Antipodal Pathways to Secondary Metabolites in the Same Eukaryotic Organism

Graziano Guella* and Francesco Pietra

In memory of D. H. R. Barton

Abstract: Rogioldiol B ((−)-**3**) and rogioldiol C ((−)-**7**) were isolated from the red seaweed *Laurencia microcladia*, which grows at Il Rogiolo along the coast of Tuscany, as the first examples of 15,14-friedoobtusane diterpenes. The absolute configuration at their brominated moiety was assumed to be identical to rogiolal (**2**), already isolated from the same seaweed as a putative biodegradation product of rogioldiol A ((−)-**1**). Failure to define the absolute configuration through investigation of Mosher's esters reflects a so far unrecognized problem of sterically crowded secondary alcohols in acyclic systems. However, the absolute configuration at C9, defined by exciton-coupling techniques, could be extended to C10 by extensive NMR experimentation and molecular mechanics calculations. Surprisingly (−)-**3** and (−)-**7** have the opposite configuration at C10, which suggests that they are formed along two different biogenetic pathways involving mirror-image folding of a geranyl geraniol-derived precursor.

Keywords: conformation analysis · diterpenes · Mosher's esters · natural products · NMR spectroscopy

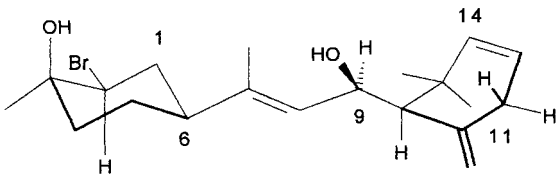
Introduction

Mosher's method for the assignment of the absolute configuration of chiral secondary alcohols is based on NMR spectroscopic data ($\delta_S - \delta_R$) for diastereomers obtained from the antipodal forms of an optically active esterifying agent possessing an anisotropic group, such as a phenyl ring, commonly methoxytrifluoromethylphenyl acetyl chloride (MTPA-Cl).[1] This method has gained tremendous importance in the assignment of the absolute configuration of structurally complex natural products since a) secondary hydroxyl groups can be created in derivatives[2] (which are needed in very small amounts and can be recovered by ester hydrolysis), and b) when—as normally occurs—the CF_3 and −O−C=O groups lie in the same plane as the carbinyl H atom, it is expected that $\delta_S - \delta_R \approx 0$, since the anisotropic effects of the phenyl group must be the same on the protons at either the front side or the rear side of the molecule; this affords a criterion for testing the self-consistency of the methodology.

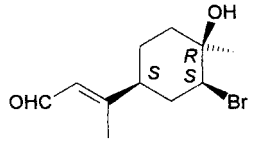
Axial or pseudoaxial OH groups at ring positions may not attain this ideal conformation, thus hindering the application of Mosher's methodology.[2a, 3] That this also applies to sterically encumbered *sec*-OH group in acyclic compounds is shown here on dealing with the first examples of 15,14-friedoobtusane diterpenes. Their stereostructures have profound biogenetic implications.

Results and Discussion

The relative configuration: The NMR spectra (Table 1) for rogioldiol B ((−)-**3**, Scheme 1, p. 1695) closely resemble those for rogioldiol A ((−)-**1**), which was isolated, together with its putative biodegradation product rogiolal (**2**), from the red seaweed *Laurencia microcladia* growing at Il Rogiolo on the coast of Tuscany.[4] This fact suggested, in line with the MS spectra (Experimental Section), an isomeric relationship between (−)-**3** and (−)-**1**. In particular, the brominated ring and inter-ring chain for (−)-**3** could be assigned by comparison of the NMR spectra of (−)-**1** and (−)-**3** (Table 1).[4] However, rogioldiol B lacks both the *gem*-dimethyl group and the C(13)=C(14) double bond of (−)-**1**, which are replaced by MeC(14)=C(15)Me. This and the presence of an *exo*-methylene group are fully supported by three sets of long-



(-)-**1**



2

[*] Prof. F. Pietra, Dr. G. Guella
Laboratorio di Chimica Bioorganica
Facoltà di Scienze MFN, Università di Trento
I-38050 Povo-Trento (Italy)
Fax: (+39) 461-881536
E-mail: fpietra@discat.unipi.it

  0947-6539/98/0409-1692 $ 17.50+.50/0

Table 1. ^{1}H and ^{13}C NMR data (C_6D_6) for rogioldiol B ((−)-**3**) and rogioldiol C ((−)-**7**).

Atom	(−)-**3**		(−)-**7**	
	^{1}H[a]	^{13}C	^{1}H[b]	^{13}C
1	ax 2.24 q (12.3); eq 1.94 dddd (2.1, 3.4, 4.5, 12.3)	38.82 t	ax 2.33 q (12.3); eq 2.02 ddd (3.3, 4.3, 12.3)	38.93 t
2	3.60 dd (4.5, 12.3)	65.79 d	3.65 dd (4.3, 12.3)	65.88 d
3	–	70.23 s	–	70.23 s
4	ax 0.97 dt (4.0, 13.2); eq 1.84 td (3.0, 13.2)	37.46 t	ax 0.97 br.t (13.2); eq 1.84 td (3.3, 13.2)	37.47 t
5	ax 1.79 dtd (3.6, 12.3, 13.2); eq 1.27 tdd (2.1, 3.2, 12.3)	25.68 t	ax 1.81 dtd (3.3, 12.3, 13.2); eq 1.29 ddd (2.5, 3.3, 12.3)	25.80 t
6	1.56 tt (3.3, 12.3)	48.42 d	1.66 tt (3.3, 12.3)	48.65 d
7	–	139.75 s	–	142.13 s
8	5.20 qd (1.2, 8.7)	126.29 d	5.25 qd (1.3, 8.5)	125.47 d
9	4.54 dd (5.5, 8.7)	70.03 d	4.43 dd (8.5, 9.4)	66.10 d
10	2.76 d (5.5)	55.70 d	2.82 d (9.4)	61.98 d
11	–	146.85 s	–	147.05 s
12	ax 2.39 ttd (1.8, 9.2, 13.3); eq 2.15 dddd 1.1, 3.0, 5.4, 13.3)	30.58 t	2.02 m	33.18 t
13	1.98 m	33.28 t	ax 0.90 dq (3.6, 12.3); eq 1.31 ddd (2.0, 4.4, 12.3)	36.55 t
14	–	124.48 s	2.03 dqd (2.0, 6.4, 12.3)	32.64 d
15	–	129.53 s	–	152.11 s
16	1.09 s	30.53 q	1.10 s	30.58 q
17	1.43 d (1.2)	15.03 q	1.53 d (1.3)	14.99 q
18	4.79 m	109.91 t	a) 4.60 td (1.5, 2.9); b) 4.64 br d (2.9)	111.00 t
19	1.50 br s	19.07 q	0.93 d (6.4)	18.07 q
20	1.73 br s	19.31 q	a) 4.84 br t (1.7); b) 4.76 t (1.7)	110.32 t

[a] NOE1D: 4.54 →2.76 and 1.73; 1.73 →4.54, 2.77 and 1.50; 1.43 →4.54 and 2.24; additional NOE2D 5.20 →4.54; 4.79 →2.76 and 2.15; 3.65 →1.09. [b] NOE1D: 4.84 →2.82; 4.76 →0.93; 4.64 →2.02; 4.60 →2.82; 4.43 →2.03 and 1.53; 1.53 →4.43, 2.33 and 1.81; additional NOE2D: 5.25 →2.82; 3.65 →1.10. $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] (conc [**3**]/[Eu(fod)$_3$] = 0.4): 4.43 (124), 5.25 (114), 2.82 (82), 4.84 (39), 2.03 (39), 4.60 (30), 4.76 (25), 1.53 (23), 1.66 (21), 2.33 (21), 4.64 (19), 0.93 (8), 3.65 (6), 1.10 (5), under which conditions NOE1D H9 →H14 and Me17; H8 →H10 and H6; H10 →H8, Ha18 and Ha20.

range heteronuclear correlation (HMBC) data: of H10 with C8, C9, C11, C15, and C18, of Me20 with C10, C14, and C15, and of Me19 with C14 and C15. Additional structural support is afforded by positive NOE between Me19 and Me20. Differential decoupling and COSY experiments complete the picture, allowing a thorough assignment of all proton and carbon resonances. This forms the basis for the stereochemical and conformational elucidation of this compound.

The relative configurations of (−)-**3** at the brominated ring are the same as for (−)-**1**.[4] The equatorial position of bromine is supported by the coupling pattern of H2, whose positive NOE with Me16 supports the *cis* position of Br and OH at 2,3, while the axial position of H6 rests on its coupling pattern (Table 1). An *E* configuration at C7=C8 is determined by the upfield resonance of Me17. The pseudoequatorial position of H10, and a preferential conformation with dihedral angle H6–C6–C7–C17 of approximately 180°, are based on a strong positive NOE of Me17 with $H_{ax}1$, $H_{ax}5$, and H9. The assignment of the relative configurations at C9 and C10 proved much more difficult; a moderate $J_{9,10}$ coupling of 5.5 Hz suggested equilibration among several conformers. A conformational-space search through the molecular mechanics (MM) program GMMX allowed us to restrict the analysis to the conformers generated by rotation around the bonds C8–C9 and C9–C10. This was carried out in 30° increments, and revealed four significant minimum-energy conformers within an energy window of 2.5 kcal mol^{-1}. These are the C9–C10 rotamers **3a** (dominant), **3b**, and **3c,** and the very minor C8–C9 rotamer **3d** (Figure 1 and Table 2), leading to an averaged $J_{9,10}$ = 6.1 Hz, in fair agreement with the experimental value of 5.4 Hz. The appropriate choice of this model is also proven by a satisfactory agreement between experimental and mean calculated values of $J_{8,9}$ (Table 2). Further support is given by the positive NOE of H10 with H9, H_a18,

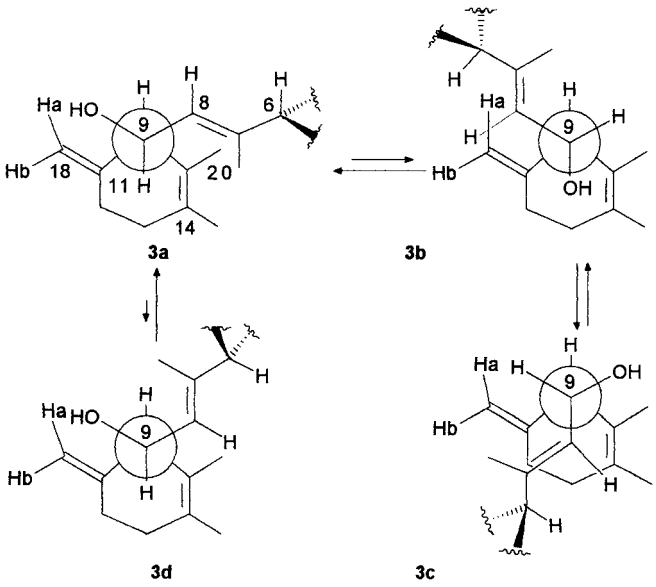


Figure 1. The four relevant conformations of rogioldiol B, **3a** (major), **3b**, and **3c** for rotation around the C9–C10 bond, and **3d** for rotation around the C8–C9 bond, as inferred from MM calculations.

and Me20, and of Me20 with H9. For the C6–C7 bond, both coupling values and MM calculations indicated that in the preferred conformer H6 and Me-17 take antiperiplanar positions. The pseudoaxial chain at C10 reflects allylic strain[5] by the *exo*-methylene group to equatorial chain attachment, resulting in a MM-calculated high-strain-energy half-chair conformation. This constitutes a suitable model to judge shift–reagent effects since vicinal *J* values remained unaltered on progressively adding [Eu(fod)$_3$]. Data in Figure 2 and in the experimental section show that [Eu(fod)$_3$], when added in less than the stoichiometric amount, binds mainly to

Table 2. Results from GMMX/MM3 calculations for rogioldiol B (−)-**3** and rogioldiol C (−)-**7**.

Conf.	E_i[a] [kcal mol^{-1}]	x_i[b]	C7-C8-C9-C10 tors. angle	H-C9-C9-C10-H-C10 tors. angle	Calculated $J_{8,9}$ [Hz][c]	Calculated $J_{9,10}$ [Hz][c]
3a	0.00	0.42	−114	+179	11.6	10.5
3b	0.31	0.25	−96	−59	11.6	1.7
3c	0.17	0.31	−112	+67	8.6	2.3
3d	1.72	0.02	+78	−176	6.4	10.6
7a	0.00	0.75	−112	+179	11.2	10.5
7b	0.90	0.17	−97	−62	9.9	1.4
7c	1.71	0.04	−106	+63	10.2	2.3
7d	1.63	0.04	+85	−179	5.8	10.6

[a] Relative strain energy of conformers as obtained from GMMX conformational space search and MM3 (block diagonal and full matrix minimization mode) energy minimization of more stable conformers. [b] Conformer molar ratio as evaluated at rt by Boltzman distribution $x_i = \exp[(-E_i/RT)]\Sigma_i (\exp[(-E_i/RT)]$. [c] Evaluated by Altona's equation (C. A. G. Haasnoot, F. A. A. M. De Leeuw, C. Altona, *Tetrahedron* **1980**, *36*, 2783) as a subroutine in PCMODEL program.

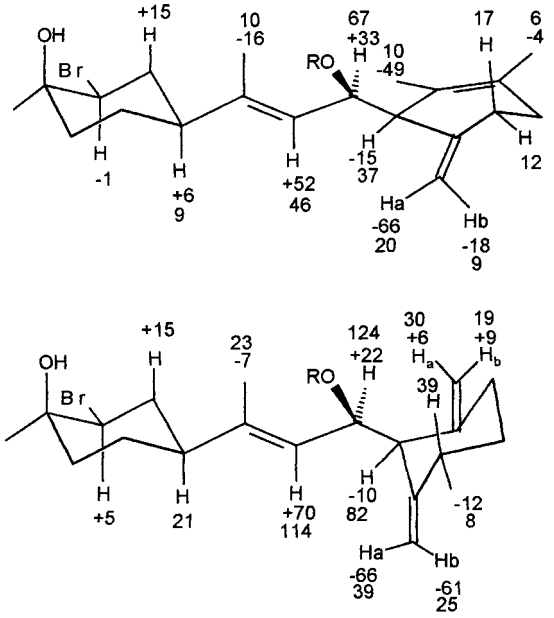


Figure 2. Values preceded by an algebraic sign represent the difference in hertz between the corresponding resonances of (*S*)-MTPA ester **4** and (*R*)-MTPA **5** (top structure) and the analogues **8** and **9**, respectively (bottom structure) (R = MTPA). The other values ($\Delta\delta$(ppm × 100)) represent [Eu(fod)$_3$]-induced deshielding in $CDCl_3$ for (−)-**3** (top) and (−)-**7** (bottom) (R = H).

HO9, inducing larger shifts on both H_a18 and H_{ax}12 than on Me20. This implies that on average the hydroxyl group is closer to these two groups than to Me20, and it is also closer to C18 than to C19, as approximately represented by the dominant conformer **3a** (Figures 1 and 2). For conformer **3c** opposite effects would be predicted, but **3c** is less populated than **3a**, while for conformer **3b** the observed changes in chemical shift would be expected to be the same for all the groups since they all have similar interatomic distances.

Data for rogioldiol C ((−)-**7**, Scheme 2) (Table 1 and Experimental Section) support the presence of the same brominated ring and inter-ring chain as in (−)-**3**. The other ring differs from that of (−)-**3** in that both double bonds are in the form of *exo*-methylene groups. This assignment was based on the coupling pattern of H14, selective homonuclear decoupling experiments, and long-range HMBC of Me19 with C13, C14, and C15, as well as COSY experiments. Conformational analysis disclosed that, within an energy window of 3 kcal mol^{-1}, (−)-**7** exists in four minimum-energy conformations, **7a** (dominant), **7b**, **7c**, and **7d** (Figure 3 and Table 2), leading to an average $J_{9,10} = 10$ Hz in good agreement with the experimental value of 9.4 Hz. Added [Eu(fod)$_3$] becomes bound mainly to HO9, inducing larger shifts on both H_a20 and H_{ax}14 than on H_a18. This implies that on average the hydroxyl group is closer to these two groups than to H_a18, and is also closer to C(20) than to C(18), as approximately represented by the dominant conformer **7a** (Figures 2 and 3). Both **7c** (which, per se, is expected to have opposite shifts) and **7d** are minor conformers, while **7b** must be neutral for the same reasons illustrated above for **3b**.

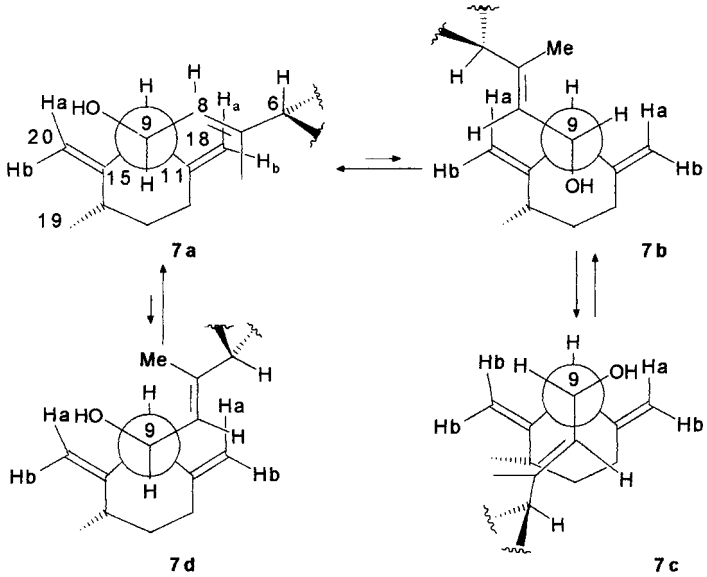


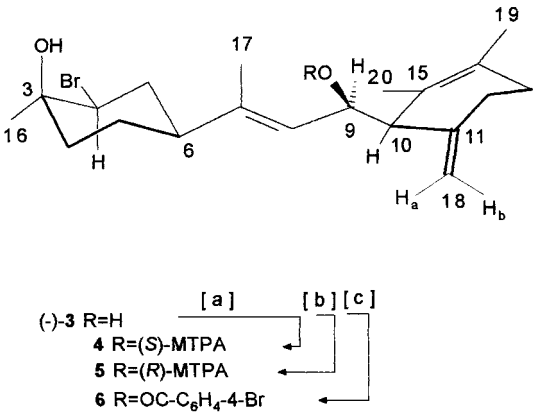
Figure 3. The four relevant conformations of rogioldiol C, **7a** (major), **7b**, and **7c** for rotation around the C9–C10 bond, and **7d** for rotation around the C(8)–C(9) bond, as inferred from MM calculations.

These conclusions are in accordance with both PCMODEL and MM3 calculations, while PM3 semiempirical calculations failed to simulate these systems correctly. Although we took care to account for nonbonded interactions between hydrogens with the Spartan package (Wavefunction), PM3 calculations pointed to very similar ratios for the three conformers **7a**, **7b,** and **7c** (Figure 3) or their C10 epimers, while **7d** was not evaluated. This pattern does not fit the NMR observa-

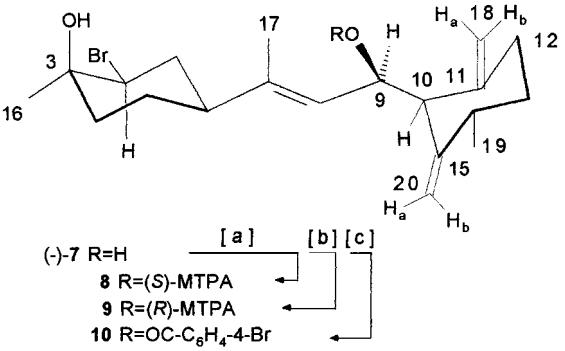
0947-6539/98/0409-1694 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 9

tions, reflecting the well-known difficulty of semiempirical calculations in accounting for the relative heats of formation of conformers.

The absolute configuration: The above results imply that (−)-**3** and (−)-**7** differ in absolute configuration at either C9 or C10. Mosher's MTPA esters, **4**/**5** from (−)-**3** (Scheme 1) or **8**/**9** from (−)-**7** (Scheme 2), proved unsuitable to deal with this question. As shown in Figure 2, the sign of $(\delta_S - \delta_R)$ is not



Scheme 1. Synthesis of MTPA and 4-bromobenzoate esters. [a] (−)-(*R*)-MTPA-Cl, pyr, DMPA, rt, 3 h; [b] (+)-MTPA-Cl in place of (−)-(*R*)-MTPA-Cl. [c] 4-Br-C_6H_4-COCl, pyr, cat. DMAP, rt, overnight.



Scheme 2. Synthesis of MTPA and 4-bromobenzoate esters; [a], [b] and [c] as in Scheme 1.

conserved along the rear side and the front side of the molecule, that is, along the sides flanking the ideal Mosher's plane, the deviations being worse for **8**/**9** than for **4**/**5**. This failure was unexpected on the basis of current literature but is not surprising in view of the large deviations from Mosher's ideal conformation observed for these compounds.

We then turned to the exciton-coupling technique,[6] relying on the facile esterification of HO9 and the adjacency of C7=C8. Phenyl esters **6** and **10** showed a positive Cotton effect while the relevant dihedral angle O−C9−C8−C7 was also positive (Figure 4), thus indicating *R* configuration at C9 in both cases. Combining these observations with those in the previous section, it must be concluded that (−)-**3** and (−)-**7** have opposite configuration at C10, *R* and *S*, respectively. It should be appreciated that conformers **6a** (Figure 4A) and **10a** (Figure 4B) are the equivalent of the preferred conformers **3a** (Figure 1) and **7a** (Figure 3) of the natural products, respectively. Thus, **6a** and **10a** fulfill the condition

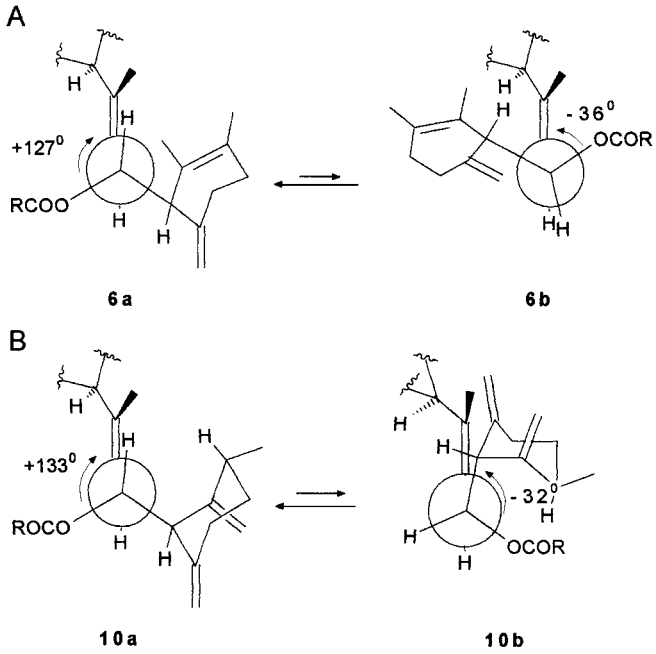
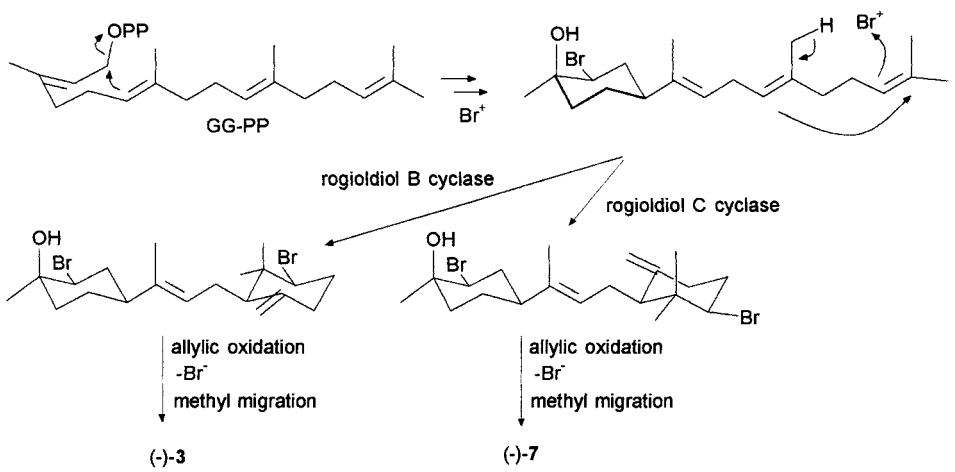


Figure 4. A): Major (**6a**) and minor (**6b**) conformations for rotation around the C8−C9 bond of rogioldiol B *p*-bromobenzoate, as inferred from MM calculations; B): major (**10a**) and minor (**10b**) conformers for rotation around the C8−C9 bond of rogioldiol C *p*-bromobenzoate; the relevant torsional angle C7−C8−C9−O for CD analysis is shown.

of the exciton-coupling technique that there must be a preferred conformer for rotation around the C8−C9 bond. Eclipsing of C7=C8 with H9 is justified by the effect of 1,3-allylic strain and the smallness of the proton. According to MM3 calculations, the population ratios **6a**/**6b** and **10a**/**10b** are approximately 80:20, corresponding to about 1 kcal mol^{-1}. Thus, the observed Cotton effect might well reflect the average of two opposite contributions.

Conclusions and Outlook

The discovery of the first 15,14-friedoobtusanes, (−)-**3** and (−)-**7**, has had the practically interesting side-effect of demonstrating the failure of Mosher's NMR methodology for the assignment of the absolute configuration of sterically crowded acyclic secondary alcohols. However, the surprising finding of opposite chirality at the pivotal center C10 of these metabolites is the most challenging aspect of this work. We envisage the intervention on a monocyclogeraniol intermediate of cyclases with opposite chirality at the active center, that is, the existence of antipodal pathways (Scheme 3). The production of enantiomeric metabolites from the same nominal species collected in different locations is not unusual,[7, 8] although the taxonomic assignment is rarely described in detail and thus remains to be proven. Also rather frequent is the detection of quasiracemic or enantiomerically impure



Scheme 3. Hypothetical biogenetic scheme from geranyl geranyl pyrophosphate to rogioldiol B ((−)-**3**) and rogioldiol C ((−)-**7**).

metabolites from an organism, in particular monoterpenes from terrestrial plants,[9a] products of phenol oxidative coupling,[9b] and alkaloids from possible involvement of vestigial enzymes.[9c] In contrast, the formation of diastereomers that require distinct antipodal pathways is quite uncommon, the most clear-cut case being clavulanic acid and clavam metabolites from actinobacteria.[10] Formation of rogioldiol B ((−)-**3**) and C ((−)-**7**) may be seen as an example of the latter category[10] for eukaryotes. The presence of (−)-**3** and (−)-**7** at similar concentrations in algal extracts makes it unlikely that either one of these metabolites was produced by a contaminating different species of *Laurencia* which had escaped our attention during the collection.

Laurencia microcladia, which grows at Il Rogiolo, is atypical of its genus and of a nominally like species[11] also for its production of oxepanes.[12] Since secondary metabolites are an expression of functional genes, this speaks for a wide genetic variability. Therefore, the population of *L. microcladia* studied here should be considered as a border-zone population[13] which may supply great genetic diversity, relevant to the evolutionary process. For this reason, preservation of border-zone populations of lower eukaryotic organisms is more important than preservation of the last rhinoceros, which, with its few offspring, has little to offer to the palette of evolution.

Experimental Section

General methods: See ref. [4]; in addition: COSY $^1H-^1H$,[4] NOE2D,[14] HMQC,[4] and HMBC[4] were carried out on both (−)-**3** and (−)-**7**. NOE1D stands for differential NOE, reported as irradiated proton →observed proton. MM calculations were carried out by the programs GMMX and PCMODEL (based on the MMX forcefield) from Serena Software and MM3(96) from QCPE.

Isolation of compounds: The residue (0.12 g) obtained from evaporation of fraction 36 of the 54 fractions previously derived from *L. microcladia* extracts[4] was purified by reverse-phase HPLC with MeCN/H_2O (65:35), followed by nitrile HPLC with hexane/(*i*Pr)OH (97:3), and several fractions were collected. The residue (0.03 g), from evaporation of fraction 7, was purified by Si-60 HPLC with hexane/Et_2O (1:1), which yielded rogioldiol B ((−)-**3**) ($t_R = 7.8$ min, 11 mg, 0.02%) and rogioldiol C ((−)-**7**) ($t_R = 8.9$ min, 6 mg, 0.01%).

Rogioldiol B ((−)-3): $[\alpha]_D^{20} = -195$ ($c = 0.45$, MeOH); ^1H NMR ($CDCl_3$): $\delta =$ 2.15 (q, $J = 12.1$ Hz, $H_{ax}1$), 2.01 (dddd, $J = 2.1, 3.7, 4.1, 12.1$ Hz, $H_{eq}1$), 4.14 (dd, $J = 4.1, 12.1$ Hz, H2), 0.97 (dt, $J = 4.0, 13.2$ Hz, $H_{ax}4$), 1.84 (td, $J = 3.0, 13.2$ Hz, $H_{eq}4$), 2.10 (dq, $J = 3.6, 12.4$ Hz, $H_{ax}5$), 1.45 (tdd, $J = 2.1, 3.2, 12.4$ Hz, $H_{eq}5$), 1.93 (tt, $J = 3.1, 12.0$ Hz, H6), 5.13 (br d, $J = 9.1$ Hz, H8), 4.53 (dd, $J = 5.5, 9.1$ Hz, H9), 2.76 (d, $J = 5.5$ Hz, H10), 2.37 (ttd, $J = 1.8, 9.2, 13.3$ Hz, $H_{ax}12$), 2.24 (dddd, $J = 1.1, 3.0, 5.4, 13.3$ Hz, $H_{eq}12$), 2.05 (m, $H_2$13), 1.31 (s, $H_3$16), 1.59 (d, $J = 1.2$ Hz, $H_3$17), 4.75 (t, $J = 1.9$ Hz, H_a18), 4.82 (t, $J = 1.9$ Hz, H_b18), 1.61 (br s, $H_3$19), 1.73 (br s, $H_3$20); NOE1D: 5.13 →1.93; 4.82 →2.15; 4.75 →2.76; 4.54 →2.76, 1.73, 1.59; 2.76 →4.75, 4.53, 1.73; $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] (conc. [**3**]/[Eu(fod)$_3$] = 0.2): 4.53 (49), 5.13 (28), 2.74 (25), 4.75 (13), 2.37 (12), 1.73 (7), 1.59 (6), 2.24 (8), 1.61 (4), 1.31 (2); ^{13}C NMR ($CDCl_3$):$\delta = 38.82$ (t, C1), 65.79 (d, C2), 70.23 (s, C3), 37.46 (t, C4), 25.68 (t, C5), 48.42 (d, C6), 139.75 (s, C7), 126.29 (d, C8), 70.03 (d, C9), 55.70 (d, C10), 146.85 (s, C11), 30.58 (t, C12), 33.28 (t, C13), 124.48 (s, C14), 129.53 (s, C15), 30.53 (q, C16), 15.03 (q, C17), 109.91 (t, C18), 19.07 (q, C19), 19.31 (q, C20); $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] (conc [**3**]/[Eu(fod)$_3$] = 0.2): 70.03 (79), 126.29 (25), 139.75 (19), 109.91 (19), 146.85 (16), 129.53 (13), 55.70 (13), 15.03 (10), 30.58 (8), 48.42 (7), 33.28 (7), 30.53 (7), 19.07 (5), 19.31 (5), 124.48 (4), 38.82 (4), 70.23 (4), 25.68 (3), 37.46 (0); MS (70 eV, EI): *m*/*z* (%) = 364/366 (10/10, $[M - H_2O]^+$), 349/351 (4/4, $[M - Me - H_2O]^+$), 284 (30, $[M - H_2O - HBr]^{\cdot+}$, 269 (13), 245 (15), 225 (15), 163 (17), 122 (100).

MTPA esters of (−)-3: Rogioldiol B ((−)-**3**; 4 mg) was treated with 3 equiv (+)-(*S*)-MTPA-Cl and 4-dimethylaminopyridine (1.0 mg) in dry pyridine (0.5 mL). The same procedure was followed with (−)-(*R*)-MTPA-Cl. In each case, the reaction was quenched after 3 h with saturated aq. $CuSO_4$ (1 mL) followed by Et_2O (4 mL) and the mixture was passed through a Whatman phase-separation filter. The organic phase was evaporated and subjected to Si60 HPLC with *n*-hexane/*i*PrOH (98:2) to give **4** ($t_R =$ 5.5 min) from (−)-(*R*)-MTPA-Cl or **5** ($t_R = 6.8$ min) from (+)-(*S*)-MTPA-Cl.

Data for 4: $[\alpha]_D^{20} = -113$ ($c = 0.14$, MeOH); ^1H NMR ($CDCl_3$): $\delta = 2.12$ (q, $J = 12.0$ Hz, $H_{ax}1$), 1.99 (m, $H_{eq}1$), 4.13 (dd, $J = 4.7, 11.8$ Hz, H2), 2.05 and 1.45 (m, $H_2$4), 170 and 1.58 (m, $H_2$5), 1.94 (tt, $J = 3.3, 12.0$ Hz, H6), 5.17 (quint. d, $J = 1.2, 9.7$ Hz, H8), 5.87 (dd, $J = 5.4, 9.7$ Hz, H9), 2.75 (br d, $J =$ 5.4 Hz, H10), 2.16 and 2.03 (m, $H_2$12), 2.05 (m, $H_2$13), 1.32 (s, $H_3$16), 1.69 (d, $J = 1.2$ Hz, $H_3$17), 4.67 (dt, $J = 0.7, 2.1$ Hz, H_b18), 4.43 (m, H_a18), 1.53 (br s, $H_3$19 and $H_3$20), 7.6–7.3 (series of m, aromatic CH); 3.56 (q, $J = 1.3$ Hz, OMe); NOE1D: 5.86 →2.75, 1.69, 1.53; 4.43 →2.75; 4.67 →2.16; 2.75 →5.88, 5.17, 4.43, 1.53; ^{13}C NMR ($CDCl_3$): $\delta = 33.08$ (t, C1), 65.45 (d, C2), 70.19 (s, C3), 37.39 (t, C4), 25.83 (t, C5), 48.21 (d, C6), 143.81 (s, C7), 127.12 (d, C8), 75.82 (d, C9), 52.75 (d, C10), 144.76 (s, C11), 30.11 (t, C12), 38.56 (t, C13), 124.15 (s, C14), 129.95 (s, C15), 30.57 (q, C16), 15.63 (q, C17), 110.76 (t, C18), 19.33 (q, C19), 18.72 (q, C20), [165.92 (s, CO), 128.27 (d), 129.43 (d), 120.67 (d), 132.71 (s), 55.55 (q), MTPA signals].

Data for 5: $[\alpha]_D^{20} = -47$ ($c = 0.12$, MeOH); ^1H NMR ($CDCl_3$): $\delta = 1.92$ (tt, $J = 3.1, 12.1$ Hz, H6), 5.00 (quint d, $J = 1.2, 9.6$ Hz, H8), 5.76 (dd, $J = 6.3, 9.6$ Hz, H9), 2.80 (br d, $J = 6.3$ Hz, H10), 1.32 (s, $H_3$16), 1.71 (d, $J = 1.2$ Hz, $H_3$17), 4.73 (dt, $J = 0.7, 2.1$ Hz, H_b18), 4.65 (m, H_a18), 1.58 (br s, $H_3$19), 1.67 (br s, $H_3$20), 7.6–7.3 (series of m, aromatic CH), 3.48 (q, $J = 1.1$ Hz, OMe); NOE2D: 5.76 →2.80, 1.71; 4.65 →2.80; ^{13}C NMR ($CDCl_3$): $\delta = 33.15$ (t, C1), 65.54 (d, C2), 70.22 (s, C3), 37.41 (t, C4), 25.87 (t, C5), 48.18 (d, C6), 143.73 (s, C7), 127.55 (d, C8), 75.82 (d, C9), 52.79 (d, C10), 144.92 (s, C11), 29.85 (t, C12), 38.59 (t, C13), 124.62 (s, C14), 129.84 (s, C15), 30.57 (q, C16), 15.68 (q, C17), 110.63 (t, C18), 19.33 (q, C19), 19.23 (q, C20), [166.08 (s), 128.33 (d), 129.53 (d), 120.79 (d), 132.20 (s), and 55.37 (q), MTPA signals]; the C9 signal was overlapped by the $CHCl_3$ residual signal.

0947-6539/98/0409-1696 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 9

Rogioldiol B *p*-bromobenzoate (**6**): 4-Bromobenzoylchloride (5 equiv) and a catalytic amount of 4-dimethylaminopyridine were added to a solution of (−)-**3** (1.4 mg) in dry pyridine (0.2 mL). The mixture was stirred overnight at room temperature and then MeOH (0.5 mL), sat. aq. $CuSO_4$ (2 mL), and *n*-hexane (4 mL) were added in sequence. The mixture was allowed to percolate through a Whatman phase-separation filter. The filtrate was evaporated, and the resulting residue was purified by Si-60 HPLC with hexane/*i*PrOH (97:3) to obtain **6** ($t_R = 6.5$ min, 1.0 mg, 43 %). UV (MeOH): λ_{max} (ε) = 244 (22000 $mol^{-1}Lcm^{-1}$); CD (MeOH): $\Delta\varepsilon_{max}$ (246 nm) = +5.8; ^{1}H NMR ($CDCl_3$, reporting only signals significantly different from (−)-**3**): $\delta = 5.18$ (br d, $J = 9.5$ Hz, H8), 5.90 (dd, $J = 5.9$, 9.5 Hz, H9), 2.93 (br d, $J =$ 5.9 Hz, H10), 1.70 (br s, $H_3$17), 4.82 (m, H_b18), 4.73 (m, H_a18), 1.76 (s, $H_3$19), 1.70 (s, $H_3$20); MS (70 eV, EI): m/z (%) = 364/366 (21/21, [M − BrC_6H_5COOH]$^{\cdot+}$), 349/351 (2/2, [M − BrC_6H_5COOH − H_2O]$^+$), 243/245 (13/13), 225/227 (5/5), 183/185 (100/100), 173 (16), 145 (12), 121 (42), 105 (31), 91 (23), 43 (47).

Rogioldiol C (**(−)-7**): $[\alpha]_D^{20} = -61$ ($c = 0.18$, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 2.19$ (q, $J = 12.1$ Hz, H_{ax}1), 2.03 (ddd, $J = 3.4$, 4.4, 12.1 Hz, H_{eq}1), 4.16 (dd, $J = 4.4$, 12.1 Hz, H2), 1.00 (br t, $J = 13.2$ Hz, H_{ax}4), 1.86 (td, $J = 2.5$, 13.2 Hz, H_{eq}4), 2.21 (dq, $J = 3.4$, 12.4 Hz, H_{ax}5), 1.49 (ddd, $J = 2.5$, 3.4, 12.4 Hz, H_{eq}5), 1.98 (tt, $J = 3.4$, 12.1 Hz, H6), 5.09 (qd, $J = 1.3$, 8.9 Hz, H8), 4.43 (dd, $J = 8.9$, 9.6 Hz, H9), 2.79 (d, $J = 9.6$ Hz, H10), 2.20 (m, $H_2$12), 1.68 (dq, $J = 2.7$, 12.3 Hz, H_{ax}13), 1.49 (m, H_{eq}13), 2.32 (tdq, $J = 2.0$, 6.5, 12.3 Hz, H14), 1.31 (s, $H_3$16), 1.61 (d, $J = 1.3$ Hz, $H_3$17), 4.60 (t, $J = 2.0$ Hz, H_a18), 4.67 (t, $J = 2.0$ Hz, H_b18), 1.11 (d, $J = 6.5$ Hz, $H_3$19), 4.91 (br t, $J = 1.8$ Hz, H_a20), 4.89 (t, $J = 1.8$ Hz, H_b20); NOE1D: 5.09 →2.79 (4 %), 1.98 (5 %); 4.91 →2.79 (12 %); 4.89 →1.11 (4 %); 4.67 →2.20; 4.60 →2.79 (4 %); 4.43 →2.34 (6 %), 1.62 (6 %); 1.62 →4.43 (7 %), 2.19; 1.31 →4.16 (6 %); 1.11 →4.89 (5 %), 1.49; $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] (conc [**3**]/[Eu(fod)$_3$] = 0.3): 4.43 (83), 5.09 (78), 2.79 (54), 4.91 (25), 2.32 (23), 4.60 (20), 4.67 (13), 1.61 (15), 4.89 (15), 1.11 (5), 3.65 (4), under the same conditions NOE1D : H9 →H14 and Me17; H8 →H10 and H6; H10 →H8, Ha18, and Ha20; ^{13}C NMR ($CDCl_3$): $\delta = 38.93$ (t, C1), 65.88 (d, C2), 70.23 (s, C3), 37.47 (t, C4), 25.80 (t, C5), 48.65 (d, C6), 142.13 (s, C7), 125.47 (d, C8), 66.10 (d, C9), 61.98 (d, C10), 147.05 (s, C11), 33.18 (t, C12), 36.55 (t, C13), 32.64 (d, C14), 152.11 (s, C15), 30.58 (q, C16), 14.99 (q, C17), 111.00 (t, C18), 18.07 (q, C19), 110.32 (q, C20); $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] (conc [**3**]/[Eu(fod)$_3$] = 0.8): 66.10 (d, (360)), 125.47 (d, (151)), 110.32 (t, (31)), 111.00 (t, (24)), 61.98 (d, (23)), 14.17 (q, (18)), 144.17 (s, (17)), 31.77 (t, (15)), 48.41 (d, (14)), 26.92 (q, (14)), 137.34 (d, (12)), 36.48 (s, (12)), 123.28 (d, (12)), 70.24 (s, (10)), 31.72 (q, (8)), 38.67 (t, (7)), 37.41 (t, (6)), 25.48 (t, (6)), 30.61 (q, (5)), 65.84 (d, (4)); MS (70 eV, EI): m/z (%) = 364/366 (2/2, [M − H_2O]$^{\cdot+}$), 284 (13, [M − H_2O − HBr]$^{\cdot+}$), 269/267 (7/7), 243/245 (14/14), 225/227 (14/14), 163 (58), 145 (41), 135 (22), 119 (29), 107 (96), 93 (56), 71 (100).

MTPA esters of (−)-7: Following the procedure described above for (−)-**3**, treatment of (−)-**7** (2 mg) with (−)-(*R*)-MTPA-Cl yielded **8** ($t_R = 6.0$ min), while treatment of (−)-**7** with (+)-(*S*)-MTPA-Cl gave **9** ($t_R = 7.5$ min).

Data for 8: $[\alpha]_D^{20} = -10$ ($c = 0.14$, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 2.14$ (q, $J = 12.0$ Hz, H_{ax}1), 2.01 (m, H_{eq}1), 4.14 (dd, $J = 4.6$, 11.9 Hz, H2), 2.20 and 1.97 (m, $H_2$4), 1.85 and 2.20 (m, $H_2$5), 2.13 (tt, $J = 3.3$, 12.0 Hz, H6), 5.20 (quint.d, $J = 1.2$, 9.7 Hz, H8), 6.04 (dd, $J = 9.7$, 10.4 Hz, H9), 3.11 (br d, $J =$ 10.4 Hz, H10), 2.39 (qd, $J = 6.5$, 10.7 Hz, H12), 1.65 and 1.42 (m, $H_2$13), 1.95 and 2.03 (m, $H_2$14), 1.33 (s, $H_3$16), 1.74 (d, $J = 1.2$ Hz, $H_3$17), 4.51 (dt, $J =$ 0.7 Hz, 2.1, H_b18), 4.59 (m, H_a18), 1.01 (d, $J = 6.5$ Hz, $H_3$19), 4.68 (t, $J =$ 1.8 Hz, H_b20), 4.64 (m, H_a20), 7.6–7.3 (series of m, aromatic CH), 3.49 (q, $J = 1.3$ Hz, OMe); NOE1D: 6.04 →2.39 and 1.74; 3.11 →5.20, 4.64 and 4.59; 2.39 →6.04.

Data for 9: $[\alpha]_D^{20} = +12$ ($c = 0.11$, MeOH); ^{1}H NMR ($CDCl_3$): $\delta = 2.08$ (q, $J = 12.0$ Hz, H_{ax}1), 2.00 (m, H_{eq}1), 4.13 (dd, $J = 4.8$, 11.8 Hz, H2), 4.96 (quint.d, $J = 1.2$, 9.6 Hz, H8), 5.97 (dd, $J = 9.6$, 10.5 Hz, H9), 3.13 (br d, $J =$ 10.5 Hz, H10), 2.38 (qd, $J = 6.5$, 10.7 Hz, H12), 1.31 (s, $H_3$16), 1.76 (d, $J =$ 1.2 Hz, $H_3$17), 4.72 (dt, $J = 0.9$, 2.0 Hz, H_b18), 4.81 (m, H_a18), 1.05 (d, $J =$ 6.5 Hz, $H_3$19), 4.65 (t, $J = 1.8$ Hz, H_b20), 4.62 (m, H_a20), 7.5–7.3 (series of m, aromatic CH), 3.49 (q, 1.3, OMe); NOE1D: 5.97 →2.38 and 1.76; 3.13 →4.96, 4.81 and 4.62; 2.38 →5.97.

Rogioldiol C *p*-bromobenzoate (**10**): 4-Bromobenzoylchloride (5 equiv) and a catalytic amount of 4-dimethylaminopyridine were added to a solution of (−)-**7** (1.4 mg) in dry pyridine (0.2 mL). The mixture was stirred overnight at room temperature and then MeOH (0.5 mL), sat. aq. $CuSO_4$ (2 mL), and *n*-hexane (4 mL) were added in sequence. The mixture was percolated through a Whatman phase-separation filter. The filtrate was evaporated, and the resulting residue was purified by Si-60 HPLC with hexane/*i*PrOH (97:3) to give **10** ($t_R = 4.2$ min, 1.0 mg, 43 %). UV (MeOH): λ_{max} (ε) = 243 (20400 $mol^{-1}Lcm^{-1}$); CD(MeOH): $\Delta\varepsilon_{max}$ (246) = + 6.0; ^{1}H NMR ($CDCl_3$, reporting only signals significantly different from (−)-**7**): $\delta = 5.18$ (br d, $J = 9.5$ Hz, H8), 6.00 (dd, $J = 9.5$, 10.3 Hz, H9), 3.17 (br d, $J =$ 10.3 Hz, H10), 1.74 (br s, $H_3$17), 4.63 (m, H_b18), 4.78 (m, H_a18), 0.99 (d, $J =$ 7.0 Hz, $H_3$19), 4.72 (m, H_b18), 4.68 (m, H_a18); MS (70 eV, EI): m/z (%) = 364/366 (8/8, [M − BrC_6H_5COOH]$^{\cdot+}$), 349/351 (1/1, [M − BrC_6H_5COOH − H_2O]$^+$), 243/245 (20/20), 225/227 (8/8), 200/202 (4/4), 183/185 (100/100), 173 (10), 145 (12), 121 (11), 105 (18), 81 (23), 69 (37).

Acknowledgments: We thank A. Sterni for recording the mass spectra and MURST (40 %) and CNR (including Progetto Strategico), Roma, for financial support.

Received: January 5, 1998 [F F951]

[1] a) J. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.* **1969**, *34*, 2543–2549; b) J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512–519; c) G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem.* **1973**, *38*, 2143–2147.

[2] a) M. D'Ambrosio, A. Guerriero, C. Debitus, F. Pietra, *Helv. Chim. Acta* **1996**, *79*, 51–60; b) G. Guella, F. Dini, F. Pietra, *Helv. Chim. Acta* **1996**, *79*, 710–717.

[3] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

[4] G. Guella, F. Marchetti, F. Pietra, *Helv. Chim. Acta* **1997**, *80*, 684–694.

[5] a) F. Johnson, *Chem. Rev.* **1968**, *68*, 375–413; b)R. W. Hoffmann, *Chem. Rev.* **1989**, *89*, 1841–1860; c) J. L. Broeker, R. W. Hoffmann, K. N. Houk, *J. Am. Chem. Soc.* **1991**, *113*, 5006–5017.

[6] a) N. Harada, K. Nakanishi, *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*, Oxford University Press, Oxford, **1983**; b) N. Harada, J. Iwabuchi, Y. Yokota, H. Uda, K. Nakanishi, *J. Am. Chem. Soc.* **1981**, *103*, 5590–5591; c) N. C. Gonnella, K. Nakanishi, V. S. Martin, K. B. Sharpless, *J. Am. Chem. Soc.* **1982**, *104*, 3775–3776.

[7] G. Guella, A. Öztunç, I. Mancini, F. Pietra, *Tetrahedron Lett.* **1997**, *38*, 8261–8264.

[8] F. Pietra, *Chem. Soc. Rev.* **1995**, 65–71.

[9] a) A. Wibe, A-K Borg-Karlson, M. Persson, T. Norin, H. Mustaparta, *J. Chem. Ecol.* **1998**, *24*, 273–287; b) A. Guerriero, M. D'Ambrosio, P. Traldi, F. Pietra, *Naturwissenschaften*, **1984**, *71*, 425–426; c) I. Mancini, G. Guella, P. Amade, C. Roussakis, F. Pietra, *Tetrahedron Lett*. **1997**, *38*, 6271–6274.

[10] L. A Egan, R. W. Busby, D. Iwata-Reuyl, C. A. Townsend, *J. Am. Chem. Soc.* **1997**, *119*, 2348–2355.

[11] D. J. Kennedy, I. A. Selby, H. J. Cowe, P. J. Cox, R. H. Thomson, *J. Chem. Soc. Chem. Commun.* **1984**, 153–155.

[12] a) G. Guella, I. Mancini, G. Chiasera, F. Pietra, *Helv. Chim. Acta* **1992**, *75*, 310–322; b) G. Guella, I. Mancini, G. Chiasera, F. Pietra, *Helv. Chim. Acta* **1992**, *75*, 303–309.

[13] a) N. Myers, *Science* **1997**, *278*, 597–598; b) J. B. Hughes, G. C. Daily, P. R. Ehrlich, *Science* **1997**, *278*, 689–692; c) S. Nee, R. M. May, *Science* **1997**, *278*, 692–694.

[14] D. L. Turner, *J. Magn. Reson*. **1985**, *61*, 28–51.