seco-Dolastanes from the Marine Brown Alga *Dictyota dichotoma* (Huds.) Lamour

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Dedicated to Prof. Atta-ur-Rahman (Director, H. E. J. Res. Inst. of Chem.) on receiving N.I. (Nishan-e-Imtiaz), the biggest civil award of Pakistan

Brown alga *Dictyota dichotoma* (Dictyotaceae) collected from Karachi coast of Arabian Sea yielded two new *seco*-dolastanes (diterpenoids) named: dichotone and dichotodione along with isolinearol acetate of the same skeleton as a new source. Their structures have been characterized with the aid of 2D-NMR spectroscopic techniques.

Key words: Dictyota dichotoma, Dictyotaceae, Brown Alga, Arabian Sea, Diterpenoids, *seco*-Dolastanes

Introduction

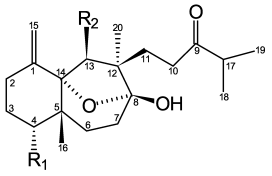
In recent years, research on chemistry of seaweed (or more generally marine organisms) has experienced a tremendous increase, due to the need of compounds possessing bioactivities of possible pharmaceutical application or other potential economic properties. To this end, a variety of species has been assayed for their activity and a number of biodynamic molecules including anthelmintic compounds [1], feeding deterrents [2], inhibitors of mitosis [3], ichthyotoxic compounds [4], cytotoxic, antibacterial [5] and antiviral compounds [6] have been isolated having unique skeleta different from those of terrestrial plants [7]. Such secondary metabolites that perhaps function as defensive compounds in the natural habitat [8].

Out of the 18 genera of brown seaweed (Phaeophyta) of the family Dictyotaceae found in the world, eight occur at the coasts of Pakistan, among them *Dictyota* Lamour. is quite common. Members of the genus *Dictyota* are common inhabitants of shallow water and inter-tidal communities in tropical and subtropical areas. This is the most extensively studied genus of the family Dictyotaceae. Hundreds of diterpenoids belonging to more than 16 skeletal classes have been isolated from several species. Brown algae of Dictyotaceae are characterized by the production of diterpenoids with different carbon skeleta containing mono-, bi- and tricycles [7 – 9], specifically based on dolastane ring system [10 – 11]. Only few *seco*-dolastanes (ring-C broken dolastane) have also been reported in the literature [12 – 15]. Among them the first was isolated by Ochi *et al.* in 1981 [14] and the last was publishes in 1990 by Ahmad *et al.* [13]. Unfortunately, the biological importance of *seco*-dolsatanes has not been explored yet by any research group. Diterpenoids of *Dictyota* species may possibly act as feeding deterrents against herbivorous marine animals [16].

D.dichotoma (Hudson) Lamour. is the only species of the genus *Dictyota* reported in all the oceans and the alga is considered, therefore, by various authors as the only cosmopolitan species of this genus. Studies on *D.dicotoma* have noted a wide range of variation among its constituents depending upon time and locations of collection [7]. In this article we wish to report two new (**1**, **2**) *seco*-dolastanes named: dichotone (**1**) and dichotodione (**2**) along with isolinearol acetate (**3**) [12] of the same skeleton as a new source. Their detailed structure elucidation is described in this report.

Results and Discussion

The ethanol soluble part of fresh brown alga *D. dichotoma* collected from Karachi coast of Arabian Sea, yielded two new *seco*-dolastanes [dichotone (**1**) and dichotodione (**2**)] along with isolinearol acetate (**3**) of the same skeleton as a new source. Such skeletal type of diterpenoids was expected from *Dictyota* species.



	R_1	R_2	
1	H	OH	(dichotone)
2	ketone	H	(dichotodione)
3	OAc	H	(isolinearol acetate)

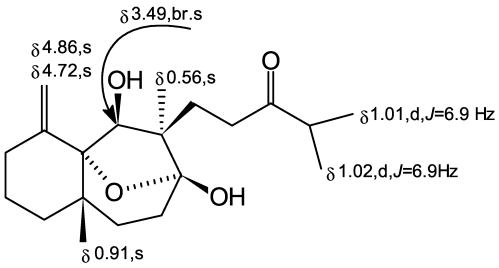


Fig. 1. ^{1}H NMR data of dichotone (**1**).

Clues for *seco*-dolastane type skeleton were taken from the exhibition of two secondary (Me-18 and Me-19) and two quaternary (Me-16 and Me-20) methyl together with a converted methyl as an *exo*-cyclic double bond (Me-15) signals in the NMR spectra [13]. Further, the characteristic signal of C-8 (at about δ 105) in ^{13}C NMR spectra of **1**-**3** [13] gave an idea that all three metabolites belong to the ring-C broken type dolastane (*seco*-dolastane) [13]. However, the key points of compounds **1** and **2** are summarized below:

Dichotone (**1**): Presence of hydroxyl, olefinic and ketonic functions in **1** were attested by their signals at 3400, 1648 and 1708 cm^{-1}, respectively, in the infrared spectrum. The molecular mass was determined through field desorption (FD) spectrum as 336 a.m.u. The first fragment appeared in the EI and HR spectra in the same mode at *m/z* 318 due to the loss of water molecule from the molecular ion peak. The formula associated with this peak (*m/z* 318) was determined *via* HRMS as $C_{20}H_{30}O_3$ confirming the presence of six degrees of unsaturation in the fragment appeared after the removal of a water molecule revealed that **1** should have five degrees of unsaturation. Other observable fragments in the mass spectra (EI and HR) are quoted in the experimental section.

The ^{1}H NMR spectrum of **1** (Fig. 1) displayed two secondary methyls associated with isopropyl moiety at

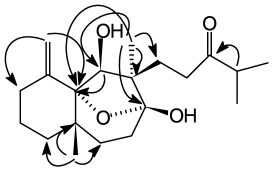


Fig. 2. HMBC connectivities in **1**.

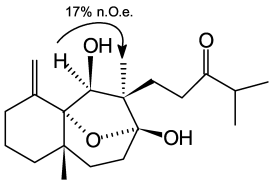


Fig. 3. n.O.e. measurements in **1**.

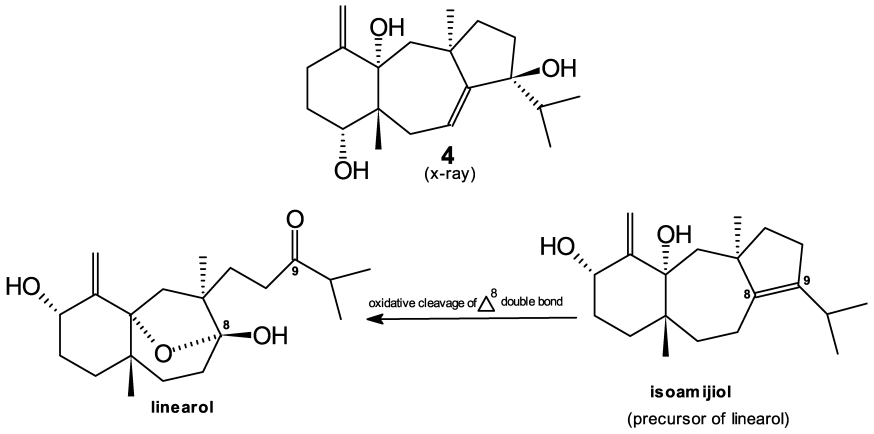
δ 1.01, 1.02 (J = 6.9 Hz) and two tertiary methyls at δ 0.56, 0.91 due to Me-20 and Me-16, respectively. A broad singlet at δ 3.49 was found to correlate with the carbinylic carbon at δ 78.7 in the HMQC spectrum. Chemical shifts of this carbon and proton inferred the situation of a hydroxyl function in the molecule, which was counter checked from IR spectrum. Position of this hydroxyl was depicted at C-13 via HMBC experiments (Fig. 2) and the stereochemistry as β was decided with the aid of n.O.e. experiment (Fig. 3) showing the 17% n.O.e. effect on Me-20 when the broad singlet of H-13α at δ 3.49 was irradiated.

The other signals in the ^{1}H NMR spectrum were at δ 4.72 and 4.86 as singlets were attested for *exo*-cyclic double bond derived from Me-15 in *seco*-dolastane skeleton.

A characteristic carbon resonance of *seco*-dolastane skeleton having an oxygen bridge across C-8 and C-14 appeared at δ 105.5 due to the C-8. Other significant signals in the carbon spectrum were at δ 146.4 (C-1) and 110.0 (C-15) associated with olefinic function, at δ 18.3 and 18.4 related to isopropyl unit, at δ 22.6 (Me-16) and 22.5 (Me-20) due to tertiary methyls, at δ 86.1 due to C-14 and at δ 215.0 assigned to ketonic function.

The above spectral information has led to the conclusion that the discussed compound must have structure **1** and can be named dichotone. This is a new addition in the diterpenoids of *D. dichotoma.*

Dichotodione (**2**): In comparison with compound **1**, ^{13}C NMR of dichotodione (**2**) had an extra ketonic signal at δ 211.8 simultaneously with the absence of carbinylic carbon and proton signals in the NMR spectra. The molecular mass of **2** was determined through FDMS as 334 a.m.u. and the formula $C_{20}H_{30}O_4$ of this mass was obtained from HREI-MS (*m/z* 334.21444). A



Scheme 1.

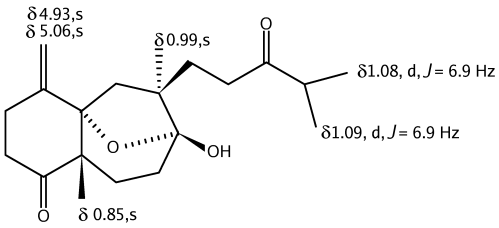


Fig. 4. ^{1}H NMR data of dichotodione (**2**).

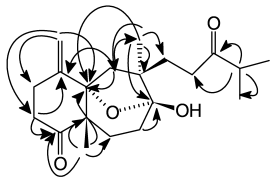


Fig. 5. HMBC connectivities in **2**.

broad absorption at 1707 cm^{-1} in the IR spectrum was due to the ketonic functions. Broadness of this band may be due to the merger of two ketonic functions in the molecule.

Compound **2** (Fig. 4) had usual two secondary [δ 1.08 (d, J = 6.9 Hz, H-18), 1.09 (d, J = 6.9, H-19); 18.3 (C-18 and C-19)] and two tertiary methyls [δ 0.85 (H-16); 21.5 (C-16) and 0.99 (H-20); 22.9 (C-20)] with olefinic signals [δ 4.93 (s, H-15A), 5.06 (s, H-15B); 110.9 (C-15) and 145.1 (C-1)]. The characteristic signal of C-8 resonated at δ 105.1 whereas; the signal of other carbon associated with the oxygen bridge was found at δ 85.1 (C-14) in the spectrum. Signals at δ 211.8 and 214.5 in the broad band spectrum were assigned to two ketonic functions due to C-4 and C-9, respectively, which were distinguish with the ad of HMBC connectivities (Fig. 5). The structure was thus concluded as **2** which is also a new addition in *Dictyota* metabolites and named dichotodione.

Isolinearol acetate (**3**): This was isolated before from *D. cervicornis* by Teixeira and Tomassini [12], therefore, only spectral data are given in the experimental section.

Stereochemistry: The newly isolated compounds **1** and **2** having five and four chiral carbon centers, respectively, and the relative configurations of all these centers in both the compounds have been determined on the basis of spectral data of previously reported same class of compounds [12 – 15]. However, the relative stereochemistry of the first *seco*-dolastane (linearol) [14] was defined by chemical correlation to isoamijiol (a representative of dolastane class) [12]. It has been suggested that the isoamijiol is the precursor of linearol [12,17] and the *seco*-dolastane skeleton could derive from the dolastane by oxidative cleavage of a Δ^8 double bond [15]. The absolute stereochemistry of dolastane **4** obtained from *Dictyota* species had already been established via chemical means and x-ray diffraction analysis [18]. Therefore, the described stereochemistry in compounds isolated by us is justified (Scheme 1).

Experimental Section

General

The ^{1}H and ^{13}C-NMR spectra were recorded at 400 and 300 MHz on Bruker AM 400 and 300, respectively, in $CDCl_3$ and in a mixture of $CDCl_3$ / CD_3OD.

Collection and identification

Alga (wet. weight 12 kg) was collected in March 2001, from Buliji near Karachi coast (Arabian Sea) by hands and

identified by Ms. Shaista Hameed, Institute of Marine Biology, University of Karachi, where a voucher specimen (# MBB-55) has been deposited.

Extraction and isolation

The collected material was washed with plenty of tap water to remove the sea salts and immediately soaked in ethanol (16 l) for a period of eight days. After filtration, the filtrate was concentrated at low pressure to avoid thermal decomposition. The crude oily concentrated extract (321 g) was subjected to silica gel column chromatography using hexane, hexane-ethyl acetate, ethyl acetate, and ethyl acetate-methanol as mobile phase.

Elution with 25% ethyl acetate in hexane was further purified *via* C. C. Compound **1** was obtained as an oil (26.3 mg).

Dichotone (**1**): (26.3 mg, 8.19×10^{-3}%, **yield**). – $[\alpha]_D^{24}$: – 51.3° (c 0.931, $CHCl_3$). – **IR**(**CHCl**$_3$): $\nu_{max} = 3400$ (O-H), 1708 (ketonic CO), 1648 (C=C) cm^{-1}. – ^{1}**H-NMR** ($CDCl_3$, 300 MHz): See Fig. 1 – ^{13}**C-NMR** ($CDCl_3$, 100 MHz): $\delta = 146.4$ (C-1), 35.9 (C-2), 32.0 (C-3), 29.7 (C-4), 43.3 (C-5), 40.9 (C-6), 27.3 (C-7), 105.5 (C-8), 215.0 (C-9), 31.7 (C-10), 33.2 (C-11), 39.6 (C-12), 78.7 (C-13), 86.1 (C-14), 110.0 (C-15), 22.6 (C-16), 40.9 (C-17), 18.3 (C-18), 18.4 (C-19), 22.5 (C-20). – **MS** (**El**, 70 eV): *m/z* = 318 [M-H_2O]$^+$, 275 [318-isopropyl]$^+$, 237 [M-side chain]$^+$. – **MS** (**FD**): *m/z* = 336. – **MS** (**HREI**): *m/z* = 318.2198 [$C_{20}H_{30}O_3$ calcd. 318.219482], 275.1195 [$C_{17}H_{23}O_3$ calcd. 275.164709] and 237.1152 [$C_{14}H_{21}O_3$ calcd. 237.149060].

Elution with the same polarity (25% ethyl acetate in hexane), **2** was obtained as a semi- pure sample which on further purification through repeated C. C. afforded **2** as an yellow oil (7.2 mg).

Dichotodione (**2**): (7.2 mg, 2.2×10^{-3}%, yield). – $[\alpha]_D^{24}$ – 55.5° (c 0.144, $CHCl_3$). – **IR**($CHCl_3$): $\nu_{max} = 3400$ (O-H), 1707 (br., ketonic CO), 1656 (C=C) cm^{-1}. – ^{1}**H-NMR**($CDCl_3$, 400 MHz): See Fig. 4 – ^{13}**C-NMR**($CDCl_3$, 100 MHz): $\delta = 145.1$ (C-1), 30.2 (C-2), 37.2 (C-3), 211.8 (C-4), 52.5 (C-5), 27.6 (C-6), 36.0 (C-7), 105.1 (C-8), 214.5 (C-9), 30.8 (C-10), 28.8 (C-11), 44.5 (C-12), 41.7 (C-13), 85.1 (C-14), 110.9 (C-15), 21.5 (C-16), 41.0 (C-17), 18.3 (C-18), 18.3 (C-19), 22.9 (C-20). – **MS**(**EI**,70 eV): *m/z* = 334 [M]$^+$, 317 [M-OH]$^+$, 316 [M-H_2O]$^+$, 301 [316-Me]$^+$, 298 [316-H_2O]$^+$, and 291 [M-Isopropyl]$^+$. – **MS** (**FD**): *m/z* = 334. – **MS (HREI)**: *m/z* = 334.21444 [$C_{20}H_{30}O_4$ calcd. 334.21439], 317.20586 [$C_{20}H_{29}O_4$ calcd. 317.21166], 316.20366 [$C_{20}H_{28}O_3$ calcd. 316.20383], 301.17951 [$C_{19}H_{25}O_3$ calcd. 301.18036], 298.18715 [$C_{20}H_{26}O_2$ calcd. 298.19327] and 291.16643 [$C_{17}H_{23}O_4$ calcd. 291.15962].

Again with the same polarity (25% ethyl acetate in hexane), **3** was obtained as a mobile oil (17.9 mg).

Isolinearol acetate (**3**): (17.9 mg, 5.5×10^{-3}%, yield). – **IR** ($CHCl_3$): $\nu_{max} = 3445$ (O-H), 1735 (ester C-O), 1712 (ketonnic C-O), 1651 (C=C) cm^{-1}. – ^{1}**H-NMR** ($CDCl_3$+CD_3OD, 400 MHz): $\delta = 0.75$ (3H, s, H-16), 0.99 (3H, s, H-20), 1.08 (3H, d, $J = 6.9$ Hz, H-18), 1.09 (3H, d, $J = 6.9$ Hz, H-19), 2.11 (3H, s, OAc), 4.79 (1H, s, H-15A), 4.83 (1H, t, $J = 2.6$, 2.6 Hz, H-4β), 4.98 (1H, s, H-15B). – ^{13}**C-NMR** ($CDCl_3$+CD_3OD, 75 MHz): $\delta = 146.8$ (C-1), 31.0 (C-2), 27.9 (C-3), 78.3 (C-4), 39.4 (C-5), 32.1 (C-6), 29.2 (C-7), 104.2 (C-8), 215.8 (C-9), 29.1 (C-10), 36.1 (C-11), 43.5 (C-12), 40.9 (C-13), 83.3 (C-14), 109.5 (C-15), 23.0 (C-16), 40.9 (C-17), 18.1 (C-18), 18.1 (C-19), 21.4 (C-20), 22.6 (**C**H_3CO), 171.7 (CH_3**C**O). – **MS** (**EI**, 70 eV): *m/z* = 378 [M]$^+$, 360 [M-H_2O]$^+$, 335 [M-isopropyl]$^+$, 318 [M-AcOH]$^+$, 301 [318-OH]$^+$. – **MS** (**FD**): *m/z* = 378. – **MS** (**HREI**): *m/z* = 378.24070 [$C_{22}H_{34}O_5$ calcd. 378.24060], 360.22915 [$C_{22}H_{32}O_4$ calcd. 360.23004], 335.18737 [$C_{19}H_{27}O_5$ calcd. 335.18583], 318.22001 [$C_{20}H_{30}O_3$ calcd. 318.21948] and 301.21321 [$C_{20}H_{29}O_2$ calcd. 301.21675].

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