Syntheses and Anti-inflammatory Action of $(\eta\text{-}C_5H_5)_2Ti(Cin)_2$ and $(\eta\text{-}C_5H_5)_2Ti(Tzea)_2$

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Two new complexes $(Cp)_2Ti(Cin)_2$ and $(Cp)_2Ti(Tzea)_2$ (Cp = Cyclopentadienyl η^5-C_5H_5) have been synthesized in THF by the reaction of HCin (Cincofen, 2-phenylquinoline-4-carboxylic acid) or HTzea (5-phenyltetrazolyl-2-ethanoic acid) with $(Cp)_2TiCl_2$, and characterized by elemental analyses, IR, ^1H NMR and ^{13}C NMR, UV spectra, molar conductivity, TG-DTA. In the complexes the carboxyl groups are coordinated to Ti(IV) in a monodentate manner. The inhibitory actions of the complexes on mice ear tumefaction caused by croton oil and the rat foot granulation growth produced by cotton wool are higher than those of the corresponding ligands HCin, HTzea and $[(Cp)_2TiCl_2]$, while their toxicities are lower than those of the free ligands.

Keywords cyclopentadienyltitanium, complexes, anti-inflammatory

Introduction

Since Köpf discovered that dicyclopentadienyltitanium dichloride exhibited antitumour action in 1979,[1] a large number of cyclopentadienyltitanium complexes bearing different substituents have been synthesized.[2,3] The experimental data revealed that $(Cp)_2TiX_2$ (X = halogen, pseudohalogen or carboxyl) complexes have fairly strong antitimour, antiviral and anti-inflammatory action. Their toxicity and side effects are also fairly low.[4] In this paper it was reported that 2-phenylquinoline-4-carboxylic acid (HCin) and 5-phenyl-tetrazolyl-2-ethanoic acid (HTzea) having anti-inflammatory action as ligands reacted with dicyclopentadienyltitanium dichloride in anhydrous THF in the presence of triethylamine to give two new solid ternary organometallic complexes. Their chemical compositions and structures were studied, and confirmed as $[(Cp)_2Ti\text{-}(Cin)_2]$ (**A**) and $[(Cp)_2Ti\text{-}(Tzea)_2]$ (**B**) in which the carboxyl groups were coordinated to titanium ion in a monodentate mode shown in Fig.1. The inhibition of the mice ear tumefaction caused by croton oil and the rat foot granulation growth produced by cotton wool have been studied as well. The anti-inflammatory actions of the two new complexes and their toxicities are better than those of the corresponding free ligands. Especially, the median lethal dose (LD_{50}) of $(Cp)_2Ti(Tzea)_2$ is 252.24 mg/kg which is 2.21 times higher than that of corresponding ligand HTzea, which is 114.17 mg/kg.

Experimental

Reagents

All chemicals were of analytical grade and used with further purification. Benzene and petroleum ether were dried over anhydrous $CaCl_2$ for 24 h, and THF was dried with KOH. These solvents were then refluxed with sodium diphenylketone until a stable blue-purple solution was formed. The Et_3N was dried with KOH.

Mixed reagent of croton oil producing inflammatory disease consists of 2% croton oil, 20% EtOH, 5% H_2O and 73% Et_2O. Croton oil was provided by Pharmacology Laboratory, Lanzhou Medical College. The mice and rats for experiment were purchased from the Animal Laboratory, Lanzhou Medical College.

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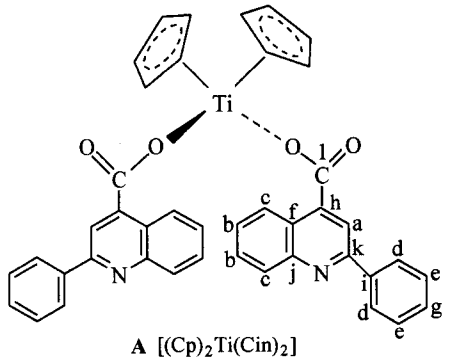
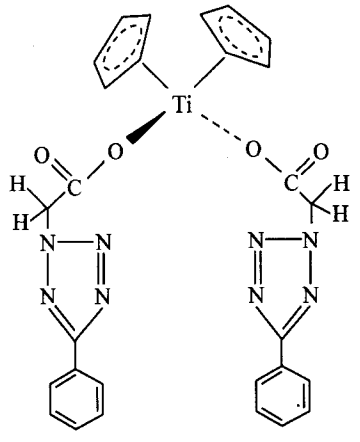


A $[(Cp)_2Ti(Cin)_2]$



B $[(Cp)_2Ti(Tzea)_2]$

Fig. 1 Proposed structure of the complexes.

Preparation of ligands

$(Cp)_2TiCl_2$ was prepared by the literature method.[5] The product is red crystals, yield 80%, m.p. 285 ℃; R_f 0.90; Molar conductance (Λ_m) 0.27 $s \cdot cm^2 \cdot mol^{-1}$; UV-vis ($CHCl_3$) λ_{max}: 255.2 (1.822) nm; ^1H NMR ($CDCl_3$) δ: 6.60 (s, 5H, η^5-C_5H_5); IR (KBr) ν: 3101, 1440, 1019, 819 (η^5-C_5H_5), 368 (Ti – Cl) cm^{-1}; Anal. calcd for $C_{10}H_{10}Cl_2Ti$: C 48.2, H 4.05, Cl 28.5, Ti 19.2; found C 48.2, H 4.03, Cl 28.1, Ti 19.2.

HCin was purchased from Xinxin Pharmaceutical Factory of Tianjin and recrystallized from ethanol; yield 90%, m.p. 216 ℃ (lit.[5] 2.8 ℃); UV-vis ($CHCl_3$) λ_{max}: 267.4 (1.739), 341.4 (0.524) nm; ^1H NMR ($CDCl_3$) δ: 7.2—9.0 (m, 10H), 8.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 119.6 (C_a), 123.9 (C_b), 125.7 (C_c), 127.2 (C_d), 128.8 (C_e), 129.7 (C_f), 129.9 (C_g), 137.1 ($C_{h,I}$), 138.3 ($C_{h,I}$), 148.8 (C_j), 156.0 (C_k), 167.7 (C_l); IR (KBr) ν: 1710, 932 (C = O), 3050, 1580, 1541 (quinoly group) cm^{-1}; Anal. calcd for $C_{16}H_{11}N_1O_2$: C 77.1, H 4.45, N 5.62; found C 76.9, H 4.52, N 5.52.

HTzea was synthesized according to the following procedure. HTz (5-phenyl-2-hydrogen-tetrazole) was prepared by literature method.[6] Equimolar mixture of HTz and sodium bromoacetate ($NaOOCCH_2Br$) was heated under reflux in anhydrous EtOH. After adding an equimolar sodium ethylate, a white precipitate was produced. The mixture was refluxed about 3 h and then filtered. The filtrate was distilled under reduced pressure to remove part of solvent, then stored over night, filtered to isolate the white precipitate. An excess of dilute HCl acid (0.1 mol/L) was added to the precipitate under stirring, then filtered. The precipitate was dried in a vacuum desiccator with P_2O_5 to constant weight; yield 80%; m.p. 180—181 ℃ (lit.[6] 181 ℃); UV-vis ($CHCl_3$) λ_{max}: 243.8 (1.702), 275.6 (0.194), 283.2 (0.154) nm; ^1H NMR ($CDCl_3$) δ: 2.84 (s, 2H, CH_2), 7.3—8.3 (m, 5H, ArH), 10.96 (s, 1H, $-CO_2H$); IR (KBr) ν: 1709.5 (C = O), 1674, 1530, 1451 (tetrazolyl group) cm^{-1}; Anal. calcd for $C_9H_8N_4O_2$: C 52.9, H 3.95, N 27.4; found C 52.8, H 3.74, N 27.5.

Preparation of complexes

HCin (Htzea) and $[(Cp)_2TiCl_2]$ were dissolved in anhydrous THF (the molar ratio of HCin with $[(Cp)_2TiCl_2]$ is 2∶1), forming a clear deep red solution. Et_3N was then added under nitrogen atmosphere. The mixture was stirred for 6 h at 40 ℃. The orange solution was filtered, then the filtrate was distilled under reduced pressure to remove the solvent. The orange solid obtained was recrystallized using THF-petroleum ether (volume ratio = 1∶1) as solvent, and dried in a vacuum desiccator to constant weight.

$[(Cp)_2Ti(Cin)_2]$ (**A**) Yield 75%; R_f 0.73; Molar conductance (Λ_m) 5.41 $s \cdot cm^2 \cdot mol^{-1}$; UV-vis ($CHCl_3$) λ_{max}: 263.4 (1.445), 338.4 (0.431) nm; ^1H NMR($CDCl_3$) δ: 6.84 (s, 10H, η^5-C_5H_5), 6.1—8.0 (m, 20H, ArH); ^{13}C NMR ($CDCl_3$) δ: 119.2 (C_a), 124.1 (C_b), 125.6 (C_c), 126.9 (C_d), 127.5 (C_e), 128.7(C_f), 129.7 (C_g), 136.7 ($C_{h,I}$), 138.5 ($C_{h,I}$), 149.9 (C_j), 156.9(C_k), 175.8 (C_l); IR (KBr) ν:

1646 (ν_{as}), 1391 (ν_s), 3105, 421, 1027, 805 (η^5-C_5H_5), 3053, 1582, 1541 (quinolyl), 567 (Ti－O) cm^{-1}; Anal. calcd for $C_{42}H_{30}N_2O_4Ti$: C 74.77, H 4.49, N 4.15, Ti 7.10; found C 74.74, H 4.60, N 4.13, Ti 7.36.

$[(Cp)_2Ti(Tzea)_2]$ Yield 75%; R_f 0.51; Molar conductance (Λ_m) 6.29 s · cm^2 · mol^{-1}; UV-vis ($CHCl_3$) λ_{max}: 244.0 (1.813), 272.0 (0.131), 283.0 (0.156) nm; ^1H NMR($CDCl_3$) δ: 3.00 (s, 4H, CH_2), 6.44 (s, 10H, η^5-C_5H_5), 7.1—8.0 (m, 20H, ArH); IR (KBr) ν: 1611 (ν_{as}), 1360 (ν_s), 3108, 1401, 1025, 823 (η^5-C_5H_5), 1674, 1529, 1449 (tetrazolyl), 592 (Ti－O) cm^{-1}; Anal. calcd for $C_{28}H_{24}N_8O_4Ti$: C 57.5, H 4.14, N 19.2, Ti 8.20; found C 57.6, H 4.19, N 18.9, Ti 8.24.

Chemical and physical analyses

Titanium was determined by gravimetric method.[7] C, H, N analyses were carried out using a Carlo-Erba 1106 elemental analyzer. Molar conductivity Λ_m was measured in 1.5×10^{-4} mol/L in acetone solution at 10 ℃ using a DDS-IIA conductometer. IR spectra were taken on a Nicolet 170SX IR spectrometer (KBr discs) in the range of 5000—200 cm^{-1}. The UV spectra of the complexes were recorded on a DJ-240 UV-vis spectrophotometer using chloroform as solvent (1.5×10^{-4} mol/L). The ^1H NMR spectra were recorded on a Bruker AC-80 NMR spectrometer and ^{13}C NMR spectra were recorded on a Bruker AM-400 NMR spectrometer, using $CDCl_3$ as solvent and TMS as an internal standard. TG-DTA were monitored on a Dupont 1090 thermo-gravimetric analyser.

Median lethal dose (LD_{50})

Mice (Kunming species) weighing 25—30 g were selected as experimental animals. The median lethal dose (LD_{50}) of the complex and ligand was determined according to the literature.[8]

Anti-inflammatory action

$[(Cp)_2TiCl_2]$, HCin, HTzea, $[(Cp)_2Ti(Cin)_2]$ and $[(Cp)_2Ti(Tzea)_2]$ were added to physiological saline containing a little Tween-80, then grounded to homogeneous suspension. The suspension was diluted with physiological saline to a concentration of 5.0%.

Method I was used to investigate the effect of drug on the mice ear tumefaction caused by croton oil: sixty male mice (Kunming species), weighing 30—35 g, were divided stochastically into 6 groups, ten in each group. Physiological saline, $[(Cp)_2TiCl_2]$, HCin, HTzea, $[(Cp)_2Ti(Cin)_2]$ and $[(Cp)_2Ti(Tzea)_2]$ were injected into the abdominal cavities of mice in each group; 2 h later, a mixed reagent of croton oil was painted on the left ear, and 5 h later, all mice were killed. The swollen ears were measured and weighed, so that swollen extent and inhibition ratios were calculated.[8]

Method II was used to study the effect of the complexes on the rat foot granulation growth produced by cotton wool: in the same way six groups of rats weighing 120—150 g were taken. Cotton wool, about 20 mg in weight, was planted in the feet of the rats; 2 h later, the drugs were injected into abdominal cavities of rats. After 7 days, the rats were killed. The granulation was dried at 60 ℃ for 12 h and weighed, and the inhibition ratio was calculated.[8]

Results and discussion

The elemental analyses and some physical properties of ligands and complexes are showed that the complex has chemical composition of dicyclopentadienyl titanium dicarboxylate. All complexes are soluble in organic solvents, such as acetone, benzene, chloroform, THF, DMF, DMSO *etc.*, but they are not soluble in water and petroleum ether. Their fairly low molar conductivities (Λ_m) in acetone solution (2×10^{-4} mol/L) show that they exist as non-electrolytes at 10 ℃. With mixed solvents of acetone/benzene ($V:V = 1:1$) as developing agent, these compounds were developed on the thin layer plates of GF 254 silica gel. The R_f values of the complexes reveal that their polarities are higher than that of $(Cp)_2Ti(IV)Cl_2$.

IR *spectra*

The characteristic absorption peaks [ν(C = O), 1710, 1709 cm^{-1}] of carboxyl groups in HCin and HTzea disappeared from the complexes. However, the characteristic absorption peaks appeared at 1646 (or 1611) and 1391(or 1360) cm^{-1}, which can be attributed to asymmetrical stretching vibration $\nu_{as}(CO_2^-)$ and symmetrical stretching vibration $\nu_s(CO_2^-)$ of the carboxyl groups re-

spectively. The differences ($\Delta\nu$) between ν_{as} and ν_s are 255 cm^{-1} and 251 cm^{-1}, which revealed that the carboxyl groups of ligands are coordinated to Ti(IV) ion in a monodentate form.[9] Secondly, two new synthesized complexes still retained the characteristic absorption peak of cyclopentadienyl group, but shifted slightly. This illustrated that the cyclopentadienyl titanim group is present, but it suffers the effect of carboxyl group to a certain extent. The IR spectrum of $[(Cp)_2Ti(Cin)_2]$ exhibits the characteristic bands of the quinolyl group (C_9H_5N) at 1582 ($\nu_{C=C}$), 1541($\nu_{C=N}$) and 3053(ν_{C-H}) cm^{-1}. The characteristic peaks of the tetrazolyl group of $[(Cp)_2Ti(Tzea)_2]$ are at 1674, 1529 and 1449 cm^{-1}, which are similar to those of the ligands HCin and Htzea respectively. These data illustrated that the Ti(IV) only coordinated with carboxyl group, while the nitrogen atoms did not take part in coordination. The characteristic peak [ν(Ti-Cl)] of $[(Cp)_2TiCl_2]$ at 368 cm^{-1} disappeared after coordination. The stretching vibration peaks for Ti – O in the complexes occured at 567 and 592 cm^{-1}, which revealed that the oxygen atom of carboxyl group coordinated to Ti(IV).

^{1}H NMR *and* ^{13}C NMR *spectra*

Compared with the ligands HCin, HTzea and $[(Cp)_2TiCl_2]$, the chemical shifts of η-C_5H_5 changed in the complexes, peaks presenting carboxyl groups in Hcin and HTzea vanished, and the chemical shifts of the quinolyl group and the phenyl group in complex moved towards low field. These indicated that the oxygen atoms of carboxyl groups coordinated to Ti(IV), substituting chloride to form the complex. The chemical shift data of ^{13}C NMR of HCin and $[(Cp)_2Ti(Cin)_2]$ revealed that the chemical shift of carbon atom (C_1) in complex moved δ 8.1 towards lower field as compared with the free ligand. And the chemical shifts of other carbon atoms (C_a – C_k) are no more than δ 2, which is identical with the coordination of ordinary carboxylic acids. Also, the insignificant chemical shifts of C_j and C_k showed that the N atom between them did not have coordination of Ti(IV). The single peak of η-C_5H_5 radical appeared at δ 120.6, showing five carbon atoms are equivalent (Fig. 1).

UV *Spectra*

The UV data of different compounds showed that after forming complex the transition absorption peaks (267.4 and 341.4 nm) of π-π^* and n-π^* of Hcin all caused blue shifts (to 263.4 and 338.4 nm), which was the effect of coordination on molecular orbital energy level. In $[(Cp)_2Ti(Tzea)_2]$, the above shifts did not occur due to the very stable conjugated system of the tetrazolyl group in ligand, and the coordination of oxygen atom in carboxyl group was difficult to vary the orbital energy level apparently.

TG-DTA

Thermograms of TG-DTA revealed that the complexes decomposed before melting at 110 ℃, then burned. The TG shows obvious mass loss and there exist exothermic peaks in the DTA. Above 550 ℃, the complex burned completely, and the weight tended to a constant value. The residual weight obtained from the TG curve cooresponds to the content of TiO_2 in the complex, which indicated the residue is TiO_2.

Table 1 Toxicity and anti-inflammatory action of compounds

Compound	Method I			Method II		
	Dose (mg/kg)	Swollen extent (mg)[a]	Inhibition ratio (%)	Wt of granulation (mg)[b]	Inhibition ratio (%)	LD_{50} (mg/kg)
Physiolog. saline	50.0	14.8 ± 3.1		28.5 ± 8.2		
$(Cp)_2TiCl_2$	500.0	7.4 ± 4.1	51.8	23.3 ± 7.8	28.9	326.67
Hcin	500.0	6.8 ± 2.5	54.2	18.6 ± 7.3	35.2	96.88
$(Cp)_2TiCin)_2$	500.0	6.1 ± 2.8	64.5	16.2 ± 6.7	42.3	171.13
Htzea	500.0	8.2 ± 3.2	48.4	19.8 ± 7.4	32.7	114.17
$(Cp)_2TiTzea)_2$	500.0	6.3 ± 3.5	62.7	14.9 ± 6.1	51.1	252.24

[a] Average of mice ear weight ± standard deviation, probability less than 0.05.

[b] Average of rats granulation weight ± standard deviation, $p < 0.05$.

Anti-inflammatory action and toxicity

The data of anti-inflammatory action and LD_{50} are presented in Table 1, which showed that the toxicityies of complexes are apparently lower than those of corresponding ligands Hcin and Htzea, and the anti-inflammatory action of the former is higher than that of the latter. The course is that the very low toxic cyclopentadienyltitanium group in complex hydrolyzes to polynuclear polymer *in vivo*, so the release velocities and the toxicities of Cin or Tzea decrease.[10] Secondarily, cyclopentadienyl titanium group has pharmacological actions, such as anti-inflammatory action *et al*. The synergic action between cyclopentadienyltitanium group and the free ligand having anti-inflammatory action, makes the complex increasing the anti-inflammatory action.

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