[18] Synthesis of the GlcNAc-β-Asn bond without use of noble metals: a) A. Y. Khorlin, S. E. Zurabayan, R. G. Macharadze, *Carbohydr. Res.* **1990**, *85*, 201–208; b) T. Inazu, K. Kobayashi *Synlett.* **1993**, 869–870; c) S. T. Cohen-Anisfield, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1993**, *115*, 10531–10537.

[19] a) A. Bayley, D. N. Standring, J. R. Knowles, *Tetrahedron Lett.* **1978**, *19*, 3633–3634; b) According to this procedure an anthracene sulfonamide was cleaved with concomitant reduction of an azido group: J. Y. Roberge, X. Beebe, S. J. Danishefsy, *Science* **1995**, *269*, 202–204.

[20] a) L. J. Berliner, M. E. Davis, K. E. Ebner, T. A. Beyer, J. E. Bell, *Mol. Cell Biochem.* **1984**, *62*, 37–42; b) C. Augé, R. Fernandez-Fernandez, C. Gautheron, *Carbohydr. Res.* **1990**, *200*, 257–268; c) J. Lehmann, *Carbohydr. Res.* **1994**, *252*, 325–332.

[21] a) C. Unverzagt, H. Kunz, J. C. Paulson, *J. Am. Chem. Soc.* **1990**, *112*, 9308–9309; b) C. Unverzagt, S. Kelm, J. C. Paulson, *Carbohydr. Res.* **1994**, *251*, 285–301; c) We were informed that the influence of alkaline phosphatase in galactosylations was also examined by A. Redlitz (Diplomarbeit, Berlin, **1988**). d) Glycosyl transferases and nucleotide sugars were obtained from Sigma, alkaline phosphatase (calf intestine) from Boehringer Mannheim.

[22] Review: H. Schachter, *Biochem. Cell. Biol.* **1986**, *64*, 163–181.

[23] **1**: 5.6 mg yield (86% based on **13**); $[\alpha]_D^{23} = -9.1°$ (0.5, H_2O); ^1H NMR (500 MHz, D_2O): $\delta = 5.19$ (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^A), 5.12 (d, $J_{1,2} = 9.7$ Hz, 1H, H-1^1), 5.01 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1$^{A'}$), 4.83 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^3), 4.69–4.65 (m, 3H, H-1^2, H-1^5, H-1$^{5'}$), 4.505, 4.502 (2d, $J_{1,2} = 7.8$ Hz, 2H, H-1^6, H-1$^{6'}$), 4.31 (dd, $J_{2,3} = 1.9$ Hz, H-2^3), 4.25 (dd, $J_{2,3} = 1.9$ Hz, H-2^4), 4.17 (dd, $J_{2,3} = 1.9$ Hz, H-2$^{4'}$), 3.00 (dd, $J_{gem} = 17.2$ Hz, $J_{vic} = 4.2$ Hz, 1H, β-CHa-Asn), 2.92 (dd, $J_{vic} = 7.0$ Hz, 1H, β-CHb-Asn), 2.76–2.71 (m, 2H, H-3eq^N, H-3eq$^{N'}$), 2.14, 2.13, 2.09, 2.07, 2.06 (5s, 18H, NAc), 1.78 (t, $J_{gem} = 12.1$ Hz, 2H, H-3ax^N, H-3ax$^{N'}$). ^{13}C NMR (125 MHz, D_2O, [D_6] DMSO as internal standard; the chemical shifts were determined by an HMQC spectum): $\delta = 104.8$ C-1^6, C-1$^{6'}$, 102.6 C-1^2, 101.8 C-1^3, 100.7 C-1^4, 100.6 C-1^5, C-1$^{5'}$, 98.1 C-1$^{4'}$, 81.9 C-4^5, C-4$^{5'}$, 81.8 C-3^3, 80.9 C-4^2, 80.2 C-4^1, 79.4 C-1^1, 77.8 C-2^4, 77.6 C-2$^{4'}$, 77.5 C-5^1, 75.7 C-5^2, C-5^3, C-5^5, C-5$^{5'}$, 75.0 C-5^6, C-5$^{6'}$, 74.9 C-5^4, 74.1 C-5$^{4'}$, 74.0 C-3^1, 73.8 C-3^6, C-3$^{6'}$, C-3^N, C-3$^{N'}$, 73.3 C-3^2, C-3^5, C-3$^{5'}$, 73.0 C-8^N, C-8$^{N'}$, 72.0 C-2^6, C-2$^{6'}$, 71.4 C-2^3, 70.7 C-3^4, C-3$^{4'}$, 69.7 C-7^N, C-7$^{N'}$, 69.6 C-4^6, C-4$^{6'}$, 69.4 C-4^N, C-4$^{N'}$, 68.6 C-4^4, C-4$^{4'}$, 67.1 C-6^3, 67.0 C-4^3, 64.6 C-6^6, C-6$^{6'}$, 64.0 C-9^N, C-9$^{N'}$, 62.9 C-6^4, C-6$^{4'}$, 61.5 C-6^5, C-6$^{5'}$, 61.2 C-6^2, 61.1 C-6^1, 56.2 C-2^2, 55.9 C-2^5, C-2$^{5'}$, 54.9 C-2^1, 53.2 C-5^N, C-5$^{N'}$, 52.1 αC-Asn, 39.0 C-3^N, C-3$^{N'}$, 36.3 βC-Asn, 23.9, 23.8, 23.7 NAc.

[24] L. Dorland, J. Haverkamp, B. L. Schut, J. F. G. Vliegenthart, *FEBS Lett.* **1977**, *77*, 15–20.

[25] ESI-MS: (50% 0.01 M NH_4OAc, 50% acetonitrile): $C_{88}H_{144}N_8O_{64}$ M_r (calcd) = 2336.8; M_r (found) = 2337.8 ($M+1$).

[26] R. R. Townsend, E. Hilliker, Y.-T. Li, R. A. Laine, W. R. Bell, Y. C. Lee, *J. Biol. Chem.* **1982**, *257*, 9704–9710.

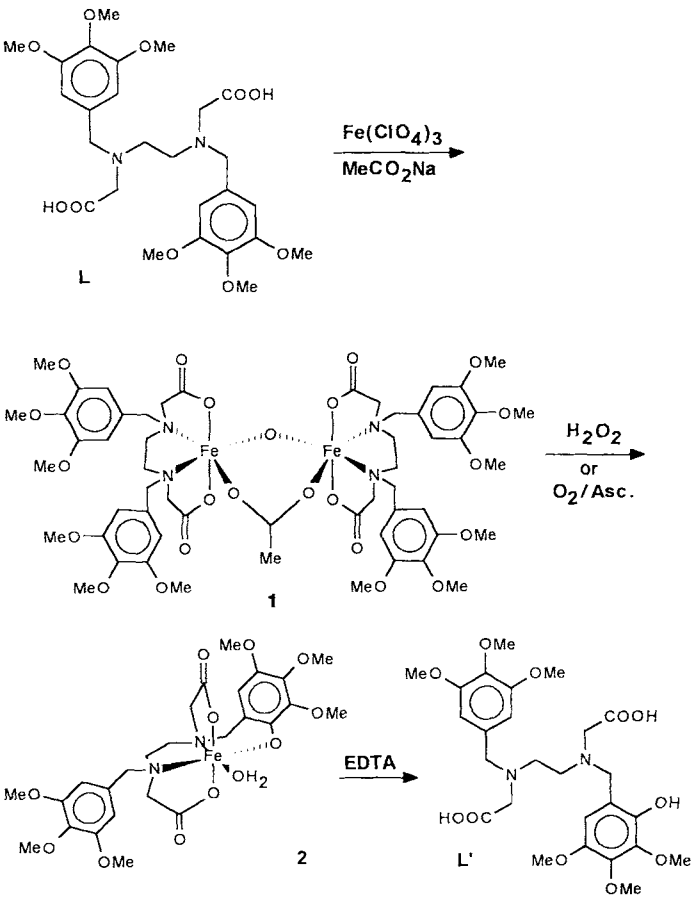
Aromatic Hydroxylation by H_2O_2 and O_2 Catalyzed by a μ-Oxo Diiron(III) Complex

Stéphane Ménage, Jean-Baptiste Galey, Georges Hussler, Michel Seité, and Marc Fontecave*

Recently a new class of enzymes, the diiron–carboxylato proteins, has emerged in which the active center contains two ferric ions linked by an oxo or hydroxo bridge and one or two carboxylato bridges of glutamate or aspartate residues.[1] Methane monooxygenase converts not only methane into methanol but reacts with a great variety of alkanes, alkenes, and aromatic compounds as well.[2] Ribonucleotide reductase oxidizes an endogeneous tyrosine to a tyrosyl radical, which initiates the conversion of ribonucleotide to deoxyribonucleotide.[3] The iron center of ribonucleotide reductase also has the potential to catalyze hydroxylation reactions as shown by the transformation of a tyrosine residue, brought into proximity of the active iron center by site-directed mutagenesis, into 3,4-dihydroxyphenylalanine (dopa).[4]

Many model compounds have been prepared but few are capable of displaying enzymatic activity such as hydroxylation of C–H bonds by molecular oxygen or hydrogen peroxide.[5, 6] Our approach here was to mimic quite closely the active center of methane monooxygenase as far as the carboxylato-rich environment of iron and the vicinity of a substrate site are concerned. An ethylenediamine tetraacetic acid (EDTA) derivative has been designed in which two carboxylato moities have been replaced by two phenyl groups which serve as substrates for the oxidation reaction.

Mixing a 1 mM solution of *N,N'*-bis-(3,4,5-trimethoxybenzyl)ethylenediamine *N,N'*-diacetic acid (ligand L)[7] with four equivalents of triethylamine in water/acetonitrile (1:1), one equivalent of $Fe(ClO_4)_3 \cdot 9H_2O$, and ten equivalents of sodium acetate led to the formation of the μ-oxo μ-acetato diferric complex $[Fe_2OL_2(CH_3CO_2)]^-$ (**1**). Its characterization in solution



was based on its UV visible spectrum,[8] which is indeed typical of μ-oxo μ-carboxylato diferric complexes.[9] Its ESI-MS spectrum[10] displays one negative ion peak at m/z 1255.5 (100%) corresponding to the pseudo-molecular ion $[Fe_2OL_2(CH_3CO_2)]^-$.[11] Assignments were confirmed by a comparison to the calculated isotopic pattern (Fig. 1). The solution was found to be EPR silent suggesting that the ferric ions are antiferromagnetically coupled through the oxo bridge. The ^1H NMR (200 MHz, CD_3CN/D_2O) spectrum of **1** in solution displays few resonances. Of interest is the resonance at $\delta = 12.5$, which is attributed to the acetate methyl protons. Such a chemical shift

[*] Prof. M. Fontecave, Dr. S. Ménage
L. E. D. S. S., UMR 5616, Université J. Fourier
BP 53X, F-38052 Grenoble, Cedex 09 (France)
Fax: Int. code + 76514382
Dr. J.-B. Galey, Dr. G. Hussler, M. Seité
L'Oreal Research Center
1 avenue Eugène Schueller
F-93600 Aulnay sour bois (France)

  0570-0833/96/3520-2353 $ 15.00+.25/0

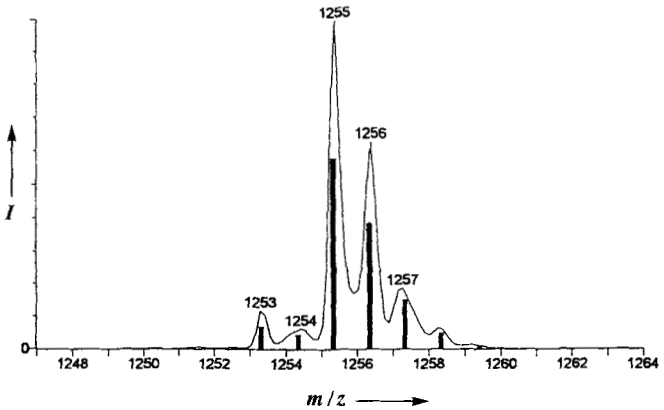


Fig. 1. Negative ionization electrospray mass spectrum of **1**. The calculated isotopic pattern for $C_{26}H_{36}O_{11}Fe$ is indicated by the bars.

indicates that the acetate is in a bridging coordination mode.[12] With a propionate as the bridging carboxylate group, the methylene protons resonated at $\delta = 11.3$ and the ring *m*-protons at $\delta = 9.03$ in the case of a benzoato bridge. All attempts to isolate and crystallize **1** were unsuccessful. However, based on the above data **1** is proposed to be a dinuclear ferric complex with one acetato and one oxo bridge. The coordination sphere for each iron is completed by two oxygen and two nitrogen atoms from the carboxylato and tertiary amino moieties of the ligand, respectively.

When three equivalents of hydrogen peroxide were added to a 1 mM solution of **1**, either in aqueous acetate buffer (pH 5.4) or acetonitrile/water (1:1), the solution turned deep blue as a result of the formation of a new chromophore with maximum absorption at 560 nm (Fig. 2). After saturation of the solution with NaCl, the new species **2** could be extracted with CH_2Cl_2. It was shown to be a monomeric ferric complex in which the ligand L is hydroxylated at position 2 of one of the phenyl groups. Monohydroxylation of L was assessed by ^1H and ^{13}C NMR spectroscopy after reduction of **2** with dithionite. The

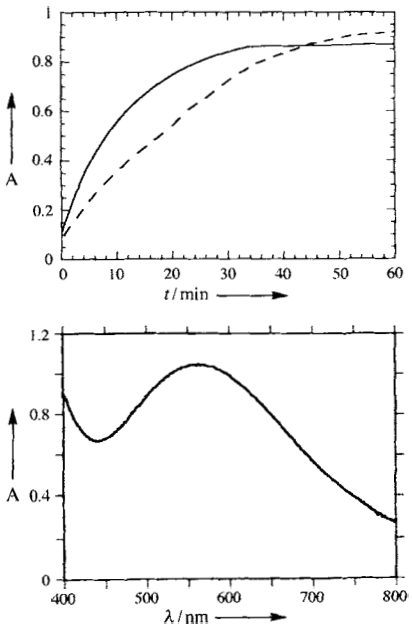


Fig. 2. Top: Absorbance at 560 nm versus time for the reaction of **1** [0.75 mM] with H_2O_2 (3 equiv, —) or O_2 (diffusing into the UV cell) and 15 equiv of ascorbate (– – –). Bottom: UV/Vis spectrum recorded at the end of the reaction.

positive mode ESI-MS analysis of **2** reveals only a signal at *m/z* 606 (90%) with the appropriate peak pattern for the pseudomolecular ion $[Fe(L') + H]^+$. No evidence was found for an ion corresponding to a binuclear species. After workup of the reaction mixture with excess EDTA to remove the metal ion, the modified ligand L′ was analyzed by HPLC/ESI-MS and confirmed to be *N*-(2-hydroxy-3,4,5-trimethoxybenzyl)-*N*′-(3,4,5-trimethoxybenzyl)ethylenediamine *N*,*N*′diacetic acid.[13] The yield of the oxidation reaction, based on the concentration of L, approached 80%. Attempts to crystallize the product were again unsuccessful due to its very high solubility in all solvents. The very intense transition at 560 nm in the visible region of the absorption spectrum of **2** can be attributed to a phenolato-to-iron charge transfer band ($\varepsilon = 1500\ M^{-1}cm^{-1}$).[14] The EPR spectrum of **2** displays a transition centered at $g = 4.3$ at 100 K for a mononuclear, hexacoordinated, high-spin iron(III) ion.[15] It is noteworthy that the EPR feature at $g = 4.3$ and the 560 nm absorption band simultaneously increased during the conversion of **1** into **2**. We thus propose that in **2** the iron(III) center is coordinated by two nitrogen atoms and four oxygen atoms (two from the carboxylato moities, one from the phenolate, and one from a water molecule).[16]

In the presence of acetate, L coordinates to iron to generate a dinuclear complex in which the ferric ions are doubly bridged by an oxo and an acetato group. The ligand L provides phenyl groups adjacent to the iron center as substrates for oxidation. Because of the well-established lability of acetato bridges,[17] reaction with H_2O_2 is possible and actually leads to aromatic hydroxylation. At the end of the reaction, the dinuclear unit is not recovered. Instead a mononuclear complex is obtained with the new phenolate moiety bound to iron. The ligand L is probably only monohydroxylated because the resulting iron–phenolato complex **2** is unable to bind and activate H_2O_2 as a consequence of the greatly decreased acidity of the ferric ion.

While alkylhydroperoxides, sodium hypochlorite, and *m*-chloroperbenzoic acid do not oxidize **1** under the conditions used with H_2O_2, monohydroxylation occurred in the absence of H_2O_2 but in the presence of molecular oxygen and excess ascorbate as a reductant (Fig. 2). The kinetics and yield of the reaction are comparable to those of the H_2O_2-dependent reaction. No reaction occurred in the absence of ascorbate. Treating **1** with ascorbate for half an hour under anaerobic conditions partially bleached the solution. Addition of O_2 then generated the 560 nm-absorbing complex **2**. This strongly indicates that ascorbate is involved in the formation of a ferrous complex which can bind and activate dioxygen. It is unlikely that the reaction depends on the formation of H_2O_2, since it is not affected by the addition of catalase.

To our knowledge, this is the first report of reductive activation of molecular oxygen resulting in aromatic hydroxylation which is promoted by a dinuclear ferric complex. This is a model for methane monooxygenase- or ribonucleotide reductase-dependent reactions. The hydroxylation of a tyrosine residue within a mutant of ribonucleotide reductase is here elegantly mimicked in that, as in the enzyme, the hydroxylated reaction product is bound to the iron center.[4] It must be noted that such chemistry is well established in the case of copper enzymes and their model compounds.[18]

Received: April 19, 1996 [Z9052IE]
German version: *Angew. Chem.* **1996**, *108*, 2535–2537

Keywords: complexes with oxygen ligands · enzyme models · iron compounds · oxidations

  0570-0833/96/3520-2354 $ 15.00+.25/0

[1] a) A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, P. Nordlund, *Nature* **1993**, *366*, 537; b) P. Nordlund, H. Eklund, B.-M. Sjöberg, *ibid.* **1990**, *345*, 593; c) L. Que, Jr., A. E. True, *Prog. Inorg. Chem.* **1990**, *38*, 97; d) J. Sanders-Loehr in *Iron Carriers and Iron Proteins* (Ed.: T. M. Loehr), VCH, New York, **1989**, 375.

[2] J. Green, H. Dalton, *J. Biol. Chem.* **1989**, *264*, 17698.

[3] M. Fontecave, P. Nordlund, H. Eklund, P. Reichard, *Adv. Enzymol.* **1993**, *65*, 147.

[4] A. Åberg, M. Ormö, P. Nordlund, B.-M Sjöberg, *Biochemistry* **1993**, 9845.

[5] D. Kurtz, *Chem. Rev.* **1993**, 586.

[6] L. Que, Jr. in *Bioinorganic Catalysis* (Ed.: J. Reedjik), Dekker, **1993**.

[7] J. B. Galey, J. Dumats, S. Génard, O. Destrée, P. Pichaud, P. Catroux, L. Marrot, I. Beck, B. Fernandez, G. Barre, G. Hussler, M. Seité, M. Hocquaux, *Biochem. Pharmacol.* **1996**, *51*, 103.

[8] UV/Vis (CH_3CN/H_2O): λ_{max} (ε) = 640 (160), 495 (530), 435 (1250) 350 (shoulder), 325 nm (10000). These bands are attributed to oxo–Fe^{III} ligand-to-metal charge transitions.

[9] S. Ménage, L. Que, Jr., *New. J. Chem.* **1991**, 431.

[10] ESI-MS analysis has proven to be a powerful technique in characterizing iron and manganese polynuclear complexes: U. N. Andersen, C. J. Mackenzie, G. Bergesen, *Inorg. Chem.* **1995**, *34*, 1435.

[11] Two more positive mode peaks were present at *m/z* (%) 1257.3 (100) and 1279.2 (10) for $[Fe_2OL_2(CH_3CO_2)+2H]^+$ and $[Fe_2OL_2(CH_3CO_2)+H+Na]^+$, respectively, with the expected isotopic patterns.

[12] D. Kurtz, *J. Am. Chem. Soc.* **1989**, *111*, 6563.

[13] The blue solution was treated with 3 mM EDTA and heated at 40 °C for 40 min. HPLC analysis was performed on a Waters Pursil C18 120 column [7].

[14] A. B. P. Lever *Inorganic Electronic Spectroscopy*, Elsevier, New York, **1984**, 311.

[15] EPR titration were done at 100 K with $[Fe^{III}(L'')(H_2O)]$ as a reference.

[16] An analog of **2**, $[Fe^{III}(L'')(H_2O)]$ (L'' = *N*-(2-hydroxybenzyl)-*N'*-(benzyl)-ethylenediamine *N,N'*diacetic acid), which shows similar spectroscopic characteristics has been isolated as a powder. A high-spin ferric EPR signal was observed and a phenolato-to-Fe^{III} CT band was present at 520 nm (1500) in its electronic spectrum. Elemental analysis for $[Fe(L'')(H_2O)]\cdot 1.5\ H_2O$: calcd for $FeC_{20}H_{26}N_2O_{7.5}$ (M_r = 470.1): C 51.10, H 5.53, N 5.96, Fe 11.88; *found*: C 51.23, H 5.43, N 6.05, Fe 12.00.

[17] S. Ménage, J.- M. Vincent, C. Lambeaux, G. Chottard, A. Grand, M. Fontecave, *Inorg. Chem.* **1993**, *32*, 4766.

[18] a) S. Itoh, T. Kondo, M. Komatsu, Y. Ohshiro, C. Li, N. Kanehisa, Y. Kai, S. Fukuzumi, *J. Am. Chem. Soc.* **1995**, *117*, 4714, and references therein; b) M. S. Nasir, K. D Karlin, D. McGowty, J. Zubieta, *ibid.* **1991**, *113*, 698.

A New In_4 Cluster with Short In–In Bonds in Trigonal-Planar $In(InTrip_2)_3$**

Penelope J. Brothers,* Klaus Hübler, Ute Hübler, Bruce C. Noll, Marilyn M. Olmstead, and Philip P. Power*

Homocatenation is an important feature of the chemistry of Group 14 elements, but in Group 13 it appears widely only for the lightest element, boron. Nonetheless, the synthesis and structural characterization of metal–metal bonded organoelement compounds of the heavier Group 13 metals has undergone a very rapid development since the late 1980s, and new structural and bonding types continue to be reported frequently. The simplest of these are the dimetallanes, R_2M-MR_2 (M = Al, Ga, In, Tl; R = alkyl, aryl, silyl)[1–6] and their derivatives $[R_2M-MR_2]^-$ obtained by one-electron reduction.[7] Cluster compounds $(MR)_n$ exhibit tetrahedral ($[AlCp^*]_4$, $[MC(SiMe_3)_3]_4$, M = Ga, In), octahedral ($[AlBu]_6^-$, $[InCp^*]_6$), or icosahedral ($[AlBu]_{12}^{2-}$) geometry.[1c, 8, 9] An Al_4 ring is found in $[Al_4Br_4(NEt_3)_4]$ whereas the cyclic trigallane dianion $[Ga(2,6\text{-}Mes_2C_6H_3)]_3^{2-}$ (Mes = 2,4,6-$Me_3C_6H_2$) is a cyclopropenium ion analog.[10, 11] Chlorogallium compounds $[Ga_4Cl_4\{Si(SiMe_3)_3\}_4]$ and $[Ga_5Cl_7(OEt_2)_5]$ consist of a Ga_4Cl_4 cage containing two Ga–Ga bonds[12] and a neopentane-like $GaGa_4$ skeleton,[13] respectively.

The tetraaryl dialane and digallane $Trip_2M-MTrip_2$ (Trip = 2,4,6-$iPr_3C_6H_2$) have been shown to be useful substrates for reduction by lithium metal to yield the one-electron π-bonded compounds $[Trip_2M\cdots MTrip_2]^{\bullet -}$ with an M–M π-bond order of 0.5.[7] We undertook the synthesis of the indium analog $Trip_2In-InTrip_2$ (**1**) to investigate its reduction by similar methods. Reaction of the tetrabromodiindane $[In_2Br_4(tmeda)_2]$[4] (tmeda = tetramethylethylenediamine) with TripMgBr at low temperature afforded **1** as large, bright orange crystals [Eq. (a)].

$$(tmeda)Br_2In-InBr_2(tmeda) \xrightarrow[Et_2O,\ -15°C]{TripMgBr} \underset{\mathbf{1}}{Trip_2In-InTrip_2} \qquad (a)$$

The compound is thermally and photochemically sensitive; above −10 °C decomposition results in the deposition of indium metal. The crystal structure of **1** (Fig. 1)[14] shows it to be a highly symmetrical compound, lying on a site of 222 symmetry in the unit cell. The In–In distance of 2.775(2) Å is comparable to that of the small number of related three-coordinate diindanes (*Table 1*). The angle between the normals to the C-In-C planes is 47.8° and each aryl ring is oriented at 63° with respect to this plane at the corresponding indium atom.

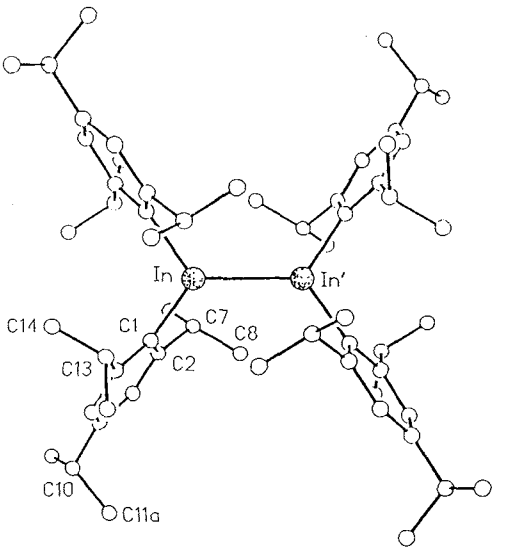


Fig. 1. Molecular structure of **1**. Selected bond lengths [Å] and angles [°]: In–In' 2.775(2), In–C1 2.184(7); In'-In-C1 121.6(2), C1-In-C1' 116.8(4).

[*] Dr P. J. Brothers, Dr K. Hübler, Dr U. Hübler
Department of Chemistry, University of Auckland
Private Bag 92019, Auckland (New Zealand)
Fax: Int. code +9 373 7422
email: p.brothers@auckland.ac.nz
Prof. P. P. Power, Dr B. C. Noll, Dr M. M. Olmstead
Department of Chemistry, University of California
Davis, CA 95616 (USA)

[**] This work was supported by the National Science Foundation. P. J. B. thanks the University of Auckland for granting sabbatical leave and K. H. thanks the Alexander von Humboldt Foundation (Lynen Fellowship).

  0570-0833/96/3520-2355 $ 15.00+.25/0