Biosynthesis of Protoporphyrin-IX from Coproporphyrinogen-III

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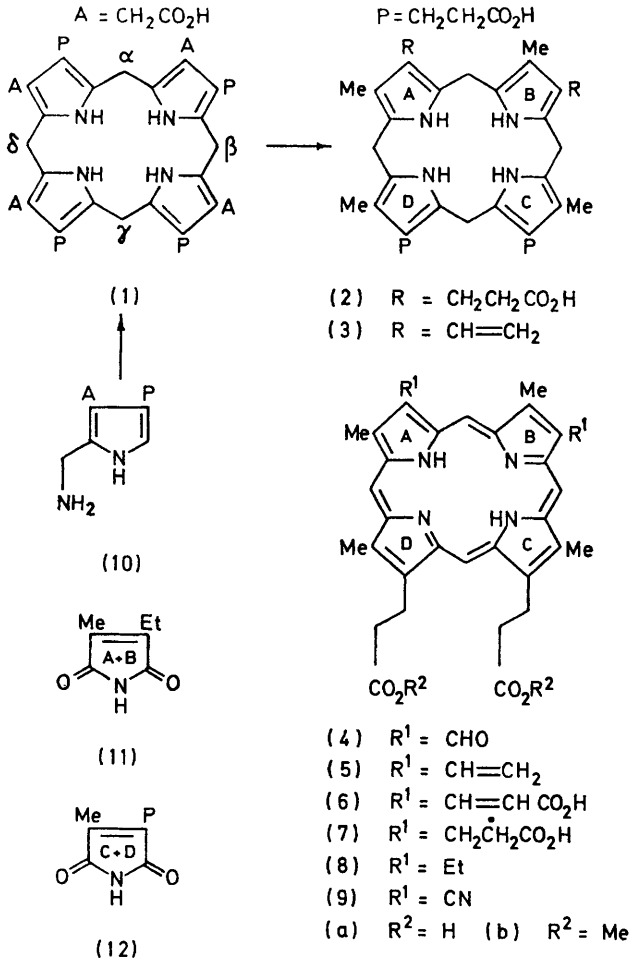
Summary Biochemical scrambling of the type-III porphyrinogen system is eliminated by showing that specifically labelled coproporphyrinogen-III (**2**) is enzymically converted into protoporphyrin-IX (**5a**) labelled at the corresponding sites.

THE sequence porphobilinogen, PBG (**10**) → uroporphyrinogen-III (**1**) → coproporphyrinogen-III (**2**) → protoporphyrinogen-IX (**3**) leading to protoporphyrin-IX (**5a**) and so to haem is supported by enzymic and mutant studies.[1] Confirmation was not available by isotopic labelling for the stages beyond PBG. Further, it was important for parallel researches to establish that the type-III isomer (*e.g.* **2** and **3**) once formed does not undergo biochemical scrambling. This report is prompted by the recent demonstration of Franck *et al.*[2] that [$\alpha\gamma$-$^{14}C_2$]uroporphyrinogen-III (**1**) is



converted enzymically into [$\alpha\gamma$-$^{14}C_2$]protoporphyrin-IX (**5a**). Our studies are complementary both as to precursor and labelling position.

The dialdehyde (**4b**), derived[3] from (**5b**), was converted by [2-^{14}C]malonic acid into the diacrylic acid (**6b**); hydrogenation and hydrolysis yielded ^{14}C-coproporphyrin-III (**7a**) labelled at ● in the propionate side-chains of rings A and B. Reduction of (**7a**) with sodium amalgam[4] gave (**2**) which was incubated in darkness and air with an enzyme system from *Euglena gracilis*[5] to form ^{14}C-protoporphyrin-IX (isolated as (**5b**) (1·5% incorporation from short incubation). This was hydrogenated and the product (**8b**) was hydrolysed and oxidised affording (**11**) and (**12**). The former contained 98% of the original activity and the latter $< 2\%$.

The foregoing partial synthesis was repeated using [^{3}H]malonic acid derived by exchange with HTO. That some ^{3}H-exchange had occurred at the *meso*-positions[6] in the Knoevenagel step was shown by oxidation of the final product (**7a**) to (**12**) which carried 78·5% of the original activity. The ^{3}H-coproporphyrinogen-III (**2**) derived from (**7a**) was incubated for longer than previously with the enzyme system from *Euglena* to give (**5b**, 15% incorporation). Degradation of (**5b**) to (**4b**) caused 80% of the ^{3}H-activity to be lost; there was no further loss on conversion of (**4b**) into (**9b**) *via* the dioxime. Degradation as before of the labelled (**5b**) to maleimides gave (**11**) carrying 78% of the activity of (**5b**) whereas (**12**) contained $< 2\%$.

It is thus proved that specifically labelled coproporphyrinogen-III (**2**) yields protoporphyrin-IX (**5a**) labelled at the corresponding sites. Our results interlock with those of the Münster group[2] to eliminate *scrambling* within the type-III system; they render improbable any isomerisation.

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