Aplydactone, a New Sesquiterpenoid with an Unprecedented Carbon Skeleton from the Sea Hare *Aplysia dactylomela*, and Its Cargill-Like Rearrangement

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Received September 5, 2000

A plethora of halogenated sesquiterpenoids have been isolated from opistobranch molluscs, belonging to the genus *Aplysia*.[1] Some contain rearranged carbon skeletons. As an example, we have recently described the isolation of a rearranged chamigrane-type sesquiterpene with two sp_2-hybridized carbons in α-positions to the spiro-atom from the extracts of the sea hare *Aplysia dactylomela*.[2] In this paper, we report the isolation,[3] structure elucidation, and unusual rearrangement of the unique sesquiterpenoid ketone **1** from the same animal. Extensive NMR analysis[4] suggested a highly strained cyclic system for **1**. We used X-ray analysis[5] to establish the structure and absolute stereochemistry of **1**. A crystal, suitable for this X-ray diffraction study, was obtained from the hexane−ethyl acetate (25:1) solution. Analysis of **1**, named aplydactone, has established that this sesquiterpenoid has an unprecedented skeleton consisting of three six-membered rings and two fused four-membered rings. It also demonstrated a dramatic aberrance in the lengths of carbon−carbon bonds and dihedral angles in comparison with usual limits. For example, the lengths of 1(6), 2(3), and 3(11) bonds are 1.608, 1.571, and 1.588 Å in contrast with 1.548 Å in cyclobutane, while C(11)−C(1)−C(6), C(2)−C(3)−C(11), C(1)−C(6)−C(12), and C(1)−C(11)−C(3) dihedral angles are equal to 85.3, 87.3, 87.5, and 87.3°, respectively. The structure of (1*R*,3*S*,6*S*,8*R*,11*S*)-8-bromo-3,7,7-trimethyl-tetracyclo[4.4.2.0^{1,6}.0^{3,11}]dodecan-4-one, established for **1** corresponded also to EIMS, IR, and CD spectra as well as to combustion analysis data.[6] The computer-generated perspective drawing of the aplydactone is given on the Figure 1. It was hard to imagine before the isolation of **1** that such compounds could be stable enough to exist in nature.

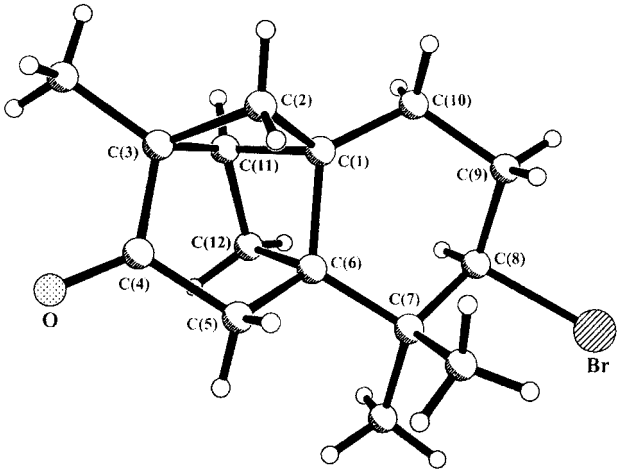
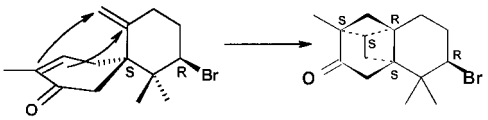


Figure 1. Computer-generated perspective drawing of **1**.

Scheme 1. Hypothetical Pathway for Biosynthesis of **1** from **2**



2 1

A hypothetical pathway for aplydactone biosynthesis is represented in Scheme 1**.** The chamigrane sesquiterpenoid **2**, previously described by us from the same mollusc,[7] may be a biosynthetic precursor of aplydactone (**1**)**.** In this case, the formation of the four-membered rings in **1** may be the result of enzymatic transformation involving a [2 + 2]-cycloaddition in **2**.

Presumably, similar biosynthetic transformations resembling [2 + 2]-cycloaddition are realized in many marine organisms that contain cyclobutane-containing secondary metabolites. The marine natural products, postulated previously as formally derived by a [2 + 2]-cycloaddition, may be exemplified by sceptrin, which is a symmetric dimer of 2-debromooroidin (= hymenidin) from the sponge *Agelas oroides*, related oxysceptrin, debromosceptrin, dibromosceptrin, debromooxysceptrin, and cycloanchinopeptolide from some sponges, belonging to the genera *Agelas* and *Anchinoe*.[8] Moreover, natural products with the same skeleton system as **1** might be predicted to be isolated from other molluscs feeding on algae, especially on *Laurencia*, due to the abundance of

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(3) The ethanol extract of *Aplysia dactylomela* (1 kg) was evaporated in vacuo. The obtained dark brown solid was repeatedly chromatographed on columns with Si gel, using the hexane−EtOAc (15:1) solvent system, to give 520 mg of terpenoid fraction. The fraction was subjected to HPLC on an Ultrasphere Si column with hexane−EtOAc (25:1) as eluent at a flow rate of 0.6 mL/min. As a result, the sesquiterpene **1** (44 mg) was obtained as an individual substance, mp 195−196 °C, $[\alpha]^{20}_{D}$ +33° (*c* 0.2, EtOH).

(4) ^{1}H NMR (300 MHz, C_6D_6) δ 0.70 (s, 3H, (C7)−C*H*$_3$ eq), 0.75 (s, 3H, (C7)−C*H*$_3$ ax), 1.05 (d, 1H, *H*12, $J_{H12,H'12}$ = 11.3 Hz), 1.09 (s, 3H, (C3)−C*H*$_3$), 1.29 (d, 1H, *H*2, $J_{H2,H'2}$ = 11.0 Hz), 1.30 (m, 2H, $H_2$10), 1.58 (d, 1H, *H*11, $J_{H11,H'12}$ = 5.6 Hz), 1.69 (m, 2H, $H_2$9), 1.72 (ddd, 1H, *H*′12, $J_{H12,H'12}$ = 11.3 Hz, $J_{H'12,H11}$ = 5.6 Hz, $J_{H'12,H'5}$ = 2.7 Hz), 1.81 (d, 1H, *H*′2, $J_{H'2,H2}$ = 11.0 Hz), 2.12 (d, 1H, *H*5, $J_{H5,H'5}$ = 16.5 Hz), 2.51 (dd, 1H, *H*′5, $J_{H'5,H5}$ = 16.5 Hz, $J_{H'5,H'12}$ = 2.7 Hz), 3.90 (m, 1H, *H*8); ^{13}C NMR (75 MHz, C_6D_6) δ 18.3 (q, (C7)−*C*H$_3$ ax), 18.6 (q, (C3)−*C*H$_3$), 22.9 (q, (C7)−CH$_3$ eq), 31.0 (t, *C*9), 31.7 (t, *C*12), 33.8 (t, *C*10), 38.2 (s, *C*7), 40.2 (s, *C*1), 40.6 (t, *C*2), 42.9 (t, *C*5), 45.5 (d, *C*11), 47.1 (s, *C*6), 48.9 (s, *C*3), 65.7 (d, *C*8), 210.4 (s, *C*4).

(5) Crystal data: $C_{15}H_{21}BrO$, M_r = 297.23, monoclinic, space group $P2_1$, a = 7.189(1) Å, b = 12.397(2) Å, c = 7.638(1) Å, β = 93.202(3)°, V = 679.6(2) Å^3, Z = 2, D_x = 1.452 Mg m^{-3}, Mo Kα radiation, λ = 0.71073 Å, cell parameters from 698 reflections, θ = 2.67−23.27°, μ = 3.01 mm^{-1}, T = 293 (2) K, box, dimensions 0.31 × 0.18 × 0.15; data collection: Bruker SMART-1000 CCD diffractometer, ω scans, absorption correction multiscan (SADABS.; Bruker, 1998), T_{min} = 0.598, T_{max} = 0.801, 3199 measured reflections, 1837 independent reflections, 1737 reflections with $I > 2\sigma(I)$, R_{int} = 0.027, θ_{max} = 23.27°, $h = -7 \rightarrow 7$, $k = -13 \rightarrow 13$, $l = -8 \rightarrow 8$; refinement: on F^2, $R[F^2 > 2\sigma(F^2)]$ = 0.027, $wR(F^2)$ = 0.067, S = 1.012, 1837 reflections, 157 parameters, H atoms constrained, $w = 1/[\sigma^2(F_o^2) + (0.0425P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$, $(\Delta/\sigma)_{max} < 0.001$, $\Delta\rho_{max}$ = 0.206 e Å^{-3}, $\Delta\rho_{min}$ = −0.166 e Å^{-3}, scattering factors from *International Tables for Crystallography* (Vol. C), absolute structure: Flack 1983, Flack parameter = 0.06 (1).

(6) EIMS *m*/*z* (%) 298/296 (11/11) $[M]^+$, 283/281 (16/16) $[M - CH_3]^+$, 217 (14) $[M - Br]^+$, 105 (100); IR ($CHCl_3$) ν/cm^{-1} 1704 (C=O); CD $[\theta]_{287}$ = +42.8 × 10^5. Anal. ($C_{15}H_{21}OBr$) C: calcd, 60.61; found, 60.47; H: calcd, 7.12; found, 7.06.

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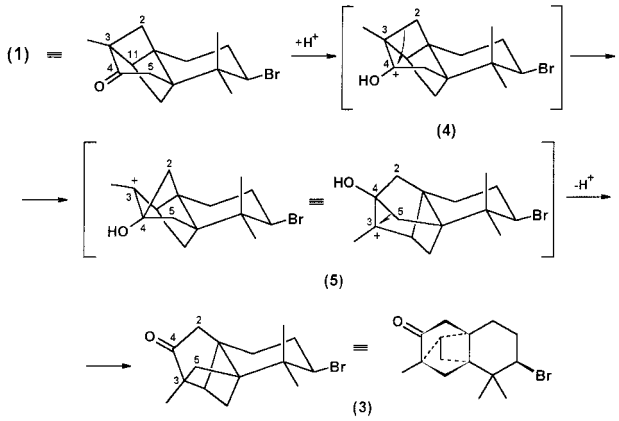
10.1021/ja003254t CCC: $20.00
Published on Web 12/23/2000

β-chamigrane sesquiterpenoids having an additional double bond in the position suitable for [2 + 2]-cycloaddition in these red algae.[9]

It is well-known that many [2 + 2]-cycloaddition products may be synthesized under conditions of UV-irradiation of the corresponding di-unsaturated compounds. However, attempts to obtain **1** by long-term UV-irradiation of **2** have failed. This suggests the in vivo formation of **1** but not during the isolation and storage of it.

We hypothesized that **1**, having an nucleophilic site, might be rearranged into a more stable compound by the action of a proton donor. In fact, reaction of **1** with *p*-TsOH gave almost quantitative yield of the isomeric compound **3.**[10] The structure of **3** was established by extensive NMR analysis, including ^{1}H−^{1}H COSY, HMQC, HMBC, and 1D-NOE experiments. A characteristic feature of these spectra consisted of a decrease of the geminal coupling constant of CH_2-12 protons to 7.9 Hz. Similar coupling constants were observed for analogous methylene group protons in bicyclo[2,1,1]hexanes.[11] A methyl group was located at a quaternary carbon adjacent to the carbonyl. These spectra indicated also a methine group in a four-membered ring and two methylene groups located between quaternary carbons. The halogen-containing ring was unchanged. On this basis and using EIMS, the structure with only one four-membered, two five-membered, and two six-membered rings was assigned to **3**. The

Scheme 2. Acid-catalyzed Rearrangement of **1** into **3**



CD spectrum confirmed the stereochemical features of **3** showed in Scheme 2. It demonstrated a positive Cotton effect with $[\theta]_{289} = +36 \times 10^5$.

In accordance with Scheme 2, the cation **4** is generated first as a result of the action of *p*-TsOH on the carbonyl group in the proposed rearrangement mechanism. The X-ray analysis data established that there are three highly strained bonds [1(6), 3(11), and 2(3)] in **1**. One of them, namely 2(3), is cleaved, followed by the formation of a new 2(4) bond (**4**→**5**). Alternative processes with the formation of the 6(4) or 4(11) bonds are less probable because of strong deformations of the remaining rings in these cases. Finally, cleavage of the 4(5) bond followed by the loss of a proton and formation of a new 3(5) bond completes the rearrangement (**5**→**3**).

The observed rearrangement is a variant of the Cargill rearrangement.[12] As it is known, the Cargill rearrangement consists of acid-catalyzed transformation of highly strained β,γ-unsaturated ketones with the cleavage of two bonds: one in the strained ring and another adjacent to a carbonyl. Our results have shown the analogous rearrangement may take place in highly strained systems such as **1**, even though they do not contain a double bond.

Acknowledgment. We thank Dr. Sergey G. Ilyin for his help in preparing the crystallographic data for publication.

Supporting Information Available: Tables of atomic coordinates, bond distances, dihedral angles, hydrogen coordinates, and anisotropic displacement parameters for **1**; the additional information on collection, extraction, and storage of the *Aplysia dactylomela* specimens and isolation of **1**; ^{1}H and ^{13}C NMR spectra of **1** in $CDCl_3$; survey ^{1}H and ^{13}C NMR spectra of **1** and **3** in C_6D_6 (PDF). An X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

JA003254T

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(10) The solution of aplydactone (20 mg) in benzene (3 mL) in the presence of 5 mg *p*-TsOH was heated to boiling. After the reaction mixture was boiling for 1 min, it was left at room temperature for 24 h, washed with water (3 × 3 mL), and evaporated in vacuo to dryness. The reaction product (**3**) was purified using HPLC as it was described above [3] and pure **3** (17 mg, 85% yield) was obtained. Colorless crystal, mp 112−113 °C; ^{1}H NMR (C_6D_6) δ 0.72 (s, 3H, (C7)−CH_3 eq), 0.75 (s, 3H, (C7)−CH_3 ax), 0.77 (d, 1H, *H*12, $J_{H12,H'12}$ = 7.9 Hz), 0.87 (dd, 1H, *H*′5, $J_{H'5,H5}$ = 11.5 Hz, $J_{H'5,H10}$ = 0.7 Hz), 0.97 (brdt, 1H, *H*10, $J_{H10,H'10}$ = 14.2 Hz, $J_{H10,H9}$ = 3.9 Hz, $J_{H10,H'9}$ = 3.9 Hz), 1.10 (s, 3H, (C3)−CH_3), 1.23 (dd, 1H, *H*5, $J_{H5,H'5}$ = 11.5 Hz, $J_{H5,H'12}$ = 2.9 Hz), 1.28 (d, 1H, *H*′2, $J_{H'2,H2}$ = 18.8 Hz), 1.40 (d, 1H, *H*11, $J_{H11,H'12}$ = 2.9 Hz), 1.42 (td, 1H, *H*′10, $J_{H'10,H10}$ = 14.2 Hz, $J_{H'10,H9}$ = 14.2 Hz, $J_{H'10,H'9}$ = 4.6 Hz), 1.51 (dt, 1H, *H*′12, $J_{H'12,H12}$ = 7.9, $J_{H'12,H11}$ = 2.9 Hz, $J_{H'12,H5}$ = 2.9 Hz), 1.63 (m, 1H, *H*9), 1.72 (m, 1H, *H*′9), 2.18 (d, 1H, *H*2, $J_{H2,H'2}$ = 18.8 Hz), 3.84 (dd, 1H, *H*8, $J_{H8,H9}$ = 11.9 Hz, $J_{H8,H'9}$ = 4.0 Hz); ^{13}C NMR (C_6D_6) δ 14.0 (q, (C3)−CH_3), 18.5 (q, (C7)−CH_3 ax), 23.4 (q, (C7)−CH_3 eq), 28.8 (t, C10), 31.7 (t, C9), 31.7 (t, C12), 37.5 (s, C7), 39.0 (t, C5), 43.1 (t, C2), 47.4 (s, C1), 48.5 (d, C11), 55.4 (s, C3), 57.8 (s, C6), 65.6 (d, C8), 214.0 (s, C4). Anal. ($C_{15}H_{21}OBr$) C: calcd, 60.61; found, 60. 41; H: calcd, 7.12; found, 7.07.

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