

a formula of $C_{15}H_{24}$ and a relatively small peak for $[M-15]^+$ 189. The IR-spectrum showed a typical carbon-carbon double bond absorption. The NMR-spectrum of this compound was compared with **1**. There were distinguishing differences. The multiplet at δ 0.45 present in **1** had disappeared, the singlet at δ 1.15 became a doublet at δ 1.73 ($J = 1.5$ Hz) and a vinyl proton was noted [δ 5.62 (m, $J = 1.5$ Hz)]. The rest of the NMR-spectrum was relatively unchanged.

Since cupric acetate is known to cause isomerization in tricyclic systems^{8,10}, we concluded from the spectral data that the 3 membered ring in **1** had been opened to form a tricyclic sesquiterpene. This same compound was formed by reaction of **1** with gaseous HCl in ether followed by dehydrohalogenation.

While this work was in progress GOVINDACHARI et al.^{11,12} reported the isolation of a tetracyclic sesquiterpene, ishwarone **3** and ishwarane, from *Aristolochia indica* (Aristolochiaceae). The stereochemistry has been recently proven¹³. During their structural elucidation of ishwarone the authors formed isoishwarane **2**. From their NMR-data we suspected our tricyclic sesquiterpene to be identical to isoishwarane. An authentic sample of **2** has superimposable IR-, NMR-mass spectra, and retention time on GLC.

The spectral data reported for ishwarane is identical with our tetracyclic sesquiterpene from the oil of orejuela.

Therefore the identity of these compounds was unambiguously established.

Résumé. De l'éther de pétrole extrait de l'huile de l'Orejuela (*Cymbopetalum penduliflorum* Dunal, Baill) fut examiné pour son contenu en terpènes. On a isolé les sesquiterpènes tétracycliques (ishwarone). L'évidence chimique et spectral appuie la constitution chimique et sa conversion à isoishwarone.

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Isolation and Characterization of Hallachrome, a Red Pigment from the Sea Worm *Hallaparthenoidea*¹

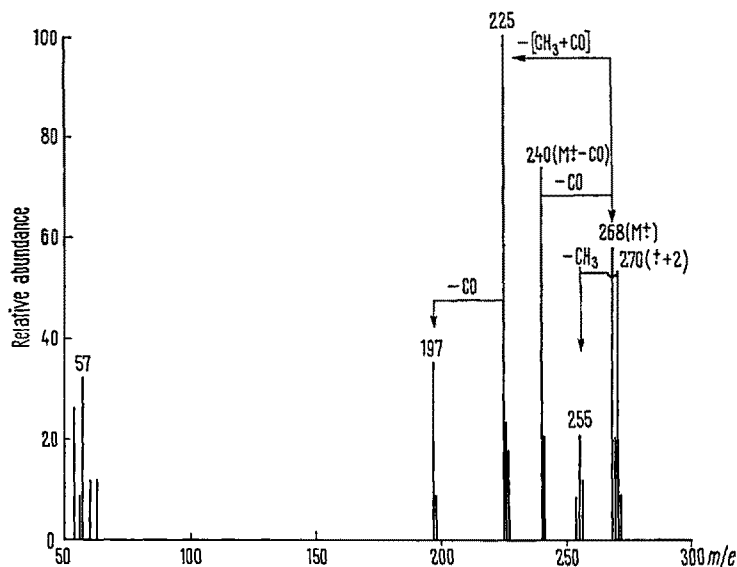
Hallachrome is a red pigment occurring in the epidermal epithelial cells of *Halla parthenopeia*², a rare polychaete found chiefly in the Bay of Naples. Controversial publications on this pigment, which was originally thought³ to have the structure of 2,3-dihydroindole-5,6-quinone-2-carboxylic acid, have appeared in the literature⁴⁻⁹.

Being so fortunate as to have access at the Zoological Station of Naples to a number of specimens of *Halla parthenopeia*, we took the opportunity to examine Hallachrome anew. Considering the discrepancies in the literature

and the reported instability of Hallachrome, our attention was initially directed to find the mildest conditions for the extraction of the natural form of the pigment. To achieve this purpose, pieces of living worms were plunged into different solvents and the resulting extracts were analyzed at regular intervals by TLC on silica (F_{254} , Merck), using as eluents chloroform-ethanol 95:5, v/v and *n*-butanol-acetic acid-water (60:20:20, v/v).

By this procedure it was found that the red pigment could be selectively extracted without modification with chloroform at room temperature. Consequently, the following procedure was developed for the isolation of Hallachrome in almost quantitative amount: 8 living specimens of *Halla parthenopeia*, between 20 and 30 cm in length, were plunged into chloroform (100 ml) and after 30 min the resulting red extract was decanted and the worms re-extracted a few times until the chloroform assumed a pale pink colour. The combined extracts were filtered and, after addition of benzene (15 ml), were concentrated under reduced pressure to a volume of about 10 ml. The solution was then chromatographed on a 3×40 cm polyamide column (Macherey, Nagel and Co.), using $C_6H_6-CHCl_3$ (20:80, v/v) as the eluent. On concentration to a small volume and standing overnight at 4°C, the red band gave 68 mg of Hallachrome, as deep-red prisms, mp 224–226° (dec), slightly soluble in ethanol, insoluble in water and in aqueous sodium bicarbonate.

The purified pigment showed no optical activity and displayed absorption maxima



Mass spectrum of Hallachrome.

(MeOH) at 500, 312 and 250 nm ($\log \epsilon$ 3.75, 4.52, 4.51): on addition of alkali the colour changed reversibly to green with λ_{max} at 650, 377, 316 and 271 nm. Elemental analyses and mass spectrometry¹⁰ revealed that the pigment did not contain both nitrogen and sulphur and suggested the molecular formula $C_{18}H_{12}O_4$, including one methoxyl group (Found: C, 71.74; H, 4.60; OCH_3 , 12.03. $C_{18}H_{12}O_4$ requires: C, 71.61; H, 4.47%). Apart from the molecular ion peak at m/e 268, the mass spectrum of Hallachrome (Figure 1) showed a prominent $[M+2]^+$ ion peak and diagnostic fragment ions at m/e 255, 240, 225 and 197, suggesting a quinonoid structure.

In agreement with this view, Hallachrome showed redox properties and, under very mild conditions, reacted with *o*-phenylenediamine to give a crystalline quinoxaline derivative, $C_{22}H_{16}N_2O_2$ (M^+ at m/e 340). Moreover, on reductive acetylation, the pigment afforded a leuco triacetate, $C_{22}H_{20}O_7$ (M^+ at m/e 396), mp 148–149°, which had an anthracene-type absorption spectrum [λ_{max} ($\log \epsilon$): 389 (3.71), 369 (3.79), 351 (3.67), 335 (3.45), 253 (4.97)]. From these results it was concluded that Hallachrome was a hydroxy-methoxy-methyl-1,2-anthraquinone.

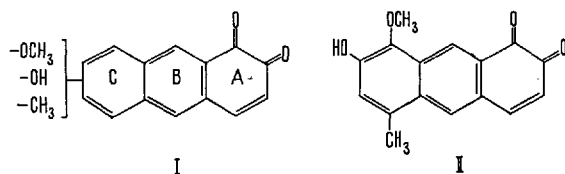
Further information on the structure of the pigment was obtained from the NMR-spectrum¹¹ (DMSO- d_6) which showed the presence in the aromatic region of an AB quartet centred at δ 6.31 and 7.68 (J, 10 Hz) and three 1H singlets at δ 7.50, 7.80 and 8.42. Comparison of the NMR data with those of some synthetic 1,2-anthraquinones, recently described by BOLDT^{12,13}, confirmed the chemical nature of the chromophore and suggested for the pigment the partial structure I, in which ring A and B are unsubstituted. Thus Hallachrome is the first anthraquinone pigment which is unsubstituted at positions 9 and 10. Further experiments are now required to

ascertain the positions of the substituents which are probably arranged as shown tentatively in II¹⁴.

Riassunto. Viene descritto l'isolamento e la caratterizzazione dello Hallachrome, il pigmento epiteliale del verme marino *Halla parthenopeia* che è stato oggetto di numerose ricerche fin dal 1931. Sulla base dei risultati ottenuti si può concludere che il pigmento, contrariamente a quanto riportato da altri Autori, è un metilmetossi-ossi-1,2-antrachinone (I). Sono in corso ulteriori ricerche per giungere alla definizione completa della struttura del pigmento, che è il primo antrachinone rinvenuto in natura non sostituito nelle posizioni 9 e 10.

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Identity of RW-47 and Venoterpine and Determination of their Absolute Configuration

Interest in the structure and stereochemistry of the naturally occurring 1,2,3-trisubstituted cyclopentanoid monoterpenes has been heightened by recent discoveries dealing with the biosynthesis of the non-aromatic portion of the complex indole alkaloids¹. In this respect, the monoterpene alkaloids of the actinidine group are especially interesting because of their intermediate complexity and their occasional co-occurrence in plants rich in indole bases. Recent papers have described the isolation and structural characterization of RW-47² and venoterpine³. These alkaloids were immediately recognized to be very similar in properties and, although there were some initial questions^{3,4}, we have now been able to perform a direct comparison and are convinced that they are identical. Their IR-spectra (Nujol) differ only in very minor respects and a mixture melting point was not depressed. Their UV-spectra were identical and the samples could not be resolved in 3 quite different TLC

systems⁵. Their NMR-spectra, initially thought to differ³, were found to be virtually identical when both samples were run at 60 MHz (CDCl₃- d_2 O). Diastereoisomeric substances would necessarily differ more substantially in properties and ORD-CD studies (vide infra) demonstrate that the alkaloids are not enantiomeric.

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