mine concentration in the range $0.25 \cdot 10^{-4}$ to $3 \cdot 10^{-4}$ mol/l, corresponding to a content of thiamine between 8.4 and 101 μg/ml. Such results suggest that this colour reaction may be used in the spectrophotometric dosage of thiamine, because of the rapidity and simplicity of the procedure with respect to other methods[8–11]. Similar investigations for thiamine pyrophosphate are in progress.

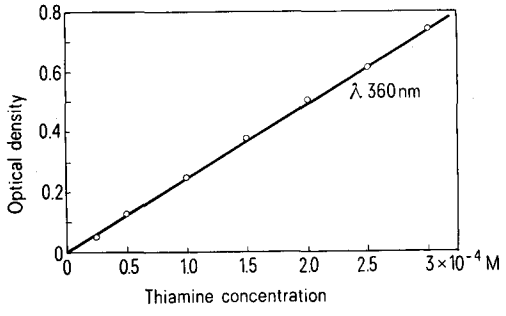
Experimental. Visible spectra were recorded using a Rank Precision Uvichem H 1600 S.T. Spectreophotometer; 1-cm stoppered fused silica cells were used. Thiamine chloride hydrochloride and 2,3-dichloro-1,4-naphtoquinone (Fluka AG, Buchs, Switzerland) were reagent grade for analysis. Dimethylformamide (DMF) was reagent grade for spectrophotometry.

Preparation of adduct. A DFM solution (5 ml) of 2,3-dichloro-1,4-naphtoquinone (0.45 g; 0.02 mol/l was added to the solution of 1.35 g (0.04 mol) of thiamine chloride hydrochloride in 10 ml of methanol. This mixture, treated with 2 ml of ammonium hydroxide, (26°Bé) immediately assumed a deep red colour. The reation product begins to separate at room temperature, and within 2 h the precipitation is almost complete. The collected product, washed with ethanol, was crystallized a few times from warm methanol and small red crystals were obtained (m.p. 235°C). UV λ_{max} at 235, 280, 470 nm (ε = 36200, 33000, 3400 respectively). IR-bands at 1669 cm^{-1} and 1650 cm^{-1} (νCO); 732 cm^{-1} (δC–H); 690 cm^{-1} (νC–S). The presence of the quinone nucleus has been detected as follows: a small amount of the adduct was suspended in methanol and a small quantity of $NaBH_4$ was added. To the decolourized solution, a few drops of H_2SO_4, 0.1N were added to destroy the excess $NaBH_4$. The mixture was gently warmed for a few min and then treated with a benzene solution of dehydroindacum: immediately it assumed the intense blue coloration due to indacum[7].

Calibration curve. Solutions of thiamine chloride hydrochloride and of 2,3-dichloro-1,4-naphtoquinone in DMF, both 10^{-3} M were prepared. The DMF solution of thiamine contained also 12% by volume of water to facilitate dissolution. The absorption spectrum of the DMF solution of each component was transparent, in the range 360–500 nm, whereas the ammonia DMF solution of the quinone showed appreciable absorbance. The wave length of 360 nm was selected as the most appropriate for the calibration curve. To this purpose a series of samples was prepared by adding known volumes (in the range 0.25 to 3 ml) of DMF 10^{-3} M thiamine solution to 4 ml of DMF 10^{-3} M 2,3-dichloro-1,4-naphtoquinone solution. Each of these samples was treated with 0.1 ml of ammonium hydroxide (26°Bé) and diluted to a constant volume of 10 ml with DMF. The samples were allowed to stand for 5 min before reading the optical densities. The reference sample was made adding 0.1 ml of ammonium hydroxide (26°Bé) to 4 ml of DMF 10^{-3} M 2,3-dichloro-1,4-naphtoquinone solution and diluting to 10 ml with DMF. The figure shows the linear correlation between optical density and thiamine concentration in the range $0.25 \cdot 10^{-4}$ to $3 \cdot 10^{-4}$ mol/l, corresponding to a content of thiamine chloride hydrochloride between 8.4 and 101 μg/ml.

Colour reactions of thiamine chloride hydrochloride with several quinones in the presence of ammonium hydroxide

Benzoquinone	red-orange
Toluquinone	brown
1,4-naphtoquinone	red-orange
2-methyl-1,4-naphtoquinone	yellow-orange
2,3-dichloro-1,4-naphtoquinone	red
1,2-naphtoquinone	wine-red
Chloranil	red-orange
Cacotheline	red-brown



Plots of optical density vs. thiamine chloride hydrochloride concentration according to the law of Lambert-Beer.

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Clausarin – a novel coumarin from Clausena pentaphylla (Roxb.) DC.

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Summary. Clausarin (**1**), a novel coumarin, has been isolated along with methyl linolenate, dentatin, clausenidin, β-sitosterol and heptaphylline from the roots of Clausena pentaphylla. Based on spectroscopic evidence, its structure has been established as 3,10-bis (1,1-dimethylallyl)-8,8-dimethyl-5-hydroxy-2H,8H-benzo (1,2-b:5,4-b′) dipyran-2-one.

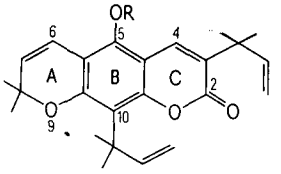
The ethanolic extract of the roots of Clausena pentaphylla (Roxb.) DC. (Rutaceae) on fractionation and column chromatography over neutral alumina afforded 6 compounds. Of these, 5 were characterized as methyl linolenate (GLC), dentatin[1,2] (major component), clausenidin[2], β-sitosterol and heptaphylline[3]. The sixth one, obtained as a minor constituent is a new coumarin and has been named as clausarin. The present communication is concerned with the elucidation of its structure as 3,10-bis(1,1-dimethylallyl)-8,8-dimethyl-5-hydroxy-2H,8H-benzo (1,2-b:5,4-b′) dipyran-2-one (**1**).

Clausarin, $C_{24}H_{28}O_4$, M^+ 380, m.p. 208 °C (d) showed absorption at ν_{max}^{KBr} 3220 (OH), 1670 (C=O), 1610 and 1590 cm^{-1} (unsaturation); $\lambda_{max}^{CH_3OH}$ 232 (log ε 3.33), 282 (3.44) and 338 nm (3.21) respectively similar to nordentatin[2]. Addi-

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

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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.