52. Rogioldiol A, a New Obtusane Diterpene, and Rogiolal, a Degraded Derivative, of the Red Seaweed *Laurencia microcladia* from Il Rogiolo along the Coast of Tuscany: a Synergism in Structural Elucidation

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The structure of rogioldiol A ((−)-**1**), isolated from the red seaweed *Laurencia microcladia*, was determined. Employing the exciton-coupling technique for rogioldiol A *p*-bromobenzoate (**2**), the absolute configuration at C(9) of (−)-**1** was assigned, and, together with extensive NMR experiments, the absolute configuration at C(10) and preferred conformations of (−)-**1** were determined. The absolute configuration of the hetero-substituted cyclohexane ring was deduced in analogy from the X-ray structure of **4**, a derivative of the aldehyde **3**, which was isolated from the same seaweed and is believed to be a degradation product of (−)-**1**.

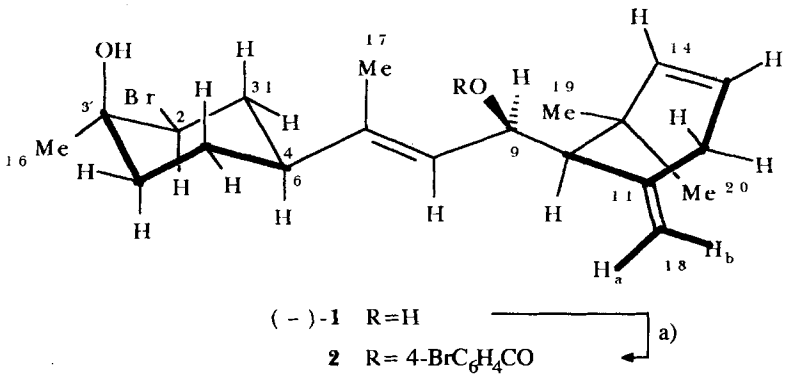
1. Introduction. – Red seaweeds in the genus *Laurencia* are the most studied of all seaweeds by natural-product chemists for their extraordinarily varied production of unusual secondary metabolites [1]. *Laurencia microcladia* from Il Rogiolo is a case in point for acetogenins and sesquiterpenes[1]). We report here that this seaweed produces also diterpenoids and offer a structure elucidation for one of them, rogioldiol A ((−)-**1**), which defeats both NMR and X-ray diffraction techniques taken singly.

2. Results and Discussion. – 2.1. *Rogioldiol A* ((−)-**1**). Five unsaturations for rogioldiol A ((−)-**1**, *Scheme 1*) were immediately detected from NMR (*Table 1*) and MS (*m/z* 364/366 1:1 doublet of composition $C_{20}H_{29}BrO$, which can be taken as $[M - H_2O]^{+\cdot}$)[2]). On assigning three unsaturations to isolated C=C bonds, two cycles were left, and extensive selective, differential decoupling and COSY experiments proved them as the trisubstituted cyclohexane and the cyclohexadiene ring depicted in (−)-**1**. COSY Data directly support the portion C(8)–C(10), which could be extended to include C(6) from long-range coupling of 3*H*–C(17) with C(6), C(8), and *H*–C(8). Interconnection of these portions, as shown in (−)-**1** (*Scheme 1*) was firmly established by HMBC experiments, in particular by the correlations H–C(8) ↔ C(6) and C(17), 2H–C(18) ↔ C(10) and

[1]) Included are *a*) β-chamigrenes of rogiolane type [2d, e, f, g], whose reactivity and conformational behavior were compared to α-chamigrenes [2h], *b*) oxepanes, branched [2a–d] or not [2c, d, i], in contrast with the oxocanes of most *Laurencia* spp. or even *L. microcladia* collected elsewhere [3], and which, like those above, are furnished to a nearby growing sponge [2c], and *c*) rare C_{15}, twelve-membered, cyclic ethers, a class of compounds that were previously only found in specimens of *Laurencia obtusa* from Turkish waters [2l].

[2]) Arbitrary C-atom numbering; systematic numbering and names are only used for retrieval purposes (see *Exper. Part*, which also contains semisystematic names).

Scheme 1



a) 1) 4-BrC_4H_4COBr, Pyridine, cat. 4-(dimethylamino)pyridine, r.t., overnight; 2) MeOH, sat. aq. $CuSO_4$, hexane.

C(12), as well as 3 H–C(19) ↔ C(10), C(14), C(15), and C(20). The configuration of the cyclohexyl ring of (–)-**1** rests on typical axial *J* coupling for both H–C(6) and H–C(2) and NOE enhancement of the latter with Me(16), which, therefore, must be *cis*-related to H–C(2) and accordingly equatorial. (*E*)-Configuration for C(7)=C(8) was established by typically high-field shift (δ(C) = 14.17 ppm) for Me(17); in further support, no NOE could be noticed between Me(17) and *H*–C(8). Strong NOE enhancement for H–C(10) with both H_a–C(18) and Me(20) indicated a preferential conformation as depicted in (–)-**1**, where H–C(10) is *cisoid* to the pseudoequatorial *exo*-methylidene group, and Me(20) is pseudoaxial. Assigning the relative configuration at C(9) proved more difficult, since the intermediate value *J*(9,10) = 7.4 Hz suggested averaging among various conformers for rotation around the C(9)–C(10) bond. However, molecular-mechanics (MM) calculations[3]), in combination with NOE data, allowed us to analyze this conformational bias. Thus, strong NOE enhancements between Me(17) and both H_{ax}–C(1) and H_{ax}–C(5), as well as between H–C(6) and H–C(8), established the preferred conformation represented by (–)-**1**, where the dihedral angle C(17)–C(7)–C(6)–H(6) is close to 180°. Moreover, strong NOE enhancements between H–C(8) and H–C(10), as well as between H–C(9) and Me(17), confirmed the preference for a conformation having nearly synperiplanar protons at C(6), C(8), and C(10) (*Fig. 1*, **1a**; calculated *J*(9,10) = 10.5). These calculations also suggested a significant contribution by other conformers, in particular 15% by a conformer having HO–C(9) *anti* to H–C(10) (*Fig. 1*, **1b**; calculated *J*(9,10) = 1.3, accounting, with a good approximation, for the observed averaged *J*(9,10) = 7.4 Hz). Preference for pseudoequatorial H–C(10) (and thus pseudoaxial attachment of the alkyl side chain) reflects the 1,3-allylic strain [4] imposed by the *exo*-methylidene group. This analysis allowed us also to rationalize the effects of an added shift reagent, [Eu(fod)$_3$], which, in less than stoichiometric amounts (*Table 1* and *Fig. 1*), becomes bound preferentially to the secondary OH group.

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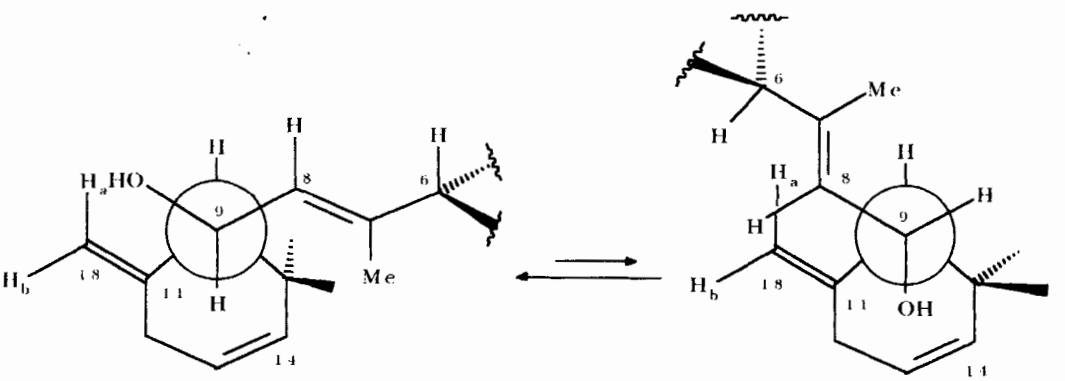
[3]) MM Calculations were carried out with MMX force field (PC MODEL 4.0, *Serena Software*, Indiana) where the C=C–C–H and C=C–C–C torsional parameters were changed from (V1 = 0.0, V2 = 0.0, V3 = –0.24) and (V1 = –0.44, V2 = 0.24, V3 = 0.06), to (V1 = 0.0, V2 = 0.0, V3 = –0.30) and (V1 = –0.54, V2 = 0.44, V3 = –0.60), respectively, according to [4b].

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *for Rogioldiol A* ((−)-**1**)

C-Atom	^{1}H-NMR[a])	^{13}C-NMR[b])
C(1)	H_{ax}: 2.15(*q*, *J* = 12.1); H_{eq}: 2.00(*dddd*, *J* = 2.1, 3.7, 4.1, 12.1)	38.67(*t*)
C(2)	4.15(*dd*, *J* = 4.1, 12.1)	65.84(*d*)
C(3)	–	70.24(*s*)
C(4)	H_{ax}: 0.97(*dt*, *J* = 4.0, 13.2); H_{eq}: 1.84(*td*, *J* = 3.0, 13.2)	37.41(*t*)
C(5)	H_{ax}: 2.10(*dq*, *J* = 3.6, 12.4); H_{eq}: 1.45(*tdd*, *J* = 2.1, 3.2 12.4)	25.48(*t*)
C(6)	1.92(*tt*, *J* = 3.1, 12.0)	48.41(*d*)
C(7)	–	139.25(*s*)
C(8)	5.29(*quint. d*, *J* = 1.2, 9.3)	126.99(*d*)
C(9)	4.44(*dd*, *J* = 7.4, 9.3)	67.21(*d*)
C(10)	2.17(br. *d*, *J* = 7.4)	59.77(*d*)
C(11)	–	144.17(*s*)
C(12)	H_{ax}: 2.57(br. *d*, *J* = 18.0); H_{eq}: 2.48(*ddd*, *J* = 1.5, 2.8, 18.0)	31.77(*t*)
C(13)	5.49(*td*, *J* = 2.8, 10.1)	123.28(*d*)
C(14)	5.42(*qd*, *J* = 1.5, 10.1)	137.34(*d*)
C(15)	–	36.48(*s*)
C(16)	1.31(*s*)	30.61(*q*)
C(17)	1.66(*d*, *J* = 1.2)	14.17(*q*)
C(18)	a) 4.78(br. *t*, *J* = 2.2); 4.87(*dt*, *J* = 1.2, 2.2)	112.64(*t*)
C(19)	1.16(*s*)	26.92(*q*)
C(20)	0.99(*s*)	31.72(*q*)

[a]) $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] ([**1**]/[Eu(fod)$_3$] = 0.3): 4.44(70), 5.29(47), 2.17(40), 4.77(19), 2.57(18), 1.16(15), 1.66(13), 4.87(11), 0.99(8), 4.15(6), 1.31(5).

[b]) $\Delta\delta$ (ppm × 100) observed on addition of [Eu(fod)$_3$] ([**1**]/[Eu(fod)$_3$] = 0.3): 67.21*d* (104), 126.99*d* (34), 139.25*s* (30), 112.64*t* (26), 59.77*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).



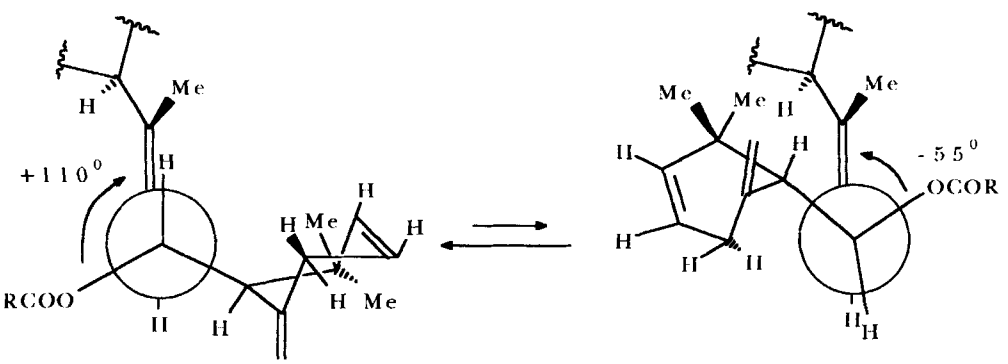
1a **1b**

Fig. 1. *Major* (**1a**) *and minor* (**1b**) *conformations, for rotation around the C(9)–C(10) bond, of rogioldiol A, as inferred from MM calculations*

^{1}H-NMR Spectra thus revealed that both H_a–C(18) and H_{ax}–C(12) are closer to HO–C(9) than to Me(19) and Me(20), while ^{13}C-NMR spectra showed that C(18) undergoes a larger shift than C(19) and, particularly, C(20). A careful quantitative

analysis of the shifts induced by [Eu(fod)$_3$] revealed that the conformation **1b** plays a minor but non-negligible role. Independence of J values from the state of binding/no-binding of the substrate to the shift reagent gave confidence in using these shift data to treat the conformational problem. Nothing, however, is implied about the diastereoisomeric assignment of rogioldiol A, since there is no way from NMR spectra to correlate the two halves of the molecule.

The absolute configuration at C(9) of (–)-**1** was assigned from a positive *Cotton* effect for the *p*-bromobenzoate **2** (*Scheme 1*). MM Calculations suggested that the conformer having H–C(9) eclipsed to C(7)=C(8) dominates, as required for successful application of exciton-coupling techniques [5]. The sign of the *Cotton* effect shows that the overall chirality between benzoate and double-bond chromophores is positive in agreement with the preferred conformation **2a** (*Fig. 2*). (*R*)-Configuration at C(9) could thus be assigned. MM Calculations suggested, however, that the measured *Cotton* effect is probably an average for the major (**2a**) and the minor conformer (**2b**) having positive (+ 110°) and negative (– 55°) values, respectively, of the O–C(9)–C(8)–C(7) dihedral angle (*Fig. 2*).



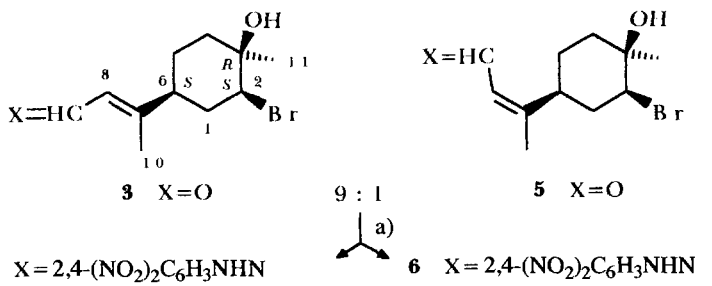
2a **2b**

Fig. 2. *Major* (**2a**) *and minor* (**2b**) *conformations, for rotation around the C(8)–C(9) bond, of rogioldiol A* p-*bromobenzoate, as inferred from MM calculations.* The relevant torsional angle C(7)–C(8)–C(9)–O for CD analysis is shown.

2.2. *The Absolute Configuration of a Co-occurring Aldehyde, Rogiolal* (**3**). Lack of definition of the absolute configuration at the brominated ring renders two diastereoisomeric structures possible for rogioldiol A. Unable to obtain suitable crystals of this compound for X-ray analysis, and lacking enough material to work with derivatives, we circumvented the problem by focussing our attention on a α,β-unsaturated C_{11} aldehyde, rogiolal (**3**)[4]), co-occurring with the isomeric **5** and rogioldiol A in our seaweed

[4]) The NMR coupling pattern and NOE experiments (*Exper. Part*) show that both H–C(2) and H–C(6) occupy the axial position, and that the bromohydrin systems has *cis*-configuration. (*E*)-Configuration at C(7)=C(8) for **3** was established by strong NOE effects within the couples H–C(9)/3H–C(7) and H–C(8)/H–C(6), other than by typically deshielded δ(C) for C(10). NOE Effects between 3H–C(7) and H–C(8) and typically deshielded C(10) were measured for **5**. Deshielding of H–C(6) by the C=O group is unusually large, *i.e.*, 1.2 ppm. The two aldehydes in solution tend to equilibrate, markedly in favor of **3**.

Scheme 2



a) 2,4-Dinitrophenylhydrazine, MeOH, cat. H_2SO_4, r.t., stirring for 2 h.

(*Scheme 2*). Rogiolal (**3**) may be reasonably envisaged as a product of biodegradation of rogioldiol A ((−)-**1**) by oxidative C(9)–C(10) bond cleavage, thus offering an alternative to assign tentatively the absolute configuration for the brominated moiety of the latter. To this end, suitable crystals of the 2,4-dinitrophenylhydrazone **4** could be obtained. The unit cell (*Fig. 3*) consists of a triclinic packing of a couple of molecules held together by a weak H-bond between the OH group of a molecule and an O-atom of a NO_2 group of the other molecule, other than by *van der Waals* forces. Bond lengths and angles are summarized in *Table 2*. This packing forces the two molecules to take different conformations by different rotations around the C(6)–C(7) bond, resulting in C(1)–C(6)–C(7)–C(8) dihedral angle values −118.6° for molecule A and 94.9° for molecule B. Molecules A and B are viewed along the C(6)–C(7) direction in the *Newman* projection in *Fig. 4*. In each molecule, the substituted, chiral cyclohexane ring is linked to an almost planar system, characterized by an extended conjugation of π electrons in

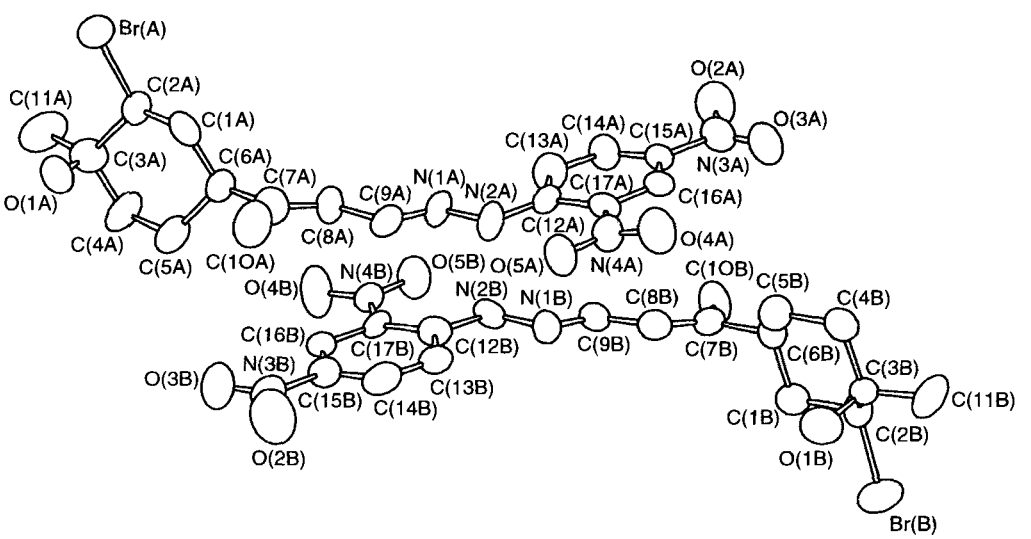


Fig. 3. *ORTEP Projection of the two molecules of* **5** *forming the asymmetric unit.* Thermal ellipsoids are represented at 50% probability.

Table 2. *Bond Lengths* [Å] *and Angles* [deg] *for* **4**

C(1A)–C(2A)	1.484(13)	C(1B)–C(2B)	1.532(14)
C(1A)–C(6A)	1.532(14)	C(1B)–C(6B)	1.52(2)
C(2A)–C(3A)	1.495(14)	C(2B)–C(3B)	1.466(12)
C(2A)–Br(A)	1.979(10)	C(2B)–Br(B)	1.935(10)
C(3A)–O(1A)	1.431(14)	C(3B)–O(1B)	1.434(11)
C(3A)–C(4A)	1.50(2)	C(3B)–C(4B)	1.53(2)
C(3A)–C(11A)	1.50(2)	C(3B)–C(11B)	1.525(14)
C(4A)–C(5A)	1.51(2)	C(4B)–C(5B)	1.506(14)
C(5A)–C(6A)	1.480(14)	C(5B)–C(6B)	1.510(13)
C(6A)–C(7A)	1.51(2)	C(6B)–C(7B)	1.53(2)
C(7A)–C(8A)	1.32(2)	C(7B)–C(8B)	1.33(2)
C(7A)–C(10A)	1.49(2)	C(7B)–C(10B)	1.494(14)
C(8A)–C(9A)	1.47(2)	C(8B)–C(9B)	1.40(2)
C(9A)–N(1A)	1.282(13)	C(9B)–N(1B)	1.254(13)
N(1A)–N(2A)	1.379(12)	N(1B)–N(2B)	1.414(12)
N(2A)–C(12A)	1.368(14)	N(2B)–C(12B)	1.355(13)
C(12A)–C(13A)	1.43(2)	C(12B)–C(13B)	1.39(2)
C(12A)–C(17A)	1.428(14)	C(12B)–C(17B)	1.421(13)
C(13A)–C(14A)	1.35(2)	C(13B)–C(14B)	1.394(14)
C(13A)–N(3A)	1.442(14)	C(13B)–N(3B)	1.431(14)
C(14A)–C(15A)	1.344(14)	C(14B)–C(15B)	1.37(2)
C(15A)–C(16A)	1.39(2)	C(15B)–C(16B)	1.37(2)
C(15A)–N(4A)	1.461(14)	C(15B)–N(4B)	1.426(14)
C(16A)–C(17A)	1.35(2)	C(16B)–C(17B)	1.35(2)
N(3A)–O(3A)	1.217(11)	N(3B)–O(3B)	1.220(11)
N(3A)–O(2A)	1.235(11)	N(3B)–O(2B)	1.210(11)
N(4A)–O(4A)	1.204(12)	N(4B)–O(4B)	1.224(13)
N(4A)–O(5A)	1.238(13)	N(4B)–O(5B)	1.221(13)
C(2A)–C(1A)–C(6A)	110.1(9)	C(2B)–C(1B)–C(6B)	111.3(9)
C(1A)–C(2A)–C(3A)	116.0(9)	C(1B)–C(2B)–C(3B)	114.9(8)
C(1A)–C(2A)–Br(A)	108.8(7)	C(1B)–C(2B)–Br(B)	108.2(7)
C(3A)–C(2A)–Br(A)	110.7(7)	C(3B)–C(2B)–Br(B)	111.3(7)
O(1A)–C(3A)–C(2A)	107.1(9)	O(1B)–C(3B)–C(2B)	111.1(8)
O(1A)–C(3A)–C(4A)	105.9(11)	O(1B)–C(3B)–C(4B)	104.8(8)
C(2A)–C(3A)–C(4A)	107.5(10)	C(2B)–C(3B)–C(4B)	107.4(9)
O(1A)–C(3A)–C(11A)	110.9(12)	O(1B)–C(3B)–C(11B)	108.2(9)
C(2A)–C(3A)–C(11A)	113.0(11)	C(2B)–C(3B)–C(11B)	115.2(9)
C(4A)–C(3A)–C(11A)	112.1(10)	C(4B)–C(3B)–C(11B)	109.7(9)
C(3A)–C(4A)–C(5A)	112.5(11)	C(3B)–C(4B)–C(5B)	115.4(10)
C(4A)–C(5A)–C(6A)	112.9(11)	C(4B)–C(5B)–C(6B)	109.0(8)
C(5A)–C(6A)–C(7A)	115.5(10)	C(5B)–C(6B)–C(7B)	116.1(9)
C(5A)–C(6A)–C(1A)	108.2(8)	C(5B)–C(6B)–C(1B)	111.1(8)
C(1A)–C(6A)–C(7A)	111.4(10)	C(1B)–C(6B)–C(7B)	110.5(9)
C(10A)–C(7A)–C(8A)	125.1(11)	C(10B)–C(7B)–C(8B)	123.9(11)
C(6A)–C(7A)–C(8A)	117.2(10)	C(6B)–C(7B)–C(8B)	121.8(11)
C(6A)–C(7A)–C(10A)	117.6(11)	C(6B)–C(7B)–C(10B)	114.2(10)
C(7A)–C(8A)–C(9A)	126.9(11)	C(7B)–C(8B)–C(9B)	126.9(12)
N(1A)–C(9A)–C(8A)	115.4(10)	N(1B)–C(9B)–C(8B)	121.0(11)
C(9A)–N(1A)–N(2A)	113.6(9)	C(9B)–N(1B)–N(2B)	114.3(9)
C(12A)–N(2A)–N(1A)	115.7(9)	C(12B)–N(2B)–N(1B)	119.7(9)
N(2A)–C(12A)–C(13A)	123.7(10)	N(2B)–C(12B)–C(13B)	124.5(10)
N(2A)–C(12A)–C(17A)	121.2(10)	N(2B)–C(12B)–C(17B)	118.3(11)
C(13A)–C(12A)–C(17A)	115.1(11)	C(13B)–C(12B)–C(17B)	117.2(11)

Table 2 (cont.)

C(14A)–C(13A)–C(12A)	122.4(11)	C(12B)–C(13B)–C(14B)	122.0(10)
C(14A)–C(13A)–N(3A)	116.6(11)	C(14B)–C(13B)–N(3B)	115.6(10)
C(12A)–C(13A)–N(3A)	120.8(11)	C(12B)–C(13B)–N(3B)	122.4(10)
C(15A)–C(14A)–C(13A)	119.6(10)	C(15B)–C(14B)–C(13B)	118.9(11)
C(14A)–C(15A)–C(16A)	121.9(10)	C(14B)–C(15B)–C(16B)	120.2(11)
C(14A)–C(15A)–N(4A)	118.9(11)	C(14B)–C(15B)–N(4B)	118.3(11)
C(16A)–C(15A)–N(4A)	119.2(11)	C(16B)–C(15B)–N(4B)	121.5(11)
C(17A)–C(16A)–C(15A)	119.4(11)	C(17B)–C(16B)–C(15B)	121.9(12)
C(16A)–C(17A)–C(12A)	121.6(11)	C(16B)–C(17B)–C(12B)	119.9(11)
O(3A)–N(3A)–O(2A)	121.2(9)	O(2B)–N(3B)–O(3B)	120.9(10)
O(3A)–N(3A)–C(13A)	118.8(11)	O(3B)–N(3B)–C(13B)	119.5(10)
O(2A)–N(3A)–C(13A)	119.9(10)	O(2B)–N(3B)–C(13B)	119.4(10)
O(4A)–N(4A)–O(5A)	124.4(11)	O(5B)–N(4B)–O(4B)	123.5(11)
O(4A)–N(4A)–C(15A)	118.4(11)	O(4B)–N(4B)–C(15B)	119.6(11)
O(5A)–N(4A)–C(15A)	117.1(12)	O(5B)–N(4B)–C(15B)	116.9(12)

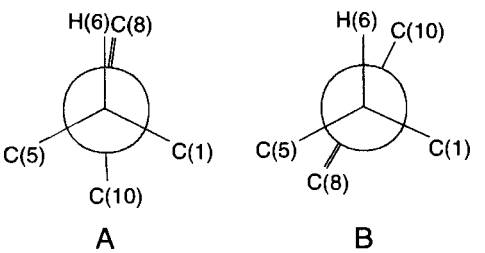


Fig. 4. Newman *projections of the C(6)–C(7) bonds in the two moieties of the asymmetric unit, showing the different conformations*

the 2,4-dinitrophenylhydrazone system and alternate C–C and C=C bonds in the C(7)–C(12) chain. Despite relatively high standard deviations in bond lengths, some degree of localization of the C=C bonds emerged clearly: for the 2,4-dinitrophenyl ring in molecule A, differences in C,C bond lengths are greater than 10σ, like for a quinoid form. In contrast, molecule B shows typical delocalization of π bonds. The planar portions of the two molecules are related to each other by approximately an inversion centre located midway between N(2A) and N(2B). This approximate symmetry is removed by the asymmetry of the substituted cyclohexane rings, with the two Br-atoms interrelated through the same symmetry centre[5]). A *Flack*'s index value close to zero gave confidence as to the choice of the absolute configurations as in **4** and, therefore, in **3**. The closest structural example from X-ray diffraction analysis is (4*R*,3*S*,6*S*,10*S*)-10-bromo-4-chloro-3,11,11-trimethyl-7-methylidenespiro[6.6]undecan-3-ol known as hurgadol [6].

3. Conclusions. – If rogiolal (**3**) and rogioldiol A have a common biogenesis – which is most likely – this work has shown that the latter is defined by (–)-**1** also with respect to the absolute configuration. The strategy applied here in assigning structure (–)-**1** at

[5]) We were initially mislead by such *quasi*-centrosymmetry, and it was only by carefully refining the analysis that all atoms could be correctly localized.

the diastereoisomeric level, *i.e.*, through the absolute configuration – no matter how determined – of a portion of (–)-**1** occurring as a co-metabolite (**3**), may find application in the frequent cases of natural products constituted of NMR non-interrelated moieties, and for which single crystals are difficult to obtain. In any case, our technique has the advantage with respect to X-ray diffraction alone to arrive also at a description of the preferred conformations in solution.

Two obtusane diterpenoids had already been described, one, unnamed, by X-ray diffraction techniques [7] and the other one, obtusadiol, from mostly NMR spectra in solution without taking into account the problem of the interconnecting flexible chain [8]: its structure remains, therefore, undefined at the diastereoisomeric level.

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Experimental Part

1. *General.* See [2a, e]. Moreover: ^{1}H-NMR at 299.94 MHz (the coupling pattern of many protons has been elucidated by differential spin decoupling [9]); 90/120 ^{1}H,^{1}H COSY [10] were carried out with compounds (–)-**1** and **2**. NOE stands for differential NOE. ^{13}C-NMR at 75.43 MHz; multiplicities from DEPT [11]; H-bearing C-atoms for compounds (–)-**1** and **2** were assigned from ^{13}C,^{1}H-COSY [12]. Inverse detection on rogioldiol A (–)-**1**: *via* the heteronuclear multiple-quantum coherence pulse sequence (HMBC) [13a] with a dedicated *Varian* probe [13b]. Differential NOE data (obtained with 5-s preirradiation) are reported as 'irradiated proton → NOE on the observed proton(s)'.

2. *Product Isolation.* The residue (0.12 g) from evaporation of fraction 36 out of 54 fractions obtained before from *L. microcladia* extracts [2f] was subjected to reversed phase HPLC with MeCN/H_2O 65:35, followed by HPLC *CN* with hexane/i-PrOH 97:3, collecting various fractions. The residue (0.03 g) from *Fr. 8* was subjected to *Si-60* HPLC with hexane/AcOEt 4:1 to give *rogioldiol A* ((–)-**1**) (t_R 9.2 min; 5.5 mg, 0.01%). The residue (0.11 g) from the combined *Fr. 37–43* (0.18 g) was subjected to HPLC *CN* with hexane/i-PrOH 95:5 to give the known obtusane diterpene obtusadiol [8] (t_R 10.5; 22 mg, 0.04%, $[\alpha]_D^{20} = -40$ ($c = 0.6$, MeOH)), *isorogiolal* (**5**; further purified by *RP-18* HPLC with MeCN/H_2O 65:35; t_R 5.0; 4.0 mg, 0.008%) and *rogiolal* (**4**; further purified by *RP-18* HPLC with MeCN/H_2O 65:35, t_R 5.2; 20 mg, 0.04%).

3. *Rogioldiol A* (= *(2S,3R,6S,9R,10R)-2-Bromoobtusa-7,11(18),14-triene-3,9-diol* = *2-Bromo-4-[3-(2,2-dimethyl-6-methylidenecyclohex-3-enyl)-3-hydroxy-1-methylprop-1-enyl]-1-methylcyclohexan-1-ol*; (–)-**1**). $[\alpha]_D^{20} = -43$ ($c = 0.10$, MeOH). NMR: *Table 1*. NOE: 5.29 → 2.17, 1.92, 2.10; 4.44 → 1.65, 1.16; 4.78 → 2.17; 4.87 → 2.48; 1.66 → 4.44, 2.15, 2.10; 1.16 → 4.44, 5.42; 0.99 → 2.17. EI-MS: 364/366 (1.2/1.2, $[M - H_2O]^+$), 349/351 (0.8/0.8, $[M - Me - H_2O]^+$) 261/263 (8/8), 243/245 (14/14), 225/227 (14/14), 163 (58), 145 (41), 135 (22), 119 (29), 107 (96), 93 (56), 71 (100).

4. *Rogioldiol A* p-*Bromobenzoate* (**2**). To a soln. of **1** (2.1 mg) in dry pyridine (0.5 ml) was added a catal. amount of 4-(dimethylamino)pyridine and 4-bromobenzoyl chloride (5 equiv.). The mixture was stirred at r.t. overnight and then, in sequence, MeOH (0.5 ml), sat. aq. $CuSO_4$ (2 ml), and hexane (4 ml) were added, followed by percolation through a *Whatman* phase-separation filter. The filtrate was evaporated, and the residue was subjected to HPLC (*Si-60*; hexane/i-PrOH 97:3): **2** (t_R 4.2 min; 1.3 mg, 43%). UV (MeOH): 244 (20400). CD (MeOH): $\Delta\varepsilon_{max}(245) = +13.0$. ^{1}H-NMR ($CDCl_3$): 2.11 (*q*, 12.0, H_{ax}–C(1)); 2.02 (*m*, H_{eq}–C(1)); 4.13 (*dd*, $J = 4.5$, 12.0, H–C(2)); 2.05, 1.45 (*m*, 2H–C(4)); 170, 1.58 (*m*, 2H–C(5)); 1.90 (*tt*, $J = 3.3$, 12.0, H–C(6)); 5.18 (*quint. d*, $J = 1.5$, 10.2, H–C(8)); 5.73 (*dd*, $J = 8.1$, 10.2, H–C(9)); 2.53 (br. *d*, $J = 8.1$, H–C(10)); 2.57, 2.49 (*m*, 2H–C(12)); 5.55 (*td*, $J = 3.3$, 10.0, H–C(13)); 5.41 (*qd*, $J = 1.5$, 10.0, H–C(14)); 1.30 (*s*, 3H–C(16)); 1.81 (*d*, $J = 1.2$, 3H–C(17)); 4.89 (*m*, H_b–C(18)); 4.83 (*m*, H_a–C(18)); 1.00 (*s*, 3H–C(19)); 0.98 (*s*, 3H–C(20)). NOE: 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 ($CDCl_3$): 38.53 (*t*, C(1)); 65.78 (*d*, C(2)); 70.23 (*s*, C(3)); 37.42 (*t*, C(4)); 25.52 (*t*, C(5)); 48.30 (*d*, C(6)); 141.64 (*s*, C(7)); 122.63 (*d*, C(8)); 71.69 (*d*, C(9)); 56.87 (*d*, C(10)); 143.36 (*s*, C(11)); 31.40 (*t*, C(12)); 123.55 (*d*, C(13)); 136.74 (*d*, C(14)); 36.78 (*s*, C(15)); 30.57 (*q*, C(16)); 14.77 (*q*, C(17)); 112.99 (*t*, C(18)); 26.44 (*q*, C(19)); 31.28 (*q*, C(20)). EI-MS: 364/366 (1.2/1.2, $[M - C_6H_5COOH]^+$), 349/351 (3/3, $[M - C_6H_5COOH - H_2O]^+$), 243/245 (9/9), 225/227 (4/4), 200/202 (9/9), 183/185 (100/100), 173 (46), 145 (41), 135 (22), 119 (29), 107 (96), 93 (56), 71 (100).

5. *Rogiolal* (= *(E)-3-[(1S,3S,4R)-3-Bromo-4-hydroxy-4-methylcyclohexyl]but-2-enal*; **3**). ^{1}H-NMR ($CDCl_3$): 2.22 (*q*, *J* = 12.4, H_{ax}–C(1)); 2.13 (*td*, *J* = 3.6, 12.4, H_{eq}–C(1)); 4.14 (*dd*, *J* = 4.6, 11.6, H–C(2)); 2.10 (*td*, *J* = 3.0, 13.0, H_{eq}–C(4)); 1.50 (*ddt*, *J* = 2.4, 3.8, 13.0, H_{ax}–C(4)); 1.79 (*ddt*, *J* = 3.8, 12.4, 13.2, H_{ax}–C(5)); 1.57 (*ddd*, *J* = 2.5, 3.3, 13.2, H_{eq}–C(5)); 2.12 (*tt*, *J* = 3.3, 12.4, H–C(6)); 5.88 (*qdd*, *J* = 1.3, 2.1, 7.8, H–C(8)); 9.99 (*d*, *J* = 7.8, H–C(9)); 2.14 (*d*, *J* = 1.3, 3H–C(10)); 1.33 (*s*, 3H–C(11)); 1.96 (*d*, *J* = 2.4, OH). ^{13}C-NMR ($CDCl_3$): 37.64 (*t*, C(1)); 64.21 (*d*, C(2)); 70.01 (*s*, C(3)); 37.06 (*t*, C(4)); 25.24 (*t*, C(5)); 49.00 (*d*, C(6)); 164.73 (*s*, C(7)); 126.54 (*d*, C(8)); 191.43 (*d*, C(9)); 15.65 (*q*, C(10)); 30.39 (*s*, C(11)). MS: 245/247 (20/20, $[M - Me]^+$), 190/192 (9/9, $[M - C_4H_6O]^{+\cdot}$), 163 (73, $[M - Br - H_2O]^+$), 147 (26), 135 (20), 119 (27), 105 (22), 93 (85), 71 (74), 43 (100).

6. *Isorogiolal* (= *(Z)-3-[(1S,3S,4R)-3-Bromo-4-hydroxy-4-methylcyclohexyl]but-2-enal*; **5**). ^{1}H-NMR ($CDCl_3$): 2.39 (*q*, *J* = 12.4, H_{ax}–C(1)); 2.04 (*td*, *J* = 3.6, 12.4, H_{eq}–C(1)); 4.19 (*dd*, *J* = 4.6, 11.6, H–C(2)); 1.94 (*td*, *J* = 3.0, 13.0, H_{eq}–C(4)); 1.55 (*ddt*, *J* = 2.4, 3.8, 13.0, H_{ax}–C(4)); 1.99 (*ddt*, *J* = 3.8, 12.4, 13.2, H_{ax}–C(5)); 1.48 (*ddd*, *J* = 2.5, 3.3, 13.2, H_{eq}–C(5)); 3.35 (*tt*, *J* = 3.3, 12.4, H–C(6)); 5.84 (*qdd*, *J* = 1.3, 2.1, 7.8, H–C(8)); 9.98 (*d*, *J* = 7.6, H–C(9)); 1.93 (*d*, *J* = 1.3, 3H–C(10)); 1.35 (*s*, 3H–C(11)). ^{13}C-NMR ($CDCl_3$): 37.80 (*t*, C(1)); 63.88 (*d*, C(2)); 69.91 (*s*, C(3)); 36.95 (*t*, C(4)); 25.59 (*t*, C(5)); 41.14 (*d*, C(6)); 164.70 (*s*, C(7)); 128.06 (*d*, C(8)); 189.44 (*d*, C(9)); 20.85 (*q*, C(10)); 30.48 (*s*, C(11)). MS: substantially superimposable to that of **3**.

7. *Rogiolal 2',4'-Dinitrophenylhydrazone* ((–)-**4**) *and Isorogiolal 2',4'-Dinitrophenylhydrazone* (**6**). To a soln. of 2,4-dinitrophenylhydrazine (25 mg) in MeOH (1 ml) were added 0.05 ml of aq. conc. H_2SO_4 and 3 ml of a MeOH soln. of **3** (15 mg, containing 10% of **5**). The mixture was stirred at r.t. for 2 h and then filtered obtaining an amorphous red powder. This was subjected to HPLC (*Si-60*; hexane/i-PrOH 98:2): **6** (t_R 10.1 min; 2.1 mg, 9%) and **4** (t_R 12.5 min; 22 mg, 85%). Crystals, m.p. (hexane/Et_2O 4:1) 164–166°, suitable for X-ray diffraction analysis were obtained from slow evaporation of a sat. EtOH soln. of **4**.

Table 3. *Crystal Data and Structure Refinement for* **4**

Empirical formula	$C_{17}H_{21}BrN_4O_5$
Formula weight	441.29
Temp.	293 (2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*1 (*n*.1)
Unit cell dimensions	
a [Å]	7.763 (1)
b [Å]	10.069 (1)
c [Å]	13.338 (1)
α [°]	80.77 (1)
β [°]	73.42 (1)
γ [°]	74.01 (1)
Volume	956.8 (2) Å^3
Z	2
Density (calculated)	1.532 Mg/m^3
Absorption coefficient	2.184 mm^{-1}
F(000)	452
Crystal size	0.10 × 0.36 × 0.38 mm
θ Range for data collection	2.11 to 29.15 deg.
Index ranges	$-7 \le h \le 7$, $-10 \le k \le 10$, $-13 \le l \le 13$
Reflections collected	4185
Absorption correction	Semi-empirical from ψ scans
Max. and min. transmission	0.5323 and 0.3528
Refinement method	Full-matrix least-squares on F^2
Data/restrains/parameters	4185/3/513
Goodness-of-fit on F^2	1.070
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0463$, $wR_2 = 0.0909$
R indices (all data)	$R_1 = 0.0696$, $wR_2 = 0.1014$
Absolute structure parameter	−0.033 (13)
Largest diff. peak and hole	0.275 and −0.377 e Å^{-3}

Data of (−)-**4**. $[\alpha]_D^{20} = -62$ ($c = 0.5$ MeOH). UV (MeOH): 216 (14500), 255 (13400), 280 (8000), 383 (24300). ^{1}H-NMR ($CDCl_3$): 2.25 (*q*, $J = 12.4$, H_{ax}–C(1)); 2.16 (*td*, $J = 3.6$, 12.4, H_{eq}–C(1)); 4.18 (*dd*, $J = 4.6$, 11.6, H–C(2)); 2.10 (*td*, $J = 3.0$, 13.0, H_{eq}–C(4)); 1.50 (*ddt*, $J = 2.4$, 3.8, 13.0, H_{ax}–C(4)); 1.79 (*ddt*, $J = 3.8$, 13.2, H_{ax}–C(5)); 1.57 (*ddd*, $J = 2.5$, 3.3, 13.2, H_{eq}–C(5)); 2.12 (*tt*, $J = 3.3$, 12.4, H–C(6)); 6.17 (*qdd*, $J = 1.3$, 2.1, 9.6 H–C(8)); 8.05 (*dd*, $J = 0.7$, 9.6, H–C(9)); 9.12 (*d*, $J = 2.4$, H–C(3′)); 8.29 (*ddd*, $J = 0.7$, 2.4, 9.6, H–C(5′)); 7.92 (*d*, $J = 9.6$, H–C(6′)); 1.95 (*d*, $J = 1.3$, 3H–C(10)); 1.36 (*s*, 3H–C(11)); 11.15 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$): 37.03 (*t*, C(1)); 64.56 (*d*, C(2)); 70.03 (*s*, C(3)); 37.03 (*t*, C(4)); 25.47 (*t*, C(5)); 48.58 (*d*, C(6)); 152.48 (*s*, C(7)); 126.54 (*d*, C(8)); 146.79 (*d*, C(9)); 15.79 (*q*, C(10)); 30.40 (*s*, C(11)), and, for the aromatic portion, 144.42 (*s*), 138.20 (*s*), 129.80 (*d*), 120.16 (*d*), 116.43 (*d*).

Data of **6**. ^{1}H-NMR ($CDCl_3$): 2.40 (*q*, $J = 12.4$, H_{ax}–C(1)); 2.00 (*td*, $J = 3.6$, 12.4, H_{eq}–C(1)); 4.25 (*dd*, $J = 4.6$, 11.6, H–C(2)); 1.94 (*td*, $J = 3.0$, 13.0, H_{eq}–C(4)); 1.55 (*ddt*, $J = 2.4$, 3.8, 13.0, H_{ax}–C(4)); 1.99 (*ddt*, $J = 3.8$, 12.4, 13.2, H_{ax}–C(5)); 1.48 (*ddd*, $J = 2.5$, 3.3, 13.2, H_{eq}–C(5)); 2.82 (*tt*, $J = 3.3$, 12.4, H–C(6)); 6.09 (*qdd*, $J = 1.3$, 2.1, 10.2, H–C(8)); 8.09 (br. *d*, $J = 10.2$, H–C(9)); 9.13 (*d*, $J = 2.4$, H–C(3′)); 8.29 (*ddd*, $J = 0.7$, 2.4, 9.6, H–C(5′)); 7.91 (*d*, $J = 9.6$, H–C(6′)); 1.92 (*d*, $J = 1.3$, 3H–C(10)); 1.38 (*s*, 3H–C(11)); 11.15 (br. *s*, NH).

8. *Crystal Structure of (−)*-**4**. Crystals of **4** were red, lance-tip shaped, several mm long. A nearly tabular splinter was broken from a side of one of them and glued at the end of a glass fibre. Its X-ray diffraction ability was first studied on *Weissenberg* photographs. It showed triclinic symmetry with well shaped spots. Accurate unit cell dimensions were measured on a four-circles *Siemens P4* diffractometer by centring 25 strong reflections with diffraction angle 2θ 23–25°. The intensity data collection was made following the experimental conditions summarized in *Table 3*. To limit the crystal decay, data collection was performed rather rapidly (9 deg/min) until a maximum θ of only 20°. 4187 reflections were collected observing only *ca.* 3% decay on 3 standard reflections controlled every 97 measurements. Correction was made for *Lorentz* and polarization effects as to both decay and absorption, owing to the relatively high linear absorption factor and the dimensional anisotropy of the crystal. By means of a fitting procedure the crystal shape was approximated following reproduction by the ellipsoid of the trend observed on the intensity of 50 strong reflections scanned at different ψ angles [14]. The structure was solved by the direct TREF procedure contained in the SHELXTL package [15]. The space group was set to *P*1 on the basis of the chirality of the molecule. All the heavy atoms were located on difference *Fourier* map, and their positions were refined by a full-matrix least-squares procedure. The H-atoms were introduced in calculated positions and refined with constraints. In the final cycles the heavy atoms were refined with anisotropic thermal parameters. Details on the refinement and some statistical reliability data are also listed in *Table 3*. The absolute configuration was assigned on the basis of the enantiomorph-polarity parameter [16] (equal to zero within 3σ (−0.035(13) for our enantiomer). Tables of final atomic coordinates were deposited with the *Cambridge Crystallographic Database*[6]), while anisotropic thermal parameters and coordinates for the H-atoms can be obtained from the authors (*F.M.*). Crystallographic calculations and structure drawings were performed with the SHELXTL, PARST95 [17], and ORTEPII [18] programs.

REFERENCES

[1] K. L. Erickson, in 'Marine Natural Products. Chemical and Biological Perspectives', Ed. P. J. Scheuer, Academic Press, New York, 1983, Vol. 5, p. 131.

[2] a) G. Guella, F. Pietra, *Helv. Chim. Acta* **1991**, *74*, 47; b) G. Guella, I. Mancini, G. Chiasera, F. Pietra, *ibid.* **1992**, *75*, 303; c) G. Guella, I. Mancini, F. Pietra, *Comp. Biochem. Physiol., B* **1992**, *103*, 1019; d) F. Pietra, *Gazz. Chim. Ital.* **1993**, *123*, 343; e) G. Guella, G. Chiasera, I. Mancini, F. Pietra, *Helv. Chim. Acta* **1990**, *73*, 1612; f) *ibid.* **1991**, *74*, 774; g) G. Guella, G. Chiasera, F. Pietra, *ibid.* **1992**, *75*, 2012; h) *ibid.* **1992**, *75*, 2026; i) G. Guella, I. Mancini, G. Chiasera, F. Pietra, *ibid.* **1992**, *75*, 310; l) G. Guella, A. Öztunç, G. Chiasera, I. Mancini, F. Pietra, to be published.

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

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

[5] N. Harada, K. Nakanishi, 'Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry', Oxford University Press, Oxford, 1983; N. Harada, J. Iwabuchi, Y. Yokota, H. Uda, K. Nakanishi, *J. Am.*

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[6]) As supplementary publication No. CCDC-10/46. Copies can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44(0)1223336033 or e-mail: teched@chemcrys.cam.ac.uk.)

Chem. Soc. **1981**, *103*, 5590; N. C. Gonnella, K. Nakanishi, V. S. Martin, K. B. Sharpless, *ibid.* **1982**, *104*, 3775.

[6] S.-E. N. Ayyad, A.-A. M. Dawidar, H. W. Dias, R. A. Howie, J. Jakupovic, R. H. Thomson, *Phytochemistry* **1990**, *29*, 3193.

[7] F. J. Schmitz, K. H. Hollenbeak, D. C. Carter, M. B. Hossain, D. van der Helm, *J. Org. Chem.* **1979**, *44*, 2445.

[8] B. M. Howard, W. Fenical, *Tetrahedron Lett.* **1978**, 2453.

[9] J. K. M. Sanders, J. D. Mersch, *Progr. N.M.R. Spectrosc.* **1982**, *15*, 353.

[10] A. Bax, R. Freeman, G. Morris, *J. Magn. Reson.* **1981**, *42*, 164.

[11] D. H. Doddrell, D. T. Pegg, H. R. Bendall, *J. Magn. Reson.* **1982**, *48*, 323; D. T. Pegg, D. M. Doddrell, M. R. Bendall, *J. Chem. Phys.* **1982**, *77*, 2745.

[12] A. Bax, *J. Magn. Reson.* **1983**, *53*, 517; H. Kessler, C. Griesinger, J. Zarbock, H. R. Loosli, *ibid.* **1984**, *57*, 331.

[13] a) L. Müller, *J. Am. Chem. Soc.* **1979**, *101*, 4481; b) G. Gray, *Magn. Moments* **1987**, *III(2)*, 6.

[14] A. C. T. North, C. Phillips, F. S. Mathews, *Acta Crystallog., Sect. A* **1968**, *24*, 351.

[15] G. M. Sheldrick, SHELXTL. Release 5.03. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, 1992.

[16] H. D. Flack, *Acta Crystallog., Sect. A* **1983**, *39*, 876.

[17] M. Nardelli, *Comput. Chem.* **1983**, *7*, 95.

[18] C. K. Johnson, ORTEPII, Report ORNL-5138, Oak Ridge National Laboratory, Tennessee, 1976.