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(15) Crystal data: $FeS_2N_4C_{54}H_{48}ClO_4\cdot 3CHCl_3$, orthorhombic, $P2_12_12_1$, $Z = 4$; a = 17.830 (3), b = 18.781 (3), c = 18.187 (3) Å; $\rho_{calcd} = 1.45$, $\rho_{expll} = 1.45$ g/cm³; 5778 unique observed data, $R = 0.077$.
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(18) Crystal data: $FeSN_7OC_{56}H_{47}\cdot C_6H_6$, monoclinic, $P2_1/c$, $Z = 4$; a = 13.225 (6), b = 15.138 (10), c = 25.652 (11) Å; $\beta = 90.42$ (2)°; $\rho_{calcd} = 1.29$, $\rho_{expll} = 1.26$ g/cm³; 3594 unique observed data, $R = 0.127$.
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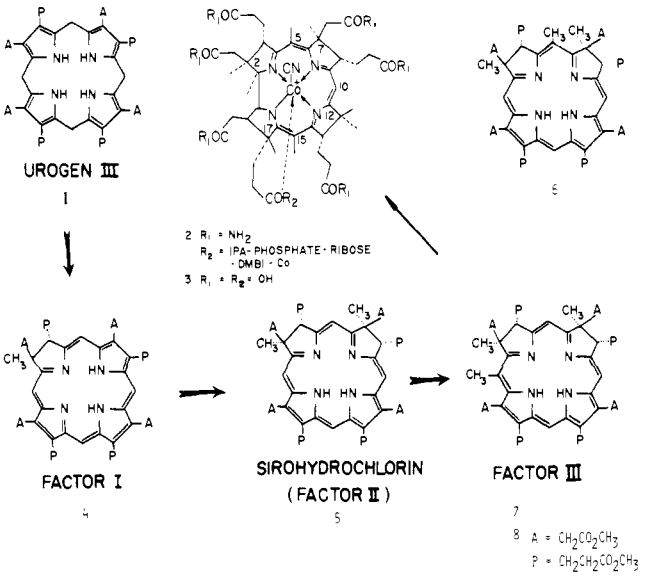
20-Methylsirohydrochlorin. A Revised Structure for a Trimethylisobacteriochlorin Intermediate (Factor III) in the Biosynthesis of Vitamin B_{12}

Sir:

With the establishment of the intermediacy of uro'gen III (**1**) in corrin biosynthesis,[1,2] a search for partially methylated metabolites as potential precursors of vitamin B_{12} (**2**) has so far led to the isolation and characterization[3,4] of three isobacteriochlorins from *Propionibacterium shermanii* and *Clostridium tetanomorphum* which undergo bioconversion to cobyrinic acid (**3**) and have been designated factors I–III (**4**–**6**).

The importance of these substances stems from their sequential position in post-uro'gen III metabolism and from the proven correspondence of factor II with sirohydrochlorin (**5**),[5-8] the iron-free prosthetic group of the sulfite and nitrite reductase enzymes[9] (siroheme). The proven[5] structural and stereochemical features of **5** led to the working structure **4** for factor I.[10] On the basis of ^1H NMR data for the bislactone of factor III (= corriphyrin-3),[6] structure **6** was proposed for the latter.[7,11] We now provide spectroscopic and biochemical evidence which requires that the "extra" methyl group is added



to sirohydrochlorin (**5**) at C-20 rather than at C-5, leading to the revised structure (**7**) for factor III, i.e., 20-methylsirohydrochlorin, which suffers a remarkable loss of C-20 with its attached methyl group on the way to vitamin B_{12}.

Factor III was isolated from δ-aminolevulinic acid (ALA) supplemented cobalt-free incubations of *P. shermanii* (ATCC 9614) and from a B_{12}-deficient mutant[12] of this organism. High resolution FD mass spectrometry of the octamethyl ester (**8**) established the formula $C_{51}H_{64}N_4O_{16}$ (988.4281), while the mass spectrum of the octa ester isolated from incubation with [*methyl*-$^2H_3$]-L-methionine revealed peaks at *m/e* 997, 994, 991, and 988 corresponding to enrichment with a maximum of three (M + 9) CD_3 groups. Analysis of the ^1H NMR spectrum (300 MHz) revealed only three signals at δ 6.43, 7.21, and 8.33 ppm in contrast to the four signals in this region in the spectrum of **5** which have been assigned to the four meso protons at C-5, C-10/C-20, and C-15 (δ 6.78, 7.36/7.46, and 8.54, respectively).[5] Factor III is therefore 10- or 20-methylsirohydrochlorin. In order to decide between these alternatives, a specimen of factor III (400 μg) was prepared from a suspended-cell incubation[5] of *P. shermanii* containing [$^{13}CH_3$]methionine and [5-^{13}C]-δ-aminolevulinic acid. When this ^{13}C-enriched species (as the octamethyl ester) was examined by ^{13}C NMR spectroscopy (Figure 1), it became

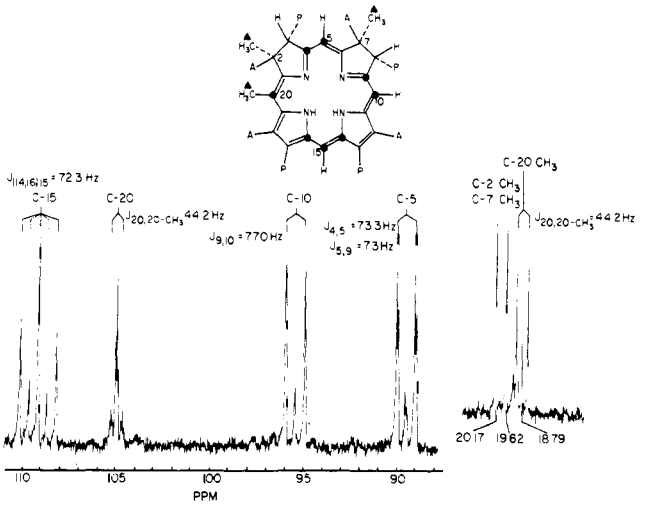


Figure 1. The meso carbon resonances of the proton noise decoupled ^{13}C FT spectrum of [5-^{13}C]-ALA- and [$^{13}CH_3$]methionine-labeled 20-methylsirohydrochlorin octamethyl ester. Inset: the methyl carbon resonances of the same sample; the spectrum was obtained in C_6D_6 at 75 MHz.

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Table I

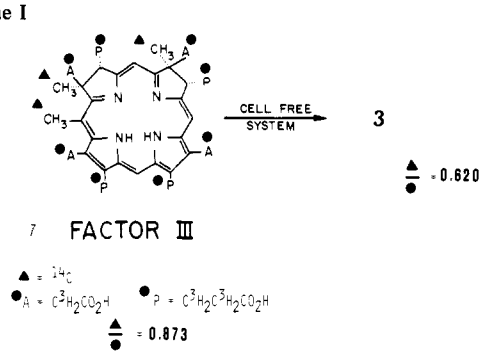
	A. double labeling of cobyrinic acid intermediates				B. incorporation of intermediates into cobyrinic acid (3)			
			radioactivity ratio of intermediate		added radioactivities of 4 and 7,		radioactivity ratio of cobyrinic acid	
expt	intermediate	added substrate	$^{14}C/^{3}H$	with ref to methyl groups	dpm	incorpn, %	$^{14}C/^{3}H$	with ref to methyl groups
1	**5**	[*methyl*-^{14}C]Met [2,3-^{3}H]ALA	0.600	2.00	1.84×10^6	15.3	0.620	2.00
	7		0.873	2.91	1.57×10^6	16.1	0.620	2.00
	7		0.873	2.91	1.65×10^6	14.3	0.660	2.13
2	**5**	dto	0.092	2.00	1.63×10^6	4.0	0.109	2.00
	7		0.141	3.06	1.62×10^6	9.9	0.102	1.87
3	**5**	dto	0.376	2.00	1.27×10^6	4.9	0.400	2.00
	7		0.537	2.86	1.20×10^6	11.5	0.405	2.03

possible to deduce the complete structure (**8**). First, the *downfield* position of the C-15 meso-carbon triplet at δ 108.98 (J = 72 Hz) confirms that rings A and B are methylated,[5] and, since the meso-carbon signals at δ 89.5 and 95.4 each show ^{13}C-^{13}C coupling to an enriched sp^2 neighbor ($J \approx 70$ Hz), these are assigned to C-5 and C-10, respectively, by analogy with the corresponding resonances in sirohydrochlorin derived by biochemical enrichment with [5-^{13}C]-ALA.[5] Thus, the remaining meso-carbon resonance at δ 104.8 consists of a doublet (J = 44.2 Hz) and must correspond to C-20, with additional fine structure due to long-range coupling with C-4 and C-16. That the ^{13}C-^{13}C coupling constant of 44.2 Hz for C-20 is due to substitution by a methionine-derived methyl group is confirmed by inspection of the *methyl* region of the ^{13}C NMR spectrum which displays three enriched species consisting of singlets at δ 20.17 and 19.62 and a doublet at δ 18.79 (J = 44.2 Hz). It can be seen that, owing to different efficiencies of incorporation of [^{13}C]methionine and of [5-^{13}C]-ALA, the enrichments in the methyl groups and in the ALA-derived sp^2 carbons are not identical, the satellite intensities reflecting a greater enrichment in C-20 than in its pendant methyl group. Hence factor III is **7**, i.e., 20-methylsirohydrochlorin rather than the C-5 methylated isobacteriochlorin (**6**), contrary to what had previously been thought.[7,11] The absolute stereochemistry of factor III and its relationship to cobyrinic acid was obtained by the following biochemical experiments.

Incorporation into cobyrinic acid of **7** labeled from [4-^{14}C]-ALA was previously demonstrated in cell-free extracts of *C. tetanomorphum*.[4] In order to trace the fate of the methyl groups during this biotransformation, specimens of **5** and **7** were prepared from a *P. shermanii* incubation with [*methyl*-^{14}C▲]-L-methionine and [2,3-^{3}H$_4$●]-ALA. In this way **7** is labeled as shown in Scheme I (where ● = ^{3}H and ▲ = ^{14}C) and the $^{14}C/^{3}H$ ratios are in good agreement for the presence of two and three methyl groups in **5** and **7**, respectively (expt 1A, Table I). However, the cobyrinic acid, derived from both of these substrates in excellent yield (15-16%) using the *C. tetanomorphum* system,[3] contains in each case only *two* methionine-derived methyl groups (Scheme I; Table I, expt 1B). Separate samples of doubly labeled **5** and **7** were again chromatographed to constant, but *different* radioactivity ratios (expt 2A, 3A). Pure factor III of expt 2 was mixed with sirohydrochlorin (factor II) from expt 3 (and vice versa) and the methyl esters were separated, purified, and again administered (after hydrolysis) to the cobyrinic acid synthesizing system (expts 2B, 3B). Again, in each experiment the isolated cobyrinic acid contains a $^{14}C/^{3}H$ ratio compatible with only *two* ^{14}C methyl groups. These experiments show that (a) there is no possibility of cross-contamination of factors II and III during isolation, (b) factor III is converted to cobyrinic acid in good radiochemical yield (10-15%) with the loss of C-20 *and* its attached methyl group (the nature of the "C_2" fragment remains to be determined[13]), and (c) the absolute stereochemistry of factor III (as **7**) follows from its relationship to cobyrinic acid.

It thus appears that, in order to achieve the intercorrin A → D ring junction, the biosynthetic route requires not only the specific formation and subsequent disruption of the type III uro'gen macrocycle but the sacrifice of at least one methionine-derived methyl group and the carbon to which it is attached (C-20), since *both* of these must be excised from the species undergoing (or having undergone) secocorrin → corrin[2] closure. This apparently prodigal series of events can be accommodated within several hypotheses consonant with published data. Further work is required to clarify the details of post-factor-III metabolism in vitamin B_{12} biosynthesis and this is now in hand.

Scheme I



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(13) In earlier experiments it was clearly shown[1a,14] that, under carefully controlled conditions, [^{14}C]formaldehyde could be trapped from the C-20 position of uro'gen III. The data can be interpreted in several ways: (a) methylation at C-20 is followed by loss of a "C_2" unit which is further cleaved to "C_1" units, one of which is trapped as formaldehyde; (b) the formaldehyde is released (under enzymic control[15]) *only* from the uro'gen III molecule and *not* from factor III; (c) more than one pathway exists for the biotransformation of uro'gen III to cobyrinic acid.
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(15) This interpretation would entail the fortuitous correspondence of the stoichiometry of formaldehyde release (3%) from ^{14}C-20 and bioconversion of the ^{14}C-5 and ^{14}C-15 labels in uro'gen III to these positions in cobyrinic acid (~3%).[14]

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Planar *s-cis*-1,3-Butadiene

Sir:

It has long been recognized that 1,3-butadiene exists in two conformations.[1] A number of calculations and experiments on this compound have been reported,[2] and the geometry of the more stable rotamer has been shown to be planar s-trans.[3] Both planar and twisted (gauche) geometries have been proposed for *s-cis*-1,3-butadiene, but the actual geometry has not been established. The energy barrier separating *s-cis*- from *s-trans*-1,3-butadiene and the relative energies of these two compounds are in question. We wish to describe the preparation of *s-cis*-1,3-butadiene by thermal trapping and photochemical generation, the spectroscopic (IR and UV) characterization of this molecule, and a direct measurement of the barrier separating *s-cis*- from *s-trans*-1,3-butadiene.

We have previously shown that it is possible to thermally generate and trap at low temperature a mixture of conformers rich in higher energy forms.[4] When 1,3-butadiene is passed as a vapor through a hot tube (400-900 °C) and allowed to impinge on a CsI plate cooled to 30 K, infrared bands can be observed which are not present in a spectrum of 1,3-butadiene deposited from room temperature.[5] When the CsI window is warmed to 60 K, these new bands rapidly disappear, the bands corresponding to s-trans increase, and the remaining spectrum is identical with that of a sample deposited from room temperature. On the basis of this rapid conversion to *s-trans*-1,3-butadiene, the additional bands[6] in the high-temperature deposition can be assigned to the s-cis conformer.

A 1,3-butadiene/argon mixture (1:1000) was also deposited from high temperatures (400-900 °C) onto a CsI window cooled to 20 K (Figure 1). Bands were again observed which were not present in the infrared spectrum obtained of this mixture deposited from room temperature.[7] These bands[8] correspond well to those found in the spectra derived from neat high-temperature depositions. The additional bands produced in the high-temperature depositions are, therefore, due to the s-cis conformer rather than to any crystalline effects. Broadband irradiation of the conformer mixture matrix isolated in argon, using a 1000-W high-pressure mercury-xenon lamp with quartz optics, resulted in the rapid disappearance of the s-cis infrared bands with a concomitant increase in the intensity of the *s-trans*-1,3-butadiene bands.[9] Prolonged irradiation of the matrix containing *s-trans*-1,3-butadiene slowly produced vinylacetylene. Neither cyclobutene nor bicyclobutane was observed.[10]

The UV spectrum of the high-temperature conformational mixture of 1,3-butadiene matrix isolated (1:2000) in argon at

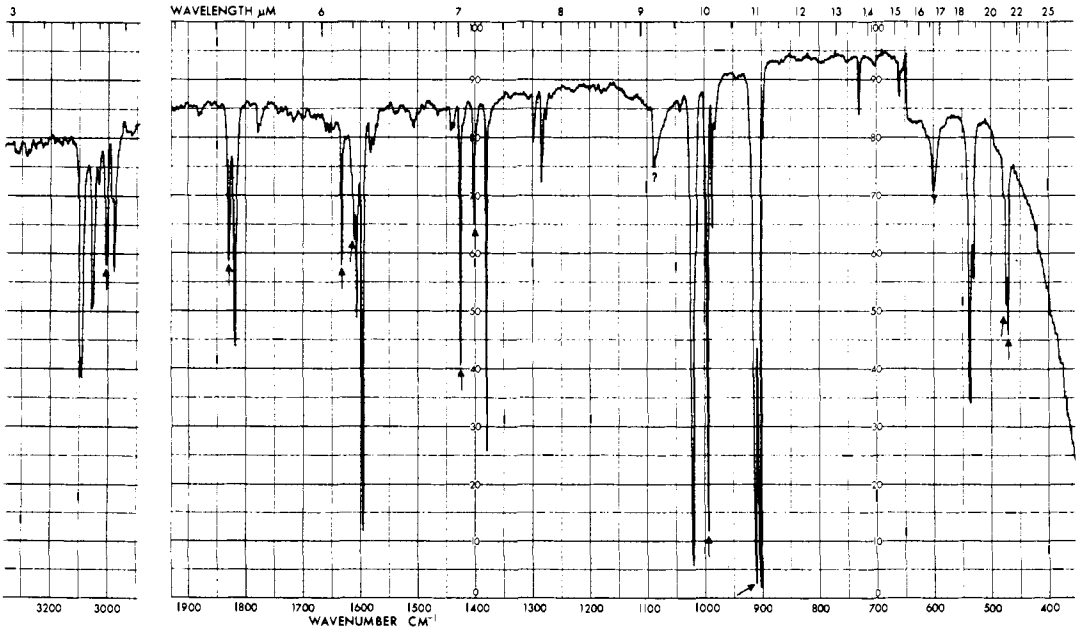

Figure 1. Infrared spectrum between 3400 and 2900 cm^{-1} and between 1930 and 350 cm^{-1} at 20 K of matrix-isolated 1,3-butadiene (1:700 in argon) deposited from 850 °C. The arrows designate bands assigned to the s-cis form. The bands at 1089 and 600 cm^{-1} are rather broad, but these bands are probably also due to *s-cis*-1,3-butadiene.

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