

HYDROAZULENOID DITERPENES FROM THE BROWN ALGA *DICTYOTA DICHOTOMA* VAR. *IMPLEXA**

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Key Word Index—*Dictyota dichotoma*; Dictyotaceae; hydroazulene diterpenes; guaiane diterpenes.

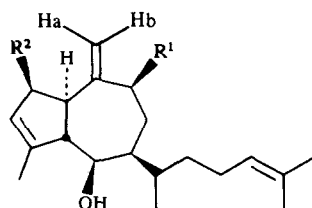
Abstract—Together with the previously described pachydictyol A and dictyol B acetate, a new hydroazulenoide diterpene, dictyol I acetate, was isolated from the brown alga *Dictyota dichotoma* var. *implexa* obtained from the Northern Adriatic sea. The structure of the new diterpenoid was proposed on the basis of its spectral data, in comparison with known related compounds. The use of 2D-NMR spectroscopy allowed us to establish its relative stereochemistry and to correct some ^1H NMR data previously reported. The difference in secondary metabolites between the same alga collected in the Northern Adriatic sea and the Tyrrhenian sea are shown.

INTRODUCTION

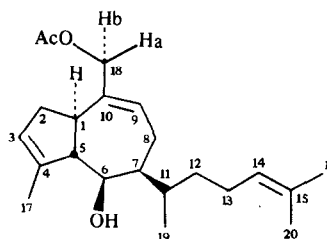
We started to study the algae of the North Adriatic sea because it is characterized by a peculiar ecological habitat in the Mediterranean area, especially characterized by lower salinity and larger temperature variations.

Continuing our studies, we have investigated the brown alga *Dictyota dichotoma* var. *implexa* (Desf.) J. Ag. collected near Rovinj, Yugoslavia. The isolation of dictyols A (4), B (5), C (6) and D (7) from the same alga collected in the Tyrrhenian sea are described elsewhere [2, 3]. We wish to describe herein the isolation from the alga of two previously known compounds, pachydictyol A (1) [4] and dictyol B acetate (2) [5] and a new diterpenoid named dictyol I acetate (3).

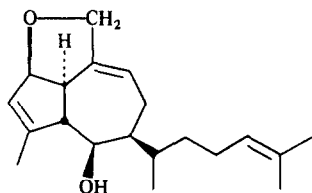
*Part 2 in the series "Chemical Studies of North Adriatic Seaweeds". For part 1 see ref. [1].



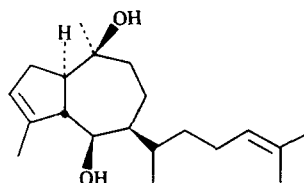
- 1 $\text{R}^1 = \text{R}^2 = \text{H}$
2 $\text{R}^1 = \text{OAc}, \text{R}^2 = \text{H}$
5 $\text{R}^1 = \text{OH}, \text{R}^2 = \text{H}$
7 $\text{R}^1 = \text{H}, \text{R}^2 = \text{OH}$



3



4



6

RESULTS AND DISCUSSION

The chloroform-soluble material from chloroform-methanol extracts of dried *D. dichotoma* var. *implexa* was chromatographed on silica gel, using increasing concentrations of diethyl ether in petrol as the eluent. Three diterpenes were isolated. In order of polarity, these were pachydictyol A (**1**) (0.24% of dry weight), dictyol B acetate (**2**, 0.07%) and dictyol I acetate (**3**, 0.05%). The spectral data of **1** and **2** were in excellent agreement with published values [4, 5].

Dictyol I acetate (**3**), obtained as a colourless unstable oil, had the molecular formula $C_{22}H_{34}O_3$ (MS in connection with NMR data). Its IR spectrum contained hydroxyl (3500 cm^{-1}) and carbonyl (1740 cm^{-1}) absorption bands. A three-proton resonance at $\delta 2.06$ (s) in the ^1H NMR spectrum, together with two signals in the ^{13}C NMR spectrum at 21.1 (q) and 165.3 (s), indicated the presence of an acetate ester. The ^1H NMR and ^{13}C NMR spectra (Table 1) of **3** were strongly reminiscent of those of **1** and **2**. In fact the ^1H NMR spectrum contained a doublet at $\delta 0.98$ ($J = 6.2\text{ Hz}$) assigned to a secondary methyl group, three broad singlets at 1.60, 1.68 and 1.85 assigned to vinyl methyl groups, a signal due to an α -hydroxy proton at 3.95 (dd, $J = 6.85, 1.8\text{ Hz}$) and a geminal AB double doublet at 4.40 and 4.64 ($J = 12.2\text{ Hz}$), respectively, for the non-equivalent acetoxy methylene protons, and three vinyl protons at 5.12 (br t, $J = 6.5\text{ Hz}$), 5.36 (br s) and 5.85 (br d, $J = 8.1\text{ Hz}$). The signals at 142.6 (s), 137.3 (s), 131.8 (d), 131.4 (s), 124.8 (d) and 124.2 (d) in the ^{13}C NMR spectrum

confirmed the presence of three trisubstituted double bonds.

The occurrence of signals at 74.5 (d) and 68.9 (t) in the ^{13}C NMR spectrum afforded additional proof for the proposed secondary alcohol and primary acetate. Moreover, the ^{13}C NMR spectrum showed four signals due to methylene groups at 34.9, 34.4, 25.7 and 24.7 and four methine groups at 58.0, 46.5, 43.7 and 33.9. Since the molecular formula of **3** requires six degrees of unsaturation, we could conclude that **3** must be bicyclic. Considering the NMR spectra of **1** and **2**, it was easy to establish that **3** has the same carbon skeleton. In fact, the absence of the exomethylene group in the NMR spectra of **3** and the presence of an acetoxy methylene, indicated that the acetoxy group was on C-18 as depicted.

The proton-proton correlation (COSY) NMR spectrum [8–11], identifying the pairs of resonances that are coupled together, provided additional confirmation of the proposed structure, and allowed the assignment of all the chemical shifts in the ^1H NMR spectrum.

From two dimensional NOE spectroscopy (NOESY) [12–14] of **1–3** as reported in Table 2, it was possible to assign the same stereochemistry for all the compounds at C-1, C-5, C-6 and C-7 carbon atoms.

Only the relative stereochemistry as depicted is in accord with results of NOESY experiments. Otherwise, an inversion of one of the asymmetric centres could result in a positive NOE between two or more protons under investigation. The stereochemistry at C-9, for **2**, as tentatively assigned by Fattorusso *et al.* [2], was confirmed by the absence of NOE between the α -acetoxy proton and the exomethylene group in the NOESY experiment.

The presence of NOE between H-14 and one of the vinyl methyls ($\delta 1.68$) suggested a *cis*-conformation. Therefore the signal at $\delta 1.68$ in the ^1H NMR spectra of all

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

Carbon	Carbon-13			Proton 3
	1	2	3	
1	46.3	43.3	43.7 d	2.82 m
2	34.0	33.8	34.4 t	2.10–2.36 m
3	124.0	123.9	124.2 d	5.36 br s
4	141.5	141.4	142.6 s	—
5	60.6	61.2	58.0 d	2.58 m
6	75.3	74.7	74.5 d	3.95 dd (6.85, 1.8)
7	47.8	43.9	46.5 d	1.65† m
8	23.6	30.3	24.7 t	2.00–2.36 m
9	40.6	77.2	131.8 d	5.85 br d (8.1)
10	152.6	161.4	137.3 s	—
11	35.0	34.9	33.9 d	1.55† m
12	35.2	35.1	34.9 t	1.20–1.50† m
13	25.7	25.7	25.7 t	1.95–2.05† m
14	124.9	124.6	124.8 d	5.12 br t (6.5)
15	131.4	131.4	131.4 s	—
16	25.8	25.6	25.8 q	1.68† br s
17	15.8	15.5	16.0 q	1.85 br s
18	107.2	104.9	68.9 t	4.40, 4.64 ABq (12.2)
19	17.6	17.4	17.6 q	0.98 d (6.2)
20	17.7	17.6	17.7 q	1.60 br s
COMe	—	177.2	165.3 s	—
COMe	—	21.1	21.1 q	2.06† s

* The ^{13}C chemical shifts were assigned by 2D-heteronuclear correlation [6, 7]. Only the multiplicities of **3** are reported. Coupling constants (Hz) are in parentheses.

† Overlapped with other signals.

Table 2. Magnetization exchange by cross relaxation (NOE) for compounds **1–3** in CDCl_3 , as obtained from NOESY*

Cross peak co-ordinates below the diagonal $\delta_x - \delta_y$	Protons correlated
1	
1.80–5.33	Me-4; H-3
2.50–4.74	H-2 β ; H-18s
2.60–4.74	H-9 β ; H-18s
1.68–5.12	H-16s; H-14
2	
1.79–5.33	Me-4; H-3
2.58–4.92	H-2 β ; H-18a
1.68–5.11	H-16s; H-14
3	
1.85–5.36	Me-4; H-3
5.85–4.40	H-9; H-18a
1.68–5.12	H-16s; H-14

* ^1H NOESY spectra of **1–3** in CDCl_3 were recorded at 500 MHz, with the mixing time $t_m = 1\text{ sec} \pm 150\text{ m sec}$ (randomly modulated) [11, 14]. Only the cross-peaks not sensitive to strong filtering are reported.

compounds is attributable to the C-16 methyl group, contrary to the assignments reported in the literature [3, 4, 15] for this class of compounds. Finally the presence of NOE between the H-9 and H-18a (δ 4.40) in **3** indicated a partially hindered rotation along the C-10–C-18 bond probably due to an intramolecular H-bond between the H2- β and the acetoxy group.

The diterpenes found in *D. dichotoma* var. *implexa* collected in the Northern Adriatic sea are not substantially different from those found in the same alga collected in the Tyrrhenian sea [2, 3].

EXPERIMENTAL

TLC was carried out using pre-coated silica gel F₂₅₄ plates (Merck). Kieselgel 60 (Merck) was used for chromatography. MS were recorded at 70 eV on an AEI MS-30 spectrometer. IR spectra were recorded on a Perkin–Elmer Model 257 Infracord. ¹H NMR and ¹³C NMR spectra were recorded at 500 and 125 MHz, respectively, in CDCl₃ with TMS as int. standard on a Bruker WM 500, under ASPECT 2000 control. The COSY-90 spectra were obtained by co-addition of 16 or 32 scans with appropriate phase cycling program [11] at each of 512 t_1 values. A 1024 × 2048 data matrix had been Fourier transformed with sine-bell filters in both domains, using DISNMR software. The digital resolution was 2.64 Hz/point in both domains. The 2D-heteronuclear correlation were obtained by co-addition of 80 or 160 scans at each of 256 t_1 values, with $J_{CH}^1 = 150$ Hz for polarization transfer. A 512 × 1024 data matrix had been Fourier transformed with Lorentz–Gauss (LB = –4.0, GB = 0.2) filters in both domains.

Extraction and isolation of compounds. The alga *Dictyota dichotoma* var. *implexa* (Desf.) J. Ag., collected in June 1984 at Lone Bay near Rovinj (YU), was dried at 60°. A voucher specimen of this alga has been deposited in the herbarium of the Center for Marine Research, Rudjer Bošković Institute, Rovinj. The dried alga (90 g after extraction) was extracted in a Soxhlet with CHCl₃–MeOH (1:1). The extract was evaporated *in vacuo* to obtain a brown gum (12.5 g) that was dissolved in CHCl₃. The CHCl₃ soluble part (8.0 g) after evaporation of solvent was applied on a column (5 × 100 cm) of silica gel. The column was eluted with a solvent gradient system from petrol (40–70°) to Et₂O. Fractions of 40 ml were collected.

The fractions containing a single compound were combined and rechromatographed to obtain pure compounds. From the fractions 34–37 was obtained pachydictyol A (**1**) (220 mg, 0.24% dry wt). The fractions 57–60 contained dictyol B acetate (**2**,

83 mg, 0.07%). All spectral data of **1** and **2** were in excellent agreement with published values [4, 5]. From the fractions 61–64 was recovered, as colourless unstable oil, dictyol I acetate (**3**, 60 mg, 0.05%) IR $\nu_{\text{max}}^{\text{film}}$ cm^{–1}: 3500, 1740, 1220 and 1050; EIMS m/z (rel. int.): 346 [M]⁺ (4), 328 [M – H₂O]⁺ (4), 286 [M – HOAc]⁺ (5), 271 [M – HOAc – Me]⁺ (5), 149 (47) and 109 (100); ¹H NMR and ¹³C NMR: see Table 1.

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REFERENCES

1. De Rosa, S., De Stefano, S., Macura, S., Trivellone, E. and Zavodnik, N. (1984) *Tetrahedron* **40**, 4991.
2. Fattorusso, E., Magno, S., Mayol, L., Santacroce, C., Sica, D., Amico, V., Oriente, G., Piattelli, M. and Tringali, C. (1976) *J. Chem. Soc. Chem. Commun.* 575.
3. Danise, B., Minale, L., Riccio, R., Amico, V., Oriente, G., Piattelli, M., Tringali, C., Fattorusso, E., Magno, S. and Mayol, L. (1977) *Experientia* **33**, 413.
4. Hirschfeld, D. R., Fenical, W., Lin, G. H. Y., Wing, R. M., Radlick, P. and Sims, J. J. (1973) *J. Am. Chem. Soc.* **95**, 4049.
5. Faulkner, D. J., Ravi, B. N., Finer, J. and Clardy, J. (1977) *Phytochemistry* **16**, 991.
6. Maudsley, A. A., Müller, L. and Ernst, R. R. (1977) *J. Magn. Reson.* **28**, 463.
7. Bax, A. and Morris, G. (1981) *J. Magn. Reson.* **42**, 501.
8. Aue, W. P., Bartholdi, E. and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 2229.
9. Nagayama, K., Wüthrich, K. and Ernst, R. R. (1979) *Biochem. Biophys. Res. Commun.* **90**, 305.
10. Bax, A. and Freeman, R. (1981) *J. Magn. Reson.* **42**, 164.
11. Wider, G., Macura, S., Kumar, A., Ernst, R. R. and Wüthrich, K. (1984) *J. Magn. Reson.* **56**, 207.
12. Jeener, J., Meier, B. H., Bachmann, P. and Ernst, R. R. (1979) *J. Chem. Phys.* **71**, 4546.
13. Macura, S. and Ernst, R. R. (1980) *Mol. Phys.* **41**, 95.
14. Macura, S., Huang, Y., Suter, D. and Ernst, R. R. (1981) *J. Magn. Reson.* **43**, 259.
15. Pathirama, C. and Andersen, R. J. (1984) *Can. J. Chem.* **62**, 1666.