Cisplatin. Synthesis of some 2,3-diaminopropionic ester analogues: dichloro(hexadecyl 2,3-diaminopropionato)platinum(II) and dichloro(cyclohexyl 2,3-diaminopropionato)platinum(II)

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The title compounds were prepared from ethyl cyanoacetate by the four-step procedure of conversion into the corresponding 2-hydroxyimino-derivative, transesterification with either hexadecanol or cyclohexanol, catalytic hydrogenation of the respective products to afford the diamino-dihydrochlorides, and complex formation of the latter pair with potassium tetrachloroplatinate. Assignment of the structures of the *cis*-platinum complexes was confirmed by infrared spectrometry.

Die titelverbindings is vanaf etielsianoasetaat deur middel van 'n vierstapprosedure berei. Die uitgangstof is na die ooreenstemmende 2-hidroksi-imino-derivaat omgeskakel, wat dan met óf heksadekanol óf sikloheksanol getransesterifiseer is. Daaropvolgende katalitiese hidrogenering van die onderskeie produkte het die diamino-dihidrochloriede gelewer, wat met kalium-tetrachloroplatinaat na die gesogte komplekse omgeskakel is. Die strukture van die *cis*-platinumkomplekse is deur infrarooispektrometrie vasgestel.

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The platinum complex, dichlorodiammineplatinum(II) ('cisplatin') (**1**), is known[1] for its antitumour activity, although its toxicity detracts from its value as a chemotherapeutic drug in the treatment of cancer. Certain derivatives of the compound in which the two ammine groups form part of a 2,3-diaminopropanoic acid ligand have been synthesized.[2] While the acid (**2**) was inactive, the corresponding ethyl ester (**3**) showed some activity. In an attempt to improve on the activity of the system and at the same time reduce its toxicity, we have established a method to synthesize some alternative esters of the compound. This paper reports on the preparation of the derivatives (**4**) and (**5**).

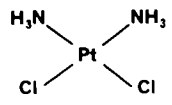
Results and Discussion

One method for the synthesis of ethyl 2,3-diaminopropionate dihydrochloride (**13**) is to convert ethyl cyanoacetate (**6**) into the 2-hydroxyimino-derivative (**7**)[3] followed by hydrogenation with Adam's catalyst to afford (**13**).[4] In order to obtain alternative ester derivatives, the hydroxyimino-ester (**7**) was transesterified using titanium tetra-isopropoxide in toluene containing an excess of the alcohol which is involved in the transesterification.[5] Ethanol was removed by azeotropic distillation with toluene, the volume of the latter solvent being kept constant by concurrent addition of more toluene. The transesterification of **7** was initially investigated with benzyl alcohol, the product (**8**) being obtained in a yield of 51%.

Transesterification was then accomplished with the alcohols, hexadecanol, cyclohexanol, and cholesterol, to form the related esters (**9**), (**10**), and (**11**) respectively, the yield of the latter (**11**) being much poorer than for the two former compounds. Amination of the ester could also be performed by this method to give rise to the amide (**12**). That such reactions had taken place was confirmed by ^{1}H n.m.r. spectrometry, the ethoxy-signals being replaced by those appropriate to the alcohol or amine concerned.

Transesterification of the ester (**7**) was also attempted with the primary alcohols, 2,3-isopropylidene glycerol (**17**) and 1,2,3,4-di-isopropylidene galactose (**18**), but the products could not be fully characterized as they were never obtained pure.

Subsequent hydrogenation of the two esters (**9**) and (**10**)



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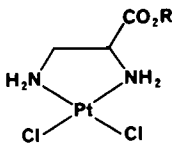
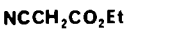


2 R = H
3 R = CH_2CH_3
4 R = $CH_2(CH_2)_{14}CH_3$
5 R = cyclohexyl



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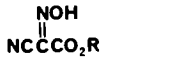
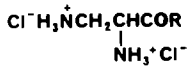
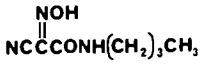


7 R = CH_2CH_3
8 R = CH_2Ph
9 R = $CH_2(CH_2)_{14}CH_3$
10 R = cyclohexyl
11 R = 3-(cholest-5-enyl)



13 R = OCH_2CH_3
14 R = $OCH_2(CH_2)_{14}CH_3$
15 R = O-cyclohexyl
16 R = $NH(CH_2)_3CH_3$



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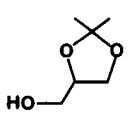

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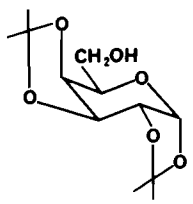

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and the amide (**12**) was achieved in the presence of palladium on carbon and hydrochloric acid, the products formed being the diamino-dihydrochlorides (**14**), (**15**), and (**16**) respectively. The appearance of the characteristic methylene and methine protons in the ^{1}H n.m.r. spectrum of each product confirmed the assignments.

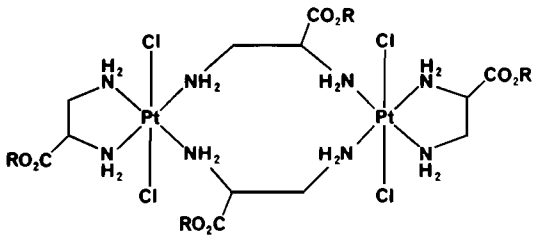
Of the three compounds (**14**), (**15**), and (**16**), attempts were made to convert only (**14**) and (**15**) into the platinum complexes (**4**) and (**5**), and each of these transformations occurred satisfactorily with potassium tetrachloroplatinate in water, in the presence of aqueous sodium hydroxide. The free bases liberated from **14** and **15** in the presence of sodium hydroxide are, like 1,2-diaminoethane, not expected to be able to span *trans*-positions in square planar platinum(II), and mononuclear *cis*-complexes are therefore expected. That the cream-coloured products (**4**) and (**5**) were the *cis*-compounds was established by two strong infrared absorptions in each case for the platinum–chlorine stretching frequencies (νPt–Cl). These appeared at 331 and 311 cm^{-1} in the case of the hexadecyl ester (**4**), and at 327 and 310 cm^{-1} for the cyclohexyl ester (**5**). To confirm the stereochemistry, the two platinum–nitrogen stretching frequencies were established through isotopic labelling of the amine hydrogens by deuterium exchange. These occurred at 426 cm^{-1} (406 cm^{-1} on labelling) and 397 cm^{-1} (382 cm^{-1} on labelling) in the case of the hexadecyl ester (**4**). For the cyclohexyl ester (**5**), the asymmetric platinum–nitrogen stretching frequency is hidden by a ligand band at 417 cm^{-1} and moves to 404 cm^{-1} on labelling, while the symmetric platinum–nitrogen stretching frequency is at 386 cm^{-1} (367 cm^{-1} on labelling).

In addition to the cream products (**4**) and (**5**), derived by reaction between (**14**) and (**15**) and potassium tetrachloroplatinate, a purple compound was isolated from each reaction. Microanalytical data corresponded to those of **4** or **5**, thereby confirming the same ratio of metal to ligand. These could be either the dinuclear complex (**19**), in which coordination about platinum(II) is octahedral, or the salt $[PtL_2][PtCl_4]$ (**20**). The lower solubility compared with **4** or **5** in, for instance, *N*,*N*-dimethylformamide, and the less intense infrared spectrum of the purple solid are consistent with both structures.

Two platinum–nitrogen stretching frequencies are theoretically required for both possible structures. Two are observed at 423 and 401 cm^{-1} in the spectrum of the hexadecyl ester complex, whereas, in the cyclohexyl ester complex, the antisymmetric component is masked by a ligand band at 417 cm^{-1} and the symmetric vibration gives rise to absorption at 387 cm^{-1}.

What establishes the structure of the purple solid as **20** rather than **19** is the position of the single νPt–Cl band, which is at 311 cm^{-1} [*i.e.* within the range 310–331 cm^{-1} established for νPt–Cl in the four-coordinate complexes (**4**) and (**5**)]. Six-coordinate Pt^{II}, as in **19**, would yield much lower values of νPt–Cl. The purple solid is therefore considered to be the salt $[PtL_2][PtCl_4]$. Whether this is one of the two possible isomers (**20a**) or (**20b**) or a mixture of both cannot be decided by infrared spectroscopy, and the insolubility of the salt does not permit an n.m.r. study. The purple compounds were not further investigated.

The two compounds (**4**) and (**5**) were tested by the US National Cancer Institute in the murine L1210 lymphoid leukemia system according to standard protocols.[6] The hexadecyl analogue was found to be inactive (T/C 98—



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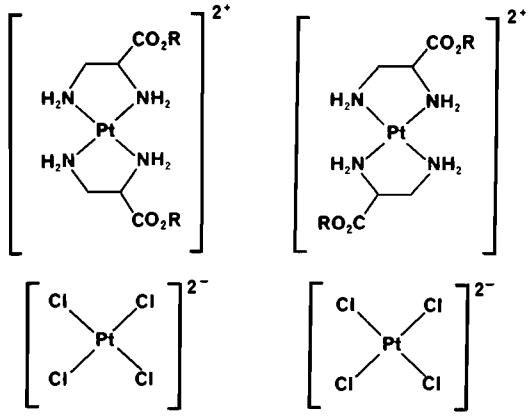
R = $CH_2(CH_2)_{14}CH_3$

R = cyclohexyl



20a **20b**

R = $CH_2(CH_2)_{14}CH_3$

R = cyclohexyl

107*) over a dose range of 3—200 mg kg^{-1}, but the cyclohexyl analogue exhibited confirmed activity ($T/C \geqslant 125$) at dose levels of 100 and 200 mg kg^{-1}, with the optimal dose being 200 mg kg^{-1} (T/C values of 260 and 180). The observed activity, however, does not match the potency exhibited by the clinically effective cisplatin.

Experimental

Unless otherwise stated, infrared spectra were obtained from Nujol mulls and n.m.r. spectra from solutions in deuteriochloroform with tetramethylsilane as internal reference. Where deuterium oxide was solvent, 3-(trimethylsilyl)propane sulphonic acid sodium salt was used as internal reference. Column chromatography was carried out on dry columns with Merck Kieselgel 60 (70—230 mesh) as adsorbent. Light petroleum refers to the fraction of b.p. 60—80°C. The phrase 'residue obtained upon work-up' refers to the residue when the organic layer was separated, dried ($MgSO_4$), and the solvent evaporated under reduced pressure.

Benzyl 2-cyano-2-hydroxyiminoethanoate (**8**)

The ester (**7**) (250 mg; 1,76 mmol) and benzyl alcohol (0,73 ml; 7,04 mmol) were dissolved in dry toluene (20 ml). Titanium tetra-isopropoxide (0,02 ml) was added and the solution was boiled for 30 min. Any ethanol produced was removed by azeotropic distillation of toluene while further toluene was added at such a rate that the volume of the

* T/C is the ratio of the median survival of the treated mice (T) to that of the controls (C) expressed as a percentage, and is the basis for an evaluation of a meaningful increase in life span of the treated mice.

solution was kept constant. As the R_F of the major component in the mixture was the same as starting material, boiling was continued overnight (16 h). The residue obtained upon work-up was chromatographed (eluent: 5—10% ethyl acetate in light petroleum). Earlier fractions afforded a minor unidentified compound, followed in later fractions by the *product* (**8**) (184 mg; 51%), m.p. 107—108°C (from toluene) (Found: C, 58,8; H, 3,9; N, 13,7. Calc. for $C_{10}H_8N_2O_3$: C, 58,6; H, 4,0; N, 13,8%); ν_{max} 3270 (OH) and 1730 (C:O) cm^{-1}; δ 5,37 (2H, s, CH_2), 7,2—7,55br (1H, s, OH), and 7,38 (5H, m, ArH); *m/z* 204 (M^+, 1%), 187 (12), 143 (12), 107 (80), and 91 (100). This product had the same R_F as starting material.

Hexadecyl 2-cyano-2-hydroxyiminoethanoate (**9**)

The ester (**7**) (3,00 g; 0,021 mol) and hexadecanol (10,25 g; 0,042 mol) in toluene (80 ml) containing titanium tetra-isopropoxide (0,3 ml) was azeotropically distilled as above for 5 h and then boiled for 16 h. The residue obtained upon evaporation was chromatographed (eluent: 20—25% ethyl acetate in light petroleum). The major component gave the *product* (**9**) (3,62 g; 56%), m.p. 80—82°C (light petroleum) (Found: C, 67,6; H, 10,05; N, 8,4. Calc. for $C_{19}H_{34}N_2O_3$: C, 67,45; H, 10,05; N, 8,3%); ν_{max} 3170 (OH) and 1733 (C:O) cm^{-1}; δ 0,80 (3H, t, *J* 6 Hz, CH_3), 1,15 (28H, sharp m, CH_2), 4,36 (2H, t, *J* 6 Hz, OCH_2), and 5,73br (1H, s, OH); *m/z* 321 (M^+−OH, 10%), 294 (6), 110 (16), 97 (38), 69 (62), 57 (90), and 43 (100).

Cyclohexyl 2-cyano-2-hydroxyiminoethanoate (**10**)

The ester (**7**) (3,00 g; 0,021 mol) and cyclohexanol (4,5 ml, 0,042 mol) in toluene (80 ml) containing the titanium alkoxide (0,3 ml) was distilled as above for 8 h, during which time the distillate (*ca* 100 ml) was collected. T.l.c. indicated some residual starting material, and the solution was boiled overnight (16 h). Distillation was repeated for a further 8 h. The residue obtained by evaporation was chromatographed (eluent: 40% ethyl acetate in light petroleum) to afford the *product* (**10**) (1,82 g; 44%), m.p. 99—100°C (from ether–light petroleum) (Found: C, 55,3; H, 6,15; N, 14,1. Calc. for $C_9H_{12}N_2O_3$: C, 55,1; H, 6,1; N, 14,3%); ν_{max} 3360 (OH), 1728, and 1711 (C:O) cm^{-1}; δ 1,0—2,7 (10H, m, CH_2), 5,13 (1H, m, CHO), and 9,60br (1H, s, OH); *m/z* 179 (M^+−OH, 3%), 99 (11), 83 (55), and 82 (100).

Cholest-5-en-3β-yl 2-cyano-2-hydroxyiminoethanoate (**11**)

The ester (**7**) (2,00 g; 0,0141 mol) and cholesterol (6,8 g; 0,0176 mol) in toluene (80 ml) containing the titanium alkoxide (0,3 ml) was azeotropically distilled as above for 6 h and then boiled for 16 h. Distillation was repeated for a further 7 h. The residue obtained by evaporation was chromatographed (eluent: 30—50% ethyl acetate in light petroleum) to give the *product* (**11**) (1,35 g; 15%), m.p. 165—167°C (from ether–light petroleum) (Found: C, 74,6; H, 9,45; N, 5,6. Calc. for $C_{30}H_{46}N_2O_3$: C, 74,7; H, 9,55; N, 5,8%); ν_{max} 3370 (OH), 1730, and 1719 (C:O) cm^{-1}; δ 0,45—2,9 (43H, m, cholesteryl H), 4,93 (1H, m, CHO), 5,60 (1H, m, C:CH), and 7,60br (1H, s, OH); *m/z* 482 (M^+, 3%), 385 (42), and 368 (100).

N-*Butyl 2-cyano-2-hydroxyiminoethanamide* (**12**)

The ester (**7**) (2,00 g; 0,014 mol) was dissolved in n-butylamine (50 ml), titanium tetra-isopropoxide was added, and the solution was boiled for 5 h. T.l.c. indicated no starting material. The solution was poured into water, which was acidified with dilute hydrochloric acid and extracted with ether (4 × 100 ml). The organic layer was washed with water and the residue obtained upon work-up afforded the *product* (**12**) (1,42 g; 60%), m.p. 122—124°C (from ether–light petroleum) (Found: C, 49,5; H, 6,6; N, 24,6. Calc. for $C_7H_{11}N_3O_2$: C, 49,7; H, 6,5; N, 24,85%); ν_{max} 3378 (s), 3210 and 3080 (OH and NH), and 1645 (C:O) cm^{-1}; δ 0,96 (3H, distorted t, *J* 6 Hz, CH_3), 0,8—2,0 (4H, m, 2- and 3-CH_2), 3,2 (2H, q, *J* 6 Hz, 1-CH_2), and 8,53 (1H, m, NH, slowly D_2O exchangeable); *m/z* 170 (M^+ + 1, 9%), 169 (M^+, 7), 152 (16), 126 (100), and 110 (37).

Hexadecyl 2,3-diaminopropanoate dihydrochloride (**14**)

The ester (**9**) (1,00 g; 2,95 mmol) was dissolved in absolute ethanol (60 ml) containing conc. hydrochloric acid (0,65 ml; 6,5 mmol) and 10% palladium on carbon (0,2 g). The mixture was hydrogenated at a pressure of 4 atm. for 16 h. The reaction mixture was filtered, and the filtrate was concentrated and chilled in ice, whereupon crystallization of the *product* (**14**) occurred. Ethanol and a little water were added to the residue from the above filtration to dissolve product which was mixed with the catalyst. This was filtered hot, and the cooled filtrate was concentrated to afford more product, the combined yield being 0,71 g (60%). A small portion was recrystallized for analysis, m.p. 175—177°C decomp. (from ethanol) (Found: C, 57,0; H, 10,25; N, 7,0. Calc. for $C_{19}H_{42}Cl_2N_2O_2$: C, 56,85; H, 10,5; N, 7,0%); ν_{max} 1750 (C:O) cm^{-1}; δ 0,86 (3H, distorted t, *J* 6 Hz, CH_3), 1,22 (28H, sharp m, CH_2), 3,30 (2H, d, *J* 6 Hz, CH_2N), 4,12 (2H, t, *J* 6 Hz, CH_2O), and 4,23 (1H, t, *J* 6 Hz, CHN).

Cyclohexyl 2,3-diaminopropanoate dihydrochloride (**15**)

The ester (**10**) (3,00 g; 15,3 mmol) was dissolved in absolute ethanol (100 ml) containing conc. hydrochloric acid (3,35 ml; 33,5 mmol) and 10% palladium–charcoal (0,6 g). The mixture was hydrogenated at 4 atm. for 40 h. The reaction mixture was diluted with water and filtered hot. The filtrate was reduced in volume, chilled in ice, and filtered to yield the *product* (**15**) (2,05 g; 51%), m.p. 211—215°C decomp. (Found: C, 41,7; H, 7,6; N, 10,75. Calc. for $C_9H_{20}Cl_2N_2O_2$: C, 41,7; H, 7,7; N, 10,8%); ν_{max} 1740 (C:O) cm^{-1}; δ (D_2O) 1,1—2,2 (10H, m, CH_2), 3,66 (2H, d, *J* 7 Hz, CH_2N), 4,53 (1H, t, *J* 7 Hz, CHN), and 4,92 (1H, m, CHO).

N-*Butyl 2,3-diaminopropanamide dihydrochloride* (**16**)

The amide (**12**) (250 mg; 1,48 mmol) was dissolved in absolute ethanol (35 ml) containing the above catalyst (100 mg) and acid (0,35 ml; 3,5 mmol), and hydrogenated at 4 atm. for 40 h. The reaction mixture was filtered and the filtrate evaporated to dryness. The crude material (250 mg) was recrystallized to give the *product* (**16**) (40 mg; 10%, yield not optimized), m.p. 188—190°C (from methanol) (Found: C, 35,95; H, 8,1; N, 18,1. Calc. for $C_7H_{19}Cl_2N_3O$: C, 36,2; H, 8,2; N, 18,1%); ν_{max} 3340 (NH) and 1690 (C:O) cm^{-1}; δ (DMSO-d_6) 0,88 (3H, distorted t, *J* 7 Hz, CH_3), 1,05—1,65 (4H, m, 2- and 3-CH_2), 2,85—3,7 (4H, m, 2 × CH_2N), 4,23 (1H, t, *J* 6 Hz, CHN), and 8,25—9,35 (7H, m, NH).

Dichloro(hexadecyl 2,3-diaminopropionato)platinum(II) (**4**)

A solution of potassium chloroplatinate (1,24 g; 2,99 mmol)

in water (150 ml) was added to a solution of the ester (**14**) (1,20 g; 2,99 mmol) in ethanol (350 ml). A solution of sodium hydroxide (0,24 g; 6 mmol) in water (50 ml) was added dropwise with stirring over a period of 20 h (overnight). The resultant precipitate was collected by filtration and added to hot dimethylformamide. A purple precipitate formed and was removed by filtration, washed well with ethanol, and dried under vacuum over silica gel. On addition of water to the dimethylformamide solution, a yellow precipitate formed, which was collected by filtration, washed well with ethanol, and dried as above to give the cis-*complex* (**4**) (1,12 g; 63%), m.p. 273°C decomp. (Found: C, 38,65; H, 6,8; N, 4,8. Calc. for $C_{19}H_{40}Cl_2N_2O_2Pt$: C, 38,4; H, 6,8; N, 4,7%); ν_{max} 1740 (C:O), 331(s) and 311(s) (*cis* Pt–Cl), and 426(w) and 397(w) (*cis* Pt–N) cm^{-1}. The purple precipitate was considered to be the salt (**20**, R = hexadecyl) (0,39 g; 22%), m.p. 235°C decomp. (Found: C, 39,8; H, 7,0; N, 4,9. Calc. for $C_{19}H_{40}Cl_2N_2O_2Pt$: C, 38,4; H, 6,8; N, 4,7%); ν_{max} 1744 (C:O), 311(s) (*trans* Pt–Cl), and 423(w) and 401(w) (Pt–N) cm^{-1}.

Dichloro(cyclohexyl 2,3-diaminopropionato)platinum(II) (**5**)

A solution of potassium chloroplatinate (2,50 g; 6,02 mmol) in water (100 ml) was added to a solution of the ester (**15**) (1,56 g; 6,02 mmol) in aqueous ethanol (1:1; 150 ml). A solution of sodium hydroxide (0,48 g; 12 mmol) in water (50 ml) was added dropwise with stirring over a period of 20 h. Work-up as above provided the cis-*complex* (**5**) (1,07 g; 39%), m.p. 325°C decomp. (Found: C, 24,1; H, 4,0; N, 6,3. Calc. for $C_9H_{18}Cl_2N_2O_2Pt$: C, 23,9; H, 4,0; N, 6,2%); ν_{max} 1736 (C:O), 327(s) and 310(s) (*cis* Pt–Cl), and 417 (masked) and 386 (*cis* Pt–N) cm^{-1}. The purple precipitate was considered to be the salt (**20**, R = cyclohexyl) (0,91 g; 33%), m.p. 269°C decomp. (Found: C, 24,15; H, 4,1; N, 6,2. Calc. for $C_9H_{18}Cl_2N_2O_2Pt$: C, 23,9; H, 4,0; N, 6,2%); ν_{max} 1736 (C:O), 311(s) (*trans* Pt–Cl), and 417 (masked) and 387 (Pt–N) cm^{-1}.

Acknowledgements

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