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CONSTITUENTS OF THE DIGESTIVE GLAND OF THE MOLLUSCS OF THE GENUS APLYSIA - I.

NOVEL DITERPENES FROM APLYSIA DEPILANS

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A variety of novel natural products, most of them containing bromine and chlorine, have been isolated from the digestive gland of the sea hares[1], and recent experiments have revealed that the chemical constituents of the digestive (midgut) gland depend on the algal diet of the individual Aplysia[2]. Because also to the possible role of the digestive gland in the chemical defense system of the sea hares[3] we became interested in Aplysia and we have firstly examined the three more common mediterranean species: A. depilans, A. limacina and A. punctata.

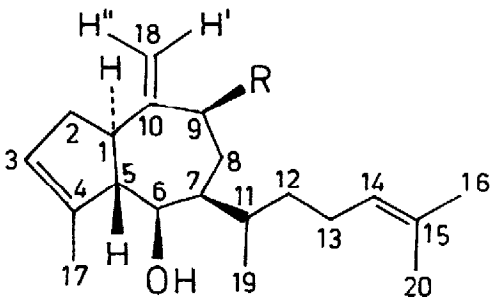
This paper reports on the isolation from the digestive gland of A. depilans of two novel guaiane-diterpenes, dictyol A (2) and B (3), found in addition to pachydictyol A (1), previously described from the brown alga Pachydicton coriaceum[4], and provides with further evidence that compounds found in Aplysia must be located as algal metabolites.

Homogenized digestive glands from six adult sea hares, collected off Naples, were extracted with acetone and the residue was partitioned between ether and water. Repeated silica gel chromatography of the ether-soluble portion (14 g) gave, in order of polarity, pachydictyol A (1, 30 mg), $\{\alpha\}_D$ + 104° (cyclohexane) (Lit[4] $\{\alpha\}_D$ + 106°), identical with an authentic sample (^{1}H-n.m.r., t.l.c.), dictyol A (2, 830 mg), m.p.84-86° (hexane), $\{\alpha\}_D$ + 79.6° (chloroform), $C_{20}H_{30}O_2$ (M^+/e 302.22457, required 302.22456), and dictyol B (3, 350 mg), m.p. 112-115° (hexane), $\{\alpha\}_D$ + 76.0° (chloroform), $C_{20}H_{32}O_2$ (M^+/e 304.24026, required 304.24021). Some related minor compounds have also been isolated and are now currently investigated.

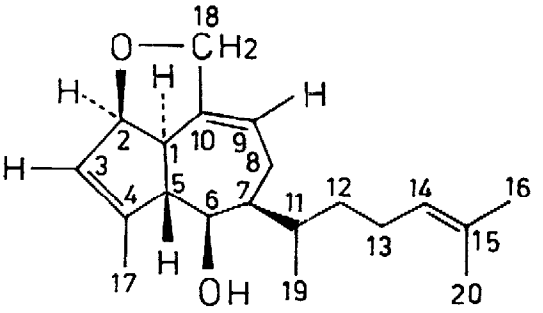
Dictyol B (3). The consecutive loss of two molecules of water in the m.s. (intense peaks at m/e 286 and 268) suggested that the two oxygen atoms in 3 are present in the form of hydroxyl functions, but the close relationship between pachydictyol A (1) and dictyol B (3) was clearly evidenced by ^{1}H-n.m.r.: both spectra include signals for three vinyl methyl's (δ-$CDCl_3$ 1.62, 1.70 and 1.78 for H-16, H-20 and H-17, respectively, in 3), a secondary methyl (δ 1.03, J 6 Hz) and two olefinic protons (t with J 6.5 Hz at δ 5.10 and m at δ 5.30 for H-14 and H-3, respectively). The exomethylene broad singlet seen in the spectrum of 1 at δ 4.72 (2H) is splitted in the spectrum of 3 into two broad singlets at δ 4.94 and 5.20, converted to more sharp signals on irradiation at δ 3.94 (2H, m, 2 CHOH), and this suggested to locate at C-9 the new hydroxy group. Indeed, the identity of carbon skeleton of 1 and 3 was established by dehydrogenation (10% Pd-C at 160°,

20 h) to the same 1,4-dimethyl-7(1',5'-dimethyl)exyl-azulene (t.l.c., g.l.c., u.v. and m.s.). The comparison of the ^{13}C-n.m.r. spectrum of dictyol B with that of 1 (Table I) unambiguosly proved the assigned structure 3, except for the stereochemistry at C-9. The assignements of the C-7 side chain shifts were based on published data on the isooctene side chain resonances in similar molecular environment[5,6]. Consideration of the known substituent effects for an additional hydroxy group[7] led to the shift assignements of C-2, C-8 and C-9 in 1, while the methine carbon at C-1 was distinguished from C-5 and C-7 by examining the effects, upon conversion of 1 into the trichloroacetyl urethane (by direct addition of trichloroacetyl isocyanate,TAI, to the alcohol in the n.m.r. tube), on the resonance positions for the carbons adjacent (C-5 and C-7) to the carbinol atom, which moved upfield by 3.17 and 1.97 p.p.m., respectively[8] . The differentiation between C-5 and C-7 was made on observing that the introduction of the 9-OH in 3 causes C-7 (γ-carbon) to move upfield by 4.02 p.p.m. Moreover, for 3, upon addition of TAI, the C-10 is shifted upfield by 6.79 p.p.m., while the C-18 is shifted downfield by 3.34 p.p.m. and this definitively located at C-9 the new hydroxy group. Differentiation between the carbinol carbons C-6 and C-9 was also made on considering the effects induced by TAI: the carbinol carbon of saturated secondary alcohols is reported to shift downfield by about 6.0-8.0 p.p.m., but the range of shift for the carbinol carbon of allylic alcohols is about 2.5-4.5 p.p.m.[8] . The stereochemistry 9β-OH assigned to dictyol B (3) is required to explain the relative intensities of the paramagnetic shifts induced by Eu(fod-d_9)$_3$ on the signals of H-18, H-5, H-1 and H-7 in its ^{1}H-n.m.r. spectrum ($\Delta\delta_{Eu}^{n=1}$: 9.00, 7.54, 4.52, 4.27 and 3.40 for H-18', H-5, H-7, H-1 and H-18''; the steric hindrance of the 6-OH suppresses the complex formation at this site; $\Delta\delta_{Eu}^{n=1}$: 11.92 and 2.90 for H-9 and H-6).

Dictyol A (2). On 10% Pd-C dehydrogenation it also gave the 1,4-dimethyl-7-(1',5'-dimethyl)exyl-azulene. Carbon-13 resonance data (Table I) revealed that there are six sp^2 carbons and hence only three double bonds. Therefore the remaining three degrees of unsaturation implied by the formula 2 must be due to rings. The carbon-13 data further showed that one oxygen atom in dictyol A is present in the form of a secondary alcohol and the second one is present in the form of ether and that the carbons directly attached to the oxygen carry one and two hydrogens, respectively. Like to pachydictyol A, dictyol A (2) formed an acetate with difficulty and was oxidized with Jones reagent to a ketone (i.r. 1700 cm^{-1}, 7-ring C=O). In the ^{1}H-n.m.r. spectrum of 2 (Table II) the CHOH resonates at δ 4.07 (shifted at δ 5.4 on acetylation) as dd coupled to H-5 and H-7 (J 9, 3 Hz; decoupling) while the ethereal methine proton resonates at δ 4.64 as bd coupled to H-1 (J 6 Hz; decoupling). The ^{1}H-n.m.r. signals are in Table II. Addition of Eu(fod-d_9)$_3$ caused downfield shifts (Table II) indicating that the ethereal oxygen and not the sterically hindered hydroxy group is involved in complex formation. Only in few cases to date has the donor property of a hydroxyl function been exceeded by competing groups[9]. Analysis of the Eu-n.m.r. spectrum by decoupling evidenced further couplings between H-2 and H-3 and established the existence of allylic couplings between the latter and the C-17 methyl protons. Allylic couplings



1 ; R = H
3 ; R = OH



2

TABLE I - Carbon shifts (p.p.m., TMS = 0) of diterpenes 1, 2, and 3

	1	2	3
C-1	46.54 (+0.48)	48.44 (+0.41)	43.03 (+0.75)
C-2	34.25	85.76 (±0)	33.84 (b) (+0.31)
C-3	124.01	123.87	123.83
C-4	141.53	142.84	140.90
C-5	60.76 (-3.17)	61.33 (-2.51)	61.25 (-2.73)
C-6	75.41 (+8.05)	74.61 (+6.55)	74.91 (+7.24)
C-7	47.85 (-1.97)	45.02 (-1.19)	43.83 (-1.54)
C-8	23.78	25.66	33.44 (b) (-3.50)
C-9	40.46	121.37	76.43 (+4.42)
C-10	152.55	151.20	154.48 (-6.79)
C-11	35.19	34.21	34.97
C-12	35.40	35.26	35.13
C-13	25.91	26.43	25.70
C-14	124.94	124.59	124.57
C-15	131.25	131.95	131.54
C-16	25.61	25.66	25.70
C-17	15.74	15.79	15.61
C-18	107.17	75.00	104.04 (+3.34)
C-19 (a)	17.63	17.38	17.46
C-20 (a)	17.60	17.67	17.70

Spectra were determined in $CDCl_3$ at 25.20 MHz on a XL-100 Varian F.T. spectrometer, operating in proton-noise decoupled and off-resonance decoupled modes; TAI-induced shifts[8] in parentheses.
(a) The C-20 high field methyl resonance was differentiated from the C-19 one by its typically lower signal intensity connected with a relatively longer T_1 relaxation time[5].
(b) Assignements may be reversed and accordingly the TAI-shift for C-8 should be -3.94.

between H-9 and H-18 were also evidenced. All this spectral evidence coupled with ^{13}C-n.m.r.data (Table I), wich moreover showed the presence of the isooctene side chain, led to the structure 2 for dictyol A. The ^{13}C chemical shifts assignements, which were straightforward when compared to those of 1 and 3, have been confirmed by a series of selective $^{13}C-\{^1H\}$ decoupling experiments for all protonated carbon atoms.

In order to find the algal source of these unusual diterpenes, we examined the gut contents of

TABLE II - Eu(fod-d_9)$_3$ induced shifts and chemical shifts (p.p.m., TMS = 0) in 2

Proton position	1	2	3	5	6	9
$\delta(CDCl_3)$ (a)	3.14(bm)	4.64(bd;6)	5.40(m)	2.81(bm)	4.07(dd;9,3)	5.48(m)
$\Delta\delta_{Eu}^{n=1}$ (b)	3.53	8.33	3.45	3.96	2.32	2.32
Proton position	**14**	**16**	**17**	**18**	**19**	**20**
$\delta(CDCl_3)$ (a)	5.10(t,7)	1.62(s)	1.87(s)	4.30,4.40(ABq,11)	0.99(d,6)	1.70(s)
$\Delta\delta_{Eu}^{n=1}$ (b)	0.40	0.29	0.93	7.06,7.68	0.82	0.21

(a) multiplicities and coupling constants (Hz) in parentheses.

(b) $\Delta\delta_{Eu}^{n=1} = \delta_{Eu}^{n=1} - \delta_{Eu}^{n=0}$ ($CDCl_3$); $\delta_{Eu}^{n=1}$ was obtained by simple extrapolation of the concentration lines to the point where the molar ratio complex to substrate is one (n=1).

A. depilans and found that it had been eating mainly the brown alga Dictyota dichotoma. When we started to extract samples of the alga we became aware that Fattorusso, Piattelli and colleagues had just completed the examination of this alga and found dictyol A and B as the major components. Examination of the gut contents of A. limacina and A. punctata, the digestive glands of them have proved to be deprived of any guaiane diterpenes, revealed that these sea hares had been eating mainly red algae, but not any Dictyota.

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