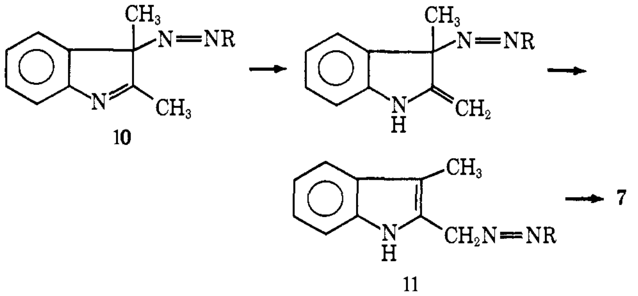

shift with alkali, confirming speculation in the literature that such shifts observed with 3-azo products from indole[16] or 2-methylindole[14] arise from removal of the indole N-H proton.

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A New Boron Hydride, Pentadecaborane(23)

Sir:

A new crystalline boron hydride has been prepared in high yield by reaction of i-B_9H_{15} with B_6H_{10}. The air sensitive compound has moderate thermal stability.

In a typical reaction KB_9H_{14} (2.06 mmol) was treated with excess HCl at −78° for 30 min. The unreacted HCl was then distilled from the reactor by pumping for 3 hr at −78° yielding a mixture of solid i-B_9H_{15} and KCl as described elsewhere.[1] Hexaborane(10) (10.20 mmol) was condensed into the reactor and melted onto the frozen i-B_9H_{15} mixture, being certain to cover each portion of the solid with liquid B_6H_{10}. The mixture was warmed to 0° for 20 min. Hydrogen (2.00 mmol) and B_6H_{10} (8.12 mmol) were removed at −196 and 0°, respectively. The solid remaining in the reactor was extracted with CH_2Cl_2 and separated from KCl by filtration. Evaporation of the clear colorless solution gave crystalline $B_{15}H_{23}$ (2.01 mmol). The KCl was dissolved in water and precipitated with $AgNO_3$ yielding AgCl (1.86 mmol). Therefore, the reaction proceeded according to the following stoichiometry assuming quantitative yields of i-B_9H_{15}.

$$(1.00)\ i\text{-}B_9H_{15} + (1.01)\ B_6H_{10} = (0.97)\ H_2 + (0.98)\ B_{15}H_{23}$$

Hydrolysis of the borane with dilute HCl and titration of the boric acid as the D-mannitol complex resulted in hydrolytic H_2 to $B(OH)_3$ ratios of 2.265 and 2.265, calcd for $B_{15}H_{23}$, 2.267. Six determinations of the molecular weight by vapor pressure depression in CH_2Cl_2 solvent at 0 and +5° gave an average molecular weight of 204, calcd for $B_{15}H_{23}$, 185. The value is probably high due to slow evolution of hydrogen from the sample during the determinations.

The 70.6-MHz ^{11}B nmr spectrum measured in $CDCl_3$ is shown in Figure 1. The sets of peaks have integrated intensities of 1 (a):3 (b + c):3 (d + e):2 (f):2 (g + 2h):2 (i + j) in agreement with a borane molecule containing 15 boron atoms. Resolution of peaks d and e was accomplished by "artificial line narrowing" as described elsewhere.[2]

In view of the established basicity of B_6H_{10}[3,4] and the preparation of $B_9H_{13}\cdot$ligand compounds from i-B_9H_{15},[1,5] we picture the hydride as an acid–base adduct, possibly one in which the two boron frameworks are held together by a three-center bond.

An X-ray crystallographic study, isotopic labeling, and other nmr studies, as well as a study of the chemistry of this hydride, are in progress. Furthermore, attempts to extend the reaction of B_6H_{10} to other boron hydride Lewis acids have already given encouraging results.

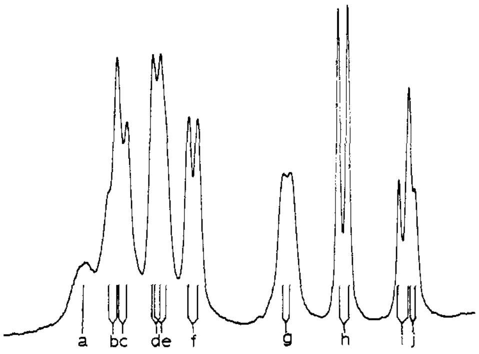

Figure 1. The 70.6-MHz ^{11}B nmr spectrum of $B_{16}H_{23}$ measured in $CDCl_3$. Chemical shifts (ppm referenced to $BF_3\cdot Et_2O$) and coupling constants (+10 Hz) are as follows: a (−20.9), b (−14.3, 140), c (−12.0, 146), d (−4.5, 128), e (−3.1, 160), f (+3.6, 139), g (+24.4, 113), h (+36.9, 153), i (+50.5, 155), j (+52.4, 101).

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Resonance Raman Spectra of Vitamin B_{12} Derivatives[1]

Sir:

Resonance Raman spectroscopy offers promise as a sensitive structural probe for biological chromophores.[2] Excitation within an electronic absorption band can produce large enhancements of certain of the Raman bands of the absorbing molecule.[3] We have obtained Raman spectra of several vitamin B_{12} derivatives (Figure 1), in dilute solution (10^{-3}–10^{-4} *M*) using laser excitation (4880 Å) within the visible absorption bands of the molecules (Figure 2). The technique may be useful

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(1) This investigation was supported by Public Health Service Grants GM-13498 and HL-12526.

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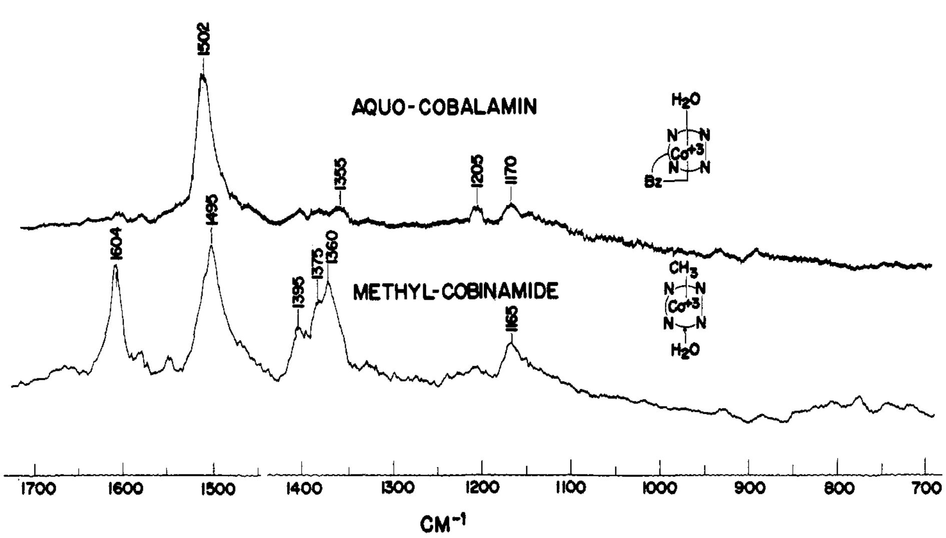

Figure 1. Raman spectra of aquocobalamin and methylcobinamide obtained with 4880-Å excitation (~50 mW) from an Ar^+ laser. The samples were contained in 1-mm glass capillary tubes and spectra were recorded with transverse excitation. Instrument conditions: slit width, 10 cm^{-1}; sensitivity, 10^{-9} Å; scan rate, 50 cm^{-1}/min; time constant, 3 sec.

in monitoring B_{12} coenzyme for evidence of structural change during enzymatic reactions. Our spectra corroborate those obtained recently by Mayer, *et al.*,[4] of cyano- and dicyanocobalamin, and extend to more derivatives.

The complex and interesting chemistry of vitamin B_{12} has been well reviewed.[5,6] A series of derivatives (cobalamins) can be prepared by interchanging ligands at the sixth (axial) coordination site of the central cobalt atom. We have examined aquo-, cyano-, methyl- and 5′-deoxyadenosyl- (B_{12} coenzyme) cobalamin. Despite some shifts in the electronic absorption spectrum, they all give essentially the same resonance Raman spectrum as that shown in Figure 1 for aquocobalamin. This spectrum is quite similar to resonance Raman spectra obtained for conjugated polyenes,[7] which also show a single strong band near 1500 cm^{-1} and several weaker bands at lower frequencies. A frequency of ~1500 cm^{-1} is consistent with the separation between the first two visible absorption bands of several B_{12} derivatives.[8] No bands assignable to vibrations of the sixth ligand (*e.g.*, Co–C or C–N stretching in the cyano derivative) are seen in the cobalamin spectra, nor are in-plane Co–N stretching modes observed. In this regard the vitamin B_{12} derivatives behave similarly to heme proteins.[2c] In both cases the electronic absorption bands are assigned to in-plane π–π^* transitions[9] of the corrin or porphyrin ring, and apparently only relatively high-frequency ring vibrations are brought into resonance.

Cobalamins have 5,6-dimethylbenzimidazole as the fifth (axial) ligand, which is tied to the corrin ring through a ribose phosphate group. X-Ray crystallography has revealed that this constraint induces substantial ruffling of the corrin ring, both cobyric acid (a benzimidazole-free derivative) and nirrin (a synthetic nickel corrin) having a more planar ring than vitamin B_{12} or B_{12} coenzyme.[10] It seems likely that a similar increase in planarity accounts for the dramatically altered resonance Raman spectrum shown by methylcobinamide (Figure 1), which is methylcobalamin with the benzimidazole nucleotide removed. The same Raman spectrum is given by "base-off" methylcobalamin, in which the benzimadazole is protonated and unable to bind to the cobalt atom.[8] In addition to the strong band near 1500 cm^{-1}, found as well in the cobalamin spectra, there are strong bands at 1604 and 1360 cm^{-1}, and several new weak bands. Remnants of some of these new features are detectable in the cobalamin spectra, and it is likely that, aside from small frequency shifts, we are seeing changes in Raman in-

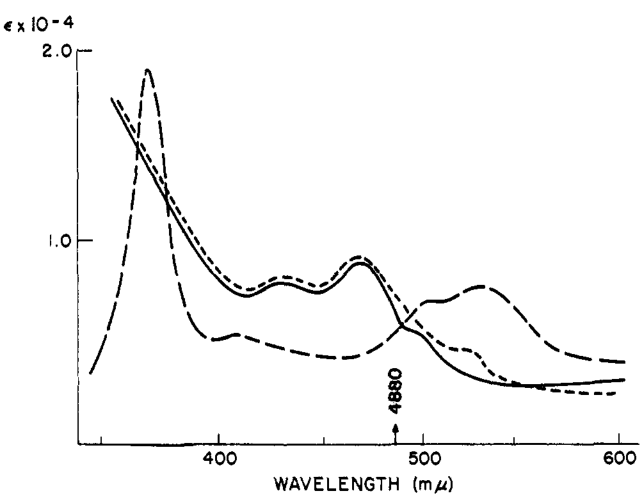

Figure 2. Absorption spectra of aquocobalamin and methylcobinamide in water and of Co(II) cobalamin (obtained by $SnCl_2$ reduction) in 0.1 *M* HCl: (— —) aquocobalamin; (——) methylcobinamide; (- - - - -) Co(II) cobalamin.

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tensity rather than the appearance of new vibrational modes. Apparently the greater planarity of the corrin ring in methylcobinamide or "base-off" methylcobalamin allows more ring vibrations to come into resonance with the electronic transitions. Intensity changes could also arise from changes in the overlap of corrin and cobalt orbitals, accompanying the displacement of benzimidazole, with the possible formation of an essentially five-coordinate square-pyramidal complex.[8,11]

When aquocobalamin is reduced in acid solution to the Co(II) derivative, vitamin B_{12r}, the benzimidazole ligand is protonated and dissociates.[12] We find that this product gives the same Raman spectrum as does methylcobinamide. Addition of an electron to the Co(III) center has no discernible influence on the resonance Raman spectrum, nor is there much change in the electronic spectrum (see Figure 2). In neutral solutions of vitamin B_{12r}, the benzimidazole is coordinated.[12] Curiously, however, the resonance Raman spectrum is the same as that of the "base-off" derivatives. We note that the electronic spectrum of "base-on" vitamin B_{12r} is also similar to those of the cobinamides.[13] It is conceivable that the corrin ring structure is also closer to that of the "base-off" derivatives, in view of the weakness of the Co(II)–benzimidazole bond.[9]

All of the bands, in both the cobalamin and the cobinamide-type resonance Raman spectra, are polarized, with depolarization ratios, ρ_l ranging from 0.2 to 0.6. The phenomenon of inverse polarization,[14] exhibited by the resonance Raman spectra of hemoglobin and cytochrome *c*, is not observed here. Inverse polarization ($\rho_l = \infty$) arises when a vibration whose scattering tensor is antisymmetric ($\alpha_{ij} = -\alpha_{ji}$) is vibronically active.[14] Antisymmetric tensors only arise when the molecular symmetry is axial (threefold or higher rotation axis). Since the highest symmetry available to the corrin ring is C_{2v},[15] it is not surprising that no inverse polarized bands are found in vitamin B_{12} spectra. The A_2 vibration in the C_{2v} point group does have an asymmetric tensor[16] and should show anomalous polarization ($\rho_l > {}^3/_4$).[14] Since all observed bands are polarized, A_2 modes are evidently not vibronically active in vitamin B_{12} derivatives.

There is considerable current interest in structural alternations of B_{12} coenzyme which may be involved in enzymatic mechanisms. Both reduction of the cobalt[17] and dissociation of the benzimidazole ligand with attendant changes in corrin conformation[18,19] are possibilities. Inasmuch as the latter alteration has profound effects on the resonance Raman spectrum, it is apparent that Raman spectroscopy may prove useful in probing vitamin B_{12}–enzyme reactions.

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Transient Absorption Spectra of the Chlorophyll a Anion and Cation

Sir:

Chlorophyll plays an important role in photosynthesis by virtue of its ability to produce a charge separation and catalyze specific electron transfer reactions in the chloroplast.[1] However, little information has been obtained on the transient ionic intermediates of chlorophyll, though there are a few investigations of the cationic intermediate produced by oxidation with ferric chloride[2] or ferric perchlorate,[3] and by the electrolytic method.[4] We wish to report here the optical absorption spectra of both the anion and cation of chlorophyll a (Chl a) observed by the pulse radiolysis technique at room temperature as well as in the rigid glasses irradiated with γ-rays at 77°K. The decay rates of these ions were also determined by the pulse radiolysis.

Chl a was extracted with methanol from spinach leaves and isolated chromatographically by using a microcrystalline cellulose column and *n*-hexane as a developing solvent. Electron pulses from a Mitsubishi Van de Graaff generator were 2.7 MeV in energy and 0.5–1.0 μsec in duration. Absorption spectra in the γ-irradiated glasses were recorded on a Cary 14R spectrophotometer by using a thin (0.5–1.5 mm thickness) Suprasil cell.[5,6]

The absorption spectrum at 77°K in Figure 1 was obtained with an irradiated *sec*-butyl chloride glass containing Chl a (7 m*M*). This spectrum is regarded as being due to Chl a cation (Chl a^+). In the butyl chloride glass, only solute cations are formed, since all the electrons ejected by γ irradiation are stabilized as Cl^- and any excited molecules or neutral radicals cannot migrate to the solutes.[7] The absorption spectrum obtained by the pulse radiolysis is also shown in Figure 1. This spectrum was observed at 20 μsec after pulse for aerated Chl a solutions (0.1–0.2 m*M*) in benzonitrile containing excess biphenyl (0.3 *M*). The aerated benzonitrile solutions have been found to give only solute cations on pulse irradiation, other possible intermediates such as triplet states and anions being completely removed by oxygen.[8] Therefore, the absorp-

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