Orthometalation in calixarenes: Synthesis and structural characterization of a novel orthopalladated calix[4]arene bisphosphite

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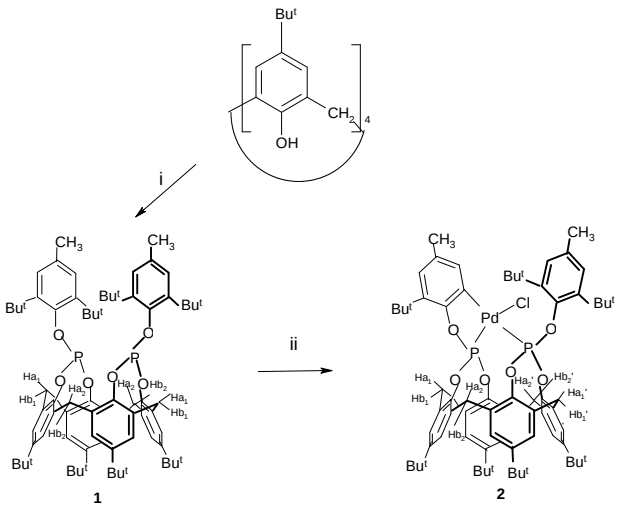
The treatment of a symmetrically bridged *p*-Bu^t-calix[4] arene bisphosphite with $PdCl_2(NCPh)_2$ yields a novel orthopalladated derivative by a C–C bond scission of a *t*-butyl group attached to an aryl ring. The structure of this orthopalladated calix[4]arene derivative has been established by X-ray crystallography.

CALIXARENES[1], a class of phenolic macrocycles, continue to find applications in diverse fields, especially in catalysis and solvent extraction of metal ions. One attractive feature of calixarene molecular architecture is that it is readily amenable to multiple functionalization. Calixarenes appended with phosphorus-containing[2] moieties at either the lower or upper rim offer further opportunities to design macrocyclic ligands with enhanced complex-forming ability. The transition metal chemistry of P(III) functionalized calixarenes[3] and the importance of phosphorus donor ligands in catalysis are well documented[4]. In the context of recent reports on calixarene phosphites as catalysts[5], and in continuation with our ongoing studies on phosphorus functionalized calixarenes and their coordination chemistry[6], we report, in this communication, an unprecedented orthopalladation reaction in calixarene chemistry. Lewis *et al.*[7] have reported that orthometalated complexes derived from phosphorus ligands show enhanced catalytic activity. Recent studies[8] reinforce this conclusion and are also directed towards understanding the mechanism of C–C and C–H bond activation by transition metal complexes[9].

The calixarene bisphosphite (**1**) was prepared by the deprotonation of the corresponding *p*–Bu^t-calix[4]arene with NaH and subsequent reaction with two mole equivalents of $Cl_2P(OR)$ (R = C_6H_2 – 2,6-Bu^t_2, 4-Me) in toluene[10]. The pure compound was isolated in 15% yield after column chromatography over silica gel. The calixarene bisphosphite (**1**) is a potential bidentate ligand to transition metals. The reaction of (**1**) (50 mg, 4.36×10^{-5} mol) in dichloromethane (20 ml) with $[PdCl_2(NCPh)_2]$ (ref. 11a) (17 mg, 4.36×10^{-5} mol) at 25°C gives the novel orthopalladated calixarene bisphosphite (**2**) in 40% yield (Scheme 1). Variable results were obtained when **2** was subjected to elemental analysis, which can be attributed to the solvent inclusion ability associated with calixarene derivatives[1]. The structure of (**2**) is supported by spectroscopic data[12] and confirmed by a single-crystal X-ray diffraction study[13]. The orthopalladated compound (**2**) is also formed in the reaction of $[PdCl_2(1,5\text{-cyclooctadiene})]$[11b] with the ligand (**1**) in boiling toluene, as shown by ^{31}P NMR spectroscopy.

The ^{31}P NMR spectrum of (**2**) shows an AX pattern with a coupling constant of 77.0 Hz, in contrast to a singlet (123 ppm) observed for (**1**). The ^1H NMR spectrum of (**2**) in the methylene region shows three pairs of doublets with a coupling constant typical of geminal protons, whereas two pairs of doublets are observed for (**1**). This non-equivalence arises as a result of formation of a 10-membered metallacycle (e.g. C21–C20–C15–O3–P1–Pd1–P2–O4–C22–C23) in addition to the two 8-membered rings containing phosphorus (C13–C8–O2–P1–O3–C15–C16–C14 and C2–C1–O1–P2–O4–C22–C27–28, see Figure 1). The formation of the palladacycle does not affect the protons *exo* {H(a, b)$_2$} to the phosphacycle, whereas the protons *endo* {H(a, b)$_1$} to the phosphacycle are rendered non-equivalent on complexation ('a' and 'b' refer to protons away from the aryl rings of calixarene and those close to the aryl rings as shown in Scheme 1; subscripts 1 and 2 refer to protons *endo* and *exo* to the phosphacycle). Accordingly, three pairs of doublets are observed at 3.40, 3.45, 3.58 ppm for Hb-type protons (i.e. Hb_2, Hb_1, Hb_1') and at 4.75, 4.80, 5.13 for Ha-type protons (i.e. Ha_2, Ha_1, Ha_1') respectively. The MALDI mass spectral data do not show the molecular ion peak corresponding to the orthopalladated derivative, but the major peak observed at 1180.2 Da corresponds to a fragmented species resulting from the loss of a methyl group and a chlorine from (**2**).

Colourless crystals of (**2**) suitable for X-ray analysis were obtained by slow evaporation of a concentrated



Scheme 1. Reagents and conditions: (i) NaH/Cl_2P(O–C_6H_2–2, 6-BU^t_2, 4-Me)/toluene/80°C/column chromatography; (ii) $PdCl_2(PhCN)_2/CH_2Cl_2$/25°C/48 h.

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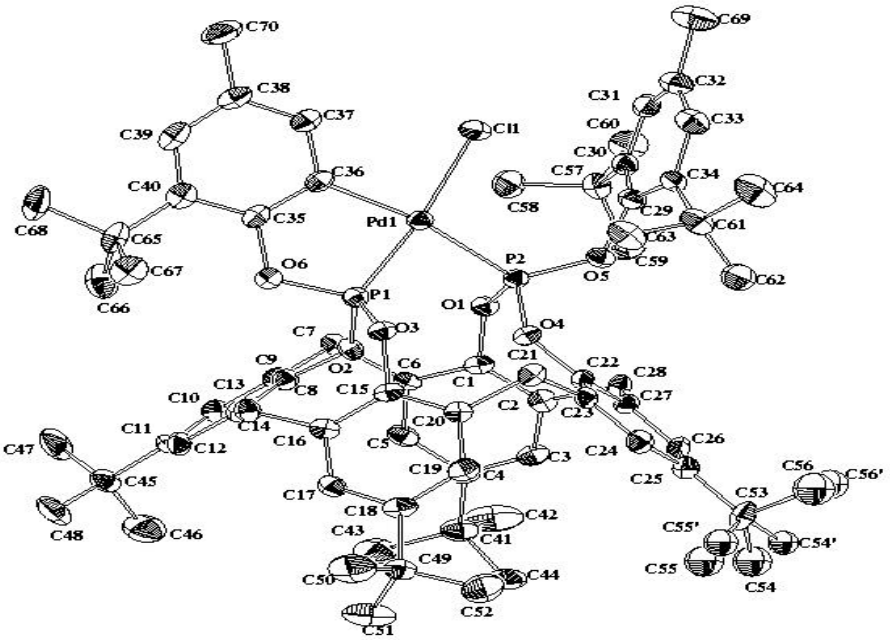

Figure 1. Molecular structure of orthopalladated complex (**2**) showing the atom numbering scheme. Methyl groups attached to C53 are disordered. Hydrogen atoms and solvent molecules are omitted for clarity.

solution of (**2**) in benzene/dichloromethane mixture (1 : 1 ratio). The compound (**2**) crystallizes with two molecules of benzene and one water molecule. The needle-shaped crystals were sealed in a glass capillary for data collection. The structure was solved by Patterson method (Fourier synthesis) and refined on F_o^2 by full-matrix least squares. All non-hydrogen atoms were refined anisotropically. The terminal methyl groups C54–C56 were found to be highly disordered. The disorder was resolved by refining the atoms in two positions with site occupancy factor of 0.7 and 0.3 respectively. All hydrogen atoms were included in calculated positions and allowed to ride on the parent carbon atoms with isotropic thermal parameters. The solvent molecules were subjected to only isotropic refinement. All calculations were carried out using SHELX programs[14].

The molecular structure of (**2**) is shown in Figure 1. Selected bond distances and angles and crystallographic data are given in Tables 1 and 2. The geometry around palladium is distorted square planar. The Pd–C distance of 2.064 Å is in the range observed for ortho metallated palladium complexes[8]. The Pd–P(1) distance is shorter than the Pd–P(2) distance. The structure of (**1**) also has been determined by X-ray crystallography[10]. A striking difference between the structures of (**1**) and (**2**) is that, in the latter, the aromatic rings of the phenolic substituents on the phosphorus are oriented perpendicular to each other to minimize strain. The torsion angle of 95.5° for P2–O5–C29–C34 found in complex (**2**) is the same as the corresponding value for the ligand (**1**), whereas the torsion angle of 178.6° about P1–O6–C35–C40 shows a distinct

Table 1. Selected bond distances (Å) and bond angles (deg) for (**2**)

Bond distance		Bond angle	
Pd(1)–C(36)	2.064(8)	P(1)–Pd(1)–P(2)	91.6(8)
Pd(1)–Cl(1)	2.319(2)	Cl(1)–Pd(1)–P(2)	96.4(8)
Pd(1)–P(1)	2.165(2)	C(36)–Pd(1)–P(1)	79.7(3)
Pd(1)–P(2)	2.340(2)	C(36)–Pd(1)–Cl(1)	92.5(3)
P(1)–O(2)	1.590(6)	O(3)–P(1)–O(2)	101.8(3)
P(1)–O(3)	1.580(6)	O(5)–P(2)–Pd(1)	126.0(2)
P(1)–O(6)	1.592(6)	O(6)–P(1)–Pd(1)	108.9(2)
P(2)–O(1)	1.602(5)	O(3)–P(1)–O(2)	101.8(3)
P(2)–O(4)	1.598(5)	O(5)–P(2)–Pd(1)	126.0(2)
P(2)–O(5)	1.592(6)	O(6)–P(1)–Pd(1)	108.9(2)

distortion from that of the ligand framework. The conformation of the calixarene may be described in terms of the dihedral angles between the aryl rings of the calixarene and that of the mean plane defined by the methylene protons[1,15]. Alternatively, the conformation of (**2**) may be specified in terms of the torsion angles (f and c) about each of the independent C(aromatic)–CH_2 bonds[16]. A scrutiny of these values shows that the calixarene framework in (**2**) adopts a distorted cone conformation; the distortion is more pronounced in the orthopalladated complex (**2**) in comparison to that of the calixarene bisphosphite (**1**)[10,15].

The loss of a *t*-butyl group by C–C scission in orthopalladation observed in the present study, is quite unusual. Although C–C bond activation is not unprecedented in orthometalation reactions, it is generally restricted to 'PCP' or 'PCN'-pincer-type ligands, as shown by Milstein and coworkers[17]. The driving force for

Table 2. Crystallographic and refinement data for (**2**)

Formula	$C_{82}\ H_{103}\ O_7\ P_2\ Cl\ Pd$
Fw	1404
Crystal size (mm)	0.5 × 0.2 × 0.2
Crystal system	Monoclinic
Space group	$P2_1/n$
a, Å	15.5977(17)
b, Å	16.921(10)
c, Å	30.009(3)
a, deg	90.0
b, deg	93.149(9)
g, deg	90.0
V, Å^3	7908(5)
Z	4
d_{calc}, mg m^{-3}	1.160
F (000)	2884
m (Mo–K*a*), mm^{-1}	0.358
l, (Mo–K$_a$), Å	0.71070
T, K	293
2*q* range, deg	1.36 to 22.48
Data collected	+ *h*, + *k*, ± *l*
No. of reflections collected	10559
No. of unique reflections	10309 [*R* (int) = 0.0100]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	10309/0/790
Final *R* indices [*I* > 2 sigma(*I*)]	$R1^a$ = 0.0694, $wR2^b$ = 0.2027
R indices (all data)	*R*1 = 0.1251, *wR*2 = 0.2465
Gof on $F^{2\,c}$	1.114
Residual density	1.400 and – 0.676 e. Å^{-3}

[a] $R1 = \Sigma\ |\ |F_o| - |F_c|\ |/\Sigma\ |F_o|$; [b] $wR2 = [\Sigma\ [w(F_o^2 - F_c^2)^2]/\Sigma\ [w(F_o^4)\ ]]^{1/2}$; [c] $S = [\Sigma\ [w(F_o^2 - F_c^2)^2]/(n-p)]^{1/2}$; *n*, Number of reflections; *p*, Total number of parameters defined; $w = [s^2(F_o^2) + (0.0517P)^2]^{-1}$; where $P = [\mathrm{Max}(F_o^2, 0) + 2F_c^2]/3$.

the loss of *t*-butyl group in the present instance is probably the result of the formation of a strong M–C_{aryl} bond and also the relief of steric strain induced by the phosphorus substituents on the calixarene framework. It is also conceivable that $PdCl_2$ acts as a Friedel–Crafts-type catalyst to bring about the cleavage of a *t*-butyl group from an aromatic ring[18]. Further studies are required to establish the generality of this type of reaction in calixarene chemistry.

The present study brings to light an interesting and novel facet of calixarene chemistry, viz. orthometalation reactions[19]. The blending of macrocyclic, phosphorus and organometallic chemistry would open up new synthetic strategies and will be a forerunner for the synthesis of a variety of new compounds with prospective applications in various fields[20].

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12. ^{1}H NMR ($CDCl_3$, 400 MHz): *d* = 1.08 (s, 9 H; C(C*H*$_3$)$_3$), 1.14 and 1.12 (2s, 36 H; C(C*H*$_3$)$_3$), 1.36 (s, 18 H; C(C*H*$_3$)$_3$), 2.35 (s, 6 H; C*H*$_3$), 3.40 (d, 14 Hz), 3.45 (d, 15 Hz), 3.58 (d, 15 Hz), [4 H; ArC*H*$_A$*H*$_B$Ar], 4.75 (d, 13.6 Hz), 4.80 (d, 14 Hz) and 5.13 (d, 14.9 Hz), [4 H, ArC*H*$_A$*H*$_B$Ar], 6.89 (s), 6.94 (br), 7.15 (s), 7.25 (m), (12 H; ArC*H*); ^{31}P NMR (161 MHz, $CDCl_3$) : *d* = 120.6 (d), 103.6 (d) (J_{pp} = 77 Hz); MALDI mass spectrum (Kratos PC-Kompact MALDI 4 V1.0.3 mass spectrometer) [M ($C_{70}H_{89}O_6P_2PdCl$) = 1230]: $(m/z)_{observed}$ = 1180.2 [M–(Cl + CH_3)], 1215.3[M–CH_3], 1200.3 [M–2CH_3].
13. An Enraf Nonius MACH3 diffractometer was used for data collection. Crystallographic data (excluding the structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 133305. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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15. The dihedral angles of the aromatic rings (C1–C6, C8–C13, C15–C20, and C22–C27) with the mean plane defined by the methylene carbons for (**2**) are 71.1, 37.1, 65.7 and 45.9° respectively and the corresponding dihedral angles for (**1**) are 65.4, 51.4, 65.4 and 51.4°, respectively.
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ACKNOWLEDGEMENTS. We thank the Sophisticated Instruments Facility, IISc, Bangalore for the NMR measurements, Prof. P. Balaram, Molecular Biophysics Unit, IISc, for MALDI mass spectral measurements and the National Single Crystal Facility (Dr K. C. Kumaraswamy) at the University of Hyderabad funded by the Department of Science and Technology, New Delhi for the X-ray data collection for compound (**2**).

Received 23 May 2002; revised accepted 14 September 2002

Purification of cytochrome P-450 in mycobacteria

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Purification of cytochrome P-450 from *Mycobacterium smegmatis, M. chelonae, M. fortuitum and M. tuberculosis* $H_{37}R_V$ was undertaken. The electrophoretic pattern revealed a single band corresponding to a molecular weight of 66 kDa in all the four species. Cytochrome P-450 purified from drug-resistant *M. tuberculosis* showed a different pattern from that of the sensitive bacteria, and the former was similar to the purified product obtained from phenobarbital-induced cytochrome P-450 in *M. tuberculosis* $H_{37}R_V$. It therefore appears that different forms of cytochrome P-450 are present in drug-sensitive and resistant *M. tuberculosis*, and that there is similarity in the pattern between drug-resistant and phenobarbital-induced *M. tuberculosis*.

A wide variety of drugs, chemical carcinogens and xenobiotics are metabolized by enzymes which belong to a family of hemoproteins with the collective name, cytochrome P-450. *M. tuberculosis*, the causative agent of tuberculosis (TB), has re-emerged as a global threat to human health. An unusual feature of the proteome of this bacteria is the large number of cytochrome P-450 enzymes, about 22 in number, that is more than in any other bacterial genome to date[1].

The role of cytochrome P-450 in the development of drug resistance has been well established in bacteria[2], insects[3] and other living species[4–6]. This phenomenon usually involves increased activity of cytochrome P-450, which brings about biotransformation of the active drug. In an attempt to elucidate the association between cytochrome P-450 and drug resistance in *M. tuberculosis*, we had previously isolated this protein in certain mycobacterial species, including *M. tuberculosis* $H_{37}R_V$ and demonstrated enhanced cytochrome P-450 activity in isoniazid-resistant and isoniazid and rifampicin-resistant *M. tuberculosis*, implicating a role for this protein in causing drug resistance in *M. tuberculosis*[7].

Cytochrome P-450 is known to exist as multiple isozymes which differ functionally. Sequencing of the *Aspergillus fumigatus CYP51* gene encoding cytochrome P-450 sterol 14α-demethylase in azole-susceptible and resistant forms showed point mutations in the latter[8], thereby demonstrating that different forms of cytchrome P-450 might exist in drug-susceptible and resistant bacteria. Since drug-resistant *M. tuberculosis* had increased cytochrome P-450 activity, it is likely that different isoforms of this protein might exist in sensitive and resistant *M. tuberculosis*.

In an attempt to investigate this aspect, we purified to homogeneity cytochrome P-450 in the standard strain of *M. tuberculosis* $H_{37}R_V$ that is sensitive to all anti-TB drugs and compared its protein profile with that obtained from isoniazid-resistant and isoniazid and rifampicin-resistant *M. tuberculosis*. In addition, cytochrome P-450 was purified in *M. smegmatis, M. chelonae* and *M. fortuitum.*

Cytochrome P-450 levels in hepatic microsomes are known to increase markedly in the presence of substances such as phenobarbital, 3-methyl cholanthrene, β-naphthoflavone, dexamethasone, ethanol, etc.[9]. We also conducted induction studies with phenobarbital on cytochrome P-450 in *M. smegmatis*, *M. tuberculosis* $H_{37}R_V$, *M. chelonae* and *M. fortuitum*, and purified the induced protein in *M. tuberculosis* $H_{37}R_V$.

The mycobacterial strains used in this study were *M. smegmatis* (ATCC 607), *M. tuberculosis* $H_{37}R_V$ (standard strain), *M. fortuitum* (TMC 1529), *M. chelonae* (clinical isolate) and clinical isolates of *M. tuberculosis* resistant to isoniazid alone and to isoniazid and rifampicin.

The organisms were maintained on Lowenstein–Jensen slopes by regular sub-culturing. For experimental purposes, they were grown in Sauton's liquid medium at 37°C. *M. smegmatis*, *M. fortuitum* and *M. chelonae* were harvested at the end of 3–4 days and *M. tuberculosis* at the end of 6–8 weeks.

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