A SYNTHESIS OF PORPHOBILINOGEN-11-^{13}C

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SUMMARY

Porphobilinogen-11-^{13}C was prepared by using benzyl 3-(β-methoxycarbonylethyl)-4-(methoxycarbonylmethyl)-2-pyrrolecarboxylate as a starting material. A Vilsmeier-Haak formylation with N,N'-dimethylform-^{13}C-amide gave the 2-formylpyrrole, which was transformed into its oxime, and the latter was hydrogenated to the hydrochloride of 5-carboxyporphobilinogen dimethyl ester. The hydrochloride cyclized to 5-carboxyporphobilinogen lactam at pH 9, and the latter was first decarboxylated and then saponified to give the title compound.

Key Words: Porphobilinogen, N,N'-dimethylformamide-^{13}C, Vilsmeier-Haak.

INTRODUCTION

Porphobilinogen (PBG) 1 is the biosynthetic precursor of all the natural porphyrins (1). As such it has been the target of many chemical synthesis (2-6), to prepare it both as unlabelled and as labelled material. Of special importance is the synthesis of PBG-11-^{13}C, which has been very useful in biosynthetic studies (7,8), since the 11-aminomethyl residue gives rise to the meso-methine bridges of the porphyrins (1). The synthesis of PBG-11-^{13}C has been described (4), and it has also been prepared (9) by using one of the aforementioned synthetic approaches (5). We found however, that neither method gave entirely satisfactory results for the synthesis of PBG-11-^{13}C and we therefore want to report here a synthetic approach to PBG-11-^{13}C which gave good and reproducible yields at all the stages of the sequence of labelled intermediates.

The starting material was the by now easily available pyrrole 2 (10), which was transformed into the 2-formylpyrrole 3 with a Vilsmeier reaction using N,N'-dime-

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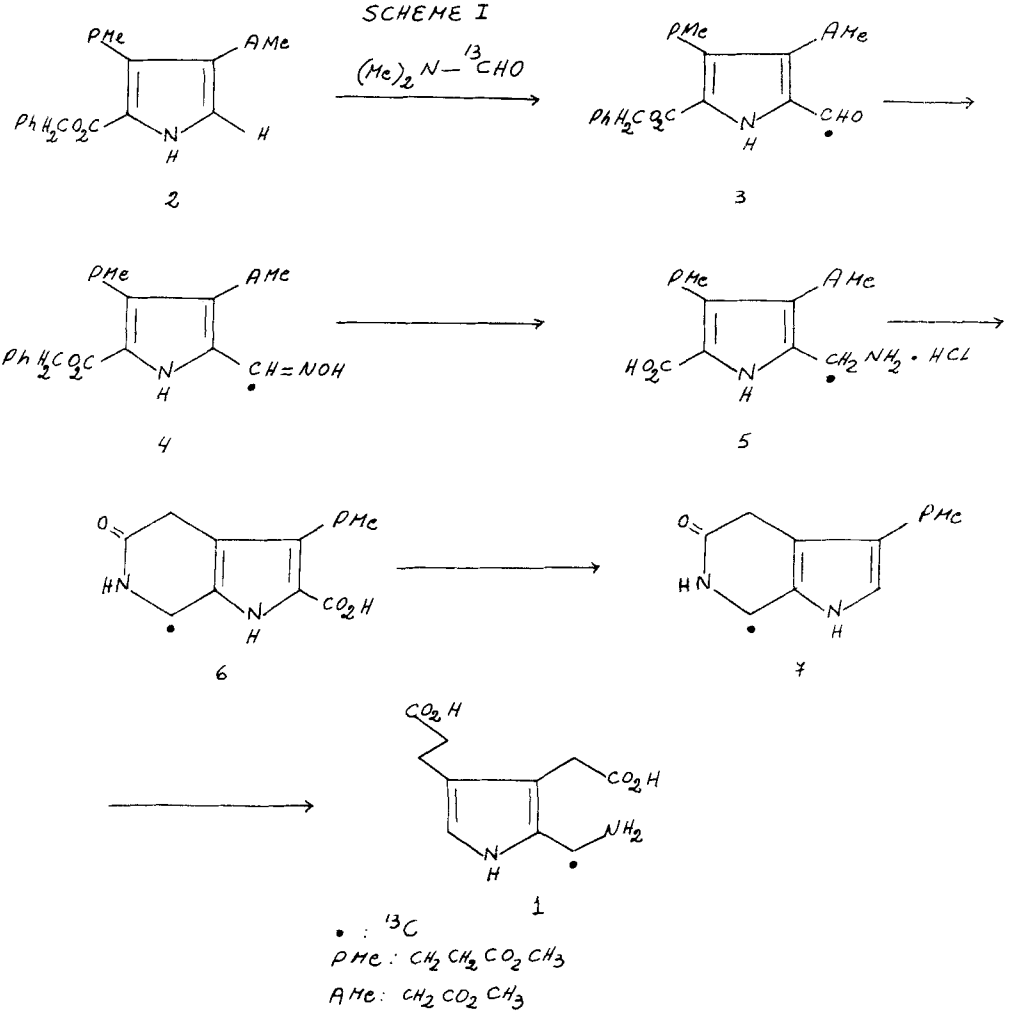
thylformamide-^{13}C (Scheme I). The formylpyrrole 3 was then transformed into its oxime 4, and the latter was reduced with hydrogen over 10% palladium on charcoal to the hydrochloride 5. A simultaneous hydrogenolysis of the benzyl ester also took place during the reaction. The hydrochloride of the aminoacid 5, when dissolved in anhydrous methanol, spontaneously cyclized to the lactam 6 (88% yield) when the solution was adjusted to pH 8.5 with base.

SCHEME I



The final transformation of the 5-carboxylactam 6 in 1 was achieved by two well known reactions (3,11). By dissolution of 6 in boiling water it was decarboxylated to the PBG-11-^{13}C lactam 7, which was saponified to PBG-11-^{13}C 1 by 2N potassium hydroxide at 20°C. This sequence of reactions also provides a simple method of porphobilinogen 1 synthesis.

A value of approximately 35-36 ppm was determined for the 2-aminomethyl-^{13}C in the pyrrole Mannich bases 1 and 5, that shifted to 41-41.5 ppm in the cyclic lactams 5 and 6 derived from them.

EXPERIMENTAL

All melting points were taken on the Kofler block and are uncorrected. ^1H-NMR spectra were recorded on a Perkin-Elmer R-12 spectrometer; CMR were recorded on a Varian FT-80A spectrometer. Me_4Si was used as internal standard.

Microanalysis were performed by the Alfred Bernhardt Mikroanalytisches Laboratorium (Elbach).

Benzyl 2-formyl-^{13}C-3-(methoxycarbonylmethyl)-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate 3. N,N'-Dimethylform-^{13}C-amide (90%^{13}C, 1 mL, 12 mmol) and 1.1 mL (12 mmol) of phosphorous oxychloride were mixed at 5°C. After keeping the mixture at 20°C during 15 min, it was again cooled to 5°C, and a solution of 1.4 g of 2 in 8 mL of 1,2-dichloroethane was slowly added. The mixture was heated and stirred at 85°C during 90 min, and then cooled and adjusted to pH 9 with a concentrated sodium hydroxide solution. The mixture was poured over 200 mL of water, the solution was extracted with chloroform (3 x 80 mL), the extracts were washed with water (40 mL), dried (Na_2SO_4), and evaporated to dryness in vacuo. The residue was dissolved in a small volume of 3% methanol in chloroform, adsorbed on a column (2 x 30 cm) of TLC silica gel (Fluca AG) packed with the same solvent, and the aldehyde 3 was eluted by using the former solvent and applying a slight nitrogen pressure; 1.26 g (82%) of 3 were obtained, mp 79-80°C (ethanol-water); ^1H NMR($CDCl_3$), 2.5, 2.9 (m,m,4H,$CH_2CH_2CO_2$), 3.5, 3.6 (s,s,6H,OCH_3), 3.8 (s,2H,CH_2CO_2), 5.3 (s,2H,CH_2Ph), 7.4 (b,5H,Ph), 9.7 (s,1H,CHO); CMR($CDCl_3$), 181.1 (d,CHO). Anal. Calcd.for $C_{20}H_2NO_7$: C,62.0; H,5.4; N,3.6. Found: C,62.1; H,5.5; N,3.7.

Oxime of benzyl 2-formyl-^{13}C-3-(methoxycarbonylmethyl)-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylate 4. A solution of 0.6 g of hydroxylamine hydrochloride in 25 mL of anhydrous methanol was added to a second solution of 0.2 g of sodium in 30 mL of the same

solvent. The aldehyde 3 (1.26 g) was added to the mixture, and the latter was then heated under reflux for 30 min, cooled, and poured over 200 mL of ice-water. The precipitated 4 was filtered, dried, and crystallized from ethanol-water; 1.25 g (96%); mp 88-90°C, with crystallization and remelting at 118-124°C; ^{1}H NMR (Cl_3CD), 8.2 (b,1H,CHNOH). Anal.Calcd.for $C_{20}H_{22}N_2O_7$: C,59,7; H,5.5; N,7.0. Found: C,59.6; H,5.6; N,6.9.

Hydrochloride of 2-aminomethyl-3-(methoxycarbonylmethyl)-4-(β-methoxycarbonylethyl)-5-pyrrolecarboxylic acid 5. A solution of 1.25 g of oxime 4 in 150 mL of dry methanol and 1.5 mL of concentrated hydrochloric acid, were reduced with hydrogen at 50 psi during 3.5 h over 1.5 g of 10% Pd on charcoal. The catalyst was then filtered, the solution was evaporated in vacuo at 30°C, and the residue was dried in vacuo overnight over alkali. The hydrochloride 5 was crystallized from dry methanol-ether; 0,87 g (84%); mp 206-208°C; ^{1}H NMR(D_2O); 2.7,3.0 (m,m,4H,$CH_2CH_2CO_2$); 3.7, 3.85 (s,s,6H,OCH_3), 3.75 (s,2H,CH_2CO_2), 4.35 (s,2H-$CH_2NH_3^+$), CMR(D_2O): 34.8 (t,$CH_2NH_3^+$). Anal.Calcd.for $C_{13}H_{19}ClN_2O_6$: C,61.3, H,7.5; N,11.0. Found: C,61.2; H,7.4; N, 11.2.

5-Cargoxy porphibilinogen-11-^{13}C-lactam methyl ester 6. A solution of 0.87 g of hydrochloride 5 in 140 mL dry methanol (freshly distilled over CaH_2), was adjusted to pH 8.5 with sodium methoxide in methanol. The mixture was stirred overnight, then poured over an equal volume of water, and the carboxylactam 6 was precipitated by addition of concentrated hydrochloric acid. The precipitate was filtered, washed with water, and dried; 0.6 g (88%); mp 236-238°C (methanol); ^{1}H NMR(DMSO), 2.5, 2.7 (m,m,4H,$CH_2CH_2CO_2$), 3.1(b,2H,CH_2CONH), 3.5(s,3H,OCH_3), 4.2(b,2H,CH_2NH), 7.65 (b, 1H,CONH), 11.2(b,1H,CO_2H); CMR(M-DONa), 41.18(t,CH_2NH).

Porphobilinogen-11-^{13}C lactam methyl ester 7. A suspension of 600 mg of 6 in 90 mL of water was heated under reflux until all the solid dissolved, and the heating was then continued for a further 1 h. The solution was evaporated to dryness in vacuo, and the residue was crystallized from methanol; 360 mg (70%); mp 244-245°C; ^{1}H-NMR (DMSO), 2.8 (m,4H,$CH_2CH_2CO_2$), 3.6 (s,3H,OCH_3), 3.9 (b,2H,CH_2CO), 4.6 (b,2H,CH_2NH), 6.8 (b,1H,H-5), 8.0 (b,1H,NH); CMR(DMSO), 41.58 (t,CH_2NH). Anal.Calcd.for $C_{11}H_{14}N_2O_3$: C,59.4; H,6.3; N,12.7. Found: C,59.3; H,6.3; N,12.7.

Porphobilinogen-11-^{13}C 1. The lactam ester 7 (360 mg) was dissolved with stirring in a mixture of 5 mL of methanol and 5 mL of 4N potassium hydroxide. The solution was kept at 20°C during 72 h, the methanol was evaporated with a stream of nitrogen,

was filtered off, washed with cold water, then methanol, dried, and stored at 20°C: 296 mg (75%); ^{1}H NMR(0.5M-NaOD) δ 2.1,2.6(m,m,4H,CH_2CH_2CO), 3.2(s,2H,CH_2CO), 3.5(s, 2H,$\underline{CH_2}NH_2$), 6.6(b,1H,H-5); CMR (M-NaOD), δ 35.9(t,CH_2NH_2). The PBG-11^{13}C was pure when compared by paper chromathography with an authentic synthetic sample (3) and was completely consumed when incubated with PBG deaminase (1).

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