la néobiliverdine δ, n'est pas observé dans le DMSO; le proton N_D–H serait le seul à fournir un signal dans ces conditions (10,59 ppm). Avec la forme tautomère **2b** on devrait s'attendre à observer les singulets des 2 protons N_A–H et N_B–H. La cyclisation de la phorcabiline en sarpédobiline ne peut avoir lieu qu'à partir du moment ou la liaison C-4′ C-5′ a pris une configuration E. La géométrie des ponts méthines des verdines en solution n'est pas connue. Cependant l'étude de la bilirubine par les rayons X montre que pour ce pigment la liaison des cycles A et B d'une part, C et D d'autre part, conserve la configuration Z qui est celle existant chez les porphyrines[6]; elle est très favorisée pour la phorcabiline, étant donné l'encombrement des substituants en 3′ et 7′; en outre Falk et al.[7], ont montré que l'isomérisation Z→E des ponts méthines peut être obtenue par irradiation.

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Interaction of molybdate with copper(II)-histidine

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Summary. Molybdate and copper(II)-histidine form an insoluble complex of empirical formula $Cu_2(His)_3(MoO_4)_2(H_2O)_2$. ESR-spectroscopy indicated that the complex had tetragonal symmetry. IR-spectroscopy showed the presence of a carboxylate anion and suggested that the molybdate ion formed an ammonium-type salt with the nitrogens of the imidazole. The complex did not form following dissociation of the protonated imidazole (above a pH of approximately 6).

During studies directed towards understanding molybdenum toxicity in ruminants an in vitro interaction between copper(II)-histidine and molybdate was observed. This report describes the partial characterization of the novel compound formed following this interaction.

Experimental. Copper(II)-L-histidine molybdate (**1**) was prepared by mixing (10.0 ml) of a solution of L-histidine (200 mM) with an equal volume of $CuSO_4$ (100 mM) or $CuCl_2$ (100 mM). To the deep blue solution 10.0 ml of Na_2MoO_4 (200 mM) was added dropwise. The precipitate formed was filtered, washed with water, absolute ethanol and dried over P_2O_5 in vacuo. (Found C, 21.4; H, 3.02; N, 12.1; Cu, 12.2; Mo, 21.2; H_2O, 3.75%. Cu:Mo:His:H_2O, 1.00:1.15:1.55:1.08. Histidine was calculated from the carbon content. Calculated for $Cu_2(C_6H_9O_2N_3)_3(MoO_4)_2(H_2O)_2$: C, 22.8; H, 3.29; N, 13.3; Cu, 13.4; Mo, 20.2; H_2O, 3.80%.) Bis-L-histidine-copper(II) dinitrate dihydrate (**2**) was prepared as described previously[2,3]. (Found C, 26.8; H, 4.05; N, 20.8; Calculated for $Cu(C_6H_9O_2N_3)_2(NO_3)_2(H_2O)_2$: C, 27.0; H, 4.15; N, 21.0%.) For ESR studies **1** was prepared in which Cu^{2+} was magnetically diluted with Zn^{2+}. An aliquot (5 ml) of $CuSO_4$ (2 mM) was mixed with 10 ml of L-histidine (200 mM). To this 5 ml of $ZnSO_4$ (200 mM) was added followed by the dropwise addition of 10 ml Na_2MoO_4 (200 mM). The precipitate was filtered, washed with water, absolute ethanol and dried over P_2O_5 in vacuo. The above substance contained 0.22% Cu. Cu and Mo were determined by atomic absorption spectroscopy. Attempts to detect Na were carried out by atomic absorption spectroscopy and by precipitation of sodium zinc uranyl acetate and SO_4^{2-} by the precipitation of $BaSO_4$ in the presence of MnO_4^-(Feigl[4]).

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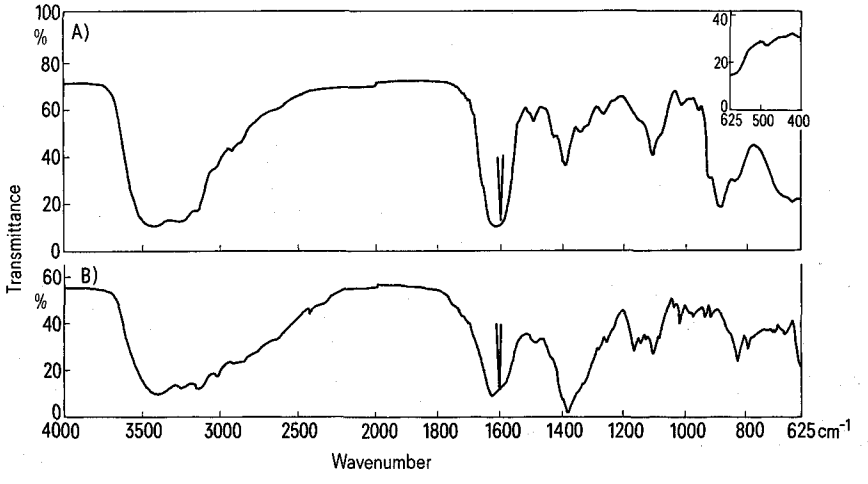
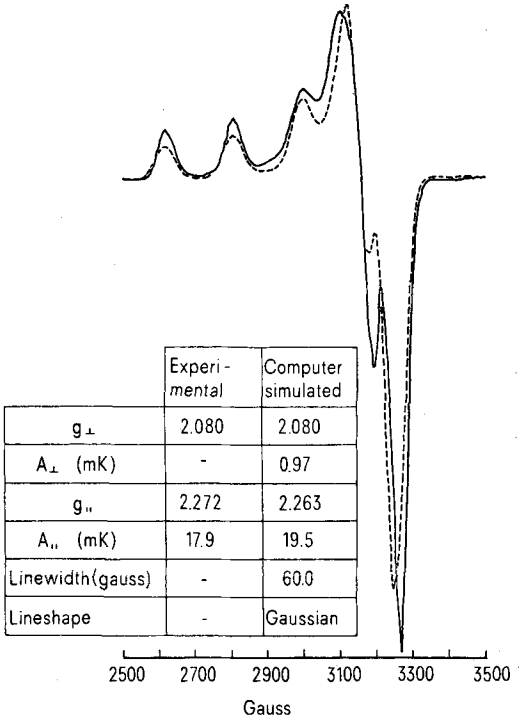


Fig. 1. IR-spectra of copper(II)-L-histidine molybdate (A) and bis-L-histidine-copper(II) dinitrate dihydrate (B). Spectra contain the absorption band of polystyrene at 1603 cm^{-1} as calibration.

IR spectra were recorded using pressed KBr discs. The absorbed water bands of KBr were identified by comparison with spectra of materials in liquid paraffin mulls. ESR spectra were recorded at a frequency of 9.48 GHz (room temperature) and 9.19 GHz (77 °K). The frequency at 77 °K was accurately determined as 9.1968 GHz using a microwave frequency counter and the magnetic field was calibrated with a proton magnetometer for each g-value. The field was modulated at a frequency of 100 kHz.

To identify copper(II)-L-histidine in **1**, the complex was suspended in water and 0.1 M NaOH added until it dissolved. Copper(II)-L-histidine was separated by TLC and identified with HCl/ninhydrin[5] and Na_2S/NH_3[6]. To identify histidine in **1**, the complex was suspended in water and NH_4OH (0.88 sp.gr.) added until the suspension dissolved. The solution was treated with 0.1% sodium diethyldithiocarbamate until no more precipitate resulted. Following extraction with CCl_4 the aqueous phase was concentrated by rotary evaporation and chromatographed on silica gel G thin layers. The solubility of **1** in water was determined at 25 °C. Excess of **1** was allowed to come to equilibrium with water and Cu determined in the filtrate. The solubility of **1** was calculated on the basis of it containing 12.2% Cu.

Results and discussion. Copper(II)-L-histidine molybdate is a light blue complex, the analysis of which approximates to a composition of $Cu_2(His)_3(MoO_4)_2(H_2O)_2$. Analyses carried out on a batch of the complex, prepared using $CuSO_4$, failed to detect the presence of Na or SO_4^{2-} in significant quantities. All attempts to crystallize **1** were unsuccessful. The complex degraded in hot DMSO, was insoluble in hot acetonitrile or hot methanol and was hydrolyzed in hot water. Precipitation and hydrolysis was prevented by adding hot Na_2MoO_4 or $(NH_4)_6Mo_7O_{24}$ to hot copper(II)-histidine containing a few drops of acetic acid. However, on cooling an amorphous precipitate resulted.



	Experimental	Computer simulated
$g_\perp$	2.080	2.080
$A_\perp$ (mK)	-	0.97
$g_\parallel$	2.272	2.263
$A_\parallel$ (mK)	17.9	19.5
Linewidth(gauss)	-	60.0
Lineshape	-	Gaussian

Fig. 2. ESR-spectra of copper(II)-L-histidine molybdate. —, Experimental; – – –, computer simulated. mK, Millikaisers (1000th of a wavenumber).

Copper(II)-L-histidine was identified in **1** compared with a standard of copper(II)-L-histidine prepared by mixing equal volumes of $CuSO_4$ and L-histidine solutions in a molar ratio of L-His: $CuSO_4$ of 2:1. In the TLC system used copper(II)-L-histidine separated into L-histidine (R_f = 0.44) and copper(II)-L-histidine (R_f = 0.32). L-Histidine was identified in **1** compared with a standard on the basis of colour with ninhydrin and R_f(0.38 with 96% ethanol: water 70:30 (v/v) and 0.42 with $CHCl_3$: MeOH: 17% NH_4OH 40:40:20 (v/v/v) as solvent systems). Copper(II)-L-histidine molybdate did not form above a pH of approximately 6. A small amount of precipitate was observed at pH 5.9 but none at pH 6.2. A graph of NaOH added versus pH of solutions containing copper(II)-L-histidine and molybdate suggested that ionization of the imidazole function of histidine (pk_a = 6.0 at 25 °C) prevents the formation of the complex.

The principle IR active bands and their assignments for **1** (figure 1A) are 3250 and 3140 cm^{-1} (N–H stretch); 1610 and 1395 cm^{-1} (COO^- stretch) and 890 cm^{-1} (MoO_4^{2-} stretch). The principal IR active bands and their assignments for **2** (figure 1B) are 3250 and 3150 cm^{-1} (N–H stretch); 1620 and 1380 cm^{-1} (COO^- stretch) and 1350 cm^{-1} shoulder (NO_3^- stretch). The high intensity of the carboxylate anion symmetric stretch relative to the antisymmetric stretch could be accounted for by the strong nitrate band. The ESR spectrum of **1** (figure 2) is similar in form to many copper proteins and model copper compounds exhibiting tetragonal symmetry[7,8]. The values of $g_\perp$, $g\parallel$ and $A\parallel$ are similar to those of copper(II)-L histidine in frozen solutions[7]. The ESR-spectrum of **1** at room temperature was of the same form as that at 77 °K. The spin Hamiltonian parameters were obtained from the observed powder spectrum by comparison with a computer simulated spectrum (figure 2). The simulated spectrum was obtained by the histogram method with energies taken to second order in perturbation. No account was taken of possible anisotropy in line width in the simulated spectrum. ESR spectra of **1** in which copper was not magnetically diluted and of **2** showed single asymmetric signals at room temperature and 77 °K. The solubility of **1** in water at 25 °C was 0.38 ± 0.02 mg/ml. The value is the mean ± SD of 3 determinations. The empirical formula of **1** of $Cu_2(C_6H_9O_2N_3)_3(MoO_4)_2(H_2O)_2$ indicates that the complex may be a binuclear copper complex. The ESR spectrum of **1** and the computer simulation of the spectrum (figure 2) indicated that the complex has tetragonal symmetry. In this symmetry ligands are situated in a square planar arrangement around the copper with axial ligands at a different distance. In **2**, X-ray crystallographic studies[3,9], have shown that histidine is coordinated to the copper atom by an amino nitrogen and a carboxyl oxygen with the imidazole function not coordinated to the copper atom. By analogy with the structure of **2** it is proposed that in **1** the imid-

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azole is not coordinated to the copper atom. On electrostatic grounds, it is likely that in **1** the carboxylate anion ligands to the copper atom and the protonated imidazole does not ligand to copper. The frequency and shape of the bands between 950 and 550 cm^{-1} of the IR spectrum of **1** are similar in frequency and shape to the bands of ammonium paramolybdate[10]. This suggests that in **1** the molybdate anion forms an ammonium-type salt with the nitrogens of the imidazole. In contrast to ammonium paramolybdate most molybdates absorb close to 800 cm^{-1} [10,11].

As well as copper(II)-L-histidine, copper(II)-threonine, copper(II)-glutamine and histidine-copper(II)-threonine have been identified in normal serum[5]. Also histidine-copper(II)-glutamine and histidine-copper(II)-serine have been observed to exist in vitro at physiological pH[12]. The addition of molybdate to copper(II)-threonine, copper(II)-glutamine or copper(II)-serine resulted in no precipitate being formed. However, precipitates resulted from the addition of molybdate to histidine-copper(II)-threonine, histidine-copper(II)-serine or histidine-copper(II)-glutamine. These precipitates were identified as **1** on the basis of IR-spectroscopy. The addition of molybdate to all the above copper-amino acid complexes raised the pH of these solutions (or filtrates where precipitates occurred) and also caused a shift in the absorption maxima to shorter wavelengths. These shifts in absorption maxima were a consequence of the pH change because they could also be produced by the addition of NaOH.

The present work reports the occurrence of an interaction between copper(II)-histidine and molybdate and partially characterizes this interaction. The formation of copper- and molybdenum-containing compounds, such as $2CuMoO_4 \cdot Cu(OH)_2$ and $Cu(NH_4)MoS_4$, has been proposed as a mechanism for the interaction between molybdenum and copper in vivo[13–16]. Unlike these compounds the interaction described in the present work is one between molybdate and a physiological form of copper, copper(II)-histidine being a component of serum[5]. As **1** dissociates above pH 6, the formation of this complex in serum would require its stabilization by a means such as being bound to protein. In molybdenum poisoning alterations in the distribution and metabolism of copper in serum occur such as an increase in direct-reacting copper, the de novo formation of a copper- and molybdenum-containing protein fraction and a reduced rate of clearance of copper[17]. The question of whether **1** is formed in serum in molybdenum poisoning is currently being investigated in this laboratory.

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Synthese und Eigenschaften von Bromocriptin[1]

Synthesis and properties of bromocriptine

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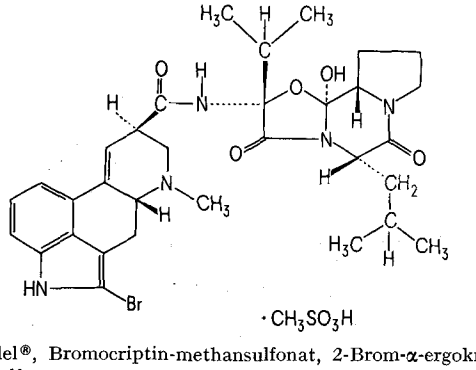
Summary. The bromination of α-ergokryptine, a genuine ergot alkaloid of the peptide type, in position 2 of the indol nucleus to 2-bromo-α-ergokryptine is described. Its transformation to the methanesulfonate led to the prolactin inhibitor bromocriptine-methanesulfonate, Parlodel®.

Die steigende klinische Bedeutung des Prolactin-Sekretionshemmers[3] Parlodel®, (**1**; Bromocriptin-methansulfonat, 2-Brom-α-ergokryptin-methansulfonat), veranlasst uns, die Synthese sowie die chemischen und physikalischen Eigenschaften dieses Präparates hier kurz zu beschreiben.



1 Parlodel®, Bromocriptin-methansulfonat, 2-Brom-α-ergokryptin-methansulfonat.

Die Halogenierung von Lysergsäurederivaten ist vor etwa 20 Jahren von F. Troxler und A. Hofmann systematisch erforscht worden; die Resultate wurden in einer zusammenfassenden Arbeit publiziert[4]. Dabei wurde gezeigt, dass Lysergsäurederivate durch Erwärmen mit 1,2 bis 1,5 Äquivalenten N-Bromsuccinimid in Dioxanlösung mit mittleren Ausbeuten in die entsprechenden 2-Bromlysergsäurederivate übergeführt werden können. Im Zuge dieser Arbeiten wurden auch die 2-Bromderivate der damals bekannten genuinen Mutterkorn-Peptidalkaloide mit Ausnahme des 2-Brom-ergokryptins hergestellt[4].

Der Grund dafür, dass 2-Brom-ergokryptin in der zitierten Arbeit[4] nicht beschrieben worden war, lag darin, dass das damals zur Verfügung stehende Ergokryptin nicht eine chemisch einheitliche Verbindung darstellte, sondern, wie sich später herausstellte, aus einem Gemisch zweier sehr nah verwandter Verbindungen (α- und β-Ergokryptin) bestand[5]. Die Bromierung eines solchen Gemisches (eine schon bei reinen Mutterkorn-Peptidalkaloiden heikle Reaktion) hatte verständlicherweise damals zu keinen definierten, einheitlichen Bromierungsprodukten geführt, weshalb in der Liste der 2-Bromderivate von Mutterkorn-Peptidalkaloiden eine Lücke offenblieb.