Studies of Porphyrin Biosynthesis by ^{13}C-Nuclear Magnetic Resonance; Synthesis of [^{13}C]Porphobilinogen and its Incorporation into Protoporphyrin-IX

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Summary [11-^{13}C]Porphobilinogen is synthesised from [^{13}C]methanol and is converted biochemically into [*meso*-^{13}C]protoporphyrin-IX; assignments are made of signals in the ^{13}C-n.m.r. spectra of protoporphyrin-IX and of other porphyrins.

LABELLING with ^{13}C offers advantages over ^{14}C for biosynthetic work on porphyrins provided that the n.m.r. signals from the carbon atoms of interest can be assigned (**1**)—(**4**) and by off-resonance decoupling.[2] The carbon atoms of the macrocycle give two sets of signals (Table), the weakness of the set near δ 140 p.p.m. being due to saturation under the conditions of measurement (no directly bonded hydrogen[3]). The four sharp signals at *ca.* 97 p.p.m. are assigned on this basis to the *meso*-carbon atoms [α, β, γ and δ on (**1**)]; *cf.* the spectrum of (**3**) which shows a signal at δ 128 p.p.m. from the two additional nuclear carbon atoms bonded to hydrogen. These assignments and the

^{13}C-Chemical shifts ($CDCl_3$) for porphyrins at 25·2 MHz; δ values in p.p.m. downfield from Me_4Si

Porphyrin	Central signal of $CDCl_3$	Ar-CH_2-CH_3[a]	Ar-CH_2-CH_2-CO-OMe[a]	Ar-CH=CH_2[a]	Ar-CH_3	*meso*-Carbon atoms[b]	Carbon atoms of macrocycle (other than *meso*-C)
(**4**)	76·8	19·8, 18·5	—	—	—	96·2	141·2—143·5[c]
(**2**)	76·9	19·7(t), 17·6(q)	21·9, 37·0, 173·5, 51·5	—	11·5	96·4	135—147[c]
(**3**)	76·8	—	21·6, 36·8, 173·2, 51·5	—	11·4, 13·5	95·5, 96·7, 99·0, 99·8	133·5—145·5[c]; 128·0[b]
(**1**)	76·9	—	21·7, 36·8, 173·2, 51·6	130·0(d), 120·2(t)	11·5, 12·5	95·7, 96·7, 97·0, 97·6	136—145[c]
[*meso*-^{13}C]-(**1**)	76·8	—	—	—	—	96·0, 97·0, 97·3, 97·9	—

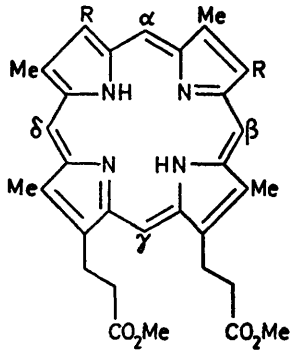
[a] Signal is assigned to the carbon set directly over δ-value; (d), (q), and (t) refer to multiplicity when observed with off-resonance decoupling. [b] Strong sharp signal(s). [c] Weak signals.

and that sufficient ^{13}C-labelled porphyrin for spectroscopic study can be produced biologically.

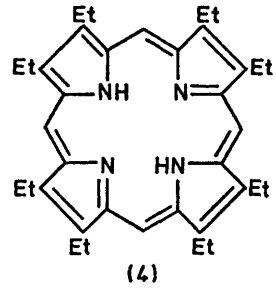
The ^{13}C-chemical shifts determined at natural abundance using proton noise decoupling and Fourier transform techniques for the methyl esters of protoporphyrin-IX (**1**), mesoporphyrin-IX (**2**), and deuteroporphyrin-IX (**3**), and for octaethylporphyrin (**4**) are listed in the Table. Assignment of the sharp signals from the side-chains was from data on chemical shifts,[1] by comparisons within the group recent ones of Doddrell and Caughey[4] agree, with one exception;† however, the signals from the *meso*-carbon atoms of (**1**) as its diethyl ester which are of crucial importance for our biosynthetic studies, appeared as one broad band under their conditions rather than as four distinguishable signals (see Table).

[11-^{13}C]Porphobilinogen (**5**, PBG) was synthesised by reductive methylation[5] of (**6**) with [^{13}C]formaldehyde (60% enrichment) to give (**7**) which was chlorinated and the

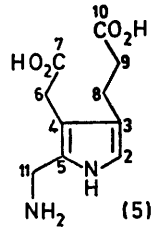
† Assignment of signals from the *C*-methyl groups of mesoporphyrin-IX methyl ester (**2**).



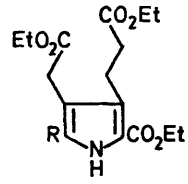
(1) R = CH=CH$_2$
(2) R = Et
(3) R = H



(4)



(5)



(6) R = H
(7) R = $^{13}CH_3$
(8) R = $^{13}CH_2Cl$
(9) R = $^{13}CH_2N_3$
(10) R = $^{13}CH_2\overset{+}{N}H_3Cl^-$

product (**8**) was converted by sodium azide[6] into (**9**). This was hydrogenated to provide (**10**) which was converted by way of carboxy-PBG lactam and PBG lactam[7] into [11-^{13}C]-PBG. Protoporphyrin-IX was isolated as its methyl ester (**1**) after incubating the labelled PBG with an enzyme system from *Euglena gracilis*.[8] The n.m.r. spectrum of the [^{13}C]-protoporphyrin-IX ester showed (in addition to the three peaks from $CDCl_3$) four sharp signals of equal intensity near δ 97 p.p.m., there being insufficient sample to allow ^{13}C at natural abundance to be observed.

The incorporation of 5-amino[5-^{14}C]laevulinic acid[9] and [^{14}C]PBG[10] into protoporphyrin-IX is known. Degradation of the porphyrin from the former precursor[9] yielded carbon dioxide (representing the four *meso*-carbons in *admixture*) which carried half of the original activity. Since the protoporphyrin-IX biosynthesised from [11-^{13}C]PBG shows *four* ^{13}C-signals of similar chemical shift, it follows that these signals can be assigned unambiguously to the *meso*-carbon atoms. The ^{13}C-studies also establish that the biosynthesis of protoporphyrin-IX from [11-^{13}C]PBG (**5**) leads, within the accuracy of the spectroscopic technique, to equal labelling of all four *meso*-positions.

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