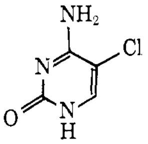
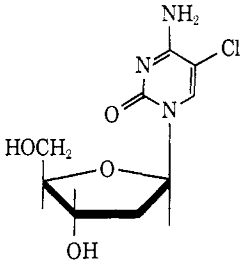
DNA was isolated from a 900 kg lot of salmon sperm by the method of Emanuel and Chaikoff[4,5] and hydrolyzed to the nucleoside level using snake venom diesterase and intestinal phosphatase. From the resulting hydrolysate, fractions enriched in each of the four principal nucleosides were obtained by ion exchange chromatography.[5] Each of these fractions was concentrated by distillation at 50° under reduced pressure and followed by crystallization of the principal nucleoside present in the respective fractions. The crystalline nucleosides were removed and the resulting filtrates were concentrated further and analyzed[6] by paper chromatography and electrophoresis.[7]


I


II

The filtrate obtained after removal of the bulk of deoxycytidine was examined using a number of chromatographic systems and was shown to contain several uv-absorbing components. Two of the components were successfully isolated and purified using ascending paper chromatography[8] and were characterized by spectrophotometric and chemical techniques. A mass spectrum of the compound with $R_f$ values of 0.76, 0.34, and 0.36 in solvent systems A, B, and C,[8] respectively, exhibited molecular ions at *m/e* 145.0040 (calcd for $C_4H_4N_3O^{35}Cl$, 145.0042) and *m/e* 147.0010 (calcd for $C_4H_4N_3O^{37}Cl$, 147.0013) and a prominent fragment ion at *m/e* 110.0360 (calcd for $C_4H_4N_3O$, 110.0354) consistent with a chlorinated cytosine structure. The uv spectra of this compound, pH 1 $\lambda_{max}$ 293.5 nm and pH 13 $\lambda_{max}$ 295 nm, and paper chromatographic mobilities (see above) were identical with those of authentic 5-chlorocytosine[9] (I). Further confirmation was provided by deamination of the isolated compound with nitrous acid to 5-chlorouracil,[10] pH 1 $\lambda_{max}$ 273 nm and pH 13 $\lambda_{max}$ 289 nm.

The second isolated compound showing $R_f$ values of 0.43, 0, and 0.21 in solvent systems A, B, and D,[8] respectively, exhibited uv spectra (pH 1 $\lambda_{max}$ 297 nm; pH 13 $\lambda_{max}$ 286 nm) consistent with assignment as 5-chlorodeoxycytidine (II).[11,12] A mass spectrum exhibited intense ions at *m/e* 110, 145, and 147[13] confirming the assignment of the base structure as 5-chlorocytosine (I). However, no molecular ions corresponding to 5-chlorodeoxycytidine (II) were observed; instead apparent molecular ions were present at *m/e* 225 and 227[13] corresponding to loss of two molecules of water from the expected molecular ions (*m/e* 161 and 163).

Although the complexities involved in processing 900 kg of salmon sperm to isolate very small samples (<1 mg) of minor DNA constituents make hazardous estimates of the quantities present, the amount of 5-chlorocytosine (I) in the DNA hydrolysate appears to be less than 0.01% of the cytosine content. It is exceedingly difficult to rule out absolutely the possibility that such small amounts of material are formed artifactually. However, during the process of isolation of DNA, enzymatic hydrolyses, and fractionation of the resulting constituents,[4,5,7,8] the material was never subjected to conditions known to effect chlorination of the pyrimidine nucleus.

The biological significance of the occurrence of 5-chlorocytosine (I) in salmon sperm DNA cannot be assessed at present. While "minor" bases are numerous in RNA[14] only 5-methylcytosine and $N^6$-methyladenine (6-methylaminopurine) are known to be widely distributed in DNAs.[15] 5-Chlorocytosine (I) may be a disfunctional derivative of cytosine resulting in some way from the high chloride concentration in the marine environment. However, the possibility that 5-chlorocytosine (I) or other minor bases occur as functional constituents of DNA cannot be ruled out. Prior to the present investigation, fractionations of DNA for the purpose of detecting minor bases have been carried out on sufficiently small scales that detection of a base occurring with a frequency of one or two residues per DNA molecule would be unlikely.[15–17]

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(4) C. F. Emanuel and I. L. Chaikoff, *J. Biol. Chem.*, 203, 167 (1953). Some modifications were required because of scale; however, the basic method and all reagents used were as described.

(5) The preparative steps involving the handling of the bulk substrate were carried out by Calbiochem Inc. of La Jolla, Calif. 92037. Their help and cooperation are gratefully acknowledged.

(6) A. W. Lis and D. I. McLaughlin, 15th Annual Meeting, Biophysical Society, New Orleans, La., Feb 16–18, 1971.

(7) A. W. Lis and W. E. Lis, *Biochim. Biophys. Acta*, **61**, 799 (1962); A. W. Lis and W. E. Passarge, *Arch. Biochem. Biophys.*, **114**, 593 (1966); S. J. Hayes and A. W. Lis, *Physiol. Chem. Phys.*, 3, 517 (1971); A. W. Lis and S. J. Hayes, *ibid.*, **4**, 377 (1972).

(8) Solvent systems used were: A, isobutyric acid–0.5 *M* ammonium hydroxide (65:39); B, *n*-butyl alcohol–water (86:14), C, *n*-butyl alcohol–water–ammonia (86:14:5) D, *tert*-amyl alcohol–formic acid–water (90:19.2:39).

(9) I. Wempen and J. J. Fox, *J. Med. Chem.*, **6**, 688 (1963).

(10) I. Wempen and J. J. Fox, *J. Amer. Chem. Soc.*, **86**, 2474 (1964).

(11) D. M. Firsch and D. W. Visser, *J. Amer. Chem. Soc.*, **81**, 1756 (1959).

(12) Reference samples of synthetic 5-chlorocytidine and 5-chlorouridine were generously supplied by Dr. D. W. Hutchison, Department of Molecular Sciences, University of Warwick, Coventry CV4 7 Al, England.

(13) High-resolution studies confirm the assigned elemental compositions.

(14) R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, N. Y., 1971.

(15) See ref 14, Chapter 5.

(16) A highly sensitive immunochemical method for the detection of minor bases in DNA has been described: D. L. Sawicki, B. F. Erlanger, and S. M. Beiser, *Science*, **174**, 70 (1971).

(17) This work was supported in part by the Medical Research Foundation of Oregon.

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## An Unusual Chelated *o*-Carborane Transition Metal Complex

*Sir:*

Lithium adducts of certain icosahedral carborane derivatives react with various transition metal salts to give σ-bonded transition metal complexes comprised of unusually stable carbon–metal bonds.[1] We would like to report the structure of a unique transition metal complex which contains a chelating *o*-carboranyl group and includes a three-membered ring having two carbon–metal σ bonds.

Treatment of 1,2-dilithio-*o*-caborane (the dilithio adduct of 1,2-dicarba-*closo*-dodecaborane(12)) in ether with 1 mol equiv of $[(C_6H_5)_3P]_2NiCl_2$ as an ether slurry for 1 hr gives a yellow-brown precipitate which on repeated recrystallization from diethyl ether gives 25–35% yields of a diamagnetic yellow-orange crystalline solid, $[(C_6H_5)_3P]_2NiC_2B_{10}H_{10}$, mp 190–192°. *Anal.* Calcd for $C_{38}H_{40}B_{10}NiP_2$ (724.7 g/mol): C, 62.94; H, 5.51; B, 14.90; P, 8.54. Found: C, 63.36; H, 5.63; B, 15.01; P, 8.54. The 36.43-MHz $^{31}P$ nmr, broad band decoupled, spectrum in dimethyl sulfoxide exhibits a resonance at −31.8 ppm relative to 85% $H_3PO_4$, in toluene −32.9 ppm, and in benzene −32.2 ppm. The 60-MHz $^1H$ nmr spectrum in $CDCl_3$ gives the ratio of aromatic protons to B–H protons 3.00:0.95; theoretically, 3:1. There were no C–H resonances exhibited by the carborane. The infrared spectrum as a Nujol mull contained absorptions at 2900, 2550, 1575, 1450, 1425, 1375, 1325, 1300, 1175, 1150, 1080, 1060, 1025, 995, 950, 920, 845, 820, 780, 740, and 690 $cm^{-1}$. The molecular weight determination (calcd 724.7; found 706 in tetrahydrofuran) is consistent with the compound being monomeric in solution.

Analytical, infrared, and nmr data indicate the possibility that the nickel atom is σ bonded to the two carbons of the chelating *o*-carborane unit, and the color and magnetic moment suggest a planar configuration about the metal. In order to obtain further structural information about this novel complex, we have initiated an X-ray analysis.

The three-dimensional X-ray analysis is virtually complete and is currently in the last stages of refinement: monoclinic; $a = 14.77 \pm 0.01$, $b = 16.63 \pm 0.02$, $c = 16.07 \pm 0.07$ Å; $\beta = 92.75 \pm 0.3°$; $Z = 4$; space group $P2_1/n$. Data from three needle-shaped crystals were photographically collected for $hKl$, $0 < K < 11$, and for $Hkl$, $0 < H < 11$ with the use of Weissenberg geometry and Cu K$\alpha$ radiation. Visual methods were used to estimate the intensities of 2040 independent reflections. The Ni atom, the two P atoms, and 12 of the 36 phenyl carbons were located by the procedure of symbolic addition. An electron density map phased by these atoms revealed the 24 phenyl carbons and part of the expected icosahedral cage. Using the Ni, two P, and the phenyl carbon atom positions, another electron density map was calculated from which all the cage atoms were located. The distinction between carbon and boron in the carborane cage was based upon chemical evidence (particularly the $^1H$ nmr) although the electron density map did exhibit maxima which were higher for those atoms in the carborane cage which were assigned as carbons.

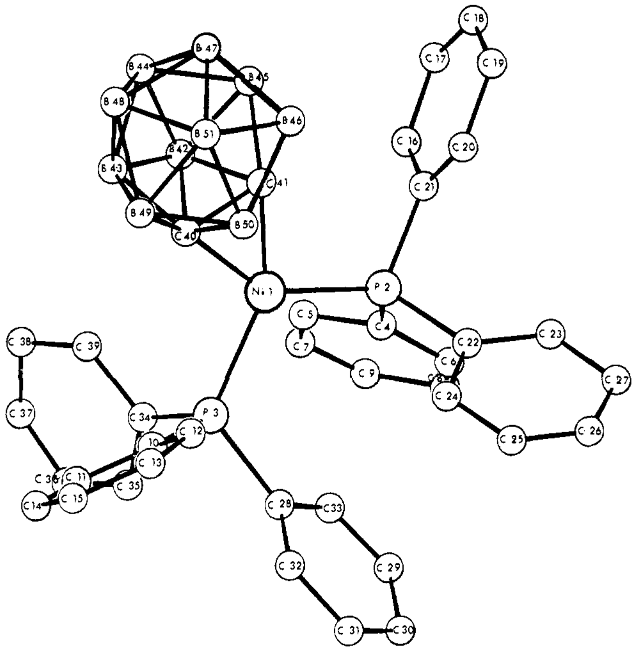

Figure 1. Molecular structure of $[(C_6H_5)_3P]_2NiC_2B_{10}H_{10}$.

Four cycles of refinement of position and isotropic temperature parameters for the 51 Ni, P, B, and C atoms led to $R_F = 0.137$. Four more cycles of refinement of position and anisotropic temperature parameters for all the atoms led to $R_F = 0.120$. A block-diagonalized least-squares refinement was used and the weighting scheme in the final stages of refinement was $w = 1/(a + |F_o| + b\,|F_o|^2 + c|F_o|^3)$ where $a = 2F_{min}$, $b = 2/F_{max}$, and $c = 5/F_{max}^2$. A three-dimensional difference map exhibited no peaks of any significance which were not residuals of known atoms. The magnitude of the highest peak in this map corresponds to 0.98 e/Å$^3$.

No significant dimensional changes are now expected and the molecular structure and essentially planar configuration about Ni atom are illustrated in Figure 1. Small deviations from strict planarity have been observed in related complexes.[2] In $[P(C_6H_5)_3]_2Ni(C_2H_4)$, $[P(C_6H_5)_3]_2Pt[(C_6H_5)C_2(C_6H_5)]$, and $[P(C_6H_5)_3]_2Pt(CS_2)$, the C–Ni–C, C–Pt–C, and C–Pt–S planes are inclined at angles 12, 14, and 6°, respectively, to the P–metal–P plane. The corresponding inclination in our case is 3.8°.

Although the coordination about nickel is essentially planar, it is severely distorted from a square. The C–Ni–C angle is compressed to 47.2 ± 0.8° whereas the P–Ni–P angle is opened up to 108.9 ± 0.2°. The Ni–C bond lengths are 2.00 ± 0.02 and 1.91 ± 0.02 Å; the Ni–P bond lengths are 2.29 ± 0.01 and 2.20 ± 0.01 Å. Of particular interest is the short C–C distance in the *o*-carboranyl cage. This distance is only 1.57 ± 0.03 Å compared with 1.64–1.67 Å found in other ordered crystal structures of *o*-carborane derivatives.[3]

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[1] J. C. Smart, P. M. Garrett, and M. F. Hawthorne, *J. Amer. Chem. Soc.*, **91**, 1031 (1969); D. A. Owen and M. F. Hawthorne, *ibid.*, **92**, 3194 (1970); **93**, 873 (1971); D. A. Owen, J. C. Smart, P. M. Garrett, and M. F. Hawthorne, *ibid.*, **93**, 1362 (1971); R. A. Love and R. Bau, *ibid.*, **94**, 8277 (1972); S. Bresadola, P. Rigo, and A. Turco, *Chem. Commun.*, 1205 (1968); C. M. Mitchell and F. G. A. Stone, *ibid.*, 1263 (1970); J. L. Spences, M. Green, and F. G. A. Stone, *J. Chem. Soc., Chem. Commun.*, 1178 (1972); S. Bresadola, G. Cecchin, and A. Turco, *Gazz. Chim. Ital.*, 100, 682 (1970); R. Rogowski and K. Cohn, *Inorg. Chem.*, 11, 1429 (1972).

[2] C. D. Cook, C. H. Koo, S. C. Nyburg,, and M. T. Shiomi, *Chem. Commun.*, 426 (1967), and references cited therein.

[3] D. Voet and W. N. Lipscomb, *Inorg. Chem.*, 3, 1679 (1964); J. A. Potenza and W. N. Lipscomb, *ibid.*, 3, 1673 (1964); 5, 1471, 1483 (1966).

Copies of the final atomic parameters, bond angles, and bond distances are available.[4] The standard deviations reported were estimated from the elements in the matrix inverse. These estimated standard deviations are probably unrealistically low for several reasons. The block-diagonal method does not necessarily give correct estimates of standard deviation. Furthermore, the omission of the hydrogen atoms can cause systematic errors. The rms estimates of standard deviation based on bond distances which should be chemically equivalent are probably more realistic estimates of the actual errors. Using this approach the overall rms deviation of the light atom distances is ±0.05. This value should be considered with regard to the short C–C distance mentioned above.

A fuller discussion of the bonding in this unusual complex and the stabilization of the Ni–carbon bond will be given on completion of the structural study.

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**Supplementary Material Available.** A listing of final atomic parameters, bond angles, and bond distances will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W,, Washington, D. C. 20036. Remit check or money order for \$4.00 for photocopy or \$2.00 for microfiche, referring to code number JACS-73-5790.

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[4] See paragraph at end of paper regarding supplementary material.

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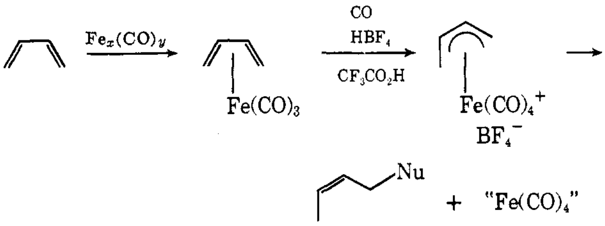
## π-Allyl Iron Cations. Iron-Moderated Carbonium Ions as Organic Reagents

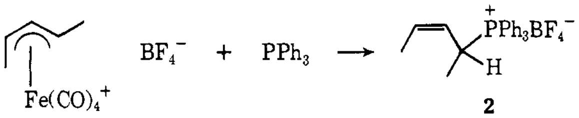
*Sir:*

We wish to report a preliminary investigation of an organometallic system which promises to be a versatile tool for the synthesis of organic species, particularly *cis*-allylphosphonium salts, from conjugated dienes. This system involves the use of π-allyliron tetracarbonyl cations, readily available in high yield from the appropriate diene iron tricarbonyl complex by protonation ($HBF_4$) in the presence of carbon monoxide.[1] These cationic species are subject to attack by a wide variety of nucleophiles, to give, in yields varying from 30 to 90%, the products of 1,4 addition across the diene. The overall transformation is shown in eq 1.



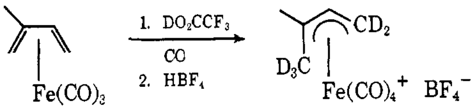
(1)

There are several features of this procedure which make it highly desirable from a synthetic standpoint. First, as shown in eq 1, the cisoid stereochemistry of the diene complex is retained in the product allyl species. Thus, this route allows the synthesis of allyl species containing cis double bonds. Even when there is a choice between the formation of a cis or a trans double bond, the cis species is favored. For example, *syn,anti*-(1,3-dimethylallyl)iron tetracarbonyl cation (**1**), prepared from *trans*-piperyleneiron tricarbonyl, gives rise to the allylphosphonium salt **2** (74%) when treated with triphenylphosphine.



**1** **2**

Secondly, as we have shown previously,[3] it is a simple matter to obtain stereospecifically deuterium labeled allyliron cations by use of deuterated acid. This observation allows us to prepare stereospecifically labeled tetracarbonyl cations by using deuterated acid in the above preparation.[4] This fact, coupled with the stereospecificity of the nucleophilic reactions, allows the synthesis of stereospecifically labeled species in high yield. Of particular interest in this connection is the species derived from isoprene, (1,1-dimethylallyl)iron tetracarbonyl cation **3**. This species undergoes preferential attack by nucleophiles at the less hindered 3 position, and the position of the label is not scrambled. Thus, this route provides a source of stereospecifically labeled isoprenyl units for use in biosynthetic studies of terpenes.



**3**

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[1] These species have been previously characterized by Gibson and Vonnahme;[2a] yields in their preparation are considerably increased (to >90% in some cases) by inclusion of CO during the protonation step.[2b]

[2] (a) D. H. Gibson and R. L. Vonnahme, *J. Amer. Chem. Soc.*, **94**, 5090 (1972); (b) G. E. Emerson, Ph.D. Thesis, University of Texas, 1964; *Diss. Abstr.*, **25**, 4955 (1965).

[3] T. H. Whitesides and R. W. Arhart, *J. Amer. Chem. Soc.*, **93**, 5296 (1971).

[4] Exchange can also be carried out after isolation of the tetracarbonyl cation; preparation of 3, among other iron cations, has been reported (D. H. Gibson and R. L. Vonnahme, *J. Chem. Soc., Chem. Commun.*, 1021 (1972)) by this route. Exchange is, however, slow in the tetracarbonyl compounds ($T_{1/2} \gg 0.5$ hr) and exchange at the tricarbonyl stage[3] is much more convenient.