Preparation and antiinflammatory action of thiocarbazone derivatives and their complexes with Cu(II) and Au(III)

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5-Nitro-2-furfural thiosemicarbazone, 2-acetylpyridine thiocarbazone (HApt), and their complexes with Cu(II) and Au(III) have been prepared and characterized by elemental analyses, IR, UV, conductivity measurements, and thermal analyses. The complexes are nonelectrolytes in methanol and dimethylformamide. Furthermore, the complex $Cu(HApt)Cl_2 \cdot H_2O$ was found to be biologically active as demonstrated by antiinflammatory test.

The bactericidal, antitumour, and antivirus actions of 5-nitro-2-furfural thiosemicarbazone compounds have been reported [1—3]. Recently, some experiments [4—6] show that their biological activity and pharmacological action are closely related to the chelation with metal ions. In this work four complexes of 5-nitro-2-furfural thiosemicarbazone (HNft) and 2-acetylpyridine thiocarbazone (HApt) with Cu(II) and Au(III) were synthesized. The structure and properties of these complexes were elucidated by elemental analyses, IR, UV, molar conductance, and thermal analysis data. Their antiinflammatory action was examined, too.

Experimental

Human serum gamma globulin (98%) was made in Hong Kong and diluted to an aqueous solution (5 mg cm^{-3}). The Hasting—Sendroy buffer solution was the mixture of 0.06 M-Na_2HPO_4 (80 cm^3) and 0.06 M-KH_2PO_4 (19.2 cm^3), and its pH = 7.40. Ox serum globulin was electrophoretically pure. The chemical reagents were anal. grade. The ligands were prepared by the methods described in literature [7, 8]. Albino rats were of Kunming species.

The elements C, H, N were analyzed by a C. Erba elemental analyzer, model 1106. The content of copper was determined by a complexometric titration using EDTA, and that of Au by photometric titration with HApt as a developer [9]. The chlorine was analyzed by a potentiometric method. IR spectra were measured with a Nicolet 170 SX spectrophotometer using KBr discs and sodium chloride optics. UV spectra were recorded on a Shimazu-240 spectrometer. Thermogravimetry (TG) and differential thermal

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analysis (DTA) were carried out by a microthermoanalyzer Thermoflex (Japan), and molar conductances of the complexes were obtained using a conductometer DDS-IIA (China) in methanol or dimethylformamide.

Complex Cu(Nft)Cl *(I)*

To a solution of 0.5 g of HNft in 20 cm^3 of THF aqueous solution of 0.2 M-$CuCl_2$ (50 cm^3) was added dropwise at 40—50 °C. A dark brown precipitate was produced. The mixture was stirred continuously for 1 h, filtered, washed with water until it was free of Cl^-, and dried.

Complex $Au(HNft)_2Cl_3$ *(II)*

The mixture of HNft (0.5 g) and $AuCl_3$ (0.3 g) in THF (20 cm^3) was heated under reflux for 7 h, concentrated to 10 cm^3 under reducing and set down. The red precipitate thus obtained was collected on a filter, washed with water until it was free of Cl^-, and dried.

Complexes $Cu(HApt)Cl_2\ \ H_2O$ *(III) and* $Au(HApt)_2Cl_3$ *(IV)*

HApt and $CuCl_2$ or $AuCl_3$ were solved in a small amount of methanol in the mole ratio $CuCl_2$ (or $AuCl_3$) : HApt = 1 : 2 (or 3) and left to react for 2 h under reflux. The precipitate produced on setting down and cooling was filtered, washed with ethanol to be free of Cl^-, and dried.

Antiinflammatory tests

When determining the inhibitory action on heat denaturation of human serum gamma globulin, the complexes were ground in the presence of surfactant Tween-80 and then diluted to 10^{-3} mol cm^{-3} suspension. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mm^3 of the suspension were taken in ten centrifuge tubes, respectively, and 1 cm^3 of human serum globulin with the concentration 5.0 mg cm^{-3} was added. Then it was diluted to 2 cm^3 with the Hasting—Sendroy buffer and shaken for 10 min in water bath at 90 °C. 5.0 cm^3 of glacial acetic acid was added for cooling, the mixture was shaken to homogenization, separated by centrifugation after setting down for 15 min, the supernatant layer was poured out, and the precipitate was washed three times with the buffer. Then 1.0 cm^3 of buffer and biuret reagent were added. After 30 min their absorbance at $\lambda = 540$ nm is determined and the content and denaturation ratio of protein is calculated

$$\text{Denaturation ratio} = \frac{\text{Amount of denatured protein}}{\text{Amount of protein added}}$$

Inhibitory action on the ear edema of albino rats caused by xylene was followed with 30 male rats, the mass of each being (21 ± 1) g, which were divided into three groups. The physiological saline was injected for group A (control group), the HApt for group B, and the complex *III* for group C on abdominal cavity. After 1 h, xylene was injected on the left ear to cause inflammatory edema. Sections on the left and right ear were taken at the same place with a punch to be weighed. The mass difference of these sections is the swelling capacity. Percentage inhibition was calculated using the following equation

$$\text{Inhibition} = \frac{m_{ec} - m_{et}}{m_{ec}} 100\%$$

where m_{ec} is the ear edema mass found in the control group, m_{et} is the ear edema mass found in the group receiving the ligands or their complexes.

Results and discussion

All complexes were insoluble in benzene and soluble in methanol, ethanol, THF, DMF, and DMSO. Complexes *III* and *IV* were sparingly soluble in water. Their molar conductances (Table 1) show the complexes are nonelectrolytes [10].

Table 1

Elemental analyses, molar conductance Λ, and thermal analysis data (TG and DTA) of complexes

Complex Appearance	w_i(calc.)/% w_i(found)/%					Λ	θ_1	θ_2	θ_3
	M	Cl	C	H	N	S cm^2 mol^{-1}	°C	°C	°C
I	19.30	11.34	23.06	1.60	17.94	6.00	75	185	480
brown	19.44	11.08	23.43	2.04	17.92	(in MeOH)			
II	26.92	14.54	19.68	1.64	15.14	12.04	80	175	615
red	27.04	14.05	19.67	1.83	14.44	(in DMF)			
III	18.31	20.43	27.69	3.46	16.16	30.05	110	222	510
green	18.33	20.37	27.71	3.04	15.96	(in MeOH)			
IV	28.49	15.38	27.78	2.90	16.20	61.16	80	218	540
yellow	28.28	15.42	27.82	3.69	15.98	(in MeOH)			

IR and UV spectra of the ligands and their complexes are shown in Table 2. It follows from them that HNft is coordinated to Cu ions in enolic form in *I* and to Au ions in keto form in *II*. The IR spectra of *I* show that the characteristic bands at $\tilde{\nu} = 843$ and 1536 cm^{-1} due to vibrations ν(C═S) and δ(N—H)

disappeared, but a new band was observed at $\tilde{\nu} = 655\ cm^{-1}$, which can be due to the ν(C—S) vibrations, while the vibrations ν(C=N) shifted to a higher wavenumber because of forming conjugate system —C=N—N=C—. The HApt is coordinated to the central ions only in keto form. In addition, a greater change of UV spectra and elemental analysis of *I* also shows that the HNft is coordinated to Cu ions in enolic form.

The thermal analysis (TG and DTA; Table 1) shows that there are mass losses at temperatures θ_1, θ_2, and θ_3. The mass loss at θ_1 was less than those at θ_2 and θ_3, and the heat effect was not apparent, while losses of masses at θ_2 and θ_3 were greater and accompanied by the extensive release of heat which can be attributed to the rupture and combustion of molecular skeleton. Finally, the residue was black copper oxide and yellow golden element.

The inhibitory action of the complexes on heat denaturation of human serum globulin has been studied. The insoluble part of gamma globulin denatured by heat at 90°C was a polymer formed by cross-linking among molecules, which

Table 2

IR and UV[a] spectra of the ligands and their complexes

Ligand or complex	$\tilde{\nu}/cm^{-1}$					ε
	ν(C=S)	ν(C—S)	ν(C=N)	ν(N—H)	δ(N—H)	$m^2\ mol^{-1}$
HNft	843 m		1604 s	3478 s	1536 s	760
				3320 s		510
				3097 m		110
I		655 m	1596 m	3486 m		720
				3334 s		410
				3120 w		90
II	806 s		1615 s	3445 m	1546 s	740
				3305 m		490
				3140 w		100
HApt	848 m		1605 s	3374 m	1502 s	880
	782 m			3261 m		
				3122 s		
III	828 m		1606 s	3345 m	1475 m	510
	772 m		1554 s	3221 m		76
				3118 br, m		
IV	857 m		1618 m	3429 w	1511 s	820
	775 s		1587 m	3349 m		120
				3291 br, m		
				3085 m		

a) The solvents in which the UV spectra of compounds were determined were methanol for HNft, *I*, and *II*, and DMF for HApt, *III*, and *IV*

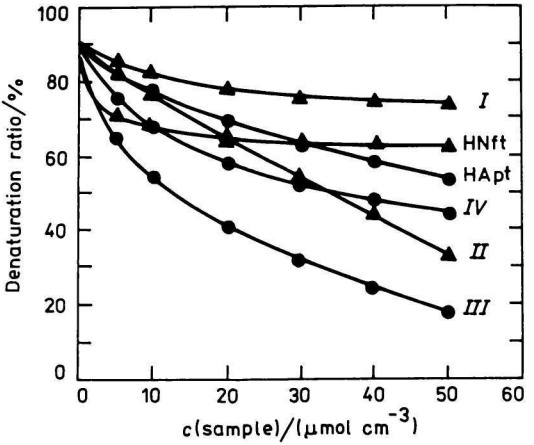

Fig. 1. Inhibitory action of ligands and their complexes on heat denaturation of human serum gamma globulin.

exhibits antigenicity and can make the antibody in a body cause inflammation [11]. Thus the substance which may inhibit heat denaturation of gamma globulin shows an inflammatory action and makes the insoluble part reducing, *i.e.* the denaturation ratio is lower.

The denaturation curve of inhibition ratio of two ligands and their complexes with different concentration with regard to human serum gamma globulin is shown in Fig. 1. It can be seen from the figure that the complexes *II*—*IV* have stronger action than their ligands except the complex *I*.

Table 3

The inhibitory action of HApt and complex *III* on the ear edema of the rats

Group Injection	Dose mg kg^{-1}	Swelling capacity/mg	Inhibition %	*P*
A — control				
Physiological saline	—	16.4 ± 3.2		
B				
HApt	17.6	9.7 ± 2.4	41.0	< 0.01
C				
$Cu(HApt)Cl_2 \cdot H_2O$	34.7	7.8 ± 1.6	52.4	< 0.05

The inhibitory action of HApt and complex *III* on the ear edema of ten albino rats caused by xylene is shown in Table 3. It is obvious that the inhibitory action of the complex *IV* is stronger than that of its ligand.

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