The First 6,8-Cycloeduesmane Sesquiterpene from a Marine Organism: The Red Seaweed Laurencia microcladia from the Baia di Calenzana, Elba Island

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Reported here is the isolation and structural elucidation of a new 6,8-cycloeduesmane sesquiterpene, 5-bromo-1-isopropyl-2,5a-dimethyl-decacyclopropa[a]inden-2-ol (5), alongside the known sesquiterpenes (-)-δ-cadinene (7) and (+)-α-cadinol (8), from the red seaweed Laurencia microcladia Kützing of the Baia di Calenzana, Elba Island. Other than belonging to a rare sesquiterpene class, 5 is the first 6,8-cycloeduesmane sesquiterpene of marine origin.

1. Introduction

Red seaweeds in the genus Laurencia are a prolific source of secondary metabolites of varied, and often unusual structure [1–3]. From this genus we present here the first example of marine origin of 6,8-cycloeduesmane sesquiterpenes (skeleton 1, Fig. 1), which are otherwise rare from terrestrial green plants [4–6]. Most cycloeduesmanes so far reported are cycloeduesmanolides belonging to the 1,3- (2) and 2,4-class (3) (Fig. 1), isolated from terrestrial plants [7]. The only cycloeduesmane of marine origin, cycloeudesmol [8], isolated from Laurencia nipponica, belongs to the 5,7-cycloeduesmane class (4, Fig. 1).

This new 6,8-cycloeduesmane sesquiterpene (5) was isolated from a strain of Laurencia microcladia Kützing collected in the Baia di Calenzana, Elba Island (which is already known for the unique calenzanane sesquiterpene 6 [3]) alongside the known

Fig. 1. 6,8-Cycloeduesmane type of skeleton (1) and other known cycloeduesmane skeletons (2–4).

Fig. 2. Brominated 6,8-cycloeduesmane isolated from L. microcladia from the Baia di Calenzana.
sesquiterpenes (-)-δ-cadinene (7) and (+)-α-cadinol (8) (Fig. 2).

2. Results and Discussion

Mass spectrometry (EI-MS and HR-EI-MS) of the new metabolite from L. microcladua (5) established the molecular composition C_{15}H_{25}BrO, implying three unsaturations or cycles, one less than calenzanol (6) [3]. Since NMR spectra did not reveal any double bond, the molecule must be tricyclic. A 3,4-dehydrocalenzanol structure, conceivable from the presence of a bromomethine group (δ_{H} = 3.86 dd) and a cyclopropyl ring (high field methine proton signal, δ_{H} = 0.52 td) bearing an isopropyl group, was ruled out by COSY and HMQC NMR experiments. These data, obtained in two different deuterated solvents, CDCl_{3} and C_{6}D_{6}, support a 6,8-cycloesudesmane skeleton (1) for the new compound.

The position and axial (β) orientation of the tertiary hydroxyl group at C-4 for 5 was defined by heteronuclear correlation NMR experiments in C_{6}D_{6} as a solvent, where the proton chemical shifts of two tertiary methyl protons are better resolved than in CDCl_{3}. Defining the position and orientation of the bromine atom required a more extensive analysis. First, a long range small homonuclear coupling was detected, in CDCl_{3} as a solvent, between the methyl group at C-4 and the equatorial (α) proton at C-3 (δ_{H} = 1.62 ddd). The alternative isomeric structure bearing the bromine atom at C-3 could not have given rise to this coupling. Moreover, 13C NMR chemical shifts are in agreement with bromine at C-1 and are consistent with those of 6,8-cycloesudesma-1,4-diol [4], whereas quite different carbon resonances would be expected in the alternative structure with bromine at C-3; in particular C-4 would be expected at 10 ppm downfield due to a β-bromine effect. Substitution of OH by Br at C-1 should induce a change in all 13C chemical shifts at the γ positions C-3, C-5, C-9, and C-14 of this new compound, while the weaker γ shielding effect of Br (4 ppm) with respect to OH (6 ppm) should be accompanied by a downfield effect of about 2 ppm. The C-1 position for Br fits also biogenetic hypotheses, as discussed below.

As to the relative stereochemistry of 5, the equatorial (α) position of 15-Me, and the axial (β) position of 14-Me, rest on δ_{C} values in CDCl_{3}, 30.31 ppm and 17.76 ppm, respectively. Axial 15-Me is expected to resonate at δ_{C} = 20 - 22 ppm and equatorial 14-Me at δ_{C} = 22 - 23 ppm [6]. Since the proton at C-1 shows up as a classical axial proton (1α-H, dd, J = 4.1, 12.3 Hz), the bromine atom must occupy the equatorial position (1β-Br, in the arbitrarily chosen enantiomer 5 shown here). A small W coupling between the 14-Me and 1α-H, 5-H, and 9α-H (detected in C_{6}D_{6} as a solvent, both in COSY experiments optimized for long range couplings and in differential decoupling experiments) established the α position for these three protons. A strong positive NOE, in CDCl_{3} as a solvent, between 9α-H and both 1α-H (δ = 3.86) and 5-H (δ = 0.84 ppm) supports the trans fused ring junction and the above spectral assignments. The stereochemistry of the cyclopropane ring of 5 was also deduced from NMR experiments, selecting data in CDCl_{3} or C_{6}D_{6} as solvents so as to circumvent the problem of overlapping signals. Thus, 7-H showed up at high field (δ = 0.24), strongly coupled with 11-H (9.3 Hz) and weakly coupled with both 6-H and 8-H (3.2 Hz).

The latter feature established the trans relationship of 7-H with both 6-H and 8-H. Extensive differential decoupling experiments allowed us to define the coupling patterns of all the protons in this region (Experimental). In particular, 5-H (δ = 0.84, br.d, J = 5.7 Hz) is coupled with 6-H (δ = 1.24 ppm, dd, J = 3.2, 5.7, 8.5 Hz, in CDCl_{3}), long-range W coupled with both 14-Me and 9α-H, and is linked to 15-Me by NOE enhancement. All these assignments were aided by molecular mechanics calculations for the preferred conformer of 5 (Fig. 3), which reproduced nicely the vicinal coupling constants (Table). Only the J couplings for the cyclopropyl protons could not be satisfactorily reproduced, lacking parametrization in the modified Karplus equation used in our minimization program.

The biosynthesis of eudesmane and guaiane sesquiterpenes is believed to occur via transannular cyclization of (E,E)-germacrenes, such as germacrene-B or its 1,10- and 4,5-epoxides [9 - 11]. In contrast, biosynthetic routes for the closely related, albeit rare, cycloesudesmanes have been scarcely considered [4, 6, 7]. Based on synthetic studies for 1α-hydroxy-6,8-cycloesudesmane [6], it was proposed that the eudesmane metabolites of a higher plant in the Apiaceae, Torilis japonica, including 6,8-cycloesudesmane-type of metabolites, originate from epoxystergermacrene-D [5]. If so, and
Table. $^1$H NMR experimental and calculated (strain energy minimized, Fig. 3) coupling constants for the 6,8-cycloedusmane (5).

<table>
<thead>
<tr>
<th>Vicinal coupling</th>
<th>Exp. J (Hz) (CDCl$_3$ or C$_6$D$_6$)</th>
<th>Calcd. J$^a$ (Hz)</th>
<th>Torsional angle (°)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-H, 2-H</td>
<td>4.1</td>
<td>4.1</td>
<td>173</td>
</tr>
<tr>
<td>1-H, 2-H</td>
<td>12.3</td>
<td>11.7</td>
<td>56</td>
</tr>
<tr>
<td>2-H, 3-H</td>
<td>4.6</td>
<td>4.6</td>
<td>−52</td>
</tr>
<tr>
<td>2-H, 3-H</td>
<td>2.5</td>
<td>2.4</td>
<td>63</td>
</tr>
<tr>
<td>2-H, 3-H</td>
<td>13.9</td>
<td>13.2</td>
<td>−167</td>
</tr>
<tr>
<td>2-H, 3-H</td>
<td>4.6</td>
<td>4.7</td>
<td>−51</td>
</tr>
<tr>
<td>5-H, 6-H</td>
<td>5.7</td>
<td>7.8</td>
<td>140</td>
</tr>
<tr>
<td>6-H, 7-H</td>
<td>3.2</td>
<td>7.5</td>
<td>140</td>
</tr>
<tr>
<td>6-H, 8-H</td>
<td>8.5</td>
<td>10.3</td>
<td>−1.0</td>
</tr>
<tr>
<td>7-H, 8-H</td>
<td>3.2</td>
<td>8.1</td>
<td>−142</td>
</tr>
<tr>
<td>7-H, 11-HF</td>
<td>9.3</td>
<td>11.7$^c$</td>
<td>−179$^c$</td>
</tr>
</tbody>
</table>

$^a$ Calculated using the PCMODEL subroutine; $^b$ from molecular-mechanics calculated minimum strain-energy conformer; $^c$ calculated on the most stable rotamer around C10-C11.

Fig. 4. Hypothetical biogenetic origin of the 6,8-cycloedusmane sesquiterpene 5.

Both 6,8-cycloedusmane and cadinene sesquiterpene were previously known as widespread metabolites in terrestrial vascular plants [4, 6, 13, 14]. Our work has shown that sesquiterpene in these classes are also found in red seaweeds (Rhodophyta), which appeared much earlier than vascular plants [15]. It is difficult to imagine a gene transfer from red seaweeds to vascular plants, which probably descend from green seaweeds [16]. Rather, genes coding for the cyclases leading to these sesquiterpenoids may have evolved twice, earlier in the red seaweeds and later in vascular plants. Given the large phylogenetic distance between red seaweeds and vascular plants, the two groups of genes may be different, which means that different biogenetic routes are expected to the same types of products in the two groups of organisms [15]. We suggest that convergence resulted from the chemical propensity to these skeletal types [15, 16].

3. Experimental

3.1. General

We carried out thin-layer chromatography (TLC) on Merck Kieselgel 60 PF$_{254}$ plates, flash-chromatography (FC) on Merck Si-60, 15 - 25 μm, silica gel, reversed-phase flash chromatography (RP-FC) on Merck LiChrosorb RP18 bonded silica gel, 20 - 50 μm, high-performance liquid chromatography (HPLC) on Merck-
3.2. Isolation of the metabolites

The residue (0.12 g) from evaporation of previous fractions 1-8 (out of 40 fractions obtained before from L. microcladia extract) [3] was subjected to HPLC with n-hexane, flow gradient from 5 to 8 ml min⁻¹ during 20 min) obtaining compound 7 (R: 5.1 min; 2.5 mg). The residue (0.15 g) from evaporation of previous fractions 14 - 16 [3] was subjected to HPLC with n-hexane/i-PrOH (99:1) under refractometric detection, to give crude compound 5 (R: 13.7 min, 12.0 mg). The latter was subjected to reversed-phase HPLC with MeCN/H₂O 70:30 to give pure 5 (R: 7.5 min, 6.2 mg). The residue (0.145 g) of previous fractions 17 - 19 [3] was subjected to HPLC with n-hexane/i-PrOH (98:2) obtaining compound 8 (R: 15.2; 7.5 mg).

3.3. 5a,7α(H)-1β-bromo-6,8-cycloedemane-4β-ol or 1β-bromo-cyclopropa[α]inden-2-ol (5)

Colorless oil. - [α]D = +10.0 (MeOH, c = 0.13). - 1H NMR (299.94 MHz, CDCl₃): δ = 0.52 (td, J₃,₅ · J₅,₆ = 3.2, J₁,₁₁ = 9.1 Hz, 1H, 7-H), 0.84 (br, J₆,₈ = 5.7 Hz, 1H, 5-H), 0.91 (m, 1H, 9a-H), 0.93 (s, 6H, 12-Me and 13-Me), 0.94 (m, 1H, 11-H), 1.14 (dd, J₆,₁₀ = 3.2, J₆,₉a = 6.7, J₆,₈ = 8.5, J₅,₉b = 11.2 Hz, 1H, 8-H), 1.16 (br, J₅,₈ = 5.7 Hz, 1H, 6-H), 1.24 (dd, J₆,₇ = 3.2, J₆,₅ = 5.7, J₆,₈ = 8.5 Hz, 1H, 12-H), 1.29 (s, 3H, 14-Me), 1.32 (br, 3H, 15-Me), 1.33 (dt, J₃,₆a = 4.6, J₃,₇a = 4.6, J₅,₃,₆a = 13.9 Hz, 1H, 30-H), 1.62 (dd, J₃,₂a = 2.5, J₅,₃,₂a = 4.6, J₇,₃,₆a = 13.9 Hz, 1H, 3,₂-H), 1.84 (dd, J₃,₈,₅ = 6.7, J₅,₃,₉a = 12.8, 1H, 9,β-H), 1.95 (dd, J₆,₁₀ = 4.1, J₆,₁₁,₁₂ = 4.6, J₅,₉a,₉b = 13.9 Hz, 1H, 2a-H), 2.25 (dd, J₇,₁₂ = 13.3, J₇,₁₃,₁₂ = 4.6, J₇,₁₃,₁₂ = 13.9 Hz, 1H, 2β-H), 3.86 (dd, J₆,₁₂ = 4.1, J₆,₁₂ = 12.3 Hz, 1H, 2β-H); (CDCl₃): δ = 0.24 (td, J₁₇,₁₇ = 3.2, J₁₇,₁₁ = 9.3 Hz, 1H, 7-H), 0.36 (br, J₆,₁₂ = 5.4 Hz, 1H, 5-H), 0.68 (d, J₁₁,₁₁ = 9.3, J₁₁,₁₁ = 8.5 Hz, 1H, 11-H), 0.82 (m, 1H, 9a-H), 0.86 (m, 1H, 3a-H), 0.89 (m, 1H, 8-H), 0.91 and 0.95 (2 x d, J₁₂,₁₃,₁₁ = 6.7 Hz, 6H, 12-Me and 13-Me), 0.92 (s, 3H, 15-Me), 0.94 (m, 1H, 6-H), 1.25 (dd, J₇,₁₂ = 2.2, J₇,₁₃,₁₂ = 4.6, J₇,₁₃,₁₂ = 13.9 Hz, 1H, 3β-H), 1.49 (s, 3H, 14-Me), 1.82 (dd, J₆,₁₂ = 4.0, J₆,₁₂ = 2.4, J₆,₁₃,₁₂ = 4.8, J₆,₁₂,₁₂ = 13.9 Hz, 1H, 2a-H), 1.88 (dd, J₇,₈,₅ = 6.5, J₇,₈,₉αβ = 12.6 Hz, 1H, 9,β-H), 2.31 (dd, J₇,₁₂ = 12.4, J₇,₁₃,₁₂ = 4.6, J₇,₁₃,₁₂ = 13.6 Hz, 1H, 1β-H), 3.60 (dd, J₆,₁₂ = 4.0, J₁,₁₂ = 12.4 Hz, 1H, 1β-H); - 13C NMR (75.43 MHz, CDCl₃): δ = 17.6 (q, C-14), 21.76 (q, C-12 or C-13), 21.87 (q, C-13 or C-12), 23.11 (d, C-8), 25.24 (d, C-6), 30.31 (q, C-15), 31.16 (t, C-2), 32.42 (d, C-11), 41.63 (t, C-3), 45.95 (t, C-9), 49.90 (d, C-7), 58.13 (s, C-10), 61.96 (d, C-1), 62.18 (d, C-5), 71.18 (s, C-4); (CDCl₃): δ = 18.43 (q, C-14), 22.44 (q, C-12 or C-13), 22.57 (q, C-13 or C-12), 23.78 (d, C-8), 25.94 (d, C-6), 30.73 (q, C-15), 32.10 (t, C-2), 32.42 (d, C-11), 42.17 (t, C-3), 46.60 (t, C-9), 50.08 (d, C-7), 59.50 (s, C-10), 62.44 (d, C-1), 62.52 (d, C-5), 70.97 (s, C-4). MS (EI, 70 eV): m/z (%): 300/302 (1) [M⁺], 282/284 (2) [M⁺ - H₂O], 267/269 (2) [M⁺ - H₂O - Me], 203 (40) [M⁺ - H₂O - Br], 161 (37), 43 (100). HR-EL-MS: m/z = 300.1080 ± 0.006, calc. for C₁₅H₂₃C₁₄H₂O₃BrO = 300.1083.

3.4. (S)-6-Cadinene (= 1-isopropyl-4,7-dimethyl-1,2,3,5,6,8-hexahydropaphthalene (7))

Colorless oil. - [α]D = -16.3 (MeOH, c = 0.09). - 1H and 13C NMR data: matching those reported [13].

3.5. (+)-α-Cadinol (= 4-isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-ol) (8)

Colorless oil. - [α]D = +13.0 (MeOH, c = 0.30). - 1H and 13C NMR data: matching those reported [14].

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