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Diterpenes from the brown algae *Dictyota dichotoma* and *Dictyota linearis*

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Abstract

Nineteen secondary metabolites of the brown alga *Dictyota dichotoma* (Huds.) Lam. and fifteen metabolites of the brown alga *D. linearis* (Ag.) Grev. were isolated and their chemical structures were elucidated on the basis of their NMR and mass spectral data. The diterpenes isopachydictyolal (**1**) from *D. dichotoma* and 4α-acetyldictyodial (**2**) from *D. linearis* are new natural products. The antiviral activity of metabolites isolated in adequate amounts was evaluated in laboratory assays against *Herpes simplex virus* I (HSV I) and *Poliomyelitis Virus I*, using Vero cells as hosts.

Keywords: *Dictyota dichotoma*; *Dictyota linearis*; Isopachydictyolal; 4α-Acetyldictyodial

1. Introduction

The genus *Dictyota* is represented by more than 40 species, thus being the richest genus of the family Dictyotaceae. It is also one of the most abundant seaweeds in tropical marine habitats such as the Atlantic and Indian Oceans, where plenty of *Dictyota* species are found. In the Greek seas and in the Mediterranean in general, only the species *D. dichotoma* and *D. linearis* are found.

Brown algae of the family Dictyotaceae produce a significant number of secondary metabolites, especially diterpenes. Generally, these diterpenes have three types of carbon skeletons: xenicanes; dolabellanes and "extended sesquiterpenes". Many members of the family though, produce cyclic diterpenes, unique in the structural variety of marine natural products.

Biological studies have shown a significant number of *Dictyota* secondary metabolites to possess cytotoxic, anti-bacterial, ichthyotoxic and anti-feedant activities (Amico et al., 1980; Duran et al., 1997; Sun et al., 1983).

In the course of our investigations towards the isolation of bioactive metabolites from marine sources (Iliopoulou et al., 2002a,b,c; Mihopoulos et al., 2001) we investigated the chemical composition of the brown alga *D. dichotoma* collected from the coasts of Saronikos gulf in Athens and *D. linearis* from the south coasts of Chios island.

2. Results and discussion

2.1. Isolation and structure elucidation

Nineteen terpenoid metabolites, including natural product **1**, stated below in order of increasing polarity as resulted from their chromatographic isolation from the crude extract of *Dictyota dichotoma*, have been identified on the basis of their NMR, MS spectral and comparison

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with the literature values: bicyclosesquiphellandrene (De Rosa et al., 1994), germacrene D (De Rosa et al., 1994), dictyoxide (Amico et al., 1979), pachydictyol A (Danise et al., 1977; Hirschfeld et al., 1973), isopachydictyol A (Duran et al., 1997), axenol (De Rosa et al., 1994), acetyldictyolal (Enoki et al., 1982), dictyol B acetate (Faulkner et al., 1977; Vazquez et al., 1988), isopachydictyolal (**1**), 10-acetoxy-18-hydroxy-2,7-dolabelladiene (Ireland and Faulkner, 1977; Sun and Fenical, 1979), dictyol E (Danise et al., 1977), fucosterol (Sheu et al., 1997), acetoxycrenulide (Sun et al., 1983), hydroxyacetyldictyolal (Enoki et al., 1982), isodictyohemiacetal (Enoki et al., 1982), dictyol A (Minale and Riccio, 1976; Fattorusso et al., 1976), dictyol B (Minale and Riccio, 1976; Fattorusso et al., 1976), dictyol C (Danise et al., 1977), and hydroxycrenulide (Sun et al., 1983). The sesquiterpenoids bicyclosesquiphellandrene, germacrene D and axenol, previously isolated from the brown algae *Taonia atomaria* (De Rosa et al., 1994), are reported for the first time as *D. dichotoma* metabolites.

Repetitive fractionations of *Dictyota linearis* crude extract by vacuum column chromatography on silica gel and HPLC purifications yielded the new natural product (**2**), as well as fourteen previously reported diterpenes and vitamin E (Pouchert and Behnke, 1993). The previously described natural products are dictyoxide, isopachydictyol A, pachydictyol A, acetals a and b (Ishitsuka et al., 1982b; Patil et al., 1993), dictyodial (**3**) (Finer et al., 1979; Kirkup and Moore, 1983; Enoki et al., 1982), 18-hydroxy-2,7-dolabelladiene (König et al., 1991; Ireland and Faulkner, 1977; Sun and Fenical, 1979; Ochi et al., 1980a), neodictyolactone (**4**) (Ishitsuka et al., 1984), acetylsanadaol (**5**) (Ishitsuka et al., 1982a), acetyldictyolal, 10-acetoxy-18-hydroxy-2,7-dolabelladiene, dictyol E, 10,18-dihydroxy-2,7-dolabelladiene (Sun and Fenical, 1979; Piatelli et al., 1995) and dictyol C. The above diterpenes are all reported for the first time as constituents of *D. linearis*, while diterpenes **4** and **5** are reported for the first time as constituents of the genus *Dictyota*. The structure determination of **2** is based on spectroscopic methods (NMR and MS), and comparison with the literature data. Up-to-date, only a secodolastane (linearol) (Ochi et al., 1981) and several dolastanes (Ochi et al., 1980a,b, 1986; Crews et al., 1982) have been reported from *D. linearis*.

Isopachydictyolal (**1**) was isolated as colorless oil and the proposed structure is based on spectral analyses. Characteristic features in the NMR of metabolite **1** include the presence of an aldehyde, three trisubstituted double bonds, one aliphatic and three vinyl methyls, as well as an α-hydroxy methine. The aliphatic methyl at δ 0.97 (*d*)/17.5, the two vinyl methyls at δ 1.58 (*s*)/17.7 and 1.67 (*s*)/25.7, the two methylenes at δ 25.3 and 34.6, the methines at δ 33.3 and 124.4, along with the quaternary carbon at δ 132.0 are assigned to the 1,5-dimethyl-4-hexenyl side chain. The presence of the 6-hydroxy-azulene is supported by the α-hydroxy proton at δ 3.98 (*m*) and the ^{1}H–^{1}H COSY and HMBC correlations. Characteristic also in the ^{1}H NMR is the aldehydic proton at δ 9.35 (*s*) attached to a carbonyl resonating at δ 194.6 (*d*) and correlated with the C-1 (δ 40.9) of the pachydictyane carbon backbone. The correlation of the aldehyde carbon with the deshielded olefinic proton at δ 6.78 (*dt*) suggests the presence of a conjugated system containing the aldehyde carbonyl and the $\Delta_{9,10}$ double bond of the pachydictyane carbon backbone. The relative stereochemistry of the chiral centres depicted in **1** was assigned on the basis of the NOESY experimental data that revealed correlations between H-1, H-6 and H-7.

4α-Acetyldictyodial (**2**), obtained as a slightly green oil, was assigned to the molecular formula $C_{22}H_{32}O_4$ (LREIMS, *m/z* 360; FABHRMS, *m/z* 361.2372 $[M+H]^+$). The NMR data of compound **2** were very similar to those of metabolite **3**, and the structure of 4α-acetyldictyodial was proposed by comparison of the ^{1}H and ^{13}C NMR spectral data with those of dictyodial (**3**) and the literature values for 4β-hydroxydictyodial (Kirkup and Moore, 1983). The most significant spectral differences between metabolites **2** and **3** are the resonances of H-2, H-3, H-4, H-5, H-8 and H-20, which for **2** appear at lower fields and the chemical shifts of C-2, C-4, C-5, C-8 and C-20, and the additional resonances for the acetoxy-group (one methyl singlet in the ^{1}H NMR spectrum at δ 2.01 and two resonances in the ^{13}C NMR spectrum at δ 21.2 and 169.7).

Characteristic are the similarities in the ^{1}H NMR spectra of metabolites **2** and **3**, more important being the two aldehydic protons at δ 10.16 (*s*) and 9.27 (*s*), the olefinic doublet at δ 6.96, the doublet at δ 5.33 (showing correlation in the ^{1}H–^{1}H COSY spectrum with a methyl singlet at δ 1.83). HMQC and HMBC correlations, clearly establish the position of the acetoxy-group on C-4. Information derived from ^{1}H–^{1}H COSY and NOESY experiments were used for the stereochemistry determination of compound **2**. As reported in literature (Finer et al., 1979; Kirkup and Moore, 1983) X-ray crystallography of compounds possessing this skeleton has shown that the relative stereochemistry is $2R^*$, $3R^*$, $10S^*$. Spectral data were adequate in the present case for confirmation of the stereochemistry at C-2 and C-3. The NOESY spectrum revealed correlations between H-4 and H-11, H-4 and H-17, while no correlation was observed between H-4 and H-3. There is also spatial correlation between the C-20 protons and H-5β, resonating at δ 2.55, and between H-5β and H-4. Based on the above correlation the relative stereochemistry was proposed as $4R^*$.

The assignments of protons and carbons for metabolites neodictyolactone (**4**) and acetylsanadaol (**5**), iso-

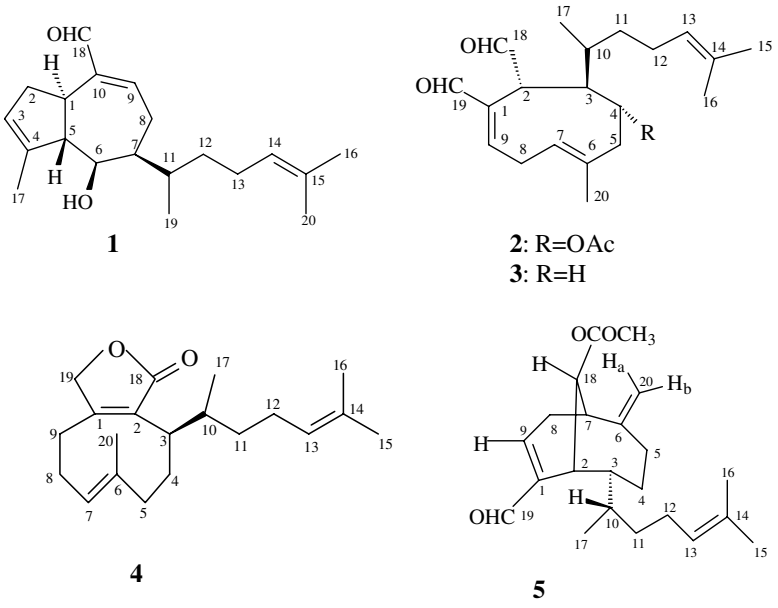

Fig. 1. Metabolites of *D. dichotoma* (**1**) and *D. linearis* (**2**–**5**).

lated from *D. linearis*, were established by 2D NMR and are described in the present investigation, since they have not been reported in earlier studies (see Fig. 1).

Table 1
Results of antiviral screening

Metabolite	HSV I	POLIO I
Pachydictyol A	100–25[a]:T 10–0.1:RF1	10–0.1:RF1
Isopachydictyol A	100–10:T 5: T/4 2.5–0.1:RF1	10–0.1:RF1
Dictyol A	100–0.1: T 0.01:RF1	10–2: T 1:RF1
Dictyol B	100–0.5:T 0.25-0.01:RF1	0.1–0.001:RF1
Dictyol C	100:N 100–10:T 1:T/2, RF1	100–50:N 100–10:T 1:RF1
Dictyol E	100–10:T 1:RF1	100–25:T 10-1:RF1
Dictyol B acetate	100–10:T 1:RF1	100–50:T 25-1:RF1
Isopachydictyolal	100–10:T 1:RF1	100:T/2
Dictyoxide	100–50:N 100–10:T 1:RF1	100:T 50:T/2 50–1:RF1

T = cell monolayer disrupted (complete lysis of cells, cell death); T/2 = cell monolayer affected (complete growth stop); T/4 = cell monolayer intact (cell growth and/or morphology affected); N = precipitation of substance(s) in growth medium; RF = titer reduction factor.

[a] Range of tested concentrations in μg/ml (e.g. 100–25 μg/ml).

2.2. Antiviral activity

The isolated metabolites were tested but they did not exhibit significant antiviral activity against *Poliomyelitis virus I* and *Herpes simplex virus I* in concentrations lower than their maximal non-toxic dose (MNTD) and proved to be toxic for Vero cells in different dilutions. Every concentration was tested in duplicate and the highest assayed concentration was 100 μg/ml. The MNTD for the metabolite **1** was found to be 10 μg/ml (see Table 1).

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded using Bruker AC 200MHz and DRX 400MHz spectrometers. Chemical shifts are given in δ (ppm) scale using TMS as internal standard. High-resolution FAB mass spectral data were recorded on a JEOL AX505HA mass selective detector and were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana. Low-resolution EI mass spectral data were recorded on a HP 5890 mass selective detector. Column chromatography was performed with Kieselgel 60 and 60H (Merck). HPLC was conducted using a Cecil 1100 Series model, equipped with a refractive index detector RI GBC LC 1240 and a Spherisorb HPLC normal phase column, 25 cm × 10 mm, S10W, 64340 plates/meter, and an Agilent 1100 Series model, equipped with a refractive index detector and a Supelco SPLC-Si normal phase column, 5 μm, 25 cm × 10 mm.

TLCs were performed with Kieselgel 60 F_{254} aluminum support plates (Merck). For IR spectrometry a FTIR Perkin–Elmer model Paragon 500 was used. Optical rotation measurements were performed in a Polarimeter 341, Perkin–Elmer ($t = 20$ °C). UV spectra were determined in spectroscopic grade C_6H_{14} on a Shimadzu UV-160A spectrophotometer.

3.2. Plant material

D. dichotoma was collected at Saronicos gulf in the Aegean Sea, Greece, at a depth of 10 m, in December of 1999. A voucher specimen is kept at the Herbarium of the Pharmacognosy Laboratory, University of Athens (ATPH/MO/147). *D. linearis*, collected from shallow habitats (1–5 m depth) in the south shores of Chios island (Vroulidia), Greece, in May of 2000, was kept frozen until analyzed. Specimens are deposited in the herbarium of the Laboratory of Pharmacognosy and Chemistry of Natural Products (ATPH/MO/215).

3.3. Extraction and separation

D. dichotoma was initially freeze dried (219.0 g dry weight) and then exhaustively extracted with mixtures of CH_2Cl_2–MeOH (3/1) at room temperature. The organic extract (18.5 g) after evaporation of the solvents, was subjected to vacuum column chromatography (VCC) on silica gel. A total of 14 fractions were eluted with cyclohexane-EtOAc mixtures of increasing polarity. Combined fractions A1–A2 were further subjected to normal phase HPLC chromatography, to yield pure bicyclosesquiphellandrene (11.5 mg), germacrene D (8.9 mg), dictyoxide (2.1 mg), pachydictyol A (14.9 mg), isopachydictyol A (4.1 mg) and axenol (5.9 mg). Further chromatographic separations (VCC) of the combined fractions A3-A4 over silica gel yielded 11 fractions, eluted with cyclohexane–EtOAc mixtures of increasing polarity. Fractions B3 and B6 were identified as acetyldictyolal (10 mg) and dictyol B acetate (11.3 mg), respectively. Combined fractions B8–B9 were further subjected to normal phase HPLC chromatography, to yield natural product isopachydictyolal (3.5 mg), 10-acetoxy-18-hydroxy-2,7-dolabelladiene (6.8 mg), dictyol E (14.3 mg) and fucosterol (11.9 mg). Combined fractions A3–A4 further purified by VCC over silica gel, yielded 11 fractions, eluted with cyclohexane–EtOAc mixtures of increasing polarity. Combined fractions C8–C9 were further subjected to normal phase HPLC chromatography, to yield pure acetoxycrenulide (1.9 mg), hydroxyacetyldictyolal (3.9 mg) and isodictyohemiacetal (3.6 mg). Combined fractions C10–C11 were further subjected to normal phase HPLC chromatography, to yield pure dictyol A (17.6 mg), dictyol B (8.7 mg), dictyol C (5.2 mg) and hydroxycrenulide (2.1 mg).

D. linearis was initially freeze dried (415.0 g dry weight) and then exhaustively extracted at room temperature with mixtures of CH_2Cl_2–MeOH (3/1). Part of the organic extract (10 g) after evaporation of the solvents, was subjected to VCC on silica gel using cyclohexane–EtOAc mixtures of increasing polarity, and finally MeOH, to yield 15 fractions. Fraction 2 was further chromatographed on a silica gel column using cyclohexane–EtOAc mixtures of increasing polarity, to yield 8 fractions. Fraction 2–2 was once more subjected to VCC on silica gel and yielded 8 fractions, eluted with cyclohexane-EtOAc mixtures of increasing polarity. Fraction 2–2–2 was further purified by normal phase HPLC column chromatography, to yield dictyoxide (1.3 mg), isopachydictyol A (2.4 mg), pachydictyol A (3.0 mg) and vitamin E (1.5 mg). Fraction 2–2–3 was further purified by normal phase HPLC column chromatography, to yield acetal a (7.4 mg). Fraction 2–2–4 was subjected to normal phase HPLC column chromatography, to yield dictyodial (1.3 mg). Fraction 2–5 was subjected to normal phase HPLC to afford acetal b (8.9 mg), 18-hydroxy-2,7-dolabelladiene (16.2 mg) and neodictyolactone (4.3 mg). Fraction 2–6 was further purified by normal phase HPLC column chromatography, to yield acetylsanadaol (3.2 mg) and acetyldictyolal (12.3 mg). Fraction 2–7 was subjected to normal phase HPLC column chromatography, to yield 10-acetoxy-18-hydroxy-2,7-dolabelladiene (3.7 mg) and dictyol E 91.6 mg). Fraction 8 was further chromatographed on a silica gel column and yielded 16 fractions. Combined fractions 8–5 and 8–6 were further purified by normal phase HPLC column chromatography, to yield dictyol C (8.5 mg). Combined fractions 8–7, 8–8 and 8–9 were identified as 10,18-dihydroxy-2,7-dolabelladiene (15.2 mg). Further chromatographic separations (VCC) of fraction 9 over silica gel yielded 13 fractions. Combined fractions 9–2, 9–3 and 9–4 were further purified by normal phase HPLC column chromatography, to yield 4α-acetyldictyodial (3.3 mg).

3.4. Isopachydictyolal (*1*)

Colorless oil; $[\alpha]_D^{20}$: −85.5° (*c* 0.30, CH_2Cl_2); UV (*n*-hexane): λ_{max}nm $\log(\varepsilon) = 206$ (3.01), 228 (3.05); IR (CH_2Cl_2): $\nu_{max} = 3606$, 2927, 1685, 1435, 1288 cm^{-1}; EIMS: m/z (rel. int.) = 302 $[M]^+$(74), 284 (20), 217 (88), 173 (62), 148 (84), 91 (81), 41 (100); HRFABMS $m/z = 303.2343$ $[M+1]^+$, Calcd: for ($C_{20}H_{31}O_2$) 303.2325. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.97 (3H, *d*, $J = 6.2$ Hz, H-19), 1.20 (1H, *m*, H-12a), 1.51 (1H, *m*, H-12b), 1.55 (1H, *m*, H-11), 1.58 (3H, *s*, H-20), 1.59 (1H, *m*, H-7), 1.67 (3H, *s*, H-16), 1.83 (3H, *d*, $J = 1.0$ Hz, H-17), 1.92 (1H, *m*, H-13a), 2.05 (1H, *m*, H-13b), 2.05 (1H, *m*, H-2a), 2.23 (1H, *m*, H-8a), 2.52 (1H, *dd*, $J = 10.0$, 10.0 Hz, H-5), 2.65 (1H, *m*, H-8b), 2.81 (1H, *m*, H-1), 2.95 (1H, *m*, H-2b), 3.98 (1H, *m*, H-6), 5.09 (1H, *t*,

$J = 7.2$ Hz, H-14), 5.39 (1H, *br s*, H-3), 6.78 (1H, *dt*, $J = 7.5$, 2.4 Hz, H-9), 9.35 (1H, *s*, H-18); ^{13}C-NMR (50,3 MHz, $CDCl_3$): $\delta = 16.0$ (C-17), 17.5 (C-19), 17.7 (C-20), 25.3 (C-13), 25.7 (C-16), 26.1 (C-8), 33.3 (C-11), 34.6 (C-12), 35.6 (C-2), 40.9 (C-1), 45.7 (C-7), 57.3 (C-5), 73.7 (C-6), 124.4 (C-14), 125.7 (C-3), 132.0 (C-15), 140.7 (C-4), 147.4 (C-10), 156.5 (C-9), 194.6 (C-18).

3.5. 4α-Acetyldictyodial (**2**)

Colorless oil; $[\alpha]_D^{20}$: −163.6° (*c* 0.30, CH_2Cl_2); IR (CH_2Cl_2): $\nu_{max} = 3068$, 2992, 2927, 1735, 1712, 1684, 1430 cm^{-1}; EIMS: *m/z* (rel. int.) = 360 $[M]^+$, 300, 282, 160, 108, 69, 43 (100); HRFABMS *m/z* = 361.2372 $[M + 1]^+$, Calcd: for ($C_{22}H_{33}O_4$) 361.23016. ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.92$ (3H, *d*, $J = 6.8$ Hz, H-17), 1.12 (1H, *m*, H-11a), 1.25 (1H, *m*, H-11b), 1.54 (3H, *s*, H-15), 1.64 (3H, *s*, H-16), 1.8 (2H, *m*, H-12), 1.83 (3H, *s*, H-20), 2.01 (3H, *s*, H-COMe), 2.09 (1H, *s*, H-3), 2.15 (1H, *dd*, $J = 13.7$, 2,7 Hz, H-5*β*), 2.47 (1H, *m*, H-10), 2.55 (1H, *brd*, $J = 13.7$Hz, H-5α), 3.05 (1H, *m*, H-8a), 3.38 (1H, *ddd*, $J = 13.7$, 13.7, 2.1 Hz, H-8b), 3.54 (1H, *s*, H-2), 5,0 (1H, *m*, H-13), 5.28 (1H, *s*, H-4), 5.33 (1H, *d*, $J = 11.6$ Hz, H-7), 6.96 (1H, *d*, $J = 6.5$ Hz, H-9), 9.27 (1H, *s*, H-19), 10.16 (1H, *brs*, H-18); ^{13}C-NMR (50,3 MHz, $CDCl_3$): $\delta = 17.6$ (*q*, C-17), 17.7 (*q*, C-15), 19.8 (*q*, C-20), 21.2 (*q*, C-MeCO), 25.6 (*q*, C-16), 25.8 (*t*, C-12), 29.4 (*t*, C-8), 32.9 (*d*, C-10), 38.4 (*t*, C-11), 44.9 (*t*, C-5), 48.8 (*d*, C-3), 51.1 (*d*, C-2), 76.2 (*d*, C-4), 124.2 (*d*, C-13), 126.5 (*d*, C-7), 131.7 (*s*, C-14), 135.9 (*s*, C-6), 150.1 (*s*, C-1), 156.7 (*d*, C-9), 169.7 (*s*, C-CO), 194.2 (*d*, C-19), 203.1 (*d*, C-18).

3.6. Neodictyolactone (**4**)

$[\alpha]_D^{20}$: −35° (*c* 0.30, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.75$ (1H, *m*, H-11a), 0.95 (3H, *d*, $J = 6.6$ Hz, H-17), 1.12 (1H, *m*, H-11b), 1.24 (3H, *s*, H-20), 1.55 (3H, *s*, H-16), 1.62 (3H, *s*, H-15), 1.86 (1H, *m*, H-3), 1.9 (2H, *m*, H-12), 1.96 (1H, *m*, H-5a), 1.96 (2H, *m*, H-8), 2.0 (1H, *m*, H-10), 2.2 (2H, *m*, H-4), 2.2 (1H, *m*, H-5b), 2.27 (2H, *m*, H-9), 4.54 (1H, *d*, $J = 17.1$ Hz H-19a), 4.59 (1H, *d*, $J = 17.1$ Hz H-19b), 4.95 (1H, *t*, $J = 7$ Hz, H-13), 5.20 (1H, *dd*, $J = 11.6$, 3.5 Hz, H-7); ^{13}C-NMR (50,3 MHz, $CDCl_3$): $\delta = 15.8$ (C-20), 17.6 (C-16), 18.0 (C-17), 25.0 (C-12)*, 25.7 (C-8)*, 25.8 (C-15), 28.4 (C-9), 32.1 (C-4), 33.0 (C-10), 35.1 (C-11), 40.4 (C-5), 43.0 (C-3), 71.1 (C-19), 123.5 (C-7), 124.7 (C-13), 131.2 (C-14), 133.2 (C-2), 140.1 (C-6), 157.6 (C-1), 174.0 (C-18).

3.7. Acetylsanadaol (**5**)

$[\alpha]_D^{20}$: +12.86° (*c* 0.30, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 0.73$ (3H, *d*, $J = 6.8$ Hz, H-17), 0.96 (2H, *m*, H-11), 1.14 (2H, *m*, H-4), 1.4–1.8 (1H, *m*, H-3), 1.4–1.8 (1H, *m*, H-10), 1.59 (3H, *s*, H-16), 1.67 (3H, *s*, H-15), 1.97 (2H, *m*, H-12), 2.05 (3H, *s*, H-COMe), 2.25 (2H, *m*, H-5), 2.48 (1H, *dd*, $J = 21.2$, 3.4 Hz, H-8a), 2.81 (1H, *m*, H-8b), 3.06 (1H, *m*, H-7), 3.22 (1H, *brd*, $J = 4.8$ Hz, H-2), 4.75 (2H, *d*, $J = 8.2$ Hz, H-20) 4.84 (1H, *t*, $J = 4.8$ Hz, H-18), 5.12 (1H, *t*, H-13), 6.80 (1H, *t*, ($J = 3.8$ Hz, H-9), 9.44 (1H, *s*, H-19); ^{13}C-NMR (50,3 MHz, $CDCl_3$): $\delta = 17.4$ (C-17), 17.6 (C-16), 21.3 (C–Me), 24.5 (C-11), 25.2 (C-12), 25.7 (C-15), 30.4 (C-5), 32.7 (C-8), 35.8 (C-10), 35.9 (C-4), 37.2 (C-2), 39.5 (C-3), 42.3 (C-7), 72.4 (C-18), 115.4 (C-20), 125.1 (C-13), 131.0 (C-14), 143.2 (C-6), 146.9 (C-1), 150.4 (C-9), 169.0 (C–CO), 192.5 (C-19).

3.8. Antiviral activity

The antiviral activity was observed as inhibition of cytopathic effects on a VERO cell monolayer infected with a virus, using the end-point titration method as described by Vanden Berghe and Vlietinck (Vanden Berghe and Vlietinck, 1991). *Herpes simplex virus I* (HSVI) and *Poliomyelitis virus I* were subcultures of ATCC isolates and were used as test viruses. Results are expressed at MNTD as a reduction factor (RF), i.e., the ratio between the virus titer of control and sample dilution. Acyclovir and 3-methylquercetin were used as positive controls for anti-HSV-1 (RF of 10^4 at a MNTD of 0.1 μg/ml) and anti-polio-1 activity (RF of 10^7 at a MNTD of 5 μg/ml), respectively.

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References

Amico, V., Oriente, G., Piattelli, M., Tringali, C., 1979. Dictyoxide, a new diterpene from the brown alga *Dilophus ligulatus*. Phytochemistry 18, 1895–1897.

Amico, V., Oriente, G., Piattelli, M., Tringali, C., 1980. Diterpenes based on the dolabellane skeleton from *Dictyota dichotoma*. Tetrahedron 36, 1409–1414.

Crews, P., Klein, T.E., Hogue, E.R., Myers, B.L., 1982. Tricyclic diterpenes from the brown algae *Dictyota divaricata* and *Dictyota linearis*. Journal of Organic Chemistry 47, 811–815.

Danise, B., Minale, L., Riccio, R., Amico, V., Oriente, G., Piattelli, M., Tringali, C., Fattorussso, E., Magno, S., Mayol, L., 1977. Further perhydroazulene diterpenes from marine organisms. Experientia 33, 413–415.

De Rosa, S., De Giulio, A., Iodice, C., Zavodink, N., 1994. Sesquiterpenes from the brown alga *Taonia atomaria*. Phytochemistry 37, 1327–1330.

Duran, R., Zubia, E., Ortega, M.J., Salva, J., 1997. New diterpenoids from the alga *Dictyota dichotoma*. Tetrahedron 53, 8675–8688.

Enoki, N., Ishida, R., Matsumoto, T., 1982. Structures and conformations of new nine-membered ring diterpenoids from the marine alga *Dictyota dichotoma*. Chemistry Letters, 1749–1752.

Fattorusso, E., Magno, S., Mayol, L., Santacroce, C., Sica, D., Amico, V., Oriente, G., Piattelli, M., Tringali, C., 1976. Dictyol A and B, two novel diterpene alcohols from the brown alga *Dictyota dichotoma*. Journal of the Chemical Society Chemical Communications, 575–576.

Faulkner, D.J., Ravi, B.N., Finer, J., Clardy, J., 1977. Diterpenes from *Dictyota dichotoma*. Phytochemistry 16, 991–993.

Finer, J., Clardy, J., Fenical, W., Minale, L., Riccio, R., Battaile, J., Kirkup, M., Moore, R.E., 1979. Structure of dictyodial and dictyolactone, unusual marine diterpenoids. Journal of Organic Chemistry 44, 2044–2047.

Hirschfeld, D.R., Fenical, W., Lin, G.H.Y., Wing, R.M., Radlick, P., Sims, J.J., 1973. Marine natural products. VIII. Pachydictyol A, an exceptional diterpene alcohol from the brown alga *Pachydictyon coriaceum*. Journal of the American Chemical Society 95, 4049–4050.

Iliopoulou, D., Roussis, V., Pannecouque, C., De Clercq, E., 2002a. Halogenated sesquiterpenes from the red alga *Laurencia obtusa*. Tetrahedron 58, 6749–6755.

Iliopoulou, D., Vagias, C., Galanakis, D., Argyropoulos, D., Roussis, V., 2002b. Brasilane-type sesquiterpenoids from *Laurencia obtusa*. Organic Letters 4, 3263–3266.

Iliopoulou, D., Vagias, C., Harvala, C., Roussis, V., 2002c. C_{15} Acetogenins from the red alga *Laurencia obtusa*. Phytochemistry 59, 111–116.

Ireland, C., Faulkner, D.J., 1977. Diterpenes from *Dolabella californica*. Journal of Organic Chemistry 42, 3157–3162.

Ishitsuka, M., Kusumi, T., Kakisawa, H., 1982a. Acetylsanadaol, a diterpene having a novel skeleton, from the brown alga *Pachydictyon coriaceum*. Tetrahedron Letters 23, 3179–3180.

Ishitsuka, M., Kusumi, T., Tanaka, J., Chihara, M., Kakisawa, H., 1984. New diterpenes from the brown alga *Pachydictyon coriaceum*. Chemistry Letters, 151–154.

Ishitsuka, M., Kusumi, T., Tanaka, J., Kakisawa, H., 1982b. New diterpenoids from *Pachydictyon coriaceum*. Chemistry Letters, 1517–1518.

Kirkup, M.P., Moore, R.E., 1983. Two minor diterpenes related to dictyodial A from the brown alga *Dictyota crenulata*. Phytochemistry 22, 2539–2541.

König, G., Wright, A., Sticher, O., 1991. Diterpenes from the brown alga *Dictyota divaricata*. Phytochemistry 30, 3679–3682.

Mihopoulos, N., Vagias, C., Mikros, E., Scoullos, M., Roussis, V., 2001. Prevezols A and B: new brominated diterpenes from the red alga *Laurencia obtusa*. Tetrahedron Letters 42, 3749–3752.

Minale, L., Riccio, R., 1976. Constituents of the digestive gland of the molluscs of the genus *Aplysia*—I. Novel diterpenes from *Aplysia depilans*. Tetrahedron Letters, 2711–2714.

Ochi, M., Asao, K., Kotsuki, H., Miura, I., Shibata, K., 1986. Amijitrienol and 14-deoxyisomijiol, 2 new diterpenoids from the brown seaweed *Dictyota linearis*. Bulletin of the Chemical Society of Japan 59, 661–662.

Ochi, M., Miura, I., Tokoroyama, T., 1981. Structure of linearol, a novel diterpenoid from the brown seaweed *Dictyota linearis*. Journal of the Chemical Society Chemical Communications, 100.

Ochi, M., Watanabe, M., Miura, I., Taniguchi, M., Tokoroyama, T., 1980a. Amijiol, isoamijiol and 14-deoxyamijiol, new diterpenoids from the brown seaweed *Dictyota linearis*. Chemistry Letters 10, 1229–1232.

Ochi, M., Watanabe, M., Kido, M., Ichikawa, Y., Miura, I., Tokoroyama, T., 1980b. Amijidictyol, a new diterpene from the brown seaweed *Dictyota linearis*. X-ray crystal and molecular structure. Chemistry Letters 10, 1233–1234.

Patil, A.D., Berry, D., Brooks, D.P., Hemling, M.E., Kumar, N.V., Mitchell, M.P., Ohlstein, E.H., Westley, J.W., 1993. A diterpene epoxide from the brown alga *Dictyota* sp.: possible Vasopressin V1 receptor antagonist. Phytochemistry 33, 1061–1064.

Piatelli, M., Tringali, C., Neri, P., Rocco, C., 1995. Stereochemistry and conformation of *Dolabellane diterpenes*: an NMR and molecular mechanics study. Journal of Natural Products 58, 697–704.

Pouchert, C., Behnke, J., 1993. In: The Aldrich Library of ^{13}C and ^{1}H NMR Spectra, vol. II, first ed. Aldrich Chemical Company Inc., USA. p. 297.

Sheu, J.H., Wang, G.H., Sung, P.J., Chiu, Y.H., Duh, C.Y., 1997. Cytotoxic sterols from the Formosan brown alga *Turbinaria ornata*. Planta Medica 63, 571–572.

Sun, H.H., McEnroe, F.J., Fenical, W., 1983. Acetoxycrenulide, a new bicyclic cyclopropane-containing diterpenoid from the brown seaweed *Dictyota crenulata*. Journal of Organic Chemistry 48, 1903–1906.

Sun, H.H., Fenical, W., 1979. Diterpenoids of the brown seaweed *Glossophora galapagensis*. Phytochemistry 18, 340–341.

Vanden Berghe, D.A., Vlietinck, A.J., 1991. Screening method for antibacterial and antiviral agents from higher plants. In: Methods in Plant Biochemistry, vol. 6. Academic Press Ltd., London, pp. 47–70.

Vazquez, J.T., Chang, M., Nakanishi, K., 1988. Structure of hydroazulenoid diterpenes from a marine alga and their absolute configuration based on circular dichroism. Journal of Organic Chemistry 53, 4797–4800.