BioMetals 1996, **9**, 249–257

Relationship between the structure of diamine platinum(II) complexes and their cytostatic activity as measured on plant roots

V. B. Ivanov, M. J. Bloemink*, P. A. Cheltsov, E. I. Bystrova, T. N. Fedotova & J. Reedijk*

*N. S. Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Moscow, Russia and *Leiden Institute of Chemistry, Gorlaeus Laboratoires, Leiden University, Leiden, The Netherlands*

Received 23 October 1995; accepted for publication 18 January 1996

The amine substituent effect in compounds [Pt(diamine)Cl_2] on inhibition of maize and cucumber root growth and branching has been investigated. The diamines used were ethylenediamine (en), *N*-methylethylenediamine (men), *N,N*-dimethylethylenediamine (*N,N*-dmen), *N,N'*-dimethylethylenediamine (*N,N'*-dmen), *N,N,N',N'*-tetramethylethylenediamine (tmen), 1,2-propanediamine (1,2-pn), 2-methyl-1,2-propanediamine (ibn), 2,3-dimethyl-2,3-butanediamine (C-tmen), 1,3-propanediamine (1,3-pn), 2,2-dimethyl-1,3-propanediamine (C_2-dm-1,3-pn), *N,N*-dimethyl-1,3-propanediamine (*N,N*-dm-1,3-pn). Increased substitution of hydrogen atoms of the amine part with CH_3 groups reduces the cytostatic activity of complexes. The substitution of hydrogen atoms of NH_2 and vicinal CH_2 groups displays similar results. C-2 dimethylation (C-dm-1,3-pn) does not change the activity of the complex compared with (1,3-pn). It was observed that maize and cucumber roots differ in their relative sensitivity to various complexes. All complexes containing pn and their substituted analogs inhibited cucumber root growth weaker than that in maize. A comparison of obtained data with earlier published results concerning antitumor activity of complexes shows that they correlate in a similar manner with increased substitution of amino groups. Therefore, roots may be used as cheap test objects for primary screening of cytostatics. The general tendency of a decrease in cytostatic activity goes parallel with the number of N- or vicinal C-methyl groups and seems to arise from a decrease in hydrogen-bonding potential; however, some other possible reasons are also discussed. The activity discrimination by different species in our experiments and clearly different results for *N,N*-dimethylation depending on the chelate ring size (en and pn derivatives) on maize cannot be attributed to slower ligand-exchange kinetics from methylation. It is possible to assume that the major role in cytostatic activity of platinum complexes belongs to a cell repair system, i.e. the ability to eliminate platinum diamine fragments from DNA, depending on the number and strength of hydrogen bonds formed by the *cis*-diamine fragment.

Keywords: cisplatinum, cytostatic, platinum compounds, plant roots

Introduction

The antitumor activity of some platinum *cis*-diamine complexes (DDP) is generally assumed to result from an interaction with cell DNA. Like many other DNA-binding reagents, these complexes induce mutations, chromosomal aberrations and retard cell proliferation (Lippard 1987, Reedijk 1992, Bloemink & Reedijk 1996); they act as typical cytostatics and are inhibitors of DNA synthesis. These complexes form kinetically inert links primarily with guanine-N^7 of DNA. However, analogical *trans* complexes can also bind to DNA but do not exert the cancerostatic activity. These effects are manifested only by complexes containing *cis*-amines and, for reasonable activity, they need to contain at least one NH on each amine group (Cleare and Hoeschele 1973, Cleare 1974, Beamont *et al.* 1976, Reedijk 1992). There are some facts which permit us to suggest that the formation of hydrogen bonds between the amine part of complexes and DNA is important for the overall activity of platinum compounds (Van Kralingen & Reedijk 1978, Broomhead *et al.* 1980, Ivanov *et al.* 1981a, Mattern *et al.* 1982, Sherman *et al.* 1985); however, the role

Address for correspondence: V. B. Ivanov, N. S. Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Leniniskii Prospekt 31, Moscow 117907, Russia. Tel: 7(095) 952 2020, Fax: 7(095) 954 1279.

such NH groups play in drug binding to DNA is still not understood in detail.

A constructive approach to this problem is a detailed study of changes in biological activities of complexes in which hydrogen atoms in the amine part are substituted with CH_3 groups, and comparison of the data thus obtained for various test objects. Such studies were published for tumor cells (Cleare and Hoeschele 1973, Cleare 1974, Beamont *et al.* 1976, Broomhead *et al.* 1980), microorganisms (Mattern *et al.* 1982, Das Sarma *et al.* 1983) and animals (Broomhead *et al.* 1980). In all cases a decrease in hydrogen-bonding potential with the substitution of hydrogen in the amine part of complexes with CH_3 groups resulted in decreasing cytostatic, mutagenic and toxic activities of complexes.

In the present study, the growth-inhibiting activities of N- and C-substituted analogs of en and pn complexes are described. The biological activities of some complexes (C-substituted en and N- and C-substituted pn) have not been studied. As test objects, we used the roots of seedlings.

The action of platinum complexes on plant and animal cells may be different. As a rule, plant cells were treated in solution with lower ion concentrations than in plasma blood, especially with regard to chloride ions. Under these conditions, the rate of hydrolysis of complexes is increased. A preliminary hydrolysis is essential for the reaction of DDP and analogical complexes with DNA. In most plant species, the DNA content of the diploid nucleus is higher than in animals. The mechanism of the biological effects of DNA-tropic substances on higher DNA content in plants and the fraction of repeated DNA sequences is still unknown. At least, plant and animal cells may differ in their ability to repair their DNA.

The actions of most complexes on root growth which were studied in the present paper have not been studied previously. We have used growing roots as a test system and have screened over 500 platinum complexes (Ivanov *et al.* 1981a, 1976, 1988, 1989, 1992, Ivanov 1994, Shevchenko *et al.* 1983, Ivanov & Cheltsov 1991). All platinum complexes manifesting antitumor activity also exerted cytostatic activity: the delayed deceleration of root growth and a cessation of root branching. Therefore, plant roots can be used for the detection and efficient screening of such compounds.

The roots of plants represent a very convenient screening model for these purposes because the effect of these drugs can be evaluated by routine measurements of root length and observations on root branching. Independent of their chemical structure, the inhibitors of DNA synthesis exhibit specific patterns of root growth inhibition. The root grows by cell division and cell elongation. At low concentrations, cytostatics inhibit cell division, but do not affect cell growth and metabolism. For these reasons they do not affect the root growth rate immediately after the treatment is commenced, as the root growth rate at the moment of the observation is determined by cell elongation. Only at high drug concentrations can the growth rate decline as a result of the non-specific action of cytostatics (e.g. reaction with SH groups of enzymes, damage to membranes, etc.).

However, the root can grow at a constant rate only when new cells start to elongate at a constant rate. When cell division is hindered by cytostatics, the rate of cell transition to elongation is always decreased. Therefore, the root growth rate decreases as a function of time. Our earlier experiments with different cytostatics have shown that this phenomenon determines the delayed decrease in root growth by cytostatics (Ivanov & Cheltsov 1991, Ivanov 1994). Other possible reasons for this phenomenon, e.g. slow penetration of cytostatics into root cells, metabolic events of cytostatics, are not significant. Therefore, simple measurements of root growth directly manifest cytostatic activity of the compound tested. Moreover, all cytostatics prevent lateral root emergence at concentrations far from lethal ones, although other toxic substances lacking cytostatic activity do not delay the appearance of lateral roots.

Materials and methods

Two-day-old maize seedlings, cv. *Sterling*, were uniformly distributed in Petri dishes ($d = 150$ mm) on filter paper dampened with distilled water (untreated roots) and with a solution of the test compound, and were incubated in darkness at 27°C. Root length was measured with a ruler daily during three consecutive days. The inhibitory action was evaluated using the following equation:

$$I = \frac{L_c - L_i}{L_c} \times 100\%,$$

where I is the index of growth inhibition, L_c is the daily increment in length of control roots and L_i is the daily increment in length of roots growing in inhibitor solution. Each compound was tested in two to four experiments, and for each concentration, 10 seedlings were sampled in two Petri dishes (five seedlings in one dish) in each experiment.

Air-dry cucumber seeds, cv. *Muromski*, were uniformly distributed in Petri dishes on filter paper dampened with distilled water (untreated roots) or with an aqueous solution of the compound to be tested, and were incubated in darkness at 27°C. The root length and lateral root emergence were recorded on the third day. In cucumber radicles, some of the lateral root primordia are preformed during seed development and therefore the lateral roots readily develop by the third day of germination. However, a series of cell divisions is necessary to provide for their development. In solutions of cytostatics, no lateral roots appear, though in the solution of a compound exerting a total toxic effect, their appearance is not inhibited even at almost lethal concentrations (Ivanov *et al.* 1986, Ivanov 1994).

Complexes **3**, **4**, **6** **8**, **9**, **10** and **13** in Table 1 were synthesized in the N. S. Kurnakov Institute of General and Inorganic Chemistry RAS. A concentrated water solution of $K_2[PtCl_4]$ (concentration 10–15%) interacted with a 10% excess of the corresponding amine and the obtained precipitate was purified by recrystallization from 0.1 M hydrochloric acid. Complexes **1** and **2** were synthesized as previously described (Chernyaev 1964). Complexes **5**, **7**, **11**

Table 1. Average increment of root length (in mm) for 72 h

Number	Ligands	0 mg l^{-1}	1 mg l^{-1}	10 mg l^{-1}	100 mg l^{-1}
Maize					
1	$2NH_3$	166.6±8.0	78.2±2.8	28.1±2.2	6.2±1.3
2	$2CH_3NH_2$	207.9±4.0	189.4±7.9	77.7±2.9	30.0±2.2
3	en	194.4±5.3	183.3±8.2	75.4±3.7	19.3±2.3
4	men	172.3±4.6	184.2±5.1	68.7±4.5	35.8±1.8
5	*N,N*-dmen	190.8±5.0	152.9±8.2	75.2±2.5	46.1±4.0
6	*N,N'*-dmen	172.5±3.9	176.4±4.4	139.8±4.9	44.2±2.4
7	tmen	176.5±3.1	176.0±4.7	169.4±5.7	72.5±4.1
8	1,2-pn	187.3±3.8	183.7±7.6	131.1±10.6	34.3±3.4
9	ibn	172.3±4.6	172.3±6.3	119.4±7.4	42.4±3.3
10	C-tmen	192.8±7.3	169.1±11.6	157.8±8.5	55.9±5.0
11	1,3-pn	190.8±5.6	182.8±7.0	80.2±6.2	14.9±2.1
12	C_2-dm-1,3-pn	207.9±4.0	152.9±8.2	75.2±2.5	46.1±4.0
13	*N,N*-dm-1,3-pn	172.5±3.4	176.3±10.4	157.1±7.1	93.9±5.7
Cucumber					
1	$2NH_3$	76.0±1.4	48.3±1.7	21.4±0.6	
2	$2CH_3NH_2$	78.3±2.5	47.9±3.8	18.4±1.1	5.6±0.7
3	en	77.3±3.0	48.6±3.1	28.0±1.9	
4	men	68.6±1.5	61.8±2.4	30.7±2.1	6.4±0.5
5	*N,N*-dmen	78.3±2.5	47.1±3.7	41.5±3.6	14.3±1.6
6	*N,N'*-dmen	70.0±1.9	65.4±2.5	47.5±1.5	19.2±1.1
7	tmen	66.4±2.7	58.7±4.0	70.4±3.0	40.0±3.4
8	1,2-pn	59.3±2.0	66.6±4.5	35.4±1.7	10.4±1.1
9	ibn	69.9±1.8	49.3±3.0	25.1±1.0	7.5±1.3
10	C-tmen	68.9±1.7	69.8±2.6	50.1±2.0	25.4±1.0
11	1,3-pn	69.4±2.8	55.9±3.4	43.2±2.4	23.0±0.9
12	C_2-dm-1,3-pn	69.4±2.7	52.3±2.4	41.1±1.9	20.3±1.6
13	*N,N*-dm-1,3-pn	71.9±1.6	82.0±3.2	63.3±2.7	53.6±2.5

See Figure 1 for ligand abbreviations and structures.

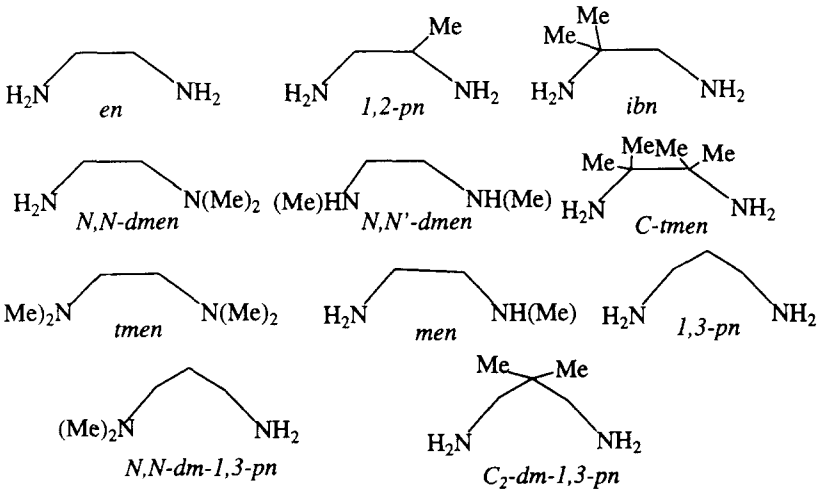

Figure 1. Structure scheme.

and **12** were prepared at Leiden University, as previously described (Bloemink *et al.* 1992). The compounds were checked for purity by spectroscopic and analytical methods. See Figure 1 for structure nomenclature.

Results

Inhibition of root growth by cis- *and* trans-*DDP*

To demonstrate the use of roots as test objects for cytostatic screening, root-growth responses to DDP and its *trans*-isomer, which does not manifest any cancerostatic and cytostatic activities, were compared. Figure 2 shows the growth inhibition curves of these two isomers. As a typical selective cytostatic, *cis*-DDP at low concentrations does not change the growth rate during the first day. However, on the second and especially on the third day, the growth rate sharply decreases, and at the concentration 1 mg l^{-1}, the growth ceases by the third day. At high concentrations,

cis-DDP blocks root growth even on the first day, directly affecting the root growth, directly inhibiting cell elongation by various mechanisms of toxicity (e.g. by inhibiting SH enzymes). As the growth inhibition by *cis*-DDP strongly increased with time, especially at low concentrations, an equal growth inhibition could be observed at various concentrations after various time periods. For example, concentrations at which inhibition equals 50% were found to be 33.3 μM on the first day and 0.8 μM on the third day. For this reason, curves demonstrating *cis*-DDP action on the second and the third days were clearly shifted to the left in Figure 2. On the other hand, the curves of *trans*-DDP effects showed virtually no shift and no sharp inhibition of growth with time was observed at a concentration of 1 mg l^{-1} at which the *cis*-isomer affected the growth (Figure 2). At concentrations above 100 mg l^{-1}, both isomers similarly affect cell elongation and, for this reason, the root growth rate decreases on the first day. At low concentrations, *cis*-DDP inhibits mitoses even during the first 3 h after treatment commences; *trans*-DDP does not inhibit mitoses even at higher concentrations (Ivanov *et al.* 1981b, Ivanov & Cheltsov 1991, Ivanov 1994). No lateral roots developed in *cis*-DDP solution at a concentration of 1 mg l^{-1} and higher. *trans*-DDP does not prevent lateral root emergence

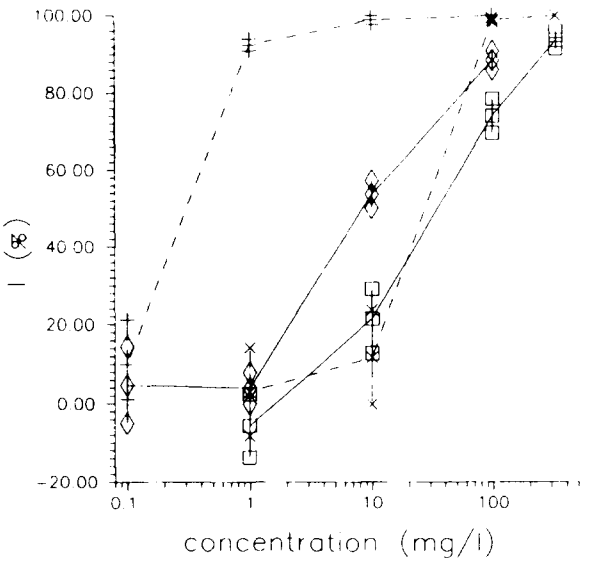

Figure 2. Inhibition curves of maize root growth for *cis*- and *trans*-DDP. Solid lines: increment of root length during 0–24 h; ◇, *cis*-DDP; □, *trans*-DDP. Dashed lines: increment of root length during 48–72 h; +, *cis*-DDP; ×, *trans*-DDP.

Table 2. Inhibition of lateral root formation (I_r in %)[a] by complexes

Number	Amine	1 mg l^{-1}	10 mg l^{-1}	50 mg l^{-1}	100 mg l^{-1}	500 mg l^{-1}
Maize						
1	$2NH_3$	64±4	97±2	—	100	—
2	$2CH_3NH_2$	58±7	86±4	95±2	97±2	—
3	en	34±7	91±2	—	100	—
4	men	13±8	82±4	95±2	95±3	—
5	*N*,*N*-dmen	3±6	75±4	—	94±2	—
6	*N*,*N'*-dmen	2±6	55±5	84±3	91±2	—
7	tmen	−16±4	14±6	—	68±6	—
8	1,2-pn	19±9	70±7	96±2	97±2	—
9	ibn	34±8	76±6	100	98±2	—
10	C-tmen	3±6	27±5	74±4	82±3	97±2
11	1,3-pn	37±11	92±3	—	97±2	—
12	C_2-dm-1,3pn	52±10	88±5	—	97±2	—
13	*N*,*N*-dm-1,3pn	−13±6	7±7	7±9	64±4	90±3
Cucumber						
1	$2NH_3$	64±5[c]	100			
2	$2CH_3NH_2$	50±8	99±1	—	100	—
3	en	40±8	96±2	100	—	—
4	men	25±8	67±6	100	100	—
5	*N*,*N*-dmen	36±12	34±10	100	100	—
6	*N*,*N'*-dmen	−6±9	32±5	85±4	97±2	—
7	tmen	16±11	−19±11	—	42±8	81±7[b]
8	1,2-pn	−16±17	59±7	100	100	—
9	ibn	42±6	88±4	100	100	—
10	C-tmen	−2±9	23±7	80±6	100	100
11	1,3-pn	29±10	32±8	77±11	88±4	—
12	C_2-dm-1,3pn	21±9	37±7	91±4	89±5	—
13	*N*,*N*-dm-1,3pn	16±12	9±7	11±12	5±8	54±7

[a] $I_r = [(L_c - L_i)/L_c] \times 100\%$, where L_c is the length of branching part of untreated roots and L_i is the length of the branching part of roots growing in solution of the complex. Negative values indicate $L_i > L_c$.
[b] At a concentration of 200 mg l^{-1}.
[c] At a concentration of 5 mg l^{-1}.

at any concentration up to a lethal level. Hence, the differences in the patterns of growth inhibition by both isomers are determined by their different action on cell division. The earlier experiments showed that a delayed reduction of root growth rate by *cis*-DDP is not due to gradual intracellular accumulation of inhibitors (Ivanov & Cheltsov, 1991).

The growth-inhibiting activity of [Pt(en)Cl_2] [Pt(1,3-pn)Cl_2] and their C- and N-substituted analogs

[Pt(en)Cl_2] inhibited root growth as a typical cytostatic but at higher concentrations than DDP (Tables 1 and 2, and Figure 3). [Pt(CH_3NH_2)$_2Cl_2$] also inhibited root growth at a higher concentration than DDP (Tables 1 and 2). By the first day, its effect was less than that of DDP, but at the third day these compounds acted similarly. Most substituted analogs of [Pt(en)Cl_2] also displayed cytostatic activity, and their activities varied considerably and were found to depend on the number and location of introduced CH_3 groups (Tables 1 and 2, and Figures 3–5).

The results obtained for N-substituted analogs of [Pt(en)Cl_2] are represented in Figure 3. During the first 24 h all substituted complexes inhibited root growth to a lesser extent than [Pt(en)Cl_2]. The complex in which all hydrogen atoms in both NH_2 groups are substituted, [Pt(tmen)Cl_2], did not influence the root growth rate even at the highest concentration tested (0.1 mg ml^{-1}). With the substitution of one hydrogen atom into each NH_2 group, [Pt(*N*,*N*'-dmen)Cl_2], the growth-inhibiting activity of the complex decreased more than with the substitution of two hydrogen atoms at the same NH_2 group: [Pt(*N*,*N*-dmen)Cl_2]. The last complex displayed a similar effect to [Pt(men)Cl_2] in which only one hydrogen atom in one NH_2 group was substituted with a CH_3 group.

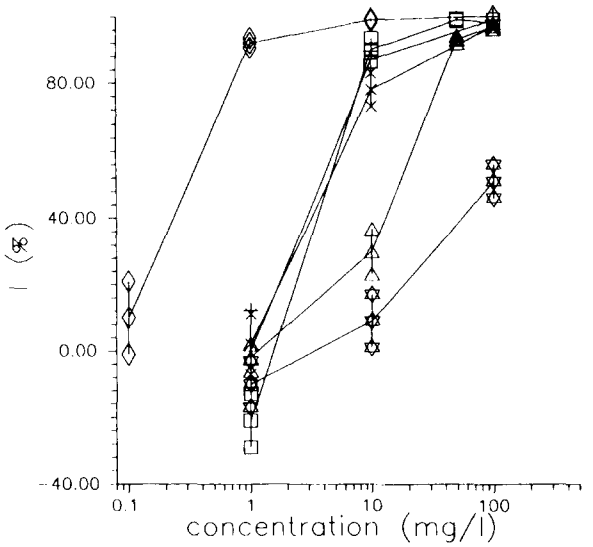

Figure 3. Inhibition curves of maize root growth for N-substituted analogs of [Pt(en)Cl_2] during 48–72 h. ◇, *cis*-DDP; +, [Pt(en)Cl_2]; □, [Pt(men)Cl_2]; ×, [Pt(*N*,*N*-dmen)Cl_2]; △, [Pt(*N*,*N*'-dmen)Cl_2]; *, [Pt(tmen)Cl_2].

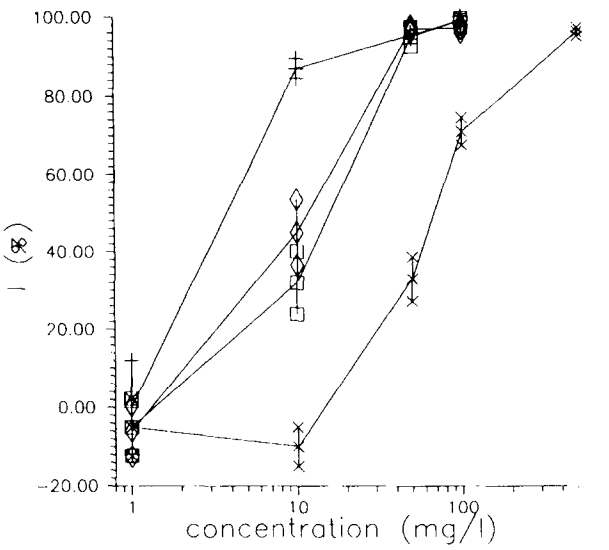

Figure 4. Inhibition curves of maize root growth for C-substituted analogs of [Pt(en)Cl_2] during 48–72 h. +, [Pt(en)Cl_2]; □, [Pt(1,2-pn)Cl_2]; ◇, [Pt(ibn)Cl_2]; ×,[Pt(C-tmen)Cl_2].

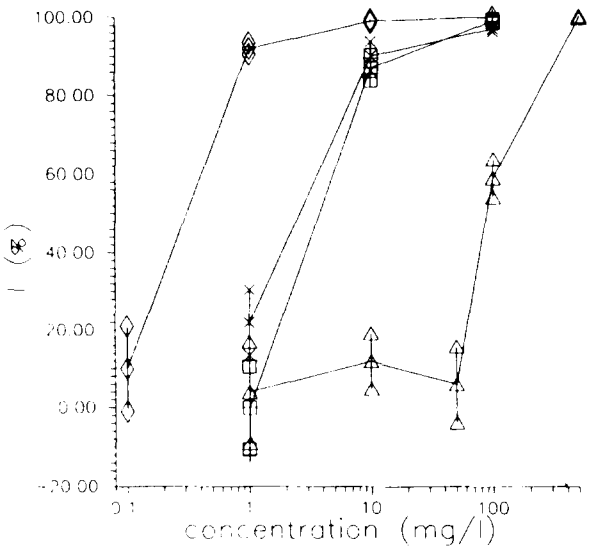

Figure 5. Inhibition curves of maize root growth for substituted analogs of [Pt(1,3-pn)Cl_2] during 48–72 h. ◇, *cis*-DDP; +, [Pt(en)Cl_2]; □, [Pt(1,3-pn)Cl_2]; ×, [Pt(C_2-dm-1,3-pn)Cl_2]; △, [Pt(*N*,*N*-dm-1,3-pn)Cl_2].

By the third day the growth-inhibiting effect of all complexes became more prominent. As already mentioned above, the decreasing root growth rate results from the inhibition of cell division. During the first day after the beginning of the experiment, the inhibition of cell division only slightly influences the root growth rate. It clearly depends on the cellular organization of the root growth. DDP enhanced the inhibition of root growth to a greater extent than [Pt(en)Cl_2] and its substituted analogs (Figure 3). [Pt(tmen)Cl_2] inhibited root growth only at the concentration of 100 mg l^{-1} and inhibition equalled 50%.

DDP displayed the similar effect at about 300 times lower concentration. [Pt(en)Cl_2], [Pt(men)Cl_2] and [Pt(*N*,*N*-dmen)Cl_2] inhibited root growth practically in a similar fashion. [Pt(*N*,*N'*-dmen)Cl_2] inhibited root growth to a lesser extent and at the concentration of 10 mg ml^{-1} the *I* value was about 2.5 times lower than in the case of the latter three complexes. Similar results were observed when the lengths of root parts possessing lateral roots were compared (Table 2). Slightly different results were obtained in the case of cucumber roots (Table 2). [Pt(men)Cl_2] and especially [Pt(*N*,*N*-dmen)Cl_2] inhibited root branching weaker than [Pt(en)Cl_2]. In contrast to maize roots, distinctions between the action of [Pt(*N*,*N*-dmen)Cl_2] and [Pt(*N*,*N'*-dmen)Cl_2] were only slightly manifested. [Pt(tmen)Cl_2] inhibited root branching significantly weaker than other complexes and even at the highest concentration tested (200 mg l^{-1}) the root branching was not suspended.

The growth-inhibiting activities of C-substituted analogs of [Pt(en)Cl_2] (Tables 1 and 2, and Figure 4) will now be considered. The substitution of one or two hydrogen atoms in one CH_2 group resulted in the evident decrease in growth-inhibiting activity. In contrast with the N-substituted analogs (Figure 3), the *I* value for maize roots decreased not only on the first day, but on the third day as well. The complex with all the hydrogen atoms substituted in both CH_2 groups (i.e. [Pt(C-tmen)Cl_2]) inhibited maize root growth only at the highest concentration. On the first day, the latter compound inhibited root growth stronger than its N-substituted analog ([Pt(tmen)Cl_2]). However, on the third day at concentrations of 1–100 mg l^{-1} these complexes inhibited the root growth similarly. These complexes inhibited maize root branching stronger than root growth. [Pt(ibn)Cl_2] was found to inhibit cucumber root branching stronger than [Pt(1,2-pn)Cl_2] and similar to [Pt(en)Cl_2] (Table 2). The effect of [Pt(C-tmen)Cl_2] was found to be considerably weaker, but this complex acted stronger than its N-substituted analogs. At the concentration of 100 mg l^{-1}, [Pt(C-tmen)Cl_2] completely stopped the formation of lateral roots.

The lengthening link between NH_2 groups in [Pt(en)Cl_2] in one CH_2 group did not practically change the action of the complex on maize root growth but decreased the effect on the growth and branching of cucumber roots (Figure 5 and Table 2). The substitution of two hydrogen atoms in one NH_2 group of [Pt(1,3-pn)Cl_2] on CH_3 groups resulted in a sharp decrease of growth-inhibiting activity for maize and cucumber roots. This effect was considerably higher than for the complex with en (Figures 3 and 5). The substitution of two hydrogen atoms of the second CH_2 group in [Pt(1,3-pn)Cl_2] on CH_3 groups did not practically change the growth-inhibiting activity of the complex (Figure 5 and Table 2).

Discussion

The obtained results show that substitution of hydrogen atoms of NH_2 and CH_2 groups in cancerostatic platinum complexes results in a decrease in their root growth-inhibiting activities. Analogical data were obtained in the studies of the action of substituted platinum complexes on tumors (Cleare & Hoeschele 1973, Cleare 1974, Beamont *et al.* 1976).

The antitumor effects of most of the complexes described in this paper were investigated earlier by Cleare & Hoeschele (1973) on Sarcoma 180 implanted in white mice. Exact comparison of these data and our results is difficult as the published data contain only the maximal therapeutic results for every compound which was obtained at different amounts of complex administered to animals. Nevertheless, it is clear that the substituted complexes require a higher concentration and as a rule produce a lower effect than [Pt(en)Cl_2]. This complex was found to be less active than DDP. Our data show similar results; however, we found some discrepancies of results for roots and tumors when the action of different substituted analogs of [Pt(en)Cl_2] was compared. Unlike in roots, the toxic levels of [Pt(men)Cl_2] and [Pt(*N*,*N*-dmen)Cl_2] for mice differ about 2-fold. The maximal effect of the first complex (the ratio of weights of tumors in treated and untreated animals, T/C) was lower (51%) than that of the latter (26%) when administered in a lower dose (15 and 30 mg kg^{-1} accordingly). The maximal effect of [Pt(en)Cl_2] was found to be 27% at a dose of 12 mg kg^{-1}. [Pt(men)Cl_2] and [Pt(*N*,*N*-dmen)Cl_2] inhibited the growth and branching of maize roots similarly, but the first complex inhibited the growth and branching of cucumber roots weaker than the last one. [Pt(*N*,*N'*-dmen)Cl_2] and [Pt(tmen)Cl_2] were found to act on tumors in a similar manner and appear considerably less active than [Pt(en)Cl_2]. However, the first complex inhibited root growth considerably stronger than [Pt(tmen)Cl_2]. [Pt(1,2-pn)Cl_2] and [Pt(1,3-pn)Cl_2] display similar antitumor effects (T/C = 62 and 58% at a dose of 12 mg kg^{-1}), but the latter complex inhibits maize root growth practically similarly to [Pt(en)Cl_2] and is more active than the former. The opposite situation occurred for cucumber roots: [Pt(1,3-pn)Cl_2] is less active than [Pt(en)Cl_2] and [Pt(1,2-pn)Cl_2].

It is necessary to carefully assess the influence of the substitution on antitumor activities of platinum complexes when only one type of tumor cell is used as a test system. For example, it has been shown (Bloemink *et al.* 1992) that the ID_{50} values (the drug concentration producing 50% inhibition of cell growth) of unsymmetric *cis*-DDP derivatives tested against human cell lines *in vitro* differed for various cell lines and the changes in the activity also varied for various lines. For instance, the ID_{50} values of [Pt$(NH_3)(NH(CH_3)_2)Cl_2$] and DDP for rhabdomyosarcoma cells differ about 2.8-fold (1118 and 402 ng ml^{-1} respectively) for melanoma cells they differ whereas 8.4-fold (3665 and 434 ng ml^{-1}, respectively). Various plant cells may also differ in their sensitivity to platinum complexes. Little is known about the relative sensitivity of various cells to a series of substituted analogs of cancerostatic platinum complexes. These results would be very important in elucidating the reasons for the changes of the biological activities of platinum complexes resulting from the different

substitution and for a more complete understanding of the role of the amine part of complexes.

The causative factors determining the changes of the biological activities of substituted analogs of $[Pt(en)Cl_2]$ can be different. The available literature has emphasized the close connection between the antitumor activity of complexes and their hydrogen-bonding potentials (Cleare & Hoeschele 1973, Cleare 1974, Beamont *et al.* 1976, Broomhead *et al.* 1980). Some facts may be interpreted as representing the formation of hydrogen bonds when platinum complexes interact with DNA; they show that the NH group in the amine is important for determining the total activity of the platinum compound (Cleare *et al.* 1978, Van Kralingen & Reedijk 1978, Ivanov *et al.* 1981a,b, Sherman *et al.* 1985,). Earlier, we reported the growth-inhibiting activity of DDP analogs of the type *cis*-$[PtNH_3LCl_2]$ (L = dmso, ethylene, pyridine, CH_3CN) (Ivanov *et al.* 1981b, Ivanov & Cheltsov 1991) containing only one ligand capable of hydrogen-bond donation. From this series of complexes, only $[Pt(NH_3)(py)Cl_2]$ demonstrated slight cytostatic activity on corn roots. All other complexes were found to be inactive at all concentrations, despite the very high reactivity of the complexes with dmso and C_2H_4 arising from the high *trans*-influence of these ligands. These complexes and their analogs did not exhibit antitumor activity (Ivanov *et al.* 1981b, Ivanov & Cheltsov 1991). On the other hand, the analog with the hydrogen-donating benzylamine ($L = C_6H_5CH_2NH_2$) clearly exhibits cytostatic activity. The antitumor and cytostatic activities of $[Pt(N,N\text{-dmen})Cl_2]$ observed in the present study are of special interest as a relatively rare example of an active complex with only one hydrogen-bond-donating NH_2 group. However, the minimum changes in the structure (substitution of en for 1,3-pn) were found to be sufficient to sharply reduce its activity.

Apart from the hydrogen-bonding ability of the amine ligands, the biological activity of complexes can be influenced by other factors, such as the rate of loss of the *cis*-chloro groups, steric hindrances exerted by methyl groups of substituted diamines in the reactions of ligand substitutions, cell permeability of these platinum complexes and the ability of DNA repair when linked to platinum complexes.

Our experiments have shown that the biological activity of the DDP solutions increased during the first day after dissolving the complex. NaCl reduces this effect. These results allow the suggestion that the products of the hydrolysis of DDP display a stronger growth inhibition than DDP. Also, they were absorbed by roots with a greater rate than DDP (Fok & Ivanov 1988). Because of this, the differences in the hydrolysis rate can influence the various biological activities of tested complexes. The hydrolysis rates of some complexes presently studied were published earlier (Cleare *et al.* 1978, Das Sarma *et al.* 1983).

Although results from different authors vary, it is evident that the hydrolysis rates do not appreciably vary with a change in amine—the maximum change in the rates under study was found to be less than a factor of 3. No direct relationship was found between the hydrolysis rates and the biological activities of various complexes. For example, the hydrolysis rate of $[Pt(en)Cl_2]$ is higher than DDP, although the growth-inhibiting activity of DDP is much higher. The hydrolysis rates of $[Pt(N,N'\text{-dmen})Cl_2]$ is higher than of DDP, $[Pt(men)Cl_2]$ and $[Pt(N,N\text{-dmen})Cl_2]$, although the biological activity of the first complex is significantly lower than other complexes. Hence the variations in the hydrolysis rates do not determine the differences in the growth-inhibiting activities of the investigated complexes. Similar conclusions are reached when the rates of reactions of complexes with dmso (Cleare *et al.* 1978, Das Sarma *et al.* 1983) are compared. The data obtained for similar compounds testifies that the steric hindrances exerted by methyl groups of substituted diamines determine the slower kinetics of ligand substitution on platinum (Inagaki *et al.* 1988, Arpalahti & Lippert 1990, Bloemink *et al.* 1992). In our experiments, the substitution of one or two hydrogen atoms of one CH_2 group in en results in a more significant decrease in growth-inhibiting activity than the substitution of hydrogen atoms in the NH_2 group. $[Pt(1,2\text{-pn})Cl_2]$ displays a significantly lower antitumor activity than $[Pt(en)Cl_2]$ (Cleare & Hoeschele 1973). Substitution of all hydrogen atoms of both CH_2 groups of en resulted in a sharp decrease of the growth-inhibiting activity. However, at very high concentrations $[Pt(C\text{-tmen})Cl_2]$ was found to completely inhibit cucumber root branching (in contrast to $[Pt(tmen)Cl_2]$). The antitumor activity of this complex on L-1210 tumors was very low when compared with the en analog (Tang *et al.* 1988). The close location of the fragments of $C(CH_3)_2$ to the NH_2 groups might produce a kinetic effect on ligand substitution and create steric hindrances for hydrogen-bond formation. The substitution of the two hydrogen atoms at C-2 in $[Pt(1,3\text{-pn})Cl_2]$ slightly increases the action of the complex on maize root growth and does not affect the inhibition of cucumber root growth and branching. In this case the fragment $C(CH_3)_2$ is distant from the NH_2 groups and cannot induce steric effects on hydrogen-bond formation. The antitumor activity of this complex is as yet unknown.

The antitumor activity of some platinum *cis*-diamine complexes is generally assumed to result from an interaction with cellular DNA. These aspects call for a great deal of attention. However, the reactions of complexes with other cellular components can be modified by the substitution of hydrogen atoms of NH_2 and CH_2 groups on CH_3 groups. The toxic effect of complexes for animals increases with the number of substituted hydrogen atoms (Cleare & Hoeschele 1973, Cleare 1974, Broomhead *et al.* 1980). The toxic level clearly is not determined only by the cytostatic effect of complexes. The nephrotoxic and gastrointestinal effects of complexes decrease as the number of substituted hydrogen atoms increases (Broomhead *et al.* 1980).

Equimolar ratios of Pt(II) complex to malate dehydrogenase, necessary to produce 50% enzyme inhibition, increased with the number of substituted hydrogen atoms in the amine part and were found to be equal to 1400 for DDP, 4300 for $[Pt(en)Cl_2]$, 5500 for $[Pt(eten)Cl_2]$, 7500 for $[Pt(etmeen)Cl_2]$ and 23000 for $[Pt(et(me)_3en)Cl_2]$ (Friedman *et al.* 1978). In our experiments (Figure 3), the concentrations of $[Pt(en)Cl_2]$ and $[Pt(tmen)Cl_2]$ or DDP and $[Pt(en)Cl_2]$ necessary for

the equal effects differ more significantly than in these experiments. However, a comparison of $[Pt(en)Cl_2]$ and $[Pt(men)Cl_2]$ produced similar results.

It will be of interest to study the influence of the substitution of hydrogen atoms on the ability of complexes to penetrate into the cell and the ability of cells to repair DNA linked to different complexes. Until now such data is missing from the literature for the presently studied complexes.

Clear differences were observed in the relative effects of complexes exerted on roots of different species. For example, all complexes with pn inhibited cucumber root growth weaker than that in maize. These discrepancies can be explained only on the basis of some relatively simple properties of complexes such as rate of hydrolysis, reactivity, etc. It seems likely that the compromise of the structural and chemical properties of complexes and peculiarities of different cell types determine the relative sensitivity of various cells. Similar examples may be found among animal cells. Drug concentrations for 50% inhibition of cell growth were found to be 271 ng ml^{-1} of Ptmea and 402 ng ml^{-1} of DDP (i.e. 1.5 times higher) for rhabdomyosarcoma, whereas the respective concentrations for bladder tumor were 970 and 381 (2.5 times lower) (Bloemink *et al.* 1992). Understanding the principles of these discrepancies is of great importance for the synthesis of new selective and effective cancerostatic complexes.

The activity discrimination by different species in our experiments and clearly different results of *N,N*-dimethylation, depending on the chelate ring size (en and the 1,3-pn derivative), on maize cannot be attributed to slower ligand-exchange kinetics arising from methylation. It has been assumed that an essential role in cytostatic activity of platinum complexes belongs to a cell repair system, i.e. the ability to eliminate platinum diamine fragments from DNA. This might well depend on the number and strength of hydrogen-bonds formed by *cis*-diamine fragment.

Acknowledgements

The authors are indebted to Dr E. L. M. Lempers for assistance with the synthesis of some of the platinum compounds. The authors wish to thank Johnson & Matthey (Reading, UK) for their generous loan of K_2PtCl_4. The authors have obtained a grant from INTAS to support and maintain the collaborative study (grant number INTAS 93-2850).

References

Arpalahti J, Lippert B. 1990 Coordination of aquated *cis*-platinum(II) diamines to purine nucleosides. Kinetics of complex formation. *Inorg Chem* **29**, 104–110.

Beamont KP, McAuliffe CA, Cleare MJ. 1976 Platinum complexes of substituted ethylenediamines and their anti-tumor activity. *Chem-Biol Interact* **14**, 179–193.

Bloemink MJ, Reedijk J. 1996 Cisplatin and derived anti-cancer drugs; mechanism and current status of DNA binding. In: Sigel H, Sigel A, eds. *Metal Ions in Biological Systems*. New York: Dekker; **32**: 641–685.

Bloemink MJ, Heetebrij RJ, Inagaki K, Kidani Y, Reedijk J. 1992 Reactions of unsymmetrically substituted derivatives of cisplatin with short oligodeoxynucleotides containing a -GpG- sequence: H-bonding interactions in pGpG moieties cross-linked by an asymmetric platinum complex enhancing the formation of one geometrical isomer. *Inorg Chem* **31**, 4656–4661.

Broomhead JA, Fairlie DP, Whitehouse MW. 1980 *cis*-Platinum(II) amine complexes: some structure–activity relationships for immunosuppressive, nephrotoxic and gastrointestinal (side) effects in rats. *Chem-Biol Interact* **31**, 113–132.

Chernyaev II, ed. 1964 *Syntheses of Platinum Metals Complexes*. Moscow: Nauka (in Russian).

Cleare MJ. 1974 Transition metal complexes in cancer chemotherapy. *Coord Chem Rev* **12**, 349–405.

Cleare MJ, Hoeschele JD. 1973 Studies on the antitumor activity of group VIII transition metal complexes. Part I. Platinum(II) complexes. *Bioinorg Chem* **2**, 187–210.

Cleare MJ, Hydes PC, Malerbi BW, Watkins DM. 1978 Anti-tumour platinum complexes: relationships between chemical properties and activity. *Biochimie* **60**, 835–850.

Das Sarma B, Daley SK, Elespuru RK. 1983 Platinum complexes with anticancer potential and their evaluation by a colorimetric λ prophage induction assay. *Chem-Biol Interact* **46**, 219–232.

Fok EM, Ivanov VB. 1988 Change of biological activity of solutions of *cis*-dichloro-diammineplatinum(II) and products of its hydrolysis with time. *Dokl Akad Nauk SSSR* **301**, 757–760 (in Russian).

Friedman MI, Melius P, McAuliffe CA, Teggins JE. 1978 Interactions of *cis*- and *trans*-platinum(II) complexes with dehydrogenase enzymes in the presence of different mono- and polynucleotides: evidence for a ternary complex. *Bioinorg Chem* **8**, 341–353.

Inagaki K, Dijt FJ, Lempers ELM, Reedijk J. 1988 Bulky ligand substituent effect on the reaction of 5'-GMP with Pt(1,3-Diamine). Rotation of 5'-GMP about the Pt–N bond and kinetic effects. *Inorg Chem* **27**, 382–387.

Ivanov VB. 1994 Root growth responses to chemicals. *Sov Scient Rev, Sect D (Physicochem Biol Rev)* **13**, part 2, 1–70.

Ivanov VB, Cheltsov PA. 1991 The effect of platinum complexes and other heavy metal salts on root growth. In: McMichael B, Person H, eds. *Plant Roots and Their Environment*. Dordrecht: Kluwer; 106–112.

Ivanov VB, Cheltsov PA, Shchelokov RN. 1976 Relationship between growth inhibition by platinum complexes and their structure. *Dokl Akad Nauk SSSR* **229**, 484–487 (in Russian).

Ivanov VB, Cheltsov PA, Shchelokov RN. 1981a Biological effect of platinum complexes with two different neutral ligands of type *cis*-$[PtNH_3LCl_2]$. *Izv Akad Nauk SSSR, Ser Biol* **N4**, 511 (in Russian).

Ivanov VB, Litinskaya TK, Cheltsov PA, Shchelokov RN. 1981b Biological effect of dichloro and chloronitrodiammine platinum(II) complexes. *Izv Akad Nauk SSSR, Ser Biol* **N1**, 104–114 (in Russian).

Ivanov VB, Bystrova EI, Dubrovskii IG. 1986 Cucumber seedlings as a test system for screening the efficient cytostatics. *Sov Plant Physiol* **33**, 158–162.

Ivanov VB, Bystrova EI, Larina LP. 1988 Growth-inhibiting effects of triamine and tetraamine complexes of platinum(II) containing purines and pyrimidines and their nucleosides. *Izv Akad Nauk SSSR, Ser Biol* **N5**, 746–751 (in Russian).2

Ivanov VB, Mogilevkina MF, Bystrova EI, Larina LP. 1989

Biological activity of methionine–platinum complexes: antitumor effect and inhibition of root growth by complexes of different composition and structure. *Izv Akad Nauk SSSR, Ser Biol* **N3**, 469–473 (in Russian).

Ivanov VB, Bystrova EI, Yakovlev KI, Rochkova ND, Stetsenko AI. 1992 Growth-inhibiting and cytostatic activities of binuclear triamine complexes of platinum(II) with heterocyclic amines. *Dokl Akad Nauk SSSR* **323**, 580–584 (in Russian).

Lippard SJ. 1987 Chemistry and molecular biology of platinum anticancer drugs. *Pure Appl Chem* **59**, 731–742.

Mattern IE, Cocchiarella L, van Kralingen CG, Lohman PHM. 1982 Prophage induction and mutagenicity of a series of anti-tumour platinum (II) and platinum (IV) co-ordination complexes. *Mutat Res* **95**, 79–93.

Reedijk J. 1992 The relevance of hydrogen bonding in the mechanism of action of platinum antitumor compounds. *Inorg Chim Acta* **198–200**, 873–881.

Sherman SE, Gibson D, Wang AH-J, Lippard SJ. 1985 X-ray structure of the major adduct of the anticancer drug cisplatin with DNA: *cis*-[$Pt(NH_3)_2${pGpG}]. *Science* **230**, 412–417.

Shevchenko VV, Grinikh LI, Cheltsov PA, Ivanov VB, Shchelokov RN. 1983 Relationship between the cytogenetic effect of platinum(II) complexes and their structure. *Mutat Res* **122**, 329–335.

Tang WX, Yun Q, Anbang D. 1988 Structure, activity and mode of action of antitumor platinum compounds. *Pure Appl Chem* **60**, 1271–1278.

Van Kralingen CG, Reedijk J. 1978 Antitumour activity of Pt(II) complexes with heterocyclic ligands. *Biochemie* **60**, 1057.