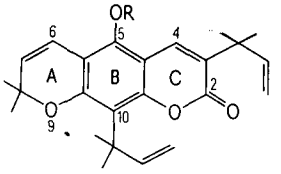
tion of alkali resulted in a bathochromic shift in the UV-spectrum. A first order analysis of NMR-spectrum (60 MHz; $CDCl_3$) of **1** revealed the presence of 2 sets of overlapping signals of the ABX-type arising from vinyl groups attached to quaternary carbons. 2 sharp singlets at τ 8.59 and 8.39 (6H each) due to 2 pairs of $>C<^{CH_3}_{CH_3}$ groups, permitted the assignment of 2 C_5H_9 fragments as 1,1-dimethylallyl groups in clausarin. A singlet at τ 8.54 for 2 equivalent methyl groups together with 2 olefinic protons forming an AB quartet at τ 4.37 and 3.34 (J = 10.0 Hz) indicated the presence of a dimethylchromene nucleus as a part of **1**. 1 proton resonating at τ 2.05 was identified as C_4–*H*, thus suggesting the attachment of one of the C_5H_9 chains at C_3 of the clausarin nucleus.

On acetylation with pyridine-acetic anhydride, clausarin gave an acetate (**2**), $C_{26}H_{30}O_5$, M^+ 422, m.p. 120 °C, ν_{max}^{KBr} 1770 (OAc), 1715 (C=O), 1615 and 1598 cm^{-1} (unsaturation). The upfield shifts of C_6–*H* at τ 3.73 and C_4–*H* at τ 2.69 in its NMR-spectrum[4] decided a) the location of the OH-function at C_5 and b) the linear fusion of the ring A with the coumarin nucleus. This mode of formation of the chromene ring and the placement of the remaining C_5H_9 unit at C_{10} got additional support from the failure of clausarin to yield a furan derivative under acid catalysed conditions. This established the structures **1** and **2** for clausarin and its acetate respectively.

The proposed structre **1** for clausarin received further support from EI mass spectral fragmentation pattern which showed the expected molecular ion peak at m/e 380. The cracking pattern was characterized by the loss of a methyl radical (m/e 365) and the subsequent loss of other functional groups thus showing fragment ions at m/e 337 [M^+–(Me+CO)], 312 (M^+–C_5H_8), 311 (M^+–C_5H_9), 309 [M^+–(Me+2CO)], 297 [M^+–(Me+C_5H_8)], 283 [M^+–(C_5H_9+CO)], etc. The other feature of the spectrum was the abundance of doubly charged ions at m/e 190.5 and below which indicated the aromatic nature of clausarin[5].



(**1**) R = H
(**2**) R = Ac

1 T. R. Govindchari, B. R. Pai, P. S. Subramaniam and N. Muthurkumaraswamy, Tetrahedron *24*, 753 (1968).
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4 A. Arnone, G. Cardillo, L. Merlini and R. Mondelli, Tetrahedron Lett. 4201 (1967).
5 CDRI Communication No. 2209.

Further perhydroazulene diterpenes from marine organisms

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Summary. 3 novel diterpenes, **4**–**6**, having the perhydroazulene skeleton already found in pachydictiol A (**1**) and dictyol A (**2**) and B (**3**), have been isolated from both the digestive gland of Aplysia depilans and algae of the family Dictyotaceae.

In 1973 Hirschfeld et al.[1] reported the isolation from the Pacific brown alga Pachydictyon coriaceum of a diterpene alcohol (pachydictyol A, **1**) with a perhydroazulene skeleton previously unknown among diterpenes. More recently, we isolated 2 related compounds, dictyol A (**2**) and B (**3**), from the digestive gland of Aplysia depilans[2] and the alga Dictyota dichotoma[3], on which the sea hare is known to feed.

In pursuing the investigation of the chemical constituents of the mollusc and the brown algae of the family Dictyotaceae, we have now isolated 3 further diterpenes based on the same perhydroazulene skeleton, and accordingly named dictyol C (**4**), D (**5**) and E (**6**). Compounds **4** and **5** have been isolated as minor components from both the digestive gland of A. depilans and the alga D. dichotoma, while the diol **6** has been obtained as the major diterpene of the alga Dilophus ligulatus (Dictyotaceae).

Isolation of the compounds from the ether-soluble portion (20 g) of an acetone extract of the digestive glands from 3 sea hares, collected near Naples, was accomplished by saponification and repeated silica-gel chromatography (increasing concentrations of ether in light petroleum) of the unsaponifiable fraction. The metabolites from the algae were separated by chromatography of the chloroform extracts of the dried material, collected near Catania, without prior saponification[4].

Material and methods. Dictyol C (**4**), m.p. 68 °C (from n-hexane), $[\alpha]_D$ −16.6 °C (c 1, $CHCl_3$) has molecular formula $C_{20}H_{34}O_2$ (accurate mass measurement). The consecutive losses of 2 molecules of water in its mass spectrum suggest that both oxygen atoms in **4** are present in hydroxyl groups. Furthermore, the spectrum of **4** is characterized by the same pattern of peaks already observed in the spectrum of **3** and corresponding to the loss of the iso-

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4 20 g of crude extract from the digestive glands of 3 adult sea hares yielded 170 mg of dictyol C (**4**) and 250 mg of dictyol D (**5**); **4** and **5** were also obtained from D. dichotoma in 0.1 and 0.01% yield of the dry material, respectively; D. ligulatus furnished dictyol E (**6**) in 0.5% yield.

Table 1. ^{1}H-NMR-spectra of dictyol C, D, E[a]

Proton position	Pachydictyol A[b] (1)	Dictyol C (4)	Dictyol D (5)	Dictyol E (6)
1		2.21 (b)	2.62 (b)	–
2		–	4.50 (b, $W_{1/2}$ 10)	–
3	5.30 (m)	5.26 (b, $W_{1/2}$ 5)	5.56 (b, $W_{1/2}$5)	5.32 (b, $W_{1/2}$5)
5		2.74 (b, $W_{1/2}$ 14)	2.94 (bt,10)	–
6	3.85 (bd)	3.87 (dd; 9,3)	3.86 (bd, 9)	4.18 (bd, 9)
14	5.10 (t)	5.14 (bt, 6)	5.11 (b)	5.14 (bt, 6)
16	1.60 (s)	1.62 (s)	1.61 (s)	1.62 (s)
17	1.75 (s)	1.85 (s)	1.92 (s)	1.82 (s)
18	4.72 (s)	1.22 (s)	5.11; 4.86 (each bs)[c]	4.75 (s)
19	0.97 (d)	1.00 (d, 6)	0.98 (d, 6)	1.23 (s)
20	1.67 (s)	1.70 (s)	1.70 (s)	1.70 (s)

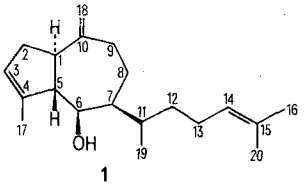
[a] Run at 100 MHz in $CDCl_3$ using TMS as internal standard. Values are in ppm (δ-scale). Multiplicities are indicated by the usual symbols. Figures in parentheses are coupling constants in Hz. Assignments were confirmed by decoupling. [b] Added for comparison[1]. [c] H-14 and H″-18 overlaps; H′-18 and H″-18 could be distinguished only after addition of Eu(fod)$_3$. –, Obscured signal.

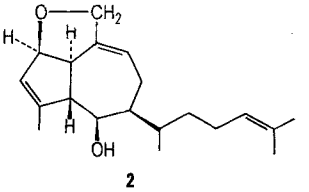
octene side chain, without or with transfer of 2 hydrogens from M^+-H_2O and M^+-$2H_2O$[5]. The ^{1}H-NMR-spectrum is strongly reminiscent of that of pachydictyol A (**1**) (table 1), apart from the replacement of the exomethylene signal by a 3H singlet at δ 1.22, indicative of a tertiary methyl group on oxygen-bearing carbon. These results led to structure **4** for dictyol C, whose ^{13}C chemical shifts compare very closely with those for pachydictyol A (table 2), taking into account the known substituent effects for an additional hydroxyl group[6]. Confirmatory evidence was provided by dehydration ($POCl_3$/pyridine at 0 °C for 20 min), which afforded a mixture of monohydroxy compounds from which pachydictyol A could be isolated and identified by comparison with an authentic sample. The stereochemistry of the C-10 hydroxyl (β) has been assigned on the basis of the relative magnitudes of the paramagnetic shifts induced by Eu(fod)$_3$ on the signals of 1-H and 5-H in its ^{1}H-NMR-spectrum [$\Delta\delta^{n=0.5}_{Eu}$ 1.68 (5-H), 0.68 (10-Me), 0.66 (1-H) and 0.48 (6-H); the steric hindrance of the 6-OH suppresses the complex formation at this site, as previously observed for **2** and **3**[2]].

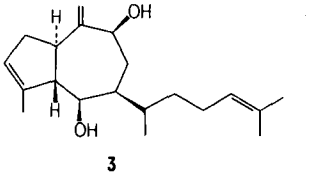
Table 2. ^{13}C chemical shifts in dictyol C, D and E[a]

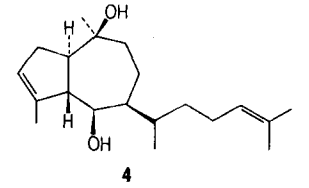
Carbon	Pachidyctyol A[b] (1)	Dictyol C (4)	Dictyol D (5)	Dictyol E (6)
1	46.54	50.04[c]	52.58[c]	46.23
2	34.25	32.95	74.47[a]	33.85
3	124.01	123.27	125.65	124.38[c]
4	141.53	142.70	147.79	140.88
5	60.76	52.77[c]	52.43[c]	60.34
6	75.41	74.47	75.06[d]	74.37
7	47.85	49.15	44.43	48.71
8	23.78	19.73	22.74	21.67
9	40.46	46.63	35.07[e]	40.55[d]
10	152.55	72.46	150.28	151.88
11	35.19	34.54	34.32	76.23
12	35.40	34.84	35.56[e]	40.99[d]
13	25.91	25.56	25.52	23.31
14	124.94	124.72	124.62	124.24[c]
15	131.25	131.27	131.37	131.67
16	25.61	25.69	25.67	25.68
17	15.74	16.28	15.81	15.81
18	107.17	30.01	111.80	107.50
19	17.63	17.48	17.68	25.38
20	17.60	17.67	17.34	17.65

[a] Spectra were run in $CDCl_3$ at 25.20 MHz on a XL-100 Varian F.T. spectrometer, operating in proton-noise and off-resonance decoupled modes. [b] Added for comparison[2]. [c–e] Assignment may be reversed.

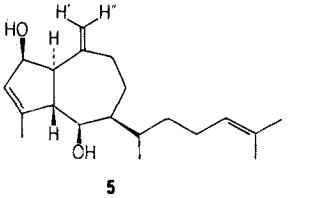


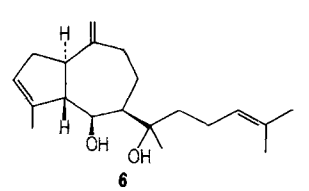






Dictyol D (**5**), oily product, $[\alpha]_D$ – 80 °C (c 2, $CHCl_3$), $C_{20}H_{32}O_2$ (precision mass spectrometry), shows in the mass spectrum the same fragmentation pattern as that of **3** or **4**[5]. The most important features in the ^{1}H-NMR-spectrum of **5** (table 1), when compared with that of **1**, are a) the appearance of an allylic carbinol methine proton as a multiplet at δ 4.50 which on irradation at





δ 5.56 (3-H) was converted to a sharp doublet (J 4.5 Hz), b) the splitting of the exomethylene signal into 2 broad singlets, and c) the downfield shifts of the 3-H and 4-Me signals. These data accord well with structure **5**. Chemical confirmation was obtained by lithium-ethylamine re-

5 Mass spectral data: **4**, m/e 306 (2, M^+), 288 (18), 270 (49), 177 (71), 175 (51), 159 (47) and 69 (100); **5**, m/e 304 (3, M^+), 286 (2), 268 (3), 175 (7), 173 (6), 157 (10) and 69 (100).

6 J. B. Stothers, in: Carbon-13 NMR Spectroscopy. Academic Press, New York 1972; C. Konno and H. Hikino, Tetrahedron *32*, 325 (1976).

duction of dictyol D to a dihydrodehydroxy derivative (M^+/e 290) which was identical (GLC, MS and NMR) with 10,18-dihydropachydictyol A, prepared from authentic **1** by the same treatment. The ^{13}C chemical shifts assignment for **5** was straightforward. The upfield shifts of the resonances of carbons 7 and 9 (ca 3 and 5 ppm, respectively) in comparison with those of the corresponding carbon atoms in **1** are probably due to the crowded environment caused by the presence of the hydroxyl group at C-2, which constrains the 7-membered ring in a somewhat rigid conformation. The stereochemistry at C-2 was deduced from the ^1H-NMR-spectra after gradual addition of $Eu(fod)_3$: since 5-H experiences a paramagnetic shift larger than 1-H, it must be nearer to the hydroxyl group [$\Delta\delta\ ^{n=0.5}_{Eu}$ 3.23 (2-H), 2.33 (5-H), 1.68 (3-H), 1.51 (18′-H), 1.41 (1-H), 0.99 (6-H) and 0.81 (18″-H)]; as already observed for dictyol C, the complex formation at 6-OH is suppressed by steric hindrance.

Dictyol E (**6**), oily product, $[\alpha]_D$ + 26.8 °C (c 1, $CHCl_3$), has molecular formula $C_{20}H_{32}O_2$ (accurate mass measurement). In its mass spectrum diagnostically important peaks are seen at m/e 286 (21%, M^+-H_2O), 268 (5, M^+-$2H_2O$), 177 (6, M^+-side chain), 175 (11, M^+-2H-side chain), 157 (24, M^+-H_2O-side chain) and 155 (10, M^+-H_2O-2H-side chain). The salient difference between the ^1H-NMR-spectrum of **6** and that of **1** resides in the absence of the methyl doublet at δ 0.97, which is replaced by a 3H singlet at δ 1.23. This located the hydroxyl group in the side chain and led to formulation **6** for dictyol E. The ^{13}C-NMR-data (table 2) definitely confirmed the proposed structure and permitted the assignment of the stereochemistry at the chiral centres in the nucleus.

Effects of increased intracellular Ca^{2+} on cyclic nucleotides production by liver tissue[1]

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Summary. During hepatointoxication, the increase of intracellular Ca^{2+} is accompanied by an increase of cAMP. This reversible phenomenon suggests that the production of cAMP is likely to be a response of the cell in order to activate the exclusion of Ca^{2+}.

Calcium ion and cyclic 3′,5′-adenosine monophosphate (cAMP) are interrelated in the control of a variety of functions of the mammalian cell[3,4]. They are considered the second messenger which translates event at the cell surface into modified activity within the cell[5]. On the other hand, there is evidence that cyclic 3′,5′-guanosine monophosphate (cGMP) is also involved in the regulation of a number of metabolic and mitogenic processes[6].

A convenient model for the study of the in vivo effects of Ca^{2+} concentration on cAMP and cGMP syntheses is the acute hepatointoxication with thioacetamide (TAA) which produces a reversible manifold increase in intracellular Ca^{2+}[7]. On this basis, and in order to correlate the variations of intracellular liver calcium with the changes in rate of cAMP and cGMP syntheses, male Wistar rats (body weight: 200–240 g) were given orally 100 mg/kg b.wt of TAA as a 2% aqueous solution. 5-animal-groups were sacrificed at different times after TAA administration. The livers were sliced with scissors and aliquots of the sliced tissue were ashed at 700 °C, the ashes dissolved in 1 N HCl, and calcium and magnesium determined by atomic absorption spectrometry. In addition to this, 1 g samples of liver tissue were homogenized and deproteinized with trichloroacetic acid[8]. Cyclic AMP and cGMP were determined using radioimmunoassays kits obtained from The Radiochemical Centre, Amersham, England. Similar determinations were performed on liver tumors (cholangiocarcinomas) induced by prolonged feeding of rats with 4-dimethylaminoazobenzene[9].

The administration of TAA provoked a reversible increase of calcium in liver tissue with a maximum of approximately 24 times the normal value at 24 h after the administration of the hepatotoxic substance (figure 1). The concentration of cAMP showed a fairly similar pattern of increase, but its maximum value was observed after 48 h. At this moment, the concentration of Ca^{2+} was only 4

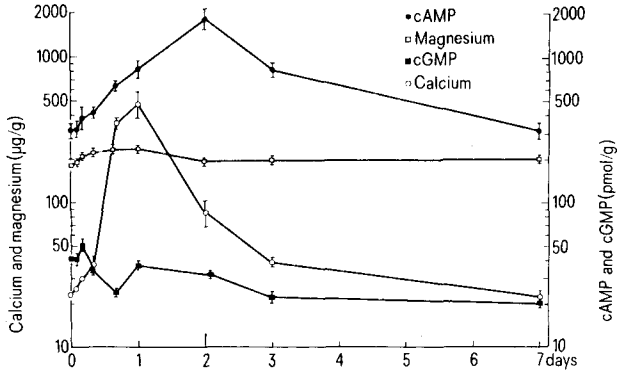

Fig. 1. Calcium, magnesium, cAMP and cGMP concentrations in liver of rats given thioacetamide. Each point corresponds to the mean value ± SE of a 5-animal-group.

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2 Request for reprints to be sent to Dr Anghileri. Present address: New England Nuclear Radiopharmaceutical Division, Atomlight Place, North Billerica, Mass. 01862, USA.

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