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PREPARATION, ISOLATION AND PROPERTIES OF DIASTEREOMERIC COPPER(II) CHELATES OF THE SCHIFF BASES OF 2-FORMYL(*N*,*N*-DIMETHYLAMINOMETHYL)CYMANTRENE WITH DIPEPTIDES

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Summary

The optical isomers of 2-formyl(*N*,*N*-dimethylaminomethyl)cymantrene (I) can be separated by liquid chromatography of Cu^{2+} chelates of the Schiff bases formed from I and a number of dipeptides, in those cases where no racimization of the amino acid residues in the initial dipeptide occurs. Hydrolysis of the chelates gives optically pure enantiomers of I. Under the conditions of the preparation of the chelate from L-Ala-L-Ala ($NH_2C(CH_3)HCONHC(CH_3)$-$HCOOH$) slow epimerization of the dipeptide into D-Ala-L-Ala takes place. Formation of Cu^{2+} chelates of the Schiff bases from L-Ala-L-Ala, D-Ala-L-Ala and L-AlaGly ($NH_2C(CH_3)HCONHCH_2COOH$) with (+) — and (—) —I proceeds stereospecifically.

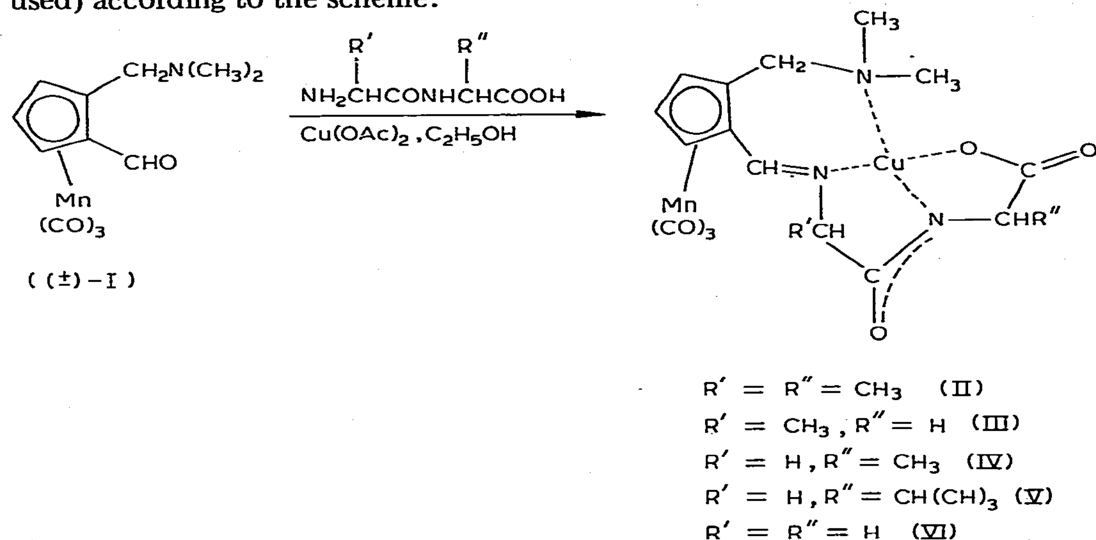
Introduction

It has been reported [1] that 2-formyl(*N*,*N*-dimethylaminomethyl) (I) can be separated into optical isomers due to planar chirality by means of diastereomeric Cu^{2+} chelates of the Schiff bases from the aminoaldehyde I with a number of dipeptides. This paper reports a new method of preparation of diastereomeric chelates of this type, their isolation and analysis of their diastereomeric purity by means of liquid chromatography (LC), and also physico-chemical parameters of individual diastereomers.

Results and discussion

The chelates studied were prepared by interaction of I with dipeptides and $Cu(OCOCH_3)_2 \cdot 2\ H_2O$ in abs. ethanol at 23—28°C (no inert atmosphere was

used) according to the scheme:



The resulting solutions were analysed, without pre-treatment, with a liquid chromatograph, on columns filled with silica gel (elution with abs. ethanol). Individual fractions were collected and were examined for dipeptides, I, Cu^{2+} ions and Cu^{2+} chelates of the schiff bases of I with dipeptide. As a result a succession was revealed in the retention times of the components of the reaction mixture, which was found to be the same in all cases, and can be illustrated by the chromatographic pattern for the reaction of (±) —I with L-Ala-L-Ala ($NH_2C(CH_3)HCONHC(CH_3)HCOOH$) and $Cu(OCOCH_3)_2$ (Fig. 1).

The chelate fractions (A and B) were purified by LC and, after removing the solvent, the chelates were isolated as blue-green crystalline substances. After hydrolysis with 3 N HCl the chelates were shown by LC and GLC to contain no acetate, with a 1/1 ratio of I and dipeptide in each case. An elemental analysis,

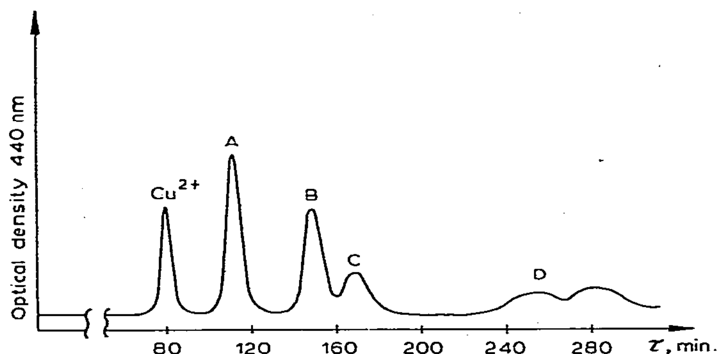


Fig. 1. The reaction mixture chromatogram for the reaction of 2-formyl(*N,N*-dimethylaminomethyl)-cymantrene (±)I with L-Ala-L-Ala and $Cu(OCOCH_3)_2$ in abs. ethanol (20 cm^3 h^{-1}, 25°C, 24 h). Fraction A; the chelate of (+)I/Cu^{2+}/L-Ala-L-Ala and D-Ala-L-Ala composition; fraction B; the chelate of (—)I/Cu^{2+}/L-Ala-L-Ala; fraction C; (±)I; fraction D. Cu^{2+}/L-Ala-L-Ala.

TABLE 1

CHARACTERISTICS AND COMPOSITION OF Cu^{2+} CHELATES OF SCHIFF BASES OBTAINED FROM 2-FORMYL(*N,N*-DIMETHYLAMINOMETHYL)CYMANTRENE AND DIPEPTIDES

Chelate	Initial dipeptide	Chelate fraction	Yield of the fraction after 24 h (%)	Retention time (min) [a]	$[\alpha]_{436}^{22}$ [b]	Chelate composition	
						dipeptide	amino-aldehyde
II	L-Ala-L-Ala	A	25	108	—1007	L-Ala-L-Ala	(+)I
		A	10	108	—2990	D-Ala-L-Ala	(+)I
		B	42	150	+3810	L-Ala-L-Ala	(—)I
III	L-AlaGly	A	0.6	120	(—) [c]	L-AlaGly	(+)I
		B	25	136	+1680	L-AlaGly	(—)I
IV	Gly-L-Ala	A	29	120	—1720	Gly-L-Ala	(+)I
		B	30	147	+1500	Gly-L-Ala	(—)I
V	Gly-L-Val	A	24	90	—2140	Gly-L-Val	(+)I
		B	24	110	+1560	Gly-L-Val	(—)I
VI	GlyGly		20	159	0	GlyGly	(±)I

[a] L.C. was performed on a 80 × 0.7 cm column with SiO_2. Ethanol was used as the eluent at 20°C. [b] Specific rotation of chelates II—V was determined in abs. ethanol at a concentration of chelate of 0.05%. [c] The sign of rotation at λ = 436 nm.

taking into account water and alcohol of crystallization, corresponded to chelates of general formula: I · dipeptide · Cu^{2+}. The IR and UV spectra of the chelates were identical to those previously reported [1]. This allowed us to assign to the chelates the structures II—VI, as suggested earlier [1] for the chelates obtained from (±) —I and dipeptides in the presence of $CuSO_4$ and CH_3ONa.

The number of fractions which contained chelates II—VI depended on the structure of the initial dipeptide. As was expected, in the case of chelates with GlyGly (VI) ($NH_2CH_2CONHCH_2COOH$) there was only one peak in the chromatogram which correspond to the chelate, whereas, with other dipeptides, after the reaction had proceeds for 24 hrs, there were two peaks of chelates.

Table 1 and Figs. 2 and 3 illustrate the physico-chemical properties of A and B fractions of the chelates II—VI and their components which were isolated after hydrolysis with 3 N HCl. The chromatographic analysis shows that for chelates II—V, fractions having a shorter retention time (fractions A) gave similar optical rotatory dispersion curves. Also similar were the curves observed for chelates II—V with longer retention times (fractions B). Moreover the signs of the Cotton effect at the same wavelength were opposite for those two groups of chelates. It was also found that for fractions A the chelates II—V (II-A—V-A) contained (+) —I, whereas chelates II-B—V-B contained isomers with the opposite planar configuration ((—) — I). The optical parameters of the isolated (+) — and (—) — isomers of I were in agreement with those reported [1] for the corresponding optically pure isomers.

Moreover, the optical rotatory dispersion curves for chelates II-B, III-B, and IV-A were identical in shape and size with those of Cu^{2+} chelates of the schiff bases of I with L-Ala-L-Ala, L-Ala-Gly ($NH_2C(CH_3)HCONHCH_2COOH$) and Gly-

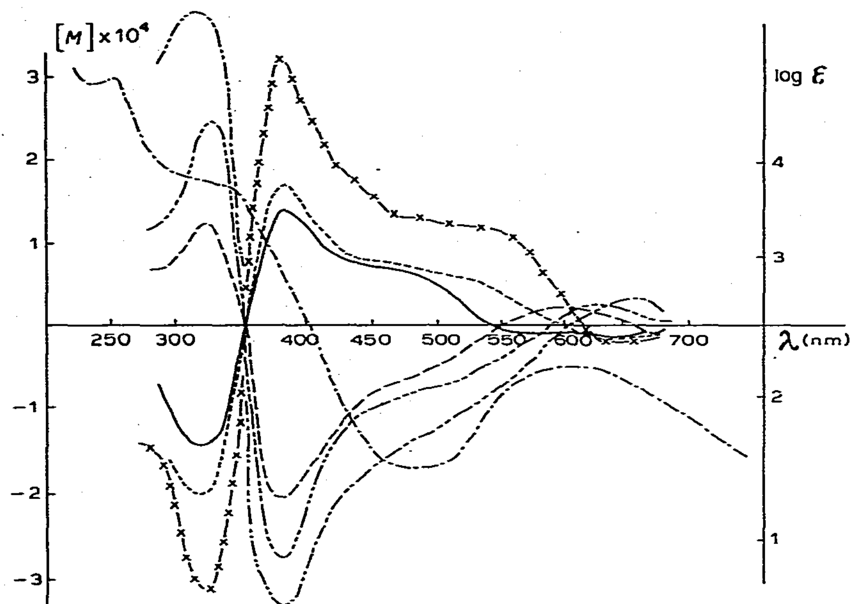


Fig. 2. Electronic spectrum (— · — · —) of chelate II-B and optical rotary dispersion spectra of chelates II-B (— X — X —), II-A-D,I (— ·· — ·· —), III-B (- - - - - -), IV-a (— — —), IV-B (———), V-A (— - - - —), V-B(———) in 96% ethanol.

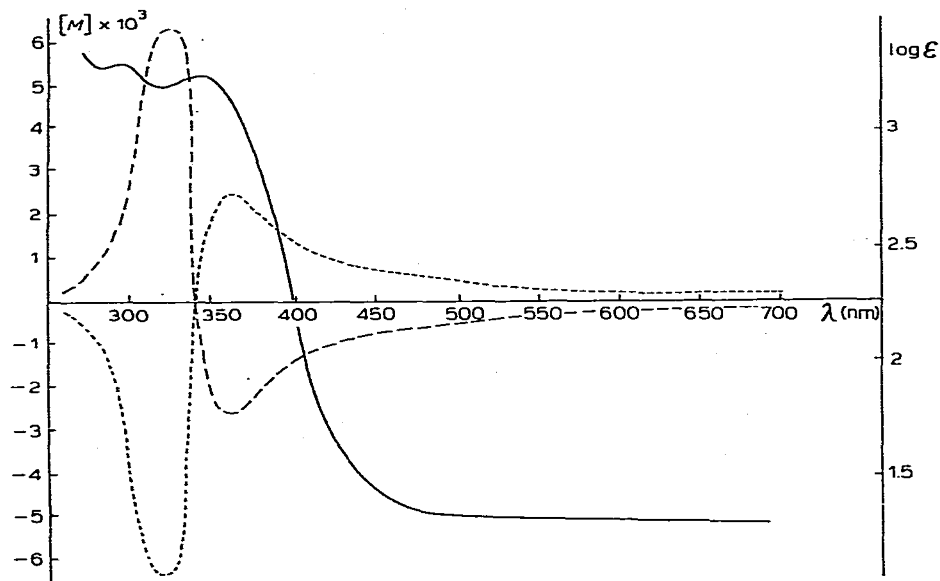


Fig. 3. Electronic spectrum (———) of racemic I in $CHCl_3$ and optical rotary dispersion spectra of $(+)_{436}$I (············) and $(-)_{436}$I (— — —) in $CHCl_3$ ($c = 1.08 \times 10^{-3}$ mol l^{-1}).

L-Ala ($NH_2CH_2CONHC(CH_3)HCOOH$), respectively [1], from which the corresponding optically pure isomers of I were isolated. Hence, LC allows separation of diastereomeric Cu^{2+} chelates of the schiff bases of aminoaldehyde I with dipeptides.

The data obtained indicated that formation of chelates with Gly-L-Ala and Gly-L-Val ($NH_2CH_2CONHC(C(CH_3))HCOOH$) did not involve racemization of the *C*-terminal amino-acid residue. Otherwise each A and B fraction would contain chelates IV and V with (+) — and (—) —I, which was not observed. For this reason we also excluded racemization of the *N*-Ala-fragment in the formation of III.

As is clear from Table 1, the reaction of I with L-Ala-L-Ala and Cu^{2+} acetate for 24 h under the conditions used involves epimerization of one of the amino acid residues of the dipeptide (probably the *N*-terminal one [2]). In our opinion the mechanism of the L-Ala-L-Ala epimerization involves radical cleavage of the C—H bond of the *N*-terminal residue of the dipeptide, which is facilitated under the conditions of the chelate preparation (presence of air and lack of a strong base).

Fraction B consisted only of a chelate of Cu^{2+}/(—) —I/L-Ala-L-Ala composition, and fraction A contained chelates of Cu^{2+} and (+) —I both with L-Ala-L-Ala and D-Ala-L-Ala, with a ratio of 1/2.5, respectively. Therefore the retention times of Cu^{2+}/(+) —I/L-Ala-L-Ala and Cu^{2+}/(+) —I/D-Ala-L-Ala are the same, and differ from that of Cu^{2+}/(—) —I/L-Ala-L-Ala. Detailed studies of the formation of diastereomeric chelates II have shown that for the first four hours no epimerization of L-Ala-L-Ala takes place and that fraction A of chelate II contained only (+) —I and L-Ala-L-Ala (II-A-L,L). But when the reaction mixture was left for along time (more than 72 h) fraction A contained only chelate II of Cu^{2+}/(+) —I/D-Ala-L-Ala structure (II-A-D,L). The optical parameters of individual chelates II-A-L,L and II-A-D,L are listed in Table 1. Comparison of the optical rotatory dispersion spectra of these diastereomeric chelates with those of II-B shows that for the chelates they depend mainly on the configuration of I, whereas the configuration of amino acid residues is less important. A similar conclusion can be made from the analysis of the curves for fractions A and B of chelates III—V. To put it in other way, the optical rotatory dispersion curves provide evidence only for the configuration of aminoaldehyde I involved into the chelate and cannot used as evidence for epimerization of the amino acid residues in peptides in the course of chelate formation.

From the data reported here we can conclude that in those cases when no racimization of the amino acid residues in peptides occurs the method developed can be used for analysis of the diastereomeric composition of chelates II—V and the enantiomeric purity of the (+) — and (—) — forms of I. It can also be used for preparative isolation of diastereomeric chelates II—V followed by isolation of enantiomers of I.

Equal peak areas for the diastereomeric chelates IV-A and IB-B and also for V-A and V-B provide evidence for nonstereospecific formation of chelates in the case of Gly-L-Ala and Gly-L-Val with enantiomers of I (Table 1). As for the formation of diastereomeric chelates with (±) —I involving L-AlaGly, the ratio of peak areas (1/42) after 24 h provides evidence for predominant formation of chelate (—) —I involving L-Ala-Gly, i.e. the process is highly stereospecific.

Time dependent composition of diastereomeric chelates II provides convincing evidence for stereospecific formation of chelates from (+) —I and (—) —I both in the case of L-Ala-L-Ala and D-Ala-L-Ala. But the data obtained so far do not allow a final conclusion to be drawn concerning the value of stereospecifity of chelate II formation.

Experimental

Racemic 2-formyl(*N*,*N*-dimethylaminomethyl)cymantrene was prepared as previously reported [1].

Synthesis of diastereomeric chelates

Chelates II—VI were obtained according to a standard procedure illustrated by the synthesis of chelate IV.

To a solution of 1.65×10^{-3} mol dipeptide in 10 cm^3 abs. ethanol at 25°C was added 300 mg of molecular sieve ("WolfenZbosorb", 3 Å), 360 mg (1.65×10^{-3} mol) copper acetate and 480 mg (1.65×10^{-3} mole) (+) I. The reaction mixture was stirred for 24 h at room temperature, filtered and evaporated to 3 cm^3. The resulting solution was placed on a 50 × 0.9 cm column filled with SiO_2 ("Silperal" (Ch SSR)) and eluted with abs. ethanol. The eluate was analyzed with a flow photometer, λ 440, 570 and 640 nm, and separated into fractions. Each fraction was treated with 3 N HCl and analyzed for dipeptide content with an amino-acid analyzer. Aminoaldehyde I was determined by TLC ("Silufol"-acetone) and Cu^{2+} ions from the electronic spectra in the region 440—640 nm. The composition of the fractions was verified by comparison of the chromatographic pattern of the reaction mixture with that of pure samples of $Cu(OCOCH_3)_2$, I, dipeptide and a Cu^{2+} dipeptide complex. After repeated chromatographic purification of fractions A and B (fraction A, peak with shorter retention time, fraction B, peaks with longer retention time, see Fig. 1), the solvent was evaporated to give green-blue crystals of the chelates. The yield of IV-A was 29%; that of IV-B was 30%. The m.p. 180°C with decomposition).

The diastereomeric purity of the chelates was examined analytically on a 80 × 0.7 cm column with SiO_2 ("G" Berlin (GDR)) with abs. ethanol, at 20°C. The rate of eluent supply was 20 cm^3 h^{-1}. For II-A-L,L: Found: C, 41.86; H, 4.95; Cu, 11.27; Mn, 9.88; N, 7.53. $C_{18}H_{20}CuN_3MnO_6 \cdot H_2O$. Calc.: C, 42.31; H, 4.34; Cu, 12.43; Mn, 10.75; N, 8.22%. For II-B, Found: C, 42.26; H, 4.16; Cu, 11.41; Mn, 10.05; N, 7.62. $C_{18}H_{20}CuN_3MnO_6 \cdot H_2O$. Calc.: C, 42.31; H, 4,34; Cu, 12.43; Mn, 10.75; N, 8.22%. For III-A, Found: C, 41.51; H, 4.02; Cu, 11.69; Mn, 10.53; N, 7.30. $C_{17}H_{18}CuN_3MnO_6 \cdot H_2O \cdot \frac{1}{2} C_2H_5OH$. Calc.: C, 41.66; H, 4.27; Cu, 12.25; Mn, 10.59; N, 8.09%. For IV-B, Found: C, 41.47; H, 4.24; Cu, 11.46; Mn, 10.62; N, 7.76. $C_{17}H_{18}CuN_3MnO_6 \cdot H_2O \cdot \frac{1}{2} C_2H_5OH$. Calc.: C, 41.65; H, 4.27; Cu, 12.25; Mn, 10.59; N, 8.09%.

General procedure for analysis of the composition of chelates II—VI

5 ml 3 N HCl was added to 0.5 g chelate in 5 cm^3 ethanol and 20 cm^3 water. The solution was neutralized with excess Na_3CO_3, and aldehyde I extracted with CCl_4 or $CHCl_3$. The yield of I, determined spectrophotometrically, was 90%. The structure of the isolated I was revealed by NMR, IR, UV and optical rotatory dispersion spectroscopy [1].

Qualitative and quantitative analysis of the dipeptide composition in the aqueous layer was made with an amino acid analyzer AAA-881 (Ch.SSR), 40 × 0.8 cm column, with "Aminex" A-5, eluent 0.2 N Na-citrate buffer, pH = 4.23, at 54°C. Retention time: L-Ala-L-Ala, 47 min; D-Ala-L-Ala, 40 min; Gly-L-Ala, 44 min; Gly-L-Val, 58 min; L-Ala-Gly, 43 min. The rate of eluent supply was 88 $cm^3 h^{-1}$.

Analysis of the acetate ion content in the acidified aqueous solution obtained after chelate decomposition was made with GLC on a 1 m × 4 mm column filled with chromotrone N-AW 0.16—0.20 mm with 15% Carbowax-TPA.

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