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Preliminary communication

Addition of imidazoles and aminoacids to the ethylenic bond in $(\eta^5\text{-}C_5H_5)Fe(CO)_2(\eta^1\text{-}N\text{-maleimidato})$

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Abstract

$(\eta^5\text{-}C_5H_5)Fe(CO)_2(\eta^1\text{-N-maleimidato})$ **1** reacts in water solution at pH = 7 with imidazole, histidine methyl ester and carnosine to give the products of the addition of the heterocyclic moiety to the ethylenic bond of the maleimidato ligand. Addition of the primary amino group of glycine and β-alanine to **1** takes place at pH = 10 and 60°C. These results are relevant for labelling of peptides and proteins with **1**.

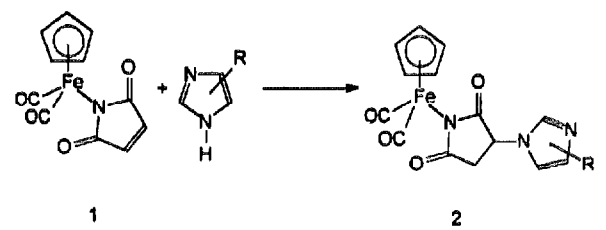
Keywords: Iron; Carbonyl; Maleimide anion; Amino acid; Imidazole; Addition to the ethylenic bond

The labelling of specific sites of proteins by organometallic complexes attracts continuously increasing attention [1]. Transition metal carbonyl tracers are of special interest since the labelled protein can be easily detected in biological samples by FTIR spectroscopy in the region around 2000 cm^{-1} (stretching vibrations of coordinated CO) even at very low concentrations ($< 10^{-9}$ M) [2]. This offers a new entry, for example, the study of protein receptors, affinity labelling and immunochemistry. Recent developments in this field include application of labelled immunogens or haptens in immonoassays (CMIA-carbonylmetalloimmunoassays) [1(a),3].

This elegant approach combines high sensitivity with the possibility of simultaneous analysis of several labelled species (multi-assays) [4]. However, for the future development of CMIA it is essential to design susceptible labelling agents to introduce organometallic tracers into proteins under mild, physiological conditions and to examine their selectivity toward typical functional groups present in proteins.

We recently reported that the air-stable and water-soluble complex $(\eta^5\text{-}C_5H_5)Fe(CO)_2\text{-}(\eta^1\text{-N-maleimidato})$ **1** reacts with cysteine (used as methyl ester hydrochloride) and a tripeptide, glutathione to give products of the addition of the HS– group to the carbon–carbon double bond of the maleimidato ligand [5]. This reaction takes place under mild conditions (in water at pH = 7 and 35°C) and therefore is of interest as a potential method of labelling of more complex systems of proteins. The question that arises at this stage concerns the selectivity of **1** toward other functional groups present in peptides and proteins. In fact, despite widely accepted opinion that maleimido function is selective for HS– groups [6], it has recently been shown that it also reacts with the imidazole moiety of histidine [7].

In this communication we report that **1** reacts with imidazole and some imidazole-containing biomolecules: L–histidine (used as methyl ester) and L–carnosine (β–alanylo–L–histidine) as is shown in Scheme 1.



1 **2**

(a) R=H
(b) R=$CH_2CH(NH_2)COOMe$
(c) R=$CH_2CH(NHCOCH_2CH_2NH_2)COOH$

Scheme 1.

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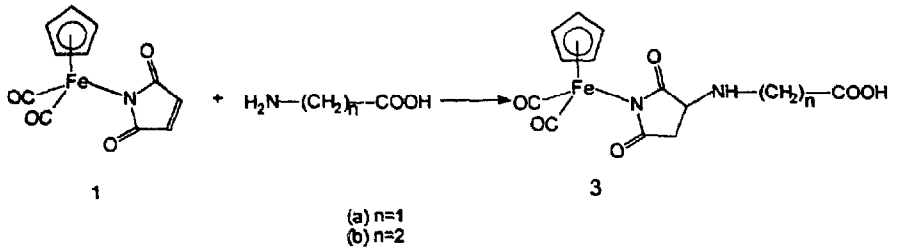

Scheme 2.

The reaction of **1** with imidazole takes place slowly in aqueous solutions (pH = 7) at 35°C. After 5 days **2a** was formed in 30% yield and was separated from the unreacted **1** by column chromatography and purified by crystallization from ether–dichloromethane [8]. When the reaction of **1** with imidazole was carried out at 60°C for 24 h the yield of **2a** was improved to 55%. However, another unidentified brown complex was also formed. The reaction of **1** with imidazole is practically stopped at pH = 5; but the raising of pH to 9 does not seem to influence the reaction.

In the existing literature there is only one report on the reaction of imidazole with a maleimide, namely with N-ethylmaleimide [9]. This reaction is described as leading to a pinky, polymeric material, presumably formed by the attack of imidazole on the carbonyl group of imide. Contrastingly, **1** with imidazole gives the product of the addition to the carbon–carbon double bond. The attachement of the Fp unit to maleimide presumably deactivates carbonyl functions by the d_{π}–p_{π} repulsion [10] (note that **1** displays ν(CO) of imide carbonyls at 1655 cm^{-1} in comparison with 1730 cm^{-1} found for maleimide) and directs the attack of imidazole on carbon–carbon double bond.

The addition of the imidazole ring to the olefinic bond in **1** was also observed when this complex reacted with L-histidine methylester and the dipeptide, L-carnosine (β-alanylo-L-histidine). The bioconjugates **2b** and **2c** were formed in good yields (50%–60%) [8]. The ^{1}H NMR indicated formation of two regioisomers, N_{τ} and N_{π}, typical for histidine alkylation reactions and due to the azole tautomerism [7,11]. This fact, together with the presence in **2b** and **2c** of the second center of chirality make ^{1}H NMR spectra of these species very complex and the completion of assignment was impossible.

We have also found that **1** reacts with aminoacids: glycine and β-alanine to afford complexes **3a–b** as in Scheme 2.

This reaction requires more vigorous conditions (temperature 60°C and pH = 10–11 to assure the presence of nonprotonated amino groups). Under these conditions the isolated yield of **3a** was 64% after 20 h [8]. In reaction of **1** with β-alanine **3b** was isolated in 72% yield after 3 days [8]. These reactions can be regarded as models indicating reactivity of **1** towards N–terminal and lysine–type amino groups in peptides or proteins.

In conclusion, we have found that **1** can be used as a metallocarbonyl label not only for cysteine-containing peptides and proteins but also for histidine residues in the biomolecules. Moreover, in a basic medium **1** can react with free amino groups present in peptides or proteins (N–terminal or lysines). Presumably, the selectivity of **1** towards different functional groups can be finely-tuned by changing reaction conditions. Nevertheless, in our opinion one should critically revise the widely accepted opinion concerning selectivity of maleimides towards HS– containing molecules.

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[8] Complexes **2a** and **3a** gave satisfactory elemental analyses (C, H, N) and showed peaks corresponding to $(M+H)^+$ and $(M+H-2CO)^+$ in the FAB mass spectra. Other new complexes synthesized in this work showed peaks corresponding to $(M+H)^+$ and $(M+H-2CO)^+$ in the FAB mass spectra. However, due to their tendency to retain residual solvent or to extremely high hygroscopicity we were unable to obtain correct elemental analyses. Selected spectral data are given below.
2a: IR ν (cm^{-1}) ($CHCl_3$): 2050, 2005 (M–CO); 1620 (imide). ^1H NMR ($CDCl_3$, δ): 7.56 (s, 1H), 7.10 (s, 1H), 6.85 (s, 1H), imidazole; 5.07 (s, 5H), Cp; 4.98 (dd, $J = 8.5$ Hz, 5.4 Hz, 1H) C*H*–N; 3.26 (dd, $J = 18.5$ Hz, 8.5 Hz, 1H) and 2.85 (dd, $J = 18.5$ Hz, 5.4 Hz, 1H), succinimide CH_2. $^{13}C\{^1H\}$ NMR ($CDCl_3$, δ): 211.7 M–CO; 185.7, 185.3 CO(imide); 136.5, 130.3, 116.9, imidazole; 85.0 (Cp); 57.2, 39.5, succinimide.
3a: IR ν (cm^{-1}) (KBr): 3180 (NH); 2050, 2005 (M–CO); 1720 (COOH); 1640 (imide). ^1H NMR (D_2O + HCl, δ): 5.21 (s, 5H), (Cp); 4.40 (dd, $J = 8.6$ Hz, 5.0 Hz, 1H) C*H*–N; 4.21 (d, $J = 17.2$ Hz and 4.06 (d, $J = 17.2$ Hz), N– CH_2–; 3.03 (dd, $J = 17.7$ Hz, 8.6 Hz, 1H) and 2.81 (dd, $J = 17.7$ Hz, 5.0 Hz) succinimide CH_2. $^{13}C\{^1H\}$ NMR (D_2O + HCl, δ): 213.5, 213.4, M–CO; 190.0, 187.2, CO(imide); 169.8, COOH; 57.2 *C*H– N; 47.4 N– *C*H_2; 35.8, succinimide *C*H_2.
3b: IR ν (cm^{-1}) (KBr): 3180 (NH); 2050, 2005 (M–CO); 1710 (COOH); 1640 (imide). ^1H NMR (D_2O + CF_3COOH, δ): 5.18 (s, 5H), Cp; 4.33 (dd, $J = 8.0$ Hz, 5.5 Hz, 1H) C*H*–N; 3.43 m, 2H), N– CH_2; 3.15 (dd, $J = 17.9$ Hz, 8.0 Hz, 1H) one of succinimide CH_2, 2.87 (t, $J = 6.0$ Hz, 2H), 2.29 (m, partially overlapped with the previous signal, 1H), one of succinimide CH_2. $^{13}C\{^1H\}$ NMR (D_2O + CF_3COOH, δ): 213.3, 213.2 M–CO; 189.8, 187.0 CO(imide); 174.9, COOH; 86.5, Cp; 57.9, succinimide C*H*– N; 43.1, N– *C*H_2–CH_2–; 35.5, succinimide; 31.1, N– CH_2–*C*H_2–.

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