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NEW ANTIMICROBIAL DITERPENES, DICTYOL F AND EPIDICTYOL F, FROM THE BROWN ALGA DICTYOTA DICHOTOMA

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The methanol extract of the brown seaweed Dictyota dichotoma inhibited the growth of microbes. Three active constituents were found to be diterpene alcohols having the hydroazulene skeleton.

In the course of studies[1] on chemical constituents of the Japanese alga Dictyota dichotoma, collected at Oshoro Bay, Hokkaido, in July 1981, we became aware that crude methanol extract of this alga showed antimicrobial activity against Staphylococcus aureus, Micrococcus luteus, and Bacillus subtilis at 500 μg/ml. After the crude methanol extract was partitioned between ether and water, concentration of the ether layer yielded a dark brown oil. The oil was separated by being passed through a silica gel column (hexane-EtOAc) and then through a prepacked Merck Size C silica gel 60 column (hexane-ether). The active fraction was further purified by extensive high performance liquid chromatography to give three bioactive compounds **1**, **2**, and **6**, together with inactive **8**, **9**, and **10**. Unexpectedly the compound **1** with the highest bioactivity (Table 1) was identified as dictyol C by comparison of the spectral data.[2] Diol **2**, oil, $[\alpha]_D^{25}$ + 48.2° (c 1.5, $CHCl_3$), had molecular formula $C_{20}H_{32}O_2$ (m/z M^+ found 304.2419, calcd 304.2504). Successive loss of two moles of water in its mass spectrum suggested that both oxygen atoms in **2** are present as hydroxyl groups and the ^{1}H-NMR spectrum[3] confirmed the presence of two secondary hydroxyl groups [δ 3.98 (1H, dd, J=8, 3 Hz) and 4.12 (1H, dd, J=8, 7 Hz)]. The ^{1}H-NMR spectrum indicated further that diol **2** has a structure very similar to that of pachydictyol A (**3**)[4] except for the side chain moiety. The absence of the isopropylidene group and the presence of a isopropenyl group in the side chain [δ 1.73 (3H, brs), 4.84 (1H, s) and 4.96 (1H, s)] were clear from the spectrum. In order to locate the position of the unknown hydroxyl group, **2** was oxidized with PDC to yield an α,β-unsaturated ketone **4** [δ 1.86 (3H, brs), 5.78 (1H, s) and 6.01 (1H, s); IR (neat) 1775 cm^{-1}]. Diol **2** thus turned out to be an allyl alcohol was then converted into its allyl mono-p-bromobenzoate **5**. The CD spectrum of **5** in methanol showed a negative Cotton effect at 240 nm (Δε -2.2). Therefore compound **2** has the 14-R absolute configuration.[5] Biogenetic consideration and comparison of ^{13}C-NMR data (Table 2) with

those of pachydictyol A suggested that its structure is expressed by formula 2.[6] Compound 6, mp 62-63 °C, $[\alpha]_D^{25}$+37.5 (c 1.1, $CHCl_3$), had molecular formula $C_{20}H_{32}O_2$ (m/z M^+ found 304.2415, calcd 304.4415), and its ^{1}H- and ^{13}C-NMR spectral data were strongly reminiscent those of 2. Oxidation of 6 with PDC yielded the α,β-unsaturated ketone 4. The CD spectrum of mono-p-bromobenzoate 7 in methanol showed a positive Cotton effect at 240 nm (Δε +2.3). Therefore compound 6 has the 14-S absolute configuration and is the C-14 epimer of 2. Compound 8, $C_{20}H_{32}O_2$ (m/z M^+ found 304.2411, calcd 304.2404), showed IR absorption band at 3200-3600 cm^{-1} and ^{1}H-NMR peaks at δ 1.01 (1H, d, J=7 Hz), 1.27 (3H, s), 1.31 (3H, s), 1.81 (3H, s), 2.74 (1H, t, J=7 Hz), 3.93 (1H, brd, J=8 Hz), 4.75 (1H, brs), 4.76 (1H, brs) and 5.34 (1H, brs). The ^{13}C-NMR spectrum (Table 2) revealed the presence of two double bonds, of which the one was trisubstituted (123.8 d and 141.4 s) and other was an exomethylene group (107.1 t and 152.3 s). Comparison of the ^{1}H- and ^{13}C-NMR (Table 2) spectra of 8, 2 and 3 suggested that one of the oxygen atoms constituted an ether linkage and, moreover, 8 was 14,15-epoxide of 3. In order to establish the position and stereochemistry of the epoxide function, epoxide 8 was treated with 1) $LiAlH_4$-$TiCl_3$ in dry THF[7] and 2) LDA in dry ether to yield two alcohols. The resulting alcohols were identical in all respects with pachydictyol A (3) and 2 respectively. Thus, the structure of 8 is established as depicted. The MIC values for representative microbes are listed in Table 1. It should be noted that antimicrobiological activity of this type of compounds, hydroazulenoid diols, is hitherto unknown. Compound 9 showed $C_{20}H_{32}O$ (m/z M^+ found 288.2437, calcd 288.2455). The nature of the five degree of unsaturation inherent in this molecular formula was well defined by the ^{1}H- and ^{13}C-NMR spectra. Two trisubstitute double bonds were present in this molecule. The remaining three degree of unsaturation were then assigned to a tricyclic skeleton. The absence of hydroxyl and carbonyl groups and the presence of 1070 cm^{-1} absorption in the IR spectrum of 9 suggested that the oxygen atom of 9 formed a cyclic ether. The ^{1}H- and ^{13}C-NMR (Table 2) spectra indicated the presence of the eight-carbon side chain containing a trisubstitute double bond [δ 0.84 (3H, d, J=7 Hz), 1.60 (3H, brs), 1.67 (3H, brs), 5.09 (1H, t, J=7 Hz); 16.9 q, 17.7 q, 25.7 q, 29.6 t, 34.3 t, 37.1 t, 124.6 d and 131.3 s]. The ^{13}C-NMR peaks at δ 77.2 (d) and 74.0 (s) are due to carbon atoms bearing ethereal oxygen. An importnat feature in the ^{1}H-NMR spectrum of 9 was the presence of singlet methyl peak at δ 1.25 and double doublet peak at 3.97 (1H, J=4, 2 Hz) and the absence of the exocyclic double bond. These data could be readily explained by the structure 9 with a C-6, C-10 ether linkage. In fact, ether 9 was converted with $BF_3 \cdot OEt_2$ at room temperature in dry ether to pachydictyol A. Therefore the structure of the ether is 9. Compound 10 oil, $[\alpha]_D^{25}$ -40.1° (c 4.2, hexane), $C_{21}H_{36}O$ (m/z M^+ found 304.2755, calcd 304.2768), exhibited in the IR spectrum a band at 1080 cm^{-1}. The significant difference between the ^{1}H-NMR spectrum of pachydictyol A (3) and that of 10 resided in the absence of the broad doublet at δ 3.85 and the 2H exomethylene singlet at 4.72 in 10, which exists in 3, and the presence of 3H singlets at 1.06 and 3.20 in 10. Therefore formula 10 is inferred for this compound. Comparison of the ^{13}C-NMR spectrum of 10 (Table 2) with those of appropriate hydroazulenoid compounds

supported this formulation. Compound 10 could be an artifact.

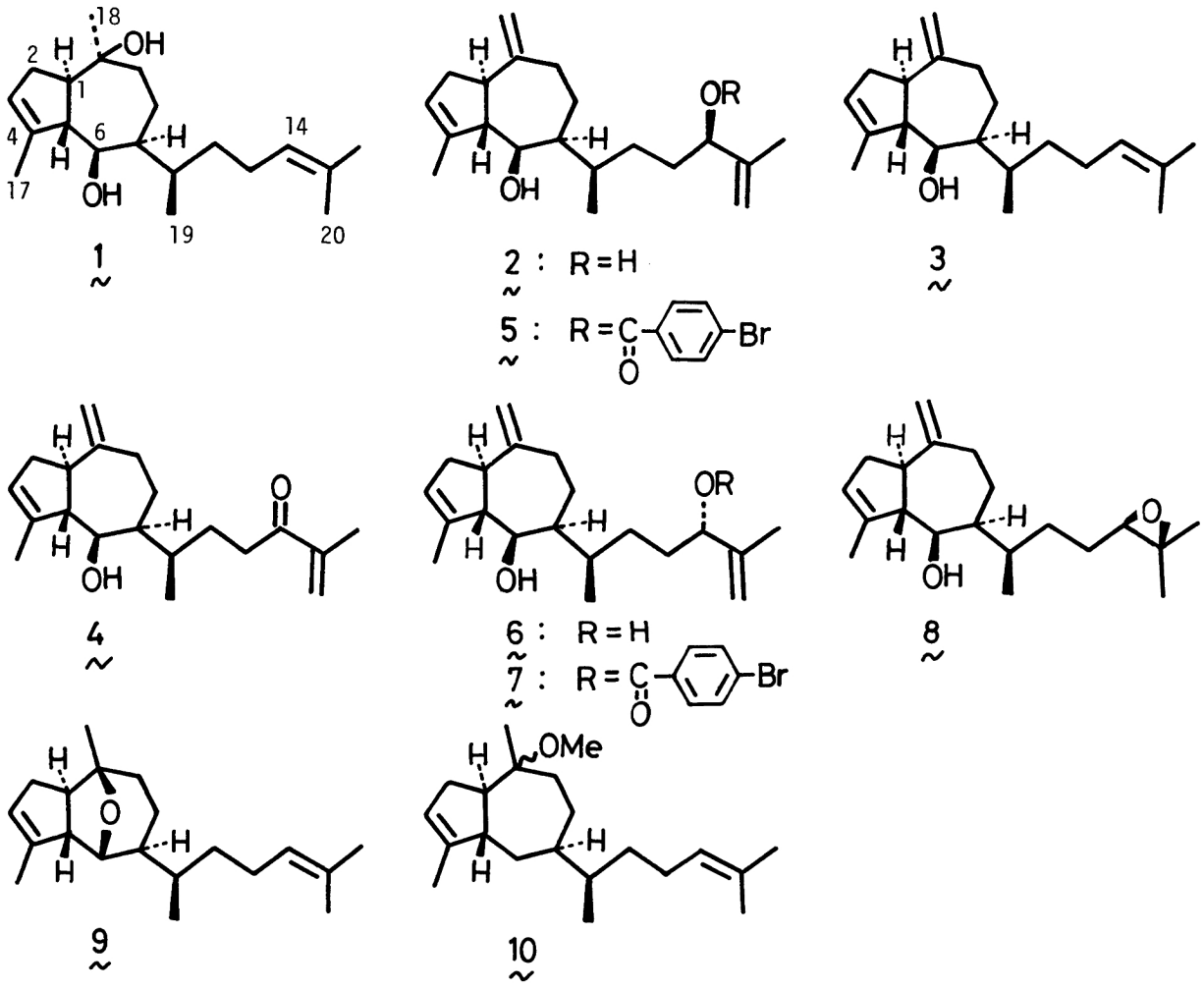

Table 1. Antimicrobial activity of 1, 2, and 6.

Test organism	MIC (μg/ml)		
	1	2	6
Staphylococcus aureus ATCC 6538P	39	241	>190
Bacillus subtilis ATCC 6633	39	241	>190
Micrococcus luteus ATCC 9341	39	483	>190
Mycobacterium smegmatis ATCC 607	313	483	190
Escherichia coli NIHJ	156	241	190
Candida albicans	>313	483	190

Nutrient agar, 37 °C, 24-48 h.

Table 2. ^{13}C-NMR data[a] of 2, 6, 8, 9, and 10.

Carbon	Compounds 2	6	8	9	10
1	45.9,d	46.0,d	46.1,d	61.1,d	46.7,d
2	33.8,t	33.9,t	33.8,t	26.1,t	30.9,t
3	123.6,d	123.9,d	123.8,d	130.5,d	124.8,d
4	141.7,s	141.4,s	141.4,s	141.4,s	131.0,s
5	59.6,d	60.2,d	60.4,d	62.8,d	44.9,d
6	74.4,d	76.2,d	74.9,d	77.2,d	26.3,t
7	48.5,d	48.1,d	47.8,d	38.0,d	46.8,d
8	23.8,t	23.7,t	23.5,t	20.3,t	23.8,t
9	40.6,t	40.5,t	40.6,t	38.6,t	35.5,t
10	152.4,s	152.4,s	152.3,t	74.0,s	76.1,s
11	35.4,d	35.2,d	34.8,d	37.1,d	30.9,d
12	33.8,t	33.9,t	31.5,t	34.3,t	35.3,d
13	31.5,t	30.6,t	26.3,t	26.3,t	26.3,t
14	74.2,d	74.6,d	64.3,d	124.6,d	124.8,d
15	147.7,s	147.7,s	58.6,s	131.3,s	131.0,s
16	18.0,q	17.8,q	18.7,q	25.7,q	25.7,q
17	16.0,q	16.0,q	15.9,q	16.1,q	17.4,q
18	106.9,t	107.1,t	107.1,t	22.3,q	22,5,q
19	17.9,q	17.4,q	17.6,q	16.4,q	17.4,q
20	110.4,t	111.1,t	24.8,q	17.7,q	17.7,q
				OMe	48.0,q

a) δ Values are relative to TMS in $CDCl_3$.

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8) The following names are suggested for the new compound. 2: Dictyol F, 6: Epidictyol F, 8: Epoxypachydictyol A, 9: Dictyoxide, 10: Methoxydictydiene.

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