Acta Poloniae Pharmaceutica – Drug Research. Vol. 55 No. 6 pp. 467–472, 1998

ISSN 0001-6837
Polish Pharmaceutical Society

ESTERS OF CEPHALOSPORINS. PART VIII. COMPARISON OF THE REACTIVITIES *IN VITRO* OF THE **R** AND **S** DIASTEREOISOMERS OF 1–ACETOXYETHYL ESTER OF CEFUROXIME

Bożena Tejchman[1], Irena Oszczapowicz[1], Andrzej Zimniak[2], and Halina Szatyłowicz[3]

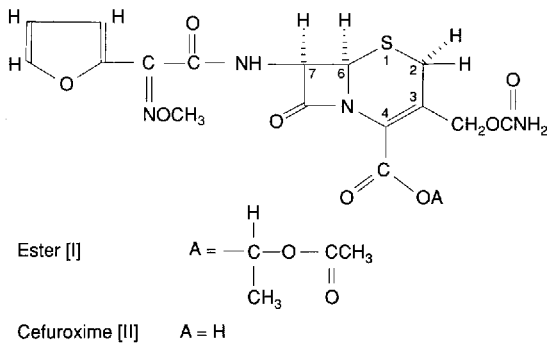
[1] Institute of Biotechnology and Antibiotics, 5, Starościńska Street, 02–516 Warsaw, Poland
[2] Medical Academy, Faculty of Pharmacy, Warsaw
[3] Warsaw University of Technology, Department of Chemistry, Warsaw

Abstract: 1–Acetoxyethyl ester of cefuroxime is an oral prodrug of cefuroxime, an injectable second generation cephalosporin with an excellent antibacterial activity. This ester is applied in therapy as a mixture of the R and S diastereoisomers. In the previous works higher efficiency and faster absorption from gastrointestinal tract as well as higher microbiological activity of the isomer R in comparison to the isomer S were found. In order to explain these facts hydrolysis of both isomers in human blood *in vitro* as well as in Sörensen's buffer with human blood have been investigated. Distinct differences in the reactivities of the R and the S isomers were observed. A larger amount of biologically active isomer of cefuroxime and a small amount of inactive isomer of this antibiotic are formed from the isomer R. This result and the better solubility of the isomer seem to explain the higher antibiotic effectiveness of this isomer.

Keywords: Cephalosporin, cefuroxime axetil, diastereoisomers R and S, enzymatic hydrolysis in vitro, esterase.

1–Acetoxyethyl ester of cefuroxime **[I]** is a prodrug of cefuroxime **[II]**, an injectable second generation cephalosporin with an excellent activity against Gram–positive and Gram–negative organisms.

After oral administration, such prodrugs as **[I]** are rapidly hydrolyzed by non–specific esterases to give high concentrations of free acid **[II]** in blood and in body tissues (1,2). Such orally active esters are applied in the case of low absorbtion from the gastrointestinal tract of antibiotics in the form of free acids or salts (3). Esterification of the carboxyl group of cefuroxime renders the molecule more lipophilic and, in contrast to the parent substance, a significant proportion is absorbed after administration *per os*.



The ester **[I]** is microbiologically inert and owes its activity to the liberation of cefuroxime by hydrolysis of the ester linkage[1].

In the molecule of the ester **[I]** three kinds of isomerism are encountered. Two of them are due to the presence of double bonds:

- E, Z isomerism on the C=N double bond in the side chain,
- Δ^3 and Δ^2 isomerism in the dihydrothiazine ring; in Δ^2 , the double bond is located in position 2.

It is important that the biological activity of the isomers E and Δ^2 of the ester **[I]** is many times lower than that of the isomers Z and Δ^3. Therefore synthesis of **[I]** should be carried out under conditions ensuring the formation of the latter isomers only.

Another kind of isomerism of the ester **[I]** is caused by the presence of an asymmetric carbon atom at position 1 of the ester group. So far the ester **[I]** has been applied in therapy as a mixture of

the R and S diastereomers (1'R, 6R, 7R and 1'S, 6R, 7R) used in ratios of 0.9:1 up to 1.1:0.9 (4, 5). In view of the increasing tendency, observed during the past decades, of using only pure isomers (6) in therapy, separation methods of the R and S diastereomers **[1R** and **1S]** and their properties have been investigated (7,8). It was found that **[1R]** is more efficiently and faster absorbed from the gastrointestinal tract of rats and has the higher microbiological activity than **[1S]** (9). In this paper, differences in activity *in vitro* of (1R) and (1S) are studied.

EXPERIMENTAL

Materials

1. 1–Acetoxyethyl ester of cefuroxime (9):
a) **[1R]** in the crystalline form, purity 94.3%,
b) **[1S]** in the crystalline form, purity 96.2%,
c) **[1R]** in the amorphous form: **[1R]**–1, purity 93.1%; **[1R]**–2, purity 92.3%,
d) **[1S]** in the amorphous form: **[1S]**–1, purity 98.0%, **[1S]**–2, purity 97.0%,
e) Amorphous form of the mixture 1 : 1 of R and S isomers, purity 96.8%.

2. Standards:
a) Δ^3 1–acetoxyethyl ester of cefuroxime , purity 96.3%, b) Δ^2 1–acetoxyethyl ester of cefuroxime, purity 70%, c) Cefuroxime sodium salt, purity 92.2% (as cefuroxime).

Preparation of samples

Conditions of hydrolysis: a water bath with shake, temperature 37.0°C ± 0.5°C, time of hydrolysis 5, 10, 15, 30, 60, 120 and 240 minutes.

Environment: a) the whole human blood, a 500 μg sample was suspended in 1 ml of blood, b) Sörenson's phosphate buffer, pH = 7.0, containing human blood in the ratio 9 : 1, concentration of sample in solutions 8 μg/ml, c) Sörenson's phosphate buffers, pH = 7.4 and pH = 7.0, concentration of sample in the solution, 8 μg/ml.

Sörenson's phosphate buffer, pH 7.4; 818 ml of 1/15 M solution of $Na_2HPO_4 \cdot 12\ H_2O$ (x) and 182 ml of 1/15 M solution of KH_2PO_4 (y);

Sörenson's phosphate buffer, pH pH 7.0 ; 612 ml of (x) and 388 ml of (y).

Test – tubes containing an appropriate medium were placed in the water bath. Hydrolysis was carried out for two hours at a temperature of 37.0°C. Then, to 1 ml of sample, 0.5 ml of 10% solution of trichloroacetic acid and 0.5 ml acetonitrile were added; the sample was shaken and centrifuged. The supernatant was diluted and chromatographed.

Method of analysis

High performance liquid chromatography HPLC/1 system was applied for the determination of **[II]** and **[IIA]**.
Column: Super Pack Cartridge LKB, Spherisorb ODS 2,3 μm; 4.0 mm x 100 mm;
detection: UV 273 nm; mobile phase: acetate buffer (pH 3.4) – MeCN (95 : 5 v/v); flow rate: 1 ml/min.
HPLC/2 system was used for the determination of **[1R], [1S], [1RA]** and **[1SA]**.

Column: Nova Pack C 18, 4 μm, 150 mm x 3.9 mm; flow rate: 1 ml/min; detection: UV 278 nm; mobile phase: 0.04 M KH_2PO_4 – MeOH (62 : 38 v/v).

Recovery of **[II], [IR]** and **[IS]** was quantitative. Methods were validated.

RESULTS AND DISCUSSION

Enzymatic hydrolysis of **[IR]** and **[IS]** of the ester **[I]** was investigated in vitro by using human blood at 37°C. The amorphous as well as the crystalline forms of **[1R]** and **[1S]** were suspended in blood (500 μg/ml) and at stated intervals the amount of cefuroxime was determined by the HPLC method. For detection of the two isomers of Δ^2 and Δ^3, differing in the position of the double bond in the dihydrothiazine ring, two separate procedures were applied, one (HPLC/1) for analysis of cefuroxime free acid, and the second (HPLC/2) for analysis of its ester. In this work, the active Δ^3 and the inactive Δ^2 isomers of cefuroxime are denoted as **[II]** and **[IIA]** and, for Δ^3 and Δ^2 isomers of the ester **[I]** as **[IR], [1RA], [1S]** and **[1SA]** respectively.
The data in Table 1 show that the efficiency of hydrolysis to **[II]** for the amorphous forms of both isomers is higher with **[IR]** than with **[1S]**. With **[1R]** and **[1S]**, this reaction was apparently terminated in 30 and 60 min., yielding 82% and 47% of **[II]**, respectively.

Isomerizations of **[1R]** to **[1RA]** and of **[1S]** to **[1SA]** were found to proceed in blood simultaneously with the process of hydrolysis with the yield of the last one respectively ca. 10% and 30% of **[2A]**. All four isomers of the ester **[1R, 1RA, 1S, 1SA]** hydrolyze to yield cefuroxime **[II]** or **[IIA]**, but no conversion from **[II]** to **[IIA]** has been observed.
All the above results for the crystalline form of **[IR]** and **[IS]** are analogous but lower in value. With **[IR]** and **[IS]**, hydrolysis was complete in 120 and 240 min. to yield ca. 46% of **[IIA]** and ca. 20% of **[II]**, respectively. The yields of the inactive isomers **[IRA]** and **[ISA]** were respectively ca. 5% and 15%.

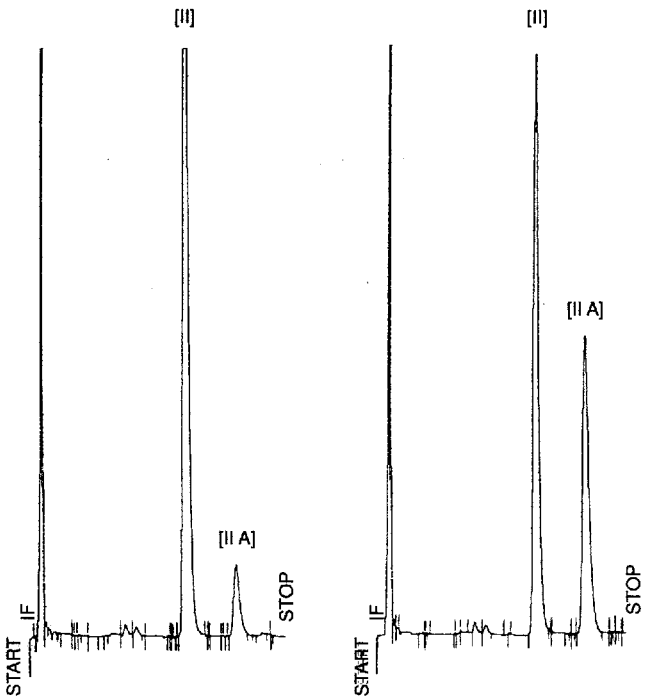


Figure 1. Chromatogram (HPLC/1) illustrating formation of Δ^3 cefuroxime [**II**] and Δ^2 cefuroxime [**II**] during hydrolysis *in vitro* in human blood of the isomer R [**IR**] and the isomer S [**IS**] of the 1-acetoxyethyl ester of cefuroxime [**I**].

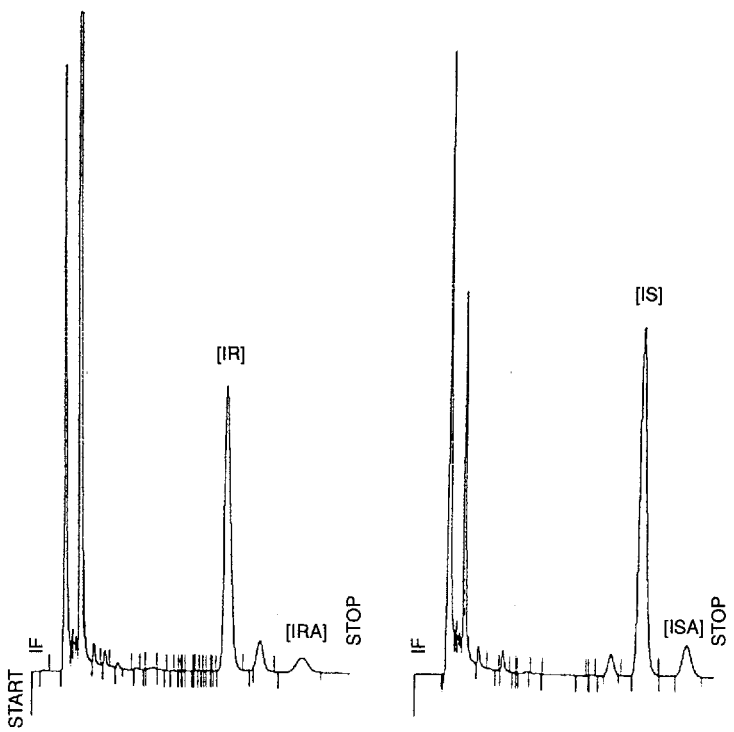


Figure 2. Chromatogram (HPLC/2) illustrating isomerization of the isomer R [**IR**] to [**IRA**] and of the isomer S [**S**] to [**ISA**] during hydrolysis *in vitro* in human blood of the isomers [**IR**] and [**IS**] of the 1-acetoxyethyl ester of cefuroxime [**I**].

These results have led to the conclusion that, for either the amorphous or the crystalline form of ester **[IR]** and **[IS]**, the levels of active cefuroxime **[II]** in blood gained by enzymatic hydrolysis are about three times as high in the case of **[IR]** relative to **[IS]**, what could be one of the reasons for the higher activity of **[IR]** in vivo. The lower levels for the crystalline forms are probably due to their poorer solubility with respect to the amorphous ones (9).

Furthermore, in order to compare the enzymatic hydrolysis of pure isomers and commercial pharmaceutical composition, three amorphous samples: **[IR]**, **[IS]** and commercial **{I[R+S]}** were investigated in the above conditions.

It was found (Table 2) that, after 5 min., the yields of active cefuroxime **[II]** were respectively *ca.* 51%, 12% and 42%, and after 240 min., *ca.* 83%, 44% and 69%, whereas the yields of inactive **[IIA]** after the latter time were *ca.* 11%, 34%, and 24%. Thus, the isomer **[IS]** seems to be responsible for lowering the activity of the commercial product in comparison to isomer **[IR]**.

The better general solubility of **[IR]** as compared with **[IS]** (9), may well have some influence on the above results. To exclude this effect on reactions investigated, solutions of isomers **[IR]** and **[IS]** in Sörenson's buffer were prepared at concentrations of 8 μg/ml (similar maximum concentrations have been observed in blood after oral administrations in rats 9). Sörenson's buffer was used to stabilize pH at 7.4, a value typical for blood. After addition of 10% (v/v) of fresh human blood to the solution, substantial discrimination between **[IR]** and **[IS]** in the course of hydrolysis was also observed (Table 3). After 5 min. of hydrolysis of **[IR]** the yields of cefuroxime **[II]** and **[IIA]** were respectively *ca.* 26% and 6%, the values for **[IS]** being *ca.* 12% and 35%. Similarly, in 120 min. the results were: for **[IR]** *ca.* 64% and 25%, whereas for **[IS]** *ca.* 31% and 59%. One can see that in this experiment the yield of hydrolysis to cefuroxime **[IIA]** is for **[IR]** more than twice as high as that for **[IS]**.

As reference solutions of **[IR]** and **[IS]** in Sörenson's buffer at pH 7.0 and 7.4 without addition of blood were prepared at above concentrations (8 μg/ml) and kept for 120 min. Isomerisations to **[IRA]** and **[ISA]** as well as non enzymatic hydrolysis were observed, but no considerable differences in generally low yields were noted.

Comparison of the enzymatic rate constants for hydrolysis of both diastereomers in phosphate buffer (pH 7.4) in the presence of dog or rat serum or intestine esterases has been described (10). Cefuroxime and the diastereoisomers R and S have

Table 1. The changes of contents [a] of Δ^3 cefuroxime **[II]** and Δ^2 ceforuxime **[IIA]** during hydrolysis of amorphous and crystalline forms of the isomers **[IR]** and S **[IS]** of 1-acetoxyethyl ester of cefuroxime **[I]** in blood *in vitro*, as determined by HPLC

Time of hydrolysis, min.	Contents (%) in the hydrolyzate								
	of amorphous form				of crystalline form				
	IR		IS		IR		IS		
	II	IIA	II	IIA	II	IIA	II	IIA	
10	72.7 (± 1.7)	7.3 (± 0.5)	22.3 (± 2.1)	8.6 (± 0.7)	24.0 (± 2.7)	1.1 (± 0.3)	1.2 (± 0.2)	<0.05	
30	80.9 (± 2.1)	10.3 (± 0.5)	37.9 (± 3.0)	24.8 (± 2.4)	39.5 (± 0.9)	3.9 (± 0.4)	4.6 (± 0.5)	1.2 (± 0.2)	
60	81.1 (± 2.0)	10.4 (± 0.6)	45.9 (± 2.7)	30.7 (± 3.0)	42.7 (± 1.3)	4.0 (±0.3)	8.4 (± 0.2)	4.0 (± 0.4)	
120	82.4 (± 1.9)	10.4 (± 0.5)	46.4 (± 2.9)	30.8 (± 2.9)	46.5 (± 1.6)	4.6 (± 0.2)	12.5 (± 0.6)	8.3 (± 0.9)	
240	82.3 (± 2.0)	10.1 (± 0.7)	47.0 (± 3.2)	30.5 (± 3.1)	46.4 (± 1.9)	4.6 (± 0.3)	20.3 (± 1.1)	14.6 (± 1.0)	

[a] Each result is the mean of five determinations ($\bar{x} \pm t \cdot S_{\bar{x}}$, $p=0.95$).

Table 2. The changes of contents of Δ3 cefuroxime **[II]** and Δ2 cefuroxime **[IIA]** during hydrolysis of amorphous form of isomer R **[IR]**, isomer S **[IS]** and a (1:1) mixture of R and S isomers of 1-acetoxyethyl ester of cefuroxime **[I]** in blood in vitro, as determined by HPLC.

Time of hydrolysis, min.	Contents (%) in the hydrolizate					
	of amorphous form of **IR**		of amorphous form of **IS**		of amorphous form **R+S**	
	II	**IIA**	**II**	**IIA**	**II**	**IIA**
5	50.9 (± 1.5)	3.5 (± 0.5)	12.5 (± 3.0)	2.4 (± 0.5)	42.3 (± 2.0)	6.0 (± 0.3)
10	74.4 (± 3.5)	6.8 (± 0.5)	24.3 (± 2.9)	9.0 (± 0.6)	61.5 (± 3.1)	12.9 (± 0.5)
30	80.4 (± 0.4)	10.0 (± 0.3)	35.8 (± 3.5)	26.8 (± 0.9)	68.7 (± 0.9)	19.2 (± 0.6)
60	81.3 (± 2.1)	10.2 (± 0.4)	42.6 (± 2.7)	33.2 (± 2.5)	69.4 (± 0.8)	22.3 (± 0.7)
120	82.9 (± 1.6)	10.5 (± 0.6)	43.8 (± 3.4)	33.2 (±2.1)	69.7 (± 3.2)	23.9 (± 1.6)
240	82.9 (± 1.9)	10.6 (± 0.6)	44.0 (± 3.1)	33.5 (± 2.7)	69.7 (± 3.0)	23.7 (± 1.5)

Each results is the mean of five determinations ($x \pm t \cdot Sx$, p=0.95).

Table 3. The changes of contents of Δ^3 cefuroxime **[II]** and Δ^2 cefuroxime **[II A]** during hydrolysis of amorphous form of isomers R **[IR]** and S **[IS]** of 1-acetoxyethyl ester of cefuroxime **[I]** in a modified Sörensen's phosphate buffer pH 7.4 containing blood (9:1)

Time of hydrolysis, min.	Contents (%) in the hydrolizate			
	IR		IS	
	II	II A	II	II A
5	26.0 (± 0.7)	5.5 (± 0.4)	11.6 (± 1.4)	35.2 (± 2.6)
15	29.9 (± 2.5)	8.4 (± 0.5)	14.3 (± 2.0)	38.6 (± 3.0)
30	40.4 (± 2.6)	11.7 (± 0.6)	20.7 (± 1.7)	39.0 (± 2.7)
60	56.9 (± 3.0)	21.4 (± 2.0)	25.9 (± 2.1)	49.5 (± 2.1)
120	64.0 (± 2.9)	25.3 (± 2.3)	30.7 (± 2.5)	59.1 (± 2.9)

Each redult is the mean of five determinations ($x \pm t \cdot Sx$, p=0.95).

been detected by HPLC analysis (10). In each casey the S isomer was found to hydrolyze faster than the R isomer did, and the specificity of the ester **[I]** esterase, present in blood, decreased in the order rat>dog>human.

Differences between these and our results may well be due to the minor specificity of the human esterase and to the different conditions (mobile phase, column) of HPLC method. Cefuroxime **[II]** and **[IIA]** according to Moscher 10) was detected as one peak, but in our method **[II]** and **[IIA]** were separated, which made it possible to follow the process of hydrolysis occurrng simultaneously with isomerisation.

Therefore, taking into consideration the formation of **[II]** and **[IIA]** during a 30 min. hydrolysis of the R and S isomers in the phosphate buffer (pH 7.4) in the presence of blood, this process is seen to be faster for the S isomer, whereas the rate of formation of the active **[II]** is considerably higher for the R isomer.

In order to estimate possible differences in the spatial crowding in the area of the ester carbonyl group in **[IR]** and **[IS]**, a semiempirical PM3 (11) calculation was applied. After the geometries of the two diastereoisomers have been optimized, the ester group was rotated around the COO–R bond by steps of 20 deg. No significant difference was observed in the rotation barriers when the isomers **[IR]** and **[IS]** were compared; the value was 35 kJ/mol for each. This result was related to the calculated barrier in n–butane (18 kJ/mol), and to

the literature value (18.4–25.5 kJ/mol) (12). The conclusion is that spatial crowding is rather not a critical factor for the differences observed in reactivity. This is consistent with the finding that in the course of non–enzymatic hydrolysis there are practically no differences between the two isomers. Thus, the observed distinction in activity between the isomers **[IR]** and **[IS]** appears to be attributable to the well–known selectivity of enzymes toward enantiomeric substrates (13).

CONCLUSIONS

The present results indicate that, in the enzymatic hydrolysis occurring in the presence of blood, **[IR]** and **[IS]** have manifested different reactivities. A much larger amount of biologically active Δ^3 cefuroxime **[II]** and a very small amount of inactive Δ^2 isomer **[IIA]** are formed from **[IR]**. This variance is most probably due to the higher solubility of **[IR]** and the well–known selectivity of enzymes towards enantiomeric substrates (13).

Thus, **[IR]** may be much more appropriate for therapy than is the commercial racemic mixture still used for this purpose. Application of **[IR]** would correspond with the last decade tendency of applying single isomers in therapy (6).

REFERENCES

1. A. M. Emmerson: J. Antimicrob. Chemother. 22, 101 (1988).
2. S.M. Harding, P.E.O. Wiliams, J. Ayrton: Antimicrob. Agents Chemother. 25, 1, 78 (1984).
3. E. Ridgway, K. Stewart, G. Rai, M.C. Kalsey, C.C. Bielawska: J. Antimicrob. Chemother. 27, 663 (1991).
4. USP XXII, First Supplement, p. 2107.
5. Polish Patent 156,001 (1983).
6. S. Gorog, M. Gazdag: J. of Chromatography, B 659, 51 (1994)
7. British Patent No. 1,571,683 (1997).
8. USA Patent 5,063,224 (1990).
9. I. Oszczapowicz, E. Malafiej et al.: Acta Polon. Pharm. 52, 471 (1995).
10. G.L. Mosher, J. Mc Bee, D. B. Shaw, Pharmaceutical Research 9, 5, 687 (1992).
11. M.J.S. Dewar, Int. J. Quant. Chem. 44, 427 (1992); P. Kollman, Chem. Rev. 1993, (93), 2395.
12. R.T. Morrison, R.N. Boyd, Organic Chemistry, 3rd Ed., 109 (1973), Allyn and Bacon, Inc., Boston 1973.
13. A. J. Hutt, S.C. Tan, Drugs, 52, Supplement 5, 4 (1996).

Received: 21.04.1998