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Coproporphyrin III and its Zn^{2+} and Ni^{2+} chelates from cultures of Streptomyces sp. A-305

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Chimica. — *Coproporphyrin III and its* Zn^{2+} *and* Ni^{2+} *chelates from cultures of* Streptomyces sp. A–305 (*). Nota di MARIA L. BRUZZONE (**), CARLO G. CASINOVI, CORRADO GALEFFI, ANTONIO TONOLO e ANTONIO TRILLI (**), presentata (***) dal Corrisp. G. B. MARINI–BETTOLO.

RIASSUNTO. — Da culture di *Streptomyces sp. A–305* contenenti ioni Zn^{2+} e Ni^{2+} sono stati isolati la coproporfirina III ed i due chelati Zn^{2+} e Ni^{2+} coproporfirina III. È discussa l'azione inibitrice dello ione Ni^{2+} nel metabolismo porfirinico.

La conformazione dei due nuovi chelati tetradentati, entrambi diamagnetici, è esaminata in base agli spettri RMN, alla configurazione elettronica degli ioni Zn^{2+} e Ni^{2+} ed alla struttura delle porfirine.

I tetrametilesteri dei due chelati sono preparati anche per introduzione degli ioni corrispondenti nella tetrametilcoproporfirina III.

Streptomyces sp. A–305 is known to produce an iron-containing, green pigment, called ferroverdin [1], when grown in presence of Fe^{2+} ions, and a cobalt-containing, pink pigment, when grown in presence of Co^{2+} ions. In both pigments the ions are chelated by the *p*-vinyl-phenyl ester of the 3-nitroso-4-hydroxy-benzoic acid.

In the course of investigations on the ability of this organism to chelate other metal ions, coproporphyrin III (I) and its Zn^{2+} (II) and Ni^{2+} (III) chelates were isolated from the culture filtrates in presence of Zn^{2+} and Ni^{2+} ions. No accumulation of pigments could be detected in the absence of Ni^{2+} in the medium. However the Ni^{2+} ion coordinated the coproporphyrin III (I) less easily than Zn^{2+}.

Compounds containing the porphyrin ring are widespread in nature [2]. Other porphyrin compounds have been found in several organisms, both prokaryots and eukaryots. Some of these porphyrins have been recognized to be precursors of the functional molecules (i.e. chlorophylls, heme and cobalamins). This is the case with the coproporphyrinogen which yields protoporphyrin after decarboxilation and removal of hydrogens by means of the enzyme coproporphyrinogen oxidase. Coproporphyrins I, II, III and IV (fig. 1), obtained by removal of hydrogens from the corresponding coproporphyrinogens, have been isolated from many microrganisms and also appear in several diseases of man [3]. The accumulation of such compounds is currently considered to be the result of disorders in the regulation of the enzymes involved in the biosynthetic pathway.

In *Streptomyces sp. A–305* the Ni^{2+} ion may be thought as the inhibitor of the enzyme coproporphyrinogen oxidase, which has been showed to be iron-

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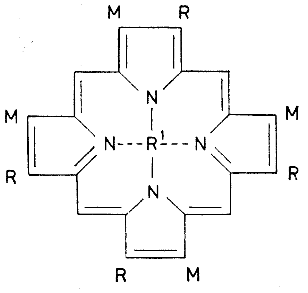
dependent in *Propionibacterium arabinosum* [4]. A similar mechanism has been proposed for this microrganism, which accumulates Co^{2+} coproporphyrin III (IV) if Co^{2+} ions are in the growth medium [5].

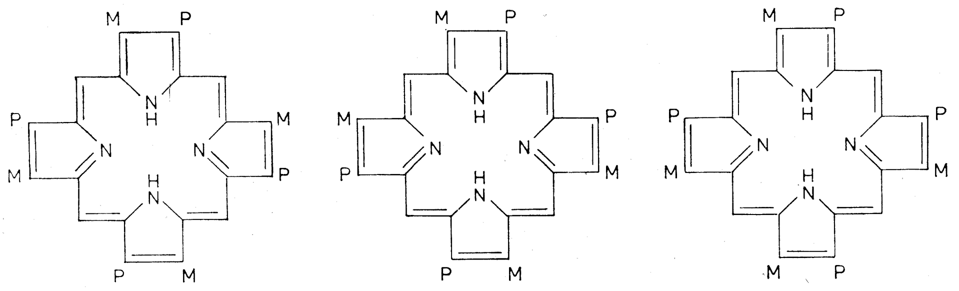
The production of coproporphyrin III (I) and its Zn^{2+} (II) and Ni^{2+} (III) chelates by *Streptomyces sp. A–305* was achieved in a medium of the following composition: yeast extract 20 g/l, mannitol 30 g/l, $NiCl_2$ 1 g/l, $ZnCl_2$ 0.1 g/l at pH 7. This medium has been developed after a series of experiments on the effect of the C– and N–source, as well as of the Zn^{2+} and Ni^{2+} concentration, on the pigment production (fig. 2). The pigments (g 1.2 from l 100) were extracted with ethyl acetate from the supernatant of the culture grown for 5 days and adjusted to pH 3, then fractionated by counter-current distribution using ethyl acetate, tetrahydrofurane (THF) (2 : 1, v : v) and phosphate



M = CH_3

	R	R^1	
I	CH_2CH_2COOH	2 H	coproporphyrin III (*);
II	CH_2CH_2COOH	Zn	Zn^{2+} coproporphyrin III;
III	CH_2CH_2COOH	Ni	Ni^{2+} coproporphyrin III;
IV	CH_2CH_2COOH	Co	Co^{2+} coproporphyrin III;
V	$CH_2CH_2COOCH_3$	Zn	Zn^{2+} tetramethylcoproporphyrin III;
VI	$CH_2CH_2COOCH_3$	Ni	Ni^{2+} tetramethylcoproporphyrin III;
VII	$CH_2CH_2COOCH_3$	2 H	tetramethylcoproporphyrin III (*).

(*) Without the dative bonds of the figure.



Coproporphyrin I   Coproporphyrin II   Coproporphyrin IV

M = CH_3 P = CH_2-CH_2-COOH

Fig. 1.

buffer at pH 6.1 ($K_r \cdot K_a$ $1.7 \cdot 10^{-7}$ for (II), $4.2 \cdot 10^{-7}$ for (III) and $1.2 \cdot 10^{-6}$ for (I), as monoprotic acids) [6].

The chelates (II) and (III) were methylated with diazomethane (V and VI), the metal ions were eliminated with acids (more easily the Zn^{2+} than the Ni^{2+} ion) and the obtained tetramethyl esters were identified as tetramethylcoproporphyrin III (VII). On reverse Zn^{2+} and Ni^{2+} tetramethylcoproporphyrin III (V and VI) were prepared from the corresponding acetate and tetramethylcoproporphyrin III (VII).

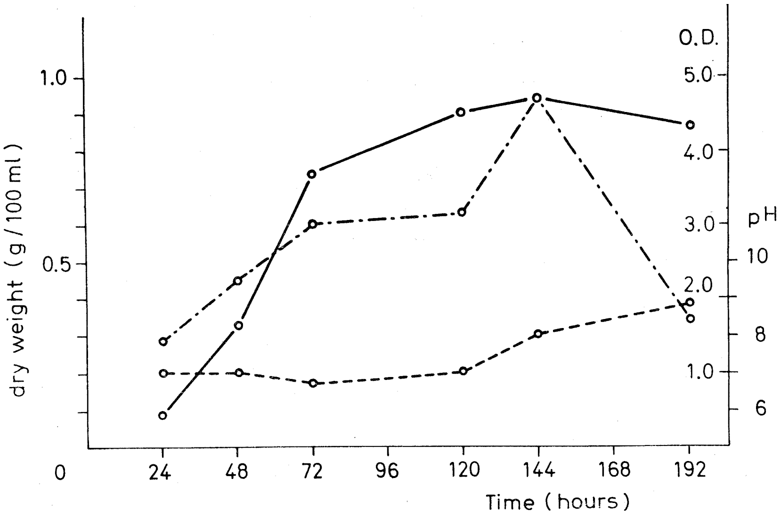


Fig. 2. – Growth curve and pigment production by *Streptomyces sp.A-305* at 25 °C. —— dry weight, ·—·—· pigment concentration, – – – pH. The culture was carried out in a 90 l stainless steel fermentor containing 50 l of medium, with vortex aeration 400 rpm, 1 *v/v* min.air, at 1 atm. The culture was inoculated with 5% of a fully grown culture in the fermentation medium.

Zn^{2+} *coproporphyrin III (II)*. Red blades from THF and ethyl acetate which carbonize without melting; raw formula $C_{36}H_{36}N_4O_8Zn$, λ_{max}(THF) 575 nm (log ε 4.30), 539 (4.24) and 410 (5.62). NMR spectrum (pentadeuteropyridine, TMS) δ in ppm: 3.50 (*t*, 6 Hz, four $C\underline{H}_2COOH$), 3,63 (*s*, four β CH_3), 4.64 (*t*, 6 Hz, four $C\underline{H}_2CH_2COOH$), 9.45 (broadened, four COOH), 10.23, 10.45, 10.46 and 10.68 (four methynes).

Zn^{2+} *tetramethylcoproporphyrin III (V)*. M.p. 231–3 °C from benzene/hexane, i.r. (CCl_4) $\nu_{C=O}$ 1755 cm^{-1}, raw formula $C_{40}H_{44}N_4O_8Zn$, λ_{max} ($CHCl_3$) 573 and 536 nm. NMR spectrum ($CDCl_3$, TMS, *c* $6 \cdot 10^{-2}$ M) δ in ppm: 2.85 (*t*, 7 Hz, four $C\underline{H}_2COOCH_3$), 2.95, 3.03, 3.09 and 3.27 (4 *s*, four β CH_3), 3.58, 3.60, 3.63 and 3.65 (4 *s*, four OCH_3), 3.90 (*t*, 7 Hz, four $C\underline{H}_2CH_2COOCH_3$), 8.56, 8.70, 8.84 and 8.94 (4 *s*, four methynes).

Ni^{2+} *coproporphyrin III (III)*. Red blades from THF and ethyl acetate which carbonize without melting, raw formula $C_{36}H_{36}N_4O_8Ni$; λ_{max} (THF) 553 and 520 nm. NMR spectrum (pentadeuteropyridine) δ in ppm: 3.60 (four

$C\underline{H}_2COOH$), 3.84 (*s*, four β CH_3), 5.37 (four $C\underline{H}_2CH_2COOH$). The band shapes are much wider than in Zn^{2+} coproporphyrin III (II). The methyne hydrogens are undistinguishable.

Ni^{2+} *tetramethylcoproporphyrin III (VI)*. M.p. 196.5–198 from benzene/hexane, raw formula $C_{40}H_{44}N_4O_8Ni$; λ_{max} ($CHCl_3$) 554 and 520 nm. NMR spectrum ($CDCl_3$, *c* $5.5 \cdot 10^{-2}$ M) δ in ppm: 3.11 (four $C\underline{H}_2COOCH_3$), 3.33, 3.36 and 3.41 (3 *s*, four β CH_3, 1 : 2 : 1), 3.66, 3.67 and 3.68 (3 *s*, four OCH_3, 1:2:1), 4.14 (four $C\underline{H}_2CH_2COOCH_3$), 9.53 and 9.58 (2 *s*, four methynes, 2 : 2). All the band shapes are very wide.

Coproporphyrin III (I). NMR spectrum (pentadeuteropyridine) δ in ppm: 3.22 (*t*, 7 Hz, four $C\underline{H}_2COOH$), 3.30 (*s*, four β CH_3), 4.34 (*t*, 7 Hz, four $C\underline{H}_2CH_2COOH$), 10.20, 10.46, 10.50 and 10.78 (*s*, 4 methynes).

In the porphyrins the π electrons are very delocalized and thus account for the aromaticity. The ring current is responsible for the high chemical shifts of the protons, in the NMR spectra, and for the moving to lower fields on dilution [7]. This latter effect, observed also for the Zn^{2+} and Ni^{2+} complexes (V) and (VI), can be explained by monomer-dimer equilibrium. In the dimer two molecules are piled one above the other: the degree of association is reduced on dilution and thus the shielding effect of one ring on all the protons of the second ring.

In these dianionic tetradentate chelates the bonds between the nitrogens and the metal ion are electrostatic and covalent. Therefore in the complexes (V) and (VI) the magnetic field due to the ring current is reduced in comparison with the free porphyrin (VII) (for the NMR spectrum of tetramethylcoproporphyrin III see Abraham *et al.*, ibid). However, with aromatic solvents the opposite effect occurs, and the protons of the chelates (II) and (III), in pentadeuteropyridine, resonate at lower fields than the protons of (I) [8].

The Zn^{2+} ion of the Zn^{2+} coproporphyrin III (II) has a complete $3d^{10}$ level. The closed shell ion offers the sp^2d orbitals [9] to the ligands with a preferred tetrahedrical configuration. The metal-nitrogen bonds, as a consequence, will be weakened, on account of the square-planar conformation of the porphyrin. This causes the dissociation with hydrochloric acid, in contrast with the Ni^{2+} coproporphyrin III.

The former complex is diamagnetic ((V), magnetic susceptibility $\mu = 0$ B.M.) as it lackes unpaired electrons. The same chelate may acquire a further ligand perpendicular to the porphyrinic plane as similar Zn^{2+} complexes [10]. A pentacoordinated square-pyramidal complex will be obtained, whose existance may be proved by fluorescence observable in polar solvents (λ 579 nm in methanol, dioxane 1 : 1).

The Ni^{2+} ion of the Ni^{2+} coproporphyrin III (III) has an uncompleted $3d^8$ outer level. Six of the eight electrons belong to t_{2g} non bonding orbitals (*xy*, *xz* and *yz*) whilst the remaining two may fill the d_{z^2} but not the $d_{x^2-y^2}$ of the e_g orbitals. The $d_{x^2-y^2}$ is the least stable orbital in the porphyrin complexes, because being cross shape, it would be directed towards the four nitrogens.

The Ni^{2+} ion should form a complex through the square-planar *dsp*^{2} bonds. However, all the NMR signals of (III) and (VI) are very broad in comparison with the Zn^{2+} complexes (II) and (V). Therefore, in Ni^{2+} coproporphyrin III an irregular tetragonal *dsp*^{2} bond sistem must be postulated. Ni^{2+} should be in the plane of the four methyne groups with the four "pyrrolic" rings alternatively above and below this plane. This was in agreement with the crystallographic [11] and emission [12] data obtained for Ni^{2+} etioporphyrin II.

Ni^{2+} tetramethylcoproporphyrin III is diamagnetic ($\mu = 0$ B.M.). Towards acids (III) and (VI) were very stable. They eliminated Ni^{2+} only with concentrated sulphuric acid, and were not fluorescent in polar solvents. It could be inferred thus that they should not bond with extra-ligands.

It was reported that the order of thermodynamic stability was the reverse of the kinetic [13] in the metalporphyrins. The Zn^{2+} ion was thus complexed more easily than Ni^{2+}, e.g. Zn^{2+} coproporphyrin III was produced by *Streptomyces sp.A–305* with nickel chloride in the broth from Zn^{2+} impurity of the medium more easily than Ni^{2+} coproporphyrin III.

According to known correlations [14], [15] the different stability of the chelates could be correlated with the wavelenght and the intensity of the absorption maxima in the visible. Ni^{2+} coproporphyrin III, which is the most stable, has the first band at lower wavelenght. Further, the ratio between the first and the second band was higher in the more stable complex. (II) $\varepsilon_1/\varepsilon_2$ 1.14, (V) 1.18, (III) 2.96, (VI) 2.80.

In the mass spectra of the tetramethyl esters (V), (VI) and (VII) the stability of the molecular ion (base peak) could be due to the high level of electron delocalisation of these sistems.

CONCLUSIONS

Streptomyces sp. A–305 accumulates coproporphyrin III (and its Zn^{2+} and Ni^{2+} chelates) if Ni^{2+} ions are in the growth medium. This fact can be explained by the competitive inhibition of the iron-dependent coproporphyrinogen oxidase by means of the Ni^{2+} ions.

In the square-planar configuration of Zn^{2+} coproporphyrin III there is strain at the nitrogen-metal bonds. Instead, the Ni^{2+} coproporphyrin III has an irregular tetragonal bonding with the four "pyrrole" rings displaced above and below the four methyne plane alternatively.

Apparatus. AEI MS–902 (mass spectra), Varian HA 100 (NMR spectra), Gouy balance (magnetic susceptibility), spectrophotofluorimeter Aminco-Bowman (emission spectra, excitation at 410 nm).

BIBLIOGRAPHY

[1] A. BALLIO, H. BERTHOLDT, A. CARILLI, E. B. CHAIN, V. DI VITTORIO, A. TONOLO and L. VERO BARCELLONA (1964) – « Rend. Accad. Naz. XL » (IV), *13*, 1.

[2] B. F. BURNHAM (1969) – *Metabolic Pathways III*, ed. D. M. Greenberg. Academic Press, 403.

[3] J. THOMAS (1938) – « Bull. Soc. Chim. Biol. », *20*, 471.

[4] A. M. DEL, C. BATLLE, A. BENSON and C. RIMINGTON (1965) – « Biochem. J. », *97*, 731.

[5] N. SONE (1971) – « J. Biochem. Tokio », *69*, 753.

[6] C. GALEFFI (1974) – « J. Chromatog. », *92*, 1.

[7] R. J. ABRAHAM, P. A. BURBIDGE, A. H. JACKSON and D. B. MACDONALD (1966) – « J. Chem. Soc., B », 620.

[8] W. S. CAUGHEY and W. S. KOSKI (1962) – « Biochemistry », *1*, 923.

[9] D. P. CRAIG, A. MACCOLL, R. S. NYHOLM, L. E. ORGEL and L. E. SUTTON (1954) – « J. Chem. Soc. », 332.

[10] J. E. FALK and J. N. PHILLIP (1964) – *Chelating Agents and Chelate Compounds*, eds. D. P. Mellor and F. P. Dwyer. Academic Press, New York.

[11] M. CRUTE (1959) – « Acta Cryst. », *12*, 24.

[12] J. B. ALLISON and R. S. BECKER (1960) – « J. Chem. Phys. », *32*, 1410.

[13] J. E. FALK (1964) – *Phorphyrins and Metalloporphyrins*, « Elsevier », *35*.

[14] J. E. FALK and R. S. NYHOLM (1958) – *Current Trends in Heterocyclic Chemistry*, eds. A. Albert, G. M. Badger and C. W. Shoppee. Butterworths, London, 130.

[15] G. D. DOROUGH, J. R. MILLER and F. M. HUENNEKENS (1951) – « J. Am. Chem. Soc. », *73*, 4315.