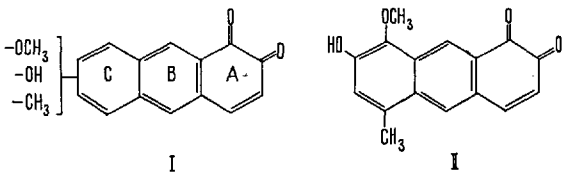
(MeOH) at 500, 312 and 250 nm (log ε 3.75, 4.52, 4.51): on addition of alkali the colour changed reversibly to green with λ_{max} at 650, 377, 316 and 271 nm. Elemental analyses and mass spectrometry[10] revealed that the pigment did not contain both nitrogen and sulphur and suggested the molecular formula $C_{16}H_{12}O_4$, including one methoxyl group (Found: C, 71.74; H, 4.60; OCH_3, 12.03. $C_{16}H_{12}O_4$ 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 ε): 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-spectrum[11] (DMSO-d_6) which showed the presence in the aromatic region of an AB quartet centrated 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 II[14].



I

II

Riassunto. Viene descritto l'isolamento e la caratterizzazione dello Hallacromo, il pigmento epiteliale del verme marino *Halla parthenopeia* che è stato oggeto 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 alkaloids[1]. 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-47[2] and venoterpine[3]. 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 systems[5]. Their NMR-spectra, initially thought to differ[3], were found to be virtually identical when both samples were run at 60 MHz ($CDCl_3$–^+D_2O). 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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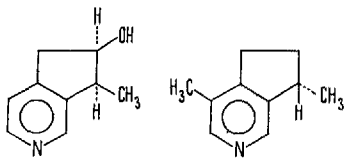
The curves (all measurements in methanol) are in fact identical within experimental limits. The very minor differences in properties are most likely due to the presence of slight impurities, poor stability of the alkaloid, and the different histories of the 2 samples. Unfortunately, the 2 materials are rare and sufficient quantities are not available for a more detailed comparison.

Comparison of the ORD-CD spectra with that of L-(−)-actinidine (II), whose absolute configuration is unambiguously known[6], demonstrated an enantiomeric relationship at the benzylic position. The stereochemistry of the benzylic position in diastereoisomeric aryl compounds is known to determine the sign of the derived Cotton effects, and asymmetry at more distant centers affect the intensity of the peaks[7]. Since detailed NMR studies on RW-47 clearly demonstrate a *cis* relationship between the methyl and hydroxyl groups, the ORD-CD findings require expression I for the absolute configuration of RW-47-venoterpine[2]. The poor asymmetry of II relative to its extinction coefficients prevent obtention of accurate CD values. The signs of the peaks are qualitatively consistent with the assignment.



I II

I. $[\Phi]_{300}$ +300, $[\Phi]_{265}$ −860, $[\Phi]_{260}$ −360, $[\Phi]_{258}$ −710, $[\Phi]_{235}$ +2060. $[\theta]_{264}$ +900, $[\theta]_{253}$ −4840.

II. $[\Phi]_{300}$ −2, $[\Phi]_{270}$ +4.5, $[\Phi]_{265}$ −5, $[\Phi]_{255}$ −12.5, $[\Phi]_{235}$ −6.5, $[\Phi]_{225}$ −12. $[\theta]_{268}$ neg., $[\theta]_{260}$ pos., $[\theta]_{240}$ neg.

It is interesting to note that the actinidine alkaloids occasionally have the opposite absolute configuration to the glycosides implicated in indole biosynthesis and, presumably, present in these same plants. The potential significance of this finding is not clear, especially as current thinking about the detailed pathways between the glycosides and the complex indole alkaloids involves destruction of the stereochemistry at both centers corresponding to those in RW-47-venoterpine during the course of their further biological elaboration[1].

Further details of these experiments and stereochemical relationships of the complex indole bases of *Alstonia venenata* will be reported in a full paper in preparation[8].

Zusammenfassung. RW-47 und Venoterpin sind identisch. Die absolute Konfiguration ist durch ORD-CD-Vergleiche mit L-(−)-Actinidin ableitbar.

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Acidolytic Cleavage of Peptide Bonds During Acetylation

Gas-chromatographic determination of amino acids was performed by JOHNSON et al.[1] using N-acetyl amino acid *n*-butyl esters. The derivatization was carried out by esterification followed by acetylation. In the present experiments acetylation was carried out first in order to allow quantitative methyl ester formation by diazomethane. Derivatization of some simple dipeptides led to formation of considerable quantities of the N-acetyl methyl esters of the constituent amino acids (Figure), presumably through oxazolone formation. A similar observation recently published[2] has made us communicate the results presented below.

For calibration purposes a number of N-acetyl amino acids described in the literature[3] were synthesized and characterized by CHN-analysis. The flame-ionization detector response to each derivative was determined by dissolving a weighed quantity of N-acetyl amino acid in a measured volume of methanol containing an internal standard. Methylation was carried out quantitatively[4] by adding an excess of ethereal diazomethane. 1–5 µl of the resulting solution was injected into the gas-chromatograph (Panchromatograph, Pye Scientific Instruments Ltd., Cambridge, England).

Values within ± 5–10% were found for the derivatives of Gly, Ala, Val, Ile, Leu, Pro, Lys, Glu, Met, Phe, Tyr and Trp. Acetylation of the individual free amino acids was effected by boiling for 1 h in acetic acid containing by volume 10% acetic anhydride and 10% pyridine. After evaporation of the solvents and several evaporations with water to decompose residual anhydride, the

Yields of N-acetyl amino acids from peptides

Peptide	Derivative of	Yield (%)[a]	Peptide	Derivative of	Yield (%)[a]
Val-Leu	Val	37	Gly-Phe	Gly	78
	Leu	36		Phe	85
Glu-Val-Phe	Glu	ca. 50	Gly-Lys-HCl	Gly	105
	Val	68		Lys	88
	Phe	100	Gly-Trp	Gly	105
				Trp	88
Cys-Gly / Glu	Glu	ca. 80	Leu-Tyr	Leu	66
	Cys	–		Tyr	80
	Gly	69			

Reaction time: 1 h. For Glu-Val-Phe the reaction time was 4 h.
[a] Average of 2 determinations.