P_c by P_σ, where P_c was the probability of collision between balls and powder particles and P_σ was the probability for powder particles to be crushed by the collision with balls.[6] In the previous paper, $J_b^{\alpha'} J_s^{-\beta'}$ was considered to be related to P_c and $(\rho_c \cdot J_b/J_s)^\gamma$ was considered to be related to P_σ.[4] Probably, in equation (5), $\rho_b \cdot J_b/J_s$ is related to P_σ and $J_s^{-0.1}$ is related to P_c, when f is constant. The probability of collision between balls and powder particles is considered to be very high in case of vibro-milling.

6) T. Tanaka, *Ind. Eng. Chem. Process Design Develop.*, **5**, 353 (1966).

[Chem. Pharm. Bull. 22(1) 236—238 (1974)]

UDC 547.92.057 : 591.05

Marine Sterols. III.[1] Synthesis of Asterosterol, a Novel C_{26} Sterol from Asteroids

MASARU KOBAYASHI, KAGEMI TODO, and HIROSHI MITSUHASHI

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received June 21, 1973)

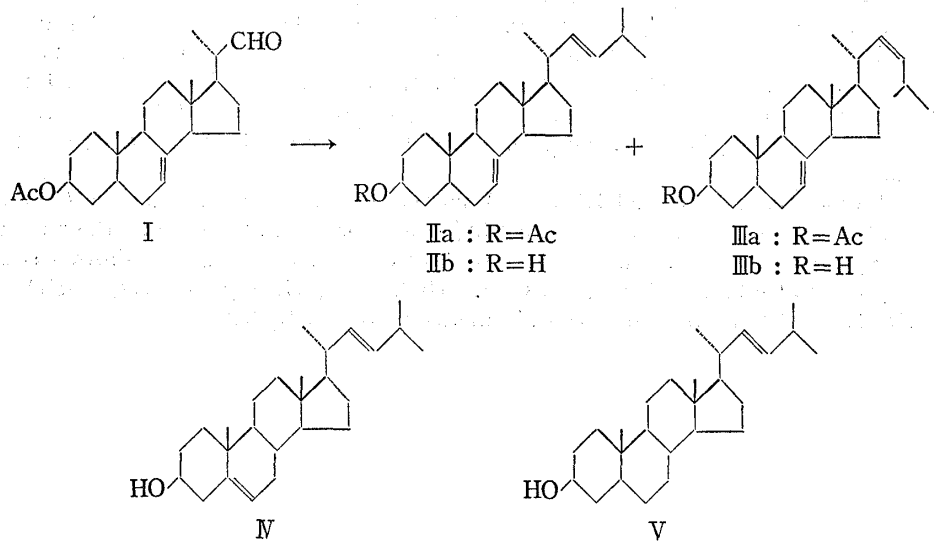
In the preceding paper we reported isolation of asterosterol, a minor C_{26} sterol from the asteroid *Asterias amurensis* and proposed the structure of 22-*trans*-24-nor-5α-cholesta-7,22-dien-3β-ol from spectral data.[1] This sterol was also found in trace amounts in other asteroids and japanese holothurian, *Stichopus japonicus*.[3] Recently, Nomura, Barbier and co-workers suggested occurrence of asterosterol in the tunicate *Halocynthia roretzi*.[4] We could not detect this sterol in another japanese tunicate, *H. aurantium* though it contained 4.4% of 22-*trans*-24-norcholesta-5,22-dien-3β-ol (IV). We now confirm the structure of asterosterol by synthesis through Wittig reaction.

The 20S aldehyde (I) obtained from 5,6-dihydroergosterol acetate[5] was treated in hexane at room temperature with the ylide generated from isobutyltriphenyl phosphonium bromide and butyl lithium and gave a mixture of 22-*trans* and -*cis* isomers (IIa and IIIa) in about 1:1 ratio (60%). The configuration at C-20 was confirmed as R by ozonolysis of the mixture which gave only the starting 20S aldehyde (I).[6] At higher temperature, I gave mainly IIa and appreciable amount of by-product. It was shown to be 20S isomer of IIa from the fact that Wittig reaction of 1:1 mixture of 20S and 20R aldehyde gave these two compounds in the same ratio. It showed retention time relative to 20R isomer (IIa) of 1.16 by gas–liquid chromatography (GLC) on 1.5% SE-30 column (1.8 m) at 250°. Interestingly, 20S-22-dehydrocholesterol was reported to show shorter retention time than natural 20R isomer.[7]

1) Part II: M. Kobayashi, R. Tsuru, K. Todo, and H. Mitsuhashi, *Tetrahedron*, **29**, 1193 (1973).
2) Location: *Kita-12-jo, Nishi-5-chome, Sapporo, 060, Japan.*
3) M. Kobayashi, R. Tsuru, K. Todo, and H. Mitsuhashi, *Tetrahedron Letters*, **1972**, 2935.
4) T. Nomura, M. Ito, J. Viala, A. Alcaide, and M. Barbier, *Bull. Jap. Soc. Sci. Fish.*, **38**, 1365 (1972); J. Viala, M. Devys, and M. Barbier, *Bull. Soc. Chim. France*, **1972**, 3626.
5) K. Sakai and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **11**, 529 (1963).
6) W. Sucrow, *Chem. Ber.*, **100**, 259 (1967).
7) N. Ikekawa, R. Watanuki, K. Tsuda, and K. Sakai, *Anal. Chem.*, **40**, 1139 (1968); K. Tsuda, K. Sakai, and N. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 835 (1961).

Column chromatography of 20R mixture over silver nitrate-silicic acid with hexane–benzene (10: 3) gave at first *trans* isomer (IIa), identical with natural asterosterol acetate, mp 143—145°, mixed mp 136—140°,[8] $[\alpha]_D$ −7.9° ($CHCl_3$), ν_{max} (Nujol) 1663, 967 cm^{-1}, δ ($CDCl_3$), 0.535 (18-Me), 0.807 (19-Me), 0.937 (6H, d, J=6.7 Hz, terminal dimethyl), 5.05—5.30 (3H, m, 7- and 22,23-H). Hydrolysis of IIa gave the free sterol (IIb), mp 130—131°, mixed mp 129—130°,[8] $[\alpha]_D$ −6.4° ($CHCl_3$), ν_{max} (Nujol) 3300, 970 cm^{-1}, δ ($CDCl_3$), 0.540 (18-Me), 0.795 (19-Me), 0.937 (6H, d, J=6.7 Hz, terminal dimethyl), 1.00 (21-Me). The identity was also confirmed by GLC on 1.5% OV-17[9] and by mass spectra.

Further elution with the same solvent gave *trans–cis* mixture then *cis* isomer (IIIa), mp 136—138°, ν_{max} (Nujol) 770 cm^{-1}, δ ($CDCl_3$) 0.56 (18-Me), 0.81 (19-Me), 0.93 (terminal dimethyl), 0.96 (21-Me), 4.96 and 5.03 (each 1H, 22,23-H). Hydrolysis of IIIa gave the free sterol (IIIb), mp 132—133.5°.



Till the present time, three C_{26} sterols (IIb, IV[10] and V[4,10c]) have been isolated from marine invertebrates. We observed by GLC the peak which correspond to IV in each two species of Rhodophytae, Phaeophytae and Chlorophytae though in very trace amounts. Most marine invertebrates show far more abundant level of C_{26} sterols independently of their ability of sterol biosynthesis. It is suggested that marine invertebrates accumulate dietary C_{26} sterol which is inconvertible to cholestane skeleton.

Experimental

Melting points were determined on a Kofler hot-stage and are not corrected. NMR spectra were recorded in $CDCl_3$ solution at 100 MHz, with Me_4Si as internal standard.

Wittig Reaction of I——To a suspension of 1.6 g of isobutyltriphenyl phosphonium bromide in 15 ml of dry hexane, 1 ml of 20% butyl lithium in hexane dispersion was added and the mixture was refluxed for 5 min then cooled to 30°. A suspension of 300 mg of I in 10 ml of dry hexane was added and the mixture was stirred for 1 hr. The mixture was diluted with hexane and water and the organic layer was washed with water and sat. NaCl solution and the solvent was evaporated off. The residue was crystallized from

8) Slightly impure sample from *A. amurensis* showed mp 129—130° for the free sterol and 134—136.5° for the acetate.[1]

9) GLC by 1.5% OV-17 (3 m) at 250° showed that *trans* isomer has slightly longer retention time than *cis* isomer.

10) *a*) D.R. Idler, P.M. Wiseman, and L.M. Safe, *Steroids*, **16**, 451 (1970); *b*) S. Teshima, A. Kanazawa, and T. Ando, *Comp. Biochem. Physiol.*, **41B**, 121 (1972); *c*) T.R. Erdman and R.H. Thomson, *Tetrahedron*, **28**, 5163 (1972); *d*) M. Kobayashi, M. Nishizawa, K. Todo, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **21**, 323 (1973).

MeOH to give 192 mg of steryl acetate mixture. The mixture (132 mg) was applied on a column of 125 g of silver nitrate-impregnated silicic acid (1:4) and eluted with a mixture of hexane and benzene (10:3). The fractions (10 ml) were collected automatically and monitored by GLC on 1.5% OV-17 column at 250° and combined accordingly.

Fraction 1 (42 mg)——This was found to be composed of *trans* isomer. The residue was recrystallized from MeOH to give IIa, mp 143—145°, mixed mp 136—140°,[8] $[\alpha]_D$ −7.9° (c=1.52, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1735, 1663, 967, 845, 830, 800. NMR δ: 0.535 (18-Me), 0.807 (19-Me), 0.937 (6H, d, J=6.7 Hz, terminal dimethyl), 5.05—5.30 (3H, m, 7- and 22, 23-H). *Anal.* Calcd. for $C_{28}H_{44}O_2$: C, 81.56; H, 10.75. Found: C, 81.54; H, 10.81. Hydrolysis of IIa by refluxing in 3% KOH–MeOH for 10 min, followed by usual work-up gave the free sterol (asterosterol), mp 130—131°, mixed mp 129—130°,[8] $[\alpha]_D$ −6.4° (c=1.00, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300, 970. NMR δ: 0.54 (18-Me), 0.795 (19-Me), 0.937 (6H, d, J=6.7 Hz, terminal dimethyl), 1.00 (21-Me), 5.10—5.30 (3H, m, 7- and 22, 23-H). Mass Spectrum m/e: 370 (M^+), 355 (M^+-Me), 352 (M^+-H_2O), 337 (M^+-Me-H_2O), 271 (M^+-side chain-2H). *Anal.* Calcd. for $C_{26}H_{42}O \cdot 1/2H_2O$: C, 82.26; H, 11.42. Found: C, 82.76; H, 11.46.

Fraction 2 (35 mg)——This was found to be composed of a mixture of *cis* and *trans*-isomer.

Fraction 3 (43 mg)——This was found to be composed of *cis* isomer (IIIa), mp 136—138° from MeOH, $[\alpha]_D$ −19.7° (c=2.13, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 770. NMR δ: 0.56 (18-Me), 0.81 (19-Me), 0.93 (6H, d, J=6.7 Hz, terminal dimethyl), 0.96 (3H, d, J=6.7 Hz, 21-Me), 4.96 and 5.03 (each 1H, 22, 23-H), 5.14 (7-H). *Anal.* Calcd. for $C_{28}H_{44}O_2$: C, 81.56; H, 10.75. Found: C, 81.43; H, 10.73. Hydrolysis of IIIa by refluxing in 3% KOH–MeOH for 20 min, followed by usual work-up gave the free sterol (IIIb), mp 132—133.5°, $[\alpha]_D$ −16.6° (c=0.95, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300, 770. *Anal.* Calcd. for $C_{26}H_{42}O$: C, 84.26; H, 11.42. Found: C, 84.06; H, 11.53.

Ozonolysis of Sterol Mixture (IIa and IIIa)——A portion of steryl acetate mixture (50 mg) in 4 ml of CH_2Cl_2–pyridine (100: 1) at −78° was treated with 1.5% ozone for 5 min, followed by the reductive work-up by the procedure reported by Tsuda, *et al.*[5] The resultant oily aldehyde fraction showed NMR signals of starting 20S aldehyde (I) at δ 9.57 (1H, d, J=3.4 Hz, CHO), 1.13 (3H, d, J=6.8 Hz, 21-Me), 0.816 (3H, s, 19-Me) and at 0.58 (3H, s, 18-Me) but no trace of isomerized 20R aldehyde.[6]