Calenzanane Sesquiterpenes from the Red Seaweed *Laurencia microcladia* from the Bay of Calenzana, Elba Island: Acid-Catalyzed Stereospecific Conversion of Calenzanol into Indene- and Guaiazulene-Type Sesquiterpenes

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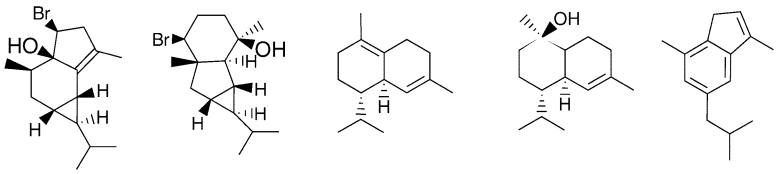
Abstract: It is shown here that calenzanane sesquiterpenes (**1** and **6**) can be isolated from organic extracts from the red seaweed *Laurencia microcladia* Kützing from the Bay of Calenzana, Elba Island, provided contact with acidic media is minimized. Such contact induces rearrangements of **1** in dry solvents to indene-type **5** and the blue-colored guaiazulenium-type ion **17**, via spectrometrically (NMR) characterized indene-type transient intermediates **10**, **14**, and **12**. Addition of NEt_3 to the reaction mixture at appropriate stages allowed the isolation of **12** (and **8** on workup on SiO_2), and guaiazulene (**18**). Prolonged contact with silica gel led to complete degradation of **1**, giving calenzanane-type epimeric enones **20a/20b** as well as indene-type epimeric carbinols **22a/22b** and fulvene **7**. The latter was also formed during silica-gel flash chromatography of the algal extracts. A unifying mechanistic view of these branching and cascade transformations may have both heuristic value, suggesting possible artefact origin of azulenoids, and synthetic applications.

Keywords: aromaticity · configuration determination · conformation analysis · reactive intermediates · terpenoids

Introduction

Red seaweeds of the genus *Laurencia* (Ceramiales, Rhodomelaceae) are a rich source of unusual secondary metabolites.[1] The first example of a calenzanane sesquiterpene, calenzanol (**1**),[2,3] is a recent addition. It was isolated as the main secondary metabolite from a strain of *Laurencia microcladia* Kützing from the Bay of Calenzana, Elba Island, which also gave a new 6,8-cycloeudesmane sesquiterpene (**2**)[4] and known sesquiterpenes, (−)-γ-cadinene (**3**) and (+)-α-cadinol (**4**). In a preliminary study, we noted that calenzanol (**1**), upon warming in either C_6D_6 or freshly base-washed $CDCl_3$, undergoes an intriguing transformation into the indene-type sesquiterpene (**5**).[2]



1 **2** **3** **4** **5**

Herein we describe reaction intermediates and branching routes for this and other decay processes, as well as a new calenzanane sesquiterpene from *L. microcladia*, in a mechanistic scenario that may have far-reaching implications for sesquiterpene chemistry.

Results and Discussion

Debromoisocalenzanol (6) and the artefact indene-type sesquiterpene 7: The unusual *cis* relationship between Br and OH in calenzanol (**1**)[2] has now found further support in its inertness toward 30% aqueous NaOH. In the less-encumbering *trans* relationship between Br and OH, epoxidation of the C3=C4 double bond should have occurred.[5]

Flash chromatographic fractions of *L. microcladia* extracts[2] were subjected anew to HPLC examination. Small

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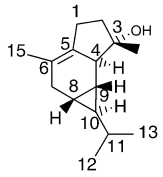
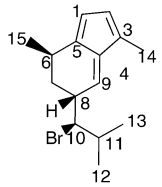
amounts of another new calenzanane sesquiterpene, debromoisocalenzanol (**6**) were isolated along with a new indene-type sesquiterpene, **7**. The composition of **6**, $C_{15}H_{24}O$, is based on HR-EI-MS data: three rings are inferred from only two singlets (C5=C6) in ^{13}C NMR spectra. The cyclopropyl ring appeared as a multiplet at $\delta_H = -0.14$ ppm, with a coupling pattern similar to that of **1**. Assuming that



6



7

the stereochemistry of **1** is conserved, the α-H4 and β-Me14 positions were derived from strong NOEs for H10/H4 and H9/Me14. The positions of both C5=C6 and the C3-OH were assigned based on differential decoupling spectroscopy (DDS), single bond correlation (HMQC), and multiple bond correlation (HMBC) data. In support, molecular mechanics (MM) calculations suggested that, as a result of a distortion imposed on the tricyclic system by the olefinic bond, the six-membered ring adopts a quasiplanar rigid conformation, with Me14 in a pseudoaxial position. This shows good agreement between observed and calculated vicinal coupling constants, in particular $J(4,9) = 1.8$ Hz, which implies that C4-H and C9-H are almost orthogonal. Biogenesis of the calenzanane skeleton may be attributed to the 1,9-cyclization of guaiane, arising from the well-established 2,6-cyclization of germacrene D.[6] The latter may also be a precursor of 6,8-cycloeudesmane (**2**) contained in our *L. microcladia*.[4]

The bicyclic nature of **7** ($C_{15}H_{21}Br$; HR-EI-MS) is based on three trisubstituted double bonds as the only unsaturation present (from ^1H and ^{13}C NMR spectra), while the fulvene-type moiety is supported both by the low-field resonances of the olefinic protons and UV absorption at $\lambda_{max} = 262.4$ nm. Bromomethine and *i*Pr groups (NMR) were connected to these groups, as in **7**, based on DDS, COSY, HMBC, and HMQC data.

Determining the stereochemistry of **7**, although complex because of the flexible side chain, is essential to the central issue of the stereospecific nature of the rearrangement reactions of **1** dealt with in the next section. The configurations at C6 and C8 are based on *J*-coupling patterns and NOE data. MM calculations suggested a preference for a boatlike six-membered ring in the main conformers (Figure 1) to maintain the planarity of the fulvene-type system. Assuming β Me15 as in **1**, the δ_H signal at 1.66 ppm is assigned to pseudoaxial $H_\beta 7$ because of the large coupling with H6 ($J =$ 10.7 Hz). In agreement, a small W coupling of $J = 0.6$ Hz occurs between H9 and pseudoequatorial H7α, while the assignment of H8β ($\delta_H = 2.96$) is based on a small coupling ($J = 4.2$ and 4.8 Hz) with C7-H_2 and a coupling of $J = 5.1$ Hz with H9 and a strong NOE with H9, which establishes an *R** configuration at both C6 and C8. C10 was assigned an *R** configuration from a conformational space search followed by strain minimization and evaluation of ^3J (Table 1) that led to three major conformers, **7a** (76%), **7b** (14%), and **7c** (10% rel. weight), in fast equilibrium (Figure 1). Measured vicinal coupling constants agree with those calculated for **7** (Table 1), whereas no agreement was found for the hypothetical (6*R**,8*R**,10*S**) epimeric structure (Table 2). Least-strained **7a** probably results from control of the side chain conformation by both the bulky bromine atom and 1,3-allylic strain between the C4=C9 bond and the C8 side chain, which is forced into a pseudoaxial position. This overrides a weaker allylic strain between C1=C5 and Me15. Therefore, in the less populated conformers **7b** and **7c**, Me15 is forced into a pseudoaxial position. No fit was obtained for either the hypothetical (6*R**,8*R**,10*S**) epimer of **7** (Table 2) or any other conceivable diastereomeric structure.

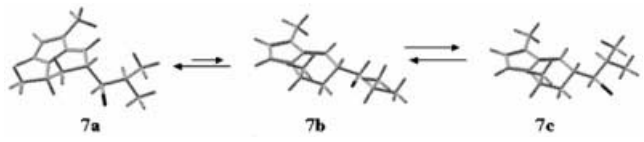


Figure 1. Major conformers (**7a**, **7b**, and **7c**, in a fast equilibrium) for compound **7** according to molecular mechanics calculations.

Table 1. Experimental and calculated (GMMX/MM3) ^3J coupling constants for compound (6*R**,8*R**,10*R**)-**7**.

Conformer type (rel. population) Vicinal protons	**7a** (76%)	**7b** (14%)	**7c** (10%)	Averaged ^3J calculated $J_{av} = \Sigma_i x_i J_i$	Experimental values [Hz]
	^3J calculated for single conformers				
6, 7α	3.5	4.9	4.7	3.8	4.2
6, 7β	12.2	2.0	2.1	9.8	10.7
8, 7α	2.2	12.2	12.2	4.6	4.2
8, 7β	4.6	3.7	3.8	4.4	4.8
8, 10	10.9	2.5	11.0	9.8	9.1
10, 11	1.9	10.6	2.2	3.2	3.8

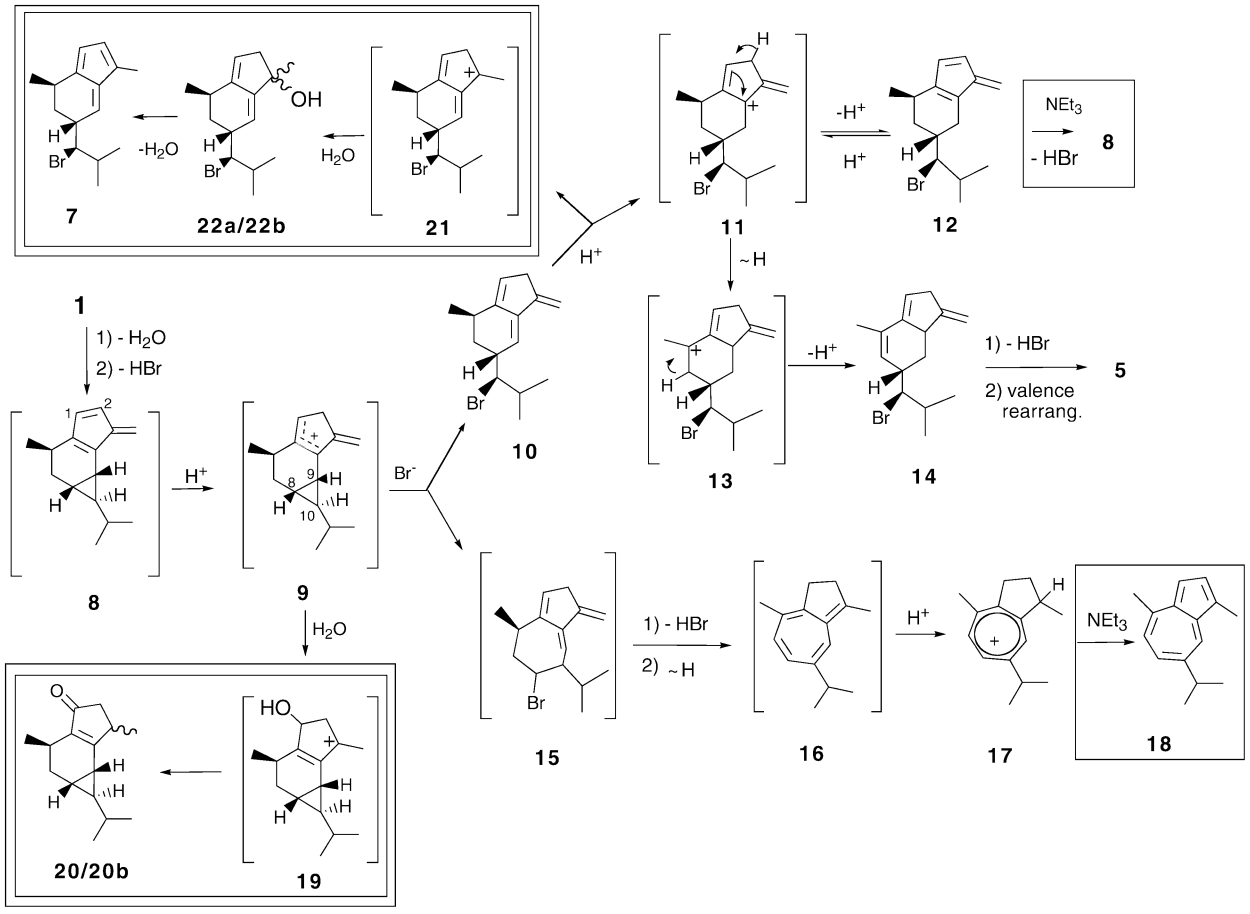
Table 2. Experimental and calculated (GMMX/MM3) ^3J coupling constants for the hypothetical (6*R**,8*R**,10*S**) epimer of **7**.

Conformer type (rel. population) Vicinal protons	**a** (44%)	**b** (24%)	**c** (16%)	**d** (14%)	**e** (2%)	Averaged ^3J calculated $J_{av} = \Sigma_i x_i J_i$	Experimental values [Hz]
	^3J calculated for single conformers						
6, 7α	3.3	5.0	4.8	4.9	4.9	4.2	4.2
6, 7β	12.3	2.0	2.1	2.0	2.0	6.5	10.7
8, 7α	2.3	12.3	12.2	12.3	12.3	7.9	4.2
8, 7β	4.5	3.6	4.0	3.6	3.6	4.0	4.8
8, 10	10.8	1.5	10.7	2.3	0.8	7.2	9.1
10, 11	2.0	10.4	2.1	10.4	1.9	5.2	3.8

Decay of calenzanol (1) in solution to form indene-type (5) and guaiazulenium ion (17) sesquiterpenes through observable intermediates 10, 12, and 14: Freshly purified, colorless calenzanol (**1**) proved to be stable, either as pure material or, at room temperature, in C_6D_6, *n*-hexane, and freshly base-washed $CDCl_3$ solution. However, warming to 40 °C in the latter medium, in the course of variable-temperature ^1H NMR experiments, induced decomposition of **1**: in a few minutes, signals for the indene-type sesquiterpene **5**[2] appeared, while those for **1** decreased. The solution also changed color to ink-blue (caused by the formation of **17**), whereby the intensity increased over a period of 1–2 h until the process was completed. Aged yellow samples of calenzanol were acidic and particularly prone to decomposition into **5** and **17**.

Decay of calenzanol (**1**) into **5** and **17** was monitored by ^1H NMR spectroscopy: three long-lived intermediates, **10**, **12**, and **14**, were detected (Scheme 1). Although these intermediates appeared in the given sequence, each in turn, their complete ^1H NMR spectral assignment required separate experiments under different conditions. Further support for the structure of **12** and **17** is given by the isolation of **8** and **18** from the respective quenching reactions by triethylamine (top-right and bottom-right boxes in Scheme 1).

The ^1H NMR spectrum of the first observable intermediate (**10**) was characterized by the absence of the high-field cyclopropyl signals of **1**; these signals were replaced by the signals for a bromomethine group (δ_H = 4.05 ppm (dd, 3.1, 9.8 Hz)) bound to the *i*Pr group (δ_H = 1.02 and 1.04 ppm, each d, J = 6.5 Hz, Me). Clearly, ring-opening of the cyclopropyl moiety of **1** had occurred, presumably induced by free HBr. Four olefinic methine signals were also detected. They are linked by strong NOE values to the two *exo*-methylene singlets at δ_H = 4.99 and 5.41 ppm. The remaining two olefinic resonances (δ_H = 5.72 and 5.84 ppm) were assigned to the terminal positions of the conjugated diene across C1/C5 and C4/C9. The transient nature of **10** made it difficult to carry out detailed NOE experiments devised to assign the configuration at the chiral centers. However, in a molecular-modeling approach as for **7** above, the relative configuration (6*R**,8*R**,10*R**) could be established from two major conformers, **10a** and **10b** (totaling 85%), with a pseudoaxial C8 side chain, and two minor conformers, **10c** and **10d** (totaling 15%), with a pseudoequatorial C8 chain (Table 3). Agreement was observed for the calculated and observed pattern of J coupling constants. In contrast, no agreement with the experimental findings was observed for the hypothetical (6*R**,8*R**,10*S**) epimeric structure (Table 4).



Scheme 1. Rearrangement of calenzanol (**1**), proceeding through intermediates **10**, **12**, and **14** and ending in the indene **5** and blue guaiazulenium ion (**17**). Addition of Et_3N allowed the isolation of guaiazulene (**18**) (lower, single-line box) and **12**. When SiO_2 chromatography was continued, compound **8** was isolated (upper, single-line box). The sequences within double-line boxes represent trapping of cations by water during workup on silica gel. Elusive intermediates are enclosed within square brackets.

Table 3. Experimental and calculated (GMMX/MM3) ^3J coupling constants for compound (6*R**,8*R**,10*R**)-**10**.

Conformer type (rel. population) Vicinal protons	**10a** (67%)	**10b** (18%)	**10c** (12%)	**10d** (3%)	Averaged ^3J calculated $J_{av} = \Sigma_i x_i J_i$	Experimental values [Hz]
	^3J calculated for single conformers					
6, 7α	3.4	4.8	4.6	4.6	3.8	4.0
6, 7β	12.3	2.1	2.2	2.1	9.0	10.7
8, 7α	2.2	12.2	12.2	12.3	5.5	4.0
8, 7β	4.6	3.7	3.9	3.5	4.3	4.5
8, 10	11.0	2.6	11.0	3.4	9.2	9.8
10, 11	1.9	10.6	2.2	1.9	3.5	3.1

Table 4. Experimental and calculated (GMMX/MM3) ^3J couplings for the hypothetical (6*R**,8*R**,10*S**) epimer of **10**.

Conformer type (rel. population) Vicinal protons	**a** (59%)	**b** (37%)	**c** (4%)	Averaged ^3J calculated $J_{av} = \Sigma_i x_i J_i$	Experimental values [Hz]
	^3J calculated for single conformers				
6, 7α	4.8	4.8	4.7	4.8	4.0
6, 7β	2.0	2.1	2.1	2.1	10.7
8, 7α	12.3	12.3	12.3	12.3	4.0
8, 7β	3.7	3.6	3.6	3.6	4.5
8, 10	1.5	2.3	0.8	1.8	9.8
10, 11	10.4	10.4	2.7	10.2	3.1

While ^{1}H NMR signals for **10** disappeared, those for **12** appeared. The latter compound was isolated from another batch of reaction mixture, made alkaline by the addition of Et_3N in small amounts. The composition of **12**, that is $C_{15}H_{21}Br$, one H_2O less than for calenzanol (**1**), is supported by EI-MS and HR-EI-MS data. Of the five unsaturations, only three (one tetra-, one 1,2-di-, and one 1,1-disubstituted C=C double bond) appeared in the ^{1}H and ^{13}C NMR spectra. This supports the presence of the two cycles. The NMR spectra further support the bromomethine substituent (δ_H = 3.94 ppm as dd, J = 5.0, 7.2 Hz and δ_C = 73.13 ppm as d) and the *i*Pr group (δ_H = 1.02 and 1.04 ppm, each d, J = 6.6 Hz; Me). Conjugation among the C=C bonds is suggested by the low-field resonance of the protons at the 1,2-disubstituted alkene moiety (δ_H = 6.09 ppm as d, J = 5.4 Hz and δ_H = 6.33 pm as d, J = 5.4 Hz). This agrees with the UV absorption at λ_{max} = 244 nm and the strong NOE enhancement between H2 (δ_H = 6.33 ppm) and one of the methylene protons of the 1,1-disubstituted alkene (δ_H = 5.65 ppm, br s, H_a14). These fragments could be assembled as in structure **12** on the basis of DDS, COSY, HMBC, and HMQC data.

A single HPLC peak and ^{13}C NMR signals for a single molecular species indicated a single **12** stereoisomer, implying that acid-catalyzed opening of the cyclopropyl ring of calenzanol (**1**) is stereoselective. Assuming, from the MM calculations discussed below, the presence of a chairlike cyclohexene ring in the least-strained conformations of **12**, the relative configurations at C6 and C8 could be assigned from the coupling pattern and NOE enhancements. Thus, the signal at δ_H = 1.73 ppm for one of the methylene protons at C7 was assigned to the axial position (β in the arbitrarily chosen enantiomer **12**) on the basis of a large coupling constant (10.5 Hz) to the neighboring methine proton H6 (δ_H = 2.66 ppm). This suggests a *trans*-diaxial relationship between the two protons, which assigns to C6 the same configuration as in **1**. A smaller coupling constant of 5.8 Hz between H7β and H8 suggests the equatorial position for the latter proton. A strong NOE enhancement between H7β and a C9 methylene proton (δ_H = 2.25 ppm) indicates an axial position for the latter. This agrees with the small coupling constant (3.2 Hz) between H9α and H8, as expected for a diequatorial relationship. No definite conclusions as to the configuration at C10 could be reached, however. The measured averaged values J(10,11) = 7.2 Hz and J(8,10) = 5.0 Hz are compatible with both the (6*R**,8*R**,10*S**) or epimeric (6*R**,8*R**,10*R**) stereochemistry. Therefore, the C10 configuration assumed for **12** was merely derived from the proposed reaction mechanism in Scheme 1. Further structural support was obtained from the isolation of a sizeable quantity of **8** from workup on SiO_2 of the NEt_3-quenched mixture (Scheme 1).

The ^{1}H NMR spectrum of the third intermediate, **14**, closely resembled that of the first intermediate **10**. In particular, the δ_H = 3.90 ppm (dd, J = 5.1 and 6.9 Hz), also observed for all other ring-opened compounds, was assigned to the bromomethine substituent at C10. However, all olefinic protons appeared at higher field, suggesting that the *exo*-methylene olefinic bonds are isolated. Molecular modeling suggested five major conformers with the side chain at C8 in a pseudoaxial position and vicinal coupling constants J(8,10) of 4.0 ± 1 Hz were in excellent agreement with the measured value, 4.1 Hz. These calculations also indicated that four of the five conformers, totaling 72% weight, have H10 and H11 in a *gauche* relationship (corresponding to small J(10,11) values), while the remaining conformer possesses these two protons in a *trans* relationship, corresponding to high J(10,11) values. Boltzman averaging led to J(10,11) = 7.8 Hz, in good agreement with the measured value 7.0 Hz. This supports the relative configuration 6*R**,8*R**,10*R** for **14**, while calculations for the hypothetical (6*R**,8*R**,10*S**) epimeric structure gave J(10,8) = 9.4 Hz and J(10,11) = 2.1 Hz, which are in sharp contrast with the measured values.

Immediately afterwards, ^{1}H NMR signals for **14** were detected, and also those for the indene-type sesquiterpene **5**[2] appeared, accompanied by three signals at low field (δ_H = 8.58, 8.90, and 9.20 ppm) for the guaiazulenium ion **17**. The latter could be isolated as an unstable, opaque royal-blue amorphous solid, on heating and then cooling concentrated **1** in benzene. The structure depicted for **17** is supported by ^{1}H NMR spectra and selective homonuclear proton decoupling data, which are in agreement with ^{1}H NMR data obtained for stabilized azulenium cations, such as 5-isopropyl-3,8-dimethyl-1*H*-azulenium tetrafluoroborate[7a] and 3-bromo- and 3,3-dibromoguaiazulenium bromide.[7b]

^{1}H NMR experiments indicated that **17** is stable with regard to the addition of water, methanol, or 2,6-di-*tert*-butylated hydroxytoluene (BHT), whereas hard nucleophiles,

such as triethylamine or potassium acetate, triggered instantaneous degradation into a complex mixture of products, from which guaiazulene **18** was obtained.

Any mechanistic hypothesis for these transformations has to take into account that calenzanol (**1**) is stable towards bases and quite sensitive to acids. Basic media and conditions include prolonged heating with 30% aqueous NaOH or Et_3N, and diazabicycloundecene (DBU),[8] even under severe conditions, for example 180°C in toluene in a sealed tube. Acidic media include Lewis acids, such as $ZnBr_2$ or $HgCl_2$, even in trace amounts, and Brønsted acids, such as residual HCl in aged $CDCl_3$. Even unneutralized Pyrex walls of the reaction apparatus induced degradation of **1** on short warming in freshly purified $CDCl_3$ or long heating in hexane or benzene. Degradation of **1**, under the above conditions, was unaffected by the addition at various times of a radical initiator, such as azoisobutyronitrile (AIBN), or an inhibitor, such as BHT.

A mechanism for the degradation of calenzanol (**1**) is proposed in Scheme 1. According to this proposal, acid-induced allylic dehydration of **1**, followed by dehydrobromination, leads to intermediate **8**, which is elusive under the reaction conditions but could be isolated on quenching the system with Et_3N just after the appearance of intermediate **12** (see Experimental Section). The low aromatic character of the fulvene ring, whose resonance energy ($\approx$6 kcal mol^{-1}) is far lower than that of a benzenoid system ($\approx$36 kcal mol^{-1}),[9] and the presence of vinylcyclopropane functionality confers unique chemical features to **8**. Semiempirical PM3 calculations for **8** indicate the highest electron density at C2, which is, therefore, assumed to be the site of protonation, leading to a second elusive intermediate **9**, in which the positive charge is mainly localized at C4. The incipient carbocation intermediate **9** is expected (and confirmed by our PM3 semiempirical calculations) to be particularly stabilized not only by two allylic double bonds but also by the conjugative effect of the bent orbitals of the cyclopropyl ring with the vacant p orbital of the cationic carbon. In **9**, in fact, the empty p orbital at C4 is almost parallel to the C8–C10 cyclopropyl σ bond, a particularly favorable position for conjugation but also for its incipient assistance to the irreversible nucleophilic attack of Br^- at C10 affording intermediate **10** through complete C8–C10 cyclopropyl σ bond migration.

In contrast, Br^- attack at C8, with ring-opening of the cyclopropyl unit through cleavage of the C8–C9 bond, followed by a 6π norcaradiene–cycloheptatriene type rearrangement, leads to the guaiazulenium ion intermediate **17** via two elusive intermediates, **15** and **16**. Intermediate **12**, which, being of the fulvene-type does not benefit from aromatic stability,[9] can be regarded as being in a cul-de-sac, in a protonation/deprotonation equilibrium with elusive **11**, which derives from the protonation of **10**. Thus, **12**, which is depleted by irreversible change of **11** into elusive **13** via a 1,3-H shift, serves as a sink to provide intermediate **14**. According to PM3 calculations, the latter is 7.1 kcal mol^{-1} more stable than **12**.

The alternative view of consecutive intermediates **10**, **12**, and **14** along the reaction path, while in accordance with the order of their appearance from the NMR spectra, contravenes least-motion principles.

Decay of calenzanol (1) on silica gel: All attempts at purifying large amounts of calenzanol (**1**) by preparative TLC on silica gel resulted in the complete degradation of this material to give the bicyclic triene **7**, and the C3 epimeric mixtures **22a/22b** (in a 3:2 ratio) and **20a/20b** (in a 7:3 ratio) (Scheme 1, double-line boxes).

A short residence time on silica gel, at low concentrations, such as on flash chromatography (FC) (0.4 g of algal extract on 40 g of silica gel; see Experimental Section) is mandatory for the isolation of **1** as well as **2**–**5**. Performing the same FC with 4 g of algal extracts on 200 g of silica gel required longer times, and thus a longer contact time of concentrated **1** with the chromatographic support. This led to **7**, a 3:2 epimeric mixture **22a/22b**, and a 7:3 mixture of C3 epimeric **20a/20b**. Similarly, preparative silica-gel TLC of **1** led to its complete conversion into **7**, and 3:2 epimeric **22a/22b**, and 7:3 epimeric **20a/20b** mixtures (see Experimental Section).

The composition of $C_{15}H_{22}O$ given for epimers **20a/20b** is based on HR-EI-MS measurements. The α,β-unsaturated keto group, detected by ^{1}H and ^{13}C NMR spectroscopy, accounts for one HBr unit less and one unsaturated bond more than calenzanol (**1**). The cyclopropyl ring finds support in a high-field methine signal at $\delta_H = 0.89$ ppm, while five-membered-ring to six-membered-ring fusion through the C=C bond is consistent with the absence of olefinic proton signals. DDS, COSY, HMBC, and HMQC experiments allowed us to determine the carbon skeletons, in particular revealing that the cyclopropyl moiety has the same stereochemistry as in calenzanol (**1**). Both ^{1}H and ^{13}C NMR spectra indicated a 7:3 molar ratio for **20a/20b**.

Isolation of triene **7** as a single stereoisomer, and carbinols **22a/22b** as a C3 epimeric mixture, provide further support to our tenet that, in the transformation of calenzanol (**1**) into **10**, **12**, **14**, and degraded products, dehydration across C5 and C6 is not involved, while opening of the cyclopropyl ring is stereoselective.

Formation of **20a/20b** and **22a/22b** is suggested in Scheme 1 (double-line boxes) from trapping of carbocations by H_2O, unavoidably present in silica gel. The first are suggested to arise from trapping of **9** to give elusive **19**, which undergoes air oxidation and reduction. To rationalize **22a/22b**, we should admit the presence of another elusive intermediate carbocation, **21**, arising, in parallel to **11**, from protonation of **10**. Carbocation **21** is trapped by H_2O to give **22a/22b**, which are immediate precursors of **7**, obtained by dehydration. That **22a/22b** were isolated during experiments of induced degradation of **1**, but not from algal extract, where **7** was present, means that the latter workup induced dehydration of **22a/22b**.

Conclusion

We have shown here that calenzanane sesquiterpenes (**1** and **6**) can be isolated from EtOH/MeOH extracts from the red seaweed *Laurencia microcladia* Kützing from the Bay of

Calenzana, Elba Island, provided that contact with acidic media is minimized. The latter trigger rearrangements of **1**. In dry solvents, indene-type **5** and the blue guaiazulenium-type ion **17** are formed through indene-type transient intermediates **10**, **14**, and **12** (Scheme 1), which were observed by ^{1}H NMR spectroscopy in separate experiments under different conditions. The latter could also be isolated on addition of NEt_3, allowing the measurement of both ^{13}C NMR and MS/HR-MS spectra. Aged **1**, degrades faster than the pure material, presumably because of HBr formed. Trapping of **17** by NEt_3 gave guaiazulene (**18**), and workup on SiO_2 of the NEt_3-quenched reaction mixture containing **12** gave **8**, which could be structurally investigated in detail by ^{1}H and ^{13}C NMR and MS/HR-MS spectra, allowing structural confirmation of the transient intermediates. On prolonged contact with silica gel, complete degradation of **1** occurred to give the calenzanane-type epimeric enones **20a/20b** as well as indene-type epimeric carbinols **22a/22b** and fulvene **7** (Scheme 1, double-line boxes), the latter was also observed during silica-gel flash chromatography of the algal extracts. Observation of reaction intermediates by NMR spectroscopy under ordinary conditions, as in this study, is uncommon. This allows us to propose a unifying mechanism on these branching and cascade transformations (Scheme 1), where elusive, hypothetical species, enclosed in square parentheses, accompany those firmly established. This may have both heuristic value, suggesting that azulenoids in nature may also result from chemical bias to aromatization, and possible synthetic applications derived from the regio- and stereospecific course of these reactions under anhydrous conditions.

Experimental Section

General methods Flash-chromatography (FC): Merck Si-60, 15–25 m. HPLC: Merck LiChrosorb Si-60 250×4.6 mm (7 μm) with hexane/*i*PrOH or Merck LiChrospher 100 RP18 (5 μm) with CH_3CH/H_2O. Preparative HPLC: 250×10 mm columns. Polarimetric data: JASCO-DIP-181 polarimeter. NMR: Varian XL-300 spectrometer equipped for inverse detection; chemical shifts are reported relative to residual solvent signals (for $CDCl_3$ δ_H = 7.260 ppm and δ_C = 77.00 ppm; for C_6D_6 δ_H = 7.150 ppm and δ_C = 128.50 ppm), coupling constants (*J*) are given in Hz. For compounds **6**, **7**, and **20**, COSY ^{1}H,^{1}H, NOE1D (differential NOE), HMQC, and HMBC experiments were carried out. EI-MS: Kratos MS80 mass spectrometer with a home-built data system. MM calculations were performed with the computer programs PCMODEL 7.0, based on the MMX force-field, from Serena Software, and MM3(96), based on the MM3 force-field, from QCPE, Indiana University. IUPAC numbering is only used in the following for retrieval purposes. Compounds **1**–**4** have been described previously.[2,4]

HPLC isolation of compounds from algal extracts: The residue (0.05 g) from evaporation of fractions 1–8 from the 40 fractions obtained before from *L. microcladia* extracts (carried out with EtOH, then MeOH),[2] was subjected to HPLC on Si 60 with *n*-hexane (flow gradient from 5 mL min^{-1} to 8 mL min^{-1} over a period of 20 min) to afford **7** (t_R = 15.8 min, 4.8 mg), along with δ-cadinene (t_R = 5.1 min, 2.5 mg), previously obtained from this seaweed.[4] The residue of fractions 9–11 (980 mg) was subjected to HPLC on Si 60 with *n*-hexane/EtOAc (98:2). The residue (150 mg) from fractions 14–16 was subjected to HPLC on Si 60 with *n*-hexane/*i*PrOH (99 :1) under refractometric detection, to give **6** (t_R = 12.9 min, 4.6 mg) and 5-bromo-1-isopropyl-2,5a-dimethyl-decahydro-cyclopropa[*a*]inden-2-ol (**2**) (t_R = 13.7 min, 12.0 mg), a 6,8-cycloeudesmane sesquiterpene previously described from this seaweed.[4]

Calenzanol (4-bromo-1-isopropyl-3,6-dimethyl-1a,2,3,4,5,6b-hexahydro-1*H*-cyclopropa[e]inden-3a-ol) (1):[2] Colorless oil; $[\alpha]_D^{20}$ = +12 (*c* = 3.00, *n*-hexane).

Calenzanane-type sesquiterpene [1-isopropyl-3,6-dimethyl-1a,2,4,5,6a,6b-hexahydro-1*H*-cyclopropa[e]inden-6-ol] (6): Colorless oil, $[\alpha]_D^{20}$ = −15.8 (*c* = 0.04, MeOH); ^{1}H NMR (300 MHz, C_6D_6): δ = −0.14 (td, ^3J(10,8) = 4.8, ^3J(10,9) = 4.8, ^3J(10,11) = 8.5 Hz, 1H; H10), 0.69 (dddd, ^3J(8,7α) = 1.8, ^3J(8,10) = 4.8, ^3J(8,7β) = 7.8, ^3J(8,9) = 9.1 Hz, 1H; H8), 0.76 (ddd, ^3J(9,4) = 1.8, ^3J(9,10) = 4.8, ^3J(9,8) = 9.1 Hz, 1H; H9), 0.88 (septd, ^3J(11,Me12) = 6.5, ^3J(11,Me13) = 6.5, ^3J(11,10) = 8.5 Hz, 1H; 11-H), 1.01 (d, ^3J(Me12,11) = 6.5 Hz, 3H; Me12), 1.03 (d,^3J(Me13,11) = 6.5 Hz, 3H; Me13), 1.38 (s, 3H; Me14), 1.53 (brs, 3H; Me15), 1.54 (ddd, ^3J = 8.9, ^3J = 10.7, ^2J(2a,2b) = 13.4 Hz, 1H; H2a), 1.71 (m, 1H; H4), 1.79 (ddd, ^3J = 2.6, ^3J = 9.1, ^2J(2b,2a) = 13.4 Hz, 1H; H2b), 1.87 (brd, ^2J(7α,7β) = 17.8 Hz, 1H; H7α), 2.07 (m, 1H; H1), 2.24 (brdd, ^3J(7β,8) = 7.8, ^2J(7β,7α) = 17.8 Hz, 1H; H7β), 2.30 (m, 1H; H1), 1.30 ppm (brs; OH); ^{13}C NMR: δ = 14.43 (d; C8), 15.33 (d; C9), 19.47 (q; C15), 21.80 (q; C12), 21.92 (q; C13), 24.66 (q; C14), 24.97 (t; C2), 30.56 (t; C7), 33.37 (d; C11), 36.15 (d; C10), 39.01 (t; C1), 50.03 (d; C4), 79.15 (s; C3), 124.89 (s; C6), 132.34 ppm (s; C5); MS (70 eV, EI): *m*/*z* (%): 220 ([*M*]$^+$, 18), 202 ([*M*−H_2O]$^+$, 9), 187 (16), 119 (100); HR-EI-MS: *m*/*z*: calcd for $C_{15}H_{24}O$: 220.1827; found: 220.1831±0.005.

Indene-type sesquiterpene 7 [6-(1-Bromo-2-methyl-propyl)-1,4-dimethyl-5,6-dihydro-4*H*-indene]: Yellow oil; CD (MeOH): −0.96 (λ_{max} = 284 nm), +0.72 (λ_{max} = 259 nm), −2.2 (λ_{max} = 210 nm); ^{1}H NMR δ = 1.02 (d, ^3J(Me13,11) = 6.5 Hz, 3H; Me13), 1.08 (d, ^3J(Me12,11) = 6.5 Hz, 3H; Me12), 1.16 (d, ^3J(Me15,6) = 6.8 Hz, 3H; Me15), 1.66 (ddd, ^3J(7β,8) = 4.8, ^3J(7β,6) = 10.7, ^2J(7β,7α) = 13.3 Hz, 1H; H7β), 1.93 (dseptet, ^3J(11,10) = 3.8, ^3J(11,Me12) = 6.5, ^3J(11,Me13) = 6.5 Hz, 1H; 11-H), 1.98 (brd, ^4J(Me14,2) = 1.4 Hz, 3H; Me14), 2.26 (dtd, ^5J(7α,9) = 0.5 Hz, ^3J(7α,6) = 4.2, ^3J(7α,8) = 4.2, ^2J(7α,7β) = 13.3 Hz, 1H; H7α), 2.82 (qdd, ^3J(6,Me15) = 6.8, ^3J(6,7α) = 4.2, ^3J(6,7β) = 10.7 Hz, 1H; H6), 2.96 (dddd, ^3J(8,7α) = 4.2, ^3J(8,7β) = 4.8, ^3J(8,9) = 5.1, ^3J(8,10) = 9.1 Hz, 1H; H8), 4.17 (dd,^3J(10,11) = 3.8, ^3J(10,8) = 9.1 Hz, 1H; H10), 5.91 (q, *J* = 1.6 Hz, 1H; H1), 6.04 (quintet, ^3J(2,1) = 2.0, ^4J(2,Me14) = 2.0 Hz, 1H; H2), 6.44 ppm (dd, ^5J = 1.6, ^3J(9,8) = 5.1 Hz, 1H; H9); ^{13}C NMR: δ = 11.64 (q; C15), 17.48 (q; C12), 19.99 (q; C14), 22.58 (q; C13), 25.96 (d; C6), 31.70 (d; C11), 37.37 (t; C7), 40.64 (d; C8), 69.13 (d; C10), 122.59 (d; C1), 128.94 (s; C3), 129.25 (d; C2), 133.54 (d; C9), 137.31 (s; C4), 146.33 (s; C5); UV (MeOH): λ_{max} (ε) = 258 nm (6200 mol^{-1} dm^3 cm^{-1}); MS (70 eV, EI): *m*/*z* (%): 282/280 ([*M*]$^+$, 10), 267/265 ([*M*−CH_3]$^+$, 1), 201 ([*M*−Br]$^+$, 7), 185 ([*M*−CH_3-HBr]$^+$, 5), 145 ([*M*−C_4H_8Br]$^+$, 100); HR-EI-MS: *m*/*z* calcd for $C_{15}H_{21}{}^{79}$Br 280.0827; found: 280.0829±0.006.

NMR observation of intermediates 10, 12, and 14, and isolation of 12 and guaiazulenium compound 17, in the degradation of calenzanol (1): The general procedure (adapted below to each particular case, allowing the isolation of **12** and **17**) consisted of taking an aliquot of freshly purified calenzanol (**1**), stored as a 0.5 mg mL^{-1} solution in *n*-hexane at −4 °C, and carefully concentrating it by repetitive evaporation in the presence of added CCl_4, so as to prevent evaporation to dryness. Freshly base-washed $CDCl_3$ was then added to the concentrated solution and the sample was flushed with argon. NMR spectra of the colorless starting solution were recorded at 14 °C, which showed only the presence of calenzanol (**1**). The solution was gently warmed for 5 min at 40 °C and then cooled to 14 °C to record the NMR spectra. Intermediates **10**, **12**, and **14** appeared consecutively and were accompanied by an intense blue coloration of the solution that increased in intensity as the rearrangement reactions proceeded to the end product, **5**.

Intermediate 10 [6-(1-bromo-2-methyl-propyl)-4-dimethyl-1-methylene-2,4,5,6-tetrahydro-1*H*-indene]: ^{1}H NMR: δ = 1.02 (d, ^3J(Me12,11) = 6.5 Hz, 3H; Me12), 1.04 (d, ^3J(Me13,11) = 6.5 Hz, 3H; Me13), 1.17 (d, ^3J(Me15,6) = 6.7 Hz, 3H; Me15), 1.44 (ddd, ^3J(7β,8) = 5.2, ^3J(7β,6) = 11.8, ^2J(7β,7α) = 13.3 Hz, 1H; H7β), 2.01 (m, 1H; 11-H), 2.16 (td, ^3J(7α,6) = 4.0, ^3J(7α,8) = 4.0, ^2J(7α,7β) = 13.3 Hz, 1H; H7α), 2.52 (m, 1H; H6), 2.75 (tdd, ^3J(8,7α) = 4.5, ^3J(8,7β) = 4.5, ^3J(8,9) = 5.4, ^3J(8,10) = 9.8 Hz, 1H; H8), 3.11 (m, 2H; H2), 4.05 (dd, ^3J(10,11) = 3.1, ^3J(10,8) = 9.8 Hz; H10), 4.99 (brs; H14a), 5.41 (brs; H14b), 5.72 (m; H1), 5.84 (brd, ^3J(9,8) = 5.4 Hz; H9).

Intermediate 12 [6-(1-bromo-2-methyl-propyl)-4-dimethyl-1-methylene-4,5,6,7-tetrahydro-1*H*-indene]: Degradation of a solution of calenzanol

(**1**) in $CDCl_3$ (4.0 mg in 0.6 mL) was initiated as described above for the general procedure; after about 5 min, at the onset of NMR signals for **12**, triethylamine (10 μL) was added, the solution was concentrated, and thereafter immediately subjected to HPLC (Merck Lichrosorb Si60, 7 μm, 1×25 cm, 100% hexane, λ = 254 nm, flow = 5 mL min^{-1}) to give **12** (t_R = 5.4 min, 2.5 mg) as a pale yellow oil. $[\alpha]_D$ = −16.5 (c = 0.9, MeOH); ^{1}H NMR: δ = 1.04 (d, ^3J(Me12,11) = 6.6, ^3J(Me13,11) = 6.6 Hz, 6H; Me12 and Me13), 1.13 (d, ^3J(Me15,6) = 7.2 Hz, 3H; Me15), 1.73 (ddd, $^3J(7\beta,8)$ = 5.8, $^3J(7\beta,6)$ = 10.5, $^2J(7\beta,7\alpha)$ = 13.4 Hz, 1H; H7β), 1.93 (br d, ^2J ($7\alpha,7\beta$) = 13.4 Hz, 1H; H7α, 2.04 (dseptet, ^3J(11,Me12) = 6.6, ^3J(11,Me13) = 6.6, ^3J(11,10) = 7.2 Hz, 1H; 11-H), 2.14 (m, 1H; H8), 2.25 (m, 1H; H9α), 2.47 (dd, $^3J(9\alpha,8)$ = 3.2, $^2J(9\alpha,9\beta)$ = 15.4 Hz, 1H; H9α), 2.66 (m, 1H; H6), 3.94 (dd, ^3J(10,8) = 5.0, ^3J(10,11) = 7.2 Hz, 1H; H10), 5.62 (s, 1H; H14b), 5.65 (br s, 1H; H14a), 6.09 (d, ^3J(1,2) = 5.4 Hz, 1H; H1), 6.33 ppm (dd, ^3J(2,H14a) = 1.2, ^3J(2,1) = 5.4 Hz, 1H; H2); ^{13}C NMR: δ = 18.61 (q; C12 or C13), 19.92 (q; C15), 22.19 (q; C13 or C12), 26.64 (t; C7), 28.36 (d; C6), 31.44 (d; C11), 34.68 (t; C9), 35.19 (d; C8), 73.13 (d; C10), 117.04 (t; C14), 124.03 (d; C2), 126.42 (s; C4), 133.27 (d; C1), 151.94 (s; C5), 152.06 ppm (s; C3); UV (MeOH) : λ_{max} (ε) = 241 nm (5500 mol^{-1} dm^3 cm^{-1}); CD (MeOH): −0.76 (λ_{max} = 278 nm), +0.40 (λ_{max} = 227 nm); MS (70 eV, EI): m/z (%): 282/280 ([M]$^+$, 0.3), 201 ([M−Br]$^+$, 0.5), 32 (43), 28 (100); HR-EI-MS: m/z calcd for $C_{15}H_{21}{}^{79}Br$: 280.0827; found: 280.0825 ± 0.006.

Intermediate 14 [6-(1-bromo-2-methyl-propyl)-4-dimethyl-1-methylene-2,6,7,7a-tetrahydro-1*H*-indene]: ^{1}H NMR: δ = 1.04 (d, ^3J(Me12,11) = 6.6, ^3J(Me13,11) = 6.6 Hz, 6H; Me12 andMe13), 1.45 (m, 1H; H9), 1.57 (s, 3H; Me15), 1.75 (m, 1H; H9), 1.80 (br s, 1H; H4), 2.02 (m, 1H; H11), 2.75 (m, 1H; H8), 3.02 (qd, ^3J(2a,1) = 1.5, ^4J(2a,14Ha) = 1.5, ^4J(2a,14Hb) = 1.5, ^2J(2a,2b) = 18.3 Hz, 1H; H2a), 3.21 (tdd, ^4J(2b,14Ha) = 1.5, ^4J(2b,14Ha) = 1.5, ^3J(2a,1) = 6.0, ^2J(2b,2a) = 18.3 Hz, 1H; H2b), 3.90 (dd, ^3J(10,11) = 7.0, ^3J(10,8) = 5.1 Hz, H10), 4.86 (br s, 1H; H14a), 4.91 (br s, 1H; H14b), 5.09 (m, 1H; H7), 5.19 (br d, ^3J(1,2a) = 6.0 Hz, 1H; H1).

Intermediate 17 [5-isopropyl-3,8-dimethyl-1,2,3,3a-tetrahydroazulenium bromide]: Degradation of a concentrated solution of calenzanol (**1**) in benzene (20.0 mg in 0.6 mL) was initiated as described above for the general procedure; degradation was allowed to proceed freely to **5**, in a blue mixture. This was cooled to −22 °C, to give tetrahydroazulenium bromide (**17**) as a blue amorphous solid. Yield: 4.95 mg (25%); ^{1}H NMR: δ = 1.45 (d, ^3J(Me12,11) = 6.6, ^3J(Me13,11) = 6.6 Hz, 6H; Me12 and Me13), 1.54 (d, ^3J(Me14,3) = 6.6 Hz, 3H; Me14), 1.97 (m, 1H; H2a), 2.66 (m, 1H; H2b), 2.98 (br s, 3H; Me15), 3.49 (m, 2H; H1), 3.51 (septet, ^3J(11,Me12) = 6.6, ^3J(11,Me13) = 6.6 Hz, 1H; H11), 3.97 (sextet, ^3J(3,Me14) = ^3J(3,2) = 6.6 Hz, 1H; H3), 8.58 (s, 1H; H10), 8.90 (d, ^3J(8,7) = 10.8 Hz, 1H; H8), 9.20 (d, ^3J(7,8) = 10.8 Hz, 1H; H7). Decomposition during acquisition prevented the recording of ^{13}C NMR spectra.

Quenching reactions with triethylamine to give 1-isopropyl-3-methyl-6-methylene-1,1a,2,3,6,6b-hexahydro-1*H*-cyclopropa[e]indene (8): To a solution of calenzanol (**1**, 4 mg in 600 μL of $CDCl_3$) that had already begun to decompose was added Et_3N (50 μL of pure reagent). ^{1}H NMR signals for only intermediates **10** and **12** were detectable. After a few minutes, triethylamine was removed in vacuo and the raw material purified by HPLC on Si60 with n-hexane (100%), to give **8** (t_R = 4.2 min. Yield: 1.2 mg (42%). Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.67 (m, 1H; H9), 0.79 (m, 1H; H10), 0.95 (m,1H; H8), 0.96 (d, ^3J(Me12,11) = 6.5 Hz, 6H; Me12 and Me13), 1.12 (d,^3J(Me15,6) = 6.5 Hz, 3H; Me15), 1.4–2.2 (series of m, 4H; H6, H7$_2$, 11-H), 5.62 (s, 1H; H14b), 5.86 (br s, 1H; H14a), 6.00 (d, ^3J(1,2) = 5.3 Hz, 1H; H1), 6.42 ppm (dd, ^3J(2,H14a) = 0.9, ^3J(2,1) = 5.3 Hz, 1H; 2-H); MS (70 eV, EI): m/z (%): 400 (2[M]$^+$, 18), 357 (5, ([M−CH(CH$_3$)$_2$]$^+$), 200 ([M]$^+$, 29), 157 (33, ([M−CH(CH$_3$)$_2$]$^+$); HR-EI-MS: m/z: calcd for $C_{15}H_{20}$: 220.1565; found: 200.1568 ±0.005.

TLC of calenzanol (1) on silica gel: Attempts to purify calenzanol (**1**, 0.045 g) by means of preparative TLC on silica gel with n-hexane/EtOAc (85:15) resulted in its complete degradation. We were able to isolate compound **7** (R_f = 0.9, 15 mg) along with a fraction consisting of **22a** and its epimer **22b** in a 3:2 ratio (R_f = 0.2, 12 mg), and the epimeric mixture **20a/20b** in a 7:3 ratio (R_f = 0.3, 7 mg).

Epimeric mixture 20/20b [1-isopropyl-3,6-dimethyl-1a,2,3,5,6,6b-hexahydro-1*H*-cyclopropa[e]inden-4-one]: The mixture from TLC was further purified by HPLC (Merck Lichrosphere Si60, 5 μm, 0.4×25 cm, hexane/EtOAc (85:15), λ = 254 nm, flow = 1 mL min^{-1}, t_R = 8.0 min) to give a diastereomeric mixture of **20a** and **20b** in 7:3 ratio as a colorless oil. ^{1}H NMR: δ = 0.89 (m, 1H; H10, **20a** & **20b**), 0.96 (d, ^3J(Me12,11) = 6.5 Hz, 0.9H; Me12, **20b**), 0.97 (d, ^3J(Me12,11) = 6.5 Hz, 2.1H; Me12, **20a**), 0.98 (d, ^3J(Me13,11) = 6.5 Hz, 3H; Me13, **20a** & **20b**), 1.10 (m, 1H; 11-H, **20a** & **20b**), 1.23 (d, ^3J(Me14,6) = 7.1 Hz, 0.9H; Me14, **20b**), 1.24 (d, ^3J(Me14,6) = 7.1 Hz, 2.1H; Me14, **20a**), 1.26 (d, ^3J(Me15,6) = 6.6 Hz, 0.9H; Me15, **20b**), 1.27 (d, ^3J(Me15,6) = 6.8 Hz, 2.1H; Me15, **20a**), 1.29 (m, 1H; H8, **20a** & **20b**) 1.35 (m, 1H; 7-Hβ, **20a** & **20b**), 1.40 (m, 1H; H9, **20a** & **20b**), 1.92 (dd, $^2J(2\alpha,3)$ = 2.0, $^2J(2\alpha,2\beta)$ = 18.3 Hz, 0.7H; H2α, **20a**), 1.94 (dd, $^3J(2\beta,3)$ = 2.0, $^2J(2\beta,2\alpha)$ = 18.2 Hz, 0.3H; H2β, **20b**), 2.09 (br dd, $^3J(7\alpha,6)$ = 1.8 Hz, $^2J(7\alpha,7\beta)$ = 13.0 Hz, 1H; 7-Hα, **20a** & **20b**), 2.16 (m, 1H; H6, **20a** & **20b**), 2.53 (dd, $^3J(2\beta,3)$ = 6.5, $^2J(2\beta,2\alpha)$ = 18.3 Hz, 0.3H; H2β, **20b**), 2.58 (dd, $^3J(2\beta,3)$ = 6.7, $^2J(2\beta,2\alpha)$ = 18.3 Hz, 0.7H; H2β, **20a**), 2.83 ppm (m, 1H; H3, **20a** & **20b**); ^{13}C NMR: δ = 17.29 (q, C15, **20a**), 17.45 (q, C15, **20b**), 19.14 (q, C14, **20b**), 19.80 (q, C14, **20a**), 20.01(d, C8, **20a** and **20b**), 21.57 (q, C12, **20b**), 21.68 (q, C13, **20a**), 21.84 (q, C13, **20a** & **20b**), 23.65 (d, C9, **20a**), 24.00 (d, C9, **20b**), 24.95 (d, C6, **20b**), 25.24 (d, C6, **20a**), 30.07 (t, C7, **20a**), 30.19 (t, C7, **20b**), 32.83 (d, C11, **20b**), 33.11(d, C11, **20a**), 34.93(d, C3, **20b**), 35.83 (d, C3, **20a**), 36.75 (d, C10, **20a**), 37.19(d, C10, **20b**), 44.01 (t, C2, **20b**), 44.19 (t, C2, **20a**), 134.22 (s, C5, **20a**), 134.65 (s, C5, **20b**), 181.29 (s, C4, **20b**), 181.75 (s, C4, **20a**), 206.61 (s, C1, **20b**), 206.84 ppm (s, C1, **20a**); UV ($CHCl_3$): λ_{max} = 254.0 nm; MS (70 eV, EI): m/z (%): 219 ([MH]$^+$, 4), 218 ([M]$^+$, 8), 203 ([M−CH$_3$]$^+$, 3), 176 ([MH−iPr]$^+$, 4), 175 ([M−iPr]$^+$, 5), 162 ([M−56]$^+$, 100), 147 (32), 120 (93), 119 (32), 105 (72), 91 (24), 41 ($C_3H_5{}^+$, 25); HR-EI-MS: m/z calcd for $C_{15}H_{22}O$ 218.1671; found: 218.1666 ± 0.006.

Diastereomeric mixture 22a/22b [6-(1-bromo-2-methyl-propyl)-1,4-dimethyl-2,4,5,6-tetrahydro-1*H*-inden-1-ol]: The material from the TLC band at R_f = 0.2 was further purified by HPLC on Si60, 7 μm, 1×25 cm, n-hexane/iPrOH (98:2), λ = 254 nm, flow = 5 mL min^{-1}, t_R = 8.5 min) to give a colorless oil composed of **22a** and **22b** in a 3:2 ratio. ^{1}H NMR: δ = 1.01 (d, ^3J(Me12, 11) = 6.5 Hz, 1.8H; Me12, **22a**), 1.02 (d, ^3J(Me12,11) = 6.5 Hz, 1.2H; Me12, **22b**), 1.03 (d, ^3J(Me13,11) = 6.5 Hz, 1.2H; Me13, **22b**), 1.04 (d, ^3J(Me13,11) = 6.5 Hz, 1.8H; Me13, **22a**), 1.14 (d, ^3J(Me15,6) = 6.6 Hz, 1.2H; Me15, **22b**), 1.15 (d, ^3J(Me15,6) = 6.6 Hz, 1.8H; Me15, **22a**), 1.37 (s, 1.2H; Me14, **22b**), 1.39 (s, 1.8H; Me14, **22a**), 1.40 (ddd, $^3J(7\beta,8)$ = 4.1, $^3J(7\beta,6)$ = 10.5, $^2J(7\beta,7\alpha)$ = 13.3 Hz, 1H; H7β), 1.55 (br s, OH), 1.97 (m, 0.4H; 11-H, **22b**), 1.99 (dseptet, ^3J(11,10) = 3.4, ^3J(11,Me12) = 6.5, ^3J(11,Me13) = 6.5 Hz, 0.6H; 11-H, **22a**), 2.12 (br td, $^3J(7\alpha,6)$ = 4.1, $^3J(7\alpha,8)$ = 4.1, $^2J(7\alpha,7\beta)$ = 13.3 Hz, 1H; H7α, **22a** & **22b**), 2.50 (m, 1H; H6, **22a** & **22b**), 2.55 (m, 2H; H2, **22a** & **22b**), 2.71 (qd, $^3J(8,7\alpha)$ = 4.1, $^3J(8,7\beta)$ = 4.1, ^3J(8,9) = 4.1, ^3J(8,10) = 9.4 Hz, 1H; H8, **22a** & **22b**), 4.02 (dd, ^3J(10,11) = 3.4, ^3J(10,8) = 9.4 Hz, 1H; H10, **22a** & **22b**), 5.60 ppm (m, 2H; H1 & H9, **22a** & **22b**); ^{13}C NMR: δ = 16.98 (q, C15, **22b**), 17.16 (q, C15, **22a**), 18.65 (q, C13, **22b**), 18.95 (q, C13, **22a**), 22.73 (q, C12, **22a**), 22.83 (q, C12, **22b**), 26.17 (d, C6, **22b**), 26.28 (d, C6, **22a**), 27.79 (q, C14, **22a**), 28.50 (q, C14, **22b**), 31.04 (d, C11, **22b**), 31.08 (d, C11, **22a**), 34.54 (t, C7, **22a**), 34.82 (d, C7, **22b**), 39.47 (d, C8, **22a**), 39.63 (d, C8, **22b**), 48.62 (t, C2, **22b**), 48.78 (t, C2, **22a**), 71.35 (d, C10, **22b**), 71.39 (d, C10, **22a**), 76.23 (s, C3, **22a**), 116.75 (d, C1, **22b**), 117.06 (d, C1, **22a**), 123.85 (d, C9, **22b**), 124.07 (d, C9, **22a**), 143.79 (s, C4, **22a**), 153.02 ppm (s, C5, **22a**). The olefinic quaternary carbons in **22b** were not detected. Because of experimental difficulties in the separation of the epimers and of the complexity of the ^{1}H NMR spectrum of the mixture, no attempt was made to establish which is which of the two epimers. UV ($CHCl_3$): λ_{max} = 262 nm; MS (70 eV, EI): m/z (%): 300/298 ([M]$^+$, 6), 285/283 ([M−CH$_3$]$^+$, 0.3), 219 ([M−Br]$^+$, 33), 163 (100), 161 (38), 43 (65); HR-EI-MS m/z calcd for $C_{15}H_{23}{}^{79}BrO$ 298.0932; found: 298.0935 ± 0.006.

Molecular modeling: For compounds **7**, **10**, **12**, and **14**, conformational space search was carried out by the GMMX computer program (allowing for either ring coordinate movements and free rotations around C8–C10 and C10–C11 bonds) from initial structures generated by the computer program PCMODEL 7.0. Structures obtained in an energy window of 2.5 kcal mol^{-1} were strain-minimized by the computer program MM3(96). All output structures obtained within an energy window of 2.0 kcal mol^{-1} were taken into account in deriving ^3J coupling constants. The relative populations of conformers were calculated according to the distribution law at 298 K, whereas the vicinal coupling constants were calculated

from the Boltzmann-averaged GMMX ensemble by means of the Altona–Karplus equation.[11] PM3 semiempirical calculations were performed with the MOPAC program as implemented in PCMODEL 7.0.

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