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Synthesis and Oral Absorption of Hetacillin and Hetamoxicillin Labile Esters

SANTIAGO GUBERT, CELIA PALACIN, AURELIO SACRISTÁN, and JOSÉ A. ORTIZ*

Centro de Investigación Grupo Ferrer, Juan de Sada 32, 08028 Barcelona, Spain

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Nine esters of hetacillin or hetamoxicillin were synthesized as ampicillin and amoxicillin derivatives with the aim of obtaining improved plasma levels after oral administration. Out of these esters, which are devoid of apparent toxic effects, the benzyloxymethyl ester of hetacillin (FI-5204) gave the best result and was chosen for subsequent studies.

Keywords——oxyhetacillin ester; hetacillin ester; benzyloxymethyl ester; prodrug; oral absorption; plasma level

The use of prodrugs is known to increase the therapeutic effectiveness of antibiotics, especially beta-lactams.[1,2] Prodrugs are chemical derivatives which are usually inactive or less active than the parent compounds, but are hydrolyzed or metabolized to release the parent compounds or active metabolites under physiological conditions. The toxicity of prodrugs as well as the released moiety should be lower than that of the parent compounds.

Ampicillin and amoxicillin are not readily absorbed when given orally. This fact has stimulated a search for new derivatives in the field of beta-lactam prodrugs that would show substantially improved oral absorption properties. Various esters of the above penicillins have been synthesized. Within this line of investigation, there are two different aspects for consideration. One aspect is the need to obtain esters that are rapidly hydrolyzed, and consequently release the starting penicillin (ampicillin or amoxicillin) quickly into the systemic circulation; this is the case with ampicillin esters such as the ethoxycarbonyloxyethyl (bacampicillin),[3,4] phthalidyl (talampicillin),[5] and pivaloyloxymethyl (pivampicillin)[6] esters. These esters are characterized by increased lipophilicity owing to the esterification of the polar carboxylic group but maintain a residual polarity (amino group) so that they are soluble in the body fluids.

The other aspect is distribution; for example, the methoxymethyl esters of hetacillin (sarpicillin)[7] and hetamoxicillin (sarmoxicillin)[8] are only partially hydrolyzed and thus appear in the portal circulation as intact esters. They are also present in tissues such as saliva, lung, *etc.* This wider distribution as compared with that of ampicillin or amoxicillin may be accounted for by the nonionizing and highly lipophilic characteristics of these esters which, however, retain the antimicrobial spectrum of the original drugs.

The purpose of the present study was to prepare hetacillin and hetamoxicillin esters. These compounds can be considered as double prodrugs. The ester group of the adduct, which is readily removable by hydrolysis in the body, improves the oral absorption properties, and the increased lipophilicity owing to the ester bond results in higher and long-lasting blood levels, a wider distribution of the drug, and a greater stability after oral administration as compared to the parent antibiotics.

The ester moieties employed in this study have the general structure $R–CH_2–O–CH_2–$,

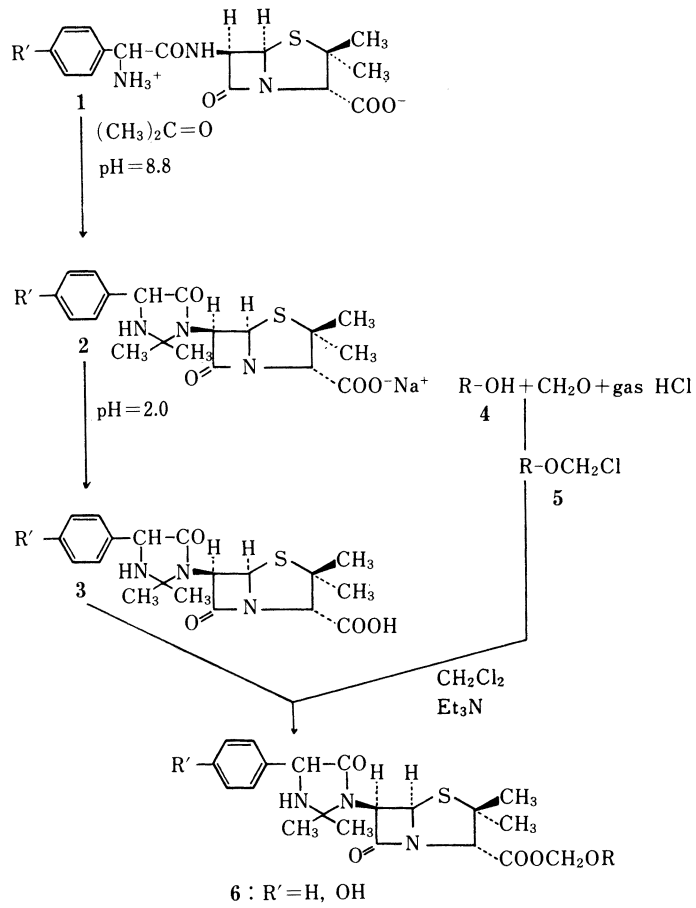


Chart 1

TABLE I. Physical Properties of the Chloromethyl Ethers (**5**)

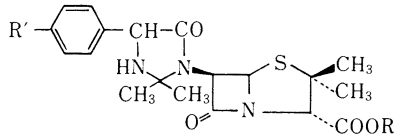
R	Yield (%)	Distillation temp. (°C)	n_D^{25}	Analysis (%) of chlorine[a]	
				Found	Calcd
	65	96—98 (9.5 mmHg)	1.5274	21.7	22.68
	39.9	84—85 (1 mmHg)	1.4599	20.8	23.54
	13.0	74—75 (0.7 mmHg)	1.4648	19.26	21.53
	14.3	30—32 (0.15 mmHg)	1.4540	20.3	29.46

a) Low values were due to unsuitable combustion conditions.

TABLE II. Physical Properties of Hetacillin (**3**: R′=H) and Hetamoxicillin (**3**: R′=OH)

Compound	mp (°C)	Acid groups (%)	IR Carbonyl β-lactam (cm^{-1})	Analysis (%) Calcd (Found)			
				C	H	N	S
Hetacillin	188—190	100.6	1790	58.61 (58.95	5.91 5.97	10.80 10.71	8.25 8.24)
Hetamoxicillin	209—210	100.9	1790	56.23 (56.70	5.67 5.80	10.36 9.91	7.93 7.90)

TABLE III. Physical Properties of Hetacillin and Hetamoxicillin Esters (**6**)



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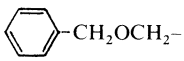
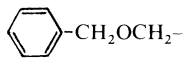
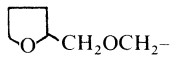
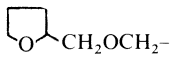
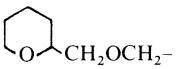
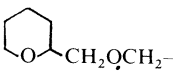
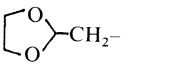
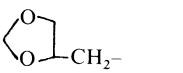
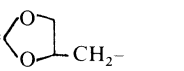
Compound	R′	R	Yield (%)	mp (°C)	Analysis (%) of sulfur		Acid groups[a]	IR Carbonyl β-lactam (cm^{-1})
					Found	Calcd		
FI-5204	H		49.4	83—86	6.27	6.3	96.0	1790
FI-5205	OH		42.0	88—94	5.96	6.1	94.2	1775
FI-5206[b]	H	$CH_3OCH_2CH_2OCH_2-$	43.5	49—54	6.75	6.73	98.5	1780
FI-5207[b]	OH	$CH_3OCH_2CH_2OCH_2-$	33.5	84—89	6.5	6.51	99.0	1780
FI-5208	H		18.6	55—58	6.25	6.37	94.9	1785
FI-5209	OH		15.0	58—63	6.0	6.05	93.1	1775
FI-5210	H		53.9	48—51	6.0	6.18	99.0	1785
FI-5211	OH		35.3	70—75	5.92	6.0	92.0	1780
FI-5214	H		15.0	—	6.69	6.74	94.5	1770
FI-5216	H		14.5	—	6.31	6.73	97.5	1770
FI-5217	OH		8.5	—	6.60	6.52	96.0	1775

a) Hydrolysis of acid groups was performed prior to titration. *b*) These compounds, which are known in the literature, have been synthesized and used in the present work for comparative purposes.

where the function of the R group (alkyl, arylalkyl, heterocycle, *etc.*) is to prevent hydrolysis to form highly toxic metabolites.

Chemistry

Chart 1 shows the reaction steps for the preparation of hetacillin and hetamoxicillin esters (**6**).

The alcohols (**4**) were treated with formaldehyde and HCl gas to give the respective chloromethyl ethers (**5**) (Table I). The pure chloromethyl ethers, obtained by fractional distillation, were reacted directly with hetacillin (**3**: R′=H) or hetamoxicillin (**3**: R′=OH) in methylene chloride to give the esters (**6**) (Table III). Preparation of hetacillin and hetamoxicillin (Table II) was carried out according to the known methods.[10,11]

Pharmacological Methods

Absorption Test——Plasma levels of the esters (**6**) listed in Table III were determined after oral administration of the test compounds to groups of 5 rats, 4 rabbits, and 2 dogs each at a single dose equivalent to 100 mg/kg of ampicillin or amoxicillin. Test compounds (**6**) were administered as a suspension (10 ml/kg volume) in glycerolformal (0.3 ml). Arlontone (0.1 ml), Tween-80 (0.6 ml) and 0.25% agar (9 ml). Blood samples were collected at 15 and 30 min and 1, 2, 4 and 6 h after dosing. Levels are expressed in ampicillin or amoxicillin values because the test compounds (**6**) are esters of hetacillin and hetamoxicillin and are rapidly converted, both *in vitro* and *in vivo*, into ampicillin and amoxicillin, respectively,[12,13] and are found in tissues and biological fluids as ampicillin and amoxicillin.

For the microbiological assessment, ampicillin trihydrate and amoxicillin trihydrate were used as standards and ATCC 9341 *Sarcina lutea* as the assay microorganism. Test curves showed a susceptibility to ampicillin ranging from 0.030 to 0.115 μg/ml and to amoxicillin from 0.020 to 0.101 μg/ml. This microorganism is susceptible to low concentrations of the tested penicillins but is not susceptible to the nonhydrolyzed esters.

Toxicity Test——Oral acute toxicity was assessed in conventional albino mice, male and female, weighing 20 ± 2 g, which were grouped homogenously. Dose levels, after a trial dosing, were set at 1, 3, 4 and 5 g/kg. Mortality was recorded daily up to 7 d. In all cases, survival was 100% at the tested doses; consequently LD_{50} is higher than 5 g/kg.

Results and Discussion

The plasma concentrations of ampicillin and amoxicillin in rats after single oral administration of 100 mg/kg of the esters are shown in Table IV, together with the concentrations measured after administering equivalent doses of ampicillin and amoxicillin; these data were used as reference values for comparing the oral absorption of the esters.

Maximum levels of ampicillin in the case of hetacillin esters were reached within half an hour after administration and were similar to those in the case of ampicillin; however, the levels for the benzyloxymethyl ester (FI-5204) and methoxyethoxymethyl ester (FI-5206)[14] were 5 times and 3 times higher than those of ampicillin, respectively. In contrast, FI-5208 and FI-5210 esters did not show any significant change. Of the hetamoxicillin esters, only FI-5211 showed similar levels, although they were rather lower than those of amoxicillin.

An explanation for the low levels of certain esters *versus* reference antibiotics would be the presence of these esters in plasma as intact (and inactive) esters. However, the literature published so far clearly indicates that the presence of the intact ester is not significant for bacampicillin-, pivampicillin-, and talampicillin-like esters[15-17] but accounts for the different pharmacokinetic distributions of the lipophilic esters of ampicillin or amoxicillin (sarpicillin or sarmoxicillin).[7,8,18]

The above considerations led us to extend oral absorption tests to other animals such as

TABLE IV. Plasma Levels of Ampicillin and Amoxicillin in the Rat after Oral Dosing of Hetacillin and Hetamoxicillin Esters (**6**)[a]

Compound	Mean plasma levels (μg/ml)			
	0.25	0.50	1	2 (h)
Ampicillin	4.26	3.34	3.47	1.6
FI-5204	21.46	17.05	10.38	2.8
FI-5206[b]	13.45	10.02	3.78	0.78
FI-5208	4.35	3.42	1.75	1.07
FI-5210	5.08	4.19	3.37	1.81
Amoxicillin	3.12	11.73	13.8	14.7
FI-5205	—	2.33	3.0	1.9
FI-5207[b]	—	3.33	3.3	1.85
FI-5209	—	1.19	1.35	1.37
FI-5211	—	9.39	6.87	4.87

a) Single oral dose of 100 mg ester/kg. *b*) References esters already described.[14] FI-5204, FI-5206, FI-5208, FI-5210; ampicillin esters. FI-5205, FI-5207, FI-5209, FI-5211; amoxicillin esters.

TABLE V. Plasma Levels of Ampicillin in the Rat and Rabbit after Oral Dosing of FI-5204

Dose group	Mean plasma levels (μg/ml)					
	0.25	0.50	1	2	4	6 (h)
Rat[a]	16.4	13.3	7.1	4.1	0.65	—
Rabbit[b]	2.66	4.25	4.60	2.77	1.77	1.5

a) FI-5204, 100 mg/kg. *b*) FI-5204, 50 mg/kg.

TABLE VI. Plasma Levels of Ampicillin in the Dog[a] after Oral Dosing of FI-5204

Compound	Mean plasma levels (μg/ml)					
	0.25	0.50	1	2	4	6 (h)
Ampicillin[b] (capsule)	—	0.31	2.1	10.96	4.7	1.05
FI-5204[c] (capsule)	0.31	0.096	0.08	—	—	—
FI-5204[d] (suspension 1)	—	0.12	1.58	3.37	2.92	0.77
FI-5204[e] (suspension 2)	0.12	0.53	2.78	5.77	1.93	0.47

a) Beagle dog. *b*) Dose: 50 mg/kg as active ampicillin. *c*) Dose: 50 mg/kg as active ampicillin. *d*) Dose: 200 mg/kg as active ampicillin. *e*) Dose: 100 mg/kg as active ampicillin; this suspension corresponds to that used for rats.

the rabbit and dog in the case of compound FI-5204 only. The results of FI-5204 plasma levels in rabbits and dogs (Tables V and VI) show that in both cases the levels obtained are lower than those in the rat (Tables IV and V) and that those when ampicillin is administered to rabbits and dogs. It should be emphasized that the kind of administration form (capsule,

suspension) in dogs did not significantly affect the plasma levels.

Experimental

Melting points were determined on a Büchi apparatus and are uncorrected. Infrared (IR) spectra were taken on a Beckman Acculab-4 spectrophotometer. Chlorine and sulfur were determined by Schöniger's method.[19,20]

Chloromethyl Ethers ($ROCH_2Cl$)——Equal amounts (1 mol each) of a hydroxymethyl derivative ($R–CH_2–OH$) and formaldehyde (37% aqueous solution) were mixed in a flask provided with a thermometer, anhydrous $CaCl_2$ tube, gas-entrance tube and gas-exit tube connected to a water-jet pump. The mixture was kept in an ice bath at 5 °C while dry HCl gas was passed for 5—6 h, and then left to stand overnight under stirring. The solvent was removed and the residue was extracted with chloroform (2 × 100 ml). The organic phase was separated and washed with water (100-ml fractions) till the wash water was neutral to litmus paper, then dried over anhydrous $MgSO_4$ and filtered. The chloroform was evaporated off under reduced pressure and the chloromethyl ether was distilled under high vacuum.

The derivatives obtained, their yields and the physicochemical data are summarized in Table I. The IR spectra and the results of thin-layer chromatography on 60-F254 silica gel indicated that the compounds are pure and free of the starting alcohol.

Hetacillin (3: R′ = H) and Hetamoxicillin (3: R′ = OH)——Ampicillin trihydrate (40.3 g, 0.1 mol) was treated with 500 ml of distilled water and 2 l of acetone; the initial pH of 5.4 was brought to 8.8 with 6 M sodium hydroxide solution to give a transparent solution, which was stirred at 35—40 °C for 3 h and at room temperature overnight. Then it was brought to pH 7.0 and the acetone was evaporated off under a vacuum. The aqueous phase was cooled in an ice-bath and acidified to pH 5.0 with 3 M hydrochloric acid to precipitate the unreacted ampicillin; the solid was filtered off and washed with water (2 × 30 ml), and the combined filtrate and washing was acidified to pH 2.0. The mixture was stirred for 2 h, then the precipitate was filtered off, washed with water (2 × 30 ml) and dried under a vacuum at 40—50 °C. Yield was 41.3%.

Hetamoxicillin was prepared in the same manner; yield 42.8%. Analysis data for hetacillin and hetamoxicillin samples are given in Table II.

Hetacillin and Hetamoxicillin Esters (6)——In a 500-ml flask provided with a thermometer and a liquid addition tube, 1 mol of either hetacillin or hetamoxicillin was dissolved in 400 ml of dry methylene chloride with the aid of triethylamine. The mixture was cooled in an ice bath at 0—5 °C and then 1 mol of chloromethyl ether dissolved in 50 ml of dry methylene chloride was added. It was kept under stirring for 5 h in an ice bath and left to stand overnight at room temperature. The reaction mixture was successively washed with distilled water, sodium bicarbonate saturated solution (2 × 80 ml) and distilled water again. The organic phase was separated, dried over anhydrous $MgSO_4$, and filtered and the methylene chloride was evaporated off. A resin was obtained and treated with anhydrous ethyl ether to give a stable solid.

The esters (**6**) obtained, their yields and the physicochemical data are summarized in Table III.

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