ORALLY ACTIVE 7-PHENYLGLYCYL CEPHALOSPORINS

STRUCTURE-ACTIVITY STUDIES RELATED TO CEFATRIZINE (SK&F 60771)

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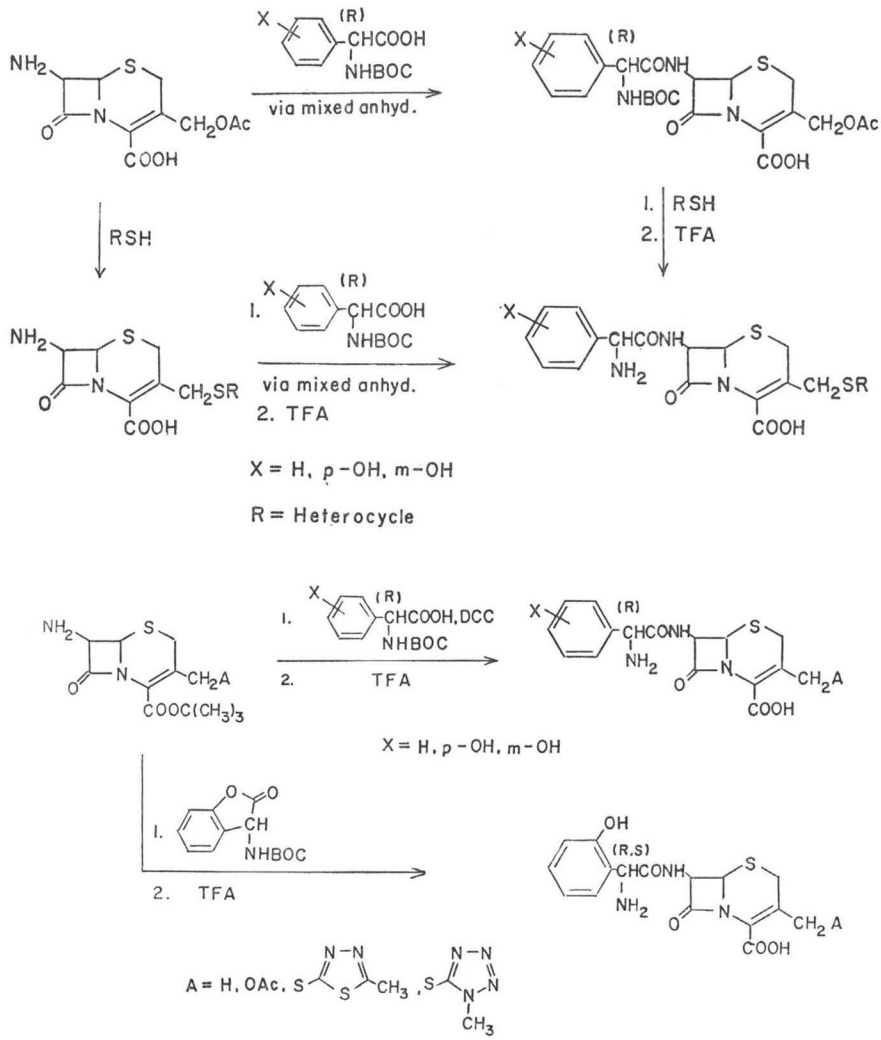
The synthesis of a series of related broad-spectrum 7-phenylglycyl cephalosporins with 3-heterocyclicthiomethyl substituents is described. The effects of benzene-ring hydroxylation and 3-substituent variation on the *in vitro* antibacterial activity, height and duration of mouse serum levels, and effectiveness in protecting against bacterial infection in the mouse are examined. Included for comparison are cephalexin, cephaloglycin and their *ortho*-, *meta*- and *para*-hydroxy derivatives. The biological properties examined were influenced by the position of the hydroxyl group and by the nature of the 3-substituent. The 7-(*p*-hydroxyphenylglycyl)-3-heterocyclicthiomethyl analogs were found to produce significantly higher serum levels on oral administration to mice than their unhydroxylated counterparts. This effect was not observed with the 7-(*m*-hydroxyphenylglycyl)-3-heterocyclicthiomethyl cephalosporins, nor with the *p*-hydroxyphenylglycyl analog of cephalexin. While *m*- and *p*-hydroxylation had little effect on *in vitro* activity the *o*-hydroxyphenylglycyl cephalosporins tested had very low antibacterial activities and were not examined further. One derivative, 7-[R-2-amino-2-(4-hydroxyphenyl)acetamido]-3-(1H-1, 2, 3-triazole-4(5)-ylthiomethyl)-3-cephem-4-carboxylic acid (SK&F 60771) was found to have outstanding *in vitro* and *in vivo* activities along with oral and subcutaneous serum levels in the mouse that were significantly higher than those obtained with cephalexin. This derivative which has been given the generic name cefatrizine was selected for extensive additional biological evaluation.

The criteria used in selecting a cephalosporin antibiotic as a candidate for clinical use require that it exhibit simultaneously a number of desirable chemotherapeutic and pharmacokinetic characteristics. Three of the characteristics examined in our laboratory for evaluating the potential utility of a cephalosporin are the *in vitro* activity (MIC) against gram-positive and gram-negative bacteria, the effectiveness in protecting small laboratory animals against bacterial infection (PD_{50}, mouse) and the serum concentrations attained when the cephalosporin is administered orally or by injection. Frequently these characteristics, and others of importance to the successful use of the cephalosporin, can be altered by modifying the chemical structure; but the alterations in biological properties do not necessarily proceed in a parallel direction. For example, recent studies[1,2] demonstrate that in certain cases *in vitro* activity can be increased against a number of bacteria by substituting an appropriate heterocyclicthiomethyl group for the methyl or acetoxymethyl group at the 3-position of the cephalosporin nucleus. The efficacy of this structural change is illustrated by cefazolin and cefamandole, two injectable cephalosporins with 3-heterocyclicthiomethyl substituents. Both

cephalosporins are more active against various bacteria, *e.g.*, the group of organisms cited in this article, than their corresponding analogs with an acetoxymethyl substituent at the 3-position. However, while the 3-position structural modification which converts 7-R-mandelamidocephalosporanic acid[3] to cefamandole produces essentially no change in the serum levels in the mouse (s.c.), the serum levels of cefazolin (a 7-tetrazoleacetamido derivative) are distinctly higher than those of its 3-acetoxymethyl analog.*

Studies on the alterations in *in vitro* activity when various heterocyclicthiomethyl groups are substituted for methyl or acetoxymethyl at the 3-position have included 7-phenylglycyl cephalosporins.[4] In some cases, but not all, this structural change results in improved *in vitro* activity. However, the serum levels attained when such analogs are administered orally to mice are significantly lower than those of cephalexin. The relatively low oral serum levels

Scheme I.



* Unpublished data from our laboratories.

demonstrated by the 3-heterocyclicthiomethyl-7-phenylglycyl cephalosporins led us to study the effects of benzene-ring hydroxylation, in addition to 3-substituent variation, on the *in vitro* antibacterial activity, the height and duration of mouse serum levels and the protective effectiveness against bacterial infection in the mouse. This article presents some results of our structure-activity studies which led to the selection of the title compound (cefatrizine, SK&F 60771) for extensive biological evaluation.[5]

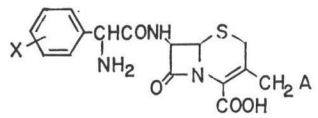
Chemistry

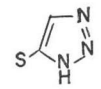
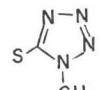
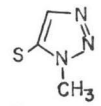
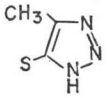
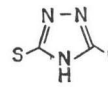
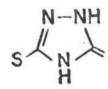
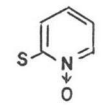
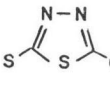
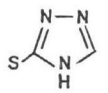
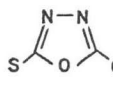
The 3-heterocyclicthiomethyl cephalosporins synthesized for use in this study are listed in Table 1. They were compared with the two commercially available 7-phenylglycyl cephalosporins, cephalexin (**1**) and cephaloglycin (**2**), and their *ortho*-, *meta*- and *para*-hydroxy analogs (**1a-c**, **2a-c**), also listed in Table 1. The synthesis of **1b** was described previously in the published literature[6]; some of the other cephalosporins in this latter group have been disclosed in patents.[7,8] A preliminary report[4] has included **4**, **4a**, **10** and **10a** but their chemical and biological properties have not been published; compound **3a** has been the subject of several recent reports.[5,9,10,11]

The cephalosporins were prepared by the four general procedures outlined in the Experimental Section (Scheme I). The methods used to synthesize each analog are indicated in Table 1. In the favored procedure (A) for preparing the 3-heterocyclicthiomethyl cephalosporins the intermediate nuclei, obtained by displacing the 3-acetoxy group of 7-aminocephalosporanic acid (7-ACA) with an appropriate heterocyclic thiol, were acylated using the mixed anhydrides derived from isobutylchloroformate and N-*t*-butoxycarbonylphenylglycine, or its corresponding *m*- or *p*-hydroxy analogs. The *t*-butoxycarbonyl (Boc) protecting group was removed using trifluoroacetic acid (TFA) or TFA-anisole (9:1) and the resulting TFA salts of the phenylglycyl cephalosporins were converted to their zwitterionic forms by treatment with a weak base ion-exchange resin or by neutralization with tri-*n*-butylamine. Alternatively, several of the 3-heterocyclicthiomethyl cephalosporins were prepared (procedure B) by first acylating 7-ACA with a Boc-protected phenylglycine as described in procedure A followed by displacement of the 3-acetoxy group with a heterocyclic thiol. The protecting group was removed and the products were converted to their zwitterionic forms by the procedures just described. The two other synthetic procedures (C and D) utilized the *t*-butyl esters of cephalosporin nuclei as intermediates. In procedure C the Boc-phenylglycine was coupled with the appropriate nucleus *t*-butyl ester using dicyclohexylcarbodiimide (DCC) and the two *t*-butyl-containing protective groups were then removed by TFA. The three *o*-hydroxyphenylglycyl cephalosporins were made by procedure D. In this case coupling was effected by refluxing a mixture of the nucleus ester and the lactone in xylene (Scheme I). Removal of the *t*-butyl groups with TFA gave in this case mixtures of the two diastereomeric products that are derived from a racemic phenylglycyl side chain.

In most cases the final cephalosporins were characterized as zwitterions, although six were characterized as TFA salts. The ir and nmr spectra for all compounds were consistent with structure. With the exception of the *o*-hydroxyphenylglycyl cephalosporins the resolved side-chain acid was used to prepare the cephalosporins. Only the phenylglycines having the

Table 1. Phenylglycyl cephalosporins.



Compounds	X	A	Method	Formula	Analysis
1a	*p*-OH	H	C	$C_{16}H_{17}N_3O_5S \cdot H_2O$	C, H, N
1b	*m*-OH		C	$C_{16}H_{17}N_3O_5S \cdot 2H_2O$*	C, H, N
1c	*o*-OH(*R*, *S*)		D	$C_{16}H_{17}N_3O_5S \cdot CF_3COOH$	C, H, N
2a	*p*-OH	OAc	A	$C_{18}H_{19}N_3O_7S \cdot 2H_2O$	C, H, N
2b	*m*-OH		C	$C_{18}H_{19}N_3O_7S \cdot 0.75H_2O$	C, H, N
2c	*o*-OH(*R*, *S*)		D	$C_{18}H_{19}N_3O_7S \cdot 0.75CF_3COOH \cdot 0.25Et_2O$	C, H, N
3	H		B	$C_{18}H_{18}N_6O_4S_2 \cdot CF_3COOH$	C, H, N
3a	*p*-OH		B	$C_{18}H_{18}N_6O_4S_2 \cdot 2H_2O$	C, H, N
3b	*m*-OH		A	$C_{18}H_{18}N_6O_5S_2 \cdot 1.5H_2O$	C, H, N
4	H		A	$C_{18}H_{19}N_7O_4S_2 \cdot 1.5H_2O$	C, H, N
4a	*p*-OH		C	$C_{18}H_{19}N_7O_5S_2 \cdot H_2O$	C, H, N
4b	*m*-OH		C	$C_{18}H_{19}N_7O_5S_2 \cdot 0.25Me_2CO \cdot 0.5H_2O$	C, H, N
5	H		A	$C_{19}H_{20}N_6O_4S_2 \cdot 2H_2O$	C, N; H[a]
5a	*p*-OH		A	$C_{19}H_{20}N_6O_5S_2 \cdot 2H_2O$	C, H, N[b]
6	H		B	$C_{19}H_{20}N_6O_4S_2 \cdot 1.5H_2O$	C, H, N
6a	*p*-OH		B	$C_{19}H_{20}N_6O_5S_2 \cdot 2.75H_2O$	C, H, N[c]
7	H		B	$C_{19}H_{20}N_6O_4S_2 \cdot 1.25H_2O$	C, H, N
7a	*p*-OH		A	$C_{19}H_{20}N_6O_5S_2 \cdot 3H_2O$	C, H, N[d]
8	H		A	$C_{18}H_{18}N_6O_3S_2 \cdot 2H_2O \cdot 0.5MIBK$	C, H, N
8a	*p*-OH		A	$C_{18}H_{18}N_6O_6S_2 \cdot 2.25H_2O$	C, H, N
9	H		B	$C_{21}H_{20}N_4O_5S_2 \cdot CF_3COOH \cdot H_2O$	C, H, N
9a	*p*-OH		A	$C_{21}H_{20}N_4O_6S_2 \cdot 2.75H_2O$	C, N; H[e]
10	H		B	$C_{19}H_{19}N_5O_4S_3 \cdot H_2O$	C, H, N
10a	*p*-OH		A	$C_{19}H_{19}N_5O_5S_3 \cdot 2H_2O$	C, H, N
10b	*m*-OH		A	$C_{19}H_{19}N_5O_5S_3 \cdot 2H_2O$	C, H, N[f]
10c	*o*-OH(*R*, *S*)		D	$C_{19}H_{19}N_5O_5S_3 \cdot CF_3COOH \cdot 0.5H_2O$	C, H, N
11	H		A	$C_{18}H_{18}N_6O_4S_2 \cdot H_2O$	C, H, N
11a	*p*-OH		A	$C_{18}H_{18}N_6O_5S_2 \cdot 3H_2O$	C, H, N
12	H		A	$C_{19}H_{19}N_5O_5S_2 \cdot 1.5H_2O$	C, H, N[g]
12a	*p*-OH		A	$C_{19}H_{19}N_5O_6S_2 \cdot 1.5H_2O$	C, H, N

a H: calcd, 4.87; found, 4.36. b N: calcd, 16.40; found, 15.74. c N: calcd, 15.97; found, 15.27.
d N: calcd, 15.84; found, 15.18. e H: calcd, 4.77; found, 4.18. f N: calcd, 13.22; found, 12.74.
g N: calcd, 14.33; found, 13.80.
* Reference 6.

R-configurations were used because earlier investigations[12] in the cephalosporin field have demonstrated that 7-phenylglycyl cephalosporins in which the 7-acyl group configuration is *R* are more active biologically than the corresponding derivatives having the *S*-configuration. The procedures for making the *R*-Boc-phenylglycines have been described previously.[6,7] Since the mixed anhydride method described here is essentially identical to that reported previously by SPENCER *et al.*[12] and by RYAN *et al.*[6] who have shown by a MOORE-STEIN analytical technique that racemization does not take place under the reaction conditions used, the cephalosporins prepared by this procedure are assumed to have side chains in which the optical integrity has been retained. The same assumption applies to the cephalosporins prepared from the nucleus *t*-butyl esters and the Boc-phenylglycines using DCC. This is based on nmr examination of the 7-aminodeacetoxycephalosporanic acid (7-ADCA) derivatives **1**, **1a** and **1b** which show only a singlet for the 3-methyl group. If racemization had taken place two narrowly separated singlets would have been observed in agreement with studies reported by RYAN *et al.* and repeated in our own laboratories. For the three *o*-hydroxyphenylglycyl cephalosporins where synthesis through the racemic lactone leads to a 1:1 mixture of diastereomers the 7-ADCA derivative **1c** shows two distinct singlets for the 3-methyl group, separated by about 5Hz, thus indicating that a roughly 1:1 mixture of diastereomers was isolated. By analogy the *o*-hydroxyphenylglycyl derivatives **2c** and **10c** are assumed to be approximately 1:1 mixtures of diastereomers although a direct indication of this is not provided by their nmr spectra.

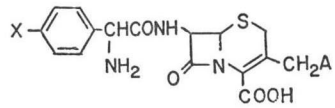
The lactone used for the synthesis of the *o*-hydroxyphenylglycyl cephalosporins was obtained by a novel sequence of reactions.[13] The reaction between phenol, glyoxylic acid hydrate and *t*-butyl carbamate results in a mixture of Boc-protected *o*- and *p*-hydroxyphenylglycines, with the *p*-hydroxy derivative being the major product. The *p*-hydroxy compound crystallized readily from the organic extract leaving the mother liquors enriched in the *o*-hydroxy derivative. Treatment of these mother liquors with DCC converted the Boc-protected *o*-hydroxyphenylglycine to its lactone which was isolated and used directly in acylation reactions as described above.

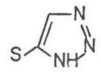
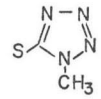
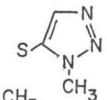
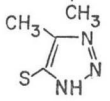
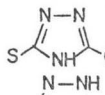
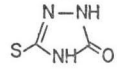
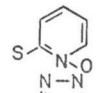
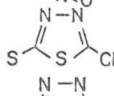
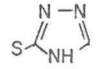
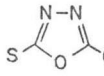
The heterocyclic thiols used in these studies were synthesized by methods described in the literature. However, for the synthesis of 1,2,3-triazole-4(5)-thiol an alternative route was developed which proved to be more practical for obtaining larger scale laboratory supplies. The key reaction in this sequence was the thiolation of 1-benzyl-1H-1, 2, 3-triazole *via* its lithio derivative to give a good yield of 1-benzyle-1H-1,2,3-triazole-5-thiol. The same procedure was used to make 1-methyl-1H-1,2,3-triazole-5-thiol. 1-Benzyl 1H-1,2,3-triazole-5-thiol was converted to the sodium salt of 1, 2, 3-triazole-4(5)-thiol by cleavage of the benzyl group with sodium in liquid ammonia.

Biological Activities

Tables 2 and 3 list minimum inhibitory concentration (MIC) values for candidate cephalosporins against one gram-positive and five gram-negative bacteria. The data presented are representative of those obtained from a larger group of four gram-positive and eleven gram-negative organisms used in our initial screen for evaluating *beta*-lactam antibiotics. In Table

Table 2. Antibacterial activities of phenylglycyl and *p*-hydroxyphenylglycyl cephalosporins

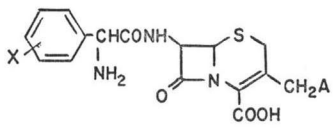


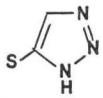
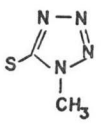
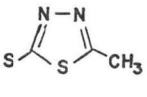
Compounds	Organism	Minimum inhibitory concentrations (mcg/ml)											
		S.a.		E.c.		*K.p.*		*Sal.p.*		*Sh.p.*		*E.a.*	
	X=	H	OH	H	OH	H	OH	H	OH	H	OH	H	OH
	A=												
1, 1a	H	3.1	3.1	6.3	13	6.3	25	6.3	13	6.3	13	13	25
2, 2a	OAc	6.3	6.3	1.6	13	3.1	6.3	1.6	6.3	1.6	3.1	3.1	6.3
3, 3a		1.6	1.6	3.1	1.6	1.6	1.6	0.8	1.6	1.6	1.6	3.1	1.6
4, 4a		1.6	3.1	1.6	1.6	0.8	1.6	0.4	0.8	0.8	1.6	1.6	3.1
5, 5a		1.6	1.6	6.3	3.1	6.3	6.3	1.6	1.6	3.1	3.1	6.3	6.3
6, 6a		1.6	6.3	6.3	3.1	6.3	6.3	3.1	3.1	1.6	1.6	1.6	6.3
7, 7a		3.1	6.3	6.3	3.1	3.1	3.1	1.6	1.6	3.1	3.1	25	6.3
8, 8a		1.6	6.3	6.3	6.3	6.3	6.3	3.1	6.3	3.1	6.3	6.3	25
9, 9a		3.1	3.1	13	13	6.3	6.3	6.3	6.3	3.1	3.1	13	13
10, 10a		3.1	3.1	6.3	6.3	3.1	6.3	3.1	6.3	3.1	3.1	6.3	25
11, 11a		3.1	3.1	13	6.3	6.3	6.3	3.1	6.3	3.1	3.1	13	13
12, 12a		3.1	6.3	13	25	6.3	13	3.1	6.3	3.1	6.3	13	25

The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MIC) in mcg/ml. The MIC's were determined by the twofold agar dilution method using TSA buffered to pH 6 by McIlvaine's citric acid-phosphate buffer. Organisms selected for inclusion in this table are *S. a. Staphylococcus aureus* HH 127 (penicillin G resistant); *E.c., Escherichia coli* 12140; *K.p., Klebsiella pneumoniae* 4200; *Sal. p., Salmonella paratyphi* ATCC 12176; *Sh. p., Shigella paradysenteriae* HH 117; *E.a., Enterobacter aerogenes* ATCC 13048. None of the compounds demonstrated significant activity against *Pseudomonas aeruginosa*.

2, MIC values are given for twenty-four cephalosporins; ten 3-heterocyclicthiomethyl derivatives and two standards (cephalexin and cephaloglycin), all with the unsubstituted phenylglycyl side chain (X=H) at the 7-position, are compared side by side with their twelve analogs in which the 7-phenylglycyl group is hydroxylated at the *para*-position (X=OH). These data

Table 3. Antibacterial activities of phenylglycyl and *ortho*-, *meta*- and *para*-hydroxyphenylglycyl cephalosporins.



Compounds	X	A	Minimum inhibitory concentrations (mcg/ml)					
			S. a.	*E. c.*	*K. p.*	*Sal. p.*	*Sh. p.*	*E.a.*
1	H	H	3.1	6.3	6.3	6.3	6.3	13
1a	*p*-OH		3.1	13	25	13	13	25
1b	*m*-OH		6.3	6.3	13	13	6.3	13
1c	*o*-OH(*R*,*S*)		25	200	100	>200	100	>200
2	H	OAc	6.3	1.6	3.1	1.6	1.6	3.1
2a	*p*-OH		6.3	13	6.3	6.3	3.1	6.3
2b	*m*-OH		3.1	3.1	1.6	1.6	3.1	3.1
2c	*o*-OH(*R*,*S*)		13	100	100	50	100	200
3	H		1.6	3.1	1.6	0.8	1.6	3.1
3a	*p*-OH		1.6	1.6	1.6	1.6	1.6	1.6
3b	*m*-OH		3.1	1.6	1.6	1.6	0.8	1.6
4	H		1.6	1.6	0.8	0.4	0.8	1.6
4a	*p*-OH		3.1	1.6	1.6	0.8	1.6	3.1
4b	*m*-OH		3.1	1.6	1.6	0.8	0.8	3.1
10	H		3.1	6.3	3.1	3.1	3.1	6.3
10a	*p*-OH		3.1	6.3	6.3	6.3	3.1	25
10b	*m*-OH		3.1	6.3	3.1	6.3	3.1	13
10c	*o*-OH(*R*,*S*)		100	>200	200	>200	200	>200

See footnote Table 2.

allow examination of the effects of 3-substituent variation, as well as the effects of *para*-hydroxylation of the phenyl group. Table 3 shows the relative influence of hydroxylation at the *ortho*-, *meta*- and *para*-positions of 7-phenylglycyl cephalosporins with a methyl, acetoxymethyl or heterocyclicthiomethyl substituent at the 3-position.

The effect of 3-substituent variation on *in vitro* activity can be seen by comparing vertically the MIC values listed in Table 2. In contrast to cephalosporins having other acyl groupings at the 7-position,[1,2] incorporation of the heterocyclicthiomethyl moiety into the phenylglycyl cephalosporin structure does not improve *in vitro* activity over that of the 3-methyl and 3-acetoxymethyl analogs with any degree of consistency. Eleven of the heterocyclicthiomethyl analogs (**3**, **3a**, **4**, **4a**, **5**, **5a**, **6**, **6a**, **7a**, **8**, **10**) have MIC values that *in toto* are lower than those of cephalexin (**1**). However, the differences in individual MIC values are slight except for four of the analogs (**3**, **3a**, **4**, **4a**). The remaining 3-heterocyclicthiomethyl analogs are about as active as cephalexin against this group of organisms, or are slightly less active. Most of the analogs that are more active than cephalexin overall have either a tetrazole or a 1,2,3-triazole moiety in the 3-substituent. The potentiating effect of the 1,2,3-triazolethio-

methyl group appears to be largely confined to the 7-phenylglycyl cephalosporins. This contrasts with our experience in placing this substituent on cephalosporins having other 7-acyl side chains.[1]

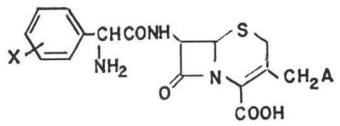
The effect of placing a hydroxyl group on the phenylglycyl side chain on *in vitro* activity is illustrated in both Tables 2 and 3. Analogously to the influence of phenyl substitution on the activities of 7-mandelamido cephalosporins,[3] hydroxylation at the *ortho*-position of the 7-phenylglycyl side chain results in a dramatic loss in *in vitro* activity.* However, hydroxylation at the *meta*- and *para*- positions produces little or no change in the *in vitro* activity. Thus, the *meta*- and *para*-hydroxylated analogs derived from 7-ADCA (A=H, Table 3) are only slightly less active than cephalexin. With the 7-ACA derived analogs (A=OAc, Table 3) the *meta*-derivative is essentially equal in activity to cephaloglycin and the *para*-derivative is slightly less active. The *meta*- and *para*-hydroxylated analogs having a heterocyclicthiomethyl moiety at the 3-position display activities equivalent to, or better than, those of the corresponding unhydroxylated parent compounds. These observations are supported further by the data in Table 2 which compare *para*-hydroxylated derivatives with their unsubstituted analogs. For the 3-heterocyclicthiomethyl analog pairs in this group the MIC values are within one twofold dilution (the variability limit of the test system) for each pair (X=H *versus* X=OH), with only six exceptions out of the 60 data pairs, where the variation is fourfold. Thus, while hydroxylation at the *ortho*-position of 7-phenylglycyl cephalosporins lowers the *in vitro* activity significantly, this substitution at the *meta*- and *para*-positions has little effect on the MIC values.

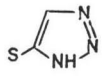
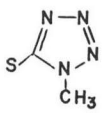
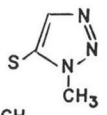
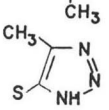
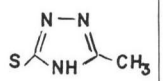
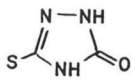
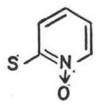
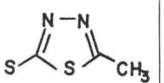
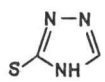
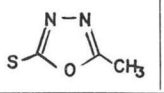
The influence of *meta*- and *para*-hydroxylation of the side chain and of 3-substituent variation on the protective effectiveness of the 7-phenylglycyl cephalosporins in mice is illustrated in Table 4. The *ortho*-hydroxy analogs were not included in these studies because of their poor *in vitro* activities. The PD_{50} values were obtained using mice challenged with *Escherichia coli* 12140, the same strain for which MIC values are given in Tables 2 and 3. The cephalosporins were administered subcutaneously or orally as indicated. Placing 3-heterocyclicthiomethyl groupings on the unhydroxylated 7-phenylglycyl cephalosporin structure results in subcutaneous PD_{50} values (Data Column 1, X=H) which tend to be slightly better than would be predicted from the PD_{50} value of cephalexin when the *E. coli* MIC values of these analogs are compared with that of cephalexin (*i.e.*, the ratio of PD_{50}/MIC for cephalexin is about three; the ratios for most of the 3-heterocyclicthiomethyl analogs are near to one; exceptions are compounds **5** and **6**).

However, in contrast to cephalexin, the 3-heterocyclicthiomethyl analogs are generally less protective when given orally than when given subcutaneously. The lower effectiveness of these cephalosporins by the oral route is readily seen by comparing the oral PD_{50} values of the individual 3-heterocyclicthiomethyl analogs (Data Column 4, Table 4) with the corresponding subcutaneous values (Data Column 1). With three exceptions (compounds **3** and **6**, both

* All phenylglycyl analogs tested had the 7-side chain in the ***R***-configuration except the *ortho*-hydroxy derivatives where the side chain was racemic (***R***, ***S***). However, compensation for an approximately 50% content of the presumed-less-active isomer having the ***S***-configuration on the side chain would not bring the activity of the presumed-more-active isomer (side chain with the ***R***-configuration) within the range of the activities of the other analogs.

Table 4. Protective activities of phenylglycyl and of *meta-* and *para*-hydroxyphenylglycyl cephalosporins.



Compounds	A	Mouse PD_{50} (mg/kg) *versus* *E. coli* 12140					
		Subcutaneous			Oral		
		X=H	X=*p*-OH	X=*m*-OH	X=H	X=*p*-OH	X=*m*-OH
1, 1a, b	H	18	32	16	20	25	19
2, 2a, b	OAc	3.8	3.5	5.5	18	18	34
3, 3a, b		3.6	0.5	1.1	4.0	0.5	3.7
4, 4a, b		1.4	0.7	1.3	10	1.4	9.5
5, 5a		35	5.2		>50	6.2	
6, 6a		18	1.8		19	3.0	
7, 7a		7.0	11		50	7.5	
8, 8a		6.3	4.0		34	9.5	
9, 9a		9.4	6.2		>50	50	
10, 10a, b		4.6	2.3	3.5	11	2.3	18
11, 11a		10	7.2		32	10	
12, 12a		22	3.0		18	3.0	

The PD_{50} values are expressed as the total dose of compound in mg/kg required to protect 50 % of the mice challenged intraperitoneally with *E coli* 12140. The compounds were administered subcutaneously or orally in equally divided portions at 1 and 5 hours postinfection. Values were calculated by the method of LITCHFIELD and WILCOXON (J. Pharmacol. Exp. Ther. 96: 99 ~113, 1949).

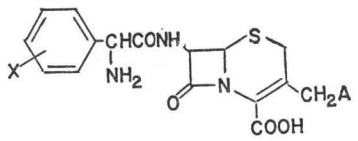
incorporating 1,2,3-triazoles without substituent on the nitrogen and compound **12**), the oral PD_{50} values are higher than the corresponding values obtained by injecting the cephalosporin.

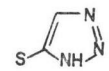
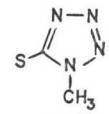
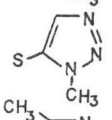
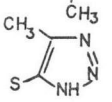
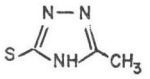
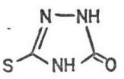
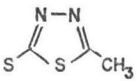
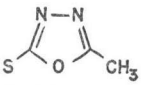
Comparison of the PD_{50} values for the hydroxylated analogs with those for the corresponding unhydroxylated derivatives provides an indication that although this structural change does not strongly influence *in vitro* activity, it can result in an improvement in the chemotherapeutic characteristics of this compound type. The data in Table 4 (Data Columns 4, 5 and 6) teach that hydroxylation of the phenylglycine side chain at the *para*-, but not the *meta*-position, consistently improves the oral infection-protection activity of the cephalosporins when the 3-substituent is a heterocyclicthiomethyl group, but not when the substituent is methyl or acetoxymethyl. Thus, hydroxylation of the phenyl group of cephalexin (**1**) and cephaloglycin (**2**), whether at the *meta*- or *para*-positions, results in analogs with activities in the mouse infection-protection test which are essentially equal to, or less than, those of the parent compounds. *meta*-Hydroxylation of those 3-heterocyclicthiomethyl cephalosporins tested results in a negligible change in the oral PD_{50} values. On the other hand, *para*-hydroxylation results in improvement of the oral PD_{50} values for all of the 3-heterocyclicthiomethyl cephalosporins examined, in contrast with the 7-ACA and 7-ADCA derived analogs. The effect of hydroxylation on the subcutaneous effectiveness is more variable (Data Columns 1, 2 and 3, Table 4).

As expected, the mouse protective effectiveness of these cephalosporins can be related in large part to their serum levels and their MIC values against *E. coli* 12140. The comparatively poor oral *versus* subcutaneous PD_{50} values for the unhydroxylated 3-heterocyclicthiomethyl analogs (Table 4; X=H) can be largely explained in terms of the serum levels obtained on oral and subcutaneous administration (Tables 5 and 6). The oral serum level values for the three 7-*meta*-hydroxyphenylglycyl-3-heterocyclicthiomethyl analogs (X=*m*-OH) reported in Table 6 are in the same range as those of their respective unhydroxylated parents (X=H). This is in agreement with the fact that the oral PD_{50} values (Table 4) are not improved by hydroxylation at the *meta*-position. However, again in general agreement with the trend in PD_{50} values, *para*-hydroxylation of the 7-phenylglycyl-3-heterocyclicthiomethyl cephalosporins consistently improves the serum levels attained, both subcutaneously and orally. The extent of increase tends to correlate inversely with the serum level of the unhydroxylated analog. Thus, the magnitudes of the increases (0.5 to twofold) for the subcutaneously administered doses, with serum peaks ranging from 11 to 35 mcg/ml for the unhydroxylated parent analog, are more modest than those (1.5 to >1,000-fold) observed with the orally administered doses, where peak serum levels range from <0.5 to 11 mcg/ml for the unhydroxylated cephalosporins.

Of the cephalosporins described in this study, six (**3**, **3a-b**, **4**, **4a-b**) emerge as tentatively worthy of further consideration based on the criteria for selection discussed earlier. The three analogs with the 1-methyltetrazolethiomethyl substituent at the 3-position (**4**, **4a-b**) display the best *in vitro* activities of the group. When administered subcutaneously all three produce mouse serum levels in the range of those seen with cephalexin, and the PD_{50} values imply a protective effectiveness significantly better than that of cephalexin. However, the serum levels attained on oral administration of the unsubstituted and the *meta*-hydroxy analogs (**4**, **4b**) are very low and this is reflected in the oral PD_{50} values. The oral serum levels of the *para*-hydroxy analog (**4a**) in this series are impressively higher than those of **4** and **4b**; the peak

Table 5. Serum levels in mice after subcutaneous administration of phenylglycyl and of *meta*- and *para*-hydroxyphenylglycyl cephalosporins.



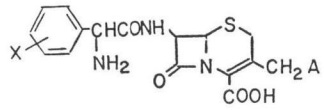
Compounds	A	Subcutaneous mouse serum levels (mcg/ml)*											
		X=H				X=*p*-OH				X=*m*-OH			
		(5)15 min.	30 min.	60 min.	120 min.	15 min.	30 min.	60 min.	120 min.	15 min.	30 min.	60 min.	120 min.
1, 1a, b	H	34	23	9.4	2.3	28	18	7.5	2.0	34	27	11	2.6
2, 2a, b	OAc	(23)	6.6	1.1	<0.5	19	17	5.8	1.1	23	14	4.3	<0.5
3, 3a, b		35	22	12	3.9	54	49	36	20	41	37	17	7.8
4, 4a, b		24	12	2.6	<0.5	38	33	17	8.6	42	30	5.8	1.0
5, 5a		21	12	2.2	<0.5	44	34	22	12				
6, 6a		25	17	5.5	1.2	47	36	24	11				
7, 7a		28	14	3.2	<0.5	47	34	14	3.7				
8, 8a		30	21	5.3	6.6	44	34	18	7.6				
9, 9a		11	11	5.5	0.5								
10, 10a, b		28	18	5.0	1.0	49	37	16	6.9	29	16	1.8	<1.0
11, 11a		12	4.3	1.0	<0.5	38	26	13	2.7				
12, 12a		21	15	5.5	1.0	38	29	14	4.2				

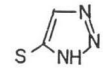
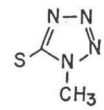
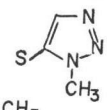
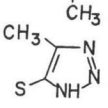
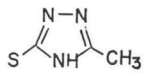
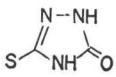
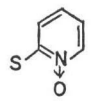
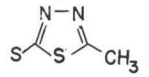
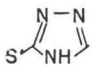
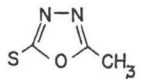
* Serum levels were determined as described by ACTOR *et al.* in reference 5.

serum level of **4a** is half of cephalexin's and the apparent half-life is longer, resulting in a 120-minute serum level that is higher than that obtained with cephalexin (5.4 mcg/ml *versus* 2.5 mcg/ml).

The enhancement of performance in these biological test systems achieved by the methyltetrazolethiomethyl grouping is carried a step further by the 1,2,3-triazolethiomethyl substituent.

Table 6. Serum levels in mice after oral administration of phenylglycyl and of *meta*- and *para*-hydroxyphenylglycyl cephalosporins.



Compounds	A	Oral mouse serum levels (mcg/ml)*											
		X=H				X=*p*-OH				X=*m*-OH			
		15 min.	30 min.	60 min.	120 min.	15 min.	30 min.	60 min.	120 min.	15 min.	30 min.	60 min.	120 min.
1, 1a,b	H	24	18	7.6	2.5	28	18	6.5	<1.0	24	21	9.0	3.1
2, 2a,b	OAc	1.3	2.7	0.6	<1.0	6.4	5.2	2.6	1.1	1.5	1.9	0.8	<1.0
3, 3a,b		11	13	7.8	3.8	44	41	26	15	5.6	7.7	6.6	5.0
4, 4a,b		0.7	0.5	<0.5	<0.5	7.6	12	9.2	5.4	0.8	0.9	0.6	<0.5
5, 5a		1.4	1.8	<0.5	<0.5	12	14	9.5	4.0				
6, 6a		1.0	2.4	2.2	<0.5	19	21	—	9.5				
7, 7a		1.2	1.6	0.9	<0.5	6.2	11	8.2	3.6				
8, 8a		1.4	2.0	0.7	<0.5	4.1	6.9	6.1	3.2				
9, 9a		<0.5	<0.5	<0.5	<0.5	<1.0	<1.0	<1.0	<1.0				
10, 10a,b		7.0	5.0	3.0	0.8	24	23	17	7.3	5.2	3.8	1.8	<0.5
11, 11a		<0.5	<0.5	<0.5	<0.5	16	15	7.2	2.6				
12, 12a		11	8.0	5.0	1.2	28	19	9.9	3.2				

* See footnote Table 5.

Thus, while compound **3** itself displays a promising profile of biological activity, its *para*-hydroxy analog **3a** combines *in vitro* activities that approach those of the methyltetrazolethiomethyl derivatives with high protective effectiveness (subcutaneous and oral PD_{50} values of 0.5 mg/kg in both cases). Its mouse serum levels, both orally and subcutaneously, are significantly higher and more prolonged than any of the other cephalosporins described in this study, including cephalexin and cephaloglycin. These data make compound **3a** (SK&F 60771,

cefatrizine) an easy choice for additional studies on its suitability as a candidate for use in humans.[5]

Experimental Section

Infrared spectra were obtained in Nujol mull using a Perkin-Elmer Infracord. Nmr spectra were obtained (unless indicated otherwise) in DMSO-d_6 or DMSO-d_6-D_2O on a Varian T-60 spectrometer using TMS as internal standard. The cephalosporins melt with extensive decomposition. Therefore, their melting points are not reported since they are not accurately reproducible. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within $\pm 0.4\%$ of theoretical values. $MgSO_4$ was used as drying agent for organic extracts.

7-Amino-3-heterocyclicthiomethyl-3-cephem-4-carboxylic acids

To a suspension of 0.1 mol of 7-ACA in 200 ml of water and 100 ml of acetone was added 0.225 mol of $NaHCO_3$ in 200 ml of water, and the resultant solution was heated to 40~50°C. The appropriate thiol (0.12~0.15 mol) in 200 ml of acetone was added and the solution was stirred under reflux. The pH was maintained at 7.4~7.8 by the addition of 5% $NaHCO_3$ or 3 N HCl if necessary. Periodically a solid sample was isolated by adjusting the pH of a small aliquot of the reaction mixture to 3.5. The reaction was judged to be complete when the ir spectrum of the solid showed no acetoxy remaining (~4 hour). When the reaction was complete the solution was cooled in an ice-bath and acidified to pH 3.5. The resulting precipitate was collected, washed with water, then with acetone and dried. The crude product was purified by suspending it in water and adding 6 N HCl until solution was effected. The acidic solution (Norit) was held at room temperature for 30 minutes to 5 hour depending on the presence of residual 7-ACA (nmr) in the crude product. After filtering through Celite the filtrate was cooled and adjusted to pH 3.5 with dilute NaOH. The precipitate was collected, washed and dried as before. These intermediates were used without further purification.

7-Phenylglycyl Cephalosporins. Procedure A

Isobutyl chloroformate (0.01 mol) was added to a cold (−10°C) solution of the Boc-protected phenylglycine (0.01 mol) in 40 ml of dry THF containing triethylamine (0.01 mol) and the mixture was stirred at −10°C for 45 minutes. To this mixture was added during 15 minutes a solution of the 7-amino-3-cephem-4-carboxylic acid (0.01 mol), which had been dissolved in 40 ml of 50% aqueous THF by adding triethylamine (0.013 mol). After stirring for one hour at −10°C and two hours without external cooling the THF was evaporated *in vacuo*. The aqueous residue was diluted with 60 ml of water and extracted with EtOAc (discarded). The aqueous phase was cooled in an ice-bath, layered with EtOAc and adjusted to pH 2.5~3.0. After separating the phases the aqueous layer was extracted twice more with EtOAc and the combined EtOAc extracts were dried and evaporated *in vacuo*. The crude Boc-protected products were purified either by reprecipitation from various solvents or by column chromatography on silica gel. Elutions were carried out, for example, with benzene - acetone (80:20) or $CHCl_3$ - MeOH - HCOOH (90:10:3). Purification was monitored by tlc (silica gel, 90:10:3, $CHCl_3$ - MeOH - HCOOH) and when the samples were sufficiently free of by-products their ir and nmr spectra were recorded.

The Boc-protecting group was removed by dissolving the Boc-derivative in cold TFA or 9:1 TFA-anisole (1 g of compound/10 ml of solvent in each case) and stirring the mixture at ice-bath temperature for 30~60 minutes. The solvent was then evaporated *in vacuo*, the residue was triturated with Et_2O and the solid TFA salt was collected. Two different methods were used to convert the salt to its zwitterionic form. In the favored method the TFA salt was dissolved in H_2O (1 g/50~100 ml), the solution was filtered to remove suspended solid and then the pH was adjusted to 5~5.6 by portionwise addition of Amberlite IR-45 ion-exchange

resin. The mixture was filtered to remove the resin and the filtrate was lyophilized to give the zwitterionic product. Alternatively, the TFA salt of the product was dissolved in H_2O (1 g/5 ml), the solution was filtered to remove suspended solid, layered with MIBK (3 ml) and cooled in an ice-bath. The pH was adjusted to 4.0 by dropwise addition of tri-*n*-butylamine and the solid was collected, washed thoroughly with MIBK, then with Et_2O or other organic solvents as necessary, and dried under vacuum.

Procedure B

A solution containing the Boc-protected cephaloglycin or its *p*-hydroxy analog (0.01 mol), thiol (0.015 mol) and $NaHCO_3$ (0.025 mol) in 75 ml of pH 6.4 phosphate buffer was heated either at 56° for 14~17 hours or at 70° for 2~4 hours. When the reaction was complete (tlc), the solution was cooled in an ice-bath, layered with EtOAc and adjusted to pH 2.5~3. Subsequent work-up and conversion to the final cephalosporins were carried out by the methods described in procedure A.

Procedure C

The appropriate Boc-protected hydroxyphenylglycine (5 mmol) was added to a solution of the appropriate *t*-butyl 7-amino-3-cephem-4-carboxylate (5 mmol) in 50 ml of dry THF containing DCC (5.5 mmol). After stirring at room temperature for 2 hours the mixture was filtered to remove DCU and the filtrate was evaporated *in vacuo* to yield the crude diprotected intermediate as a non-crystalline foam. This material was dissolved in EtOAc and extracted successively with 1N HCl, 5 % $NaHCO_3$ and finally H_2O. The solvent was evaporated *in vacuo* and the solid residue was purified further by precipitation from Et_2O-petroleum ether.

The protecting groups were removed from this intermediate by dissolving it in ice-cold TFA (1 g/10 ml) and stirring the mixture at room temperature for 1 hour. The TFA was evaporated *in vacuo* and the residue was triturated with Et_2O to give the solid TFA salt of the product. This was converted to the zwitterion by the method described in procedure A.

In one case (compound **12a**) the *t*-butyl groups were cleaved by dissolving the blocked intermediate in glacial AcOH saturated with HCl and stirring the mixture for 25 minutes at room temperature. The mixture, from which a solid had precipitated, was poured into Et_2O and stirred for 1.5 hours. The solid HCl salt was collected, dried and converted to its zwitterionic form as described in procedure A.

Procedure D

A solution of lactone (3.5 mmol) and *t*-butyl 7-amino-3-cephem-4-carboxylate (3.5 mmol) in 50 ml of xylene was refluxed for 1 hour under N_2. The solvent was evaporated *in vacuo*, the residue was dissolved in Et_2O and the solution was washed successively with 5 % $NaHCO_3$, dilute HCl, and saturated NaCl solution. The Et_2O was evaporated *in vacuo* and the residue was purified by chromatography on silica gel. The structures of these diprotected intermediates were supported by their ir and nmr spectra. Yields ranged from 40 to 60 %.

The protecting groups were cleaved with TFA as described under procedure C and the products were characterized as their TFA salts.

1-Benzyl-1H-1,2,3-triazole-5-thiol Lithium Salt Sesquihydrate (**13**)

A solution of *n*-butyllithium (30 ml, 0.07 mol) in hexane was added dropwise under N_2 to a stirred solution of 1-benzyl-1H-1,2,3-triazole[14] (9.5 g, 0.06 mol) in 120 ml of dry THF which had been cooled to −60°C. When the addition was complete the deep orange solution was stirred for another 15 minutes and then sublimed sulfur (1.95 g, 0.06 mol) was added in one portion. After stirring for 15 minutes at −60°C and 30 minutes at −40°C the dark mixture was poured into 800 ml of Et_2O. The mixture was stirred for 30 minutes and the Et_2O was decanted and replaced with fresh Et_2O. After washing 3 more times with Et_2O the solid was triturated with EtOAc, collected and dried to give 11.2 g (87 %) of the lithium salt.

Anal. ($C_9H_8N_3SLi \cdot 1.5H_2O$) Calcd: C, 48.21 H, 5.94 N, 18.74
Found: C, 48.26 H, 6.06 N, 18.94

1H-1,2,3-Triazole-4(5)-thiol Sodium Salt (**24**)

The lithium salt of 1-benzyl-1H-1,2,3-triazole-5-thiol (20 g, 0.102 mol) was dissolved in 500 ml of liquid NH_3 and small pieces of sodium (6 g, 0.26 mol) were added gradually (*ca.* 1 hour) until a permanent blue color persisted for 5 minutes. The NH_3 was allowed to evaporate and the white solid which remained was dissolved in 250 ml of H_2O. The solution was adjusted to pH 10~11 with concentrated HCl and extracted with Et_2O (3×50 ml). The separated aqueous phase was acidified to pH 2.5 with concentrated HCl, extracted with EtOAc and the combined dried extracts were treated dropwise with 75 ml of sodium 2-ethylhexanoate solution (30 % in isopropyl alcohol). The precipitated white solid was collected, washed with EtOAc then with Et_2O, and dried at 60°C to give 9.5 g (76 %) of product.

Anal. ($C_2H_2NaN_3S$) Calcd: C, 17.58 H, 2.58 N, 30.75
Found: C, 18.06 H, 3.03 N, 30.49

N-(*tert*-Butoxycarbonyl)-2-(2-hydroxyphenyl)glycine γ-Lactone (**15**)

A mixture of glyoxylic acid hydrate (10.1 g, 0.11 mol), *tert*-butyl carbamate (11.7 g, 0.10 mol) and phenol (18.8 g, 0.20 mol) was warmed gently to form a homogeneous melt. After standing for 3 days at room temperature the reaction mixture was adjusted to pH 7.0 by addition of 5 % aqueous trisodium phosphate solution and extracted with EtOAc. The aqueous phase was adjusted to pH 4.0 with 85 % H_3PO_4 and extracted with EtOAc. The pH of the aqueous phase was readjusted to 4.0 after each extraction, if necessary. (Extraction much below pH 4.0 also extracts a polar contaminant). The combined, dried pH 4.0 EtOAc extracts were evaporated *in vacuo*, the residue was dissolved in CH_2Cl_2 and hexane was added to incipient turbidity. Addition of 1.8 ml of water with vigorous stirring caused crystallization of N-(*tert*-butoxycarbonyl)-2-(4-hydroxyphenyl)glycine monohydrate (13.4 g, 50 %), mp 112~114°C dec. The filtrate from this crystallization was evaporated *in vacuo*. The residue (5.8 g) was dissolved in CH_2Cl_2 (50 ml) and added dropwise over 30 minutes to a stirred solution of DCC (3.2 g) in CH_2Cl_2 (150 ml). After standing overnight the precipitated DCU was removed and the filtrate was evaporated *in vacuo*. The residue was dissolved in Et_2O and this solution was washed with 5 % $NaHCO_3$, dried and evaporated. The solid was crystallized from EtOAc-hexane to give 2.3 g (9 %) of the lactone, mp 153~154°C.

Anal. ($C_{13}H_{15}NO_4$) Calcd: C, 62.64 H, 6.07 N, 5.62
Found: C, 62.39 H, 6.21 N, 5.52

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