Frs 20, 21 were dissolved in $CHCl_3$ and refrigeration (−20°) resulted in crystallization of 2.5 g of cnicin (**1a**) [10] whose ^1H NMR spectrum compared with that of an authentic sample.

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TERPENES FROM THE RED ALGA *SPHAEROCOCCUS CORONOPIFOLIUS* OF THE NORTH ADRIATIC SEA*

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Key Word Index—*Sphaerococcus coronopifolius*; Rhodophyta; sesquiterpene; diterpenes.

Abstract—From the red alga *Sphaerococcus coronopifolius* collected in the north Adriatic Sea, four terpenes were isolated, viz. the sesquiterpene alloaromadendrene, the diterpenes bromosphaerol, sphaerococcenol *A* and the new compound sphaeroxetane. The structure of the new diterpenoid was proposed on the basis of its spectral data, including 2D-NMR spectroscopy, in comparison with known related compounds. All ^{13}C chemical shifts of known compounds are assigned.

INTRODUCTION

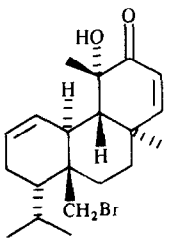
Continuing our studies on the seaweeds of the north Adriatic Sea, we have investigated the red alga *Sphaerococcus coronopifolius* collected near Plomin, Yugoslavia. From the same alga, collected in Spain, Fenical *et al.* [2] described the isolation of sphaerococcenol A (**1**), while Fattorusso *et al.* [3] reported the isolation of bromosphaerol (**2**) as the main diterpenoid together with a series of minor diterpenoids [4–6] isolated from the same alga collected in Sicily (Tyrrhenian Sea). We wish to describe herein the isolation from the alga, of a new bromoditerpenoid, named sphaeroxetane (**3**), of two previously reported related diterpenoids, sphaerococcenol A [2] and bromosphaerol [3], and of the sesquiterpene alloaromadendrene [7] (**4**).

*Part 3 in the series 'Chemical studies of north Adriatic seaweeds'. For part 2 see ref. [1].

RESULTS AND DISCUSSION

The chloroform-soluble material from chloroform–methanol extracts of dried *S. Coronopifolius* was chromatographed on silica gel, using increasing concentrations of diethyl ether in petrol as the eluent. Three bromoditerpenes and one sesquiterpene were isolated. In order of decreasing polarity, these were bromosphaerol (**2**) (0.04% dry wt), sphaerococcenol A (**1**) (0.01%), sphaeroxetane (**3**) (0.008%) and alloaromadendrene (**4**) (0.01%). The spectra of **1** and **2** were in excellent agreement with published data [2,3]. Using 2D–NMR spectroscopy, proton–proton correlation (COSY) and 2D-heteronuclear correlation we were able to assign all the ^{13}C chemical shifts, not completely assigned in the literature, for the two known diterpenoids (see Table 1).

The spectral data, including 2D-NMR spectroscopy, of **4** were in agreement with the gross structure of an aromadendrene skeleton. The stereochemistry was deduced by NOESY with the aid of a Dreiding model. The



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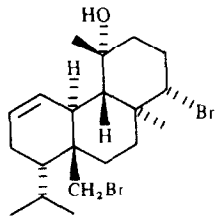

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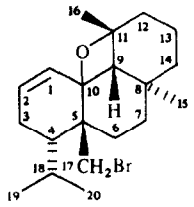

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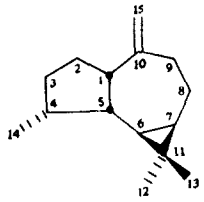

4

absence of NOEs between the exomethylene group and the proton on C-2, and between the methyl doublet (H-14) and the proton H-6, exclude the *trans* ring-junction in the hydroazulene skeleton, while the presence of NOE between the H-5 and the methyl singlet at δ 0.95 (H-13) confirms the *cis* junction. Furthermore, the absence of NOE between the methyl doublet (H-14) and the H-1 establishes also the stereochemistry at C-4, as depicted in the foumula. The value for the optical rotation $[\alpha]_D$-12, excludes the possibility of a 4-epialloaromadendrene structure [7] and thus is additional proof for the proposed structure.

Sphaeroxetane **(3)** obtained as an unstable oil had the molecular formula $C_{20}H_{31}OBr$ (mass spectral and NMR data). Its ^1H NMR and ^{13}C NMR spectra (Table 1) were strongly reminescent of those **1** and **2**. The ^1H NMR spectrum shows four methyl signals doublets at δ 0.89 and 0.94 (3H each, $J=6.9$ Hz, H-19 and H-20) and singlets at δ 1.21 and 1.23 (H-15 and H-16). The two methyls doublets are part of an isopropyl group. In fact, both are coupled with a methine at δ 1.96, that is coupled with an another methine at δ 1.78 (from COSY). The loss of 43 mu (C_3H_7) in the mass spectrum, also confirms the presence of an isopropyl group in the molecule; the fragments *m/z* 287 and 273, attributed to $[M-Br]^+$ and $[M-CH_2Br]^+$, respectively, are characteristic for a CH_2Br group linked to a quaternary carbon atom.

Moreover, the ^1H NMR spectrum showed a multiplet at δ2.74, a singlet at 2.41, a series of signals between 2.10 and 1.20, an AB system at 3.68 (2H, J=10. 3 Hz, H-17), and two vinyl protons, a broad doublet at 5.26 ($J=10.0$ Hz, H-1) and a double multiplet at 5.67 ($J=10.0$ Hz, H-2). The presence in the ^{13}C NMR spectrum of two signals, at 131.03 (*d*) and 126.95 (*d*), confirms the presence of one disubstituted double bond. The occurence of signals at 89.42 (*s*) and 81.40 (*s*) in the ^{13}C NMR spect-

Table 1. ^{13}C NMR chemical shifts for compounds 1–3 and ^1H NMR chemical shifts for compound **3***

	Carbon-13			Proton
C	1	2	3	3
1	128.31	128.65	131.03 *d*	5.26 *br d* (10.0)
2	127.67	126.88	126.95 *d*	5.67 *dm* (10.0)
3	22.38	21.91	22.92 *t*	2.05–1.98
4	42.16	42.64	45.25 *d*	1.78
5	40.31	40.76	47.10 *s*	—
6	33.21	36.52	34.35 *t*	1.80–1.58
7	24.61	25.04	23.38 *t*	1.80–1.55
8	36.90	41.89	32.00 *s*	—
9	45.75	50.67	47.08 *d*	2.41 *s*
10	35.61	37.41	81.40 *s*	—
11	75.36	72.75	89.42 *s*	—
12	203.34	46.18	41.47 *t*	1.86–1.72
13	124.51	30.16	29.71 *t*	1.26
14	161.94	68.80	34.97 *t*	2.74–1.55
15	21.07	14.07	18.25 *q*	1.21 *s*
16	31.38	35.02	27.61 *q*	1.23 *s*
17	39.82	40.36	44.41 *t*	3.68 ABq (10.3)
18	25.95	25.88	26.21 *d*	1.96
19	26.00†	20.01†	25.85 *q*	0.94 *d* (6.9)
20	19.45†	19.78†	18.75 *q*	0.89 *d* (6.9)

*The ^{13}C chemical shifts were assigned by 2D- heteronuclear correlation. Only the multiplicities of 3 are reported. Coupling constants (Hz) are in parentheses.

†Assignments may be reversed.

rum, and considering the molecular formula, it is possible to deduce the presence of an ether oxygen in the molecule; the low frequencies of these signals exclude the presence in the molecule of an epoxy group. Since the molecular formula of **3** requires five degrees of unsaturation, **3** must therefore be tetracyclic. Considering the NMR spectra of **1** and **2**, we could assume for **3** the same carbon skeleton. Bearing in mind this consideration and the spectral data quoted so far, we could conclude that the oxygen is located in an oxytane ring. The COSY experiment allowed us to assign all the chemical shifts in the ^{1}H NMR spectrum (Table 1) and provided additional proof for the proposed structure **3**.

From 2D-NOE spectroscopy (NOESY) of **3** it (Table 2) was possible to establish the unique assignment of chemical shifts of two methyls at δ1.23 and δ1.21 from the presence of NOE between the methyl at δ1.23 and the vinyl proton at δ5.26. The presence of NOE between the methine at δ2.41 and the methylene at δ3.68, shows the same stereochemistry of these groups and provides additional evidence for the presence of the oxytane ring, because only if the methine is present in position 9 it is possible to observe this NOE. Other NOEs reported in Table 2 are also in accordance with the proposed structure.

The toxicity of compounds **1–4** were tested in the *Artemia salina* shirimp bioassay [10] which is used as an in house assay substituting for 9KB and 9PS cytotoxicities. Only compound **2** showed significant activity (LC_{50} 17.9 μg/ml).

The isolation of sphaerococcenol A, bromosphaerol and the new sphaeroxetane as the main diterpenes in *S. coronopifolius* collected in the Northern Adriatic Sea, is in contrast to the same alga collected in Spain and Sicily from which the main diterpenoids were **1** and **2** respectively. This could be evidence [1, 11] that the same algal species living in different habitats can produce different secondary metabolites. It is possible, however, that such differences could be related to the growth stage of the alga.

Table 2. Magnetization exchange by cross relation (NOE) for compound **3** in $CDCl_3$, as obtained from NOESY*

Cross peak co-ordinates below the diagonal (δ_x–δ_y)	Protons correlated
5.26–1.23	H–1; Me–11
3.68–2.41	H–17; H–9
2.41–1.86	H–9; Hβ–12
2.41–1.55	H–9; Hβ–14 and or Hβ–7
2.74–1.26	Hα–14; H–13

*^{1}H NOESY spectra of **3** in $CDCl_3$ was recorded at 500 MHz, with the mixing time t_m = 1 sec ± 180 msec (randomly modulated [8,9].Only the cross-peaks not sensitive to strong filtering are reported.

EXPERIMENTAL

MS were recorded at 70 eV. TLC was carried out using precoated silica gel F_{254} plates (Merck). Kieselgel 60 (Merck) was used for other chromatography. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, in $CDCl_3$ with TMS as int. std: 2D-NMR were obtained using a Bruker microprogram. COSY-45 spectra were obtained by co-addition of 16 scans at each of 256 t_1 values. A 512 × 1024 data matrix had been Fourier transformed with sine-bell filters in both domains, using DISN86 software. The digital resolition was 8 Hz/point in both domains. The 2D-hetero nuclear correlations were obtained by co-addition of 128 or 240 scans at each of 256 t_1 values, with J_{CH}=140 Hz for polarization transfer, to obtain direct C-H correlation. A 512 × 1024 data matrix had been Fourier transformed with Lorentz-Gauss (LBI = −3.0, GB1 = 0.15, LB2 = −9.0, GB2 = 0.3) filters in both domains. The digital resolution was 5.8 Hz/point and 19.5 Hz/point in F1 and F2 domain, respectively.

Extraction and isolation of compounds. S. coronopifolius (Good. et Woodw.) C. Ag. collected in December 1985 and 1986 at Plomin (YU), was dried at 60°. A voucher specimen has been deposited in the herbarium of the Center for Marine Research, 'Rudjer Bošković' Institute, Rovinj. The dried alga (410 g after extn) was extd with $CHCl_3$–MeOH (1:1). The extract was evapd *in vacuo* to obtain a brown gum (4.5 g), that was dissolved in $CHCl_3$. The $CHCl_3$ sol part (1.1 g), after evapn of solvent was chromatographed on a column of silica gel, using a solvent gradient system from petrol (40–70°) to Et_2O as eluent. Fractions exhibiting similar TLC profiles were combined and rechromatographed to obtain **4,3,1** and **2** in order of increasing polarity.

Sphaerococcenol A. **1**, 43 mg, mp 181–183°; $[\alpha]_D$-78 ($CHCl_3$; *c* 1.0); UV λ_{max}^{MeOH} nm (log ε): 228 (3.87); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3400, 2980, 2940 and 1680; EIMS *m/z* (rel. int.). 382 $[M+2]^+$ (6), 380 $[M]^+$ (6), 339 (14), 337 $[M-C_3H_7]^+$ (14), 301 $[M-Br]^+$ (100), 287 $[M-CH_2Br]^+$ (30), 283 (10) and 269 (14); ^{1}H NMR: δ0.92 (3H, *d*, *J*=6.8 Hz, H-19 or H-20), 0.97 (3H, *d*, *J*=6.8 Hz, H-19 or H-20), 1.09 (3H, *s*, H-15), 1.33 (3H, *s*, H-16), 1.46 (1H, *m*, H-6), 1.59 (1H, *m*, H-7), 1.68 (1H, *m*, H-6), 1.76 (1H, *m*, H-7), 1.78 (1H, *m*, H-4), 1.90–2.0 (3H, *m*, H-3, H-11, H-18), 2.16 (1H, *m*, H-3), 2.90 (1H, *br d*, *J*=13.0 Hz, H-10), 3.72 (1H, *d*, *J*=10.7 Hz, H-17), 3.89 (1H, *d*, *J*=10.7 Hz, H-17), 5.75 (1H, *dm*, *J*=10.3 Hz, H-2), 6.05 (1H, *br d*, *J*=10.3 Hz, H-1), 6.07 (1H, *d*, *J*=9.8 Hz, H-13), 6.83 (1H, *d*, *J*=9.8 Hz, H-14); ^{13}C NMR see Table 1.

Bromosphaerol **2**, 170 mg; $[\alpha]_D$ 0.4 ($CHCl_3$; *c* 1.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3400; EIMS *m/z* (rel. int.): 450 $[M+4]^+$ (2), 448 $[M+2]^+$ (4), 446 $[M]^+$ (2), 433 (6), 431 (12), 429 (6), 369 (8), 367 $[M-Br]^+$ (8), 355 (100), 353 $[M-CH_2Br+H]^+$ (100), 337 (98), 335 (98), 273 $[M-CH_2Br-HBr]^+$ (53), 255 (78); ^{1}H NMR: δ0.90 (3H, *d*, *J*=6.8 Hz, H-19 or H-20), 0.96 (3H, *d*, *J* = 6.8 Hz, H-19 or H-20), 1.28 (1H, *m*, H-6), 1.30 (3H, *s*, H-15), 1.38 (3H, *s*, H-16), 1.46–1.54 (2H, *m*, H-7 and H-9), 1.65–1.88 (5H, *m*, H-4, H-6, H-7 and H-12), 1.92–2.0 (3H, *m*, H-3, H-13 and H-18), 2.13 (1H, *m*, H-3), 2.41 (1H, *ddd*, *J*=13.3, 12.4 and 4.1 Hz, H-13), 2.97 (1H, *br d*, *J*=10.2 Hz, H-10), 3.61 (1H, *d*, *J*=10.5 Hz, H-17), 3.93 (1H, *d*, *J*=10.5 Hz, H-17), 4.0 (1H, *dd*, *J*=12.4 and 3.4 Hz, H-14), 5.69 (1H, *dm*, *J*=10.5 Hz, H-2) and 6.01 (1H, *br d*, *J*=10.5 Hz, H-1); ^{13}C NMR see Table 1.

Sphaeroxetane. **3**, 33 mg, $[\alpha]_D$-9 ($CHCl_3$; *c* 0.57); IR ν_{max}^{film} cm^{-1}: 2960, 2930, 1460, 1380, 1240, 1170 and 1090; EIMS *m/z* (rel. int.): 368 $[M+2]^+$ (4), 366 $[M]^+$ (4), 353 (3), 351 $[M-Me]^+$ (4), 325 (3), 323 $[M-C_3H_7]^+$ (3), 310 (18), 308 $[M-Me-C_3H_7]^+$ (17), 287 $[M-Br]^+$ (31), 273 $[M-CH_2Br]^+$ (51), 204 (80), 189 (100), 175 (61), 173 (59) and 161 (96); ^{1}H and ^{13}C NMR see Table 1.

Alloaromadendrene. **4**, 40 mg, $[\alpha]_D -12$ ($CHCl_3$; *c* 1.65); IR ν_{max}^{film} cm^{-1}: 3080, 1675, 1460, 1375 and 890; EIMS *m/z* (rel. int.): 204 $[M]^+$ (64), 189 (41), 161 (100), 147 (71), 133 (68), 119 (93), 107 (86) and 105 (100); ^1H NMR: δ0.25 (1H, *dd*, *J*=11.0 and 9.6 Hz, H-6), 0.56 (1H, *m*, H-7), 0.94 (3H, *d*, *J*=7.2 Hz, H-14), 0.96 (3H, *s*, H-13), 1.00 (3H, *s*, H-12), 1.26 (1H, *m*, H-8), 1.32 (1H, *m*, H-3), 1.73 (2H, *m*, H-2), 1.80–1.90 (3H, *m*, H-3; H-5 and H-8), 2.07 (1H, *m*, H-4), 2.32 (2H, *m*, H-9), 2.67 (1H, *m*, H-1), 4.71 (1H, *br s*, H-15) and 4.73 (1H, *br s*, H-15); ^{13}C NMR: δ 152.5 (*s*, C-10), 109.6 (*t*, C-15), 51.0 (*d*, C-1), 42.3 (*d*, C-5), 37.8 (*d*, C-4), 35.8 (*t*, C-9), 31.3 (*t*, C-3), 28.7 (*q*, C-12), 28.3 (*t*, C-2), 25.0 (*d*, C-7), 23.7 (*d*, C-6), 22.2 (*t*, C-8), 17.3 (*s*, C-11), 16.4 (*q*, C-14) and 15.8 (*q*, C-13).

Bioassay. In the *Artemia salina* shrimp assay technique [10] samples of **1**–**4** (5 mg) were dissolved in DMSO (500 μl). Appropiate amounts of solns were transferred into vials, to obtain three vials for each concn (5, 10, 20 and 50 μl/ml) and artificial sea water was added to make 5 ml. Ten shrimps were then added to each vial. Survivors were counted after 24 hr exposure and the percent deaths at each dose and control were determined. LC_{50} were determined using the probit analysis method described in ref. [12].

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THE ABSOLUTE CONFIGURATION OF GRINDELIC ACID

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Key Word Index—*Grindelia*; Compositae; grindelic acid; 6-oxogrindelic acid; 7α,8α-epoxygrindelic acid; grindelane diterpenoids.

Abstract—Contrary to recent assignments, the labdane absolute configuration of grindelic acid, 6-oxogrindelic acid and 7α-8α-epoxygrindelic acid is confirmed through application of the CD exciton chirality method.

We have been interested in the structure of the grindelane diterpenoids isolated from the resin of *Grindelia robusta*, namely grindelic acid **1a**, 6-oxogrindelic acid **2a** and 7α,8α-epoxygrindelic acid **3a** [1–3] for some years. Their stereochemistry was defined by chemical correlation to sclareol **4** [4, 5] and was confirmed by total synthesis from sclareol itself [6]. More recently, the diterpenoid content of several *Grindelia* species has been investigated and many functional derivatives of grindelic acid have been isolated [7–16]. In one of the most recent reports [14] the isolation of grindelic acid from the aerial parts of *G. perennis*, *G. aphanactis*, *G. boliviana* and *G. chiloensis* is described and the *ent*-labdane structure **5a** is proposed for the compound. This was deduced on the grounds of