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# Caulerpin, a New Red Pigment from Green Algae of the Genus *Caulerpa**

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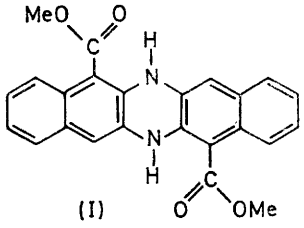
Caulperin ($C_{21}H_{18}N_2O_4$), a new red pigment, was isolated from three species of the green algal genus *Caulerpa*. On the basis of its u.v., i.r., n.m.r., and mass spectral data and chemical properties, it was assigned the structure dimethyl 6,13-dihydrodibenzo[*b,i*]phenazine-5,12-dicarboxylate (I).

THE genus *Caulerpa* of the family Caulerpaceae is represented by several green species considered as salad delicacies in the Philippines and other countries of the Pacific. The peppery nature of one of the most common edible varieties of *Caulerpa racemosa*, the var. *clavifera*, led to the undertaking of this investigation,[1,2] but the peppery principle has as yet eluded isolation. However, during the Soxhlet extraction of the powdered dry algae with ether, a red crystalline substance, which we named caulerpin, settled to the bottom of the flask. Further concentration of the ether extract caused more caulerpin to separate (total yield 0·63% dry wt.).

Caulerpin (I), $C_{24}H_{18}N_2O_4$ ($M^+$ 398),[3] forms red prisms from ether or acetone, m.p. 317°. Its u.v. and i.r. spectra [$\lambda_{max.}$ 222, 270, 292, and 317 mμ (ε 50,000, 27,000, 29,000, and 35,000); $\nu_{max.}$ 1684s, 1631s, and 1613s cm.$^{-1}$] suggested the presence of carbonyl functions in conjugation with aromatic groups. The strong




(I)

aromatic character of caulerpin is indicated by the i.r. bands at 3030, 1631, 1613, 1582, 1488, and 1445 cm.$^{-1}$, the n.m.r. signals at τ 2·4—3·0 and 1·79, and the fact that the mass spectrum shows the molecular ion as the base peak and the presence of doubly charged ions.[4] The n.m.r. spectrum indicated the presence of 18 protons: τ 6·17 [6H, s 2 × OMe], 2·4—3·0 (8H, m), 1·79 (2H, s), and —1·36 (2H, s). The aromatic proton signals at τ 2·4—3·0 and 1·79 and the i.r. bands at 730 and 920 cm.$^{-1}$ suggested the presence of two identically substituted condensed aromatic ring systems; this is substantiated by the elimination of 26 mass units (CH≡CH) [5] in the mass spectra of caulerpin, caulerpinic acid, and decarboxycaulerpinic acid.

Caulerpin contains two methoxy-groups in the form of αβ-unsaturated methyl ester systems [$\nu_{max.}$ 1685vs cm.$^{-1}$; τ 6·17 (6H)]. Its mass spectrum supports this assignment: [6] *m/e* 398 ($M^+$), 366 ($M$ — MeOH), 338 (366 — CO), 306 (338 — MeOH), 278 (306 — CO), 339 ($M$ — $CO_2Me$), and 280 ($M$ — $2CO_2Me$); *m** [7] 336·57, 312·14, 277·03, 252·56, 288·75, and 196·98.

Saponification of caulerpin (I) with alcoholic potassium hydroxide yielded caulerpinic acid, $C_{22}H_{14}N_2O_4$, $M^+$ 370, $\nu_{max.}$ 2700—2480 cm.$^{-1}$, which lacked the six-proton n.m.r. signal at τ 6·17.

The two-proton n.m.r. signal at τ —1·36 in the spectrum of caulerpin could only be due to secondary amino-groups (N, 7·05%; $\nu_{max.}$ 3380 and 1585 cm.$^{-1}$), which exchange with deuterium oxide. These groups must be in conjugation with the two methoxycarbonyl groups, as indicated by the low-frequency carbonyl absorption (1685 cm.$^{-1}$) similar to that of methyl *N*-methylanthranilate.[8] The methoxycarbonyl groups must therefore be located in the two α-positions of the two naphthalene rings, conjugated with the NH groups at the β-positions. This arrangement also accounts for the strong hydrogen bonding of the NH protons.

When heated with copper bronze in quinoline at 200—210°, caulerpinic acid yielded a decarboxy-compound, m.p. >300°, *m/e* 282 ($M^+$). The presence of a very small peak at *m/e* 300 might be due to the presence of a molecule of water which is strongly bound to one NH group.

The physical and chemical data described indicate that caulerpin is probably dimethyl 6,13-dihydrodibenzo-[*b,i*]phenazine-5,12-dicarboxylate. The stability of the compound favours the assignment of the linear structure rather than that of the geometric isomer, dimethyl-7,14-dihydrodibenzo[*a,h*]phenazine-6,13-dicarboxylate. Synthesis and *X*-ray analysis are at present under investigation.

## EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Samples for analysis were dried under vacuum ($P_2O_5$) at 100°. U.v. spectra were measured with a Cary 14M instrument for solutions in ethanol (1·295 and 1·870 mg. in 10 ml.; 1 cm. path length). I.r. spectra were determined for Nujol mulls. N.m.r. spectra were measured with Perkin-Elmer 60 MHz and Varian 100 MHz instruments, with tetramethylsilane as internal reference. Mass spectra were

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[7] J. H. Beynon, R. A. Saunders, and A. E. Williams, 'Table of Metastable Transitions,' Elsevier, London, 1966, pp. 605, 648, 669, 693, 721, 737.
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measured with an A.E.I. MS9 double-focusing spectrometer.

*Isolation of Caulerpin.*—Three species of *Caulerpa* were used: *C. racemosa*, *C. serrulata*, and *C. sertularioides*.

The air-dried algae were cut and powdered in a mill. A sample (*ca.* 1·4 kg.) of each species was Soxhlet extracted with ether (*ca.* 4 l.). The extract was concentrated to *ca.* 300 ml. and set aside to cool. Red prisms of caulerpin separated. Further concentration of the extract gave more caulerpin, m.p. 317° (from acetone) (total yield 0·55—0·63%).

*Saponification of Caulerpin.*—Caulerpin (1 g.) was saponified with potassium hydroxide (10 g.) in 60% ethanol (300 ml.). It dissolved completely only when heated. The ethanol was distilled off and the remaining basic aqueous solution was extracted with ether to remove unchanged caulerpin. It was then neutralised with 10% hydrochloric acid and extracted with ether; the extract was washed with distilled water, dried ($Na_2SO_4$), and evaporated to give dark brown crystalline caulperinic acid (835 mg.), m.p. 256° (sublimes).

*Decarboxylation of Caulerpinic Acid.*—Caulerpinic acid (600 mg.), copper bronze (60 mg.), and quinoline (4 g.) were mixed in a flask fitted with an air-condenser and heated on a metal-bath. Evolution of carbon dioxide began at 200° and heating was continued for 30 min. at 210° until gas evolution ceased. The quinoline was distilled off and the residue was extracted with ether (*ca.* 100 ml.). The extract was washed with sodium carbonate solution to remove unchanged caulerpinic acid, then with water, dried ($Na_2SO_4$), and evaporated to leave decarboxycaulerpinic acid (415 mg.), m.p. >300°. The compound was homogenous upon chromatography.

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