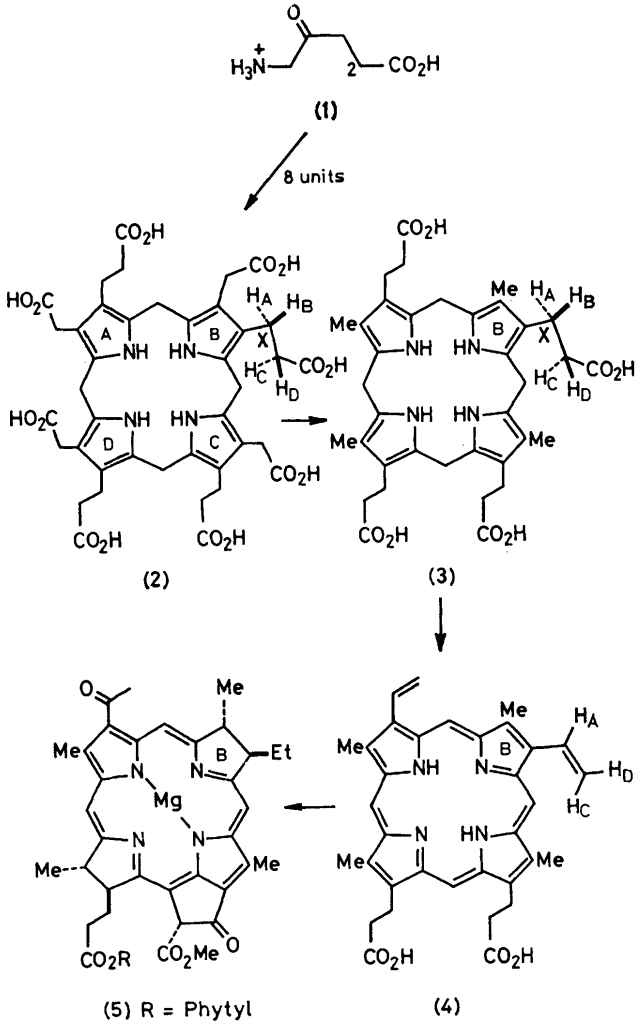
Stereochemistry of Formation of Methyl and Ethyl Groups in Bacteriochlorophyll a

By ALAN R. BATTERSBY,* ARIE L. GUTMAN, and CHRISTOPHER J. R. FOOKES
(*University Chemical Laboratory, Lensfield Road, Cambridge* CB2 1EW)

and HELMUT GÜNTHER and HELMUT SIMON
(*Lehrstuhl für Organische Chemie und Biochemie der Technischen Universität, Munich*, D8046 *Garching, Federal Republic of Germany*)

Summary δ-Aminolaevulinic acid has been synthesised carrying ^2H and ^3H isotopes at C-2 in the *S*-configuration; this has been incorporated by *Rhodopseudomonas spheroides* into bacteriochlorophyll a, degradation of which has established the mode of addition of hydrogen to the precursor vinyl group to form the ring-B ethyl group and that the decarboxylation to form the ring-B methyl group occurs with *retention*.

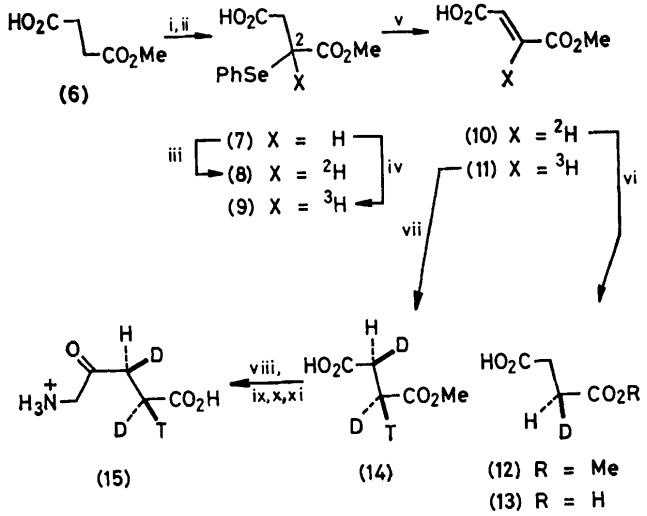
IT is known that chlorophylls-a and -b obtained from higher plants[1] and bacteriochlorophyll a[2] (**5**) obtained from photosynthetic bacteria are biosynthesised from protoporphyrin IX (**4**) *via* uro'gen III (**2**) and copro'gen III (**3**), Scheme 1.[3]



SCHEME 1

Our interest is in the stereochemistry of formation of the ethyl group on ring-B which is a characteristic feature of the plant chlorophylls and bacteriochlorophyll a (**5**).

Earlier work had proved that generation of the vinyl groups on rings-A and -B of protoporphyrin IX (**4**) from copro'gen III (**3**) involves (i) stereospecific removal of H_B from site X[4] and (ii) an overall antiperiplanar elimination.[5] The hydrogen atoms H_A, H_C, and H_D from the propionic residues of (**3**) thus appear in (**4**) as illustrated in Scheme 1. Hydrogen atoms H_C and H_D are derived from C-2 of δ-aminolaevulinic acid (**1**), ALA; synthesis was therefore undertaken of ALA (**1**), made chiral by labelling at C-2 with ^2H and ^3H. By incorporation of this precursor in H_2O into bacteriochlorophyll a (**5**), the methyl group of the ring-B ethyl residue should be produced in chiral form susceptible to configurational assay.[6]



SCHEME 2. *Reagents:* i, Lithium di-isopropylamide (LDA); ii, PhSeBr; iii, LDA, CF_3CO_2D; iv, LDA, CF_3CO_2T; v, H_2O_2; vi, 2-enoate reductase, H_2O; vii, 2-enoate reductase, D_2O; viii, $SOCl_2$, CH_2N_2, HCl; ix, NaN_3; x, H_2, Pd, HCl; xi, aqueous HCl.

The synthesis (Scheme 2) involved regiospecific bis-anion generation from monomethyl succinate (**6**) which led to the selenide (**7**).[7] The selenium not only activated 2-H for easy replacement by ^2H or ^3H to give compounds (**8**) and (**9**), but also allowed ready conversion into monomethyl [2-^2H]-fumarate (**10**) or the corresponding [2-^3H]fumarate (**11**). 2-Enoate reductase from *Clostridium sp. Lal* (DSM 1460)[8] accepted the fumarate (**10**) well and with H_2O as medium the product (**12**) was obtained, as proved by acidic hydrolysis to [2-$^2H_1$]succinic acid (**13**). This was kindly shown by Dr G. Ryback (Shell, Sittingbourne) to be the configurationally pure (±5%) (2*S*)-[2-$^2H_1$]succinic acid (**13**) (found

TABLE. Stereochemical assay on samples of acetic acid from bacteriochlorophyll a.

Expt. no.	Acetic acid (**21**) from ethyl group $^3H:^{14}C$ Ratios (% retention) Acetic acid	Malic acid	Fumaric acid[a]	Acetic acid (**19**) from C-7 $^3H:^{14}C$ Ratios (% retention) Acetic acid	Malic acid	Fumaric acid[a]	Acetic acid from random [2-$^3H_1$]ALA $^3H:^{14}C$ Ratios (% retention) Acetic acid	Malic acid	Fumaric acid[a]
1	5·11	4·54 (89)	3·63 (80)	8·22	7·74 (94)	5·34 (69)	3·95	3·83 (97)	2·06 (54)
2	5·11	4·62 (90)	3·54 (77)	8·22	7·52 (91)	5·58 (74)	3·95	3·90 (99)	2·11 (54)

[a] Usual values for retention of ^3H in fumaric acid derived from (2*R*)-[$^1H_1$, $^2H_1$, $^3H_1$]acetic acid range up to 80% (refs. 6 and 14).

by c.d. $\Delta\epsilon_{max}$ + 0·052 at λ 206 nm, after correction for D content of 84 ± 3%; standard value $\Delta\epsilon_{max}$ + 0·055).

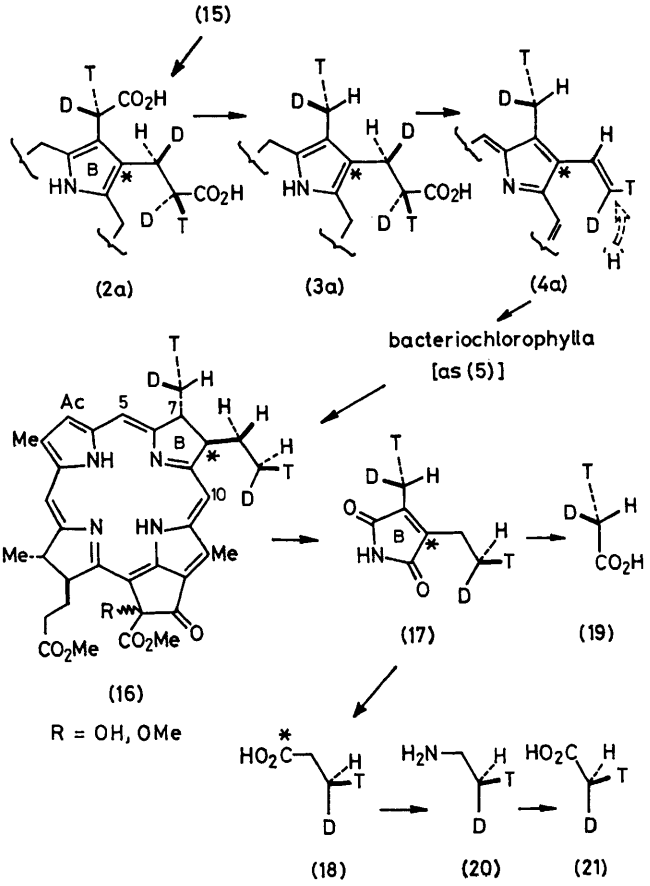
With the configurational sense of the reduction secure, the [2-^{3}H]fumarate (**11**) was enzymically reduced, but now in D_2O to yield monomethyl (2*S*,3*S*)-[2-$^2H_1$,$^3H_1$; 3-$^2H_1$]-succinate (**14**). This was converted, in part as earlier,[9] and then by a mild introduction of the amino-group into the required ALA (**15**). Assays on the various products and/or media in Scheme 2 showed < 0·5% loss of ^3H over the entire sequence; significant racemisation is thereby excluded.

This chiral product (**15**), mixed with [4-^{14}C]ALA as internal standard [$^3H:^{14}C$, 5·32], was incorporated into bacteriochlorophyll a (**5**) using *R. spheroides* (incorporation 2·5—3%). It was found that methanolysis of the pigment (**5**) to remove magnesium and the phytyl side-chain caused unimportant (for the present purposes) changes at the isocyclic ring [see (**16**)]. Dehydrogenation[10] of this material aromatised ring-B [$^3H:^{14}C$ of product, 4·27] and chromic acid oxidation then gave the methylethylmaleimide (**17**) [$^3H:^{14}C$, 5·29] which was diluted with synthetic material[11] for purification. A sample of ALA (**1**) randomly ^{3}H-labelled at C-2 was fed to *R. spheroides* in parallel to act as standard and methylethylmaleimide [as (**17**)] was isolated from (**5**) by degradation as before.

Kuhn–Roth oxidation of the imide (**17**) gave [1-^{14}C, 3-$^2H_1$, $^3H_1$]propionic acid (**18**) [^3H: ^{14}C, 5·48] from the ethyl group together with [2-$^2H_1$, $^3H_1$]acetic acid (**19**); as expected, the propionic acid carried ^{14}C, but not the acetic acid (see Scheme 3). These acids were separated and purified as their *p*-bromophenacyl esters. Schmidt degradation of the recovered propionic acid (**18**), after addition of a suitable amount of [2-^{14}C]propionic acid, gave the ethylamine (**20**) which was oxidised with permanganate[12] to the acetic acid (**21**).† This was shown by assay with malate synthetase and fumarase [6,13] to have the *R*-configuration (Table).

Knowledge of the configuration of the ALA (**15**) leads to the illustrated labelling pattern, Scheme 3, for the ring-B side-chains of the uro'gen III (**2a**) and of the propionate residue of the copro'gen III (**3a**); the arrangement shown for the vinyl group of the protoporphyrin IX (**4a**) then follows (see above). Since the acetic acid (**21**) isolated from the ethyl group has the *R*-configuration, it is proved that reduction of the vinyl group of (**4a**) has involved addition of hydrogen to the methylenic *si*-face as shown, Scheme 3.

The acetic acid (**19**), produced directly in the Kuhn–Roth oxidation of the imide (**17**), was also found to have the *R*-configuration (Table). This acid is derived mainly from



SCHEME 3

the *C*-methyl group at the original C-7, but in part from the *C*-methyl group of the ethyl residue (see Scheme 3), the latter having been shown above to have the *R*-configuration. It is fortunate that both assayed samples of the acetic acids (**19**) and (**21**) have the same configuration, since the mixed origin of one of them [(**19**)] still allows the conclusion that the *C*-methyl group at C-7 of (**16**) and so of (**3a**) has the *R*-configuration. This result is built into Scheme 3 which shows that the decarboxylation step (**2a**) → (**3a**) has

† All steps in the degradation of the imide (**17**) were checked for possible racemisation by control experiments (to be given in the full paper). The methyl group isolated as propionic acid was unaffected (< 1% racemisation); that isolated as acetic acid contained < 10% of the enantiomer.

occurred at ring-B with *retention* of configuration as found earlier for the same conversion (**2**) → (**3**) in chicken erythrocytes.[13]

We thank Dr C. Jones (Cambridge) for his valuable help with malate synthetase, Mrs Frank (Munich) for skilful technical assistance, the Royal Society for the award of the Bruno Mendel Fellowship (to A. L. G.), and the Nuffield Foundation and S.R.C. for financial support.

(*Received*, 10*th April* 1981; *Com.* 422.)

[1] G. W. Kenner, R. P. Carr, and M. T. Cox, footnote 1 in *J. Am. Chem. Soc.*, 1969, **91**, 1232; J. Shien, G. W. Miller, and M. Psenak, *Biochem. Physiol. Pflanz.*, 1974, **165**, 100.
[2] A. Gorchein, *Biochem. J.*, 1973, **134**, 829.
[3] Reviewed by A. R. Battersby and E. McDonald in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975; A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, and E. McDonald, *Nature*, 1980, **285**, 17.
[4] Z. Zaman, M. M. Abboud, and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1972, 1263; A. R. Battersby, J. B. Campbell, and E. McDonald, quoted by A. R. Battersby, *Experientia*, 1978, **34**, 1.
[5] A. R. Battersby, E. McDonald, H. K. W. Wurziger, and K. J. James, *J. Chem. Soc., Chem. Commun.*, 1975, 493.
[6] J. W. Cornforth, J. W. Redmond, H. Eggerer, W. Buckel, and C. Gutschow, *Nature*, 1969, **221**, 1212; J. Lüthy, J. Rétey, and D. Arigoni, *ibid.*, 1969, **221**, 1213.
[7] For related cases see M. W. Rathke and A. Lindert, *J. Am. Chem. Soc.*, 1971, **93**, 2318; K. B. Sharpless, R. F. Laver, and A. Y. Teranishi, *ibid.*, 1973, **95**, 6137.
[8] W. Tischer, J. Bader, and H. Simon, *Eur. J. Biochem.*, 1979, **97**, 103; H. Simon, H. Günther, J. Bader, and W. Tischer, *Angew. Chem.*, 1981, in the press.
[9] A. Neuberger and J. J. Scott, *J. Chem. Soc.*, 1954, 1820.
[10] J. H. Golden, R. P. Linstead, and G. H. Whitham, *J. Chem. Soc.*, 1958, 1725.
[11] H. M. Muir and A. Neuberger, *Biochem. J.*, 1949, **45**, 163.
[12] V. Prelog, H. H. Kägi, and E. H. White, *Helv. Chim. Acta*, 1962, **45**, 1658.
[13] G. F. Barnard and M. Akhtar, *J. Chem. Soc., Perkin Trans.* 1, 1979, 2354.
[14] *E.g.* D. Arigoni in 'Molecular Interactions and Activity in Proteins,' Ciba Foundation Symposium No. 60, Excerpta Medica, Amsterdam, 1978, p. 243.