SYNTHESIS OF PORPHOBILINOGEN-9-^{14}C

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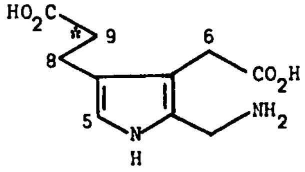
SUMMARY

Ethyl 3-dimethylaminomethyl-5-benzyloxy-6-azaindole-2-carboxylate was condensed with the sodium salt of diethyl malonate-2-^{14}C in decaline, and ethyl 5-benzyloxy-2-ethoxycarbonyl-6-azaindole-3-(2'-ethoxycarbonyl) propionate-2'-^{14}C hydrochloride was obtained in good yield. Further treatment of the labelled azaindole with acid afforded the labelled 6-azaindanone which was reduced to 5-carboxyporphobilinogen lactam. Decarboxylation and saponification of the latter afforded porphobilinogen-9-^{14}C with an overall yield of 27% in the five labelled steps.

Key Words: 6-azaindoles, diethyl malonate-2-^{14}C, porphobilinogen-^{14}C.

INTRODUCTION

Porphobilinogen 1 is the universal metabolic precursor of all the natural porphyrins, chlorins, and corrins (1). The preparation of labelled porphobilinogen 1 is of permanent interest since it is used both for research and for clinical studies. Although porphobilinogen-^{14}C can be obtained from its metabolic precursor, 5-aminolevulinic acid, by making use of immobilized aminolevulinic acid dehydratase (2), the synthetic approaches to 1 are clearly the methods of choice when specifically labelled porphobilinogen is desired.



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Porphobilinogen 1 has been the subject of several syntheses which made use of the classical Knorr synthesis of pyrroles (3-6). The most elaborate of

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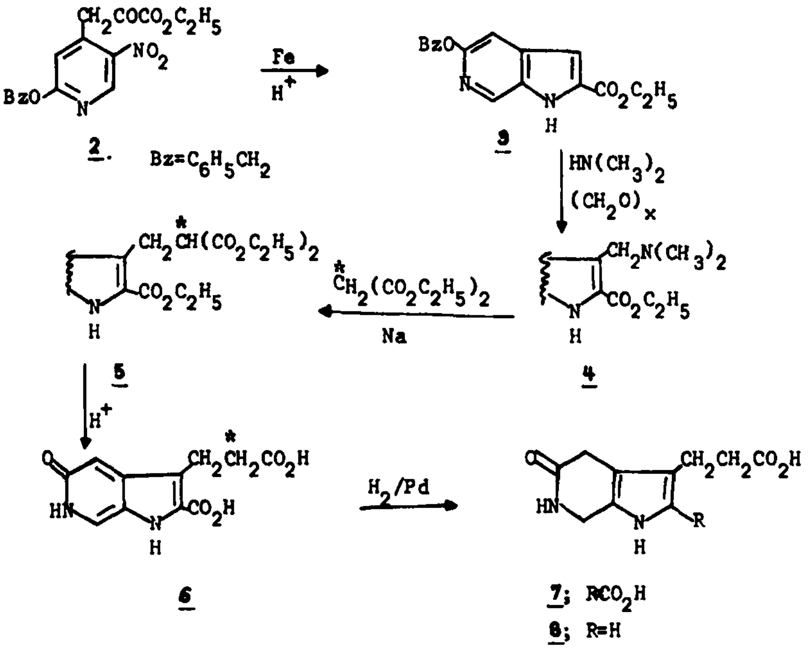
them (6) allowed the synthesis of porphobilinogen-6-^{14}C. A different route of 1 was developed by Frydman et al (7), which made use of the transformation of conveniently substituted 6-azaindoles into porphobilinogen lactam, followed by a saponification of the latter to porphobilinogen 1. This method, together with its recent modifications (8), offers an extremely versatile route for the introduction of labelled atoms in the porphobilinogen molecule. Moreover, since the synthesis of pyrroles from azaindoles can also be used to prepare porphobilinogen-like molecules (9), the method can be used to label the 2-aminomethylpyrroles at convenient sites. The original synthesis (7) has been used to prepare porphobilinogen-8-^{14}C for biosynthetic studies (10), (11).

During the course of our work on uroporphyrinogen biosynthesis (see (12) and references therein), we have mainly used porphobilinogen-9-^{14}C, which can easily be prepared by introducing several modifications in the original synthesis (7). The readily available ethyl 2-benzyloxy-5-nitro-4-pyridinepyruvate 2 (7), was reduced with iron powder in acetic acid to give the 5-benzyloxy-6-azaindole 3 in 58% yield. This procedure is a considerable improvement over the original method (catalytic hydrogenation), and avoids the use of chromathographic procedures. The azaindole 3 was then converted into its Mannich base 4 following the original procedure (7), and the latter was reacted with diethyl malonate-2-^{14}C in decaline in the presence of sodium. The 6-azaindole hydrochloride 5 was thus obtained labelled at the side chain in 50% yield. Treatment of 5 with boiling hydrochloric acid produced the hydrolysis of all the ester groups, of the 5-benzyloxy ether residue, as well as the simultaneous decarboxylation of the 2'-carboxypropionate. The resulting azaindanone-^{14}C 6 was reduced by catalytic hydrogenation to the carboxylactam-^{14}C 7. Decarboxylation of the latter in boiling water gave porphobilinogen-9-^{14}C lactam 8 which was transformed either into the methyl ester by treatment with diazomethane (for use in pyrrylmethane synthesis) (12), or into porphobilinogen-9-^{14}C by saponification with 2N potassium hydroxide (7).



EXPERIMENTAL

Melting points are uncorrected. Radioactivity was measured by the liquid scintillation technique using an ICN TRACERLAB model SC-722 spectrometer. Diethyl malonate-2-^{14}C was purchased from New England Nuclear.

Ethyl 5-benzyloxy-6-azaindole-2-carboxylate 3. A mixture of 5 g of pyridine-pyruvate 2 (7), 25 g of iron powder (Merck's Iron reduced), 100 ml of acetic acid, and 50 ml of water, was stirred and heated at 130°(external bath) during 30 min. The bath temperature was slowly raised from room temperature to 130° to avoid foaming. The mixture was then cooled, poured over 500 ml of ice water, the precipitate was filtered, dried, and extracted in Soxhlet extraction apparatus with ethyl acetate. The extracts were evaporated to dryness, and the residue was crystallized from ethanol-water; 2.5 g (58%); mp 150-152° (lit. (7), mp 150-152°).

Ethyl 5-benzyloxy-2-ethoxycarbonyl-6-azaindole-3-(2'-ethoxycarbonyl) propionate-2'-^{14}C hydrochloride 5. The 6-azaindole 3 (600 mg) was transformed into the

Mannich base dihydrochloride 4 (400 mg) following the described procedure (7). The free base was then liberated from its salt by dissolution in aqueous alkali and extraction into ethyl ether.

The crystalline base 4 (200 mg) was dissolved in 6 ml of decaline and 50 mg of sodium followed by 160 mg of diethyl malonate-2-^{14}C (2mCi, 4mCi/mmol) were added. The mixture was heated at 120° during 6 hr under anhydrous conditions. The warm decaline solution was then diluted with 10 ml of ether and 6 ml of 2N hydrochloric acid, the mixture was cooled, and the precipitate 5 was filtered, dried and crystallized from ethanol-ether; 150 mg (50%); mp 158° (lit. (7), mp 158°); specific activity, 2.7mCi/mmol.

2-Carboxy-5-oxo-5,6-1H-pyrrolo[2,3-c]pyridine-3-propionic-2'-^{14}C acid 6. The 6-azaindolylmethylmalonate-^{14}C hydrochloride 5 (150 mg) was dissolved in 2 ml of concentrated hydrochloric acid and heated under reflux for 6 hr. The solution was evaporated to dryness, the residue was dissolved in 25 ml of a 10% sodium carbonate solution, and the acid was precipitated at pH 4 with acetic acid; 70 mg (94%); specific activity, 2.2mCi/mmol; mp 280° (lit. (7); mp 280°).

2-Carboxy-5-oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic-2'-^{14}C acid (5-carboxyporphobilinogen lactam) 7. The azaindanone-^{14}C 6 (70 mg) was dissolved in 5 ml of a sodium carbonate solution (pH 7), and was reduced with hydrogen at 50 p.s.i. over 70 mg of 10% palladium on charcoal for 2 hr. The catalyst was removed and the solution was adjusted to pH 4 with acetic acid. The precipitate was collected dissolved in a small volume of N ammonium hydroxide and was filtered through an alumina column (aluminium oxid 90aktiv, neutral, E. Merck), using the same ammonium hydroxide solution for elution. The eluate was acidified to pH 4 with acetic acid and the precipitate was collected and dried; 68 mg (90%) specific activity, 1.26mCi/mmol; mp 295° (lit. (7), mp 295°).

Porphobilinogen-9-^{14}C lactam 8. 5-Carboxyporphobilinogen-9-^{14}C lactam 7 (68mg) was suspended in 10 ml of water and heated under reflux for 4 hr. The solution was evaporated to dryness and the residue was recrystallized from water; 50 mg (95%); specific activity, 1.35mCi/mmol; mp 290° (lit. (7) 295°).

Porphobilinogen-9-^{14}C **1**. The former lactam **8** -^{14}C (50 mg) was dissolved in 1 ml of 2N potassium hydroxide and the solution was kept at 20°during 72 hr. It was then adjusted to pH 4 with concentrated acetic acid, cooled, the precipitate of **1** was filtered, dried, and purified by dissolution in dilute ammonium hydroxide and precipitation with concentrated acetic acid; 40 mg (68%); specific activity 1.22mCi/mmol; mp 165°(lit. (1) 167°). It was pure by paper radiochromatography and was quantitatively transformed into uroporphyrinogen I by porphobilinogen deaminase.

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