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REVIEW

Contributions to the synthesis of some ferrocene-containing antibiotics

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This Review discusses the synthesis and characterization by our Group of new antibiotics belonging to the class of penicillins, cephalosporins and rifamycins with ferrocenyl and 1,1′-ferrocenilene residues in the molecule.

As reactants for 6-aminopenicillanic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA) the following were used: 1,1-bis(chlorocarbonyl)ferrocene, ferrocenyl sulfochloride, 1,1′-ferrocenylenedisulfochloride and thioglycolic acids *S*-modified with ferrocene. In the synthesis of rifamycins, the hydrazides of the thioglycolic acids, *S*-modified with ferrocene, were employed as nucleophilic agents. The synthesized intermediates were characterized by elemental analysis, TLC, IR, UV and ^{1}H NMR spectra. The characterization of new antibiotics was made by TLC, IR and UV spectral analysis.

Biological activity was tested on Gram-negative and Gram-positive bacteria. Good activity is reported towards Gram-positive bacteria in the case of derivatives containing residues of thioglycolic acid *S*-modified with ferrocene, the antibacterial activity being similar to that of amoxicillin, carbenicillin and cephalothin. All compounds are inactive towards Gram-negative bacteria.

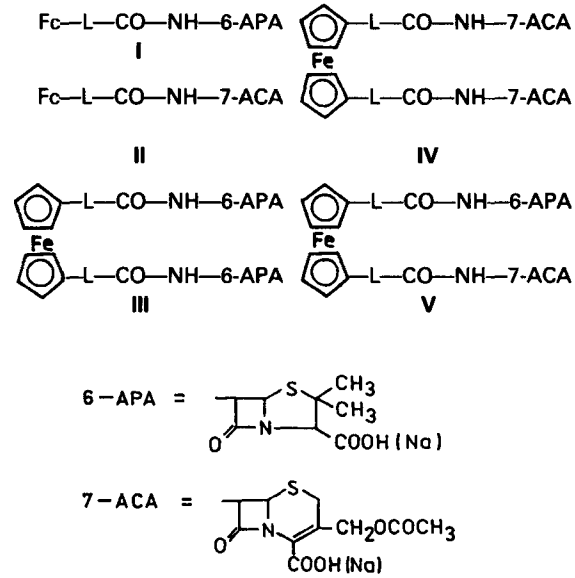
Keywords: Ferrocene, β-lactamic antibiotics, rifamycins

1 INTRODUCTION

Ferrocene and its derivatives have been used in various applications, biology and pharmacology included. The possibility of a favorable influence on organisms could arise because of the special properties of ferrocene, such as high stability, reversible change of the valency state, non-benzoid aromatic structure, low toxicity, and potential as an iron source. Ferrocene derivatives showing anti-ulcerous, anticonvulsive, antitumor or antimicrobial actions are already known.[1]

Under such circumstances, one of the tempting directions of research was that of synthesizing β-lactam antibiotics containing side chains modified with various radicals of the ferrocenyl or 1,1′-ferrocenilene type. This idea was even more interesting as there are several commercial β-lactam antibiotics containing aromatic-type structures or heterocycles in their side chains. Introduction of the ferrocene nucleus in the side chain of the antibiotic confers the possibility of the molecule being modified in three dimensions without changing the molecular profile required for biological activity.

Literature data on β-lactam antibiotics containing ferrocenyl or 1,1′-ferrocenilene residues are relatively scarce; various compounds, corresponding principally to structures of the **I–V** type[2–5] have been synthesized (Scheme 1).



Scheme 1 L represents a bond (**I**, **II**) or a bonding group (**III–V**) and Fc denotes the ferrocenyl residue

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Preparation of new β-lactamic, ferrocene-containing products involved the synthesis of mono- and 1,1′-heterodi-substituted derivatives of ferrocene, to be further employed as acylating agents of 6-aminopenicillanic (APA) and 7-aminocephalosporanic (7-ACA) acids.

2 THE INTERMEDIATES

2.1 1,1′-Bis(chlorocarbonyl)ferrocene

This was obtained from 1,1′-ferrocenyl-endicarboxylic acid.[6]

2.2 Thioglycolic acids *S*-modified with ferrocene

2.2.1 Synthesis and characterization

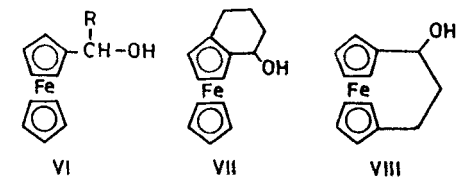
The preparation of thioglycolic acid derivatives *S*-modified with ferrocene involved, initially, application of the method of Combs and Ratajczak, who employed various α-substituted ferrocenyl alcohols of the **VI–VIII** type, using trifluoroacetic acid (TFA) as catalyst (Scheme 2).[7–9]

Ratajczak's method has been extended by us to the synthesis of **X**-type acids starting from **IX**-type derivatives, according to Scheme 3.[10]

Literature data on this topic indicate a reaction time of 12 h.[8,9] Utilization of TLC (benzene : acetone, 10/2, v/v; Silica gel FG 254) to control the conversion of the ferrocenyl derivative suggested that the reaction occurs extremely fast at temperatures ranging between 20 and 30 °C, the transformation of **IX** diols being practically total after 10–15 min.

Structural investigations (quantitative elemental analysis, IR and ^{1}H NMR analyses agree with the structures proposed. Thus, the IR spectra contain absorptions characterizing the —COOH group, while ^{1}H NMR spectra suggested the following:

(1) The 1,1′-disubstituted ferrocene nucleus has a singlet situated between 4.12 and 4.22 ppm.
(2) The methynic protons appear between 3.7 and 4.0 ppm, showing multiplicities depending on the nature of the protons from the R residue with which they couple (when R = phenyl, $\delta = 5.1$ ppm).
(3) The methylenic protons give a singlet situated between 3.16 and 3.32 ppm.
(4) The alkyl (R) radicals give characteristic signals.

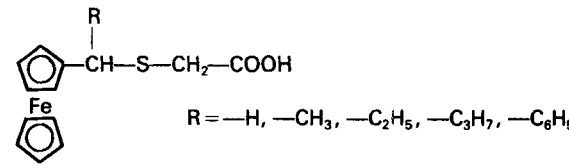


VI VII VIII

R = -H, $-CH_3$, $-C_2H_5$, $-CH(CH_3)_2$, $-C_6H_5$

Scheme 2

Compounds of the **XI** type (Scheme 4) described by Ratajczak have been synthesized from **VI**-type derivatives. This reaction is also complete after 10–15 min, the yields and structures being similar.

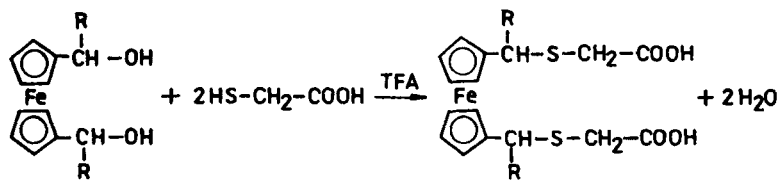


XI

Scheme 4

2.2.2 Kinetic aspects

On the basis of the high reactivity and selectivity of the mono- and di-hydroxylated ferrocene deri-



IX (mezo + dl) X (mezo + dl)

R = $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_6H_5$

Scheme 3

Table 1 Kinetic parameters of the reaction of 1-ferrocenylethanol with thioglycolic acid

T(°C)	n[a]	k (min^{-1})[b]	$t_{1/2}$ (min)[c]
0	1.065	0.04226	15.0
10	1.240	0.07853	8.0
15	1.177	0.11916	3.5
20	1.175	0.26968	1.8
30	1.122	0.78852	0.9

[a] n = reaction order. [b] k = rate constant. [c] $t_{1/2}$ = half-life.

vatives in the reactions with the thioglycolic acid performed in the presence of TFA, some kinetic aspects have been studied, in parallel with the proposal of the reaction mechanism.[11]

1-Ferrocenylethanol, for which literature data indicate a complete retention of configuration in this reaction,[9] was employed as a model substrate. Complete retention of configuration is frequently encountered in ferrocene derivatives that have had readily transferable groups at an sp^3 C substituted with a ferrocenyl unit. Retention of configuration can be explained if it is assumed that the reaction develops by S_N2 mechanism (two successive inversions of configurations) or by S_N1 mechanism; in the later case the α-ferrocenyl radical enhances nucleophilic attack on the reaction center.

Kinetic studies have shown that the reaction is first-order, which implies the appearance in the system of a ferrocenylcarbenium ion, whose high stability is generally recognized.

Plotting of the kinetic curves employed data from quantitative TLC, for temperature values of 0, 10, 15, 20 and 30 °C. The use of TLC permitted the identification of the vinylferrocene that results from a competitive elimination reaction, its concentration in the system decreasing with rising temperatures. Table 1 lists the kinetic data for the reaction of 1-ferrocenylethanol with thioglycolic acid.

The activation energy, determined from the Arrhenius equation, is 83.042 kJ mol^{-1}, which is consistent with the rapid rate of the reaction.

2.3 Functional derivatives of thioglycolic acids, *S*-modified with ferrocene

To obtain ferrocene-containing rifamycins, the synthesis of hydrazides **XII** and **XIII** has been carried out by means of methyl esters (Scheme 5).[12]

The preparation of methyl esters **XIIa** and **XIIIa** involved treatment of the corresponding acids with diazomethane or methyl chloroformate and triethylamine, the yields being about 80%, while purification was achieved through column chromatography, leading to products generally having an oily consistency. The IR spectra evidenced the presence of ester-type carbonyl between 1730 and 1745 cm^{-1}.

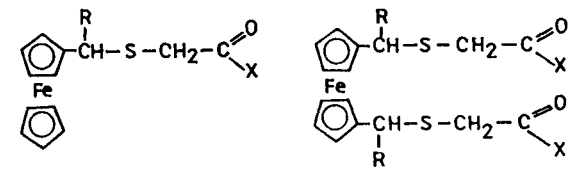
Hydrazides **XIIb** and **XIIIb** were obtained from the corresponding methyl esters, also showing oily consistency. As the presence of the CO—NH polar group did not permit purification through column chromatography, transformation of hydrazides into hydrazones **XIIc** and **XIIIc** was necessary, by reaction with acetone. The hydrazones obtained are crystalline, having clear melting points, and are easily transformed into starting compounds through acid hydrolysis.

The structure of **XIIb** and **XIIIb** hydrazides has been confirmed by IR spectra (amide 1 1645–1670 cm^{-1}, amide 2 1520–1550 cm^{-1}) and by the characterization of the corresponding hydrazones (m.p., quantitative elemental analysis, IR, ^{1}H NMR).

2.4 Mono- and di-sulfochlorinated ferrocene derivatives

Ferrocenylsulfochloride was obtained from ferrocenylsulfonic acid, according to information given in the literature.[13]

1,1′-Ferrocenylene disulfochloride was prepared by an original method involving treatment of the *p*-tolydine salt of the 1,1′-ferrocenylene disulfonic acid with PCl_3.[14]



XII (a, b, c) **XIII (a, b, c)**

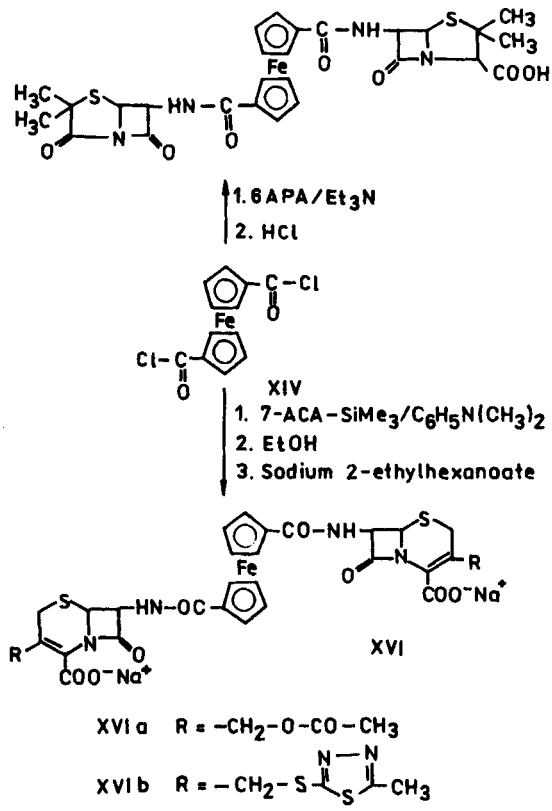
XIIa, XIIIa X = —OCH$_3$

XIIb, XIIIb X = —NH—NH$_2$

XXXc, XIIIc X = —NH—N=C(CH$_3$)(CH$_3$)

R = —CH$_3$, —C$_2$H$_5$, —C$_3$H$_7$, —CH(CH$_3$)$_2$, —C$_6$H$_5$

Scheme 5



Scheme 6

3 β-LACTAM FERROCENE-CONTAINING ANTIBIOTICS

3.1 Ferrocene-containing penicillins and cephalosporins obtained through acylation

β-Lactam ferrocene-containing antibiotics were synthesized through acylation of the amino groups of the 6-aminopenicillanic and 7-aminocephalosporanic acids with mono- or 1,1′-heterodi-substituted acids of ferrocene.

Employing 1,1′-bis(chlorocarbonyl)ferrocene (**XIV**) as acylating agent for 6-APA, 7-ACA and 7-amino-3(2-methyl-1,3,4-thiadiazol-5-thiomethyl)cephalosporanic acid (7-ADCA), we obtained 1,1′-ferrocenylene dicarboxamide penicillanic acid (**XV**) and 1,1′-ferrocenylene dicarboxamidocephalosporanic acid (**XVI**), with the following chromatographic purities: **XV** 80%, **XVIa** 98% and **XVIb** 88–90%, according to Scheme 6.[15]

Activity towards Gram-positive and Gram-negative bacteria of some clinically used antibiotics, containing substituents with sulfur atoms inserted into a thiophene, thiadiazole, thiazole heterocycle or into an ether bridge, induced us to study certain antibiotics containing in their molecules derivatives of mercaptoacetic acid, *S*-modified with ferrocene.

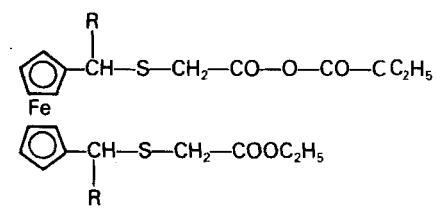
To avoid the difficulties usually met in the synthesis of acid chlorides,[16,17] **X**- and **XI**-type acids were used as acylating agents by applying the method of mixed anhydrides.

Mixed anhydrides were prepared through reaction of the triethylammonium salts of **X** and **XI** acids with ethyl chloroformate, in the presence of *N*-methylmorpholine as catalyst, at temperatures ranging between −25 and −30 °C for the **XI**-type compounds and −40 and −45 °C, respectively, for **X**-type derivatives; thus, the partial decomposition of the mixed anhydrides of the **X**-type compounds, as well as the formation of **XVII**-type structures, was avoided (Scheme 7).

Transformation of the acid into a mixed anhydride was followed chromatographically (benzene:acetone, 10/2 v/v; silica gel), the reaction being completed after 15 min.

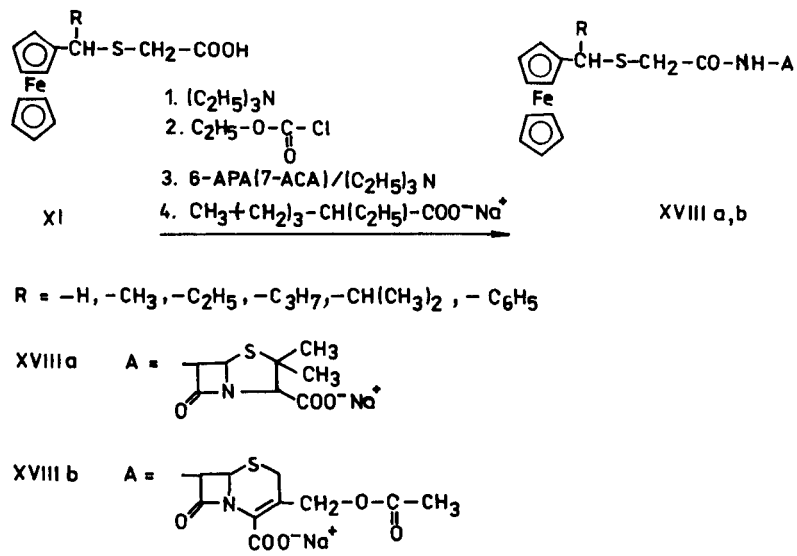
Acylation of 6-APA and 7-ACA with monosubstituted derivatives of ferrocene was conducted at temperatures between −25 and −30 °C, and between −40 and −45 °C for the disubstituted derivatives. The reaction time was 1 h for 6-APA and 1.5 h for 7-ACA. The solution of the mixed anhydride was added to the triethylammonium salt of the 6-APA or 7-ACA, dissolved in dichloromethane. Ferrocene-containing β-lactam antibiotics were isolated as sodium salts, by treating the acids with sodium ethylhexanoate in isopropanol; the sodium salts, soluble in dichloromethane were precipitated with n-hexane or petroleum ether.

The reactions performed for the preparation of penicillins and cephalosporins through acylation of 6-APA and 7-ACA with *S*-modified thioglyco-



XVII

Scheme 7



Scheme 8

lic acids are shown in Scheme 8.[16]

The same method was applied for the disbubstituted derivatives of ferrocene.[17]

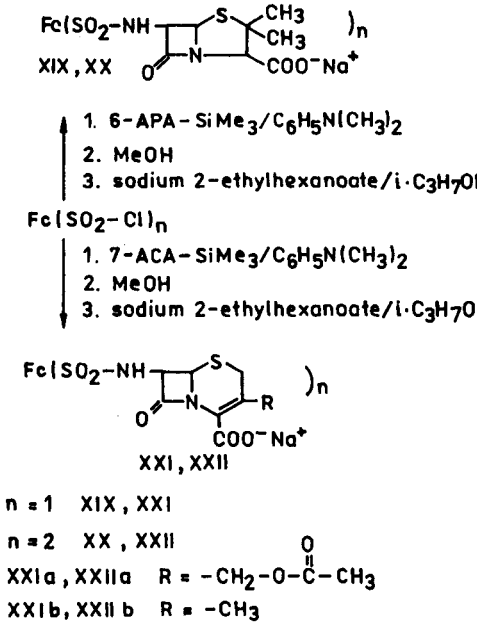
The newly synthesized β-lactam antibiotics were characterized as sodium salts, through IR and UV spectroscopy and TLC. IR spectra showed the absorptions characteristic of the β-lactam C=O groups (1772–1776 cm^{-1} in penicillins and 1756–1764 cm^{-1} in cephalosporins), C=O amide groups (1712–1740 cm^{-1} in penicillins and 1700–1704 cm^{-1} in cephalosporins), COO^- groups (1602–1616 cm^{-1}). The UV spectra were used only for the structural assignment of cephalosporins which give a typical absorption band near 260 nm, due to the conjugated system O=C—N—C=C—.[18] The existence of such an absorption band is correlated with the integrity of the β-lactam ring, as well as with the presence of the C=C double bond.

3.2 Ferrocene-containing, sulfonamidated penicillins and cephalosporins

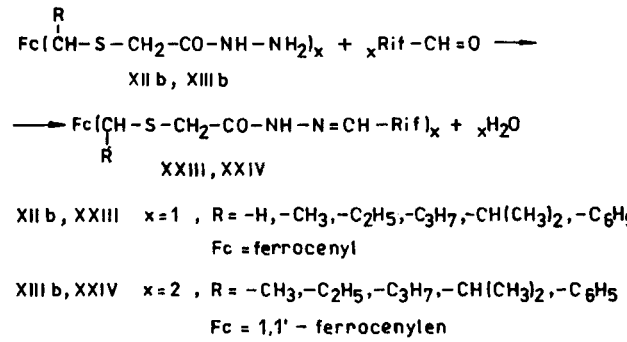
β-Lactam antibiotics containing a sulfonamide group, adjacent to the β-lactam cycle, have generally been less investigated.[19,20]

The synthesis of new penicillins and cephalosporins through the reaction of 6-APA, 7-ACA and 7-ADCA, as silicon esters, with ferrocenyl sulfochloride and 1,1′-ferrocenylene disulfochloride, leads to compounds of the **XIX**, **XX**, **XXI** and **XXII** type.[14] the reactions are presented in Scheme 9.

The compounds were characterized by TLC and IR spectra, showing the presence of the β-lactam C=O group (1740–1780 cm^{-1}), SO_2(sym) (1140–1200 cm^{-1}), SO_2(asym) (1340–1380 cm^{-1}) and COO^- (1610–1620 cm^{-1}).



Scheme 9



Scheme 10

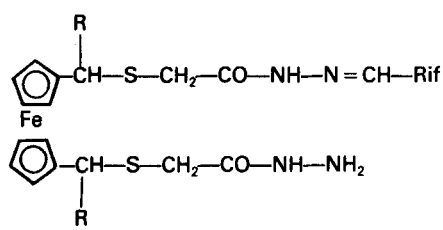
4 NEW FERROCENE-CONTAINING RIFAMYCINS

Semisynthetic rifamycins are usually obtained through condensation of 3-formylrifamycin SV (Rif-CHO) with nucleophilic agents such as amines, hydrazines, hydroxylamines, semicarbazones, thiosemicarbazones, sulfonhydrazides, etc.

Our investigations have mainly been aimed at the synthesis of new rifamycins having a ferrocenyl or 1,1'-ferrocenylene group in the molecule,[21] employing hydrazides of thioglycolic acids *S*-modified with ferrocene as nucleophilic agent, according to Scheme 10.

The reaction occurs in mild conditions (i.e. temperature 40 °C, reaction time 30 min), on employing a slight excess of hydrazide in the case of monosubstituted derivatives of ferrocene (Rif-CHO/hydrazide molar ratio 1:1.05). A molar ratio of 1:2 for 1,1'-disubstituted ferrocene derivatives was used in order to restrict the formation of the **XXV** monosubstituted hydrazides (Scheme 11).

In order to avoid the use of hydrazides (known to be oily products) we used the corresponding **XIIc** and **XIIIc** hydrazones, which are simpler to handle and known to cleave quickly in an acid medium. The reaction requires a longer reaction time, yet it might be of interest for the synthesis of compounds more difficult to isolate and purify through conventional methods.



XXV

Scheme 11

5 ANTIBACTERIAL PROPERTIES

The antibacterial activity of the new ferrocene-containing antibiotics was tested *in vitro*, by measuring the diameters of the inhibition zones. Tests have been made on Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Sarcina lutea*, *Escherichia coli*) and Gram-negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria.

The newly synthesized antibiotics are all active towards the Gram-positive bacteria and wholly inactive to the Gram-negative ones.

The biological activity of the ferrocenylsulfonamido- and arylsulfonamido-penicillanic (cephalosporanic) acids is weaker than that of the corresponding acids having a carboxylamido group.[15] This observation agrees with literature data, which indicate greater reduction in the activity of the sulfonamido-β-lactam antibiotics, as compared with the carboxamido ones.[19,22] The sulfonamido group probably increases the stability of the β-lactam cycle, reducing the antimicrobial activity.[23]

Penicillins and cephalosporins containing a residue of thioglycolic acid *S*-modified with a ferrocenyl residue show an equal or even higher activity than that of the control (i.e. amoxicillin, carbenicillin or cephalothin). The observation to be made is that the antibacterial activity is sensibly influenced by the nature of the R radical and

that a non-significant decrease of activity occurs with increasing chain length. The fact that the biological activity of the 1,1′-disubstituted ferrocenyl derivatives is not higher than that of the monosubstituted ones might be due to the complex structure, which reduces access to the reactive zones of the enzymes involved in the biosynthesis of the bacterial cell wall.

The antibacterial activity of the ferrocene-containing rifamycins is similar to, or even weaker than, that of the control sample (amoxicillin, carbenicillin, cephalothin). As in the case of penicillins and cephalosporins, the 1,1′-disubstituted derivatives of ferrocene are less active than the monosubstituted ones, probably for similar reasons.

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