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Calenzanol, the first member of a new class of sesquiterpene with a novel skeleton isolated from the red seaweed *Laurencia microcladia* from the Bay of Calenzana, Elba Island

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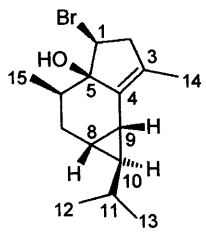
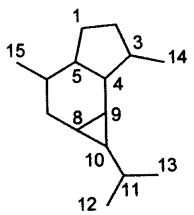
Abstract—The sesquiterpene calenzanol **1**, the major metabolite of the red seaweed *Laurencia microcladia* Kützing from the Bay of Calenzana in Elba Island, possesses a previously unreported ring system, thus establishing a new class of sesquiterpene with a novel skeleton, calenzanane **2**. Because of the special array of functionalities, **1** exhibits marked instability, readily rearranging to give the novel indene **3**. © 2001 Elsevier Science Ltd. All rights reserved.

Red seaweeds in the genus *Laurencia* (Ceramiales, Rhodomelaceae) are a rich source of secondary metabolites, including sesquiterpenes with either new skeletons, like (seco)- or (9,10-friedo)-chamigrane, guimarane, (cyclo)perforane and poitane, or skeletons previously reported from bryophytes, angiosperms and certain marine invertebrates, like aristolane, aromadendrane, bisabolane, brasilenane, cadinane, chamigrane, cuparane, eudesmane, germacrane, guaiane, guimarane, humulane, maaliane, perforane, poitane and oppositane (Fig. 1).[1]



1



2

We report here on the sesquiterpene calenzanol **1**, the major metabolite[2] isolated from *Laurencia microcladia* Kützing from the southern infralittoral zone of the Bay of Calenzana in Elba Island,[3] which possesses a previously undescribed skeleton, thus establishing a new class of sesquiterpene with a novel skeleton, calenzanane **2**.

Mass spectrometry (EI-MS and HR-EI-MS) furnished the molecular composition of calenzanol, $C_{15}H_{23}BrO$,[4] implying four unsaturations or cycles, while NMR spectra[4] revealed the presence of one tetrasubstituted carbon–carbon double bond. Since there was no evidence for another unsaturated bond, the molecule must be tricyclic. ^1H and ^{13}C NMR spectroscopy also revealed the presence of an *i*-Pr group (0.94 and 0.96 ppm, $^3J = 6.6$ Hz each, 3H each, 12/13-Me), a vinylic methyl group (1.48 ppm, 14-Me), a tertiary carbinol group (s at 78.71 ppm, C-5) and a bromomethine group (3.71 ppm, 1-H). Most distinctive for a cyclopropyl ring is an extremely high field methine proton signal (0.09 ppm, 10-H) in the ^1H NMR spectrum, equally coupled ($^3J(10,8) \approx {}^3J(10,9) = 4.5$ Hz) to two methine protons resonating at 0.87 ppm (8-H) and 1.16 ppm (9-H). The high values of the ^{13}C–^1H coupling constants for the carbon centers C-8, C-9 and C-10 (^1J(C8,8H) = 164 Hz, ^1J(C9,9H) = 160 Hz, ^1J(C10,10H) = 156 Hz) are also indicative of a cyclopropyl moiety. A combination of DDS, COSY, HMBC and HMQC NMR experiments allowed us to connect these fragments as shown in **1**.

The relative configurations of the six chiral centers were established from *J*-coupling analyses, NOE experiments and molecular modeling. The *cis*-relationship of the bromo and hydroxy groups was established by the similar vicinal H,H-coupling constants ($^3J(1,2\alpha) = 7.8$ and $^3J(1,2\beta) = 8.4$ Hz) observed between the bromomethine (1-H) and the neighboring geminal methylene protons at 2.27 and 2.62 ppm (2-Hα and 2-Hβ, respec-

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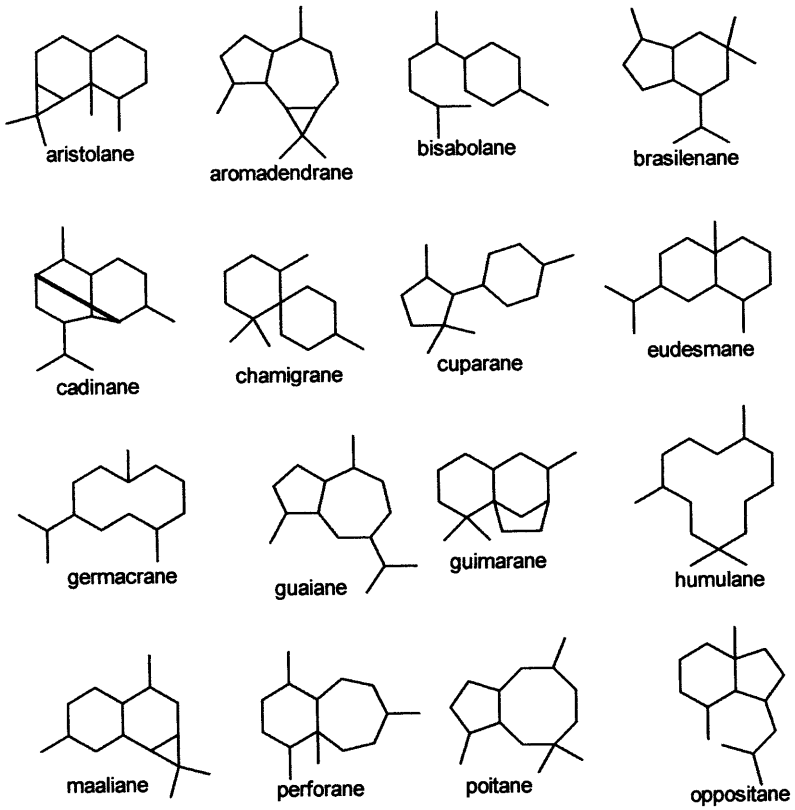


Figure 1. Sesquiterpene skeletons from *Laurencia* spp.

tively). A *trans*-relationship between these groups would be expected from molecular modeling[5] to give two quite different coupling constants. A strong NOE between 1-H and the methine signal at 1.00 ppm (6-H) placed the methine 6-H in the axial position on the cyclohexyl ring. This assignment is confirmed by the large vicinal coupling constant of 11.4 Hz observed between 6-H and the signal at 1.98 ppm (7-Hβ), indicative of a *trans*-diaxial coupling relationship. A strong NOE between 7-Hβ and both the methyl signal at 1.03 ppm (15-Me) and the cyclopropyl proton 8-H, then established a *cis* relationship between them. Furthermore, the *cis*-relationship between the cyclopropyl protons 8-H and 9-H is supported by a large vicinal H,H-coupling constant of 8.5 Hz, whereas, the *trans*-relationship between the cyclopropyl protons 8-H/10-H and 9-H/10-H was established by smaller vicinal H,H-coupling constants of 4.5 Hz each. This assignment of 10-H to the α-face (in the arbitrarily chosen enantiomer shown here) was confirmed by an NOE to the α-proton 6-H, thus completing the spatial assignment of all chiral centers. Experimental vicinal coupling constants for **1** (or the enantiomer) proved to be in excellent agreement with those calculated from the most stable conformation (Fig. 2).[5]

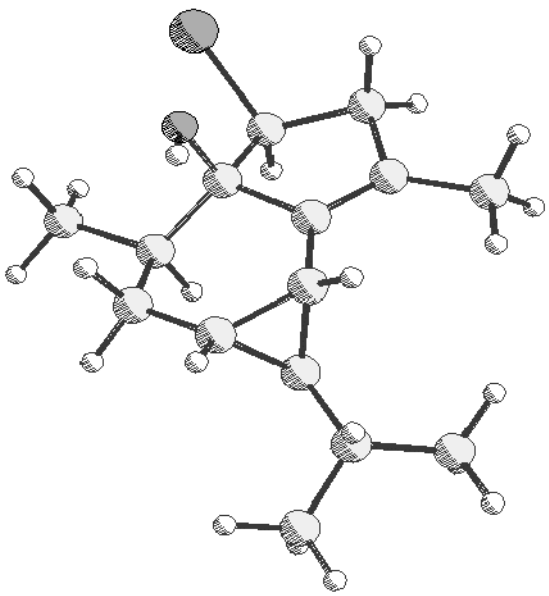
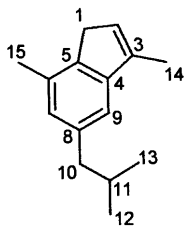


Figure 2. Strain-energy minimized conformation of calenzanol **1**.

The biosynthesis of **1** may be imagined to involve a germacrane precursor, possibly via a significant deviation from routes that lead to aromadendranes, guaianes, and poitanes.[1a,6]

While performing variable temperature NMR spectroscopy it was discovered that **1** at 40°C undergoes thermal decomposition to give predominantly the novel indene derivative **3**.[7] These processes are under study.



3

The C_{15} oxocane acetogenins from a sample of *L. microcladia* Kützing from Cap Ferrat along the Côte d'Azur,[8] the unusually dominant C_{15} oxepanes, including the sole examples of *C*-branched C_{15} acetogenins together with accompanying chamigrane sesquiterpenes from another sample of nominally the same seaweed from Il Rogiolo, south of Livorno,[9] and the novel calenzanane sesquiterpene from the present sample from Elba Island, show the inadequacy of current taxonomic keys for this algal genus. Attention to these observations should be paid by the ecologist, because *L. microcladia* has become a protagonist of the infralittoral zones of the Mediterranean, involving exchange of metabolites with both opisthobranch mollusks and sponges.[9]

Acknowledgements

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2. The ^{1}H NMR spectrum of the crude extract of *L. microcladia* comprises essentially only the sesquiterpene **1** along with common unsaturated fatty compounds.
3. The alga was immediately plunged into 95% EtOH, stored at 4°C in the dark for one year, and then extracted with MeOH. The residue after solvent evaporation (0.44 g, 0.6% of dry algal weight after extraction) was subjected to SiO_2 flash chromatography with *n*-hexane/EtOAc gradient elution, collecting 40 fractions of 0.1 L each. Fr. 12 was subjected to HPLC (Merck Lichrosorb Si60, 7 μm, 1×25 cm) with *n*-hexane/EtOAc 85:15, 5 mL/min, obtaining **1** (t_R = 5.5 min, 95 mg) as a dextrorotatory colorless oil, $[\alpha]_D$ +12, c = 3.00 mg/mL, hexane).
4. Compound **1**: EI-MS: *m/z* (%) 300, 298 (M^+, 4/4), 219 ($[M-Br]^+$, 20), 201 (49), 163 (34), 145 (100), 69 (21), 57 (41), 55 (30). HR-EI-MS: *m/z* 298.0937±0.006, calc. for $C_{15}H_{23}{}^{79}BrO$ 298.0932. ^{1}H NMR (300 MHz, C_6D_6, δ in ppm rel. to residual benzene [= 7.16 ppm] and J in Hz; some J-couplings were obtained from difference decoupling experiments): δ 3.71 (dd, $^3J(1,2\alpha)$ = 7.8, $^3J(1,2\beta)$ = 8.4, 1H, 1-H); 2.27 (quintet dd, $^4J(2\alpha,Me14) \approx {}^5J(2\beta,9)$ = 1.0, $^3J(2\alpha,1)$ = 7.8, $^2J(2\alpha,2\beta)$ = 15.7, 1H, 2-Hα), 2.62 (quintet dd, $^4J(2\beta,Me14) \approx {}^5J(2\beta,9)$ = 2.0, $^3J(2\beta,1)$ = 8.4, $^2J(2\beta,2\alpha)$ = 15.7, 1H, 2-Hβ); 1.00 (m, 1H, 6-H); 1.52 (ddd, $^3J(7\alpha,8)$ = 1.0, $^3J(7\alpha,6)$ = 4.2, $^2J(7\alpha,7\beta)$ = 13.5, 1H, 7-Hα); 1.58 (br s, OH); 1.98 (ddd, $^3J(7\beta,8)$ = 5.0, $^3J(7\beta,6)$ = 11.4, $^2J(7\beta,7\alpha)$ = 13.5, 1H, 7-Hβ); 0.87 (dtd, $^3J(8,7\alpha)$ = 1.0, $^3J(8,7\beta) \approx {}^3J(8,10)$ = 4.9, $^3J(8,9)$ = 8.5, 1H, 8-H); 1.16 (br ddd, $^5J(9,2\beta)$ = 2.0, $^3J(9,10)$ = 4.5, $^3J(9,8)$ = 8.5, 1H, 9-H); 0.09 (td, $^3J(10,8) \approx {}^3J(10,9)$ = 4.5, $^3J(10,11)$ = 9.0, 1H, 10-H); 0.74 (septet d, $^3J(11,Me12) \approx {}^3J(11,Me13)$ = 6.6, $^3J(11,10)$ = 9.0, 1H, 11-H); 0.94 (d, $^3J(Me12,11)$ = 6.6, 3H, 12-Me); 0.96 (d, $^3J(Me13,11)$ = 6.6, 3H, 13-Me); 1.48 (td, $^4J(Me14,2\alpha) \approx {}^5J(Me14,9)$ = 1.0, $^4J(Me14,2\beta)$ = 2.0, 3H, 14-Me); 1.03 (m, 3H, 15-Me). ^{13}C NMR (75 MHz, C_6D_6) (assignments based on HMBC and HMQC experiments): δ 56.04 (d, C1); 45.61 (t, C2); 134.47 (s, C3); 137.78 (s, C4); 78.71 (s, C5); 36.38 (d, C6); 28.96 (t, C7); 18.86 (d, C8); 16.52 (d, C9); 32.47 (d, C10); 33.54 (d, C11); 22.19 (q, C12); 22.45 (q, C13); 13.63 (q, C14); 14.56 (q, C15).
5. The strain-energy minimized conformation of **1** (Fig. 1) was obtained using the computer program PCMODEL V. 7.0, by Serena Software, Bloomington, Indiana, 1999.
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7. Compound **3**: Colorless, optically inactive oil. EI-MS*:* (%) *m/z:* 200 (M^+, 31), 185 ($[M-Me]^+$, 2), 158 (26), 157 ($[M-C_3H_7]^+$, 100), 142 ($[M-Me-C_3H_7]^+$, 24), 32 (31), 28 (73). HR-EI-MS *m/z:* 200.1569±0.006, calc. for $C_{15}H_{20}$ 200.1565. UV–vis (hexane) λ_{max}: 215 nm (ε 22,000), 253 nm (ε 7,000). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.16 (quintet, J = 2.0, 2H, 1-H); 6.18 (sextet, J = 1.7, 1H, 2-H); 6.81 (br.s, 1H, 7-H); 6.96 (br.s, 1H, 9-H); 2.50 (d, J = 6.7, 2H, 10-H); 1.88 (nontet, J = 6.7, 1H, 11-H); 0.92 (d, J = 6.7, 6H, 12-Me and 13-Me); 2.14 (dt, J = 1.5, 2.1, 3H, 14-Me); 2.33 (s, 3H, 15-Me). ^{13}C NMR (300 MHz, $CDCl_3$) (assignments based on HETCOR experiments): δ 36.15 (t, C1); 117.28 (d, C2); 128.45 (d, C7); 126.77 (d, C9); 45.59 (t, C10); 30.50 (d, C11); 22.51 (2×q, C12 and C13); 18.55 (q, C14); 13.21 (q, C15) and 132.15, 140.10, 140.17, 140.18, 145.75 (unassigned s for C3-6 and C8).
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