represented in Scheme I. Reversible hydrogen-bonding interactions of the type depicted have been observed spectroscopically for similar, more tightly bound, anionic ligands.[17] Displacement of MeOH from the tungsten's coordination sphere is observed, as would be expected,[18] to be dependent on the concentration of the incoming CO ligand.[19]

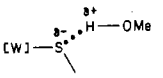
In the absence of this solvent-assisted ligand substitution process, that is when the reaction is carried out in aprotic solvents, displacement of the $Ph_2P(O)NPPh_3$ ligand is much less facile. For example, in THF at atmospheric CO pressure, substitution of $Ph_2P(O)NPPh_3$ occurs without any buildup of $W(CO)_5THF$ at a rate at least 50 times slower than the displacement of methanol in $W(CO)_5MeOH$. Therefore **dissociative ligand loss** of $Ph_2P(O)NPPh_3$ from $W(CO)_5OPPh_2NPPh_3$, a process directly related to the [W]–O bond strength, is **much slower** than the analogous processes involving the neutral O-bonded ligands THF and MeOH.

In conclusion, the observation of nucleophilic attack at the electrophilic phosphorus atoms of the PNP^+ cation by OMe^- and/or OH^- reported herein might be more widespread for good nucleophiles (including, e.g., the hydride ligand)[20] and hence needs to be considered when PNP^+ salts are used in organometallic chemistry.

Acknowledgment. The financial support of this research by the National Science Foundation (Grant CHE 83-08281) is greatly appreciated.

Registry No. [PNP]Cl, 21050-13-5; NaOMe, 124-41-4; $Ph_2P(O)NPPh_3$, 2156-69-6; $W(CO)_5OPPh_2NPPh_3$, 99618-35-6; $W(CO)_5THF$, 36477-75-5.

(17) A reversible interaction of this type



has been observed for the $W(CO)_5SH^-$ and $W(CO)_5SPh^-$ derivatives: unpublished results of Kathryn Summers from our laboratories.

(18) Darensbourg, D. J. *Adv. Organomet. Chem.* **1982**, *21*, 113.

(19) An increase in CO pressure from atmospheric to 110 psi resulted in an increase in the rate of MeOH displacement in $W(CO)_5MeOH$ by a factor of 7.9.

(20) Slater, S. G.; Lusk, R.; Schumann, B. F.; Darensbourg, M. Y. *Organometallics* **1982**, *1*, 1662.

Department of Chemistry
Texas A&M University
College Station, Texas 77843

Donald J. Darensbourg*
Magdalena Pala

Department of Chemistry
University of Delaware
Newark, Delaware 19716

Arnold L. Rheingold*

Received August 30, 1985

The Principal Components of Synthetic Vitamin B'_{12}

Sir:

An extensive literature indicates that commercial sources of B_{12} compounds contain impurities with potential health consequences.[1] Nath[1-5] has investigated these impurities and has concluded that an isomeric modification of B_{12} is present on the basis of synthetic materials having properties similar to those of the impurities. The synthetic materials also have properties very similar to those of the B_{12} analogues and thus have been called B'_{12}. However, the evidence suggests that B'_{12} contains a negatively charged group since, for example, the aquocobalamin (B_{12a}) is positively charged whereas B'_{12a} is not retained by cation-exchange resins.[1]

Slow hydrolysis of the amide functions in B_{12} compounds could account for (a) the slow changes in B_{12} species that lead to the corrresponding B'_{12} and (b) the occurrence of a negatively charged group (i.e. an ionized carboxylic acid). Therefore, we utilized the method of Hogenkamp[6] to acid hydrolyze vitamin B_{12}. The three

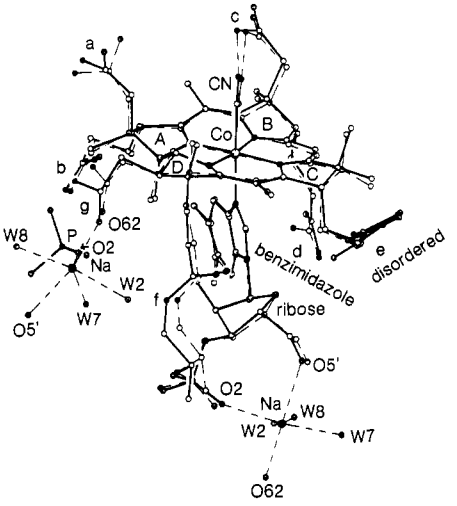


Figure 1. View of the structures of vitamin B'_{12} (solid lines) superimposed, via the four pyrrole nitrogen atoms, on the structure of air-dried vitamin B_{12} (broken lines). Broken lines are also used to show the coordination to the sodium ions (one per vitamin B'_{12}) and the internal hydrogen bond from the cyanide group to the c side chain. The e side chain is disordered in vitamin B'_{12}. P and N are denoted by solid circles, O is shown by stippled circles, and Co and C are depicted by open circles. Hydrogen atoms are not included. Note the great similarities in the two structures. Sodium ions and phosphate groups from two different asymmetric units are shown. Water molecules are designated W.

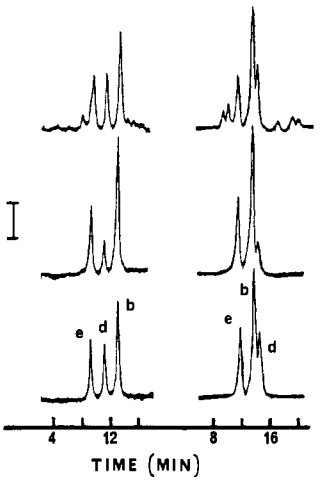


Figure 2. Traces from the HPLC experiments using CH_3CN (left) or CH_3OH (right) as solvent: (top) noncrystalline B'_{12}; (middle) crystalline B'_{12}; (bottom) mixture of d-, b-, and e-monocarboxylic acids. The vertical bar corresponds to 0.01 absorbance unit. HPLC separation conditions: 4.6 × 250 mm Microsorb C_{18} (5 μm) column; 15–20-μL injections of 0.34 mg/mL solutions (except for the crystalline B'_{12}, which was available in insufficient quantity for weighing); absorbance at 260 nm, 0.2 AUFS; flow rate 1.5 mL/min; 30-min gradients; solvent 0.1 M triethylamine/acetate buffer (pH 7.0); gradients from 10 to 15% CH_3CN (left) and from 20 to 25% CH_3OH (right). Separations were optimized by starting from conditions outlined by Jacobsen.[11]

(1) Kohli, R. K.; Nath, A. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 698.

(2) Katada, M.; Somdev, T.; Nath, A.; Petersen, R. L.; Gupta, R. J. *Biochim. Biophys. Acta* **1979**, *584*, 149.

(3) Gupta, R. K.; Goswami, P. C.; Nath, A. In "Vitamin B_{12}"; Zagalak, B., Friedrich, W., Eds.; Walter de Gruyter: New York, 1979; p 179.

(4) Mishra, P. K.; Gupta, R. K.; Goswami, P. C.; Venkatasubramanian, P. N.; Nath, A. *Biochim. Biophys. Acta* **1981**, *668*, 406.

(5) Mishra, P. K.; Gupta, R. K.; Goswami, P. C.; Venkatasubramanian, P. N.; Nath, A. *Polyhedron* **1982**, *1*, 321.

(6) (a) Yamada, R.; Hogenkamp, H. P. C. *J. Biol. Chem.* **1972**, *247*, 6266. (b) Anton, D. L.; Hogenkamp, H. P. C.; Walker, T. E.; Matwiyoff, N. A. *J. Am. Chem. Soc.* **1980**, *102*, 2215.

0020-1669/86/1325-0127$01.50/0

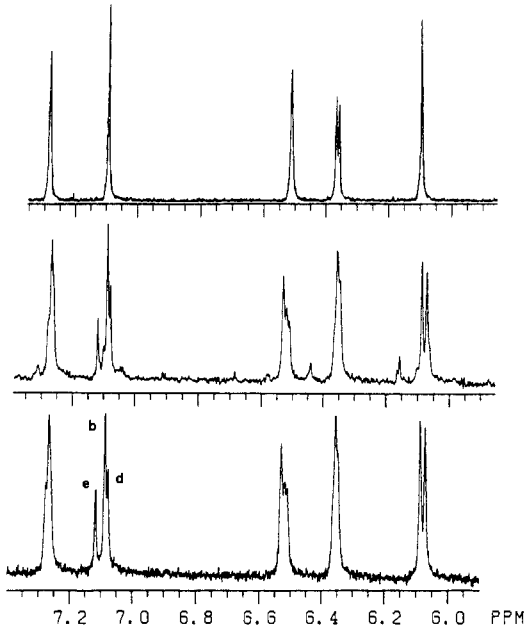


Figure 3. ^{1}H NMR spectra: (top) vitamin B_{12}, obtained from Sigma, 1.2 mg/0.5 mL; (middle) noncrystalline vitamin B'_{12}, 1.2 mg/0.5 mL; (bottom) mixture of d-, b-, and e-monocarboxylic acid derivatives of vitamin B_{12}, 2.0 mg/0.5 mL. Spectra were obtained at 361.06 MHz in D_2O and referenced to internal (trimethylsilyl)propionate.

propionamide groups at positions b, d, and e are hydrolyzed most readily (see Figure 1).[7] We then examined the resulting chromatographically separated and neutralized products by HPLC with two different gradient systems (Figure 2). Similarly, we examined two vitamin B'_{12} samples prepared by R.K.K.—a crude sample and a crystalline sample.[1] From these HPLC traces, it is clear that both samples of B'_{12} are mixtures of monocarboxylic acids. (The literature contains the term monocarboxylic acids, but the procedure involves neutralization with sodium acetate). The noncrystalline sample also contained minor impurities. The crytalline B'_{12} sample and the monocarboxylic acid mixture were found to have superimposable visible spectra (included in the supplementary material).

The HPLC retention time of each form was easily identified since the d form can be separated by anion-exchange chromatography from the e and b forms and the b form is the predominant isomer in the mixture. Hogenkamp's procedure[6] yielded the three monocarboxylic acids in a ratio of b:d:e[8] of ~2:1:1. In comparison, crystalline B'_{12} contained these species in a ratio of ~2:0.3:1 and noncrystalline B'_{12} contained a slight deficit of the e-monocarboxylic acid.

The ^{1}H NMR spectral region from 7.4 to 6.0 ppm for the B_{12} monocarboxylic mixture and the noncrystalline B'_{12} (insufficient quantity of crystalline B'_{12} was available) is illustrated in Figure 3. By separate analysis of the d and the e and b fractions, we can assign the three signals (downfield to upfield) at ca. 7.1 ppm to the H at the 2-position of the benzimidazole ring of the e, b, and d isomers, respectively. Note that the well-separated downfield e signal is relatively smaller in the B'_{12} mixture than in the mixture of monocarboxylic acids. Similarly, in the feature at ca. 6.5 ppm, which is due to the H at the 4-position of the benzimidazole ring, the assignment order is b, d, e. The e signal is clearly smaller in the B'_{12} mixture. The ^{31}P NMR spectra of B'_{12} and the monocarboxylic acid mixture both contain almost all the intensity in two signals (−3.8 and −3.9 ppm relative to trimethylphosphate). The upfield signal is identified as the e isomer as above and is relatively smaller in the B'_{12} sample. The carboxyl group of the e isomer should be closest to the phosphate group.[10] Thus, NMR spectra confirm the HPLC analysis.

We also confirmed by electrophoresis that the B'_{12} material derived from vitamin B_{12} is negatively charged. The ^{31}P[9,10] and ^{1}H NMR[10] spectra of cobalamins are sensitive to minor changes in structure at the Co center. The ^{31}P NMR signals, as well as the ^{1}H NMR signals shown in Figure 3, of B'_{12} are very similar to those of B_{12} itself. Thus, the properties of the major components of B'_{12} can be easily rationalized if the compounds are monocarboxylic acids. Such spectral characteristics would be more difficult to rationalize if an isomerization has occurred.

The crystals of synthetic B'_{12}, grown by Drs. Kohli and Nath, were orthorhombic space group $P2_12_12_1$ with unit cell dimensions a = 23.907 (4) Å, b = 22.356 (4) Å, c = 15.832 (3) Å. These values may be compared with the unit cell dimensions of air-dried vitamin B_{12} (a = 24.35 Å, b = 21.29 Å, c = 16.02 Å)[12] and "wet" vitamin B_{12} (a = 25.33 Å, b = 22.32 Å, c = 15.92 Å).[13] The structure was determined by locating the cobalt atom from a Patterson map and then computing electron density maps so that the rest of the molecule could be located. The resulting structure has been refined with isotropic temperature factors (anisotropic for cobalt) to R = 0.16.[14] The conformation of the molecule is almost identical with that of air-dried vitamin B_{12}.[12] The main difference is the presence of a high peak 2.1–2.7 Å from the two oxygen atoms of O5′ (ribose hydroxyl) and O2 (phosphate oxygen) in one B'_{12} molecule and O62 (oxygen of g side chain) of a second B'_{12} molecule. The octahedral coordination completed by H_2O meets all the characteristics of a sodium ion, i.e., each sodium ion is surrounded by three functional groups in two B'_{12} molecules and three water molecules. The stoichiometry is one sodium ion per B'_{12} molecule. No non-B_{12} counteranion in the disordered solvent is obvious, and therefore it seems likely that the compound is the sodium salt of a monocarboxylic acid. This differs from that of the monoclinic monocarboxylic previously reported.[15] For molecules of this size, carboxyl and amide groups are not readily distinguished by X-ray diffraction methods.[15] In addition there may be disorder between carboxyl groups and amide groups in some side chains; such disorder is particularly evident in the e side chain, as shown in Figure 1. The g side chain is coordinated to a sodium ion.

The general conformation of the molecule and the unit cell dimensions are close to those parameters for air-dried vitamin B_{12},[12] in which there is a hydrogen bond from the c side chain to the cyanide group. The general conformation of the corrin ring in vitamin B'_{12} does not appear to be significantly different from that of vitamin B_{12}. Coordinates for the structure at the present stage of refinement and observed and calculated structure factors have been deposited as supplementary material. Unfortunately this structure contains, as does vitamin B_{12}, large amounts of water, much of it highly disordered. We have accounted for 12 water molecules and 1 molecule of acetone of crystallization. A further analysis of solvent structure is continuing.

(7) Hydrolysis of the three acetamides in B_{12} is sterically hindered by the corrin ring: Cason, J.; Gastaldo, C.; Glusker, D. L.; Allinger, J.; Ash, L. B. *J. Org. Chem.* **1953**, *18*, 1129.

(8) Designations of the three isomers are correlated with their order of elution (I, II, III) from a column of anion-exchange resin (AG 1X2, acetate form).[6] Hogenkamp has assigned the isomers as I(d), II(b), III(e) from ^{13}C NMR studies:[6b] (a) Bratt, G. T.; Hogenkamp, H. P. C. *Arch. Biochem. Biophys.* **1982**, *218*, 225. (b) Anton, D. L.; Hogenkamp, H. P. C.; Walker, T. E.; Matwiyoff, N. A. *Biochemistry* **1982**, *21*, 2372.

(9) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 1894.

(10) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729.

(11) Jacobsen, D. W.; Green, R.; Quadros, E. V.; Montejano, Y. D. *Anal. Biochem.* **1982**, *120*, 394.

(12) Hodgkin, D. C.; Lindsey, J.; Sparks, R. A.; Trueblood, K. N.; White, J. G. *Proc. R. Soc. London, A* **1962**, *266*, 494.

(13) Brink-Shoemaker, C.; Cruickshank, D. W. J.; Hodgkin, D. C.; Kamper, M. J.; Pilling, D. *Proc. R. Soc. London, A* **1964**, *278*, 1.

(14) A total of 7805 intensities (3786 reflections with $I > 2\sigma(I)$) were measured to 2θ = 135° with Cu Kα radiation on the instrumentation and with the procedures described previously.[10] The resolution of observed data corresponded to 135° in 2θ ($(\sin\theta)/\lambda$ = 0.6 Å^{-1}).

(15) (a) Hodgkin, D. C. *Proc. Indian Acad. Sci., Chem. Sci.* **1984**, *93*, 195. (b) Waters, J. M.; Waters, T. N. M. *Proc. Indian Acad. Sci.* **1984**, *93*, 219. (c) Moore, F. H.; O'Connor, B. H.; Willis, B. T. M.; Hodgkin, D. C. *Proc. Indian Acad. Sci.* **1984**, *93*, 235.

We feel that we have established beyond question that monocarboxylic acids are the major component of synthetic vitamin B'_{12}. These could also arise quite naturally on storage of solid B_{12} compounds, which usually contain large numbers of H_2O molecules. The dicarboxylic acids from acid hydrolysis cannot be excluded as the additional species in noncrystalline vitamin B'_{12} observed by HPLC. The ^1H NMR spectrum of the dicarboxylic acid fraction contains signals that are obscured by the larger monocarboxylic acid signals. The B'_{12} spectrum contains several additional signals (e.g. at 6.15 and 6.45 ppm)—and thus, all the impurities in the noncrystalline B'_{12} sample cannot be the components in the dicarboxylic acid fractions.[6] Since "natural" vitamin B'_{12} itself is a trace impurity (0.08%),[2] the other components in "B'_{12}" are insignificant indeed. In any case, the physical and chemical measurements reported thus far on vitamin B'_{12} (e.g. the reported 360-MHz ^1H NMR spectrum[2]) must represent primarily the properties of the monocarboxylic acid mixture.

Acknowledgment. We thank Professor Nath for interesting discussions. This work was supported by NIH Grants GM29225 (to L.G.M.) and CA10925 (to J.P.G.). We thank the NSF for a departmental grant to Emory toward the purchase of the 360-MHz NMR spectrometer.

Supplementary Material Available: Comparison of the visible spectra of the mixture of monocarboxylic acids and B'_{12} and tables of positional parameters and structure factors (30 pages). Ordering information is given on any current masthead page.

Department of Chemistry, Emory University, Atlanta, Georgia 30322 — **Luigi G. Marzilli***, **Wallace O. Parker, Jr.**

Department of Chemistry, Drexel University, Philadelphia, Pennsylvania 19104 — **Rakesh K. Kohli**

The Institute for Cancer Research, The Fox Chase Cancer Center, Phladelphia, Pennsylvania 19111 — **H. L. Carrell**, **Jenny P. Glusker***

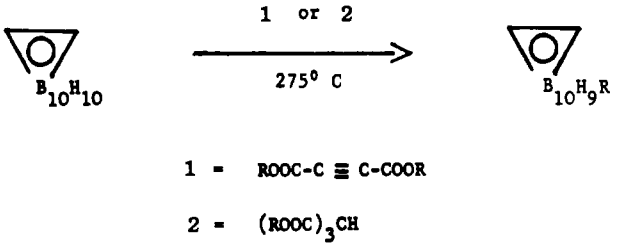
Received July 31, 1985

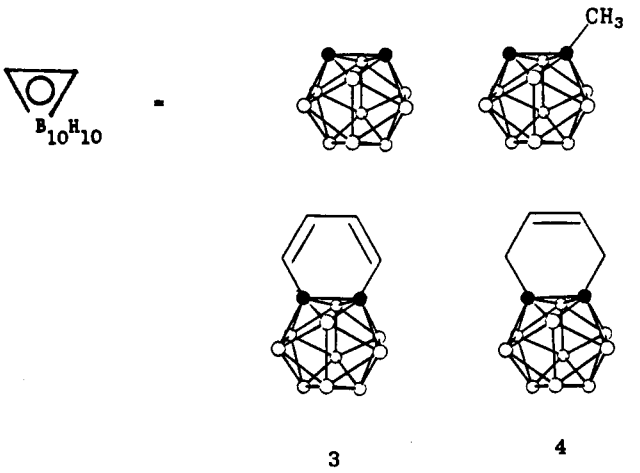
Alkylation of *o*-Carborane by Ester Pyrolysis[1]

Sir:

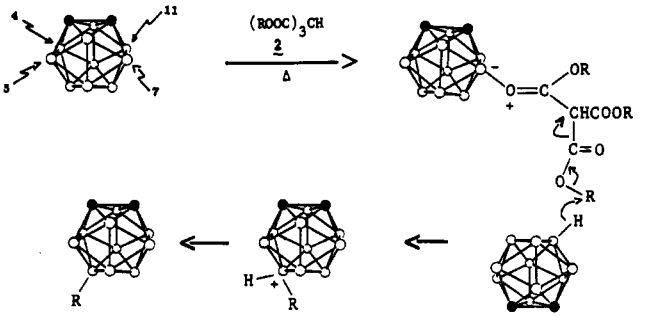
Efficient substitution of the (9,12)-position in *o*-carborane cannot be achieved through simple Friedel–Crafts alkylation. Books on boron hydride chemistry give this reaction short shrift,[2] and for good reason. The reports of such alkylation reactions are sparse and contradictory,[3,4] as remarked by Zakharkin and his co-workers[4] in the most detailed description of the reaction. They found that introduction of a single ethyl group on boron accelerated further substitution until four groups had added. As a result mixtures were obtained and the utility of simple alkylation is clearly limited. Nor are alternate procedures without difficulty. Although the reported replacement of a 9-iodo group by the alkyl group of a Grignard reagent works well,[5] at least in our hands the required synthesis of 9-iodo-*o*-carborane[6] does not.[7]

Scheme I

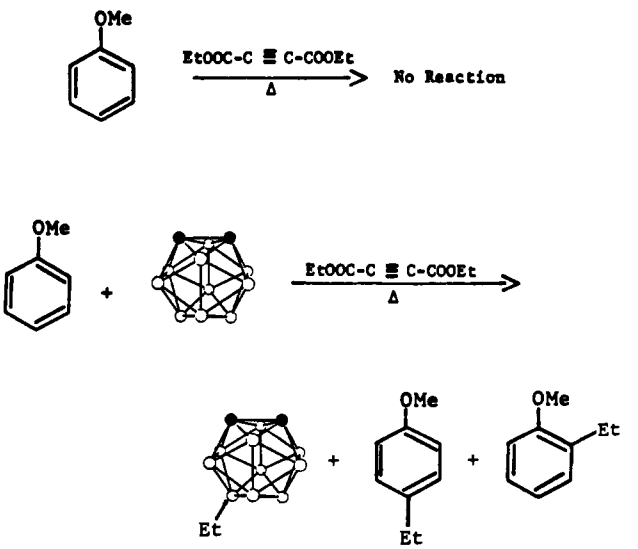




Scheme II



Scheme III



(1) We thank the National Science Foundation for support of this work through Grant CHE-8318345. We also express our appreciation for support from the donors of the Petroleum Research Fund, administered by the American Chemical Society. Dr. José Zayas kindly synthesized authentic samples of 9-*n*-propyl- and 9-isopropyl-*o*-carborane.

(2) Beall, H. In "Boron Hydride Chemistry"; Muetterties, E. L., Ed.; Academic Press: New York, 1975; Chapter 9, pp 321 ff.

(3) Fielding, T. E. Ph.D. Thesis, University of Pittsburgh, 1971; *Diss. Abstr. Int., B* **1972**, *32*, 6891.

(4) Zakharkin, K. I.; Pisareva, I. V.; Bikkineev, R. Kh. *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1977**, 577.

(5) Zakharkin, L. I.; Kovredov, A. I.; Ol'shevskaya, V. A.; Shaugumbekova, Zh, S. *J. Organomet. Chem.* **1982**, *226*, 217.

0020-1669/86/1325-0129$01.50/0