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KETOSTEROIDS FROM THE FAR-EAST MARINE PROSOBRANCH MOLLUSK *Onchidiopsis variegata*

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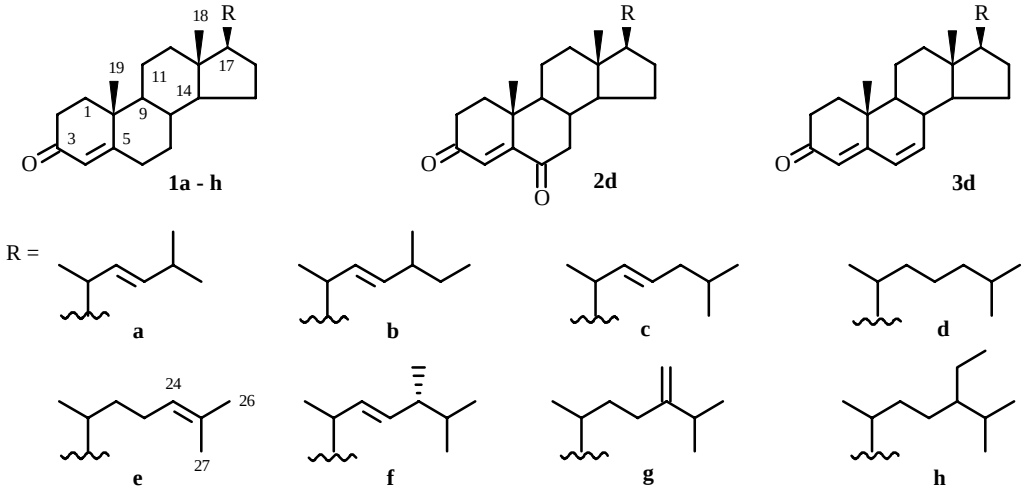
The composition of the ketosteroid fraction from the Far-East marine prosobranch mollusk Onchidiopsis variegata *was determined. The structure of a new steroidal ketone cholesta-4,24-dien-3-one* ***(1e)*** *was established by NMR, IR, UV, and mass spectroscopy. It was proposed that* O. variegata *feeds on ascidians of the genus* Styela.

Key words: *Onchidiopsis variegata*, Velutinidae, ketosteroid, cholesta-4,24-dien-3-one, *Styela*.

Chemical aspects of the ecology of marine Prosobranchia have been little studied [1] whereas the chemical composition of Far-Eastern representatives of this subclass is virtually unknown. In continuation of our research on secondary metabolites from marine mollusks [2], we established the composition of the ketosteroid fraction from the Far-Eastern prosobranch mollusk *Onchidiopsis variegata*. The fraction consisted of 10 components: Δ^4-3-ketosteroids **1a-h**, Δ^4-3,6-diketosteroid **2d**, and $\Delta^{4,6}$-3-ketosteroid **3d** (Table 1). Known compounds were identified based on GC—MS and PMR spectroscopy [3-5]. Certain ketosteroids were isolated from the total fraction using HPLC, including the previously unknown component **1e**.



According to GC—MS and ^{13}C NMR spectroscopy, the new ketosteroid **1e** had molecular formula $C_{27}H_{42}O$. The presence of signals in the ^{13}C NMR spectrum at δ 201.6 (s), 173.6 (s), and 125.8 (d) and absorption bands at 232 nm in the UV spectrum and 1678 cm^{-1} in the IR spectrum indicated that the structure contained an α,β-unsaturated ketone. The chemical shifts of the allyl proton of an enone fragment (5.72 ppm, s), CH_3-18 angular protons (0.71 ppm, s), and CH_3-19 (1.18 ppm, s) in the PMR spectrum agreed with spectral data for Δ^4-3-ketosteroids in the literature [3].

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TABLE 1. Composition of Δ^4-3-Ketosteroid Fraction from *O. variegata*

Name	RRT*	% in fraction
24-Nor-cholesta-4,22-dien-3-one (**1a**)	0.79	6.1
27-Nor-cholesta-4,22-dien-3-one (**1b**)	0.93	2.5
Cholesta-4,22-dien-3-one (**1c**)	0.95	8.7
Cholest-4-en-3-one (**1d**)	1.00	65.9
Cholest-4,6-dien-3-one (**3d**)	1.05	1.0
Cholesta-4,24-dien-3-one (**1e**)	1.06	4.4
24ξ-Methylcholesta-4,22-dien-3-one (**1f**)	1.07	2.5
24-Methylcholesta-4,24(28)-dien-3-one (**1g**)	1.17	2.2
Cholest-4-en-3,6-dione (**2d**)	1.26	3.9
24ξ-Ethylcholest-4-en-3-one (**1h**)	1.36	0.6

*Relative retention time of cholest-4-en-3-one (**1d**).

The mass spectrum of **1e** exhibited peaks with *m*/*z* 382 $[M]^+$, 298 [M - sidechain fragment lost due to cleavage of the C-20—C-22 bond]$^+$, 269 (100%) [M - sidechain - 2H]$^+$, 229 [M - 42 - sidechain]$^+$, and 124 for a Δ^4-3-ketosteroid with a double bond in the sidechain [5]. Besides signals for the enone double bond, the PMR and ^{13}C NMR spectra contained signals for another trisubstituted double bond at δ_H 5.09 (t, J = 7.1 Hz) and δ_C 125.2 (d), 131.0 (s) and two methyls at δ_H 1.60 and 1.68 (br.s) and δ_C 25.7 (q) and 17.7 (q). The chemical shifts of these signals agreed with those for a desmosterol-type sidechain [6].

Furthermore, the structure of **1e** was confirmed by DEPT, COSY, HSQC, and HMBC experiments. Thus, $^1H—^1H$ correlations revealed a spin system in ring A for protons of two methylenes at δ_H 2.39 (CH_2-2, m, 2H), 2.02 (CH_2-1a, m), and 1.70 (CH_2-1b, m); olefinic proton H-4 (5.72 ppm, s) in the COSY spectrum was coupled by a distant interaction with H-6b (2.39 ppm, m) similar to how H-24 (5.09 ppm, t, J = 7.1 Hz) was correlated with CH_3-26 (1.60 ppm, s) and CH_3-27 (1.68 ppm, s). In addition, the HMBC spectrum contained cross-peaks CH_3-19/CH_2-1, CH-9, C-5; CH_2-1/C-3, C-5; CH_2-2/CH_2-1, C-3; CH_2-6/CH-4, C-5; CH_3-26/CH-24, C-25, CH_3-27; CH_3-27/CH-24, C-25, CH_3-26; and other signals for a $\Delta^{4,24}$-3-ketosteroid. Thus, it was found that the isolated compound had the structure cholesta-4,24-dien-3-one (**1e**).

Earlier total Δ^4-3-ketosteroids with sidechains standard for benthic marine organisms were isolated from sponges [3, 4], ascidians [7], and nudibranchiate mollusks [8]. With respect to the observation of Δ^4-3,6-diketosteroid **2d** in *O. variegata*, compounds of this group were isolated previously from sponges [4, 9] and red and brown algae [10]. Monoketosteroid cholesta-4,6-dien-3-one (**3d**) is a known precursor in the biosynthesis of cholestanol [11] (we observed cholestanol among the free sterols of *O. variegata*).

The steroid composition of mollusks is known to be closely tied to their food. A significant part of their steroids have an exogenic origin [8, 12, 13 and references therein]. There are no reports on the food of *Onchidiopsis* species. It is currently thought that species of the family Velutinidae, to which the mollusk under study belongs, are predators and feed on attached animals [14, 15]. Thus, *Velutina velutina* feeds on attached ascidians [15], in particular, representatives of the generi *Phallusia* and *Styela* [16].

The rather complicated composition of the steroid mixture in *O. variegata* suggests that this mollusk feeds on invertebrate filterers. The lack in its stomach of spicules and the wide range of depths (55-1000 m) at which the mollusk resides [17], which does not correspond with the vertical distribution of spiculeless sponges of Far-Eastern seas [18, 19], indicate that sponges are not consumed by *O. variegata*. A probable biosynthetic desmosterol precursor of **1e** was observed among ascidians in two Far-Eastern species, *Styea plicata* and *S. clava* [20]. We also identified a desmosterol among free sterols of *O. variegata* and the ascidian *S. clavata* collected on the same cruise in the Kuril Islands. Considering the distribution of *Styela* in Far-Eastern seas, it can be assumed that *O. variegata*, like the other representative of the family *V. velutina*, feeds on *Styela* ascidians, accumulating desmosterol and **1e** with analogous sidechains.

EXPERIMENTAL

GC was carried out on an Agilent 6850 Series GC System chromatograph with an HP-5MS capillary column (30 m), flame-ionization detector, and He carrier gas. PMR and ^{13}C NMR spectra were obtained on a Bruker DRX-500 spectrometer using $CDCl_3$ solvent, 500 MHz for ^1H, and 125 MHz for ^{13}C. Chemical shifts are given in the δ system as ppm relative to TMS internal standard. UV spectra in hexane were recorded on a Cecil CE 7200 spectrophotometer; IR spectra, in CCl_4 on a Bruker Vector 22 FTIR spectrophotometer. Column chromatography was performed over silica gel L 40/100 μ (Chemapol, Czechoslovakia). GC—MS was carried out on a Hewlett Packard HP6890 GC system with an HP-5MS capillary column (30.0 m × 0.25 mm) at 270°C, He carrier gas, and 70 eV ionizing potential. HPLC used a Du Pont Series 8800 instrument with a RIDK-102 refractometer, Altex Ultrasphere^{TM}-Si columns (10 mm × 250 mm), and Agilent Hypersil ODS (5 μm, 4 × 250 mm).

Biological Material. Prosobranch mollusk *O. variegata* Derjugin, 1937 (class Gastropoda, subclass Prosobranchia, family Velutinidae) was collected in July 2005 at 433 m depth in Ekaterina Bay near Iturup Island (Kuril Islands) and identified by Candidate of Biological Sciences A. V. Chernyshev (A. V. Zhirmunskii Institute of Marine Biology, FED, RAS).

Extraction and Isolation. Freshly collected animals were frozen and extracted with ethanol. The extract (19.6 g) was distributed between water and hexane. Column chromatography over silica gel (hexane:ethylacetate, 5:1) of the concentrated hexane fraction (7.0 g) and normal-phase HPLC (hexane:ethylacetate, 7:1) followed by reversed-phase HPLC (80% ethanol) isolated **1a** (2.0 mg), **1d** (35.0 mg), **1e** (1.0 mg), **1g** (0.4 mg), **2d** (0.5 mg) and a subfraction containing the remaining ketosteroids.

Cholesta-4,24-dien-3-one (1e). IR spectrum (CCl_4, ν, cm^{-1}): 2936, 2871, 1678. UV spectrum (hexane, λ_{max}, nm): 232. Mass spectrum (EI, 70 eV *m*/*z*, I_{rel}, %): 382 (37) $[M]^+$, 367 (37), 298 (50), 283 (9), 269 (100), 256 (7), 245 (23), 229 (10), 147 (16), 124 (14).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3-18), 0.93 (3H, d, J = 6.5, CH_3-21), 0.93 (1H, m, H-9), 1.02 (1H, m, H-14), 1.05 (1H, m, H-22β), 1.10 (1H, m, H-15β), 1.12 (1H, m, H-17), 1.12 (1H, m, H-7β), 1.15 (1H, m, H-12β), 1.18 (3H, s, CH_3-19), 1.29 (1H, m, H-16β), 1.38-1.54 (2H, m, CH_2-11), 1.42 (1H, m, H-22α), 1.45 (1H, m, H-20), 1.53 (1H, m, H-8), 1.60 (3H, s, CH_3-27), 1.61 (1H, m, H-15α), 1.68 (3H, s, CH_3-26), 1.69 (1H, m, H-1β), 1.84 (1H, m, H-7α), 1.85 (1H, m, H-23β), 1.86 (1H, m, H-16α), 2.02 (1H, m, H-23α), 2.03 (1H, m, H-12α), 2.20 (1H, m, H-1α) 2.26 (1H, ddd, J_1 = 2.4, J_2 = 4.2, J_3 = 14.7, H-6α), 2.38 (1H, m, H-6β), 2.39 (2H, m, CH_2-2), 5.09 (1H, t, J = 7.1, H-24), 5.72 (1H, d, J = 1.2, H-4).

^{13}C NMR spectrum (125 MHz, $CDCl_3$): 12.0 (q, C-18), 17.5 (q, CH_3-19), 17.7 (q, C-27), 18.6 (q, C-21), 21.1 (t, C-11), 24.3 (t, C-15), 24.8 (t, C-23), 25.7 (q, C-26), 28.2 (t, C-16), 32.1 (t, C-7), 33.0 (t, C-6), 34.1 (t, C-2), 35.6 (d, C-20), 35.7 (d, C-8), 35.8 (t, C-1), 36.1 (t, C-22), 38.7 (s, C-10), 39.7 (t, C-12), 42.5 (s, C-13), 53.9 (d, C-9), 56.0 (d, C-14), 56.1 (d, C-17), 123.8 (d, C-4), 125.2 (d, C-24), 131.0 (s, C-25), 172.6 (s, C-5), 199.6 (s, C-3).

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REFERENCES

1. a) J. C. Coll, D. M. Tapiolas, B. F. Bowden, L. Webb, and H. Marsh, *Mar. Biol.*, **74**, 35 (1983); b) K. T. Fielman, S. A. Woodin, M. D. Walla, and D. E. Lincoln, *Marine Ecology-Progress Series*, **181**, 1 (1999).
2. a) S. N. Fedorov, O. S. Radchenko, L. K. Shubina, A. I. Kalinovsky, A. V. Gerasimenko, D. Y. Popov, and V. A. Stonik, *J. Am. Chem. Soc.*, **123**, 504 (2001); b) M. Gavagnin, F. Castelluccio, S. Di Micco, E. Mollo, Y. W. Guo, and G. Cimino, in: *Proceedings of XIth International Symposium on Marine Natural Products*, Sorrento, PC 33 (2004).

3. Y. M. Sheikh and C. Djerassi, *Tetrahedron*, **30**, 4095 (1974).
4. A. Migliuolo, V. Piccialli, and D. Sica, *J. Nat. Prod.*, **53**, 1262 (1990).
5. F. J. Brown and C. Djerassi, *J. Am. Chem. Soc.*, **102**, 807 (1980).
6. W. K. Wilson, R. M. Sumpter, J. J. Warren, P. S. Rogers, B. Ruan, and G. J. Schroepfer, *J. Lipid Res.*, **37**, 1529 (1996).
7. T. B. Tam Ha, W. C. M. C. Kokke, and C. Djerassi, *Steroids*, **40**, 433 (1982).
8. S. W. Ayer and R. J. Andersen, *Tetrahedron Lett.*, **23**, 1039 (1982).
9. a) M. Tischler, S. W. Ayer, R. J. Anderson, J. F. Mitchell, and J. Clardy, *Can. J. Chem.*, **66**, 1173 (1988); b) A. Aiello, E. Fattorusso, S. Magno, M. Menna, and M. Pansini, *J. Nat. Prod.*, **54**, 281 (1991).
10. a) J.-H. Sheu, S.-Y. Huang, G.-H. Wang, and C.-Y. Duh, *J. Nat. Prod.*, **60**, 900 (1997); b) J.-H. Sheu, G.-H. Wang, P.-J. Sung, and C.-Y. Duh, *J. Nat. Prod.*, **62**, 224 (1999).
11. M. S. Buchmann, I. Bjorkhem, and S. Skrede, *Biochem. Biophys. Acta*, **922**, 111 (1987).
12. Yasman, R. A. Edrada, V. Wray, and P. Proksch, *J. Nat. Prod.*, **66**, 1512 (2003).
13. G. B. Elyakov and V. A. Stonik, *Steroids of Marine Organisms* [in Russian], Nauka, Moscow (1988).
14. V. Fretter and A. Graham, *British Prosobranch Mollusks, Their Functional Anatomy and Ecology*, London (1962).
15. W. E. Ankel, *Prosobranchia, Die Tierwelt der Nord- und Ostee*, Akademishe Verlagsgemeinschaft, Leipzig (1936).
16. M. Diehl, *Kiel. Meeresforsch.*, **12**, 180 (1956).
17. Yu. I. Kantor and A. V. Sysoev, *Catalog of Mollusks from Russia and Adjacent Countries* [in Russian], Tovarishchestvo Nauch. Izd. KMK, Moscow (2005).
18. V. M. Koltun, *Silica-spicule Sponges of Northern and Far-Eastern Seas of the USSR* [in Russian], Nauka, Leningrad (1966).
19. V. M. Koltun, *Four-ray Sponges of Northern and Far-Eastern Seas of the USSR* [in Russian], Nauka, Leningrad (1966).
20. S. Yasuda, *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, **50**, 399 (1975).