Copper(II) Complexes with Girard-T and -D Reagents [1]

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The cupric complexes of Girard-T and -D reagents have been investigated. Potentiometric and spectrophotometric experiments showed that Girard-T formed 1 : 1 and 2 : 1 ligand–cupric ion complexes, whereas Girard-D formed only a 1 : 1 complex. Acid dissociations from the cupric complexes of both reagents were recognized. The complexes, $Cu(Gir\text{-}T)_2$ and Cu(Gir-D), were isolated in the crystalline state. The scheme for the proton dissociation of Girard reagents and hydrazones has been revised.

DURING investigations on the effects of metal ions on the hydrolysis of Girard hydrazones, some complexes were found to be formed from cupric ion and Girard reagents. Information on these complexes would afford a clue to the role of cupric ion in the hydrolysis of Girard hydrazones, so we investigated the behaviour of these complexes in aqueous solution and attempted to isolate them.

EXPERIMENTAL

Materials.—Girard-T reagent (Merck, G. R.) was recrystallized from absolute ethanol. Girard-D reagent, prepared by a known method,[2] was recrystallized from ethanol. Copper salts and other inorganic compounds were of reagent grade and were used without further purification. *N*-Deuteriated Girard-T and -D reagents were prepared by adding heavy water (99·8%) to samples of the reagents in a desiccator at room temperature, and then evaporating the solutions to dryness under reduced pressure. The sodium salts of Girard-T and -D reagents were prepared by dissolving these reagents in solution containing an equimolar amount of sodium hydroxide and evaporating the solutions to dryness under reduced pressure.

Modified Girard Reagents and Hydrazones, in which the Amide Hydrogen Atom is Substituted by a Methyl Group.—These compounds were prepared by condensation of *N*-chloroacetyl-*N*-methyl-*N'*-benzylidenehydrazine, $PhCH{:}N{\cdot}NMe{\cdot}CO{\cdot}CH_2{\cdot}Cl$ (I), with appropriate amines.

N-Chloroacetyl-N-methyl-N'-benzylidenehydrazine. To a solution of tribenzylidenemethylhydrazine [3] (15 g.) in benzene (100 ml.)–ether (80 ml.) containing a suspension of K_2CO_3 (12 g.) chloroacetyl chloride (10 g.) was added with stirring, and the mixture was refluxed for 2 hr. It was then cooled to room temperature, water (200 ml.) was added, and the ether and benzene were distilled off under reduced pressure. The solid precipitate was filtered off and recrystallized from ethanol as needles, m.p. 142° (13·5 g.) (Found: C, 57·4; H, 5·1; N, 13·3. Calc. for $C_{10}H_{11}ClN_2O$: C, 57·0; H, 5·3; N, 13·3%).

N'-Methyl-benzaldehyde Girard-T Hydrazone. To a suspension of (I) (1·7 g.) in absolute ethanol (200 ml.), dry trimethylamine (5 g.) was added and the mixture was warmed at 40° with stirring until a clear solution was obtained. After stirring the solution for 4 hr. at room temperature and leaving it overnight, the excess of trimethylamine and ethanol was evaporated off under reduced pressure. The solid material obtained was recrystallized from methanol–ethyl acetate–ether, m.p. 258—259° (decomp.) (2·1 g.) (Found: C, 57·4; H, 7·3; N, 15·5. Calc. for $PhCH{:}N{\cdot}NMe{\cdot}CO{\cdot}CH_2N^+Me_3Cl^-$: C, 57·4; H, 7·5; N, 15·6%).

N'-Methyl-benzaldehyde Girard-D Hydrazone. To a suspension of (I) (15 g.) in benzene (200 ml.)–ether (100 ml.), dry dimethylamine (10 g.) was added and the mixture was stirred at 30° for 5 hr. Evaporation of the solvent gave a solid, which was dissolved in water (100 ml.)–ether (100 ml.) and dried. This procedure was repeated several times. Finally the solid was recrystallized from ethanol–ether, m.p. 212—214° (15 g.) (Found: C, 55·9; H, 7·0; N, 15·9. Calc. for $PhCH{:}N{\cdot}NMe{\cdot}CO{\cdot}CH_2{\cdot}NMe_2HCl$: C, 56·4; H, 7·1; N, 16·4%).

N'-Methyl-Girard-D Reagent. *N'*-Methyl-benzaldehyde Girard-D hydrazone (10 g.) was treated with 2,4-dinitrophenylhydrazine according to the method of Cymerman-Craig and Willis.[4] *N'*-Methyl-Girard-D reagent crystallized from methanol–ethyl acetate as needles, m.p. 198° (8 g.) (Found: C, 29·1; H, 7·3; N, 20·2. Calc. for $NH_2{\cdot}NMe{\cdot}CO{\cdot}CH_2{\cdot}NMe_2{\cdot}2HCl$: C, 29·4; H, 7·3; N, 20·6%).

Benzaldehyde Girard-T hydrazone, m.p. 214—216°, and -D hydrazone, m.p. 214·5—215·5°, were prepared as described previously.[5]

Apparatus.—A Shimazu spectrophotometer, type QV-50, was used for u.v. spectroscopy. I.r. spectra were obtained using a Hitachi EPI-2 spectrophotometer with NaCl prisms. A Metrohm pH-meter, type E-300B, with a combined electrode was used for pH measurements. pH titrations were carried out using a Metrohm Dosimat, type E-415, connected with the above pH-meter.

Preparation of $Cu(NH_2{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}N^+Me_3)2SO_4,2H_2O$.—To a solution of $CuSO_4,5H_2O$ (1·0 g.) in water (30 ml.) Girard-T reagent (1·4 g.) was added. Just sufficient methanol was added to make the solution cloudy and the resulting mixture was stood at room temperature for 2 or 3 hr. The pale blue needles which formed were collected, washed with a small amount of water–methanol, and dried in a vacuum-desiccator, m.p. 157—160° (decomp.) (0·6 g.) [Found: C, 21·8; H, 5·8; Cu, 11·9; N, 14·9; SO_4, 34·3. Calc. for $Cu(C_5H_{14}N_3O)_2SO_4,2H_2O$: C, 21·6; H, 5·8; Cu, 11·4; N, 15·1; SO_4, 34·6%].

Even when the ligand : metal ratio was changed to 4 : 1, Girard-T reagent and cupric ion gave this same complex and a 1 : 1 complex was not obtained in spite of many efforts.

Preparation of $Cu(NH_2{\cdot}N{:}CO^-{\cdot}CH_2{\cdot}NMe_2)ClO_4,H_2O$.—Cupric perchlorate (3·7 g.) was dissolved in water (30 ml.) and the solution was filtered. To the filtrate free Girard-D reagent (not the hydrochloride) (1·1 g.) in ethanol (10 ml.) was added. Very small violet needles soon appeared.

[1] A part of this paper was read at the Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1968.

[2] M. Viscontini and J. Meier, *Helv. Chim. Acta*, 1950, **33**, 1773.

[3] C. Harries and T. Haga, *Ber.*, 1898, **31**, 62.

[4] J. Cymerman-Craig and D. Willis, *J. Chem. Soc.*, 1955, 4315.

[5] M. Masui and H. Ohmori, *J. Chem. Soc.* (*B*), 1967, 762.

These were collected, washed with water, and dried in a vacuum-desiccator, m.p. 212—213° (decomp.) (2·2 g.) (Found: C, 15·9; H, 4·0; Cu, 21·6; N, 13·9. Calc. for $Cu(C_4H_{10}N_3O)ClO_4,H_2O$: C, 16·2; H, 4·1; Cu, 21·4; N, 14·1%).

Use of cupric chloride and sulphate in place of perchlorate also gave the same complex. The same result was obtained with the hydrochloride of Girard-D reagent instead of the free base. In all cases where the ligand : metal ratio was changed from 1 : 1 to 4 : 1, the ratio of the Girard-D reagent to cupric ion in the resulting complexes was always 1 : 1.

RESULTS AND DISCUSSION

We concluded that the proton in the methylene group located between a carbonyl and a quaternary ammonium group was responsible for the acid dissociation of Girard-T reagent and its hydrazones, in view of the results of spectrophotometric studies and the electron-attracting effect of the quaternary ammonium group.[5,6] However, an additional acid dissociation, which has been observed in the complex formation of cupric ion with a dipeptide such as glycylglycine, was found to occur at the peptide nitrogen atom.[7,8] Similar proton dissociation has been reported for isonicotinoylhydrazine and related compounds.[9] These results indicate the need for reinvestigation of proton dissociation from Girard-T reagent and its hydrazones.

As shown by curves A and B in Figure 1, the absorption bands for benzaldehyde Girard-T and -D hydrazones

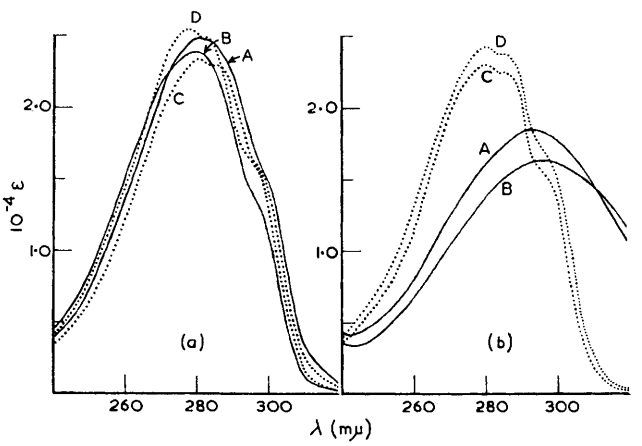


FIGURE 1 U.v. absorption spectra of $PhCH{:}N{\cdot}NR^1{\cdot}CO{\cdot}CH_2{\cdot}N^+R^2Me_2\ Cl^-$ in (a) H_2O and (b) 0·2N-NaOH containing 2% of ethanol

	A	B	C	D
R^1.....	H	H	Me	Me
R^2.....	Me	H	Me	H

in water change similarly in alkaline solution. The same results were reported previously for various different Girard-T and -D hydrazones.[6] This change indicates that a proton dissociates from these hydrazones. On the other hand, no such change is observed with modified benzaldehyde Girard-T and -D hydrazones in which the hydrogen atom attached to the nitrogen atom adjacent to the carbonyl group is substituted by a methyl group (curves C and D). Even when the concentration of sodium hydroxide is increased up to 0·5N, the u.v. absorption bands of these hydrazones were virtually unchanged. The spectrum of *N'*-methylbenzaldehyde Girard-T hydrazone in 0·2N-sodium hydroxide solution [Figure 1(b), curve C] is that obtained by extrapolation to zero time, because of the rather rapid hydrolysis of the hydrazone catalyzed by hydroxide ion. This also indicates that a proton does not dissociate from this compound in alkaline solution, which is in accordance with the mechanism of hydrolysis of Girard hydrazones previously reported.[5]

From these results, the scheme proposed for the dissociation of Girard reagents and hydrazones should be revised as shown in Scheme 1.

For Girard-T reagent and hydrazones

$$Y{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}N^+Me_3 \rightleftharpoons Y{\cdot}N{:}CO^-{\cdot}CH_2{\cdot}N^+Me_3 + H^+$$

For Girard-D reagent and hydrazones

$$Y{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}N^+HMe \rightleftharpoons Y{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}NMe_2 + H^+$$

$$Y{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}NMe_2 \rightleftharpoons Y{\cdot}N{:}CO^-{\cdot}CH_2{\cdot}NMe_2 + H^+$$

SCHEME 1

where Y represents NH_2 and $RR'C{:}N^-$ for Girard reagents and hydrazones, respectively.

Titration curves for Girard-T and -D in the presence and absence of cupric ions are shown in Figure 2. These results indicate that, in the case of Girard-T, one proton per ligand is liberated and at least two ligand molecules co-ordinate to a cupric ion. In the case of Girard-D, on the other hand, two protons are liberated and only a 1 : 1 complex is formed below pH 10.

The absorption spectra shown in Figure 3 support this view. Thus, the absorption maximum of the Girard-D–cupric ion mixture and its intensity are little affected above pH 4 by varying the ratio from 1 : 1 to 4 : 1. The spectra of the Girard-T–cupric ion system are closely related to those of a semicarbazide–cupric ion system,[10] which indicates that similar complexes are formed in the two systems.

The titration curves for *N'*-methyl Girard-D in the presence of cupric ions (Figure 4) are quite different from those for Girard-D, showing that one proton per ligand molecule is liberated and two ligand molecules co-ordinate to a cupric ion. This result also seems to support Scheme 1.

Various i.r. absorption peaks ($cm.^{-1}$) for Girard-T and -D, their sodium salts, and their copper complexes are summarized in the Table. Assignments for the individual absorption bands of Girard-T and -D were made by com-

[6] M. Masui and H. Ohmori, *Chem. and Pharm. Bull. (Japan)*, 1964, **12**, 877.
[7] S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, 1956, **52**, 1123.
[8] H. Dobbie and W. O. Kermack, *Biochem. J.*, 1955, **59**, 246.
[9] K. Nagano, H. Tsukahara, H. Kinoshita, and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1963, **11**, 797.
[10] H. El Khadem, M. F. Iskander, and S. E. Zayan, *Z. anorg. Chem.*, 1963, **320**, 261.

paring these spectra with those of their *N*-deuteriated compounds and those of monoacid hydrazides reported by Mashima.[11] The amide I and II bands of Girard-T and -D are not present in the spectra of their sodium

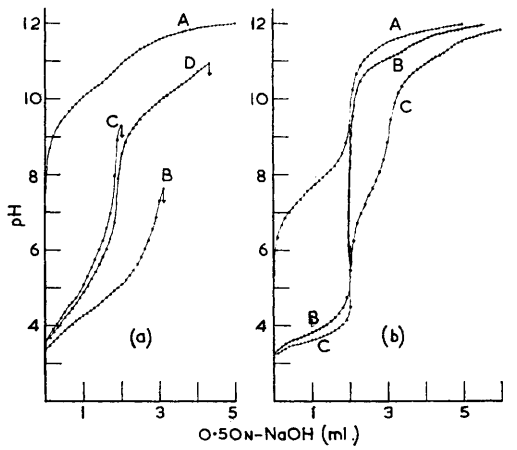


FIGURE 2 Titration curves of Girard reagents in the presence of cupric ions with 0·5N-NaOH, μ = 0·5 with KCl, at 25°

(a) Girard-T		Cu^{2+}	(b) Girard-D		Cu^{2+}
A	0·01M	—	A	0·01M	—
B	0·01	0·01M	B	0·005	0·005M
C	0·01	0·005	C	0·01	0·005
D	0·02	0·005			

(each solution initially 100 ml.)

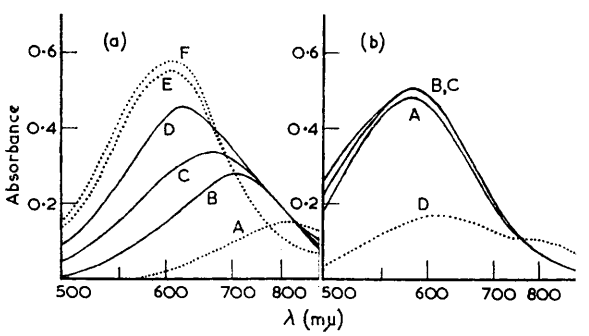


FIGURE 3 Absorption spectra of a mixture of solutions of cupric ion and Girard reagents

(a) Cu^{2+} 0·01M

Girard-T		
A	0·00M in H_2O	
B	0·01	pH 5·5
C	0·02	
D	0·04	
E	0·02	pH 6·9
F	0·04	

(light-path 1 cm.)

(b) Cu^{2+} 0·01M

Girard-D		
A	0·01M	pH 5·5
B	0·02	
C	0·04	
D	0·02	pH 3·0

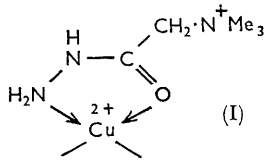
(light-path 0·5 cm.)

salts, and instead there are new intense peaks in the range of 1570—1580 $cm.^{-1}$. Although the i.r. spectrum of the Girard-T–cupric ion complex was as a whole rather different from that of the reagent, indicating the formation of a complex, the band for the C=O stretching vibration was only slightly shifted to lower frequencies.

[11] M. Mashima, *Bull. Chem. Soc. Japan*, 1962, **35**, 1882.

The co-ordination at the carbonyl oxygen, therefore, should be weak in the Girard-T–cupric ion complex just as described for the benzoylhydrazine–cupric ion and cadmium ion complexes and the *N*-methyl-*N*-isonicotinoylhydrazine–cupric ion complex.[12]

These observations along with the results of elemental analysis suggest that the Girard-T–cupric ion complex should have the partial structure (I). A



(I)

similar complex has been isolated from the semicarbazide and cupric ion and its structure was decided by *X*-ray analysis.[13]

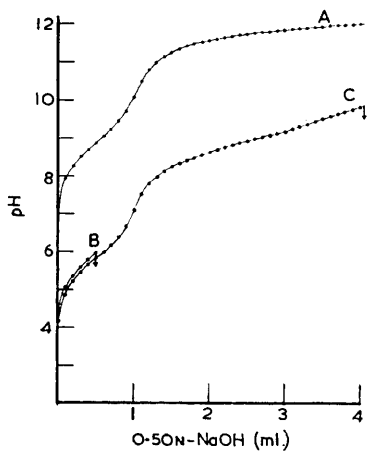


FIGURE 4 Titration curves of *N*-methyl Girard-D reagent (as monohydrochloride) in the presence of cupric ions with 0·5N-NaOH, μ = 0·5 with KCl, at 25°

	N-Me-Gir-D	Cu^{2+}
A	0·005M	—
B	0·01	0·005M
C	0·04	0·005

(initial volumes of the solutions were 100 ml. in A and B, and 50 ml. in C)

The frequency of the NH_2 bending vibration is little affected by the formation of the complex as shown in the Table. The same has also been observed in the

I.r. frequencies of Girard-T and -D, their sodium salts, and their cupric complexes

	Assignment ($cm.^{-1}$)			
Compound	Amide I	NH_2 bend.	Amide II	ν(–N=C=O–)
Gir-T	1687	1611	1548	
Gir-D	1683	1619	1547	
Gir-T-Na		1623	—	1579
Gir-D-Na		1624	—	1573
Gir-T-Cu(II) ...	1675	1612	—*	
Gir-D-Cu(II) ...		1616	—	1551

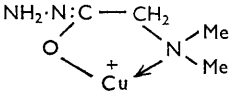
* Assignment was impossible.

[12] K. Nagano, H. Kinoshita, and A. Hirakawa, *Chem. and Pharm. Bull.* (*Japan*), 1964, **12**, 1198.

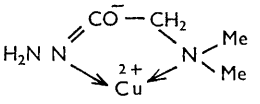
[13] M. Nardelli, G. F. Gasparri, P. Boldrini, and G. G. Battistini, *Acta Cryst.*, 1965, **19**, 491.

case of the thiosemicarbazide–cupric ion complex, where the two NH_2 bending vibrations of the thiosemicarbazide in the range of 1610—1650 cm.$^{-1}$ [14] were little affected by complex formation.[15]

The i.r. spectra of the Girard-D–cupric ion complex and of the Na-salt of Girard-T and -D resemble each other. Thus Girard-D probably has a similar form in the cupric complex and in the Na-salt. According to Rosenberg,[16] when the amide hydrogen of the dipeptide–cupric ion complex is dissociated, a new i.r. absorption band appears in the range 1520—1550 cm.$^{-1}$ attributable to the $^{-}N{=}C{=}O^{-}$ group with disappearance of the amide I band of the original dipeptide. This suggests that the band of the Girard-D–cupric ion complex near 1550 cm.$^{-1}$ may be ascribed to the $^{-}N{=}C{=}O^{-}$ stretching frequency. But this possibility was opposed by Martell *et al.*[17] on the basis of studies of the i.r. spectra of solutions of the glycylglycine–cupric ion complex in D_2O. In any case, however, the large shift of the amide I band in the Girard-D–cupric ion complex indicates a dissociation of hydrazide hydrogen from the ligand. Similar dissociations have been reported for the cupric complex of isonicotinoylhydrazine and related compounds.[12] These observations together with results of elemental analysis suggest that the Girard-D–cupric ion complex should have either the partial structure (II) or (III).



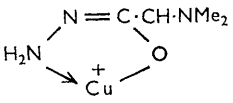
(II)



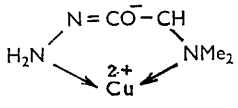
(III)

An *NN*-co-ordination structure such as (III) has been proposed for peptide–cupric ion complexes [16,17] and an *NO*-co-ordination structure such as (II) has been suggested for picolinoylhydrazine.[12] It is not possible from the present results to decide definitely which structure is correct. In the case of a cupric complex of a dipeptide, such as glycylglycine, however, an *NN*-co-ordination such as (III) would make it possible for terminal carboxy-oxygen to co-ordinate with the centre cupric ion by making an additional five-membered ring,[16,17] and the complex would become more stable, whereas with a structure such as (II) co-ordination of the carboxy-oxygen with the cupric ion would result in an unstable seven-membered ring. On the other hand, no such additional chelate-ring formation is possible with the Girard-D–cupric ion complex, and a structure such as (II) seems the more probable as in the picolinoylhydrazine–cupric ion complex.[12]

Structure (II) is more satisfactory than (III) to explain the effects of cupric ion on the hydrolysis of Girard hydrazones.[18] This study on the effects of cupric ion on the hydrolysis also excludes the possibility of predominant formation of a complex such as (IV) or (V).



(IV)

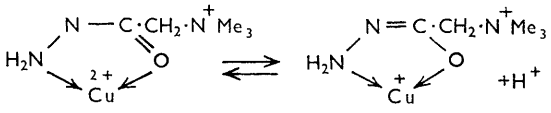


(V)

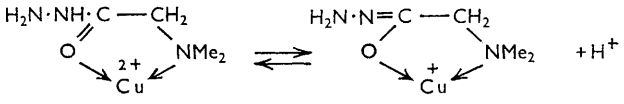
It is not clear why formation of cupric complexes which contain other than a 1 : 1 ratio is not observed with Girard-D either in solution and in the solid state. The role of the terminal NH_2 group is also obscure. The formation of a binulclear chelate complex or a polymeric chain might be considered, though present results are insufficient to demonstrate these.

The above considerations suggest that the following may be the difference between the interactions of Girard-T and -D with cupric ions in aqueous solution:

For Girard-T:



For Girard-D:



SCHEME 2

Although another form may be considered for the Girard-D as mentioned above, the initial bonding site at least would be that in Scheme 2 just as in dipeptide–cupric ion complexes.[19] Acid dissociations similar to that of the Girard-T–cupric ion complex have been reported for isonicotinoylhydrazine and related compounds.[20]

[8/1112 *Received, August* 1*st*, 1968]

[14] M. Mashima, *Bull. Chem. Soc. Japan*, 1964, **37**, 974.
[15] M. J. Campbell and R. Grzeskowiak, *J. Chem. Soc.* (*A*), 1967, 396.
[16] A. Rosenberg, *Acta Chem. Scand.*, 1957, **11**, 1390.
[17] K. Kim and A. E. Maetell, *Biochemistry*, 1964, **3**, 1169.
[18] M. Masui and H. Ohmori, unpublished results.
[19] S. P. Datta, R. Leberman, and B. R. Rabin, *Trans. Faraday Soc.*, 1959, **55**, 1982, 2141.
[20] J. Cymerman-Craig, D. Willis, S. D. Rubbo, and J. Edgar, *Nature*, 1955, **176**, 34; A. Albert, *ibid.*, 1956, **177**, 525; K. Nagano, H. Kinoshita, and Z. Tamura, *Chem. and Pharm. Bull.* (*Japan*), 1963, **11**, 999.