JOURNAL OF THE CHEMICAL SOCIETY

Chemical Communications

Number 16
1985

Syntheses Relevant to Vitamin B_{12} Biosynthesis: Synthesis of Sirohydrochlorin and of its Octamethyl Ester

Michael H. Block, Steven C. Zimmerman, Graeme B. Henderson, Simon P. D. Turner, Steven W. Westwood, Finian J. Leeper, and Alan R. Battersby*

University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Sirohydrochlorin is an isobacteriochlorin isolated (a) as the metal-free prosthetic group of sulphite reductase and (b) as the aromatised form of the di-*C*-methylated intermediate on the biosynthetic pathway to vitamin B_{12}; the synthesis is described of the natural enantiomer of sirohydrochlorin and also of its octamethyl ester using a mild photochemical route.

Sirohydrochlorin was isolated from sulphite reductase[1] as the metal-free form of the enzyme's prosthetic group. Its structure (**23**), based on the isobacteriochlorin macrocycle, was established in 1977[2] by work arising from the finding that sirohydrochlorin is important for the biosynthesis of vitamin B_{12}.[3] The true intermediate on the biosynthetic pathway to vitamin B_{12} is 15,23-dihydrosirohydrochlorin[4] but under normal work-up conditions (aerobic), it is the aromatised sirohydrochlorin (**23**) which is isolated, usually as its octamethyl ester (**24**). Future researches on vitamin B_{12} would be greatly assisted by having available far greater quantities of sirohydrochlorin than can be obtained from natural sources. We report here the synthesis of sirohydrochlorin (**23**) and of its octamethyl ester (**24**) by a route which (a) allows the introduction of specific isotopic labels and (b) can readily be scaled up.

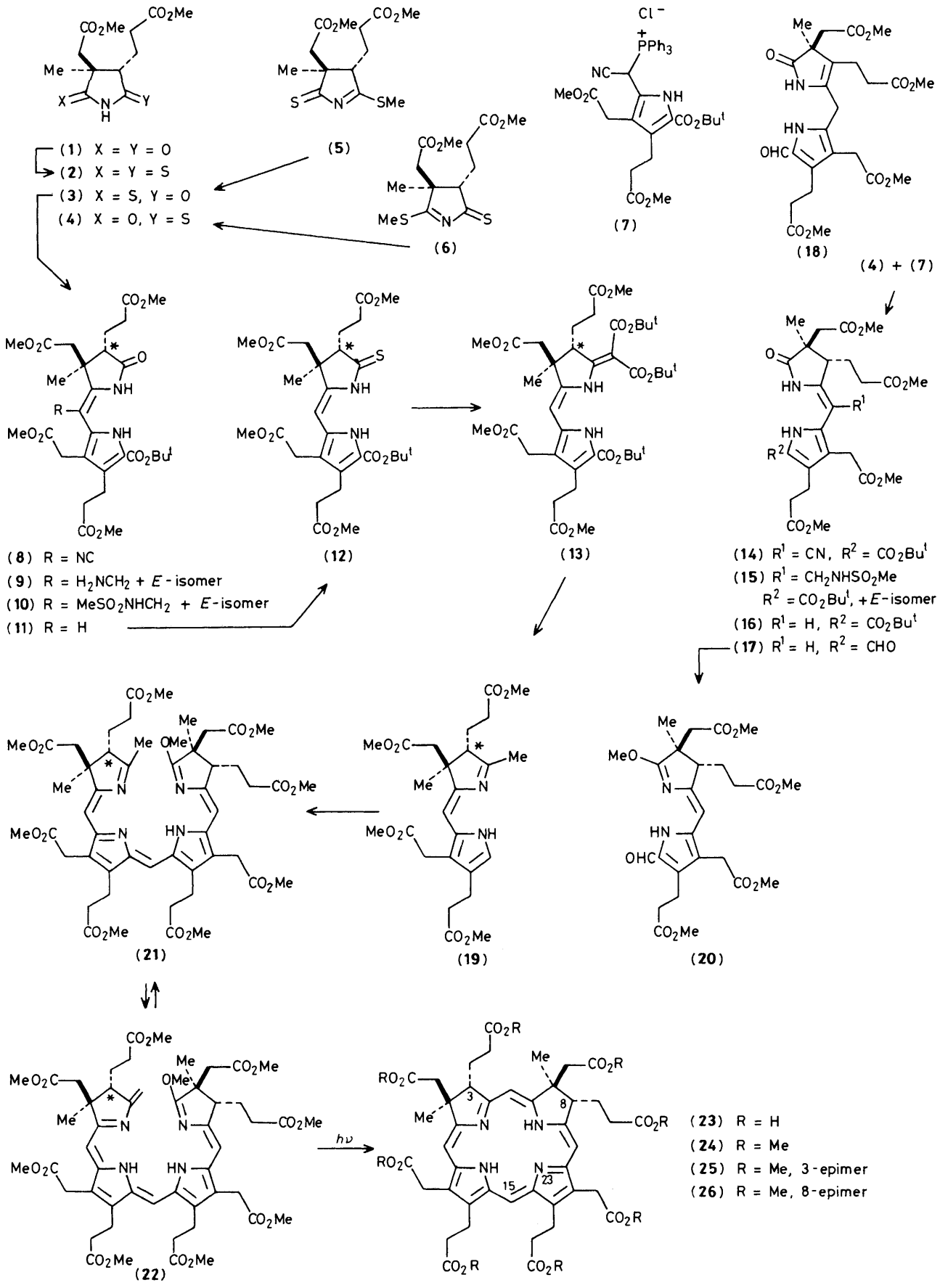
The basic strategy involved using the imide (**1**) to provide the reduced ring of both the western (**19**) and eastern (**20**) building blocks for the sirohydrochlorin molecule. These, in optically active form, were to be condensed to generate the seco-system (**21**) ready for final photochemical ring-closure,[5] presumably as the 18π-tautomer (**22**), to give sirohydrochlorin octamethyl ester (**24**). Essentially all the steps in the synthesis described below were developed using racemic materials starting from synthetic (±)-imide,[6] as (**1**); however, only the work based on optically active intermediates is outlined here.

Synthesis of the stable immediate precursor (**13**) of the labile western block (**19**) started with the conversion of the amorphous optically active imide[7] (**1**) by Lawesson's reagent[8] into the crystalline dithioimide (**2**), 81%. This was treated with trimethyl orthoformate (TMOF) and trifluoroacetic acid (TFA) to yield a mixture of the imino sulphides (**5**) and (**6**) which by hydrolysis with aqueous acid afforded a *ca.* 1:1 mixture of the monothioimide (**3**), 41%, and its regio-isomer (**4**), 49%; these were separable. In the (±)-series, both monothioimides as (**3**) and as (**4**) were obtained crystalline; their illustrated structures were confirmed by *X*-ray analysis.[9]

When the optically active monothioimide (**3**) was heated in toluene with the Wittig salt[10] (**7**) (the nitrile function was *essential* for success) and potassium t-butoxide, the *E*-nitrile was obtained, 72%, composed of the major isomer (**8**) and its epimer at the *-centre; *Z*-nitrile, 7%, was also formed and some starting material (**3**), 14%, was recovered. The nitriles (**8**) were reduced with hydrogen and W2 Raney nickel in methanol–water–methanesulphonic acid to give a mixture of the *Z*- and *E*-amines (**9**) which also contained the corresponding epimers at the asterisked position. Later experience (see below) showed that it was unnecessary for preparative work to separate the stereoisomers at this stage so the mixture was used in the next step. Treatment of the total basic product with 4-dimethylaminopyridine and methanesulphonyl chloride afforded the *Z*- and *E*- sulphonamides (**10**), 72%. These sulphonamides (**10**) were the substances of choice for a reverse-Mannich reaction brought about by heating them in anisole with *N,N'*-diethyl-1,2-diaminoethane; the reagent presumably played a role in trapping the reactive reverse-Mannich fragment ($CH_2{=}NSO_2Me$).

The major product was the desired *Z*-lactam (**11**) together with the epimer (at *), total 71%; the *E*-isomer was undetectable so considerable simplification had occurred. The *Z*-lactam (**11**) and its epimer at * were separated to allow full characterisation, but for preparative work, the two were carried forward together. They were converted by Lawesson's reagent into the thiolactams (**12**), 90%, which by reaction with di-t-butyl monobromomalonate and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) gave the *S*-malonyl system. Sulphur extrusion[11] by heating this product in toluene with triphenylphosphine and DBU then afforded the triester (**13**) and its epimer at * (total 60% for the two stages).

Synthesis of the racemic form of the starting material (**14**) required for the eastern block (**20**) has been outlined;[12] the same chemistry applied to the optically active monothioimide (**4**) gave the *E*-nitrile (**14**), 70%, together with 11% of the separable *Z*-isomer. Reduction of the nitrile (**14**) and methanesulphonation of the product as above yielded the *Z*- and *E*-sulphonamides (**15**), 67%. In this case, the reverse-Mannich reaction [as (**10**)→(**11**) above] was best run to partial


(1) X = Y = O
(2) X = Y = S
(3) X = S, Y = O
(4) X = O, Y = S
(5)
(6)
(7)
(18)
(4) + (7)
(8) R = NC
(9) R = H_2NCH_2 + E-isomer
(10) R = $MeSO_2NHCH_2$ + E-isomer
(11) R = H
(12)
(13)
(14) R^1 = CN, R^2 = CO_2Bu^t
(15) R^1 = CH_2NHSO_2Me
R^2 = CO_2Bu^t, + E-isomer
(16) R^1 = H, R^2 = CO_2Bu^t
(17) R^1 = H, R^2 = CHO
(19)
(20)
(21)
(22)
hν
(23) R = H
(24) R = Me
(25) R = Me, 3-epimer
(26) R = Me, 8-epimer

conversion (35%) cleanly to yield just the *Z*-isomer (**16**), 78% based on unrecovered starting material. When this was deprotected, decarboxylated, and formylated with TFA–TMOF the product was the aldehyde (**17**) (67%); in some reactions, isomerisation occurred to give the lactam with the *endo*-double bond (**18**) but this material is also synthetically useful as will be described in our full paper. The required eastern block (**20**) was then produced, 64%, from the aldehydolactam (**17**) by treatment with trimethyloxonium tetrafluoroborate and Hunig's base.

Treatment of the tri-t-butyl ester (**13**) with TFA removed the three t-butyl groups and catalysed three decarboxylations to generate the labile western block (**19**) which without isolation was condensed with the eastern block (**20**) in 2:3 TFA–methanol. The seco-system (**21**) $\rightleftarrows$ (**22**) so formed was cyclised photochemically[5] (3—4 days) to yield isobacteriochlorins (IBCs), 15% over the four main stages from the building blocks (**13**) and (**20**); this corresponds to an average yield for each stage of >60%. Sirohydrochlorin octamethyl ester (**24**) accounted for 84% of the total IBCs, the 3-*epi*-isomer[13] (**25**) for 8% and the 8-*epi*-isomer[14] (**26**) for 8%. These three products were separated by preparative h.p.l.c. and identified by comparison (t.l.c., h.p.l.c., u.v.–vis., 400 MHz n.m.r., field-desorption mass spectrometry) with authentic samples isolated from *Propionibacterium shermanii*. Since it has been shown[13] that the 3-*epi*-isomer (**25**) can be epimerised under basic conditions to yield sirohydrochlorin methyl ester (**24**), and this surely will also be so for the 8-*epi*-isomer (**26**), all the material generated in the synthesis can be converted into valuable product. Hydrolysis of the ester (**24**) of sirohydrochlorin to the octa-acid (**23**) has often been carried out[15] so the present work also constitutes a synthesis of sirohydrochlorin itself. Thus the structure (**23**) deduced[3] by a combination of spectroscopic methods and biosynthetic incorporation experiments is rigorously confirmed.

We thank the SERC for a Studentship (to M.H.B.), for a CASE Studentship (to S.P.D.T.), and for financial support. Grateful acknowledgement is also made to the National Science Foundation (U.S.A.) for a NATO Postdoctoral Fellowship (to S.C.Z.), to Shell Australia Ltd. for a Shell Scholarship (to S.W.W.), and to Dr. D. A. Evans (FBC Ltd.) for his interest.

Received, 8th May 1985; Com. 617

References

1 L. M. Siegel, M. J. Murphy, and H. Kamin, *J. Biol. Chem.*, 1973, **248**, 251; M. J. Murphy and L. M. Siegel, *ibid.*, p. 6911; M. J. Murphy, L. M. Seigel, H. Kamin, and D. Rosenthal, *ibid.*, p. 2801.
2 A. R. Battersby, K. Jones, E. McDonald, J. A. Robinson, and H. R. Morris, *Tetrahedron Lett.*, 1977, 2213; A. R. Battersby, E. McDonald, M. Thompson, and V. Ya. Bykhovsky, *J. Chem. Soc., Chem. Commun.*, 1978, 150; A. I. Scott, A. J. Irwin, L. M. Siegel, and J. N. Shoolery, *J. Am. Chem. Soc.*, 1978, **100**, 316 and 7987.
3 Reviewed by A. R. Battersby and E. McDonald in 'B_{12},' ed. D. Dolphin, Wiley, New York, 1982, p. 107.
4 A. R. Battersby, K. Frobel, F. Hammerschmidt, and C. Jones, *J. Chem. Soc., Chem. Commun*, 1982, 455.
5 P. J. Harrison, C. J. R. Fookes, and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1981, 797.
6 A. R. Battersby and S. W. Westwood, in preparation.
7 T. L. Bogard and A. Eschenmoser, unpublished work; A. Eschenmoser, Special Lectures, 23rd IUPAC International Congress of Pure and Applied Chemistry, Butterworths, 1971, Vol. 2, p. 69.
8 S. Scheibye, B. S. Pedersen, and O.-S. Lawesson, *Bull. Soc. Chim. Belg.*, 1978, **87**, 229.
9 W. B. Cruze and P. Raithby (Cambridge), unpublished work.
10 D. M. Arnott, A. R. Battersby, P. J. Harrison, G. B. Henderson, and Zhi-Chu Sheng, *J. Chem. Soc., Chem. Commun.*, 1984, 525.
11 M. Roth, P. Dubs, E. Gotschi, and A. Eschenmoser, *Helv. Chim. Acta*, 1971, **54**, 710.
12 S. P. D. Turner, M. H. Block, Zhi-Chu Sheng, S. C. Zimmerman, and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1985, 583.
13 A. R. Battersby, E. McDonald, R. Neier, and M. Thompson, *J. Chem. Soc., Chem. Commun.*, 1979, 960.
14 J. J. Huang, C. J. R. Fookes, and A. R. Battersby, unpublished work (Cambridge).
15 *E.g.* H. C. Uzar and A. R. Battersby, *J. Chem. Soc., Chem. Commun.*, 1982, 1204.