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INHIBITION OF THE YEAST ALCOHOLDEHYDROGENASE BY Cu(II)-COMPLEXES OF COLCHICEINE AND N-DEACETYLCOLCHICEINE

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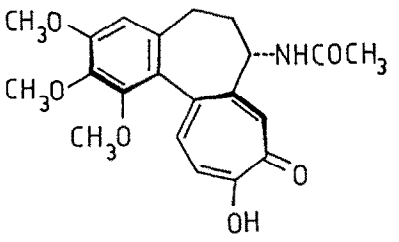
Abstract: The Cu(II)-complexes of colchiceine (1) and N-deacetylcolchiceine (2) were prepared. They have been shown to be potent inhibitors of the yeast alcoholdehydrogenase. The results indicate the formation of a ternary enzyme-Cu(II)-alkaloid complex.

Colchiceine (1) and N-deacetylcolchiceine (trimethylcolchicinic acid, 2) are 10-hydroxytropone derivatives of colchicine, which are effective in the treatment of connective tissue diseases[1]. These diseases are associated with the altered copper metabolism[2]. Colchiceines do not show antimitotic activity in contrast to that of colchicine[3]. A possible mechanism of biological activity of some mono- and bistropolones has been explained by their metal-chelating properties[4]. In this communication we wish to report the synthesis of two copper(II)-colchiceine complexes[5] and to describe their interaction with yeast alcoholdehydrogenase (YADH).



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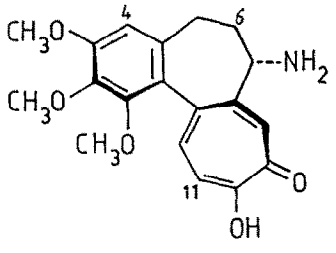

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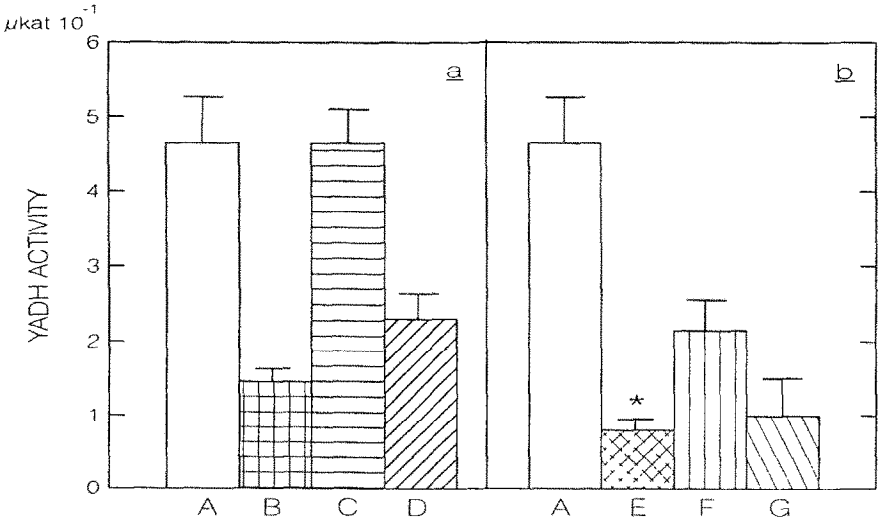
Copper complexes of 1 (Cu(II)-colchiceine-Cl.H_2O, 3) and 2 (Cu(II)-N-deacetylcolchiceine-Cl, 4) were prepared by the reaction of the alkaloids with $CuCl_2$ in ethanolic solution[6]. The complexes 3 and

4 were found to cause concentration-dependent inhibition (IC_{50} = 1.30± ±0.06 μM and 1.0±0.04 μM, respectively) of YADH[7]. The Cu(II)-ion was in comparison with 3 or 4 less potent inhibitor of YADH (IC_{50} = 2.00± ±0.08 μM), the effect of alkaloids 1 and 2 was nonsignificant. The influence of chelating agents (EDTA,D-penicillamine) on the inhibition of YADH[8] by Cu(II)-ion, 3 and 4 is shown in Figure 1.

Figure 1. The effect of EDTA and D-penicillamine on YADH inhibition by Cu(II)-ion (a) and Cu(II)-colchiceine complex 3 (b).



YADH activity without inhibitors (A)
+ Cu(II) (B)
+ Cu(II) + EDTA (C)
+ Cu(II) + D-penicillamine (D)
+ complex 3 (E)
+ complex 3 + EDTA (F)
+ complex 3 + D-penicillamine (G)

Concentration of Cu(II), complex 3 and chelating agents - 3.7 μM[8]
* $p < 0.05$ as compared with B

While chelation of Cu(II)-ion by EDTA and D-penicillamine causes the loss or decrease of Cu(II)-promoting YADH inhibition, the chelation by 1 or 2 results in stronger inhibitory effect[9].These data indicate that the specific ternary complex involving Cu(II)-ion, alkaloid and YADH is formed. A significantly lower effect of EDTA and D-penicillamine on the inhibition of YADH by 3 and 4 is in agreement with these findings.

Recently, the dissociation constants of ternary complexes Cu(II)-acid monoazodyes-YADH have been determined[10]. The formation of these complexes was suggested at the NAD^+-binding site of the enzyme. In this study, kinetic measurements[11] proved a noncompetitive character of YADH inhibition by **3**, **4** versus ethanol and NAD^+ suggesting Cu(II)-colchiceine-YADH-binding outside the enzyme active site.

The study of ternary complexes enzyme-alkaloid-metal ion can provide new insights on a mechanism of biological action of colchiceines. An investigation of complexation of these substances with Zn(II) and Fe(III) is currently underway, the results of which will be reported later.

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References and Notes

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5. Complex Cu(II)$(C_{21}H_{22}NO_6)_2.5\ H_2O$ was obtained from colchiceine by the reaction with copper(II)hydroxide: Zeisel, S. *Mh. Chemie* **1886**, *7*, 557.
6. Data for Cu-colchiceine-Cl.H_2O (3): UV/VIS [EtOH, λ_{max}, nm(ε, $M^{-1}cm^{-1}$)] 257 (26 920), 357 (23 600), 380 (38 920);
   IR (KBr, cm^{-1}) 1628, 1593, 1430, 1364, 1315, 1135, 1086, 1004;
   Anal. Calcd for $C_{21}H_{24}NO_7CuCl$: C, 50.43;, H, 4.22; N, 2.80; Cl, 7.08. Found: C, 50.11; H, 4.56; N, 3.27; Cl, 6.98.
   Data for Cu-N-deacetylcolchiceine-Cl (4): UV/VIS [EtOH, λ_{max}, nm (ε, $M^{-1}cm^{-1}$)] 257 (30 120), 357 (19 000), 380 (21 240);
   IR (KBr, cm^{-1}) 1587, 1428, 1360, 1300, 1186, 1130, 1085, 993;
   Anal. Calcd for $C_{19}H_{20}NO_5CuCl$: C, 51.58; H, 4.71; N, 3.17; Cl, 8.01. Found: C, 1.94; H, .24; N, 2.86; Cl, 7.60.

7. The ADH activity was determined as the initial rate v_o of the ethanol dehydrogenation. The time increment A_{340}/min of the increased NADH was recorded. The measurements were carried out at pH 7.0, 25°C in the total volume 2.7 ml.The concentrations in reaction mixture: ethanol 8 mM, NAD^+ 0.4 mM, enzyme 5.5 μg/ml (Merck 180 U/mg), Cu(II) and complexes 0.37 - 3.7 μM. The IC_{50} values were determined from the dependence of the inhibitors concentrations vs. the percent of inhibition (% inh = $(1-v_i/v_o).100$; v_i is the inhibited reaction rate.
8. YADH was preincubated with Cu(II), and **3**, **4**, respectively before addition of the chelating agents and substrates. EDTA and D-penicillamine were without any effect on the YADH activity.
9. Equilibrium constants in water: for Cu(II)-EDTA log β = 18.80 in Kotrlý, S.; Šůcha, L. *Chemické rovnováhy v analytické chemii*; SNTL Praha 1988. p.161; for Cu(II)-1 log β = 10.65±0.07, for Cu(II)-2 log β = 10.67±0.05, taken from Ulrichová, J.; Walterová, D.; Lasovský, J.; Vičar, J.; Šimánek, V. *Collect. Czech. Chem. Commun.*- submitted for publication.
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11. Kinetic measurements were carried out at 1) 10 μM ethanol, variable NAD^+(51.11-511.1 μM); 2) 500 μM NAD^+, variable ethanol (0.97-24.3 mM). Concentration of Cu(II), and complexes **3**, **4** were 5.0 and 25.0 μM. Double-reciprocal plots of the obtained data were fit using the computer program[12]. The results proved a noncompetitive inhibition with both ethanol and NAD^+ in all the inhibitors tested.
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