# Biosynthesis of Vitamin $B_{12}$: Structure of the Trimethylisobacteriochlorin from *Propionibacterium shermanii*

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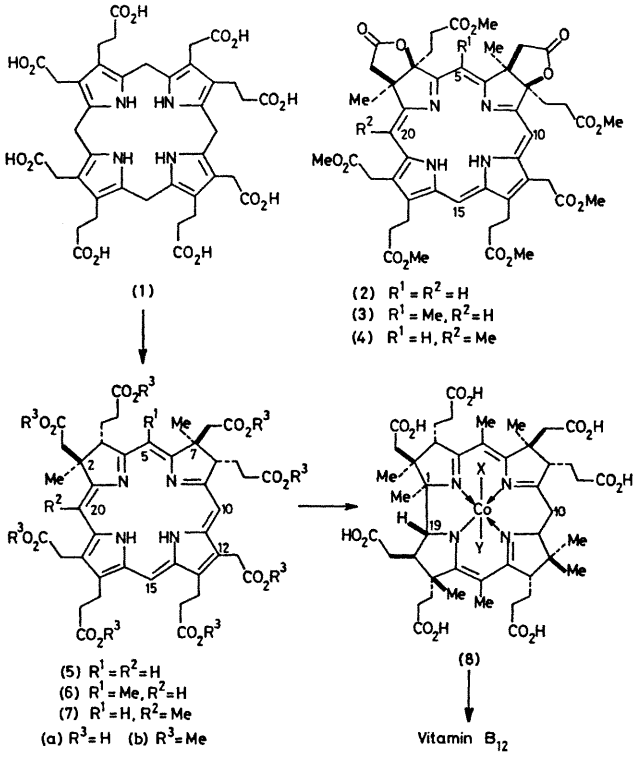
*Summary* N.m.r. and chemical studies on a family of related macrocycles lead to structure (**7a**), having *C*-methylation at C-20, for the trimethylisobacteriochlorin from *P. shermanii*.

THE detection[1] of new pigments from the vitamin $B_{12}$-producer, *P. shermanii*, led to the isolation of pure isobacteriochlorins[2] and to proof of the structures of three of them.[2,3] One of these pigments, shown[2,3] to be the 2,7-dimethylisobacteriochlorin (**5a**), was proved identical with sirohydrochlorin[4] thereby also establishing the structure of this substance. The latter had been shown by the important work of Kamin and Siegel[4] to be the metal-free form of the prosthetic group from several sulphite reductases. Further, the $^{14}C$-labelled 2,7-dimethylisobacteriochlorin (**5a**) was proved to be specifically incorporated[3] into cobyrinic acid (**8**) by a broken cell enzyme system[5] from *P. shermanii*. It was thus concluded[2,3,6] that the isobacteriochlorin itself (**5a**), or a dihydro derivative of it, lies on the biosynthetic pathway between uro'gen-III (**1**) and cobyrinic acid (**8**), the known precursor[7] of vitamin $B_{12}$, and independent work[8] was in agreement.

The 2,7-dimethylisobacteriochlorin (**5a**) is readily converted, by handling in air and esterification, into the bislactone (**2**), previously known[2] as corriphyrin-4 ester. Thus the isolation[2] of a trimethylisobacteriochlorin bislactone from *P. shermanii* pointed to the existence of a non-lactonised parent and this was isolated in Stuttgart[8a] and Cambridge.[6] The properties of the trimethyl bislactone could be accommodated by structures (**3**) or (**4**) and on the basis of information only on the bislactone, preliminary preference was given[2] to (**3**). The development of methods making the parent (unlactonised) trimethylisobacteriochlorin available in mg quantities has now allowed a fuller study.

The octamethyl ester of the trimethylisobacteriochlorin gave a parent ion by field desorption mass spectrometry at *m/e* 988·4346, corresponding to $C_{51}H_{64}N_4O_{16}$. Its $^1H$ n.m.r. spectrum showed only three signals from protons on *meso*-bridges (Table) and comparison of their chemical shifts with the four *meso*-signals from the octamethyl ester of the dimethyl analogue (**5b**) indicates that introduction of the third methyl group has eliminated one of the two resonances found at $\delta$ 7·42 and 7·30 in $CD_3CN$, for the dimethyl system (**5b**), which correspond to protons at C-10 and C-20.[2,6] Also, the spectrum of (**5b**) does not show the singlet at $\delta$ 2·77 corresponding to the third *C*-methyl group of the

trimethyl system. The signal at δ 7·42 in the dimethyl system (**5b**) and that at δ 7·20 in the trimethyl analogue were doublets (*J* 1 Hz, respectively in $CD_3CN$) which were shown to be allylically coupled to a broad 1H triplet at δ 4·0, corresponding to hydrogen at a β-position of a reduced pyrrole residue. The δ 7·42 and 7·20 signals must therefore arise in each case from H-10 to allow allylic coupling to H-8. Thus C-20 is strongly indicated as the substituted *meso*-position in the trimethylisobacteriochlorin (**7b**). The tabulated sets of shift values for derivatives and zinc complexes in the dimethyl and trimethyl series support this view.




Additional evidence comes from two angles. (a) It is known[9] that the CH at the bridge between the reduced rings of isobacteriochlorins undergoes the fastest acid-catalysed exchange, those adjacent to the reduced rings are next in rate, whilst the bridge CH of the pyrromethene residue is essentially unaffected. Here, the signal at δ 6·45 ($CD_2Cl_2$) for the trimethyl system (**7b**) was completely exchanged against neat $CF_3CO_2D$ in less than 2 h at 24 °C in agreement with assignment to position 5 whereas *ca.* 63% of the δ 7·22 and 100% of the δ 8·35 signals remained; *cf.* behaviour of the ester of the dimethyl macrocycle (**5b**) where 84% of δ 6·73 ($CD_2Cl_2$) was exchanged in 8 h at 22 °C, whilst 50, 70, and 100% respectively, of the δ 7·26, 7·38, and 8·45 signals remained. (b) The n.m.r. signals from the acetate methylenes at C-12 and C-18 of the dimethyl system (**5b**) overlap as a slightly broadened singlet at δ 4·29. In contrast, one of the C-12, C-18 acetate methylenes of the trimethyl system (**7b**) appears as a slightly broadened singlet at δ 4·22, and the other as an AB system (*J* 17 Hz) centred at δ 4·24. Although the methylene protons of the acetate groups at C-12 and C-18 are diastereotopic in both (**5b**) and (**7b**), the spectrum shows they are substantially equivalent for (**5b**); their non-equivalence for *one* methylene of the trimethyl compound can readily be understood on the basis of structure (**7b**) whereas this is not so for structure (**6b**). The sum of evidence thus allows structure (**7a**) to be assigned with considerable confidence to the trimethylisobacteriochlorin from *P. shermanii*.

TABLE. $^1H$ N.m.r. shift values (δ) for *meso*-positions of isobacteriochlorins

| Substance and solvent | C-15 | C-10 | C-20 | C-5 |
|---|---|---|---|---|
| Dimethylisobacteriochlorin (**5b**), $CD_2Cl_2$ | 8·45 | 7·38 | 7·26 | 6·73 |
| Trimethylisobacteriochlorin (**7b**), $CD_2Cl_2$ | 8·35 | 7·22 | | 6·45 |
| Dimethylisobacteriochlorin (**5b**), $CD_3CN$ | 8·53 | 7·42 | 7·30 | 6·85 |
| Trimethylisobacteriochlorin (**7b**), $CD_3CN$ | 8·37 | 7·20 | | 6·50 |
| Zn complex of (**5b**), $CD_2Cl_2$ | 8·75 | 7·67 | 7·56 | 7·00 |
| Bislactone (**2**), $CD_2Cl_2$ | 9·03 | (8·09 + 7·82) | | 7·38 |
| Bislactone (**4**), $CD_2Cl_2$ | 9·26 | 8·31 | | 7·61 |
| Zn complex of (**2**), $CDCl_3$ | 8·93 | (8·02 + 7·85) | | 7·23 |
| Zn complex of (**4**), $CD_2Cl_2$ | 8·82 | 7·90 | | 6·90 |

The biosynthetic interest of this substance stems from its isolation from a vitamin $B_{12}$-producing organism and from its incorporation in $^{14}C$-labelled form[8a] into cobyrinic acid (**8**), though as yet of unknown labelling pattern. Structure (**7a**) raises two fascinating possibilities for the biosynthesis of cobyrinic acid (**8**); (i) that C-20 may be extruded as a $C_2$-unit,[10] or (ii) that the methyl group at C-1 of cobyrinic acid (**8**) may arise by migration from C-20.§ Experimental tests are in progress.

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§ This possibility was first suggested by W.-D.W.

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