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Structure Elucidation of Oxygenated Sterols from Eggs of Sea Hare, *Aplysia juliana*

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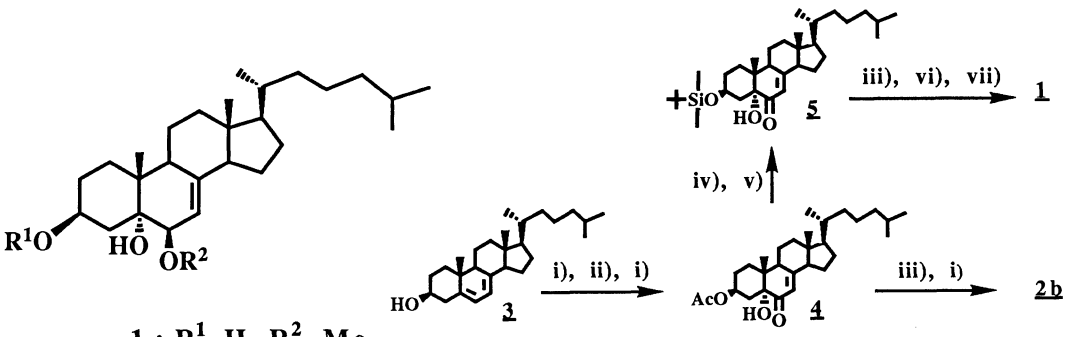
Cholest-7-en-6R-methoxy-3S,5R-diol, a new sterol, and cholest-7-en-3S,5R,6R-triol were isolated from eggs of sea hare, *Aplysia juliana*, and their structures and absolute configurations deduced by spectroscopic studies and chemical synthesis.

Polyhydroxylated sterols are common metabolites in marine invertebrates[1] and sponges.[2] Recently, Komori *et al*. isolated a cholesterol peroxide derivative[3] 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 SiO_2 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 ^{13}C-NMR spectra of 1[5] indicated the presence of three sp^3 carbons attached to oxygen atoms and two olefinic carbons. The ^{1}H-NMR spectra[5] 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 ^{1}H-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 Δ^7-sterol suggested a 7-en-6-methoxy-3β,5α-diol. The cholesterol-type side chain of 1 was deduced from ^{13}C-NMR and ^{1}H-NMR.[2] Thus, the structure of this sterol was formulated as cholest-7-en-6-methoxy-3β,5α-diol.

The ^{1}H-NMR and ^{13}C-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, Na_2CrO_4 oxidation, $NaBH_4$ reduction and reacetylation. Reduction of 4 with $NaBH_4$ 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 ^{1}H-NMR, ^{13}C-NMR, IR, EI/MS and $[\alpha]_D$ were identical with those of 1.[5]



1 : R^1=H, R^2=Me
2a : R^1=R^2=H
2b : R^1=R^2=Ac

i) Ac_2O / Py, cat. DMAP, r. t., 3 h
ii) Na_2CrO_4 / Ac_2O / AcOH, 0 °C, 4 h
iii) $NaBH_4$ / THF / IPA, r. t., 20 h
iv) 0.5 M KOH-MeOH / $CHCl_3$, 0 °C, 2 h
v) $^tBuMe_2SiCl$ / Et_3N / CH_2Cl_2, cat. DMAP, r. t., overnight
vi) NaH / DMSO, r. t., 1 h, then MeI, r. t., 1 h
vii) AcOH / H_2O / THF, r. t., 6 h

Compound 1 was isolated from a natural source for the first time. Compound 2a was reported by F. Cafieri[7] 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 D_3 may be biosynthesized from 7-dehydrocholesterol 3[8] as defense substances, since the biological significance of Vitamin D_3 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

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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 miligrams of 1 and 2a were obtained from eggs of *A. juliana* (3.5 kg, wet wt.), respectively. 1: $[\alpha]_D$ -55.6° (c 0.19, CHCl3); IR (CHCl3), ν 3300-3500; EI/MS, m/z (rel. int.) 432(M^+, 2), 414(M^+-H2O, 100), 399(M^+-H2O-Me, 24), 381(M^+-2H2O-Me, 46), 365(M^+-2H2O-OMe, 40), 364(M^+-2H2O-MeOH, 25), 251(M^+-2H2O-MeOH-side chain, 19); ^{1}H-NMR (500MHz, CDCl3), 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-Hax), 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-Heq), 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, CDCl3), 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).
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