After concentration of the solvent, the residue was recrystallized from *n*-hexane–ether (three times) to give **18** as colorless prisms, mp 182–184 °C.

1-[4(*S*)-(Isopropoxycarbonyl)-2-oxazolinyl]-5-methoxynaphthalene [(+)-20]. To a stirred suspension of amide **5** (1.65 g, 8.2 mmol) in dry 1,2-dichloroethane (80 mL) was added triethyloxonium tetrafluoroborate (2.02 g, 10.46 mmol) under argon at room temperature and the mixture stirred for 20 h. (−)-Serine isopropyl ester (2.26 g, 12.3 mmol) was added, in one portion, and the reaction mixture was heated at reflux for 20 h. After cooling, the mixture was poured into brine (50 mL), the organic layer was separated, and the aqueous layer was extracted with dichloromethane (15 mL × 2). The combined extracts were dried (Na_2SO_4) and concentrated to give a residue, which was chromatographed (silica gel; hexane–ethyl acetate, 4:1) to give the oxazoline ester **20** as colorless plates (2.42 g, 94.2%): mp 75.5–76.5 °C (*n*-hexane); $[\alpha]^{25}_D$ +77.14° (*c* 3.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.63 (d, 1 H, *J* = 8.8 Hz), 8.74 (d, 1 H, *J* = 8.5 Hz), 8.09 (dd, 1 H, *J* = 7.3 and 1.4 Hz), 7.52–7.45 (m, 2 H), 6.85 (d, 1 H, *J* = 7.7 Hz), 5.16 (m, 1 H), 5.04 (dd, 1 H, *J* = 10.5 and 7.6 Hz), 4.68 (br t, 1 H, *J* = 8.1 Hz), 4.60 (dd, 1 H, *J* = 10.5 and 8.7 Hz), 3.98 (s, 3 H), 1.34 (d, 3 H, *J* = 6.8 Hz), 1.33 (d, 3 H, *J* = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 170.8, 166.5, 155.6, 132.5, 129.8 (×2), 127.4, 126.2 (×2), 123.8, 118.9, 104.4, 69.8, 69.2, 69.0, 55.6, 21.8 (×2). IR (film): 1735, 1640, 1590, 1516, 1470, 1410 cm^{-1}.

Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.98; H, 6.11; N, 4.64. Found: C, 69.19; H, 6.08; N, 4.46.

1-[4(*R*)-(Hydroxymethyl)-2-oxazolinyl]-5-methoxynaphthalene [(+)-21]. To a suspension of lithium aluminum hydride (0.363 g, 9.57 mmol) in dry ether (20 mL) at 0 °C under argon was added a solution of **20** (2.0 g, 6.38 mmol) in dry ether (30 mL) over a period of 10 min. After the mixture was stirred for 1 h at 0 °C, the excess reagent was decomposed by the dropwise addition of water and filtered through Celite. Evaporation of the solvent gave a pale yellow gum, which was chromatographed (silica gel; hexane–ethyl acetate–methanol, 15:15:1) to give the oxazoline alcohol **21** as colorless needles (1.34 g, 81.6%): mp 88.0–89.0 °C (*n*-hexane–Et_2O); $[\alpha]^{25}_D$ +9.93° (*c* 3.03, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.52 (d, 1 H, *J* = 8.7 Hz), 8.44 (d, 1 H, *J* = 8.3 Hz), 8.04 (dd, 1 H, *J* = 7.3 and 0.7 Hz), 7.50–7.41 (m, 2 H), 6.85 (d, 1 H, *J* = 7.7 Hz), 4.58–4.52 (m, 1 H), 4.88 (br t, 1 H, *J* = 7.4 Hz), 4.30 (br t, 1 H, *J* = 7.4 Hz), 3.99 (s, 3 H), 3.94 (dd, 1 H, *J* = 11.4 and 3.7 Hz), 3.69 (dd, 1 H, *J* = 11.4 and 4.3 Hz), 2.60 (br s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 165.7, 155.6, 132.2, 129.6, 127.3, 126.2, 125.9, 124.4, 123.9, 118.7, 104.4, 68.9 (×2), 64.3, 55.6; IR ($CHCl_3$) 3600–3150 (br OH), 1636, 1585, 1502, 1460, 1402, 1348, 1245, 1010 cm^{-1}.

Anal. Calcd for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.21; H, 5.75; N, 5.33.

1-[4(*R*)-(Methoxymethyl)-2-oxazolinyl]-5-methoxynaphthalene [(+)-22]. To a stirred suspension of potassium *tert*-butoxide (0.63 g, 5.61 mmol) in dry THF (20 mL) was added a solution of **21** (1.2 g, 4.66 mmol) in dry THF (30 mL) under argon at room temperature. After the mixture was stirred for 12 h, iodomethane was added dropwise, and the reaction mixture was stirred for an additional 3 h.

Ether (50 mL) was added, and the organic phase was washed with water and dried (Na_2SO_4). Concentration in vacuo gave a yellow oil, which was chromatographed (silica gel; hexane–ethyl acetate–methanol, 15:10:1) to give the oxazoline **22** (0.934 g, 73.8%) as a colorless oil: $[\alpha]^{25}_D$ +25.76° (*c* 4.53, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.61 (d, 1 H, *J* = 8.7 Hz), 8.43 (d, 1 H, *J* = 8.4 Hz), 8.06 (d, 1 H, *J* = 7.2 Hz), 7.50–7.42 (m, 2 H), 6.81 (d, 1 H, *J* = 7.7 Hz), 4.66–4.55 (m, 1 H), 4.48 (t, 1 H, *J* = 8.3 Hz), 4.31 (t, 1 H, *J* = 8.3 Hz), 3.94 (s, 3 H), 3.74 (dd, 1 H, *J* = 9.4 and 4.3 Hz), 3.51 (dd, 1 H, *J* = 9.4 and 6.8 Hz), 3.41 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.0, 155.7, 132.5, 129.6, 127.3, 126.3, 125.8, 124.6, 123.9, 119.0, 104.3, 75.1, 69.7, 67.4, 59.3, 55.6; IR (film) 1642, 1587, 1513, 1456, 1408, 1258, 1011, 780 cm^{-1}.

Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.14; H, 6.22; N, 5.15.

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Supplementary Material Available: X-ray data for 18 (6 pages). Ordering information is given on any current masthead page.

Novel Cytotoxic Monoterpenes Having a Halogenated Tetrahydropyran from *Aplysia kurodai*

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The structures of four new halogenated monoterpenes, aplysiapyranoids A, B, C, and D, the constituents of a midgut gland of *Aplysia kurodai*, are presented. They have a 2-(2-chlorovinyl)-2,6,6-trimethyltetrahydropyran skeleton. The conformations of aplysiapyranoids A and B are mobile, and they exhibit only ambiguous NMR signals. The structure of aplysiapyranoid A was deduced from that of aplysiapyranoid B, which has been determined by X-ray analysis. On the contrary, aplysiapyranoids C and D have fixed conformations, and their structures were elucidated by means of conventional spectroscopic analysis.

Since aplysin, the first brominated sesquiterpene, was isolated from *Aplysia kurodai* Baba,[1] the constituents of the mollusc and its congeners have been attracting chemists' interest, and a variety of unique compounds have been isolated from the animals.[2] The types of compounds strongly depend on the places where the molluscs are collected, and therefore, the ingredients isolated from them are considered to originate from the seaweeds on which they feed. This paper describes the structure of a new type

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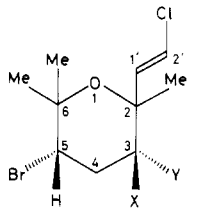
(1) Yamamura, S.; Hirata, Y. *Tetrahedron* **1963**, *19*, 1485.
(2) (a) Yamamura, S.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 2560. (b) Miyamoto, T.; Higuchi, R.; Komori, T. *Tetrahedron Lett.* **1986**, *27*, 1153.

0022-3263/87/1952-4597$01.50/0

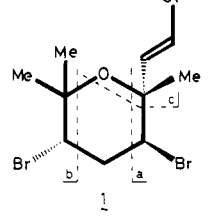
of halogenated monoterpene isolated from *A. kurodai*, which was collected at the Izu-Shimoda beach, a southeast coast of Japan.

A methanol extract of a midgut gland of *A. kurodai* was separated chromatographically to yield four new compounds, named as aplysiapyranoids A (**1**, 43 mg), B (**2**, 51 mg), C (**3**, 49 mg), and D (**4**, 139 mg).



1: X=Br, Y=H; α-chlorovinyl
2: X=Br, Y=H; β-chlorovinyl
3: X=H, Y=Cl; α-chlorovinyl
4: X=H, Y=Cl; β-chlorovinyl

Aplysiapyranoid A (**1**), $C_{10}H_{15}OBr_2Cl$, a colorless oil, $[\alpha]_D$ +4.4° (*c* 1.0, $CHCl_3$), shows IR absorptions at 1600 and 950 cm^{-1} (trans-disubstituted HC=CH) and 1120 and 1070 cm^{-1} (ether). The absence of OH and C=O bands in the spectrum indicates that the oxygen is involved only in ether linkage. The ^1H and ^{13}C NMR spectra ($CDCl_3$) suggested the occurrence of a *trans*-2-chlorovinyl group (CH=CHCl) [^1H NMR δ 6.16 (1 H, d, *J* = 14 Hz), 6.12 (1 H, d, *J* = 14 Hz); ^{13}C NMR δ 138.5 (d), 118.5 (d)], two tertiary carbons possessing a bromine atom (CHBr) [^1H NMR δ 4.45 (1 H, dd, *J* = 6, 4 Hz), 4.38 (1 H, dd, *J* = 8, 5 Hz); ^{13}C NMR δ 55.2 (d), 54.7 (d)], one methylene group [^1H NMR δ 2.65 (1 H, ddd, *J* = 15, 8, 4 Hz), 2.61 (1 H, ddd, *J* = 15, 6, 5 Hz); ^{13}C NMR δ 37.4 (t)] adjacent to both the tertiary carbons, two quaternary carbons bearing an oxygen atom [^{13}C NMR δ 76.1 (s), 75.8 (s)], and three methyl groups [^1H NMR δ 1.39, 1.37, 1.35 (each 3 H, s); ^{13}C NMR δ 29.1, 28.7, 27.4 (each q)]. On the basis of these data, structure **1** (without stereochemistry) was proposed for aplysiapyranoid A. Location of the chlorine atom on the vinyl group was confirmed from the chemical shifts (^{13}C) of the two sp^2 carbons[3] and further verified by observation of the fragments **1a**, **1b**, and **1c** in the high-resolution mass spectrum.

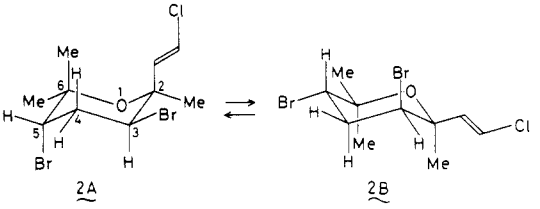


1

a: BrCH=C(Me)-CH=CHCl m/e 179.9355
b: BrCH=C(Me)-Me m/e 133.9723
c: [Me(C=OH)-CH=CHCl]$^+$ m/e 105.0118

Tetrahydropyran is known to exist in a chair conformation,[4] and the stereochemistry of C-3 and C-5 could, in principle, be determined by analyzing the coupling patterns of H-3 and H-5 in the ^1H NMR spectrum. However, the situation is more complicated in this case. Both protons appear as double doublets due to the couplings with the neighboring methylene protons (4-CH_2), but the *J* values of 6 and 8 Hz for H-3 and H-5 are too small for ordinary axial–axial couplings ($J \simeq 10$ Hz) and too big for axial–equatorial or equatorial–equatorial couplings ($J \simeq 4$ Hz). Whereas **1** exhibited sharp signals in the ^1H and ^{13}C spectra taken at 28 °C, serious broadening of the signals was observed when the temperature was lowered to −40 °C. These facts indicate that **1** exists in two or more conformations that interchange rapidly at 28 °C and relatively slowly on a NMR time scale at −40 °C. In fact, a NOESY experiment (28 °C), carried out in an attempt to deduce the relative configurations of the substituents, showed unreasonable cross peaks possibly due to saturation transfer caused by the interconversion of the conformations. The stereochemistry of **1** was eventually deduced on the basis of the structures of its congeners, aplysiapyranoids B, C, and D, as will appear later.

Aplysiapyranoid B (**2**), $C_{10}H_{15}OBr_2Cl$, $[\alpha]_D$ −27° (*c* 0.91, $CHCl_3$), exhibits spectral properties similar to those of **1**. The mass spectrum of **2** shows essentially the same fragments as those of **1**, indicating that the positions of the halogen atoms are identical in **1** and **2**. Also, the ^1H signals broadened when the spectrum was measured at low temperature (−30 °C). Fortunately, aplysiapyranoid B could be crystallized from ethanol, and its relative stereochemistry together with its absolute configuration was determined by X-ray crystallography (see **2**).[5] In the crystalline state, **2** takes the conformation **2A**, in which the chlorovinyl group at C-2 and the bromine atom on C-5 are oriented in axial positions. In solution, **2A** would equilibrate with the inverted conformation **2B**. The free energy



2A **2B**

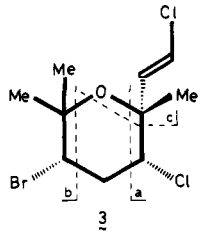
difference between the two conformations would be small because the orientation of one bromine atom is axial and the other equatorial in each conformer. This would result in an almost equal population of the two conformers in solution, besides the low-energy barrier of interconversion, and therefore cause the broadening of the NMR signals.

Aplysiapyranoid C (**3**), a colorless oil, $[\alpha]_D$ +52° (*c* 1.0, $CHCl_3$), has the molecular formula $C_{10}H_{15}OBrCl_2$ and was deduced to have the structure in which one of the bromine atoms of **1** or **2** was replaced by a chlorine atom. Indeed, the spectral features of **3** are closely related with those of **1** and **2**; *trans*-CH=CHCl [^1H NMR ($CDCl_3$) δ 6.41 (1 H, d, *J* = 13.7 Hz), 6.34 (1 H, d, *J* = 13.7 Hz); ^{13}C NMR ($CDCl_3$) δ 133.9 (d), 123.0 (d)], CH(Br)CH_2CH(Cl) [^1H NMR δ 3.87 (1 H, dd, *J* = 12.0, 4.7 Hz), 3.72 (1 H, dd, *J* = 12.0, 4.8 Hz), 2.52 (1 H, ddd, *J* = 13.1, 4.8, 4.7 Hz), 2.46 (1 H, ddd, *J* = 13.1, 12.0, 12.0 Hz); ^{13}C NMR δ 62.1 (d), 53.6 (d), 37.9 (t)], and Me_2COCMe [^1H NMR δ 1.40, 1.33, 1.30 (each 3 H, s); ^{13}C NMR δ 77.0 (s), 76.4 (s), 30.1 (q), 29.1 (q), 24.0 (q)]. The positions of the halogen atoms were confirmed by appearance of the fragments **3a**, **3b**, and **3c** in the mass spectrum. In contrast with **1** and **2**, the ^1H and ^{13}C NMR signals of **3** remain sharp even at −90 °C,

(3) (a) Stierle, D. B.; Wing, R. M.; Sims, J. J. *Tetrahedron* **1979**, *35*, 1261 and 2855. (b) Crews, P. J. *J. Org. Chem.* **1977**, *42*, 2634. (c) Gonzalez, A. G.; Arteaga, J. M.; Martin, J. D.; Rodriguez, M. L.; Fayos, J.; Ripolls, M. M. *Phytochemistry* **1978**, *17*, 947. (d) Higgs, M. D.; Vanderah, D. J.; Faulkner, D. J. *Tetrahedron* **1977**, *33*, 2775. (e) Norton, R. S.; Warren, R. G.; Wells, R. J. *Tetrahedron Lett.* **1977**, *18*, 3905. (f) Castedo, L.; Garcia, M. L.; Quinoa, E.; Riguera, R. *J. Nat. Prod.* **1984**, *47*, 724.

(4) Katritzky, A. R.; Rees, C. W. *Comprehensive Heterocyclic Chemistry*; Boulton, A. J., McKillop, A., Eds.; Pergamon: New York, 1984; Vol. 3, p 579.

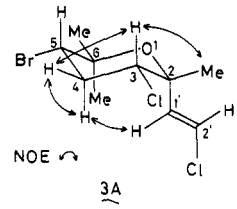
(5) Inouye, Y.; Uchida, H.; Kusumi, T.; Kakisawa, H. *J. Chem. Soc., Chem. Commun.* **1987**, 346.



3

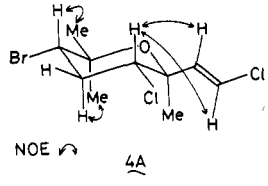
a: ClCH=C(Me)-CH=CHCl m/e 135.9798
b: BrCH=C(Me)-Me m/e 133.9734
c: [Me(C=OH)-CH=CHCl]$^+$ m/e 105, 107

and only one set of signals are found in the spectra. Moreover, the coupling patterns of the ^{1}H signals do not change from 25 to −90 °C. This indicates that aplysiapyranoid C (**3**) takes on only one fixed conformation within a wide range of temperatures. The axial orientations of H-3 and H-5 were obvious from their coupling constants ($J_{3,4ax}$ = 12.0 Hz, $J_{5,4ax}$ = 12.0 Hz), and this infers that the tetrahydropyran skeleton of **3** assumes the chair conformation. The presence of NOEs between 4-H_{ax} and 1′-H (see **3A**) reveals that the chlorovinyl group is axially oriented. The other NOEs depicted in **3A** confirmed the relative configurations of the substituents as in **3A**.



3A

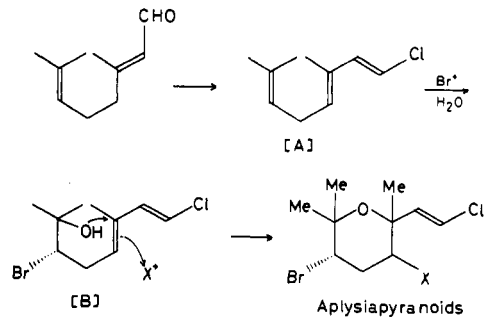
The structure of aplysiapyranoid D (**4**), $C_{10}H_{15}OBrCl_2$, a colorless oil, $[\alpha]_D$ +3.4° (*c* 1.1, $CHCl_3$), was determined essentially in the same manner as described above. The positions of the halogen atoms were confirmed by the mass spectrum, which exhibited the fragments at *m/e* 284.9508 (M^+ − Me), 135.9739 [ClCH=C(Me)CH=CHCl] and 133.9719 [BrCH=C(Me)Me]. The axial orientations of H-3 (δ 3.08) and H-5 (δ 3.19) were determined from their coupling patterns ($J_{3,4ax}$ = 12.7 Hz, $J_{5,4ax}$ = 12.7 Hz) in the ^{1}H NMR spectrum (C_6D_6). Also, the relative configurations of the substituents were determined by observation of the NOEs (see **4A**). This fact indicates that the principal factor preventing the conformation of the tetrahydropyran ring from inversion is the presence of the equatorial halogen atoms at C-3 and C-5. If the ring is converted into another chair conformation, these halogen atoms are oriented in a 1,3-diaxial relationship, which would result in serious steric repulsion.



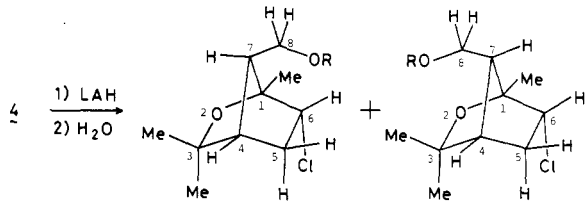
4A

The stereochemistry of aplysiapyranoid A (**1**) was deduced at this stage. The flexibility of the conformation of **1** indicated that one of the two bromine atoms possesses an axial configuration and the other is oriented equatorially as in aplysiapyranoid B (**2**). Because the 2*S*,3*S*,5*S* configurations of **2** were firmly established, the only possible structure of aplysiapyranoid A that fulfills the anti relationship of the bromine atoms must be **1** (2*S**,3*R**,5*R**).[6]

Scheme I



In attempts at chemical transformations of the aplysiapyranoids, we found a unique reaction. When aplysiapyranoid D (**4**) was treated with lithium aluminum hydride in ether under a nitrogen atmosphere, the unexpected products **5a** and **6a**, separable as their acetates **5b** and **6b**, were obtained after aqueous workup. The



5a: R = H
5b: R = Ac
6a: R = H
6b: R = Ac

structures of the compounds were determined by spectroscopic analysis. Although the other analogue of **4** produced the same type of compounds, the reactions were more complicated.

A possible biosynthetic pathway of the aplysiapyranoids is described in Scheme I. The chlorotriene [A], which would be derived from farnesal, is hydroxybrominated to give the bromohydrin [B]. Attack of a chloro or a bromo cation would result in cyclization to produce the aplysiapyranoids. An attempted synthesis of aplysiapyranoids through the hypothetical precursor [A] is now in progress.

Aplysiapyranoids exhibit moderate cytotoxicities against Vero, MDCK, and B_{16} cells (IC_{50} = 19–96 μg/mL). Among the aplysiapyranoids, aplysiapyranoid D (**4**) is most active, and it also shows activity against human tumor cells (Moser; IC_{50} = 14 μg/mL).

Experimental Section

High-resolution mass spectra were recorded on a JEOL JMS-DX-300 spectrometer. Low-resolution mass spectra were taken on a Hitachi RMU-6M spectrometer. ^{1}H and ^{13}C NMR spectra were measured on JEOL FX-90Q and Bruker AM-400 and AM-500 spectrometers. Optical rotations were measured on a JASCO DIP-181 polarimeter. Infrared spectra were recorded on a Hitachi Grating 215 infrared spectrophotometer.

Materials. *A. kurodai* Baba was collected at the Nabeta Bay of Izu-Shimoda coast in June, 1985. (The reference specimen is preserved at the Shimoda Institute of Marine Biology, the University of Tsukuba.) The midgut gland was immediately taken out and soaked in MeOH. After 12 days of extraction, the gland

(6) Aplysiapyranoids have three asymmetric centers. If one neglects the absolute configuration and the kind of halogen atoms, the four possible sets of stereochemistry can be described as (a) 2*S**,3*S**,5*S**, (b) 2*S**,3*R**,5*R**, (c) 2*S**,3*S**,5*R**, and (d) 2*S**,3*R**,5*S**. Of these, (c) and (d) correspond to aplysiapyranoids C (**3**) and D (**4**), respectively, in which the two halogen atoms are located in a syn manner. The sets (a) and (b) fulfill the anti relationship of the halogen atoms, and aplysiapyranoid B (**2**) has the 2*S*,3*S*,5*S* configuration (a). Therefore, the only possible set that is left for **1** is (b).

was homogenized and further extracted with MeOH for 3 months. The methanol extract was concentrated to afford an oily residue, which was successively washed with CH_2Cl_2 and EtOAc. The CH_2Cl_2 layer was concentrated to give a dark green residue (935 mg). A part (215 mg) of this material was chromatographed on silica gel (Wakogel C-300, 10 g). The fraction (147 mg) eluted with CH_2Cl_2 was further separated by silica gel (15 g) chromatography. Elution with hexane–CH_2Cl_2 (8:2) afforded a fraction, concentration of which resulted in a colorless oil (106 mg). Flash chromatography on silica gel [40 g, hexane–CH_2Cl_2 (100:1)] followed by separation with preparative TLC gave aplysiapyranoid A (**1**, 15 mg). Aplysiapyranoids B (**2**, 18 mg), C (**3**, 17 mg), and D (**4**, 40 mg) were obtained in essentially the same manner as described above from the CH_2Cl_2 extract (215 mg).

Aplysiapyranoid A (1): High-resolution MS (Br* and Cl* correspond to ^{81}Br and ^{37}Cl, respectively), *m/e* 312.9486 (M^+ – Cl, $C_{10}H_{15}OBr*_2$), 310.9473 ($C_{10}H_{15}OBrBr*$), 308.9472 ($C_{10}H_{15}OBr_2$), 183.9330 ($C_5H_6Br*Cl*$), 181.9374 (C_5H_6Br*Cl/C_5H_6BrCl*), 179.9355 (**1a**; C_5H_6BrCl), 135.9686 (C_4H_7Br*), 133.9723 (**1b**; C_4H_7Br), 107.0073 (C_4H_6OCl*), 105.0118 (**1c**; C_4H_6OCl); CI MS (CH_4), *m/e* 351, 349, 347, 345 ($M^+ + 1$).

Aplysiapyranoid B (2): mp 46–49 °C (EtOH); high-resolution MS, *m/e* 312.9455 (M^+ – Cl; $C_{10}H_{15}OBr*_2$), 310.9437 ($C_{10}H_{15}OBr*Br$), 308.9466 ($C_{10}H_{15}OBr_2$), 266.9912 (M^+ – Br; $C_{10}H_{15}OBr*Cl/C_{10}H_{15}OBrCl*$), 265.0008 ($C_{10}H_{15}OBrCl$); CI MS ($CH_4$), *m/e* 351, 349, 347, 345 ($M^+ + 1$); low-resolution MS (EI), *m/e* 333, 331, 329 (M^+ – Me), 313, 311, 309 (M^+ – Cl), 267, 265 (M^+ – Br), 184, 182, 180, 136, 134, 107, 105; IR (CCl_4) 2980, 2930, 1610, 1450, 1370, 1120, 1060, 980, 930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, –30 °C) δ 6.30 (1 H, d, *J* = 13.3 Hz), 6.12 (1 H, d, *J* = 13.3 Hz), 4.32 (1 H, br dd, *J* = 7.2, 5.1 Hz), 4.31 (1 H, br dd, *J* = 6.4, 4.6 Hz), 2.65 (1 H, ddd, *J* = 15.1, 7.2, 4.6 Hz), 2.61 (1 H, ddd, *J* = 15.1, 6.4, 5.1 Hz), 1.49, 1.35, 1.34 (each 3 H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$, 28 °C) δ 137.3 (d), 121.0 (d), 76.1 (s), 75.7 (s), 56.1 (d), 54.3 (d), 37.1 (t), 29.5 (q), 28.9 (q), 28.5 (q).

Aplysiapyranoid C (3): High-resolution MS, *m/e* 286.9519 (M^+ – Me; $C_9H_{12}OBr*Cl_2/C_9H_{12}OBrCl*Cl$), 284.9512 ($C_9H_{12}OBrCl_2$), 266.9909 ($M^+$ – Cl; $C_{10}H_{15}OBr*Cl/C_{10}H_{15}OBrCl*$), 265.0016 ($C_{10}H_{15}OBrCl$), 139.9778 ($C_5H_6Cl*_2$), 137.9806 ($C_5H_6ClCl*$), 135.9798 (**3a**; $C_5H_6Cl_2/C_4H_7Br*$), 133.9734 (**3b**; C_4H_7Br); CI MS (CH_4), *m/e* 307, 305, 303, 301 ($M^+ + 1$); IR (CCl_4) 2980, 2920, 1610, 1460, 1370, 1300, 1140, 1090, 980, 940 cm^{-1}.

Aplysiapyranoid D (4): High-resolution MS, *m/e* 288.9399 (M^+ – Me; $C_9H_{12}OBr*Cl*Cl/C_9H_{12}OBrCl*_2$), 286.9478 ($C_9H_{12}OBr*Cl_2/C_9H_{12}OBrCl*Cl$), 284.9508 ($C_9H_{12}OBrCl_2$), 265.0001 ($M^+$ – Cl, $C_{10}H_{15}OBrCl$), 139.9703 ($C_5H_6Cl*_2$), 137.9771 (C_5H_6ClCl*), 135.9739 ($C_5H_6Cl_2$), 133.9719 (C_4H_7Br); CI MS (CH_4), *m/e* 307, 305, 303, 301 ($M^+ + 1$); IR (CCl_4) 2980, 2930, 1640, 1620, 1460, 1450, 1370, 1340, 1210, 1120, 1070, 960, 930 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 28 °C) δ 6.20 (1 H, d, *J* = 13.1 Hz), 6.03 (1 H, d, *J* = 13.1 Hz), 3.19 (1 H, dd, *J* = 12.7, 4.3 Hz), 3.08 (1 H, dd, *J* = 12.7, 4.3 Hz), 2.08 (1 H, ddd, *J* = 12.7, 12.7, 12.7 Hz), 1.99 (1 H, ddd, *J* = 12.7, 4.3, 4.3 Hz), 1.09, 1.08, 1.03 (each 3 H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$, 28 °C) δ 137.6 (d), 119.7 (d), 77.6 (s), 76.6 (s), 61.3 (d), 53.5 (d), 37.6 (t), 30.4 (q), 23.4 (q), 21.1 (q).

Reduction of Aplysiapyranoid D (4). To a solution of **4** (11 mg, 0.037 mmol) in absolute ether (1 mL) was added lithium aluminum hydride (15 mg, 0.39 mmol) at 0 °C. The solution was stirred at 0 °C under nitrogen for 45 min. Water (1 mL) was added to the reaction mixture (hydrogen evolution), and the resultant mixture was extracted with five 10-mL portions of ether. The ether layer was washed with brine and dried over Na_2SO_4. Evaporation of the ether gave a colorless oil (10 mg). This substance (4 mg), an inseparable mixture of **5a** and **6a** (3:2), showed, after purification by preparative TLC [Merck, Kieselgel 60 GF_{254}, hexane–EtOAc (1:1)], the following spectral properties: MS, *m/e* (relative intensity) 206 (3), 204 (8) (M^+), 175 (1), 173 (3) (M^+ – CH_2OH), 141 (44) (M^+ – CH_2=CHCl – H), 120 (5), 118 (15) (Me_2C=$CHCH_2CH_2Cl$), 111 (19) (M^+ – Me_2C=O), 82 (100) (Me_2C=CHCH=CH_2), 87 (68) [$HOCH_2CHC$(=O)Me]; IR (film) 3400, 2870, 1460, 1380, 1160, 1090, 1030 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) (assignment of the signals was performed for the mixture and verified by the COSY spectrum) [**5a**] δ 3.61 (dd, *J* = 11, 3 Hz, H-6), 3.00 (dd, *J* = 13, 8 Hz, H-8), 2.93 (dd, *J* = 13, 8 Hz, H-8), 2.23 (t, *J* = 8 Hz, H-7), 1.93 (dd, *J* = 14, 3 Hz, H-5), 1.79 (m, H-6), 1.62 (d, *J* = 2 Hz, H-4), [**6a**] δ 3.61 (dd, *J* = 11, 3 Hz, H-6), 3.51 (dd, *J* = 12, 8 Hz, H-8), 3.42 (dd, *J* = 12, 7 Hz, H-8), 1.83 (dd, *J* = 13, 3 Hz, H-5), 1.70 (d, *J* = 2 Hz, H-4), 1.60 (m, H-5), 1.35 (overlapped with impurity signal, H-7), [methyl signals of **5a** and **6a**] δ 1.37 (s), 1.21 (s), 1.11 (s). This mixture (4 mg) was treated with acetic anhydride (50 μL) and pyridine (50 μL) at room temperature for 18 h and worked up. Separation of the products (4 mg) with prepative TLC [hexane–CH_2Cl_2 (3:7), eight times development] afforded the acetates **5b** (1 mg) and **6b** (2 mg). **5b**: MS, *m/e* 246.0977 (M^+; $C_{12}H_{19}O_3Cl$), 211.1399 (M^+ – Cl; $C_{12}H_{19}O_3$), 189.0772 (M^+ – AcO; $C_{10}H_{16}OCl*$), 187.0807 ($C_{10}H_{16}OCl$); ^1H NMR (500 MHz, C_6D_6) δ 3.78 (1 H, dd, *J* = 11.6, 7.5 Hz, H-8), 3.66 (1 H, dd, *J* = 11.6, 7.5 Hz, H-8), 3.56 (1 H, dd, *J* = 10.6, 3.9 Hz, H-6), 2.42 (1 H, br dd, *J* = 7, 7 Hz, H-7), 1.89 (1 H, ddd, *J* = 14.7, 3.9, 1.0 Hz, H-5), 1.81 (1 H, ddd, *J* = 14.7, 10.6, 4.2 Hz, H-5), 1.64 (3 H, s), 1.55 (1 H, dd, *J* = 4.2, 1.6 Hz, H-4), 1.31, 1.15, 1.05 (each 3 H, s); ^{13}C NMR (125 MHz, C_6D_6) δ 170.2 (s), 85.5 (s), 79.5 (s), 62.7 (d), 61.5 (t), 52.0 (d), 48.1 (d), 32.3 (t), 29.4 (q), 25.1 (q), 20.3 (q), 16.4 (q). **6b**: MS, *m/e* 183.1015 (M^+ – CHCl=CH_2; $C_{10}H_{15}O_3$), 189.0856 (M^+ – AcO; $C_{10}H_{16}OCl*$), 187.0881 ($C_{10}H_{16}OCl$); ^1H NMR (500 MHz, C_6D_6) δ 4.26 (1 H, dd, *J* = 11.1, 8.3 Hz, H-8), 4.17 (1 H, dd, *J* = 11.1, 5.1 Hz, H-8), 3.54 (1 H, dd, *J* = 10.7, 2.8 Hz, H-6), 1.80 (1 H, dd, *J* = 14.2, 2.8 Hz, H-5), 1.67 (3 H, s), 1.65 (1 H, d, *J* = 3.9 Hz, H-4), 1.57 (1 H, ddd, *J* = 14.2, 10.6, 3.8 Hz, H-5), 1.53 (1 H, br dd, *J* = 8.3, 5.1 Hz, H-7), 1.32, 1.14, 0.99 (each 3 H, s); ^{13}C NMR (125 MHz, C_6D_6) δ 169.8, 86.4, 79.9, 63.7, 62.5, 52.3, 49.7, 35.2, 29.6, 27.1, 20.4, 15.4. The correlations of the signals of **6b** were confirmed by the COSY spectra.

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