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3-Acyloxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylic Acids. An Improved Synthesis and Biological Properties

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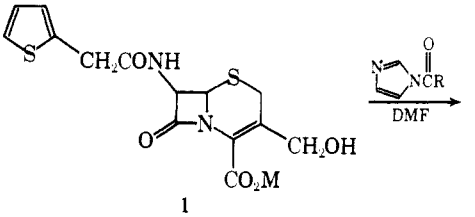
3-Acyloxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylic acids have been prepared by an improved method utilizing the acylation of desacetylcephalothin with acid imidazolides. Their in vitro and in vivo antibacterial activities have been determined and compared with those of cephalothin.

Cephalothin (2, R = CH_3), a commercially important cephalosporin, has good in vitro activity against a broad spectrum of gram-positive and gram-negative bacteria, but its in vivo activity is diminished by enzymatic hydrolysis of the acetate group to give the less active 3-hydroxymethyl compound 1.[1] Testing the hypothesis that increasing the bulk of the R group in 2 would decrease susceptibility to enzymatic hydrolysis, Kukolja[2] found only a small increase in in vivo activity relative to cephalothin for a series of cephalosporins in which R ranged from ethyl to cyclobutyl. Van Heyningen[3] reported a significant drop in in vitro activity when R was an aromatic ring. Nevertheless, when R was 2-thienyl, the in vivo activity in mice was superior to that of cephalothin. With an interest in derivatives which possess in vitro activity equivalent to that of cephalothin but with greater metabolic stability, the variation of R (in particular, the introduction of heteroatoms) has been examined further.

It has been reported that aroyl chlorides but not aliphatic acid chlorides acylate 7-acylaminodesacetylcephalosporanic acids.[3] Aliphatic acid derivatives have been made by acylation of desacetylcephalosporanic acid esters with subsequent regeneration of the 4-carboxyl[4] and by acylating 2-cephem acids followed by double bond isomerization.[2]

In contrast to the acid chlorides, acylation of sodium 3-hydroxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylate (1) with aliphatic acid imidazolides[5] in an inert solvent (DMF) produced respectable yields[6] of the 3-acyloxymethyl compounds 2. Lactonization and double bond migration, reported to be serious problems with the acid chloride acylations,[7] were not observed with the acid imidazolides.



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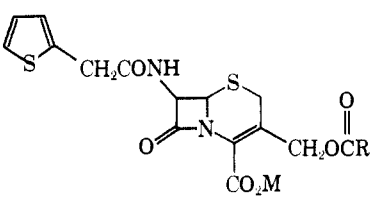

2

The new cephalosporins, which are summarized in Table I, were characterized by elemental analysis and ir and NMR spectroscopy.

Table I. Cephalosporins

Compd	R	M	Yield, %	Formula[a]
2a	$C(CH_3)_3$	Na	18	$C_{19}H_{21}N_2NaO_3S_2 \cdot H_2O$
2b	CH_2OCH_3	Na	60	$C_{17}H_{17}N_2NaO_7S_2 \cdot 0.5H_2O$
2c	CH_2NHCO_2-$C(CH_3)_3$	Na	59	$C_{21}H_{24}N_3NaO_8S_2 \cdot 0.5H_2O$
2d[b]	CH_2NH_2	H	38	$C_{16}H_{17}N_3O_6S_2 \cdot H_2O$

[a]Elementary analyses for C, H, and N were within 0.4% of the theoretical value with one exception. N for 2d: calcd, 9.74; found, 9.21. [b]Obtained by trifluoroacetic acid treatment of the free acid of 2c.

The biological evaluation of the new cephalosporins is shown in Table II. The *tert*-butyl derivative **2a** extends Kukolja's series. While its gram-positive activity was roughly equivalent to that of cephalothin, it was much less active than cephalothin against gram-negative bacteria. It was less active than cephalothin in a mouse protection test.

The derivatives substituted by nitrogen or oxygen on the methyl group of the acetate (**2b–d**) were generally less active in vitro than cephalothin. However, **2b** was several times better in vivo than would have been predicted from its MIC's. The potencies of **2c** and **2d** were not great enough to give meaningful mouse protection data.

In terms of facility, yield, and generality the acylation of desacetylcephalothin with acid imidazolides has proved superior to previously reported methods for the preparation of analogous compounds. While the products have shown reduced in vitro activity, the unexpected in vivo activity of **2b** may reflect greater stability toward enzymatic hydrolysis although this could also be due to other factors which affect the pharmacokinetic properties of the compound.

Experimental Section

Infrared spectra were obtained in Nujol mull using a Perkin-Elmer Infracord. NMR spectra were obtained in Me_2SO-d_6, Me_2SO-d_6–D_2O, or TFA-*d* on a Varian T-60 spectrometer using Me_4Si as internal standard.

Sodium 3-Acyloxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylates (2a–c). A mixture of 0.012 mol of the appropriate carboxylic acid and 0.012 mol (1.96 g) of *N,N'*-carbonyldiimidazole in 20 ml of dry DMF was stirred at room temperature for 0.5–1.5 hr while protected from atmospheric moisture, and then 0.010 mol (3.76 g) of sodium 3-hydroxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylate (1)[8] was added as a solid or in DMF solution. The reaction's progress was followed by TLC on fluorescent silica gel plates using an 8:2:1 mixture of $CHCl_3$–*i*-PrOH–HCOOH. When the reaction had reached completion (26–45 hr), the mixture was poured into Et_2O (0.5–2.0 l.), and the insoluble sodium salt of the product was isolated. Unreacted hydroxymethyl compound 1, if present, was converted to its lactone by stirring a MeOH solution of the crude product with Amberlite IR-120H ion-exchange resin. The resin was removed and the MeOH evaporated in vacuo. The residue was dissolved in Me_2CO and treated with 30% sodium 2-ethylhexanoate in *i*-PrOH to precipitate the product as its sodium salt (2).

3-Glycyloxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylic Acid (2d). To 20 ml of cold TFA was added with stirring 1.71 g (0.0033 mol) of 3-(*N-tert*-butoxycarbonylglycyloxymethyl)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid.[9] After 1 hr at 0°, the reaction mixture was allowed to warm to room temperature over 0.5 hr and then poured slowly into 250 ml of rapidly stirred Et_2O. The resulting solid was dissolved in H_2O containing a small amount of Me_2CO and 5% aqueous $NaHCO_3$ was added to raise the pH to 3.0. The resulting gel was washed with H_2O and then with Me_2CO and dried to give 0.54 g (38%) of **2d**.

Table II. Biological Activities

Compd	MIC, μg/ml[a]					ED_{50}, mg/kg[b]		
	S.a.	*S.f.*	*S.p.*	*E.c.*	*K.p.*	*S.a.*	*E.c.*	*K.p.*
2a	0.8	50	0.1	200	100	13.5		
2b	2.4	75	0.4	50	13		42	200
2c	1.2	50	<0.1	75	25			>200
2d	3.1	100	0.8	200	75	>50		
Cephalothin	0.4	25	<0.1	6.3	3.1	2.9	46	116

[a]The in vitro antibacterial activities, reported as minimum inhibitory concentrations (MIC's) and the average of two tests, were determined in twofold dilution by the agar inclusion method [T. Jen, B. Dienel, J. Frazee, and J. Weisbach, *J. Med. Chem.*, 15, 1172 (1972)]. The organisms selected for inclusion in this table are *S.a.*, *Staphylococcus aureus* HH 127 (penicillin resistant); *S.f.*, *Streptococcus faecalis* HH 34358; *S.p.*, *Streptococcus pyogenes* C203; *E.c.*, *Escherichia coli* 12140; *K.p.*, *Klebsiella pneumoniae* 4200. [b]The ED_{50} values are expressed as the total dose of compound which afforded protection to 50% of the mice challenged. The doses were administered subcutaneously in equally divided portions at 1 and 5 hr postinfection.

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