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Preliminary communication

Cobalt and molybdenum carbonyl clusters in immunology. Synthesis and binding properties of mycotoxin derivatives of zearalenone

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Abstract

Transition metal cluster haptens of zearalenone derivatives having high binding affinity values for antibodies specific for this mycotoxin series have been synthesised. This provides the basis for a new type of non-isotopic immunoassay.

Recent work involving transition metal complexation of bioligands has widened interest in these species beyond the field of organic chemistry and into bio-organometallics [1]. Although the idea of using metallolabels has appeared several times in the literature in the last few years [2–4], demonstration of the real usefulness of this concept is still in its infancy [5,6]. The key feature, in addition to the analytical methodology used for the metal detection [6–10], concerns the degree of molecular recognition between the metal modified bioligand and the binding macromolecule under investigation (receptor, antibody, enzyme).

We now report the synthesis of transition metal carbonyl clusters coupled to zearalenone and zearalanol derivatives and on their binding properties with respect to the specific antibodies produced for these bioligands. We are not aware of any previous work in this area.

Zearalenone **1** is a natural mycotoxin produced by *Fusarium graminearum* on wet corn and other cereal grains [11,12]. This compound causes hyperestrogenism [13], while the reduction product α-zearalanol, (**2**), is 5 times more estrogenic [14], exhibits anabolic properties [15], and is implanted in cattle to promote rapid weight gain [15]. There has been considerable concern that these compound may be passed to humans, and banning of their use in animal feed is planned in Europe. Consequently the development of a non-radioisotopic immunoassay, necessary for veterinary diagnosis, is of immediate importance for these mycotoxins [16].

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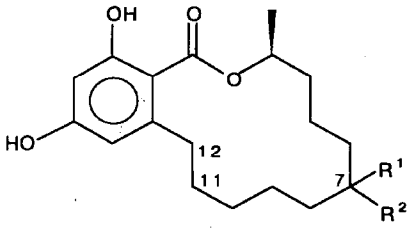
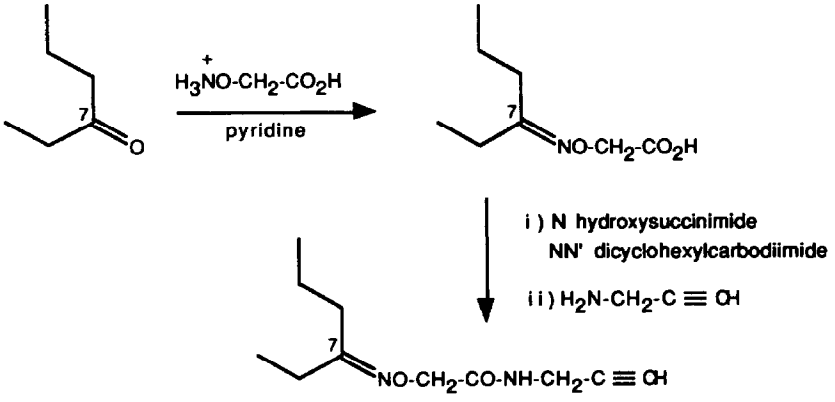


Fig. 1. Structure of the Zearalenone derivatives.

Compound	C(11)–C(12)	R^1	R^2
Zearalenone (**1**)	*trans*	O	
α,β-Zearalanol (**2**)	saturated	α,β-OH	α,β-H
Zearalanone (**3**)	saturated	O	
4α, **4**β	saturated	α,β OH	α,β-C≡CH
5α, **5**β	saturated	α,β OH	α,β-C≡CH $Co_2(CO)_6$
6	*trans*	$NOCH_2CONHCH_2C{\equiv}CH$	
7	*trans*	$NOCH_2CONHCH_2C{\equiv}CH$	$Co_2(CO)_6$
8	saturated	$NOCH_2CONHCH_2C{\equiv}CH$	
9	saturated	$NOCH_2CONHCH_2C{\equiv}CH$	$Co_2(CO)_6$
10	saturated	$NOCH_2CONHCH_2C{\equiv}CH$	$Mo_2Cp_2(CO)_4$

We have synthesised two types of metallohaptens in this series (Fig. 1). In one type complexes **5** retain the hydroxy function at the 7 position for the other, this position has been modified by a spacer bearing at its end a cobalt or molybdenum cluster group (**7**, **9**, **10**). In both cases, the resorcylic part of the molecule was left untouched, since this region was recognized as crucial for binding with the antibodies [17]



Scheme 1

Table 1

Cross-reaction yields of the organometallic complexes and zearalenone and zearalanol with [6,8-^3H]-zearalenone binding to antibodies [a]

Compound	5 [c]	7	9	10	Zearalenone [d]	α-Zearalanol [e]
CR_{50} [b] (%)	5	57	39	31	100	53

[a] Cross-reaction was performed in presence of 0.86 ng of [6,8-^3H]-zearalenone (specific activity 2.26 Ci/mmol) as described in ref. 17. [b] CR_{50} = [(ng of compounds displacing 50% label)/(ng of zearalenone displacing 50% of label)]×100. [c] Cross-reaction was performed using a mixture of **5α** and **5β**. [d] Value by definition. [e] Data from ref. 17.

Compounds **5α** and **5β** were prepared as follows. After protection of the phenolic functions of zearalanone **3** with the t-butyldimethylsilyl group, compounds **4(α,β)** were obtained by addition of the acetylene Grignard reagent in THF. The two isomers were then separated by thin layer chromatography (silica gel; ether/pentane 1/1). The phenolic functions were then deprotected by use of tetrabutylammonium fluoride in THF, and the triple bond was complexed by use of $Co_2(CO)_8$ in CH_2Cl_2 solution to give **5α** and **5β** (yields: 60%).

Compounds **6** and **8** were prepared by the procedure, adapted from ref. 17, shown in Scheme 1, starting from zearalenone for **6** and zearalanone for **8**. The triple bond was then complexed by use of $Co_2(CO)_8$ or $Mo_2Cp_2(CO)_4$ in CH_2Cl_2 solution [18,19]. The complexes **7, 9, 10**, were purified by thin layer chromatography (silica gel; ether, pentane 2/1) and recrystallized from an ether/pentane mixture. The complexation yields were of the order of 60%.

The compounds **5, 7, 9, 10**, have been fully characterized by NMR, ^1H (1D and 2D COSY), at 250 MHz, ^{13}C (DEPT), and IR spectroscopy, and by mass spectrometry with chemical desorption using NH_3 as reactant gas. Elemental analysis was performed on the alkyne compounds **4, 6, 8** prior to complexation.

The new complexes were tested for displacement of [6,8-^3H]-zearalenone bound to the antibodies obtained as described by Thouvenot and Morfin (17). The cross-reaction results are shown in table 1.

All the complexes displaced the antibody-bound [^3H]-zearalenone but not to the same extent. Compound **5** competes rather weakly, although all he original functions are present in the molecule. However, complexes **7, 9, 10** are more effective despite the removal of the 7-hydroxy function, and the cross-reaction values come close to or exceed 50%.

The poor recognition shown by **5** is attributable to the deformation of the flexible lactone ring due to the presence at the 7 position of the bulky cluster group which introduces steric strains. The remoteness of the cluster in **7, 9, 10** means that the conformation of the molecule is not changed from that for the free ligand and allows an excellent hapten antibody interaction. Thus the cross reaction yields appear very satisfactory, even with bulky clusters, provided that a spacer chain permits fitting of the metallohaptens to the antibody association sites. We are currently working on generalization of this observation, but the good molecular recognition found here already augurs well for the development of a sensitive infrared immunoassay (IRIA) with these species (6).

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