Biosynthesis of Vitamin B_{12}: Isolation and Proof of Structure of 3-Episirohydrochlorin

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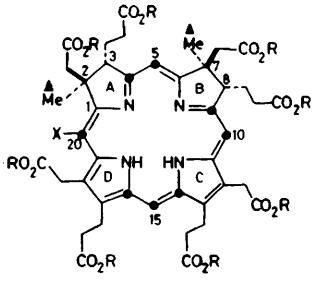
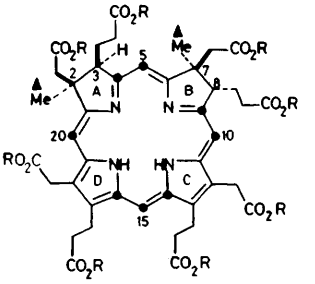
Summary The isomer isolated from *Propionibacterium shermanii* cultures together with sirohydrochlorin (**1a**) is shown by ^{13}C-labelling and nuclear Overhauser effect studies to have structure (**5a**), *i.e.* 3-episirohydrochlorin.

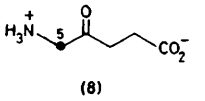
FIVE different isobacteriochlorins have been isolated so far from resting *P. shermanii* cells and their structures have been established.[1-3]. Three of them are lactones formed by air oxidation of the two key pigments which have structures 2,7-dimethylisobacteriochlorin (**1a**) and 2,7,20-trimethylisobacteriochlorin (**4a**). The former was shown[1,3] to be identical with sirohydrochlorin isolated from sulphite reductases.[4] Both dimethyl-, and trimethyl- systems (**1a**) and (**4a**) were found to be incorporated into cobyrinic acid (the precursor of vitamin B_{12}) by a broken cell system from *P. shermanii*.[1-3]

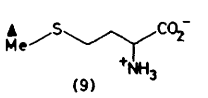
Always during the isolation of sirohydrochlorin (**1a**) from *P. shermanii*, a sixth pigment was detected by h.p.l.c. in varying amounts and was isolated as its octamethyl ester. This was found to be an isobacteriochlorin by u.v.–visible spectroscopy and it was isomeric (M^+ 974) with sirohydrochlorin octamethyl ester by field desorption mass spectroscopy; the problem was to determine whether the new pigment is a structural- or stereo-isomer. The studies below show it to have structure (**5a**).

Sirohydrochlorin ester (**2b**) and the isomer (**6b**) were isolated from *P. shermanii* cells fed with [5-^{13}C]-δ-aminolaevulinic acid (**8**). The ^{13}C-labelling pattern is fixed as illustrated for (**2b**) with respect to rings A, B, C, and D by the earlier stages of biosynthesis.[5] Sirohydrochlorin ester (**2b**) from this experiment showed ^{13}C-signals for the *meso*-bridges characteristic of this labelling pattern[1b,3a] (C-15, triplet; C-20, singlet, C-5 and C-10, doublets). The ^{13}C-spectrum of the isomer (**6b**) showed the same signal pattern and it was confirmed for this spectrum that the signal (triplet) at lowest field (δ 108·1 p.p.m.) did arise from C-15 by selective decoupling at δ 8·49 in the ^1H-range (corresponding to H at C-15); only the δ 108·1 p.p.m. signal was then fully decoupled, the other three signals from C-5, C-10, and C-20 showing residual ^1H–^{13}C coupling. It follows that the isomer is *C*-methylated on rings A and B (**5b**).






(8)


(9)

Sirohydrochlorin ester (**3b**) and the isomer (**7b**) were prepared biosynthetically from [*methyl*-^{13}C]methionine (**9**) and the Table shows that the signals from the two ^{13}C-methyl groups of sirohydrochlorin ester (**3b**) have similar δ-values whereas for the isomer (**7b**), one ^{13}C-methyl signal has moved considerably downfield (*ca.* 7 p.p.m.), a change understandable by loss of one γ-effect.[6,7]

To discover which *C*-methyl group had been affected, separate irradiations were made at the ^1H-resonances of the

two *C*-methyl groups of (**1b**) and (**5b**) checking for nuclear Overhauser effect enhancement of the ^{1}H-signals for 5-H and 20-H. This allowed the ^{1}H-signal assignments for the *C*-methyl groups of sirohydrochlorin ester (**1b**) given in the Table.

TABLE. ^{13}C and ^{1}H n.m.r. shift values (δ) for sirohydrochlorin ester and the ester of 3-epi-isomer.

Assignment	Sirohydrochlorin ester (**2b**) and (**3b**) ^{13}C	3-Epi-isomer (**6b**) and (**7b**) ^{13}C
C-5	89·4	89·9
C-20	93·4	92·4
C-10	95·4	96·5
C-15	107·5	108·1
2-Me	20·4 (5·6 Hz)[a]	27·3 (8·4 Hz)[a]
7-Me	19·7 (7·4 Hz)[a]	19·6 (4·3 Hz)[a]
	^{1}H [for (**1b**)]	^{1}H [for (**5b**)]
2-Me	1·83	1·55
7-Me	1·78	1·79

[a] Size of residual ^{1}H–^{13}C coupling with decoupler set at 2·12 p.p.m. in ^{1}H-n.m.r. spectrum.

It remained to correlate the ^{13}C and ^{1}H signals for the *C*-methyl groups. Since the ^{1}H-signal from 7-Me appears at δ 1·78—1·79 for both esters (**3b**) and (**7b**), selective irradiation at 2·12 p.p.m. (^{1}H-scale) with observation of the ^{13}C-spectrum should leave a larger residual coupling for 7-Me than 2-Me for sirohydrochlorin ester (**3b**) whereas the reverse should hold for the isomeric ester (**7b**). The results allowed the assignments given in the Table. Thus, the configuration of the propionate residue has changed relative to the *C*-methyl group in ring-A of the isomer (**5b**) and not in ring-B.

To prove that the absolute configurations at C-2, C-7, and C-8 in the ester of the isomer are as in sirohydrochlorin (**1b**), the former was epimerised under basic conditions to give the latter, the product being identified with authentic sirohydrochlorin ester by t.l.c., h.p.l.c., u.v.–visible, ^{1}H-n.m.r., and, decisively, by c.d. spectra.

The isomer is thus 3-episirohydrochlorin ester (**5b**). It was shown not to be produced during acid-catalysed esterification of sirohydrochlorin (**1a**) and so the acid (**5a**) must be present in the cultures or formed during the early isolation steps.

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[1] (a) A. R. Battersby, E. McDonald, H. R. Morris, M. Thompson, D. C. Williams, V. Ya. Bykhovsky, N. I. Zaitseva, and N. V. Bukin, *Tetrahedron Letters*, 1977, 2217; A. R. Battersby, *Experientia*, 1978, **34**, 1; A. R. Battersby, E. McDonald, M. Thompson, and V. Ya. Bykhovsky, *J.C.S. Chem. Comm.*, 1978, 150; A. R. Battersby, G. W. J. Matcham, E. McDonald, R. Neier, M. Thompson, W.-D. Woggon, V. Ya. Bykhovsky, and H. R. Morris, *ibid.*, 1979, 185; (b) N. G. Lewis, G. W. J. Matcham, R. Neier, E. McDonald, and A. R. Battersby, *ibid.*, 1979, 541.

[2] R. Deeg, H.-P. Kriemler, K.-H. Bergmann, and G. Müller, *Z. Physiol. Chem.*, 1977, **358**, 339, 1315; G. Müller, R. Deeg, K. D. Gneuss, G. Gurzer, and H.-P. Kriemler, Proc. Third European Symposium on Vitamin B_{12}, Zürich, 1979.

[3] (a) A. I. Scott, A. J. Irwin, L. M. Siegel, and J. N. Shoolery, *J. Amer. Chem. Soc.*, 1978, **100**, 316, 7978; (b) G. Müller, K. D. Gneuss, H. P. Kriemler, A. I. Scott, and A. J. Irwin, *J. Amer. Chem. Soc.*, 1979, **101**, 3655.

[4] L. M. Siegel, M. J. Murphy, and H. Kamin, *J. Biol. Chem.*, 1973, **248**, 251, 6911; M. J. Murphy, L. M. Siegel, H. Kamin, and D. Rosenthal, *ibid.*, p. 2801.

[5] A. R. Battersby, G. L. Hodgson, E. Hunt, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1976, 273.

[6] B. V. Cheney and D. M. Grant, *J. Amer. Chem. Soc.*, 1967, **89**, 5319; D. K. Dalling and D. M. Grant, *ibid.*, 1972, **94**, 5318 and refs therein.

[7] *Cf.* A. I. Scott, C. A. Townsend, and R. J. Cushley, *J. Amer. Chem. Soc.*, 1973, **95**, 5759.