Assignment of ^{13}C-Signals from the *meso*-Carbons by Syntheses of ^{13}C-Protoporphyrin-IX Dimethyl Esters

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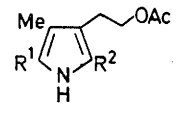
Summary [β-^{13}C]-, [γ-^{13}C]-, and [δ-^{13}C]-Protoporphyrin-IX dimethyl esters have been synthesised by unambiguous routes from [^{13}C]formaldehyde to allow assignment of the four n.m.r. ^{13}C-signals from the *meso*-carbons; the dimethyl ester of diacetyldeuteroporphyrin-IX has been similarly studied.

IT was essential for our biosynthetic studies[1] to assign the four n.m.r. ^{13}C-signals near δ 97 arising[2] from the *meso*-carbons (α, β, γ, δ) of protoporphyrin-IX dimethyl ester (**10**). This has been achieved by the following syntheses[3] of specifically ^{13}C-labelled porphyrins which, for the β-, and δ-labelled samples, were based on Fischer's pyrromethene route[3a] using Johnson's *a,c*-biladiene method[4].

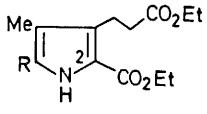
The 5-methyl of pyrrole[3d] (**1**) was converted into carboxy and decarboxylation gave the α-free pyrrole (**2**) which was reductively methylated[5] with [^{13}C]formaldehyde (90 atom% in all experiments) yielding [5-*Me*-^{13}C]-(**1**). Similarly, pyrrole[6] (**5**) afforded the [5-*Me*-^{13}C]pyrrole (**6**). The former was converted[7] into the [^{13}C]pyrromethene (**8**) [69% from pyrroles (**3**) and (**4**)] and the latter into the [^{13}C]pyrromethene (**9**) (55%) which was known[4] in unlabelled form. Reaction of the [^{13}C]pyrromethene (**8**) with unlabelled (**9**) in AcOH–$CHCl_3$–$SnCl_4$ followed by methanolic HBr gave the *a,c*-biladiene system. Cyclisation of this total material in Me_2SO–pyridine[4] and treatment of the products with acidic methanol gave the known diol[3d] (**11**) in 69% yield from the pyrromethenes. Diol (**11**) was converted essentially by Jackson and Kenner's halide-elimination method[3d] into [β-^{13}C]protoporphyrin-IX dimethyl ester (**10**). The foregoing sequence affords the porphyrin (**10**) in 42% yield overall from the dipyrrolic units. [δ-^{13}C]Protoporphyrin-IX dimethyl ester (**10**) was similarly synthesised from ^{13}C-pyrromethene (**9**) and unlabelled (**8**).

The route to [γ-^{13}C]protoporphyrin-IX dimethyl ester (**10**) made use of the ring C–ring D symmetry by condensation of ^{13}C-formaldehyde with the *a,c*-biladiene (**17**); this approach has not been widely used[8] and proof that it yields one pure isomer was first obtained by synthesis in this way[9] of coproporphyrin-II tetramethyl ester. The route to the diene (**17**) started with the pyrromethane[7] (**18**) synthesised from the pyrroles (**15**) and (**16**) each being prepared from (**14**); diborane reduction[10] of (**18**) and acetylation gave (**19**). The pyrromethane (**20**) obtained from (**19**) by deprotection and decarboxylation condensed with the aldehyde (**7**) to yield the unstable (**17**). Acid-catalysed ring closure using [^{13}C]formaldehyde followed by methanolysis gave the [γ-^{13}C]diol (**11**) [7% overall yield from (**19**)]; this was converted as before into [γ-^{13}C]protoporphyrin-IX dimethyl ester (**10**).

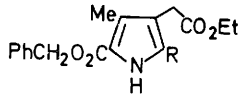
The three labelled samples of (**10**) were diluted with (**10**) from natural sources to give about 5 atom% ^{13}C. The ^{13}C-spectra then showed in each case *one* strongly enhanced signal from the ^{13}C-enriched *meso*-site and three small signals from the three other *meso*-carbons. The results allow the following unambiguous assignments:† α-*meso* at δ 97·7, β-*meso* at 97·1, δ-*meso* at 96·7, and γ-*meso* at 95·8.



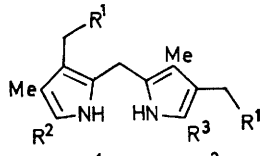
(1) R^1=$PhCH_2O_2C$, R^2=Me
(2) R^1=$PhCH_2O_2C$, R^2=H
(3) R^1=H, R^2=CHO
(4) R^1=H, R^2=Me



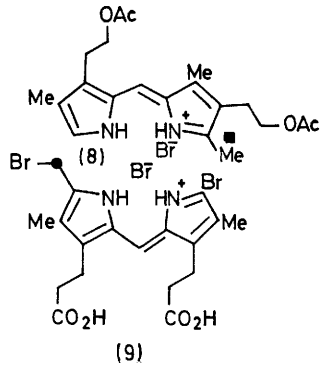
(5) R = H
(6) R = Me
(7) R = CHO, CO_2H in place of 2-CO_2Et



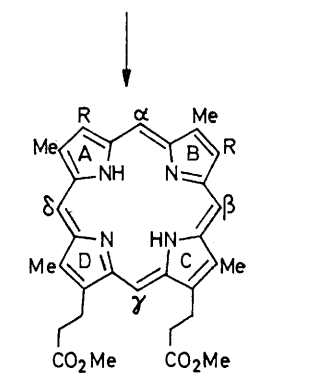
(14) R = Me
(15) R = CH_2OAc
(16) R = CO_2Bu^t



(18) R^1 = CO_2Et, R^2 = $PhCH_2O_2C$, R^3 = CO_2Bu^t
(19) R^1 = CH_2OAc, R^2 = $PhCH_2O_2C$, R^3 = CO_2Bu^t
(20) R^1 = CH_2OAc, R^2 = R^3 = H

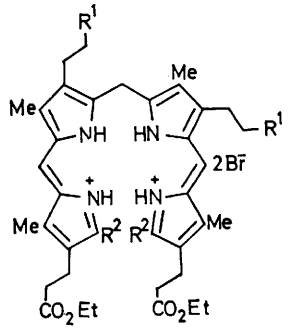


(8)



(9)

(10) R = $CH=CH_2$
(11) R = CH_2CH_2OH
(12) R = $CH(OH)Me$
(13) R = COMe



(17) R^1 = OH or OAc
R^2 = CO_2H

† At 0·01–0·02M in $CDCl_3$; the precise chemical shifts are significantly affected by concentration.

Each ^{13}C-labelled sample of (**10**) was converted[11] into haematoporphyrin dimethyl ester (**12**) and this then oxidised with Jones' reagent to afford the diketone (**13**). The ^{13}C-spectra so obtained allowed the well spread ^{13}C-signals[12] from the *meso*-carbons to be assigned: α-*meso* at δ 102·3, β-*meso* at 99·9, δ-*meso* at 97·3, and γ-*meso* at 95·4.

The foregoing results are of key importance for current[1] and future research on the biosynthesis of the porphyrin macrocycle.

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[12] For spectrum of (**13**) at natural abundance see D. Doddrell and W. S. Caughey, *J. Amer. Chem. Soc.*, 1972, **94**, 2510.