Intermediates in Porphyrin Biosynthesis: Studies with ^{14}C- and ^{13}C-Labelled Pyrromethanes

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Summary Chemical and enzymic studies with the labelled aminomethylpyrromethanes (**3**) and (**4**) are described which have bearing on research into the biosynthesis of type-III porphyrins.

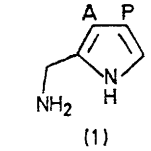
THE biosynthesis of natural porphyrins and chlorins involves at a key stage the conversion of four molecules of porphobilinogen, PBG (**1**) into uroporphyrinogen-III (**2**) in which the side-chains of ring D appear with reversed sequence. This process requires the co-operative action of two enzyme systems, PBG deaminase (synthetase) and uroporphyrinogen-III cosynthetase;[1] no intermediates have been identified. Our studies using PBG doubly labelled with carbon-13 have led to the discovery of the nature of this important rearrangement.[2] Further understanding requires research on possible intermediates and rational syntheses have been described[3] of the pyrromethanes (**3**) and (**4**) in specifically labelled form for studies of their chemistry and their possible enzymic incorporation into uroporphyrinogen-III (**2**). Recent papers from other groups[4] dealing with work in this area prompt us to outline our findings.

The pyrromethanes (**3**) and (**4**), ^{14}C-labelled at ●, were incubated separately with enzyme preparations from (a) *Euglena gracilis*[5] and (b) duck's blood;[6] each pyrromethane was incubated alone and in admixture with radioinactive PBG (**1**). Protoporphyrin-IX (**5**) and, in the case of blood, haemin (**6**) were isolated; the former and part of the latter were converted into protoporphyrin-IX dimethyl ester which was purified to constant activity. The Table shows (Expts. 1—7) that only from the pyrromethane (**3**) was significant radioactivity found in the porphyrin ester [ester of (**5**)]. The remaining haemin (**6**) from Expts. 4 and 5 was degraded by Bonnett's method[7] to a separable mixture of the esters of biliverdin-IXα, -IXβ, -IXγ, and -IXδ; biliverdin-IXα lacks the α-*meso* carbon atom of the original macrocycle (**6**), biliverdin-IXβ lacks the β-*meso* carbon, and so on. Only biliverdin-IXα dimethyl ester (**7**) is illustrated. The activities of the individual crystalline biliverdin esters show that in both experiments the protoporphyrin-IX dimethyl

TABLE. Radioactivity[a] of porphyrins and biliverdins derived from ^{14}C-pyrromethanes

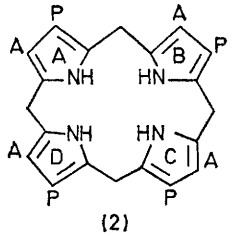
Pyrromethane	*Euglena gracilis* Protoporphyrin-IX dimethyl ester		Duck's blood enzyme Protoporphyrin-IX dimethyl ester		Haemin[b]	Percentage[c] of total activity at *α-meso*	*β-meso*	*γ-meso*	*δ-meso*
^{14}C-(**3**)	Expt. 1	0·54	Expt. 3	0·09	1·5[d]				
			Expt. 4	0·96	8·1	52 ± 5	0 ± 7	51 ± 4	11 ± 7
^{14}C-(**3**) with PBG(**1**) ..	Expt. 2	0·19	Expt. 5	1·2	4·1	35 ± 5	21 ± 6	56 ± 3	0 ± 7
^{14}C-(**4**)		—	Expt. 6	<0·03	<0·01[d]				
^{14}C-(**4**) with PBG(**1**) ..		—	Expt. 7	<0·03	<0·02[d]				
^{14}C-(**4**) with ^{12}C-(**3**) ..		—	Expt. 8	<0·03	0·25				
^{14}C-PBG(**1**)		2·8		14·7	28·4				

[a] Percentage of total radioactivity in administered pyrromethane appearing in porphyrin. [b] Converted into protoporphyrin dimethyl ester for purification. [c] Calculated from activities of the four derived biliverdins. [d] The same enzyme preparation of low activity used for these three runs.

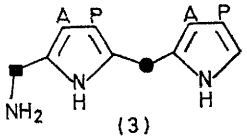


(1)

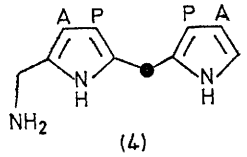
A = CH_2CO_2H
P = $CH_2CH_2CO_2H$



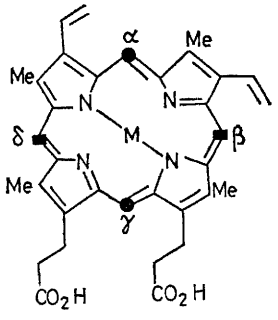
(2)



(3)

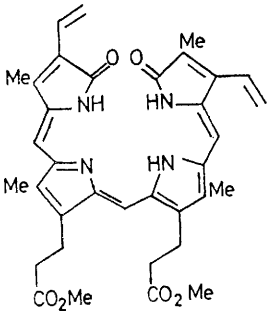


(4)

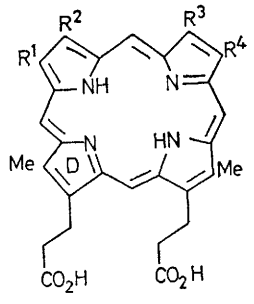


(5) M = H_2

(6) M = Fe^{III} Cl



(7)



(8) $R^1 = R^4$ = Me, $R^2 = R^3$ = P
(9) $R^1 = R^3$ = Me, $R^2 = R^4$ = P
(10) $R^1 = R^4$ = P, $R^2 = R^3$ = Me

ester is labelled predominantly at the α- and *γ-meso*-positions (Table). When pyrromethane (**3**) labelled at ● with ^{13}C was incubated alone with the enzyme system from *Euglena gracilis*, protoporphyrin-IX (**5**) was isolated as its dimethyl ester. This was found to be labelled specifically at the α- and *γ-meso*- positions by inspection of its proton-decoupled ^{13}C–FT spectrum using our unambiguous signal assignments.[8] Furthermore, the pyrromethane (**3**) labelled with ^{13}C at ■ similarly afforded (**5**) which was specifically enriched at the complementary β- and *δ-meso* carbons.

The foregoing specific conversions of the pyrromethane (**3**) into protoporphyrin-IX (**5**) cannot be explained by some fragmentation process yielding labelled monopyrrole units which are then incorporated enzymically. However, it is conceivable that uroporphyrinogen-III (**2**) could be formed from (**3**) by a specific chemical rearrangement (*i.e.* non-enzymic). If this occurred, the product (**2**) would then be converted enzymically into protoporphyrin-IX (**5**). The pyrromethanes (**3**) and (**4**), ^{14}C-labelled at ●, were therefore treated separately, as above but without enzyme, at pH 7·4 and in other runs at pH 8·2. The resultant uroporphyrin isomers were isolated in each case and were decarboxylated[9] to yield the corresponding coproporphyrins (as **8**, **9**, **10**) which were analysed by t.l.c. on cellulose.[10] At both pH 7·4 and 8·2, the pyrromethane (**4**) gave only coproporphyrin-II (**8**). However, the pyrromethane (**3**) gave in addition to the expected coproporphyrin-I [(**9**) with substituents on ring D interchanged], 10%, a fraction in 2—3% yield containing coproporphyrin-III (**9**) and/or coproporphyrin-IV (**10**); these isomers do not separate under the conditions used. The latter fraction was further analysed by reduction to the coproporphyrinogen level followed by incubation with the enzyme preparation from duck's blood. The incorporation of radioactivity into the multiply recrystallised protoporphyrin-IX dimethyl ester (constant activity) indicated that at least 62% of this fraction is the type-III isomer.†

The difference in chemical behaviour between the pyrromethanes (**3**) and (**4**) is remarkable and the mechanism by which (**3**) or products derived from it undergo rearrangement is of considerable interest. One chemical possibility that (**3**) partially rearranges first to (**4**) is excluded by the finding (Expt. 8) that incubation of a mixture of radioinactive (**3**) and radioactive (**4**) with the duck's blood enzyme system yielded haemin (**6**) and protoporphyrin-IX (**5**) of far

† Projected work using analysis by liquid–liquid chromatography is expected to increase the accuracy of this experiment.

lower activity than had been obtained from radioactive (**3**) alone (Expts. 3 and 4).

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