Journal of Natural Products
Vol. 49, No. 4, pp. 570-575, Jul-Aug 1986

DOLASTANE AND SECODOLASTANE DITERPENES FROM THE MARINE BROWN ALGA, *DICTYOTA CERVICORNIS*

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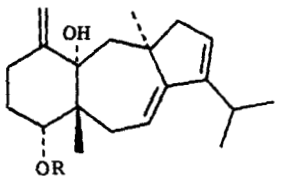
ABSTRACT.—Seven dolastane (**1-7**) and three secodolastane (**8-10**)-diterpenes have been isolated from the marine brown alga *Dictyota cervicornis*. Isolinearol (**9**) and isolinearol acetate (**10**) are new compounds. Their structures have been determined on the basis of spectral and chemical data.

Marine brown algae of the family Dictyotaceae are rich sources of monocyclic, bicyclic, and tricyclic diterpenes (1). Of about ten main genera of the family, the genus *Dictyota* has been the most extensively studied, and more than 90 diterpenes belonging to 16 skeletal classes have been isolated from some 20 species. A number of these compounds have an antibiotic (2,3), antifungal (4), ichthyotoxic (5), cytotoxic (6), and antitumor (6) activities. This prompted us to investigate *Dictyota cervicornis* Kützing (Dictyotaceae), abundant near Rio de Janeiro. From the crude hexane extract, we have isolated seven dolastane (**1** to **7**) and three secodolastane (**8** to **10**) diterpenes.

The seven dolastanes (**1** to **7**) were known compounds previously isolated from *Dictyota linearis* (7,8), *Dictyota divaricata* (9), and an unidentified *Dictyota* species from the Canary Islands (10). Identification of these diterpenes came from their spectrophysical properties ($[\alpha]_D$, ir, uv, ms, ^{1}H nmr and mp when crystalline) identical with literature data (7-10). Among these diterpenes, the conjugated dienes **1** and **2** might be artifacts generated during the extraction/purification steps because we observed very easy dehydration of **3** and **4** to **1** and **2**, respectively.

The secodolastane skeleton had been described only for linearol (**8**), and the relative stereochemistry defined by chemical correlation to isoamijiol (**11**) (11). We isolated, along with linearol (**8**), two new secodolastanes which we have named isolinearol (**9**) and isolinearol acetate (**10**). Here we report their structural assignments.

Isolinearol (**9**) was obtained, by repetitive silica gel column chromatography, as a noncrystalline gum ($[\alpha]^{25}_D$ −52.1). The molecular formula $C_{20}H_{32}O_4$ was established by hrms (observed: 336.2315; required: 336.2300). The ^{13}C-nmr spectrum showed the presence of four CH_3, seven CH_2, one CH, one CH(O), two C, and one C(O), together with one hemiketal, one exomethylene, and one ketonic carbon atom (Table 1). These data strongly suggested that **9** possesses the very rare secodolastane skeleton. The ^{1}H-nmr spectrum (Table 2) was in agreement with this assumption and exhibited four methyl groups, two on quaternary carbons and two that are part of an isopropyl group whose methine hydrogen appeared deshielded at δ 2.63. Characteristic of this spectrum are the exomethylene hydrogens, more shielded than in **8** (Table 2), and the carbinol hydrogen which appeared as a triplet at δ 3.57. All these data showed that **8** and **9** are position isomers of the secondary OH group. The ms of **9** displayed intense fragment ions at *m/z* 275.1654 ($C_{17}H_{23}O_3$), 233.1537 ($C_{15}H_{21}O_2$), and 219.1378 ($C_{14}H_{19}O_2$) corresponding to the loss of the entire side chain by successive losses of isopropyl, ketene, and methylene groups from ion at *m/z* 318.2218 ($C_{20}H_{30}O_3$). This indicated



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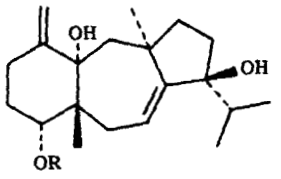

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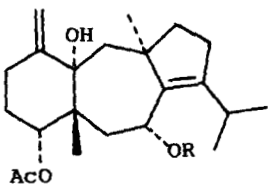

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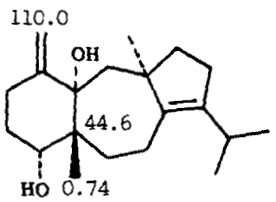

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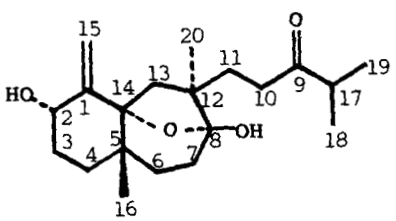

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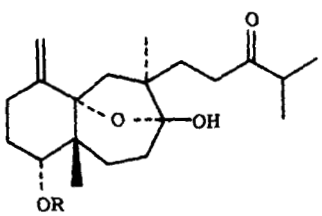

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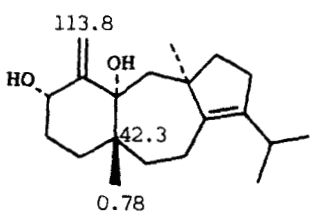
10 R=Ac



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that the secondary hydroxyl group was located in the cyclic part of the molecule. ^{13}C-nmr data of the sidechain carbon atoms, assigned using suitable models from the literature (12,13), corroborated this conclusion (Table 1). From the possible positions for the hydroxy group, two (C-3 and C-13) could be discarded on the basis of the triplet nature of the carbinol hydrogen in ^{1}H-nmr (Table 2), and one (C-7) was incompatible with the carbon chemical shift of C-8 almost identical in **8** and **9** (Table 1). Indeed, comparison of the ^{13}C nmr of **8** and **9** (Table 1), showed major differences only for the six-membered ring carbon atoms, suggesting that the secondary OH group could be attached at C-4. The latter position, currently functionalized in the related dolastane series (**1** to **7**), was further confirmed by ^{1}H nmr (small protection of Me-16) and by the ^{13}C-nmr spectrum of **9** in which C-5 is deshielded by ca 2 ppm (β effect) and C-15 shielded by ca 4 ppm. These effects are identical to those observed in the ^{1}H- and ^{13}C-nmr spectra of isoamijiol (**11**) and amijiol (**7**) (7). Finally, the equatorial (β) orientation of the carbinol hydrogen and, hence, the axial (α) orientation of the hydroxyl group at C-4 were deduced from the coupling constants of H-4 ($J_{3\alpha/4}=J_{3\beta/4}=4.5$ Hz).

Isolinearol acetate (**10**) (gum $[\alpha]^{25}$D −50.0; ms M^{+} −60=*m/z* 318) showed an OH band (3570 cm^{-1}) in the ir spectrum and two carbonyl absorptions, one of an ester

TABLE 1. ^{13}C-nmr Data for Compounds **8** (11), **9**, and **10**

Carbon Atom	Compound		
	8(11)	**9**	**10**
1	147.1 s	146.58 s	146.98 s
2	75.1 d	33.31*t	31.22*t
3	30.1*t[a]	32.20*t	32.26*t
4	34.4*t	78.68 d	78.19 d
5	37.5 s	39.76 s	39.57 s
6	31.4*t	31.88*t	29.31*t
7	28.6*t	29.82*t	29.31*t
8	106.5 s	105.47 s	104.34 s
9	215.9 s	214.48 s	214.49 s
10	27.9 t	27.37 t	28.21 t
11	36.4 t	35.91 t	36.02 t
12	43.9 s	43.44 s	43.70 s
13	41.8 t	41.03 t	41.18 t
14	86.2 s	86.04 s	83.20 s
15	113.5 t	109.82 t	109.32 t
16	21.6 q	22.55 q	22.61+q
17	41.3 d	41.03 d	40.99 d
18	18.46+q[a]	18.33 q	18.33 q
19	18.49+q	18.33 q	18.33 q
20	23.2 q	22.55 q	23.16+q
$OCOCH_3$	—	—	170.88 s
$OCOCH_3$	—	—	21.82 q

[a]*, +: signals may be reversed

function (1735 and 1250 cm^{-1}), the other of a saturated ketone group (1715 cm^{-1}). The fragmentation in ms was very similar to that of **9** and suggested that **10** was structurally related to **9**. The ^{1}H-nmr spectrum of **10** (Table 2) was characterized by a 3H singlet at δ 2.14 and by the 4βH deshielded to δ 4.84 and indicated that **10** is the monoacetate of **9**. This was further confirmed spectroscopically by the ^{13}C-nmr spectrum of **10** (Table 1) and chemically by acetylation, in poor yield, of **9** to a compound identical with **10** in all aspects ([α], uv, ir, ms, ^{1}H nmr, and ^{13}C nmr).

Among the dolastanes reported here, the occurrence of amijiol (**7**) is of biosynthetic relevance since it is the likely precursor of isolinearol (**9**), in the same way as isoamijiol (**11**) has been suggested to be the precursor of linearol (**8**) (11). Finally, the presence of dolastanes and secodolastanes in *D. cervicornis* suggests that this species is closely related to *D. linearis* (7,8,11) and *D. divaricata* (8,9), a conclusion in good agreement with morphological observations (14). Hence, *Dictyota* diterpenes might be useful as chemotaxonomic markers, and this will be the subject of a further publication.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured with a Perkin-Elmer 241 polarimeter at ambient temperature in $CHCl_3$ solutions. Ir spectra were recorded as films with a Perkin-Elmer 137 apparatus. Uv spectra were obtained in MeOH solutions on a Beckman DB-GT grating spectrophotometer coupled to a Beckman 10″ recorder. Low resolution ms (70 eV) were determined on a Micromass MM12F instrument; intensities of the fragments are expressed as percentages of the base peak (100%). Hrms were determined on a Varian CH 5DF instrument. ^{1}H-nmr and ^{13}C-nmr spectra were obtained on a Varian XL100 apparatus at 100 and 25.2 MHz, respectively; spectra were recorded in $CDCl_3$ solutions using TMS as internal standard; shifts are expressed in the δ scale; the following abbreviations are used: s=singlet, b=broad, d=doublet, t=triplet, q=quadruplet, m=multiplet, h=heptuplet, no=not observed; coupling constants are expressed in Hertz (Hz). Analytical tlc was performed on Merck Kieselgel

TABLE 2. ^{1}H-nmr Data for Compounds **8** (11), **9**, and **10**

Proton	Compound		
	8(11)	**9**	**10**
H-2	4.24 ddd[a]	—	—
H-4	—	3.57 t[b]	4.84 t[b]
H-15	4.90 bs	4.82 bs	4.80 bs
H'-15	5.14 bs	4.97 bs	4.94 bs
H-17	2.60 qq[c]	2.63 h[c]	2.62 h[c]
Me-16	0.71 s	0.66 s	0.78 s
Me-18	1.10* d[c,d]	1.12 d[c]	1.11 d[c]
Me-19	1.11* d[c,d]	1.12 d[c]	1.11 d[c]
Me-20	1.03 s	1.02 s	1.02 s
OH-2	4.34 d[e]	—	—
OR-4	—	3.54 m[f,g]	2.14 s
OH-8	2.86 s	3.54 m[f,g]	n.o.

[a]J=10, 3 and 3 Hz
[b]J=4.5 Hz
[c]J=7 Hz
[d]Signals may be reversed
[e]J=10 Hz
[f]Disappear on D_2O addition
[g]W1/2=10 Hz

G plates, spot detection was obtained by spraying with a 2% solution of $Ce(SO_4)_2$ in 2N H_2SO_4 followed by heating 5 min at 150°. Column chromatography was performed on Merck Kieselgel 60 (70-230 mesh). All solvents were distilled prior to use.

PLANT MATERIAL.—*D. cervicornis* was collected by snorkling at a depth of 6-10 m at Baía da Ribeira, Angra dos Reis, State of Rio de Janeiro, Brazil, during February 1984. Intact samples of alga were cleaned from epiphytic organisms and washed with sea water. Identification was made by one of us (VLT). Various specimens were deposited in the Herbarium of Phycology of the Faculdade de Biologia e Psicologia Maria Thereza, Niterói, RJ, Brazil.

EXTRACTION AND ISOLATION OF COMPOUNDS **5**, AND **8**, **9**, AND **10**.—Air-dried material (1.2 kg) was extracted successively with hexane, CH_2Cl_2, EtOAc, and MeOH (3×3 liters each). Evaporation of the hexane crude extract afforded 39 g of residue of which 10.5 g were subjected to rapid filtration on silica gel eluted with hexane, $CHCl_3$, EtOAc, and MeOH. Fractions of 100 ml were collected, and those exhibiting similar profiles by tlc were combined. Fractions eluted with EtOAc (1.4 g) contained contaminated secodolastanes **8-10**. This fraction was submitted to silica gel column chromatography (hexane/EtOAc); the fraction eluted with 50% EtOAc in hexane afforded 185 mg of a mixture of **5**, **9**, and **10**, and the fraction eluted with 60% EtOAc in hexane, 209 mg of crude diterpenes **8** and **9**. Further purification of the latter mixture by silica gel column chromatography (CH_2Cl_2/EtOAc) afforded 80 mg of unseparated **8** and **9**, in relative proportions 2:3. The above cited mixture of **5**, **9**, and **10** (185 mg) consisted of a solid embedded in an oil. The oil was soluble in hexane; the remaining solid material was filtered off and crystallized from hexane/EtOAc. This afforded 6 mg of a colorless solid identified as **5** by mp [149-150°, lit. 150° (10)], ir and ^{1}H nmr (10). Finally, the hexane-soluble oil was rechromatographed on a silica gel column using as eluent increasing concentrations of $CHCl_3$, then of EtOAc in hexane. Compound **10** (isolinearol acetate, 26 mg) was eluted in 75% $CHCl_3$ in hexane, and compound **9** (isolinearol, 65 mg) in pure $CHCl_3$ and 10% EtOAc in $CHCl_3$.

ISOLINEAROL (**9**).—Compound **9** was isolated as 0.05% from dry plant material; gum $[\alpha]^{25}$=−52.1 (589 nm), −54.1 (578), −61.5 (546), −106.2 (436), and −171.0 (365) (c=1.00, $CHCl_3$); uv (MeOH) end absorption; ir ν max (film) 3520, 3020, 1715, 1625, 1470, 1450, 1410, 1380, 1220, 1140, 1090, 1065, 1030, 970, 955, 940, 910, 885 cm^{-1}; ms *m/z* (rel. int.) 336 (M^+, 3), 318 (14), 308 (5), 303 (4), 300 (5), 285 (3), 279 (3), 275 (12), 257 (6), 251 (20), 247 (9), 245 (10), 238 (11), 237 (10), 233 (15), 232 (12), 227 (5), 219 (60), 217 (10), 206 (11), 201 (7), 193 (52), 173 (22), 159 (44), 149 (20), 147 (25), 145 (18), 133 (27), 125 (51), 71 (55), 55 (43), and 43 (100); hrms 336.2315 ($C_{20}H_{32}O_4$, required 336.2300), 318.2218 ($C_{20}H_{30}O_3$, required 318.2193), 316.2041 ($C_{20}H_{28}O_3$, required 316.2038), 275.2013 ($C_{18}H_{27}O_2$, required 275.2011), 275.1654 ($C_{17}H_{23}O_3$, required 275.1647),

251.1644 ($C_{15}H_{23}O_3$, required 251.1647), 233.1537 ($C_{15}H_{21}O_2$, required 233.1542), and 219.1378 ($C_{14}H_{19}O_2$, required 219.1385); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1.

ISOLINEAROL ACETATE (**10**).—Compound **10** was isolated as 0.01% from dry plant material; gum; $[\alpha]^{25}=-50.0$ (589 nm), −52.3 (578), − 59.5 (546), −101.4 (436) and −161.7 (365) (c=1.00, $CHCl_3$); uv (MeOH) end absorption; ir ν max (film) 3570, 3030, 1735, 1715, 1640, 1455, 1430, 1365. 1250, 1175, 1140, 1115, 1085, 1065, 1040, 990, 960, 940, 910 cm^{-1}; ms *m/z* (rel. int.) 318 (M^+ −60, 11), 300 (5), 293 (7), 285 (3), 280 (4), 275 (9), 257 (8), 247 (8), 245 (10), 238 (6), 233 (28), 227 (10), 219 (100), 194 (18), 173 (40), 159 (73), 157 (35), 149 (31), 147 (27), 145 (28), 133 (26), 125 (65), 119 (48), 71 (95); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1.

ACETYLATION OF **9**.—Isolinearol (**9**, 50 mg) in pyridine (1 ml) was treated, at room temperature, with Ac_2O (1 ml) for 48 h. Extraction of the reaction mixture in the usual way afforded crude **10**, which was purified by silica gel column chromatography (eluent: 10% EtOAc in CH_2Cl_2). Pure isolinearol acetate (**10**) was obtained in ~50% yield, and was found identical with natural **10** by $[\alpha]_D$, uv, ir, ms, ^{1}H nmr, and ^{13}C nmr.

ISOLATION OF DOLASTANES **1-4**, **6**, and **7**.—The crude hexane extract (27 g) was partitioned between 10% aqueous MeOH and hexane. The MeOH soluble part (13 g) was filtered through a 100 g silica gel column eluted with hexane (M-1), hexane-EtOAc (85:15) (M-2), hexane-EtOAc (60:40) (M-3), and EtOAc-MeOH (50:50) (M-4). Fraction M-1 (7 g) was purified by careful silica gel column chromatography (eluent: gradient of EtOAc in hexane from 0 to 15%). Fractions 6-9 contained 74 mg of an oil identified as **2** by $[\alpha]_D$, uv ir, ms, and ^{1}H nmr (8-10). Fractions 17-28 contained 180 mg of an oil identified as **6** $[\alpha]_D$, ir, ms, and ^{1}H nmr (9). Fractions 29-32 furnished 15 mg of colorless needles from hexane/EtOAc [mp 178-179°, lit. 180° (10)] identified as **7** by mp, ir, ms, and ^{1}H nmr (7,8,10). Fractions 33-40 were further purified by silica gel column chromatography (eluent: CH_2Cl_2); this furnished 350 mg of an oil identified as **4** by $[\alpha]_D$, ir, ms, and ^{1}H nmr (8,10), and 3 mg of compound **1** [mp 142-143°, lit. 145° (10)] identified by mp, uv, ir, and ^{1}H-nmr (8,10). Finally, fraction M-2 (3 g) was again submitted to filtration on a silica gel column eluted with hexane, $CHCl_3$, EtOAc, and EtOH. The $CHCl_3$ fraction (1.14 g) consisted of a solid embedded in an oil. The oil was soluble in hexane and contained secodolastanes **8-10**; the solid was filtered off and crystallized from MeOH to furnish 28 mg of compound **3** [mp 216-218°, lit. 220° (10)] identified by $[\alpha]_D$, ir, ms, and ^{1}H nmr. Confirmation of the structures of compounds **1** to **6** came by chemical correlations.

HYDROLYSIS OF **4**.—Dolastane **4** (35 mg) was treated with a saturated methanolic solution of K_2CO_3 (5 ml) at room temperature. After 5 h, the reaction medium was diluted with H_2O (10 ml), extracted with EtOAc (3×10 ml), dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure to furnish pure **3** (28 mg) identical with the natural compound.

HYDROLYSIS OF **2**.—Dolastane **2** (18 mg) was treated as above to yield pure **1** (12 mg) identical with the natural compound.

DEHYDRATION OF **3**.—A methanolic solution (5 ml) of **3** (24 mg) was acidified with 1 ml 2 N HCL at room temperature, immediately diluted with H_2O, and extracted with EtOAc (3×10 ml). The organic layer was dried over $MgSO_4$ and evaporated to dryness to furnish pure **1** (22 mg) identical with the natural compound. Alternatively, dehydration of **3** also occurred in $CHCl_3$ solution at room temperature and was completed after about 1 h.

DEHYDRATION OF **4**.—Dolastane **4** (43 mg) was treated as above during 45 min. Compound **2** (39 mg) was obtained pure and was found identical to the natural product. Alternatively, dehydration of **4** was also observed in $CHCl_3$ solution at room temperature and was completed after several days [see also Crews *et al.* (8)].

CONVERSION OF **6** TO **2**.—Dolastane **6** was treated as described by Sun *et al* (9). Extraction of the reaction medium and purification of the crude extract by silica gel column chromatography (eluent: hexane-EtOAc, 90:10) furnished 28 mg of an oil identical to natural **2**.

ACKNOWLEDGMENTS

We are grateful to the Laboratórios Silva Araújo Roussel S.A. (SARSA) for optical rotation measurements, to Mr. C. Moulard (Free University of Brussels, Belgium) for low and high resolution mass spectrometry. This work is supported by FINEP and CNPq grants.

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Received 22 August 1985