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MARINE STEROLS—IV[1]

STRUCTURE AND SYNTHESIS OF AMURESTEROL, A NEW MARINE STEROL WITH UNPRECEDENTED SIDE CHAIN, FROM *ASTERIAS AMURENSIS* LÜTKEN

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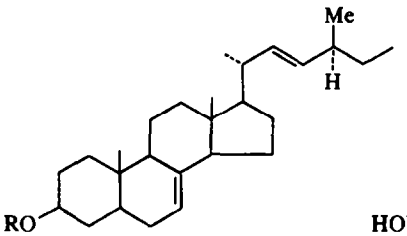
Abstract—Amuresterol, a new marine C_{27} sterol, was isolated from an asteroid *Asterias amurensis*, and its structure was confirmed as biogenetically unprecedented 22-*trans*-27-*nor*-(24S)-24-methylcholesta-7, 22-dien-3β-ol (**1**) from the spectral data and by synthesis. Gas-liquid chromatography indicated that **1** is a common sterol in six species of asteroids collected in Hokkaido.

A previous paper reported the structure of four diunsaturated sterols (**3**, **4**, **5**, and **7**) isolated from an asteroid, *Asterias amurensis*.[1,2] In addition to the sterols reported, we found an unidentified sterol, amuresterol (**1**), which showed a slightly shorter retention time in GLC than 22-*trans*-cholesta-7, 22-dien-3β-ol (**5**) and amounted to *ca* 1·8% of total sterol mixture in *A.* amurensis. It was found to be a common component of sterols in other asteroids, *A. pectinifera*, *Certonardoa semiregularis*, *Lysastrosoma anthostictha*, *Distolasterias sticantha*, and *Solaster paxillatas* collected in the same district (Table 1). We now report the isolation and structure of amuresterol, which turned out to be the first member of a class of C_{27} sterols having a migrated cholestane-type side chain.

The crude sterol acetates of *A. amurensis* was separated by column chromatography over silver nitrate-impregnated silicic acid as reported previously.[2] Cholesterol, cholest-7-en-3β-ol, and 24-ethylcholesta-7,22-dien-3β-ol (**11**) were isolated as their acetate from the less polar fraction. The configuration of **11** at C-24 was not clear due to the minute amount isolated, though its pattern in NMR spectrum suggested that it was α-spinasterol rather than its 24 epimer, chondrillasterol.[3] Amuresterol acetate (**2**) was more polar on argentation chromatography than 22-*trans*-24-methylcholesta-7,22-dien-3β-ol acetate (**8**) but less polar than **6**. Repetition of chromatography of the diunsaturated sterol fraction gave **2** found to be pure by GLC, m.p. 161–163°, $[\alpha]_D + 1 \pm 2°$. Hydrolysis of **2** gave the free sterol (**1**), m.p. 151–152°, $[\alpha]_D + 4 \pm 2°$, $C_{27}H_{44}O$, from the elemental analysis and mass spectrum.

Compound **1** showed a deep green color in the Lieberman-Burchard test in accordance with other $\Delta^{7,22}$-sterols (**3**, **5**, **7**, **9** and **11**). Its mass spectrum showed a molecular ion (M^+) at *m/e* 384 and other prominent ions at 369 (M^+-Me), 351 (M^+-Me and H_2O), 273 (M^+-side chain), 255 (M^+-side chain and H_2O), 246 (M^+-side chain and C-16 to C-17), 229 (M^+-side chain and C-16 to C-17 and HO), 231 (M^+-side chain and ring D cleavage), and 213 (M^+-side chain and H_2O, and ring D cleavage) in a typical pattern of Δ^7-monounsaturated sterol ring having an unsaturated side chain.[4a] The ions at *m/e* 271 (base peak, M^+-side chain, and 2H) and 300 (allylic cleavage of C-20 and C-22 bond with one hydrogen transfer) indicate the presence of a double bond in the side chain at C-22.[4b] This cracking pattern and its relative intensity were identical with those of **5**. The IR spectrum of **1** was also superimposable with that of **5** showing a strong absorption of *trans*-disubstituted side-chain double bond at 970 cm^{-1} and other absorptions at 800, 835, and 850 cm^{-1} (Δ^7). The possibility that **1** is a 22-*cis* isomer of **5**, was excluded by the absence of corresponding absorption at 750 to 770 cm^{-1} and its faster mobility than **5** on argentation chromatography.[1,5] Although the IR and mass spectra and LB reaction do not allow the differentiation between **1** and **5**, the key difference was noticed in its NMR spectrum (100 MHz, Fig 1). It showed signals of 18-Me (δ 0·530), 19-Me (0·783), 21-Me (0·991, 3H, d, $J = 6·7$ Hz), Hydroxy-methine (3·45–3·75, 1H, m), and olefinic protons (3H, centered at δ 5·15 as an ill-defined multiplet, 7-and 22,23-H) but no terminal dimethyl signals as other sterols. In their stead, three peaks, equal to 6H, were present at δ 0·818, 0·878, and 0·943. The peaks at δ 0·878 and 0·943 were found to be due to secondary methyl doublet ($J = 6·7$ Hz), collapsing by irradiation at δ 1·9 in the region expected for allylic protons. These observations indicate that amuresterol bears a secondary methyl at C-24 and accordingly, an ethyl



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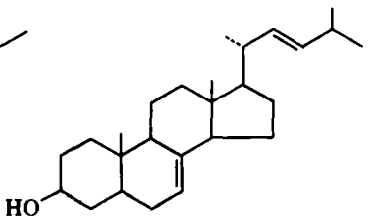
2: R = Ac



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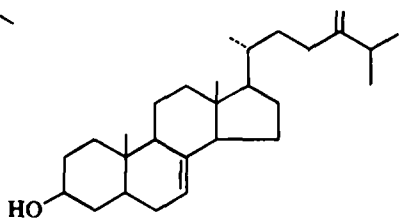

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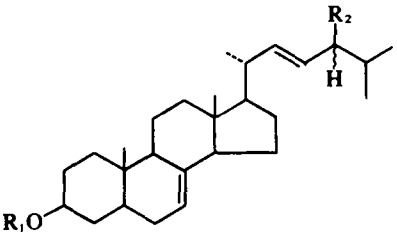
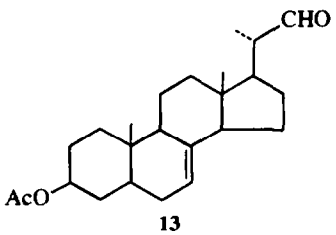


5: R_1 = H, R_2 = H
6: R_1 = Ac, R_2 = H
7: R_1 = H, R_2 = Me, 24S
8: R_1 = Ac, R_2 = Me, 24S
9: R_1 = H, R_2 = Me, 24R
10: R_1 = Ac, R_2 = Me, 24R
11: R_1 = H, R_2 = Et
12: R_1 = Ac, R_2 = Et



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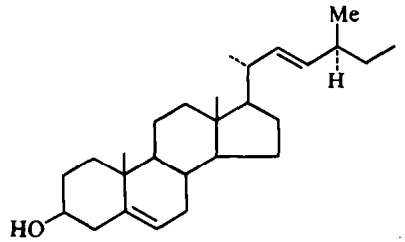

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group in the same position as that in **1**, and rules out those structures in which n- or tertiary butyl group is attached at C-23. The peak at δ 0·818 should form a part of a triplet signal of a primary methyl at C-26, enveloped by other signals. The broad nature of hydroxy-methine signal indicates 3β-hydroxy-A/B-*trans* or 3α-hydroxy-A/B-*cis* system. Confirmatory evidence for the structure of **1** for amuresterol was provided by its partial synthesis by an unequivocal route.

Bromination of (-)-2-methylbutan-1-ol,[6] followed by refluxing with triphenyl phosphine in benzene, gave a phosphonium salt. The Wittig reaction of 20S-3β-acetoxy-5α-bisnorchol-7-en-3β-ol-21-al (**13**)[7] with the ylide generated from the phosphonium salt and butyllithium in hexane at room temperature gave, after purification by chromatography, a sterol acetate, m.p. 161·5–163·5°, $[\alpha]_D$ 0 ± 2°, m.p., underpressed on admixture with natural amuresterol acetate (**2**). The condition of the Wittig reaction used is known to afford 22-*trans* isomer predominantly and leave the C-20 configuration intact.[8] Hydrolysis of the acetate gave the free sterol, m.p. and mixed m.p. 151–153°, $[\alpha]_D$ + 3 ± 2°. The IR, NMR, and mass spectra and the retention time in GLC (1·5% OV-17, 3m, 250°) of the synthetic sterol and its acetate were completely identical with those of natural amuresterol (**1**) and its acetate (**2**). Amuresterol, therefore, is 22-*trans*-27-*nor*-24-methylcholesta-7,22-dien-3β-ol (**1**), the first member of a class of sterols having a migrated cholestane-type side chain. The configuration at the asymmetric center of (-)-2-methylbutan-1-ol derived from fusel oil has been established as S by correlating to L-(+)-isoleucine.[9] Although the discrepancies in the melting point, specific rotation, and IR and NMR spectra between 24S and R epimers of C-24 alkylated sterols are generally small and their mass spectrum and retention time in GLC make no differences,[10] the observed identity of synthetic and natural sterol supports the configuration of amuresterol at C-24 as S.

At present, the biogenesis of amuresterol is quite uncertain. It may have been derived in asteroids by conversion from the corresponding digested $\Delta^{5,22}$-C_{27} sterol (**14**) as the most of asteroid sterols,[11] or by internal methylation of asterosterol (**3**) or demethylation of 24S ergostane-type sterol such as **7**. It is noteworthy that *S. paxillatas*, *A. pectinifera*, and *L. anthostictha* contain 22-*trans*-24-methylcholesta-7,22-dien-3β-ol (**7**) as the most abundant sterol and **1** as the major sterol than 22-*trans*-cholesta-7,22-dien-3β-ol (**5**) compared with relatively low level of **7** and **1** in other three species (Table 1). There is a discrepancy in the specific rotations between **7** (m.p. 162·5°–164·5°, $[\alpha]_D$ + 8·0; acetate (**8**): m.p. 176–178°, $[\alpha]_D$ + 7·0°) isolated from *A. amurensis* and authentic 22-*trans*-(24R)-24-methylcholesta-7,22-dien-3β-ol (**9**, m.p. 173·5–174°, $[\alpha]_D$-22·8°; acetate (**10**): m.p. 183·5–184°, $[\alpha]_D$ − 23·4°) prepared

Table 1. Composition of sterols[a] (%)

Peak No.[b]	1	2	3	4	5	6	7	8	9	10	11	12	13
Retention time relative to cholesterol[c]	0·67	0·78	0·94	1·00	1·06	1·10	1·17	1·34	1·55	1·60	1·70	1·93	2·17
A. amurensis	trace	2·3	trace	1·5	1·8	5·3	37·1	10·3	28·0			5·6	1·9
D. sticantha	trace	0·7	trace	4·8	2·4	5·9	41·9	13·0	18·7		2·5	9·6	trace
C. semiregularis		1·3	trace	3·2	3·2	4·9	36·2	17·3	19·7		3·8	9·7	trace
A. pectinifera		0·5	trace	0·7	2·8	1·3	11·7	31.3	8·8		13·3	29·4	trace
L. anthostictha		0·8	trace	1·0	5·8	4·1	26·4	33·8	8·4		7·0	12·0	trace
S. paxillatas				0·5	3·7	2·0	9·4	38·8	9·3		7·4	28·2	3·1

[a] Characterized by combined GLC-mass spectromety analysis of the sterols of *D. sticantha*.[12]

[b] 1: 24-Norcholesta-5,22-dien-3β-ol; 2: asterosterol (3); 3: cholest-22-en-3β-ol; 4: cholesterol and cholestanol; 5: amuresterol (1); 6: 22-*trans*-cholesta-7,22-dien-3β-ol (5); 7: cholest-7-en-3β-ol; 8: 22-*trans*-(24S)-24-methylcholesta-7,22-dien-3β-ol (7); 9: 24-methylcholest-7-en-3β-ol; 10: episterol (4); 11: 24-ethylcholesta-7,22-dien-3β-ol (11); 12: 24-ethylcholest-7-en-3β-ol; 13: 24-ethylidenecholest-7-en-3β-ol.

[c] 1·5% OV-17 (3m × 3mm) at 250°.

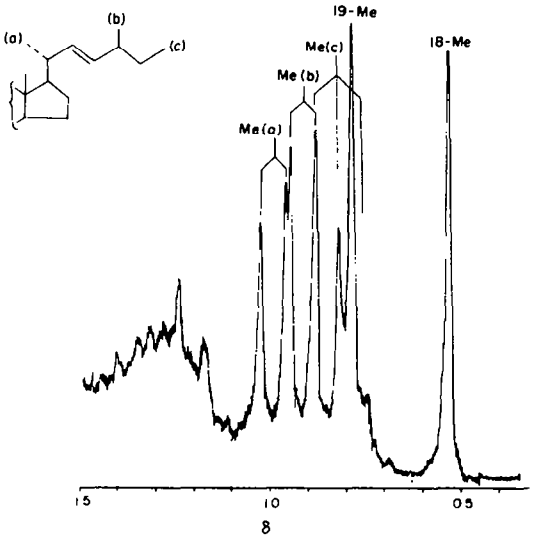

Fig 1.

from ergosterol, though IR, mass, and NMR spectra and GLC retention time were almost indistinguishable. The dextrorotatory specific rotation of 7 was confirmed by the sample isolated from *A. amurensis* collected in the same district in another year (m.p. 159·5–161°, $[\alpha]_D$ +7·8; acetate (8): m.p. 181–182°, $[\alpha]_D$ +6·4°)[2] so that it is reasonable to assume that the configuration of 7 at C-24 is S or it is at least a mixture of epimers in which 24S epimer predominates. It should be emphasized that a conclusive proof for the biogenetic process can come only through feeding experiments using labeled precursors.

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 an a Shimadzu GC4BPF gas chromatograph using a glass column (3m × 3mm i.d.) packed with 1·5% OV-17 on 80–100 mesh Shimalite W at 250°, with N_2 carrier gas flow-rate of 60 ml/min. Hydrolysis of sterol acetate was carried out by refluxing in 3% KOH–MeOH for 20 min, followed by usual work-up throughout.

Isolation of crude sterol The dried and powered internal organs (1·5 kg) of the starfish, *A. amurensis*, collected in the coast of Tokoro, Hokkaido, in July 1971, was used as the material. The ether extract (300g) was refluxed in 1·5 l of 10% KOH in 80% EtOH for 3 h, concentrated to *ca* 1 l, then diluted with 2 l of H_2O. The nonsaponifiable matter was extracted with ether, the extract was washed with H_2O, and sat NaCl aq, and evaporated. The residue was dissolved in hot MeOH (50 ml), then left standing at r.t. for several hours. The ppt was collected, washed with cold MeOH, and recrystallized from MeOH. The crude sterol thus obtained was acetylated in the usual manner with Ac_2O in pyridine and then recrystallized twice from $CHCl_3$–MeOH, giving the crude acetate (2·8g) found to be pure by TLC on silica gel (benzene).

Separation of sterol acetate Chromatography of the sterol acetate over a column of $AgNO_3$–silicic acid (Mallinckrodt, 100 mesh), as reported previously,[2] gave: (1, 38 mg) stanols associated with a trace of Δ^7-sterol; (2, 635 mg) a mixture of cholesterol acetate, cholest-7-en-3β-ol acetate, and its C-24 alkylated analogs; (3, 1·07g) a mixture of acetates of Δ^7- and $\Delta^{7,22}$-sterols; (4, 630 mg) mainly acetate of $\Delta^{7,22}$-sterols; (5, 130 mg) a mixture of acetate of $\Delta^{7,22}$-sterols and 4. Fractional recrystallization of the fraction 2 from $CHCl_3$–MeOH by a triangular procedure gave cholest-7-en-3β-ol acetate m.p. and m.m.p. 122°, and a tar containing 40% of cholesterol acetate (50 mg). The tar was treated in 5 ml of ether with 5 drops of bromine and 3 mg of AcONa in 2 ml of AcOH for 5 min and stirred with 500 mg of Zn powder for 10 min. After dilution with H_2O, the mixture was extracted with ether and the ether layer was washed with H_2O and sat NaCl aq. The evaporation residue was submitted to preparative TLC (silica gel, hexane–benzene (3:1), developing twice, and bands were detected by spraying H_2O. The upper band (Rf 0·6) was extracted with $CHCl_3$ and recrystallized from MeOH to 5 mg of slightly impure cholesterol acetate,

m.p. 105–110°, underpressed on admixture with an authentic specimen with identical spectral and GLC proprties.

Isolation of Amuresterol (1) Fraction 4 was applied on a column of $AgNO_3$–silicic acid (1:4, 500g) and eluted with benzene–hexane (1:5). Elution with 6·5 l of the solvent gave a mixture of Δ^5-and Δ^7-sterols. Further elution with 0·8 l of the solvent gave an equal mixture of acetate of cholesterol, cholest-7-en-3β-ol, and **12**. Five recrystallizations from $CHCl_3$–MeOH gave 10 mg of a sample of **12** containing *ca* 5% of cholest-7-en-3β-ol acetate, m.p. 174–176°, IR: 1665, 973, 850, 835, 805 cm^{-1}. Hydrolysis of **12** gave **11**, as fine needles from MeOH, m.p. 162–165°, $[\alpha]_D$ 0 ± 2° (*c*, 0·79). NMR (δ): 0·53 (18–Me), 1·00 (3H, d, J = 6·7 Hz, 21–Me), 5·05–5·20 (3H, m, 7- and 22,23–H). Mass spectrum: *m/e* 454 (M^+), 439 (M^+-Me), 394 (M^+-AcOH), 342 (M^+-C-22 to C-29 and 1H), 313 (base peak, M^+-side chain and 2H), 255 (M^+-side chain and AcOH), 288 (M^+-side chain and C-16 to C-17). Further elution with 4·8 l of the solvent gave **8** (250 mg), m.p. 176–178°, $[\alpha]_D$ + 7·0 (*c*, 1·68). Hydrolysis of **8** gave 7, m.p. 162·5–164·5°, $[\alpha]_D$ + 8·0° (*c*, 2·14). Spectral properties of **7** and **8** were identical as reported previously.[2] Further elution with 1·6 l of the solvent gave a mixture of **2** and **6** (4:1). Further elution gave a mixture of **6** and acetate of **3** and **4** from which **6** was purified from appropriate fraction, m.p. 143–145°, with identical spectral properties as reported previously.[2] The fraction containing **2** (45 mg) was purified again by a column of $AgNO_3$–silicic acid to give a sample of **2** (35 mg) as plates from $CHCl_3$–MeOH, m.p. 161–163°, $[\alpha]_D$ + 1 ± 2 (c, 2·0). (Found: C, 81·48; H, 10·94. $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·87); IR: 1665, 970, 848, 833, 803 cm^{-1}; NMR: (δ): 0·535 (3H, s, 18-Me), 0·803 (3H, s, 19-Me), 1·003 (3H, d, J = 6·7 Hz, 21-Me), 0·920 (3H, d, J = 6·7 Hz, sec Me), 4·5–4·9 (3α-H), 5·15 (3H, m, 7- and 22,23-H); Mass spectrum: *m/e* 426 (M^+), 411 (M^+-Me), 397 (M^+-Et), 366 (M^+-AcOH), 351 (M^+-AcOH and Me), 342 (M^+-C-22 to C-28 and H), 315 (M^+-side chain), 313 (base peak, M^+-side chain and 2H), 288 (M^+-side chain and C-16 to C-17), 255 (M^+-side chain and AcOH). Hydrolysis of **2** gave **1** as long needles from MeOH, m.p. 151–152°, $[\alpha]_D$ + 4 ± 2° (*c*, 1·2); (Found: C, 80·89; H, 10·99. $C_{27}H_{44}O \cdot H_2O$ requires: C, 80·54; H, 11·52); IR, NMR and mass spectrum: see Discussion.

Synthesis of amuresterol (1) A mixture of 4 ml of bromide prepared from commercial (-)-2-methylbutan-1-ol by Crombie's method[6] and 3g of triphenylphosphine in 10 ml of dry benzene was refluxed for 8 days and the precipitated phosphonium salt was collected, washed with benzene, and dried (3·1 g). To a suspension of 600 mg of powdered phosphonium salt in 15 ml of dry hexane, 1 ml of 20% butyllithium in hexane dispersion was added and the mixture was refluxed for 5 min, then cooled to 40°. A suspension of the aldehyde (**13**, 220 mg) in 10 ml of dry hexane was added and the mixture was stirred for 1 h. The mixture was diluted with hexane and H_2O, and the organic layer was washed with H_2O, sat. NaCl solution, and the solvent was evaporated. Acetylation of the evaporation residue in the usual way gave 118 mg of crude acetate. It was found by GLC to be composed of mainly three compounds (3:3:1) and the retention time of two major compounds corresponded to **2** and **6**. The crude acetate in hexane was applied to a column of 125 g of $AgNO_3$–silicic acid (1:4) and eluted with a mixture of hexane and benzene. The fractions (10 ml) were collected automatically and nomitored by GLC and combined accordingly. The proportion of 22-*cis* and -*trans* sterols was estimated from GLC and NMR spectra of the combined fractions which showed slightly deshielded 18-methyl signal of 22-*cis* sterols at δ 0·56 compared with 0·54 of 22-*trans* sterols.[1,5] Elution with hexane-benzene (5:1) gave a mixture (50 mg) which consisted of *trans* isomers of **2** and **6** (3:2). Further elution gave 20 mg of a mixture of **6** and *trans* and *cis* isomers of **2** (5:1:1). Recrystallizations from $CHCl_3$-MeOH gave a sample of **6** (10 mg), assumed to be derived from contaminated isoamyl alcohol in the starting "active amyl alcohol", m.p. and m.m.p. 143–145°, with identical spectral properties as reported.[2] Further elution gave 45 mg of a mixture of *cis* and *trans* isomers of 6, and another unidentified compound (1:1:2) whose retention time by GLC is remarkably shorter than **6**. It was found by mass spectrum to be a C_{26} (M^+ *m/e* 412) but did not correspond to the acetate of **3** so that it is a product derived from contaminated n-butanol in the starting alcohol. The fractions containing **2** (50 mg) was purified again by a column of $AgNO_3$–silicic acid to afford a sample (12 mg) found to be pure by GLC, m.p. and m. m.p. 161·5–163·5°, $[\alpha]_D$ 0 ± 2° (*c*, 0·64), completely identical in spectral and GLC properties with amuresterol acetate (**2**). Hydrolysis gave the free sterol, long needles from MeOH, m.p. and mixed m.p. 151–153°, $[\alpha]_D$ + 3 ± 2° (*c*, 0·44), also completely identical in all respects with natural amuresterol (**1**).

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