144. Rogiolol Acetate: a Novel β-Chamigrene-Type Sesquiterpene Isolated from a Marine Sponge[1])

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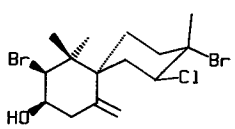
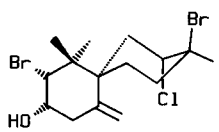
(8.V.90)

The marine sponge *Spongia zimocca* SCHMIDT, 1862, collected in front of the torrent Il Rogiolo, south of Livorno, contains the sesquiterpene rogiolol acetate (= *(+)-(2R,3S,6R,8R,9R)-2,8-dibromo-9-chloro-1,1,9-trimethyl-5-methylidenespiro[5.5]undec-3-yl acetate*; (+)-**3a**), which represents the first chamigrane isolated from a sponge. Although compounds of this class are common in red seaweeds of the genus *Laurencia*, and our sponge actually contains 9-bromochamigrene and a variety of other metabolites of nearby growing *Laurencia* sp., (+)-**3a** is unique to our sponge.

Introduction. – A plethora of halogenated chamigrane-type sesquiterpenes have been recently isolated from red algae of the genus *Laurencia* as well as from opisthobranch mollusks of the genus *Aplysia* [1] which feed on such algae [2]. Of our present interest are β-chamigrenes-type sesquiterpenes, such as obtusol ((+)-**1**), isolated from *Laurencia obtusa* (HUDS) LAMOROUX [3], and isoobtusol ((+)-**2**), isolated from the same alga and, as the acetate, from *Aplysia dactylomela* collected near La Parguera, Portorico [4]. In fact,



(+)-**1**



(+)-**2**

we report here on a novel halogenated β-chamigrene-type sesquiterpene isolated from the sponge *Spongia zimocca* SCHMIDT, 1862, which grows in a marine coastal area densely colonized by red algae of the genus *Laurencia*. *S. zimocca* is interesting from the ecological point of view: it is in fact a commercial sponge which is fished in Tunisia and the eastern Mediterranean, and there was no clear documentation, before this case, about the existence of this sponge in the western Mediterranean. More relevant form the biochemical viewpoint is that this is the first case that a chamigrene-type compound is found in a sponge.

Results and Discussion. – *The Gross Structure.* Both the ^{1}H- and the ^{13}C-NMR spectrum of rogiolol acetate at room temperature at 299.94 *(Fig., B)* and 75.43 MHz, respectively, revealed several broad resonances which proved to be temperature-depen-

[1]) We use the chamigrene numbering for the structural formulae and spectroscopic data; IUPAC nomenclature and numbering are used for retrieval purposes.

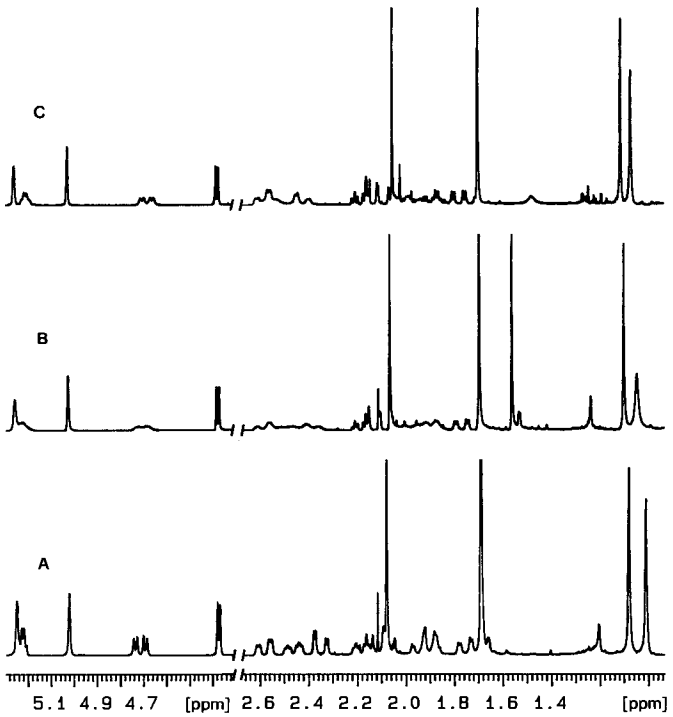
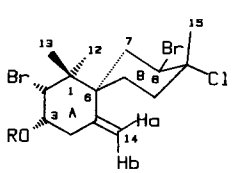


Figure. *300-MHz ^{1}H-NMR Spectra* ($CDCl_3$) *of rogiolol acetate* ((+)-**3a**) *at −30° (A), +21° (B), and +50° (C)*

dent. Thus, either on raising the probe temperature to 50° *(Fig., C)* or lowering it to −30° *(Fig., A)*, a considerable sharpening of these signals was observed, mainly in the ^{1}H-NMR spectra. Although similar phenomena occurred with most of the ^{13}C-NMR signals of rogiolol acetate, a few of these signals remained considerably broad even on raising the temperature to +50°.

Such phenomena of broadening were at first puzzling, as the ^{1}H-NMR spectra in the *Figure* for rogiolol acetate could be interpreted in terms of the single conformer (+)-**3a**, as will become clear later. It was clarifying that, when the ^{1}H-NMR spectrum of rogiolol acetate was taken at 80 MHz at r.t., all signals were sharp, implying that line broadening at higher frequency must result from a classical phenomenon of chemical exchange among chemically and magnetically non-equivalent sites. We have, thus, to admit that, in the 'low-exchange limit', the observed conformer (represented by (+)-**3a**) thermodynamically prevails over all other ones. Similar phenomena were observed with rogiolol ((−)-**3b**), obtained by mild saponification of (+)-**3a**.



(+)-**3a** R = Ac
(−)-**3b** R = H
(+)-**3c** R = CH_3SO_2

The mass spectrum of rogiolol acetate shows the highest-mass fragment (*m/z* 315) with the typical pattern for 1 Br and 1 Cl, whereas with rogiolol M^+ is observed at *m/z* 412 with the typical pattern for 2 Br and 1 Cl. This, taking into account ^{1}H- and ^{13}C-NMR data at low temperature for rogiolol acetate, as well as its chemical transformation into rogiolol ((−)-**3b**), allows us to define the molecular formula $C_{15}H_{23}Br_2ClO$ for rogiolol and $C_{17}H_{25}Br_2ClO_2$ for rogiolol acetate.

The *β*-chamigrene structure of rogiolol acetate ((+)-**3a**) (which, with two carbocycles and a C=C bond, accounts for the unsaturations implied in the above formula) can be deduced from a simple inspection of its ^{1}H-NMR spectrum. The '*exo*'-methylidene group is revealed by the *δ*(H) 5.02 and 5.25 ppm (br. *s*), the *gem*-dimethyl group by the *δ*(H) 1.05 (br. *s*) and 1.10 (*s*), and the spiro center by *δ*(C) 50.69 ppm.

Table 1. *^{13}C-NMR Data* ($CDCl_3$) *for Rogiolol Acetate* ((+)-**3a**) *and Rogiolol* ((−)-**3b**)[a])

C-Atom	(+)-**3a**	(−)-**3b**	C-Atom	(+)-**3a**	(−)-**3b**
C(1)	44.42 (*s*)	44.12 (*s*)	C(10)	38.22 (*t*)	38.73 (*t*)
C(2)	62.39 (*d*)	70.13 (*d*)	C(11)	25.43 (*t*)	25.53 (*t*)
C(3)	73.40 (*d*)	71.92 (*d*)	C(12)	24.33 (*q*)	24.16 (*q*)
C(4)	37.26 (*t*)	38.55 (*t*)	C(13)	20.03 (*q*)	20.63 (*q*)
C(5)	140.44 (*s*)	141.00 (*s*)	C(14)	118.36 (*t*)	117.81 (*t*)
C(6)	50.69 (*s*)	48.77 (*s*)	C(15)	24.21 (*q*)	24.16 (*q*)
C(7)	38.52 (*t*)	38.47 (*t*)	CH_3CO	24.50 (*q*)	–
C(8)	61.29 (*d*)	60.97 (*d*)	CH_3CO	170.63 (*s*)	–
C(9)	72.31 (*s*)	71.70 (*s*)			

[a]) Spectrum recorded at −30°.

Table 2. *^{1}H-NMR Data for Rogiolol Acetate* ((+)-**3a**) *and Rogiolol* ((−)-**3b**)[a])[b])

H-Atom	(+)-**3a**	(−)-**3b**
H–C(2)	4.37 (*d*, *J*(2,3) = 3.7)	4.46 (*d*, *J*(2,3) = 3.1) [4.66]
H–C(3)	5.21 (*φq*, *J*(3,2) ≈ *J*(3,4ax) ≈ *J*(3,4eq) = 3.7)	4.10 (*m*) [4.65]
H_{ax}–C(4)	2.58 (br. *dd*, *J*(4ax,4eq) = 14.8, *J*(4ax,3) = 4.2)	2.62 (br. *d*, *J* = 14.1)
H_{eq}–C(4)	2.35 (*dd*, *J*(4eq,4ax) = 14.8, *J*(4eq,3) = 3.4)	2.47 (br. *dd*, *J* = 14.1, 2.8)
H_{ax}–C(7)	2.10 (*φt*, *J*(7ax,7eq) ≈ *J*(7ax,8ax) = 14.3)	2.08 (*dd*, *J* = 12.8, 14.3)
H_{eq}–C(7)	2.45 (*ddd*, *J*(7eq,7ax) = 14.3), *J*(7eq,11eq) = *J* ((7eq,8) = 4.6)	2.50 (br. *d*, *J* = 14.3)
H–C(8)	4.71 (*dd*, *J*(8,7ax) = 12.9, *J*(8,7eq) = 4.6)	4.72 (*dd*, *J* = 12.8, 4.5) [4.78]
H_{ax}–C(10)	2.03 (*m*)	2.16 (*m*)
H_{eq}–C(10)	2.18 (*dt*, *J*(10eq, 10ax) = 13.9, *J*(10eq, 11eq) ≈ *J*(10eq, 11ax) = 3.5)	2.18 (*ddd*, *J* = 13.3, 3.4, 3.4) [2.22]
H_{ax}–C(11)	1.74 (*td*, *J*(11ax, 11eq) ≈ *J*(11ax, 10ax) = 13.9, *J*(10eq, 11ax) = 3.5)	1.73 (*td*, *J* = 14.2, 2.3) [1.82]
H_{eq}–C(11)	1.88 (br. *dt*, *J*(11eq,11ax) = 13.9, *J*(11eq, 10ax) ≈ *J*(11eq, 10eq) = 3.5)	1.90 (br. *d*, *J* = 14.2) [2.01]
H_a–C(14)	5.02 (br. *s*)	5.05 (br. *s*) [5.16]
H_b–C(14)	5.25 (br. *s*)	5.38 (br. *s*) [5.53]
3H–C(12)	1.09 (*s*)	1.06 (*s*) [1.27]
3H–C(13)	1.00 (*s*)	1.02 (*s*) [1.32]
3H–C(15)	1.68 (*s*)	1.69 (*s*) [1.74]
Ac	2.08 (*s*)	–
OH	–	2.28 (br. *s*)

[a]) Spectrum recorded at −30°. [b]) Data within square brackets refer to [(−)-**3b**]/[Eu(fod)$_3$] = 0.2. *φt* and *φq*: *pseudo-triplett* and *pseudo-quadruplet*, resp.

The position of the substituents at the β-chamigrene skeleton of both rogiolol acetate ((+)-**3a**) and its derivative rogiolol ((−)-**3b**) can be assigned as follows. The RO group is located at C(3) on the basis of selective $^1H,^1H$ decouplings and the examination of the δ(C) values for these two compounds *(Table 1)* in comparison with data in the literature for related compounds [5] [6]. Two of the halogen atoms can be located at the other two CH groups on the basis of their δ values. Thus, H−C(2)−Br can be assigned from the close similarity of δ values of both (+)-**3a** and (−)-**3b** (δ(H−C(2)) 4.37, 4.46, and δ(C(2)) 62.39, 70.13) with obtusol ((+)-**1**) [3a] [5]. The other CH group must be H−C(8)−Br (or H−C(10)−Br) on the basis of a similar analogy of chemical shift values (*Tables 1* and *2*) with caespitol [7]. That the other quaternary center must bear both the Me group, and the Cl-atom is suggested by the analogy of δ values of rogiolol acetate and rogiolol (δ(C) 72.31 and 71.70 *s*'s, δ(H) 1.68 and 1.69 *s*'s) with caespitol [7]. The assignment of all protons of (+)-**3a** and (−)-**3b** was confirmed and completed on the basis of COSY and $^1H,^{13}C$-correlation experiments[2]).

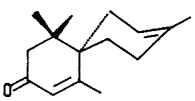
The Relative Configuration. The relative configurations at the five chiral centers of (+)-**3a** can be derived from J(H,H) values and mono- and bidimensional NOE experiments, mainly carried out at low temperature. The RO group is assigned to the axial position, since H−C(3) shows up as a pseudo *q* with J values typical for an equatorial proton coupled to three adjacent protons. The Br-atom at C(2) must occupy the equatorial position in order to account for a marked NOE between H−C(2) and H_{eq}−C(11).

According to the discussion above, the other Br-atom can be located at either C(8) or C(10). Now, on the basis of a marked NOE between H−C−Br and H_a−C(14), position H−C(8)−Br is firmly indicated. Moreover, that, this Br-atom occupies the equatorial position, is suggested by the appearance of H−C(8) as a *dd* with both a *trans*-diaxial and an axial-equatorial coupling. Finally, the axial position for CH_3(15) is based on a marked NOE with H_{ax}−C(11).

The effects of gradual additions of Eu(fod)$_3$ to (−)-**3b** *(Table 2)* indicate that the rare-metal cation becomes bound, as expected, to the OH group; all paramagnetic shifts observed are in accordance with the above structural analysis. In particular, CH_3(12), undergoing a downfield shift comparable to H_{ax}−C(2), can be assigned to the axial position. Moreover, the br. *s* at 5.25 ppm can be assigned to H_b−C(14) on the basis of a larger downfield shift than with H_a−C(14).

Finally, further evidence for the configuration at the spiro center, which is implied in the above data, is based on marked NOE's within the couples CH_3(12)/H_{eq}−C(7), H_{ax}−(4)/H_{eq}−C(11), and H−C(2)/H_{eq}−C(11).

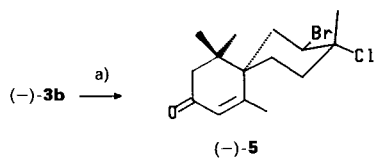
The Absolute Configuration. To solve the problem of the absolute configuration of rogiolol acetate ((+)-**3a**), we first focussed our attention on enone (+)-**4**, or its enantiomer, of known absolute configuration [3]. Our attempts at chemical correlations failed,



(+)-**4**

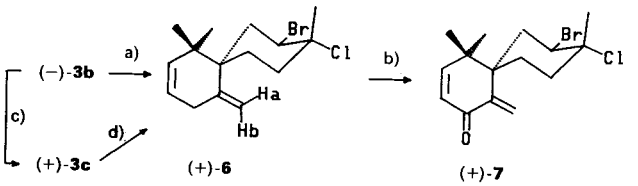
[2]) Some of the patterns of (+)-**3a** are better identified from experiments in $(CD_3)_2CO$ as solvent (*Exper. Part*).

Scheme 1



a) 3% KOH/MeOH, r.t., 24 h; 70% yield.

Scheme 2



a) Zn/Et_2O/AcOH, r.t., 48 h, then reflux (2 h); 73% yield. b) PDC/*t*-BuOOH, *Celite*, benzene, r.t., 24 h; 66% yield. c) Pyridine/MsCl, r.t., 24 h; 60% yield. d) $LiAlH_4$/THF, r.t., 3 h; 75% yield.

however. Thus, the halogenated enone (−)-**5**[3]), obtained from rogiolol ((−)-**3b**) on alkali treatment *(Scheme 1)*, was recovered unchanged from prolonged treatment with Zn/AcOH. Moreover, rogiolol ((−)-**3b**) failed to eliminate the ring-*B* halogen atoms on the treatment with Zn/AcOH, leading to (+)-**6**[4]) instead *(Scheme 2)*. This contrasts with the behavior of both (+)-**1** and (+)-**2** which eliminate all halogen atoms, giving the expected trienes, on the treatment with Zn/AcOH at r.t. [3a].

We then turned our attention to an analog of (+)-**3a** with CH_2(3) in place of H–C(3)–OR, the absolute configuration of which is known [8b]. Our efforts to transform chemically (−)-**3b** into this chamigrene (or its enantiomer) *via* the mesylate (+)-**3c** failed, however. Thus, (+)-**3c** proved inert towards both $NaBH_3CN$ (at r.t.) and $LiAlH_4$ (at +80°) in HMPA, although it reacted with $LiAlH_4$ in excess in THF at r.t., giving the diene (+)-**6** *(Scheme 2)*.

These experiments have shown that rogiolol ((+)-**3a**) and its derivatives (−)-**3b** and (+)-**3c** are peculiar in their compound class, insofar as ring *A* is reactive while ring *B* is inert toward both reducing and basic reagents. Although this has thwarted our aim to establish the absolute configuration of rogiolol acetate *via* chemical correlations intended at transforming ring *B*, we are in the position for inferring the absolute configuration of (−)-**5** from CD data. In fact, the CD spectrum (−)-**5** shows a long-wave, low-amplitude positive *Cotton* effect ($\Delta\varepsilon(350) = 0.0023$) and a short-wave, high-amplitude negative *Cotton* effect ($\Delta\varepsilon(232) \approx 0.024$), in qualitative agreement with the CD spectrum of (+)-**4** and in specular relationship with the CD spectrum of (−)-**4**, the absolute configurations

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[3]) The structural assignment of (−)-**5** rests on the enone UV absorption at λ_{max} 325 and 240 nm and on NMR data for two conformers resulting from flipping of the enone ring (*Exper. Part*).

[4]) The ^{1}H-NMR spectra are fully assigned for both conformers of (+)-**6**, resulting from flipping of the diene ring *(Exper. Part)*.

of which are known [3a]. This suggests that the perturbing groups (Br- and Cl-atoms in our chamigrene) occupy the same spatial position with respect to the enone chromophore as the isolated olefin group in (+)-**4**. On this basis, structural formula (−)-**5**, and, therefore, (+)-**3a**, represent absolute configurations.

Encouraged by these results, we have sought for confirmatory chemical evidence of the absolute configuration of (+)-**3a**, turning our attention to the dienone (+)-**7**, the absolute configuration of which is known from X-ray diffraction data [8a]. We found that (+)-**6** can be oxidized to (+)-**7** with PDC/*t*-BuOOH in benzene on *Celite* [9] *(Scheme 2)*, thus, confirming the assignment (from CD spectra[5]) of the (2*R*,3*S*,6*R*,8*R*,9*R*)-configuration for rogiolol acetate, which is, thus, represented by (+)-**3a**.

The Biogenesis. Rogiolol acetate ((+)-**3a**) represents the first documented case of polyhalogenated chamigrene which, in the biogenetic scheme of *Sakai et al.* [8a], can be viewed to arise *via* a 4-*D*-type pathway. Moreover, dienone (+)-**7**, as a natural product of the opisthobranch mollusc *Aplysisa dactylomela* [8a], can be now viewed to arise *via* the same biogenetic pathway (the authors were unable to indicate the biogenetic pathway for (+)-**7** in the absence of the Br-marker at ring *A* [8a]).

Whichever the biogenetic route, it must be noted that, in this work, a chamigrane-type sesquiterpene is found for the first time in a sponge. Compounds of this class are commonly found in red seaweeds of the genus *Laurencia* [1] [3], and actually our sponge contains 9-bromochamigrene and other compounds of a nearby growing *Laurencia* sp. Although a transfer of these metabolites from the *Laurencia* sp. to the sponge is implied, one wonders how. Marine sponges are well known to accumulate metabolites of microalgae by either filter-feeding or association with the algae themselves [2], while no case was known, before 9-bromochamigrene and the other metabolites alluded to above, of incorporation of seaweed metabolites in a sponge[6]). A route for such a transfer in the present case might be *via* filter-feeding by the sponge on the spores of the *Laurencia* sp., which are extremely abundant during the sporulation season[7]). Formation of (+)-**3a** might well involve a modification of algal metabolites by our sponge.

We thank Prof. *M. Zandomeneghi* for the CD spectra, Dr. *M. Verlaque* for stimulating discussions, Dr. *J. Vacelet* for the sponge identification, Mr. *L. Ziller* for excellent technical aid, and Mr. *A. Sterni* for recording the mass spectra. Financial support by *M.P.I.* (40%) and *C.N.R.*, Roma, is gratefully acknowledged.

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[5]) Compounds (−)-**5**, (+)-**6**, and (+)-**7** appear, by ^{1}H-NMR spectroscopy, as mixtures of two conformers. In the *Exper. Part*, the NMR assignments are reported for these species – formally indicating non-integer proton and C-numbers –, while a study of the dynamics of the conformational changes involved is under way. NMR data for laurencenone D (a metabolite isolated from *Laurencia obtusa* of Jamaica and formulated as the enantiomer of our (−)-**5**, although no chiroptical data were reported [10a]) differ from our NMR data in the *Exper. Part.* Laurencenone D (which, contrary to our (−)-**5**, undergoes elimination of the halogen atoms on the treatment with Zn/AcOH [10a]) must be a diastereoisomer of our (−)-**5**. Similar problems – as concerns NMR data – arise with nidifidiene (a metabolite isolated from *Laurencia nidifica* of Hawaii and formulated as the enantiomer of our (+)-**6**, although no chiroptical data were reported [10b]). The NMR data of nidifidiene [10b] differ from our data for our (+)-**6** in the *Exper. Part.*

[6]) Other cases have been reported of sponge products (for example meroditerpenes) which are similar to products of seaweeds [11].

[7]) We thank Dr. *Marc Verlaque* (who found no trace of *Laurencia* in our sponge) for suggesting us this possibility.

Experimental Part

General. All evaporations were carried out at reduced pressure at r.t. and yields are given on reacted material. TLC: *Merck Kieselgel 60 PF_{254}*. Flash chromatography (FC): *Merck Kieselgel Si60*, 15–25 μm. HPLC: *Merck LiChrosorb Si60* (7 μm). Reverse-phase HPLC: *Merck-LiChrosorb RP-18* (7 μm; 25 × 1 cm column). M.p.: *Kofler* hot-stage microscope. UV spectra: *Perkin-Elmer Lambda-3* spectrophotometer ($\lambda_{max.}$ in nm, ε in $dm^3\,mol^{-1}\cdot cm^{-1}$). CD: *JASCO J-40AS* dichrograph. Polarimetric data: *JASCO-DIP-181* digital polarimeter. ^1H- and ^{13}C-NMR spectra: *Varian XL300* (299.94 or 75.43 MHz, resp.) (80-MHz ^1H-NMR spectra on a *Varian CFT20* modified for proton); δ(ppm) rel. to internal Me_4Si (= 0 ppm) and J in Hz; probe temp. 21°, unless otherwise indicated. Carbon multiplicities: DEPT [12]. Both δ and J values in ^1H-NMR spectra were drawn from differential double irradiations; with compounds (+)-**3a**, (+)-**3c**, (−)-**5**, and (−)-**7**; these assignments were confirmed by COSY [13] experiments. With rogiolol acetate ((+)-**3a**), ^{13}C-NMR-^1H-NMR shift-correlation experiments [14] were also carried out. Differential NOE were obtained with 5-s preirradiation at *ca.* −35°. The probe temp. was calibrated on the chemical-shift difference between the residual OH and CD_2H protons of 99.8% CD_3OD [15]. EI-MS: home-built quadrupole mass spectrometer based on the *ELFS-4-162-8 Extranuclear* quadrupole [16].

1. *Collection and Isolations.* The sponge was collected in May 1989 by SCUBA diving at small depth in front of the torrent Il Rogiolo near Quercianella, south of Livorno, and immediately plunged into 95% EtOH. The sponge was identified by Dr. *J. Vacelet* as *Spongia zimocca* SCHMIDT, 1862 (Dictyoceratida, Spongiidae). No algal symbionts are known for this sponge. In July 1989, the sponge was homogenized and extracted several times with fresh EtOH. Filtration, evaporation of EtOH, and extraction with hexane and then AcOEt led to 4.6 and 0.32 g of organic residue, resp.; the dry sponge residue was 90 g.

The hexane extract was subjected to FC with hexane/Et_2O gradient elution, completing the elution with AcOEt and MeOH. Overall, 38 fractions of 100 ml each were collected. The residue from evaporation of *Fractions 20–25* (1.10 g) was subjected to FC with hexane/CH_2Cl_2 gradient elution, collecting 23 fractions of 50 ml each. The residue from evaporation of *Fractions 11* and *12* (0.12 g) was subjected to HPLC with hexane/$(i\text{-}PrO)_2O$ 83:17 (λ 225 nm) to get impure (+)-**3a** (t_R 19 min; 0.030 g) which was further purified by reverse-phase HPLC with MeCN/H_2O 4:1 (λ 225) (t_R 11 min; 0.022 g, 0.03% of dry sponge weight).

2. *Rogiolol Acetate* (= *(+)-(2*R,*3*S,*6*R,*8*R,*9*R)*-2,8-Dibromo-9-chloro-1,1,9-trimethyl-5-methylidenespiro[5.5]undec-3-yl Acetate;* (+)-**3a**). Colorless prisms. M.p. (hexane) 129–130°. $[\alpha]^{20}$ (λ[nm]) = +24.1 (589), +51.1 (435), +85.4 (365; c = 0.70, $CHCl_3$). UV ($CHCl_3$): 240 (530). ^1H-NMR ($(CD_3)_2CO$): 5.33 (br. *s*, Hb−C(14)); 5.23 (*m*, H−C(3)); 5.07 (*s*, H_a−C(14)); 4.74 (br. *d*, J = 12.8, H−C(8)); 4.73 (*d*, J = 3.5, H−C(2)); 2.84 (br. *d*, J = 14.7, H_{ax}−C(4)); 2.52 (br. *d*, J = 14.0, H_{eq}−C(7)); 2.36 (br. *d*, J = 14.7, H_{eq}−C(4)); 2.24 (*dt*, J = 14.0, 4.0, H_{eq}−C(10)); 2.20 (*dd*, J = 14.0, 12.8, H_{ax}−C(7)); 2.04 (*s*, Ac); 1.96 (*m*, H_{ax}−C(10)); 1.84 (*td*, J = 14.0, 3.2, H_{ax}−C(11)); 1.72 (*s*, 3H−C(15)); 1.16 (*s*, 3H−C(13)); 1.08 (br. *s*, 3H−C(12)). ^{13}C-NMR ($(CD_3)_2CO$): 169.77 (*s*, CH_3CO); 142.62 (*d*, C(5)); 117.66 (*t*, C(14)); 74.22 (*d*, C(3)); 72.99 (*s*, C(9)); 64.05 (*d*, C(2)); 62.19 (*d*, C(8)); 51.82 (*s*, C(6)); 44.93 (*s*, C(1)); 39.60 (*t*, C(10)); 39.16 (*t*, C(7)); 37.70 (*t*, C(4)); 25.97 (*t*, C(11)); 24.48 (*q*, C(15)); 24.35 (*q*, C(13)); 20.85 (*q*, CH_3CO); 20.34 (*q*, C(12)). MS: 318/317/315 (22, 100, 75, $[M - Br - AcOH]^+$), 281 (8), 279 (8), 201 (24), 200 (15), 199 (39), 173 (6), 159 (11), 157 (22), 145 (19), 143 (15).

3. *Treatment of (+)-***3a** *with Mild Base.* Compound (+)-**3a** (22 mg) was stirred at r.t. with 36 mg of K_2CO_3 in 2.6 ml of MeOH, until complete disappearance of (+)-**3a** (24 h). The mixture was then acidified to pH ≈ 5 with dil. HCl, evaporated, and extracted with Et_2O. The residue from evaporation of Et_2O was subjected to reverse-phase HPLC with MeCN/H_2O 3:2 to obtain (−)-**5** (t_R 9.5 min, 1 mg, 6%) and *(−)-(2*R,*3*S,*6*R,*8*R,*9*R)*-2,8-dibromo-9-chloro-1,1,9-trimethyl-5-methylidenespiro[5.5]undecan-3-ol* ((−)-**3b**; t_R 12.2 min; 18 mg, 90%). Colorless oil. $[\alpha]^{20}$(λ [nm]) = −7.7 (589), −9.1 (546), −14.6 (435), −23.1 (365; c = 0.86, $CHCl_3$). MS: 416/414/412 (4,6,2, M^+), 336/334/332 (15,66,45, $[M - HBr]^+$), 299/297 (91,91, $[M - HBr - Cl]^+$), 281 (48), 278 (18), 276 (15), 133 (100), 119 (65), 105 (82), 91 (95).

4. *Treatment of* (−)-**3b** *with Strong Base.* Compound (−)-**3b** (5 mg) was stirred at r.t. in 3% KOH/MeOH (1.5 ml) at r.t. for 24 h. Workup as described in *4* led to unreacted (−)-**3b** (1 mg) and *(+)-(6*S,*8*R,*9*R*)-8-bromo-9-chloro-1,5,5,9-tetramethylspiro[5.5]undec-1-en-3-one* ((−)-**5**; 2.3 mg, 70%). Colorless oil. $[\alpha]_{20}$ (λ[nm]) = −52.4 (589), −62.9 (546), −102.3 (435), −65.9 (365; c = 0.27, $CHCl_3$). UV ($CHCl_3$): 240 (9800), 325 (50). ^1H-NMR (−20°): 5.87 (*q*, J = 1.4, H−C(4)); 4.87 (*dd*, J = 13.0, 5.5, 0.65 H, H−C(8)); 4.53 (*dd*, J = 13.2, 4.3, 0.35 H, H−C(8)); 2.57 (*dd*, J = 13.8, 13.2, 0.35 H, H_{ax}−C(7)); 2.52 (*m*, H_{psdax}−C(2)); 2.02 (*m*, H_{psdeq}−C(2)); 2.32 (*dd*, J = 14.6, 5.5, 0.65 H, H_{eq}−C(7)); 2.25 (br. *s*, 1.95 H, 3H−C(14)); 2.24 (*dd*, J = 13.8, 4.3, 0.35 H, H_{eq}−C(7)); 2.22 (br. *s*, 1.05 H, 3H−C(14)); 2.20–1.70 (series of *m*, 2H−C(10), 2H−C(11)); 1.72 (*s*, 3H−C(15)); 1.13 (br. *s*,

3H–C(13)); 0.96 (br. *s*, 1.95 H, 3H–C(12)); 0.94 (br. *s*, 1.05 H, 3H–C(12)). ^{13}C-NMR: 127.93 (*d*, C(4)); 62.06 (*d*, 0.65 C, C(8)); 60.89 (*d*, 0.35 C, C(8)); 48.77 (*t*, C(2)); 39.88 (*t*, C(10)); 37.42 (*d*, C(7)); 31.73 (*q*, C(15)); 26.65 (*t*, C(11)). The remaining C could either not be observed or assigned. MS: 336/334/332 (1,5,4, $M^{+\cdot}$), 298/296 (24,24, $[M^+ - HCl]^+$), 280 (12), 278 (57), 276 (43, $[M-(CH_3)_2=CH_2]^+$), 199 (34), 197 (100, [278 – HBr]), 161 (47, [278 – HCl – Br]), 151 (23).

5. *Treatment of* (–)-**3b** *with Zn/AcOH.* Rogiolol ((–)-**3b**) (7.4 mg) was stirred with 37.8 mg of freshly activated Zn powder [17] in 0.5 ml of Et_2O and 30 µl of AcOH at r.t. for 48 h. This mixture was then heated at reflux for 2 h. The resulting mixture was neutralized with sat. $NaHCO_3$, filtered, evaporated, and the residue was subjected to FC (hexane/Et_2O) to obtain unreacted (–)-**3b** (2.9 mg) and *(+)-(6R,8R,9R)-8-bromo-9-chloro-1,1,9-trimethyl-5-methylidenespiro[5.5]undec-2-ene* ((+)-**6**; 2.5 mg, 73%). Colorless oil. $[\alpha]^{20}(\lambda[nm])$ = +4.0 (589), +8.3 (546), +15.7 (435), +20.5 (365; *c* = 0.22, $CHCl_3$). ^{1}H-NMR (–20°): 5.46 (*dt*, *J* = 9.8, H–C(3)); 5.23 (*dt*, *J* = 9.8, 2.3, H–C(2)); 5.23 (br. *s*, H_b–C(14)); 4.82 (br. *s*, H_a–C(14)); 4.78 (*dd*, *J* = 12.5, 4.9, 0.5 H, H–C(8)); 4.27 (*dd*, *J* = 13.2, 3.6, 0.5 H, H–C(8)); 2.68 (*m*, 2H–C(4)); 2.42 (*dd*, *J* = 13.5, 3.1, 0.5 H, H_{eq}–C(7)); 2.60–2.30 (*m*, 2H–C(10)); 2.18 (*m*, 0.5 H, H_{eq}–C(11)); 1.98 (*ddd*, *J* = 13.5, 12.5, 3.2, H_{ax}–C(7)); 1.80 (*m*, 0.5 H, H_{eq}–C(11)); 1.70 (*s*, 3H–C(9)); 1.64 (*m*, 0.5 H, H_{ax}–(11)); 1.58 (*ddd*, *J* = 14.3, 13.0, 3.3, 0.5 H, H_{ax}–C(11)); 0.96 (very br. *s*, 3H–C(13)); 0.82 (very br. *s*, 3H–C(12)). ^{13}C-NMR: 137.21 (*d*, C(2)); 122.71 (*d*, C(3)); 112.74 (*t*, C(14)); 72.45 (*d*, C(9)); 61.95 (br. *d*, C(8)); 40.55 (*t*); 39.61 (*t*); 37.34 (*t*); 33.94 (*t*); 24.06 (br. *q*, C(12)); 22.96 (br. *q*, C(13)); 22.60 (*q*, C(15)). MS: 320/318/316 (2,14,11, $M^{+\cdot}$), 277/275/273 (10,38,24, $[M - C_3H_7]^+$), 239 (6), 237 (18, $[M - Br]^+$), 213 (1), 211 (4), 209 (3), 201 (60), 185 (35), 107 (100), 93 (79), 91 (84).

6. *Rogiolol Methanesulfonate (+)-***3c**. A mixture of (–)-**3b** (4.2 mg) and 6 mol-equiv. of MsCl in 2 ml of dry pyridine was stirred for 24 h at r.t. The mixture was then quenched with H_2O and extracted with Et_2O. The Et_2O extract was freed of pyridine by shaking with aq. sat. $CuSO_4$, evaporated, and the residue was subjected to reverse-phase HPLC with MeCN/H_2O 3:2 to obtain 3.2 mg (60%) of *(+)-(2R,3S,6R,8R,9R)-2,8-dibromo-9-chloro-1,1,9-trimethyl-5-methylidenespiro[5.5]undec-3-yl methanesulfonate* ((+)-**3c**). $[\alpha]^{20}(\lambda[nm])$ = +25.7 (589), +31.0 (546), +51.9 (435), +75.7 (365; *c* = 0.26, $CHCl_3$). ^{1}H-NMR: 5.46 (br. *s*, H_b–C(14)); 5.13 (br. *s*, H_a–C(14)); 4.98 (*q*, *J* = 3.3, H_b–C(3)); 4.69 (br. *d*, *J* = 13.1, H–C(8)); 4.39 (*d*, *J* = 3.3, H–C(12)); 3.10 (*s*, CH_3SO_2); 2.69 (*AB*, J_{AB} = 15.7, 2H–C(4)); 2.49 (br. *d*, *J* = 14.6, H_{eq}–C(7)); 2.09 (*dd*, *J* = 14.6, 13.1, H_{ax}–C(7)); 2.18 (*dt*, *J* = 13.6, 3.4, H_{eq}–C(10)); 1.98 (*m*, H_{ax}–C(10)); 1.90 (*m*, H_{eq}–C(11)); 1.75 (*td*, *J* = 14.0, 3.2, H_{ax}–C(11)); 1.69 (*s*, 3H–C(9)); 1.10 (*s*, 3H–C(13)); 1.02 (br. *s*, 3H–C(12)). MS: 400/398/396 (0.5,3,2, $[M - CH_3SO_3H]^+$), 319/317/315 (21,100,75, $[M - Br - CH_2SO_3H]^+$), 281 (16), 279 (16, $[M - Br - CH_3SO_3H - HCl]^+$), 201 (37), 200 (25), 199 (52), 185 (22), 173 (14), 171 (17), 159 (24), 157 (45).

7. *Treatment of (+)-***3c** *with LiAlH.* A mixture of (+)-**3c** (3 mg) and 5 mol-equiv. of $LiAlH_4$ in 1 ml of dry THF was stirred at r.t. for 3 h and then quenched with H_2O. Extraction with Et_2O followed by evaporation led to a mixture (+)-**6**/(+)-**3c** 4:1 (^{1}H-NMR).

8. *Treatment of (+)-***6** *with PDC/*t*-BuOOH.* Compound (+)-**6** (2.5 mg) was stirred with pyridinium dichromate (PDC) (11.8 mg) and 70% *t*-BuOOH (5 µl) in 0.5 ml of dry C_6H_6 over *Celite* (10 mg) at r.t. for 24 h. The mixture was then evaporated and the residue was subjected to column chromatography on *Kieselgel Si60* with some *Celite* at the top to adsorb unreacted PDC (eluent Et_2O). The residue from evaporation of this eluate was subjected to semiprep. TLC with hexane/Et_2O 75:25. The band at R_f 0.25 gave *(+)-(6S,8R,9R)-8-bromo-9-chloro-5,5,9-trimethyl-1-methylidenespiro[5.5]undec-3-en-2-one* ((+)-**7**; 1.7 mg, 66%). Colorless oil. $[\alpha]^{20}(\lambda[nm])$ = +17 (589), +25 (546), +54.6 (435), + 41.7 (365; *c* = 0.108, $CHCl_3$). The MS data and the NMR spectrum proved superimposable to the one in [8a].

REFERENCES

[1] D.J. Faulkner, *Tetrahedron* **1977,** *33,* 1421; J.D. Martín, J. Darias, in 'Marine Natural Products', Ed. P.J. Scheuer, Academic Press, New York, 1983, Vol. I, p. 125; K.L. Erickson, in 'Marine Natural Products', Ed. P.J. Scheuer, Academic Press, New York, 1983, Vol. 5, p. 131; D.J. Faulkner, *J. Nat. Prod. Rep.* **1984,** *1,* 551; D.J. Faulkner, *ibid.* **1986,** *3,* 3; D.J. Faulkner, *ibid.* **1987,** *4,* 539; D.J. Faulkner, *ibid.* **1988,** *5,* 613.

[2] F. Pietra, 'A Secret World. Natural Products of Marine Life', Birkhäuser Verlag, Basel, in press.

[3] a) A.G. Gonzáles, J. Darias, A. Diaz, J.D. Fourneron, J.D. Martín, C. Perèz, *Tetrahedron Lett.* **1976,** 3051; b) A.G. Gonzáles, J.D. Martín, V.S. Martín, M. Martinez-Ripoll, J. Fayos, *ibid.* **1979,** 2717; c) A. Perales, M. Martinez-Ripoll, J. Fayos, *Acta Crystallogr., Sect. B* **1979,** *35,* 2771; d) J.D. Martin, P. Caballero, J.J. Fernandez, M. Norte, R. Peréz, M.L. Rodriguez, *Phytochemistry* **1989,** *28,* 3365.

[4] F.J. Schmitz, D.P. Michaud, P.J. Schmidt, *J. Am. Chem. Soc.* **1982,** *104,* 6415.

[5] A. G. González, J. Darias, J. D. Martín, M. Norte, *Tetrahedron Lett.* **1979,** 2719.
[6] Y. Terui, K. Tori, N. Tsuji, *Tetrahedron Lett.* **1976,** *8,* 621.
[7] A. G. González, J. Darias, J. D. Martín, C. Pérez, *Tetrahedron Lett.* **1974,** 1249.
[8] a) R. Sakai, T. Higa, C. W. Jefford, G. Benardinelli, *Helv. Chim. Acta* **1986,** *69,* 91; b) M. Suzuki, M. Segawa, T. Suzuki, E. Kurosawa, *Bull. Chem. Soc. Jpn.* **1983,** *56,* 3824.
[9] N. Chidambaram, S. Chandrasekaran, *J. Org. Chem.* **1987,** *52,* 5048.
[10] a) D. J. Kennedy, I. A. Selby, R. H. Thomson, *Phytochemistry* **1988,** *27,* 1761; b) S. M. Waraszkiewicz, K. L. Erickson, *Tetrahedron Lett.* **1974,** 2003.
[11] J. C. Braekman, D. Daloze, G. Hulot, B. Tursch, J. P. Declercq, G. Germain, M. Van Meerssche, *Bull. Soc. Chim. Belg.* **1978,** *87,* 917; W. H. Gerwick, W. Fenical, *J. Org. Chem.* **1981,** *46,* 22; A. G. González, J. Darias, J. D. Martín, *Tetrahedron Lett.* **1971,** 2729; A. G. González, J. Darias, J. D. Martín, C. Pascual, *Tetrahedron* **1973,** *29,* 1605.
[12] D. M. Doddrell, D. T. Pegg, M. R. Bendall, *J. Magn. Reson.* **1982,** *48,* 323; D. T. Pegg, D. M. Doddrell, M. R. Bendall, *J. Chem. Phys.* **1982,** *1977,* 2745.
[13] V. Piantini, O. W. Sorensen, R. R. Ernst, *J. Am. Chem. Soc.* **1982,** *104,* 6800; A. Bax. R. Freeman, *J. Magn. Reson.* **1981,** *44,* 542.
[14] H. Kessler, C. Griesinger, J. Zarbock, H. R. Loosli, *J. Magn. Reson.* **1984,** *57,* 331.
[15] E. W. Hansen, *Anal. Chem.* **1985,** *57,* 2993.
[16] A. Slomp, G. Chiasera, C. Mezzena, F. Pietra, *Rev. Sci. Instrum.* **1986,** *57,* 2786.
[17] L. F. Fieser, M. Fieser, 'Reagents for Organic Synthesis', J. Wiley, New York, 1967, Vol. I, p. 1276.