 10·75. $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·87%); IR and NMR: see Discussion. Hydrolysis of **8** gave **7** as needles from MeOH, m.p. 129–130·5°, $[\alpha]_D$ −4·2° (*c*, 2·89); IR: 3300, 970, 847, 832, 800 cm^{-1}; Mass spectrum: *m/e* 384 (M^+), 369 (M^+-Me), 351 (M^+-Me-H_2O), 300 (M^+-C-22 to C-27 and 1H), 271 (base peak, M^+-side chain and 2H), 255 (M^+-side chain and H_2O), 246 (M^+-side chain and C-16 to C-17). Further elution with the same solvent gave a mixture (75 mg) containing 60% of **2**. This fraction was purified twice over a column of 100 g of $AgNO_3$-silicic acid which was eluted with benzene-hexane (1·5:10), and *ca* 30 mg of a mixture containing 82% of **2** was obtained. It was fractionally recrystallized several times from $CHCl_3$-MeOH to give pure sample of **2** (8 mg) as plates, m.p. 134–136·5°, $[\alpha]_D$ −2·8° (*c*, 0·71). (Found: C, 80·90; H, 10·67. $C_{28}H_{44}O_2$ requires: C, 81·56; H, 10·75%); IR and NMR: see Discussion; Mass spectrum: *m/e* 412 (M^+), 397 (M^+-Me), 352 (M^+-AcOH), 342 (M^+-C-22 to C-26), 337 (M^+-AcOH and Me), 313 (M^+-side chain and 2H, base peak), 288 (M^+-side chain and C-16 to C-17), 255 (M^+-side chain and AcOH). Hydrolysis of **2** gave **1** as needles from MeOH, m.p. 129–130°, $[\alpha]_D$ ±0° (*c*, 0·65). (Found: C, 81·15; H, 11·07. $C_{26}H_{42}O$ 3/4 H_2O requires: C, 81·29; H, 11·42%); IR: 3300, 967, 843, 827, 794 cm^{-1}; NMR and Mass spectrum: see Discussion.

Acknowledgement—We are grateful to Mr. Y. Tsuji of Hokkaido Fisheries Experimental Station for the collection of asteroids.

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