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Five-membered [C,N] and [N,O] metallocyclic complexes of palladium(II) with monoalkyl [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonates: synthesis, characterization and antitumour activity

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Abstract

The synthesis, spectroscopic and biological properties of the novel palladium(II) complexes with monoethyl (HL1) and monobutyl (HL2) esters of [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid, have been prepared and studied. These potential polydentate ligands form two types of metallocyclic compounds, those with [C,N] and [N,O] five-membered chelate rings. The former are cyclopalladated chloro-bridged binuclear complexes, $[PdL(\mu\text{-}Cl)]_2$, in which the deprotonated ligand undergoes palladation at the azo nitrogen and the *ortho*-carbon, while the latter mononuclear complexes, PdL_2, contain the organophosphorus ligand bonded through the aniline nitrogen and the deprotonated phosphonic acid oxygen. The complexes were identified and characterized by elemental analysis, magnetic and conductance measurements as well as by IR, ^1H, ^{13}C and ^{31}P nuclear magnetic resonance and ESI-mass spectroscopic studies. The in vitro antitumour activity of all the complexes was evaluated against the human KB cell line as a preliminary screening for their biological activity. The coordination behaviour of monoalkyl benzeneazophosphonates as well as the spectral and biological properties of their complexes were compared with the results reported for the corresponding dialkyl phosphonates and their palladium(II) complexes.

Keywords: Palladium(II) complexes; Metallocycles; Aminophosphate complexes; Spectroscopy; Antitumour activity

1. Introduction

Organopalladium chemistry is one of the most extensive and varied areas of transition metal chemistry. Special attention has been paid to metallocyclic complexes with nitrogen donor ligands, such as various alkyl and aryl substituted amines and imines, azo, hydrazo and heterocyclic compounds. The chelate ring generally possesses three to seven members, with the five-membered ring being most favoured. These compounds are used successfully in organic synthesis [1–3], homogeneous catalysis [4], asymmetric synthesis [5], photochemistry [6] and optical resolution [7,8], and are rather promising as liquid crystals [9,10] and potential biologically active materials [11–13]. Complexes with O-bonding ligands are less abundant because palladium as a soft metal has a strong preference for N-donor ligands. Monodentate ligands of this type readily undergo substitution reactions [14–16], however, the chelate effect is important in favouring the final stability of a complex and there is a well developed chemistry for chelating ligands that combine a soft and a hard donor centre. There are known cyclometallated complexes in which the ligand is bonded through the carbon, nitrogen and oxygen atoms [17,18]. Derivatives of aminocarboxylic and aminophosphonic acids as [N,O] chelate ligands are of particular interest owing to their relevance to the natural systems and promising biological activity [19–22].

Our systematic investigation has been directed to the synthesis and characterization of new biological active palladium(II) complexes with dialkyl and monoalkyl esters of phosphonic acids derived from quinoline [14–16,21–23] and aniline [13,24,25]. It was found that most of these complexes

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displayed a certain in vitro antitumour activity against human and animal tumour cell lines. The greatest activity was found for complexes of dialkyl esters of [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid [13]. These ligands with several donor atoms serving as potential sites for metal coordination, i.e. the anilino and azo nitrogen as well as the phosphoryl oxygen, form two types of palladium(II) halide complexes: adducts and cyclometallated derivatives. The former are square planar with *trans*-bonded ligands through the azo nitrogen, while in the latter the deprotonated ligand undergoes palladation at the azo nitrogen and the *ortho*-carbon forming the binuclear organopalladium complexes. The anilino and phosphoryl groups are not involved in metal coordination and are free to be involved in hydrogen bonding, which is known to have some importance in metal–DNA approaching and binding interactions [26]. In the present paper we describe the synthesis as well as spectroscopic analyses and biological properties of new palladium(II) complexes of monoethyl (HL1) and monobutyl (HL2) esters of [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid. In the course of these investigations, the results obtained are compared with those obtained for palladium complexes of the corresponding dialkyl benzeneazophosphonates [13], and are discussed with respect to their structure–stability and structure–activity relationships. Differences in their complex-forming behaviour mainly arise from the presence of the phosphonic acid group in monoester derivatives, which enables these ligands to coordinate to the palladium ion in either their molecular or anionic form.

2. Results and discussion

2.1. Synthesis and properties

Investigations of the chelating behaviour of monoalkyl [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonates (HL1 and HL2) towards the palladium(II) ion have shown that these ligands act in a bidentate manner forming two types of metallocyclic complexes, those with [C,N] and [N,O] five-membered chelate rings (Fig. 1). The reaction of HL1 and HL2 with $PdCl_4^{2-}$ (molar ratio M:L = 1:1) in methanol affords cyclopalladated binuclear complexes with metal–metal chloro bridge, $[PdL(\mu\text{-}Cl)]_2$ (**1** and **3**), in which the deprotonated ligand undergoes palladation at the azo nitrogen and the *ortho*-carbon. In basic media the phosphonate acid group is deprotonated and, thus, the reaction of sodium salts of monoalkyl phosphonates (NaL1 and NaL2) with $PdCl_4^{2-}$ in water (M:L = 1:2) gives [N,O] chelate complexes, PdL_2 (**2** and **4**), with coordinated ligand through the aniline nitrogen and the phosphonic acid oxygen. It should be noted that in an unsymmetrically substituted azobenzene derivative, orthopalladation can occur in either benzene ring with the possibility of obtaining an isomeric mixture, but in general it has been noticed that the palladium–carbon σ-bond is formed preferentially with the benzene ring having an electron-donating group [27,28]. Our results presented here, as well as those obtained for the cyclopalladated complexes of the corresponding dialkyl phosphonates [13], are in accordance with this assumption. In both cases the palladation occurred in the anilinobenzyl-substituted aromatic ring. It may be presumed that the simplified mechanism of cyclopalladation includes the initial coordination of azo nitrogen to metal ion followed by electrophilic substitution at the *ortho*-carbon atom. It is worth noting that in the case of dialkyl phosphonates, the complexes with monodentate azo N-bonded ligands could easily be isolated, while all efforts to prepare dihalide adducts of the monoester ligands were unsuccessful.

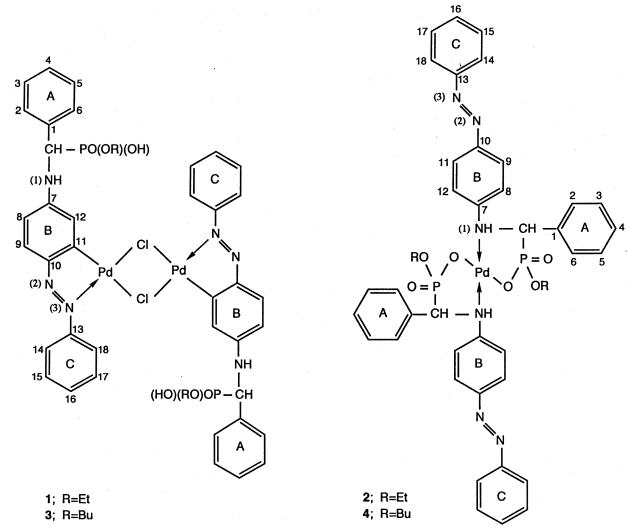


Fig. 1. Proposed chemical structures and numbering scheme of palladium complexes with monoalkyl [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonates (HL1 and HL2).

The prepared complexes are red powder like or microcrystalline compounds, stable in the solid state under normal laboratory conditions. Their diamagnetic behaviour suggests square-planar stereochemistry around the palladium(II) ion. The molar conductance values (below 15 S cm^2 mol^{-1} in DMF) correspond to those of non-electrolytes. The complexes are insoluble in water, slightly soluble or soluble in common organic solvents, and very soluble in DMF and DMSO. In general, the binuclear complexes are more soluble than the mononuclear complexes, as are complexes of butyl ester with respect to complexes of its ethyl analogue. Because of the insolubility of the prepared complexes in suitable solvents, or because of their instability, we could not grow crystals for X-ray analysis. The structure of the complexes, as well as their stability and mode of decomposition, were deduced mainly from their IR, multinuclear magnetic resonance and mass spectroscopic studies. A detailed spectroscopic analysis of the free organophosphorus ligands has also been described.

2.2. Infrared spectra

There are great differences in the IR spectra of the two types of complexes, supporting different metal–ligand inter-

actions in these compounds. Significant differences may be noticed between 1600 and 1530 cm^{-1} where the NH deformation modes along with the benzene ring stretching vibrations are found. A very strong band at 1576 cm^{-1} with a shoulder at 1555 cm^{-1} is characteristic for the cyclopalladated complexes **1** and **3** with an aryl carbon–metal σ bond, while the chelate complexes **2** and **4** in this frequency region are characterized by two very intense bands near 1600 and 1585 cm^{-1}. There are marked differences also between 1250 and 950 cm^{-1} where absorption bands appear associated with the acidic monoalkyl phosphonate group PO(OR)(OH) [29,30]. In the cyclopalladated complexes two bands at 1237 and 1198 cm^{-1} could be ascribed to the P=O stretching absorption, while the PO–H stretching vibration is superimposed on the PO–C stretching, giving broad and complex bands between 1030 and 950 cm^{-1}.

In the spectra of the mononuclear complexes **2** and **4** there is no evidence of the bands associated with P–O–H vibrations, since in these complexes the phosphonate ligands act in the anionic form. This is confirmed by the presence of the $\nu(PO_2^-)$ absorptions as medium or strong bands between 1230 and 1200 cm^{-1} for the antisymmetric and between 1070 and 1025 cm^{-1} for the symmetric mode of this vibration. In the latter frequency range the ν(PO–C) absorption is superimposed upon that of the symmetric PO_2^- vibration [31,32].

The chloro-bridged cyclopalladated complexes **1** and **3** in the far-IR region show two Pd–Cl stretching bands. The higher-frequency band at 307 cm^{-1} is attributed to the vibration of the Pd–Cl bond *trans* to the nitrogen atom, while the lower-frequency band at 255 cm^{-1} is ascribed to the vibration of the Pd–Cl bond *trans* to the σ-bonded carbon. This is a consequence of a greater *trans* influence of a σ-bonded carbon compared to that of a nitrogen atom [33].

2.3. Mass spectra

Electrospray ionization mass spectrometry (ESI-MS) is a soft ionization technique which has recently been widely used in the structural analysis of non-volatile and thermally labile species such as large biomolecules [34,35] and various coordination metal compounds, especially polynuclear complexes [36,37]. This method allows pre-existing ions in solution to be very gently transferred to the gas phase with minimal fragmentation, followed by conventional mass spectrometry. The ESI mass spectrometric investigation of monoalkyl anilinobenzylphosphonates and their palladium(II) complexes has shown that their mass fragmentation pattern is much less extensive than that obtained for the corresponding dialkyl phosphonates and their palladium complexes studied under fast atom bombardment (FAB) conditions [24,25]. In the ESI negative-ion mass spectra of the free non-coordinated organophosphorus ligands, the deprotonated molecular ion was observed as the base and the only intense peak, since the phosphonic acid group could easily be deprotonated to give an anion. In the corresponding spectra obtained in the positive-ion mode, the ligand fragmentation is more pronounced and the most abundant fragment ion is formed by cleavage of the C–P bond and complete loss of the phosphonate ester group. The abundance of the protonated molecular ion is only about 5% as is shown in Fig. 2 for HL2, as an example of the common decomposition pattern for both alkyl monoesters in positive/negative ESI mass spectra. All palladium complexes have been investigated by spectrometric measurements carried out under positive/negative-ion ESI conditions with different cone voltages, using methanol as the solvent and the mobile phase. The negative-ion mass spectra give more relevant structural information than the spectra obtained in the positive-ion mode. The molecular ion as well as the fragment ions containing palladium are identified by the presence of the characteristic clusters of isotopic peaks covering about 10 *m/z* units, due to the presence of the numerous palladium isotopes. The fragmentation pathway depends mainly on the type of complex and the major structurally informative fragments in negative-ion mass spectra are summarized in Table 1. The first point to be noted is that all complexes exhibit the deprotonated molecular ion and its relative abundance is about 3% for the cyclopalladated binuclear complexes **1** and **3** and about 1% for the mononuclear palladium complexes **2** and **4**. There are great differences in mass spectroscopic behaviour between these two types of complexes.

The fragmentation pathways of the cyclopalladated complexes, including possible structures of Pd-containing ions, proposed on the basis of the accurate mass measurements and isotopic cluster analysis are reported in Scheme 1 and Fig. 3(a). The loss of $Cl^{\bullet}$ produces the fragment ion which corresponds to binuclear monobridged species. In this respect it

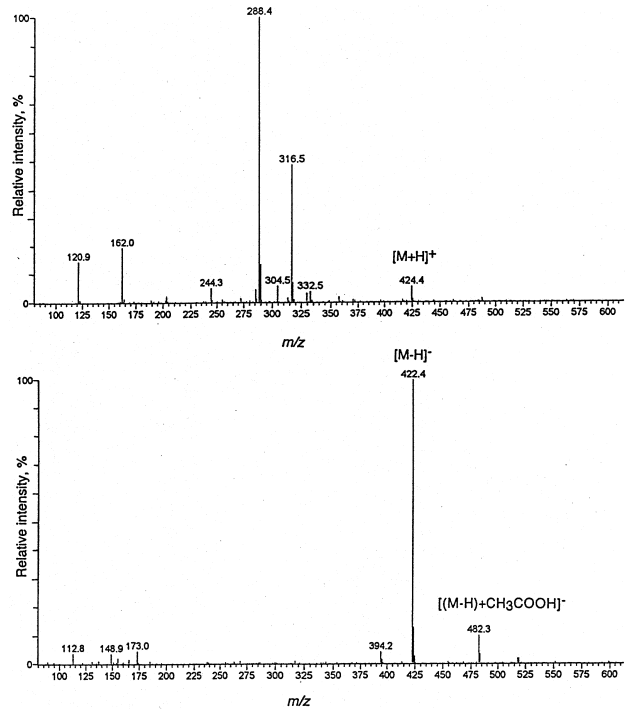


Fig. 2. Positive-ion and negative-ion ESI mass spectra of HL2 in a mixture of acetonitrile and water (50:50) with 0.1% acetic acid at a cone voltage of 50 V.

Table 1
Major ion fragments in negative-ion mass spectra of Pd(II) complexes [a]

Ionic species [b]	*m/z* (relative intensity %) [c]			
	1	**2**	**3**	**4**
$[(M–2H)+Na]^-$	1091 (7)		1147 (4)	
$[M–H]^-$	1069 (3)	893 (1)	1125 (3)	949 (0.9)
$[(M–2H)–Cl+Na]^{-\bullet}$	1056 (3.5)		1112 (3.5)	
$[(M–H)–Cl]^{-\bullet}$	1034 (9)		1090 (11)	
$[(M–H)–Cl–HCl]^{-\bullet}$	998 (11)		1054 (5)	
$[(HL)_2–2H+Na]^-$		811 (1)		867 (0.9)
$[(HL)_2–H]^-$		789 (0.4)		845 (0.5)
$[PdLCl_2+Me]^-$	585 (5)		613 (5)	
$[PdLCl_2]^-$	570 (12)		598 (17)	
$[(PdLCl)–2H+Na]^-$	556 (15)		584 (15)	
$[(PdLCl)–H+Me]^-$	549 (8)		577 (10)	
$[(PdLCl)–H]^-$	534 (100)		562 (100)	
$[(PdL)–H+MeOH]^{-\bullet}$	531 [d]	531 (0.8)	559 [d]	559 (1)
$[(PdL)–H]^{-\bullet}$	499 (14)	499 (0.7)	527 (10)	527 (0.3)
$[L+MeOH]^-$	426 (6)	426 (0.6)	454 (3)	454 (0.6)
$[L+Me]^-$	409 (29)	409 (0.7)	437 (11)	
$[L]^-$	394 (63)	394 (100)	422 (79)	422 (100)

[a] In methanol, cone voltage 50 V.
[b] L = L1, L2.
[c] Ion masses referenced to isotopes ^{106}Pd and ^{35}Cl.
[d] Partly overlapped with $[(PdLCl)–H]^-$ ion.

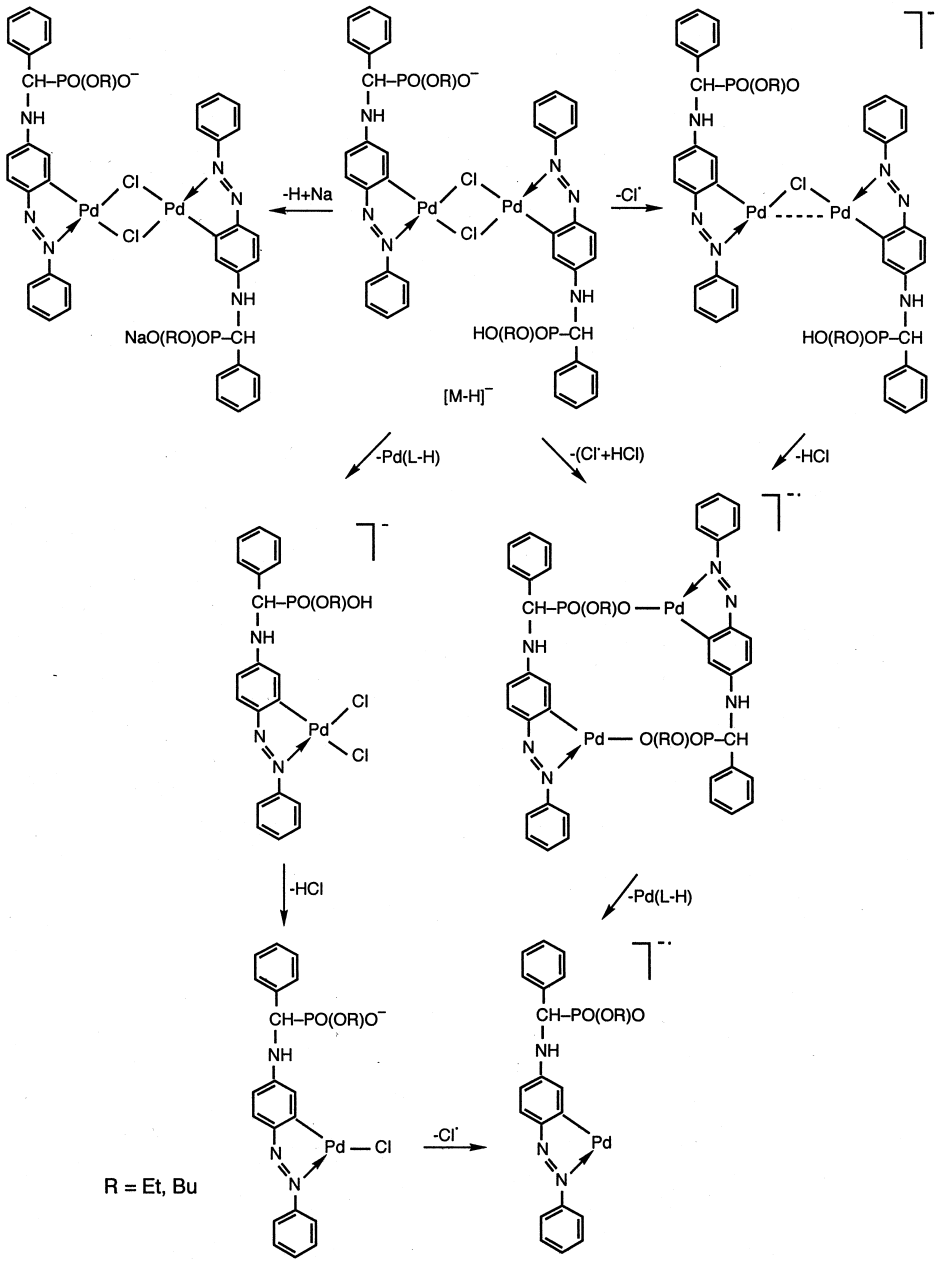
could be pointed out that some monohalide-bridged binuclear palladium complexes have been isolated and characterized [38,39]. The further loss of hydrogen chloride involves structural rearrangement of the species giving a dinuclear complex in which are two cyclopalladated moieties bonded through the deprotonated phosphonic acid groups. The loss of one palladium and one ligand molecule from the parent complex ion leads to a deprotonated dichloro monopalladated complex $[PdLCl_2]^-$, which by the sequential losses of HCl and $Cl^\bullet$ leads finally to the orthometallated species $[(PdLCl)–H]^-$ and $[(PdL)–H]^{-\bullet}$, respectively. The former mono-chloropalladium fragment ion is the base peak in the spectra of these complexes. The high stability of such metallated systems is in agreement with the reported high stability of palladium(II) five-membered cyclometallated systems having nitrogen donor ligands [24,40,41]. Of the organic fragments, the most intense peak (60–80%) corresponds to the deprotonated ligand molecule.

Investigations of decomposition properties of the mononuclear complexes **2** and **4** indicate much less stability of these compounds with respect to the binuclear cyclopalladated complexes. The predominant pathway for their fragmentation is complete dissociation of the phosphonate ligand molecules (Fig. 3(b)). The deprotonated ligand ion is the major and the only intense fragment ion in the spectra of these complexes. The relative abundance of all other fragment ions is very small amounting up to 1%. The most abundant palladium containing fragment ions are the parent molecular ion $[(PdL_2)–H]^-$, and the ion containing only one [N,O] bonded organophosphorus ligand. Dimerization of the free organophosphorus ligand gives rise to $[(HL)_2–H]^-$ ions at *m/z* 798 and 845, respectively. The sodium adducts are also present.

It is worth noting that the spectra of complexes show some ions based on addition of methanol as solvent molecules as well as addition of the sodium ion either to the parent complex or to the ligand molecules or their fragments; this is in agreement with the ESI mass results obtained for various complex compounds [37,42]. Some of the most abundant adduct ions are given in Table 1.

2.4. NMR spectra

^{1}H, ^{13}C and ^{31}P NMR data of free ligands HL1 and HL2, sodium salt NaL2 and both types of palladium complexes derived from the butyl ester (complexes **3** and **4**), obtained in $CDCl_3$ solution, are summarized in Tables 2 and 3, and presented in Figs. 4–7. The numbering scheme is shown in Fig. 1. Complexes of ethyl ester are not sufficiently soluble for spectral analysis. The results obtained are compared and discussed with those reported for dialkyl [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonates and their palladium complexes [13,43]. The broadening of H/C aromatic absorptions in the free monoesters with respect to those observed in the corresponding diesters could be connected with their dimeric structure in chloroform solution [30]. The most pronounced broadening could be observed for protons H-9,11 and H-15,17, as shown in Figs. 4 and 5(a). The slight upfield shift (up to 0.25 ppm) of most of the aromatic protons with respect to those in diesters could be attributed to mutual interligand shielding between two hydrogen bonded monoester molecules lying parallel to one another. The ligand



Scheme 1. ESI negative-ion fragmentation pathway for complexes **1** and **3**.

association is ascribed to the strong intermolecular hydrogen bonding between the phosphonic acid groups, which is confirmed by the presence of a very broad absorption of the acidic OH resonance around 10 ppm. In the case of sodium salt (Fig. 5(c)) the signal broadening of the aromatic protons is not visible, as well as in the spectra of monoesters in DMSO solution (Fig. 5(b)), in which there is no ligand association. The assignment of all aromatic H/C resonances was confirmed unambiguously by two-dimensional homonuclear and heteronuclear NMR experiments. As examples, Fig. 4 shows a ^{1}H–^{1}H COSY spectrum of HL1 and Fig. 6 shows a part of the long-range ^{1}H–^{13}C HMBC spectrum of sodium salt NaL2. In addition, the signal assignment of the carbons C(1)–C(6) was supported with the long-range phosphorus–carbon couplings.

The spectra of both types of complexes are rather complex, caused either by the removal of the degeneracy of resonances due to metal coordination (complex **3**) or by the presence of more isomeric forms (complex **4**). The most pronounced chemical shift changes with respect to the uncomplexed ligand could be noticed for the atoms involved in metal binding and those in the vicinity of the ligation site. In the binuclear complex **3**, with palladation at the azo nitrogen and the *ortho*-carbon, there are significant differences in the B-phenyl and C-phenyl rings of the ligand molecule. The proton NMR spectrum shows a great broadening of all signals and an extensive overlapping of the aromatic resonances (Fig. 5(c)) due to the dimeric nature of this complex. The non-equivalence of the aromatic protons H(8) and H(12) and their upfield shifts of 0.41 and 0.19 ppm, respectively, supports

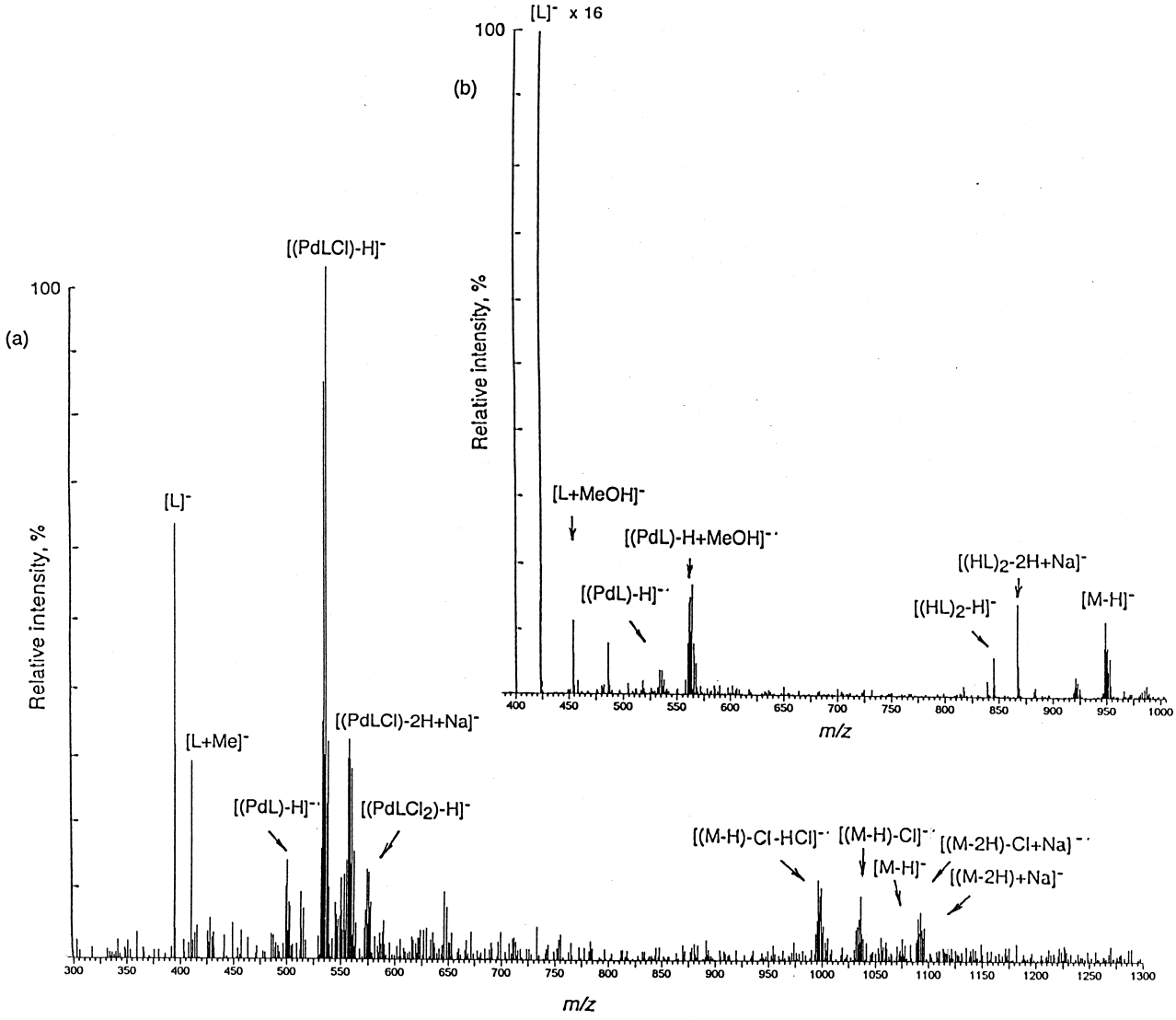


Fig. 3. Negative-ion ESI mass spectra of complexes **1** (a) and **4** (b) in methanol at a cone voltage of 50 V.

the metallation at C-11 of the B-phenyl ring. The shielding of these protons is caused by the flow of electron density from the d orbital of the palladium atom into this aromatic ring. Resonances of the other aromatic protons could not be clearly resolved owing to complex overlap of signals between 7.20 and 7.80 ppm. The ^{31}P NMR spectrum of this complex shows a broad signal at 21.88 ppm (Fig. 7(a)). The ^{13}C NMR spectrum of this complex gives much more spectral information. Comparing with the spectrum of the free ligand, the most important changes appear in resonances related to carbons C-7-C-12, as an identification of changes in the electron density of this aromatic ring due to *ortho*-metallation. For this reason none of these carbons are still equivalent. The palladium-bound C(11) is greatly shifted downfield by 31.66 ppm, C(9) and C(12) are shifted to a lesser extent by 5.87 and 2.93 ppm, respectively, while an upfield shift of 7.07 ppm for the C(8) atom, *para* to the Pd atom, clearly indicates the existence of some metal-to-ligand π-back-bonding. As expected, the overall spectral pattern of complex **3** is very similar to that of the cyclopalladated complex of dibutyl benzeneazophosphonate owing to the same palladium–ligand bonding [43].

The spectra of mononuclear complex **4** show a great complexity arising from the existence of more isomeric species in dynamic equilibrium with different magnetic environment. This can be interpreted by the presence of a new chiral centre on the nitrogen atom due to its coordination to palladium, in addition to that at the adjacent PCH asymmetric carbon and phosphorus atoms, as well as by the restricted rotation around the C–N linkage. As a consequence, the proton NMR spectrum of this complex shows a great broadening of all proton resonances (Fig. 5(d)), while its ^{13}C NMR spectrum is characterized by extensive signal splitting and multiple overlap of signals, which prevent signal assignments for almost all aromatic proton and carbon atoms. The existence of more isomeric species is visible in the ^{31}P NMR spectrum of this complex. There are few signals in the range 5–25 ppm as was shown in Fig. 7(b). The two most intense signals are at 14.46

Table 2
^{1}H NMR data [a]

	HL1	HL2 [b]	NaL2	**3**	**4**
H-2,6	7.54 d J(HH) = 7.5	7.54 d (7.56) J(HH) = 7.6 (7.0)	7.37 d J(HH) = 7.0	[c]	[c]
H-3,5	7.35 t J(HH) = 7.3	7.35 t (7.36) J(HH) = 7.3 (7.2)	7.21 t J(HH) = 7.0	[c]	[c]
H-4	7.29 t J(HH) = 7.4	7.29 t [d] (7.32) J(HH) = 7.4 (7.1)	7.17 t J(HH) = 7.6	[c]	[c]
H-8,12	6.75 d J(HH) = 8.8	6.72 d (6.76) J(HH) = 8.2 (8.8)	6.66 d J(HH) = 8.4	6.53, 6.31 br s [e]	6.62 br s
H-9,11	7.73 br s	7.72 br s (7.81 d) J(HH) = (8.7)	7.69 d J(HH) = 8.6	[c]	[c]
H-14,18	7.61 d J(HH) = 7.1	7.63 d (7.85) J(HH) = 6.5 (7.6)	7.78 d J(HH) = 7.5	[c]	[c]
H-15,17	7.21 br m [f]	7.22 br m [f] (7.47 d) J(HH) = (7.3)	7.43 t J(HH) = 7.5	[c]	[c]
H-16	7.31 t [g] J(HH) = 7.3	7.31 t [g] (7.39) J(HH) = 7.4	7.36 t [h] J(HH) = 7.2	[c]	[c]
PCH	4.97 d ^2J(PC) = 22.8	4.95 d (4.92) ^2J(PC) = 22.9 (23.9)	4.46 dd ^2J(PC) = 23.2 J(NH) = 6.4	4.91 br s	4.80 br d ^2J(PC) = 23.8
NH	7.18 [i]	7.20 [i] (5.83)	6.22 br s	6.09 br s	[j]
OH	9.80 [k]	10.40 [k]	3.28 br s [l]	[j]	
OCH_2	3.99, 3.82 m [m]	3.94, 3.76 m [m] (3.64; 3.91; 4.12) [o]	3.58, 3.53 m [n]	3.90, 3.65 br s [n]	3.70 br m [n]
$OCH_2CH_2CH_2CH_3$		1.52 m [p] (1.42; 1.63) [o]	1.36 m [p]	1.46 br s	1.40 br s
$OCH_2CH_2CH_2CH_3$		1.31 m [r] (1.23; 1.38) [o]	1.12 m [r]	1.25 br s	1.15 br s
CH_3	1.21 t J(HH) = 7.1	0.85 t (0.84; 0.90) [o] J(HH) = 7.3 (7.3)	0.72 t J(HH) = 7.2	0.83 br s	0.75 br s

[a] Spectra recorded in $CDCl_3$ (δ in ppm, coupling constants in Hz). Multiplicities: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; br, broad signal.
[b] Data for dibutyl [α-(4-benzeneazoanilino]-*N*-benzyl]phosphonate from Ref. [43] are given in parentheses for comparison.
[c] Resonance not resolved owing to extensive broadening and overlapping of signals between 6.8–8.0 ppm.
[d] Partly overlapped with H16.
[e] No equivalent protons. The first value is related to H-8 and the second to H-12.
[f] Overlapped with NH. Value obtained from a ^{1}H–^{1}H COSY experiment.
[g] Partly overlapped with H-4. Value obtained from a ^{1}H–^{1}H COSY experiment.
[h] Value obtained from a ^{1}H–^{13}C HMBC experiment. Signal partly overlapped with H-2,6.
[i] Overlapped with H-15,17. Tentative value obtained from a ^{1}H–^{13}C HMBC experiment.
[j] Resonance overlapped with aromatic protons.
[k] Very broad signal due to strong hydrogen bonding.
[l] OH resonance from H_2O.
[m] Centres of two multiplets. Separate resonances for two geminal protons.
[n] Centres of two multiplets. Resonances for two geminal protons are partly overlapped.
[o] Values in parentheses related to protons of two diastereotopic butyl groups of dibutyl ester.
[p] Appears as quintet with J(HH) ~ 7.2 Hz.
[r] Appears as sextet with J(HH) ~ 7.4 Hz.

and 18.90 ppm. The fact that the ^{31}P NMR chemical shifts of the complex do not differ much from that observed for the free ligand and its sodium salt at 18.59 and 19.19 ppm, respectively, supports the conclusion that coordination of phosphonate to metal ion is predominantly ionic and scarcely affects the electron density of the phosphonate phosphorus [44].

The NMR spectral studies have shown that complexes retain their integrity in chloroform solution, but decompose in DMSO. This solvent with strong complexing ability initiates dissociation of the complex moiety by displacing the organophosphorus ligand. As could be expected, the decomposition process is much more pronounced in the [N,O] chelate complexes.

In conclusion, the results obtained by NMR studies are in agreement with those of mass spectrometry, indicating that the five-membered [C,N] chelate ring in the binuclear chloro-bridged complexes is much more stable than the five-membered [N,O] ring in the mononuclear chelate complexes. This behaviour is in accordance with the assumption that the chelate ring where the cyclopalladation is assisted by the coordination of a nitrogen atom has a remarkable stability.

Table 3
^{13}C NMR data [a]

	HL1	HL2 [b]	NaL2	**3**
C-1	136.30	136.42 (135.50)	138.52	135.15
			^{2}J(PC) = 3.5	
C-2,6	127.89	128.00 (127.79)	127.47	127.82
	^{3}J(PC) = 4.9	^{3}J(PC) = 5.0 (5.5)	^{3}J(PC) = 4.1	
C-3,5	128.49	128.57 (128.54)	128.44	128.69
	^{4}J(PC) = 2.3	^{4}J(PC) = 2.3 (2.4)	^{4}J(PC) = 2.3	
C-4	127.69	127.76 (127.95)	127.27	128.15
	^{5}J(PC) = 2.9	^{5}J(PC) = 2.7 (3.1)	^{5}J(PC) = 3.4	
C-7	147.60	148.43 (149.08)	150.12	147.90
			^{3}J(PC) = 14.0	
C-8	114.55	114.57 (113.31)	113.86	107.50
C-12	114.55	114.57 (113.31)	113.86	117.50
C-9	124.20	124.04 (124.79)	124.90	129.91
C-11	124.20	124.04 (124.79)	124.90	155.70
C-10	141.42	141.59 (145.19)	145.21	144.50
C-13	154.28	153.74 (152.88)	152.97	150.28
C-14,18	120.48	120.68 (122.16)	122.30	123.62
C-15,17	128.86	128.96 (128.77)	128.90	128.05
C-16	129.45	129.55 (129.52)	129.66	129.01
PCH	56.86	56.92 (55.99)	56.82	56.10
	^{1}J(PC) = 146.2	^{1}J(PC) = 146.2	^{1}J(PC) = 139.3	^{1}J(PC) = 147.1
OCH_2	62.87	66.64 (66.99; 67.10) [c]	66.47	67.46
	^{2}J(POC) = 7.1	^{2}J(POC) = 7.3 (7.1)	^{2}J(POC) = 6.0	
CH_3	16.42	13.60 (13.39; 13.43) [c]	13.77	13.56
	^{3}J(POCC) = 6.2			
$OCH_2CH_2CH_2CH_3$		32.61 (32.19; 32.40) [c]	32.73	32.31
		^{3}J(POCC) = 6.2 (5.5)	^{3}J(POCC) = 6.9	
$OCH_2CH_2CH_2CH_3$		18.69 (18.37; 18.52) [c]	18.74	18.50

[a] Spectra recorded in $CDCl_3$ (δ in ppm, coupling constants in Hz).
[b] Data for dibutyl [α-(4-benzeneazoanilino]-*N*-benzyl]phosphonate from Ref. [43] are given in parentheses for comparison.
[c] Values of dibutyl ester are related to two diastereotopic butyl groups.

Furthermore, it is known that palladium as a soft metal prefers nitrogen bonding over that of oxygen and therefore the coordination of oxygen is more easily displaced. On the other hand, it is worth noting that our recently reported investigations of the coordination of monoethyl 2-quinolylmethylphosphonate to the palladium(II) ion have shown that this monophosphonate ligand forms a very stable PdL_2 complex with a six-membered [N,O] chelate ring via the quinoline nitrogen and the phosphonic acid oxygen [14], in spite of the general belief that the six-membered ring is less favoured. The differences observed in stability between the six-membered [N,O] ring in the quinoline complex and a five-membered ring in the benzeneazoanilino phosphonate complex could be ascribed on one side to the relatively low basicity of the benzalaniline nitrogen compared with that of the quinoline nitrogen, caused by participation of its electron pair in resonance with the adjacent azobenzene π-system, and, on the other side, to the influence of steric factors. The benzeneazoaniline ligands are more bulky than the quinoline derivative. The geometric and steric requirements of the ligand containing two or more donor atoms are very important in determining the number of donor atoms that will coordinate, the final geometry, and the stability of the metallic species.

2.5. In vitro antitumour activity

The antitumour activity of the complexes was determined in vitro on the KB cell line derived from a human oral epidermoid carcinoma as a preliminary screening for their biological activity. The results obtained have shown that the concentration of the complex required to inhibit tumour cell growth by 50% was higher than 10^{-5} mol l^{-1}. There are no significant differences in response between complexes of ethyl and butyl esters, nor between the two types of complexes in spite of their differential structural properties and most probably different DNA binding and nicking ability. When comparing results of the in vitro assays related to the cyclopalladated complexes **1** and **3** with those obtained for the corresponding palladated azobenzene complexes of dialkyl phosphonates [13], it could be seen that diester complexes show about ten times higher activity ($< 5 \times 10^{-6}$ mol l^{-1}) than the monoester complexes which may be ascribed to their greater lipophilicity and solubility. In this regard it is worth noting that similar effects were observed for the antitumour activity of palladium(II) complexes with diethyl and monoethyl esters of quinolylmethylphosphonic acids [15,16,21]. The lipophilicity of the complexes increases with increasing bulkiness from monoester to diester derivatives

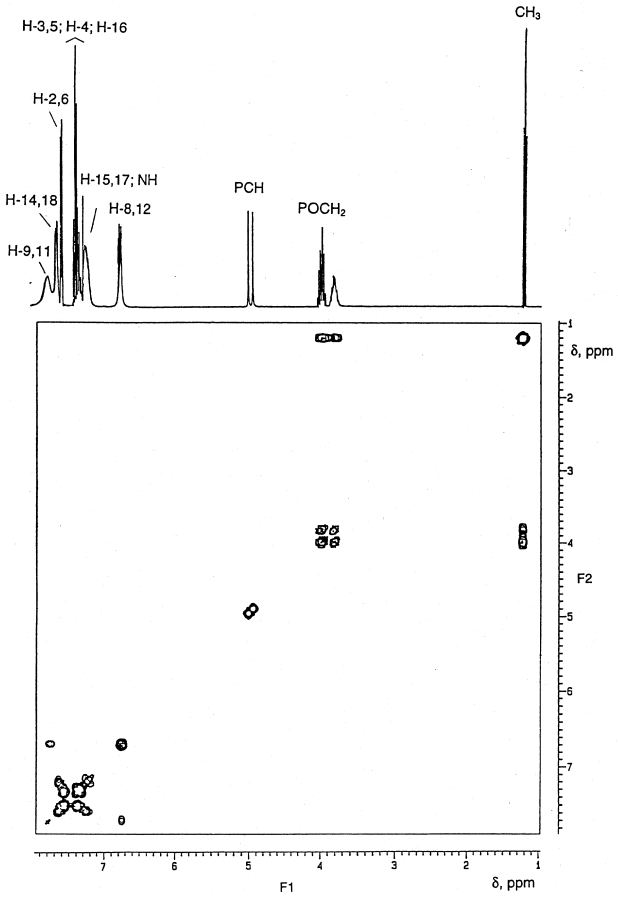


Fig. 4. ^1H–^1H COSY spectrum of HL1 in $CDCl_3$.

and may facilitate transport across the cellular membrane [45,46].

3. Experimental

3.1. Materials

Monoethyl and dibutyl [α-(4-benzeneazoanilino)-*N*-benzyl]phosphonates (HL1 and HL2) and their sodium salts (NaL1 and NaL2), prepared as previously described [47], were purified by recrystallization from absolute ethanol prior to use. All other reagents and solvents were analytical grade products and were used without purification.

3.2. Preparation of complexes

3.2.1. $[Pd(L1)Cl]_2$ (1)

The reaction of HL1 (0.205 g, 0.52 mmol) and $Na_2[PdCl_4]$ (0.160 g, 0.54 mmol) in methanol (10 ml) at room temperature gave after stirring for 5 days a precipitate, which was separated, washed with cold methanol, and dried under vacuum. The dried complex was dissolved in hot chloroform (5 ml), and after filtering to remove some black decomposition products, the solution was evaporated to dryness yielding a dark red glassy solid which was stored overnight under vacuum. Yield 0.156 g (56%). *Anal.* Found: C, 47.13; H, 4.17; N, 8.09; Pd, 19.11. Calc. for $C_{42}H_{42}N_6O_6Pd_2P_2Cl_2$: C, 47.03; H, 3.95; N, 7.84; Pd, 19.84%. IR: ν(C=C), δ(N–H), 1576vs, 1554sh; ν(P=O), 1237w, 1198w-m; ν(PO–C), ν(P–OH), 1031s, 997m; ν(Pd–Cl), 307w, 255vw.

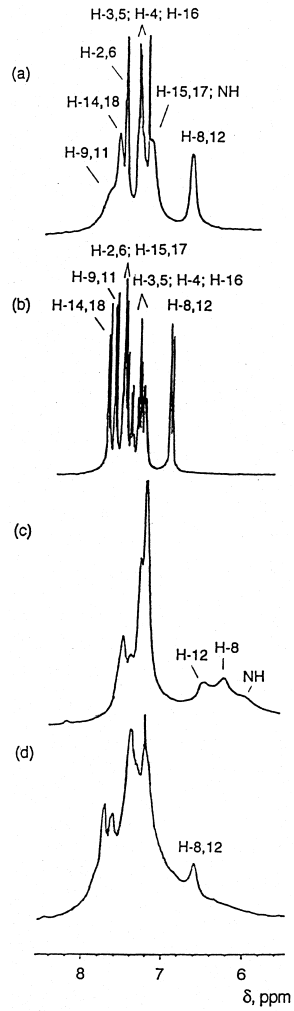


Fig. 5. Aromatic part of ^1H NMR spectra: (a) HL2 in $CDCL_3$, (b) HL2 in DMSO-d_6, (c) **3** in $CDCl_3$ and (d) **4** in $CDCl_3$.

3.2.2. $Pd(L1)_2$ (2)

A concentrated aqueous solution of $K_2[PdCl_4]$ (0.081 g, 0.25 mmol) was added dropwise to a stirred solution of NaL1 (0.203 g, 0.49 mmol) in water (10 ml). The reaction mixture was stirred continuously for 3 h at room temperature. The red precipitate formed was filtered off, washed with cold water and dried under vacuum over P_2O_5 for 24 h. Yield 0.214 g (52%). *Anal.* Found: C, 56.41; H, 4.97; N, 9.37; Pd, 12.41. Calc. for $C_{42}H_{42}N_6O_6PdP_2$: C, 56.35; H, 4.73; N, 9.40; Pd, 11.89%. IR: ν(C=C), δ(N–H), 1601vs, 1583vs; $\nu_{as}(PO_2^-)$, 1224m, 1207m; $\nu_{sym}(PO_2^-)$, ν(PO–C), 1070m, 1046s.

3.2.3. $[Pd(L2)Cl]_2$ (3)

This complex was prepared in a similar manner as complex **1** by stirring the reaction mixture of HL2 (0.204 g, 0.48

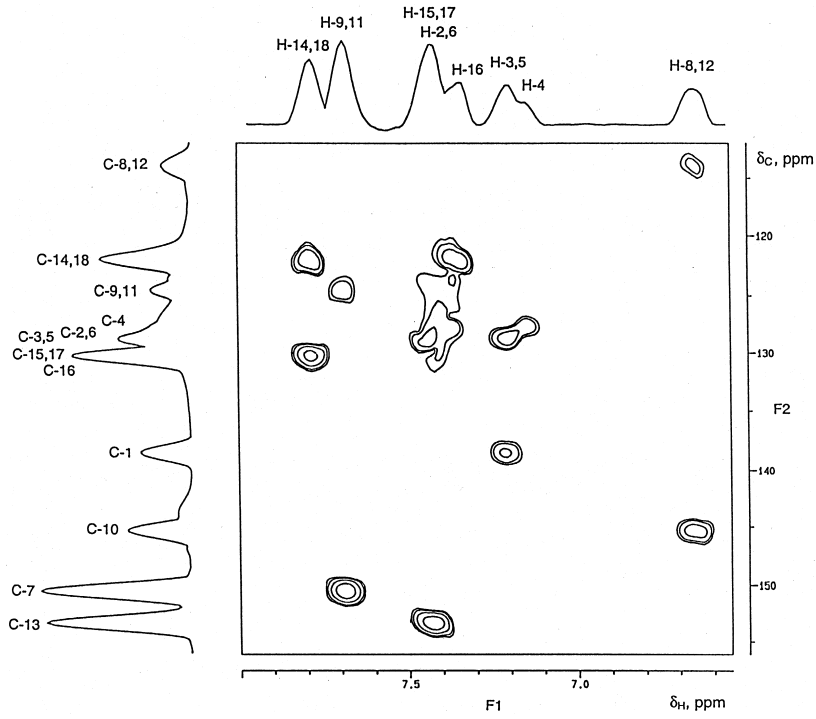


Fig. 6. Aromatic part of the long-range ^{1}H–^{13}C HMBC spectrum of NaL2.

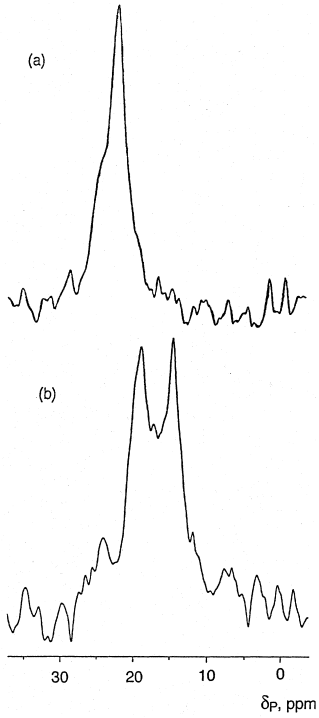


Fig. 7. ^{31}P NMR spectra of complexes **3** (a) and **4** (b).

mmol) and $Na_2[PdCl_4]$ (0.145 g, 0.49 mmol) in methanol (5 ml) for 3 days. Yield 0.114 g (42%). *Anal.* Found: C, 48.65; H, 4.49; N, 7.17; Pd, 18.87. Calc. for $C_{46}H_{50}N_6O_6Pd_2P_2Cl_2$: C, 48.95; H, 4.47; N, 7.45; Pd, 18.86%. IR: ν(C=C), δ(N–H), 1576vs, 1555sh; ν(P=O), 1235w, 1198m; ν(PO–C), ν(P–OH), 1023m-s, 997m-s; ν(Pd–Cl), 306w, 255vw.

*3.2.4. Pd(L2)$_2$ (**4**)*

This complex was prepared by almost the same procedure as complex **2** by reaction of sodium salt of L2 (0.206 g, 0.46 mmol) and $K_2[PdCl_4]$ (0.081 g, 0.25 mmol) in water (5 ml). Yield 0.175 g (80%). *Anal.* Found: C, 57.96; H, 5.39; N, 8.96; Pd, 11.40. Calc. for $C_{46}H_{50}N_6O_6PdP_2$: C, 58.08; H, 5.30; N, 8.84; Pd, 11.19%. IR: ν(C=C), δ(N–H), 1598vs, 1589vs; $\nu_{as}(PO_2^-)$, 1205s br; $\nu_{sym}(PO_2^-)$, ν(PO–C), 1063s, 1025s.

3.3. Physical measurements and analyses

Melting points were determined on a hot stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 580 B spectrophotometer using KBr (4000–250 cm^{-1}) and polyethylene (400–200 cm^{-1}) pellets.

The electrospray mass spectrometric measurements were performed on a Navigator (Finnigan, San Jose, CA) instrument equipped with ESI ion source. The ligands were recorded in positive and negative ion modes using methanol or a mixture of acetonitrile and water (50:50) with 0.1% acetic acid as the solvent and the mobile phase. Complexes were recorded in methanol in both modes. The sample solution (10 μl of 10^{-5} mol l^{-1}) was introduced into the ion source, at a flow rate of 0.3 ml min^{-1}, using a Rheodyne external loop injector coupled to a Thermo Separation (San Jose, CA) spectra series HPLC pump. The measurements were performed using nitrogen as nebulizing gas, and using different cone voltages (30, 50 and 120 V). Expected natural abundance isotope clusters patterns for various ion clusters were calculated with the Isotopic Distribution Program

(IDS) contained in the FTMS 7.01 application program of the NICOS operating system using polynomial expansion based on natural abundances of the Pd and Cl isotopes.

The ^{1}H and ^{13}C one- and two-dimensional NMR spectra were performed with Varian broadband Gemini 300 and Bruker AMX 360 spectrometers, operating at 75.46 and 90.56 MHz for the ^{13}C resonance, respectively. Spectra were recorded in $CDCl_3$ or DMSO-d_6 containing $SiMe_4$ as an internal standard. Digital resolution in ^{1}H NMR spectra was 0.25 Hz, while in ^{13}C NMR it was 0.60 Hz per point. The narrower spectral regions of special interest were measured also with a smaller spectral width and greater digital resolution (down to 0.2 Hz). The following techniques were used: proton-noise decoupling, attached proton test (APT), ^{1}H–^{1}H COSY, NOESY and ^{1}H–^{13}C COSY, long-range ^{1}H–^{1}H COSY, HMQC and HMBC. The ^{1}H–^{1}H COSY spectra were obtained in the magnitude mode, while NOESY spectra were obtained in the phase-sensitive mode. All two-dimensional experiments were performed by standard pulse sequences, using Gemini Data System software Version 6.3 Revision A (for VXR-4000) and Bruker microprograms of UXNMR software Version 940501.3 [48]. For proton decoupling Waltz-16 modulation was used. ^{31}P NMR spectra were measured at 145.79 MHz on a Bruker AMX 360 spectrometer in $CDCl_3$ with external 85% H_3PO_4 in a coaxial capillary.

Conductance measurements were carried out at room temperature using a CD 7A Tacussel conductance bridge for 10^{-3} mol l^{-1} solutions in DMF. Magnetic susceptibilities in the solid state were measured at room temperature by the standard Gouy method using a Cahn-Ventron RM-2 balance and $CuSO_4 \cdot 5H_2O$ as the susceptibility standard. Elemental analyses were performed in the Ruđer Bošković Institute.

3.4. In vitro antitumour assays

Antitumour activity was assayed with the human epidermoid KB cell line [49] according to previously described methods [21,22,50]. The cells were maintained in Eagle's minimum essential medium (MEM) supplemented with glutamine, non-essential amino acids (1%) and newborn calf serum (10%). The cell population doubling time was ca. 24 h. For the cytostatic assay a cell culture in the exponential growth phase was used. The compounds were dissolved immediately before use in sterile DMSO, and these solutions were diluted with the growth medium to the desired concentrations. The final DMSO concentration in the culture medium, 0.5%, showed no cytostatic effect in preliminary tests. At least five concentrations of each compound were used and each agent was assayed on at least three separate occasions. The incubation time was 72 h. The cell growth was determined by the sulforhodamine B (SRB) method [51]. The cytostatic activity was evaluated from the inhibition of cell growth in the treated cultures with respect to the controls. The significance of the results was evaluated by use of classical Student's t test ($p < 0.01$). The activity was expressed as the concentration of the complex at which tumour cell growth showed 50% inhibition (IC_{50}), and was calculated by linear regression analysis [52]. The activity threshold was set at 10^{-5} mol l^{-1}, since this appears to be a fairly realistic cutoff point for most compounds [53].

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