Some New Complexes of Co(III) with Hypoxanthine, Inosine and Purine Nucleotides

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Abstract

Co(III) complexes with 5′-IMP, 5′-GMP, 5′-AMP, inosine and hypoxanthine were prepared by reaction of *trans*-$[Co(en)_2Cl_2]Cl$ with the nucleotide, nucleoside and the base in water medium. The complexes were characterized by their elemental analysis, conductivity, UV, NMR and IR data. In the case of nucleotide complexes, there was total substitution of the coordinated chlorines of the starting complex and entry of two nucleotides, but in the case of the inosine and hypoxanthine derivatives only one Cl was substituted.

In all cases, bonding is inferred through the heterocyclic bases, probably through the N(7), except in the 5′-AMP derivative in which indirect bonding seems to occur.

Introduction

Derivatives of Co(III) with nucleotides have been obtained and used as activators or inhibitors substituting the Mg(II) or Ca(II) nucleotide natural complex of allosteric enzymes [1–3], revealing important facts for understanding these activation mechanisms. $[Co(NH_3)_6]^{3-}$ stabilizes the structure of Z-DNA by interacting via hydrogen bonds with phosphate groups and the N(7) and O(6) of guanine residues [4]. These facts have increased interest in well characterized Co(III)–nucleotide complexes [3, 5–7]. In the present paper, the synthesis and characterization of some new ternary complexes Co(III)–en–puric nucleotides, inosine and hypoxanthine derivatives is described.

Experimental

The analyses of carbon, hydrogen and nitrogen were carried out on a Carlo Erba Model 1106 microanalyser at the Institute of Bioorganic Chemistry in Barcelona and on a Perkin-Elmer 240.B at the Faculty of Chemistry, Tarragona.

The chlorine analysis was determined by the Schoniger method using a 638 Metrohm Titro Processor, by burning the sample in O_2 over sodium disulphite. Dissolution was assessed by the Volhard method.

Cobalt was determined by atomic absorption in a Perkin-Elmer 703 spectrophotometer. The working conditions were: $\lambda = 240.7$ nm and slit = 0.2 nm with an acetylene–air flame oxidant. The sodium content was determined by flame photometry in a PE703 spectrophotometer. The working conditions were: $\lambda = 590$ nm and 0.2 nm slit with an acetylene–air flame oxidant. The phosphoros content was determined by using the colorimetric method of phosphomolybdovanadate. The measurements were carried out on a Perkin-Elmer 552 UV–Vis spectrophotometer at 390 nm and slit 2 nm.

The conductivities were measured with a Crisom 525 conductimeter. The cell constant was determined by using a water solution of KCl 10^{-2} N ($k = 1.04$ cm^{-1}).

The infrared spectra were registered in the solid state (KBr pellets) on a Perkin-Elmer 683 infrared spectrophotometer with an infrared data station PE1600. The reflectance spectra were recorded in the solid state on a PE552 UV–Vis spectrophotometer with an integrating sphere attachment.

^{13}C NMR spectra were obtained on a Varian FT-80A NMR spectrometer operating in a Fourier transform mode with proton noise decoupling at frequency 20 MHz. Chemical shifts were measured relative to internal dioxane and converted to the TMS scale using δ(dioxane) = 67.4 ppm.

cis- and *trans*-$[Co(en)_2Cl_2]Cl$ (0.5 mmol) were dissolved in 5 ml water and 1 mmol disodium salt of the nucleotide in 5 ml water (or 1 mmol of inosine and hypoxanthine in 10 ml of hot water). Both solutions were mixed and the resulting solution was placed in a thermostatted bath at 52 °C with constant stirring for 6–7 h for the nucleotides and 72 h for the

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TABLE I. Analytical Data and some Properties of the Complexes

Compound	Found(calculated) (%)					Melting point (°C)	Λ_M (Ω^{-1} cm^2 mol^{-1}) 10^{-3} M in H_2O (20 °C)	Colour
	C	H	N	Co	P			
$Na[Co(en)_2(5'\text{-IMP})_2]\cdot 7H_2O$	28.04 (28.22)	5.12 (5.09)	17.13 (16.46)	7.04 (5.77)	6.87 (6.06)	214(d)[a]	100	red
$Na[Co(en)_2(5'\text{-GMP})_2]\cdot 8H_2O$	27.30 (26.95)	5.27 (5.24)	18.73 (18.35)	6.19 (5.51)	6.15 (5.80)	229(d)	160	red
$Na[Co(en)_2(5'\text{-AMP})_2]\cdot 8H_2O$	27.91 (27.80)	5.55 (5.44)	17.99 (18.91)	6.46 (5.68)	6.99 (5.97)	223(d)	103	violet
$[Co(en)_2(Ino)Cl]Cl_2\cdot 3H_2O$	27.18 (27.66)	5.46 (5.59)	18.08 (18.43)	9.15 (9.69)		147(d)	262	red
$[Co(en)_2(Hyp)Cl]Cl_2\cdot 7H_2O$	19.51 (19.73)	5.56 (6.20)	20.04 (20.46)	12.02 (10.76)		248(d)	275	red

[a]d, decomposition.

inosine and hypoxanthine derivatives. The final pH was 6–6.5 for the nucleotides and 3.6 (hypoxanthine) and 4.8 (inosine). The solution was concentrated at 50 °C to a 5 ml volume and passed through a Sephadex G-15 column in order to purify the products. The complexes were isolated by addition of ethanol for the nucleotides and evaporation to dryness in the other cases.

No differences in the elemental analysis and spectroscopic properties were observed between the compounds obtained from *cis* and *trans* starting complexes. For this reason, in this paper we only refer to the complexes obtained from the *trans*-$[Co(en)_2Cl_2]Cl$.

The composition of the complexes and the analytical results appear in Table I.

Results and Discussion

The nucleotide complexes obtained were soluble in water an it was possible to measure Λ_M and to obtain ^{13}C NMR spectra. The low solubility of the inosine and hypoxanthine compounds in water and DMSO did not prevent clear ^{13}C NMR spectra being obtained.

The Λ_M values are consistent with electrolytes 1:1 for the nucleotides and 1:2 for the inosine and hypoxanthine derivatives. In the latter cases, a dimeric structure, also consistent with the measurements of conductivity and solubility, cannot be ruled out.

The infrared band that appears near 290 cm^{-1}, tentatively assigned to $\delta(NCoCl) + \delta(NCoN)$ [8], diminishes in intensity in comparison with the starting complexes in the inosine and hypoxanthine derivatives. In the $Co(py)_2Cl_2$ complex [9] a dramatic drop in intensity for the band at 306 cm^{-1} (ν(Co–Cl)) is observed in the polymeric form in this complex in comparison with the monomeric form. In the $[Co(en)_2(Ino)Cl]Cl_2$ and $[Co(en)_2(Hyp)Cl]Cl_2$ derivatives this decrease in intensity may be consistent with a dimeric structure.

Table II records the infrared data for the compounds obtained with inosine and hypoxanthine. The assignment has been carried out according to the literature [10, 11]. In both complexes bands appear at 585, 514 and 471 cm^{-1}, assignable to ν(Co–N), due mainly to the two coordinated ethylenediamines, as well as bands related to $\delta(NH_2)$ (1594 cm^{-1}, 1366 cm^{-1}) and ν(C–C) + ν(C–N) (1054 cm^{-1}) of the 'en' group [12].

For the inosine derivative there are many bands corresponding to vibrations of the sugar that overlap with the 'en' bands making it difficult to study them.

The C=O bands show slight variations in both complexes. In the case of the 'hyp' complex the peak appearing at 1671 cm^{-1} shifts to 1667 and 1663 cm^{-1}. These variations could be due to the participation of the carbonyl group in hydrogen bonds with neighbouring groups (NH_2 of 'en').

For the inosine complex there are significant changes in the bands corresponding to ν(ring). The noticeable shift in the 1546 cm^{-1} band, assignable to ν(C–N) + $\nu(C_6{=}O)(C_4{=}C_5)$ [10, 11] seems to indicate that the heterocyclic ring is involved in coordination with the Co(III). The 1412 cm^{-1} band, assignable to $\nu(N_7{-}C_5)$ [10], is shifted to lower frequencies by about 10 cm^{-1} and the 1257 cm^{-1} band, due to $\nu(C_6{-}N_1) + \nu(N_7{-}C_5)$ [11], increases its frequency to 1289 cm^{-1}, although in the latter case there is some contribution from the starting complex 1274 cm^{-1} band, mainly in its broad form. Other bands corresponding to ν(ring), *i.e.* 1374, 1306 and 1200 cm^{-1}, are shifted to 1366, 1319 and 1215 cm^{-1}, respectively, and this seems to indicate interaction of Co(III) with the ring, probably through the N(7) atom.

In the case of the hypoxanthine complex, there are three shifts of special interest with the following

TABLE II. Infrared Data for the Inosine and Hypoxanthine Complexes (cm^{-1})

Tentative assignment	Inosine	$[Co(en)_2(Ino)Cl]Cl_2 \cdot 3H_2O$	Hypoxanthine	$[Co(en)_2(Hyp)Cl]Cl_2 \cdot 7H_2O$
$\nu(C_6{=}O) + \nu(C_6{-}C_5)$	1703s, 1681s	1679s	1671s	1667s, 1663s
$\nu(C{=}N) + \nu(C_4{=}C_5)$ $+ \delta(N{-}H) + \nu(C_6{-}N_1)$	1583s	1594s[a]	1581m	1593vs[a]
		1560sh[a]		
	1546m	1527m		
	1503m	1497w		
$\delta(C_8{-}H) + \nu(N_7{-}C_8)$	1457w, sp	1458m, 1455m	1470m	1455m
$\nu(C_4{-}N_9) + \nu(N_7{-}C_5)$			1422m	1399m
ν(ring)	1412m	1402w		
	1374m	1366w[a]	1368m	1367m[a]
	1337w	1339vw	1349m	
	1306m	1319w[a]		
$\nu(C_6{-}N_1) + \nu(N_7{-}C_5)$	1257m	1289w[a]	1275m	1288m[a]
	1200s	1215s[a]	1215m	1208m[a]
			1153m	
	1123s	1131s	1138m	1138m
ν(ring) + ν(sugar)		1087sh		
	1058s	1058vs[a]		1058s[a]
	1034s			
$\nu(N_3{-}C_2)$			966m, 893m	
ν(Co–N)		585w, 515w, 471w[a]		587w, 514w, 473w[a]
ν(NCoCl) + δ(NCoN)		295w[a], 269s		287w[a], 274m

[a]Bands with contribution of those of the starting complex. For the *trans*-$[Co(en)_2Cl_2]Cl$ complex bands at 1592s, 1566sh, 1367m, 1315m, 1274m, 1209, 1119s, 1103s, 1054s, 591m, 514m, 475m, 293s.

bands: 1470, 1422 and 1275 cm^{-1}, which are related to ν(C=N) and C–N (N(7) and N(9), mainly). This implies coordination of Co(III) with the 5-membered ring, but we cannot discern from the IR data the N(7) or N(9) binding site.

The infrared data of the nucleotide derivatives (Table III) show different facts.

The 5′-IMP derivative presents changes for the base bands and no changes for the phosphate absorptions. The $\nu(C_6{=}O)$ and ν(C–C) + ν(C–N) peaks from the ethylenediamine show a small increase in frequencies which may indicate the participation of these groups in hydrogen bonding. The ring bands present a shift of frequencies, although some of these receive vibrations due to the starting complex groups. The 1483 cm^{-1} band ($\nu(C_8{=}N_7)$) [11] splits into two peaks at 1469 and 1465 cm^{-1}, decreasing in intensity. Moreover, the stretching ring band at 1330 cm^{-1}, assigned to $\nu(C_8{=}N_7) + C_8{-}N_9$, shifts to a lower frequency, appearing at 1322 cm^{-1}.

All these data agree with bonding between the metal ion and the base, probably Co(III)–N(7).

For the 5′-GMP compound the infrared data show similar facts. Both the ν(C=O) peak and the ethylenediamine band appearing at 1060 cm^{-1} slightly increase in frequency, indicating possible hydrogen bonding between carbonyls and the $-NH_2$ group of 'en'. The 1484 cm^{-1} band ($\nu(C_8{=}N_7) + \delta(C_8{-}H)$) also splits into two peaks at 1486 cm^{-1} and 1460 cm^{-1}, but the 1331 cm^{-1} band disappears. Other purine ring absorptions are equally altered in the spectrum of the complex, such as the 1361 cm^{-1} band of the nucleotide which increases its frequency, appearing as a broad absorption, possibly coupling with the 1367 cm^{-1} peak of the starting complex. On the other hand, no significant changes are found for the phosphate group stretching bands. All these data suggest binding between the Co(III) and the heterocyclic ring, but perhaps not exactly identical as in the case of the 5′-IMP derivative.

The infrared data for the 5′-AMP derivative show only slight variations in frequencies for the 1584 cm^{-1} (ν(ring)) and 977 cm^{-1} $\nu(PO_{3symm})$ bands, but in general these are not noticeable changes and no clear conclusion can be drawn from these data.

From the UV data (Table IV) we can deduce variations in frequency and intensity for the bands due to the purine ring ($\pi \longrightarrow \pi^*$). In comparison with the expected values of the ring bands – 5′-IMP (248.5 nm), 5′-GMP (253 nm), 5′-AMP (260 nm), Ino (249 nm) and Hyp (248 nm) – there is in all cases a shift to higher frequencies (lower λ). Moreover, the extinction molar coefficient (ϵ) undergoes change from values of $10^4 - 1.5 \times 10^4$ in the ligand to $2-3 \times 10^4$ in the complex. These modifications are probably a consequence of coordination Co(III)–base and subsequent restructuring of the ring charge. The three nucleotide complexes show similar D_q values calculated by considering a pseudo-octahedral geometry, which agree with bonding through the heterocyclic

TABLE III. Infrared Data[a] for the Nucleotide Complexes (cm^{-1})

Tentative assignment	$Na_2$5′-IMP	$Na[Co(en)_2(5'\text{-IMP})_2]\cdot 7H_2O$	$Na_2$5′-GMP	$Na[Co(en)_2(5'\text{-GMP})_2]\cdot 8H_2O$	$Na_2$5′-AMP	$Na[Co(en)_2(5'\text{-AMP})_2]\cdot 8H_2O$
$\nu(C_6{=}O) + \nu(C_6{-}C_5)$	1690s, 1681s	1694s	1694s	1696s		
$\delta(NH_2) + \nu(C_2{-}N_2)$[b]			1661sh	1671s, 1662s, 1646s	1663s	
					1646s	1649s
$\delta(NH_2) + \nu(C_5{-}C_6)$					1608s	1606s
ν(ring)	1592m	1593m[c]	1577w			
$\nu(C_4{=}C_5) + \nu(N_3{-}C_4{=}C_5)$					1584s	1579s
ν(ring)	1551m	1554m	1536m	1535m		
$\delta(C_8{-}H) + \nu(C_8{-}N_7)$	1483m	1469w, 1465w	1484m	1486m, 1460sh	1484m	1481m
					1425m	1422m
ν(ring)	1383m	1375w				
	1371m		1361s	1377s, br; 1373s, br[c]		
	1347m	1348w				
$\nu(C_8{=}N_7) + \nu(C_8{-}N_9)$	1330m	1322w, sh	1331w			
$\delta(C_8{-}H) + \nu(N_7{=}C_8)$					1307m	1305m
$\nu(N_7{=}C_8{-}N_9) + \nu(C_2{-}H)$					1255m	1252m
$\nu(PO_3)_{asymm}$ +	1128s, br	1119s, br[c]	1122s, br	1110s, br[c]	1120s, br	1114s, br[c]
$\nu(C{-}O)_{sugar}$	1098–1070s, br	overlap.	1097–1066s, br	1090–1075s, br	1094s, br	overlap.
$\nu(C{-}C) + \nu(C{-}N)$(en)		1061s[c]		1061s[c]		1060s[c]
$\nu(PO_3)_{symm}$	980s	980s	975s	977s	977s	981s
$\nu(N_7{-}C_5) + \delta(N_1{-}C_2{=}N_3)$	830s	815m				
$\nu(P{-}O)$	793m	792m	781m	781m	797m	799m

[a]s = strong, m = medium, br = broad, sh = shoulder, w = weak; ν = stretching; δ = bending. [b]Assignment for Na_2(5′-AMP) and its complex: $\delta(NH_2) + \nu(C_5{-}C_6) + \nu(C_6{-}NH_2)$. [c]Bands with contribution from those of the *trans*-$[Co(en)_2Cl_2]Cl$ complex.

TABLE IV. UV–Vis Spectra of the Complexes[a] (bands in nm)

Compound	$\pi \longrightarrow \pi^*$	$(\nu_2)^1T_{2g} \longrightarrow {}^1A_{1g}$	$(\nu_1)^1T_{1g} \longrightarrow {}^1A_{1g}$	$10D_q$ (cm^{-1})
$Na[Co(en)_2(5'\text{-IMP})_2]\cdot 7H_2O$	245(28734)	351(129)	502(104)	21960
$Na[Co(en)_2(5'\text{-GMP})_2]\cdot 8H_2O$	249(31000)	345(220)	498(98)	22178
$Na[Co(en)_2(5'\text{-AMP})_2]\cdot 8H_2O$	257(34877)	367(113)	517(90)	21322
$[Co(en)_2(Ino)Cl]Cl_2\cdot 3H_2O$	222(19660)		501(85)	
$[Co(en)_2(Hyp)Cl]Cl_2\cdot 7H_2O$	221(33064)		497(95)	

[a]For the *trans*-$[Co(en)_2Cl_2]Cl$ complex, bands occur at 617(34), 454(24) and 354(54).

TABLE V. ^{13}C NMR Spectra of the Complexes[a]

Compound	C(6)	C(2)	C(4)	C(8)	C(5)	C(1′)	C(4′)	C(3′)	C(2′)	C(5′)	C(en)
Na_2(5′-IMP)	159.4	147.5	149.4	140.6	124.4	88.3	85.7	71.4	75.7	64.5	
							85.3			64.3	
$Na[Co(en)_2(5'\text{-}IMP)_2]\cdot 7H_2O$	160.0	147.5				89.6	86.4	71.2	74.9	64.5	46.5
		147.1						71.0			45.7
											45.0
Na_2(5′-GMP)	159.6	154.8	152.2	138.3	116.8	87.8	88.4	71.1	75.1	65.5	
							84.9			64.3	
$Na[Co(en)_2(5'\text{-}GMP)_2]\cdot 8H_2O$	158.6	154.1				89.9	85.2	71.5	75.2	65.5	46.4
						89.1	84.3	71.1	74.3		45.7
											45.0
Na_2(5′-IMP)	155.6	153.1	149.0	140.6	118.7	88.0	85.4	71.4	75.5	64.6	
							84.9			64.4	
$Na[Co(en)_2(5'\text{-}AMP)_2]\cdot 8H_2O$	155.9	152.7		140.5		88.8	84.5	71.1	74.8	67.0	45.9
							84.4				45.7
											45.4
$[Co(en)_2(Ino)Cl]Cl_2\cdot 3H_2O$		147.6				90.6	86.2	71.0	74.4	62.1	45.9
											45.8
											45.6
$[Co(en)_2(Hyp)Cl]Cl_2\cdot 7H_2O$	162.8	147.6									45.9
											45.8
											45.7

[a]Chemical shifts were measured relative to dioxane and converted to the TMS scale using δ(dioxane) = 67.4 ppm.

base, although in the 5'-AMP compound this value is slightly lower, indicating a different coordination site.

The ^{13}C NMR spectra were registered in H_2O solutions (0.1–0.05 M) with D_2O for lock (Table V). The relatively low solubility of the complexes does not allow some of the signals of the spectra to be distinguished. The assignments were made by comparison with values in the literature [13–15]. All the ^{13}C NMR spectra confirm the presence of ethylenediamine in a mixture of *cis* and *trans* geometry with respect to Co(III), the *trans* signal being the most intense. The direction in chemical shift of the C(6) signal is different for the 5'-IMP and 5'-AMP derivatives in comparison with the 5'-GMP compound. The shifts are higher for the C(6) signal of the 5'-GMP derivative. This fact suggests a different type of binding to the base for the 5'-IMP and 5'-GMP derivatives.

The variation in chemical shift for the 5'-AMP compound is very low for the signal of C(8), discarding bonding in this complex with the N(7). The other nitrogen atoms N(1), N(3) and the $-NH_2$ group are alternative sites of binding for this complex. Nevertheless, we cannot discard the possibility of indirect bonding of N(7) through one water molecule, as occurs in the ATP–Co(II) complex described by Torreilles *et al.* [16]. In addition, some signals of the ribose ring present significant shifts to downfield, suggesting some kind of interaction (perhaps hydrogen bonding) of the $-OH$ groups or the phosphate group with the $-NH_2$ of ethylenediamine. For the 5'-GMP derivative, the C(6) signal shifts to upfield, suggesting participation of the $C_6{=}O$ group in the bonding. The shift of the C(2) signal is also significant. The signals corresponding to the C(1'), in the case of 5'-GMP and 5'-IMP compounds are downfield shifted by about 1–2 ppm, suggesting coordination with N(7), but unfortunately the C(8) signal shift cannot be observed [15].

The low solubility of inosine and hypoxanthine in water and the corresponding complexes in DMSO and other common solvents made a comparison between complexes and ligand signals not possible.

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