Semisynthetic Cephalosporins. Synthesis and Structure–Activity Relationships of 7-Mandelamido-3-cephem-4-carboxylic Acids

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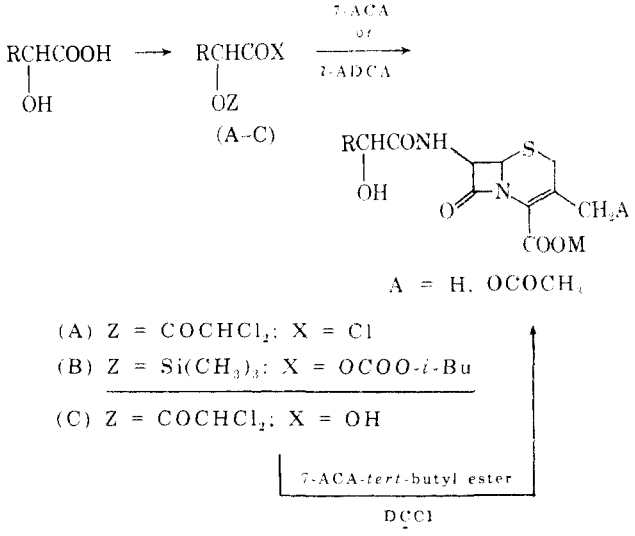
The synthesis, microbiological profile, and *in vivo* effectiveness in laboratory animals of a broad-spectrum cephalosporin, 7(*R*)-mandelamidocephalosporanic acid (**1**), are described. A number of derivatives and analogs of **1** were prepared and their structure–activity relationships are discussed.

Most of the several cephalosporins that have been found to be clinically useful incorporate at the 7 position an acyl moiety derived from acetic acid, monosubstituted with an appropriate grouping such as thiophene, tetrazole, pyridylthio, or cyano. Structure–activity studies on cephalosporins teach that homologation or substitution of the acetic acid by a second grouping is generally undesirable from the standpoint of level and breadth of biological activity. The most notable exception to this principle is the (*R*)-phenylglycine side chain which has found great utility, first on the penicillin structure (ampicillin) and later on the cephalosporins (cephaloglycin and cephalexin).

In early studies on the cephalosporins we reinforced this principle by synthesizing and testing the cephalosporin analog (compound **13**)[1] of 3,4-dichloro-α-methoxybenzylpenicillin, an antibiotic developed in Europe for treating staphylococcal infections.[2] While active against gram-positive organisms *in vitro* (Table V) this cephalosporin had poor activity against various gram-negative bacteria. However, the parent structure of this series of compounds, *viz.*, 7(*R*)-mandelamidocephalosporanic acid[1] (compound **1**), was found to possess significant broad-spectrum antimicrobial activity *in vitro* and *in vivo*. The antimicrobial profile of this cephalosporin and structure–activity relationships for a large number of related analogs are presented here.

Chemistry. The cephalosporins were prepared by acylating 7-aminocephalosporanic acid (7-ACA) or 7-aminodesacetoxycephalosporanic acid (7-ADCA) using one of the three general procedures listed in the Experimental Section (Scheme I). In each case the α-hydroxyl group of

Scheme I



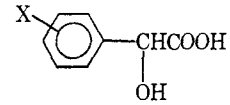
the side-chain acid to be attached was protected prior to the coupling. The dichloroacetyl group was used most frequently for this (procedures A and C). Stable to mild acid conditions, it was readily removed after completion of the acylation by controlled hydrolysis at pH 9–9.5. For acids which were unstable under the conditions of procedure A, or which contained a complicating functional group, the hydroxyl group was protected using the trimethylsilyl ether which was readily formed by treating the salt of the hydroxy acid with trimethylsilyl acetamide. In this case the carboxyl group was activated by conversion to the mixed carbonic-carboxylic acid anhydride using isobutyl chloroformate. The trimethylsilyl group was readily removed by brief exposure to dilute aqueous acid. With the dichloroacetyl-protected acids the acylation was accomplished by conversion to the acid chloride or by coupling the free acid with the *tert*-butyl ester[3] of the nucleus using DCCI. In the latter case the protective groups were subsequently removed by treatment with neat TFA followed by aqueous Na_2CO_3. The cephalosporins with an amino group on the benzene ring were obtained by catalytic hydrogenation of the fully blocked nitro-substituted cephalosporins which had been prepared by procedure C, with subsequent deblocking as already indicated.

In most cases the final cephalosporins were characterized as their sodium, cyclohexylamine, or triethylamine salts. The ir and nmr data of all compounds were consistent with structure. Each cephalosporin was checked for retention of the optical integrity of the side chain after the acylation and deblocking. This was done using the N–H absorption of the side-chain amide (δ 8.15–9.00) from the nmr spectrum of the compound. We observed that when the nmr spectra (DMSO-d_6) of the diastereomers **1** and **2**, differing only in the configuration of the benzylic carbon atom, were compared, the N–H doublet for **1** (*R* isomer) appeared at δ 8.54 while that for **2** (*S* isomer) appeared at δ 8.38. With an artificial 1:1 mixture of **1** and **2**, the N–H absorption appeared as a symmetrical triplet (actually two overlapping doublets) at δ 8.45. Surprisingly, the remaining absorptions in the spectra of **1** and **2** were nearly superimposable, with minor variation in the multiplet absorptions around δ 5–5.2 due to the benzylic hydrogen, H-6, and the 3-position methylene. This symmetrical triplet feature was also present in each of the products obtained by acylating with a racemic side-chain acid (compounds **5–8**, **11**, **29–31**, and **40**), indicating that roughly a 1:1 mixture of diastereomers had been isolated. Using artificial mixtures of the diastereomers, as little as 10% of one isomer could be detected in the presence of the other. By this criterion all of the final cephalosporins prepared from resolved side-chain acids were free of their diastereomers, with the exception of compounds **33**, **44**, and **49**. These proved to be approximately 1:1 mixtures of the diastereomers when made by the acid chloride procedure. Compound **44** was made without racemization of the side chain using the mixed anhydride method (procedure B).

Most of the side-chain acids used in the synthesis of cephalosporins have already been reported in the literature as the racemic mixtures. Those that are new are included in the Experimental Section. Since our data clear-

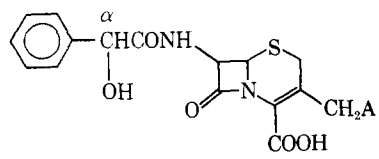
†This article is dedicated to Professor Alfred Burger, a longtime consultant to Smith Kline & French Laboratories, a source of encouragement and advice, a close friend, and a respected colleague.

Table I. (*R*)-(−)-Mandelic Acids



X[a]	Resolving base	$[\alpha]^{25}D$, deg	Mp, °C	Ref
2-F	*b*	−141 (*c* 1, EtOH)	86–88	*c*
3-F	(−)-Ephedrine	−123 (*c* 0.3, EtOH)	123–125	*c*
4-F	(+)-α-Methylbenzylamine	−134 (*c* 0.3, EtOH)	153–156	*c*
2-Cl	(−)-Ephedrine	−158 (*c* 0.3,' EtOH)	119–121	*d*
3-Cl	*b*	−116 (*c* 4, EtOH)	103–105	*c*
4-Cl	(+)-α-Methylbenzylamine	−134 (*c* 0.3, EtOH)	119–121	*c*
4-Br	(−)-Ephedrine	−110 (*c* 1, H_2O)	130–132	*c*
2-NO_2	Brucine	−453 (*c* 1, Me_2CO)	99–101	*e*
3-NO_2	*b*	−120 (*c* 2.2, H_2O)	133–134	*f*
4-NO_2	(+)-α-Methylbenzylamine	−129 (*c* 1, EtOH)	93–95	*g*
3-OCH_3	(+)-α-Methylbenzylamine	−117 (*c* 0.3, EtOH)	78–79	*c, d*
4-OCH_3	(+)-α-Methylbenzylamine	−141 (*c* 0.3, H_2O)	102–104	*c, d*
2-CH_3	*b*	−175 (*c* 2, EtOH)	61–63	*h*
3-CH_3	(+)-α-Methylbenzylamine	−144 (*c* 0.3, EtOH)	109–112	*h*
4-CH_3	(+)-α-Methylbenzylamine	−153 (*c* 0.3, EtOH)	132–134	*i*
3-CF_3	(+)-α-Methylbenzylamine	−102 (*c* 0.3, EtOH)	50–54	*h*
4-CF_3	(−)-Ephedrine	−105 (*c* 0.3, EtOH)	146–150	*h*
4-CH_2NH_2	*b*	−124 (*c* 1.5, 1 *N* HCl)	>200	*h*
2,4-Cl_2	*b*	−141 (*c* 1.5, EtOH)	121–123	*h*
3,4-Cl_2	(+)-α-Methylbenzylamine	−113 (*c* 0.3, H_2O)	117–118	*h*
2,6-Cl_2	(−)-α-Methylbenzylamine	−90 (*c* 0.3, EtOH)	145–147	*h*
3,4-OCH_2O	(−)-Ephedrine	−127 (*c* 0.3, EtOH)	132–135	*d*

[a] All compounds gave satisfactory C and H analyses. [b] These compounds were custom prepared by the Niels Clauson-Kaas Chemical Research Laboratory, Rugmarken 28, Farum, Denmark. [c] O. Korver, *Tetrahedron*, **26,** 5507 (1970). [d] D. G. Neilson, U. Zakii, and C. M. Scrimgeouv, *J. Chem. Soc. C*, 898 (1971). [e] A. McKenzie and P. Stewart, *J. Chem. Soc.*, 104 (1935). [f] A. Fredga and E. Andersson, *Ark. Kemi, Mineral. Geol.*, **14,** No. 18 (1940); *Chem. Abstr.*, **35,** 39939 (1941). [g] A. Fredga and E. Andersson, *ibid.*, **14,** No. 38 (1941); *Chem. Abstr.*, **35,** 69471 (1941). [h] Only the racemic compound has been reported in the literature. [i] S. Fujise and M. Iwakiri, *Biochem. Z.*, **283,** 298 (1936).



1, A = $OCOCH_3$; α = *R*
2, A = $OCOCH_3$; α = *S*
3, A = H; α = *R*
4, A = H; α = *S*

ly indicated that the side chain with the *R* configuration is the one required for maximum broad-spectrum activity, the majority of the mandelic acids were resolved by classical methods and the *R* enantiomer only was used in the subsequent acylation. The data pertaining to the preparation of the resolved (*R*) acids are listed in Table I. Although some of the *R*-mandelic acids listed there were reported previously, an optical rotation value was not always available. The heterocyclic-substituted glycolic acids, *trans*-styrylglycolic acid, and lactic acid were used in the racemic form.

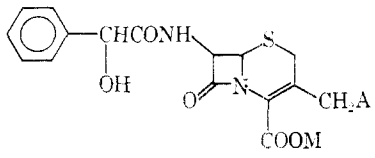
Compounds **52** and **54** were prepared from compound **1** by direct displacement of the 3-acetoxy group using pyridine or 2-mercapto-5-methyl-1,3,4-thiadiazole. Compound **53** was made by acylating 7-amino-3-azidomethyl-3-cephem-4-carboxylic acid[4] using procedure A.

Biological Activities. The broad-spectrum *in vitro* activity of 7(*R*)-mandelamidocephalosporanic acid (**1**) is apparent from its minimum inhibitory concentration (MIC) values against the four gram-positive and eleven gram-negative microorganisms listed in Table II. Against the gram-negative organisms compound **1** is slightly more active *in vitro* than cephalothin and cephaloglycin. This difference has been observed consistently in many additional tests not reported here. The *in vitro* tests were carried out using buffered media. While the buffer had little effect on the MIC's of compound **1** and cephalothin, it was employed because of the inherent instability[5] of cephaloglycin when tested in unbuffered media (*vide infra*). Both **1** and cephalothin are more active than cephaloglycin against the cephalosporin-sensitive gram-positive bacteria; we have frequently observed this when cephalosporins with a neutral side chain are compared with those having an ionizable (amino, carboxyl) substituent on the α position. In all cases cephalexin displays similar but slightly lower *in vitro* activity. None of these cephalosporins have significant activity against the strains of pseudomonas, serratia, and indole-positive proteus in the test group. The same is true for the methicillin-resistant strain of *Staphylococcus aureus* (70390) and for *Streptococcus faecalis*.

Upon intramuscular (im) injection **1** produces serum levels in the dog comparable to those obtained with cephalothin (Table III). In the rabbit the serum levels after im injection of **1** are higher than those produced by an equivalent dose of cephalothin; they are also higher than cephalothin in the mouse (subcutaneously). The *in vitro* activity (Table II) and serum levels (Table III) of **1** are reflected in its ability to protect mice infected with gram-positive (*S. aureus* HH 127) and gram-negative (*Escherichia coli* 12140, *Klebsiella pneumoniae* 4200) bacteria (Table IV). The slight advantage of cephalothin over **1** in protecting mice against *S. aureus* HH 127 is consistent with the slightly better MIC of cephalothin against this penicillin-resistant organism. On the other hand, **1** is more active than cephalothin in protecting mice against the *E. coli* and *K. pneumoniae* organisms in keeping with the comparative MIC and serum level values for the two compounds. The relatively higher serum levels which cephaloridine produces in the mouse[6] are reflected in its ED_{50} values in Table IV.

7(*R*)-Mandelamidocephalosporanic acid and cephaloglycin have similar structures and they display similarities in

Table II. Antibacterial Activities



Microorganism	MIC, μg/ml[a] A = OCOCH₃ R[b] (1)	A = OCOCH₃ S[b] (2)[c]	A = H R[b] (3)	A = H S[b] (4)[c]	Cephalothin	Cephaloglycin	Cephalexin
Staph. aureus (HH 127)	0.8	6.3	6.3	50	0.4	3.1	3.1
Staph. aureus (23390)	0.8	6.3	6.3	50	0.2	3.1	6.3
Staph. aureus (70390)	25	200	200	>200	12.5	25	100
Strept. faecalis (HH 34358)	50	200	200	>200	12.5	50	100
E. coli (12140)	1.6	50	12.5	>200	3.1	3.1	6.3
E. coli (33779)	1.6	200	50	>200	12.5	3.1	12.5
K. pneumoniae (4200)	1.6	200	12.5	>200	3.1	3.1	6.3
K. pneumoniae (1200)	1.6	50	12.5	200	3.1	3.1	6.3
Sal. paratyphi (ATCC 12176)	0.8	50	6.3	200	1.6	1.6	6.3
Shig. paradysenteriae (HH 117)	0.8	25	12.5	200	6.3	3.1	6.3
P. aeruginosa (HH 63)	>200	>200	>200	>200	>200	>200	>200
Ser. marcescens (ATCC 13880)	200	>200	200	>200	>200	200	100
Prot. morganii (179)	200	>200	>200	>200	>200		>200
Ent. aerogenes (ATCC 13048)	12.5	200	25	>200	25	3.1	12.5
Ent. cloacae (HH 31254)	3.1	200	12.5	>200	12.5		6.3

[a]The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MIC's) in μg/ml. The MIC's 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)]. [b]*R* and *S* designate the absolute configuration at the benzylic carbon. [c]Prepared by procedure A using (*S*)-(+)-mandelic acid as starting material. Both compounds gave satisfactory C, H, and N analyses.

Table III. Serum Levels for Compound 1 and Cephalothin in Three Species of Animals

Species	Time, min	Serum level, μg/ml 1	Cephalothin
Rabbit (NZW, 2–2.5 kg), dose, 20 mg/kg im	15	39	31
	30	28	16
	60	14	6
Dog (Beagle, 8–14 kg), dose, 10 mg/kg im	15	18	13[a]
	30	13	13[a]
	60	5	7[a]
Mouse (Swiss–Webster), dose, 20 mg/kg sc	5	25	14
	30	12	6
	60	2.1	0.5

[a]Serum levels from C. Lee, E. B. Herr, Jr., and R. C. Anderson, *Clin. Med.*, **70**, 1123 (1963), using mongrel dogs (10.6–14.7 kg, dose 10 mg/kg im).

Table IV. Protective Activity in Mice

Compound	ED_{50}, mg/kg[a] *E. coli* 12140 sc	*E. coli* 12140 po	*K. pneumoniae* 4200 sc	*K. pneumoniae* 4200 po	*S. aureus* HH 127, sc
1	6.2	100	60	168	3.8
Cephalothin	46	>200	128	>400	2.9
Cephaloridine	3.6	60	17	132	0.8
Cephaloglycin	6.2[b]	25[b]	ND	ND	2.5[c]

[a]The ED_{50} values are expressed as the total dose of compound which afforded protection to 50% of the mice challenged (ip) with 6 LD_{50}'s of *E. coli* 12140, 60 LD_{50}'s of *K. pneumoniae* 4200, or 200 LD_{50}'s of *S. aureus* HH 127. The doses were administered subcutaneously in equally divided portions at 1 and 5 hr postinfection except in the *S. aureus* HH 127 test where a single dose at 1 hr postinfection was given. Values were calculated by the method of L. J. Reed and H. Muench, *Amer. J. Hyg.*, **27**, 493 (1938). [b]Data from separate experiment. Cephaloridine as a control gave ED_{50} values essentially the same as that reported in the table. [c]See footnote *b*; ED_{50} from oral dosing, 5.7 mg/kg.

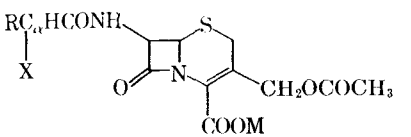
their *in vitro* activities (Table II). However, they have significant differences in their chemical and pharmacokinetic properties. Thus, in contrast to cephaloglycin, which is sufficiently unstable to require special techniques for bioassay,[5] the chemical stability of 1 is roughly equivalent to that of cephalothin. A second important difference between 1 and cephaloglycin is seen in the absorption of these cephalosporins on oral administration. As the ED_{50} values in Table IV imply, 1 is not appreciably absorbed when given by mouth and thus resembles more closely cephalothin and cephaloridine than cephaloglycin.

For the cephalosporins derived from the mandelic acids the effect of side-chain configuration on activity parallels that observed with the phenylglycine analogs.[7] The congeners having the *S* configuration on the side chain are considerably less active than their diastereomers having the *R* configuration, against both gram-positive and gram-negative bacteria (Table II).

Deletion of the acetoxy grouping from the 3 position reduces the *in vitro* activity of both the (*R*)-mandelic acid and (*R*)-phenylglycine analogs but the effect is more pronounced with the cephalosporins having the mandelic acid side chain. Thus, compound 1 compares favorably with cephaloglycin *in vitro*, but 7(*R*)-mandelamidodesacetoxycephalosporanic acid (3) is generally less active than cephalexin.

The broader structural requirements for antibacterial activity can be deduced from the data in Tables II and V. While introduction of an α-methoxyl group on the side chain of cephaloram (compound **15**) does not appreciably affect the activity against gram-positive bacteria, it severely lowers the activity against gram-negative organisms (compounds **13** and **14**, Table V). Introduction of a free-hydroxyl group (*R* configuration) at the α position (compound 1) likewise does not appreciably affect the activity against gram-positive organisms, but in this case the activity against gram-negative organisms is increased significantly. This differs from the introduction of an amino group at the α position (cephaloglycin) where the increase

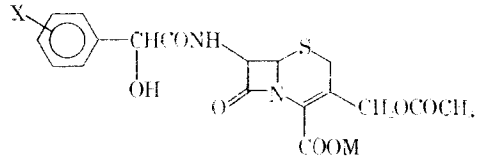
Table V



Compd	R[c]	X	Config at C_α	Procedure	Formula	Analyses	MIC, μg/ml[a] S.p.	S.a.	E.c.	K.p.	ED_{50}, mg/kg,[b] *E. coli* 12140
1, 2		OH	*RS*				**0**.2	1.6	3.1	3.1	
5		OH	*RS*	B	$C_{16}H_{15}N_2NaO_8S \cdot H_2O$	C, H, N	0.2	1.6	3.1	3.1	17
6[d]		OH	*RS*	B	$C_{16}H_{15}N_2NaO_7S_2 \cdot 2H_2O$	C, H, N	0.2	3.1	3.1	3.1	12.5
7		OH	*RS*	C	$C_{15}H_{15}N_3O_7S_2 \cdot C_6H_{13}N \cdot 1.5H_2O$	C, N; H[e]	<0.1	1.6	6.3	6.3	25
8	$C_6H_5CH{=}CH-$	OH	*RS*	A	$C_{20}H_{20}N_2O_7S \cdot C_6H_{13}N \cdot 0.5H_2O$	C, H, N	**0**.4	1.6	>200	>200	NT
9	$C_6H_5CH_2-$	OH	*R*	A	$C_{19}H_{19}N_2NaO_7S \cdot 0.5H_2O$	C, H, N	0.8	0.8	>200	>200	NT
10	$C_6H_5CH_2-$	OH	*S*	A	$C_{19}H_{19}N_2NaO_7S \cdot 1.5H_2O$	C, H, N	**0**.8	**6**.3	>200	>200	NT
11	CH_3-	OH	*RS*	A[f]	$C_{13}H_{16}N_2O_7S \cdot 0.5H_2O$	C, H; N[j]	**0**.8	**6**.3	50	50	>200
12	H–	OH		A[f]	$C_{12}H_{14}N_2O_7S \cdot 0.5H_2O$	C, H; N[k]	0.8	12.5	**200**	**200**	NT
13	3,4-Cl_2-C_6H_3-	OCH_3	*R*	A	$C_{19}H_{17}Cl_2N_2NaO_7S \cdot 0.5H_2O$	C, H; N[g]	**0**.2	0.4	>200	>200	NT
14	C_6H_5-	OCH_3	*R*	A	$C_{19}H_{19}N_2NaO_7S$	C, H, N		0.8	200	200	>200
15	C_6H_5-	H			Cephaloram[h,i]			**0**.4	12.5	12.5	>200

[a]See footnote *a*, Table II. Organisms selected for inclusion in this table are *S.p.*, *Streptococcus pyogenes* C203; *S.a.*, *Staphylococcus aureus* 23390 (Smith); *E.c.*, *Escherichia coli* 12140; *K.p.*, *Klebsiella pneumoniae* 4200. MIC's for *S. aureus* HH 127 (penicillin resistant), *E. coli* 33799, and *K. pneumoniae* 1200 were within one tube dilution of those reported for the organism of the same species. [b]See footnote *a*, Table IV. [c]Side-chain acids not referenced were obtained from commercial sources. Those cited here were prepared by published procedures as follows: **5**, H. Normant, *C. R. Acad. Sci.*, **225**, 580 (1947); **6**, S. Gronowitz, *Ark. Kemi*, **11**, 519 (1957); **7**, see Experimental Section; **8**, F. Nerdel and H. Rachel, *Chem. Ber.*, **89**, 671 (1956); **13**, see ref 2; **14**, D. G. Neilson and D. A. V. Peters, *J. Chem. Soc.*, 1519 (1962). [d]L. D. Crast, Jr., U. S. Patent 3,422,099 (1969). [e]H: calcd, 5.75; found, 5.11. [f]90% aqueous Me_2CO and Et_3N used to dissolve 7-ACA instead of the mixture described in procedure A. [g]N: calcd, 5.38; found, 4.30. [h]R. R. Chauvette, *et al.*, *J. Amer. Chem. Soc.*, **84**, 3401 (1962). [i]M. Jago, *Brit. J. Pharmacol.*, **22**, 22 (1964). [j]N: calcd, 7.93; found, 7.40. [k]N: calcd, 8.37; found, 7.87.

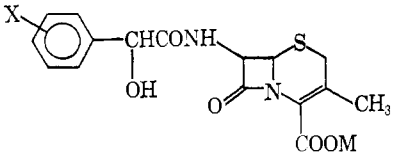
Table VI



Compd	X	Procedure	Formula	Analyses	MIC, μg/ml[a] S.p.	S.a.[c]	E.c.	K.p.	ED50, mg/kg,[b] E. coli 12140
1	H	A	$C_{18}H_{17}N_2NaO_7S \cdot 1.5H_2O$	C, H, N	<0.1	0.8	1.6	1.6	6.2
16	2-F	A	$C_{18}H_{16}FN_2NaO_7S \cdot 1.5H_2O$	C, H; N[c]	0.8	0.8	3.1	3.1	5.5
17	3-F	A	$C_{18}H_{16}FN_2NaO_7S \cdot H_2O$	C, H, N	<0.1	0.4	3.1	3.1	
18	4-F	A	$C_{18}H_{16}FN_2NaO_7S \cdot 2H_2O$	C, H, N	<0.1	0.8	3.1	6.3	22
19	2-Cl	A	$C_{18}H_{17}ClN_2O_7S \cdot C_6H_{13}N \cdot 0.5H_2O$	C, H, N	0.4	1.6	25	25	116
20	3-Cl	A	$C_{18}H_{16}ClN_2NaO_7S \cdot H_2O$	C, H, N	<0.1	0.4	12.5	12.5	94
21	4-Cl	A	$C_{18}H_{17}ClN_2O_7S \cdot C_6H_{15}N \cdot H_2O$	C, H, N	<0.1	0.4	25	25	200
22	4-Br	A	$C_{18}H_{16}BrN_2NaO_7S \cdot H_2O$	C, H, N	<0.1	0.8	50	50	
23	2-NO_2	A	$C_{18}H_{17}N_3O_9S \cdot C_6H_{13}N \cdot H_2O$	C, H; N[d]	0.2	1.6	25	25	>200
24	3-NO_2	A	$C_{18}H_{17}N_3O_9S \cdot C_6H_{13}N \cdot H_2O$	C, H, N	<0.1	0.4	25	25	70
25	4-NO_2	A	$C_{18}H_{17}N_3O_9S \cdot C_6H_{15}N \cdot 1.25H_2O$	C, H, N	0.2	0.4	50	25	
26	2-NH_2	C, D	$C_{18}H_{19}N_3NaO_7S \cdot 2H_2O$	C, H, N	0.2	1.6	12.5	6.3	
27	3-NH_2	C, D	$C_{18}H_{18}N_3NaO_7S \cdot 3.5H_2O$	C, H, N	0.4	3.1	3.1	3.1	7.4
28	4-NH_2	C, D	$C_{18}H_{18}N_3NaO_7S \cdot 2H_2O$	C, N; H[e]	0.2	1.6	25	12.5	140
29	3-OH[f]	B	$C_{18}H_{15}N_2O_8S \cdot C_6H_{15}N \cdot 0.5H_2O$	C, H; N[d]	0.8[f]	3.1[f]	3.1[f]	3.1[f]	22[f]
30	4-OH[f]	B	$C_{18}H_{18}N_2O_8S \cdot C_6H_{15}N$	C, H, N	0.2[f]	1.6[f]	3.1[f]	3.1[f]	29[f]
31	2-OCH_3[f]	A	$C_{19}H_{19}N_2NaO_8S \cdot 1.5H_2O$	C, H, N	1.6[f]	6.3[f]	200[f]	200[f]	
32	3-OCH_3	A	$C_{19}H_{19}N_2NaO_8S \cdot H_2O$	C, H, N	<0.1	0.8	12.5	6.3	32
33	4-OCH_3[g]	A	$C_{19}H_{20}N_2O_8S \cdot C_6H_{15}N \cdot 0.75H_2O$	C, H, N	0.2[h]	3.1[h]	25[h]	25[h]	11.6[h]
34	2-CH_3	A	$C_{19}H_{20}N_2O_7S \cdot C_6H_{13}N \cdot 0.5H_2O$	C, H, N	0.4	3.1	200	25	
35	3-CH_3	A	$C_{19}H_{19}N_2NaO_7S \cdot H_2O$	C, H, N	<0.1	0.8	25	12.5	88
36	4-CH_3	A	$C_{19}H_{20}N_2O_7S \cdot C_6H_{13}N \cdot H_2O$	C, H, N	<0.1	0.8	12.5	6.3	>50
37	3-CF_3	A	$C_{19}H_{16}F_3N_2NaO_7S \cdot H_2O$	C, H; N[i]	<0.1	0.4	100	50	
38	4-CF_3	A	$C_{19}H_{16}F_3N_2NaO_7S \cdot 0.5H_2O$	C, H, N	<0.1	0.4	200	100	
39	4-CH_2NH_2	B	$C_{19}H_{21}N_3O_7S \cdot 1.25H_2O$	C, N; H[j]	<0.1	1.6	12.5	3.1	9.6
40	3-$CONH_2$	C	$C_{19}H_{19}N_3O_8S \cdot C_6H_{17}N \cdot 3.25H_2O$	C; H,[k] N[k]	<0.1[f]	1.6[f]	12.5[f]	12.5[f]	72[f]
41	2,4-Cl_2	A	$C_{18}H_{15}Cl_2N_2NaO_7S \cdot 0.75H_2O$	C, H, N	0.2	0.8	200	200	
42	3,4-Cl_2	A	$C_{18}H_{16}Cl_2N_2O_7S \cdot C_6H_{15}N \cdot H_2O$	C, H, N	0.1	0.2	100	100	
43	2,6-Cl_2	A	$C_{18}H_{15}Cl_2N_2NaO_7S \cdot H_2O$	C, H, N	1.6	25	>200	>200	
44	3,4-OCH_2O	B	$C_{19}H_{18}N_2O_9S \cdot C_6H_{15}N \cdot 0.5H_2O$	C, H, N	<0.1	0.8	6.3	12.5	100
Cephalothin					<0.1	0.2	3.1	3.1	46

[a] See footnote *a*, Table V. [b] See footnote *a*, Table IV. In these tests the mice were challenged ip with 4–350 LD_{50}'s of *E. coli* 12140. [c] N: calcd, 5.92; found, 5.44. [d] N: calcd, 9.84; found, 9.34. [e] H: calcd, 4.62; found, 4.12. [f] The acylation was carried out with the racemic side-chain acid. The product was approximately a 1:1 mixture of diastereomers according to the nmr spectrum. [g] N: calcd, 7.89; found, 7.28. [h] The resolved side-chain acid racemized during acylation giving a 1:1 mixture of diastereomers (see footnote *f*). [i] N: calcd, 5.44; found, 4.97. [j] H: calcd, 4.94; found, 5.41. [k] H: calcd, 6.33; found, 5.88. N: calcd, 9.19; found, 8.68.

Table VII. 7-[(*R*)-(−)-Mandelamido]-3-desacetoxycephalosporanic Acids



Compd	X	Formula[b]	Analyses	MIC, μg/ml[a] *S. pyogenes*	*S. aureus*	*E. coli*	*K. pneumoniae*
3	H	$C_{16}H_{15}N_2NaO_5S \cdot H_2O$	C, H, N	0.4	3.1	12.5	6.3
45	4-F	$C_{16}H_{14}FN_2NaO_5S \cdot 1.5H_2O$	C, H, N	0.8	6.3	50	50
46	4-Cl	$C_{16}H_{15}ClN_2O_5S \cdot C_6H_{15}N \cdot H_2O$	C, N; H[c]	0.1	3.1	200	200
47	4-Br	$C_{16}H_{15}BrN_2NaO_5S \cdot H_2O$	C, H, N	<0.1	1.6	200	>200
48	4-NO_2	$C_{16}H_{15}N_3O_7S$	C, H, N	0.1	3.1	>200	>200
49	4-OCH_3[d]	$C_{17}H_{18}N_2O_6S \cdot C_6H_{15}N \cdot H_2O$	C, N; H[e]	1.6[d]	6.3[d]	>200[d]	>200[d]
50	2,4-Cl_2	$C_{16}H_{14}Cl_2N_2O_5S \cdot C_6H_{13}N \cdot H_2O$	C, H, N	1.6	12.5	>200	>200
51	3,4-Cl_2	$C_{16}H_{14}Cl_2N_2O_5S \cdot C_6H_{15}N \cdot H_2O$	C, N; H[f]	0.1	1.6	>200	>200

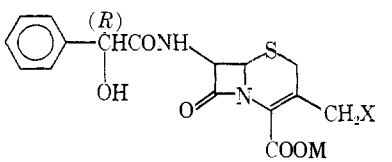
[a] See footnote *a*, Table V. [b] All cephalosporins in this table were prepared by procedure **A**. [c] H: calcd, 6.51; found, 5.90. [d] See footnote *h*, Table VI. [e] H: calcd, 7.08; found, 6.51. [f] H: calcd, 5.82; found, 5.36.

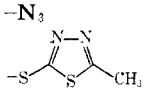
in activity against gram-negative organisms is accompanied by a decrease against the gram-positive ones. The low antibacterial activities of the lactic and glycolic acid derivatives of 7-ACA (compounds **11** and **12**) indicate the need for an electron-rich moiety such as a benzene or aromatic heterocyclic ring (compounds **5**–**7**). However, the conjugated aromatic styrene system cannot serve this purpose (compound **8**). As with other 7 substituents which confer broad-spectrum activity on the structure, homologation of the acetic acid moiety results in loss of the gram-negative activity (compounds **9** and **10**). It should be noted that several of the cephalosporins used in this comparison (Table V) were synthesized from the racemic side-chain acids, and the products were obtained as 1:1 mixtures of the resulting diastereomers. From the biological activity of **1** and **2** the MIC and ED_{50} values of these mixtures would be expected to be approximately double those of the pure *R* diastereomer since the diastereomer with the *S* configuration in the side chain is, in all cases known to us, significantly less active than its isomer. However, in order to make these comparisons as accurate as possible, the appropriate test values obtained from an artificial 1:1 mixture of **1** and **2** are included in Table V.

Because of the encouraging biological activity of **1**, a number of derivatives and analogs of this cephalosporin were made and tested. The 7-mandelamidocephalosporanic acids with substituents on the benzene ring are listed in Table VI. All but five had side chains in the *R* configuration only. For the five tested as diastereomeric mixtures, the preceding considerations regarding the effect on the biological test values apply. In general, the test results from these compounds indicate that most substitutions, whether ortho, meta, or para, lower the activity against the gram-negative organisms; the activity against the gram-positive bacteria is less affected. Ortho substitution is the most deleterious. With nonionizable substituents (CH_3, OCH_3, F, Cl, Br, CF_3, etc.) the variation in activity appears to be more closely related to bulk of the substituent than its electronic contribution to the chemical system. Thus, the fluoro derivatives of **1** are the most active of these analogs, ranking with the hydroxy (meta and para) and *m*-amino derivatives. Although these several latter analogs have activities approaching that of **1**, none are better in overall profile (MIC and ED_{50}).

The effects of 3-substituent variation on biological activity are summarized in Tables VII and VIII. Table VII merely documents further the relatively large differences in activity observed between the 7-ACA and 7-ADCA derivatives for this series of compounds. The combination of deletion of the 3-acetoxy group and benzene ring substitution drastically lowers the activity of this chemical system against gram-negative organisms. On the other hand, replacement of the 3-acetoxy group by other commonly used substituents results in analogs with activities comparable to **1** (Table VIII). The relatively good ED_{50} against *E. coli* of the 3-pyridinium derivative **52** in spite of its poor MIC

Table VIII



Compd	X	MIC, μg/ml[a] *S.p.*	*S.a.*	*E.c.*	*K.p.*	ED_{50}, mg/kg,[b] *E. coli* 12140
1	–OAc	<0.1	0.8	1.6	1.6	6.2
3	–H	0.4	3.1	12.5	6.3	25
52	$-{}^{+}NC_5H_5$	<0.1	0.4	12.5	12.5	8.8
53	$-N_3$	<0.1	0.4	3.1	1.6	7.0
54		<0.1	0.4	1.6	0.8	6.2

[a] See footnote *a*, Table V. [b] See footnote *a*, Table IV.

implies improved pharmacokinetic handling often seen with this compound type (*e.g.*, cephaloridine[6]), although we have not verified this by serum level determinations. Substitution of azide for the acetoxy group produces a cephalosporin almost identical with 1 in its antimicrobial activities, while displacement of the acetoxy group by selected heterocyclic thiols results in an improvement in activity as exemplified by compound **54**. This improvement in the latter case will become more evident as additional data are reported for this compound type.[8]

Experimental Section

Melting points were determined in open capillary tubes using, of course, a Thomas-Hoover Uni-Melt apparatus. The melting points of the cephalosporins are not accurately reproducible because these compounds melt with extensive decomposition. 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. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values. $MgSO_4$ was used as drying agent for organic extracts.

Preparation and Resolution of Side-Chain Acids. The resolved mandelic acids used in these syntheses are listed in Table I. With two exceptions (the 4-aminomethyl and 3-carboxamido derivatives) all of the racemic acids had been described previously in the literature. The (*R*)- and (*S*)-mandelic acids are commercially available. The 2-F, 3-Cl, 3-NO_2, 2-CH_3, 4-CH_2NH_2, and 2,4-Cl_2 mandelic acids were acquired as the *R* enantiomers (Table I, footnote *b*). The others were purchased as racemic mixtures or were synthesized from the corresponding aldehyde *via* the cyanohydrin.[9] Resolutions were accomplished by serial recrystallizations of the salt of the desired acid with the optically active base indicated in Table I. The optical rotations of the freed acids are reported in the table. Corresponding values were not always available from the literature. When rotations were reported there was good agreement with the values obtained.

With the exception of 5-isothiazoleglycolic acid, described below, the side-chain acids used in the synthesis of the cephalosporins listed in Table V were either obtained from commercial sources or prepared by published procedures. References to the latter are included in the footnotes to the table.

4-Aminomethylmandelic Acid. A mixture of α-bromo-*p*-tolualdehyde (15.4 g, 0.078 mol) and potassium phthalimide (26.4 g, 0.14 mol) in 140 ml of dry DMF was heated on a steam bath for 1.75 hr. After cooling to room temperature the solution was diluted with H_2O (180 ml) and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with 5% $NaHCO_3$ and then with H_2O, dried, and evaporated *in vacuo* to give after trituration with ether 13.5 g (65%) of **4-formylbenzylphthalimide**, mp 143–146°. This was dissolved in 130 ml of acetone cyanohydrin containing 2 ml of Et_3N and the mixture was stirred for 18 hr at room temperature. The solid was collected, washed with Et_2O, and dried *in vacuo* to give 12.8 g (86%) of the cyanohydrin which was suspended in 130 ml of concentrated HCl and the mixture heated at reflux for 8 hr. The solution was cooled to room temperature and extracted with EtOAc, and the aqueous layer was evaporated *in vacuo* to give 21.7 g of a white solid. This solid was dissolved in 50 ml of H_2O, and the cold solution was adjusted to pH 4.6 with 5% $NaHCO_3$ and, after stirring at 5° for 4 hr, the solid was collected and dried to give 5.0 g (52%) of **4-aminomethylmandelic acid**, mp >260°. *Anal.* ($C_9H_{11}NO_3$) C, H, N.

4-*tert*-Butoxycarboxamidomethylmandelic Acid. A stirring mixture of 4-aminomethylmandelic acid (12.2 g, 0.067 mol), *tert*-butylazidoformate (19.4 g, 0.14 mol), and MgO (5.5 g, 0.14 mol) in 120 ml of H_2O and 200 ml of dioxane was heated at 44° for 23 hr. The mixture was cooled to room temperature, diluted with H_2O (700 ml) and EtOAc (700 ml), and filtered. When no product was obtained following work-up of the aqueous phase the filter cake of magnesium salts was suspended in a mixture of H_2O (750 ml) and EtOAc (750 ml) and acidified to pH 1.5 with 3 *N* HCl. After separation, the aqueous layer was extracted twice more with EtOAc. The combined, dried EtOAc extracts were evaporated *in vacuo* and the crude solid was recrystallized from 1:1 $CHCl_3$–CCl_4 to give 15.5 g (82%), mp 165–167° dec. *Anal.* ($C_{14}H_{19}NO_5$) C, H, N.

5-Isothiazoleglycolic Acid Cyclohexylamine Salt. Lithium tri-*tert*-butoxyaluminum hydride (11.4 g, 0.045 mol) was added gradually over a 30-min period under N_2 to a cold (−35°) solution of ethyl 5-isothiazoleglyoxylate (5.6 g, 0.03 mol) in diglyme (90 ml) and Et_2O (90 ml). Stirring was continued for an additional 30 min at −25°. Water (75 ml) was added slowly and the mixture was stirred for 1 hr without cooling. The reaction mixture was poured into H_2O (450 ml) and the mixture was extracted with Et_2O. The aqueous layer was acidified with dilute HCl and extracted with Et_2O and the combined, dried extracts were evaporated *in vacuo*. The residual yellow oil was dissolved in Me_2CO and treated with cyclohexylamine to give 3.85 g of crude salt. This was recrystallized from MeOH–Me_2CO–petroleum ether to give 2.3 g (38%) of pure salt, mp 197–199°. *Anal.* ($C_5H_5NO_3S$·$C_6H_{13}N$) C, H, N. For acylation the salt was converted to the free acid and the crude product was used without further purification.

Preparation of Dichloroacetylmandeloyl Chlorides. A mixture of the appropriate mandelic acid (0.01 mol) and dichloroacetyl chloride (0.03 mol) was heated at 80–85° for 1.5 hr and the excess dichloroacetyl chloride was then removed *in vacuo*. The residue was dissolved in 2.5 ml of $SOCl_2$ and the solution refluxed for 1.5 hr. After removing the excess $SOCl_2$ *in vacuo*, dry C_6H_6 was added and the evaporation was repeated. The residual acid chloride was kept under high vacuum for several hours to ensure complete removal of dichloroacetyl chloride (absence of peak at δ 6.72 in the nmr spectrum). Purity and identity were verified *via* the ir and nmr spectra of the sample which was used for the subsequent acylation without further purification.

Preparation of 7-Mandelamidocephalosporanic Acids. Procedure A. The appropriate dichloroacetylmandeloyl chloride (0.01 mol) in 15 ml of Me_2CO was added over a 15-min period to a cold (−20°) stirred solution of 7-ACA (0.01 mol) in 50 ml each of Me_2CO and 5% $NaHCO_3$. The reaction mixture was stirred at −20° for 30 min and then allowed to warm to room temperature (with stirring) over a period of 2 hr. The solution was brought to pH 9–9.5 and maintained at that pH for 30 min (room temperature) by adding 5% Na_2CO_3 as required. After extraction with Et_2O the aqueous phase was cooled to 5°, layered with EtOAc, and acidified (pH 1.5) with 6 *N* HCl. The aqueous layer was extracted twice more with EtOAc and the combined EtOAc extracts were dried and evaporated *in vacuo*. The residual syrup was precipitated three times from acetone using petroleum ether (to remove residual dichloroacetic acid) to give a semisolid residue. Crude yields at this stage ranged from 35 to 80%.

The crude acid was purified by conversion to an appropriate salt which was subsequently reprecipitated from various solvents when necessary. The sodium salts were obtained by titrating a cold (10°) methanol solution of the acid to pH 6.8 with 5% $NaOCH_3$ in methanol. After dilution with 10 vol of ether the solid was collected, washed with Et_2O, and dried over P_2O_5 *in vacuo*. When needed, as indicated by tlc, ir, or nmr, the salt was dissolved in methanol and reprecipitated with ether. In a few cases the sodium salt was obtained by adding a 1.4 *N* solution of sodium 2-ethylhexanoate in isopropyl alcohol to the acid dissolved in the alcohol with subsequent addition of ether to complete the precipitation.

The triethylamine salts were prepared by treating the crude acid in MIBK with an excess of dry Et_3N followed by excess ether. Subsequent purification, when necessary, was accomplished by recrystallization from $CHCl_3$–Et_2O or $CHCl_3$–petroleum ether. The cyclohexylamine salts were made by an analogous procedure sometimes using ethyl acetate in place of the MIBK.

Procedure B. A solution of the appropriate mandelic acid (0.018 mol), *N*-trimethylsilylacetamide (0.02 mol), and Et_3N (0.018 mol) in 50 ml of dry THF was refluxed (under N_2) for 2 hr. Isobutyl chloroformate (0.018 mol) was added dropwise to the cooled (−10°) solution over a 20-min period. After an additional 20 min at −10° a solution of 7-ACA (0.018 mol) and Et_3N (0.018 mol) in THF and H_2O (30 ml each) was added over 30 min (−5 to −10°). The mixture was stirred at 0° for 1 hr and then at room temperature for 1.5 hr. After concentration *in vacuo* the aqueous residue was treated with 3% $NaHCO_3$ (to dissolve precipitated 7-ACA), diluted with H_2O (50 ml), and extracted with Et_2O. The aqueous solution was cooled (5–10°), layered with EtOAc, and adjusted to pH 1.5. The aqueous phase was reextracted twice with EtOAc and the combined extracts were dried and evaporated *in vacuo* to give the crude acid (20–50% yield) as a gum. This was converted to the appropriate salt as described in procedure A.

Procedure C. A mixture of the mandelic acid (0.01 mol) and dichloroacetyl chloride (3 ml) was heated at 80–85° for 3 hr. The resulting clear solution was evaporated *in vacuo* and the residue was pumped under high vacuum at 45° for 18 hr. The protected

acid was dissolved in Et_2O (80 ml); 7-ACA *tert*-butyl ester (3.3 g, 0.01 mol) was added, followed by DCCI (2.1 g, 0.01 mol). The mixture was stirred at room temperature for 5 hr. The precipitated DCU was removed and the filtrate was evaporated *in vacuo*. The semisolid residue was dissolved in Et_2O and precipitated with petroleum ether; the resulting solid was dried *in vacuo* to give the 7-mandelamidocephalosporanic acid protected as the *tert*-butyl and dichloracetyl diester (75–95% crude yield). The diester (0.005 mol) was dissolved in cold TFA (25 ml) and the solution was stirred at room temperature for 45 min. The TFA evaporated *in vacuo* and the residue was treated with 5% aqueous Na_2CO_3 (pH 9.5) for 1 hr and worked up as described under procedure A.

Procedure D. Preparation of 7-(Aminomandeloyl)cephalosporanic Acids (Compounds 26–28). The appropriate 7-(dichloroacetylnitromandeloyl)cephalosporanic acid *tert*-butyl ester (0.01 mol) prepared by procedure C was dissolved in MeOH (300 ml) containing 10 g of prereduced 5% Pd/C and hydrogenated on a Parr apparatus at 3.5 kg/cm² for 3 hr. The mixture was filtered and evaporated *in vacuo* and the residue was triturated with Et_2O. The resulting solid was dissolved in 20 ml of TFA for 30 min at room temperature. The excess TFA was evaporated and the residual TFA salt of the product was triturated with Et_2O. It was converted to the sodium salt with NaOMe as described in procedure A.

7-[(*R*)-(−)-Mandelamido]-3-(*N*-pyridiniummethyl)-3-cephem-4-carboxylate (52). This compound was prepared by displacement of the acetoxy group of 1 with pyridine using the method described by Arkley, *et al.*[10] *Anal.* ($C_{21}H_{19}N_3O_5S \cdot 2.5H_2O$) C, H, N.

Sodium 7-[(*R*)-(−)-Mandelamido]-3-azidomethyl-3-cephem-4-carboxylate (53). This compound was prepared in 35% yield by procedure A substituting 7-amino-3-azidomethyl-3-cephem-4-carboxylic acid[4] for 7-ACA. *Anal.* ($C_{16}H_{14}N_5NaO_5S \cdot 1.25H_2O$) C, H; N: calcd, 16.14; found, 15.66.

Sodium 7-[(*R*)-(−)-Mandelamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-3-cephem-4-carboxylate (54). A mixture of 1 (4.55 g, 0.01 mol) and 5-methyl-1,3,4-thiadiazole-2-thiol (1.32 g, 0.01 mol) in 100 ml of pH 6.4 phosphate buffer was heated at 60° with stirring for 21 hr. The cooled (25°) mixture was layered with EtOAc and acidified to pH 2 with dilute HCl. The aqueous phase was extracted twice more with EtOAc and the combined dried extracts were evaporated to give 4.9 g solid. Chromatography on silica gel using a $CHCl_3$–MeOH gradient gave 2.91 g of the cephalosporin free acid. Treatment of an acetone solution of this acid with a slight excess of 30% sodium 2-ethylhexanoate in isopropyl alcohol gave 2.3 g (44%) of the sodium salt. *Anal.* ($C_{19}H_{18}N_4NaO_5S_3 \cdot H_2O$) C, H, N.

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References

(1) J. R. E. Hoover, U. S. Patent 3,167,549 (1965).
(2) H. Vanderhaeghe, P. van Dijck, M. Claesen, and P. DeSomer, *Antimicrob. Ag. Chemother.*, 581 (1961).
(3) R. J. Stedman, *J. Med. Chem.*, **9**, 444 (1966).
(4) L. A. Wetherill, W. Graham, and M. J. Covil, British Patent 1,104,938 (1968).
(5) W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 248 (1965).
(6) J. P. Currie, *Postgrad. Med. J., Suppl.*, **43**, 22 (1967).
(7) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746 (1966).
(8) W. E. Wick and D. A. Preston, *Antimicrob. Ag. Chemother.*, **3**, 221 (1972).
(9) B. B. Corson, R. A. Dodge, S. A. Harris, and J. S. Yeaw, "Organic Syntheses," Collect. Vol. I, H. Gilman, Ed., Wiley, New York, N. Y., 1941, p 336.
(10) V. Arkley, S. Eardley, and A. G. Long, British Patent 1,028,563 (1966).

Differential Effects of Benzodioxane, Chroman, and Dihydrobenzofuran Analogs of Clofibrate on Various Parameters of Hepatic Drug Metabolism†,‡

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The influence of pretreatment with clofibrate (1) and related analogs on various parameters of hepatic drug metabolism in rats was investigated. Of the analogs tested, only ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (4) and ethyl 6-chlorochroman-2-carboxylate (6) possessed inductive properties similar to 1. Increases were noted in the activities of ethylmorphine *N*-demethylase and/or aniline hydroxylase and cytochrome P-450 in liver microsomes obtained from animals pretreated with 1, 4, and 6. Deschloro analogs (5 and 7) of 4 and 6 were ineffective as hepatic enzyme inducers. Presence of a chloro substituent was favorably correlated to the inductive properties noted within this series of compounds.

We recently described a comparative analysis of the hypocholesterolemic and hypotriglyceridemic activities of a number of cyclic ethyl ester analogs of clofibrate [ethyl 2-methyl-2-(4-chlorophenoxy)propionate, 1].[1] A rat model was utilized in which hyperlipemia was induced by intraperitoneal injection of Triton WR-1339. Under these conditions, clofibrate (1), ethyl 1,4-benzodioxane-2-carboxylate (2), ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (4), and ethyl 6-chlorochroman-2-carboxylate (6) significantly reduced serum cholesterol levels. The deschloro analogs 5 and 7 were inactive. In these same hyperlipemic rats, esters 1, 2, and 6 significantly reduced plasma triglyceride levels. Deschloro analogs 5 and 7 were inactive and the apparent hypotriglyceridemic effect of chloro analog 4 was not statistically significant. Chlorochroman ana-

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