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The (η^5-cyclopentadienyl)Fe(CO)$_2$ complex of the (L)- and (D)-tryptophan methyl ester N-anion: synthesis and acylation of the amino function

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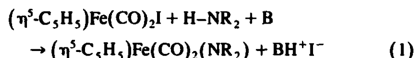
Abstract

The photochemical reaction of (η^5-C_5H_5)Fe(CO)$_2$I with (L)- and (D)-tryptophan methyl ester in the presence of diisopropylamine in benzene leads to complexation of the (η^5-C_5H_5)Fe(CO)$_2$ moiety to the deprotonated indole nitrogen. This reaction takes place without detectable (< 1%) racemization at C^α. The amino group of the complex formed was acylated by treatment with p-chlorobenzoic acid and Fmoc-(L)-leucine in the presence of dicyclohexylcarbodiimide.

Keywords: Iron; Carbonyl; Cyclopentadienyl; Photochemical synthesis; Tryptophan; Acylation

1. Introduction

We have earlier reported that irradiation with visible light of (η^5-C_5H_5)Fe(CO)$_2$I with compounds containing 'acidic' N–H bonds such as pyrrole, indole, cyclic imides and uracils in the presence of diisopropylamine (B) brings about the substitution of iodide by the corresponding N-anion [1–4]:



$$(\eta^5\text{-}C_5H_5)Fe(CO)_2I + H\text{–}NR_2 + B \rightarrow (\eta^5\text{-}C_5H_5)Fe(CO)_2(NR_2) + BH^+I^- \quad (1)$$

We thought that it would be interesting to look at whether this reaction could be applied for chemospecific coordination of the (η^5-C_5H_5)Fe(CO)$_2$ moiety (hereafter denoted Fp) to the indole system in tryptophan. It is now well established that tryptophan itself and many of its derivatives, including tryptophan-containing peptides, display interesting biological activity (e.g. cholecystokinin, endothelin, neurotensin and substance P receptor antagonists) [5–11]. Organometallic complexes of these compounds containing the tryptophan system coordinated to a transition metal carbonyl moiety are of potential importance for the study of receptor interactions and for immunoassays of tryptophan-containing haptens or antigens.

The labelled molecules could easily be detected and quantified at sub-nanomole range by Fourier transform IR spectroscopy using very strong *M*–CO bands appearing in the approximately 2000 cm^{-1} region, free of the absorption of the peptide or the protein.

This idea, put forward by Jaouen et al. [12], was succesfully applied in immunochemistry (carbonylmetalloimmunoassays (CMIA)) [13] and studies of hormone–receptor interactions [14]. It is also worth noting that some studies [10] have shown that substitution of the indolyl hydrogen of tryptophan by alkyl or acyl groups can retain the biological activity of the tryptophan-containing peptides. We think therefore that the same could be true for substitution of this hydrogen by metal-containing groups, and biological activity of peptides containing tryptophan residue complexed to the Fp moiety via deprotonated indolyl nitrogen cannot be excluded. Another reason that prompted us to study the complexation of the Fp moiety to the tryptophan system was the recent interest in organometallic derivatives of aminoacids and peptides, see for example Ref. [15]. It should be noted that recently an η^6-organometallic ruthenium complex of tryptophan has been synthesized [16]. However, to our knowledge there was no report on

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coordination of this aminoacid to an organometallic unit via deprotonated indole nitrogen.

In this paper we report the chemo- and stereospecific synthesis of the Fp complex of deprotonated tryptophan and some of its N_b-acylated derivatives related to biologically active compounds: p-chlorobenzoyltryptophan (benzotript) and phosphamidon [11].

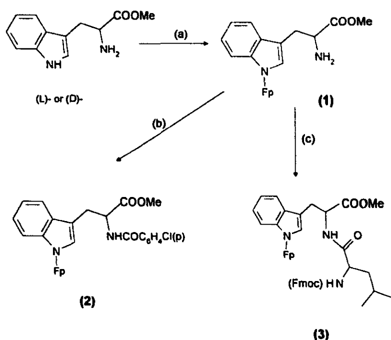
2. Results and discussion

Irradiation with visible light of FpI with (L)- or (D)-tryptophan methyl ester hydrochloride and an excess of diisopropylamine in benzene results in formation of the red complex 1 (Scheme 1).

As previously noted for the similar reaction with uracils [4], for unknown reasons this reaction stops before completion. Consequently, when stoichiometric amounts of FpI and L-tryptophan methyl ester were used, the isolated 1 was contaminated with unreacted tryptophan methyl ester (around 10–15%). We were unable to remove this contamination, either by column chromatography or crystallization. We therefore decided to use an excess of readily accessible FpI, which can easily be separated from 1. When the photolysis was carried out in the presence of a four-fold excess of this complex, the contamination of 1 was reduced to less than 1% and its isolated yield was 71–79%.

To confirm the coordination of the Fp moiety to deprotonated indolyl nitrogen, we compared the ^{1}H and ^{13}C chemical shifts of 1 with those of L-tryptophan methyl ester, and also compared the ^{1}H and ^{13}C chemical shifts of Fp (η^1-indolyl) [1] with those of the parent indole.

The spectral assignment was done on the basis of HETCOR and HMBC spectra. Fig. 1 shows the changes



Scheme 1. $Fp = CpFe(CO)_2$: (a) FpI/diisopropyloamine/$h\nu$; (b) p-chlorobenzoic acid/DCC; (c) Fmoc-(L)-leucine/DCC.

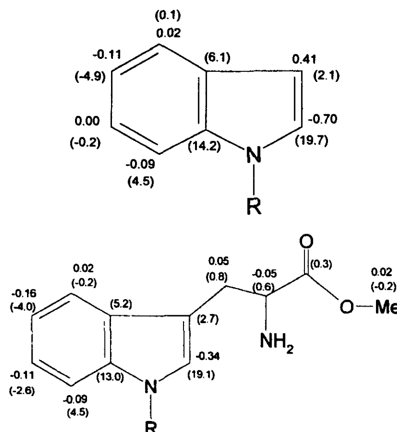


Fig. 1. The changes in chemical shift due to the replacement of R = H by R = Fp: $\Delta\delta = \delta(R = Fp) - \delta(R = H)$. Values without parentheses correspond to ^{1}H, whereas those with parentheses correspond to ^{13}C.

in ^{1}H and ^{13}C chemical shifts observed on going from tryptophan methyl ester and indole to their Fp complexes, i.e. $\delta(R = Fp) - \delta(R = H)$.

These data clearly show that changes of chemical shifts due to the introduction of the Fp moiety to indole and tryptophan methyl ester are similar. In the ^{1}H NMR spectra of both systems, H-2 is shifted upfield (0.7 and 0.34 ppm for the indole and tryptophan methyl ester respectively). Among aromatic protons the most influenced are H-5 and H-7 (in the case of tryptophan methyl ester also H-6), but the changes in their chemical shifts are smaller than that of H-2. ^{13}C NMR spectra show that the introduction of the Fp moiety to indole and tryptophan causes in both systems a strong downfield shift of signals of carbons directly bound to the indolyl nitrogen and a slighter shift of signals of carbons bound to these carbons. A significant downfield shift is also observed in both systems for C-5 and C-7, i.e. for carbons which should be influenced by mesomeric delocalization of the indolyl nitrogen lone pair. In our opinion, the data presented provide unambiguous evidence that in both indole and tryptophan methyl ester systems the Fp moiety is coordinated to the same site, the deprotonated indolyl nitrogen. (Note that for the indole system this mode of coordination clearly results from the synthetic procedure and from 18-electron counting around Fe.) Additional evidence for coordination of the Fp moiety to deprotonated indole nitrogen is provided by the IR spectrum of 1, showing stretching

vibrations of the NH_2 (at 3390 and 3280 cm^{-1}) and of the ester group (at 1730 cm^{-1}), i.e. at approximately the same values as those found for the uncomplexed tryptophan methyl ester. This means that in **1** there is no interaction between the iron centre and the lateral chain of the tryptophan.

Many non-peptide N^a-acylated derivatives of tryptophan display biological activity, e.g. p-chlorobenzoyltryptophan (benzotript), which was used as a model in the development of the CCK-A (cholecystokinin) antagonist [11]. It therefore seemed interesting to look at whether **1** can be acylated at N^a with p-chlorobenzoic acid. We have found that such an acylation can be achieved by the treatment of **1** with p-chlorobenzoic acid and dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperatue. The isolated yield of **2** was 83% and its structure was confirmed by spectroscopic as well as elemental analysis data.

A very important problem in syntheses of peptides utilizing chiral aminoacids in basic media is overcoming racemization [17]. In order to find out whether coordination of the Fp moiety proceeds with or without racemization, we decided to prepare the Fp complex **1** starting from (D)-tryptophan methyl ester. Then the samples obtained from each tryptophan enantiomer were treated with Fmoc-(L)-leucine (Fmoc denotes 9-fluorenylmethoxycarbonyl group) in the presence of DCC in CH_2Cl_2 at room temperature. This reaction afforded dipeptide **3** in 84 and 97% yield respectively for (L)- and (D)-tryptophan enantiomers. ^1H NMR spectra of both products clearly showed that products obtained from tryptophan enantiomers are diastereoisomers, Fmoc-(L)-Leu-Fp-(L)-Trp-OMe and Fmoc-(L)-Leu-Fp-(D)-Trp-OMe respectively. The most significant difference in the NMR spectra of these products was observed for the indole H-2 signals (6.68 and 6.62 respectively). By addition of the known amount of the second diastereoisomer to the first one, we have estimated that the possible contamination by this diastereoisomer of 1% would have been easily detected. This means that complexation of the Fp moiety to deprotonated indolyl nitrogen of tryptophan methyl ester and typical peptide coupling procedure using **1** proceed practically without racemization (< 1%) of the tryptophan system. This approach is therefore of interest for syntheses of peptides containing Fp-labelled tryptophan residues.

3. Experimental

All experiments were carried out under an argon atmosphere. Benzene was distilled from sodium–benzophenone. Other solvents were pure for analysis grade and were used without purification. Column chromatography was carried out using Kieselgel 60 (230–400 mesh ASTM) (Merck) and chloroform as eluent. (D)- and (L)-tryptophan methyl ester hydrochlorides, diisopropylamine, p-chlorobenzoic acid, Fmoc-leucine and DCC (1.0 M solution in dichloromethane) were of reagent grade and used as received from Aldrich. FpI and Fp (η^1-indole) were prepared by the procedures described earlier [1,13]. (L)-tryptophan methyl ester was prepared from its hydrochloride by treatment with aqueous potassium carbonate and extraction with dichloromethane. NMR spectra were recorded on a Bruker WM 400 (400 MHz for ^1H) and a Varian Gemini 200BB (200 MHz for ^1H) spectrometer, using TMS as external reference. IR spectra were taken on a Specord 75 IR spectrometer.

3.1. *Fp-(L)-Trp-OMe (1)*

A solution of FpI (1.22 g, 4.0 mmol), (L)-tryptophan methyl ester hydrochloride (0.255 g, 1.0 mmol) in benzene (25 ml) containing diisopropylamine (2 ml) was photolysed (4 × 150 W domestic tungsten lamps) with magnetic stirring and an external cooling with water-ice during 5 h. The photolyte was washed with 20 ml of 5% aqueous KOH, water (2 × 20 ml) and dried over Na_2SO_4. The solvent was evaporated to dryness and the residue chromatographed. The deep-red fraction eluted after unreacted FpI (black band) was collected to afford Fp-(L)-Trp-OMe as a red foam. The ^1H NMR spectrum of this material in DMSO-d_6 showed the presence of residual $CHCl_3$ (0.16 molecule). Anal. Found: C, 55.35; H, 4.34; N, 7.06; Cl, 4.07. Fp-(L)-Trp-OMe · 0.16$CHCl_3$ Calc.: C, 55.59; H, 4.42; N, 6.76; Cl, 4.28%. Yield 0.33 g (79%). ^1H NMR ($CDCl_3$, δ [ppm]): 7.53 (d, $J = 7.5$ Hz, 1H, H-4); 7.32 (d, $J = 7.5$ Hz, 1H, H-7); 7.08 (t, $J = 7.5$ Hz, 1H, H-6); 6.92 (t, $J = 7.5$ Hz, 1H, H-5); 6.69 (s, 1H, H-2); 5.11 (s, 5H, Cp); 3.80–3.73 (m, 4H, H_α and CH_3); 3.29 (dd, $J = 3.8$ Hz, $J = 14.1$ Hz) and 3.02 (dd, $J = 7.8$ Hz, $J = 14.1$ Hz, CH_2); 1.6 (broad s, 2H, NH_2). ^{13}C NMR ($CDCl_3$, δ [ppm]): 213.04 and 213.00 (Fe–CO); 176.1 (CO–ester); 149.4 (C-7a); 142.6 (C-2); 132.6 (H-3a); 119.0 (H-7); 118.3 (H-6); 116.0 (H-5); 115.1 (H-4); 112.6 (H-3); 55.5 (C^α); 51.8 (Me); 31.5 (C^β). IR ($CHCl_3$, cm^{-1}): 3390, 3280 (NH_2); 2060, 1980 (Fe–CO); 1730 (CO–ester).

3.2. *Fp-(D)-Trp-OMe*

Synthesized using the above procedure starting from (D)-tryptophan methyl ester hydrochloride. Yield 0.280 g (71%).

3.3. *Fp-(N^α-p-Chlorobenzoyl-(L)-Trp-OMe) (2)*

To a solution of Fp-(L)-Trp-OMe (**1**) (0.098 g, 0.25 mmol) and p-chlorobenzoic acid (0.039 g, 0.25 mmol) in dichloromethane (5 ml), dicyclohexylcarbodiimide (0.25 ml of 1.0 M solution in

dichloromethane, 0.25 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and the dicyclohexylurea formed was filtered off. The product was isolated by column chromatography and crystallized from ether–pentane. Yield 0.110 g (83%). Anal. Found: C, 58.62; H, 3.97; N, 5.26; Cl, 6.65. $C_{26}H_{21}N_2O_5FeCl$ Calc.: C, 58.61; H, 3.93; N, 5.27; Cl, 6.57%. ^1H NMR ($CDCl_3$, δ [ppm]): 7.6–6.8 (m, 9H, ArH); 6.66 (s, 1H, H-2); 6.59 (d, $J = 8$ Hz, 1H, NH); 5.08 (s, 5H, Cp); 5.08–4.98 (m, 1H, proton H^α); 3.75 (s, 3H, Me); 3.41 (d, $J = 6.5$ Hz, 2H, CH_2). IR ($CHCl_3$, cm^{-1}): 3400 (NH); 2040, 1990 (Fe–CO); 1720 (CO–ester); 1650 (CO–amide).

3.4. Fmoc-(L)-Leu-Fp-(L)-Trp-OMe

To a solution of Fp-(L)-Trp-OMe (1) (70 mg, 0.18 mmol) and Fmoc-(L)-Leu (64 mg, 0.18 mmol) in dichloromethane (5 ml), DCC (0.18 ml of 1.0 M solution in dichloromethane, 0.18 mmol) was added and the resulting mixture was stirred at room temperature for 1 h. The white solid formed (N,N'-dicyclohexylurea) was filtered off and the solvent evaporated to dryness. The residue was purified by column chromatography and crystallization from dichloromethane–ether–pentane. Yield 110 mg (84%). Anal. Found: C, 64.30; H, 5.62; N, 5.68. $C_{40}H_{41}FeN_3O_8$/Fmoc-(L)-Leu-Fp-(L)-Trp-OMe · H_2O/ Calc.: C, 64.26; H, 5.53; N, 5.62%. ^1H NMR ($CDCl_3$, δ [ppm]): 7.8–6.8 (m, 12H, ArH–indole + FMOC); 6.68 (s, 1H, H-2); 6.28 (d, $J = 8.4$ Hz, 1H, NH); 5.07 (s, 5H, Cp); 4.82 (m, 1H, tryptophan H^α); 4.36 (m, 2H, CH_2 (Fmoc)); 4.20 (m, 2H, Fmoc + leucine H^α); 3.71 (s, 3H, Me); 3.31 (d, $J = 5.4$ Hz, 2H, tryptophan CH_2); 1.58 (m, 3H, leucine CH_2 + CH); 0.87 (d, $J = 5.4$ Hz, 6H, leucine Me). IR ($CHCl_3$, cm^{-1}): 3320 (NH); 2040, 1980 (Fe–CO); 1715, 1680 (CO).

3.5. Fmoc-(L)-Leu-Fp-(D)-Trp-OMe

This compound was prepared from Fp-(D)-Trp-OMe using the present procedure. Yield 97%. Anal. Found: C, 65.83; H, 5.90; N, 6.16. $C_{40}H_{39}FeN_3O_7$ Calc.: C, 65.85; H, 5.39; N, 5.76%. ^1H NMR ($CDCl_3$, δ [ppm]): 7.8–6.8 (m, 12H, indole + Fmoc); 6.62 (s, 1H, H-2); 6.33 (d, $J = 7.8$ Hz, 1H, NH); 5.08 (s, 5H, Cp); 4.78 (m, 1H, tryptophan H^α); 4.36 (m, 2H, CH_2 Fmoc); 4.20 (m, 2H, Fmoc leucine H^α); 3.69 (s, 3H, CH_3); 3.31 (d, $J = 5.4$ Hz, 2H, tryptophan CH_2); 1.58 (m, 3H, leucine CH_2 + CH); 0.84 (d, $J = 5.4$ Hz, 6H, leucine Me). IR ($CHCl_3$, cm^{-1}): 3320 (NH); 2040, 1980 (Fe–CO); 1715, 1680 (CO).

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