New Cytotoxic Sterol Glycosides from the Octocoral Carijoa (Telesto) riisei

Lenize F. Maia,‡ Rosângela de A. Epifanio,§,* and William Fenical

Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de J aneiro, 21941-590, Rio de Janeiro, RJ, Brazil, Departamento de Quimica Orgânica, Instituto de Química, Universidade Federal Fluminense, Campus do Valonguinho, 24020-150, Niterói, RJ, Brazil, and Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California–San Diego, La Jolla, California 92037-0204

Two new steroidal glycosides, 3\(^{-}\)-O-(3\(^{-}\)-O-acetyl-\(\beta\)-D-arabino-pyranosyl)-25\(^{-}\)-cholestane-3\(^{\beta}\),5\(^{\alpha}\),6\(^{\alpha}\),26-tetrol-26-acetate (riisein A, 2) and 3\(^{-}\)-O-(4\(^{-}\)-O-acetyl-\(\beta\)-D-arabino-pyranosyl)-25\(^{-}\)-cholestane-3\(^{\beta}\),5\(^{\alpha}\),6\(^{\alpha}\),26-tetrol-26-acetate (riisein B, 3), were isolated from extracts of the Brazilian teleostaecean octocoral Carijoa (Telesto) riisei collected near Rio de Janeiro. The new glycosides co-occur with the polyhydroxy sterol, 25\(^{-}\)-cholestane-3\(^{\beta}\),5\(^{\alpha}\),6\(^{\alpha}\),26-tetrol-26-acetate (1), an inseparable diastereomeric mixture previously reported from Telesto riisei collected in Micronesia. The structures of the new glycosides were assigned by spectroscopic methods and by comparison with spectral data for sterol 1. Riiseins A and B showed in vitro cytotoxicity toward HCT-116 human colon adenocarcinoma with IC\(_{50}\) values of 2.0 \(\mu\)g/mL.

Octocorals or soft-corals (subclass Octocorallia) are common, soft-bodied invertebrates found throughout the world's oceans. This group of marine invertebrates is recognized to be an extremely rich source of bioactive secondary metabolites, and because these animals lack physical defenses, these compounds are generally hypothesized to function as chemical defenses. Octocorals within the order Telestoideae are rare, and chemical investigations have been suggested the molecular formula C\(_{29}\)H\(_{50}\)O\(_{5}\). This molecular formula was confirmed by HRFABMS mass measurement of the fragment ion at m/z 460.3549 (calcd for [C\(_{29}\)H\(_{50}\)O\(_{5}\) - H\(_{2}\)O\(\cdot\)H\(_{2}\)O], 460.3553). NMR measurements revealed that 1 was cholestane-3\(^{\beta}\),5\(^{\alpha}\),6\(^{\alpha}\),26-tetrol-26-acetate, a sterol previously isolated by Lyngage and Schmitz from Telesto riisei collected in Micronesia. This was confirmed by direct comparison of the NMR data from sterol 1 with that derived from an authentic sample provided by Professor Francis Schmitz. Although we conducted our NMR experiments in a different solvent, we observed the same diastereomeric mixture as C-25 as reported earlier.

Spectroscopic analyses of riiseins A and B (2 and 3) revealed that they had almost identical structures possessing the same molecular formulas and exhibiting similar NMR features. Riisein A analyzed for the molecular formula C\(_{29}\)H\(_{50}\)O\(_{10}\) on the basis of HRFABMS and \(^{13}\)C NMR data. The IR spectrum showed absorption bands due to hydroxyl (3436 cm\(^{-1}\)) and ester carbonyl groups (1734 cm\(^{-1}\)). Analysis of 2D NMR experiments (\(^{1}H\) COSY, HMQC, and HMBC) revealed that riisein A was a pentose glycoside derivative of 1. This conclusion was supported by \(^{1}H\) signals for the pentose pyranoside between \(\delta\) 3.0 and 5.0 and by corresponding \(^{13}\)C NMR bands between 60 and 73 ppm. NMR bands for the characteristic sugar anomic carbon (97.8 ppm) and its corresponding proton (\(\delta\) 5.0) were clearly observed (Table 2). \(^{1}H\) NMR coupling-constant analysis of the pyranose ring indicated the presence of a pyranoarabinoside sugar linked to the steroidal polyol by a \(\beta\)-glycoside linkage (Table 2). The attachment of the sugar moiety at C-3 of the aglycone 1 in riisein A was based on \(^{3}\)J and \(^{3}\)J correlations observed in the HMQC spectrum. The sugar anomic carbon C-1 (97.8 ppm) and the aglycone carbon C-3 (74.6 ppm) showed correlations with the H-3 proton (\(\delta\) 4.06) and H-1' protons (\(\delta\) 5.03), respectively. NMR data also indicated the presence of an additional acetate ester positioned at C-3 (\(\delta_{\text{H}}\) 5.07, dd, \(\delta_{\text{C}}\) 3.0 and 9.8 Hz; and \(\delta_{\text{C}}\) 73.1 ppm). Acetylation of riisein A yielded the triacetate 4. This was an unexpected reaction that apparently occurred by acid-catalyzed hydrolysis of the arabinopyranoside ring and subsequent acetylation at C-3 and C-6. Triacetate 4, produced by acetylation of riisein A, was
Table 1. NMR Data for the Sterol 1 and the Steroidal Aglycon Components of Rilisins A (2) and B (3) in CDCl₃

<table>
<thead>
<tr>
<th>position</th>
<th>₁³C (DEPT)</th>
<th>₄H (m, J in Hz)</th>
<th>₂⁺³C (DEPT)</th>
<th>₂⁺₄H (m, J in Hz)</th>
<th>₃⁺³C (DEPT)</th>
<th>₃⁺₄H (m, J in Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>32.3 (CH₂)</td>
<td>1.40 (m)</td>
<td>32.3 (CH₂)</td>
<td>1.42 (m)</td>
<td>31.7 (CH₂)</td>
<td>1.37 (m)</td>
</tr>
<tr>
<td>2</td>
<td>30.7 (CH₃)</td>
<td>1.86 (m)</td>
<td>28.7 (CH₂)</td>
<td>1.86 (m)</td>
<td>28.2 (CH₂)</td>
<td>1.85 (m)</td>
</tr>
<tr>
<td>3</td>
<td>67.6 (CH)</td>
<td>4.08 (m)</td>
<td>74.6 (CH)</td>
<td>4.06 (m)</td>
<td>74.8 (CH)</td>
<td>4.01 (m)</td>
</tr>
<tr>
<td>4a</td>
<td>40.6 (CH₂)</td>
<td>1.63 (m)</td>
<td>37.3 (CH₂)</td>
<td>1.67 (m)</td>
<td>36.8 (CH₂)</td>
<td>1.64 (m)</td>
</tr>
<tr>
<td>4b</td>
<td>2.08 (m)</td>
<td>2.08 (m)</td>
<td>2.10 (m)</td>
<td>2.08 (m)</td>
<td>2.08 (m)</td>
<td>2.08 (m)</td>
</tr>
</tbody>
</table>

* Data included as a reference for NMR data of compounds 2 and 3 in CDCl₃.  

Rilisins A (2) and B (3) were examined for their in vitro cytotoxic properties toward HCT-116 human colon tumor cells. Both compounds showed significant cytotoxicity, with ED₅₀ values of 2.0 µg/mL.  

The generic nomenclature Carrijoa and Tejesto are synonymous in the literature. The variability of chemical components found from geographically diverse collections of
this animal is not fully understood. Certainly, different approaches in extraction and purification of natural products can lead to the isolation of different classes of natural products. The variability of secondary metabolites found in C. riisei, however, may indicate temporal or geographic variations in the production of such chemicals as a consequence of environmental conditions, phenotype differences, or other biotic factors. Typically, the systematics of octocorals represent difficulties not yet clarified by gene sequence-based systems.

Although different classes of compounds have been isolated from Pacific specimens, the southwest Atlantic populations of this animal appear closely related to those populations studied from the southwest Pacific Ocean. The isolation of compounds 1–3, as well as the previously described pregnanes from the Brazilian Carioja (Tetesto) riisei, indicates the similarity of the Brazilian, Enneatakis, and Chuuk Atoll collections, all of which contain 18-acetoxypregna-1,4,20-trien-3-one and 25α-cholestane-3β,5α,6β,26-tetrol-26-acetate (1).

**Experimental Section**

**General Experimental Procedures.** Corrected melting points were observed on a Thomas-Hoover capillary apparatus. IR spectra (film, CHCl3) were recorded on a Perkin-Elmer model 1600 FTIR spectrometer. Optical rotations were measured on a Perkin-Elmer 243B (D2O = 589 nm, c 1.0, CHCl3 or MeOH). Mass measurements were obtained on a HP5989A spectrometer. NMR spectra were recorded on a Varian Unity-500 (1H and 13C spectra) spectrometer. NMR spectra were recorded on a Varian Unity-500 (1H and 13C spectra) spectrometers (with CDCl3 solutions using TMS as internal standard). GC analyses were performed on a Varian model 3400 equipped with DB-5 glass capillary column (30 m) using hydrogen as carrier gas and temperature programming from 120 to 240 °C at a rate of 2 °C/min. Normal (Si gel) and reversed-phase (C18) HPLC separations were carried out using semipreparative columns (4 mm i.d.) using a Waters model M6000 pump and a model R401 refractive index detector.

**Extraction and Isolation.** Colonies of Carioja (Tetesto) riisei were collected by surface-air-supply diving, in July 1993, at Mangaratiba, Rio de Janeiro state, Brazil, at a depth of 6 m. After collection, the specimens were immediately frozen. The animals were freeze-dried (235 g) and subsequently extracted at room temperature 3 times with MeOH–CH2Cl2 (1:1). After removal of the solvents from the combined extracts under reduced pressure, 15.0 g of a brownish gum was obtained. The CH2Cl2-soluble portion of the crude extract (5.64 g) was fractionated by flash chromatography on Si gel (230–400 mesh, Merck) employing a gradient of 0–100% of EtOAc in isooctane and pure MeOH as eluents. The EtOAc-soluble compounds obtained from the MeOH fraction (983 g) were subjected to semipreparative normal-phase HPLC separation, with EtOAc as eluent, to yield 1–3 as impure compounds.

Compounds 1–3 were further purified by reversed-phase (C18) HPLC using MeOH–H2O (94:6, flow rate of 2.5 mL/min) to furnish sterol 1 (19 mg, 0.008% dry wt), riisein A (2, 8 mg, 0.003% dry wt), and riisein B (3, 12 mg, 0.005% dry wt).

**25α-Cholestan-3β,5α,6β,26-tetrol-26-acetate (1):** amorphous white powder; mp 187–189 °C; [α]D20 −61° (c 0.1, CHCl3); IR (film, CHCl3) 1717, 1707 cm−1; 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 75 MHz) see Table 1. EI mass (rel int) [M]+ 478 (4), 460 (30), 442 (100) 173, 383 (24), 360 (5); HRMS (70 eV) [M − H2O]− obsd 460.3549, calcd for C25H40O11: 460.3553.

**Riisein A (2), 3β-3-O-Acetyl-5β-cholestan-3β,5α,6β,26-tetrol-26-acetate:** white powder; mp 187–189 °C; [α]D20 −30° (c 0.1, CHCl3); IR (film, CHCl3) 1717, 1707 cm−1; 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 75 MHz) see Tables 1 and 2; 1H COSY (CDCl3, 500 MHz) see Table 1; EIMS (70 eV) [M]+ 675 (10), 443 (12), 425 (10) 154 (100); [M − CHO]− obsd 657.10 (4), 425 (10), 383 (4), 154 (100); RFABMS (70 eV) [M + Na]+ 965 (10), 443 (12), 425 (10), 383 (4), 154 (100); RFABMS (70 eV) [M + Na]+ 675.4058, calcd for C36H58O12Na, 675.4058.
H-26ab,-27; C-26: H-25,-27; C-27: H-24,-25,-26ab; C-28: H-29,-26ab; for arabinosyl moiety see Table 2; FABMS m/z (rel int) [M + Na]+ 675 (5), 460 (12), 443 (22), 425 (25), 383 (15), 154 (100).

Acetylation of Compounds 1, 2, and 3. In separate experiments, a solution of 2 mg of compounds 1, 2, or 3 were combined with Ac2O-pyridine (0.5 mL each) and allowed to stand at room temperature for 24 h. The solvents were removed under reduced pressure, and the reaction products were chromatographed by Si gel HPLC, eluting with EtOAc, to give the triacetate 4. Compound 4, which was also prepared by Lyanage and Schmitz as part of their structure analysis of sterol 1, provided the following NMR data (in CDCl3): 1H NMR (500 MHz) δ 0.67 (3H, s), 0.90 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.5 Hz), 0.92 (3H, d, J = 7.0 Hz), 1.18 (3H, s), 2.01 (3H, s), 2.05 (3H, s), 3.84 (1H, m), 3.95 (1H, m).

Absolute Configuration of Arabinose. Riisein B (2, 1 mg) was treated with (-)-2-butanol (0.5 mL) in 0.5 M HCl at 80 °C for 18 h. The solution was washed with heptane (3 x 2 mL) and evaporated to dryness under N2. Bistrimethylsilyl-trifluoroacetamide (25 μL) in dry pyridine (25 μL) was added to the resulting residue. After 1 h at room temperature, the solution was analyzed by GC. The same procedure was used to prepare the authentic trimethylsilylated (±)-2-butyl glycosides of α-(-)-arabinose. The chromatograms obtained were compared with literature data, which indicated the preparation of the corresponding α-arabinose derivatives.

Acknowledgment. This research was supported by National Brazilian Research Council (CNPq), International Foundation for Science (IFS, Sweden), and by a collaborative CNPq-NSF project. L.F.M. thanks CAPES for award of a DSc fellowship. We also thank Clovis B. e Castro for identification of the animals, Orlando Agrellos Filho for GC analysis, and Marquis Cummings for performing the cytotoxicity assays. We thank Professor Francis Schmitz, University of Oklahoma, for kindly providing an authentic sample of sterol 1 for NMR comparison.

References and Notes