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SYNTHETIC SUBSTRATES FOR COPROPORPHYRINOGEN OXIDASE: MESOPORPHYRINOGEN-VI REVISITED

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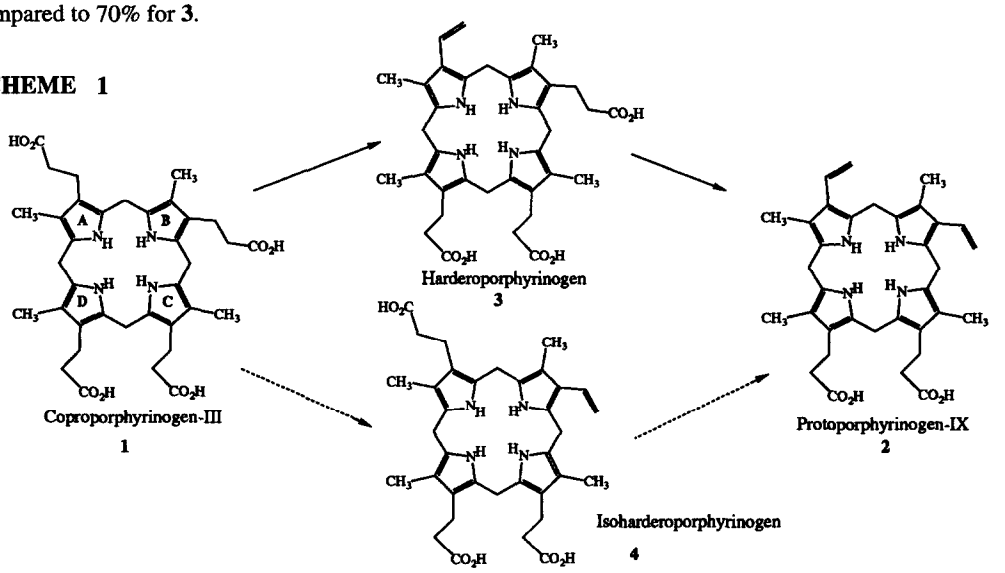
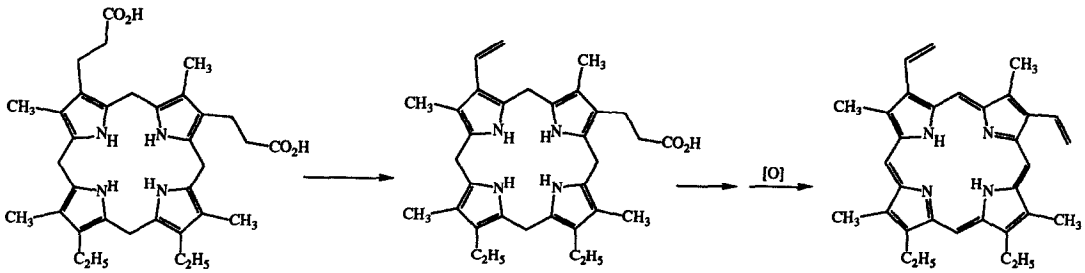
Abstract: In contradiction to a previous study, mesoporphyrinogen-VI, a dicarboxylate analog of coproporphyrinogen-III, has been shown to be converted to a monovinylic product by chicken red cell hemolysate preparations of coproporphyrinogen oxidase.

Coproporphyrinogen oxidase (C.O.) is a mitochondrial enzyme that catalyses the conversion of coproporphyrinogen-III (**1**) to protoporphyrinogen-IX (**2**) (Scheme 1), an important intermediate in heme and chlorophyll biosynthesis.[1] The A and B ring propionate moieties undergo oxidative decarboxylations to give vinyl groupings. In principle, the transformation of **1** to **2** could take place via two different pathways (Scheme 1), depending on whether the A or B ring propionate moiety is converted to a vinyl substituent first. The conversion of coproporphyrinogen-III to protoporphyrinogen-IX appears to take place via a single, specific pathway involving initial oxidative decarboxylation of the A ring propionate residue to give harderoporphyrinogen (**3**).[2-4] No trace of the B ring modified isomer, isoharderoporphyrinogen (**4**), has been detected in enzyme incubation studies using HPLC[3] or mass spectrometry using specifically deuteriated coproporphyrinogen-III.[4] Isoharderoporphyrinogen (**4**) is a very poor substrate for C.O. Enzyme preparations from chicken red cell hemolysates gave only a 1.5% incorporation of radiolabelled **4** into protoporphyrin-IX, compared to 70% for **3**.

SCHEME 1

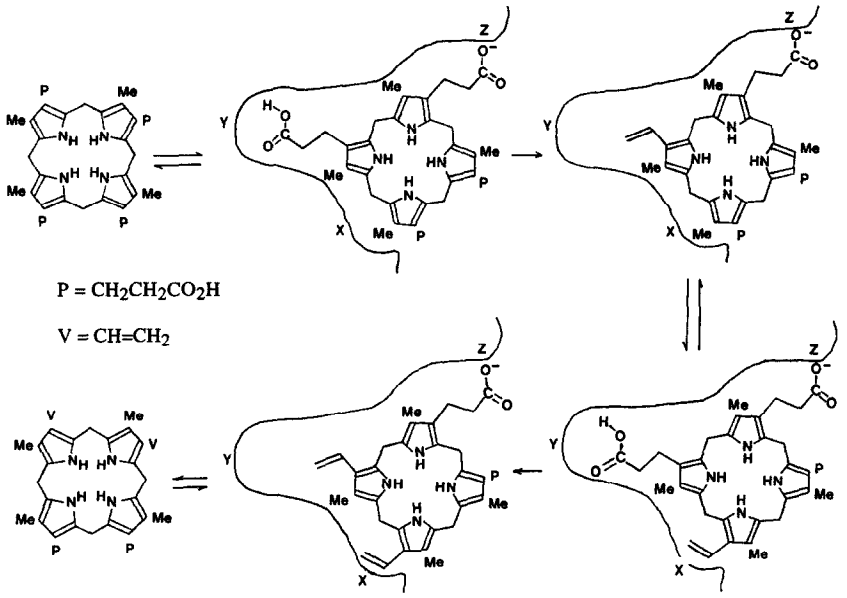


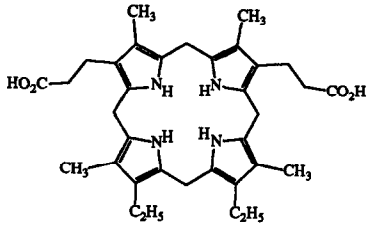
SCHEME 2



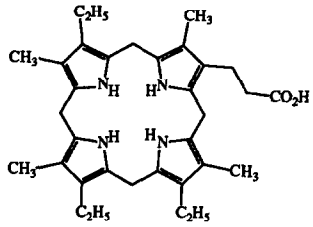
When **1** is degraded by C.O., only the propionate moieties at the A and B rings are affected. In the 1970's, it was unclear whether the C and D ring propionate groupings were necessary for a porphyrinogen to be a substrate for the enzyme.[5] Mesoporphyrinogen-VI (**5**)[6] is an analog of **1** where the C and D ring propionate residues have been replaced by ethyl groups. Mesoporphyrin-VI was synthesized by the MacDonald route[7] and reduction with sodium amalgam gave the corresponding porphyrinogen.[6] Porphyrinogen **5** proved to be a good

SCHEME 3





Mesoporphyrinogen-XII
8



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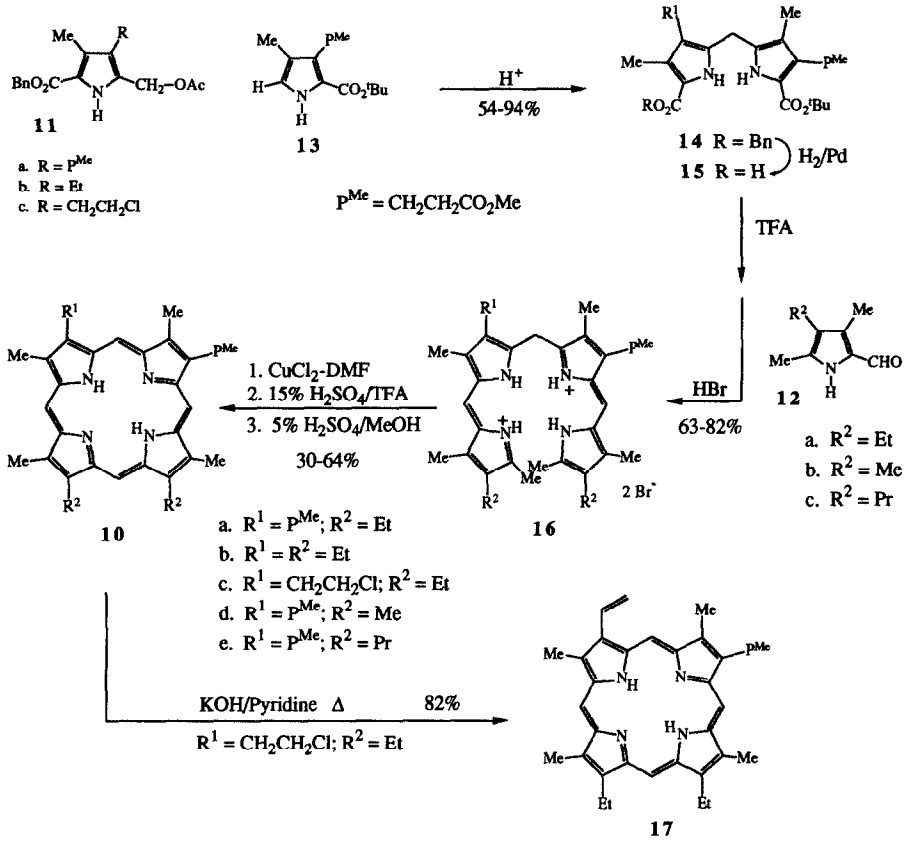
substrate for C.O. In incubations with chicken red cell hemolysates, a monocarboxylate porphyrinogen accumulated initially, but this was further converted into protoaetioporphyrin-IX (**6**; Scheme 2).[6] Mesoporphyrinogen-XII (**8**), the diethyl analog of coproporphyrinogen-IV, was subsequently shown to not be a substrate for C.O.[8] An analysis of the known substrates for C.O. indicated that a specific sequence of peripheral substituents was required for a given porphyrinogen to be metabolized. Initially, it was proposed that the sequence R Me-P Me, where R = Me, V or Et, was necessary in order for the propionate residue (P) to be degraded.[6] It was later recognized that a second propionate grouping was needed as part of the extended sequence R Me-P Me-P in order for the porphyrinogen to be a good substrate for the enzyme.[9-11] A model for the active site of C.O. is shown in Scheme 3.[9] Three regions on the enzyme (**X**, **Y** and **Z**) appear to be important in enzyme-substrate recognition, where **Y** is the region where oxidative decarboxylation occurs. Region **X** has a steric requirement where R can be Me, V or Et but not a bulky propionate grouping. Region **Z** is presumably involved in selectively binding the substrate via the second propionate unit, possibly due to an interaction with a positively charged amino acid residue.[9]

The metabolism of mesoporphyrinogen-VI is somewhat of an enigma, in that it is the only example of a substrate for C.O. that does not easily fit the model detailed in the previous paragraph. This has been overlooked by previous workers in the field. Mesoporphyrinogen-VI does have the correct ordering of peripheral substituents for the A ring propionate group to be converted to a vinyl group and one must assume, although this was not proven, that the intermediary porphyrinogen is the structure (**7**) shown in Scheme 2. However, this structure does not have a second propionate residue to bind to region **Z** and on the basis of this analysis one would not expect further metabolism to occur. Indeed, the monocarboxylate porphyrinogen is the major product from incubations of mesoporphyrinogen-VI with rat liver homogenates.[6] However, analysis of the kinetic profile for the metabolism of mesoporphyrinogen-VI by chicken red cell hemolysates indicates that the monocarboxylate porphyrinogen is as good a substrate for this system as harderoporphyrinogen (**3**).[6] One possibility is that region **X** has a high affinity for the vinyl moiety and this may provide an alternate mode by which the intermediate can associate with the active site. The affinity of vinyl groupings with region **X** may be responsible for inducing the 90° rotation needed during the metabolism of coproporphyrinogen-III. If this idea is correct, the vinyl monocarboxylate porphyrinogen should be a good substrate for avian erythrocyte C.O., whereas the corresponding ethyl substituted porphyrinogen (**9**) would not be metabolized. In order to test this hypothesis, the corresponding porphyrins have been synthesized by the a,c-biladiene route.

Porphyrins **10a-c** were synthesized from the pyrrolic intermediates shown in Scheme 4. Pyrroles **11a-c** and **12a** were prepared using literature procedures.[12-14] The *tert*-butyl ester **13** was prepared using the Barton-Zard isocyanoacetate methodology[15] from methyl 4-oxobutanoate.[16] Condensation of **11a-c** and **13** in the presence of Montmorillonite clay[17] gave the dipyrrylmethanes **14**. Hydrogenolysis of the benzyl esters over 10% palladium-charcoal yielded the related carboxylic acids **15**, and subsequent treatment with TFA and condensation with two equivalents of the pyrrole aldehyde **12a** in the presence of hydrobromic acid afforded the a,c-biladienes **16**. Cyclization of the a,c-biladienes with copper(II) chloride in dimethylformamide,[18] followed by demetallation with sulfuric acid-trifluoroacetic acid and reesterification with 5% sulfuric acid-methanol, yielded the required porphyrins **10a-c** (Scheme 4). The 2-chloroethylporphyrin **10c** was dehydrohalogenated with potassium hydroxide in refluxing pyridine to give the related vinyl porphyrin **17**.

SCHEME 4



The porphyrin methyl esters **10a**, **10b** and **17** were hydrolyzed with 25% hydrochloric acid and the resulting carboxylic acids were reduced with sodium amalgam to give the corresponding porphyrinogens. The porphyrinogens were incubated with chicken red cell hemolysates and the resulting porphyrin products[19] were analyzed by TLC, HPLC, and mass spectrometry. Coproporphyrinogen-III was metabolized by these enzyme

preparations to give protoporphyrin-IX. Mesoporphyrinogen-VI (**6**) was rapidly metabolized by C.O., but contrary to the earlier literature report,[6] the only product of metabolism was the monovinyl porphyrin **17**. TLC and HPLC (Figure 1) showed no trace of a zero-carboxylate porphyrin (etioporphyrin-IV was used as a standard in our study). The identity of the major product was also established by isolating the porphyrin methyl ester using flash chromatography and carrying out further structural studies. The 300 MHz proton NMR spectrum of the monocarboxylate metabolite (Figure 2) clearly showed the presence of a vinyl grouping and a single propionate residue. High resolution FAB mass spectrometry also gave a molecular ion that was consistent with the monovinylic porphyrin **17** (Calc. for $C_{34}H_{38}N_4O_2$ + H: 535.3075. Found: 535.3072). The porphyrinogens derived from **10b** and **17** were repeatedly shown to not be substrates for C.O.

The results obtained in this study contradict the earlier findings.[6] In order to provide further support for these observations, two additional substrates for C.O. were synthesized. Porphyrins **10d** and **10e** were synthesized using the a,c-biladiene route (Scheme 4). The corresponding porphyrinogens were found to be good substrates for C.O. and afforded monocarboxylate products by TLC and HPLC. Hence, when the C and D ring propionate units of coproporphyrinogen-III are replaced by methyl, ethyl or propyl groups, only the A ring propionate residue is metabolized by C.O.

Further synthetic and biosynthetic studies are underway to further investigate the activity and substrate specificity of C.O. The results obtained by Jackson et al.[6] may be due to the presence of isozymes or even variations in the C.O. present in various breeds of chickens, and these possibilities are currently under evaluation.

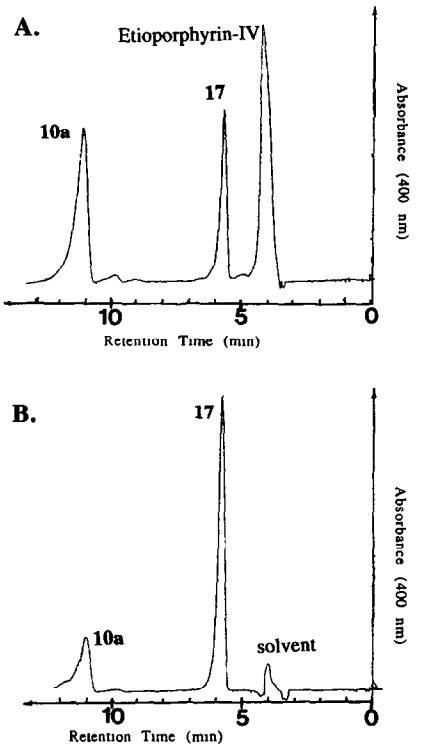


FIGURE 1:
A. HPLC analysis for a standard mixture of **10a**, **17** and etioporphyrin-IV.
B. HPLC analysis for the esterified products obtained from incubating **5** with chicken red cell hemolysates at 37°C for 20 min. Vinylporphyrin **17** is the major product, and no trace of a zero-carboxylate porphyrin can be detected.
HPLC analyses of porphyrin mixtures were carried out on a 5μ partisil HPLC column (250 mm x 5 mm I.D.) eluting with 20% (v/v) ethyl acetate/ cyclohexane at a flow rate of 1 mL/min.

Acknowledgements

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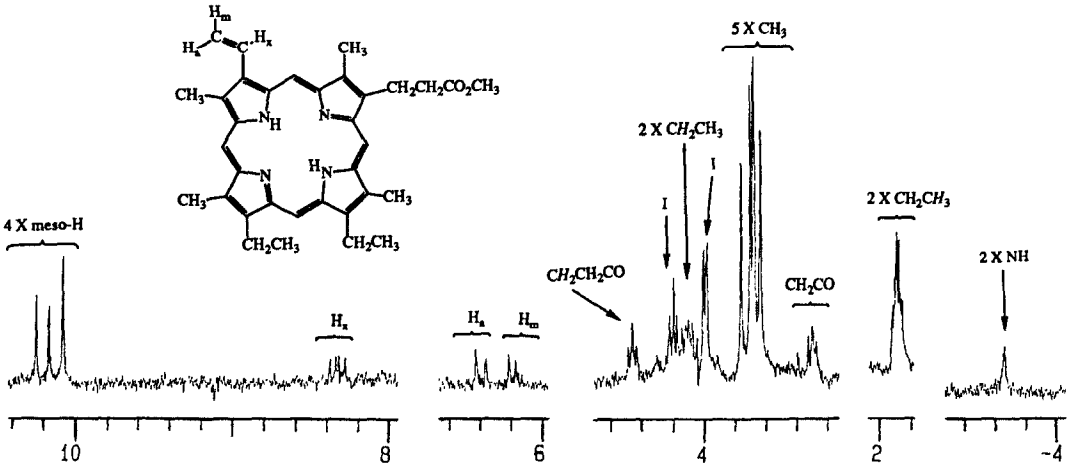
FIGURE 2: 300 MHz proton NMR spectrum of the monocarboxylate product from the incubation of mesoporphyrinogen-VI with chicken red cell hemolysates.



REFERENCES

1. Battersby, A.R.; McDonald, E. In *Porphyrins and Metalloporphyrins*; Smith, K.M., Ed.; Elsevier: Amsterdam, **1975**, pp 61-122.
2. Kennedy, G.Y.; Jackson, A.H.; Kenner, G.W.; Suckling, C.J. *FEBS Lett.* **1970**, *6*, 9.
3. a. Games, D.E.; Jackson, A.H.; Jackson, J.R.; Belcher, R.V.; Smith, S.G. *J. Chem. Soc., Chem. Commun.* **1976**, 187. b. Elder, G.H.; Evans, J.O.; Jackson, J.R.; Jackson, A.H. *Biochem. J.* **1978**, *169*, 205.
4. Jackson, A.H.; Jones, D.M.; Philip, G.; Lash, T.D.; Batlle, A.M. del C.; Smith, S.G. *Int. J. Biochem.* **1980**, *12*, 681.
5. Jackson, A.H.; Games, D.E. *Ann. N.Y. Acad. Sci.* **1975**, *244*, 591.
6. Jackson, A.H.; Jones, D.M.; Smith, S.G.; Elder, G.H. *J. Chem. Soc., Chem. Commun.* **1976**, 322.
7. Arsenault, G.P.; Bullock, E.; MacDonald, S.F. *J. Am. Chem. Soc.* **1960**, *82*, 4384.
8. Jones, D.M., Ph.D. Thesis, Cardiff, **1978**.
9. Lash, T.D., Ph.D. Thesis, Cardiff, **1979**.
10. Yoshinaga, T.; Sano, S. *J. Biol. Chem.* **1980**, *255*, 4727.
11. Robertson, J.A.; McDonald, E.; Battersby, A.R. *J. Chem. Soc., Perkin Trans. I* **1985**, 1699.
12. Johnson, A.W.; Kay, I.T.; Markham, E.; Price, R.; Shaw, K.B. *J. Chem. Soc.* **1959**, 3416.
13. a. Jackson, A.H.; Smith, K.M. In *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley: New York, **1973**; Vol. 1, pp 143-234. b. Ibid. **1984**, Vol. 6, pp 237-280.
14. a. Al-Hazimi, H.M.G.; Jackson, A.H.; Knight, D.W.; Lash, T.D. *J. Chem. Soc., Perkin Trans. I* **1987**, 265. b. Jackson, A.H.; Lash, T.D.; Ryder, D.J. *J. Chem. Soc., Perkin Trans. I* **1987**, 287.
15. a. Barton, D.H.R.; Zard, S.Z. *J. Chem. Soc., Chem. Commun.* **1985**, 1098. b. Barton, D.H.R.; Kervagoret, J.; Zard, S.Z. *Tetrahedron* **1990**, *46*, 7587. c. Lash, T.D.; Bellettini, J.R.; Bastian, J.A.; Couch, K.B. *Synthesis*, **1994**, 170.
16. Drinan, M.A.; Lash, T.D. *J. Heterocyclic Chem.* **1994**, *31*, 255.
17. Jackson, A.H.; Pandey, R.K.; Rao, K.R.N.; Roberts, E. *Tetrahedron Lett.* **1985**, *26*, 793.
18. Smith, K.M.; Minnetian, O.M. *J. Chem. Soc., Perkin Trans. I* **1986**, 277.
19. The incubation products were isolated and analyzed as the porphyrin methyl esters. See reference 4.

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