n-pentane. Subsequent fractionation of the methanolic phase was monitored by tasting the fractions in order to locate the exceedingly bitter taste of the beetles[3] and by testing their efficiency as repellent against the ant *Myrmica rubra*.

Repeated column chromatography on alumina, followed by several recrystallizations afforded 135 mg of a compound $C_{13}H_{23}NO$ as white needles charring at 235° without melting and for which the name coccinellin was coined. The empirical formula was established by high-resolution mass spectrometry: a molecular ion was measured at *m/e* 209.1777 (calculated for $C_{13}H_{23}NO$: 209.1779), with fragmentation peaks at *m/e* 193 and 192. Coccinellin shows only end-absorption in UV and its NMR-spectrum shows the absence of vinyl protons. The compound is soluble both in water and in methylene chloride. Coccinellin forms a hydrochloride that gives the same mass spectrum as the original product and displays prominent IR-bands at 2550 (broad) and 1530 cm^{-1} [4].

Our fractionation procedure also afforded 10 mg of another, less polar, amorphous compound $C_{13}H_{23}N$ (by mass spectrometry), precoccinellin. Our data strongly suggested that coccinellin is the N-oxide of precoccinellin and that relationship was confirmed by reduction of the N-oxide with ferrous sulphate.

Coccinellin is responsible for the bitter taste of *C. septempunctata* but not for its peculiar smell. Its efficiency as a defensive compound could be demonstrated by its activity towards ants. Water containing 0.5% coccinellin was almost completely refused by thirsty *Myrmica rubra* and even a concentration of 0.1% markedly prevented drinking. These concentrations are much smaller than that of the compound in the haemolymph of the beetle. Coccinellin can repel ants without direct contact with their mouthparts: evaporation of 2 mg from methylene chloride on a filter paper around their food prevented hungry ants from reaching it.

Coccinellin could be detected directly in the haemolymph of *C. septempunctata* by TLC on alumina plates. Neither coccinellin nor precoccinellin could be found in the aphids that constitute the prey of the beetle. Both compounds were detected in *C. undecimpunctata* L. but not in *Adalia bipunctata* L. nor in *Propylaea quatuordecimpunctata* L. Other coccinellid chemical defenses and the chemical structure of coccinellin are currently being investigated in our laboratories.

Résumé. Le composé utilisé pour la défense chimique de *Coccinella septempunctata* (Coleoptera, Coccinellidae) a été isolé. Il s'agit de la coccinelline $C_{13}H_{23}NO$, qui est un N-oxyde de l'alcaloïde précoccinelline $C_{13}H_{23}N$, également présent dans l'hémolymphe de l'insecte.

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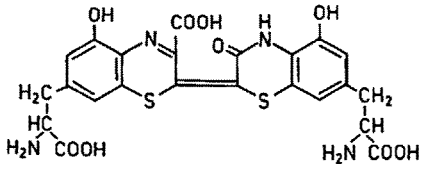
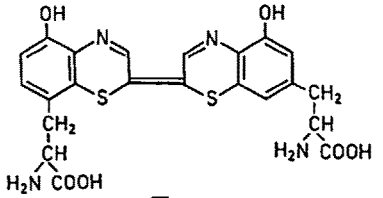
The Isolation and Structure of Trichosiderin B[1]

The trichosiderins[2] are a group of phaeomelanic pigments occurring in human and mammalian red hair and in the feathers of many species of birds.

In previous reports[3–5] we have shown that a suitable source for the extraction of these pigments are the feathers of the New Hampshire chicken, from which 3 homogenous trichosiderins (C, E and F) have been isolated and characterized as $\Delta^{2,2'}$-bi(2H-1,4-benzothiazine) derivatives (I, II, III)[6]. From the same source we have now isolated an isomer of trichosiderin C which has been called trichosiderin B (IV), since it corresponds to the fraction designated B2 in earlier chromatographic work[7].



I



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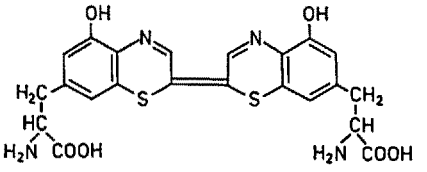

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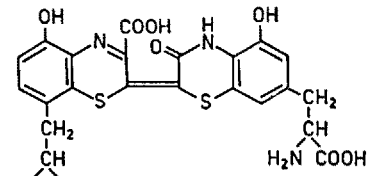
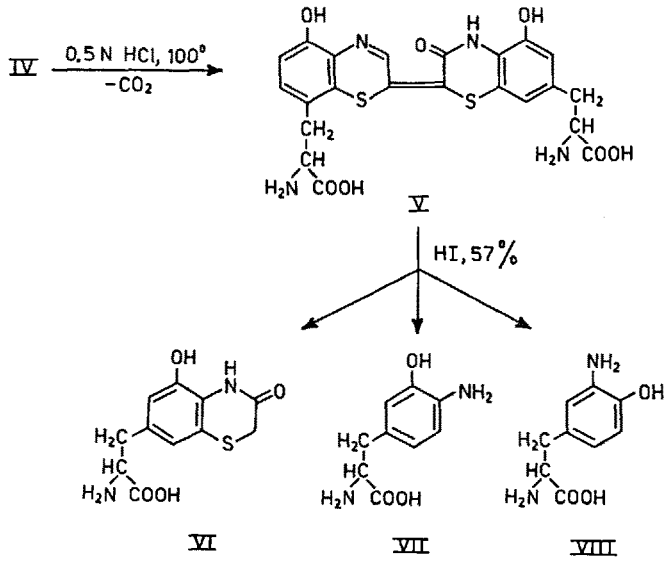


IV



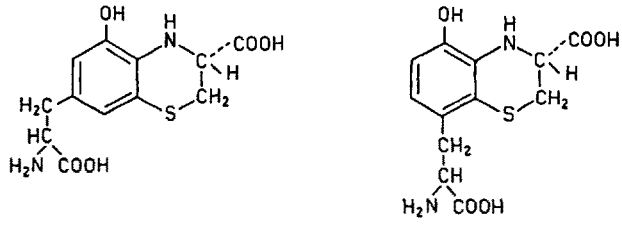
Alkaline extracts (0.1 *N* NaOH) of feathers (2.5 kg), cooled to 4 °C, were adjusted to pH 1 with 6 *N* HCl and centrifuged to remove the acid-insoluble gallophaeomelanins[8] and proteins. The yellow supernatant was then passed on to a column (2.5 × 10 cm) of Dowex 50W-X2 (100–200 mesh, H^+ form), kept at 4 °C. After washing with 1 *N* HCl and distilled water, trichosiderins were eluted from the resin with 0.5 *N* NaOH and separated into the individual components by column chromatography on Sephadex G-25 (5 × 94 cm), using as the eluent phosphate buffer at pH 11. The fraction containing trichosiderin B, which emerged from the column after about 3 l, was re-chromatographed on Sephadex twice and eventually concentrated by resin treatment as described above. Acidification of the concentrated solution to pH 3 gave the pigment (65 mg) as an amorphous red-orange precipitate, insoluble in water and in any organic solvent.

The microanalyses suggested that the molecular formula was isomeric with that of trichosiderin C (Found: C, 49.51; H, 4.02; N, 9.70; S, 11.31. $C_{23}H_{20}N_4O_9S_2$ requires: C, 49.28; H, 3.57; N, 10.00; S, 11.42). Moreover, like trichosiderin C, the pigment showed aminoacidic properties and gave 4.7% Van Slyke nitrogen, corresponding to 2 primary amino groups.

Further structural similarity of the pigment to trichosiderin C was revealed by their absorption spectra which coincide in the UV and visible region (Table), indicating that the same chromophore must be present in both pigments.

On brief heating with 0.1 *N* HCl the pigment lost CO_2 (1 mole) to give descarboxytrichosiderin B (V; scheme I), obtained as an amorphous red powder, $C_{22}H_{20}N_4O_7S_2$ (Found/calc. C, 51.06/51.16; H, 4.03/3.87; N, 10.90/10.85; S, 12.07/12.40), by column chromatography of the reaction mixture on Sephadex G-25 (eluent 0.1 *N* NaOH). The UV-spectrum of V (in 0.2 *N* NaOH) displayed absorption maxima at 462, 313 and 245 nm (log 4.19, 4.15 and 4.49) with a large bathochromic shift on acidification ($\lambda_{max}^{H^+}$ 533, 358 and 299 nm), as expected for a $\Delta^{2,2'}$-bibenzothiazine chromophore without a carboxyl group at C-3[4].

Treatment of descarboxytrichosiderin B with HI and red phosphorus under mild conditions (30 min at 100 °C) gave β-7-(3-oxo-5-hydroxy-3,4-dihydro-2H-1,4-benzothiazinyl)-alanine (VI) and a mixture of 3-hydroxy-4-amino-



Absorption spectra λ_{max} nm (log ε_{max}) in 0.2 *N* NaOH

Trichosiderin B	454	329	243	(3.98	4.01	4.60)
Trichosiderin C	452	327	240	(4.13	4.04	4.56)

[1] This work was supported by a grant from Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Napoli.

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[6] The stereochemistry of the $\Delta^{2,2'}$-bibenzothiazine skeleton in I, II and III has not yet been defined. However, studies of model compounds (F. GIORDANO, L. MAZZARELLA, G. PROTA, C. SANTACROCE and D. SICA, J. chem. Soc., in press) have shown that the interconvention of geometrical isomers in this series occurs rather readily and the isolated pigments may well be equilibrium mixtures of the 2 forms.

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[8] Under these conditions trichosiderin C, which is only slightly soluble in aqueous dilute acids, co-precipitates in part with gallophaeomelanins.

[9] The identification was carried out using a Beckman Amino Acid analyzer (mod. 116) with a column (22 cm) of PA 35 resin operating under the standard conditions for the analysis of basic amino acids.

phenylalanine (VII, minor component) and 3-amino-4-hydroxyphenylalanine (VIII), identified[9] by comparison of their chromatographic properties with those of authentic samples[10,11].

Considering that 3-hydroxy-4-aminophenylalanine is a secondary product arising from further degradation of the amide VI[4], the results of the degradative experiment prove unequivocally the nature and the relative positions of the aromatic substituents attached to the $\Delta^{2,2'}$-bibenzothiazine chromophore of descarboxytrichosiderin B, which accordingly can be formulated as V. Hence structure IV can be derived for trichosiderin B.

Therefore it appears that trichosiderin B differs from trichosiderin C only in the position of attachment of an alanine residue to the $\Delta^{2,2'}$-bibenzothiazine chromophore. This structural difference is consistent with recent biosynthetic studies[12] according to which trichosiderins are considered as a new group of animal pigments deriving from tyrosine and cysteine by the intermediacy of either IX or X or of both.

Riassunto. Un nuovo pigmento feomelanico, denominato tricosiderina B, è stato isolato dalle piume di pollo di razza New Hampshire. Sulla base delle proprietà chimiche e spettrali a tale pigmento, $C_{23}H_{20}N_4O_9S_2$, è stata assegnata la struttura $\Delta^{2,2'}$-bibenzotiazinica IV.

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Alcaloide des Canneberges II

Les extraits des feuilles des Canneberges possèdent l'application dans la médecine traditionelle (l'agent antithermique) et dans la thérapie de certaines formes du cancer.

Méthode. L'extraction des feuilles des Canneberges (*Vaccinium oxycoccus*) du Nouveau-Brunswick donne une fraction basique (1%)[1]. La chromatographie sur couches minces (CCM) de la fraction basique a révélé la présence d'un minium de 7 produits basiques différents.

Une des bases – Rf 0.44 (benzène-éthanol 9:1) – a été isolée par la CCMP (MN Silica Gel G, épaisseur: 1 mm). On a obtenu 53 mg d'une base que nous avons appelé Cannivonine (1). La structure partielle de la Cannivonine a été déterminée par l'étude des spectres et la dégradation.

Identification de la structure de la Cannivonine (1). Le spectre de masse de la Cannivonine (**1**) a donné le pic moléculaire a 163, correspondant a la composition $C_{11}H_{17}N$ (analyse: calcule C, 80.9; H, 10.5; N, 8.6. Trouvé C, 81.1, H, 10.7; N,8.3). Cette composition suggère pour une base **1** la structure tricyclique avec une liaison double.

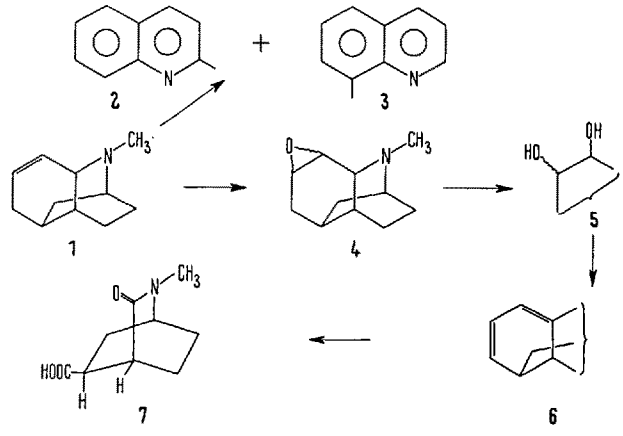
Le spectre IR (Nujol) ne révèle pas d'absorption NH mais l'absorption a 1620 cm^{-1} confirme la présence d'une liaison double. Le spectre RMN (Varian T-60, $CDCl_3$, δ, ppm) donne: 2 protons olefinique (5.3), 5 protons en α d'azote (2.3–3.7) dont un singulet N-CH_3 (3.3) et 3 protons allyliques (2.0–2.3).

La dégradation de Hofmann sur le produit **1** donne quelques produits dont un diène conjugé (λ_{max} 278 nm). La présence de ce produit elimine la possibilité d'existance du cycle à 7 dans la molécule.

La deshydrogenation catalytique (Pd, C, 250°) donne méthyl-2 (**2**) et méthyl-8 chinolines (**3**). Les deux chinolines prouvent la substitution du système azabicyclo-[2,2,2]-octane de la cannivonine.

L'époxydation de la liaison double (acide m-chloroperbenzoïque, $CHCl_3$) suivie de l'hydrolyse du mélange des époxydes (**4**) (HCl à 5%) donne les diols (**5**) facilement deshydraté au milieu basique (KOH a chaud). Ce diène (λ_{max} 273 nm) a été oxydé (KMnO4, a chaud, pH 8–9 en donnant la petite quantité d'acide N-méthylazabicyclo-[2,2,2]-octanone-2 carboxylique-4 (**7**) (Schéma I), qui a été identifié par la comparaison avec le produit connu pour la serie des degradations des alcaloïdes de la famille Ibogae[2–4]. Le spectre de masse (Hitachi RMU 6D) du produit **1** a révélé juste quelques pics importants. Le pic moléculaire est assez important (37%).

D'abord la voie la plus importante – les pics à 149 et 148 peuvent resulter à une perte de CH_2 ou CH_3 du système NCH_3 aussi bien que du CH_2 du cycle. La suite de la fragmentation c'est la formation du système de la méthyl-dihydropyridine (96) et sa déméthylation (81) puis aromatisation (79). L'autre voie-minoritaire et plus



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