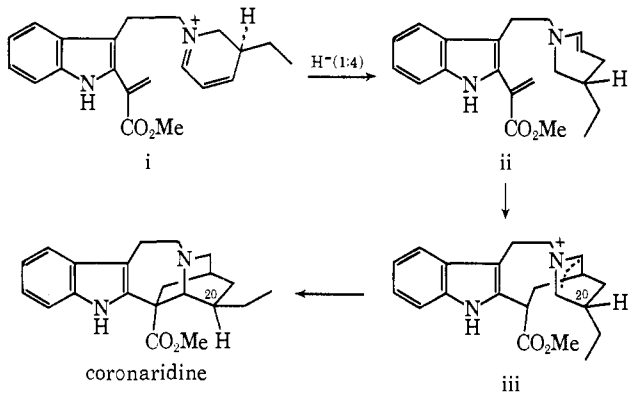
stereospecific cyclization mechanism involving dehydrosecodine A and B for the *Iboga* and *Aspidosperma* alkaloids, respectively,[11] and further, their regiospecific elaboration from stemmadenine *via* suitably modified versions of the *prestrychnos* and *precondylocarpine* systems. Further biochemical and synthetic aspects of these fascinating rearrangements are now receiving intensive study.

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation.

alkaloid stereochemistry. These aspects will be discussed in our full paper.



(10) K. Bláha, Z. Koblicová, and J. Trojánek, *Tetrahedron Lett.*, 2763 (1972).

(11) The actual *biochemical* relationship could correspond to the *reverse* of the chemical reactivity by operation of the appropriate ionic mechanisms under enzyme control. Thus the processes involving species A (**3**) and B (**8**) as specific precursors for *Aspidosperma* and *Iboga* alkaloids *in vivo* remain to be defined.

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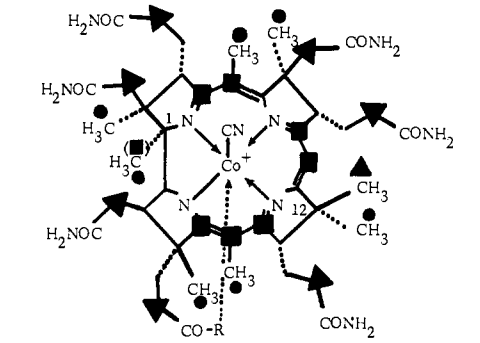
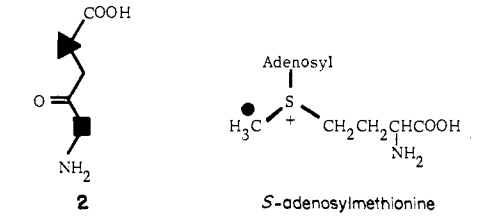
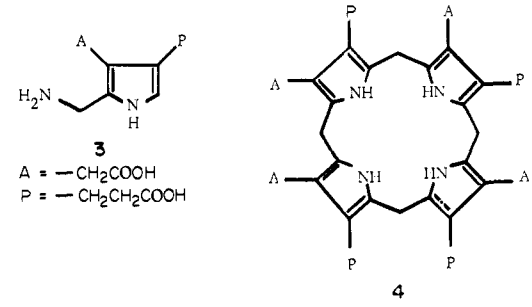
Biosynthesis of Corrinoids. Concerning the Origin of the Methyl Groups in Vitamin B_{12}

Sir:

As a result of the pioneering experiments of Shemin,[1] it has been established that the nucleus of vitamin B_{12} (**1**) is formed by the succinate–glycine pathway *via* δ-aminolevulinic acid (**2**, ALA) and thence by way of porphobilinogen (**3**, PBG) to the corrins, whose structures are reminiscent of uroporphyrinogen III (**4**, urogen III) in that the sequence of acetate and propionate functionality is "reversed" in ring D.[2] Of the eight methyl groups attached to the periphery of **1** it was suggested[3] that those at C-1 and C-12 stem from C-5 and C-2 of ALA, respectively, the latter by a well-documented[4] decarboxylation of acetate attached to the urogen system, while the derivation of the former (C-1) methyl group could be envisioned either as a reduction of a CH_2 bridge of urogen III[4,5] or as a result of direct cyclization of a linear tetrapyrrole,[2a] the six remaining methyl groups arising from methionine.[3] Support for these ideas came from Kuhn–Roth oxidation of corrinoids labeled with [5-^{14}C]- and [2,3-^{14}C]-ALA and [$^{14}CH_3$]methionine. As pointed out by Shemin[3] the yield of acetic acid from C-1 was low (10% of theoretical) and the severity of the problem of degradation of the vitamin has so far precluded rigorous definition of the preliminary results. We have now reexamined the problem using ^{13}C Fourier transform nmr and report on the fate of [2-^{13}C]- and [5-^{13}C]-labeled ALA and of [$^{13}CH_3$]methionine in *Propionibacterium shermanii*.







Administration of [2-^{13}C]-ALA[6] to *P. shermanii* afforded a sample of vitamin B_{12} in which eight high-field signals in the CH_2 and CH_3 region were enriched as shown in the proton noise-decoupled ^{13}C FT nmr spectrum (Figure 1a). Assignments of the eight ^{13}C resonances were made to the seven CH_2CONH_2 methylenes and one of the geminal dimethyl groups of ring C in full accord with earlier ^{14}C studies. It is evident, however, that the methyl signal appears at lower field

(1) D. Shemin and R. C. Bray, *Ann. N. Y. Acad. Sci.*, 112, 615 (1964); D. Shemin and G. Kikuchi, *ibid.*, 75, 122 (1958).

(2) (a) J. H. Mathewson and A. H. Corwin, *J. Amer. Chem. Soc.*, 83, 135 (1961); (b) D. Mauzerall, *ibid.*, 82, 2601 (1960); (c) L. Bogorad, *Ann. N. Y. Acad. Sci.*, 104, 676 (1963).

(3) R. C. Bray and D. Shemin, *Biochim. Biophys. Acta*, 30, 647 (1958); R. C. Bray and D. Shemin, *J. Biol. Chem.*, 238, 1501 (1963).

(4) B. F. Burnham in "Metabolic Pathways," 3rd ed, Vol. III, D. M. Greenburg, Ed., Academic Press, New York, N. Y., 1969, Chapter 18.

(5) A. I. Scott, C. A. Townsend, K. Okada, and M. Kajiwara, *Trans. N. Y. Acad. Sci.*, in press.

(6) [2-^{13}C]-ALA (60% enriched) was prepared by adaptation of the method of L. Pichat, J. Loheac, M. Herbert, and G. Chatelain, *Bull. Soc. Chim. Fr.*, 10, 3271 (1966).

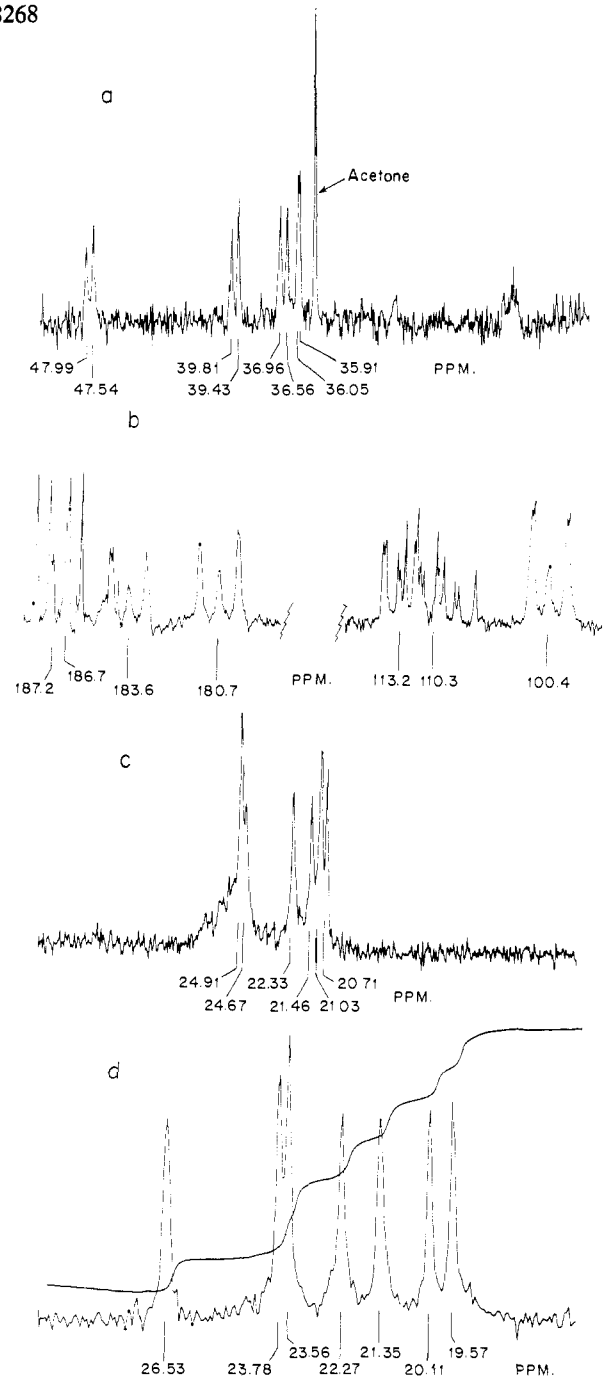


Figure 1. (a) Proton noise-decoupled ^{13}C FT spectrum of [2-^{13}C]-ALA enriched cyanocobalamin (vitamin B_{12}, 26 mg) in H_2O. [The external ^{19}F lock (C_6F_6) and ^{13}C reference (hexamethyldisilane, HMDS) were contained in a 5-mm tube mounted coaxially in a 10-mm sample tube. The ^{13}C FT spectra were determined at ambient probe temperature (40–45°) using a computer controlled Fourier transform system described previously (R. J. Cushley, D. R. Anderson, and S. R. Lipsky, *Anal. Chem.*, **43**, 1281 (1971)).] The methyl group of acetone, 35.11 ppm, provides an internal reference. Only the range 50.53–19.59 ppm downfield of external HMDS is shown: data set = 8K points; digitizing rate 10 kHz; pulse width = 50 μsec; receiver skip = 100 μsec. (b) Proton noise-decoupled ^{13}C FT spectrum of [5-^{13}C]-ALA enriched cyanocobalamin (38 mg) in H_2O. Two portions of the spectrum, 188.53–177.49 ppm (left) and 117.83–95.74 ppm (right) downfield from external HMDS are shown: data set = 4K; digitizing rate = 10 kHz; pulse width = 50 μsec; receiver skip = 100 μsec. (c) Portion, 35.11–8.60 ppm downfield of external HMDS, of the proton noise-decoupled ^{13}C FT spectrum of [$^{13}CH_3$]methionine enriched cyanocobalamin (36 mg) in H_2O; conditions as in (b). (d) Portion, 29.89–16.63 ppm downfield from HMDS, of the proton noise-decoupled ^{13}C FT spectrum of dicyanocobalamin (36 mg) in 0.1 *M* KCN; conditions as in (a).

than the methyl region assigned by Doddrell and Allerhand.[7] A sample of B_{12} enriched by feeding [5-^{13}C]-ALA[8] provided the surprising result that, of the eight anticipated enriched carbons, only seven signals appeared in the low-field region associated with sp^2 functions (four C=N resonances, Figure 1b left; three C=C resonances at higher field, Figure 1b right). The splitting pattern predicted for the distribution of label illustrated in **1** (■) was indeed obtained as shown in Figure 1b. Such an array is in harmony with current ideas[9] on the mechanism of type III urogen formation. However, there was no ^{13}C enhanced signal above 95 ppm downfield from HMDS showing that no enrichment of the C-1 methyl had occurred. This indicates that one of the $^{13}CH_2NH_2$ termini of ALA (and hence of PBG or urogen III) has been extruded in the formation of the vitamin. The origin of the "missing" C-1 methyl group has now been demonstrated to be methionine. Although the ^{13}C FT spectrum of cyanocobalamin obtained by feeding [$^{13}CH_3$]methionine (Figure 1c) revealed only six signals highly enriched above natural abundance, conversion of this sample to the dicyano form (Figure 1d) revealed seven well-defined resonances. Hence the signal at 24.91 ppm (Figure 1c) corresponds to two superimposed resonances. Inspection of the integrated spectrum (Figure 1d) leaves no doubt that seven methionine methyl groups have been incorporated.

From these data we conclude that a revision of the biosynthetic scheme is required and any rationalization of the overall mechanism must take account of the following facts. (1) An aminomethyl terminus from C-5 of one of the eight molecules of ALA utilized is lost, perhaps as formaldehyde (or its equivalent).[10] (2) Seven rather than six "extra" methyl groups are supplied by methionine: two in ring A, one in each of rings B, C, and D, and one in each of two meso positions (C-5, C-15). (3) As discussed in the accompanying communication, urogen III serves as a good precursor for vitamin B_{12}, and with the presumption[11] that urogen III contains a C-5 ALA label *in all four meso positions*, one of these (that between rings A and D) must be lost in the subsequent mechanism and its place taken (in ring A) by a methionine derived methyl group. (4) The acetate and propionate side chains of PBG are incorporated intact into the corrinoid with the exception of ring C, where it has been confirmed that a decarboxylative process led to *one* of the biochemically distinguishable methyl groups.[12]

(7) D. Doddrell and A. Allerhand, *Proc. Nat. Acad. Sci. U. S.*, 68, 1083 (1971). Details of the ^{13}C assignments in corrinoids will be published elsewhere.

(8) [5-^{13}C]-ALA (90% enriched) was prepared by the method of D. Shemin in "Organic Synthesis with Isotopes," A. Murray and D. L. Williams, Ed., Interscience, New York, N. Y., 1958.

(9) (a) The exact nature of the linear polypyrroles of porphyrin and corrinoid intermediary metabolism cannot yet be defined;[9b-e] (b) L. Bogorad in "The Chlorophylls," L. P. Vernon and G. R. Seeley, Ed., Academic Press, New York, N. Y., 1966; (c) L. Bogorad and J. Pluscec, *Biochemistry*, **9**, 4736 (1970); R. Radmer and L. Bogorad, *ibid.*, 11, 904 (1972); (d) B. Frydman, S. Reil, A. Valasinas, R. Frydman, and H. Rapoport, *J. Amer. Chem. Soc.*, **93**, 2738 (1971); (e) A. R. Battersby, *Proc. 23rd IUPAC Symp.*, **5**, 1 (1971).

(10) The origin of the small but detectable (≤ 10%) level of radioactivity in the C-1 methyl group in B_{12} from administration of [5-^{14}C]-ALA could be rationalized in terms of exchange of one of the C-5 labels with the "C_1" pool and hence with endogenous *S*-adenosylmethionine.

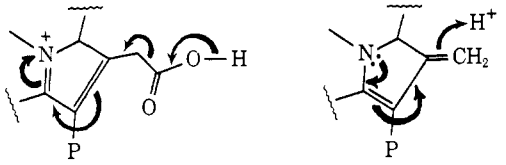
(11) *Cf.* A. R. Battersby, J. Moron, E. McDonald, and J. Feeney, *Chem. Commun.*, 920 (1972), and references cited therein.

(12) The decarboxylation of the acetate side chains in porphyrin metabolism is well known[13] and in the case of corrin biosynthesis most probably takes place *before* methylation in ring C which would other-

The implications of these results[14] in terms of mechanistic rationale are discussed in the following communication.

Acknowledgment. We thank the National Science Foundation and the National Institutes of Health (Grant RR-00356) for support of this work.

wise block a process such as, *e.g.*



(13) J. Lascelles, "Tetrapyrrole Biosynthesis and Its Regulation," W. A. Benjamin, New York, N. Y., 1964.

(14) Essentially identical results using [5-^{13}C]-ALA have been obtained by Professor D. Shemin (personal communication). See C. E. Brown, J. J. Katz, and D. Shemin, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2585 (1972).

(15) Carbon-13 Fourier Transform Nuclear Magnetic Resonance. IV.

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Biosynthesis of Corrinoids. Uroporphyrinogen III as a Precursor of Vitamin B_{12}

Sir:

In considering the plethora of mechanistic proposals[1] for the conversion[2] of porphobilinogen (**1**, PBG) to vitamin B_{12} (**2**) we have been guided by the essential simplicity, symmetry, and energetic economy of a scheme[1,3] wherein the cobalt (corrin), iron (heme), and magnesium (chlorophyll) pathways diverge *after* the formation of uroporphyrinogen III (**3**, urogen III). Regardless of the details of urogen III formation, an experimental distinction can be made between the intermediacy of **3** and the ingenious corrin synthetase mechanism of Corwin[4] (Scheme I, path A) which bypasses the urogens to form the ring A → D corrin linkage directly. At the same time many of the other interesting hypotheses[1] which involve the A → D linkage at an earlier assembly stage could be discarded if proof for the intervention of urogen III were forthcoming. Recent feeding experiments, however, with whole cells of *Propionibacterium shermanii* have indicated that

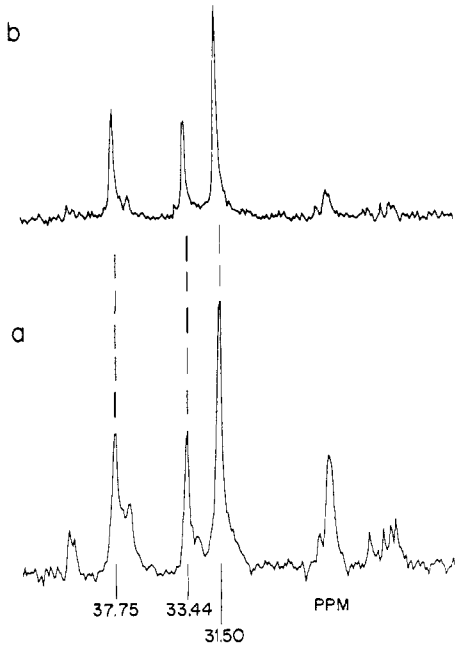
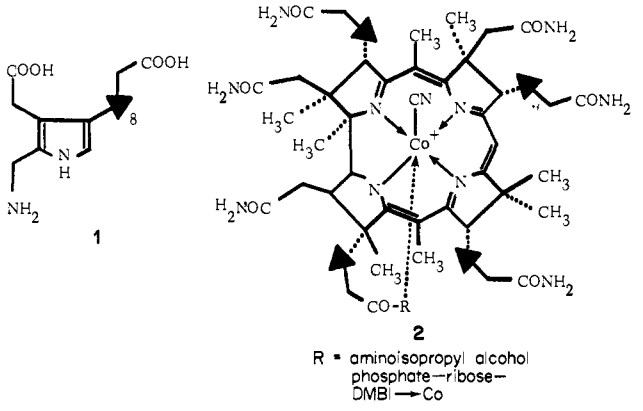


Figure 1. (a) Portion, 44.73–17.22 ppm downfield from external HMDS [the external ^{19}F lock (C_6F_6) and ^{13}C reference (hexamethyldisilane, HMDS) were contained in a 5-mm sample tube mounted coaxially in a 10-mm sample tube containing the aqueous cyanocobalamin solutions; the ^{13}C FT spectra were determined at ambient probe temperature (40–45°) using a computer-controlled FT system previously described (R. J. Cushley, D. R. Anderson, and S. R. Lipsky, *Anal. Chem.*, **43**, 1281 (1971))] of the proton noise-decoupled ^{13}C FT spectrum of 41 mg of [8-^{13}C]-PBG enriched cyanocobalamin (vitamin B_{12}, **2**) in H_2O; data set = 4K points; digitizing rate = 10 kHz; pulse width = 50 μsec; receiver skip = 100 μsec (Cushley, *et al.*). (b) Portion, 44.73–17.22 ppm downfield from external HMDS, of the proton noise-decoupled ^{13}C FT spectrum of 40 mg of [^{13}C]urogen enriched cyanocobalamin (**2**) in H_2O; same conditions as for spectrum 1a.

virtually no specific incorporation of enzymically[5] or chemically[6] synthesized [^{14}C]urogen III could be observed. We believe that these negative results may be attributed to the conditions of the feeding experiment and although valid for the concentrations and/or pH, aeration, heat treatment, and cellular ages specified, may be contrasted with the successful incorporations described in this communication.



(1) B. F. Burnham in "Metabolic Pathways," 3rd ed, Vol. III, D. M. Greenburg, Ed., Academic Press, New York, N. Y., 1969, Chapter 18.

(2) S. Schwartz, K. Ikeda, I. M. Miller, and C. J. Watson, *Science*, **129**, 40 (1959). The bioconversion of PBG to vitamin B_{12} has been assumed from the isolation of radioactive B_{12} from a feeding experiment. No degradations to locate the label were performed but the assumption seems reasonable on the basis of the specific, nonrandomized incorporation of various ^{14}C radiomers of δ-aminolevulinic acid into the corrin molecule, since the latter amino acid is a well proven precursor of PBG [D. Nandi and D. Shemin, *J. Biol. Chem.*, **243**, 1224, 1231, 1236 (1968)].

(3) B. F. Burnham and R. A. Plane, *Biochem. J.*, **98**, 13c (1966). The isolation of urogen III from vitamin B_{12} producing organisms[1] lends further support to this concept.

(4) J. H. Mathewson and A. H. Corwin, *J. Amer. Chem. Soc.*, **83**, 135 (1961).

(5) G. Müller and W. Dieterle, *Hoppe-Seyler's Z. Physiol. Chem.*, **352**, 143 (1971).

(6) B. Franck, D. Gantz, and F. Hüper, *Angew. Chem., Int. Ed. Engl.*, 11, 421 (1972).