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DOLASTANE DITERPENES FROM THE MARINE BROWN ALGA *DICTYOTA CERVICORNIS*

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Key Word Index—*Dictyota cervicornis*; Dictyotaceae; brown alga; diterpene; dolastanes.

Abstract—One known and two new dolastane diterpenes have been isolated from *Dictyota cervicornis*. The structures and absolute configurations of the new metabolites were established from spectral data and chemical correlation to known compounds.

INTRODUCTION

As a part of our continuing interest in the diterpenes from marine brown algae [1–5] we have described the isolation of seven dolastanes [3] and four secodolastanes [2, 3] from *Dictyota cervicornis*. Further examination of the hexane extract from this alga has allowed us to isolate three additional minor dolastane diterpenes, two of which (**1** and **2**) are new metabolites whereas the third one (**3**) has been obtained previously from *Dictyota divaricata* [6].

RESULTS AND DISCUSSION

The methanol-soluble material from the hexane extract of *Dictyota cervicornis*, collected at Baia da Ribeira (Angra dos Reis, State of Rio de Janeiro, Brazil), was chromatographed on silica gel. A fraction slightly less polar than the secodolastane diterpenes reported earlier [2, 3] contained at least six compounds as shown by TLC. Repetitive silica gel column chromatography yielded, in order of polarity, an unidentified hydroxylated fatty acid (0.0005 % dry wt), diterpenes **2** (0.009%), **3** (0.002%), **1** (0.004%) and the already reported **4** (0.006%) [3].

Compound **1** is a laevorotatory ($[\alpha]_D^{CHCl_3} = -80.6°$) colourless gum for which the empirical formula $C_{22}H_{34}O_4$ was established by high resolution mass spectrometry (observed: *m/z* 362.2465, required 362.2457). The IR spectrum showed the presence of hydroxyl (3425 cm^{-1}), ester (1730 and 1225 cm^{-1}) and exomethylene groups (1636 and 901 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) indicated a tricyclic skeleton bearing one terminal vinyl group, one trisubstituted olefin, two tertiary methyl groups, one isopropyl moiety, two tertiary hydroxyls and one secondary acetoxyl function. In the light of our previous results on the same plant [2, 3], these data, together with biogenetic considerations [1, 5], pointed to a dolastane diterpene. Comparison of the ^1H and ^{13}C NMR spectra of **1** with the literature data [6, 7] (Tables 1 and 2) showed that **1** was the acetyl derivative of the known diterpene **5** that had been isolated from an unidentified *Dictyota* species and for which the absolute configuration had been determined [7]. Thus, gentle alkaline hydrolysis of **1** furnished, in quantitative yield, a more polar compound identical with **5** by [α], mp, IR, ^1H NMR and mass spectra. This established the structure and the absolute stereochemistry of **1** as [4*R*,9*R*,14*S*]-4α-acetoxy-9α,14α-dihydroxydolast-1(15),7-diene.

Compound **2** (mp 196–199°; $[\alpha]_D^{CHCl_3} = -80.7°$) showed the molecular formula $C_{20}H_{34}O_4$ (*m/z* 320.2363 $[M-18]^+$ required 320.2351) compatible with the ^{13}C NMR spectral data (Table 2). The IR spectrum indicated only the existence of a terminal vinyl group (1635

Table 1. ^1H NMR (250 MHz, $CDCl_3$) spectral data of compounds **1**, **2**, **5** and **6**

	Multiplicity	**1**	**5**	**2**	**6**
H-2α	*ddd*†	2.72	2.90	2.88	2.91
H-4β	*bs*	4.85	3.49	3.46	3.45
H-6α	*dd*‡	3.06	3.35	n.o.	3.48
H-6β	*dd*§	1.62	n.o	n.o.	1.77
H-7	*dd*‖	5.61	5.69	n.o.	5.45
H-10	*bs*	n.o.	n.o.	n.o.	5.57
H-15	*s*	4.80	4.77	4.88	4.78
H′-15	*s*	4.91	4.92	4.91	4.91
H-17	*qq*¶**	1.85	1.83	n.o.	2.41
Me-16	*s*	0.92	0.82	0.97	0.81
Me-18	*d*¶	0.87*	0.88*	1.00*	1.07*
Me-19	*d***	1.03*	1.04*	1.05*	1.09*
Me-20	*s*	1.45	1.46	1.23	1.34
OR-4	—	2.15 *s*	3.79 *d*	n.o.	3.82 *d*

*Signals may be reversed in any vertical column.

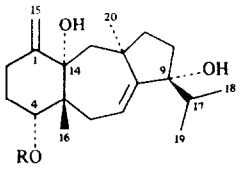
†**1**: $J = 13.7$ & 5.4; **2**: $J = 13.7$ & 6.2; **5**: $J = 13.6$ & 5.2; **6**: $J = 13.4$ & 5.7 Hz.

‡**1**: $J = 15.2$ & 4.0; **5**: $J = 15.5$ & 4.2; **6**: $J = 15.0$ & 5.0 Hz.

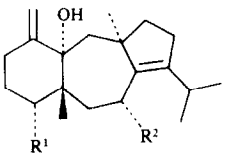
§**1**: $J = 15.2$ & 9.5; **5**: $J = 15.5$ & 9.5; **6**: $J = 15.0$ & 9.5 Hz.

‖**1**: $J = 9.5$ & 4.0; **5**: $J = 9.5$ & 4.2; **6**: $J = 9.5$ & 5.0 Hz.

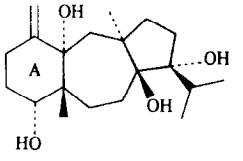
¶**1**: $J = 6.7/6.9$; **2**: $J = 6.9/7.0$; **5**: $J = 6.7/6.9$; **6**: $J = 6.7/6.7$ Hz.



1 R = H
5 R = Ac



3 R^1 = OH; R^2 = OAc
4 R^1 = OAc; R^2 = OH



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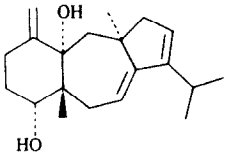

6

Table 2. ^{13}C NMR (62.5 MHz, $CDCl_3$) data of compounds **1**, **2** and **5**

C	Multiplicity	1	5[7]	2
1	*s*	151.38	152.4	152.03
2	*t*	28.20	31.2	30.76
3	*t*	26.76	27.7	26.62
4	*d*	81.93	80.0	80.84
5	*s*	43.17	43.1	44.12
6	*t*	31.00	31.9	25.31*
7	*d*	119.19	120.8	22.26*†
8	*s*	158.97	157.6	75.93†
9	*s*	85.07	85.0	73.51†
10	*t*	32.63	32.5	29.33*
11	*t*	42.19*	42.1	43.58
12	*s*	45.76	45.5	44.96
13	*t*	41.95*	43.4	37.89
14	*s*	79.75	81.6	80.98
15	*t*	109.46	109.5	109.63
16	*q*	17.14	17.2	17.97
17	*d*	37.61	34.8	29.02
18	*q*	19.03†	19.0′	18.73
19	*q*	20.20†	20.2′	18.73
20	*q*	26.97	26.6	21.97
C OMe	*s*	169.39	—	—
COMe	*q*	21.29	—	—

*†: Signals may be reversed in any vertical column.

Signals were attributed by comparison with model compounds [6–9] and by consideration of predictible substituent effects.

and 894 cm^{-1}) and hydroxyl functions (3300 cm^{-1}). The ^1H NMR spectrum (Table 1) contained signals attributable to an isopropyl group, to two tertiary methyl groups and to an exomethylene moiety. The ^{13}C NMR spectrum established the tricyclic nature of **2** and showed the presence of one secondary and three tertiary hydroxyl functions. All these spectral data strongly suggested the dolastane skeleton of **2**. In addition, the fairly good agreement observed for the signals of the six-membered ring carbon atoms in **2** and **5** (Table 2) supported their identical A-ring. Hence, the two remaining tertiary hydroxyl groups are necessarily located at carbons C-8 and C-9 of the dolastane skeleton. The 8β-hydroxy stereochemistry was deduced from the signal in ^1H NMR spectrum of Me-16 at δ0.97, deshielded by 0.25 ppm from the mean position (δ0.72) observed for that methyl group in dolastanes having a 4α,14α-dihydroxy substitution pattern [6, 9]. Indeed, examination of Dreiding models showed that the 8β-hydroxyl is spatially close (almost parallel) to Me-16, and exerts a diaxial interaction on it. Consequently, the isopropyl at C-9 should be β-oriented to account for the strong γ-gauche effect of the 8β-hydroxyl group on C-17 (compare **2** and **5**, Table 2). The *trans*-glycol system of **2** was further confirmed chemically since **2** was found to be inert to sodium periodate treatment. Finally, the dolastane skeleton and the absolute configuration of **2** were established by acid catalysed (HCl–MeOH) partial dehydration [3] of **2** affording, in high yield, conjugated diene **6** identical by $[\alpha]_D$, mp, IR, UV, ^1H NMR and mass spectra to natural **6**, of known absolute stereochemistry [7]. Compound **2** is thus [4*R*,-8*S*,9*R*,14*S*]-4α,8β,9α,14α-tetrahydroxydolast-1(15)-ene. Diterpene **2** is an unusual compound since it is the first dolastane having an oxygen function at C-8. Moreover, it is the possible missing link on the way from the dolastane to the secodolastane diterpenes which are particularly abundant in *Dictyota cervicornis* [2, 3].

EXPERIMENTAL

Low and high resolution mass spectrometry: 70 eV. ^1H NMR and ^{13}C NMR spectra were obtained at 250 and 62.5 MHz, respectively. The other equipment used for this study has been described previously [3].

Isolation of compounds **1**–**4**. *Dictyota cervicornis* Kützing was collected by snorkling at a depth of 6–10 m at Baia da Ribeira, Angra dos Reis, State of Rio de Janeiro, Brazil. The plant material (1.1 kg), free from epiphytic organisms, was washed with seawater, air-dried and extracted with *n*-hexane. Evapn of the solvent under red. pres. furnished a brownish residue (39 g) of which 27 g were partitionned between *n*-hexane and 5% aq. MeOH. The methanolic layer was evapd to dryness and the residue (13 g) fractionated by filtering on a small silica gel column. The fraction eluted with hexane–EtOAc (9:1) (7 g) was purified by repetitive silica gel CC using gradients of EtOAc in hexane. This yielded a series of dolastanes reported earlier [3] and a complex fraction (*ca* 1 g) containing at least six compounds (by TLC) slightly less polar than the secodolastanes we reported previously [2, 3]. Purification of the latter mixture by silica gel column chromatography eluted alternatively with a gradient of EtOAc in CH_2Cl_2 (from 0:1 to 1:4) or of EtOAc in hexane (from 1:19 to 3:7) yielded an unidentified hydroxylated fatty acid, and four pure diterpenes eluted in the following sequence: **2** (105 mg; 0.009% dry wt), **3** (29 mg; 0.002%), **1** (51 mg; 0.004%) and the already reported **4** (67 mg; 0.006%) [3]. Dolastane **3**, an oil, was identified with a metabolite isolated from *Dictyota divaricata* [6] by $[\alpha]_D$, IR, MS and ^1H NMR.

Compound **1**. A gum; $[\alpha]_D = -80.6°$, $c = 5.06$ in $CHCl_3$; UV λ_{max}^{MeOH} nm (ε): transparent above 210 nm; IR ν_{max}^{film} cm^{-1}: 3425, 2940, 1730, 1636, 1445, 1366, 1225, 1160, 1037, 1015, 978, 953, 918, 901 and 850; MS *m/z* (rel. int.): 362.2465 $[M]^+$ (2, $C_{22}H_{34}O_4$ required 362.2457), 344 $[M-H_2O]^+$ (4.5), 319.1910 $[M-i\text{-}Pr]^+$ (48, $C_{19}H_{27}O_4$ required 319.1909), 301.1801 $[319-H_2O]^+$ (40, $C_{19}H_{25}O_3$ required 301.1804), 284 $[344-AcOH]^+$, 269 $[284-Me]^+$ (6), 266 $[284-H_2O]^+$ (6), 259

$[319-AcOH]^+$ (34), 251 $[269-H_2O]^+$ (4.5), 241.1598 $[301-AcOH]^+$ (99, $C_{17}H_{21}O$ required 241.1592), 226 $[241-Me]^+$ (5), 223 $[241-H_2O]^+$ (13), 217.1591 $[259-CH_2CO]^+$ (57, $C_{15}H_{21}O$ required 217.1592), 199 $[217-H_2O]^+$ (35) and 43 (100); ^1H NMR: see Table 1; ^{13}C NMR: see Table 2.

Compound **2**. Mp 196–199°; $[\alpha]_D = -80.7°$ ($CHCl_3$; *c* 1.00); UV: no absorption above 210 nm; IR ν_{max}^{film} cm^{-1}: 3300, 2940, 1635, 1453, 1370, 1300, 1230, 1205, 1166, 1126, 1095, 1061, 1024, 975, 954, 905, 894, 862 and 845; MS *m/z* (rel. int.): no molecular ion, 320.2363 $[M-H_2O]^+$ (12, $C_{20}H_{32}O_3$ required 320.2351), 305.2121 $[320-Me]^+$ (10, $C_{19}H_{29}O_3$ required 305.2117), 302.2249 $[320-H_2O]^+$ (54, $C_{20}H_{30}O_2$ required 302.2246), 287 $[302-Me]^+$ (15), 284 $[302-H_2O]^+$ (12), 277.2176 $[305-CO]^+$ ($C_{18}H_{29}O_2$ required 277.2167), 277.1809 $[320-i\text{-}Pr]$ ($C_{17}H_{25}O_3$ required 277.1804, 269 $[287-H_2O]^+$ (9), 259 (37) i.e 259.2063 ($C_{18}H_{27}O$ required 259.2062, $277-H_2O$) and 259.1704 $[277-H_2O]^+$ ($C_{17}H_{23}O_2$ required 259.1698, 241 $[259-H_2O]^+$ or $[284-iPr]^+$ (16), 235 (13), 231 (13), 222 (15), 217 (18), 216 (20), 213 (16), 209 (42), 203 (60), 201 (64), 175 (47), 133 (83), 71 (88), 55 (88), 53 (50), 43 (100) and 41 (94): ^1H NMR: see Table 1; ^{13}C NMR see Table 2.

Alkaline hydrolysis of **1**. Dolastane **1** (11 mg) was treated with a saturated methanolic solution of K_2CO_3 (2 ml) at room temp. After 4 hr, the reaction mixture was diluted with H_2O (10 ml), extracted exhaustively with CH_2Cl_2 (4 × 8 ml), the organic layer dried over $MgSO_4$, and evapd to dryness under red. pres. to furnish pure **5** (10 mg): mp 157–159° (lit. 160–161° [7]); $[\alpha]_D = -105°$ (lit. −59° [7]); IR, MS and ^1H NMR (see Table 1) identical in all respects to data reported for the natural compound [7].

Dehydration of **2**. Dolastane **2** (14 mg) in MeOH (3 ml) was treated at room temp with 5 drop of conc. HCl. After 2 hr, the reaction medium was diluted with H_2O (20 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The organic layer was dried over $MgSO_4$ and evapd to dryness under red. pres. to furnish crude **6**. Purification by prep. silica gel TLC (eluent: hexane–EtOAc 7:3, 3 elutions) yielded pure **6** (10 mg): mp 140–142° (lit. 145° [6, 7]), $[\alpha]_D^{CHCl_3} = -155°$, $c = 1.00$ (lit. −190° [6]); UV λ_{max}^{MeOH} nm (ε): 243 (7040) lit. 243 (8000) [7]; IR and MS identical to reported data [6, 7]; ^1H NMR: see Table 1.

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