Cytotoxic Hydroazulene Diterpenes from the Brown Alga *Dictyota dichotoma*

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Two new hydroazulenoid (prenyl guaiane) diterpenes, dictyone acetate (**2**) and 3,4-epoxy 13-hydroxy pachydictyol A (**4**) were isolated from the petroleum ether fraction of the alcoholic extract of the brown alga, *Dictyota dichotoma* (Hudson) Lamouroux, which was collected from the Red Sea coasts at Hurgada, Egypt, together with three known ones, pachydictyol A (**1**), dictyone (**3**) and 11-hydroxypachydictyol A (dictyol *E*) (**5**). In addition, the steroidal compound, stigmasta-5,(*E*)-24(28)-dien-3-β-ol (fucosterol) (**6**) was also isolated. The structures of the isolated compounds have been determined on the basis of spectroscopic evidences as well as physical and chemical correlation with known compounds. Compounds **1**, **2**, **3** and **5** showed moderate cytotoxic activity.

Key words: Dictyota dichotoma, Cytotoxic Hydroazulene Diterpenes, Fucosterol

Introduction

Species of brown algae belonging to family Dictyotaceae have been shown to be a rich source of interesting biologically active diterpenoids of versatile chemical classes (Vashishta, 1984; Duran *et al.*, 1997). Members of the genus *Dictyota* have been chemically the most extensively investigated mainly for their diterpenoid content. *Dictyota dichotoma* afforded the greatest share of diterpenoids, at least 77 compounds belonging to 15 classes. Such versatility was claimed to either the cosmopolitan nature of this species being spreaded all over the oceans on one hand, or regarding this species as a complex of species (Teixeira *et al.*, 1990). So it was found of interest to investigate the Red Sea, *Dictyota dichotoma* (hudson) Lamouroux in order to assess the relation of its diterpenoid content to those reported. It was also selected for study since the petroleum ether extract displayed significant cytotoxic activity against two proliferating mouse cell lines, NIH3T3 and KA3IT.

Results and Discussion

The petroleum ether extract of the brown alga, *Dictyota dichotoma* was fractionated on silica gel column using a gradient of n-hexane-ethyl acetate to afford in order of elution, six compounds **1**–**6**.

Compounds **1** and **3** were identified as pachydictyol A (Hirschfeld *et al.*, 1973) and dictyone (Enoki *et al.*, 1982) respectively, on the basis of physical and spectral data (IR, MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H-cosy, HMQC, HMBC and 2D-NOESY) as reported in literature.

Compound **2** has close similarity with compound **3** where ^{1}H- and ^{13}C NMR data for both compounds (Tables I, II) revealed that the only significant difference could be rationalized in term of **2** being the 6-acetoxy derivative of **3** based on the deshielding of H-6 from δ 4.05 ppm in **3** to 5.28 ppm in **2** and C-6, from δ 73.8 ppm in **3** to δ 78.72 ppm in **2**, together with the presence of ^{1}H-, ^{13}C NMR resonance associated with C-6 acetoxy group [2.03(3H, s), 21.87(q)$\underline{CH_3}$CO, 171.08 (s), ($CH_3\underline{CO}$)]. Consequently, compound **2** is an acetate ester of dictyone. To the best of the authors knowledge, this is the first report indicating the isolation of this compound from a natural source. However, **2** was concurrently identified by two of the authors in another brown alga, *Cystosira myrica* collected from Red Sea (Ayyad *et al.*, 2002).

Compound **4** was isolated as a colorless oil. Its molecular formula was calculated via the NMR data. ^{13}C NMR revealed 20 carbon signals and the DEPT spectrum confirmed the presence of four

Table I. ^{1}H-NMR data of compounds **1**–**5**.

Proton no.	Pachydictyol A **1**	Dictyone acetate **2**	Dictyone **3**	3,4-Epoxy 13-hydro xypachydictyol A **4**	Dictyol E **5**
1	2.6 (1H, m)		2.6 (1H, m)	2.18 (1H, m)	2.56 (1H, m)
2	2.2, 2.4 (2H, m)		1.25 (1H, m); 2.15 (1H, m)	1.9, 2.04 (2H, m)	2.2, 2.46 (2H, m)
3	5.2 (1H, brs)	5.3 (1H, m)	5.30 (1H, s)	3.3 (1H, s)	5.3 (1H, s)
4	–	–	–	–	–
5	2.2 (1H, m)		2.35 (1H, m)	1.7 (1H, m)	2.35 (1H, m)
6	3.8 (1H, d, *J* = 7)	5.28 (1H, dd, *J* = 4.1, 3.6)	4.0 (1H, dd, *J* = 3.1, 8.1)	4.06 (1H, dd, *J* = 1.8, 7.3)	4.15 (1H, brd, *J* = 6.8)
7	1.5 (1H, m)		1.4 (1H, m)	1.42 (1H, m)	1.64 (1H, m)
8	1.8 (2H, m)		1.5 (1H, m), 1.6 (1H, m)	1.3, 2.1 (2H, m)	1.66, 1.74 (2H, m)
9	2.25 (2H, m)		2.1 (1H, m), 2.6 (1H, m)	2.08 (2H, m), 2.4 (1H, ddd, *J* = 2.4, 5, 13.5)	2.1, 2.65 (2H, m)
10	–		–	–	–
11	1.4 (1H, m)		1.08 (1H, m)	185 (1H, m)	–
12	1.1, 1.4 (2H, m)		2.4 (2H, m)	1.6, 1.2 (2H, m)	1.7, 1.74 (2H, m)
13	1.6, 1.8 (2H, m)		1.05 (1H, m), 2.08 (1H, m)	4.31 (1H, ddd, *J* = 4.3, 8.5, 11)	1.95, 2.04 (2H, m)
14	5.02 (1H, br. t, *J* = 7.5)		–	5.17 (1H, dq, *J* = 1.5, 8.5)	5.13 (1H, br. t, *J* = 6.8)
15	–		2.1 (1H, m)	–	–
16	1.61 (3H, s)	1.08 (3H, d, *J* = 7.0)	1.09 (3H, d, *J* = 6.9)	1.72 (3H, d, *J* = 1.6)	1.68 (3H, s)
17	1.75 (3H, brs)	1.6 (3H, brs)	1.84 (3H, brs)	1.48 (3H, s)	1.78 (3H, brs
18	4.6 (2H, br)	4.78 (1H,s), 4.75 (1H, s)	4.71 (2H, s)	4.61 (1H, d, *J* = 12) 4.7 (1H, brs)	4.72 (1H, s) 4.74 (1H, s)
19	0.94 (3H, d, *J* = 6.0)	0.87 (3H, d, *J* = 6.8)	0.94 (3H, *J* = 6.4)	1.17 (3H, d, *J* = 7.5)	1.19 (3H, s)
20	1.54 (3H, s)	1.08 (3H, d, *J* = 7.0)	1.09 (3H, d, *J* = 6.9)	1.7 (3H, d, *J* = 1.5)	1.6 (3H, s)
-OAc		2.03 (3H, s)			

methyl groups, five methylene (one of them is olifinic), eight methine (three attached to oxygenated function groups and one olifinic) and three quaternary carbons (one attached to an oxygenated function group and two olifinic). The data so far, have accounted for 30 hydrogen atoms attached directly to the twenty carbons. The remaining hydrogens are those of the hydroxyl groups. The data indicated the presence of two hydroxyl groups attached to the methine carbons at δ 73.04 and 68.45 ppm.The other oxygenated carbons resonated at a significantly high field; the quaternary one at δ 67.28 ppm while the methine carbon at 63.92 ppm and its proton at δ 3.3 ppm. Therefore, these two carbons are part of an epoxide ring, so the suggested molecular formula is $C_{20}H_{32}O_3$. Comparing the data of **4** with those of **1** revealed the close similarity except for the following differences: the disappearance of double bond signals at C-3 (δ 123.78) and C-4 (δ 141.54) and the presence of an epoxide ring instead, to account for the upfield signals of C-3 (δ 63.92) and C-4 (δ 67.28). This was confirmed from the HMBC experiments (Fig. 1) as the proton signal at δ 3.3 (1H, br. s, H-3) showed $^3J_{CH}$ and $^2J_{CH}$ correlation with C-1 (δ 39.24) and C-17 (δ 17.29) & C-2 (δ 30.81) respectively, while each of H-2_b (δ 2.04, 1H, m) and H-17 (δ 1.48, 3H, s) showed cross peaks with C-3 (δ 63.92), C-4 (δ 67.28).

The presence of additional hydroxyl group attached to C-13 (δ 68.45) was based on the COSY data. The proton at δ 4.31 (1H, ddd, *J* = 4.3, 8.5, 11.0, H-13) showed cross peaks with the protons at δ 1.2 (1H, m, H-12_a), δ 1.60 (1H, m, H-12_b) and

Table II. ^{13}C-NMR data of compounds **1**–**6**.

Carbon no.	Pachydictyol A	Dictyone acetate	Dictyone	3,4-Epoxy 13-hydroxypachy dict-yol A	Dictyol E	Fucosterol
	1	**2**	**3**	**4**	**5**	**6**
1	46.08(d)	46.19(d)	45.86(d)	39.24(d)	46.21(d)	37.3(t)
2	33.72(t)	34.09(t)	33.99(t)	30.81(t)	33.83(t)	31.9(t)
3	123.78(d)	125.20(d)	123.55(d)	63.92(d)	124.48(d)	71.8(d)
4	141.54(s)	140.19(s)	142.62(s)	67.28(s)	141.04(s)	42.3(t)
5	60.31(d)	57.47(d)	59.27(d)	53.48(d)	60.27(d)	140.8(s)
6	75.05(d)	78.72(d)	73.80(d)	73.04(d)	74.23(d)	121.7(d)
7	47.79(d)	46.39(d)	49.10(d)	43.12(d)	48.60(d)	31.6(t)
8	23.47(t)	23.04(t)	23.89(t)	30.06(t)	21.66(t)	36.4(d)
9	40.68(t)	39.95(t)	40.85(t)	40.23(t)	40.64(t)	50.08(d)
10	152.50(s)	152.09(s)	152.78(s)	151.50(s)	152.05(s)	36.9(s)
11	34.82(d)	34.58(d)	34.26(d)	34.82(d)	76.28(d)	21.06(t)
12	35.07(t)	38.79(t)	37.86(t)	33.77(t)	41.03(t)	39.7(t)
13	25.67(t)	30.52(t)	27.17(t)	68.45(t)	23.35(t)	42.3(s)
14	124.83(d)	215.14(s)	216.50(s)	126.50(d)	124.25(d)	56.6(d)
15	131.20(s)	41.02(d)	41.09(d)	136.92(s)	131.71(s)	24.3(t)
16	25.67(q)	18.45(q)	18.52(q)	25.81(q)	25.78(q)	28.2(t)
17	16.21(q)	15.51(q)	16.29(q)	17.29(q)	15.93(q)	55.8(d)
18	107.02(t)	107.80(t)	107.01(t)	107.92(t)	107.57(t)	11.8(q)
19	17.42(q)	15.91(q)	18.21(q)	19.47(q)	25.31(q)	19.4(q)
20	17.61(q)	18.45(q)	18.46(q)	18.52(q)	17.73(q)	34.8(d)
21						18.7(q)
22						35.2(t)
23						25.6(t)
24						147.0(s)
25						31.9(d)
26						22.1(q)
27						22.2(q)
28						115.5(d)
29						13.2(q)
OAc		21.87 (q), 171.08 (s)				

δ 5.17 (1H, dq, J = 1.5, 8.5, H-14). This was further confirmed from HMBC experiments (Fig. 1) as the proton signal at δ 5.17(1H, dq, J = 1.5, 8.5, H-14) showed $^{3}J_{CH}$ correlation with C-12, C-16, and C-20 and the proton signal at δ 1.60 (1H, m, H-12$_b$) showed $^{2}J_{CH}$ correlation with C-13.

Based on the above data, compound **4** is deduced to be a prenylated guaiane 3,4-epoxy-13-hydroxypachydictyol A. Reviewing the current literature, this is the first isolation of this compound from natural source.

Compound **5** has close similarity with compound **1** where IR, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, HMBC and 2D-NOESY data for both compounds (Tables I, II) revealed that the only differences reside in the following: the CH signal at δ 34.82 ppm (C-11) in **1** was replaced by an oxygenated quaternary carbon signal at δ 76.28 ppm indicated the presence of additional hydroxyl group in **5**. Also, the absence of methyl doublet at δ 0.94 (3H, C-19) in **1** and the down field shift of the carbon at C-19 from δ 17.42 ppm in **1** to 25.31 ppm in **5** together with the downfield shift of C-12 from δ 35.07 ppm in **1** to δ 41.03 ppm in **5** suggested the location of hydroxyl group in the side chain at C-11. This was further confirmed from HMBC (Fig. 1), as the proton signal at δ 1.19(H-19) showed $^{2}J_{CH}$ correlation with carbon at δ 76.28 (C-11).

The collective data of **5** were found to be identical to those of the prenylated guaiane, 11-hydroxy pachydictyol A (Dictyol E). It was isolated from the alga, *Dilophus ligulatus* (Dictyotaceae) (Danise *et al.*, 1977) and later on, from a Mediterranean *Dictyota dichotoma* (Amico *et al.*, 1980).

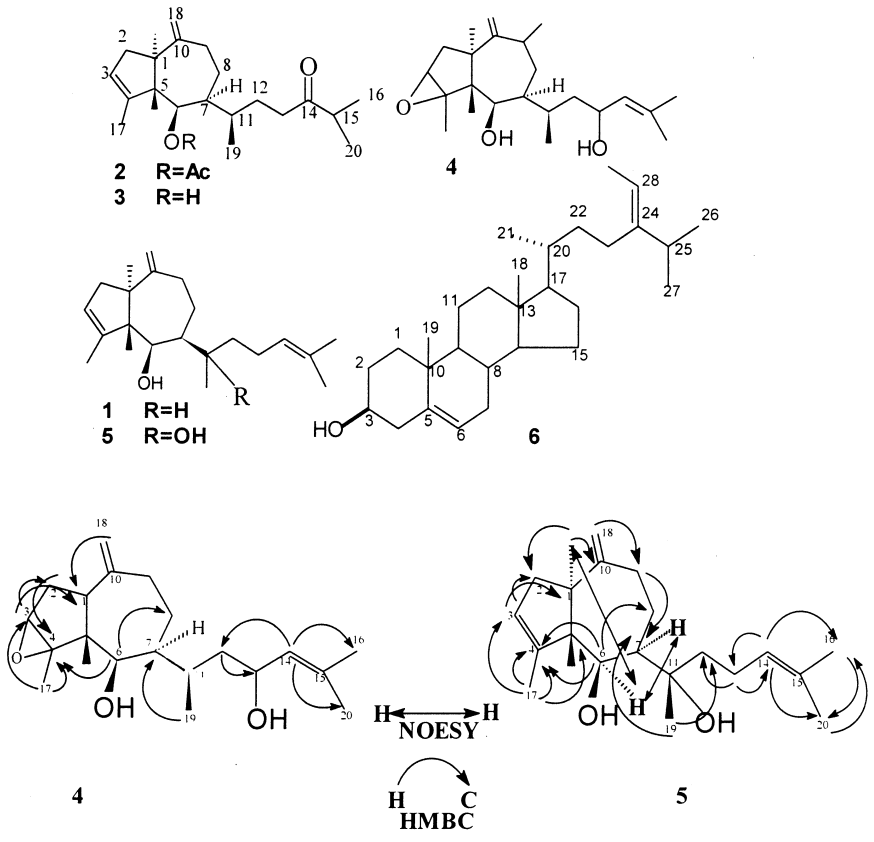

Fig. 1. Important HMBC and 2D-NOESY correlations for compounds 4 and 5.

Compound **6** was found by mass spectrometry and ^{13}C NMR spectroscopy to have the molecular formula $C_{29}H_{48}O$. Of the five degrees of unsaturation, implied by the molecular formula of **6**, two were accounted for as carbon-carbon double bonds (δ 140.8, 121.7, 147.0 and 115.5); **6** was thus tetracyclic. The presence of a secondary hydroxyl group was established by IR, NMR and MS [3520 cm^{-1}, δ_H 3.52(1H, m, H-3); δ_C 71.8 (d); *m/z* 394($[M]^+$-H_2O). The melting point and spectral data (IR, EI-MS, ^1H-NMR and ^{13}C-NMR) of **6** were in excellent agreement with those reported for **6** as stigmasta-5, (*E*)-24(28)-diene-3-β-ol (fucosterol), isolated from the brown alga, *Turbinaria ornata* (Sheu *et al.*, 1997).

Finally, cytotoxicity assays (Shier, 1983; Abbas *et al.*, 1992) for the petroleum ether extract of *Dictyota dichotoma* as well as the isolated compounds were carried out *in vitro* using two proliferating mouse cell lines, a normal fibroblast line NIH3T3 and virally transformed form of cells, KA3IT. **5** exhibited moderate cytotoxic activity on the cancer cell line KA3IT (IC_{50} 10 μg/ml) and showed reduced cytotoxicity towards the normal cells NIH3T3. The other compounds **1**–**3** also displayed moderate cytotoxic activity against the aforementioned two cell lines as reported by Ayyad *et al.*, 2002. Meanwhile, the petroleum ether extract showed substantial significant cytotoxic activity against normal cell line (IC_{50} 1 μg/ml) than on the other virally transformed forms (IC_{50} 10 μg/ml). It is worth to note that compound **6** was reported to display potent cytotoxic activity against mouse P 388 leukemia cells with IC_{50} 0.6 μg/ml (Sheu *et al.*, 1997).

Experimental

General. Mps. Uncorr. IR; thin films of $CHCl_3$ and KBr, recorded on Nicolet MX-IFT-IR, USA; ^1H NMR spectra were recorded at 400 or 500 MHz and ^{13}C NMR at 100 or 125 MHz, chemi-

cal shifts are given in δ (ppm) relative to TMS as internal standard in $CDCl_3$; EIMS: 70 ev. on a Kratos MS-25 spectrometer; TLC: Silica gel (Kieselgel 60, F254) of 0.25 mm layer thickness, the spots were visualized by spraying with anisaldhyde; PTLC was performed on silica gel plates (20 cm × 20 cm) of 0.5 mm thickness in 10% EtOAc/hexane, 15% EtOAc/hexane, 2% MeOH/$CHCl_3$ and 5% MeOH/$CHCl_3$ (v/v).

Plant material

The alga *Dictyota dichotoma* (Hudson) Lamouroux was collected in June, 1996 from Red Sea coasts at Hurgada, Egypt. A voucher sample was identified by Prof. Yahia El-Azab, Dept. of Botany, Faculty of Science, Mansoura Univ., and deposited at Pharmacognosy Dept. Faculty of Pharmacy, Mansoura University.

Extraction and isolation

The air-dried powdered sample (2.3 kg) was extracted to exhaustion with ethanol (90%) in a percolator at room temperature. After filteration, the alcoholic extract was evaporated under reduced pressure to solvent-free residue. The residue was suspended in water and extracted with petroleum ether (6 × 500 ml). The petroleum ether extract was evaporated under vacuum to afford a dark brown viscous oily residue (101 g, 4.4% dry weight). The petroleum ether extract was chromatographed on a silica-packed column using n.hexane-EtOAc gradient. Fractions of 100 ml were collected. The fractions containing a single compound were combined and further purified by repeated silica gel C. C. and preparative TLC to give the following compounds in the following order:

Pachydictyol A (**1**): Hexane fractions 5–18 were combined, purified by silica gel c.c. (1% EtOAc/hexane) and PTLC (15% EtOAc/hexane) to afford pachydictyol A (colorless oil, 265 mg, 0.0012% dry wt.) $IR\nu^{CHCl3}{}_{max}$ cm^{-1}: 3514(OH), 1644 (C = C); EIMS *m/z* (rel. int.): 288 (50)$[M]^+$ $[C_{20}H_{32}O]$, 270 (55)$[M\text{-}H_2O]^+$,159(100)$[C_{12}H_{15}]^+$. ^1H NMR Table: 1; ^{13}C-NMR: Table II. All physical and spectral properties are identical to those reported in the literature for **1** (Hirschfeld *et al.*, 1973).

Dictyone acetate (**2**): Fractions (21–25) eluted by 2% EtOAc/hexane were combined and purified by further silica gel C. C. using gradient of EtOAc/hexane. Subfractions eluted by 3% EtOAc/hexane were fractionated by PTLC (10% EtOAc/hexane) to afford dictyone acetate **2** (R_f = 0.68) and dictyone **3** (R_f = 0.56). **2** was obtained as an oil (10 mg, 0.00004% dry wt.), $IR\nu^{CHCl3}{}_{max}$ cm^{-1}: 1735(-OAc), 1707 (C = O); ^1H NMR: Table I; ^{13}C-NMR: Table II.

Dictyone (**3**): was obtained as colorless oil (50 mg, 0.0002% dry wt.) $IR\nu^{CHCl3}{}_{max}$ cm^{-1}: 3440 (OH), 1705 (C=O); EIMS *m/z* (rel. int.): 304(10)$[M]^+[C_{20}H_{32}O_2]$, 286 (60)$[M\text{-}H_2O]^+$ $[C_{20}H_{30}O]$, 159(50)$][C_{12}H_{15}]^+$, 145(19), 107(48), 71(58), 43(100)$[C_3H_7]^+$. ^1H NMR: Table I; ^{13}C-NMR: Table II. The physical and spectral properties are identical to those reported in the literature for **3** (Enoki *et al.*, 1982).

3,4-Epoxy-13-hydroxypachydictyol A (**4**): Fractions (29–31) eluted by 3% EtOAc/hexane were combined and purified by further silica gel C. C. (5% EtOAc/hexane) and PTLC (2% MeOH/$CHCl_3$) to afford **4** as colorless semisolid (12 mg, 0.00005% dry wt.), ^1H NMR: Table I; ^{13}C-NMR: Table II.

11-Hydroxypachydictyol A (Dictyol E) (**5**): Fractions (38–45) eluted by 5% EtOAc/hexane were combined and purified by further silica gel C. C. (10% EtOAc/hexane) and PTLC (5% MeOH/$CHCl_3$) to afford **5** as yellow semisolid (25 mg, 0.0001% dry wt.), UV $\lambda^{MeOH}{}_{max}$ nm: 213; $IR\nu^{KBr}{}_{max}$ cm^{-1}: 3397 (OH), 1630 (C=C); ^1H NMR: Table I; ^{13}C-NMR: Table II. The physical and spectral properties are identical to those reported in the literature for **5** (Danise *et al.*, 1977).

Stigmasta-5, (E)-24(28)-dien-3-β-ol (Fucosterol) (**6**): Fractions (50–55) eluted by 6% EtOAc/hexane were combined and purified by further Silica gel C. C. (2% MeOH/$CHCl_3$) and PTLC (2% MeOH/$CHCl_3$) to afford **6** as colorless needles, mp 122–124 ° (MeOH); (24 mg, 0.0001% dry wt.), $IR\nu^{KBr}{}_{max}$ cm^{-1}: 3520 (OH), 1590, 1620 (C = C); EIMS *m/z* (rel. int.): 412(8)$[M^+$ $C_{29}H_{48}O]$, 394 (10)$[M\text{-}H_2O)]^+$, 314(100), 299(21), 271(18, 213(14). ^1H NMR, δ, *J* in Hz: 5.35 (1H,d, *J* = 5.5, H-6), 5.17 (1H,q, = 3.5, H-28), 3.52(1H,m), 1.58 (3H, d, *J* = 3.5, H-29), 1.01 (3H, s, H-19)), 0.99 (3H, d, *J* = 6.6, H-21), 0.97 (6H, d, *J* = 6.8, H26, H-27) and 0.69 (3H, s, H-18); ^{13}C-NMR: Table II.

The physical and spectral properties are identical to those reported in the literature for **6** (Sheu *et al.*, 1997).

Cytotoxicity

Cytotoxic assay (Shier, 1983; Abbas *et al.*, 1992) was determined using two proliferating mouse cell lines, a normal fibroblast line NIH3T3 (ATCC: CRL1658) and a virally transformed form, KA3IT (3T3 mouse fibroblast cell line transformed by the K ras oncogene-containing Kirsten strain of Malony sarcoma virus). Samples of extract or pure compound (5 mg) were dissolved in 62.2 µl of DMSO, and working solutions made by diluting 20 µl of the DMSO solution into 2 ml of sterile medium (Dulbecco's modified Eagle's medium, Sigma Chemical Co. St. Louis, MO, USA). Two-fold or 2.5-fold dilutions of the extracts of pure compounds from 200 µg/ml to 0.5 µg/ml were prepared in triplicate in the wells of 96-well culture trays (Falcon Micro Test III, # 3072, Becton Dickinson Labware, Lincoln Park, NJ, USA) in 200µl of medium containing 5% (v/v) calf serum (Hyclone Laboratories, Logon, Utah, USA). An inoculum of 2×10^3 cells was added to each well in a 100 µl aliquot of 10% calf serum in medium. The 96-well trays of cells were cultured under standard conditions until uninhibited cultures (control) became confluent. The contents of the wells were decanted, and each cell layer washed with a small amount of the medium. The wells were filled with formal saline (3.7% w/v formaldehyde in 0.15 M NaCl), and allowed to stand at room temperature for at least 30 min. The trays was washed with tap water, and attached cells stained by adding two drops of 0.5% (w/v) crystal violet solution in 20% (v/v) aqueous methanol added to each well. The trays were washed with tap water, and the IC_{50} estimated visually as the approximate concentration that causes 50% reduction in the number of stained cells adhering to the bottom of the wells.

Abbas H. K., Mirocha C. J., Shier W. T. and Gunther R. (1992), Procedures for bioassay, extraction and purification of Wortmannin, the hemorrhagic factor produced by *Fusarium oxysporum* N17B grown on rice. J. Assoc. Offic. Anal. Chem. **75**, 474–480.

Amico V., Oriente G., Piattelli M., Tringali C., Fattorusso E., Magno S. and Mayol L. (1980), Diterpenes based on the dolabellane skeleton from *Dictyota dichotoma.* Tetrahedron **36**, 1409–1414.

Ayyad S. N., Abdel-Halim O. B., Shier W. T. and Hoye T. R. (2003), Cytotoxic hydroazulenoid diterpenes from the brown alga *Cystosira myrica.* Z. Naturforsch. **58c**, 33–38.

Danise B., Minale L., Riccio R., AmicoV., Oriente G., Piattelli M., Tringali C., Fattorusso E., Magno S. and Mayol L. (1977), Further perhydro-azulene diterpenes from marine organisms. Experientia **33**, 413–416.

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

Enoki N., Ishida R., Urano S., Ochi M., Tokoroyama T. and Matsumoto T. (1982), New hydroazulenoid diterpenes from the marine alga *Dictyota dichotoma.* Chem. Lett., 1837–1840.

Hirschfeld D. R., Fenical W., Lin G. H. Y., Wing R. M., Bachlick P. and Sims J. J. (1973), Marine natural products VIII. Pachydictyol A, an exeptional diterpene alcohol from the brown alga *Pachydictyon coriaceum.* J. Amer. Chem. Soc. **95**, 4049–4052.

Sheu J., Wang G., Sung P., Chin Y. and Duh C. (1997), Cytotoxic sterols from the formosan brown alga *Turbinaria ornata.* Planta Med. **63**, 571–572.

Shier W. T. (1983), The undergraduate experiment to demonstrate the use of cytotoxic drugs in cancer chemotherapy. Amer. J. Pharm. Educ. **47**, 216–220.

Teixeira V. L., Almeida S. A. and Kelecom A. (1990), Chemsystematic and biogeographic studies of the diterpenes. Biochem. Sys. Ecol. **18**, 87–92.

Vashishta B. R. (1984), Botany for degree students, Part 1 "Algae", 7th. Ed., S. Chand & Company LTD, New Delhi, p. 5.