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(η^5-Cyclopentadienyl)Fe(CO)$_2$-complexes of sulfonamide anions. Acylation of the amino group in the sulfanilamide complex and its relevance to the protein labelling

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Abstract

Illumination with visible light of [CpFe(CO)$_2$I] (Cp = η^5-C$_5$H$_5$) with NH$_2$SO$_2$C$_6$H$_4$-*p*-R (R = Me, NO$_2$, NH$_2$) in benzene in the presence of diisopropylamine gives [CpFe(CO)$_2$NHSO$_2$C$_6$H$_4$-*p*-R] in 62–91% yield. The primary amino group in [CpFe(CO)$_2$NHSO$_2$C$_6$H$_4$-*p*-NH$_2$] was acylated by treatment of this complex with neat carboxylic acid anhydrides (acetic, propionic and isobutyric) or with carboxylic acid (acetic, iodoacetic, β-maleimidopropionic) and 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide (EDC) in aqueous solutions at pH 5–6 at room temperature. The latter reaction shows that [CpFe(CO)$_2$NHSO$_2$C$_6$H$_4$-*p*-NH$_2$] can be used as an IR-detectable metallo-carbonyl reagent labelling the COOH groups in proteins and as a parent compound for a new family of reagents labelling the thiol function.

Keywords: Iron carbonyls; Cyclopentadienyl; Sulfonamide anions; *N*-acylation; Carbodiimide

1. Introduction

We are currently investigating the synthetic potential of the photochemical substitution of iodide in [CpFe(CO)$_2$I] (Cp = η^5-C$_5$H$_5$) by anions of N–H acids (Eq. (1); H–N(X)(Y) = N–H acid, B = diisopropylamine).

$$[CpFe(CO)_2I] + H{-}N(X)(Y) + B \xrightarrow[-\,BH^+I^-]{h\nu} [CpFe(CO)_2N(X)(Y)] \quad (1)$$

This reaction leads to a variety of stable CpFe(CO)$_2$-complexes of anions of pyrroles, indoles, cyclic imides and uracils [1]. Some of these complexes contain functional groups able to react with functional groups present in lateral chains of proteins and can be used for introduction of IR-detectable labels into these (and other) biopolymers. This is the case of [CpFe(CO)$_2$(η^1-*N*-maleimidato)] and [CpFe(CO)$_2$(η^1-*N*(1)-isothiocyanatophthalimides)], which were succesfully used for labeling of model aminoacids and bovine serum albumine ([1]c, f).

Sulfonamides constitute a class of N–H acidic compounds which display very interesting pharmacological properties, especially *p*-aminobenzenesulfonamide (sulfanilamide) and its derivatives [2]. However, very little is known about transition-metal complexes of sulfonamides or their anions [3], and, to our knowledge, there is no example of a complex in which such a ligand is co-ordinated to a metallo-carbonyl moiety.

We thought that it would be of interest to look if sulfonamides undergo reaction shown in Eq. (1) and afford CpFe(CO)$_2$-complexes of their anions. The derivative of sulfanilamide would be of special interest as containing NH$_2$ group which is of interest as a reactive function for coupling with the COOH func-

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tions in biomolecules. This could be easily tested in the reactions with some model carboxylic acids. Herein we report results of this study.

2. Results and discussion

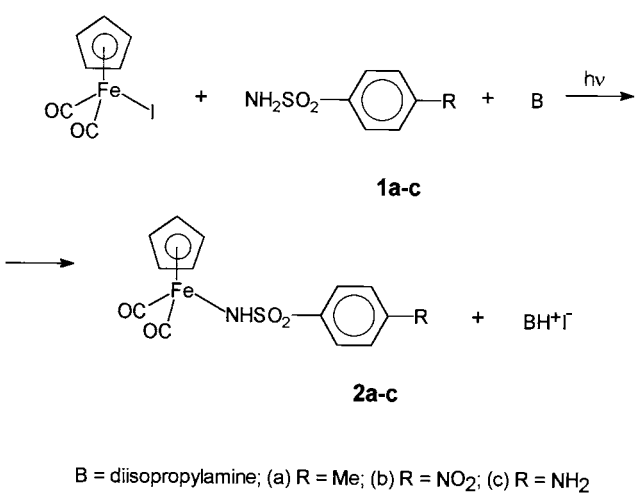
Irradiation of $[CpFe(CO)_2I]$ with sulfonamides **1a–c** and diisopropylamine in benzene brings about formation of orange, air stable complexes **2a–c** in 64–91% yield (Scheme 1).

The reaction proceeds in the same manner with **1c** as with **1a–b**, suggesting that amino group in the benzene ring is not involved, and does not hamper the reaction. We reported earlier a similar phenomenon in the case of reaction with aminophthalimides ([1]f).

The IR spectra of **2a–c** in the region 1400–1100 cm^{-1} are very similar to the spectra of **1a–c** and display characteristic, strong bands assignable to the symmetric and antisymmetric stretching vibrations of the $-SO_2-$ moiety (shifted around 50 cm^{-1} towards lower wavelengths in comparison to the same bands in **1a–c**). This is in full accord with the structure in which $CpFe(CO)_2$-moiety is bonded to deprotonated sulfonamide nitrogen causing repulsion between iron d-electrons and the lone pair at nitrogen. In the region of the N–H stretching vibrations **2a–b** show one band at 3290 cm^{-1} (νNH), whereas **2c** shows three bands: 3370, 3310 (νNH_2) and 3280 cm^{-1} (ν NH), accordingly to the presented structure.

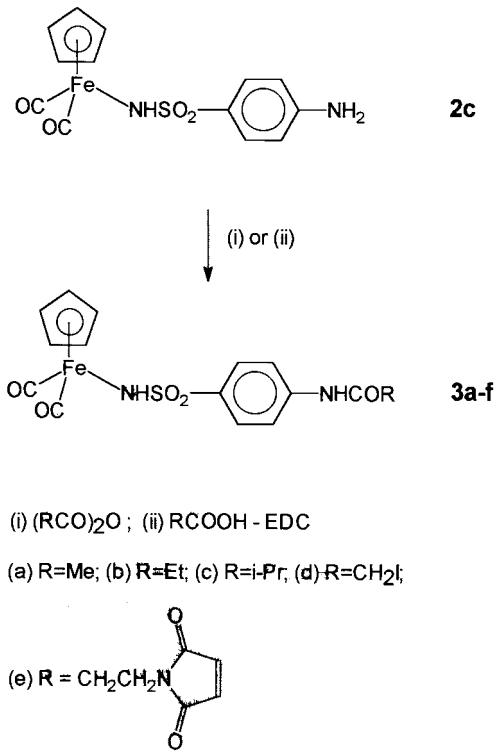
The derivative of sulfanilamide **2c** is sparingly water-soluble (ca. 1 mg ml^{-1}) and its aqueous solutions are stable providing they are stored in the dark.

Attempted reactions of **2c** with acetyl chloride and triethylamine in dichloromethane failed. However, we have found that **2c** react neat liquid anhydrides (acetic, propionic and isobutyric anhydride) at r.t. to afford corresponding amides **3a–c**, which precipitate directly



B = diisopropylamine; (a) R = Me; (b) R = NO_2; (c) R = NH_2

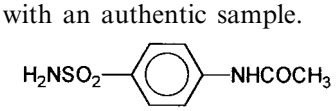
Scheme 1.



Scheme 2.

from the solutions in analytically pure form (Scheme 2).

IR spectra of these products display in the 1700–1500 cm^{-1} region bands characteristic for compounds of the type PhNHCOR: 1680–1685 cm^{-1} (Amide I) and 1540 cm^{-1} (Amide II, characteristic for *N*-substituted amides). This is consistent with the structures **3a–c**, acetylation taking place at the NH_2 nitrogen. ^{1}H-NMR spectra of **3a–c** in DMSO-d_6 show at low field two one-proton singlets: the first at 10–10.5 ppm, which can be assigned to the amide proton, and the second at 7.70–7.75 ppm, which by comparison with the value of 7.71 ppm in **2c** can be assigned to the $NHSO_2$ proton. Another evidence supporting the structure of **3a–c** is provided by their IR spectra, showing two absorption bands in the region of NH stretching vibrations. The band appearing at 3280–3290 cm^{-1} can be assigned to the NH stretching vibrations in the sulfonamide moiety, whereas the band at 3310–3320 cm^{-1} to the same vibrations in the amide moiety. Finally, we have found that illumination with visible light of an aerated solution of **3a** in methanol leads to decomposition of the complex and liberated 4-acetamidobenzenesulfonamide **4** was isolated and identified by comparison with an authentic sample.



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The above results indicate that **2c** can be of interest for modification of proteins (and other biopolymers) by coupling of their COOH groups (present in the C-terminal aminoacid and in aspartate and glutamate residues) with the NH_2-group in **2c**. However, to be applicable for biological samples such reactions should work in aqueous (or polar organic solvent-water) media in the presence of reagents activating the carboxylic function (carbodiimides, Woodward,'s reagent) [4]. Protein modification with **2c** is of interest since modified biomolecules are expected to be detectable, even at very low concentrations, by means of FTIR spectroscopy in the region of 1900–2100 cm^{-1} (region of stretching vibrations of co-ordinated CO ligands) where biomolecules and biological matrices are practically transparent.. The idea of using transition-metal complexes at IR-detectable labels of biomolecules was put forward by Jaouen [5,6] and was successfully applied for the study of hormone–receptor interactions [7] and in immunoassays (carbonylmetallo-immunoassays, CMIA) [8]. The labelling procedures applied up to now for the introduction of the organometallic label were mainly based on the reaction of organometallic active *N*-succinimidyl (NS) esters with the amino groups (lysine residues in proteins) [9]. These groups were also labeled with the oganometallic isothiocyanates ([1]f), imidoester [10] and pyrylium salts [11]. Thiol groups present in cysteine residues can be labelled by the use of [$CpFe(CO)_2(\eta^1$-N-maleimidato)] ([1]c). However, despite the potential importance [4,12] there was no example of a metallo-carbonyl label reacting with the COOH function.

Taking advantage of solubility of **2c** in water we decided to study the reactions of **2c** with carboxylic acids in the presence of a water-soluble carbodiimide, 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (EDC). We chose some simple water-soluble acids to mimic conditions used in protein labeling. The reaction was carried out at r.t. in weakly acidic solutions (pH 5–6). This fact is very important for an eventual application for labelling of proteins since in basic media ($pH > 8$) polycondensation or cyclization of these molecules due to reaction of activated carboxylic function with the nonprotonated, nucleophilic amino functions [4]. Obviously, the amino function in the labeling reagent must not be completely protonated under coupling conditions, i.e. it should be only weakly basic ($pK_a < 5–6$). We think that this condition is fulfilled for **2c**. Although we did not measure the pK_a value for this complex, we expect that will not differ very much from the value measured for **1c** (2.1 in H_2O) [13]. No detectable protonation of **2c** is therefore expected at pH 5–6. We confirmed this from ^{1}H-NMR spectra which show no pH-dependence of the chemical shifts of the signals of this complex in the pH range 5–7. We have found that **2c** can be readily acylated with carboxylic acids in aqueous solutions containing an excess of EDC. When equimolar amounts of carboxylic acid and **2c** were used the best yields were obtained in the presence of a 4-fold excess of EDC. The products **3a**,**d**,**e** crystallize directly from the reacting solution in 54–81% yield in analytically pure form. NMR spectra of mother solutions indicate that they contain some amount of the product but its separation from EDC and the product of its hydrolysis is troublesome. In reaction with acetic acid the product obtained was identical (IR and ^{1}H-NMR) with that obtained in the reaction of **2c** with acetic anhydride. The entries (**d**) and (**e**) deserve some comments. Iodoacetamides and maleimides are considered as reagents reacting specifically with the cysteine thiol groups and are frequently used in protein labeling [4]. Consequently, **3d**,**e** are potential metallocarbonyl markers for thiol-containing biomolecules. Complex **2c** can be, therefore, a starting compound for creation of a family of metallocarbonyl markers for various functional groups.

3. Experimental

3.1. General remarks

Photochemical syntheses of $CpFe(CO)_2$-complexes of sulfonamide anions were carried out under an atmosphere of argon. Chromatographic separations were carried out on silica gel 60 (230–400 mesh ASTM). Solvents were dried by using standard procedures. The ^{1}H-NMR spectra were obtained by using a Varian Gemini 200BB spectrometer at 200 MHz on solutions in DMSO-d_6 and were referenced toward internal Me_4Si. IR spectra were run on a Specord 75 IR spectrometer. FAB MS was obtained on a Finnigan MAT 95 spectrometer. The elemental analyses were determined by the Analytical Services of the Center of Molecular and Macromolecular Studies of the Polish Academy of Sciences, Łódź. All reagents are available commercially.

3.2. Synthesis of $CpFe(CO)_2$-complexes of sulfonamide anions

A magnetically stirred suspension of [$CpFe(CO)_2I$] (460 mg, 1.5 mmol), sulfonamide (1.0 mmol) in benzene (15 ml) and diisopropylamine (1 ml) was illuminated with visible light (4×150 W domestic tungsten lamps) at 0–5°C (external cooling in a water-ice bath). The colour gradually changes from black to orange. After 2.5 h (3.5 h in the case of sulfanilamide) the illumination was stopped and the reaction mixture evaporated to dryness to give an orange solid which was treated with 5% aq. K_2CO_3 (10 ml) and extracted with chloro-

form (3 × 30 ml). After drying ($MgSO_4$) and concentration to a small volume, the reaction mixture was introduced on a short silicagel column. Chloroform eluted a black band of unreacted FpI, and then the product was eluted as orange crystals with $CHCl_3$–methanol (95/5). Analytical samples were crystallized from CH_2Cl_2–ether.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-CH_3], **2a**: Yield 62%. IR (KBr, cm^{-1}): 3290 (NH); 2045, 2000 (FeCO), 1290, 1130 (SO_2). ^{1}H-NMR ($CDCl_3$, δ ppm): 7.71, d, $J = 7.9$ Hz, 2H and 7.67, d, $J = 7.9$ Hz, 2H (aromatic protons); 5.29, s, 5H (Cp); 2.65, s, 3H (CH_3). Anal.: Calc. for $C_{14}H_{13}NO_4SFe$: C 48.44, H 3.77, N 4.03, S 9.23. Found: C 48.66, H, 3.91, N 3.88, S 9.42.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NO_2], **2b**: Yield 91%. IR(KBr, cm^{-1}): 3290 (NH); 2055, 2000 (FeCO); 1530, 1345 (NO_2), 1305, 1140 (SO_2). ^{1}H-NMR ($CDCl_3$, δ ppm): 8.37, d, $J = 8.0$ Hz, 2H and 7.91, d, $J = 8.0$ Hz, 2H (aromatic protons); 5.20, s, 5H (Cp). Anal.: Calc. for $C_{13}H_{10}N_2O_6Fe$: C 41.29, H 2.67, N 7.41, S 8.48. Found: C 41.56, H 2.66, N 7.15, S 8.72.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NH_2], **2c**: Yield 87%. IR (KBr, cm^{-1}): 3370, 3310 (NH_2), 3280 (NH), 2045, 2000 (FeCO), 1290, 1125 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 7.71, bs, 1H (NH, sulfonamide), 7.31, d, $J = 8.5$ Hz, 1H, and 6.53, d, $J = 8.5$ Hz, 1H (aromatic H's); 5.52, bs, 2H (NH_2); 5.12,s, 5H (Cp). Anal: Calc. for $C_{13}H_{12}N_2O_4SFe$: C 44.85, H 3.47, N 8.05, S 9.21%. Found: C 44.48, H 3.65, N 8.01, S 9.35%.

3.3. Acylation of [$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NH_2] (**2c**) with acid anhydrides

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NH_2], **2c**,(100 mg, 0.29 mmol) was dissolved in the anhydride (1.5 ml) and stand overnight at r.t. The solid formed was filtered off, washed with diethyl ether and dried in vacuo (50°C/0.2 Tr).

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH_3$], **3a**: Yield 86%. IR (KBr, cm^{-1}): 3320 (NH, amide); 3280 (NH, sulfonamide); 2040, 1990 (FeCO) 1680 (amide I) 1540 (amide II) 1320, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 10.15, s, 1H (NH, amide); 7.75, s, 1H (NH, sulfonamide); 7.61, m, 4H (aromatic H's); 5.16, s, 5H (Cp); 2.08, s, 3H (Me). FAB MS(NBA matrix, positive ions): 391 (M + H); 335 (M + H–2 CO). Anal. Calc. for $C_{15}H_{14}N_2O_5SFe$: C 46.16, H 3.62, N 7.18, S 8.22%. Found: C 45.85, H 3.66, N 7.03, S 7.99%.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH_2CH_3$], **3b**: Yield 80%. IR (KBr, cm^{-1}): 3315 (NH, amide); 3280, (NH, sulfonamide); 2050, 1990 (FeCO) 1685 (amide I) 1540 (amide II) 1310, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 10.07, s, 1H (NH, amide); 7.73, s, 1H (NH, sulfonamide); 7.66, d $J = 8.5$ Hz, 2H and 7.56, d, $J = 8.5$ Hz, 2H (aromatic H's); 5.14,s, 5H (Cp), 2.34, q, $J = 7.4$ Hz, 2H (CH_2); 1.08, t, $J = 7.4$Hz, 3H, (CH_3). Anal. Calc. for $C_{16}H_{16}N_2O_5SFe$: C 47.54, H 3.99, N 6.93, S 7.93%. Found: C 47.63, H 4.35, N 6.99, S 7.89%.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH(CH_3)_2$], **3c**: Yield 94%. IR (KBr, cm^{-1}): 3310 (NH, amide); 3280 (NH, sulfonamide); 2040, 1990 (FeCO) 1680 (amide I) 1540 (amide II) 1320, 1140 (*n* SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 10.03, s, 1H (NH, amide); 7.74, s, 1H (NH, sulfonamide); 7.67, d, $J = 8.6$ Hz, 2H and 7.56, d, $J = 8.6$ Hz, 2H (aromatic H's); 5.15, s, 5H (Cp); ca. 2.6, m, partly obscured by the solvent signal, (CH); 1.10, d, $J = 7.5$ Hz, 6H (CH_3). Anal. Calc. for $C_{17}H_{18}N_2O_5SFe$: C 48.80, H 4.34, N 6.70, S 7.65%. Found: C 48.69, H 4.44, N 6.70, S 7.80%.

3.4. Decomplexation of 4-acetamidobenzenesulfonamide, **4**, from **3a**

An aerated solution of **3a** (77 mg, 0.2 mmol) in methanol (5 ml) was irradiated (setup as described above) at 0–5°C for 1 h. The pale yellow solution obtained was evaporated to dryness and triturated with methanol (5 ml). The insoluble yellow amorphic material was filtered of and the filtrate after evaporation of the solvent afforded **4** as a gray solid. Crystallization from water gave **4** as colorless microcrystals. M.p. 216–218°C. Yield 32 mg (75%). IR and ^{1}H-NMR spectra of this product were identical to those of an authentic sample.

3.5. Coupling of [$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NH_2] (**2c**) with carboxylic acids/EDC

To a magnetically stirred solution of [$CpFe(CO)_2NHSO_2C_6H_4$-*p*-NH_2] (**2c**) (100 mg, 0.29 mmole) in water (5 ml) were added carboxylic acid (0.30 mmol) and EDC (1.38 mmol). The pH of the resulting mixture was 5–6. After 3 h the solid formed was filtered off, washed with water (2 ml) and dried in vacuo (40–50°C/0.2 Tr).

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH_3$], **3a**: Yield 54%.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH_2I$], **3d**: Yield 81%. IR (KBr, cm^{-1}): 3310 (NH, amide); 3280 (NH, sulfonamide); 2045, 1995 (FeCO); 1690 (amide I) 1555 (amide II); 1325, 1130 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 10.52, s, 1H, (NH, amide); 7.70, s,(NH, sulfonamide) 7.61 s, 4H (aromatics); 5.16, s, 5H (Cp); 3.84, s, 2H, CH_2. Anal.: Calc. for $C_{15}H_{13}N_2O_5ISFe \cdot 0.5H_2O$: C 34.31, H 2.69, N 5.33, S 6.16, I 24.17. Found: C 34.42; H 2.50; N 5.45; S 6.24, I 23.82.

[$CpFe(CO)_2NHSO_2C_6H_4$-*p*-$NHCOCH_2CH_2NC_4H_2O_2$], **3e**: Yield 62%. IR (KBr, cm^{-1}): 3320 (NH, amide); 3280 (NH, sulfonamide); 2055, 2000 (FeCO); 1710,

1680 (sh) (imide CO + amide I) 1540 (amide II); 1315, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6, δ ppm): 10.20, 1H, (NH, amide); 7.71 s, 1H, (NH, sulfonamide); 7.58 s, 4H, aromatics; 7.03, s, 2H, (olefinic); 5.15, s, 5H, (Cp); 3.72, t, $J = 6.8$ Hz, 2H, (CH_2), (second CH_2 signal presumably obscured by the solvent signal). Anal. Calc. for $C_{20}H_{17}N_3O_7SFe$: C 48.09, H 3.43, N 8.42, S 6.41. Found: C 47.76; H 3.50; N 8.84; S 6.26.

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