# Brominated Oxylipins and Oxylipin Glycosides from Red Sea Corals

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Eight new brominated oxylipins **1**–**8**, including two glycosides, were isolated from the Red Sea invertebrates *Dendrophyllia* sp., *Dendronephthya* sp. (red variety), *Dendronephthya* sp. (yellow variety), and *Tubipora musica*. Their structures were elucidated mainly on the basis of NMR spectroscopic data. The relative and absolute configurations were determined by analysis of NOESY and CD data and by the modified Mosher method. The compounds gave positive results in a brine shrimp toxicity assay, a sea urchin eggs test (*Paracentrotus lividus*), and a crown gall tumor on potato disks test (*Agrobacterium tumefaciens*).



## Introduction

The ongoing investigation of the chemical constituents of soft corals of the Anthozoa class has resulted in the isolation of a number of different active metabolites.[1] Among them, halogenated compounds − predominantly steroids,[2] diterpenes,[3] alkaloids,[4] and prostanoids[5] − have received much attention because of their strong antiinflammatory and antitumor activities. The chemistry of prostaglandins has now entered a new stage, thanks to newly discovered, predominantly halogenated, compounds. The first paper describing the isolation of prostaglandins from a Caribbean gorgonian coral[6] was published more than 30 years ago; 16 years later, halogen-substituted prostaglandins were isolated for the first time from an octocoral.[5]

Marine prostanoids, predominantly halogenated, are distributed among a number of different marine sources and include, for example, chloro-punaglandins[7−10] from octocorals living in the Pacific ocean, chloro-epoxy prostanoids from *Clavularia viridis*,[11] punaglandins from Hawaiian octocorals,[5] and also substituted chloro-, bromo-, and iodovulones[12−16] from corals.

Further metabolites with different but similar structures − including a cyclopentenone ring − were found in *Dendrophyllia* sp. They are similar to chromomoric acids B and F, isolated from *Chromolaena morii* and *C. chasleae*.[17,18] Chromomoric acid B methyl ester has also been found in other species such as *Schistostepnium*,[19] *Montanoa*,[20] and *Inulanthera calva*,[21] and showed antibiotic and antihypertensive activities.

Chromomoric acids D were isolated as their methyl esters from *C. morii*.[17] This group of natural products includes metabolites of linolenic acid. However, they bear a structural resemblance to the marine prostanoids clavulones,[13−16] which are biosynthetically derived from arachidonic acid. This distinct feature aroused our interest in the possible biological activity of this family of new octadecanoids.

In the course of our investigation of the chemical composition of marine alga and invertebrates[22−24] we have examined soft corals from the Red Sea, collected in the Gulf of Aqaba (Eilat, Israel). Eight novel bromo-substituted cyclopentenones, including two glycosides with unusual unsaturation and substitution patterns, have been isolated from their extracts by the method of Bligh and Dyer.[25] Here we report on structure elucidation of these new brominated oxylipins, based mainly on their spectral characteristics.

## Results and Discussion

The four genera *Dendrophyllia* sp., *Dendronephthya* sp. (red variety), *Dendronephthya* sp. (yellow variety), and *Tubipora musica* were collected on August 27, 2001 in the Gulf of Aqaba in the Red Sea (Eilat, Israel). Fresh corals were placed in ethanol and stored at −10 °C. The alcoholic solution was concentrated under nitrogen and extracted separately by the method of Bligh and Dyer.[25] The $CHCl_3$-soluble fractions (lower layers) were individually separated by column chromatography on Sephadex LH-20. Further separation by RP-HPLC gave fractions featuring different compounds (see Figure 1). The aqueous MeOH (upper) layers, after extractions of lipids from *Dendronephthya* sp.

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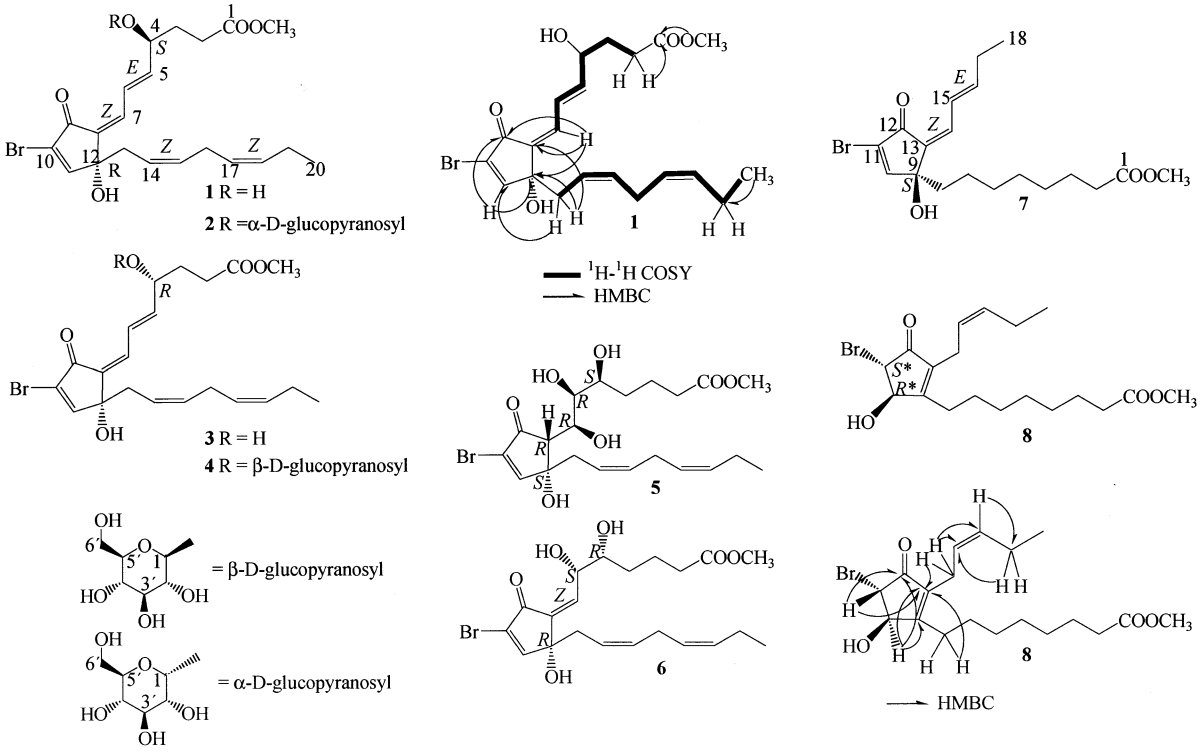


Figure 1. Structures of new bromo derivatives **1**−**8** from Red Sea corals; the HMBC and COSY correlations are included

(both varieties), were also chromatographed on a Sephadex LH-20 column and subjected to reversed-phase HPLC to give compounds **2** and **4** described in the Exp. Sect. and shown in Figure 1.

The HREIMS and $^{13}C$ NMR spectroscopic data of compound **1** indicate a bromine-containing molecular formula of $C_{21}H_{27}BrO_5$ (i.e. 438.1046 $[M]^+$). The IR spectrum of **1** shows absorptions due to hydroxy group (3490 $cm^{-1}$), methyl ester (1738, 1233 $cm^{-1}$), and α,β-unsaturated cyclopentenone (1706 $cm^{-1}$). The conjugated system in **1**, corresponding to that of bromovulone,[12] is demonstrated by absorptions at 248 (log ε = 4.08) and 313 (log ε = 4.01) nm, indicating the presence of a cross-conjugated system (α-halogenocyclopentenone and dienone).

The $^1H$ NMR spectrum of **1** (Table 1) shows signals of four olefinic protons in the cross-conjugated system at δ = 6.21 (dd, *J* = 5.7, 15.3 Hz, 1 H, 5-H), 6.73 (dd, *J* = 11.4, 15.3 Hz, 1 H, 6-H), 7.03 (d, *J* = 11.4 Hz, 1 H, 7-H), and 7.53 (s, 1 H, 11-H) ppm; of four olefinic protons on a methylene-interrupted (*Z*) double bond system at δ = 5.21 (dt, *J* = 7.6, 10.8 Hz, 1 H, 17-H), 5.30 (ddd, 1 H, *J* = 7.1, 8.2, 10.9 Hz, 14-H), 5.39 (dt, *J* = 7.4, 10.8 Hz, 1 H, 18-H), and 5.50 (dt, 1 H, *J* = 7.5, 10.9 Hz, 15-H) ppm; of a hydroxy-bearing methine group at δ = 4.29 (m, 1 H, 4-H) ppm; and of a terminal methyl group at δ = 0.96 (t, *J* = 7.5 Hz, 3 H, 20-H) ppm.

The $^{13}C$ NMR spectrum of **1** shows 21 carbon signals: two methyl, five methylene, nine methine, and five quaternary carbon atoms. The chemical shift values indicate the presence of one ester carbonyl group [174.3 (C-1)], four disubstituted double bonds [144.1 (C-5), 126.1 (C-6), 121.4 (C-14), 133.1 (C-15), 126.2 (C-17), and 132.5 (C-18)], and one oxygen-bearing carbon atom [73.6 (C-4)]. The remaining quaternary carbon atom, signal at δ = 78.6 (C-12) ppm, was thus deemed to represent a carbon atom bearing a tertiary hydroxy group. The COSY spectrum analysis (Figure 1) reveals a sequence of correlations starting from a triplet at δ = 2.36 ppm (2-H) to a doublet at δ = 7.07 (7-H) ppm, indicating the partial structure shown by the bold line in Figure 1 from 2-H to 7-H on the α-side chain. The connectivity from 13-H to 20-H on the ω-side chain is also indicated by the correlations in the $^1H$-$^1H$ COSY spectrum starting from two broad signals at δ = 2.53 (13-H) and 2.38 (13-H) ppm and ending with the methyl proton signal at δ = 0.96 (20-H) ppm.

HMBC correlations from the methine proton signal at δ = 7.07 (7-H) ppm to the carbonyl carbon signal at δ = 196.9 (C-9) ppm, and quaternary carbon signal at δ = 140.1 (C-8) ppm bearing the double bond show that C-7 is connected across C-8 to the carbonyl carbon atom (C-9) on the cyclopentenone ring. The correlations from the signals at δ = 2.53 and 2.38 (13-H) ppm to the quaternary carbon signal at δ = 78.6 (C-12) ppm, quaternary carbon signal at δ = 140.1 (C-8) ppm, and the olefinic carbon signal at δ = 162.7 (C-11) ppm reveal that the ω-side chain is connected to the C-12 carbon atom on the cyclopentenone ring. The correlations from the 2-H methylene signal at δ = 2.36 ppm and the methyl ester signal at δ = 3.65 ppm to the ester

Table 1. $^1$H NMR of compounds **1**−**8** from Red Sea marine corals

| H atom | **1**[a] | **1a**[a][b][c][d] | **1b**[a][b][c][d] | **2**[e] |
|---|---|---|---|---|
| 2 | 2.36 (m, 2 H) | 2.35 (m, 2 H) | 2.37 (m, 2 H) | 2.35 (m, 2 H) |
| 3 | 1.88 (m, 2 H) | 1.86 (m, 2 H) | 1.90 (m, 2 H) | 2.02 (m, 2 H) |
| 4 | 4.29 (m, 1 H) | 4.29 (m, 1 H) | 4.29 (m, 1 H) | 4.47 (m, 1 H) |
| 5 | 6.21 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.17 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.25 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.30 ((dd, *J* = 5.7, 15.3 Hz, 1 H) |
| 6 | 6.73 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.71 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.76 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.75 (dd, *J* = 11.4, 15.3 Hz, 1 H) |
| 7 | 7.07 (d, *J* = 11.4 Hz, 1 H) | 7.05 (d, *J* = 11.4 Hz, 1 H) | 7.09 (d, *J* = 11.4 Hz, 1 H) | 7.06 (d, *J* = 11.4 Hz, 1 H) |
| 11 | 7.53 (s, 1 H) | 7.53 (s, 1 H) | 7.53 (s, 1 H) | 7.51 (s, 1 H) |
| 13 | 2.53 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.53 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.53 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.55 (dd, *J* = 8.2, 14.2 Hz, 1 H) |
| | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.39 (dd, *J* = 7.1, 14.2 Hz, 1 H) |
| 14 | 5.30 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.30 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.30 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.31 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) |
| 15 | 5.50 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.50 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.50 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.49 (dt, *J* = 7.5, 10.9 Hz, 1 H) |
| 16 | 2.73 (m, 2 H) | 2.73 (m, 2 H) | 2.73 (m, 2 H) | 2.72 (m, 2 H) |
| 17 | 5.21 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.21 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.21 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.23 (dt, *J* = 7.6, 10.8 Hz, 1 H) |
| 18 | 5.39 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.39 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.39 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.39 (dt, *J* = 7.4, 10.8 Hz, 1 H) |
| 19 | 1.98 (m, 2 H) | 1.98 (m, 2 H) | 1.98 (m, 2 H) | 2.00 (m, 2 H) |
| 20 | 0.96 (t, *J* = 7.5 Hz, 3 H) | 0.96 (t, *J* = 7.5 Hz, 3 H) | 0.96 (t, *J* = 7.5 Hz, 3 H) | 0.98 (t, *J* = 7.5 Hz, 3 H) |
| $OCH_3$ | 3.65 (s, 3 H) | 3.65 (s, 3 H) | 3.65 (s, 3 H) | 3.64 (s, 3 H) |
| 1′ | − | − | − | 4.82 (d, *J* = 7.1 Hz, 1 H) |
| 2′ | − | − | − | 3.54 (dd, *J* = 8.9, 7.1 Hz, 1 H) |
| 3′ | − | − | − | 3.60 (t, *J* = 8.9 Hz, 1 H) |
| 4′ | − | − | − | 3.42 (t, *J* = 8.9 Hz, 1 H) |
| 5′ | − | − | − | 3.47 (m, 1 H) |
| 6′ | − | − | − | 3.75 (dd, *J* = 12.0, 5.2 Hz, 1 H) |
| | | | | 3.95 (dd, *J* = 12.0, 2.2 Hz, 1 H) |

| H atom | **3**[a] | **3a**[a][b][c][d] | **3b**[a][b][c][d] | **4**[e] |
|---|---|---|---|---|
| 2 | 2.37 (m, 2 H) | 2.36 (m, 2 H) | 2.38 (m, 2 H) | 2.33 (m, 2 H) |
| 3 | 1.87 (m, 2 H) | 1.86 (m, 2 H) | 1.90 (m, 2 H) | 2.01 (m, 2 H) |
| 4 | 4.30 (m, 1 H) | 4.30 (m, 1 H) | 4.30 (m, 1 H) | 4.48 (m, 1 H) |
| 5 | 6.22 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.19 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.24 (dd, *J* = 5.7, 15.3 Hz, 1 H) | 6.31 ((dd, *J* = 5.7, 15.3 Hz, 1 H) |
| 6 | 6.72 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.69 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.74 (dd, *J* = 11.4, 15.3 Hz, 1 H) | 6.76 (dd, *J* = 11.4, 15.3 Hz, 1 H) |
| 7 | 7.09 (d, *J* = 11.4 Hz, 1 H) | 7.07 (d, *J* = 11.4 Hz, 1 H) | 7.11 (d, *J* = 11.4 Hz, 1 H) | 7.07 (d, *J* = 11.4 Hz, 1 H) |
| 11 | 7.52 (s, 1 H) | 7.52 (s, 1 H) | 7.52 (s, 1 H) | 7.52 (s, 1 H) |
| 13 | 2.54 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.54 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.54 (dd, *J* = 8.2, 14.2 Hz, 1 H) | 2.54 (dd, *J* = 8.2, 14.2 Hz, 1 H) |
| | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.38 (dd, *J* = 7.1, 14.2 Hz, 1 H) | 2.41 (dd, *J* = 7.1, 14.2 Hz, 1 H) |
| 14 | 5.31 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.31 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.31 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) | 5.30 (ddd, *J* = 7.1, 8.2, 10.9 Hz, 1 H) |
| 15 | 5.52 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.52 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.52 (dt, *J* = 7.5, 10.9 Hz, 1 H) | 5.51 (dt, *J* = 7.5, 10.9 Hz, 1 H) |
| 16 | 2.71 (m, 2 H) | 2.71 (m, 2 H) | 2.71 (m, 2 H) | 2.73 (m, 2 H) |
| 17 | 5.22 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.22 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.22 (dt, *J* = 7.6, 10.8 Hz, 1 H) | 5.24 (dt, *J* = 7.6, 10.8 Hz, 1 H) |
| 18 | 5.38 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.38 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.38 (dt, *J* = 7.4, 10.8 Hz, 1 H) | 5.40 (dt, *J* = 7.4, 10.8 Hz, 1 H) |
| 19 | 1.99 (m, 2 H) | 1.99 (m, 2 H) | 1.99 (m, 2 H) | 2.01 (m, 2 H) |
| 20 | 0.97 (t, *J* = 7.5 Hz, 3 H) | 0.97 (t, *J* = 7.5 Hz, 3 H) | 0.97 (t, *J* = 7.5 Hz, 3 H) | 0.99 (t, *J* = 7.5 Hz, 3 H) |
| $OCH_3$ | 3.64 (s, 3 H) | 3.64 (s, 3 H) | 3.64 (s, 3 H) | 3.64 (s, 3 H) |
| 1′ | − | − | − | 4.82 (d, *J* = 7.1 Hz, 1 H) |
| 2′ | − | − | − | 3.56 (dd, *J* = 8.9, 7.1 Hz, 1 H) |
| 3′ | − | − | − | 3.59 (t, *J* = 8.9 Hz, 1 H) |
| 4′ | − | − | − | 3.43 (t, *J* = 8.9 Hz, 1 H) |
| 5′ | − | − | − | 3.46 (m, 1 H) |
| 6′ | − | − | − | 3.76 (dd, *J* = 12.0, 5.2 Hz, 1 H) |
| | | | | 3.96 (dd, *J* = 12.0, 2.2 Hz, 1 H) |

| H atom | **5**[a] | **6**[a] | **7**[a] | **8**[a] |
|---|---|---|---|---|
| 2 | 2.23 (m, 2 H) | 2.27 (m, 2 H) | 2.28 (t, *J* = 7.6 Hz, 2 H) | 2.27 (t, *J* = 7.2 Hz, 2 H) |
| 3 | 1.68[f] (m, 2 H) | 1.65[f] (m, 2 H) | 1.68 (m, 2 H) | 1.61 (m, 2 H) |
| 4 | 1.54[f] (m, 2 H) | 1.58[f] (m, 2 H) | | |
| 5 | 3.49 (ddd, *J* = 5.3, 5.3, 7.5 Hz, 1 H) | 3.35 (ddd, *J* = 4.1, 4.8, 7.7 Hz, 1 H) | 4-, 5-, 6-H: 1.29 (m, 6 H) | 4-, 5-, 6-H: 1.28 (m, 6 H) |
| 6 | 3.72 (dd, *J* = 5.3, 5.3 Hz, 1 H) | 3.98 (dd, *J* = 4.1, 9.2 Hz, 1 H) | | |
| 7 | 3.87 (dd, *J* = 4.2, 5.3 Hz, 1 H) | 6.47 (d, *J* = 9.2 Hz, 1 H) | 1.34 (m, 2 H) | 1.45 (m, 2 H) |
| 8 | 2.63 (d, *J* = 4.2 Hz, 1 H) | − | 1.98 (m, 2 H) | 2.41[f] (m, 1 H) |
| | | | | 2.33[f] (m, 1 H) |
| 10 | − | − | 7.03 (s, 1 H) | 4.56 (d, *J* = 1.8 Hz, 1 H) |
| 11 | 7.35 (s, 1 H) | 7.32 (s, 1 H) | − | 4.38 (d, *J* = 1.8 Hz, 1 H) |
| 13 | 2.41 (dd, *J* = 7.0, 14.7 Hz, 1 H) | 2.40 (dd, *J* = 7.1, 14.8 Hz, 1 H) | − | − |
| | 2.36 (dd, *J* = 8.1, 14.7 Hz, 1 H) | 2.32 (dd, *J* = 8.3, 14.8 Hz, 1 H) | | |
| 14 | 5.35 (ddd, *J* = 7.0, 8.1, 10.8 Hz, 1 H) | 5.37 (ddd, *J* = 7.1, 8.3, 10.6 Hz, 1 H) | 7.22 (d, *J* = 12.4 Hz, 1 H) | 2.91 (m, 2 H) |
| 15 | 5.53 (dt, *J* = 7.0, 10.8 Hz, 1 H) | 5.51 (dt, *J* = 7.1, 10.6 Hz, 1 H) | 6.45 (dd, *J* = 12.4, 10.8 Hz, 1 H) | 5.39 (dtt, *J* = 10.5, 6.9, 1.7 Hz, 1 H) |
| 16 | 2.68 (m, 2 H) | 2.65 (m, 2 H) | 5.97 (ddd, *J* = 10.8, 7.6, 1.0 Hz, 1 H) | 5.30 (dtt, *J* = 10.5, 7.4, 2.0 Hz, 1 H) |
| 17 | 5.39 (dt, *J* = 1.7, 10.6 Hz, 1 H) | 5.42 (dt, *J* = 1.8, 10.7 Hz, 1 H) | 2.28 (m, 2 H) | 2.11 (qdd, *J* = 7.4, 7.4, 1.8 Hz, 2 H) |
| 18 | 5.41 (dt, *J* = 7.0, 10.6 Hz, 1 H) | 5.44 (dt, *J* = 7.2, 10.7 Hz, 1 H) | 1.07 (t, *J* = 7.5 Hz, 3 H) | 0.97 (t, *J* = 7.4 Hz, 3 H) |
| 19 | 2.04 (m, 2 H) | 2.02 (m, 2 H) | − | − |
| 20 | 1.04 (t, *J* = 7.5 Hz, 3 H) | 1.00 (t, *J* = 7.2 Hz, 3 H) | − | − |
| $OCH_3$ | 3.65 (s, 3 H) | 3.64 (s, 3 H) | 3.65 (s, 3 H) | 3.66 (s, 3 H) |

[a] A $CDCl_3/CD_3OD$ (4:1) mixture was used as solvent. [b] The following signals were observed for compounds **1a**, **1b**, **3a**, and **3b**: δ = 3.54−3.57 (s, 3 H, MTPA OMe); δ = 7.51−7.57 (m, 5 H, MTPA phenyl). [c] Δδ values, in Hz, obtained for the MTPA esters **2** [Δδ = δ(*S*-MTPA ester) − δ(*R*-MTPA ester)]. [d] Resonances of H directly attached to esterified carbon atom are not analyzed or values δ < |0.005 ppm| have not been included. [e] A $CDCl_3/CD_3OD$ mixture (1:4) was used as solvent. [f] Assignments may be interchanged.

carbonyl carbon signal at δ = 174.3 (C-1) ppm indicate the connectivity from the 2-H methylene group to the methyl ester group.

The (5*E*,14*Z*,17*Z*) configurations of the three disubstituted double bonds are assigned on the basis of the $^1$H coupling constants between the olefinic protons, 5-H and 6-H (15.3 Hz), 14-H and 15-H (10.9 Hz), and 17-H and 18-H (10.8 Hz), respectively.

The configuration of the olefinic bond at C-7 is assigned on the basis of the chemical shift values of the 7-H signals of compound **1** and similar natural substances reported by Iguchi[8,9,12] [δ = 7.35 ppm for the (*E*) isomer, δ = 6.99 ppm for the (*Z*) isomer]. Because of the anisotropy effect (shielding effect) of the C-9 carbonyl moiety, the signal of proton 7-H in the (7*Z*) configuration should appear at a higher field than that of the corresponding (7*E*) isomer. The signal of the olefinic proton 7-H in our methyl ester **1** is observed at δ = 7.07 ppm, whereas in the prostanoids the chemical shift of 7-H is at δ = 7.35 ppm.[8,9,12] Our isolated methyl ester should therefore be assigned a (7*Z*) configuration.

The (*R*) configuration at C-12 in **1** is assigned by comparison of the CD spectra of this compound with that of bromovulone I, the absolute configuration of which has already been determined[12,13] as (*R*). The CD data of **1** [λ (Δε) = 232 (+6.4), 260 (−4.7), 360 (+0.9) nm] are similar to those of bromovulone I [λ (Δε) = 235 (+5.6), 260 (−3.0), 360 (+1.2) nm]. These findings establish the structures of **1**.

The absolute configuration at C-4, bearing the secondary hydroxy group, was independently clarified by the modified Mosher method.[26,27] Compound **1** was converted into its (*R*)-(+)-MTPA and (*S*)-(−)-MTPA esters **1a** and **1b**. Comparison of the $^1$H NMR spectroscopic data (i.e., Δδ values obtained for the MTPA esters {Δδ = δ[(*S*)-MTPA ester] − δ[(*R*)-MTPA ester]}; Table 1) showed a downfield shift (δ = −0.08 ppm) of the 5-H signal along with an upfield shift (δ = 0.035 Hz ppm) of the 3-H signal. These data, in conjunction with the literature data,[26,27] allowed us to assign the (*S*) configuration to C-4 in compound **1**. The structure of **1** was identified as (4*S*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-hydroxy-17,18-didehydrobromovulone-3.

The structure of compound **3** was determined on the basis of UV, IR, $^1$H NMR, $^{13}$C NMR, and mass spectra. Only small differences in the CD spectrum were seen on comparison with compound **1** (see Exp. Sect.). The optical rotation ($[\alpha]_D^{22}$ = +18.5) is the opposite of that of compound **1** and the resulting structure is a diastereomer of **1** (see also Exp. Sect. for differences in chemical shifts of the Mosher esters).

Compounds **2** and **4** were obtained as colorless powders with $[\alpha]_D^{23}$ = +112 (*c* = 0.11, MeOH) and −118 (*c* = 0.12, MeOH), respectively. The empirical formulas of **2** and **4** were determined to be $C_{27}H_{37}BrO_{10}$ on the basis of HRFABMS. The UV spectra of both **2** and **4** each have a maximum band at 313 (log ε = 3.94) nm. Their IR spectra show absorption bands due to hydroxy (3490 cm$^{-1}$), methyl ester (1738, 1233 cm$^{-1}$), and α,β-unsaturated cyclopentenone (1706 cm$^{-1}$) groups.

In the $^1$H NMR spectrum of **2**, the signals of five oxymethine protons in diaxial conformations (*J* = 7.0−9.0 Hz) and one oxymethylene group indicate the presence of a β-glucopyranosyl group. The glucosyl residue is located at the 4-*O*-position of the aglycon skeleton according to long-range HMBC correlations between the C-4 signal at δ = 80.7 ppm and the anomeric 1′-H signal at δ = 4.82 ppm, as well as the 3-H and 5-H signals at δ = 2.02 and 6.30 ppm, respectively. Subsequently, glucoside **2** was hydrolyzed by β-glucosidase, furnishing aglycon **1** and glucose.

To determine the absolute configurations of the carbohydrate components in the two glycosides, the acetylated 2-butyl derivatives, prepared from hydrolyzed glucoses, were analyzed by gas chromatography on a glass capillary column (Supelco SPB-1).[28] Each derivative was eluted as a peak with a retention time of 13.21 min, identical with that of (+)-tetraacetyl 2-butyl-D-glucoside. These results thus reveal that **2** and **4** also contain D-glucose.

In the $^1$H NMR spectrum of **2**, the unusual 4-*O*-glycosylation is also indicated by the downfield shifts of the 3-H (+ 0.14 ppm) and the 5-H (+ 0.09 ppm) signals with respect to that of methyl ester **1** serving as model compound. Similarly, in the $^{13}$C NMR spectra of **2** (Table 2), 4-*O*-glycosylation is confirmed by the diagnostic downfield shift of the C-4 signal (δ = 7.1 ppm) and an upfield shift of the related C-5 (δ = −1.9 ppm) and C-3 (δ = −3.9 ppm) carbon signals with respect to compound **1**.

Table 2. $^{13}$C NMR of compounds **1**−**8** from Red Sea marine corals

| C atom | **1**[a] | **2**[b] | **3**[a] | **4**[b] | **5**[a] | **6**[a] | **7**[a] | **8**[a] |
|---|---|---|---|---|---|---|---|---|
| 1 | 174.3 | 173.9 | 174.1 | 173.9 | 173.0 | 173.5 | 173.0 | 173.1 |
| 2 | 29.1 | 29.2 | 29.0 | 29.2 | 32.6 | 32.8 | 33.2 | 33.1 |
| 3 | 35.2 | 31.3 | 35.3 | 32.1 | 19.8 | 20.1 | 24.8 | 24.7 |
| 4 | 73.6 | 80.7 | 73.4 | 79.8 | 32.4 | 33.2 | 28.8 | 28.8 |
| 5 | 144.1 | 142.2 | 144.0 | 141.7 | 70.9 | 76.9 | 29.5 | 29.4 |
| 6 | 126.1 | 127.0 | 126.0 | 127.0 | 80.1 | 74.2 | 29.7 | 29.6 |
| 7 | 135.7 | 135.6 | 135.6 | 135.6 | 67.4 | 147.2 | 24.1 | 26.7 |
| 8 | 140.1 | 139.8 | 140.2 | 139.8 | 64.1 | 142.9 | 38.1 | 33.2 |
| 9 | 196.9 | 197.1 | 197.0 | 197.1 | 198.7 | 196.7 | 77.2 | 152.3 |
| 10 | 126.6 | 127.0 | 126.5 | 127.0 | 120.6 | 122.6 | 160.4 | 75.6 |
| 11 | 162.7 | 161.8 | 162.6 | 161.8 | 154.1 | 158.4 | 124.8 | 63.8 |
| 12 | 78.6 | 78.5 | 78.5 | 78.5 | 70.8 | 76.3 | 196.5 | 194.5 |
| 13 | 39.3 | 39.7 | 39.2 | 39.7 | 36.3 | 37.9 | 141.3 | 130.2 |
| 14 | 121.4 | 121.5 | 121.3 | 121.5 | 124.6 | 123.8 | 136.2 | 21.5 |
| 15 | 133.1 | 133.1 | 133.2 | 133.1 | 133.2 | 132.9 | 128.7 | 124.2 |
| 16 | 25.7 | 25.4 | 25.8 | 25.4 | 25.3 | 26.0 | 130.1 | 130.1 |
| 17 | 126.2 | 126.8 | 126.4 | 126.8 | 125.0 | 124.7 | 26.4 | 21.2 |
| 18 | 132.5 | 132.6 | 132.3 | 132.6 | 131.9 | 132.3 | 14.2 | 14.0 |
| 19 | 20.6 | 21.0 | 20.2 | 21.0 | 20.2 | 19.7 | – | – |
| 20 | 14.2 | 14.9 | 14.1 | 14.9 | 14.2 | 15.1 | – | – |
| $OCH_3$ | 51.6 | 52.0 | 51.7 | 52.0 | 50.9 | 51.9 | 51.8 | 51.4 |
| 1′ | – | 101.7 | – | 101.7 | – | – | – | – |
| 2′ | – | 74.8 | – | 74.8 | – | – | – | – |
| 3′ | – | 77.9 | – | 77.9 | – | – | – | – |
| 4′ | – | 72.5 | – | 72.5 | – | – | – | – |
| 5′ | – | 76.8 | – | 76.8 | – | – | – | – |
| 6′ | – | 68.1 | – | 68.1 | – | – | – | – |

[a] A $CDCl_3/CD_3OD$ mixture (4:1) was used as solvent. [b] A $CDCl_3/CD_3OD$ mixture (1:4) was used as solvent.

The absolute configuration of **2** was also established by $^{13}C$ NMR spectroscopy. Comparison of the $^{13}C$ NMR chemical shifts of glycoside **2** with those of **1** (Table 2) reveal that a larger glycosidation shift of the C-3 signal (|3.9| ppm) than that of the C-5 signal (|1.9| ppm) is observed in [$D_5$]pyridine as solvent. Application of the glycosidation shift rule[29−31] to these shifts indicates that the configuration at C-4 of the glycoside **2** is (*S*), and the glycoside **2** was thus confirmed as the 4-*O*-α-D-glucopyranoside of **1**. In the spectrum of **4**, the glycosidation shift is the same (|3.1| and |2.4| ppm), but the sugar is β-glucopyranose; the configuration in **4** must thus be (*R*) and this glycoside is a derivative of aglycon **3**. These results were confirmed by the isolation of **1** and/or **3** after enzymatic hydrolysis (see above) by α-glucosidase and β-glucosidase, respectively. Both aglycons were converted into Mosher esters **1a**, **1b** and **3a**, **3b**, and their chemical shift differences are shown in Table 1.

The UV, IR, MS, and $^1H$ NMR spectra of compound **4** are also identical with those of compound **2**. A minor difference is observed only in the $^{13}C$ NMR spectrum (see Table 2).

The structure of **2** was identified as (4*S*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-(α-D-glucopyranosyloxy)-17,18-didehydrobromovulone-3 and that of **4** as (4*R*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-(β-D-glucopyranosyloxy)-17,18-didehydrobromovulone-3.

Extraction and chromatography of *Tubipora musica* gave the two compounds **5** and **6**, with the structures depicted in Figure 1. The chloro analogues of these compounds have been described.[7] Only small differences in the chemical shifts in the neighborhood of the carbon atom carrying the bromine atom (i.e., carbon and hydrogen atoms of the cyclopentenone ring; see Table 1 and 2) are observed on comparison with previously published data.[11] Other chemical shifts display no differences. Different $^3J_{H\text{-}H}$ values are observed in the $^1H$ NMR spectrum of **6**. The magnitude of the 5-H,6-H coupling constant is about 4.1 Hz, compatible with a *threo* configuration, whereas *J* values of 6−10 Hz are typical[32,33] of an *erythro* relationship.

Compounds **5** and **6** were identified as bromopunaglandin-1 and 5-*epi*-bromo-(*Z*)-punaglandin-3, respectively.

The empirical formula of the compound **7**, obtained from *Dendrophyllia* sp., was determined to be $C_{19}H_{27}BrO_4$ (six degrees of unsaturation) by MS and NMR spectroscopic data. Analysis of $^1H$ and $^{13}C$ NMR spectroscopic data (Table 1 and 2) shows that **7** is structurally and biogenetically quite different from **1**−**6**. These data reveal the presence of two methyl groups, eight methylene units, four methine groups, two trisubstituted olefins, and five quaternary carbon atoms. These signals, together with one exchangeable proton, account for the molecular formula and imply that compound **7** is monocyclic.

Analysis of COSY and HMBC data results in the identification of structure **7**, which is very similar to chromomoric acid D-III.[17,19−21] The signals of the hydrogen and carbon atoms of the cyclopentenone ring are moved to a high field, in agreement with previously published data.[17,19−21]

The spectra obtained with compound **8** are very similar to those of compound **7**, with one exception. On the basis of spectra previously described for chromomoric acid F[18] and our compound **7** we assume that the correct structure is as shown in Figure 1. The mass spectrum of **8** shows $M^+$ peaks at *m*/*z* = 400 and 402 (1:0.98) ($C_{19}H_{29}BrO_4$). The spectroscopic data of **7** and **8** are closely similar. Only the UV spectrum is shifted ($\lambda_{max}$ = 225 nm), consistent with a simple chromophore system (i.e., α,β-unsaturated ketone in the cyclopentenone ring).

The relative stereochemistry at C-10 and C-11 was established by comparison of the proton coupling constant between 10-H and 11-H with analogous couplings observed for other cyclopentenones.[32,33] In the cyclopentenone ring, a vicinal coupling constant of 5−6 Hz normally indicates a *cis* relationship, while a coupling constant of ca. 2 Hz suggests a *trans* [*threo*; (*R*,*S*) or (*S*,*R*)] one.[34−36] Thus, in the case of **8**, the small coupling constant of 1.8 Hz is suggestive of a *trans* relationship between 10-H and 11-H. The absolute configuration of **8** is not determined.

Clavulones, punaglandins, and related compounds are of great interest, thanks to their potent antitumor properties.[37,38] Chlorovulone I shows strong antiproliferative and cytotoxic activity. The $IC_{50}$ value (for HL-60 cells) is 0.01 μg/mL.[12] Clavulone I and its halogenated analogues show remarkable antiproliferative activity and cytotoxicity to the human promyelocytic leukemia HL-60 cell line. (7*E*)-Punaglandin 3 inhibits proliferation of another leukemia cell line L 1210 with an IC value of 0.02 μg/mL,[16] which is similar to the activity of halogenated analogues of clavulones. Testing of clavulones[13] in vivo also revealed promising anticancer activity.

The two most popular primary screening bioassays are the brine shrimp lethality test and the crown gall tumor inhibition test. The first test has been used for the active antitumor agents produced in vivo by organisms and is also used to evaluate extracts for different pharmacological activities. The isolated compounds were evaluated by their ability to inhibit the growth of crown gall tumors on potato discs inoculated with *Agrobacterium tumefaciens* carrying a tumor-inducing plasmid. All compounds showed significant inhibition of the growth of crown gall tumors on potato disks, suggestive of in vivo antitumor activity.[39] All the extracts assayed demonstrated crown gall tumor inhibition, ranging from 15% for compound **6** to 74% for compound **2**. Table 3 also summarizes the other bioactivities of compounds **1**−**8**. As has been proposed,[38] clavulones may have an allelopathic role in corals. Their potent cytotoxicity may play a role in coral defense against some other marine organisms.

We have isolated eight previously undescribed bromosubstituted prostaglandins and oxylipins. The absolute or relative configurations of the compounds were determined. The discovery of glycosides containing α- and β-glucose is very recent. In contrast to glycosidic compounds from other marine organisms, this is the first example of glycosides identified from corals.

Table 3. Bioactivities of cyclopentenones **1**−**8**

| Test organism | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus*[a] | 14.3 | 11.8 | 24.1 | 12.9 | 9.6 | 5.7 | 3.2 | 5.1 |
| *Bacillus subtilis*[a] | 18.1 | 12.4 | 20.4 | 14.2 | 8.9 | 6.7 | 5.8 | 7.7 |
| *Escherichia coli*[a] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Saccharomyces cerevisiae*[a] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Artemia salina*[b][c] | 6.5 | 5.2 | 5.4 | 5.4 | 4.1 | 3.2 | 10.7 | 9.9 |
| *Paracentrotus lividus*[c][d] | 0.3 | 0.5 | 0.2 | 0.4 | 1.1 | 2.3 | 0.8 | 0.9 |
| *Agrobacterium tumefaciens*[c][e] | 45±6[f] | 74±7 | 28±5 | 30±5 | 18±6 | 15±5 | 23±7 | 37±4 |

[a] Samples were applied on 6.35 mm paper disks, values are diameters (mm) of inhibitory zones. [b] In μg/mL (minimum lethal doses). [c] Details in Exp. Sect. [d] In μg/mL ($IC_{50}$). [e] Presented values are the means of three determinations. [f] % of crown gall tumor inhibition (± S.D.).

## Experimental Section

**General Experimental Procedures:** UV spectra were measured with a Cary 118 (Varian) apparatus in EtOH in the 200−350 nm range. A Perkin−Elmer Model 1310 (Perkin−Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of compounds as neat films. Circular dichroism (CD) measurement was carried out with a Jasco-500A spectropolarimeter at 24 °C under dry $N_2$. NMR spectra were recorded with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz ($^1$H), 125.7 MHz ($^{13}$C). Both high- and low-resolution MS were recorded with a VG 7070E−HF spectrometer (70 eV). GC-MS analysis of the sugar derivatives was performed with a Finnigan 1020 B single-state quadrupole GC-MS instrument in the EI mode. A $C_{18}$ reversed-phase column (5 μm, 7.8 × 250 mm, Supelco, USA), was employed.

**Animal Materials:** The four genera of the class Anthozoa were collected by hand (scuba-diving) from rocks 10−15 m deep in the Red Sea, Gulf of Aqaba (Eilat, Israel), on August 27, 2001. The voucher specimens are deposited in the collection of the second author (V. M. D.). Fresh corals were placed in ethanol and stored at −10 °C under nitrogen.

**Extraction and Isolation:** All four genera of corals were extracted independently by the methods of Bligh and Dyer[25] and the extracts were also chromatographed separately on Sephadex LH-20 columns with chloroform/hexane (65:35) and then separated by RP-HPLC. A linear gradient from 40% $H_2O$/60% acetonitrile to pure acetonitrile over 30 min, flow rate 1.7 mL/min with different UV detection, was used to separate of compounds in the crude extract. The aqueous MeOH (upper) layers obtained after extractions of lipids from *Dendronephthya* sp. (both varieties) were also chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH mixture 1:1). The separation of glycosides was performed by the linear gradient technique over 30 min with a solution containing 99% water and 1% acetonitrile to a mixture of 50% water and 50% acetonitrile, and compounds **2** or **4** were found. In order to prove that compounds **1** and **3** are not the products of hydrolytic decomposition of compounds **2** and **4**, it was confirmed that no free glucose was present. After extraction according to Bligh and Dyer,[25] no glucose was identified in the water phase (commercially available analytical kit based on the glucose oxidase + *o*-dianisidine reaction, Sigma).

**Enzymatic Hydrolysis and Determination of Glycosides:** A solution of **2** and **4** (ca. 1 mg each) in acetate buffer (0.22 M, pH = 4.4, 10 mL) was treated with α-D-glucosidase (EC, 3.2.1.20) from yeast and/or β-D-glucosidase (EC, 3.2.1.21) from almonds, and the solution was left at 37 °C for 48 h. The reaction solutions were concentrated to dryness, and the residues were chromatographed on columns of silica gel (10 g) with $CH_2Cl_2$/MeOH/$H_2O$ (90:10:1) to afford **1** or **2** (ca. 0.6 mg). $^1$H NMR: see Table 1. The identification and the D or L configuration of the sugar (i.e., D-glucose) was determined by GC-MS (according to the methods of Gerwig et al.,[28] with some modifications, as described previously[40]) with an SPB-1 (Supelco) column (30 m × 0.25 mm i.d.).

**(*S*)-MTPA Esters:** (−)-MTPA chloride (20 μL) was added to a stirred solution of the hydroxy compound **1** (ca. 1.0 mg) in dry pyridine (0.3 mL). The mixture was stirred under $N_2$ at room temperature for 1 h and the solvent was then removed under a stream of $N_2$. The residue was redissolved in EtOAc/hexane (2 mL) and filtered through a Sep-Pak silica column. After removal of the solvent under vacuum, the residue was separated by reversed-phase HPLC (ODS column, 100% MeCN) to yield ca. 1.0 mg of (*S*)-ester as a colorless gum. $^1$H NMR: see Table 1.

**(*R*)-MTPA Esters:** Prepared as described for (*S*)-esters. An amount of ca. 1.0 mg of compound **1** and 20 μL of (+)-MTPA chloride gave 0.9 mg of (*R*)-ester as a colorless gum.

The following compounds (in mg/100 g of lyophilized coral) were isolated from Red Sea marine corals of class Anthozoa:

*Dendronephthya* sp. (red variety), subclass Hexacorallia, family Dendrophyliidae: **1** (7.4, 0.0074%), **2** (12.3, 0.0123%).

*Dendronephthya* sp. (yellow variety): **3** (5.1, 0.0051%), **4** (9.8, 0.0098%).

*Tubipora musica*, subclass Octocorallia, order Alcyonaria, family Nephtyidae: **5** (13.6, 0.0136%), **6** (2.9, 0.0029%).

*Dendrophyllia* sp. subclass Octocorallia, order Alcyonaria, family Tubiporidae: **7** (6.4, 0.0064%) and **8** (18.2, 0.0182%).

**(4*S*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-Hydroxy-17,18-didehydrobromovulone-3 (1):** Colorless oil. $[\alpha]_D^{22} = -17.5$ (*c* = 0.15, MeOH). UV (EtOH): $\lambda_{max}$ (log ε) = 248 (4.08), 313 (4.01) nm. CD (MeOH): $\lambda_{ext}$ (Δε) = 232 (+6.4), 260 (−4.7), 360 (+0.9) nm. IR (film): $\tilde{\nu}_{max}$ = 3490 (OH), 1738 and 1233 (COOMe), 1706 (C=C−C=O) $cm^{-1}$. HREIMS: 438.1046 (calcd. for $C_{21}H_{27}BrO_5$ 438.1042); LREIMS: 438 and 440 (intensity ratio 1:0.98) [$M^+$]. NMR: see Table 1 and 2.

**(4*S*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-(α-D-Glucopyranosyloxy)-17,18-didehydrobromovulone-3 (2):** Colorless powder. $[\alpha]_D^{23} = +112$ (*c* = 0.11, MeOH). The UV and IR spectra are identical with those of **1**. HRFABMS: 601.1652 [M + H]$^+$ (calcd. for [$C_{27}H_{37}BrO_{10}$ + H]$^+$ 601.1648); negative FABMS: 599 [M − H]$^-$, 437 [M − H − 162]$^-$. $^1$H and $^{13}$C NMR: see Table 1 and 2.

**(4*R*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-Hydroxy-17,18-didehydrobromovulone-3 (3):** Colorless oil. $[\alpha]_D^{22} = 18.5$ (*c* = 0.14, MeOH). CD (MeOH): $\lambda_{ext}$ (Δε) = 231 (+5.8), 259 (−3.9), 357 (+0.7) nm. HREIMS: 438.1046 (calcd. for $C_{21}H_{27}BrO_5$ 438.1042). The UV, IR and $^1$H- and $^{13}$C NMR spectra are identical with those of **1**.

**(4*R*,5*E*,7*Z*,12*R*,14*Z*,17*Z*)-4-(β-D-Glucopyranosyloxy)-17,18-didehydrobromovulone-3 (4):** Colorless powder. $[\alpha]_D^{23} = -118$ (*c* =

0.12, MeOH). HRFABMS: 601.1652 $[M + H]^+$ (calcd. for $[C_{27}H_{37}BrO_{10} + H]^+$ 601.1648). The UV, IR and $^1H$ NMR spectra are identical to those of **2**. $^{13}C$ NMR: see Table 2.

**Bromopunagladin-1 (5):** Colorless oil. $[\alpha]_D^{22} = +11.3$ ($c = 0.12$, MeOH). UV (MeOH): $\lambda_{max}$ (log ε) = 230 (3.46) nm. CD (MeOH): $\lambda_{ext}$ (Δε) = 215 (+11.3), 255 (−7.13), 330 (+1.85) nm. IR (film): $\tilde{\nu}_{max}$ = 3430 (OH), 1740 (COOMe), 1705 (C=C−C=O) $cm^{-1}$. HRFABMS: 475.1339 $[M + H]^+$ (calcd. for $C_{21}H_{31}BrO_7$ + H 475.1331); LRFABMS: 476 and 478 (intensity ratio 1:0.99) $[M + H]^+$. NMR: see Table 1 and 2.

**5-*epi*-Bromo-*Z*-punagladin-3 (6):** Colorless oil. $[\alpha]_D^{22} = +37.4$ ($c = 0.14$, MeOH). UV (MeOH): $\lambda_{max}$ (log ε) = 240 (3.91) nm. CD (MeOH): $\lambda_{ext}$ (Δε) = 230 (+ 5.7), 260 (−5.1), 360 (+1.0) nm. IR (film): $\tilde{\nu}_{max}$ = 3500 (OH), 1735 (COOMe), 1702 (C=C−C=O) $cm^{-1}$. HRFABMS: 457.1231 $[M + H]^+$ (calcd. for $C_{21}H_{29}BrO_6$ + H 457.1225); LRFABMS: 458 and 460 (intensity ratio 1:0.99) $[M + H]^+$. NMR: see Table 1 and 2.

**11-Bromochromomoric Acid D-III Methyl Ester (7):** $[\alpha]_D^{22} = -14.2$ ($c = 0.13$, MeOH). UV (MeOH): $\lambda_{max}$ (log ε) = 246 (4.28), 312 (4.03) nm. IR (film): $\tilde{\nu}_{max}$ = 3400 (OH), 1740 (COOMe), 1700 and 1635 (C=C−C=O) $cm^{-1}$. CD (MeOH): $\lambda_{ext}$ (Δε) = 232 (−10.9), 262 (+5.8), 355 (−1.1) nm. HRFABMS: 431.1075 $[M + H]^+$ (calcd. for $C_{19}H_{27}BrO_6$ + H 431.1069); LRFABMS: 430 and 432 (intensity ratio 1:0.98) $[M + H]^+$. NMR: see Table 1 and 2.

**(10*R**,11*S**)-11-Bromo-10-hydroxychromomoric Acid F Methyl Ester (8):** $[\alpha]_D^{22} = +23.8$ ($c = 0.11$, MeOH). UV (MeOH): $\lambda_{max}$ (log ε) = 225 (2.18). IR (film): $\tilde{\nu}_{max}$ = 3450 (OH), 1740 (COOMe), 1705 and 1635 (C=C−C=O) nm. CD spectrum was not measured. HRFABMS: 401.1330 $[M + H]^+$ (calcd. for $C_{19}H_{29}BrO_4$ + H 401.1327); LRFABMS: 400 and 402 (intensity ratio 1:0.98) $[M + H]^+$. NMR: see Table 1 and 2.

**Antibacterial Tests:** The test organisms were *Bacillus subtilis*, *Staphyloccocus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae* (Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried out according to the literature.[40] The amounts used were 50 μg of compound per test disk (see Table 3).

**Brine Shrimp Toxicity Bioassay:** The sample (ca. 0.05 mg) was dissolved in DMSO (50 μL) and added to a test vial of artificial seawater (3.0 mL). Approximately 20 brine shrimps, *Artemia salina*, were added to the vial. The brine shrimps were observed periodically over a 24-h period. A positive assay was the death of all brine shrimps.

**Sea Urchin Eggs Test:** Sea urchin eggs and sperms were collected from mature specimens of *Paracentrotus lividus*. Soon after fertilization, the eggs were dropped onto the test media, previously prepared from a graded concentration of compounds **1** and **2**, dissolved in EtOH and filtered seawater. After the test media had been allowed to stand, the embryos were observed under a light microscope at certain intervals. In an inhibitory test against the development of sea urchin eggs,[41] **1** and **2** were effective at $IC_{50}$ levels of 2.5 and 0.2 μg/mL, respectively.

**Crown Gall Tumors on Potato Disks Test:** The *Agrobacterium tumefaciens* potato disc assay for tumor/antitumor induction was performed by the procedure described in the literature.[42] The potatoes were sterilized by immersion in ethanol (70%) for 2 min and in sodium hypochlorite solution (50%, active chlorine 30 g/L) for 30 min. They were then rinsed several times with sterilized distilled water, in a laminar flow hood. A core of tissue was extracted from each tuber with a sterilized 1.5 cm cork borer. Discs (0.5 cm) were cut with a scalpel. The potato discs were placed in 1.5% agar Petri dishes. To each potato disc was applied 0.05 mL of a solution containing 2 mL of a broth culture of *A. tumefaciens* (48 h culture of ca. 109 cells/mL), 1.5 mL of sterile $H_2O$, and 0.5 mL of the solution test extract (8 mg of extract in 2 mL of DMSO filtered through 0.22-mm filters). Control discs were prepared with sterile DMSO in place of the test extract. A minimum of three Petri dishes (5 discs/dish) ($n = 15-25$) was used for each test compound and the control. After preparation, the Petri dishes were placed in an incubator at 27 °C for 12−21 d. To determine the number of tumors, the potato discs were stained with a solution of $I_2$ (1 g) KI (2 g) in 300 mL of distilled $H_2O$. Significant activity is indicated when two independent assays give 20% or greater inhibition.


[1] D. J. Faulkner, *Nat. Prod. Rep.* **2002**, *19*, 1−48 and the earlier references in this series cited therein.
[2] M. Iwashima, K. Nara, Y. Nakamichi, K. Iguchi, *Steroids* **2001**, *66*, 25−32.
[3] B. F. Bowden, J. C. Coll, I. M. Vasilescu, P. N. Alderslade, *Aust. J. Chem.* **1989**, *42*, 1727−1734.
[4] G. Grible, "Naturally occurring organohalogen compounds: A comprehensive review" in: *Progress in the chemistry of organic natural products*, vol. 63 (Eds.: W. Hertz, G. W. Kirby, R. E. Moore, W. Steglich, C. Tamm), Springer Verlag, Vienna and New York, **1996**.
[5] B. J. Baker, R. K. Okuda, P. T. K. Yu, P. J. Scheuer, *J. Am. Chem. Soc.* **1985**, *107*, 2976−2977.
[6] A. J. Weinheimer, R. L. Spraggins, *Tetrahedron Lett.* **1969**, 5185−5187.
[7] B. J. Baker, P. J. Scheuer, *J. Nat. Prod.* **1994**, *57*, 1346−1353.
[8] K. Iguchi, S. Kaneta, K. Mori, Y. Yamada, *Chem. Pharm. Bull.* **1987**, *35*, 4375−4376.
[9] K. Iguchi, S. Kaneta, K. Mori, Y. Yamada, A. Honda, Y. Mori, *J. Chem. Soc., Chem. Commun.* **1986**, 981−982.
[10] H. Nagaoka, K. Iguchi, T. Miyakoshi, N. Yamada, Y. Yamada, *Tetrahedron Lett.* **1986**, *27*, 223−226.
[11] H. Nagaoka, T. Miyakoshi, J. Kasuga, Y. Yamada, *Tetrahedron Lett.* **1985**, *26*, 5053−5056.
[12] K. Iguchi, S. Kaneta, K. Mori, Y. Yamada, A. Honda, Y. Mori, *Tetrahedron Lett.* **1985**, *26* 5787−5790.
[13] K. Watanabe, M. Sekine, H. Takahashi, K. Iguchi, *J. Nat. Prod.* **2001**, *64*, 1421−1425.
[14] M. Suzuki, Y. Morita, A. Yanagisawa, R. Noyori, B. J. Baker, P. J. Scheuer, *J. Am. Chem. Soc.* **1986**, *108*, 5021−5022.
[15] H. Nagaoka, H. Miyaoka, T. Miyakoshi, Y. Yamada, *J. Am. Chem. Soc.* **1986**, *108*, 5019−5021.
[16] M. Suzuki, Y. Morita, A. Yanagisawa, B. J. Baker, P. J. Scheuer, R. Noyori, *J. Org. Chem.* **1988**, *53*, 286−295.
[17] F. Bohlmann, N. Borthakur, R. M. King, H. Robinson, *Phytochemistry* **1982**, *21*, 125−127.
[18] F. Bohlmann, P. Singh, J. Jakupovic, R. M. King, H. Robinson, *Phytochemistry* **1982**, *21*, 371−374.
[19] F. Bohlmann, J. Jakupovic, M. Ahmed, A. Schuster, *Phytochemistry* **1983**, *22*, 1623−1636.
[20] F. Bohlmann, G. Schmedahirschmann, J. Jakupovic, V. Castro, J. F. Ciccio, G. Calvo, *J. Nat. Prod.* **1984**, *47*, 663−672.
[21] C. Zdero, L. Lehmann, F. Bohlmann, *Phytochemistry* **1991**, *30*, 1161−1163.
[22] V. M. Dembitsky, T. Rezanka, *Comp. Biochem. Physiol. B* **1996**, *114*, 317−320.
[23] T. Rezanka, V. M. Dembitsky, *Phytochemistry* **2001**, *57*, 607−611.
[24] T. Rezanka, V. M. Dembitsky, *Tetrahedron* **2001**, *57*, 8743−8749.
[25] E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.

[26] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092−4095.
[27] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Org. Chem.* **1991**, *56*, 1296−1298.
[28] G. J. Gerwig, J. R. Kamerling, J. F. G. Vliegenthart, *Carbohydr. Res.* **1978**, *62*, 349−357.
[29] K. Tori, S. Seo, Y. Yoshimura, H. Arita, Y. Tomita, *Tetrahedron Lett.* **1977**, 179−182.
[30] S. Seo, Y. Tomita, K. Tori, Y. Yoshimura, *J. Am. Chem. Soc.* **1978**, *100*, 3331−3339.
[31] R. Kasai, M. Suzuo, J. Asakawa, O. Tanaka, *Tetrahedron Lett.* **1977**, 175−178.
[32] F. A. L. Anet, R. Anet, in *Determination of Organic Structures by Physical Methods* (Eds.: F. C. Nachod, J. J. Zuckerman), Academic Press, New York, **1971**, p. 340.
[33] H. Booth, in *Progress in NMR Spectroscopy* (Eds.: J. W. Emsley, J. Feeney, L. H. Sutcliffe), Pergamon Press, Oxford, **1969**, pp. 215−216.
[34] A. Bianco, C. Iavarone, C. Trogolo, *Tetrahedron* **1974**, *30*, 4117−4121.
[35] B. N. Ravi, R. J. Wells, *Aust. J. Chem.* **1982**, *35*, 129−144.
[36] G. Wolczunowicz, F. G. Cocu, T. Posternak, *Helv. Chim. Acta* **1970**, *53*, 2275−2288.
[37] W. H. Gerwick, *Chem. Rev.* **1993**, *93*, 1807−1823.
[38] A. N. Grechkin, *J. Lipid Mediators Cell Signalling* **1995**, 205−218.
[39] B. Schulz, J. Sucker, H. J. Aust, K. Krohn, K. Ludewig, P. Jones, D. Doring, *Mycol. Res.* **1995**, *99*, 1007−1015.
[40] T. Rezanka, I. A. Guschina, *Phytochemistry* **2000**, *54*, 635−645.
[41] R. S. Jacobs, S. White, L. Wilson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 26−29.
[42] J. L. McLaughlin, *Methods in Plant Biochemistry* (Ed.: K. Hostettman), Academic Press, London, **1991**; vol. 6, pp. 1−30.
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