

Structure Elucidation of Oxygenated Sterols from Eggs of Sea Hare, *Aplysia juliana*Yoshihiro YAMAGUCHI,* Yukio NAKANISHI, Teruyuki SHIMOKAWA,
Shigeru HASHIGUCHI, and Akira HAYASHI

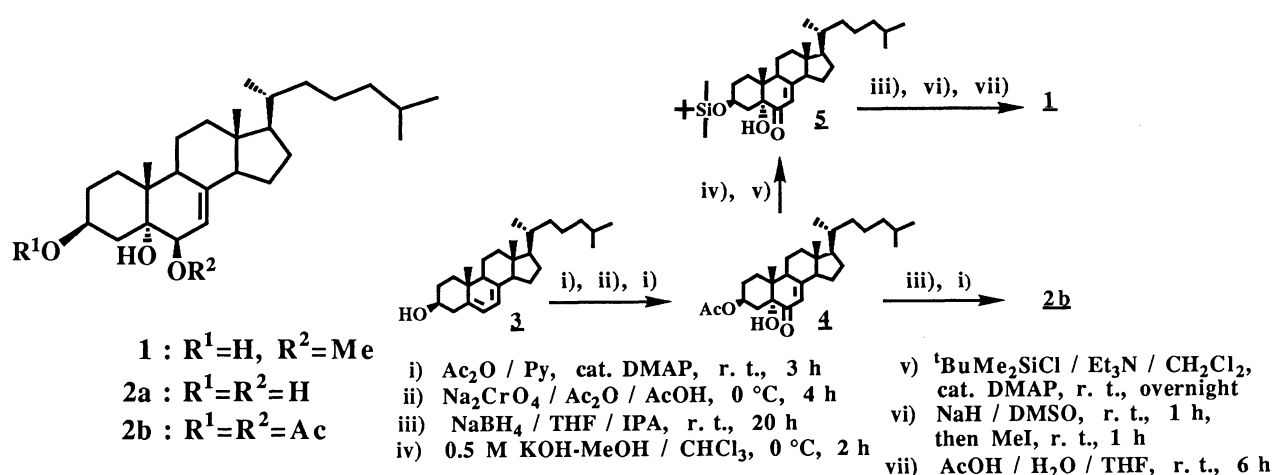
Department of Chemistry, Faculty of Science and Technology, Kinki University, Higashiosaka, Osaka 577

Cholest-7-en-6R-methoxy-3S,5R-diol, a new sterol, and cholest-7-en-3S,5R,6R-triol were isolated from eggs of sea hare, *Aplysia juliana*, and their structures and absolute configurations deduced by spectroscopic studies and chemical synthesis.

Polyhydroxylated sterols are common metabolites in marine invertebrates¹⁾ and sponges.²⁾ Recently, Komori *et al.* isolated a cholesterol peroxide derivative³⁾ from the albumen gland of sea hare (*Aplysia juliana*), and we also isolated the same compound from eggs of *A. juliana* and reported on its X-ray crystal analysis.⁴⁾ Further investigation of the lipidic extract of the same organism yielded two oxygenated sterols, cholest-7-en-6R-methoxy-3S,5R-diol (**1**) and cholest-7-en-3S,5R,6R-triol (**2a**), described in this paper.

Oxygenated sterols (**1** and **2a**) were obtained by SiO₂ column chromatography of an acetone extract of eggs of *A. juliana*.⁵⁾ The electron impact mass spectra (EI/MS) of **1**⁵⁾ showed a weak molecular ion peak (m/z 432, 2%) and fragment ion peaks at m/z 414, 399, 381, 365, 364, and 251. The fragment pattern indicated the presence of one methoxy group, two hydroxyl groups and one double bond, all of them located in rings A and B. The ¹³C-NMR spectra of **1**⁵⁾ indicated the presence of three sp³ carbons attached to oxygen atoms and two olefinic carbons. The ¹H-NMR spectra⁵⁾ of **1** showed one-proton signals at δ 4.07 and 3.17, consistent with the presence of two secondary carbinol methines. The complex methine signal at δ 4.07 showed the characteristic pattern for 3α-carbinol proton of an A/B trans-steroid. This unusually downshifted signal is typical of 3β-hydroxysterols bearing a 5α-hydroxyl group. The ¹H-NMR spectra of **1** showed, in addition, an olefinic proton at δ 5.41 coupled with the secondary carbinol methine at δ 3.17. Moreover, the singlet at δ 3.40 revealed the presence of one methoxy group. These data and the agreement of the C-18 methyl resonance at δ 0.59 with the value expected for a Δ⁷-sterol suggested a 7-en-6-methoxy-3β,5α-diol. The cholesterol-type side chain of **1** was deduced from ¹³C-NMR and ¹H-NMR.²⁾ Thus, the structure of this sterol was formulated as cholest-7-en-6-methoxy-3β,5α-diol.

The ¹H-NMR and ¹³C-NMR spectra of **2a** were very similar to those of **1**, except for the absence of the methoxy group. The structure of **2a** was determined by the following synthesis. Diacetate **2b** derived from **2a** was synthesized by acetylation of 7-dehydrocholesterol **3**, Na₂CrO₄ oxidation, NaBH₄ reduction and reacetylation. Reduction of **4** with NaBH₄ yielded a 6β-hydroxy compound as a major product,⁶⁾ whose physical data were identical with those of diacetate **2b**. The structure and absolute configuration of **2a** were determined to be cholest-7-en-3S,5R,6R-triol. The structure and absolute configuration of **1** were determined to be cholest-7-en-6R-methoxy-3S,5R-diol as follows. Compound **4** was converted into **5** by hydrolysis and silylation. Subsequently, reduction, methylation, and desilylation of **5** gave the diol methylether derivative, of which ¹H-NMR, ¹³C-NMR, IR, EI/MS and [α]_D were identical with those of **1**.⁵⁾



Compound 1 was isolated from a natural source for the first time. Compound 2a was reported by F. Cafieri⁷⁾ and D. Sica,²⁾ but this is the first report of its isolation from the eggs of *A. juliana*.

Compounds 1 and 2a instead of Vitamin D₃ may be biosynthesized from 7-dehydrocholesterol 3⁸⁾ as defense substances, since the biological significance of Vitamin D₃ playing important roles in the shell formation may be lower in Opisthobranchia such as *Aplysia sp.* which lacks the shell. The 7, 8-dihydro derivative of 2a has several bioactivities.⁹⁾ Study on biological activities of compound 1 and 2a is now in progress.

References

- 1) B. M. Jagodzinska, J. S. Trimmer, W. Fenical, and C. Djerassi, *J. Org.Chem.*, **50**, 2988 (1985).
- 2) V. Piccialli and D. Sica, *J. Nat. Prod.*, **5**, 915 (1987).
- 3) T. Miyamoto, M. Honda, S. Sugiyama, R. Higuchi, and T. Komori, *Liebigs Ann. Chem.*, **6**, 589 (1988).
- 4) K. Takahashi, Y. Yamaguchi, and A. Hayashi, *Acta Crystallogr., Sect. C*, **47**, 2581 (1991).
- 5) Seven and seventeen milligrams of 1 and 2a were obtained from eggs of *A. juliana* (3.5 kg, wet wt.), respectively. 1: $[\alpha]_D -55.6^\circ$ (c 0.19, $CHCl_3$); IR ($CHCl_3$), ν 3300-3500; EI/MS, m/z (rel. int.) 432(M^+ , 2), 414(M^+-H_2O , 100), 399(M^+-H_2O-Me , 24), 381(M^+-2H_2O-Me , 46), 365(M^+-2H_2O-OMe , 40), 364($M^+-2H_2O-MeOH$, 25), 251($M^+-2H_2O-MeOH$ -side chain, 19); 1H -NMR (500MHz, $CDCl_3$), δ 5.41(1H, ddd, $J=5.2, 2.6, 2.6, 7-H$), 4.07(1H, dddd, $J=11.5, 11.5, 4.8, 4.8, 3-H$), 3.39(3H, s, 6-OMe), 3.17(1H, ddd, $J=5.2, 2.0, 2.0, 6-H$), 2.13(1H, dd, $J=13.3, 11.5, 4-H_{ax}$), 2.08(1H, dt, $J=12.5, 3.5$), 1.86-1.92(2H, m), 1.84(1H, bd, $J=12.5$), 1.75(1H, ddd, $J=13.3, 4.8, 2.0, 4-H_{eq}$), 1.41-1.62(9H, m), 1.20-1.40(8H, m), 1.08-1.18(2H, m), 1.00(3H, s, 19-Me), 0.93(3H, d, $J=6.7, 21-Me$), 0.87(3H, d, $J=6.7, 26-Me$), 0.86(3H, d, $J=6.7, 27-Me$), 0.59(3H, s, 18-Me); ^{13}C -NMR (125MHz, $CDCl_3$), δ 143.7(s), 115.0(d), 82.5(d), 76.3(s), 67.8(d), 58.2(d), 56.2(q), 54.9(d), 44.0(s), 43.9(d), 39.6(t), 39.5(t), 39.5(t), 37.3(s), 36.2(d), 36.1(t), 32.8(t), 30.9(t), 28.0(d), 27.8(t), 23.9(t), 22.9(t), 22.8(q), 22.6(q), 22.2(t), 18.8(q), 18.4(q), 12.1(q).
- 6) C. Rufer, H. Hoffmeister, H. Schairer, and M. Traut, *Chem. Ber.*, **98**, 2383 (1965).
- 7) F. Cafieri, E. Fattorusso, M. Gavagnin, and C. Santacroce, *J. Nat. Prod.*, **48**, 944 (1985).
- 8) There was little 7-dehydrocholesterol in eggs of *A. juliana* though mollusks usually contain $\Delta^{5,7}$ -sterols in their bodies; D. R. Idler and P. Wiseman, *J. Fish. Res. Board Can.*, **29**, 385 (1972).
- 9) For example, N. A. Higley and S. L. Taylor, *Food Chem. Toxicol.*, **22**, 983 (1984).

(Received May 11, 1992)