Mass Spectrometric Study of Platinum Complexes Based on Cisplatin

P. DOLEŽEL and V. KUBÁŇ*

Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, CZ-613 00 Brno
e-mail: kuban@mendelu.cz

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Dedicated to Lidka Doleželová in honour of her birthday

Flow injection-electrospray ionization-mass spectrometric and liquid chromatography-UV spectrometry-electrospray ionization-mass spectrometric systems with the possibility of collision-induced dissociation were used for characterization of Pt(II) and Pt(IV) complexes well known as antitumour agents. Mass spectra of *cis*-platin and *trans*-platin $[Pt^{II}(NH_3)_2Cl_2]^0$ under various experimental conditions were compared. Ion clusters and adduct ions with the assumed structure are described. The process of partial ion fragmentation allows characterization of the LA12 product, $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)_2Cl_2]^0$, a new agent with enhanced cancerostatic activity. The structure of the principal impurity, $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)Cl_3]^0$, present in the final product of the LA12 synthesis, was designed after chromatographic separation and subsequent mass spectra analysis.

Pt(II) and Pt(IV) complexes are widely used compounds with cytotoxic activity. Pt(II) complexes readily react with DNA, forming either intrastrand or interstrand crosslinks [1]. Conjugates of platinum complexes with N^7 of guanine are mostly assumed [2]. Metabolic conversion of Pt(IV) complexes to Pt(II) is well known [3—6].

cis-Platin $[Pt^{II}(NH_3)_2Cl_2]^0$ has been widely used in medicine with regard to its antitumour properties, including the treatment of various solid tumours [7—11]. It has become a prototype for synthesis of new cytostatic agents on the platinum basis [12—14] showing a much stronger antitumour activity in comparison with *cis*-platin. They display cytotoxic activity also against carcinoma cell lines with acquired resistance to *cis*-platin [15, 16].

A number of analytical techniques are currently available for quantification and structure elucidation of platinum complexes in biological samples and for identification of possible impurities in commercial preparations [17]. The HPLC-MS analytical system combines advantages of a separation process with structural information on eluted components of a sample. Electrospray ionization (ESI) mass spectrometry enables species to transfer from solution to the gas phase with little decomposition, thus making it ideal for analyzing metal complexes. Analysis of *cis*-platin, *trans*-platin, and other related platinum complexes by HPLC in off-line conjunction with ESI-MS was demonstrated [17, 18].

A recent study uses a flow injection-electrospray ionization-mass spectrometric (FI-ESI-MS) on-line system for comparison of *cis*-platin and *trans*-platin mass spectra in various solvents. Mass spectra enabled identification of the new synthetic antitumour agent LA12 [19]. Moreover, the liquid chromatography-UV spectrometry-electrospray ionization-mass spectrometric on-line system HPLC-UV-ESI-MS with collision-induced dissociation (CID) facilitated determination and identification of a principal impurity (abbreviated as U1) present in the final product of LA12 synthesis.

EXPERIMENTAL

cis-Platin, *trans*-platin, and LA12 were synthesized and provided by Pliva-Lachema (Brno, Czech Republic). Sodium formate (anal. grade) was of the same origin. HPLC grade solvents were purchased from Merck. Redistilled water was of Milli-Q quality.

FI-ESI-MS and LC-UV-ESI-MS systems: A series HP 1100 MSD (Hewlett—Packard, USA) HPLC sys-

*The author to whom the correspondence should be addressed.

tem with an MSD quadrupole mass spectrometer was used. Flow injection experiments were performed by on-line connection of the six-port valve to the mass spectrometer. The connector capillary volume was 9 mm^3. The sample volume 100 mm^3 was injected into a mixture of acetonitrile and water ($\varphi_r = 60:40$) as carrier stream; its flow rate was 0.7 cm^3 min^{-1}. HPLC system with Hypersil BDS C_{18} column (250 mm × 4.6 mm, particle size 5 μm; Shandon, Cheshire, UK) operated at 1 cm^3 min^{-1}. A sample was dissolved in a mobile phase and 20 mm^3 were injected onto the column. A sample was eluted using isocratic conditions of methanol—water ($\varphi_r = 60:40$). All chromatograms were monitored at the wavelength of 206 nm.

Measurements of mass spectra were performed on an MSD quadrupole mass spectrometer with an ESI source (Hewlett—Packard) in the positive ion mode. The spray voltage was set to 4 kV. Nitrogen was used as the nebulizing gas at pressure 344.8 kPa and as the heated gas at 350 °C and the flow rate 10—13 dm^3 min^{-1}. Voltage of the skimmer ranged from 10 to 100 V. Data were acquired over the mass range m/z = 50—800, the gain was set to 12, the threshold set to 150 and the step of mass scanning to $m/z = 0.1$. The autotune function accomplished calibration of MSD for $m/z = 118.10, 622.04, 921.95, 1521.95$, and 2121.95 of the standard mixture (Hewlett—Packard). The calibration check was performed by injecting of reserpine ($m/z = 609.30$).

RESULTS AND DISCUSSION

Mass spectra of *cis*-platin $[Pt^{II}(NH_3)_2Cl_2]^0$ and its isomer *trans*-platin were acquired. A FI-ESI-MS system with acetonitrile—water (φ_r = 60:40) as the transport liquid was used. Mass spectra of *cis*-platin, *trans*-platin, and compounds containing carbon and chlorine are typical by an isotopic cluster pattern demonstrating the presence of isotopes $^{194}Pt(32.9)$, $^{195}Pt(33.8)$, $^{196}Pt(25.3)$, $^{198}Pt(7.2)$, $^{35}Cl(75.5)$, $^{37}Cl(24.5)$, $^{12}C(98.9)$, and $^{13}C(1.1)$ with the nuclide abundance in parentheses (A %). The m/z values listed in the text refer to the first left peak of the isotopic cluster corresponding to the ^{194}Pt and ^{35}Cl isotopes. The acidified sample prefers the $[M + H]^+$ ion cluster and the presence of sodium salt results in $[M + Na]^+$. But the protonated molecule $[M + H]^+$ at $m/z = 299$ is not observed after acidifying the *cis*-platin sample and the transport liquid (up to 2 mmol dm^{-3} formic acid). ESI mass spectrum of *cis*-platin contained predominantly $[M + 6]^+$ at $m/z = 304$. The structure $[M - Cl + CH_3CN]^+$ was designed [18]. The obtained isotopic distribution values (90, 89, 100, 30, 40, 1, 7), which are in good agreement with the theoretical values for PtCl compounds (Table 1), confirmed this structure (Fig. 1*a*). The existence of the $m/z = 304$ $[M - NH_3 + Na]^+$ and $[M - Cl + CH_3CN]^+$ ions was proved earlier [17, 18]. We assumed that the abundance of Na^+ in the sample and transport liq-

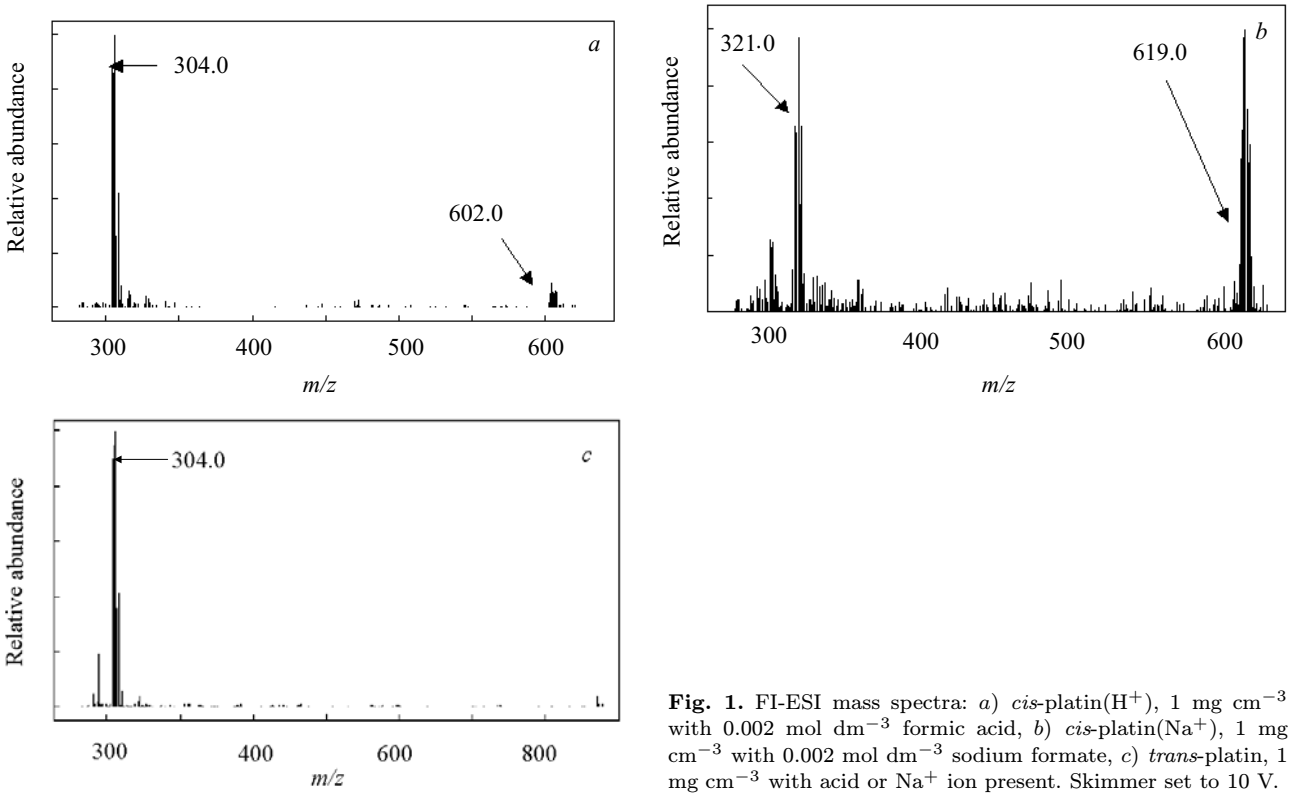

Fig. 1. FI-ESI mass spectra: *a*) *cis*-platin(H^+), 1 mg cm^{-3} with 0.002 mol dm^{-3} formic acid, *b*) *cis*-platin(Na^+), 1 mg cm^{-3} with 0.002 mol dm^{-3} sodium formate, *c*) *trans*-platin, 1 mg cm^{-3} with acid or Na^+ ion present. Skimmer set to 10 V.

Table 1. Theoretical Isotopic Distribution Values for Molecules Containing $PtCl_n$ and $PtCl_nC_m$, where $n = 1, 2, 3$ and $m = 12, 14$

Compound	A[a]	A + 1	A + 2	A + 3	A + 4	A + 5	A + 6
PtCl	91	94	100	31	43	0	6
$PtCl_2$	71	73	100	47	58	8	16
$PtCl_3$	57	58	100	58	75	20	29
$PtCl_2C_{14}$ (LA12)	63	74	100	57	59	15	16
$PtCl_3C_{12}$ (U1)	52	61	100	66	77	28	30

a) Corresponds to the ^{194}Pt isotope.

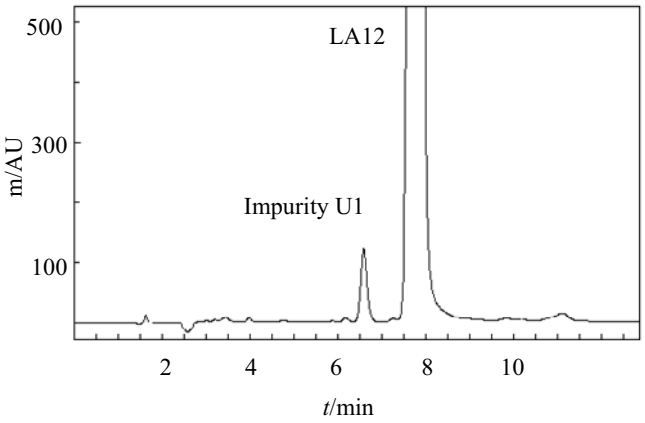

Fig. 2. Chromatogram of LA12 (5 mg cm^{-3}) with U1 impurity. Isocratic elution methanol—water ($\varphi_r = 60:40$).

uid could enhance the presence of the Na^+ ion cluster.

Addition of sodium formate (2 mmol dm^{-3}) to the *cis*-platin sample and the transport liquid causes the preference of $m/z = 321$ $[M + Na]^+$ ion (Fig. 1*b*). The existence of the $m/z = 304$ $[M - NH_3 + Na]^+$ ion cluster [17] was also proved. In addition, mass spectrum of *cis*-platin is characterized by $m/z = 602$ (Fig. 1*a*) and $m/z = 619$ (Fig. 1*b*) adduct ions. Analysis of the obtained isotopic distribution values points to the possibility of overlapping of the mass spectra with the spectra of adduct ions with the difference in AMU = 1; thus $m/z = 602$ and $m/z = 603$, respectively $m/z = 619$ and $m/z = 620$ adduct ions could be present. The structures of adducts $[(M) + (M - Cl + CH_3CN)]^+$ and $[(M - NH_3 + H_2O) + (M - Cl + CH_3CN)]^+$ or $[(M) + (M + Na)]^+$ and $[(M - NH_3 + H_2O) + (M + Na)]^+$ could correspond with the mentioned theory. We have assumed that [M] and $[M - NH_3 + H_2O]$ have zero charge. On the other hand, $[M - Cl + CH_3CN]^+$ and $[M + Na]^+$ carry the positive charge 1+.

Mass spectrum of *trans*-platin shows the $m/z = 304$ $[M + 6]^+$ cluster ion (Fig. 1*c*). The isotopic distribution values (90, 95, 100, 36, 42, 1, 6) demonstrate a chloro complex of Pt with the structure $[M - Cl + CH_3CN]^+$. The distributions are the average values calculated from ten consecutive mass spectra measurements of the same sample. This could result in a difference in the isotopic distributions in the test and in the selected real sample (see Figs. 3*a*, 3*b*). Neither adducts nor $[M + Na]^+$ ion were observed. *cis*-Isomerism and higher polarity of the Pt complex molecule are probably decisive for production of the adduct ions fully missing in the mass spectrum of *trans*-platin.

Complexes based on Pt(IV) are distinguished for their great antitumour activity and lowered toxicity. LA12 $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)_2Cl_2]^0$ is one of the most interesting compounds where $C_{10}H_{17}N$ is the ligand based on 1-aminoadamantane. An online HPLC-UV-ESI-MS system was used for the purity and structure verification of the LA12 synthesized [19]. The chromatogram under isocratic conditions (methanol—water, $\varphi_r = 60:40$) shows the presence of U1 impurity besides the LA12 main peak (Fig. 2). Its retention time is 6.6 min. The relative content of 0.3 % of the U1 impurity in LA12 was calculated from the ratio (20:5990) of U1 and LA12 peak areas.

The mass spectra acquired show typical $[M + H]^+ = 551.0$, $[M + Na]^+ = 573.0$, and $[M + K]^+ = 589.0$ ions for LA12 (Fig. 3*a*) for the skimmer set to 50 V. On the other hand, the ions $[M_{U1} + H]^+ = 527.0$, $[M_{U1} + Na]^+ = 549.0$, and $[M_{U1} + K]^+ = 565.0$ are present for the U1 impurity (Fig. 3*b*). Measurements of the isotopic distribution values of LA12 (65, 75, 100, 60, 64, 18, 18) and U1 (54, 61, 100, 67, 77, 25, 28) are in good agreement with the theoretical values for complex compounds $PtCl_2C_{14}$ and $PtCl_3C_{12}$ (see Table 1).

The identity confirmation for U1 is based on four facts: *a*) the existence of an isotopic cluster in the mass spectrum of U1 is the evidence of $PtCl_3$ molecule, *b*) a shorter retention time on reverse phase demonstrates a weaker hydrophobic nature of U1 in comparison with LA12, *c*) the relative molecular mass of $[M_{U1} + H]^+ = 527.0$ agrees very well with the equation $M - CH_3COO + Cl = 550 - 59 + 35 = 526.0$, *d*) collision-induced dissociation of the M (Fig. 3*c*) and M_{U1} (Fig. 3*d*) ions is realized for the skimmer set to 100 V. Fragment ions common for both LA12 and U1 are presented:

$[M - 2CH_3COOH - HCl + H]^+ = [M_{U1} - CH_3COOH - 2HCl + H]^+ = 395.0$;

$[M - 2CH_3COOH - HCl - NH_3 + Na]^+ = [M_{U1} - CH_3COOH - 2HCl - NH_3 + Na]^+ = 400$;

$[M - 2CH_3COOH - NH_3 + Na]^+ = [M_{U1} - CH_3COOH - HCl - NH_3 + Na]^+ = 436.0$;

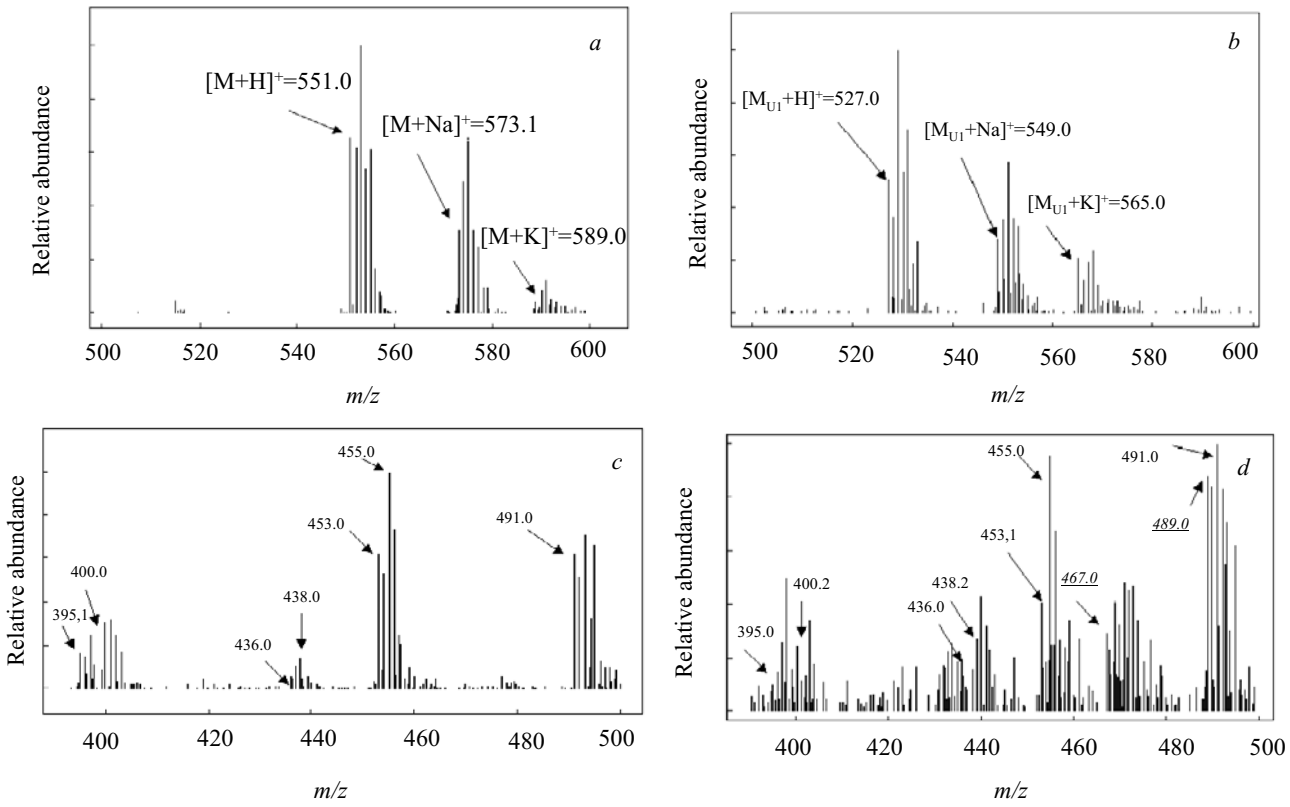

Fig. 3. LC-ESI mass spectrum of LA12 for skimmer set to *a*) 50 V, *c*) 100 V and mass spectrum of U1 for skimmer set to *b*) 50 V, *d*) 100 V.

$[M - CH_3COOH - HCl - NH_3 + H]^+ = [M_{U1} - 2HCl - NH_3 + H]^+ = 438.0$;
$[M - 2CH_3COOH + Na]^+ = [M_{U1} - CH_3COOH - HCl + Na]^+ = 453.0$;
$[M - CH_3COOH - HCl + H]^+ = [M_{U1} - 2\,HCl + H]^+ = 455.0$;
$[M - CH_3COOH + H]^+ = [M_{U1} - HCl + H]^+ = 491.0$.

The fragment ion $m/z = 467.0$, present only in the mass spectrum of U1 (Fig. 3*d*), demonstrates the existence of $[M_{U1} - CH_3COOH + H]^+$, the species which can be written in the form $[Pt(C_{10}H_{17}N)(NH_3)Cl_3 + H]^+$. In analogy, the fragment ion $m/z = 489.0$ demonstrates the presence of the $[M_{U1} - CH_3COOH + Na]^+$ ion. Its structure can be written as $[Pt(C_{10}H_{17}N)(NH_3)Cl_3 + Na]^+$. The facts emphasized by points *a*)—*d*) offer the solution of the U1 structure substituting the CH_3COO ligand by Cl. Hence the summary structure for U1 is $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)Cl_3]^0$ (Fig. 4).

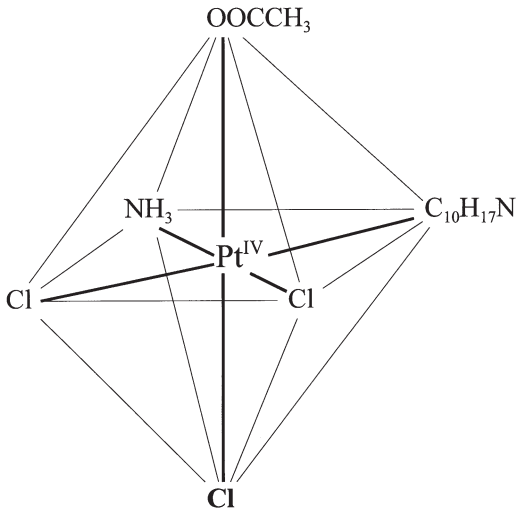

Fig. 4. The assumed structure of U1 impurity. Cl ligand replaces CH_3COO in the LA12 molecule at the top of the bipyramid (bold faced).

CONCLUSION

A flow injection-electrospray ionization-mass spectrometric (FI-ESI-MS) system was used to measure mass spectra of *cis*-platin $[Pt^{II}(NH_3)_2Cl_2]^0$ and *trans*-platin as its isomer. Ion clusters, fragment ions, and adduct ions with the assumed structure are described. In contrast to the *trans*-platin, the $[M + Na]^+$ ion and m/z = 602.0, 603.0, 619.0, and 620.0 adducts are present and the proposed structure under API-ESI (atmospheric pressure ionization-electrospray ionization) conditions was confirmed for *cis*-platin. We assumed that [M] and $[M - NH_3 + H_2O]$ have zero charge. On the other hand, [M −

$Cl + CH_3CN]^+$ and $[M + Na]^+$ bear the charge 1+.

MSD with CID possibility is an important tool for fragmentation of ions produced in the ESI source and passing the skimmer area. An LC-UV-ESI-MS system with CID was used for chromatographic separation and subsequent identification of the U1 impurity present in LA12 cancerostatic agent, $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)_2Cl_2]^0$. The mass spectrum of the U1 ion and the results of ion fragmentation indicate substitution of the CH_3COO ligand with chlorine atoms in the LA12 molecule. Hence, the structure of $[Pt^{IV}(C_{10}H_{17}N)(NH_3)(CH_3COO)Cl_3]^0$ was assigned to U1.

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